Lecture Supplements

Plant Bacteriology

Bacterial Pathogenesis-Part 2

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Pathogenicity islands (PAIs)

Pathogenenicity islands are discrete genetic loci that encode factors which make a microbe more virulent

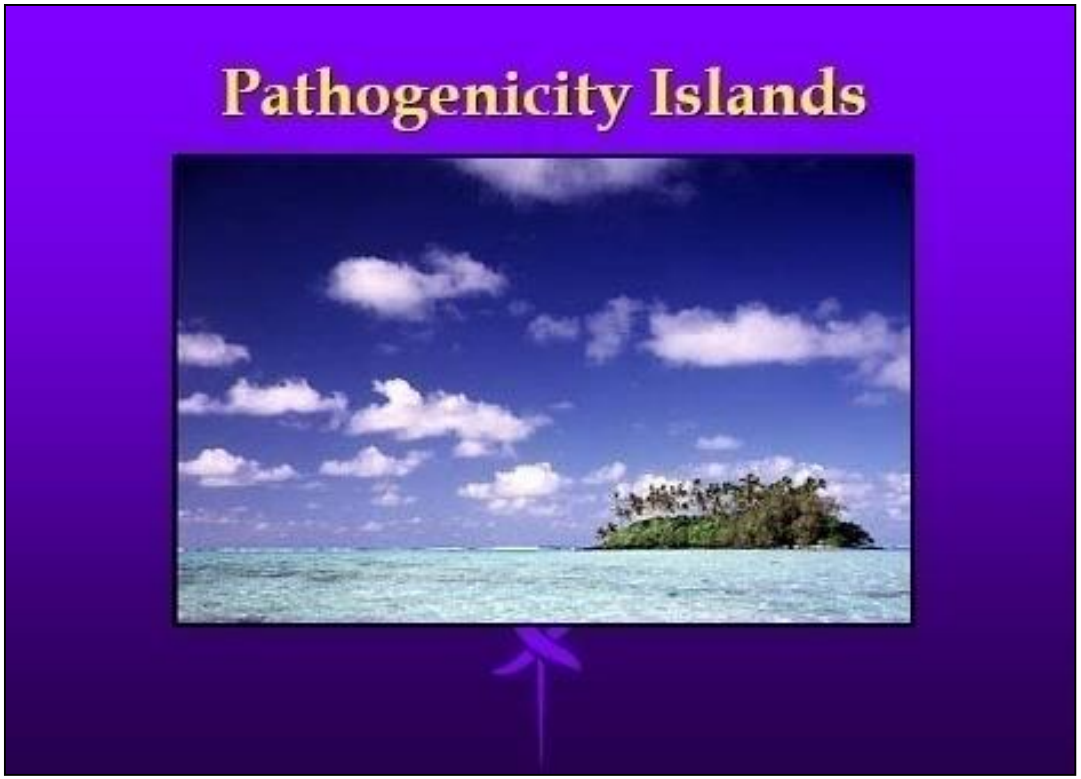
The genetic element, the "island of evil", within the genome of an organism that is responsible for its capacity to cause disease.

Identification of PAIs is essential in understanding the development of disease and the evolution of bacterial pathogenesis.

Pathogenicity Islands (PAIs)

A distinct class of genomic islands acquired by microorganisms through horizontal gene transfer

- The islands of pathogenic bacteria affecting and causing diseases on plants, animals and humans.



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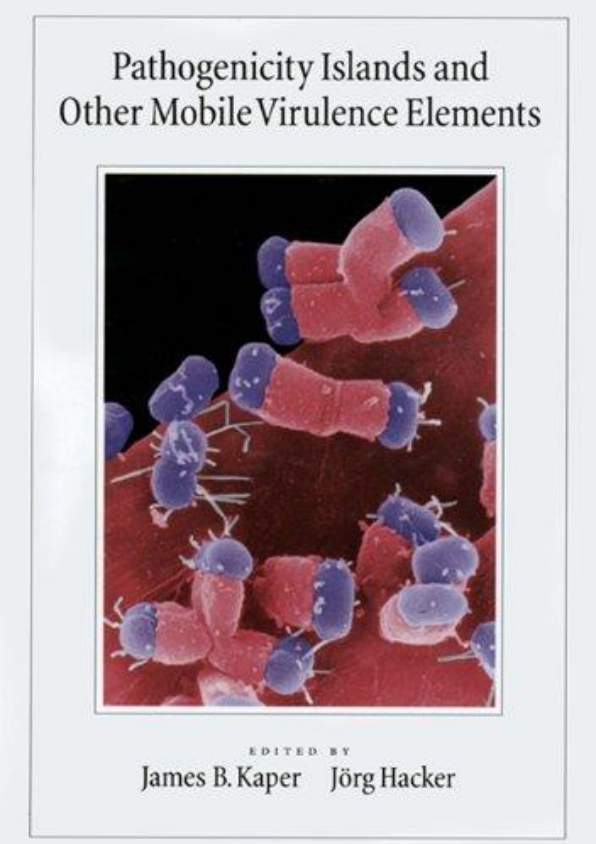

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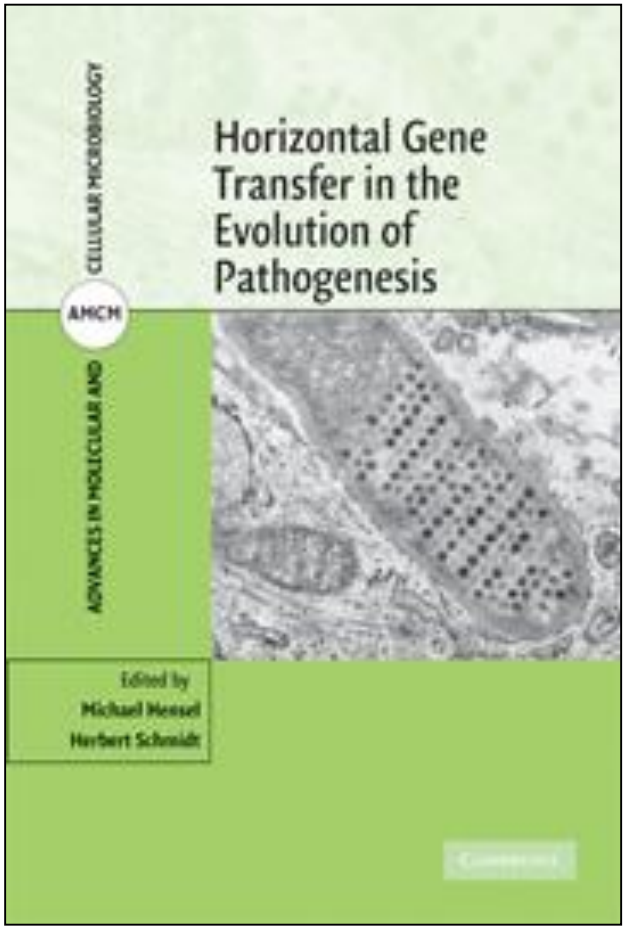

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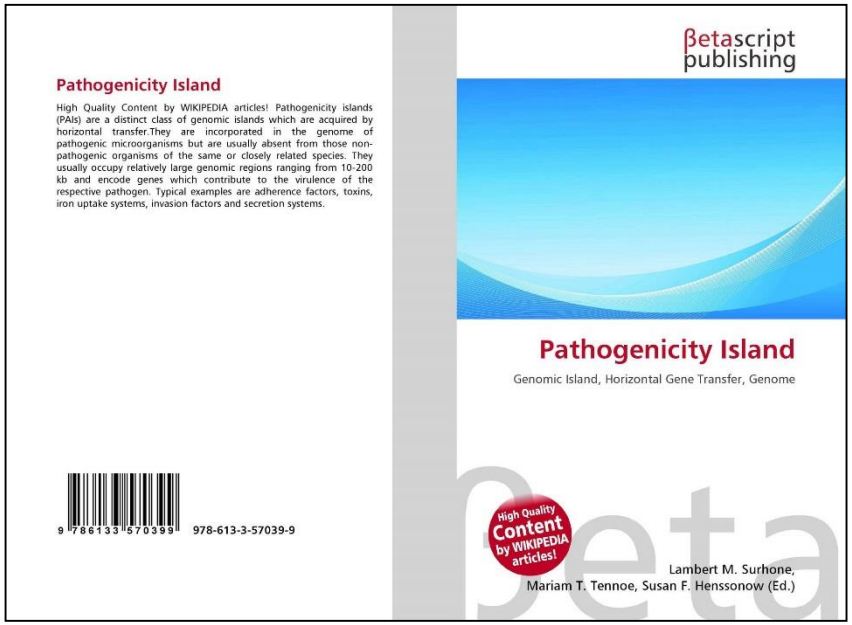

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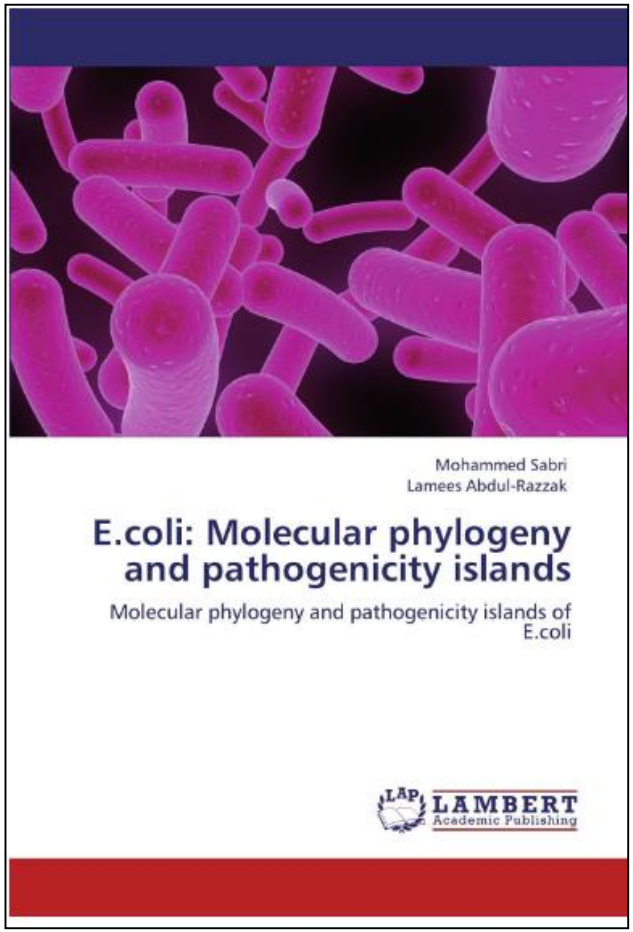

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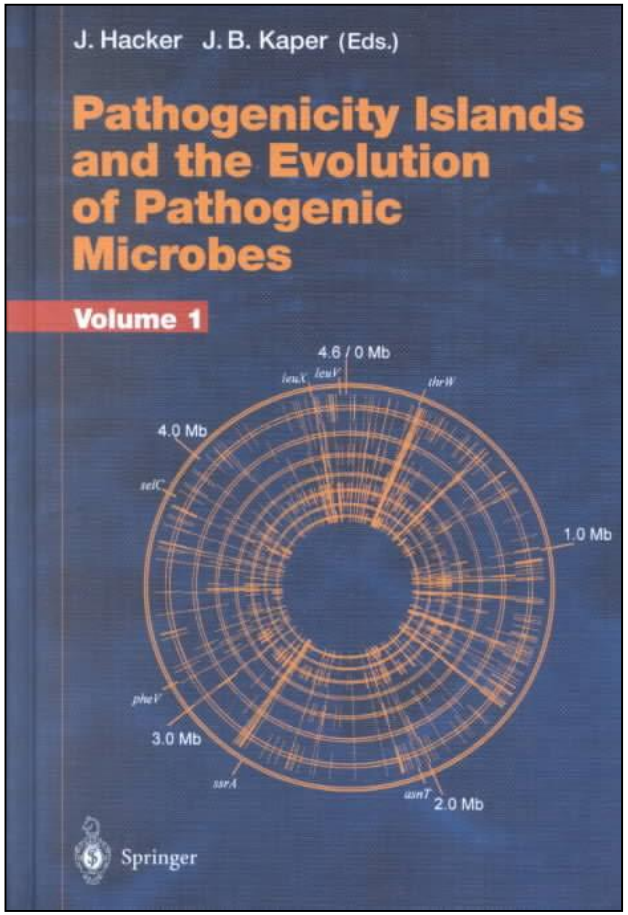

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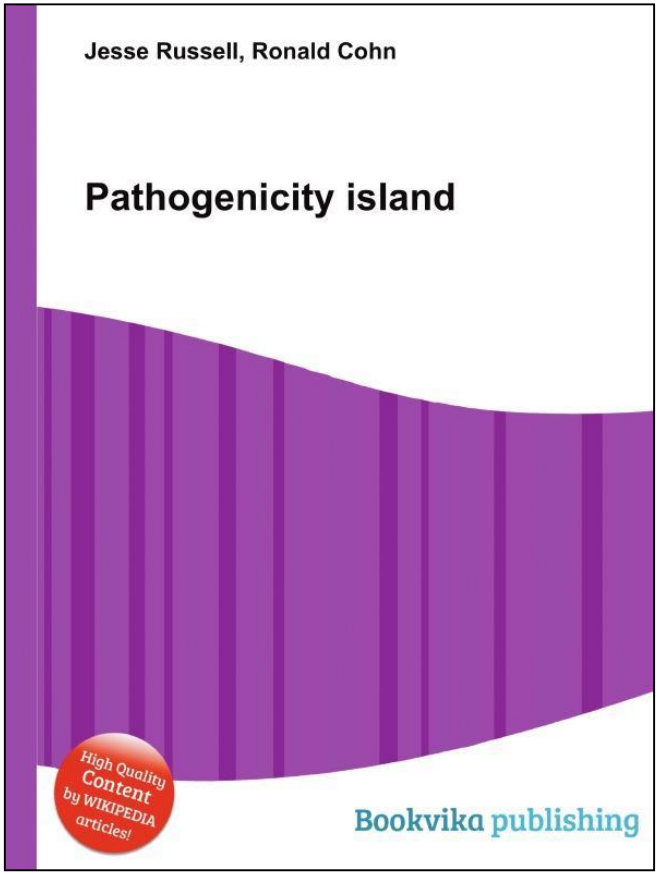

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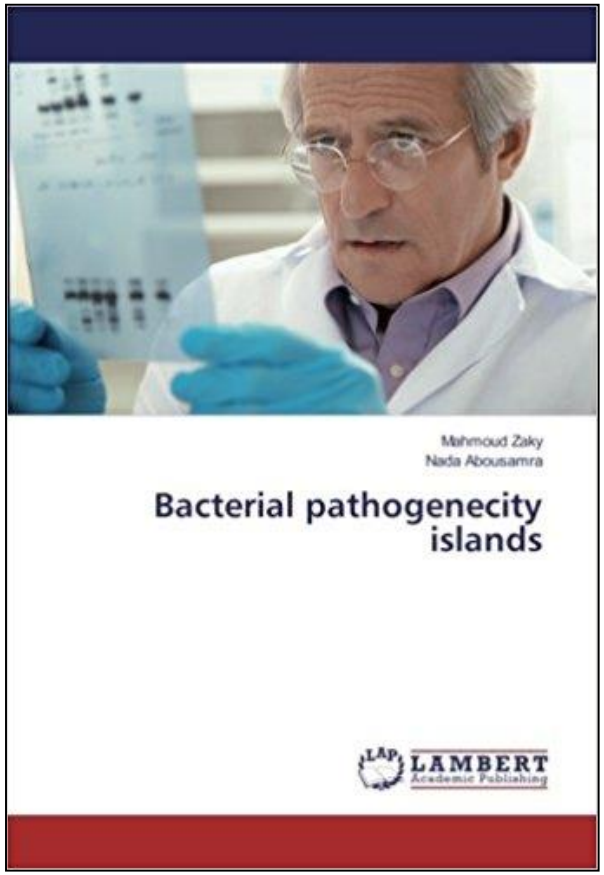

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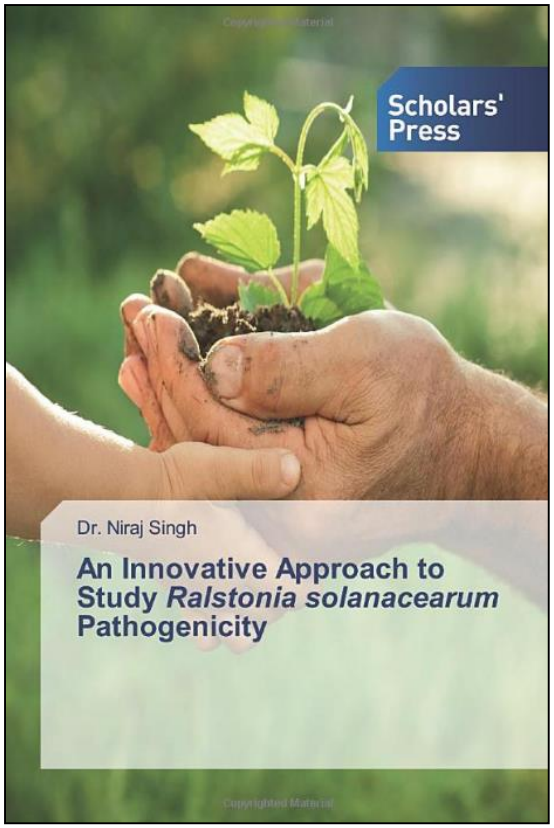

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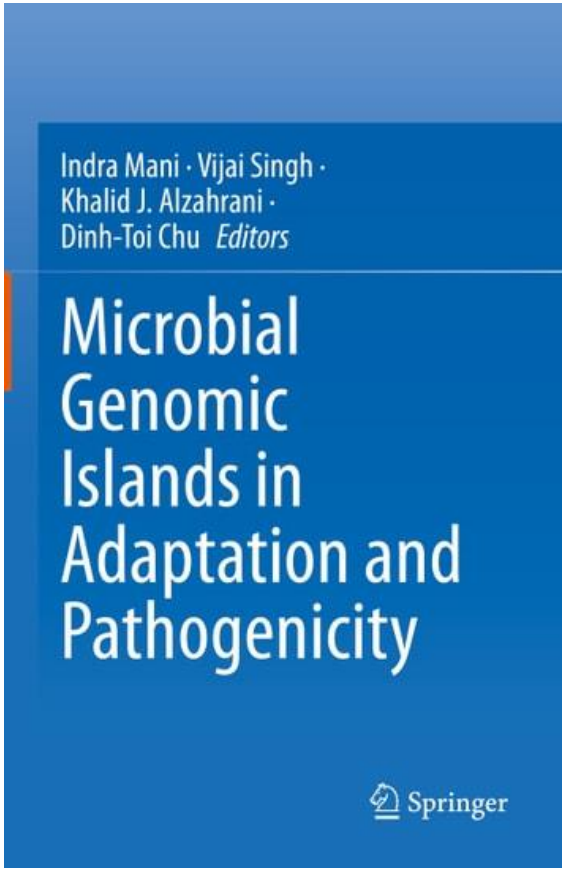
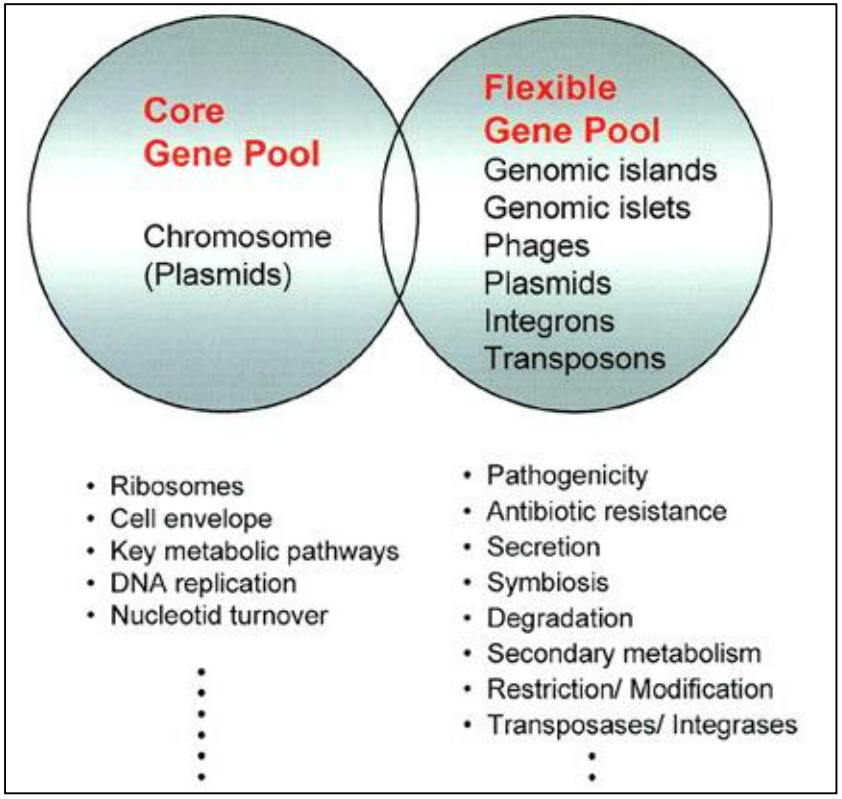


Prokaryotic genomes

DNA pools in the genomes of prokaryotes

Core and Flexible gene pools

- Proposed model of the DNA pools in the genomes of prokaryotes.
- Most of the horizontally transferred DNA is part of the "flexible" gene pool.
- Some functions encoded by the pools are given in the lower part of the diagram.
- Turnover metabolism is a dynamic process. The cell is continuously degrading and synthesizing molecules.



Model of the DNA pools in the genomes of prokaryotes. The DNA elements comprising the core as well as the flexible gene pools are presented in the circles. Functions encoded by the pools are given in the lower part of the diagram. Core genome: the genes shared by all members of a pre-defined group of bacteria or archaea. Flexible genome may also be referred to as the accessory, variable, dispensable, auxiliary, noncore, adaptive or distributed genome.

Hacker and Carniel,2001; Scortichini,2005

Genome of prokaryotes
Genomic islands (GISs)

- Genomic islands (GEIs) are clusters of genes within a bacterial genome that appear to have been acquired by horizontal gene transfer.
- GEIs often carry genes offering a selective advantage for host bacteria.
- To this date, several dozen genomic islands have been described and it is expected that many more await discovery.
- In 50% of the marine bacterial genomes analyzed the GIs accounted for approximately 3% of the genome length, with a maximum of 12%.

Genome size in *Escherichia coli* is 4.6 million base pairs (Mbp)=4600 KB.

Genome of prokaryotes
Genomic islands (GISs)

- The distinct properties of GIs and that they allow bacterial organisms to evolve and adapt to different environments.
- **The genes present in GIs are typically grouped to perform specific and advantageous functions in the bacteria.**
- PAIs, for example, can cause major changes in the bacterial phenotype. Thus, they are the most studied Gis.
- **GI number per genome was strongly and positively correlated with the total GI size.**

Genome of prokaryotes
Subgroup of genomic islands (GISs)

- The large number of sequenced genomes and analyses of genetic sequences have revealed that GIs are mosaics of genes formed by HGT.
- Genomic islands (GEIs) encoded some islands.
- These include:

1. Pathogenicity islands (PAIs)
2. Antimicrobial resistance islands (REIs)
3. Symbiosis islands
4. Pathogen/ecological fitness islands

Also, it can be of other functions like carbon and nitrogen sources utilization (metabolic islands).

Genome of prokaryotes
Genomic islands (GISs)

- Gnomic island (GI) is usually used in microbiology, especially with regard to bacteria.
- A genomic island (GI) is part of a genome that has evidence of horizontal origins.
- These "islands" are characterized by:

1. their large size (>10 Kb),
2. their frequent association with tRNA-encoding genes, and
3. A different G+C content compared with the rest of the genome.

Wikipedia,2016

Genomic islands

Genomic islands encode different functions

Genomic or metabolic islands and smaller inserts

- **Genomic islands (GIs):**

1. Large segments (10-200 kb) of foreign DNA inserted into bacterial chromosomes are often referred to as genomic islands (GEIs).
2. Smaller inserts (1-10kb) have also been found and may be termed genomic islets.

- Genomic island and its subgroup PAIs are part of that flexible gene pool.
- Genomic elements with characteristics similar to PAI but lacking virulence genes are referred to as genomic or metabolic islands.

Van Sluys *et al.*,2002; Hentschel and Hacker,2001

Genomic islands

Genomic islands encode different functions

1. Many genomic islands (GEIs) are flanked by repeat structures and carry fragments of other mobile and accessory genetic elements, such as bacteriophages, plasmids and insertion sequence (IS) elements.

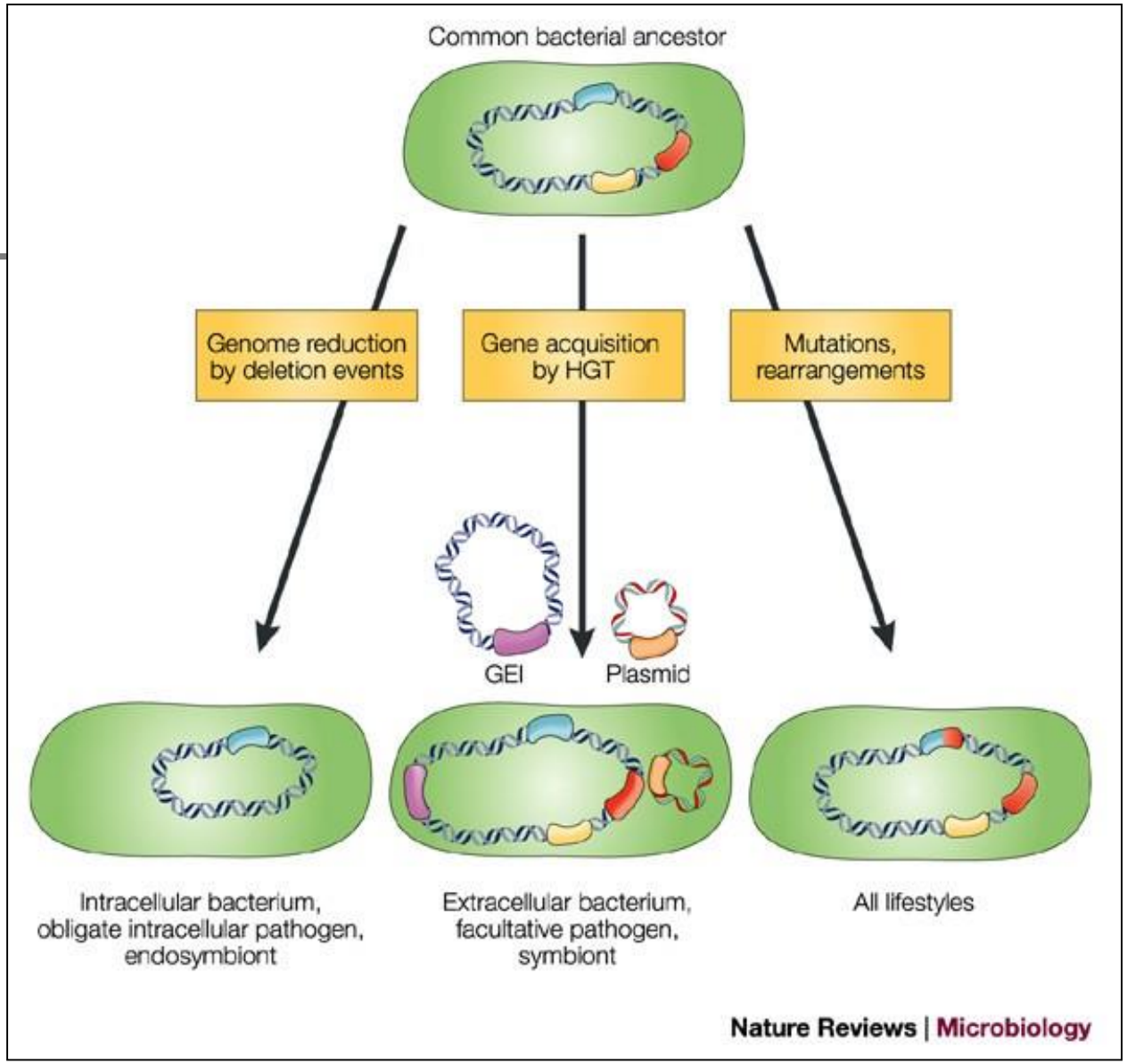
- These functional or cryptic genes encoding integrases or factors related to plasmid conjugation systems or phages involved in GEI transfer.

1. But some genomic islands can **excise themselves spontaneously from the chromosome** and can be transferred to other suitable recipients.

- A hypothetical 'life cycle' of GEIs includes the insertion of mobile genetic elements into the bacterial chromosome.

Dobrindt *et al.*,2004; Wikipedia,2016

Genomic islands encode different functions.



Dobrindt *et al.*,2004

Genomic islands

Genomic islands encode different functions

- Genomic islands present in the majority of genomes of pathogenic as well as nonpathogenic bacteria and may encode many different functions.
- The size of genomic islands may vary from a few Kb to as many as 200 Kb.

1. A different G-C content compared with the rest of the chromosome; biased codon usage.
2. often mobile (genomic islands have been acquired by horizontal gene transfer, HGT).

- GIs encode accessory functions such as metabolic activities, antibiotic resistance, or properties involved in microbial fitness, symbiosis, or pathogenesis.

Campbell and co-workers;..

Genomic islands

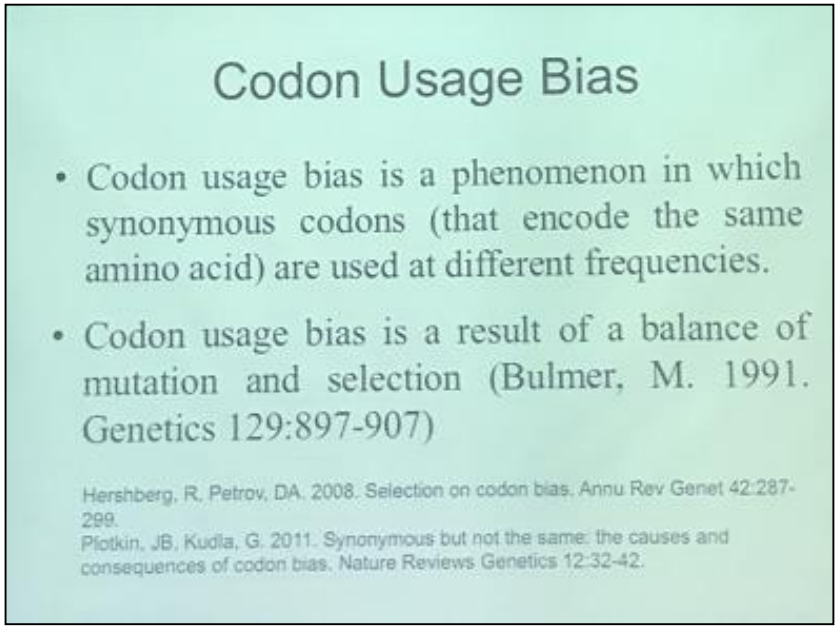
Codon usage bias is a result of a balance of mutation and selection. The degree of codon usage bias for each gene/bacterium is different

- A large amount of varied ways, all amino acids (aa) are coded by two to six synonymous codons, except Met and Trp.
- This biased use of codons has been observed in all branches of life.
- The redundancy of the genetic code is due to multiple codons which encode the same amino acid.
- Codon bias is the result of long-term selection and is presumed to confer an evolutionary advantage.
- Codon usage bias is selected to optimize the speed of protein production.

Genomic islands

Codon usage bias is a result of a balance of mutation and selection. The degree of codon usage bias for each gene/bacterium is different

- A codon is a series of three nucleotides (a triplet) that encodes a specific amino acid residue in a polypeptide chain or for the termination of translation (stop codons).
- The overabundance in the number of codons allows many amino acids to be encoded by more than one codon.
- Because of such redundancy it is said that the genetic code is degenerate.

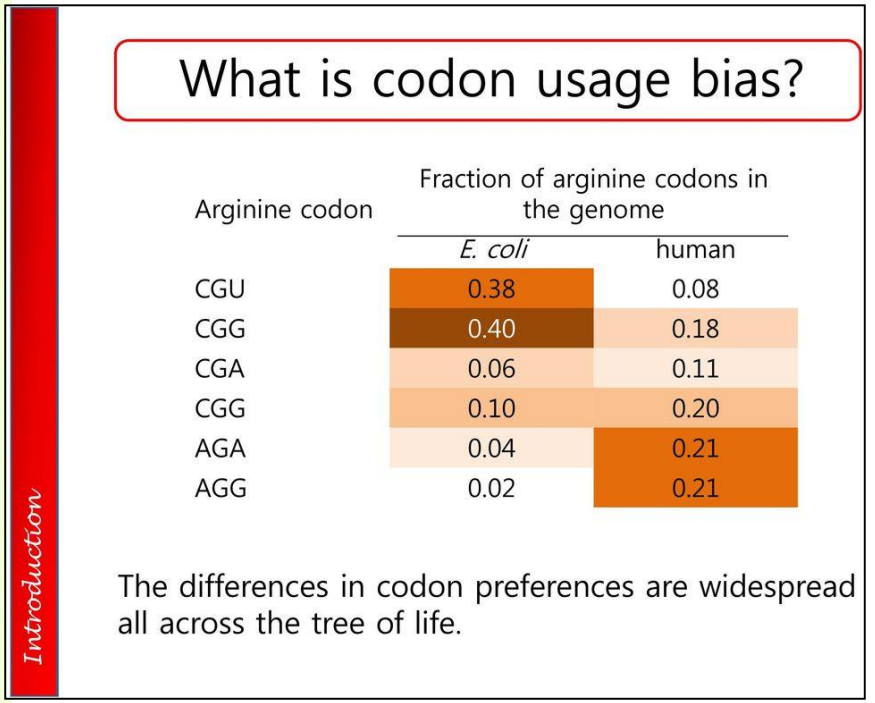


Due to the degeneracy of the genetic code, most amino acids can be encoded by multiple synonymous codons. Synonymous codons naturally occur with different frequencies in different organisms. Redundancy: the state of being not or no longer needed or useful.

Genomic islands

Codon usage bias: an important evolutionary feature in a genome

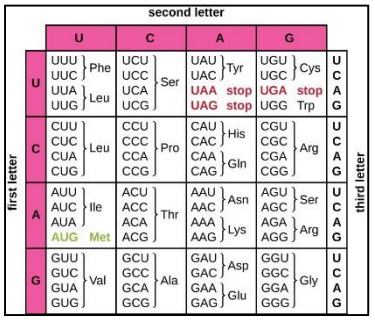
- Because a high amount of codon usage bias in bacteria is believed to be the result of selection for translational efficiency.
- Codon usage bias (CUB) is an important evolutionary feature in a genome which provides important information for studying organism evolution, gene function and exogenous gene expression.



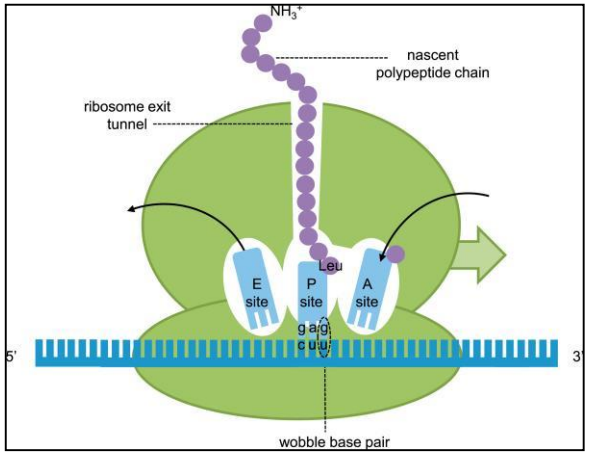
Distant phylogenetic species usually has greater variations in codon usage bias.

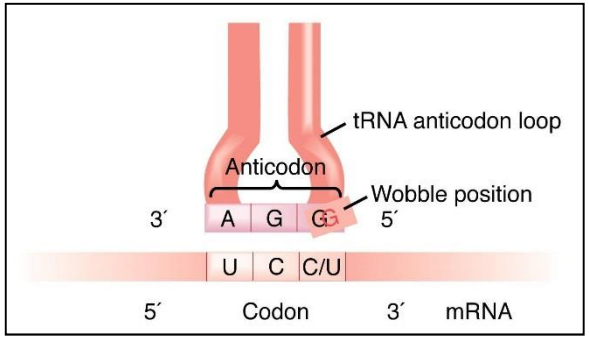
Genome of prokaryotes

Codon usage bias (CUB)



- **Translation in the ribosome and tRNA structure**
- Cartoon of the ribosome (green) during translation of a mRNA (blue) with a wobbling codon-anticodon base pair encoding a leucine amino acid.
- A site: aminoacyl-tRNA site; E site: exit site;
- P site: peptidyl-tRNA site.

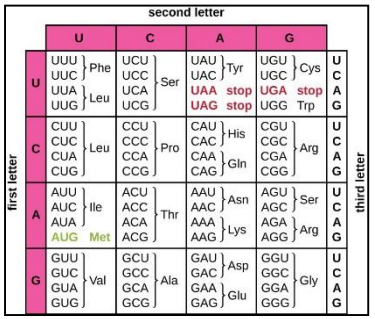




Wobble: pairing of the tRNA anticodon with the mRNA codon proceeds from the 5' end of the codon. In this example, the double-ringed G can pair with either a single-ringed U or C. Each organism seems to prefer a different set of codons over others; this phenomenon is called codon bias.

Genome of prokaryotes

Codon usage bias (CUB)



- Several important variations of codon bias have recently been discovered, such as:

1. the existence of a ramp of rare,
2. slowly translated codons at the 5′ end of protein-coding sequences, and
3. the co-occurrence of certain codons.

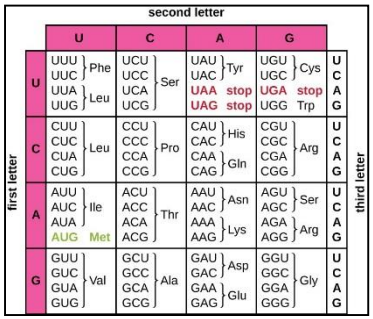
- Apart from directly affecting general protein expression levels, it has been established that codon bias also influences:

1. protein folding, and
2. differential regulation of protein expression.

Quax *et al.*,2015

Genome of prokaryotes

Codon usage

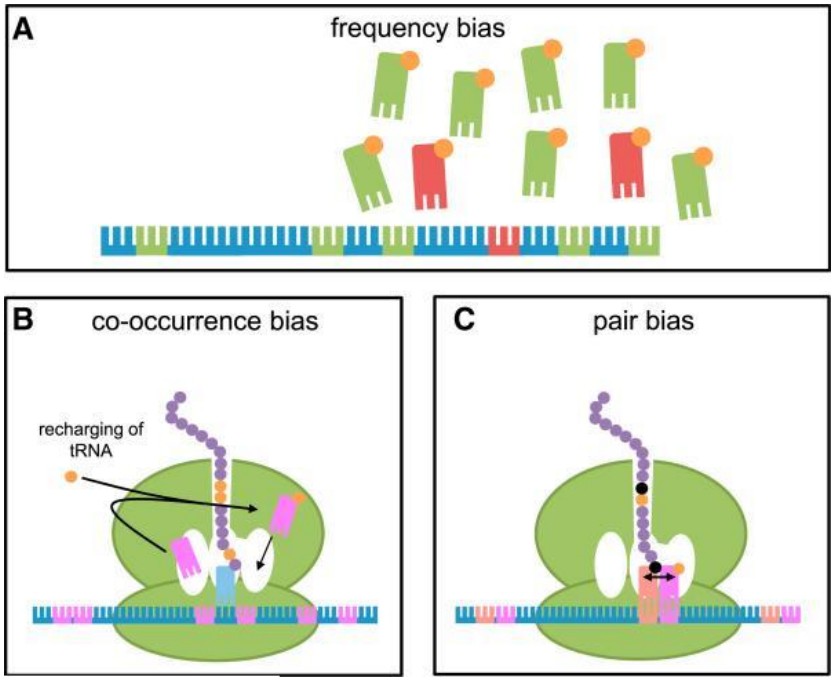


- **Different Types of Codon Bias**

A. Frequency bias will result in effective protein production when the frequency of used codons matches the cellular tRNA population.

B. Co-occurrence bias enhances protein expression, presumably because of tRNA recharging in the vicinity of the translating ribosome.

C. Pair bias is probably selected because of more optimal interactions of tRNAs in the A site and the P site.

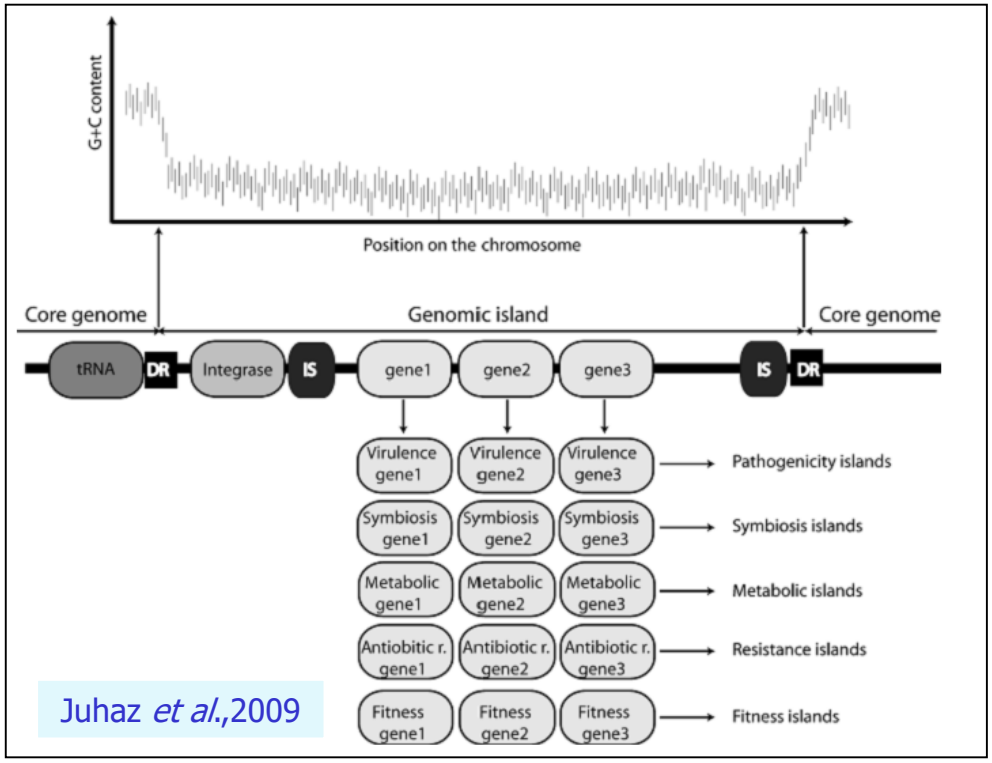


Quax *et al.*,2015

Genome of prokaryotes

Subgroup of genomic islands (GISs)

Mobility genes, such as integrases (*int*), are frequently located at the beginning of the island, close to the tRNA locus or the respective attachment site



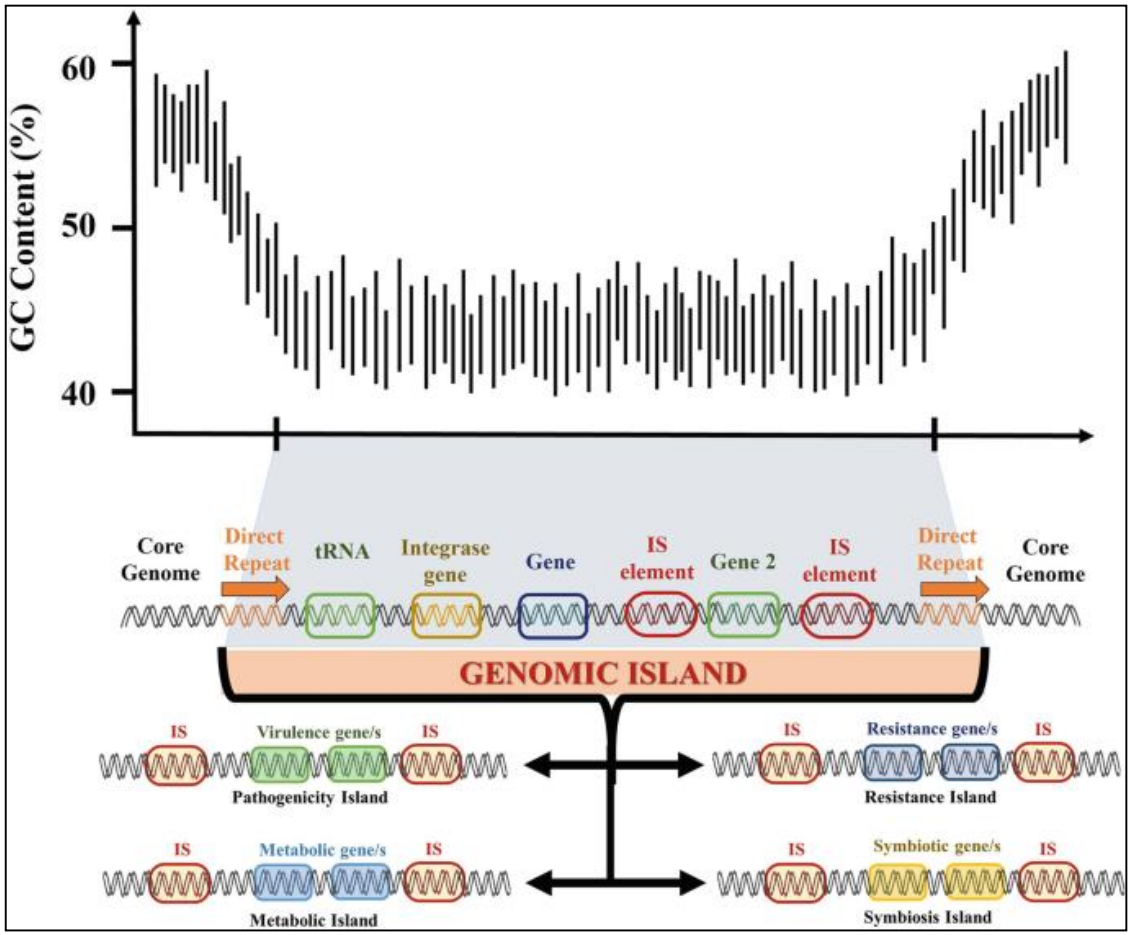
General features of GEIs. GEIs are relatively large segments of DNA whose nucleotide characteristics often differ from the rest of the chromosome. GEIs are often inserted at tRNA genes and flanked by direct repeat sequences (DR).

GEIs typically harbour genes encoding factors involved in genetic mobility, such as integrases, transposases and insertion sequences (IS). According to their gene content, GEIs can be described as pathogenicity, symbiosis, metabolic, fitness or resistance islands.

Genome of prokaryotes

Subgroup of genomic islands (GISs)

Mobility genes, such as integrases (*int*), are frequently located at the beginning of the island, close to the tRNA locus or the respective attachment site



Saini *et al.*,2023

Genomic islands
Fitness islands and saprophytic islands

- The fitness islands are classified into several subtypes based on the life style of the microbes that harbor them. E.g.

1. ecological islands: in which the islands contribute to the survival of the microbe in a environment.
2. Other fitness that are related with microbe-host interaction. These are:

- Saprophytic islands: in microorganism can persist as a saprophyte in a host.
- In many case, the fitness factor temporarily or prermanetely resides in the host either providing some benefits (symbiosis islands) or cause damage (pathogenicity islands).

Safwat Mohammad,2014;..

Genomic islands
Fitness islands

- Genomic islands increase bacterial fitness.
- The ability of a bacterial strain to infect a host, persist, proliferate and transfer to a new host in a specific niche is known as bacterial pathogen fitness.
- Fitness islands contribute to fitness and adaptation of the strains.
- A simultaneous acquisition of many genes by HGT that allow the bacteria to rapidly gain complex virulence functions and to exploit new environmental niches increasing bacterial fitness.

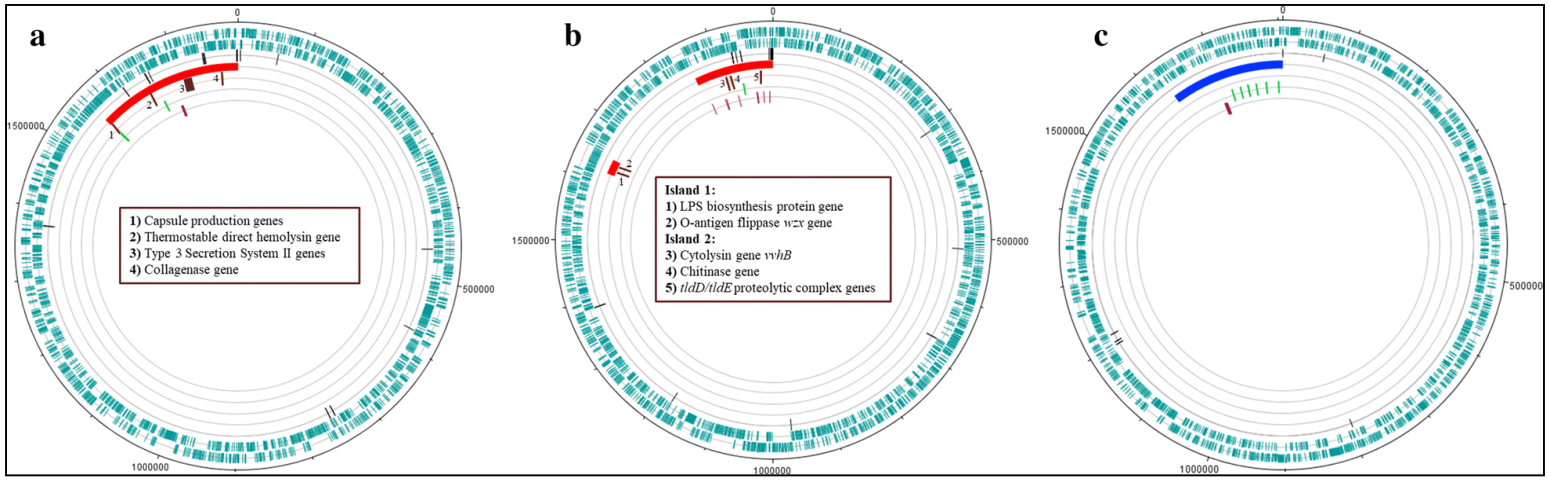
Safwat Mohammad,2014


Track

Genomic islands

Occurrence and significance of pathogenicity and fitness islands in environmental vibrios

- **a-c Circular presentation of the second chromosome of three *Vibrio* spp.**
- Track 1, forward coding sequences; track 2, reverse coding sequences; track 3, tRNA genes; track 4, red, pathogenicity islands, blue, genomic fitness islands; track 5, virulence and virulence-associated genes; track 6, genes involved in toxin–antitoxin systems; track 7, mobile genetic elements.
- Virulence and virulence-associated genes are numbered and are defined via the center text boxes.



Kelin *et al.*,2018

Genomic islands
Fitness islands

- Fitness genes are associated with entry and survival in the host.
- In nonpathogenic bacteria, these DNA segments may act as fitness islands or ecological islands.
- These encodes for:

1. iron uptake systems.
2. an exopolysaccharide (EPS) that is also involved in biofilm formation.
3. determining the identity, origin and evolution of traits that contribute significantly to fitness in plant-associated bacteria.

Genomic islands

Pathogenicity islands is a subclass of genomic islands

- Pathogenicity islands (PAIs) are a subset of genome islands.
- When the island contains pathogenicity genes it is called a pathogenicity island.
- PAIs are a distinct class of genomic islands which are also acquired by horizontal gene transfer.

Van Sluys *et al.*,2002; Hentschel and Hacker,2001

Genomic islands

Pathogenicity islands is a subclass of genomic islands

- In the early 1980s large unstable chromosomal regions carrying virulence-associated genes were identified in uropathogenic *E. coli* (pathogen of urinary tract).
- Later, such large unstable chromosomal regions were designated pathogenicity islands (PAIs).
- Pathogenicity islands (PAIs), as termed in 1990.
- These are a distinct class of genomic islands.
- They are incorporated in the genome of pathogenic bacteria.

Schneider *et al.*,2011;..

Genomic islands

Pathogenicity islands is a subclass of genomic islands

- Their instability and the high level sequence similarity of different (partial) islands suggest an exchange of PAIs between strains of the same or even different bacterial species by horizontal gene transfer (HGT).
- PAIs comprise large genomic regions (10-200 kilobases in size) that are present on the genomes of pathogenic strains but absent from the genomes of nonpathogenic members of the same or related species.

Genome size in *Escherichia coli* is 4.6 million base pairs (Mbp)=4600 KB.

Schneider *et al.*,2011;..

Pathogenicity Islands

Distinct class of genomic islands

Large, mosaic, genetic islands, found in all pathogenic bacteria

- The genomes of prokaryotes are highly diverse mosaic structures, contains:

1. conserved segments, and
2. various flexible regions (i.e. GIs).

- A core genome, with mostly homogeneous G+C content and codon usage,
- A flexible gene pool, with DNA with different G+C ratios, different codon usage and with mobile genetic elements.

Mosaic mobile genetic elements enabling dynamic lateral gene flow.

Pathogenicity Islands

Distinct class of genomic islands

Large, mosaic, genetic islands, found in all pathogenic bacteria

- PAIs, a type of mobile genetic elements, may range from 10-200 kb and encode genes which contribute to the virulence of the respective pathogen.
- Pathogenicity islands carry genes encoding one or more virulence factors, including, but not limited to, adhesins, secretion systems (like type III secretion system), toxins, invasins, modulins, effectors, superantigens, iron uptake systems, o-antigen synthesis, serum resistance, immunoglobulin A proteases, apoptosis (programmed cell death), capsule synthesis, and plant tumorigenesis via *A. tumefaciens*.

Wikipedia,2019

Pathogenicity Islands

Distinct class of genomic islands

Stable or unstable islands?

- PAIs are often unstable DNA regions.
- Like most definitions in biology, there are exceptions to each criteria, and despite their similar functional characteristics, PAIs are actually quite heterogeneous.
- Not all pathogenicity islands are genetically unstable, but each one shows an indication of foreign origin.
- These pieces of DNA are often missing in closely related, nonvirulent bacteria (Mecsas and Strauss,1996).

Kaper and Hacker,1999

Pathogenicity Islands

Distinct class of genomic islands

Stable or unstable islands?

- The presence of sequences associated with seven different PAIs, previously characterized in uropathogenic *E. coli* (UPEC), was determined:
- PAI I_{536}, II_{536}, IV_{536}, $ICFT_{073}$, $IICFT_{073}$, IJ_{96} and IIJ_{96}.
- The most prevalent pathogenicity island, in strains from hemoculture, were PAI IV_{536}, described by many researchers as a stable island in enterobacteria.

Koga *et al.*,2014

Pathogenicity Islands

Stable or unstable islands?

Detection of PAI Markers

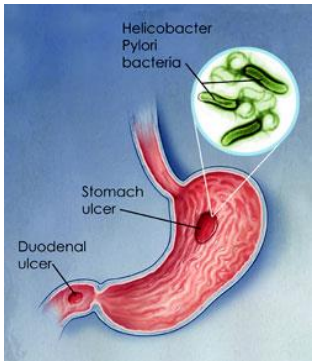
- Primers for detection of PAIs markers, phylogenetic analysis, virulence genes, and their respective virulence factors.
- The presence of sequences associated with seven different PAIs, previously characterized in uropathogenic *E. coli* (UPEC), was determined (PAI I_{536}, II_{536}, IV_{536}, $ICFT_{073}$, $IICFT_{073}$, IJ_{96} and IIJ96). This PCR contained 1U Taq DNA polymerase (Invitrogen) in 2x PCR buffer (Invitrogen), 0.2mM of each dNTP, 2.5mM $MgCl_2$, and 20 pmol/μL of each primer. The program consisted of 94°C for 5 min, followed by 30 cycles of 94°C for 1min, 55°C for 1 min, and 72°C for 1 min, with a final extension step at 72°C for 10 min. The positive control used in the PCR was J96 (Koga *et al.*,2014).

Genes	Sequence (5′ to 3′)	Size of product (bp)	Virulence factors
PAI I_{536}	TAA TGC CGG AGA TTC ATT GTC AGG ATT TGT CTC AGG GCT TT	1.800	α-Haemolysin, CS12 fimbriae, and F17-like fimbrial adhesin
PAI II_{536}	CAT GTC CAA AGC TCG AGC C CTA CGT CAG GCT GGC TTT G	1.000	α-Haemolysin and P-related fimbriae
PAI IV_{536}	AAG GAT TCG CTG TTA CCG GAC TCG TCG GGC AGC GTT TCT TCT	300	Yersiniabactin siderophore system
PAI I_{CFT073}	GGA CAT CCT GTT ACA GCG CGC A TCG CCA CCA ATC ACA GC GAA C	930	α-Haemolysin, P-fimbriae, and aerobactin
PAI II_{CFT073}	ATG GAT GTT GTA TCG CGC ACG AGC ATG TGG ATC TGC	400	P-fimbriae and iron-regulated genes
PAI I_{J96}	TCG TGC TCA GGT CCG GAA TTT TGG CAT CCC ACA TTA TCG	400	α-Haemolysin and P-fimbriae
PAI II_{J96}	GGA TCC ATG AAA ACA TGG TTA ATG GG GAT ATT TTT GTT GCC ATT GGT TAC C	2.300	α-Haemolysin, Prs-fimbriae, and cytotoxic necrotizing factor 1

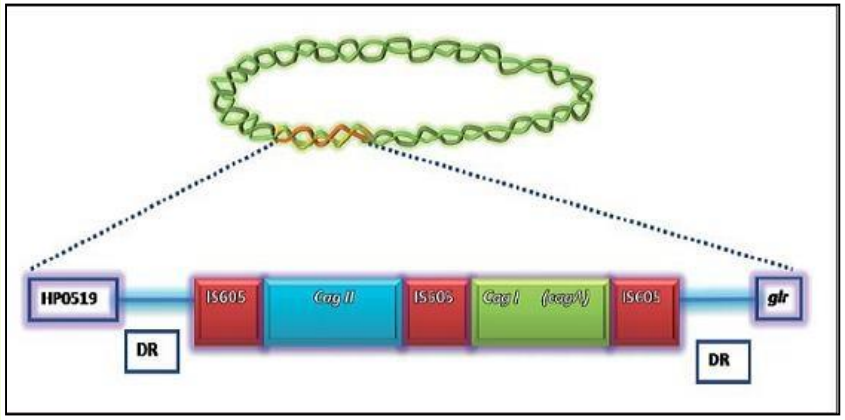
Pathogenicity Island

Cag type IV secretion system

Helicobacter pylori



- *H. pylori* colonize the body and the antrum of the human stomach causing gastritis (inflammation of the lining of the stomach) and complications such as gastric and duodenal ulcers, etc.
- The PAI is surrounded by direct repeat sequences (DR).
- It is divided into cagI and cagII by IS605 elements, the quantity of which may vary among different strains.
- *H. pylori* uses the Cag type IV secretion system to inject its effector protein CagA into gastric (intestinal) cells.



The ***cagA* gene** is part of cagPAI, and **CagA** is the primary virulence factor of ***H. pylori***. Cag is split into a right segment (cagI) and a left segment (cagII) by a novel insertion sequence (IS605).

Arévalo *et al.*,2009

Pathogenicity islands

Chromosome or plasmid borne virulence genes

- The pathogenicity islands (PAIs) are located usually on:

1. bacterial chromosome, or
2. may be a part of a plasmid.

- Genome (conserved and flexible);
- genomic islands (flexible);
- PAIs (flexible).
- Large segments (10-200 kb) are often referred to as islands and segments less than 10 kb are called islets (a very small island).

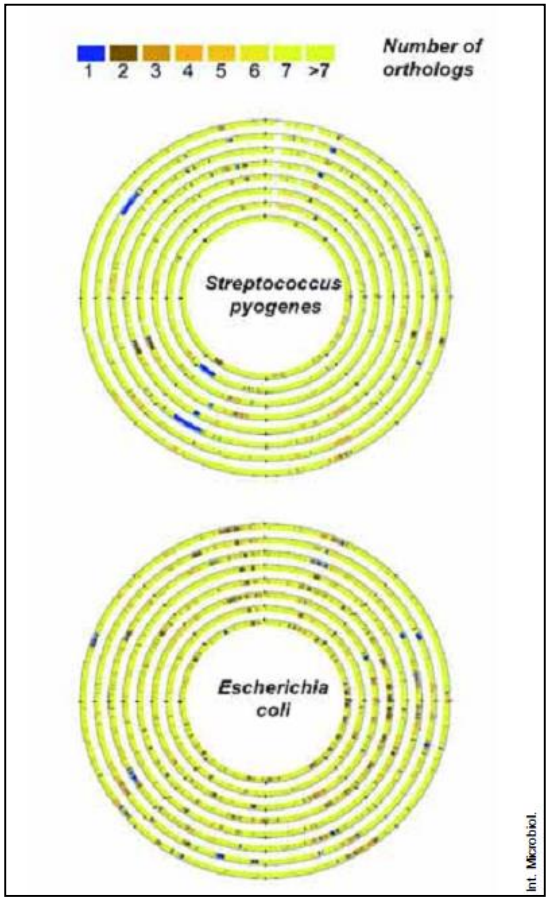
The pan-genome of prokaryotes
A core and flexible gene pools

- Even across genomes of the same species, prokaryotes exhibit remarkable flexibility in gene content.
- The **pangenome** is the entire gene set of all strains of a species. It includes genes present in all strains (core genome) and genes present only in some strains of a species (variable or accessory genome).
- The **core genome** represents the genes present in all strains of a species. It typically includes housekeeping genes for cell envelope or regulatory functions.
- The **variable or accessory genome** (also: flexible, dispensable genome) refers to genes not present in all strains of a species. These include genes present in two or more strains or even genes unique to a single strain only, for example, genes for strain specific adaptation such as antibiotic resistance.

Matthias Scholz;..

The pan-genome of prokaryotes
A core and flexible gene pools

- Core and accessory genomes of *Escherichia coli* and *Staphylococcus aureus*.
- The shared, core genome accounted for around 40% of the total gene pool in *E. coli* and it is interrupted by multiple variable regions unique to individual (or a few strains).
- Each circle represents the genome of a given strain, and the color scale indicates the number of orthologs found for each gene across the sequenced strains.
- The circles correspond to the genomes of (from outside to inside): *E. coli* (536, APEC O1, CFT073, K12 MG1655, O157 H7 EDL933, UTI89, W3110, O157 H7 Sakai); *S. pyogenes* (M1GAS, MGAS10750, MGAS2096, MGAS10270, MGAS6180, MGAS5005, MGAS10394, SSI-1).



Mira *et al.*,2010

The pan-genome of prokaryotes

A core and flexible gene pools

A core (genes shared among compared genomes) and a flexible gene pool (genes unique for each genome)

- A classic paper demonstrated this in three isolates of *Escherichia coli* that were found to have less than 40% of their genes in common.
- A considerable fraction (~40%) of accessory genomes harbours beneficial metabolic functions. i.e. metabolic adaptations underlying genome flexibility in prokaryotes.
- Indeed, this is the subject of an ongoing debate: do the majority of prokaryotic accessory genes have negligible or positive fitness effects, i.e. are they neutral or adaptive due to flexible genes?

E. coli genome size ranging from 4.5 to 5.5 Mpb

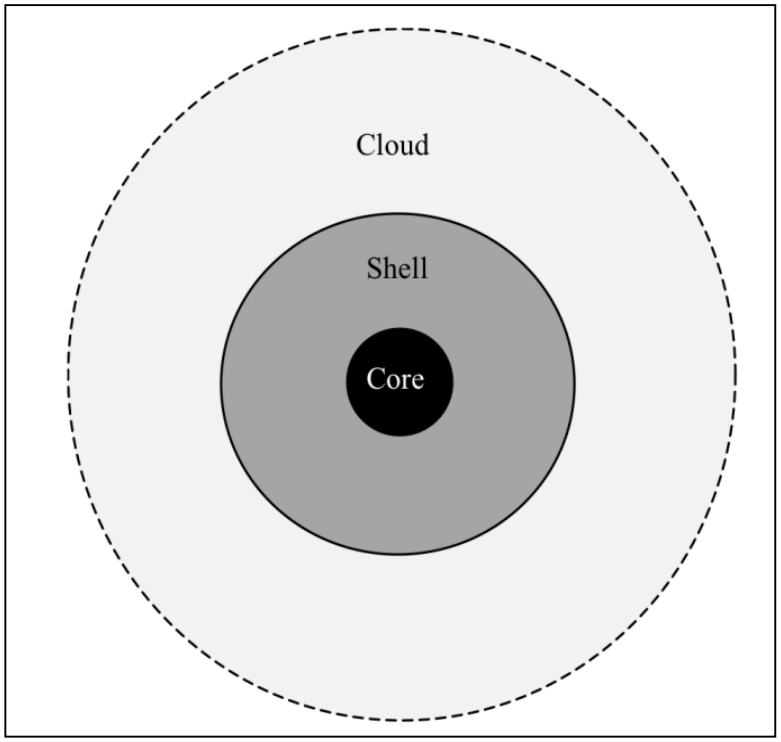
Polz *et al.*,2013; Akshit Goyal,2018;..

The pan-genome of prokaryotes

A core and flexible gene pools

A core (genes shared among compared genomes) and a flexible gene pool (genes unique for each genome)

- The bacterial pan-genome can be divided into the:
  1. core (genes always occurring in any genome inside the pan-genome)
  2. the shell (genes frequently occurring), and
  3. cloud (rarely occurring genes).
- The size of the cloud and shell can be significantly larger than the core genome, reflecting the diversity (or lack thereof) of various types of bacteria in different ecological niches.



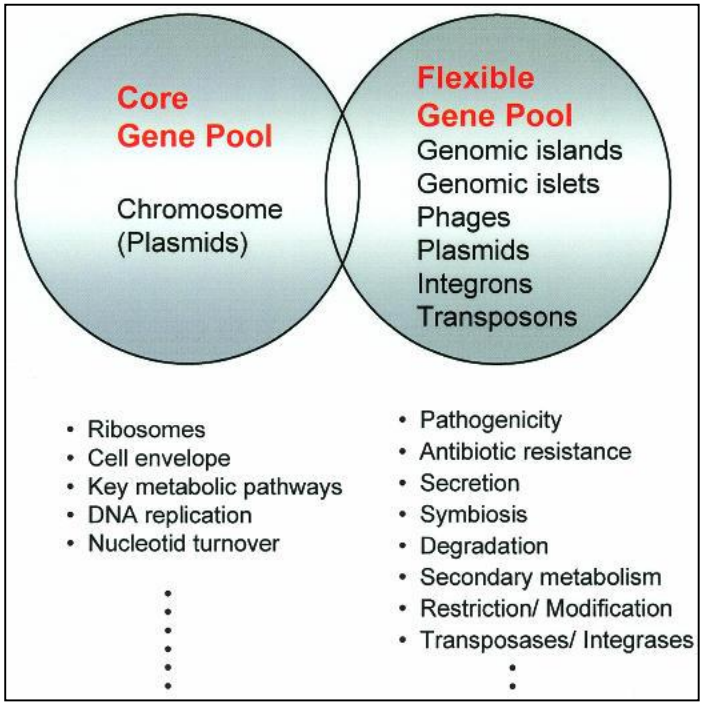
Pathogenic, parasitic and commensal species that are not routinely found in the environment could have smaller clouds.

Snipen and Ussery,2010;….

The pan-genome of prokaryotes:
The sum of core and flexible genes in a pre-defined group of bacteria or archaea

- Model of the DNA pools in the genomes of prokaryotes.
- The DNA elements comprising:

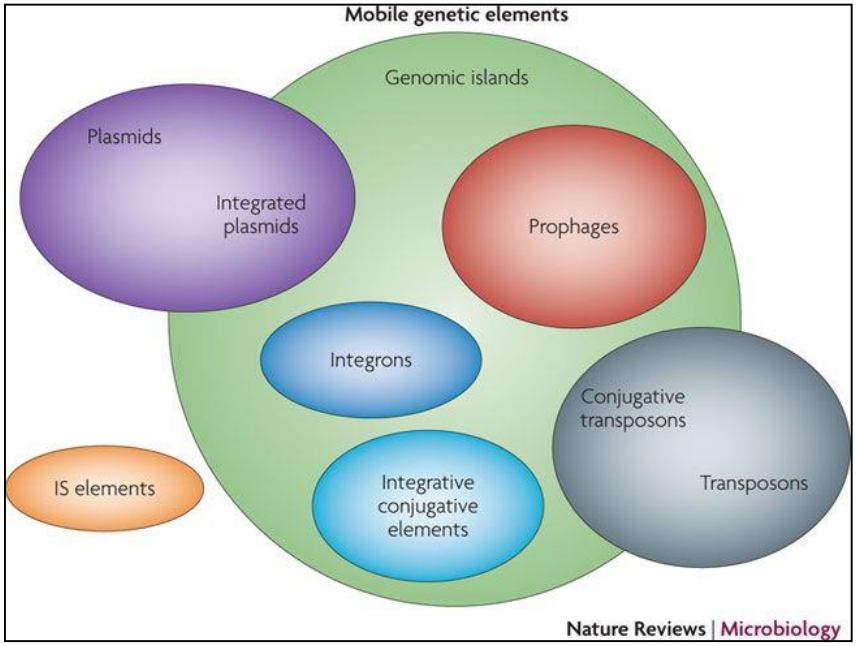
1. the core, as well as,
2. the flexible gene pools are presented in the circles.



Among related strains, this divides the genome into a "flexible" part that differs between strains, and a "core" part that is shared. Each strain is characterized by specific alleles of a set of core genes (a conserved "genomic backbone"), along with a smaller group of strain-specific flexible genes. Conserved sequences may be identified by homology search, using tools such as BLAST. Flexible gene pool clearly reflect strain-specific adaption to local environmental conditions. The majority of the horizontally transferred DNA is part of the flexible bacterial gene pool. Some genes may belong to both the core and flexible pools, but the majority belong to either one or the other. Perhaps not surprisingly, the number of genes within a cell that belong to the flexible pool may vary from 18% (*Escherichia coli* K-12) to <1% (*Mycoplasma*) of the total genome (Hacker and Carniel,2001).

The pan-genome of prokaryotes
A core genome and flexible gene pools
Genomic islands

- Genomic islands can be introduced into the bacterial core genome through a variety of mobile elements:
  1. plasmids,
  2. Phages, and
  3. Integrative and Conjugative Elements (ICEs) such as conjugative transpsons.
- Genomic islands are not colored, because most genomic islands are not mobile.



Flannery,2011; Langille *et al.*,2010

Pathogenicity islands

Gram-negative and Gram-positives bacteria

- The first GIs identified were termed pathogenicity islands.
- Those GEIs that carry virulence genes are called PAIs.
- Pathogenicity islands are found in:

1. Mainly in Gram-negative bacteria, and
2. In a few Gram-positives (cornyforms and actinomycetes).
3. PAI was not clearly identified in fastidious bacteria.
4. Usually absent from those non-pathogenic organisms.

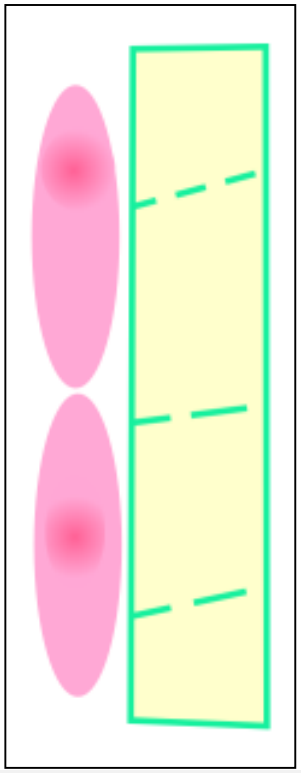
Dhillon *et al.*,2013;..

Microbial strategies for attack

Atypical mechanisms of pathogenicity

Sugar catabolism in *Spiroplasma*

- The genome of the sieve tube-restricted mollicute *Spiroplasma citri* is almost sequenced, and should neither contain genes for TTSS.
- In *Spiroplasma citri*, sugar catabolism, and more specifically fructose utilization has been shown to be a key factor in pathogenicity.

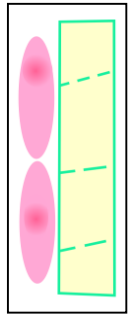


Solid green: sieve tube; dashed green: sieve tube plates; light pink: companion cell; dark pink: nucleus; yellow: nutrients

Microbial strategies for attack

Atypical mechanisms of pathogenicity

Sugar catabolism in *Spiroplasma*



Solid green: sieve tube; dashed green: sieve tube plates; light pink: companion cell; dark pink: nucleus; yellow: nutrients

- Competition for fructose between the companion cell and the *spiroplasmas* in the sieve tubes, seems to result in impaired sucrose loading into the sieve tubes and non-balanced sugar distribution between:

1. Sink organs (young leaves and roots), and
2. Source organs (leaves and stems).

Companion cell: Any of a number of specialized parenchymal cells adjacent to a sieve tube in the phloem of flowering plants, believed to regulate the flow of nutrients through the tube.
Sucrose is a disaccharide consisting of one molecule of glucose linked with one molecule of fructose.

See also *Ca.* Liberibacter asiaticus.

Pathogenicity islands

In some fungi

Ustilago hordei

- Large 'pathogenicity loci', which share common features with bacterial PAIs have been identified in the pathogenic fungus, *Ustilago hordei*, the agent of covered smut of barley.
- HGT has also been identified in the pathogenic fungus.

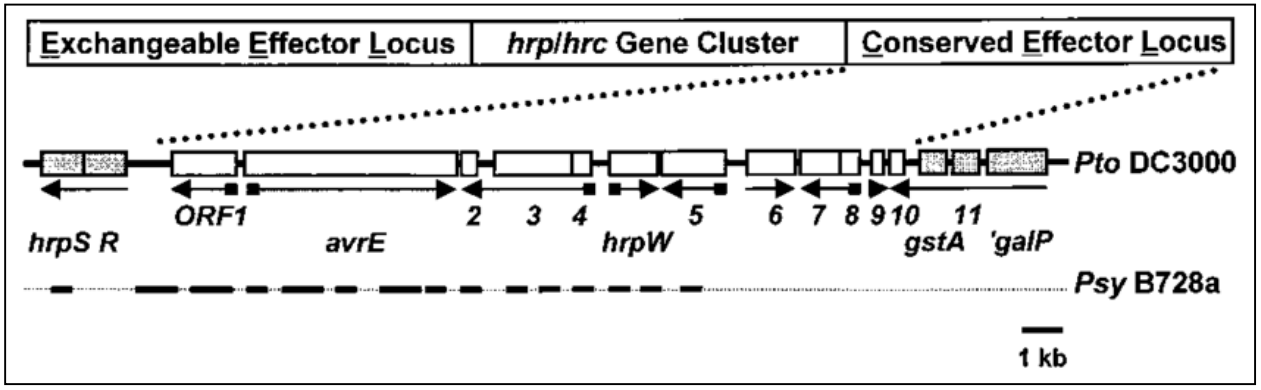
Pathogenicity Islands

Tripartite mosaic structure

Pseudomonas syringae Hrp pathogenicity island

- The *Pseudomonas syringae* Hrp pathogenicity island has a tripartite mosaic structure composed of:

1. an exchangeable effector locus (EEL),
2. a central conserved region (CCR),
3. a conserved effector locus (CEL). AvrE, HopM, and HopAA are part of the conserved effector locus.



Afano *et al.*,2003

Pathogenicity Islands
Mosaic structures

- The genomes of prokaryotes are highly diverse mosaic structures.
- During evolution, several genetic elements have been acquired independently at different time points and from different hosts.
- They may be located on a bacterial chromosome or may be transferred within a plasmid.
- PAIs carry genes encoding one or more virulence factors.
- These mobile genetic elements may range from 10-200 kb, and may encode genes contributing to the virulence of the respective pathogen.

Schmidt and Hensel,2004; The LibreTexts libraries

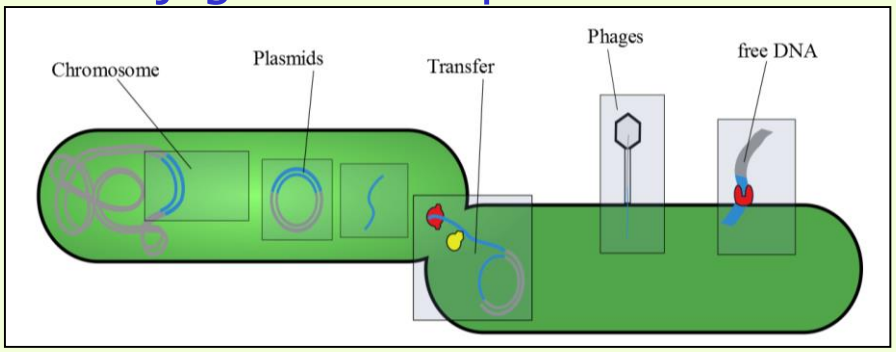
Pathogenicity Islands

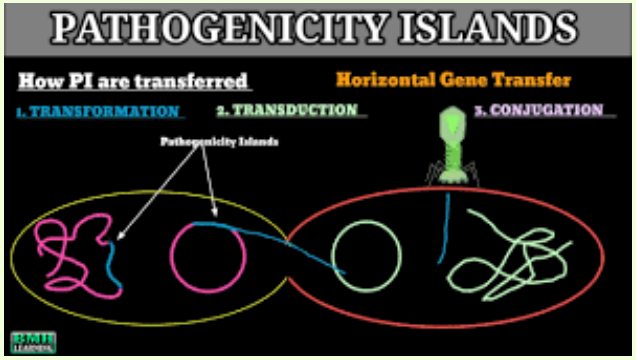
Distinct class of genomic islands

Large, mosaic, genetic islands, found in all pathogenic bacteria

- They can be transferred as a single unit to new bacterial cells, thus conferring virulence to formerly benign (not harmful) strain.
- They are transferred through horizontal gene transfer events such as transfer by:

1. a plasmid,
2. phage, or
3. conjugative transposon.





Boundless.com;…

Pathogenicity Islands
Evolution of pathogens

1. Pathogenicity islands (PAIs), and
2. Antimicrobial resistance islands (REIs) are key to the evolution of pathogens.
3. PAIs promote disease development, and
4. REIs give a fitness advantage to the host over multiple antimicrobial agents.

Microbial strategies for attack
Pathogenicity islands

- PAI are included in bacterial genome and occupy relatively large genomic regions.
- They are in range of 10 to 200 kb.
- PAIs carry genes encoding one or more virulence factors, including:

1. adherence factors,
2. toxins,
3. iron uptake systems,
4. invasion factors, and
5. Secretion systems (Types III, IV and VI).

T3SSs are typically located in pathogenicity islands (PAIs) and found to be transferred horizontally among bacterial species. T3SS is encoded by a distinct cluster of genes, termed hrp/hrc (hypersensitive response and pathogenicity/conserved) genes in the plant pathogens.

Pathogenicity islands

Mobile genetic elements

Major virulence features encoded by pathogenicity islands

Adherence factors. E.g. fimbriae	Diarrheagenic *Escherichia coli* Uropathogenic (pathogen of urinary tract) *E. coli* *Vibrio cholerae*
Toxins	Uropathogenic *Escherichia coli* *Staphylococcus aureus*
Iron uptake systems	Uropathogenic *E. coli* *Shigella flexneri* *Yersinia* spp.
Invasins (enzymes), modulins (a family of protein toxins soluble in phenols), effectors	*Salmonella* spp. *Shigella* spp. *Listeria* spp.
Type III secretion systems	*Pseudomonas syringae* *Erwinia* spp. *Yersinia* spp. *Salmonella* spp. *Shigella* spp.
Type IV secretion system	*Helicobacter pylori* *Agrobacterium tumefaciens*

Hacker and Kaper,2000

Pathogenicity islands

Collection of PAI loci which were reported in academic literatures

Species (strains)	PAI name	Insertion site	Size	GenBank Accession	Function
Pseudomonas syringae DC3000	Hrp-PAI	tRNA-leu	52.5kb	AF232004	Type III secretion system, Hop, Avr, hypersensitive response, mosaic structure (exchangeable effector locus, hrp/hrc gene cluster, conserved effector locus)
Pseudomonas syringae 464	Hrp-PAI	tRNA-leu	2.4kb	AY147017	Type III secretion system, Hop, Avr, hypersensitive response, mosaic structure (exchangeable effector locus, hrp/hrc gene cluster, conserved effector locus)
Pseudomonas cichorii Jan-83	S-PAI	-	55.6kb	DQ168848	Type III secretion system (10-kb-long insertion(avrE and avrF) in the middle of the hrp/hrc cluster)
Pseudomonas viridiflava LP23.1a	S-PAI	-	9.2kb	AY597279	Type III secretion system (10-kb-long insertion(avrE and avrF) in the middle of the hrp/hrc cluster)
Yersinia pseudotuberculosis IP 31758	YAPI	tRNA-Phe	64.6kb	NC_009708_P1	Type IV pilus
Rhodococcus equi 103S	rpl	-	8.9kb	NC_014659_P1	Biogenesis of Flp-subfamily type IVb pili
Acinetobacter baumannii AYE	AbaR1	ATPase	100.0kb	CT025832	European clone I, β-lactams aminoglycosides, fluoroquinolones, chlloramphenicol, tetracycline, rifampin resistance

PAIBD (Pathogenicity island database)

Pathogenicity islands

Mobile genetic elements

Groups of virulence factors encoded by PAI

Group	Examples of virulence factors	PAI names
Iron uptake systems	**FyuA, aerobactin, Sit, Pit2ABCD**	**HPI, SPI-1, PPI-1, SHI-2, 3, $PAII_{CFT073}$,PAI III, IV_{536}**
Adhesins: A surface structures such as EPSs (xanthan or amylovorin) that mediate the binding of a bacterium to the host cell.	**Type 4 pili, P-Pili, S- and P-fimbriae, Sap adhesin, Hek adhesin, AfaE-III, Iha, TcpA**	**Major PAI, PAI I, II_{CFT073}, PAI I-IV_{536}, PAI I, II_{J96}PAI-I_{AL863}, TAI, VPI-1**
Pore-forming toxins	**Listeriolysin, alpha-hemolysin, RTX-like exotoxin**	**LIPI-1, PAI I_{536}, PAI II_{536}, O#28**
Proteins causing apoptosis	**SipB**	**SPI-1**
Secreted lipases	**PlcA, PlcB, SmlC**	**LIPI-1, LIPI-2**
Secreted proteases	**EspC, SigA, Pic, ShetA1, Mop, BFT**	**SHI-1, EspC PAI, VPI-1, BFPAI**
O antigens	**GtrA, GtrB, Gtr**	**SHI-O**
Proteins transported by type I, III, IV, and V protein secretion systems	**Alpha-hemolysin, EspI, EspC, SigA, Cag,Tir, EspB, G, F, Map, SptP, Sse, Ste, SopD, SopE, SopE2, PipB, SifA, SpiC, EspC, CagA**	**SHI-1, PAI I, II_{536}, PAI I, PAI, II_{J96}, LPA, EspC PAI, SHI-1, SPI-1, SPI-3, SPI-5, LEE, *cag* PAI**
Antibiotic resistance phenotype	**Pse-1, FloR, AadA2, Sull, TetR, G**	**SGI-1**

Cytotoxin Associated Gene A (CagA)

Schmidt and Hensel,2004

Genomic islands

Genomic islands encode different functions

Symbiosis islands

- An analogous genomic structure in rhizobia is termed a symbiosis island.
- The symbiosis island comprises 10% of the entire genome and is therefore larger (500 kb) than any PAI known to date.
- It has also been found in the bacterial symbiont (*Mesorhizobium loti*) of leguminous plants.
- The symbiosis island has an average GC content of 59.4% compared to the genomic average of 64%.

Genomic islands

Genomic islands encode different functions

Resistance islands

- Resistance Islands (REIs) with genes responsible for antibiotic resistance:
- Another class of GIs, and are linked to pathogenesis by conferring simultaneous resistance to multiple antibiotics and facilitating the emergence of multidrug-resistant pathogens.
- *Pseudomonas aeruginosa* genomic island 1 (PAGI-1) is found in the majority of the clinical isolates.
- Acquired by horizontal gene transfer.

Genomic islands

Genomic islands encode different functions

Metabolic islands

- Islands with genes related to metabolism.
- Some bacteria such as *Salmonella senftenberg*, *E. coli* and *Yersinia* spp. have this ability to colonize plant surfaces and tissues, to metabolize plant-derived carbon sources such as sucrose uptake.
- The genome of *B. subtilis* shows many interesting features including the genes involved in the metabolism of plant derived carbon compounds, such as opines and starch.

Preston *et al.*,1998;..

Genomic islands

Genomic islands encode different functions

Metabolic islands

- They have a large metabolic versatility and are able to utilize numerous substrates as carbon and energy sources.
- *Pseudomonas aeruginosa* is well known as an opportunistic pathogen for plants, animals, and humans.

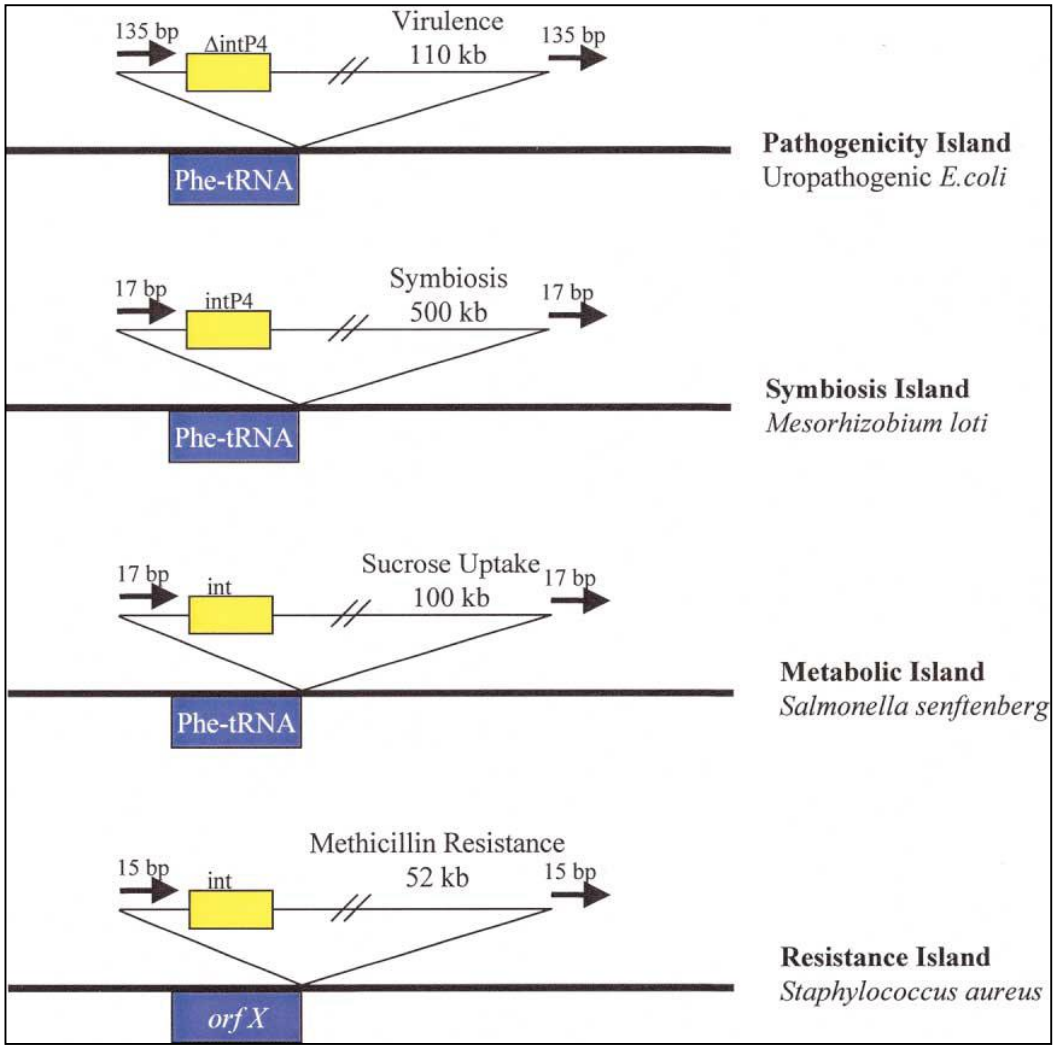
Schmidt and Hensel,2004

Genomic islands encode different functions

Genomic islands encoded some islands including antibiotic resistance functions (resistance island).

GEIs are often inserted at tRNA genes and flanked by DR. Note: The tRNA genes frequently associated with PAIs which target sites for integration and recombination of PAIs into the new bacterial DNAs (chromosome or plasmid).
One of the tRNA loci is Phe tRNA gene (phenylalanyl-tRNA synthetase), encode for integrase (int)(yellow box). Resistant island is locates within the *orfX* gene.

Hentschel and Hacker,2001;..



Examples of genomic islands

Genomic islands encode different functions

Organism	Property	Type of island	Genetic feature	Size (kb)
Mesorhizobium loti	Nitrogen fixation	Symbiosis	Integrated plasmid	500
Pseudomonas putida	Chlorocatechol Degradation	Degradation	Integrated plasmid	105
Salmonella senftenberg	Sucrose uptake	Metabolism	Conjugative transposon	100
Staphylococcus aureus	MecA protein	Resistance	Location on chromosome	52
Salmonella typhimurium DT 104	Multiresistance	Resistance	Location on chromosome	14
Various bacteria	Type III secretion Type IV secretion	Secretion	Location on chromosome or plasmid	Variable
Various bacteria	Iron uptake	Fitness	Location on chromosome or plasmid	Variable

Hacker and Kaper,2000

Genome sequencing
Bacterial genome database

- Since the first bacterial genome was completely sequenced in 1995, more than 200,000 bacterial and archaeal are completed.
- By now, approximately 126 plant pathogenic bacterial species have been sequenced. To explore the virulence mechanisms for plant bacterial pathogen, we can perform the comparative genomic analysis between different strains, such as virulent and avirulent strains, or strains across different locations and hosts.
- Genome sequencing has become the standard for the study of bacterial species.

Kishore *et al.*,2015; Xu, 2019; Zhang,2020

Genome sequencing

Bacterial genome database

PAIDB-Pathogenicity Island Database

- PAIDB is a web-based user-friendly resource and has been widely used for detecting PAIs in newly sequenced genomes and mining virulence genes from metagenome.

Metagenomics is the study of the metagenome- the collective genome of microorganisms from an environmental sample, to provide information on the microbial diversity and ecology of a specific environment.

Genome sequencing

Bacterial genome database

PAIDB-Pathogenicity Island Database

- The pathogenicity island database (PAIDB; http://www.gem.re.kr/paidb) is a comprehensive relational database of all the reported pathogenicity islands (PAIs) and potential PAI regions which were predicted by a method that combines feature-based analysis and similarity-based analysis.
- As of April 2006, PAIDB contains 112 types of PAIs and 889 GenBank accessions containing either partial or all PAI loci previously reported in the literature, which are present in 497 strains of pathogenic bacteria.

Yoon *et al.*,2014

Genome sequencing

Bacterial genome database

PAIDB-Pathogenicity Island Database

- The database also offers 310 candidate PAIs predicted from the 118 sequenced prokaryotic genomes.
- With the increasing number of prokaryotic genomes without functional inference and sequenced genetic regions of suspected involvement in diseases, this internet-based, user-friendly resource has the potential to be of significant use in pathogenomics (Genome analysis of pathogenic microbes).
- PAIDB is part of Genome Encyclopedia of Microbes (GEM; www.gem.re.kr) that is a web portal for microbial genome information.

Yoon *et al.*,2014

Genome sequencing

Bacterial genome database

PAIDB-Pathogenicity Island Database

- With the improved detection scheme, 2673 prokaryotic genomes were analyzed to locate potential:

1. Pathogenicity Islands (PAIs), and
2. Resistance Islands (REIs).

- The update encompasses dramatic increase in:

1. database contents of genomes analyzed,
2. accuracy improvement of detection of candidate regions, and
3. functionality update of web application.

http://www.gem.re.kr/paidb

Genome sequencing

Pathogenicity islands

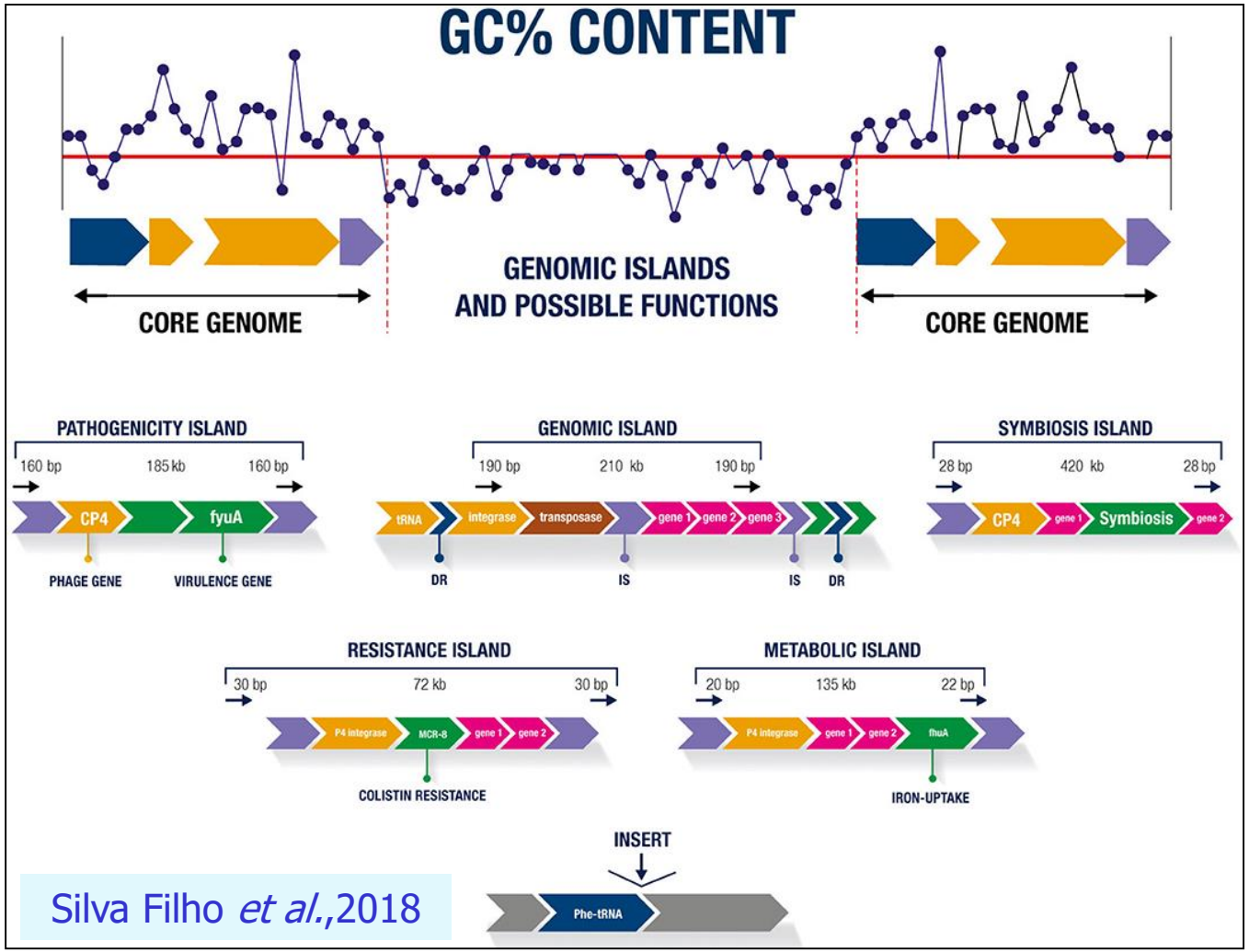
Bioinformatics soft wares

- To date, several open source and commercial software packages are available for creation and visualization of genome maps in linear, circular, or in both forms.
- Examples:
- "GenomePlot" and "GenoMap" developed for generating genome maps (Atlas).
- A novel software suite designed for the prediction of pathogenicity islands is pathogenicity island prediction software, or PIPS.

Ali *et al.*,2013; Soares *et al.*,2012

Genome sequencing

Main characteristics of genomic islands and possible functions

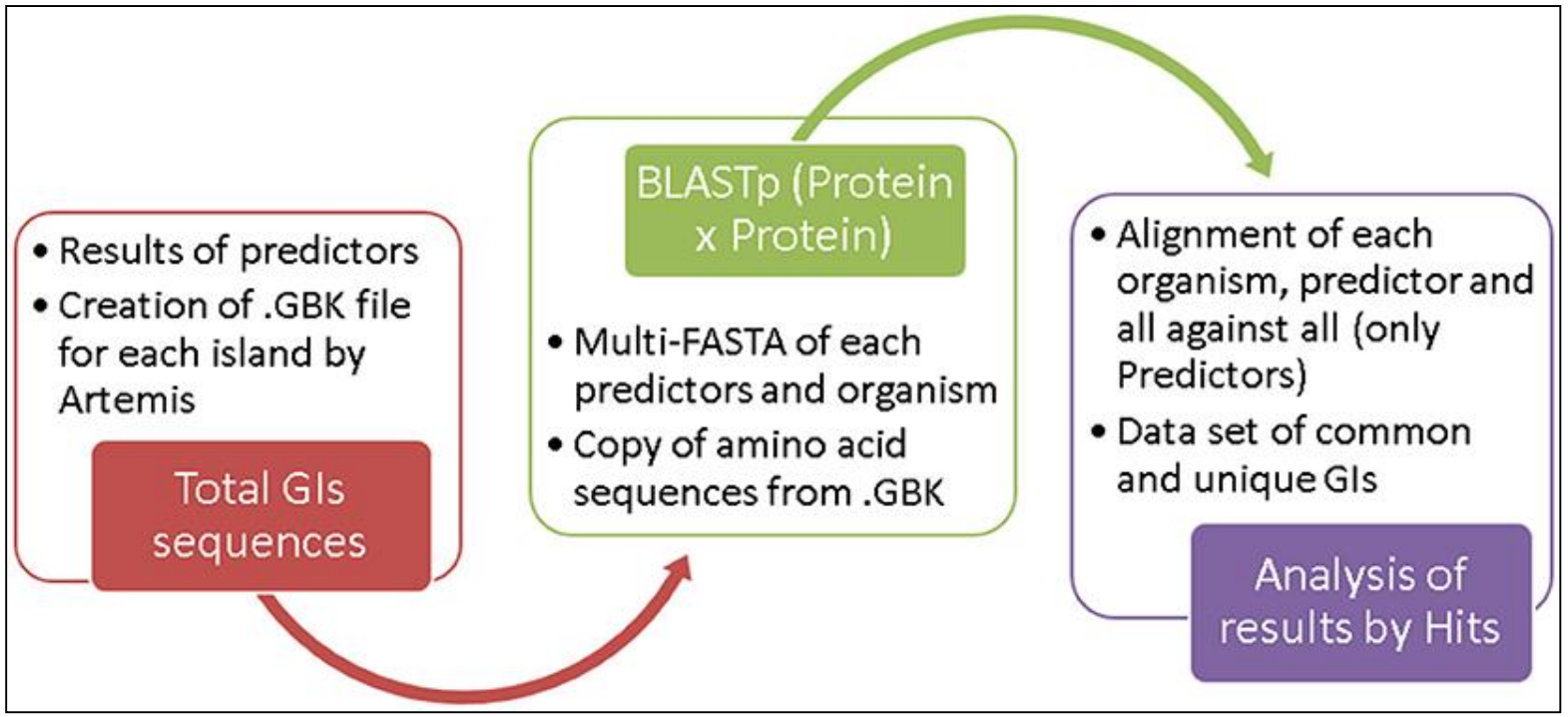


Silva Filho *et al.*,2018

Genome sequencing

Bioinformatics soft wares

Steps in dataset creation



Silva Filho *et al.*,2018

Genome sequencing

Data of GIs, PAIS, and regions with DNA of bacteriophages curated *in vitro* from the reference organism *Escherichia coli* cft073

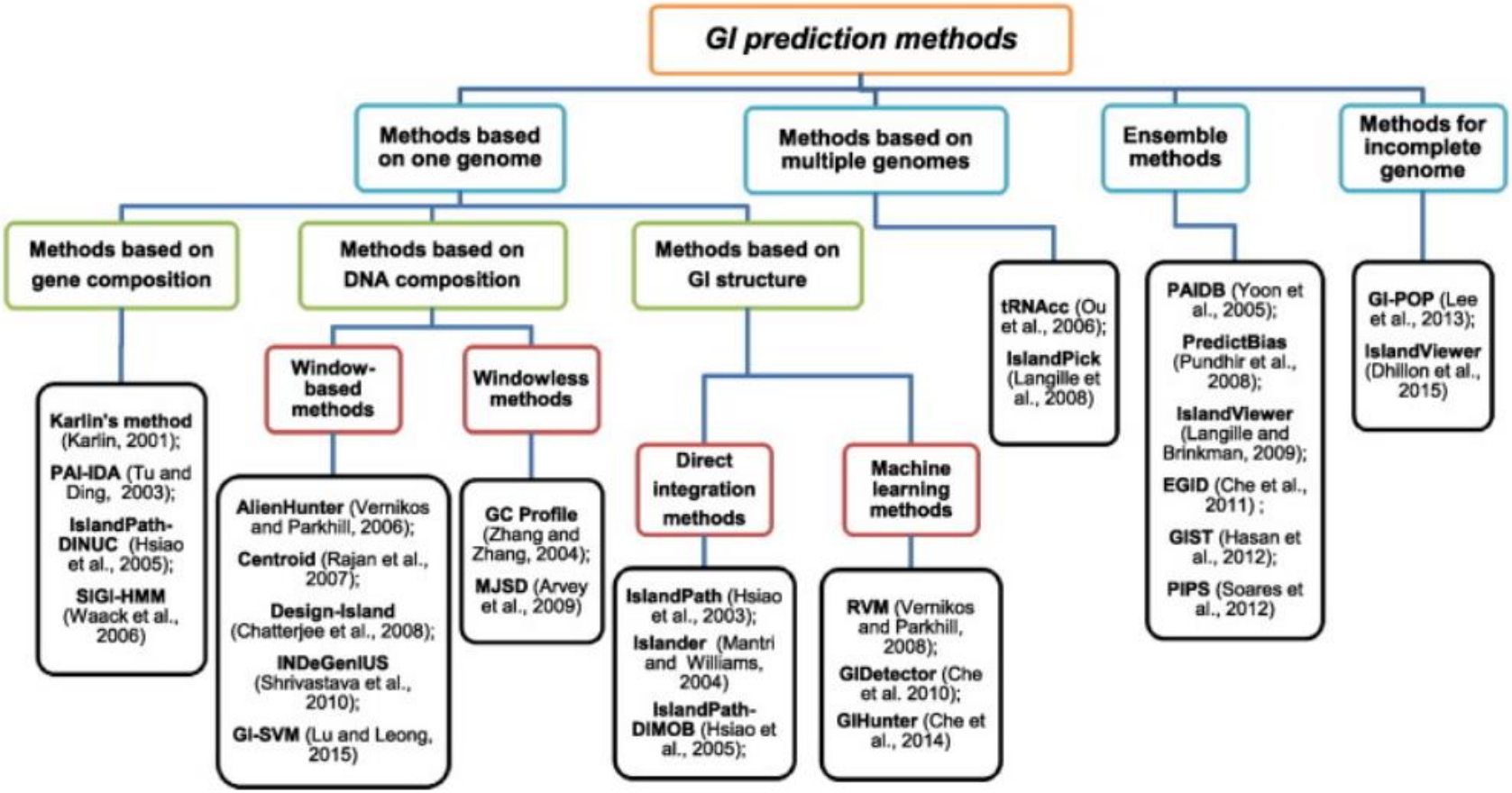
GIs[a]	GI name	Locus tag[b]	CDS[c]	tRNA[d]	GC% content[e] (%)
1	GI-CFT073-leuX	c5386–c5371	15	leuX	48.15
2	PAI-CFT073-pheU	c5216–c5143	61	pheU	47.57
3	GI-CFT073-selC	c4581–c4491	70	selC	47.04
4	PAI-CFT073-pheV	c3698–c3556	124	pheV	47.08
5	PAI-CFT073-metV	c3410–c3385	25	metV	53.37
6	ϕ-CFT073-smpB	c3206–c3143	49		49.32
7	GI-CFT073-cobU	c2528–c2482	37		49.68
8	GI-CFT073-asnW	c2475–c2449	26	asnW	53.12
9	PAI-CFT073-asnT	c2436–c2418	15	asnT	58.27
10	PAI-CFT073-serU	c2416–c2392	19	serU	37.65
11	PAI-CFT073-icdA	c1601–c1518	74		50.23
12	ϕ-CFT073-ycfD	c1507–c1481	14		49.78
13	ϕ-CFT073-potB	c1475–c1400	51		50.97
14	PAI-CFT073-serX	c1293–c1165	102	serX	48.76
15	ϕ-CFT073-b0847	c0979–c0932	42		50.45
16	PAI-CFT073-aspV	c0368–c0253	83	aspV	47.43

[a](Genomic islands), [b](Identifiers applied to each gene), [c](Number of coding sequences), [d](Transfer RNA), [e](Percentage of guanine and cytosine content in the region). GI, Genomic islands; PAI, Pathogenic islands; ϕ, Islands containing predominantly bacteriophage DNA.

Silva Filho *et al.*,2018

Genome sequencing

The hierarchical overview of computational methods for predicting genomic islands

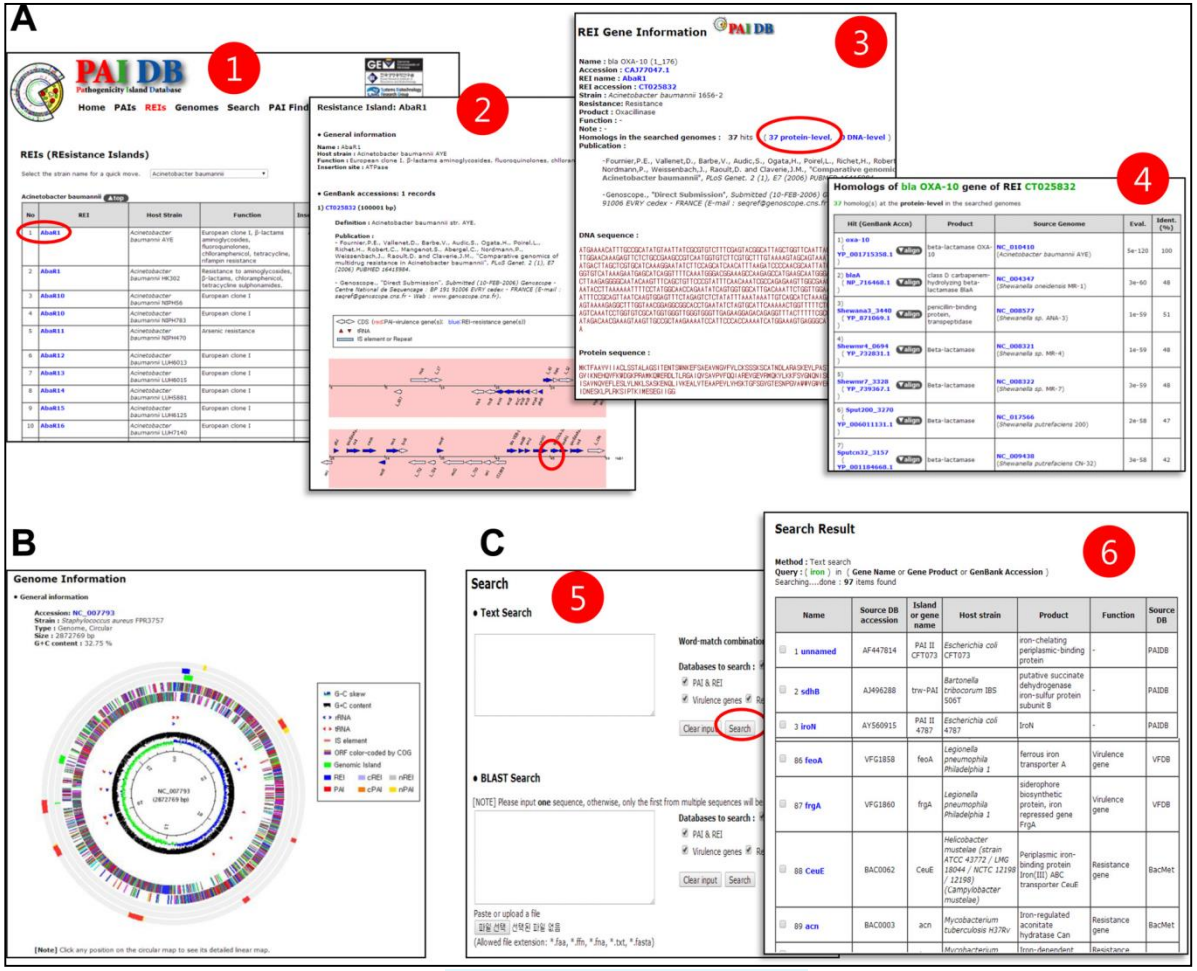


Lu and Leong,2016

Genome sequencing

Procedure for identifying candidate PAIs and REIs in a sequenced genome

Screenshots of new functional features in PAIDB v2.0



Yoon *et al.*,2014

Genome sequencing

Summary of genome sequence projects of *Enterobacter cloacae*

Strain	*E. cloacae* subsp. *cloacae* ENHKU01	*E. cloacae* subsp. *cloacae* ATCC13047	*E. cloacae* EcWSU1	*E. cloacae* subsp. *dissolvens* SDM
Size	4.73	5.6	4.8	4.97
No. of Chromosome	1	1	1	1
No. of plasmid	0	2	1	0
GC content %	55.1	54.6	54.5	55.1
Total genes	4445	5639	4740	4646
Predicted CDS	4338	5518	4619	4542
No. of tRNAs	82	24	83	53
No. of rRNA operons	8	8	8	3
Host	Plant	Human	Plant	Soil
Important feature	Endophyte	Human opportunistic pathogen	Plant pathogen	2,3-butanediol production

Coding sequence or CDS: portion of a gene's DNA or RNA that codes for protein. While the ORF may contain introns as well.

Liu *et al.*,2013

Genome of prokaryotes

Genomic islands (GISs)

Bioinformatics platform for high school students

- We utilize a bioinformatics platform that is easy to adapt, integrate, and implement into the existing biology curriculum through:

1. student centered, and
2. activity-based teaching, and
3. learning methods tailored for high school students.

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Genome of prokaryotes
Genomic islands (GISs)
Implementation

- Lessons from this module were implemented in professional development programs for high school teachers.
- Afterward, a number of teachers expressed interest in integrating select lessons from the module in their classes.
- This module presents a gateway to embracing integration of bioinformatics-based tools in the high school curriculum.
- We hope that more projects like this will result in further progress toward seamless adoption of bioinformatics in various curricula.

Bacusmo *et al.*,2019

Genome of prokaryotes
Genomic islands (GISs)
Bioinformatics

- Bioinformatics, the study of biological data using various computational techniques, is a very important aspect of biology, and its integration would greatly benefit current high school curricula.

1. We describe a bioinformatics-based module that introduces the application of genome comparison in the identification of "pathogenic islands."
2. The module also introduces foundational concepts of
    1. horizontal gene transfer and
    2. the genetic basis of virulence, with a special focus on antibiotic resistance.

A **module** is one of a set of parts from which some buildings are made.

Bacusmo *et al.*,2019

Genome of prokaryotes

Genomic islands (GISs)

Bioinformatics

- One of the constant challenges in health care is the potential inefficacy of existing antimicrobial drugs against new and emerging pathogens.
- Most pathogens evolve at an accelerated rate, thus promoting diversification and rapid distribution of virulence genes.
- These facts place emphasis on the importance of studying the epidemiology of various virulence genes.
- One of the ways to approach this problem is through rapid identification of the virulence genes in pathogenic strains using bioinformatics tools such as genome comparison.

Bacusmo *et al.*,2019

Genome of prokaryotes

Genomic islands (GISs)

Bioinformatics

- Pathogens often contain "signatures" in their genomes where accumulation of virulence and antimicrobial resistance genes are concentrated into specific regions of the genome called "genomic islands."
- Genome comparison, a bioinformatics tool, allows for easy identification of these genome signatures and provides information on the various virulence genes contained within the island.
- This leads to a targeted approach in counteracting the pathogen.

Bacusmo *et al.*,2019

Genome of prokaryotes

Genomic islands (GISs)

Bioinformatics

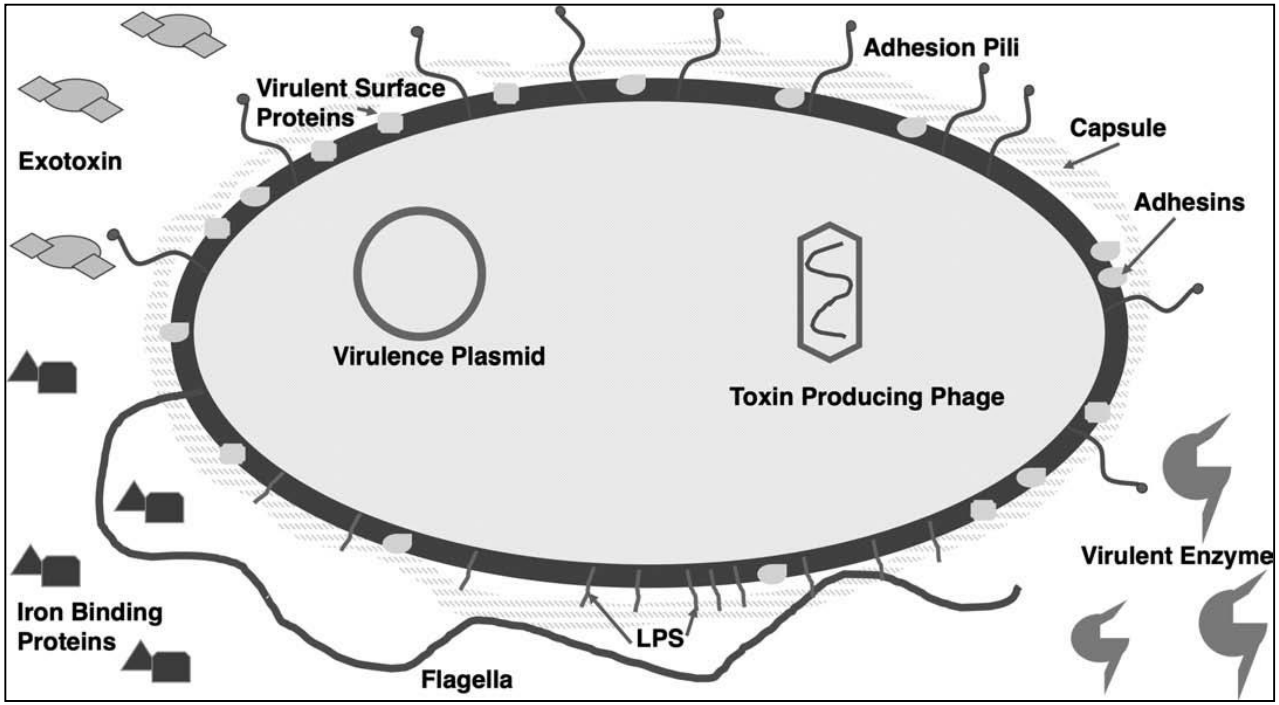
- Common virulence factors designated as either offensive or defensive features.

offensive	defensive
Flagella or ability to swim	Acid resistance (in Gram-negative enteric bacteria)
Attachment	Antibiotic resistance
Toxins	Capsule or protective coating
Secretion systems	
Effector protein injection	

Bacusmo *et al.*,2019

Genome of prokaryotes
Genomic islands (GISs)
Bioinformatics

- Example of a pathogen prototype displaying common virulence factors (adapted from https://preview.tinyurl.com/yc89aw4g).



Bacusmo *et al.*,2019

Genome of prokaryotes
Genomic islands (GISs)
Module Overview

- The "Pathogenic Islands" module consists of six lessons formatted into 50-minute sessions (Table 1).
- It was developed by a science teacher/science researcher team to align with Florida's Next Generation Sunshine State Standards for Science.
- It also embraces three-dimensional learning as espoused by the National Research Council (2012), which calls for students to be actively engaged in the practices of science while exploring disciplinary core ideas and crosscutting concepts.

Bacusmo *et al.*,2019

Genome of prokaryotes
Genomic islands (GISs)
Module Overview

- The major learning goals of the module are as follows:

1. List and describe virulence genes that cause the different modes of pathogenicity.
2. Describe the various modes of gene transfer.
3. Demonstrate the concept of gene transfer applied in the process of selection and evolution.
4. Use genome comparison tools such as PATRIC and Island Viewer to identify genomic islands.
5. Apply knowledge of virulence genes and horizontal gene transfer to identify the source of virulence in pathogenic strains.
6. Perform independent research on a set of pathogens and present the location of pathogenic islands on the genome, identify the virulence genes contained in the pathogenic islands and their function, and provide a brief history on the epidemiology of the disease caused by the pathogen.

Bacusmo *et al.*,2019

Genome of prokaryotes

Module Overview

Lesson sequencing guide and summaries. The "Pathogenic Islands" module consists of six lessons formatted into 50-minute sessions and 24 students per class

Day 1	Lesson 1	Students take a pretest over the content presented in these six lessons. They then watch short videos of a pathogenic bacterium invading a host cell to identify the behaviors and biological mechanisms (virulence factors) exhibited by the bacterium that make it successful.
Day 2	Lesson 2	Student groups build a bacterial prototype expressing virulence factors and then compare their prototype to those created by the other groups. Students assess the potential success of each prototype by voting for the most successful and least successful prototype and justifying their choices.
Day 3	Lesson 3, Activity 1	Students watch a video on horizontal gene transfer and answer three questions. The teacher can choose to discuss the answers to these questions after the worksheet has been collected.
	Lesson 3, Activity 1	Students team up and play a teacher-directed game ("Pathogen Survivor") demonstrating genome diversification via gene transfer, highlighting its impact on bacterial fitness and survival.
Day 4	Lesson 4, Activity 1	The video "The Power of Comparative Genomics" (7:07; https://www.youtube.com/watch?v=mU9ROpm6d70) introduces comparative genomics as a tool to help scientists focus their research.
	Lesson 4, Activity 2	The video "Comparison of Genomes of Eight Enteroaggregative *E. coli* O104:H4 Isolates" (2:07; https://youtu.be/6VTxmnZQXgU) shows how comparative genomics facilitates identification of genomic islands that contribute to the pathogenicity of disease outbreak strains.
	Lesson 4, Activity 3	Students complete video tutorials on the Pathosystems Resource Integration Center (PATRIC), a web-based comparative genomics tool.
Day 5	Lesson 5, Activity 1	Students work in groups, using PATRIC to research virulent genes and disease outbreaks for an assigned bacterial species.
Day 6	Lesson 5, Activity 2	Students present their research to the class and are graded according to a rubric (Figure 4).

Survivor curve being an actual representation of pathogen survival during preservation process.

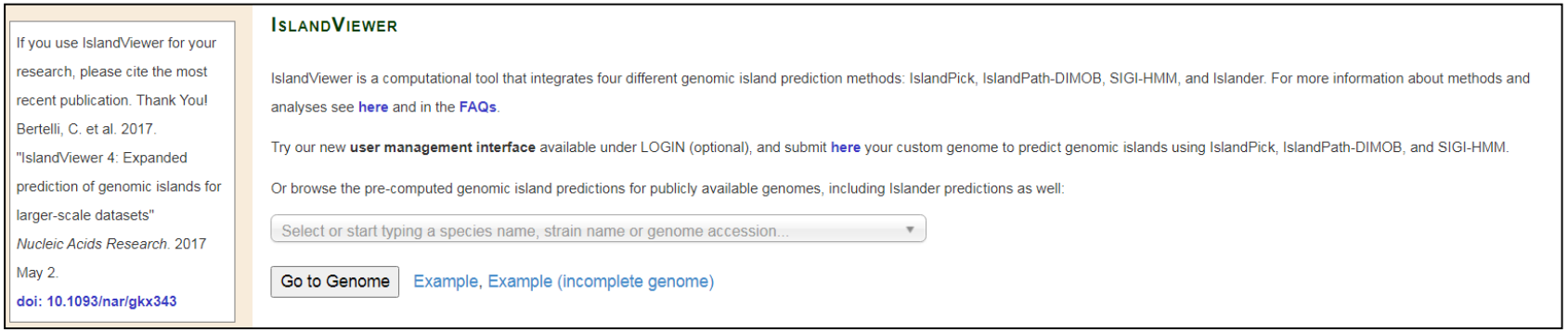
Bacusmo *et al.*,2019

Genome of prokaryotes

Module Overview

IslandViewer is a computational tool that integrates four different genomic island prediction methods: IslandPick, IslandPath-DIMOB, SIGI-HMM, and Islander. For more information about methods and analyses see here and in the FAQs.

- Select option Example and then press the Go to Genome button to browse the pre-computed genomic island predictions for publicly available genomes, including Islander predictions as well:



Genome of prokaryotes

Module Overview

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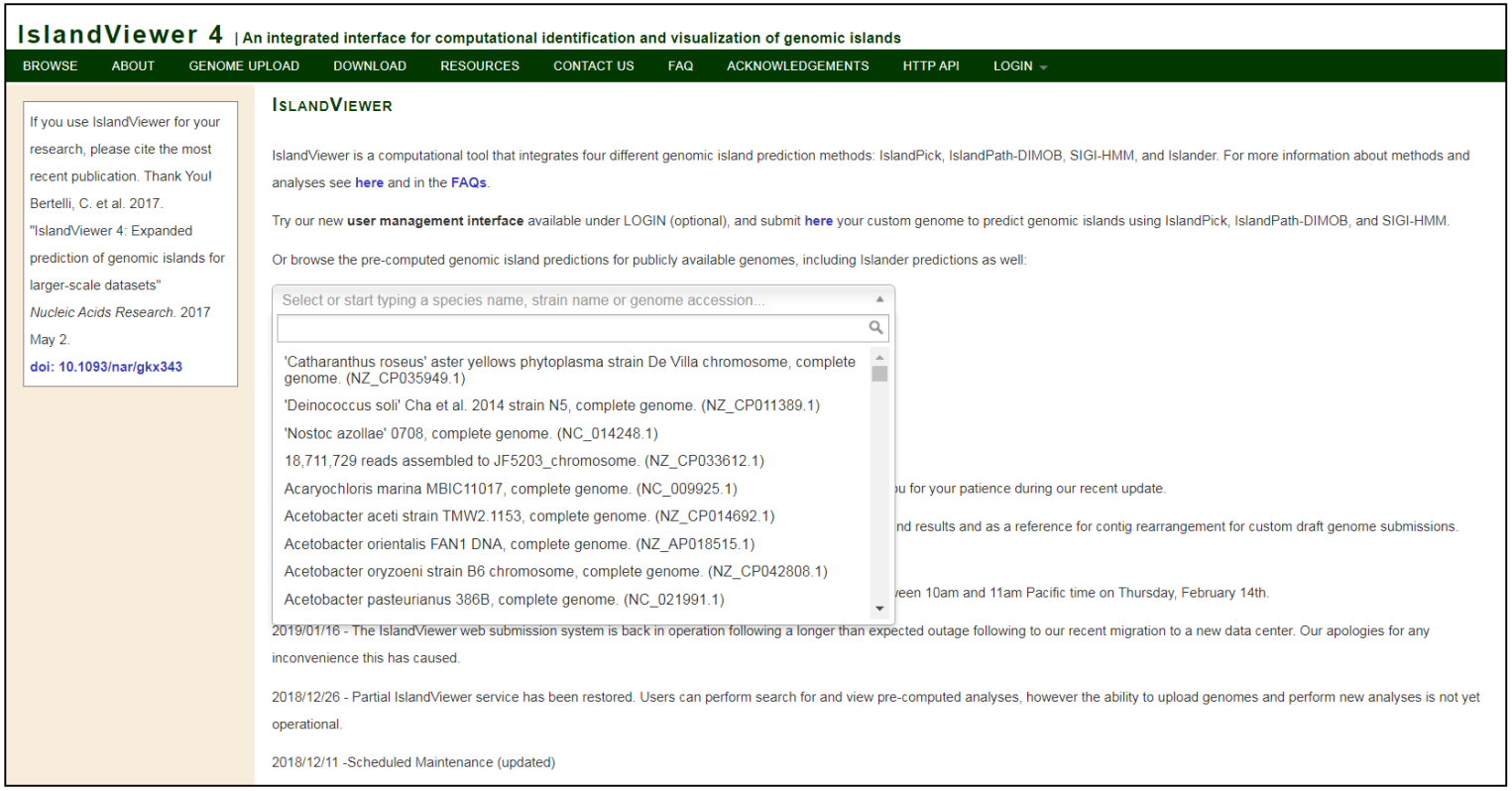
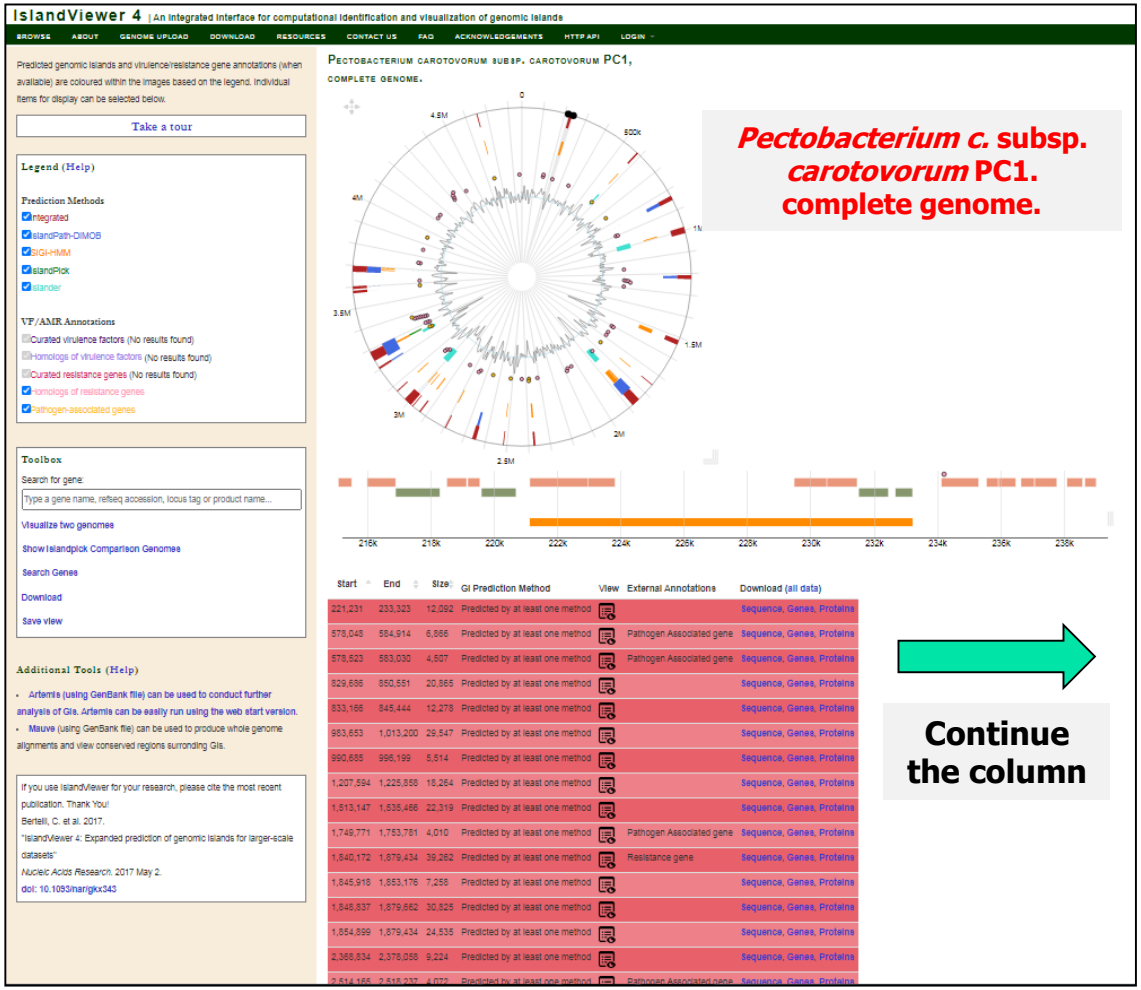


Genome of prokaryotes

Module Overview

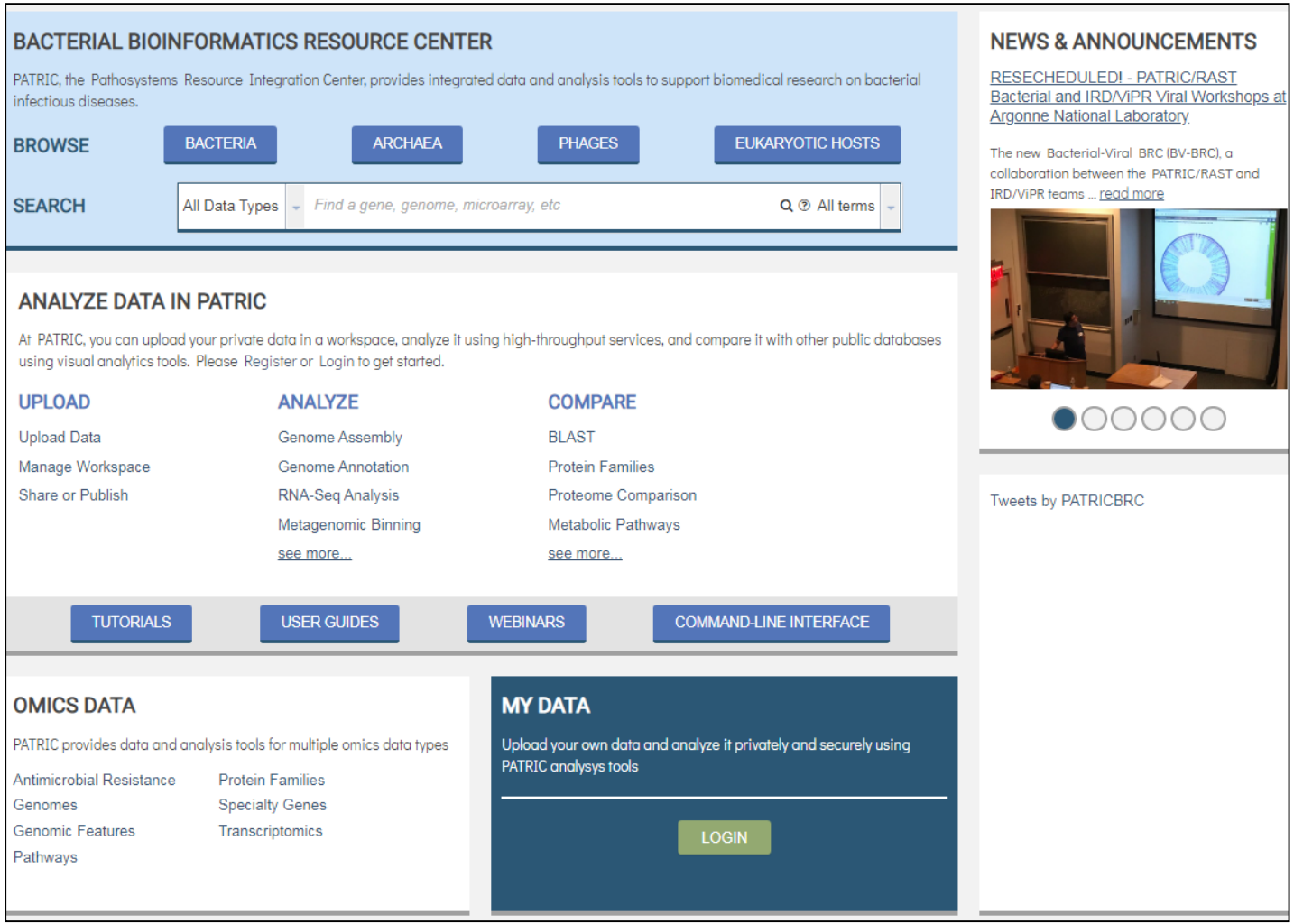
IslandViewer 4 | An integrated interface for computational identification and visualization of genomic islands



Genome of prokaryotes

Module Overview

PATRIC, the Pathosystems Resource Integration Center, provides integrated data and analysis tools to support biomedical research on bacterial infectious diseases



Genome of prokaryotes

Module Overview

Rubric used to assess student performance in the independent research and presentation

Poster and Presentation Rubric

Bacteria Name _____

Group Members Names _____

All points earned according to this rubric assume accuracy of content and can be verified by the teacher.

Criteria	Excellent (3)	Good (2)	Fair (1)	Poor (0)
Poster (9 points)	☐ Content is well organized ☐ Excellent balance of text, graphics and color ☐ No misspellings	☐ Content is reasonably organized ☐ Good balance of text, graphics and color ☐ No more than one misspelling	☐ Minimal organization of content ☐ Fair balance of text, graphics and color ☐ No more than two misspellings	☐ Lacking in organization (cluttered) ☐ Poor balance of text, graphics and color ☐ Many misspelled words
Bacteria (12 points)	☐ Morphology is complete ☐ Photo enhances content ☐ Genomic information is complete ☐ Genomic Islands are identified and thoroughly discussed	☐ Morphology is missing one item ☐ Photo relates to content ☐ Genomic information is missing one item ☐ Genomic islands are identified and discussed	☐ Morphology is missing two items ☐ Photo only tangentially related to content ☐ Genomic information is missing two items ☐ Genomic islands are minimally discussed	☐ Morphology is missing more than two items ☐ Missing photo or graphic ☐ Genomic information is mostly incomplete ☐ Genomic Islands are mentioned, but not discussed
Disease (9 points)	Thorough discussion of the following topics: ☐ Pathology ☐ Antibiotics ☐ Epidemiology	Good discussion of the following topics: ☐ Pathology ☐ Antibiotics ☐ Epidemiology	Fair discussion of the following topics: ☐ Pathology ☐ Antibiotics ☐ Epidemiology	Poor or no discussion of the following topics: ☐ Pathology ☐ Antibiotics ☐ Epidemiology
Story (6 points)	☐ Outbreak Information is complete	☐ Outbreak information is partially complete	☐ Outbreak information is mostly incomplete	☐ Missing outbreak information
Resources (9 points)	☐ Evidence of primary resource that relates directly to lesson content ☐ Main points on IslandViewer Image are complete and easy to identify ☐ Typed bibliography in APA format attached	☐ Evidence of primary resource that is mostly relatable to the content ☐ Main points on IslandViewer Image are incomplete ☐ Typed bibliography attached, but format is inconsistent	☐ Primary resource is only tangentially related to lesson content ☐ Main points on IslandViewer Image are missing ☐ Bibliography attached, but not typed and inconsistent formatting	☐ No primary resource ☐ Missing IslandViewer Image ☐ Missing bibliography
Oral Presentation (9 points)	☐ Roles are clearly defined ☐ Each student seems confident and knowledgeable and correctly pronounce all words ☐ Time limit is met, but not exceeded	☐ Roles mostly well defined ☐ Three students seem confident and knowledgeable ☐ Time is within 2-min window	☐ Roles are not clearly defined ☐ Two students seem confident and knowledgeable ☐ Time is within 4-min window	☐ Students do not seem to know their role ☐ An obvious and heavy reliance on one student for knowledge ☐ Time is within 5-min window
Total Points			Points Earned: /54	

Bibliography Grade: _____/

IslandViewer Data Table Grade: _____/

Total Points Earned: _____ / (+ 54)

Rubric: a set of instructions, especially on an exam paper, usually printed in a different style or colour.

Bacusmo *et al.*,2019

Pathogenicity islands

Variable numbers of pathogenicity islands

- A bacterium may have more than one pathogenicity islands (PAIs).
- One species of bacteria may have more than one PAI.

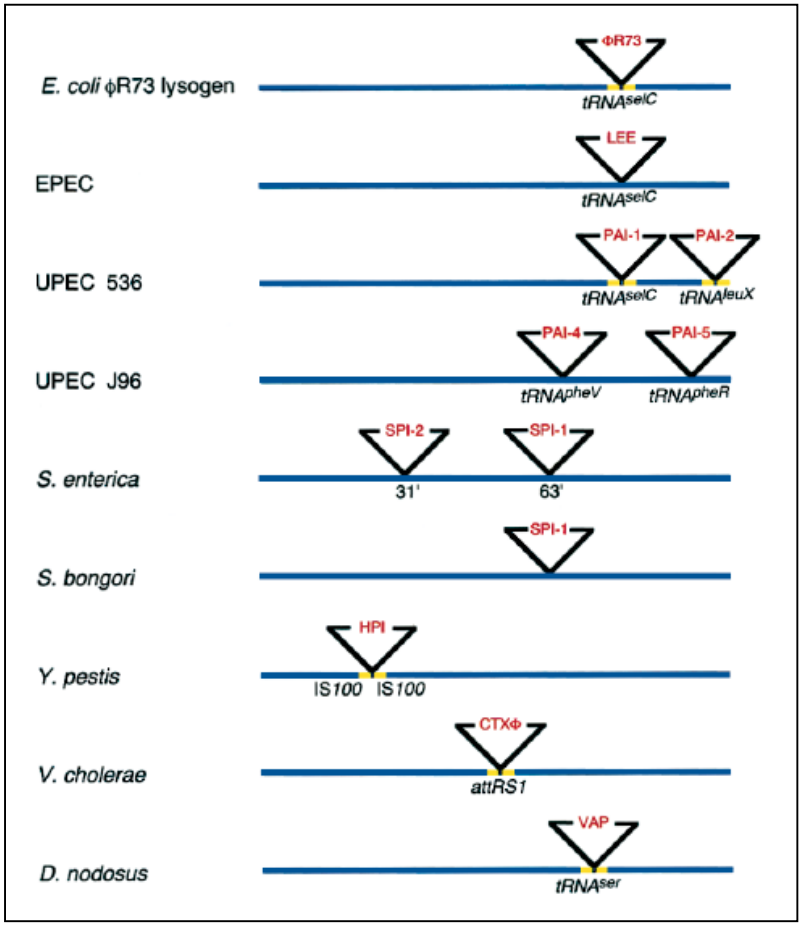
1. *Salmonella* has at least 5 PAIs.
2. *E. coli* at least 2 PAIs.
3. Frequently, PAIs encode type III and IV secretion systems which, by excretion of proteins, directly interfere with the functions of host cells.

Wikipedia,2011

Pathogenicity islands

Location of selected pathogenicity islands and phages of Gram-negative bacteria

- Chromosomes (blue lines) and pathogenicity islands (black triangles) are not drawn to scale.
- The presence of repeated sequences at the site of insertion, which is shown below the island, is indicated by short yellow lines.

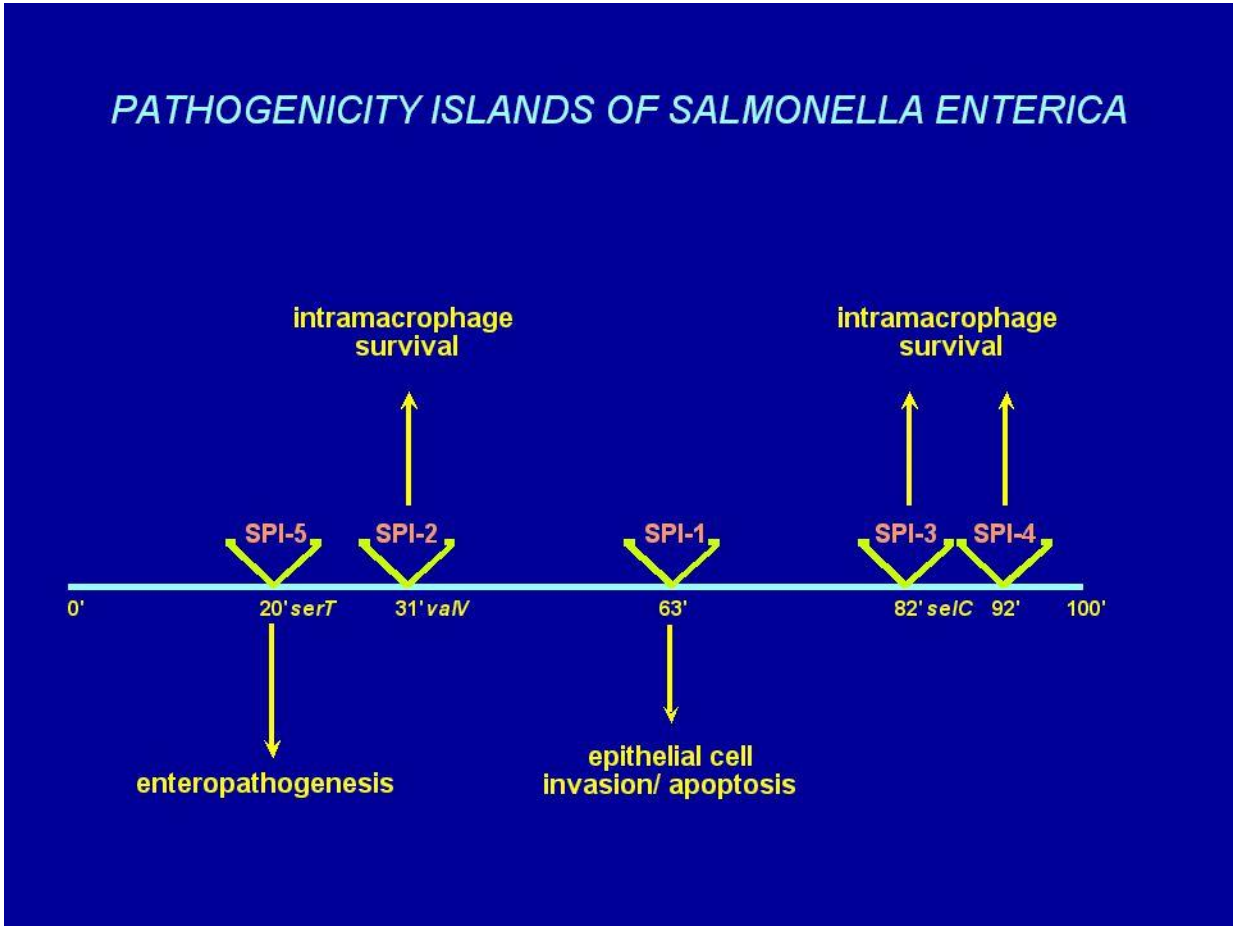


UroPathogenic *Escherichia coli* (UPEC) causes urinary tract infections (UTIs).

Groisman and Ochman,1996

Pathogenicity islands

Salmonella anterica



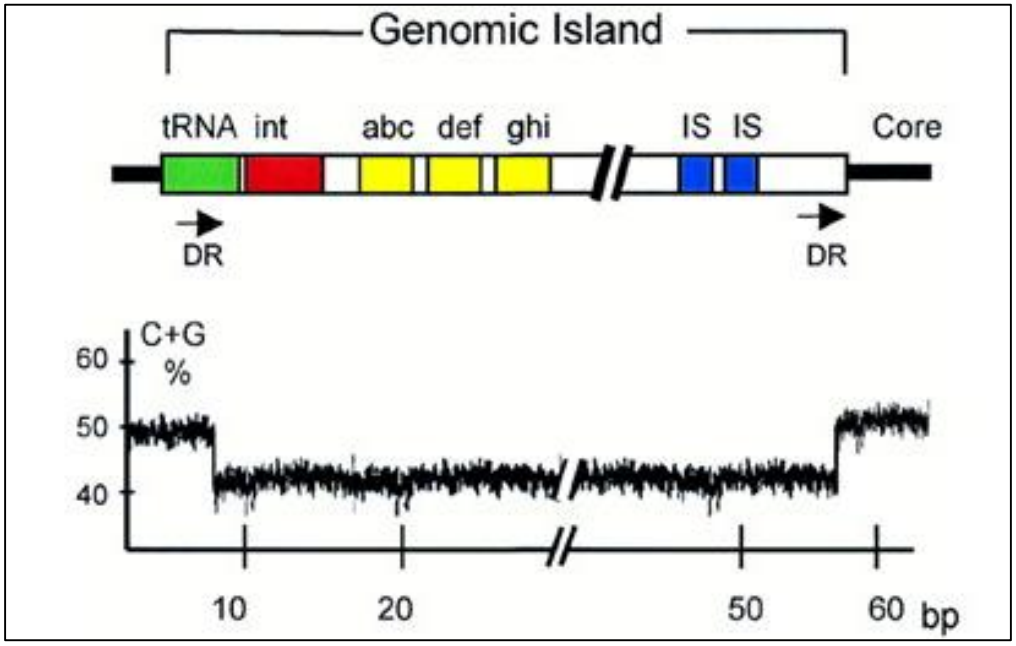
Genome sequencing
General structure and feature of PAI

1. found in a wide variety of bacterial pathogens;
2. occupy relatively large regions of the chromosome, ranging from 10-200 kb;
3. missing from related non-pathogens (e.g. *E. coli*);
4. often different G-C content; codon usage;
5. often mobile (spread easily), transferred horizontally, through plasmids or transposons.
6. often associated with tRNA loci on one side and direct repeat (DR) sequences on both sides. tRNAs act as sites for recombination into the DNA.
7. encode functions necessary for pathogenesis.

The addition of a pathogenicity island to a non-invasive species can make the non-invasive species pathogenic.

Genome sequencing

General structure and feature of genomic islands



Schematic model of a genomic island of bacteria (upper part). The formerly transferred DNA block is linked to a tRNA gene and flanked by direct repeats (DR). The guanine plus cytosine (G+C) content of the genomic island is different from that of the core genome (lower part).
Other abbreviations: int, integrase gene; abc, def and ghi, genes encoding specific functions; IS, insertion sequence element; bp, base pair.

Hacker and Carniel,2001

Pathogenicity islands

Shared and unique genes

GC-content of pathogenicity islands

- It is estimated that each bacterium has about 40% of its genome devoted to unique genes.
- The GC-content of pathogenicity islands often differs from that of the rest of the genome.
- The G+C content of pathogenicity islands often differs (below 40%) from that of the rest of the genome (overall G+C content of the organism).

The codon usage or codon preference of whole genomes is strongly biased. i.e. each individual genome uses a preferred set of codons.

Relatively low GC contents (below 40%) are assembled in a "pathogenicity island".

Pathogenicity islands
Unstable DNA regions

- PAIs often represent unstable DNA regions.
- PAIs may:

1. move from one tRNA locus to another, or
2. be deleted.

- Deletions of PAIs may occur via:

1. the direct repeats (DRs) at their ends, or
2. via IS elements or other homologous sequences located on PAIs.

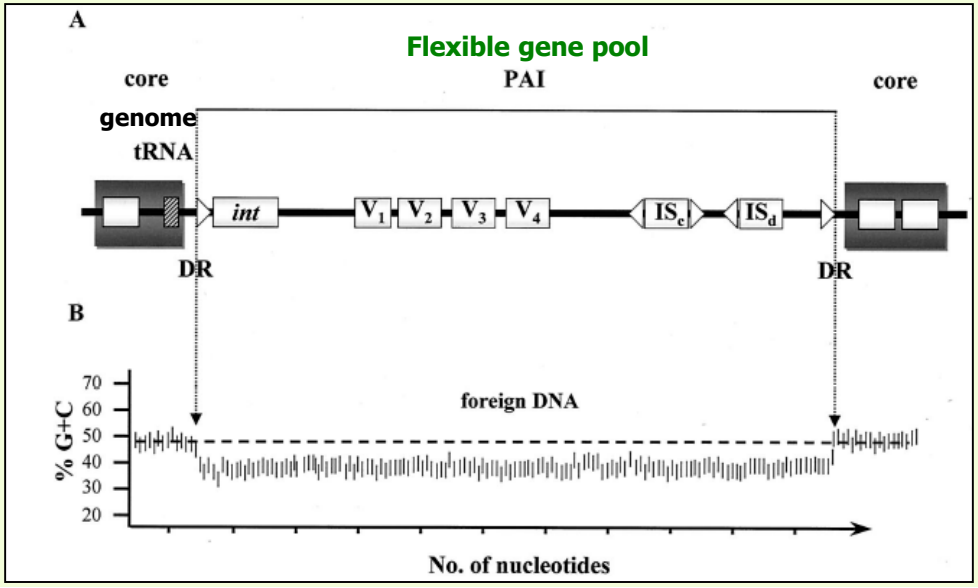
Relatively low GC contents (below 40%) are assembled in a "pathogenicity island".

Hacker and Kaper,2000

General structure of a PAI

tRNA genes may act as landmarks for the integration of foreign DNA, either of plasmids and phages. Direct repeats (DR) may save served as recognition sites for the integration of bacteriophages, and for enzymes involved in excision of mobile genetic

- The PAI boundaries are frequently determined by DRs (direct repeats), each DR is 16-20 bp long with perfect sequence repetition (triangle) at the ends of the pathogenicity island.
- DRs are used for insertion and deletion processes. *int*, integrase gene. IS, insertion sequence(simple transposon). The association of PAIs and tRNA loci led to the generation of PAIs by horizontal gene transfer.



Relatively low GC contents (below 40%) are assembled in a "pathogenicity island".

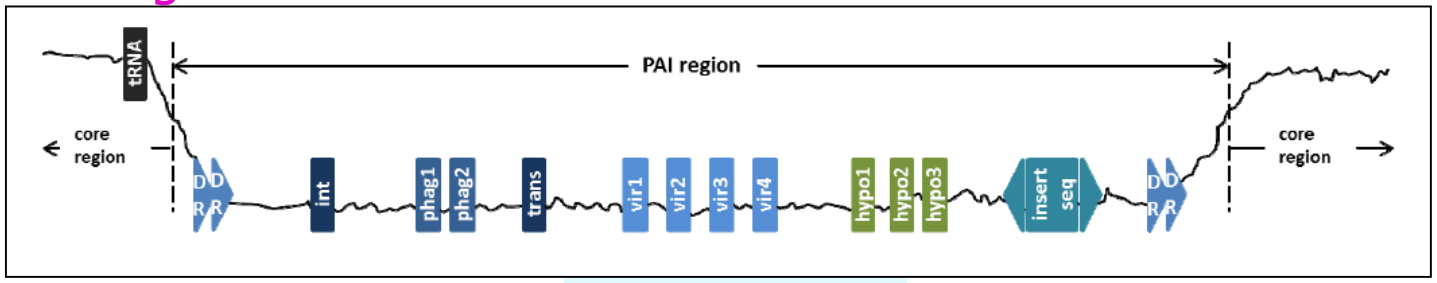
Schmidt and Hensel,2004

General structure of a PAI

Schematic view of a pathogenicity island with associated features. The PAI region has biased sequence composition

- The PAI regions are associated with:

1. virulence genes (four genes: vir1, vir2, vir3, and vir4),
2. phage-related genes (phag1 and phag2),
3. mobile genes (integrase gene and transposase gene),
4. hypothetic protein genes (proteins with unknown function) such as hypo1, hypo2, and hypo3,
5. insertion sequence elements,
6. direct repeats(DRs), and
7. tRNA gene.



Che *et al.*,2014

General structure of a PAI

In methicillin-resistant *Staphylococcus aureus*

- *Staphylococcus aureus* genome contains core genome, accessory component, and foreign genes.

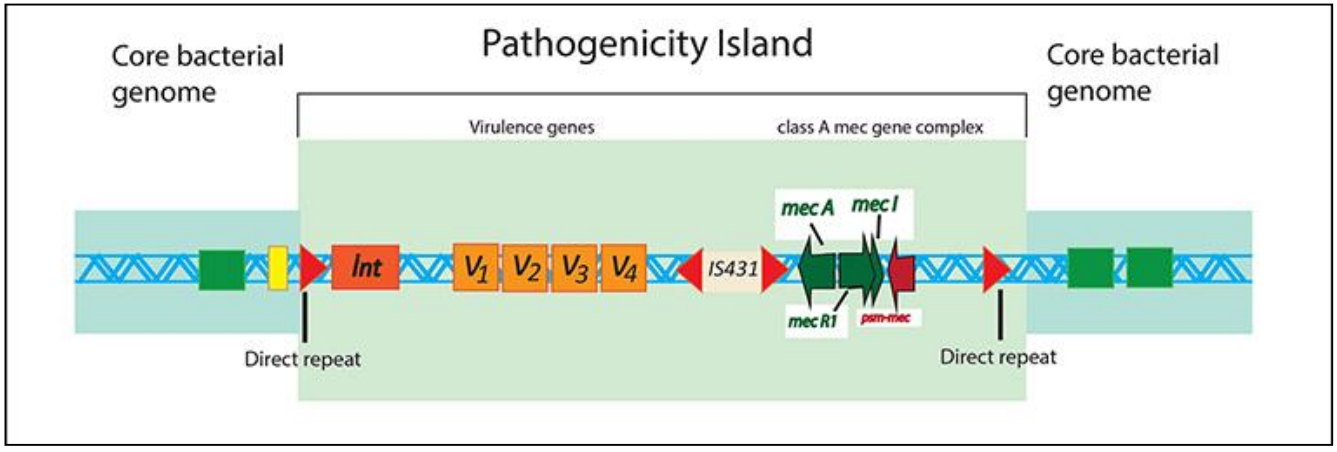
1. Core genome that constructs backbone of genome has main metabolic function. Core genome is highly conserved, and similarity of genes among isolates is ~98–100%.
2. Accessory component that constructs 25% of *S. aureus* genome contains mobile genetic elements (MGEs) such as transposons (Tn), chromosomal cassettes, pathogenicity islands (PIs), genomic islands, and prophages acquired horizontally between strain.

Kırmusaoğlu,2017

General structure of a PAI

In methicillin-resistant *Staphylococcus aureus*

- Mobile genetic elements (MGEs) carry virulence genes. These genes encode for varied virulence factors and toxins.
- MGEs in methicillin-resistant *S. aureus* (MRSA) contain the mecA gene causing methicillin resistance and affect biofilm phenotype of *S. aureus*. *mec*A gene expression is regulated by *mec*I and *mec*RI that are own regulators.



Kırmusaoğlu,2017

General structure of a PAI

Schematic view of a pathogenicity island with associated features. The PAI region has biased sequence composition

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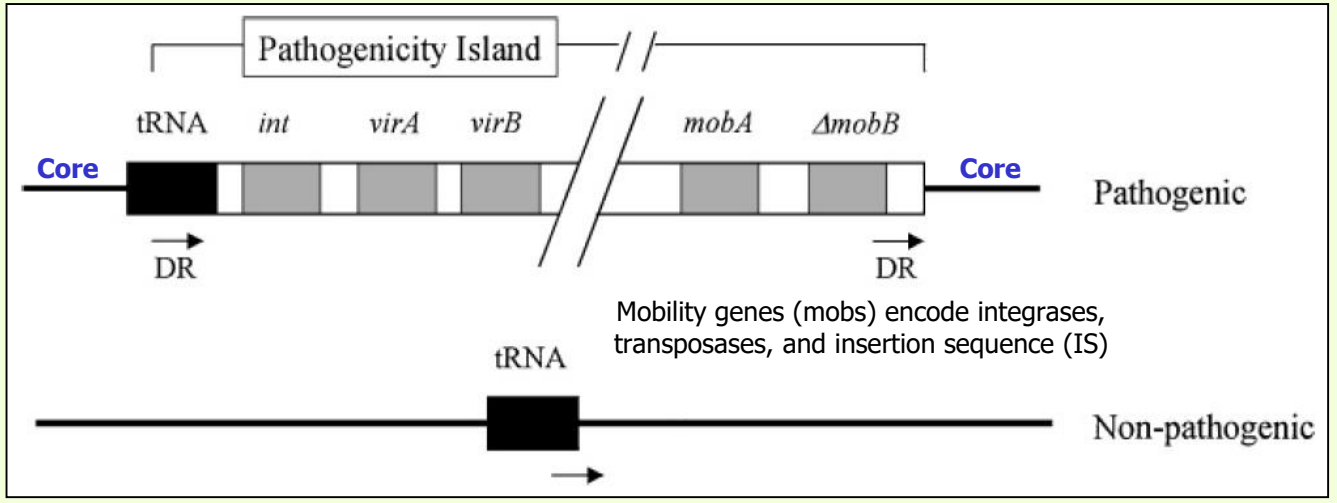
Che *et al.*,2014

Pathogenicity islands

General structure of PAI

Pathogenic vs. Nonpathogenic bacteria

- The thin bold line represents regions of the core genome; The box represent genes whose products contribute to virulence. The arrows indicate the presence of direct repeats (DRs) at the ends of the pathogenicity island.
- Abbreviations:
- DR, direct repeats; *int*, integrase gene; *vir*, virulence-associated gene; *mob*, mobility gene; *Δ*mob, pseudo-mobility gene. *mob* genes encode integrases, transposases, or other proteins involved in mobility of the prokaryotic genome.



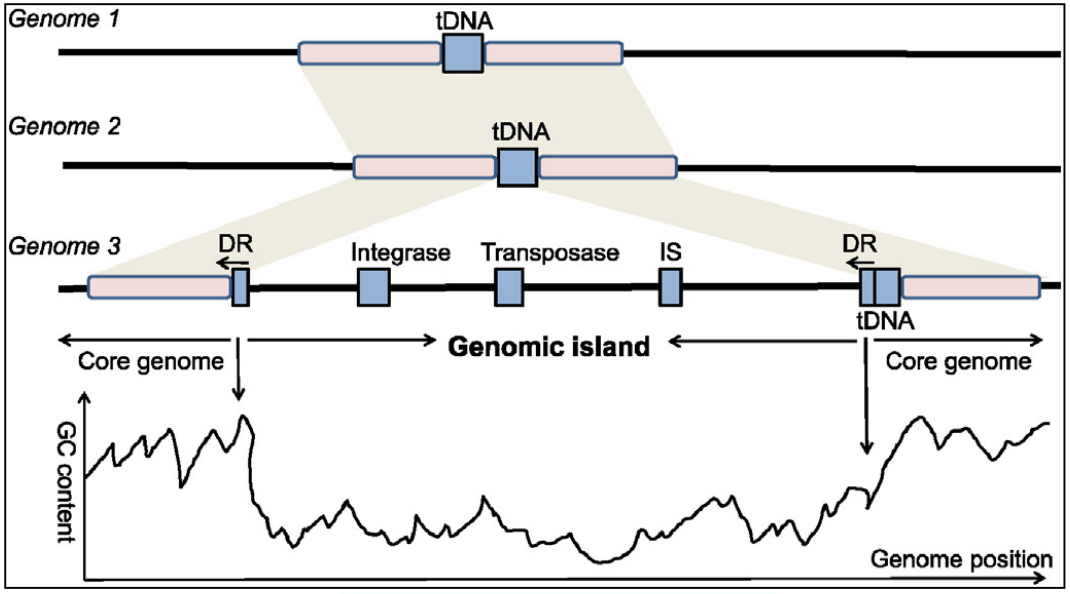
Hacker and Kaper,2000

Pathogenicity islands

General structure of PAI

A GI is often absent in closely related genomes

- The schematic representation of several GI-associated features. A GI is often absent in closely related genomes (1 and 2). It may also have atypical compositional characteristics compared with the core genome, such as lower GC content. The presence of several sequence elements is indicative of a GI: flanking conserved regions, DRs, insertion sequence (IS) elements and mobility-related genes encoding integrase and transposase..



Lu and Leong,2016

General structure of PAI
Function of different elements

- **Direct repeats (DRs):**
- The PAI boundaries are frequently determined by DRs (direct repeats).
- Direct repeats (DR) that are presented as DNA sequences of 16 to 20 bases pairs (up to 130 bp).
- Might have served as recognition sites for the integration of bacteriophages, and their integration resulted in the duplication of the DR.
- Furthermore, DR act as recognition sequences for enzymes involved in excision of mobile genetic elements, thus contributing to the instability of a PAI flanked by DR.

General structure of PAI
Function of different elements

- **Mobility genes (integrase gene and transposase gene):**
- These genes are frequently located at the beginning of the island, act as rapid DNA rearrangements, and exchange.
- Site specific integration of pathogenicity islands is mediated by an integrase recombinase.

Integrase: Any enzyme that integrates foreign DNA into that of an infected cell.
Transposon: A segment of DNA that can move to a different position within a genome. Transposons are involved in the intrabacterial movement of DNA. They may, however, be transmitted to other bacteria by transfer of plasmid or chromosomal DNA.

General structure of PAI
Function of different elements

- **Different functions of tRNA genes:**
- The main function of tRNA molecules is to bind mRNA at the ribosome for protein synthesis.
- But some tRNAs have functions unrelated to translation.
- E.g. Some tRNA genes are frequently associated with bacterial pathogenicity islands.
- tRNA genes served repeatedly for the integration of foreign DNA.

Pettersson,2009;..

General structure of PAI
Function of different elements

- **tRNA genes:**
- PAIs are often associated with transfer RNA (tRNA) genes.
- About 75% of the PAIs identified so far are associated with tRNA loci.
- The association of PAIs and tRNA loci may therefore reflect the generation of PAIs by horizontal gene transfer.

Hacker and Kaper,2000

General structure of PAI

tRNA gene structure and organization

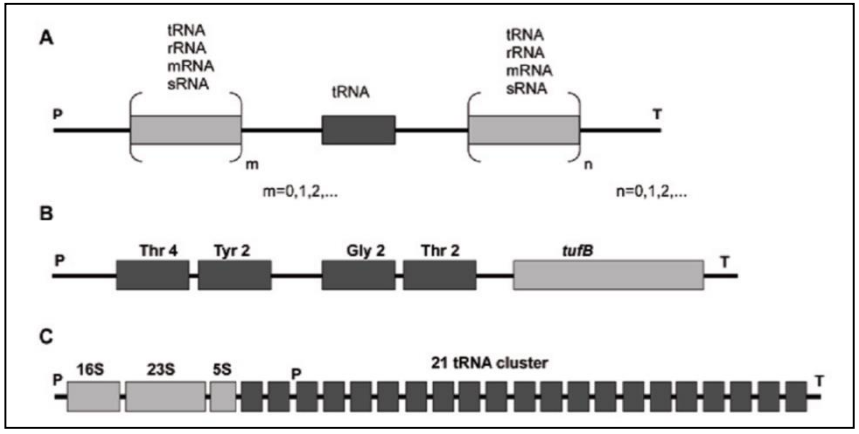
tRNA genes

A. A generalized tRNA operon is shown: A tRNA gene may be transcribed in many different contexts, including rRNA, mRNA, sRNA (bacterial small RNAs), and other tRNA genes.

B. The *E. coli tufB* operon: is shown as an example of an operon with both tRNA and protein coding genes.

C. The *B. subtilis rrnB/trnB* operon: is shown as an example of an rRNA operon encoding several tRNAs.

rrnB: an rRNA operons; P and T indicate promoter and terminator sequences, respectively.



Two genes, tufA and tufB, located at 73 and 88 minutes of the Escherichia coli linkage map, code for the polypeptide chain elongation factor EF-Tu. tufB is transcribed with four upstream tRNA genes, thrU, tyrU, glyT and thrT, into a cotranscript of approximately 1800 nucleotides.

Pettersson,2009

Pathogenicity islands

Horizontal gene transfer

Pathogenicity islands

- Pathogenicity islands were first described in human pathogens of the species *Esehерichia coil* in the late 1980s, but have recently been found in the genomes of various pathogens of humans, animals, and plants.
- Thus, rapid alterations of phenotype can occur via horizontal gene transfer (HGT).
- It is possible to recognize genes that arose by lateral gene transfer by simply examining genome sequences.

Kjemtrup *et al.*,2000; Buonaurio,2008;Van Sluys *et al.*,2002

Pathogenicity islands

Horizontal gene transfer

Integration sites of PAI

1. Pathogenicity islands are discrete genetic units flanked by direct repeats, insertion sequences or tRNA genes, which act as sites for recombination into the DNA.
2. Cryptic mobility genes may also be present, indicating the provenance as transduction.
3. They are transferred through horizontal gene transfer events such as transfer by a plasmid, phage, or conjugative transposon.

Conjugative transposons such as Tn916 are transposon-like elements, different from well-studied transposons such as Tn*5* and Tn*10*. Resistance genes need not be carried on the conjugative transposon to be transferred. See Genetic file for details.

Pathogenicity islands

Horizontal gene transfer

Integration sites of PAI

- Integration of PAI into the bacterial chromosome is a site-specific event.

1. Most PAI currently known have inserted at the 3’ end of tRNA loci.
2. Also, phage attachment sites frequently are located in this region.
3. However, certain genes, and infrequently intergenic regions in operons are used by PAI.

Schmidt and Hensel,2004

Pathogenicity islands

Horizontal gene transfer

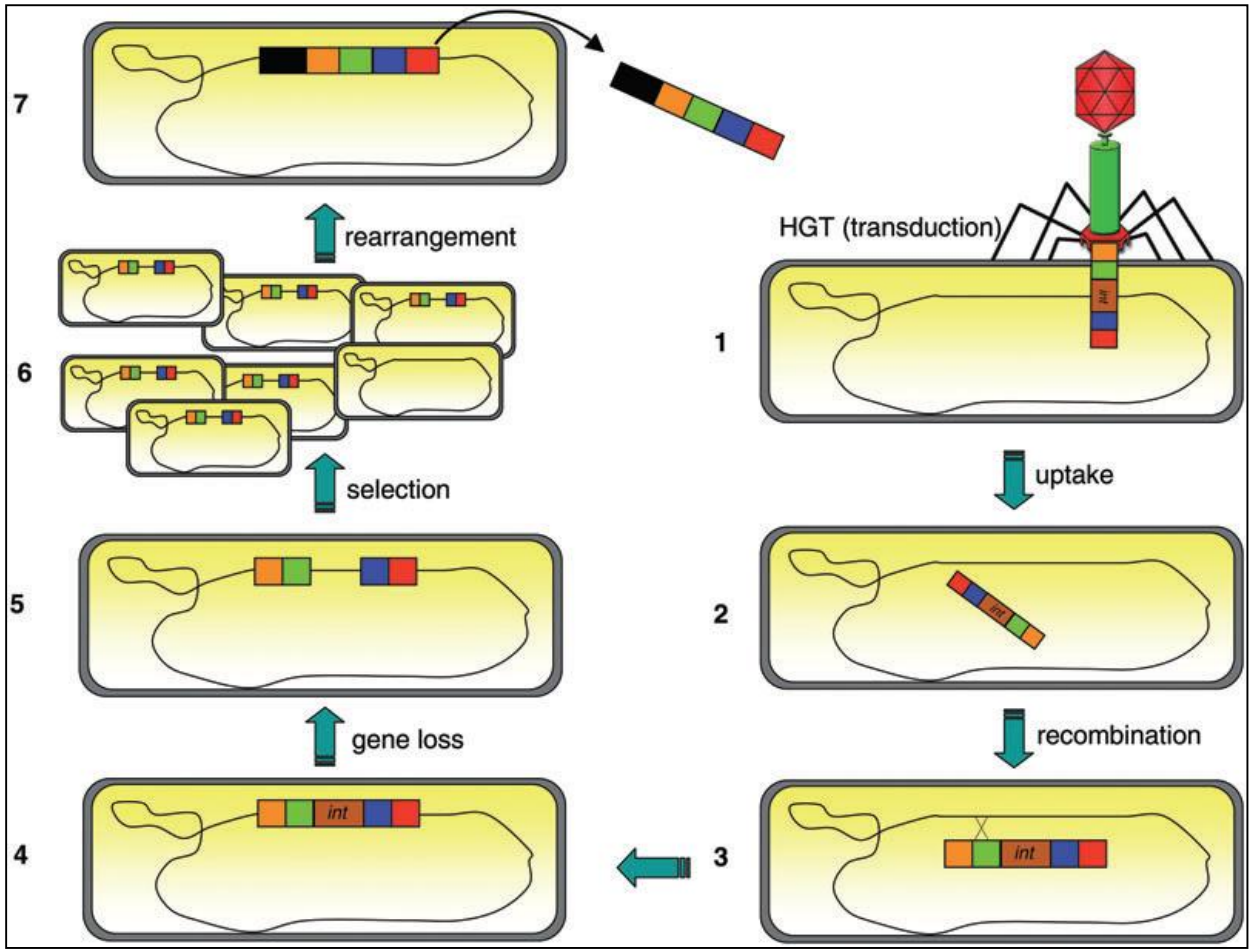
Phage transduction and prophage integration are the major mechanisms of horizontal gene transfer

- High percentage of phage-related genes has been found in PAIs.
- In actuality, phage transduction and prophage integration are the major mechanisms of horizontal gene transfer in prokaryotes.
- The food pathogen *E. coli* O156:H7 strain Sakai has been discovered to contain around 16% prophage of its own total genome sequence.

Che *et al.*,2014

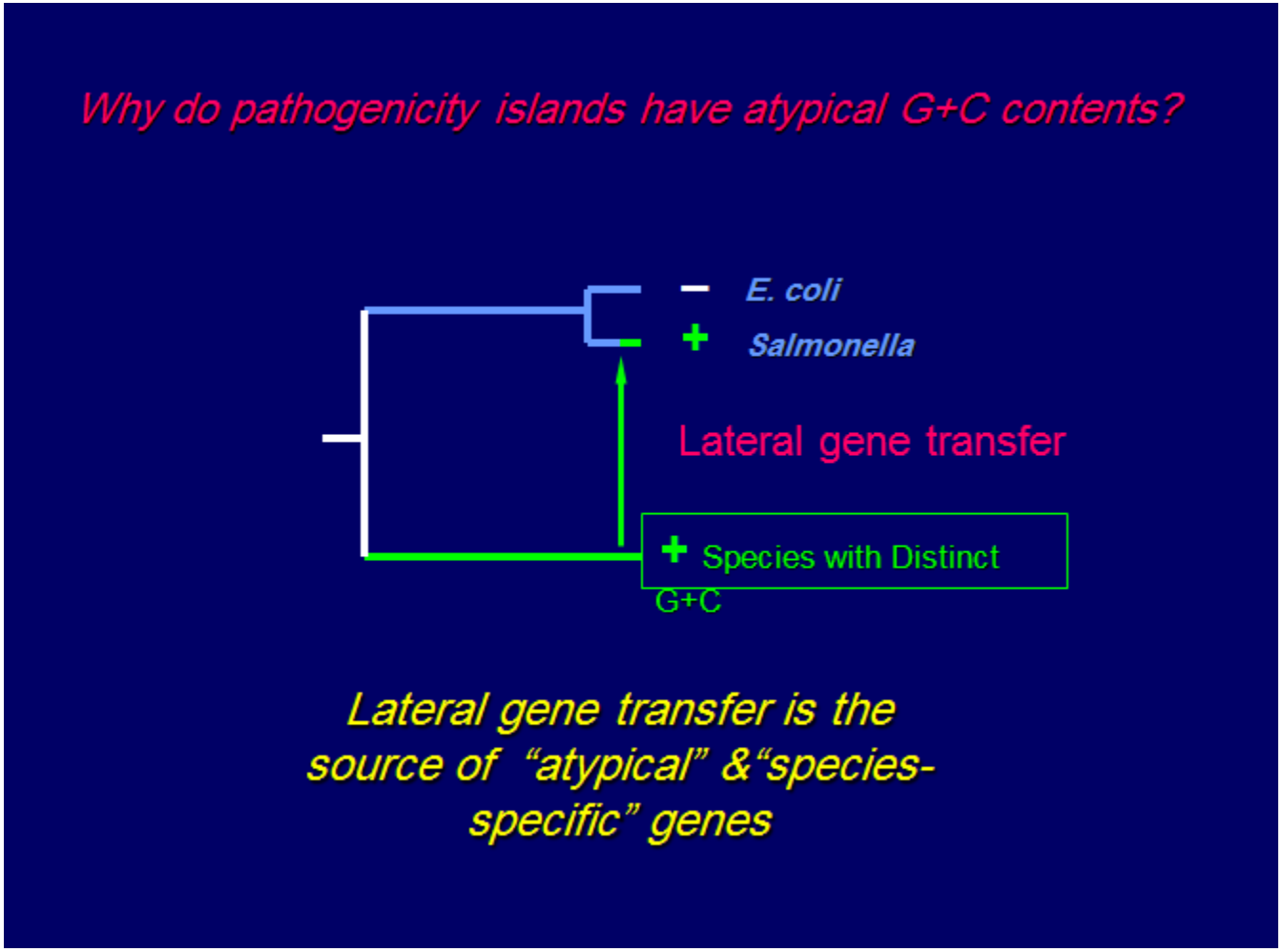
Phage transduction and prophage integration are the major mechanisms of horizontal gene transfer

Mobile genes such as integrases (*int*), are frequently located at the beginning of the island, act as rapid DNA rearrangements, and exchange



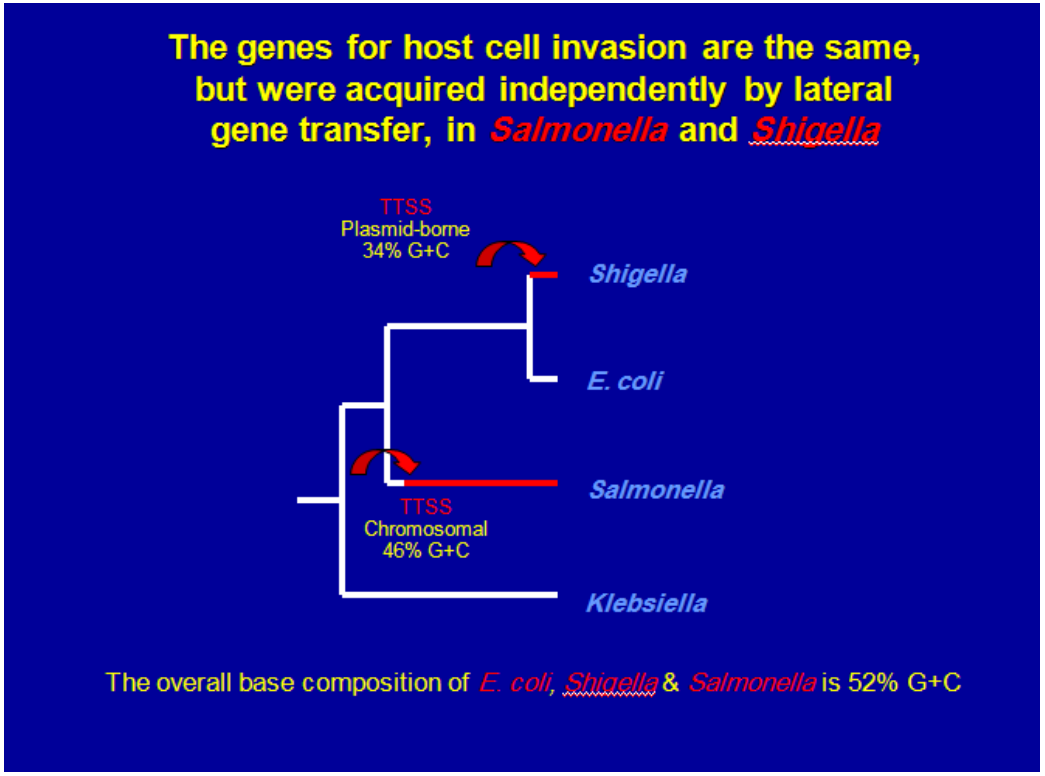
Gal-Mor and Finlay,2006

Pathogenicity islands
Horizontal gene transfer



Pathogenicity islands
Horizontal gene transfer

- Frequently, PAIs encode type III and IV secretion systems which, by excretion of proteins, directly interfere with the functions of host cells.



Pathogenicity islands

hrp-hrc regions are now designated pathogenicity islands (PAIs)

Plant pathogenic bacteria

- The *hrp-hrc* regions are now designated pathogenicity islands (PAIs) in various plant-pathogenic bacteria.
- The *hrp* cluster encodes a type III secretion system that is regulated by four proteins encoded on the *hrp* PAI.
- *hrp* and *hrc* genes are part of a PAI (located within pathogenic islands) that has been designated Hrp. e.g.

1. Hrp PAI of *E. amylovora*
2. Hrp PAI island of *Pseudomonas syringae*, or
3. Hrp PAIs of other *Xanthomonas* species (note: in *Xanthomonas* the *hrp* gene cluster was previously known as a pathogenicity island but it is reported missing from some *Xanthomonas* spp.).
4. The *hrp*/*hrc* genes within the Hrp PAI core region was highly conserved.

Genome (conserved and flexible)/genomic islands (flexible)/PAIs (flexible)/hrp/hrc regions (conserved).

Kjemtrup *et al.*,2000; Buonaurio,2008, Ali Shah *et al.*, 2019

Pathogenicity islands

Chromosome or plasmid-encoded PAIs

Plant pathogenic bacteria

1. PAIs are located on bacterial chromosome.
2. PAIs have also been localized to mobilizable plasmids.

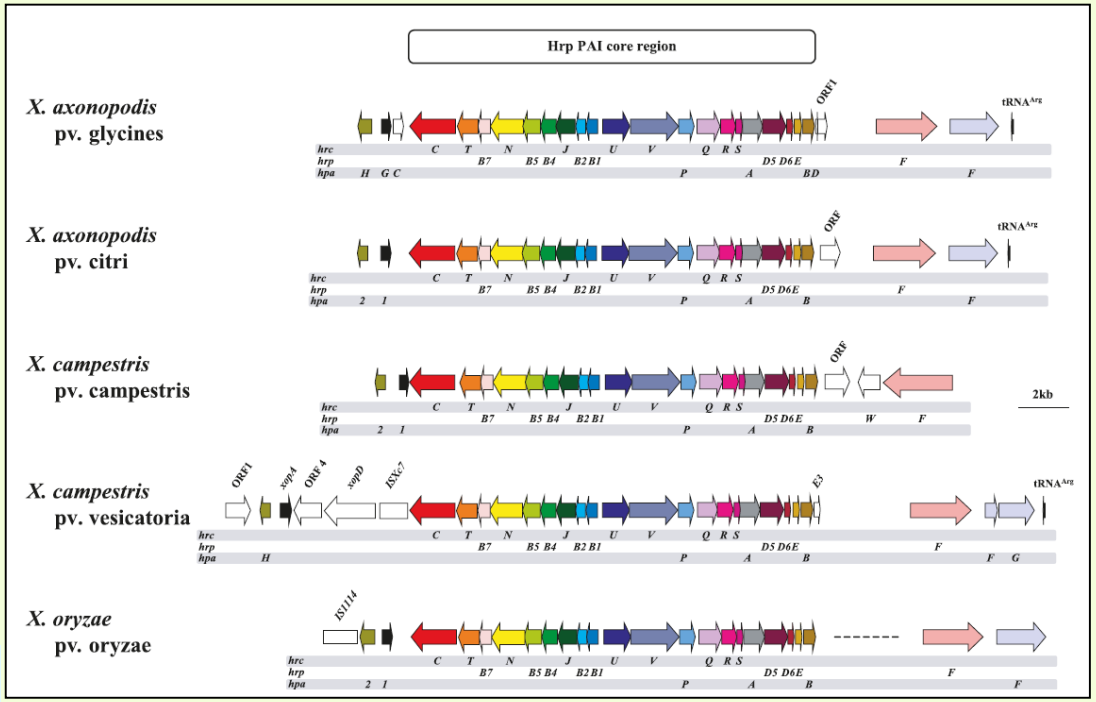
- **Plasmid-encoded PAIs:**
- Plasmid-encoded PAI was recently isolated from race 7 of *Pseudomonas phaseolicola* strain 1449B.
- Cured of a 154 kb plasmid, this strain is no longer virulent on bean, demonstrating a virulence function for this plasmid.
- **Chromosome-encoded PAIs:**
- *hrp*/*hrc* cluster of *R. solanacearum* does not seem to be a PAI and that its effector genes are distributed randomly on both the plasmid and chromosome.

Kjemtrup *et al.*,2000,...

Comparisons of Hrp PAIs of five *Xanthomonas* species

Note: in *Xanthomonas* the *hrp* gene cluster was previously known as a pathogenicity island but it is reported missing from some *Xanthomonas* spp.

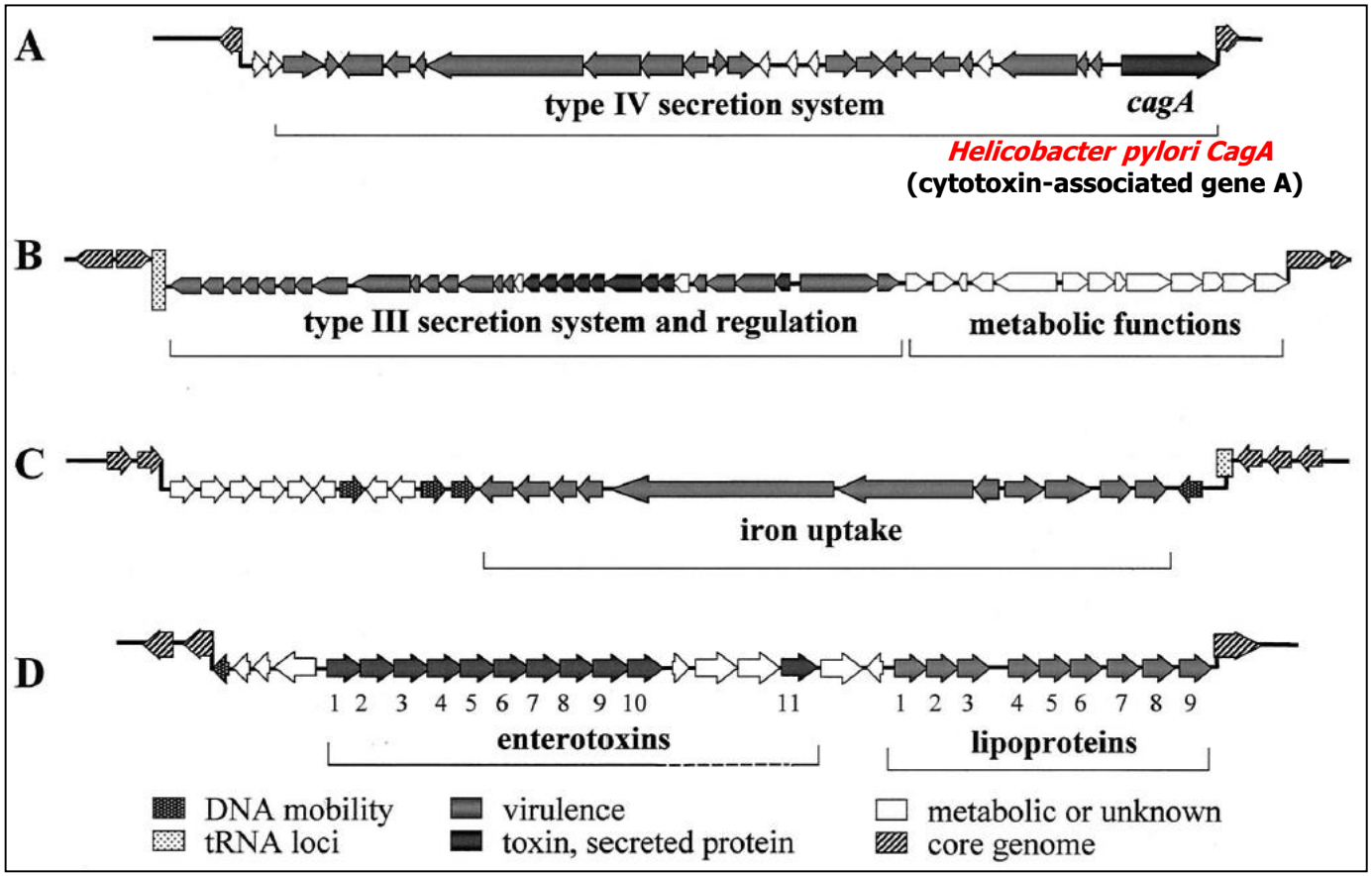
- The Hrp PAI core regions (highly conserved) and variable flanking regions of *X. axonopodis* pv. *glycines*, *X. axonopodis* pv.*citri*, *X. campestris* pv.*campestris*, *X. campestris* pv.*vesicatoria*, and *X. oryzae* pv.*oryzae* are represented.
- The direction of arrows indicate the transcription orientation.
- The Hrp PAI core region was composed of 20 genes, from *hrcC* to *hpaB*, and encoded a type III secretion system that was highly conserved (90% similarity) among xanthomonads.



Kim *et al.*,2003

Pathogenicity islands

Examples of PAI of various pathogens. The topology of PAI of various pathogens is depicted to demonstrate different features of PAI. The functional classes of the genes are as indicated in the figure



Lipoprotein serves as a gated channel for secretion of substrates to the cell surface.

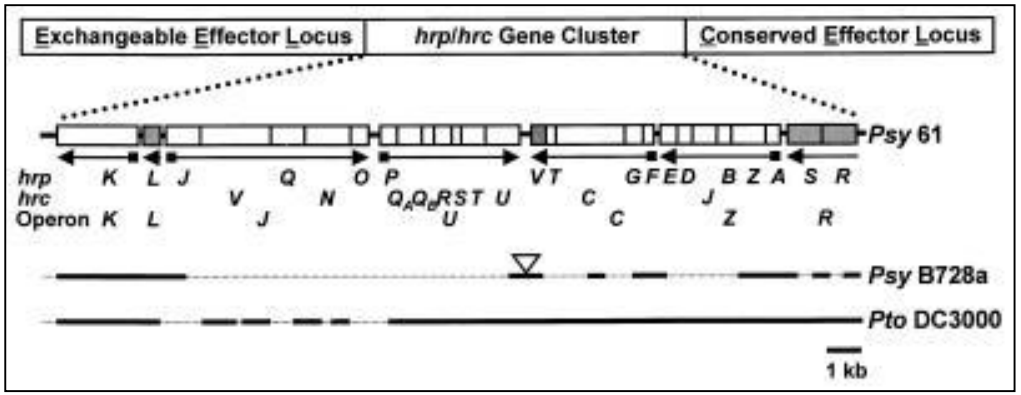
Schmidt and Hensel,2004

Pathogenicity islands

Chromosome-encoded PAI

Hrp PAI islands of *Pseudomonas syringae*

- The conserved arrangement of *hrp*/*hrc* genes within the Hrp PAIs of three strains: *Psy* 61, *Psy* B728a, and *Pto* DC3000.
- Known regulatory genes are shaded.
- Arrows indicate the direction of transcription, with small boxes denoting the presence of a Hrp box.
- The triangle denotes the 3.6-kb insert with phage genes in the B728a *hrp*/*hrc* region.

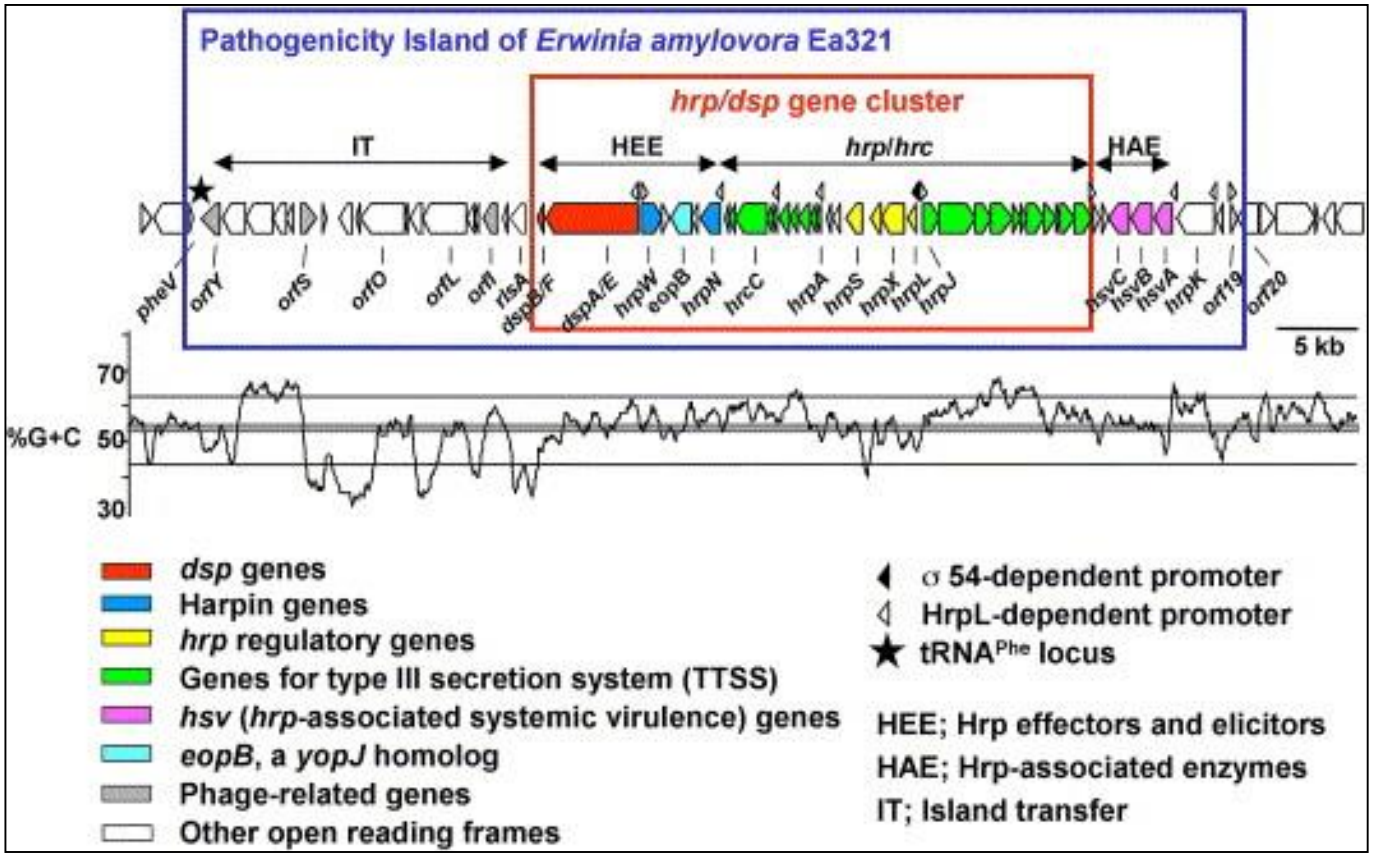


Alfano *et al.*,2000

Pathogenicity islands

Chromosome-encoded PAI

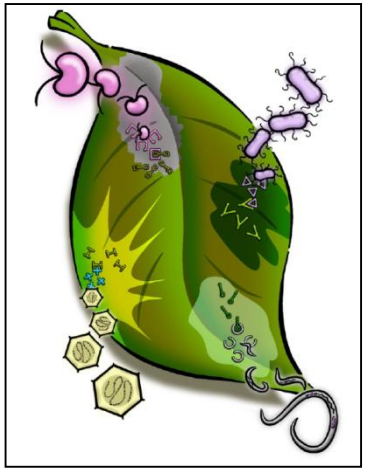
Hrp PAI islands of *Erwinia amylovora*



Oh and Beer,2005

Pathogen-generated secreted proteins
Pathogenicity-related genes/effectors

What determinants allow a bacterium to be a pathogen?



Effectors in plant–microbe interactions:
22nd New Phytologist Symposium,2009

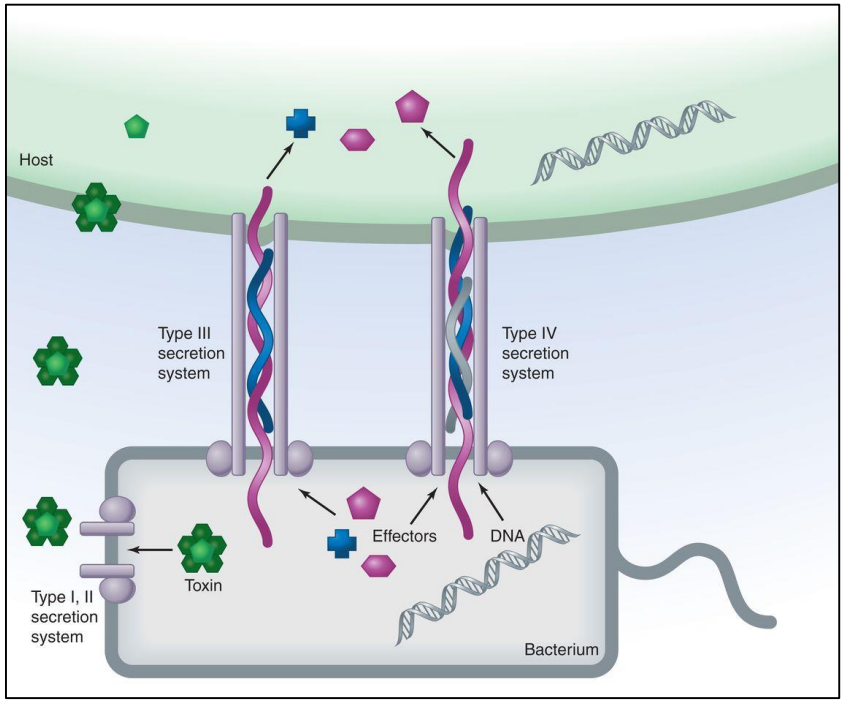
Pathogen-generated secreted proteins
Type III secretion systems (TTSSs)
Effectors of pathogens

- Effectors are pathogen molecules that:

1. manipulate host cell structure, and
2. function in order to cause/facilitate the formation of symptoms
3. often contribute quantitatively to pathogen aggressiveness, and are
4. dispensable for the pathogen life cycle.

Pathogen-generated secreted proteins
Type III secretion systems (TTSSs)
Effectors of pathogens

1. Toxins, including peptides and proteins, are typically secreted through a type I or II secretion system.
2. Effectors are translocated through a type III or IV secretion system,
3. DNA is **only** transferred through the type IV secretion system.



Alto and Orth,2012

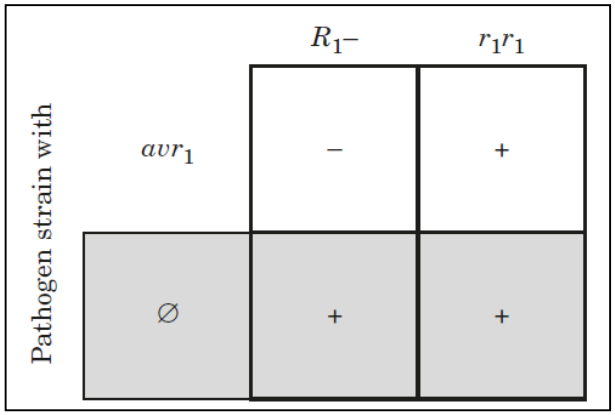
Pathogen-generated secreted proteins
Type III secretion systems (TTSSs)
Effectors of pathogens

- The type III secretion system (T3SS) is one of the most remarkably versatile pathogenicity factors yet discovered.
- A number of Gram-negative bacterial plant and animal pathogens depend on type III secretion systems (TTSSs) to inject virulence effector proteins into host cells.

1. The ability to elicit the hypersensitive response (HR),
2. A programmed cell death (PCD) associated with plant defence, as well as
3. Their pathogenic ability.

Pathogen-generated secreted proteins *avr* genes

- Pathogens possess avirulence genes whose products are involved with determining host specificity.
- The bacterial avirulence gene function is dependent on interactions with HR and pathogenicity (*hrp*) genes.



The gene-for-gene `quadratic check', as observed with phytopathogenic bacteria.
Ø=unicode name

The transcriptional activation of a number of bacterial avirulence (avr) genes is controlled by Hrp regulatory proteins.

Pathogen-generated secreted proteins *hrp* genes

- ***hrp* genes**
- *hrp* genes may be one of the most important groups of genes found in phytopathogenic bacteria in relationship to pathogenicity and host range.
- These genes/products are associated with induction of the hypersensitive response in plants.

The transcriptional activation of a number of bacterial avirulence (avr) genes is controlled by Hrp regulatory proteins.

PCR detection and identification of plant-pathogenic bacteria

Based on conserved sequences of the effectors

Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Synonyms/observations
P. savastanoi pv. *phaseolicola*	**AVR1-F/AVR1-R** Locus avrPphF **PHTE-F/PHTE-R** Locus pthE	Conventional	Bacteria (DNA extraction)	Toxigenic and nontaxigenic strains
X. citri subsp. *citri* (Pathotypes A, B and C)	**J-pth1/J-pth2** Pathotypes A, B and C Strains pthA gene (involved in virulence) **J-RXg/J-RXc2** Pathotype A strains ITS region	Conventional	Bacteria (DNA extraction)	*X. axonopodis* pv. *citri*
X.citri subsp. *citri* *X. citri* pv. *aurantifolii*	**VM1/VM2** **VM3/VM4** **VM5/VM6** pthA gene family Kingsley forward/reverse *X. citri* pv. *citri* A chromosome	Real-time (SBYR ® Green Master Mix)	Bacteria, plant (DNA extraction)	*X.citri* subsp. *citri* The *X. citri* pv. *aurantifolii* is not included in the ISPP List.
P. stewartii subsp. *stewartii*	**ES16/ES1G2c** 16S-23S rRNA/ITS region **HRP1d/HRP3r** *hrpS* ORF	Conventional	Bacteria (boiled or alkaline lysis)	Rcommended in the EPPO protocol.

The use of primers to conserved sequences flanking these hot spots provides another approach to isolating effector genes.

Genes of type III secretion system (TTSS) in bacterial plant pathogens

Pathogenicity-related genes

- Two major sets of pathogenicity-related genes:

1. *hrp* genes
2. *avr* genes

- The other genes are:

1. *hrc*
2. *dsp*, The disease-specific (*dsp*) *gene* dspA/E of *Erwinia amylovora* (see chaperon section)
3. *Pth* genes
4. *Hpa* genes
5. *Xop* genes

The *hpa* (*hrp*-associated) genes encode harpin-like proteins.

Xop, *Xanthomonas* outer protein.

Pth, pathogenicity gene necessary to condition pathogenicity on a given host.

Pathogen-generated secreted proteins **Effectors**

- At least 57 families of effectors, with each bacterial strain expressing about 15-30 effectors, have been identified in the bacterial pathogen *Pseudomonas syringae* alone.
- Effectors are produced by:

1. all the major species of pathogenic bacteria infecting plants and animals,
2. fungi,
3. Viruses, and
4. nematodes.

Surico,2013

Pathogen-generated secreted proteins Effectors

- The proteins secreted by Hrp TTSSs have been given a variety of names including:

1. Hop (Hrp outer protein) in *Pseudomonas* and many other bacteria such as *P. syringae*, *Erwinia*, *Pantoea* spp., etc.
2. Xop (*Xanthomonas* outer protein).
3. Pop (*Pseudomonas* outer protein, which actually are *R. solanacearum* proteins based on its earlier genus name),
4. Avr (for avirulence).

- Hop, Xop and Pop proteins were originally identified based on their property of limiting the host range of the pathogen.

Espinosa and Alfano,2004

Name of genes	Predicted functions of effectors
Erwinia amylovora	
avrRpt2 (EA)	AvrRpt 2(EA)-virulence factor, inducing systemic infection
avrE1	constitute Hrp pathogenicity island
hopM1	conserved effector locus (CEL)
hop AA1-1	
dspA	DspA, virulence factor
dspA/E	DspA/E virulence factor, induction of necrotic symptoms
hrpN	HrpN-virulence factor
hrpW	HrpW-a negative factor
E. chrysanthemi	
hrp/hrc	Encoding a putative mixture of virulence factors
hrpN	HrpN (harpin) facilitating colonization of host tissues
hrpC	Viulence factors
hrpG	
***P. syringae pv. phaseolicola* (*Psp*)**	
hrc C	encoding core structural components
hrc J	
hrc N	
hrc V	
hrp F	
hrp J avrPto	AvrPto suppressing a cell wall based defense
hrp P	
hrpR avrPphF	encoding secreted effectors
hrpL virPphA	
hrpY virPphC	allows evasion of HR-based immunity
hrpZ virPphF	
***P. syringae* pv. *syringae* (*Pss*)**	
syrB	Synthesis of syringomycin
sal A	LuxR regulatory protein family, production of syringopectin
syrB1	syringomycin synthetase
syr F	syrF-signal molecules
***Pseudomonas syringae* pv. *tomato* (Pst)**	
avrPto	Necrosis associated with disease; enhancement of virulence
avrPtoB	Functions as cell death inhibitor / programmed cell death (PCD)
avrPto and avrPtoB	Regulation of *LeAC01* and *LeAC02* host genes involved in biosynthesis of ethylene forming enzyme ACC oxidase
avrRpt2	Virulence factor Avirulence factor

Name of genes	Predicted functions of effectors
hrpA	HrpA, production of pilus
hrpZ	HrpZ, production of pilus
hrpW	HrpW, production of pilus
hopPtoA1	Putative effector genes
hopPtoA2	
hop Pto M	Induction of symptoms
hopPtoN	Reduction in symptom intensity
Ralstonia solanacearum	
hrpB	HrpB-transcription activator acting as a master regulatory gene
hrpG	homologous to HrpG from *Xcv* activating *hrp* genes
prhJ	Plant inducible, regulatory protein
popA	Putative effector expressed in early stages
***X. axonopodis* pv. *citri* (*Xac*)**	
pthA	pthA-division, enlargement and death of plant cells
***Xanthomonas campestris* pv. *vesicatoria* (*Xcv*)**	
avrBs1	unknown function
avrBs2	Putative glycerophosphoryl diester phosphodiesterase
avrRxv	YopJ/AvrRxv-family, putative cysteine protease
XopB	Homology to HopD1 (*Pseudomonas syringae* pv. *tomato*)
XopC	Unknown function
XopD	SUMO (small ubiquitin-like modifier) cysteine protease
XopF1	Unknown function
XopF2	Unknown function
XopJ	AvrRxy/YopJ. family, putative cysteine protease
XopN	Unknown function
XopO	Homology to HopK1 and AvrRps4 (*P. syringae*)
XopP	unknown function
XopQ	HopQ1-1 family protein, putative inosine-uiridine nucleoside N-ribohydrolase
XopX	Unknown function
Ecf	Early chlorosis factor, homology to HopAE1 (*P. syringae* pv. *syringae*)

Name of genes	Predicted functions of effectors
avrBs3	AvrBs3 – nuclear localization singal (NLS), recognition
AvrXv4	SUMO (small ubiquitin-like modifier) protease localized to plant cytoplasm
avrBs4	AvrBs4-triggers hypersensitive response (HR)
***X. oryzae* pv. *oryzae* (*Xop*)**	
avrXa7	AvrXa7-virulence factor and avirulence specificity
avrBs3	virulence factor, interaction with host DNA and transcriptional machinery

Source: Gürlebeck et al. (2006)

Genes of type III secretion system (TTSS) in bacterial plant pathogens and the predicted functions of effectors.

Narayanasamy,2008

Some phytopathogen type III effectors
T3E activities and plant targets

T3E	Species	Activity	Target
AvrB	*P. syringae* pv. *glycinea* race 0	Induces phosphorylation	RIN4/RAR1
AvrBs3	*X. campestris* pv. *vesicatoria* race 1	Transcription activator-like	Upa20 (transcription factor)/Bs3
AvrPphB	*P. syringae* pv. *phaseolicola* race 3	Cysteine protease	cleaves the Arabidopsis protein kinase PBS1
AvrPto	*P. syringae* pv. *tomato* JL1065	Kinase inhibitor	Pto/EFR/FLS2
AvrPtoB	*P. syringae* pv. *tomato* DC3000	E3 ubiquitin ligase	Fen (tomato kinase protein)
AvrRpm1	*P. syringae* pv. *glycinea* race 0	Induces phosphorylation	RIN4 protein (regulated basal defense in *Arabidopsis*)
AvrRpt2	*P. syringae* pv. *tomato* T1	Cysteine protease	RIN4 protein (regulated basal defense in *Arabidopsis*)
AvrXa27	*X. oryzae pv. oryzae* PXO99^{A}	Transcription activator-like	Rice R gene *Xa27*
AvrXv4	*X. campestris* pv. *vesicatoria* T3	DeSUMOylating cysteine protease	Unknown
GALA	*R. solanacearum* GMI1000	F-box and LRR domains	*A. thaliana* Skp1-like proteins
HopAI1	*P. syringae* pv. *tomato* DC3000	Phosphothreonine lyase	MPK3/MPK6

Some phytopathogen type III effectors
T3E activities and plant targets

T3E	Species	Activity	Target
12	*P. syringae* pv. *tomato* DC3000	Protein tyrosine phosphatase	Unknown
HopI1	*P. syringae* pv. *maculicola* ES4326	J-domain protein (possible Hsp70 cochaperone)	Unknown
HopM1	*P. syringae* pv. *tomato* DC3000	Unknown	AtMIN7 and other immunity associated *Arabidopsis* protein
HopU1	*P. syringae* pv. *tomato* DC3000	Mono-ADP-ribosyltransferase	GRP7 and other RNA-binding proteins
HsvB	*Pantoea agglomerans* pv. *betae* 4188	Transcriptional activator-like	Unknown
HsvG	*Pantoea agglomerans* pv. *gypsophilae* 824-1	Transcriptional activator-like	Unknown
PthXo1	*X. oryzae* pv. *oryzae* PXO99^{A}	Transcriptional activator-like	host gene *Os8N3*
PthXo6/7	*X. oryzae* pv. *oryzae* PXO99^{A}	Transcriptional activator-like	*OsTFX1 OsTFIIAg1*
XopD	*X. campestris* pv. *vesicatoria* 85-10	DeSUMOylating cysteine protease	Unknown

Block *et al.*,2008

T3SS substrate effector proteins

hop genes encode *P. syringae* T3SE families HopZ, HopF,...

- Effectors of *P. syringae* and other phytopathogenic bacteria are generally designated as Hop proteins (Hrp outer proteins, i.e. proteins that have the capacity to travel through the T3S system.
- Nearly 60 different type III effector families (T3SE families) encoded by *hop* genes have been identified in *P. syringae*.

1. The HopF T3SE family is broadly distributed among pathovars of *P. syringae*.
2. The HopZ T3SE family, is widely distributed among the *P. syringae* species complex.

McCann and Guttman,2007;..

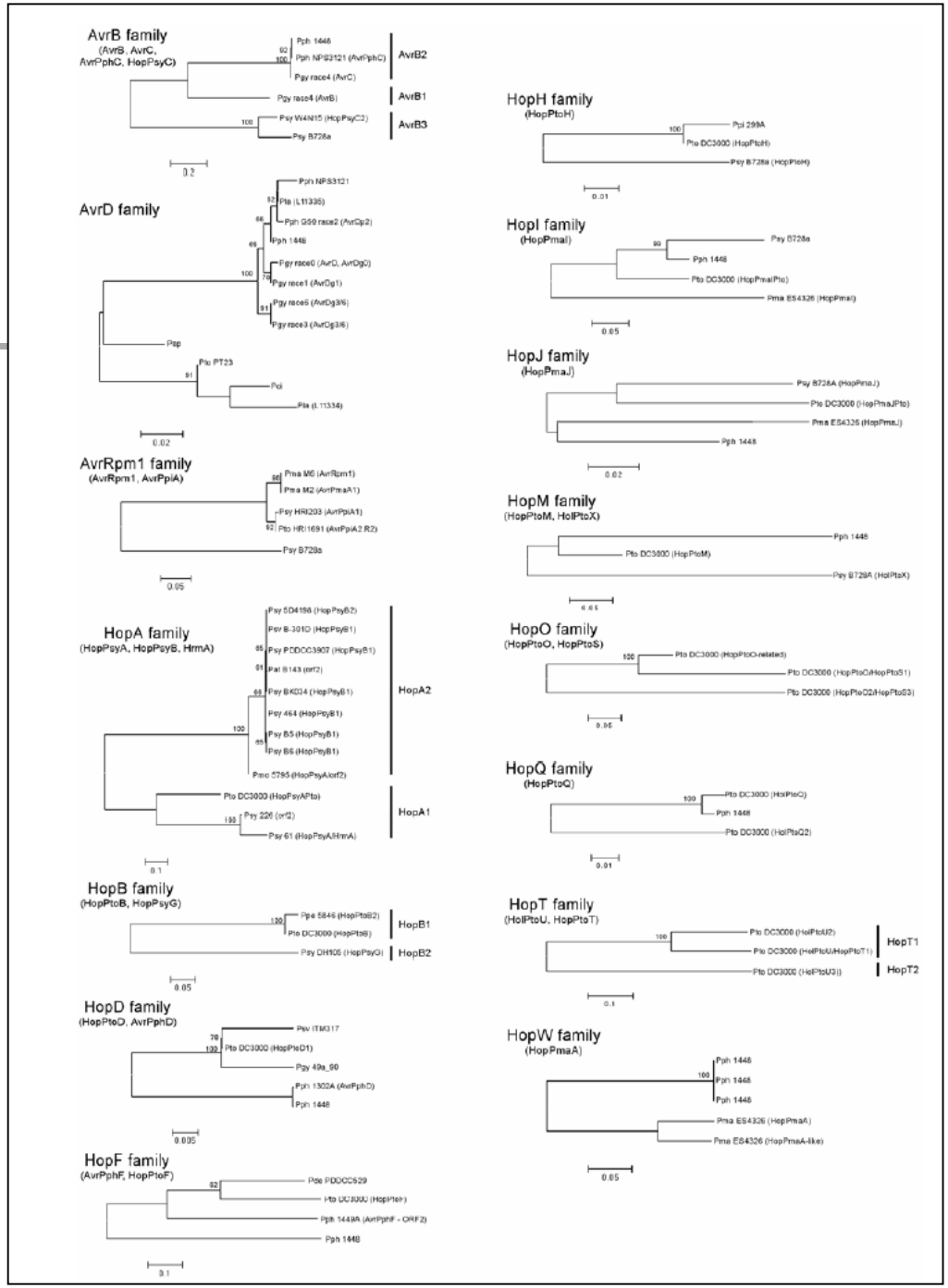
Phylogenetic trees for some Hrp outer protein (Hop) families with three or more members

Nomenclature & Phylogeny of Hops in *P. syringae*:

The phylogenetic analyses were used to determine whether homology families should be subdivided into Hop subgroups based on amino acid sequence divergence.

A homology family was subdivided if it was found to have within-group amino acid diversity <0.75 and between-group amino acid diversity >0.75.

Lindeberg *et al.*,2005



The horizontal line below each phylogeny indicated a genetic distance of 0.2.

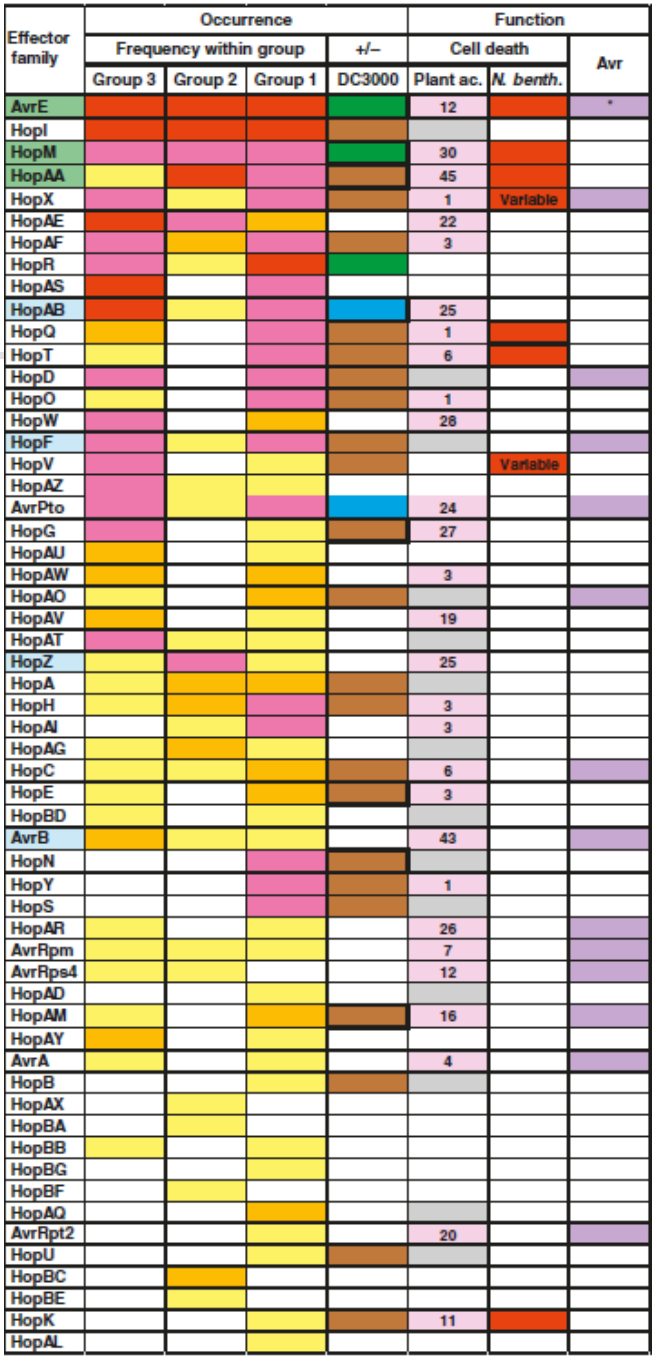
Hrp outer protein (Hop):

A generic designation for *P. syringae* type III effectors

The phylogenetic distribution of effector families in the *Pseudomonas syringae*.

Plant accessions (Plant ac.)

Nicotiana benthamiana (colloquially known as benth or benthi) is a close relative of tobacco and species of *Nicotiana indigenous* to Australia.



Lindeberg *et al.*,2012

T3SS substrate effector proteins

Xanthomonas AvrBs3 T3SE family

AvrXa7 and AvrXa10, AvrB6, and the Hax and Pth proteins

- The *Xanthomonas* AvrBs3 T3SE family, which also includes AvrXa7 and AvrXa10, AvrB6, and the Hax and Pth proteins, provides a very nice example of pathoadaptation.
- This entire family is characterized by the presence of a variable number of direct repeats in a central region of the gene.
- The repeats are highly conserved with the most consistent variation present at codons 12 and 13 of the individual repeat units.
- Deletion of one or more of these repeats results in a range of different phenotypes.

McCann and Guttman,2007; White *et al.*,2000

Structure of *avrBs3* gene products

Xanthomonas *AvrBs3 family*-type III effectors

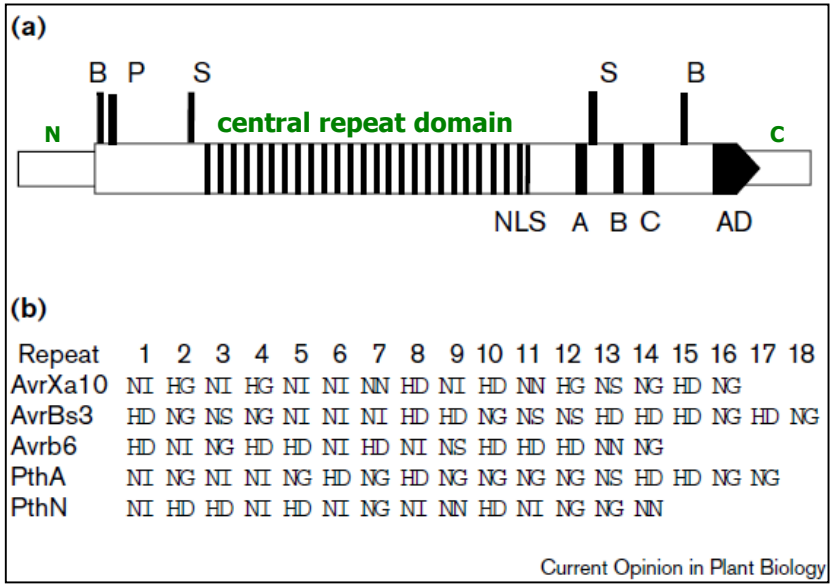
Map of the phenotype gene in the *avrBs3* family

(a) The central repeat domain (leucine rich) is represented by series of open boxes.

- NLS, nuclear (NLS) localization motif sequences A, B, and C, and
- AD/AAD, the transcriptional activation domain.
- The conserved restriction sites B, BamHI; P, PstI; and S, SphI are shown.

(b) Organization of 34-amino acid repeat units in selected members of the *avrBs3* family.

- Each individual repeat unit is represented by the amino-acid residues at positions 12 and 13.



AvrBs3 gene product has 17.5 nearly identical repeats of this motif.

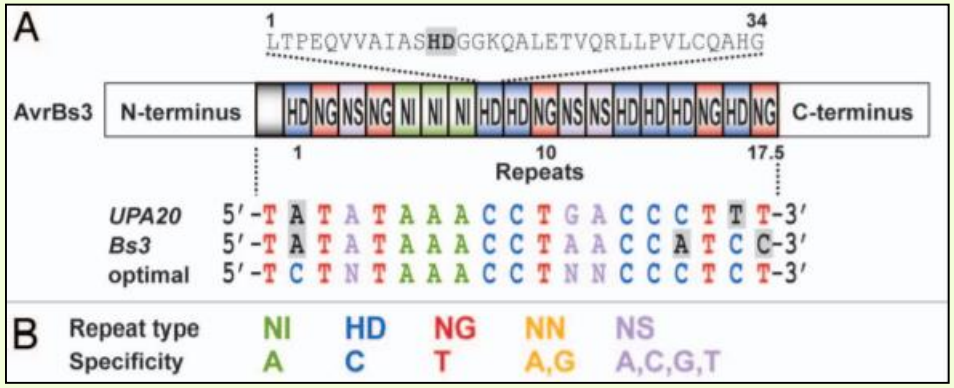
Family members differ in the number and the order of the repeats.

White *et al.*,2000

Domain architecture of the AvrBs3 effector

Xanthomonas campestris pathovars

- The amino acid sequence of the AVRBs3 protein is characterized by a central region consisting of 17.5 nearly identical repeats of 34 amino acids.
- The 17.5 repeats of AvrBs3 were found to be essential for recognition by the Bs3 resistance gene.
- The target box of AvrBs3 (17.5 repeats) is 19 bp long, differ mainly at positions 12 and 13.

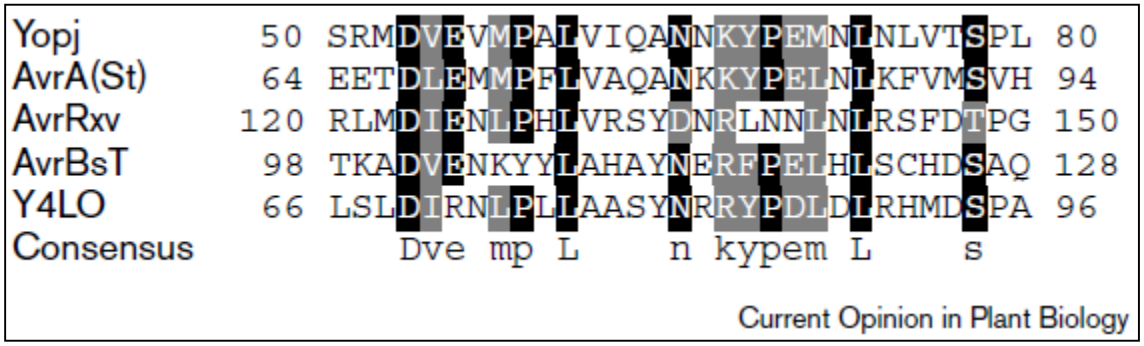


Scholze & Boch,2010;…

Structure of *avrRxv* gene products

Alignment of the amino-terminal region of members of the AvrRxv family

- The alignment shows that the residues of the amino-terminal(N terminal) portion of the protein, which are the products of five genes of the *avrRxv* family, are conserved.
- The protein product of *avrRxv* has sequence relatedness to the virulence factor YopJ from *Yersinia pseudotuberculosis*.



White *et al.*,2000

T3SS substrate effector proteins

T3SE evolution

Variability in T3SE (effectors) numbers in each strain of *P. syringae*

- The genome sequences have revealed a considerable variability in T3SE (effectors) numbers per strain and a large number of T3SE families.
- A comparison of *P. syringae* pv. *tomato* strainT1 against strain DC3000 found that the genomes were highly similar except for theT3SE repertoires, which had diverged significantly.

Arnold and Jackson,2011

T3SS substrate effector proteins

T3SE evolution

P. syringae pv. *tomato* strainT1 vs. *Pst* strain DC3000

1. Pst DC3000 is a pathogen of both tomato and *Arabidopsis thaliana*, whereas
2. Pst T1 is pathogenic on tomato but not on *A. thaliana*.

- So the differences in their respective T3SE repertoires may help to explain the different host range of these strains.

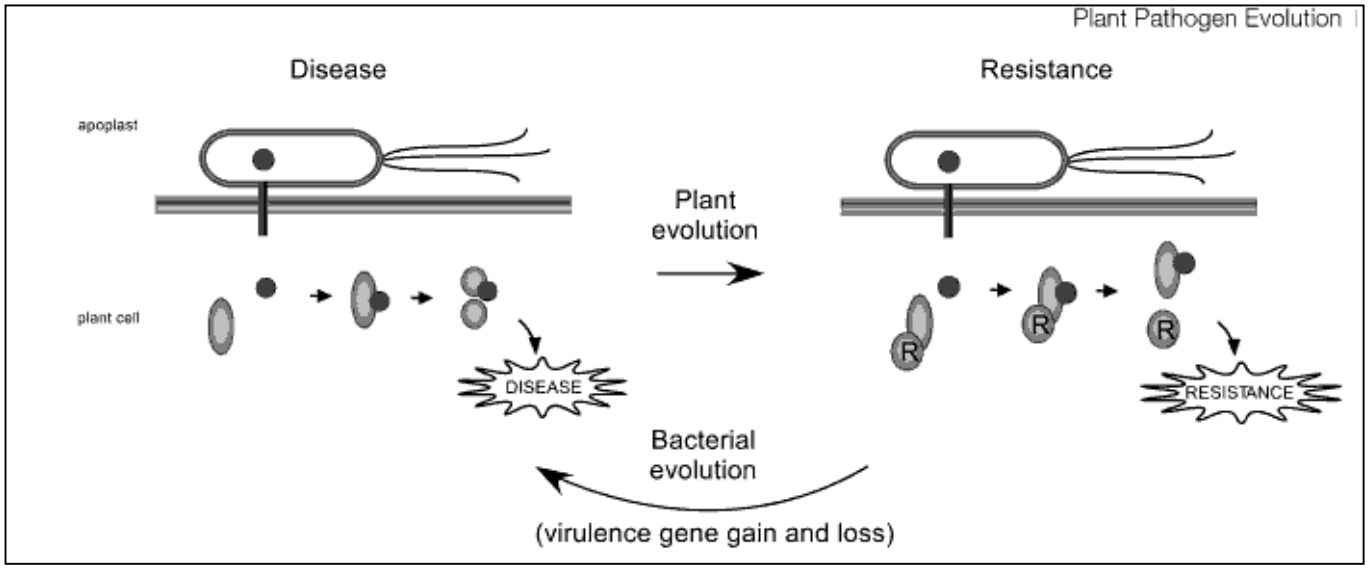
Arnold and Jackson,2011

T3SS substrate effector proteins

Coevolution of bacterium-plant interaction

Coevolving molecular dialogs between effector repertoires and plant immune systems

- The T3SS substrate effector proteins (T3SE) are secreted via a pilus from the bacterial cell to inside a plant cell and are subject to considerable change as the pathogen and host co-evolve.



Jackson,2009; Arnold and Jackson,2011;..

The zigzag model of plant-microbe interactions
Coevolution of bacterium-plant interaction
Plant immune system

- Coevolutionary model of plant–pathogen interactions, called the 'zigzag' model, which encompasses two branches of the plant immune system:

1. The first branch recognizes conserved molecules (pathogen-associated or microbe-associated molecular patterns, PAMPs or MAMPs), and is now called pattern-triggered immunity (PTI).
2. The second branch recognizes and responds to virulence factors termed effectors that, in the model, serve to suppress PTI.

Pritchard and Birch,2014

Coevolution of bacterium-plant interaction
The zigzag theory

- In the first level, the plant recognizes conserved pathogen-associated molecular patterns (PAMPs).
- Recognition at this level elicits a 'general defence response', which is weaker than the second level of active defence.
- This first level of recognition provides a type of basal immunity, PAMP-triggered immunity (PTI).
- This level of recognition was probably the first to evolve, since it responded to all microorganisms, harmful or not, that confronted the plant.

Pathogen-associated or microbe-associated molecular patterns (PAMPs or MAMPs) include molecules such as flagellin, peptidoglycan, lipopolysaccharide, the elongation factor TU, etc.

Surico,2013

Coevolution of bacterium-plant interaction
The zigzag theory

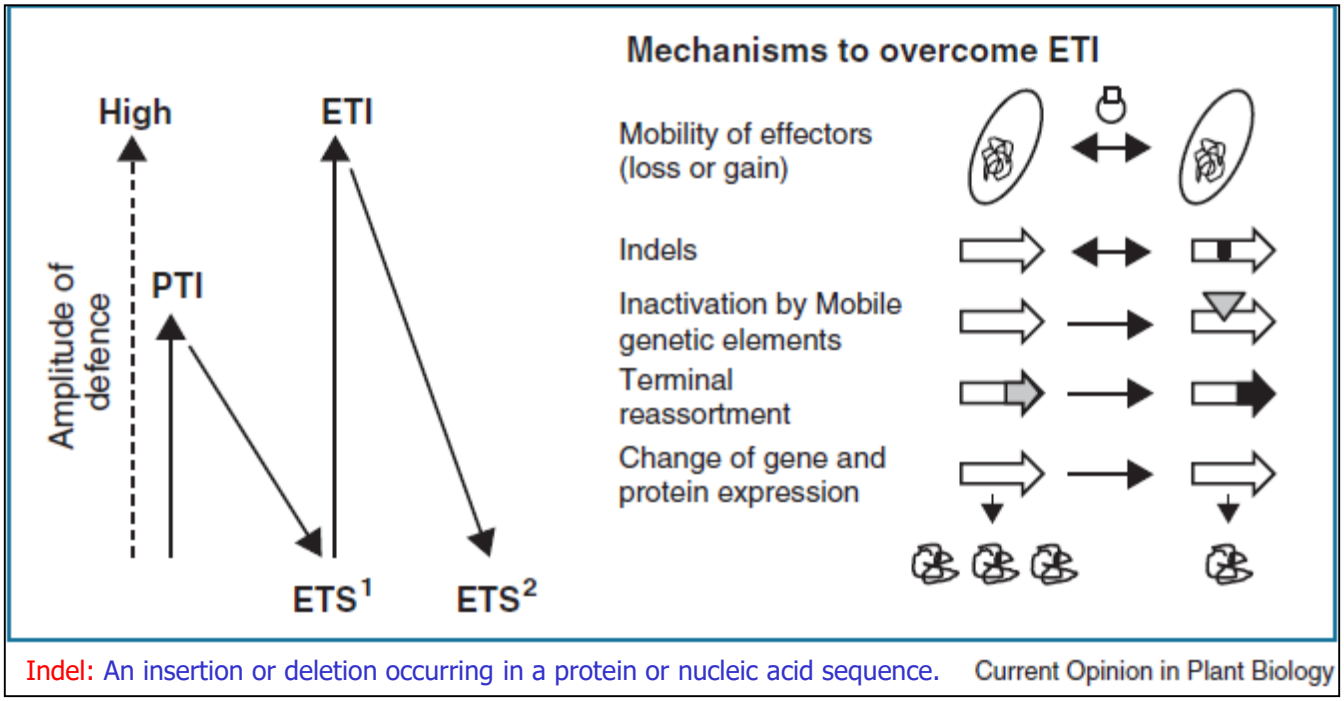
- The "zigzag model" illustrates the quantitative output of the plant immune system (from Jones and Dangl, 2006).
- PAMPs, pathogenesis-associated molecular patterns;
- PTI, PAMP-triggered immunity;
- ETS, effector-triggered susceptibility;
- ETI, effector-triggered immunity;
- Avr, avirulences;
- R, resistance proteins;
- HR, hypersensitive response.
- Plants use pattern recognition receptors (PRRs) present on their plasma membranes to recognize pathogen-associated molecular patterns (PAMPs) and this detection leads to PAMP-triggered immunity (PTI). (PAMP/PRR interaction).

McCann and Guttman,2007;..

Coevolution of plant-pathogen interaction
The zigzag theory

- The zigzag theory (Jones and Dangl,2006) is a useful model for understanding the progression of the plant-pathogen co-evolution and mode of plant immunity.

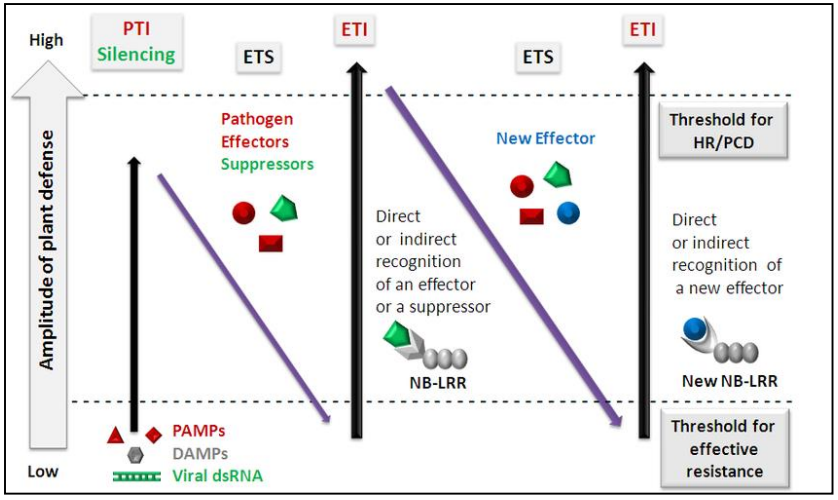
PTI : PAMP-triggered immunity ETS : effector-triggered susceptibility ETI : effector-triggered immunity



Arnold and Jackson,2011

Coevolution of bacterium-plant interaction
The zigzag theory

- PTI is PAMP-triggered immunity.
- ETI (effector-triggered immunity) a plant resistance response that is activated upon recognition of enemy effectors by R genes (NB-LRRs).
- ETS is effector-triggered susceptibility in host.
- PTI is overcome by effector (T3SE) proteins leading to disease (ETS^1);
- TheseT3SEs may then be recognized by the host resistance surveillance system leading to ETI.
- ETI is overcome by evasion of T3SE recognition enabling the pathogen to cause disease (ETS^2).

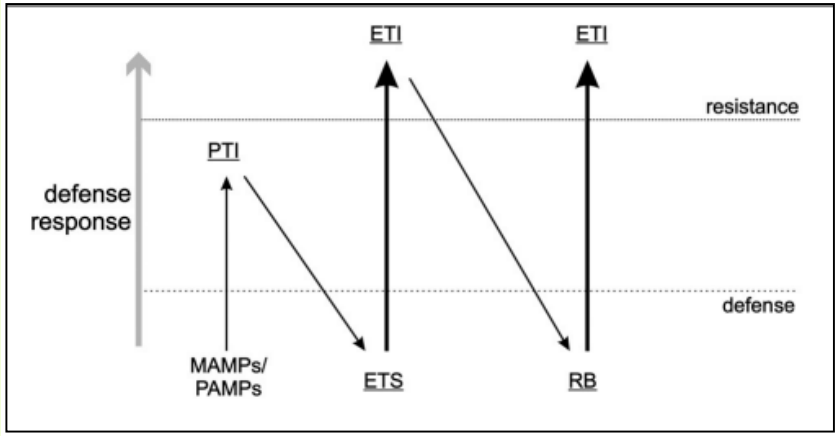


MAMPs or PAMPs: Microbial-associated or pathogen-associated molecular patterns.

Arnold and Jackson,2011;M.M Pooggin

Coevolution of bacterium-plant interaction
The zigzag theory

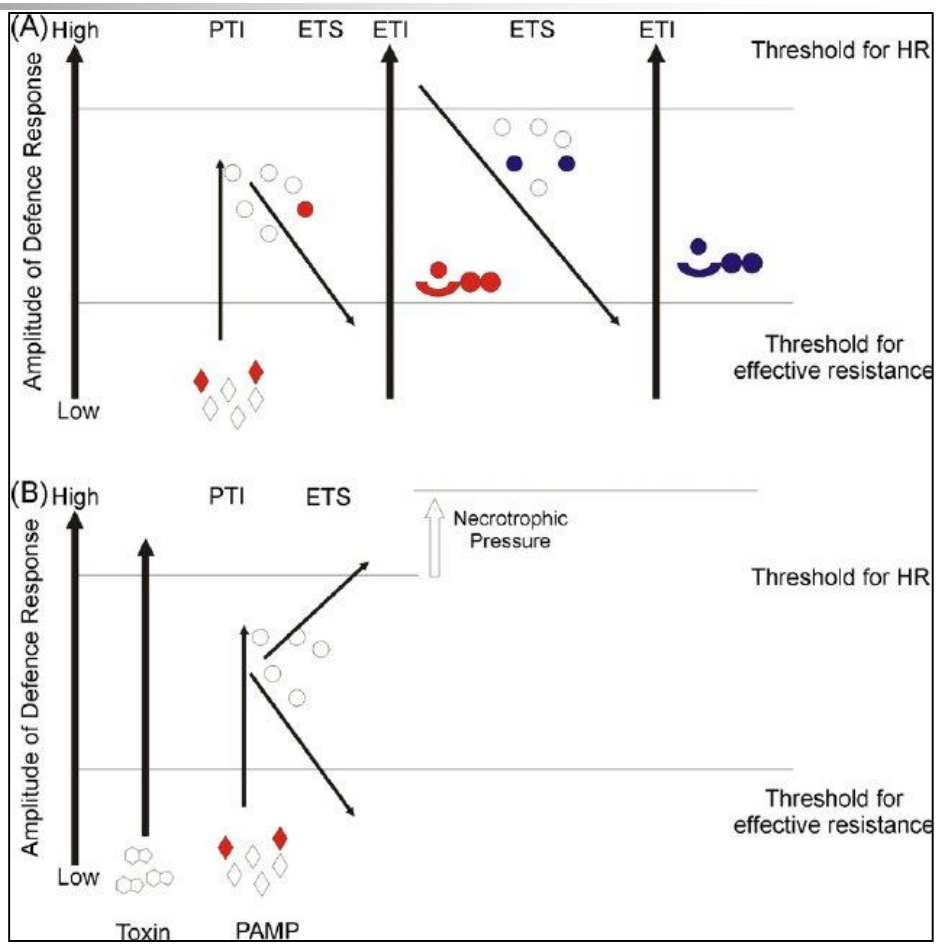
- A visual presentation of the arms race between pathogen and host according to Jones and D angl (2006).
- Here, a slightly modified version of that model is presented and as described in this review.
- MAMPs/PAMPs, Microbe/Pathogen associated molecular patterns;
- PTI, PAMP triggered immunity;
- ETS, Effector triggered susceptibility;
- ETI, Effector-triggered immunity ;
- RB, resistance breaker.



De Ronde *et al.*,2014

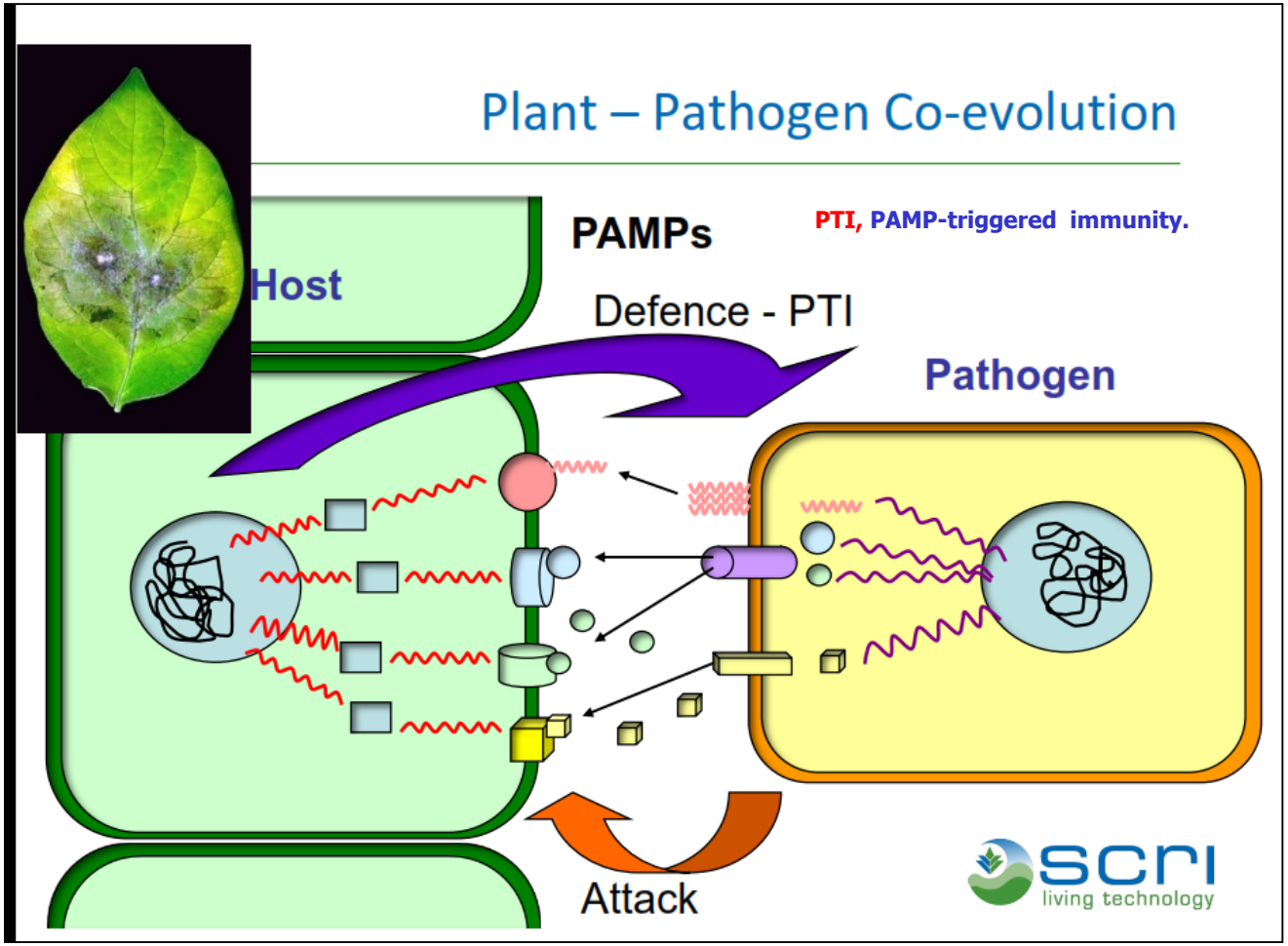
Coevolution of bacterium-plant interaction
The zigzag theory

Opportunity costs of a hypersensitive response.
(A) The proposed zigzag model of plant disease resistance as previously described by Jones and Dangl,2006.
Abbreviations are as previously classified in the published model. PTI is pathogen-triggered immunity. ETS is effector-triggered susceptibility. ETI is effector-triggered immunity.
(B) The proposed ecological costs of having a "threshold for HR" with regards to necrotroph invasion. Toxin shows the potential direct st imulation of an HR by necrotrophic toxins.
The arrow increasing the "threshold for HR" indicates the potential pressure upon plants to require a sufficiently high thres hold as to limit necrotrophs capacity to induce the HR via toxins.



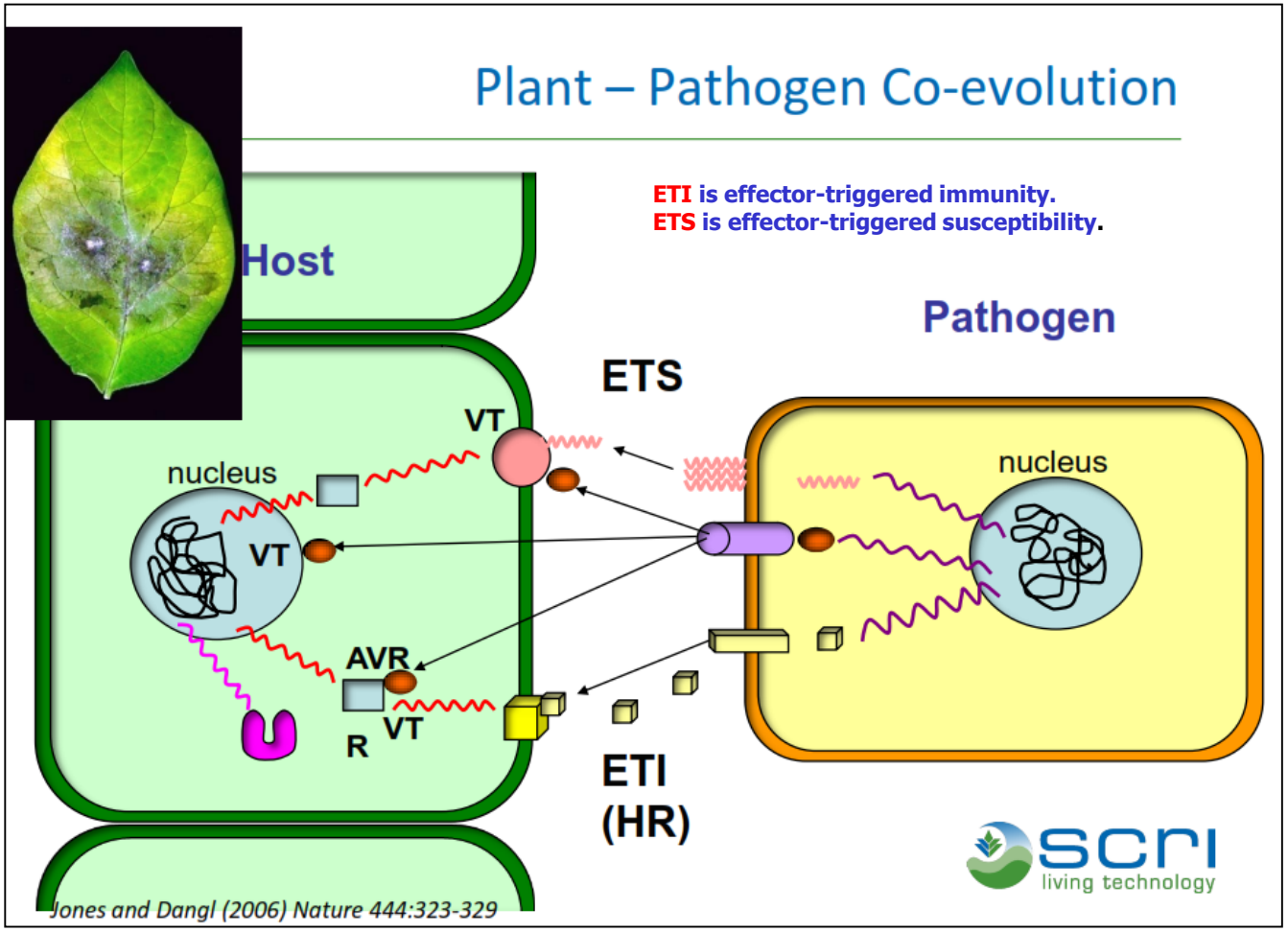
Kliebenstein and Rowe,2008

Coevolution of bacterium-plant interaction
The zigzag theory
PAMP-triggered immunity



Ingo Hein

Coevolution of bacterium-plant interaction
The zigzag theory
ET-triggered immunity



Coevolution of bacterium-plant interaction
PAMP/PRR interaction
Pattern recognition receptors against pathogen-associated molecular patterns

- To perceive potential pathogens in their environment, plants use pattern recognition receptors (PRRs) present on their plasma membranes.
- PRRs recognize conserved microbial features called pathogen-associated molecular patterns (PAMPs) and this detection leads to PAMP-triggered immunity (PTI), which effectively prevents colonization of plant tissues by non-pathogens.

Video Article presented by Chakravarthy *et al.*,2009

Coevolution of bacterium-plant interaction
PAMP/PRR interaction
Pattern recognition receptors against pathogen-associated molecular patterns

- The most well studied system in PTI is the FLS2-dependent pathway.
- FLS2 (Leu-rich repeat receptor kinase) is the PRR for flagellin recognizes.
- FLS2 recognizes PAMP flg22 (a component of bacterial flagellin) and led to PAMP/PRR interaction.

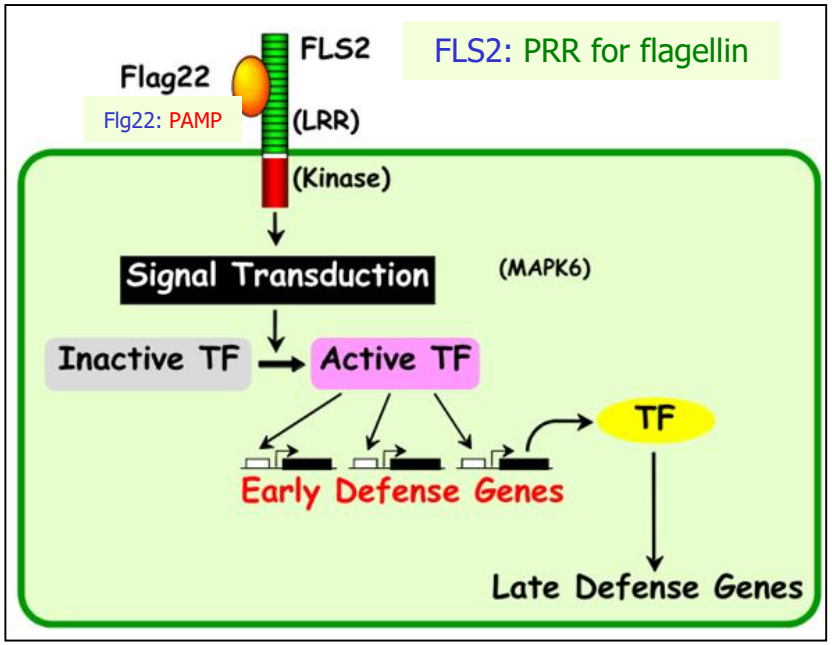
Video Article presented by Chakravarthy *et al.*,2009

FLS2-dependent pathway

A Model for defense gene induction

Bacterial FLS2-flg22 interaction (PAMP/PRR interaction)

- The FLS2, Leu-rich repeat receptor kinase (LRR-RK) is the PRR for flagellin.
- PMAPs elicit PAMP-triggered immunity (PTI) which in turn, produce:
- Reactive oxygen species (ROS),
- callose deposition,
- cell thickening, etc.



From work in T. Boller's lab;..

Coevolution of bacterium-plant interaction
The zigzag theory
Fresh look at the model

- The system is divided into two compartments:

1. extracellular (external to the cell wall), and
2. intracellular (internal to the cell wall).

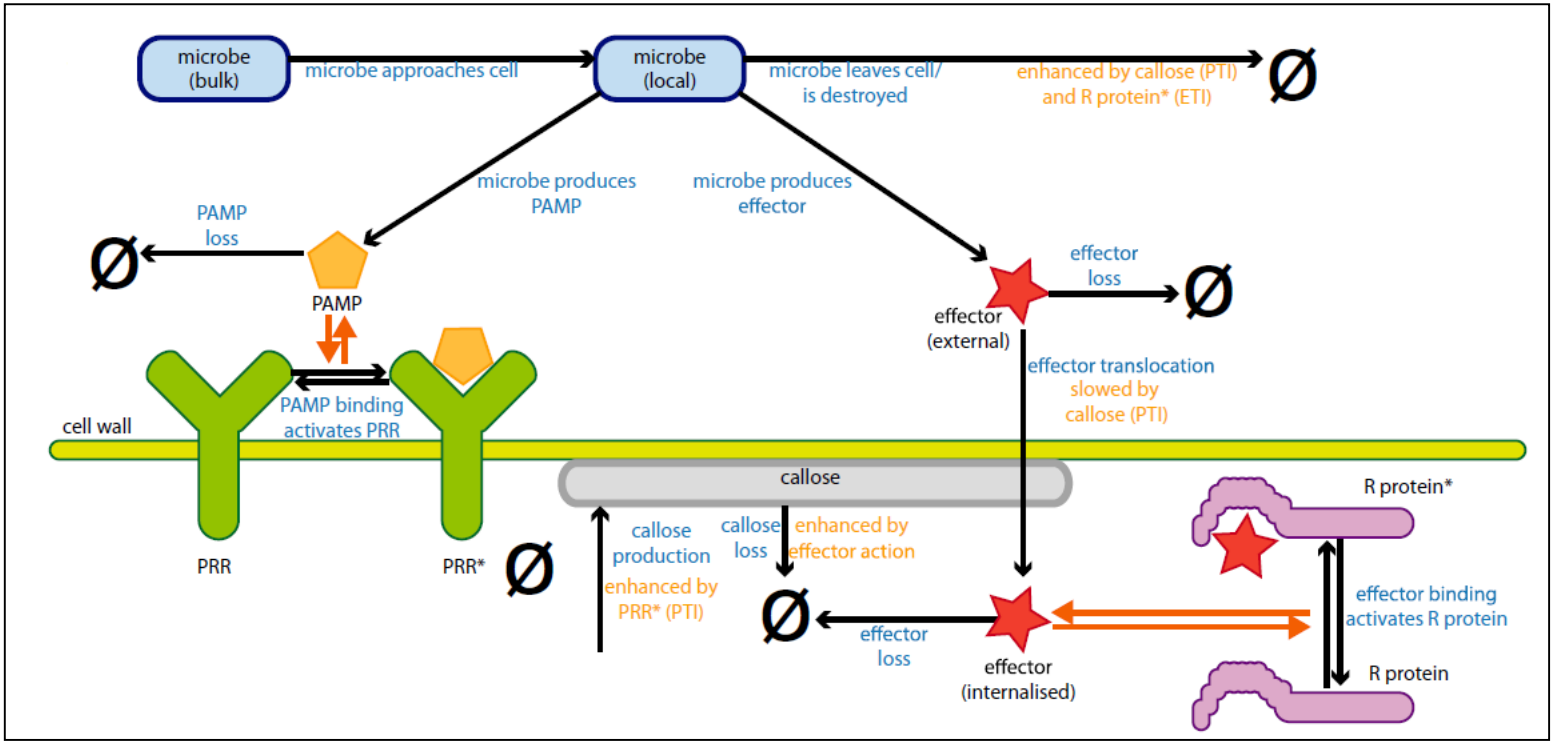
- In the extracellular compartment the local microbial population is drawn from a remote bulk population and is also 'destroyed', as indicated by the arrow pointing to the empty set symbol (ϕ).
- While local to the plant, the microbe produces two species:

1. pathogen-associated molecular patterns (PAMP), and
2. effector;

- Both species can be lost (e.g. by diffusion or destruction) in the extracellular compartment.

Pritchard and Birch,2014

Coevolution of bacterium-plant interaction
The zigzag theory
Fresh look at the model



Pritchard and Birch,2014

Bacterial *avr* genes

Dual-acting genes

As long as one pair of *avr* / *R* genes matches in pathogen and host, an HR results, while in the absence of this matching, disease is the outcome.

The *avr* genes
Virulence or avirulence genes?

- The effectors specifically recognized by 'matching' resistance proteins (termed R proteins) are termed avirulence (AVR) proteins.
- This term (avr genes without virulence properties) was coined by H. H. Flor.
- An avirulence gene can be defined as:
- any gene that codifies(organize) factors that are recognized by specific genotypes (cultivars) of a host species that contains the corresponding resistant gene.

Surico,2013

The *avr* genes

Avirulence genes

- The first avirulence gene was isolated from *Pseudomonas* bacteria infecting soybean.
- Over 50 bacterial avirulence genes have been isolated and sequenced.
- More than 30 bacterial *avr* genes in pathovars of *Pseudomonas syringae* and *Xanthomonas* spp. have been described.
- They are:

1. Chromosomal, or
2. plasmid-borne.

Function of *avr* genes

Mutation of *avr* genes

- Mutations in *avr* genes make the bacterium invisible to the host defense recognition system.
- Therefore, plant fails to develop an HR and the bacterium is able to cause disease.
- Because the lack of *avr* or *R* genes usually results in a compatible (disease) interaction.

Function of *avr* genes
Requirements for Avr activity

- Since the Avr proteins are encoded by the avr genes, it is obvious that avr genes can alter the signaling of host defense systems in resistant host plants.
- Transcription of *avr* often under *hrp* regulation (i.e. part of *hrp* regulon).
- Expression of *hrp* type III secretion system often required.
- Secretion of harpin sometimes required.
- Host must have appropriate resistance gene.

The *avr* genes
Avirulence genes

- An individual pathogen strain may have multiple avirulence genes, and the combination of *avr* genes within a particular strain specifies the physiologic *race* of the strain.
- Within a species or pathovar, avirulence genes hypothetically are the most recent genetic variations in the evolutionary adaptation process between the host and pathogen.

Leach and White,1996

The *avr* genes
Avirulence genes

- *avrA*, the first avirulence gene characterized, was cloned from a race 6 strain of the soybean pathogen *Pseudomonas syringae* pv. *glycinea* and, when transfered to other races of *P. syringae* pv. *glycinea*, conferred the ability to elicit a resistant response only on cultivars of soybean with the *Rpg2* gene for resistance.
- This study utilized four heterologous avirulence genes *avrA*, *avrD*, *avrE*, and *avrPto*, which were identified on the basis of elicitation of the HR in soybean cultivars when introduced into *P. syringae* pv. *glycinea*.

Leach and White,1996

Function of *avr* genes
In plant pathogenic bacteria

- Some of *avr* genes which play a role in pathogenicity on susceptible plants are:
- *avrBs2* from *X. vesicatoria*,
- *avrRpm1* from *P. syringae* pv. *maculicola*,
- *pthA* from *X. citri*,
- *avrBs3* family of *avr* genes in *Xanthomonas campestris*,
- *avrA* & *avrE* in *Pseudomonas syringae* pv. *tomato*,
- *X. oryzae* pv. *oryzae* has more than 12 avrBs3/pthA members, and two of them have been subcloned and well-characterized, avrXa7 and avrX10.

The *avr* genes
Bacterial avirulence genes

Pathogen	Avirulence gene	Alleles or related gene (+/− avirulence activity)
Pseudomonas syringae pv. *glycinea*	*avrA (avrPgyA1.Rpg2)*	Allele from *P. syringae* pv. *tomato* (+)
	avrB (avrPgyB1.Rpg1)	Sequence similarity to *avrC*
	avrC (avrPgvC1.Rpg3)	*avrPphC* from *P. syringae* pv. *phaseolicola* (+); similarity to *avrB*
Pseudomonas syringae pv. *tomato*	*avrD (avrPtoA1)*	Alleles from *P. syringae* pvs. *glycinea* (−), *lachrymans* 1 (+), *lachrymans* 2 (+)
	avrE (avrPtoE1)	
	avrPto (avrPtoC1)	
	avrRpt2 (avrPtoB1)	
	avrBs1	Similarity to *avrA* from *P. syringae* pv. *glycinea*
	avrBs2	Functional homologs in *X. compestris* pvs. *alfalfae*, *malvacearum*, *vignicola*, *vitians*, *campestris*, & *phaseoli*; *X. oryzae* pv. *oryzae*?
	avrBs3	Related to genes from *X. campestris* pv. *vesicatoria*, *X. oryzae* pv. *oryzae*, *X. campestris* pv. *malvacearum*, *X. citri*

Leach and White,1996

Bacterial avirulence genes

Bacterial avirulence genes with virulence or virulence-associated properties

Gene or protein	Organism	possible function	Related proteins or alleles
avrBs2	*X. c* pv. *vesicatoria*	Cytoplasmic	Agrocinopine synthase, glycerol-phosphodiesterase
avrXa7	*X. o.* pv. *oryzae*	NLS, AD, DNA binding, nucleus	*avrBs3* family
avrb6	*X. c.* pv. *malvacearum*	Nucleus	*avrBs3* family
avrPto	*P. s.* pv. *tomato* (T1)	MYM, kinase binding, IM	-
avrRpm1	*P. s.* pv. *maculicula*	MYM, inner membrane?	AvrPpiA1 (*P.s.* pv. *pisi*)
avrE	*P. s.* pv. *tomato* (PT23) *E. amylovora*	Unknown	DspA (DspE) (*E. amylovora*)
avrF	*P. s.* pv. *syringae* *E. amylovora*	Chaperone	DspB (DspF) (*E. amylovora*)
avrA	*P. s.* pv. *tomato* (PT23)	Cytoplasmic	AvrA (*P. s.*pv. *glycinea*), AvrBs1

White *et al.*,2000

The *avr* genes

An important features of *avr* genes

1. A virulence protein can become an avirulence protein if an R protein in the host recognizes it.
2. a virulence protein acts as virulence in the absence of a host *R* gene.

- An important features of *avr* genes:

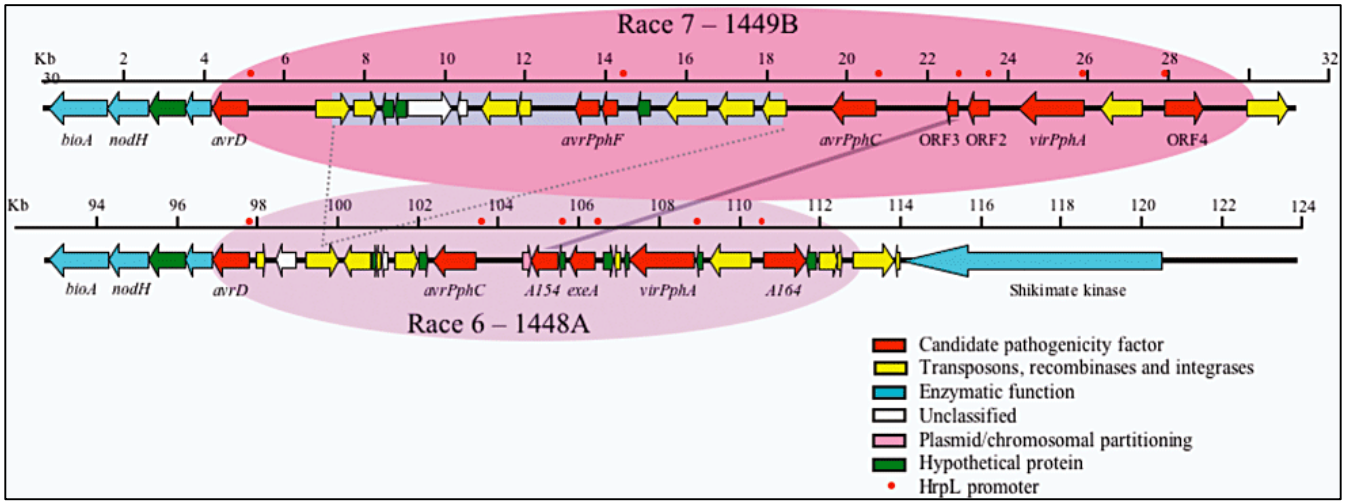
1. the *hrp* dependency of all *avr* genes;
2. the discovery of functional nuclear localization signal (NLS) sequences on some of the genes;
3. the ability of many tested *avr* genes to elicit an HR when expressed inside resistant plant cells.

Gabriel,1999

The *avr* genes

avr genes in cluster

- Several avr genes, including avrPphF, avrD and avrPphC, were also found to be clustered in pAV520.



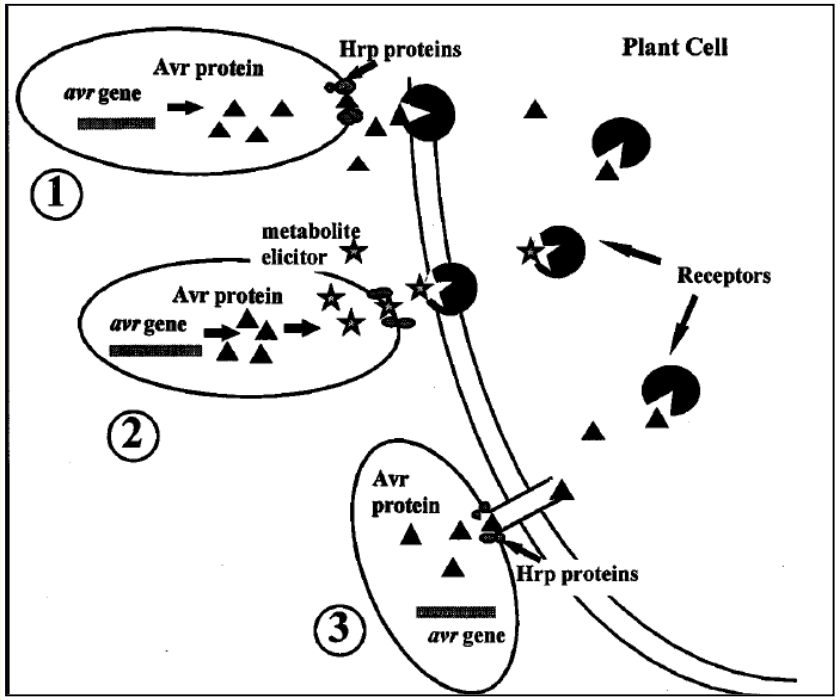
Comparison of the plasmid-borne pathogenicity island containing effector genes in *Psudomonas phaseolicola* strains 1448A (race 6) and 1449B (race 7).

Mansfield,2009

Bacterial avirulence genes

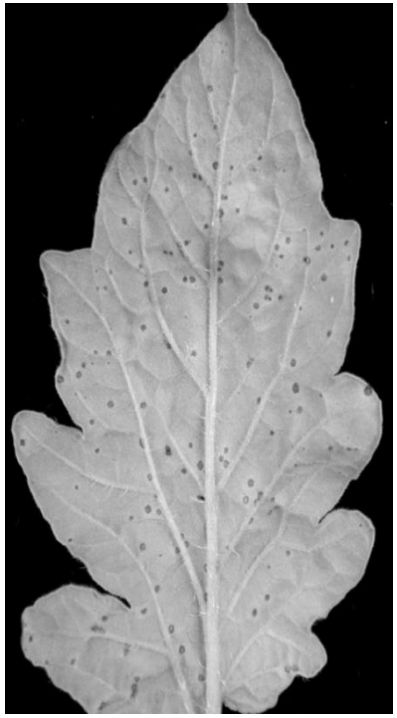
Models for avirulence gene function in the elicitation of plant defense responses.

- In Model 1, the avr gene product (Avr) is the elicitor. The elicitor is exported from the cell via the Hrp apparatus and interacts directly with the plant receptor, which is likely the product of the corresponding resistance gene.
- In Model 2, Avr directs the synthesis of or modifies a metabolite or protein, which is the race-specific elicitor.
- In both Models 1 and 2, the receptor may span in the plant cell membrane, or may be cytoplasmic.
- In Model 3, Avr is the elicitor, but it is directly delivered to the cytoplasm of the plant cell via an attachment between the bacterial and plant cell, possibly containing Hrp proteins.



Leach and White,1996

The *avr* genes of *P. syringae* pv. *tomato* Symptoms of the dual-acting avirulence genes *avrPto* in compatible interaction



Tomato leaves infected with *P. syringae* pv. *tomato* T1 without *avrPto*.

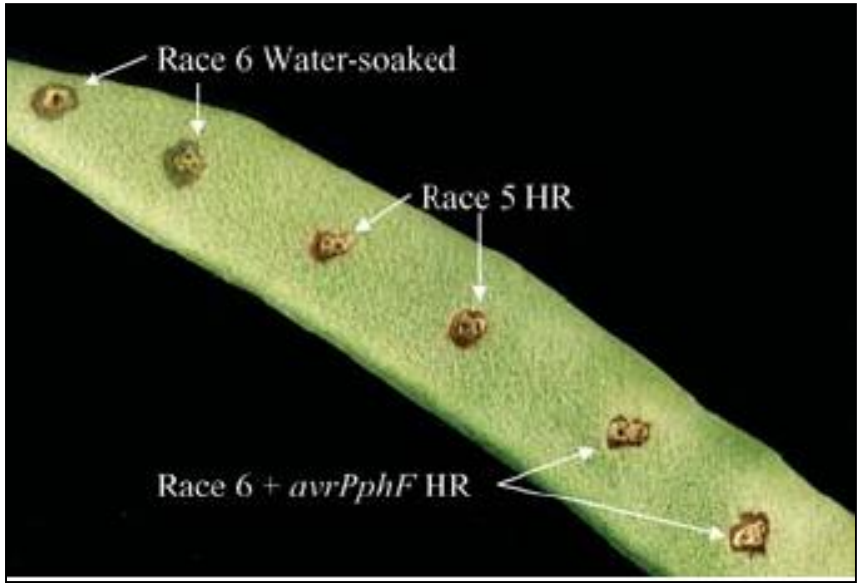
Tomato leaves infected with *P. syringae* pv. *tomato* T1 with *avrPto*. The virulence effect of *avrPto* was observed.

White *et al.*,2000

Cloning of the avirulence gene avrPphF from *P. phaseolicola* race 5

- Cloning of the avirulence gene avrPphF from *Pseudomonas phaseolicola* race 5.

1. Race 6 gives a susceptible water-soaked reaction, whereas
2. Race 5 gives a hypersensitive response (HR).
3. The HR was conferred on race 6 when transformed with a clone containing avrPphF (race 6+avrPphF).



Strange,2003

The *avr* genes

Structure and functions of avirulence genes

1. A virulence protein can become an avirulence protein if an R protein in the host recognizes it.
2. avirulence protein acts as virulence in the absence of a host *R* gene.

- An important features of *avr* genes:

1. the *hrp* dependency of all *avr* genes;
2. the discovery of functional nuclear localization signal (NLS) sequences on some of the genes;
3. the ability of many tested *avr* genes to elicit an HR when expressed inside resistant plant cells.

A likely role for avirulence determinants in conferring virulence in the absence of the corresponding resistance gene also has been established for a number of bacterial Pathogens.

Gabriel,1999; Wolpert *et al.*,2002

The *avr* genes

Xanthomonas *AvrBs3* family-type III effectors

Structure of *avrBs3* gene products

- The structure of genes within the *avrBs3* family is remarkable.
- Molecular characterization of several members of the *avrBs3* family reveals that three key structures of the gene products:

1. A central region of tandem 33-35 residue repeats;
2. Three nuclear localization signal (NLS) sequences, and
3. An acidic activation domain (AAD) have roles in avirulence and/or virulence.

The *avr* genes

Structure of *avrBs3* gene products

Xanthomonas *AvrBs3* family-type III effectors

- The central domain of these genes contains a series of 102 bp, directly repeated DNA sequences.
- Each avrBs3-homolog may contain different numbers of the 102-bp repeat. For example,

1. the avrBs3, avrBs3-2, and pthA genes contain 17.5 copies;
2. avrXa10 contains 15.5 copies, and
3. avrB6 contains 13.5 copies.

See also TAL effectors (Transcription activator-like) effectors and Plant Bacterial pathogensis-Part1.

The *avr* genes

Structure of *avrBs3* gene products

Xanthomonas *AvrBs3* family-type III effectors

- Minor differences occur within 102-bp repeats of a given gene, and most of the differences are located within a variable two-codon region (codons 12 and 13, Figure 2b
- Proteins of the AvrBs3 family consist of a central region of direct, nearly identical repeats, usually of 34 amino acids.
- Minor differences occur within 102-bp repeats of a given gene usually at amino acid positions 12 and 13 of each repeat.

See also TAL effectors (Transcription activator-like) effectors.

The *avr* genes

Structure of *avrBs3* gene products

Xanthomonas *AvrBs3* family-type III effectors

- The number and order of the repeats determine:

1. the specificity of recognition in resistant plants, and
2. the virulence function in susceptible plants

Structure of *avrBs3* gene products

Xanthomonas *AvrBs3 family*-type III effectors

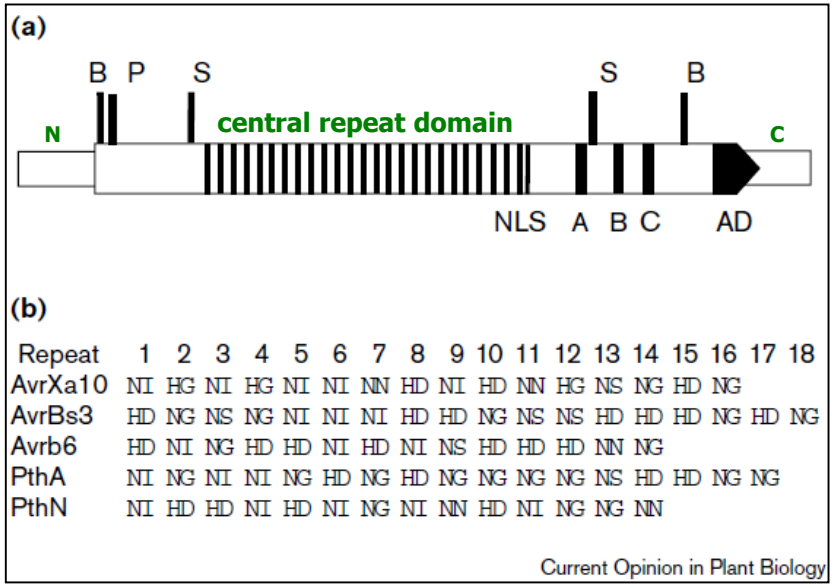
Map of the phenotype gene in the *avrBs3* family

(a) The central repeat domain (leucine rich) is represented by series of open boxes.

- NLS, nuclear (NLS) localization motif sequences A, B, and C, and
- AD/AAD, the transcriptional activation domain.
- The conserved restriction sites B, BamHI; P, PstI; and S, SphI are shown.

(b) Organization of 34-amino acid repeat units in selected members of the *avrBs3* family.

- Each individual repeat unit is represented by the amino-acid residues at positions 12 and 13.



AvrBs3 gene product has 17.5 nearly identical repeats of this motif.

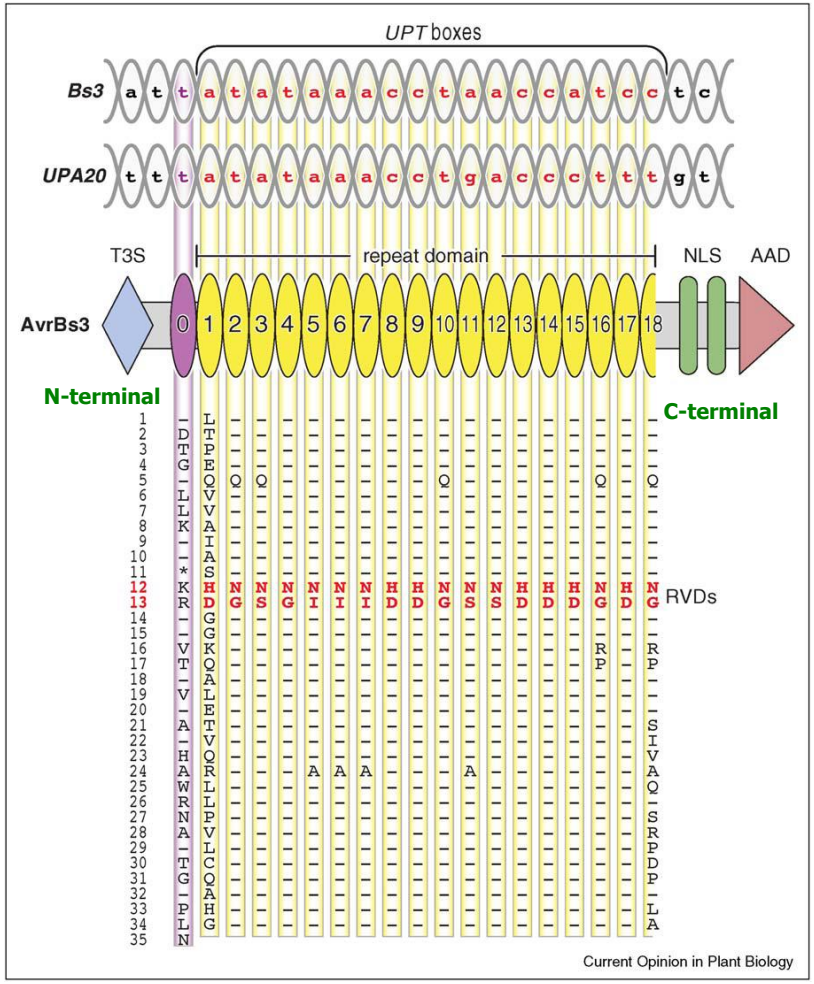
Family members differ in the number and the order of the repeats.

White *et al.*,2000

Structure of *avrBs3* gene products

Map of the phenotype gene in the *avrBs3* family

- AvrBs3 contains:
  1. A N-terminal type III secretion and translocation signal (T3S),
  2. A repeat domain consisting of 17.5 repeat units (yellow ovals),
  3. Two nuclear localization signals (NLS), and
  4. A C-terminal acidic activation domain (AAD/AD).
- Repeat polymorphism occurs largely at residues 12 and 13, the repeat-variable diresidues (RVDs, uppercase boldface red letters).

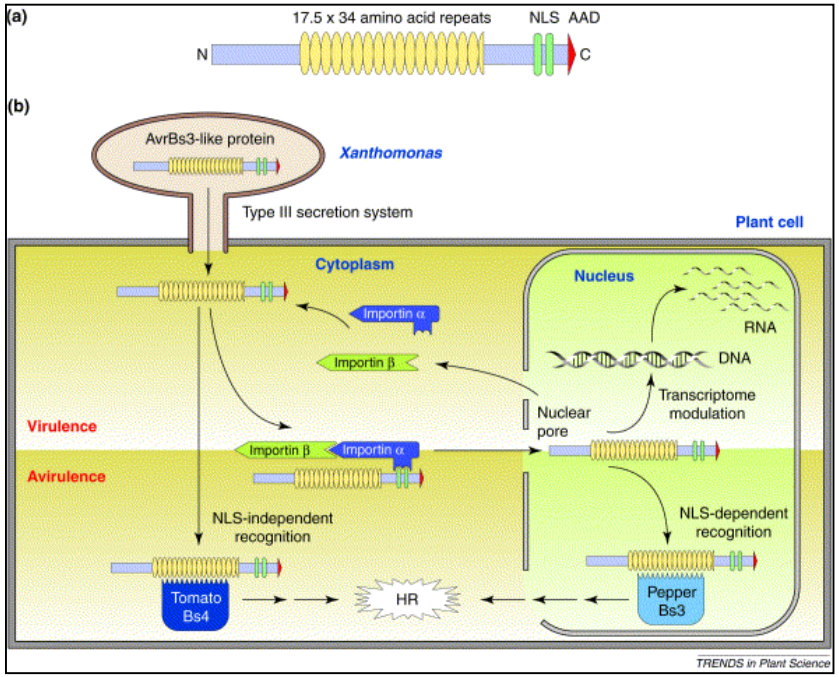


Bogdanove *et al.*,2010

AvrBs3 from *X. vesicatoria*
Localization of avirulence proteins

- Structure of AvrBs3 from *X. vesicatoria*, the prototype of the AvrBs3 protein family.
- The central domain is composed of 17.5 nearly identical 34-amino-acid repeats.
- The C-terminus contains:

1. Two functional nuclear localization signals (NLS), and
2. An acidic transcriptional activation domain (AAD).



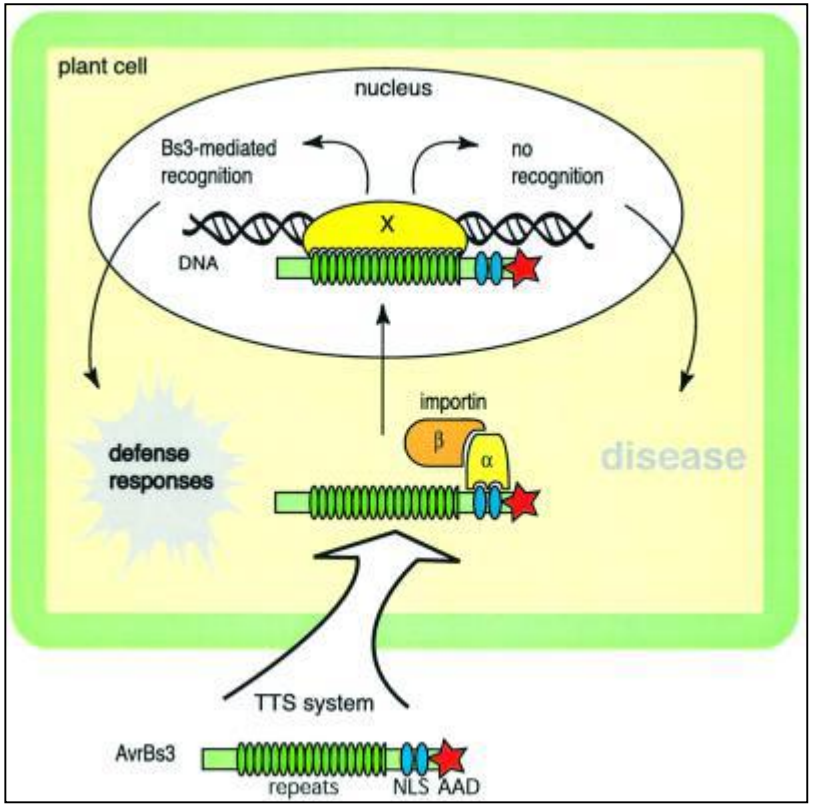
Arrival of AvrBs3 localizes to the plant cell nucleus. the two host proteins i.e. importin α which, together with importin β, mediates nuclear protein import.

AvrBs3

Xanthomonas *AvrBs3 family*-type III effectors

Localizes to the plant cell nucleus with intact NLS and the host cell α-importin protein

- The nuclear localization signals (NLS), probably provide the admission ticket for AvrBs3 to use the host's protein traffic road into the nucleus.
- AvrBs3 interacts with pepper importin α which, together with importin β, mediates nuclear protein import.



Büttner and Bonas,2002;..

Yeast two-hybrid technology

Evidence supporting gene for gene hypothesis

- The yeast two-hybrid approach has been widely used for library screening to identify and isolate genes encoding proteins that interact with a favorite protein.
- It has the capability of identifying and isolating desired clones in a relatively short time by allowing one to easily screen through tens of millions of clones.

- Yeast two-hybrid system: An experimental technique of detecting the protein-protein interactions in yeast cells.
- Two-hybrid system: The yeast or bacterial system that is employed for detecting specific protein-protein interaction; the protein of interest is used as a "bait" to "fishout" proteins that may bind to it (referred to as "prey").

Function of *avr* genes
Diversity and fitness

- Avirulence genes are:

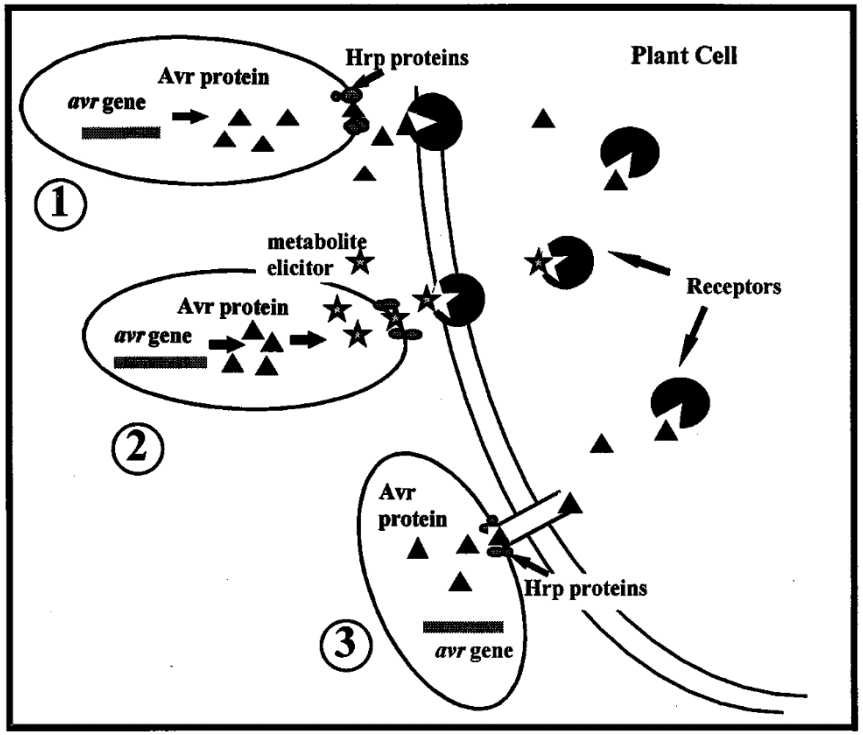
1. extremely diverse,
2. often species or isolate/strain-specific, and
3. rarely have matches in sequence databases.

- When *avr* genes submitted to resistant genes in the plants, pathogens can alter or delete their avirulence proteins to avoid defence elicitation, at risk of a fitness cost associated with loss-of-function of those effectors.

Models for avirulence gene function in the elicitation of plant defense responses

Models for *avr* + *hrp* gene function

- In Model 1, the *avr* gene product (Avr) is the elicitor. The elicitor is exported from the cell via the Hrp apparatus and interacts directly with the plant receptor, which is likely the product of the corresponding resistance gene.
- In Model 2, Avr directs the synthesis of or modifies a metabolite or protein, which is the race-specific elicitor.
- In both Models 1 and *2*, the receptor may span in the plant cell membrane, or may be cytoplasmic.
- In Model 3, Avr is the elicitor, but it is directly delivered to the cytoplasm of the plant cell via an attachment between the bacterial and plant cell, possibly containing Hrp proteins.



Leach and White,1996

Function of *avr* genes
Genes conferring fitness in planta

Gene	Fitness
avrb6	enhanced watersoaking in cotton
avrBs2	essential for growth in pepper and for homologues in alfalfa
avrA	contributes to virulence in tomato
avrE	enhanced virulence and bacterial growth in tomato
avrRpm1	essential for virulence in compatible arabidopsis acessions
avrPphF	restores virulence of *Pph* strain RW60 in soybean
pthA	functions as *avr* gene with bean and cotton: pathogenicity with citrus
virPphA	restores virulence of *Pph* strain RW60 in bean cultivars
avrXa7	functions in aggressiveness/fitness in rice

Vivian and Arnold,2000

Function of *avr* genes

Xoo avirulence genes

Some mores functions

- Avr proteins are one of the type III effectors function as determinants of race-cultivar specificity.
- e.g. Since strain *Xoo* MAFF 311018 exhibits a high degree of host-specificity at the rice cultivar level, and we therefore expected it to contain a number of avirulence genes.
- Other functions are:

1. Promoting virulence,
2. Inhibiting host defense responses,
3. Masking of other *avr* genes,
4. Increasing access to host nutrients (colonization of host plants).

Function of *avr* genes
Enzymatic functions

1. **In *P. syringae***

- Function as an enzyme that is involved in the synthesis of syringolides, which are able to elicit the hypersensitive response.
- avrD of *P. syringae* pv. *tomato* appears to encode an enzyme that makes small molecule elicitors.
- These elicitors are acyl glycosides call syringolides 1 and 2.

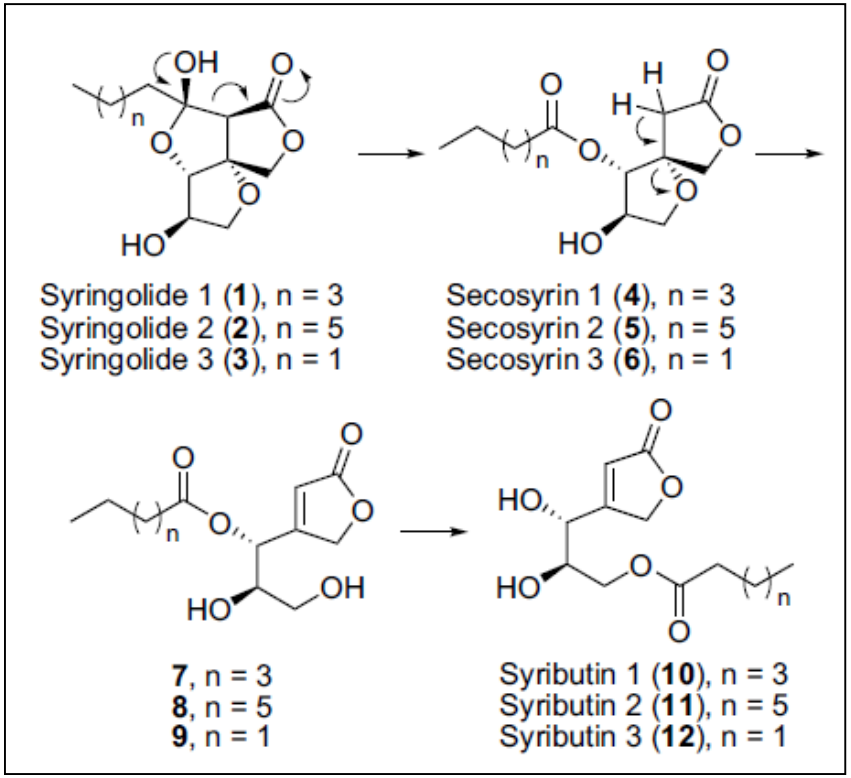
2. **In *X. campestris***

- The AvrBs2 effector of *X. campestris* may also be an enzyme and act within plant cells.

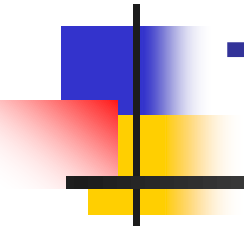
Glycoside= sugar + a small organic molecule

Structures of the syringolides

- Syringolides 1 and 2 are bacterial signal molecules (elicitors) produced by the avirulence gene D (*avrD*) of *Pseudomonas syringae pv. tomato*.
- These metabolites secosyrin 1 (4), secosyrin 2 (5), syributin 1 (10), and syributin 2 (11) were also isolated from *P. syringae* pathovars carrying the avirulence *avrD* gene and they are the major coproducts of the syringolides 1 (1) and 2 (2).



Villalobos *et al.*,2009



The *hrp* genes

Dual-acting genes

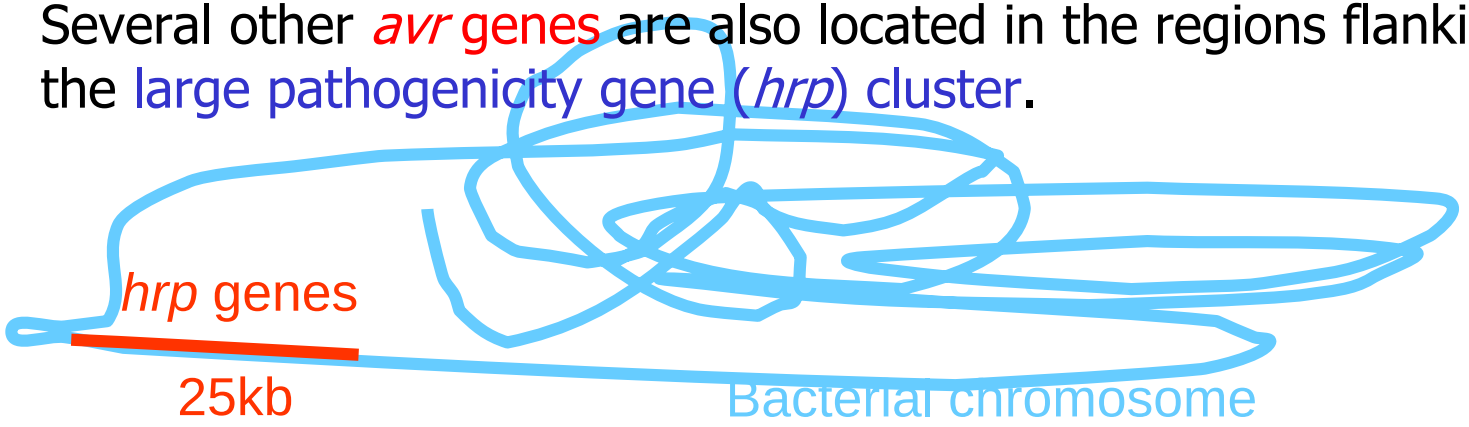
Hypersensitive/disease responses

hrp gene organization

- The *hrp* (pronounced harp) genes in plant-pathogenic bacteria are present as clusters spanning roughly 25 kb.
- *hrp* genes are always clustered and are localized either:

1. on the bacterial chromosome, or
2. on a plasmid.

- In *Pseudomonas syringae* the *hrp* cluster is flanked on both sides by effector genes.
- Several other *avr* genes are also located in the regions flanking the large pathogenicity gene (*hrp*) cluster.



hrp gene organization

- Interestingly, the regions flanking the *hrp* gene cluster also contain insertion sequences and genes for a putative transposase and a $tRNA^{Arg}$.
- These features suggest that the *hrp* gene cluster of *X. campestris* pv. *vesicatoria* is part of a pathogenicity island.

Noël *et al.*,2002

The *hrp* gene
Organization and functions

- hrp genes may be one of the most important groups of genes found in phytopathogenic bacteria in relationship to

1. Pathogenicity, and
2. host range.

Lindgren,1997

hrp/hrc genes
Organization and functions

I. Assembling Hrp system/Hrp pilus:

- At least 9 *hrp* genes (termed *hrc* for *hrp* conserved) are conserved which encode components of the TTS system.
- The TTSS pathway is encoded by:

1. hrp (HR and pathogenicity) and
2. hrc (HR and conserved) genes.

II. Secretion or translaocation of proteins:

- Translocates effector proteins (e.g. Harpins) into the plant cell.
- Harpins are glycine-rich and heat-stable proteins that are secreted through type III secretion system in Gram-negative plant-pathogenic bacteria.

The *hrp* genes
T3SS
Organization and functions

- T3SS is encoded by a distinct cluster of genes, termed hrp/hrc (hypersensitive response and pathogenicity/conserved) genes in the plant pathogens.
- hrp genes exist in clusters of about 20 genes,

1. one of which codes for a constituent of an outer membrane,
2. whereas many others code for:

- core secretion machinery (secretion systems),
- regulatory genes,
- for harpins,
- for the Hrp-pilin, which in some bacteria is required for type III secretion to function, for avirulence (avr) genes, and so on.

Agrios,2005; Prasannath,2013

The *hrp* genes
T3SS
Organization and functions

- Gene clusters encoding T3SS are found in many Gram-negative bacterial pathogens of humans, animals, plants and plant growth-promoting rhizobacteria.
- Type III gene clusters are also present in *Ralstonia*, *Erwinia*, *Xanthomonas* and *Pantoea* plant pathogens.
- T3SS genes are usually located on chromonsomes.
- Sometimes the T3SS genes are located on plasmids, for examples:
- the wilt pathogen *Ralstonia solanacearum* (the 2.09 Mb megaplasmid) and
- the gall pathogen *Erwinia herbicola* (the 150 kb pPATH plasmid).
- It revealed that the T3SS gene cluster in R. solanacearum is not a PAI origin.

Prasannath,2013

The *hrp* genes
In plant bacterial pathogens
Different types of hrp clusters in G-ve bacteria

- Phylogenetic analysis suggests that various TTSSs can be organized into five groups:

1. the Ysc group, including the plasmid-borne Yersinia Ysc TTSS, the *P. aeruginosa* Psc TTSS, the *Rhizobium* Rsc TTSS, etc.
2. the Hrp1 group, including *P. syringae* and *Erwinia* TTSSs;
3. the Hrp2 group, including *Xanthomonas* and *Ralstonia* TTSSs and one of the two *Burkholderia* TTSSs;
4. the Inv/Mxi/Spa group, including the TTSS encoded by *Salmonella* pathogenicity island 1 (SPI-1), the other *Burkholderia* TTSS,
5. the Esa/Ssa group, including the TTSS of enteropathogenic *E. coli* (EPEC).

He *et al.*,2004

Hrc proteins of plant pathogenic bacteria HrcC operon/proteins

- The hrcC gene within the hrpC operon encodes an outer membrane component of the Hrp secretion system that is conserved in all type III protein secretion systems and is required for most pathogenic phenotypes and for secretion of the HrpZ harpin to the bacterial milieu.
- HrcC proteins, which are members of the outer membrane secretin family, of *P. s.* pv. *tomato* and syringae share 80% identity at the amino acid level.

He *et al.*,2004

Hrc proteins of plant pathogenic bacteria and their animal pathogen and flagellar homologs

- The designations for Hrc (HR and conserved) homologs in various bacteria outside of the plant pathogen group.

Plant pathogen Protein	*Yersinia* protein	*Salmonella* protein	*Shigella* protein	Flagellar protein(s)
HrcC	YscC	InvG	MxiD	
HrcJ	YscJ	PrgK	MxiJ	FliF
HrcN	YscN	SpaL	Spa47	FliL
HrcQ	YscQ	SpaO	Spa33	FliN, -Y
HrcR	YscR	SpaP	Spa24	FliP
HrcS	YscS	SpaQ	Spa9	FliQ
HrcT	YscT	SpaR	Spa29	FliR
HrcU	YscU	SpaS	Spa40	FlhB
HrcV	LcrD	InvA	MxiA	FlhA

Eight hrc genes are homologous to genes involved in flagellar assembly.

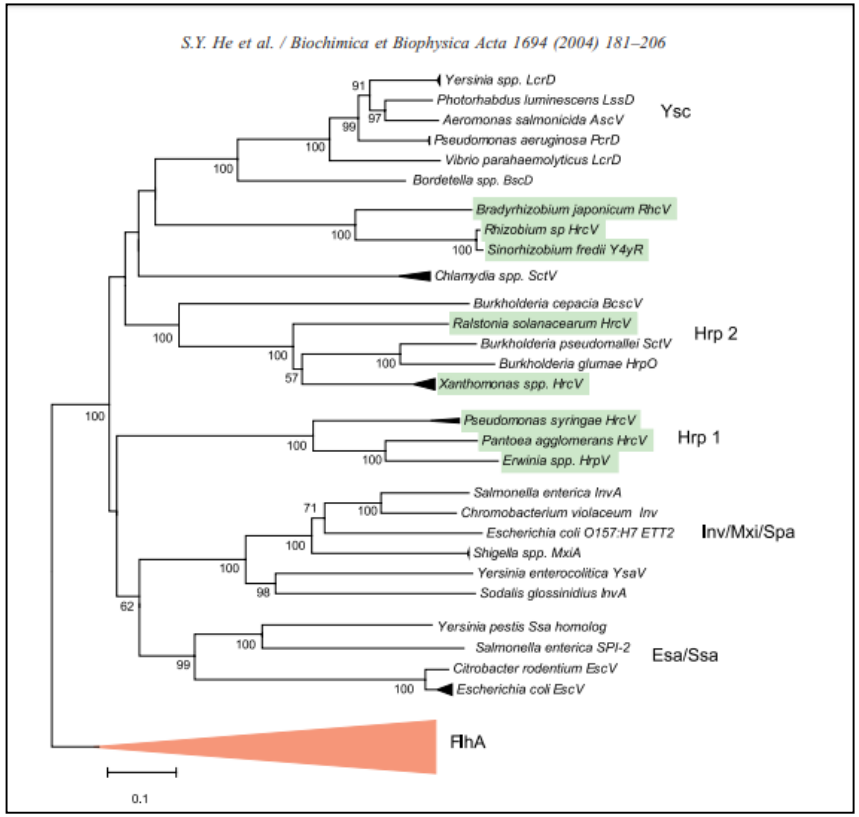
Alfano and Collmer,1997; He *et al.*,2004

The *hrp* genes

In plant bacterial pathogens

Different types of hrp clusters in G-ve bacteria

- Phylogenetic tree based on aligned amino acid sequences of HrcV/FlhA (HrcV (*Xanthomonas*, etc.)/flagellar protein) homologues.
- The numbers at each node are bootstrap confidence values from 500 replicate neighbor-joining trees.
- Plant pathogens are highlighted in green.
- The five TTSS groups are indicated on the right.



He *et al.*,2004

The *hrp* genes

In plant bacterial pathogens

Different types of hrp clusters in G-ve bacteria

- Thus, some of the hrp genes appear to be completely different.
- The arrangements of genes within some operons are characteristic of each group, and the regulatory systems are distinct.

Alfano and Collmer,1997

The *hrp* genes
In plant bacterial pathogens

- The *hrp* gene cluster itself encodes and controls the secretion of a glycine-rich protein called harpin:
- HrpZ from *P. syringae*,
- HrpN from *E. amylovora*,
- PopA from *R. solanacearum*.
- In *Erwinia amylovora*, this protein was given the name harpin and the corresponding gene designated *hrpN*.
- This was the first example of such a protein and gene identified from any bacterial species.

hrp/ hrc genes
Chromosome or megaplasmid borne

- In all genomes that have been sequenced to date, the *hrp/ hrc* genes are found clustered:

1. In a single region of the chromosome, or
2. On a 2.1-Mb megaplasmid in the case of *R. solanacearum*, or
3. A 150-kb plasmid in *Pantoea agglomerans* pv. *gypsophilae*.
4. The *hrp* cluster of *R. solanacearum* is also appear to be chromosomal.

- These clusters of *hrp/hrc* genes are typically flanked by regions that contain different effector genes in different bacterial species or pathovars.

Alfano and Collmer,2004

Hrp/hrc genes

TTSSs and the proteins they secrete have been given a variety of names depending on the species of origin.

- Those *hrp* genes that are broadly conserved in:

1. Plant pathogenic bacteria (*Pseudomonas*, *Erwinia*, *Ralstonia*, *Xanthomonas*), and
2. Animal pathogenic bacteria (*Yersinia*, *Salmonella*, *Shigella* spp.).

- The TTSSs and the proteins they secrete have been given a variety of names depending on the species of origin.
- For example,

1. The TTSS from *Yersinia* spp. is called the Ysc system (Yop secretion), and
2. The effector proteins it secretes are Yops (*Yersinia* outer protein).

hrp gene cluster
Xanthomonas

- The *Xanthomonas hrp* gene cluster contains six operons (*hrpA* to *hrpF*, composed of 22 genes) and an additional two genes outside *hrpA*15.
- Comparison of the *hrp* cluster among *Xoo*, *Xac* and *Xcc* revealed that the gene orders were similar but the clusters were located in different regions on the genomes.
- One exception to their similarity was a region between *hpaB* (*hrpE2*) and *hrpF*, which varied in terms of its length and predicted genes.

The *hpa* (*hrp*-associated) genes encode Harpin-like proteins contribute to pathogenicity and to the induction of HR in nonhost plants but are not essential for the pathogenic interactions of bacteria with plants.

The *hrp* genes

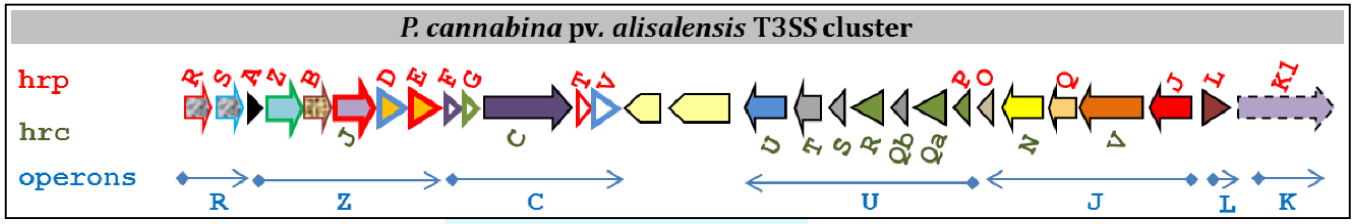
Different types of hrp clusters in G-ve bacteria

Pseudomonas cannabina pv. *alisalensis*

- Schematic representation of the *hrp/hrc* gene cluster coding for the synthesis of the type III secretion system of *Pseudomonas cannabina* pv. *alisalensis*.
- The genes were arranged in five operons organized in two main blocks having convergent transcription:

1. the *hrpRS*, *hrpZ* and *hrpC* operons in one orientation, and
2. the *hrpU* and *hrpJ* in the opposite direction.

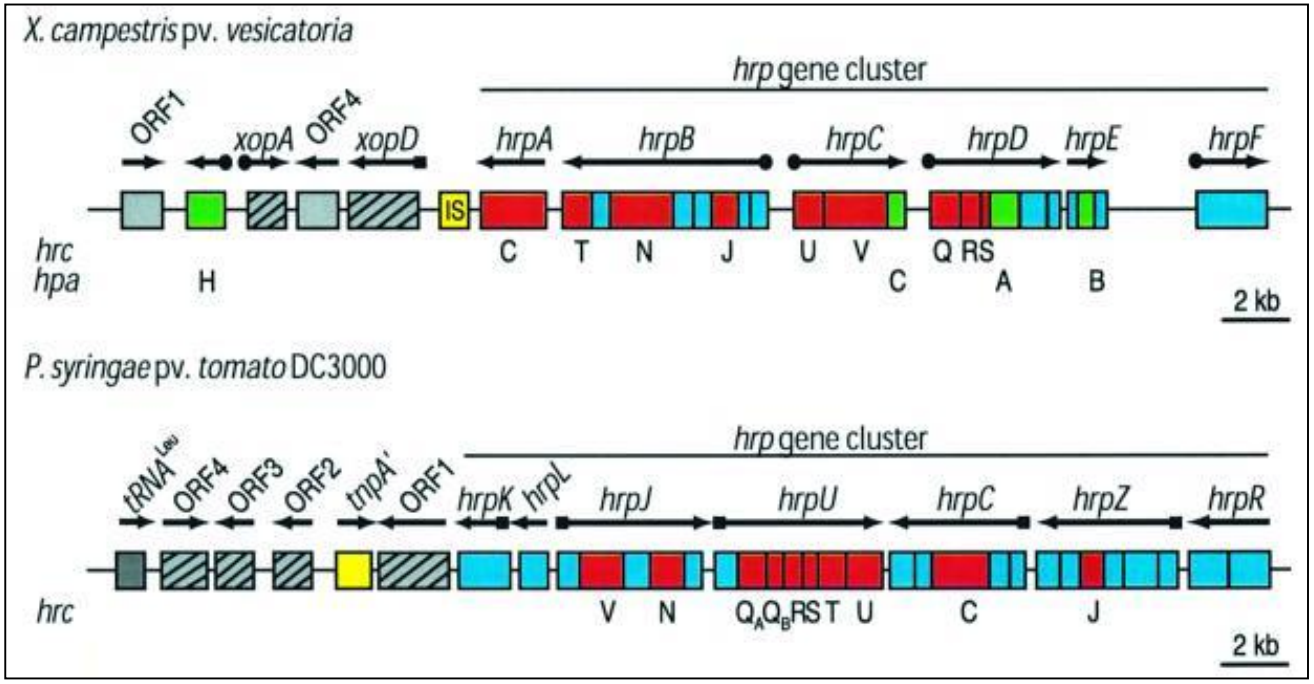
- The two blocks were separated by a hyper variable region with a very low level of conservation between closely taxonomically related bacteria.



Sarris *et al.*,2013

Organization of the *hrp/hrc* gene cluster *Xanthomonas vesicatoria*

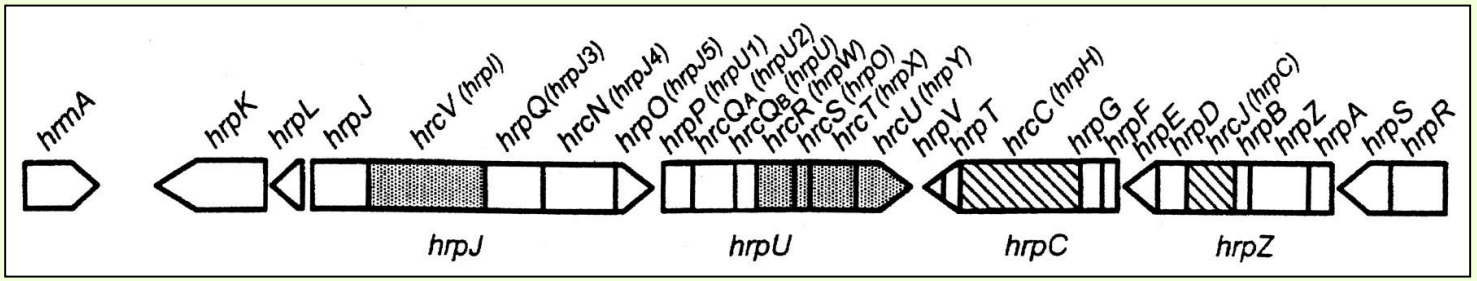
- The *hrp* region also contains so-called *hrp*-associated (*hpa*) genes, which are not essential for bacterial pathogenicity but contribute to the interaction with the host plant.



Büttner and Bonas,2002

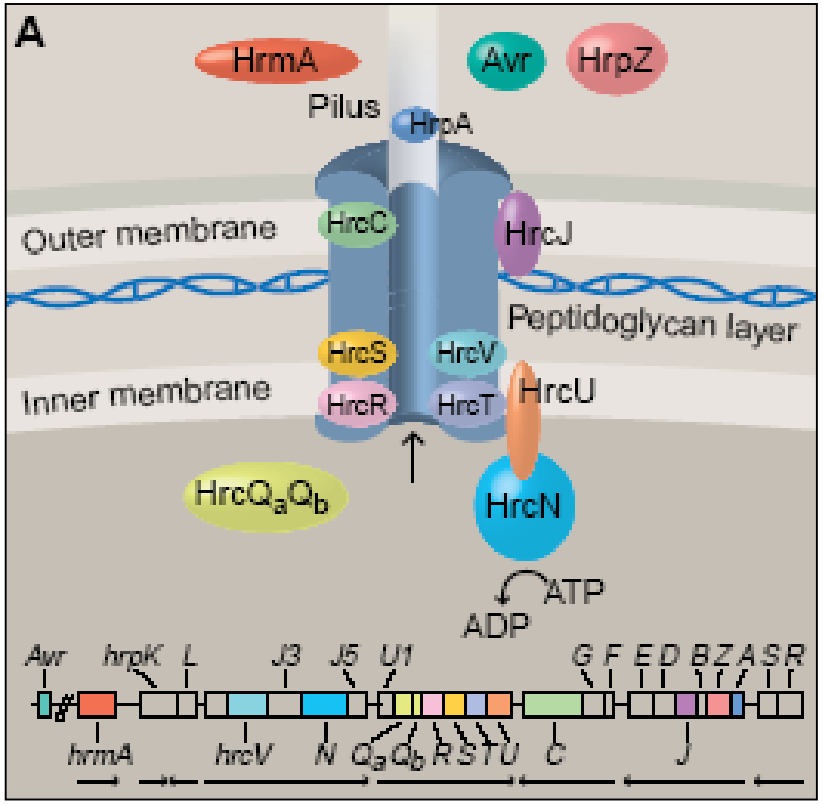
Organization of the *hrp/hrc* gene cluster *P. syringae* pv. *syringae*

- The gene designation employs the unified nomenclature for widely conserved *hrp* genes (*hrc*).
- Arrowheads indicate the direction of transcription for each operon.
- Genes encoding proteins predicted to be associated with the inner or outer membrane of the type III secretion system are stippled and hatched, respectively, but HrcJ may be associated with both membranes.



Organization of the *hrp/hrc* gene cluster
Hrp secretion system of *P. syringae*

- The genomic organization of the *hrp/hrc* genes is presented in the lower section of the panel.
- HrcN has a conserved ATPase domain, which suggests that it may play a role in providing the energy needed to actively transport these proteins into the plant cell.
- *Avr* genes are often located in or adjacent to the *hrp* gene cluster.

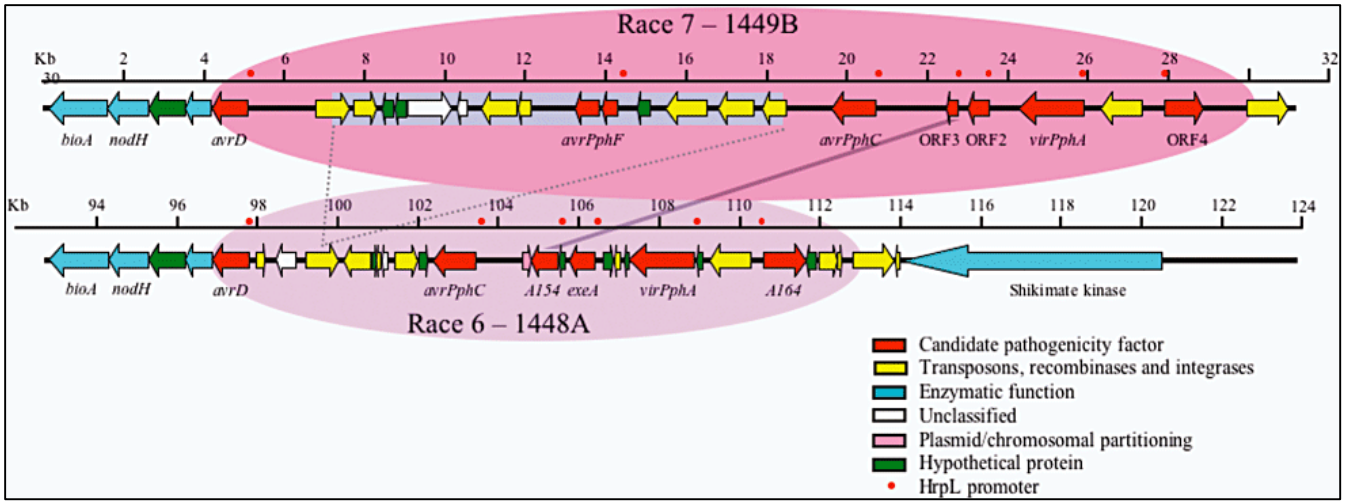


A unified nomenclature for *P. syringae* T3SS-secreted proteins was established. The detailed naming system can be found in the website: www.pseudomonas-syringae.org.

The *avr* genes

avr genes in cluster

- Several avr genes, including avrPphF, avrD and avrPphC, were also found to be clustered in pAV520.



Comparison of the plasmid-borne pathogenicity island containing effector genes in *Psudomonas phaseolicola* strains 1448A (race 6) and 1449B (race 7).

Mansfield,2009

The *hrp* genes

Harpins

Functions

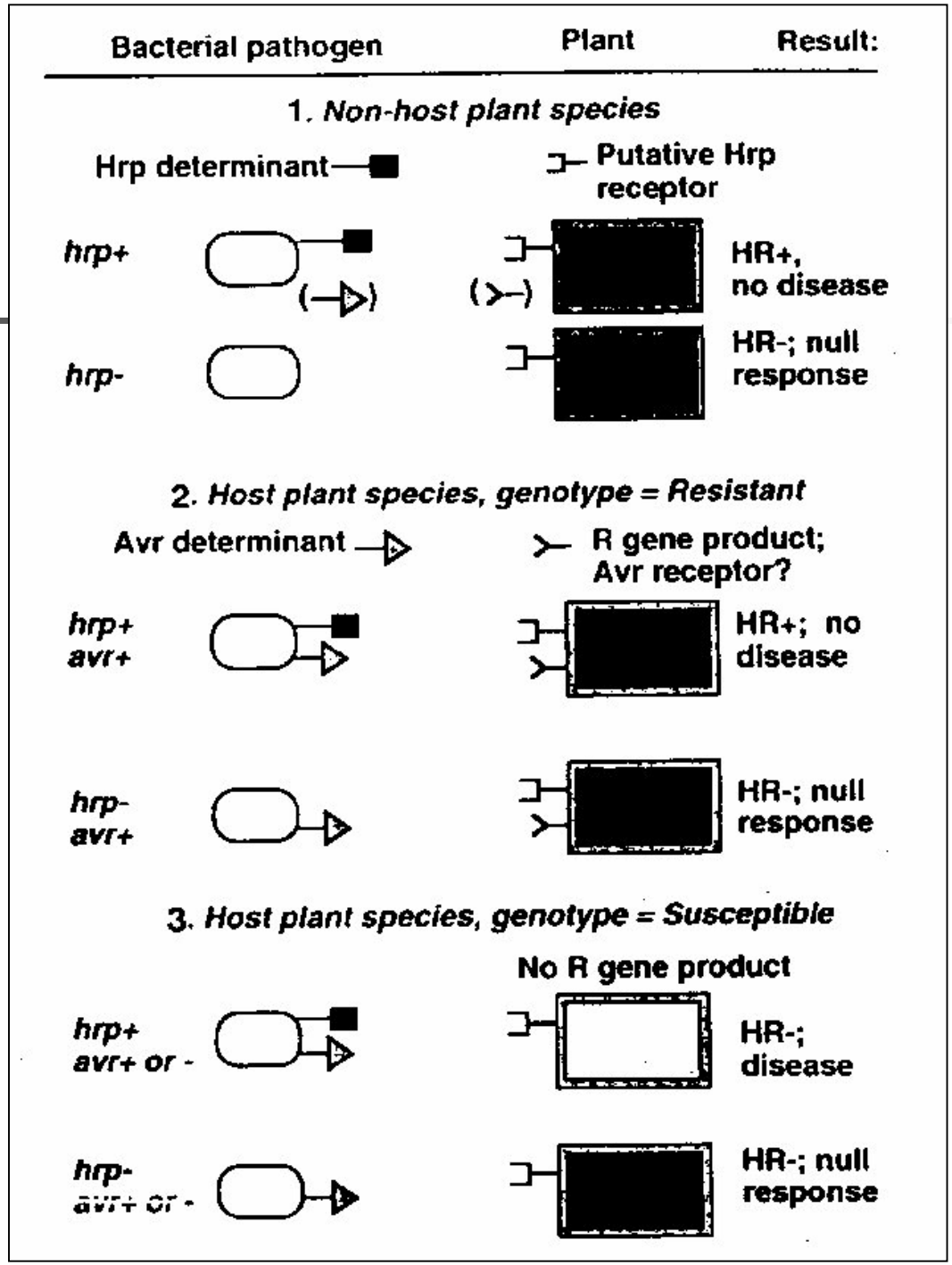
- Harpin is a single protein was identified that:

1. Elicited HR in non-host plants;
2. Necessary for pathogenesis.

- Mutations in *hrp* genes cause loss of the ability of the bacteria to:

1. Not elicit the HR in resistant plants;
2. Not cause disease symptoms.

- The mutants thus behave like non-pathogenic bacteria.


Bacterial pathogen
Plant
Result:
1. Non-host plant species
Hrp determinant
Putative Hrp receptor
hrp+
HR+, no disease
hrp-
HR-; null response
2. Host plant species, genotype = Resistant
Avr determinant
R gene product; Avr receptor?
hrp+ avr+
HR+; no disease
hrp- avr+
HR-; null response
3. Host plant species, genotype = Susceptible
No R gene product
hrp+ avr+ or -
HR-; disease
hrp- avr+ or -
HR-; null response

Harpin test in tobacco

Hrp test in tobacco

Inject bacterial suspension into tobacco leaf. If hypersensitive reaction occurs, then the bacterium possess a 'Type III secretion 'system.

Type III secretion systems are used by bacteria to inject virulence factors into host cells.

Many bacterial pathogens of both plants and animal possess a Type III system.

'Hrp' means hypersensitive response, pathogenesis-related

Bioassay method

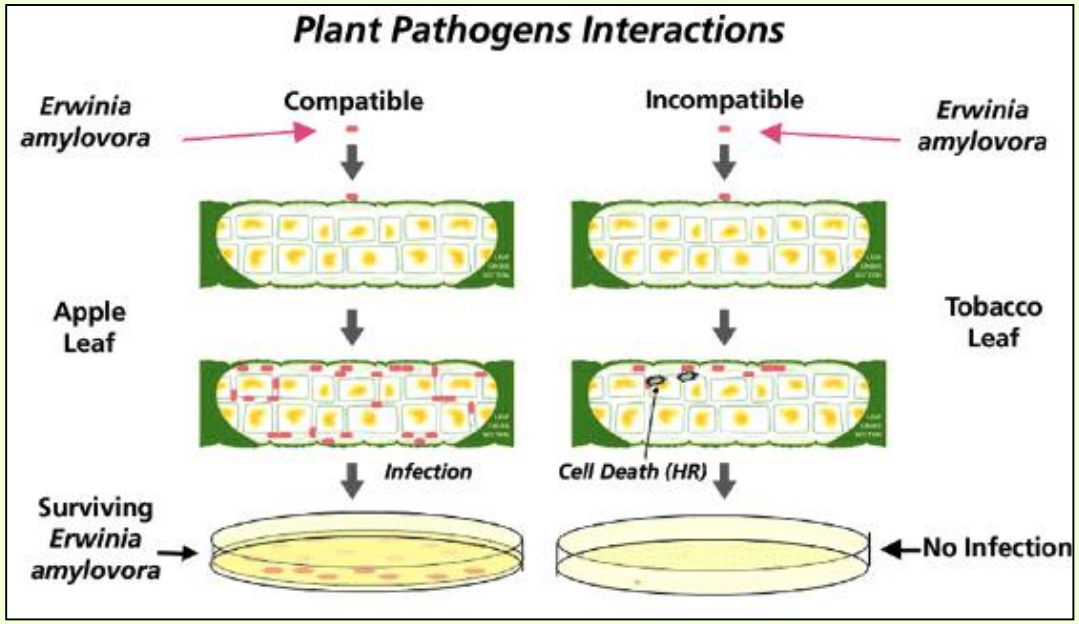
Compatible versus incompatible plant-pathogen interactions

- The difference between compatible and incompatible interactions can be visualized and quantitated with a simple bio-assay.
- To test the ability of a plant pathogen to infect specific plant species, the plants are infected with the bacteria.
- Following inoculation and an incubation period, plant tissue is disrupted and the bacteria extracted.
- The bacteria are spread onto petri dishes containing media optimal for the specific bacterial growth.
- After a period of growth, bacterial colony counts are taken.
- The plates with bacteria from a compatible host show high colony counts (left) demonstrating a compatible plant-pathogen interaction.
- The pathogen succeeds in infecting the host plant due to the lack of initiation of defense mechanisms protecting the plant from invasion.
- The plates containing bacteria from an incompatible host plant show little or no bacterial growth (right) demonstrating an incompatible plant-pathogen interaction.
- The non-host plant is able to recognize the bacteria as a signal to activate defense mechanisms that stop further infection.
- The harpin protein was identified based on its ability to elicit HR in an incompatible host plant.

Bioassay method

Compatible versus incompatible plant-pathogen interactions

- A schematic diagram comparing the differences between compatible and incompatible interactions of *Erwinia amylovora* with a host plant (apple) and a non-host plant (tobacco).



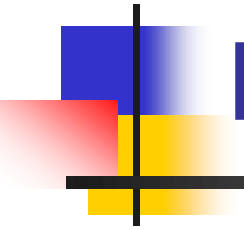
In vitro hrp genes expression

- *hrp* genes are generally only expressed in conditions like those found in the plant apoplastic fluid (low nutrients).
- That is, the bacterial pathogens do not express these genes until they find themselves in conditions that mimic the plant environment.

hrp genes
Physical characteristics of harpins

1. 403 amino acids in length;
2. Approximately 44 kD in molecular mass (small);
3. Heat stable: 100°C for 10 minutes does not eliminate biological activity;
4. Rich in glycine but lacks cysteine;
5. Water soluble;
6. Acidic;
7. With no known enzymatic activity.

- GenBank accession number for the amino acid sequence: AAC31644.



Bacterial *pth* genes

Pathogenicity genes

Pth proteins
The T3SS machinery

- A great many T3S dependent Avr proteins have been identified, probably because the resulting HR phenotype is so readily assayed, even on non-hosts.
- By contrast, only a relatively few T3S dependent Pth proteins have been identified and shown to be necessary to condition pathogenicity on a given host.

pthA, the first member of the *Xanthomonas avr/pthA* gene family to be recognized as being essential for pathogenicity, is required for the production of necrotic cankers on all species of citrus attacked by *X. citri*.

Pth proteins

- Most of the members of this gene family were first isolated as *avr* genes, and without evidence of *pth* function.
- The exceptions are:

1. All the members involved in inducing citrus canker disease (*pthA, pthB, pthC* and *pthW*), and
2. Two involved in cotton blight (*pthN* and *pthN2*).

- In *X. citri* the single most important effector is PthA. Even in the absence of the pathogen, PthA induces canker-like symptoms when transiently expressed in plants.

Pth proteins

avr/pth gene families in xanthomonads

- In xanthomonads, three *avr* gene families:

1. *avrBs1*
2. *avrBs2*
3. *avrBs3/pth*, have been reported to date.

- Four *avrBs3/pth*-like genes are found in *Xac*, on two plasmids.
- *avrBs3/pth* family found in the genome of *Xoo*.

Pth proteins in *X. citri*
Cause hyperplastic canker symptoms

- *pthA* plays the most important role in pathogenicity and is present in all *Xanthomonas* that causes canker in citrus, often in multiple copies.
- The *pthA*, *pthB*, *pthC* and *pthW* homologues are fully isofunctional and they are absolutely required for the xanthomonad that carries them to cause hyperplastic canker symptoms.

Brunings & Gabriel,2003

Pth proteins in *X. citri*

Cause hyperplastic canker symptoms

Cell division signalling molecule PthA

- *pthA*, is necessary for *X. citri* pv. *citri* A to cause citrus canker disease, and the insertion of *pthA* into other xanthomonads confers an ability to elicit cankers on citrus.
- *X. citri* induces cell divisions in citrus within 72 h of inoculation, presumably by injecting the cell division signalling molecule PthA.

Pth proteins in *X. citri*

Cause hyperplastic canker symptoms

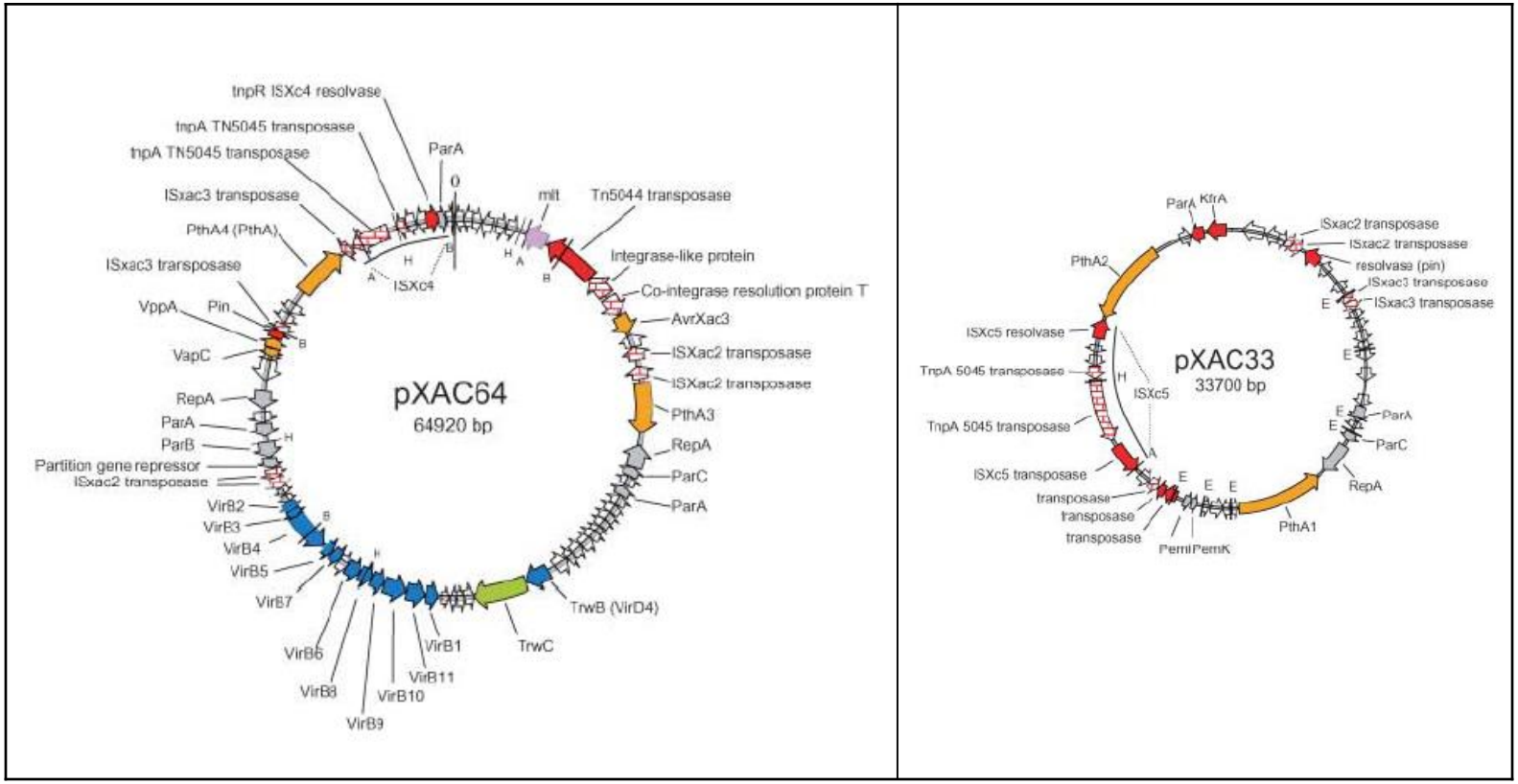
Cell division signalling molecule PthA

- The two plasmids found in *X. citri* strain 306:
- Predicted products of open reading frames are indicated as follows:
- Complete transposase or resolvase genes in red;
- Truncated or rearranged transposase or resolvase genes are red hatched,
- *pthA* homologues and *avr* genes in orange;
- Plasmid maintenance genes in grey;
- T4S genes in blue;
- trwC in green; unknown genes are open and
- Other genes in purple.

Pth proteins in *X. citri*

Cause hyperplastic canker symptoms

Cell division signalling molecule PthA

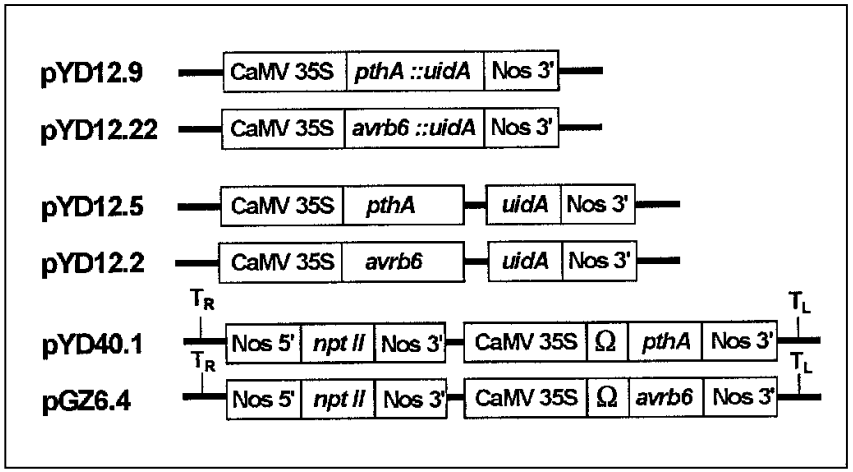


The two plasmids found in *X. citri* strain 306.

Pth proteins in *X. citri*

DNA constructions used in the transient expression assays

- *pthA* was cloned from pZit45 into pUC118 with a uracil DNA glycosylase (UDG) polymerase chain reaction (PCR) technique (Nisson *et al.*,1991) that allowed creation of multiple cloning sites at strategic locations.
- No cankers were elicited on citrus in transient expression assays in which another member of the gene family, *avrb6*, was substituted for *pthA* on the same vectors.



Duan *et al.*,1999

Pth proteins in *X. citri*

Cause hyperplastic canker symptoms

Cell division signalling molecule PthA

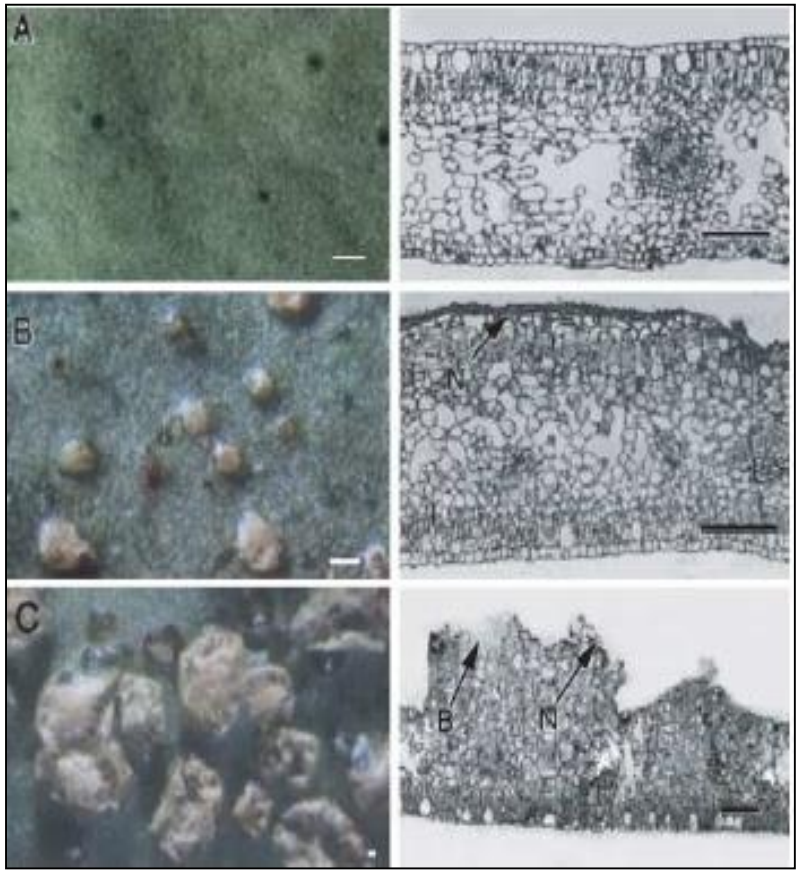
- Comparisons of canker symptoms on the citrus leaf surface and thin-sections through leaves.

A. Normal citrus leaf.

B. Citrus leaf following particle bombardment with *pthA* under control of a plant promoter,

C. Citrus leaf following artificial inoculation with *X. citri*.

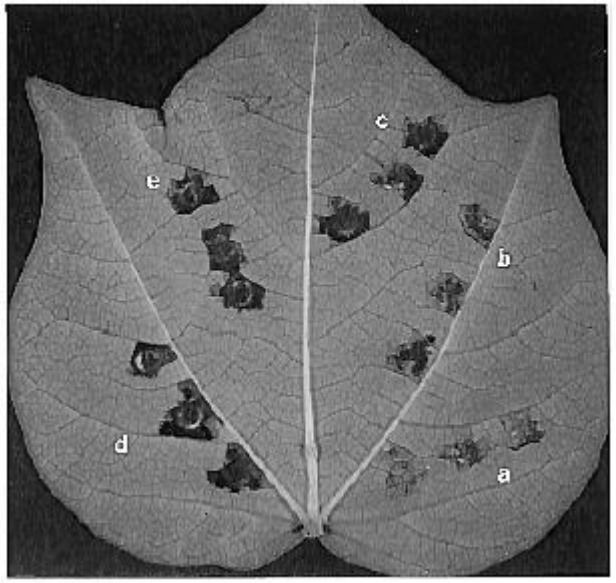
- (N) the necrosis of the abaxial epidermal layer,
- (B) *X. citri* bacteria oozing from the artificial inoculation.
- Figure excerpted from Duan *et al.*,1999.



Brunings & Gabriel,2003

Pth proteins in *X. malvacearum* *pthN* and *pthN2*

- 10 genes belong to a large family of *Xanthomonas avr/pth* (avirulence/pathogenicity genes) were found in *X. a.* pv. *malvacearum*.
- Water-soaking symptoms caused by *pthN* and *pthN2*.
- Photo taken 5 days after inoculation.



Chakrabarty *et al.*,1997

Pth proteins
Mutation

- Mutation of the third important structure of the AvrBs3/PthA family, the AAD, results in loss of both avirulence and virulence.
- Also, mutations of either *hrp* genes or of *pthA* in *X. citri* abolish canker pathogenicity.
- When *pthA* is expressed by itself in citrus cells, visible cankers are formed within 10-14 days.

Bacterial *hpa* genes

hpa (*hrp*-associated) genes

Harpin-like proteins

The *hpa*
hrp -associated genes

- The *hpa* (*hrp*-associated) genes contribute to:

1. Pathogenicity and to the induction of HR in nonhost plants, but
2. Are not essential for the pathogenic interactions of bacteria with plants.

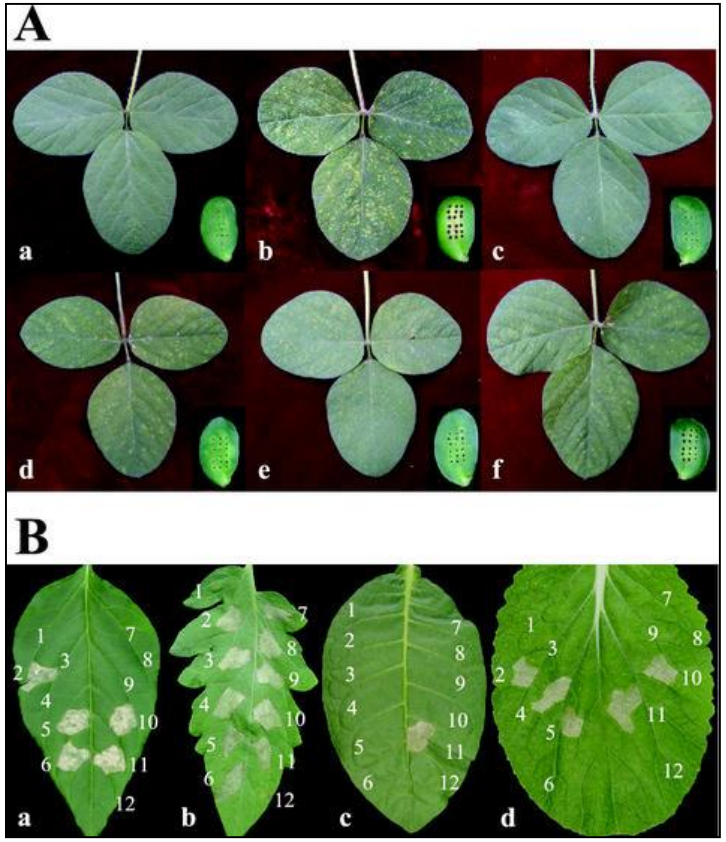
The *hpa*
hrp -associated genes

- The *hpa* (*hrp*-associated) genes that contribute to, but are not essential for the pathogenic interaction of Xcv with host plants were identified by the analysis of nonpolar mutants in the *hrp* gene cluster.
- The gene *hpaA* encodes a protein needed for the HR and full pathogenicity.
- *HpaA* secreted by the TTSS, is localized to the plant cell nucleus when expressed in planta.
- In contrast, *HpaB* remains in the pathogen to accomplish its role in the control of type III protein export.

Narayanasamy,2008

The *hpa*
hrp -associated genes

- The *hrp* and *hrc* genes tested were essential for full pathogenicity and for the induction of HR (Fig. 3A and B).
- The *hpa* mutants, which included *hpaG*, *hpaC*, and *hpaF* mutants, had significantly reduced virulence but induced HR in pepper plants (Fig. 3A and B).
- By contrast, the *hpaH* mutant showed significantly reduced virulence and did not induce HR in pepper plants (Fig. 3A and B).
- The wild-type strain 8ra and its *hrp*, *hrc*, and *hpa* mutants did not induce HR in tobacco plants(Fig. 3B).



Kim *et al.*,2003

The *hpa*
hrp -associated genes

- Harpin-like proteins have been reported in all genera of phytopathogens that have a TTSS.
- These proteins include the Hpa1/HpaG family in *Xanthomonas*, which was recently shown to have HR elicitor activity.
- In *Xac*, the *hrp* cluster is composed of 25 genes extending from *hpa2* and *hpaF*.

Alfano and Collmer,2004;..

The *hpa*

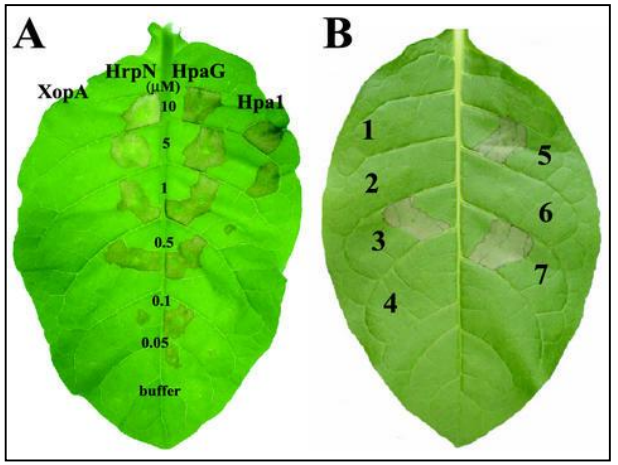
hrp -associated genes

Effect of HpaG on the ability to induce HR in tobacco leaves

- HpaG is an Hrp type III-secreted elicitor.
- Since HpaG has the features of harpins, which are found in *P. syringae* pathovars, *Erwinia* species, and *Ralstonia solanacearum*.

A. Comparison of HpaG activity with other known harpin HrpN and harpin-like XopA and Hpa1 (A).

B. Sites: 1, 50 mM Tris-HCl (pH 8.0); 2, 8ra(pLAFR3) (2 × 10^8 CFU/ml); 3, 8ra(pLGX3) (2 × 10^8 CFU/ml); 4, *hrcU* mutant 1-44(pLGX3) (2 × 10^8 CFU/ml); 5, HpaG (1 μM) in 50 mM Tris-HCl (pH 8.0); 6, *hpaG* mutant 70-1 (pLGX3) (2 × 10^8 CFU/ml); 7, 70-1 (pLGXhpaG) (2 × 10^8 CFU/ml) .



Kim *et al.*,2003

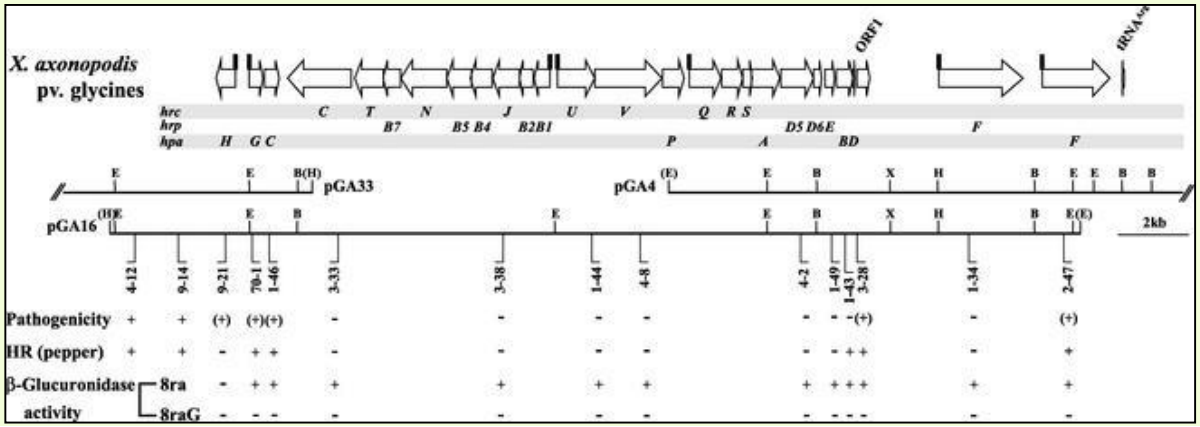
The *hpa*
hrp -associated genes

- We sequenced an approximately 29-kb region from *Xanthomonas axonopodis* pv. *glycinea* that contained the Hrp type III secretion system, and we characterized the genes in this region by Tn*3-gus* mutagenesis and gene expression analyses.
- The PAI was composed of 9 *hrp*, 9 *hrc*, and 8 *hpa* genes with seven plant-inducible promoter boxes.
- The *hpa* (*hrp*-associated) genes are generally clustered in a chromosomal region that spans 20 to 30 kb.

Kim *et al.*,2003

The *hpa*
hrp -associated genes

- Open arrows indicate the positions and orientations of the *hrp*, *hrc*, and *hpa* genes.
- Black rectangles above open arrows indicate the plant-inducible promoter (PIP) boxes.
- Vertical bars in the pGA16 map indicate the positions and orientations of the Tn*3-gus* insertions, and the major phenotypes of the mutants are represented below the restriction map.
- B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; X, *Xba*I. Enzyme sites from the vector are shown in parentheses.



Kim *et al.*,2003

The *hpa*
hrp -associated genes

- The *hrp* gene cluster in *Xoo* contains 27 genes extending from hpaZ to hpaF and the structure has similarity to those of other characterized *Xanthomonas* hrp gene clusters, exception being the *hpaB* (*hrpE2*)-*hrpF* region.
- Three novel genes were detected in the region between *hpaB* and *hrpF*.
- In addition, four tandem transposase homologs were present between hpaB and hrpF.

Narayanasamy,2008

Bacterial *xop* genes

Specific *Xanthomonas* effectors

Xanthomonas outer proteins
Xop effectors

- In plant pathogens, many virulence genes have been identified such as *avr*, *hrp*, *hrc*,... genes.
- New *xop* genes which encode *Xanthomonas* outer proteins (Xops) were also characterized.
- Xop proteins such as Xop A, XopB, and XopD were initially identified in *Xanthomonas* spp., and because of earlier genus name, these were designated as outer protein (Xop).
- Two more novel Xop proteins namely XopC and XopJ have been identified from *X.campestris* pv.*vesicatoria*.
- All these effectors were secreted by the TTS system and are regulated by *hrp*G gene.

Noël *et al.*,2003;..

Xop (*Xanthomonas* outer protein)
Xop effectors

Effector	Homology (GenBank accession no.)	BLASTP,* bits/e-value	Predicted size, aa	Insertion site, aa	Homologs in sequenced species
XopC	Type III effector XopC (AAR23832)	575/e-163	834	708	*Rs*
XopF1	*Xoo* conserved hypothetical protein ORF1 (BAD30000)	879/0.0	670	151	*Xoo*, *Xcc*†
XopF2	*Xoo* conserved hypothetical protein ORF1 (BAD30000)	517/e-145	667	108	*Xoo*, *Xcc*†
XopN	*Xac* conserved hypothetical protein XAC2786 (NP_643095)	1059/0.0	733	197	*Xac*, *Xcc*
XopO	*Psp* avirulence protein AvrRps4 (AAB51082)	119/4e-26	220	93	*Psp* *Pst DC3000*
XopP	*Xac* conserved hypothetical protein XAC1208 (NP_641544)	865/0.0	658	321	*Xac*, *Xcc*, *Rs*
XopQ	*Xac* conserved hypothetical protein XAC4333 (NP_644627)	704/0.0	464	81	*Xac*, *Pst DC3000*, *Xcc*, *Rs*

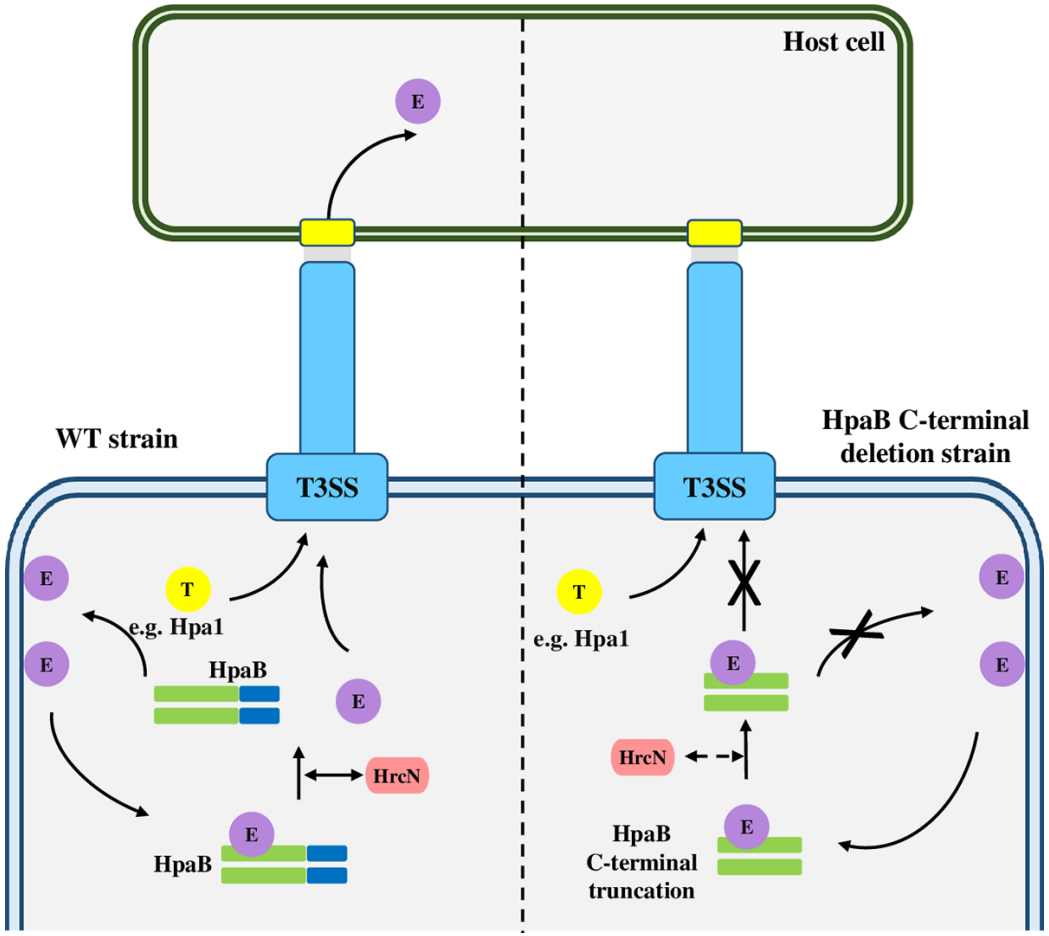
*BLASTP queries were done by using databases at the National Center for Biotechnology (23).
†BLASTN of nucleotide sequence also yields homology with *Xac*, *Xag*, and *Xcc*.

Roden *et al.*,2004

Xop (*Xanthomonas* outer protein)

Xop effectors

Xanthomonas axonopodis pv. *punicae*

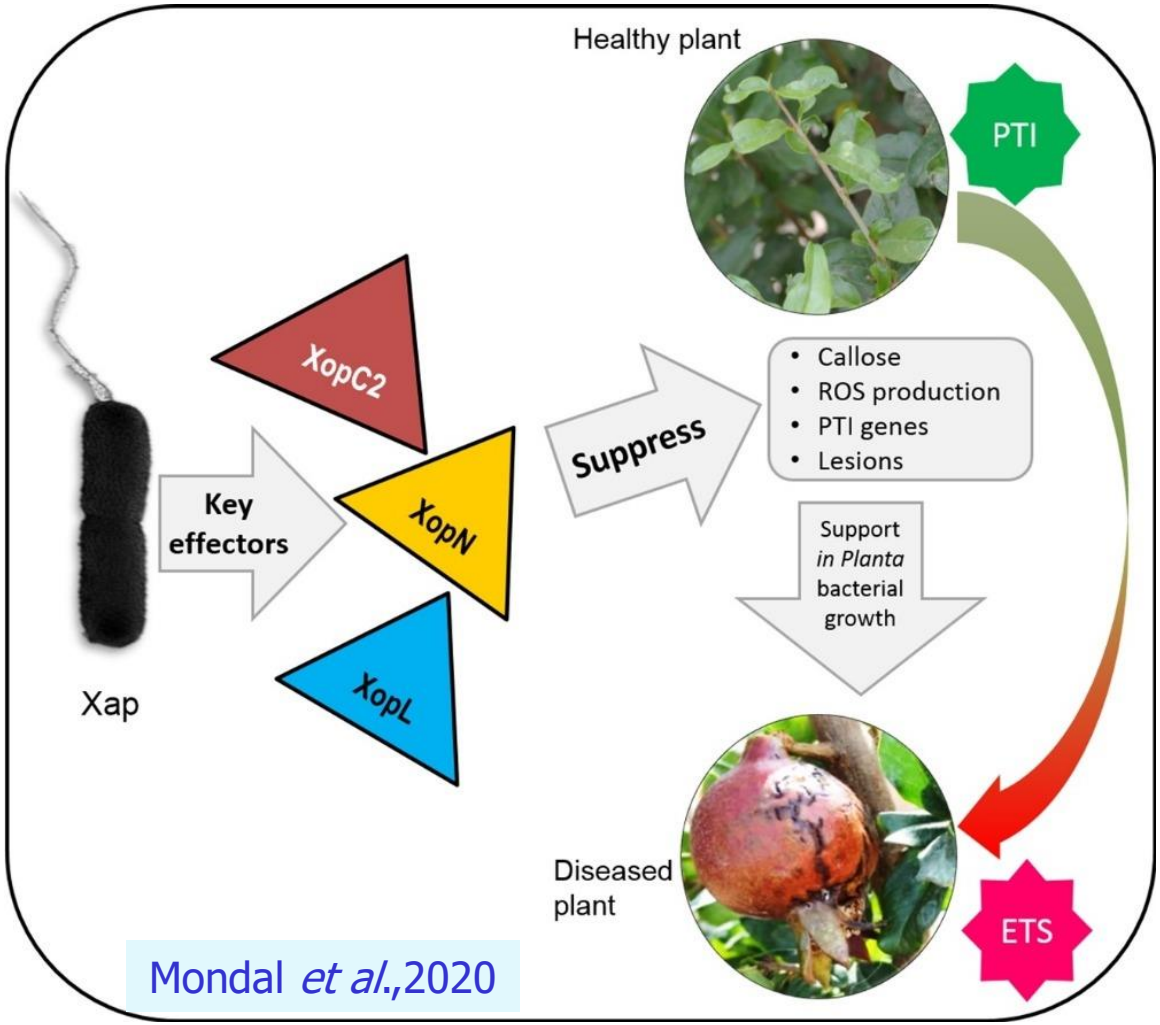


Gan *et al.*,2021

Xop (*Xanthomonas* outer protein)

Xop effectors

Xanthomonas axonopodis pv. *punicae*



Mondal *et al.*,2020

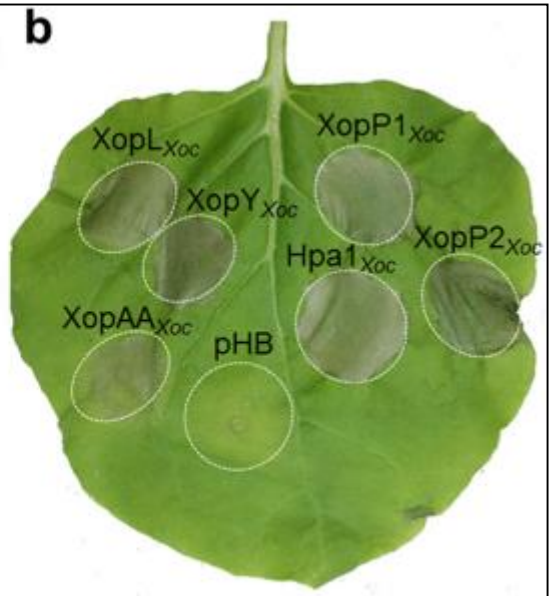
Xop (*Xanthomonas* outer protein)
Xop effectors

- Cell death responses induced by T3Es of *X. oryzae* pv. *oryzicola* RS105. Harpin protein Hpa1 was used as positive cell death inducer.

a

Effector	Cell death	Effector	Cell death
$XopC_{Xoc}$		$XopW_{Xoc}$	
$XopF_{Xoc}$		$XopX_{Xoc}$	
$XopI_{Xoc}$		$XopY_{Xoc}$	Yes/++
$XopK_{Xoc}$		$XopZ_{Xoc}$	
$XopL_{Xoc}$	Yes/++	$XopAA_{Xoc}$	Yes/+
$XopN_{Xoc}$		$XopAB_{Xoc}$	
$XopO_{Xoc}$		$XopAE_{Xoc}$	
$XopP1_{Xoc}$	Yes/+++	$XopAF_{Xoc}$	
$XopP2_{Xoc}$	Yes/+++	$XopAK_{Xoc}$	
$XopQ_{Xoc}$		$HpaA_{Xoc}$	
$XopR_{Xoc}$		$Hpa1_{Xoc}$	Control/+++
$XopV_{Xoc}$			

b



Ma *et al.*,2020

Xanthomonas outer proteins
Xop effectors

- Three xop genes are *Xanthomonas* spp.- specific, whereas homologs for the rest are found in other phytopathogenic bacteria.
- In the genome sequence of *X. campestris* pv. *vesicatoria* 85-10, two new type III effector proteins, XopE1 and XopE2 belonging to the HopX family of *Pseudomonas syringae* were recently identified.
- XopF1 and XopF2 define an effector gene family in *X. campestris* pv.*vesicatoria* (Xcv).
- XopN contains a eukaryotic protein fold repeat and is required for full Xcv pathogenicity in pepper and tomato.

Anne *et al.*,2004; Lin *et al.*,2010

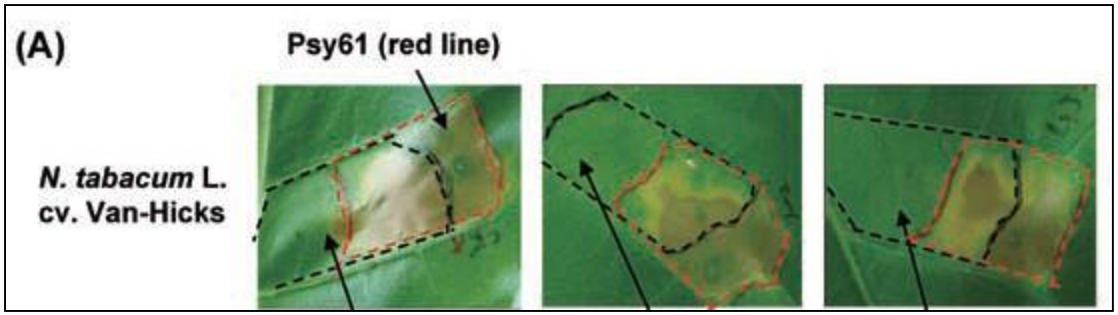
Xanthomonas outer proteins
XopE2 effector

- The XopE2 effector protein of *Xanthomonas campestris* pv.*vesicatoria* (Xcv) is involved in virulence and in the suppression of the hypersensitive response.
- Employed the AFLP technique to investigate the diversity of *X. campestris* pv.*vesicatoria* isolated in Taiwan, and consequently a XopE2 homologue was identified in all fourteen Xcv strains that have been classified into two groups.

Lin *et al.*,2010

Xanthomonas outer proteins
XopE2 effector

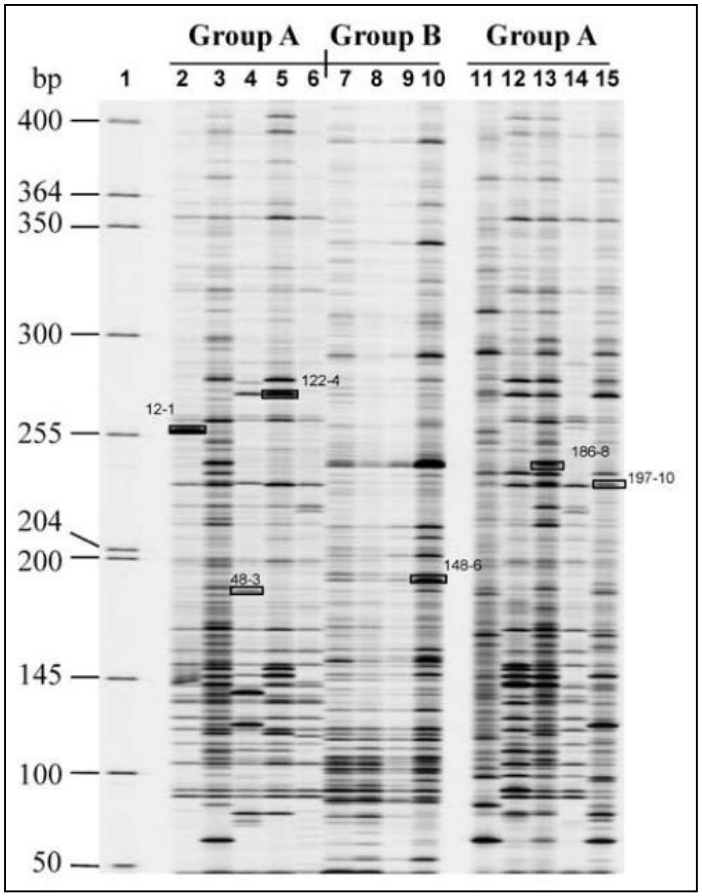
- Suppression of the HR by the XopE2 of Xcv Xvt122 or Xcv Xvt45.
- The *Agrobacterium tumefaciens* LBA4404 carrying pBI121 or xopE2 expressing plasmids was injected into the leaf of *Nicotiana tabacum* L. cv. Van-Hicks (A) and the infiltrated areas were encircled with black dashed line.
- The leaves was challenged with 5 × 10^6 cfu/ml of *P. syringae* pv. *syringae* 61 (Psy61) 24 h after the infiltration.
- The second infiltrated sites were encircled with red dashed lines and photographs were taken 6 days after the challenge inoculation.



Lin *et al.*,2010

Xanthomonas outer proteins
XopE2 effector

- AFLP fingerprints of the *Xanthomonas campestris* pv. *vesicatoria* strains.
- The genomic DNA isolated from each of the bacteria was digested with *EcoRI/MseI* and then subjected to PCR using primers IR700/MseI-GA and IR700/Mse-GT.
- The six polymorphic bands isolated for sequence determination are marked with rectangles.

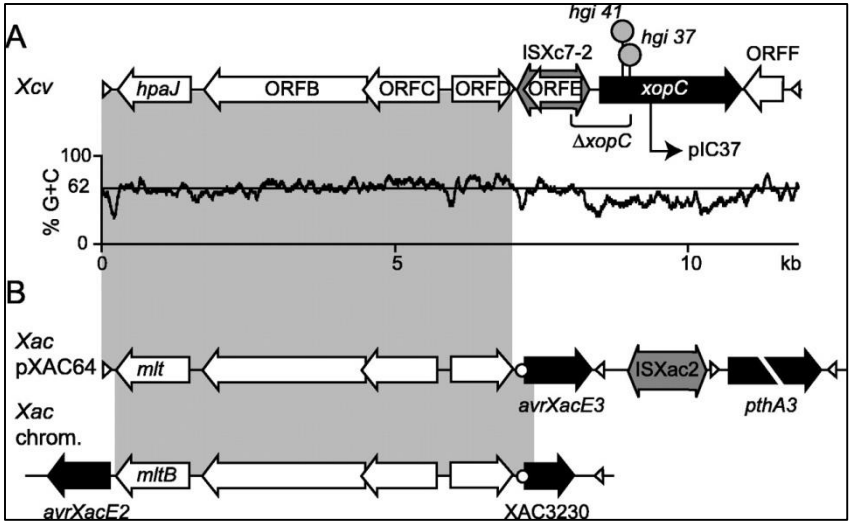


Lin *et al.*,2010

Xanthomonas outer proteins

Comparison between the xopC region from *X. campestris* pv. *vesicatoria* and corresponding regions from *X. axonopodis* pv. *citri*

- Schematic overview of the *X. campestris* pv. *vesicatoria* (A) and *X. axonopodis* pv. *citri* (B) xopC regions.
- Black arrows indicate putative effector genes.
- Both genes have a significantly lower G+C content (54 and 47%, respectively) than the genomic average of 64%, indicating that they have been acquired by horizontal gene transfer.



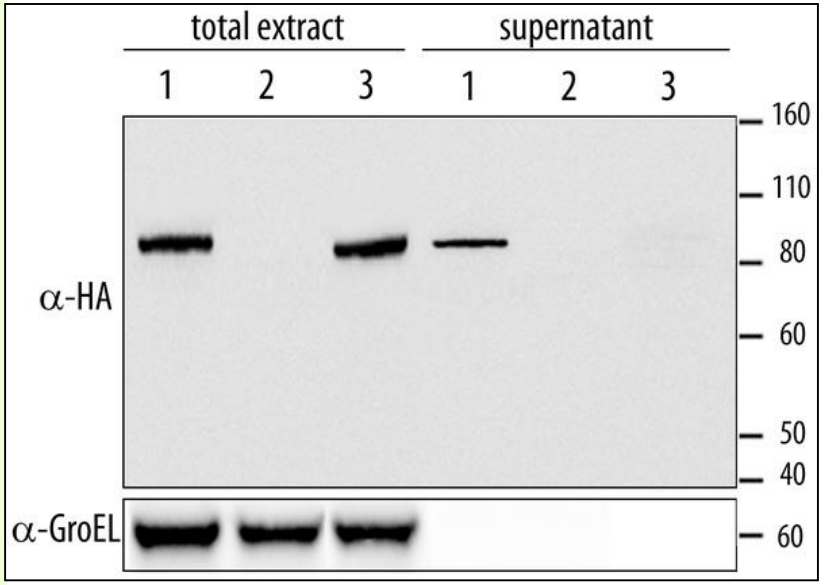
The grey area represents colinear DNA regions (more than 85% identity on the DNA level).

Noël *et al.*,2003

Expression analysis of XopD
X. campestris pv. *vesicatoria* (Xcv)

1. Strains 85* (XopD-HA)
2. 85* and
3. 85* Δ*hrcV* (XopD-HA) were incubated in MOKA rich medium (total extract, left) or secretion medium (supernatant, right).

- Total protein extracts (10-fold concentrated) and TCA-precipitated filtered supernatants (200-fold concentrated) were analyzed by
- Immunoblotting using anti-HA antibodies (upper panel) to detect the presence of XopD, or
- Anti-GroEL antibodies (lower panel) to show that bacterial lysis had not occurred.



Canonne *et al.*,2010

XopD

SUMOylation and deSUMOylation: wrestling with life's processes

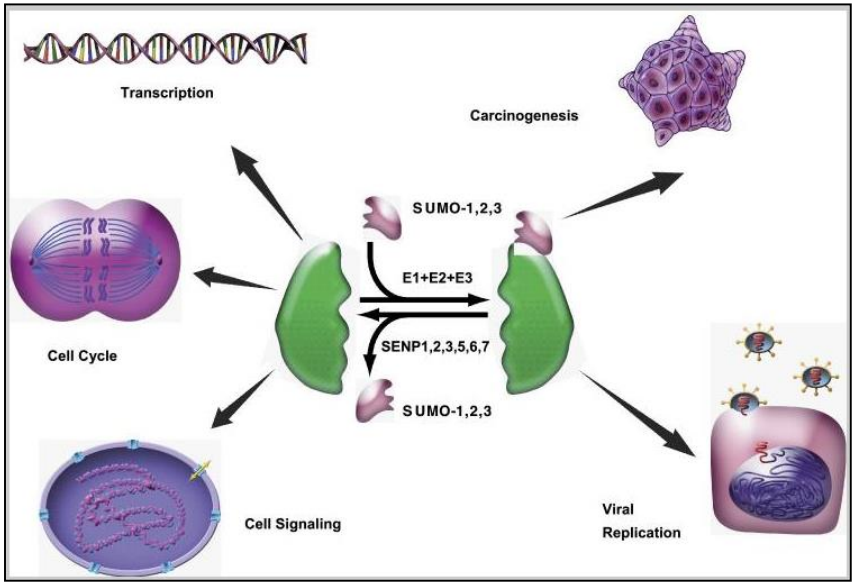
- **SUMOylation**
- SUMO proteins (Small Ubiquitin-like Modifier) are small proteins, most are around 100 amino acids in length and 12 KDa in mass.
- SUMO proteins are covalently attached to and detached from other proteins in plant cells to modify their function.
- SUMOylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle.
- **deSUMOylation**
- SUMO-specific proteases or desumoylating enzymes reverse SUMOylation. Modification by SUMOylation is rapidly reversed by the action of deSUMOylating enzymes.

Ubiquitin is a small regulatory protein that exists ubiquitously in all eukaryotic cells.

XopD

SUMOylation and deSUMOylation: wrestling with life's processes

- SUMOylation and de-SUMOylation regulate a diverse spectrum of biological responses, from transcription, cell division, and signal transduction to carcinogenesis and viral/bacterial replication.
- Fig. shows SUMOylation dynamic process in human.



Et,2009

XopD

SUMOylation and deSUMOylation: wrestling with life's processes

- In plants, SUMOylation and deSUMOylation regulate a number of processes.
- For example

1. In abiotic stress responses,
2. Pathogen defense, and
3. Flower induction.

- SUMOylation itself stabilizes proteins.
- XopD of bacteria has protease activity. C-terminal cysteine protease domain of XopD shows structural similarity with the yeast ubiquitin-like protease 1 (ULP1).
- XopD cysteine protease of pathogens removes SUMO groups from plant proteins and affects host transcription.

XopD
SUMOylation and deSUMOylation

- The XopD activities lead to the suppression of:
- Defense- and senescence-associated genes resulting in:

1. Delayed disease symptoms and
2. Increased bacterial multiplication.

XopD
SUMOylation and deSUMOylation
Mode of actions

- XopD, promote bacterial growth by targeting:

1. Plant transcription factors, and/or
2. Regulators.

- The helix-loop-helix (HLH) domain of XopD is necessary and sufficient to mediate these effects.
- EAR motif (ERF-associated amphiphillic repression) act as a transcriptional repressor.
- AvrBs3 induces the expression of a master regulator of cell size, *upa20*, which encodes a transcription factor containing a basic helix-loop-helix domain.

Model of the molecular function of the type III effectors XopD and AvrBs3

a) The protein structures of XopD

b) AvrBs3 are shown, and

c) The proposed mode of action of the effectors is illustrated.

- After translocation by the T3S system XopD is transported into the nucleus of the plant cell where it localizes to nuclear foci.
- Here, it binds to DNA unspecifically via a helix-loop-helix (HLH) domain.
- Via two EAR motifs, XopD might inhibit yet unidentified plant transcription factors (TFs).
- Furthermore, XopD possesses SUMO protease activity mediating deSUMOylation of yet unknown host plant target proteins.

Kay *et al.*,2007

Model of the molecular function of the type III effectors XopD and AvrBs3

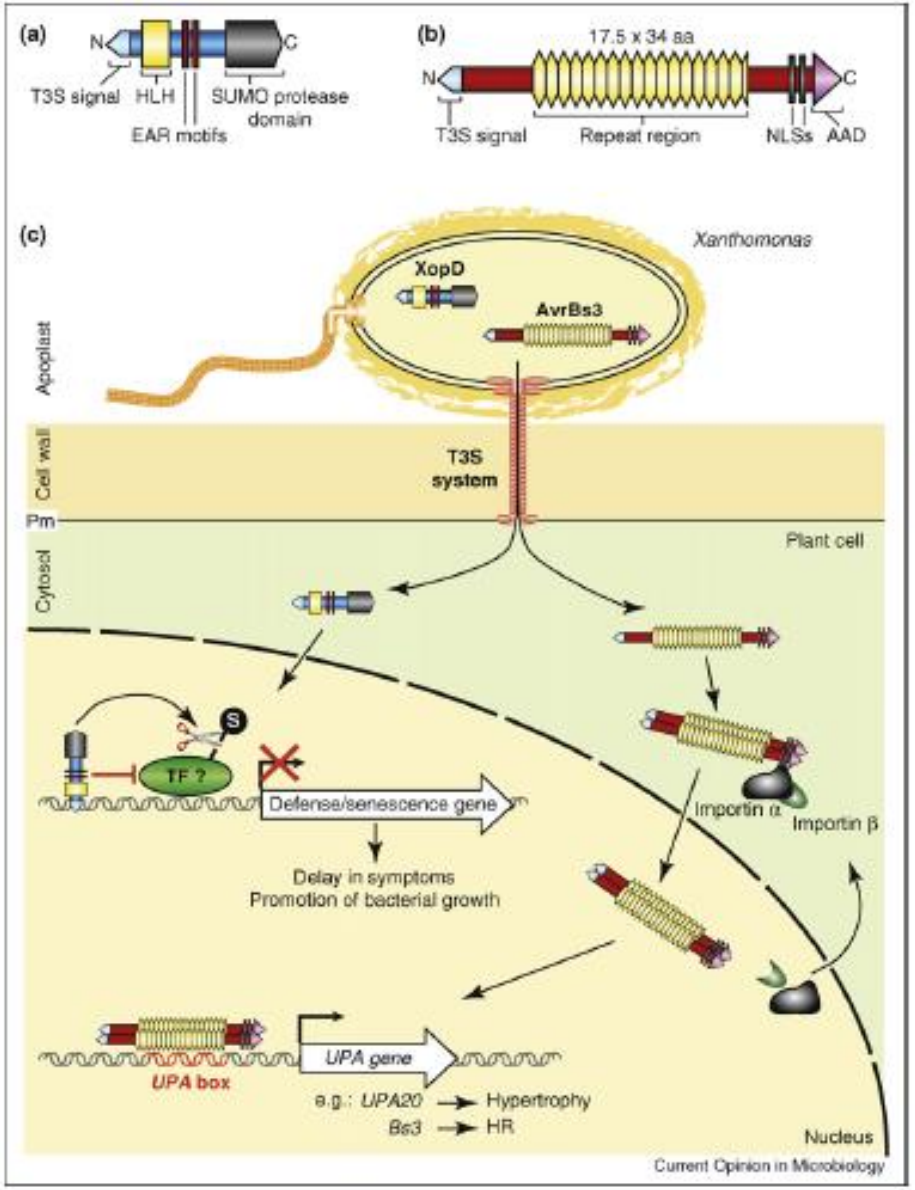
- The XopD activities lead to the suppression of defense- and senescence-associated genes resulting in delayed disease symptoms and increased bacterial multiplication.
- AvrBs3 dimerizes in the plant cell cytoplasm and interacts with importin α via its nuclear localization signals (NLSs).
- The protein complex is bound by importin β mediating nuclear import.
- Here, AvrBs3 binds to a specific DNA sequence, the *UPA* box, and activates transcription of more than 10 *UPA* genes.
- *UPA20*, one of the induced genes, is the key regulator of plant cell hypertrophy.
- In resistant pepper plants, activation of *Bs3* leads to the HR.

Kay and Bonas,2009

Model of the molecular function of the type III effectors XopD and AvrBs3 in *Xanthomonas*

The C terminus of XopD contains a small ubiquitin-like modifier (SUMO) protease
Structurally similar to yeast ubiquitin-like protease 1.

EAR motif act as a transcriptional repressor and inhibit plant transcription factors (TFs).
Helix-loop-helix (HLH) domain essential for maximal DNA-binding.



AAD, acidic activation domain; Pm, plasma membrane

Kay and Bonas,2009

Chromosome or Plasmid-borne of pathogenicity genes

Genes involved in pathogenicity and host specificity

Genes involved in pathogenicity and host specificity

Chromosome or Plasmid-borne?

- The genes involved in pathogenicity and host specificity comprise two main groups:

1. avirulence (*avr*) and virulence (*vir*) genes.
   - *avr* genes are evenly divided between:
   - Plasmid, and
   - Chromosomal locations.
1. The 'harp' (*hrc*/*hrp*) genes involved with a type III protein secretion system (chromosomally encoded).

Genes involved in pathogenicity and host specificity

Chromosome or Plasmid-borne?

- Many plant pathogens, particularly the soft-rot pathogen *Pcc* and some pathovars of *X. campestris, E. amylovora* and *P. syringae*, secrete extracellular enzymes, which are specified by chromosomal genes.
- e.g. A majority of strains of *P. syringae* pv. *maculicola* examined, the genes were chromosomal.
- In *Ralstonia solanacearum*, these are located on a megaplasmid, pVir (previously designated pWi).
- In *Pantoea* pv. *gypsophilae* the *hrp* genes are on a 150 kb plasmid, pPATH.

Plasmids found in Gram-negative phytopathogenic bacteria

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
B. cepacia					
Strain ATCC 25416	pPEC320	200	NK	*pehA* endoglucanase	González *et al.* (1997)
Er. amylovora					
Strain EA322	pCPP60	56	IncF	Cryptic, low copy, conjugative	Steinberger *et al.* (1990)
Strain CA11	pEa34	34	NK	pEa29 carrying Sm^r on Tn*5393*; conjugative	Chiou & Jones (1993)
Strain CA11	pEa29	28	NK	Loss results in thiamin prototrophy, reduced EPS and pathogenicity	Bereswill *et al.* (1992); McGhee & Jones (2000)
Strain CA3R	pEa8.7	8·7	IncQ	Su^r, Sm^r; related to RSF1010	Palmer *et al.* (1997)
Er. carotovora pv. *carotovora*	pEC3	5·8	NK	Cryptic, multicopy, non-conjugative, mobilizable by incP plasmids	Nomura *et al.* (1996)
Er. chrysanthemi					
Strain NCPPB 377	pLS1	50	NK	Cryptic	Sparks & Lacy (1980)
Strain NCPPB 377	pLS2	4·8	NK	Cryptic	Sparks & Lacy (1980)
Er. citreus					
Strain ATCC 31623	pPZG500	3·8	NK	Cryptic, multicopy	Bilic & Delic (1997)
Er. herbicola pv. *gypsophilae*	pPATH	150	NK	Gall-formation; *etz*, *hrp*, *iaa* and *pthG* genes, IS*1327*	Lichter *et al.* (1995a); Ezra *et al.* (2000)
Pa. agglomerans	ND	50–126	NK	Sm^r; associated with *Er. amylovora*, but transfer not detected	Huang & Burr (1999)
Pa. citrea	pUCD5000	5·3	NK	Related to p15a and ColE1; contributes to pink coloration in pineapple	Pujol & Kado (1998)
Pa. stewartii					
Strain SS104	pDC140	34	pDC250	Cryptic	Coplin *et al.* (1985)
Strain SS104	pDC190	68	NK	Not involved in pathogenicity	Frederick & Coplin (1986)
Strain SW2	pDC250	52	pDC250	Cryptic, conjugative; designated pSW800	Coplin *et al.* (1985)
Strain SW2	pSW100	4·3	ColE1	Cryptic, mobilizable by pDC250	Fu *et al.* (1995)
Strain SW2	pSW200	4·4	ColE1	Cryptic, *mobCBAD*, 41 × 15 bp repeats	Fu *et al.* (1998)
Strain SW2	pSW500	35	NK	Cryptic, iterons, 7 × 16 bp repeats	Fu *et al.* (1996)
Strain SW2	pSW1200	106	IncY	Cryptic, RepA related to phage P1	Fu *et al.* (1997)
Pseudomonas spp. strain PyR19	pCPP519	83	pPT23A	*strA*, *strB*; conjugative	Huang & Burr (1999)
Ps. savastanoi pv. *glycinea*					
Strain PG4180	p4180A	90	pPT23A	Coronatine	Bender *et al.* (1991)
Strain Pg83	pPg2	40	NK	Tp^r	Leary & Trollinger (1985)
Strain MAFF301683	pETH2	105	NK	Ethylene	Watanabe *et al.* (1998)
Ps. savastanoi pv. *phaseolicola*					
Strain 1302A	pAV505	140	pPT23A	Curing results in loss of pathogenicity	Jackson (1997)
Strain 1449B	pAV511	154	pPT23A	Curing results in loss of pathogenicity; contains PAI with *vir* and *avr* genes	Jackson *et al.* (1999)
Strain 1449B	pAV512	32	pPT23A	Cryptic	R. W. Jackson, unpublished
Strain LR700	pMMC7105	150	NK	Integration/excision into host chromosome via repeat sequences; can form smaller plasmids	Quant & Mills (1984); Szabo & Mills (1984a, b)

Vivian *et al.*,2001

Plasmids found in Gram-negative phytopathogenic bacteria

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
Ps. savastanoi pv. *phaseolicola* kudzu					
Strain PK2	pPSP1	68	NK	Ethylene	Fukuda *et al.* (1992)
Ps. savastanoi pv. *savastanoi*					
Strain PS93	pPS10	10	NK	Cryptic, minimum replicon (1267 bp), iterons present, used to study *rep* function	Nieto *et al.* (1990)
Strain EW2009	pIAA1	52	NK	Indoleacetic acid; oleander pathogen	Comai & Kosuge (1980)
Strain PB213	pIAA2	72	NK	Indoleacetic acid; oleander pathogen	Soby *et al.* (1994)
Strain PB213	pCK1	42	NK	Cytokinin; oleander pathogen	MacDonald *et al.* (1986)
Strain EW1006	pCK2	105	NK	Cytokinin; olive pathogen	MacDonald *et al.* (1986); Powell & Morris (1986)
Strain NA1	ND	44	NK	Cytokinin; oleander pathogen	Caponero *et al.* (1995)
Strain OC17	ND	73	NK	Cytokinin; olive pathogen	Caponero *et al.* (1995)
Strain OD21	ND	97	NK	Cytokinin; indoleacetic acid; olive pathogen	Caponero *et al.* (1995)
Ps. syringae pv. *atropurpurea*					
Strain NIAES 1309	pCOR1	88	NK	Coronatine; conjugative	Sato *et al.* (1989)
Ps. syringae pv. *cannabina*					
Strain MAFF 302256	pETH1	110	NK	Ethylene	Sato *et al.* (1997)
Ps. syringae pv. *eriobotryae*					
Strain NAE6	ND	82	NK	Curing results in loss of pathogenicity; contains *vir* gene *pvsA*	Kamiunten (1995, 1999)
Strain NAE6	ND	94·5	NK	Cryptic, curing does not affect pathogenicity	Kamiunten (1995)
Ps. syringae pv. *maculicola*					
Strain H3-6	pMAC1	83	NK	Coronatine	Zhu *et al.* (1995)
Ps. syringae pv. *morsprunorum*	ND	105	NK	Coronatine	Bender *et al.* (1991); Liang *et al.* (1994)
Ps. syringae pv. *papulans*					
Strain Psp36	pCPP501	108	NK	Sm^r; conjugative	Burr *et al.* (1988)
Strain Psp 47	PCPP511	89	NK	Sm^r; conjugative	Huang & Burr (1999)
Ps. syringae pv. *syringae*					
Strain A2	pPSR1	68	pPT23A	Cu^r, Sm^r, *rulAB* (UV^r)	Sundin & Bender (1993)
Strain B3010	pPSR12	200	pPT23A	Cu^r, Co^r, As^r; confers stable mucoid colony form	Kidambi *et al.* (1995)
Strain J900	pOSU900	80	pPT23A	Cryptic, loss does not affect pathogenicity toward bean	Mukhopadhyay *et al.* (1990)
	pOSU221	8·2	Shuttle/ pUC	Ap^r based on pOSU900	Mukhopadhyay *et al.* (1990)
	pOSU222	9·7	Shuttle/ pUC	Km^r based on pOSU900	Mukhopadhyay *et al.* (1990)
Strain HS191	pCG131	55	pPT23A	Conjugative; may contribute to virulence	González *et al.* (1984)
Ps. syringae pv. *tabaci*					
Strain BR2	pBPW1	48	NK	Cryptic, loss does not affect pathogenicity toward bean	Obukowicz & Shaw (1983, 1985)
Ps. syringae pv. *tomato*					
Strain DC3000	pDC3000A	64	pPT23A	*vir* genes	G. Tsiamis, personal communication; Sesma *et al.* (2000)

Vivian *et al.*,2001

Plasmids found in Gram-negative phytopathogenic bacteria

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
Strain PT23	pPT23A	101	pPT23A	Coronatine; conjugative; affects lesion size and *in planta* growth 4 d post-infection	Bender *et al.* (1989)
Strain PT23	pPT23B	83	pPT23A	Syringolide elicitors; affects lesion size and *in planta* growth 4 d post-infection	Sesma *et al.* (2001)
Strain PT23	pPT23C	65	NK	Cryptic	Bender & Cooksey (1986)
Strain PT23	pPT23D	35	pPT23A	Cu^r; non-conjugative	Cooksey (1987); Mills *et al.* (1993)
R. solanacearum					
Strain M4S	pJTPS1	6·6	NK	Low copy confers loss of pathogenicity and reduced EPS and endoglucanase production	Negishi *et al.* (1993)
Strain GMI1000	pVir	~ 2030	Mega-plasmid	*hrp* and *dsp* genes	Boucher *et al.* (1986); C. A. Boucher, personal communication
Strain mps5	pWI5	7·5	NK	Cryptic	Morales & Sequeira (1985)
X. campestris pv. *vesicatoria*					
Race 2 strain 81-23	pXvCu1	200	NK	Sm^r, Cu^r, *avrBs1*, conjugative	Stall *et al.* (1986); Swanson *et al.* (1988)
Strain Xv2	pXV2	14·6	IncW	Cryptic, non-conjugative	Wu & Tseng (2000)
Race 1 strain 71-21	pXV11	45	NK	*avrBs3*, conjugative	Bonas *et al.* (1989)
Race 1 strain 82-8	pXV12	ND	NK	*avrBs3-2*	Bonas *et al.* (1993)
	ND	68	NK	Sm^r	Minsavage *et al.* (1990a)

ND, No designation given.

NK, Not known.

*Strains given where known.

General features of the *Xanthomonas oryzae* pv. *oryzae* genome

No plasmid was detected in the *Xoo* genome

	X. oryzae pv. *oryzae* MAFF 311018	*X. axonopodis* pv. *citri* 306	*X. campestris* pv. *campestris* ATCC 33913
Length (bp)	4,940,217	5,175,554	5,076,187
G+C content (%)	63.7	64.7	65.0
Total number of predicted genes	4,372	4,313	4,182
Plasmid	0	2	0
Insertion sequence elements (IS)	386(225)	87	109
Proposed HrpX regulons	37	20	17
Putative avirulence genes (family)			
avrBs1	0	0	2
avrBs2	1	1	1
avrBs3 / pth	16 (genome)	4 (plasmid)	0
avrPphE	0	3	1
avrXca	0	0	2
yopJ	0	0	1
avrC	0	0	1

Ochiai *et al.*,2005

The roles of plasmids in phytopathogenic bacteria

Plasmids as mobile arsenals?

Plasmids

Plasmid-borne phenotypes

- Plasmids are extra-chromosomal elements of finite size, usually stably inherited within a bacterial cell line and potentially capable of transfer between strains, species or genera.
- Plasmids are common residents of phytopathogenic bacteria. e.g.
- *Burkholderia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Ralstonia*, *Xanthomonas*, *Agrobacterium*.
- The main role of plasmids is in pathogenicity and host specificity.
- A range of plasmid-borne phenotypes, including:
- Toxin, hormone and bacteriocin production,
- Resistance to bactericides such as copper and antibiotics, and to UV irradiation.

Plasmids from *Erwinia*, *Pseudomonas*, *Xanthomonas*, and *Xylella* in the size range 19-183 kb with complete sequences available

Organism	Strain	Plasmid	Size (bp)	% G+C		Accession	Reference
				Host	plasmid		
Erwinia amylovora							
	Ea110	pEA29	28,185	53.5	50.3	NC_005706	(52)
	LebB66	pEL60	60,145	53.5	51.5	NC_005246	(23)
	UTRJ2	pEU30	30,314	53.5	48.2	NC_005247	(23)
Erwinia pyrifoliae							
	Ep1/96	pEP36	35,904	nd	49.8	NC_004445	(51)
Erwinia sp.							
	Ejp556	pEJ30	29,593	nd	49.7	NC_004834	(51)
Pseudomonas syringae							
pv. *maculicola*	M6	pFKN	39,554	nd	53.5	NC_002759	(68)
	ES4326	pPMA4326A	46,697	nd	55.0	NC_005918	(78)
		pPMA4326B	40,110	nd	55.3	NC_005919	(78)
pv. *phaseolicola*	1448A	pPph1448A	131,950	58.0	54.1	NC_007274	(34)
		pPph1448B	51,711	58.0	56.0	NC_007275	(34)
pv. *syringae*	A2	pPSR1	72,601	nd	54.4	NC_005205	(83)
pv. *tomato*	DC3000	pDC3000A	73,661	58.4	55.1	NC_004633	(8)
		pDC3000B	67,473	58.4	56.2	NC_004632	(8)
Xanthomonas axonopodis							
pv. *citri*	306	pXAC64	64,920	64.8	61.5	NC_003922	(17)
		pXAC33	33,700	64.8	61.9	NC_003921	(17)
pv. *glycines*	8ra	pXAG81	26,721	nd	61.4	AY_780632	(40)
Xanthomonas campestris							
pv. *vesicatoria*	85–10	pXCV183	182,572	64.7	60.5	NC_007507	(89)
		pXCV38	38,116	64.7	60.7	NC_007506	(89)
		pXCV19	19,146	64.7	59.8	NC_007505	(89)
Xylella fastidiosa							
	9a5c	pXF51	51,158	52.7	49.6	NC_002490	(49)

Sundin,2007

Functional analysis of open reading frames from phytopathogenic bacterial plasmids

		No. of ORFs with various functions						
Plasmid	No. of ORFs	Plasmid	Conjugation	Virulence	Ecol. fitness	Mobile elements	Conserv. hypothet.	Hypothet.
pEA29 [E][a]	21	3	0	4	5	1 [0][b]	2	6
pEL60 [E]	68	16	22	0	6	0	11	13
pEU30 [E]	25	4	11	1	5	0	2	2
pEP36 [E]	32	5	0	5	8	6 [2]	3	5
pEJ30 [E]	22	5	0	4	7	2 [0]	2	2
pFKN [P]	26	2	0	2	9	2 [1]	0[c]	11
pPMA4326A [P]	50	9	15	0	4	3 [2]	11	8
pPMA4326B [P]	29	6	1	3	2	6 [4]	8	3
pPph1448A [P]	127	20	6	16	16	26 [18]	3	40
pPph1448B [P]	60	11	15	1	10	2 [1]	0	21
pPSR1 [P]	55	7	15	5	11	8 [3]	3	6
pDC3000A [P]	68	5	25	6	9	6 [2]	0	17
pDC3000B [P]	70	4	25	1	7	4 [4]	0	29
pXAC64 [X]	73	8	12	5	0	13 [5]	0	35
pXAC33 [X]	42	8	0	2	0	11 [4]	0	21
pXAG81 [X]	34	5	8	2	0	4 [2]	5	10
pXCV183 [X]	172	17	15	20	9	21 [9]	0	90
pXCV38 [X]	44	8	14	3	0	5 [2]	0	14
pXCV19 [X]	19	2	3	0	2	5 [3]	0	7
pXF51 [Xy]	65	8	19	1	2	0	0	35

[a]E, *Erwinia*; P, *Pseudomonas*; X, *Xanthomonas*; Xy, *Xylella*. Host bacterial information for the listed plasmids is listed in Table x.

[b]The number of mobile (intact and truncated) genetic elements on the plasmids is listed in brackets.

[c]Conserved hypothetical proteins were not included in the annotations for plasmids pFKN, pPph1448B, pDC3000A, pDC3000B, pXAC64, pXAC33, pXCV183, pXCV38, and pXCV19.

Sundin,2007

Plasmid-borne genes for plant hormone production

Gene	Pathogen	Plasmid	Hormone
efe	*Ps. savastanoi* pv. *glycinea*	pETH2	Ethylene
	Ps. savastanoi pv. *phaseolicola*	pPSP1	Ethylene
	Ps. syringae pv. *cannabina*	pETH1	Ethylene
etz	*Er. herbicola* pv. *gypsophilae*	pPATH	Cytokinin
iaaH	*Er. herbicola* pv. *gypsophilae*	pPATH	IAA
	Ps. savastanoi pv. *savastanoi*	pIAA2	IAA
iaaL	*Ps. savastanoi* pv. *savastanoi*	pIAA1	IAA
iaaM	*Er. herbicola* pv. *gypsophilae*	pPATH	IAA
	Ps. savastanoi pv. *savastanoi*	pIAA2	IAA
ipt	*Ps. savastanoi* pv. *savastanoi*	ND	Cytokinin
ptz (*ipt*)	*Ps. savastanoi* pv. *savastanoi*	pCK2*	Cytokinin

Vivian *et al.*,2001

Plasmid-borne bactericide resistance genes

Gene	Pathogen	Plasmid	Compound*	Reference
copA–D	*Ps. syringae* pv. *tomato*	pPT23D	Cu	Mellano & Cooksey (1988)
ND	*Ps. syringae* pv. *syringae*	pPSR12	Cu, As, Co	Kidambi *et al.* (1995)
strA strB	*Ps. syringae* pv. *syringae*	pPSR14	Cu, STR	Sundin *et al.* (1994)
strA strB	*X. campestris* pv. *vesicatoria*	ND (68 kb)	STR	Minsavage *et al.* (1990a)
tetB	Orchard epiphytes†	ND	TET	Schnabel & Jones (1999)
tmp	*Ps. savastanoi* pv. *glycinea*	pPg2	TMP	Leary & Trollinger (1985)

ND, Not designated.

* As, arsenate; Co, cobalt; Cu, copper; STR, streptomycin; TET, tetracycline; TMP, trimethoprim.

† Identified only as *Pa. agglomerans* (= *Er. herbicola*) with TetB or *Pseudomonas* spp. with TetA, TetC or TetG.

Vivian *et al.*,2001

Plasmid-borne *avr* effector genes

Pathogen	Effector gene	Plant interactor*	Plasmid	Plasmid size (kb)	Reference
Ps. savastanoi pv. *glycinea*	*avrC.RPG3*	Soybean	ND	150–200	Tamaki *et al.* (1988)
Ps. savastanoi pv. *phaseolicola*	*avrPphD*	Pea	pAV505	140	Wood *et al.* (1994); Arnold *et al.* (2001a)
	avrPphF.R1	Bean, soybean	pAV511	154	Jackson *et al.* (1999); Tsiamis *et al.* (2000)
	avrPphC	Soybean, bean	pAV511	154	Yucel *et al.* (1994b); Tsiamis *et al.* (2000)
	avrD5	Soybean	pAV511	154	Yucel *et al.* (1994a); Jackson *et al.* (1999)
	virPphA	Bean, soybean	pAV511	154	Jackson *et al.* (1999)
Ps. syringae pv. *eriobotryae*	*psvA*	Loquat	ND	82	Kamiunten (1999)
Ps. syringae pv. *lachrymans*	*avrD3*	Soybean	ND	90	Yucel *et al.* (1994a)
	avrD4	Soybean	ND	75	Yucel *et al.* (1994a)
Ps. syringae pv. *maculicola*	*avrRpm1.RPM1*	Arabidopsis	ND	NA	Dangl *et al.* (1992)
Ps. syringae pv. *pisi*					
Race 7	*avrPpiA2.R2*	Pea	pAV382	47	Gibbon *et al.* (1997)
Race 5	*avrPpiA3.R2*	Pea	pAV251	45	Gibbon *et al.* (1997)
Race 3	*avrPpiB1.R3*	Pea	pAV232	40	Cournoyer *et al.* (1995)
Race 1	*avrPpiB2.R3*	Pea	pAV212	66	Cournoyer *et al.* (1995)
Race 7	*avrPpiB3.R3*	Pea	pAV381	66	Cournoyer *et al.* (1995)
Race 1	*avrPpiE.RPS4*	Arabidopsis	ND	NA	Hinsch & Staskawicz (1996)
Race 4A	*avrPpiG*	Bean	pAV241	45	Arnold *et al.* (2001b)
Ps. syringae pv. *tomato*	*avrD1.RPG4*	Soybean	pPT23B	83	Kobayashi *et al.* (1990)
X. campestris pv. *vesicatoria*	*avrBsT*	Pepper	ND	41	Minsavage *et al.* (1990b)
Race 2 strain 81-23	*avrBs1*	Pepper	pXvCu1	200	Swanson *et al.* (1988)
Race 1 strain 71-21	*avrBs3*	Pepper	pXV11	45	Bonas *et al.* (1989)
Race 1 strain 82-8	*avrBs3-2*	Tomato	pXV12	NA	Bonas *et al.* (1993)
X. campestris pv. *malvacearum*					
Strain XcmH	*avrb6* plus 5 other *avr* genes	Cotton	pXcmH	90	De Feyter & Gabriel (1991); Yang *et al.* (1994, 1996)

ND, Not designated.

NA, Not available.

* Plant in which the gene has an effect.

Vivian *et al.*,2001

Effector genes other than *avrBs3*-like

Chromosome or Plasmid-borne?

- * known to be plasmid-borne;
- ** known to be chromosomal and plasmid-borne in different strains.
- –: not available;
- na: not applicable;
- nd: not determined.

Bacterium	Gene	Host plant	R gene	Peptide (kDa)	G + C %	Accession number
R. solanacearum	*avrA*	tobacco	nd	nd	nd	–
	popA	petunia	nd	33	67.4	AJ245811
P. syringae	*avrA*	soybean	*RPG2*	100	45.0	M15194
pv. *glycinea*	*avrB*	soybean	*RPG1*	36	46.3	M21965
	*avrC**	soybean	*RPG3*	39	47.5	M22219
	avrD2	soybean	*RPG4*	nd	nd	–
	*virPphA**	soybean	nd	60	54.0	AF141883
pv. *maculicola*	*avrRpm1**	arabidopsis	*RPM1*	24	43.6	X67808
pv. *phaseolicola*	*avrPphA*	bean	nd	nd	nd	–
	avrPphB.R3	bean	*R3*	38	48.1	M86401
	*avrPphC**	soybean	*RPG3*	39	47.6	U10377
	*avrPphD**	pea	nd	75	55.0	AJ277494
	avrPphE.R2	bean	*R2*	41	57.6	U16817
	*avrPphF.R1**	bean	*R1*	15/22	40.0/52.5	AF231452
pv. *pisi*	*avrPpiA.R2***	pea	*R2*	24	44.3	X67807
	*avrPpiB.R3**	pea	*R3*	31	39.7	X84843
	avrPpiC	bean	nd	29	46.9	AJ277496
	*avrPpiD.R5**	pea	*R5*	nd	nd	–
	*avrRps4**	arabidopsis	*RPS4*	24	52.3	L43559
	*avrPpiG**	bean	nd	41	48.0	AJ277495
pv. *syringae*	*hopPsyA* (≡ *hrmA*)	tobacco	nd	nd	nd	–
pv. *tomato*	*avrA*	soybean	*RPG2*	nd	nd	–
	*avrD**	soybean	*RPG4*	34	41.0	J03681
	avrE	soybean	nd	195	57.6	U16119
	avrF	soybean	nd	14	56.7	U16119
	avrPto	tomato	*PTO*	18	50.5	S35220
	avrRpt2	arabidopsis	*RPS2*	28	51.5	A40613
E. amylovora	*dspE* (*dspA*)	pear	nd	198	54.7	U97504
X. campestris	*avrBs1**	pepper	*Bs1*	50	42.2	M32142
pv. *vesicatoria*	*avrBs2*	pepper	*Bs2*	80	63.3	AF114720
	*avrBsT**	pepper	nd	39	43.0	AF156163
	avrRxv	bean	*Rxv*	42	52.3	L20423
	hpaA	pepper	na	30.4	65.0	AF05646
pv. *raphani*	*avrXca*	arabidopsis	nd	67	68.2	M99059

Vivian and Arnold,2000

Role of plasmids in bacterial virulence
P. syringae

- Horizontal transfer of virulence genes has played an important role in the evolution and divergence of *P. syringae* into the pathovars observed today.
- Most strains of *P. syringae* analyzed, regardless of pathovar, contain at least one indigenous plasmid.
- Genetic studies, originally designed to examine the relationships of plasmids from different pathovars, revealed that the large majority of plasmids in *P. syringae* shared a major replication gene *repA* that encodes an essential replication protein.

Sundin,2007

Role of plasmids in bacterial virulence
P. savastanoi pv. *phaseolicola*

- *Ps. savastanoi* pv. *phaseolicola*, which comprises nine races, eight of which harbour large plasmids of about 150 kb.
- Curing of one of these plasmids, pAV511, from a race 7 strain resulted in the loss of virulence toward bean cultivars.
- The cured strains, however, continued to elicit an HR in bean, implying that the chromosomal type III system remained functional and was delivering some signal to the plant that resulted in the elicitation of a defence reaction.

Role of plasmids in bacterial virulence
P. syringae pv. *eriobotryae*

- Curing of an approximately 82 kb native plasmid by heat shock (32°C) from a strain of *Ps. syringae* pv. *eriobotryae*, the causal agent of stem canker of loquat (*Eriobotrya japonica*) led to loss of pathogenicity on the host plant.
- A single gene, *psvA*, was capable of restoring pathogenicity.

Role of plasmids in bacterial virulence *Xanthomonas* spp.

- In contrast to *P. syringae*, plasmids are less well understood in *Xanthomonas* spp. beyond the knowledge that plasmids are carriers of important virulence/avirulence genes.
- However, six plasmid-borne effector genes that redundantly encode the ability to cause watersoaking in cotton were identified in *Xanthomonas campestris* pv.*malvacearum*.
- Plasmid-curing experiments have defined a role of indigenous plasmids in exopolysaccharide production and virulence also in *Xanthomonas campestris* pv. *malvacearum*.

Sundin,2007

Role of plasmids in bacterial virulence *Xanthomonas malvacearum*

- Gain and loss of genes on plasmids was shown to be high when compared to the chromosome (Sundin, 2007).
- Plasmids can potentially be lost during the purification procedure due to their:
- Dynamic 's nature and
- Low copy of plasmid numbers.
- Also plasmids are known to carry integrases and transposases which facilitate:

1. Rapid DNA rearrangements, and
2. Exchange.

Narra *et al.*,2011

Role of plasmids in bacterial virulence

Influence of host leaf extracts and intercellular fluid on the plasmid number *Xanthomonas malvacearum*

- Laboratory subcultured isolates contained fewer plasmids (i.e. two plasmids of size 60 and 40 kb) presumably due to loss or undetectable low copy number during subculturing.
- When these isolates were grown in the presence of leaf extract and intercellular fluid obtained from cotton differentials, the number of plasmids increased and the plasmid profile resembled those of the natural isolates.

Narra *et al.*,2011

Role of plasmids in bacterial virulence

Influence of host leaf extracts and intercellular fluid on the plasmid number

Xanthomonas malvacearum

- Based on known facts about genome rearrangement, we speculate this as an adaptation strategy for *Xam* to increase copy number of genes involved in pathogen aggressiveness which are otherwise present as single copy in bacterial chromosome and this possibly occurred by induction from host elicitors present in leaf extracts.

Narra *et al.*,2011

Role of plasmids in bacterial virulence

Influence of host leaf extracts and intercellular fluid on the plasmid number *Xanthomonas malvacearum*

- Influence of host intercellular fluid (ICF) and leaf extract from *Gossypium hirsutum* differentials with bacterial blight resistance genes (designated as B-genes) on the plasmid profile of *Xanthomonas axonopodis* pv. *malvacearum* races 27 and 32.

Plasmid size (kb)	Plasmid profile of LS races (control)		Plasmid profile of LS races after treatment with ICF from 101-102.B (B_2B_3+B_{sm})		Plasmid profile of LS races after treatment with host leaf extract from cotton differentials											
					Stoneville 2B.S9 (Bsm)		Stoneville 20 (B_7+B_{sm})		Mebane B.1(B_2+B_{sm})		1-10B (B_{in}+ B_{sm})		20-3 (B_N + B_{sm})		101-102.B (B_2B_3 +B_{sm})	
	27	32	27	32	27	32	27	32	27	32	27	32	27	32	27	32
60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.2	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+
3.7	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
1.6	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+

LS-races: Laboratory subcultured races; +, plasmid detected; -, plasmid not detected.

Narra *et al.*,2011

Role of plasmids in bacterial virulence

Influence of host leaf extracts and intercellular fluid on the plasmid number

Xanthomonas malvacearum

- Leaf extract and ICF (host intercellular fluid) contain sugars, carbohydrates, proteins which support bacterial growth (Theobald *et al*., 2005).
- Furthermore, citric, malic, shikimic and quinic acids and glucanases and chitinases present in leaf extract and ICF may also act as signal molecules involved in virulence gene activation in the pathogen leading to infection (Cavalcanti *et al*.,2006; Li *et al*.,1998).

Narra *et al*.,2011

Role of plasmids in bacterial virulence

Influence of host leaf extracts and intercellular fluid on the plasmid number

Xanthomonas malvacearum

- Rahme *et al*. 1992 observed an elevated *hrp* gene expression in *Pseudomonas syringae* pv. *syringae* grown in minimal media supplemented with high concentrations of citric acid (present in leaf extract).
- The present study provides a viable *in vitro* assay mimicking pathogen growth in a host plant.

Narra *et al*.,2011

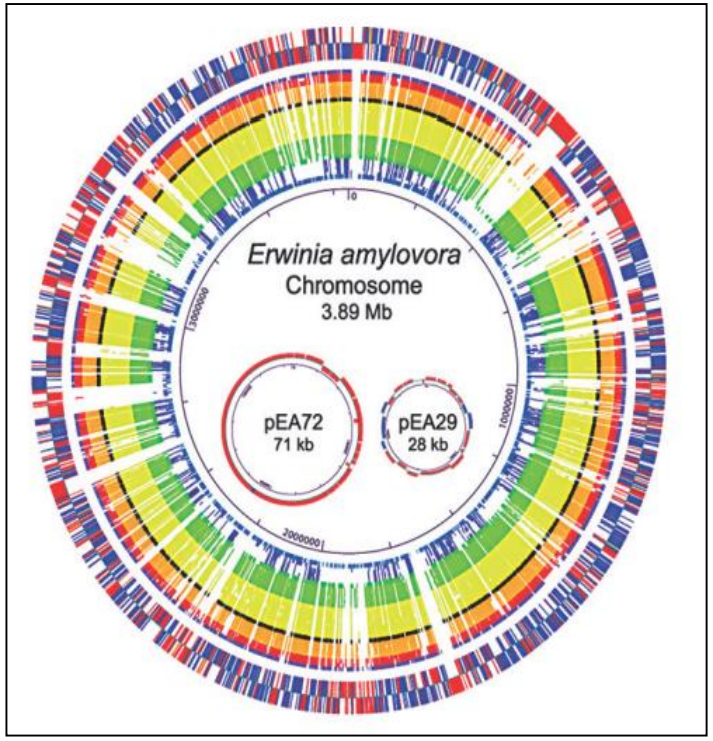
Role of plasmids in bacterial virulence
Erwinia amylovora

- All strains of *E. amylovora* isolated from diseased tissues, with few exceptions, contain a nonconjugative plasmid termed the ubiquitous plasmid pEA29.
- pEA29 can be cured in the laboratory, and cured strains were reduced in virulence and exhibited thiamine auxotrophy.
- Thiamine biosynthesis is a requirement for the high-level expression of the the exopolysaccharide amylovoran biosynthetic operon during infection.

Sundin,2007

Circular representation of the genome of *Erwinia amylovora* and comparison with related genomes

- The two outer circles depict the genes of *E. amylovora*.
- The innermost circle represents genome epnates.
- The two plasmids inside the chromosomal diagram follow the same colour scheme as the two outer circles of the chromosome genome.
- The absence of a particular colour indicates the absence of an orthologue.
- The genes in blue have predicted orthologues in *E. coli* K12, whereas the genes in red do not.
- Loci coloured orange, yellow and purple are RNA genes.



Mansfield *et al.*,2012

Role of plasmids in bacterial virulence
Race change due to loss of a plasmid

- Race change is observed when a plant pathogen extends its host range as a result of the loss or inactivation of an avirulence determinant.
- Race change due to loss of a plasmid carrying *avrPpiB* was detected in *Ps. syringae* pv. *pisi*, while a similar plasmid loss from race 1 strains of *X. campestris* pv.*vesicatoria* involving *avrBs3* resulted in extension of host range.

Role of plasmids in bacterial virulence
Race differentiation in bacteria

- Race differentiation is not clear in the plant pathogenic bacteria:
- *Agrobacterium tumefaciens*
- *Pseudomonas syringae* pv. *tomato*
- *Ralstonia solanacearum*
- *Xylella fastidiosa*, whose genome sequencing has been completed.

Role of plasmids in bacterial virulence
Race change due to loss of a plasmid

- In the pathogen *Burkholderia cepacia*, particular strains causing bulb-rot of onion produce an endopolygalacturonase which was shown to be due to a gene, *pehA* ,located on a 200 kb plasmid.
- Mobilization of this plasmid to non-soft rot strains conferred enhanced maceration of onion.

Role of plasmids in bacterial virulence
Toxins production due to plasmids

- Phytopathogenic bacteria produce a variety of toxins that affect the host plant, often causing chlorosis and stunting.
- The genetic determinants for one of these, coronatine, are generally located on plasmids.
- Coronatine production is thermo-regulated, in a manner consistent with its expression during plant infection, which is optimal at 18 °C.

Role of plasmids in bacterial virulence

Plasmid-borne *cor* genes in *Ps. syringae*

- In most strains of *Ps. savastanoi* pv. *glycinea* and *Ps. syringae* pv. *tomato*, coronatine production is specified by a 30 kb *cor* gene cluster located on large 90-100 kb plasmids.
- Coronatine is a virulence factor, enabling producer strains to form larger lesions and develop higher *in planta* populations; in this way it appears to confer a selective advantage in the natural habitat of these bacteria.

Role of plasmids in genetic diversity

Plasmid content differences among *X. fastidiosa* strains

- Plasmids were in most of the *X. fastidiosa* strains.
- The grape strains having:

1. Similar chromosomal DNA,
2. Differed in plasmid DNA content.

- *X. fastidiosa* which causes citrus variegated chlorosis comprises a circular chromosome and two plasmids of 51,158 bp and 1,285 bp.
- Plasmid might be transferable between different *X. fastidiosa* strains.

Other plasmid-borne traits
EPS production

- As explained earlier, the plasmid pEa29 (previously described as a 28 kb plasmid) plays a role in the physiology or metabolism of *E. amylovora*.
- The control of extracellular polysaccharide (EPS) production has been associated with plasmid:

1. pEa29 in *E. amylovora* and with
2. pJTPS1 in *R. solanacearum*.

- However, recent studies in *R. solanacearum* have shown that high-level transcription of *eps* requires the products of at least seven regulatory genes that are chromosomally located.
- In both cases, these traits are associated with full virulence of the pathogen.

Other plasmid-borne traits
Bacteriocin production

- Gram-negative and Gram-positive bacteria commonly harbor plasmid-borne genetic determinants of:

1. Bacteriocin production, and of
2. Host cell bacteriocin immunity.

- But some evidences (Chuang,1997) show that these genes are located on chromosomal DNA in *P. carotovorum* subsp. *carotovorum* strains.

Other plasmid-borne traits

Production of a pink coloration

- *Pantoea citrea* causes a post-harvest disease of pineapple (called pink disease, which produces a pink coloration in canned pineapple due to the production of chromogenic 2,5-diketo gluconate from glucose.
- Although the genes *gdhA* and *gdhB* are chromosomal, plasmid pUCD5000 was required for full expression of the pink colour.

Insertion sequences
ISs or IS elements

Occur in chromosomes of bacteria and plasmids

Insertion sequences (ISs) are approximately 1 kbp long jumping genes found in prokaryotes.

See also Bacterial Genetic File

Insertion sequences

ISs are the simplest autonomous mobile elements in bacterial genomes

- Insertion sequences (ISs) are small transposable elements, commonly found in bacterial genomes.
- ISs are commonly present in multiple copies in a single genome.
- Transposable elements are often associated with antibiotic resistance determinants, suggesting a role in the emergence of resistance.
- Identifying the location of IS in bacterial genomes can be useful for a variety of purposes including:

1. epidemiological tracking, and
2. predicting antibiotic resistance.

Hawkey *et al.*,2015; He *et al.*,2015

Insertion sequences
Two major characteristics of ISs

- Insertion sequences have two major characteristics:

1. they are small relative to other transposable elements (generally around 700 to 2500 bp. in length) and
2. only code for proteins implicated in the transposition activity (they are thus different from other transposons, which also carry accessory genes such as antibiotic resistance genes).

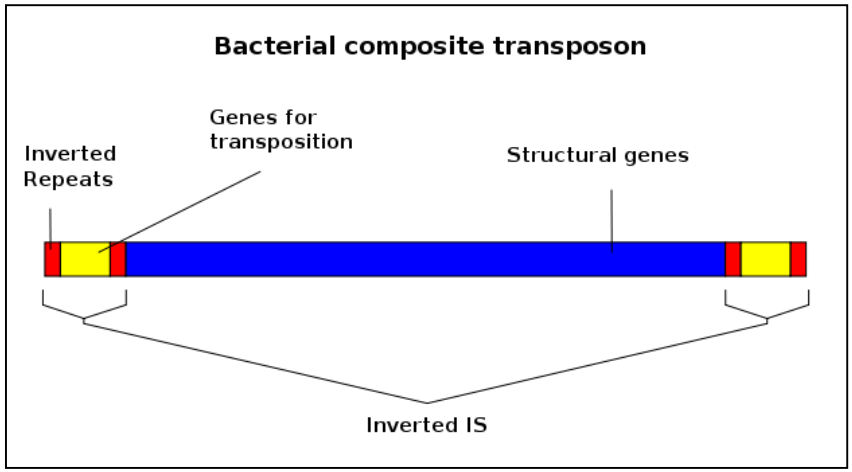
Wikipedia,2015

Insertion sequences

Diagram illustrating the role of insertion sequences in a composite transposon

- ISs are typically composed of:

1. short terminal sequences, often arranged as inverted repeats (terminal inverted repeats [TIRs]) at their ends, and
2. at least one open reading frame (ORF) that encodes the transposase, the only protein essential for mobility.



Wikipedia,2015; He *et al.*,2015

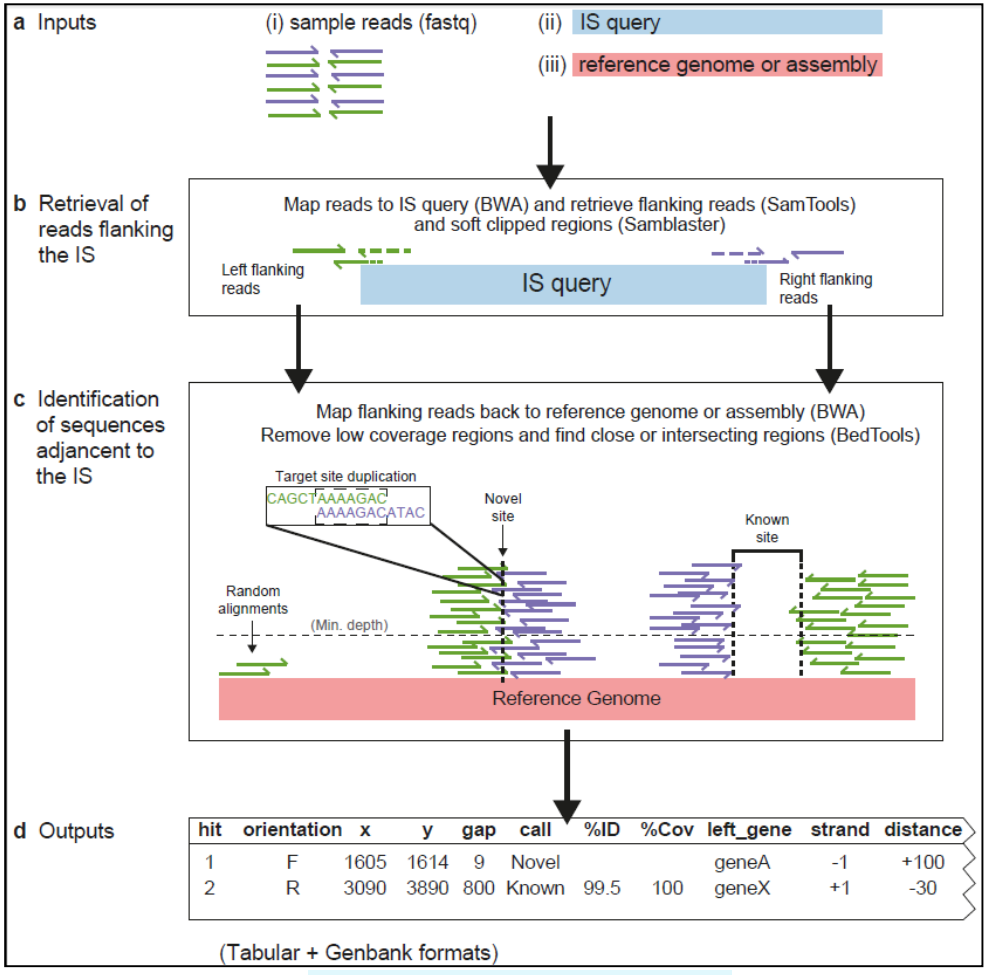
ISMapper:

Identifying insertion sequences in bacterial genomes from short read sequence data

- ISMapper, is a mapping-based tool for identification of the site and orientation of IS insertions in bacterial genomes, direct from paired-end short read data.
- ISMapper provides a rapid and robust method for identifying IS insertion sites direct from short read data, with a high degree of accuracy demonstrated across a wide range of bacteria.

Hawkey *et al.*,2015

ISMapper:
Workflow for ISMapper



Hawkey *et al.*,2015

Insertion sequences
On plasmid or chromosome

- Insertion sequence elements contribute to bacterial evolution in a number of ways.
- These sequences are mobile:

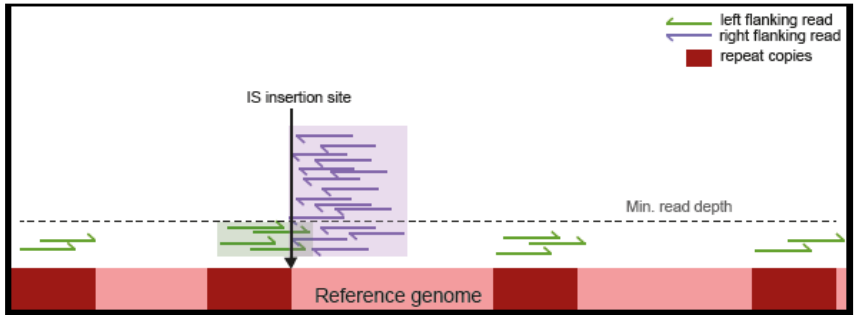
1. Within plasmids,
2. Between plasmids, and
3. Between plasmid and chromosome.

- IS elements can cause mutations by insertion within genes.

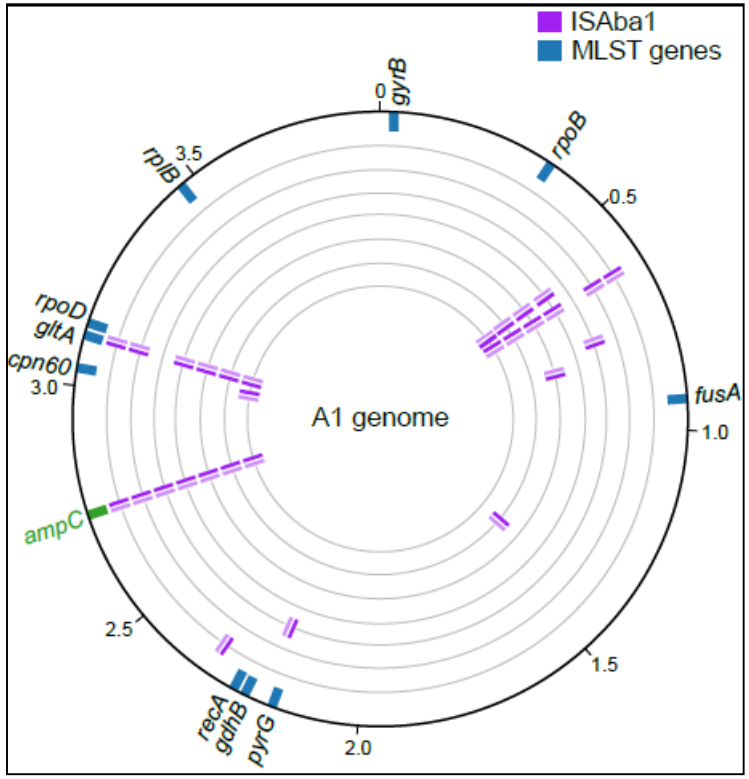
Sundin,2007

ISMapper:

Identifying insertion sequences in bacterial genomes from short read sequence data



Issues can arise in the second mapping step of ISMapper if the IS insertion is next to a region that occurs more than once in the reference genome.

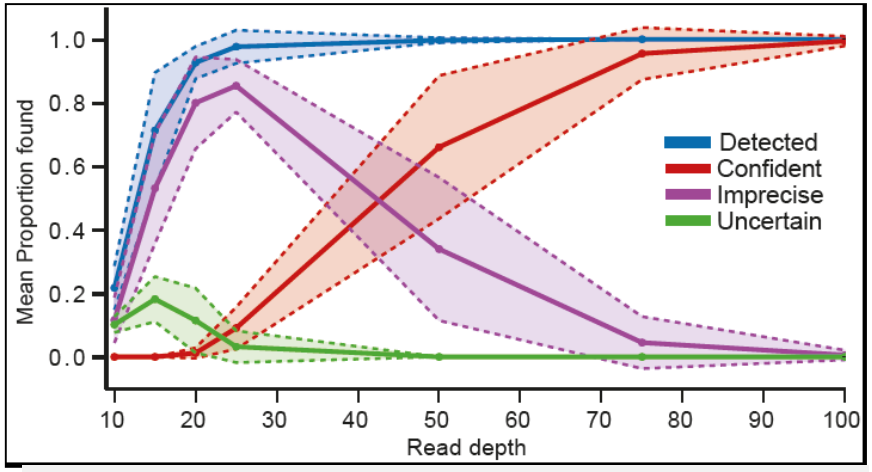


ISAba1 positions detected in seven *Acinetobacter baumannii* genomes.

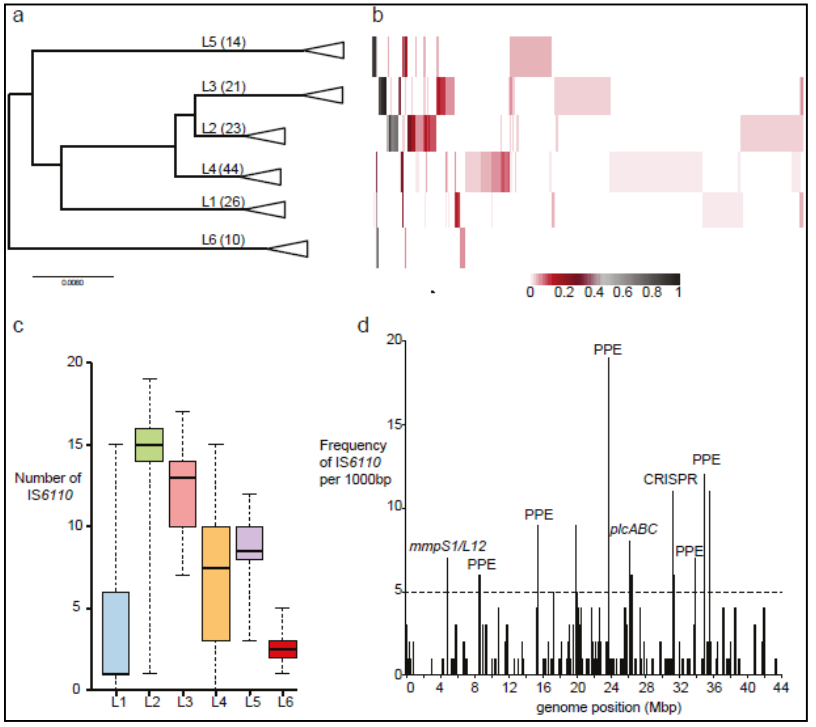
Hawkey *et al.*,2015

ISMapper:

Identifying insertion sequences in bacterial genomes from short read sequence data



ISAba1 detection rate as a function of read depth, for seven *A. baumannii* GC1 genomes sequenced with Illumina.



Analysis of IS*6110* insertions in a diverse set of *M. tuberculosis* genomes.

Hawkey *et al.*,2015

Insertion sequences
Numbers and numbering

- A particular insertion sequence may be named according to the form IS*n*, where *n* is a number.
- e.g.
- IS*1*, IS*2*, IS*3*, IS*10*, IS*50*, IS*911*, IS*26*, etc.
- The sequencing of *Shigella* genomes has revealed that a large number of insertion sequence (IS) elements (over 200 elements) reside in the genome.

Wikipedia,2015;..

Insertion Sequences

IS3 family

In gram-negative and gram-positive bacteria

- The IS3 family is one of the largest families of IS elements, with members found in diverse bacterial groups including:
- Gram-negative rods and cocci,
- Gram-positive cocci,
- Mycobacteria, and
- Mycoplasmas.

Sundin,2007

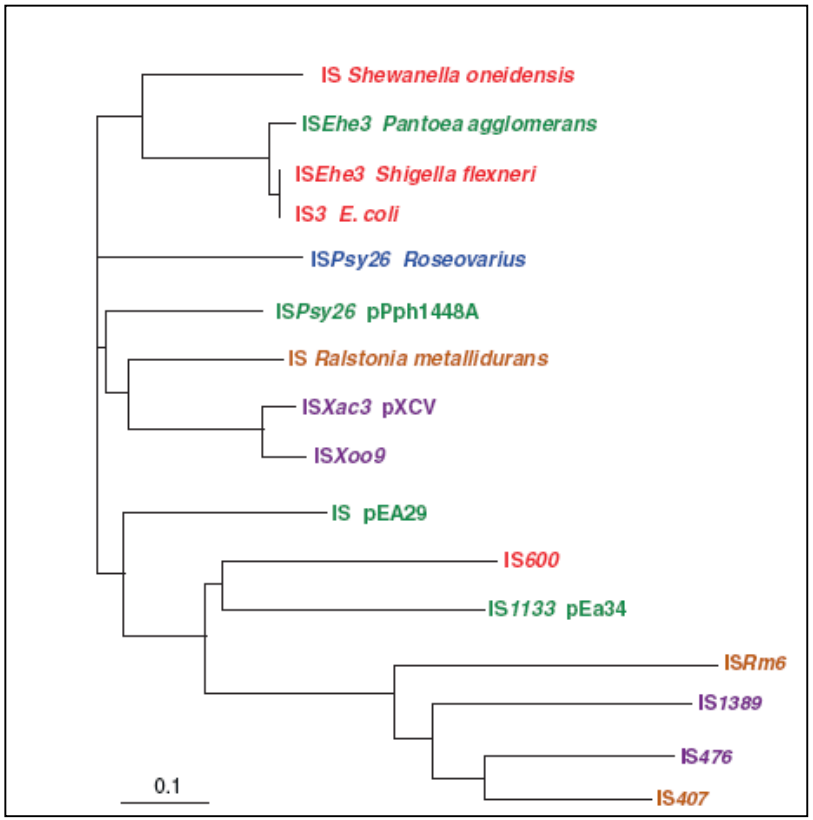
Insertion Sequences

IS3 family

In some phytopathogenic bacteria

- Several IS elements encoded on phytopathogenic bacterial plasmids are members of the IS*3* family including:
- IS*476* from *Xanthomonas campestris* pvs. *vesicatoria* and *campestris*,
- IS*1389* from *Xanthomonas campestris* pv. *amaranthicola*,
- *ISXac3* from *Xanthomonas axonopodis* pv. *citri*,
- IS*Psy26* from *P. syringae* pv. *tomato*, and
- an unnamed IS element from pEA29in *E. amylovora*.

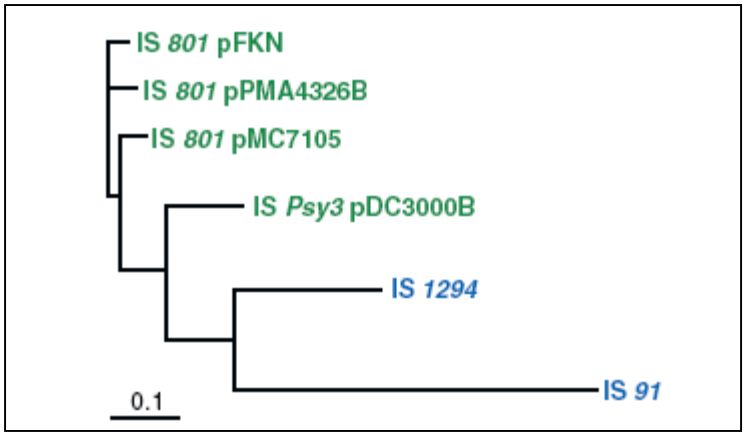
Sundin,2007



Dendrogram based on alignment of the transposase *orfB* gene from members of the IS3 family of insertion sequences including several from phytopathogenic bacteria.

An IS element: IS*801*
In *Pseudomonas syringae*

- The 1.5-kb element IS*801* appears prominently in the evolution of certain *P. syringae* pathovars and appears to be absent in other pathovars.



Dendrogram based on alignment of the transposase gene from IS*801* and the related elements IS*Psy3*, IS*91*, and IS*1294*.

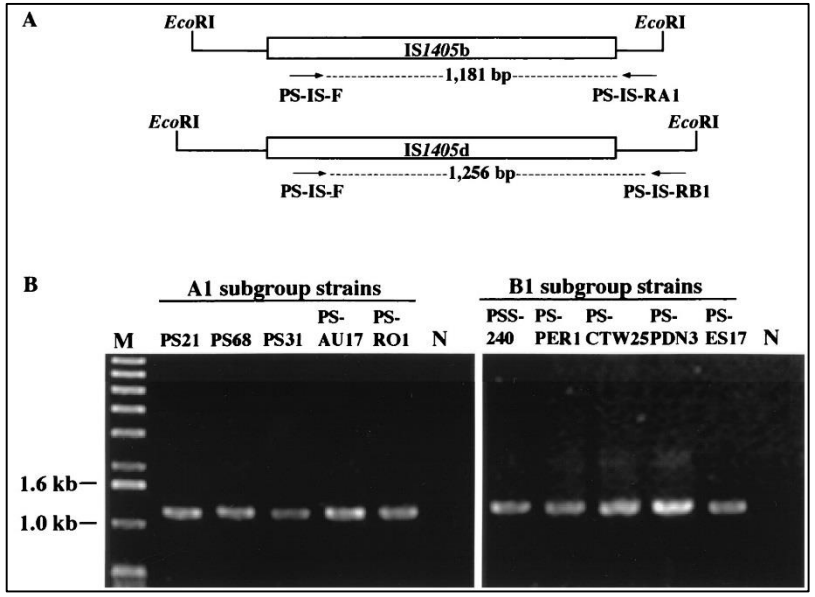
Sundin,2007

Two IS elements:
IS*1405*b and IS*1405*d
In *R. solanacearum*

A. A scheme of the subgroup-correlated insertion site of IS*1405* with the position of each primer. The locations of IS*1405*b and IS*1405*d in 1.4 and 2.0-kb EcoRI fragments are specific to the A1 and B1 subgroup strains, respectively.

B. Agarose gel electrophoresis of PCR products from genomic DNAs of five representative A1 or B1 subgroup strains of *R. solanacearum* race 1 with PS-IS-F and PS-IS-RA1 or PS-IS-F and PS-IS-RB1 primer pairs, respectively.

- M, molecular size marker (1-kb DNA ladder, sizes (base pairs) are on the left. Lane N, a negative control.



Lee *et al.*,2001

IS elements

In *Xanthomonas oryzae* pv. *oryzae*

- Genome sequence of *Xanthomonas oryzae* pv.*oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity.
- Total of 611 ISs of 25 types were found in the genome of *Xoo*.
- Their ratio in relation to the whole genome was approximately 10%.
- This percentage was remarkably high compared with that of other sequenced plant-pathogenic which may be a characteristic feature of Xoo.
- ISs were located throughout the genome, and they were repeated tandemly in many loci.
- In some cases, multiple IS regions (IS islands) that encompassed about 30 kb were also present.

IS elements in xoo

Table 2. IS elements present in *X. oryzae* pv. *oryzae* MAFF 311018

Name	Family	Group	No. of full-length copies	No. of partial (truncated) copies	Structure
IS*Xo1*	IS*5*	IS*5*	42	2	orfA
IS*Xo2*	IS*NCY*		7	3	orfA
IS*Xo3*	IS*5*	IS*1031*	30	10	orfA / orfB
IS*Xo5*	IS*NCY*		47	21	orfA
IS*Xo7*	IS*630*		7	0	orfA
IS*Xo8*	ISI		36	34	orfA
IS*1112*	IS*30*		20	12	orfA
IS*1113*	IS*NCY*		8	2	orfA
IS*Xoo2*	IS*630*		11	15	orfA
IS*Xoo3*	IS*3*	IS*407*	16	26	orfA / orfB
IS*Xoo4*	IS*5*	IS*5*	11	41	orfA
IS*Xoo5*	IS*5*	IS*5*	10	2	orfA
IS*Xoo6*	IS*5*	IS*5*	10	2	orfA
IS*Xoo7*	IS*5*	IS*5*	2	0	orfA
IS*Xoo8*	IS*4*		11	8	orfA
IS*Xoo9*	IS*3*	IS*51*	1	1	orfA / orfB
IS*Xoo10*	IS*NCY*		1	0	orfA
IS*Xoo11*	unknown		46	0	orfA
IS*Xoo12*	unknown	IS*4*	42	0	orfA
IS*Xoo13*	IS*3L*		19	6	orfA
IS*Xoo14*	IS*5*		5	1	orfA
IS*Xoo15*	IS30		2	16	orfA
IS*Xoo16*	IS*630*		2	7	orfA
Others	IS*3*		0	8	
	unknown		0	8	

Comparison of IS, proposed HrpX regulons and putative *avr* genes among the three *Xanthomonas* spp.

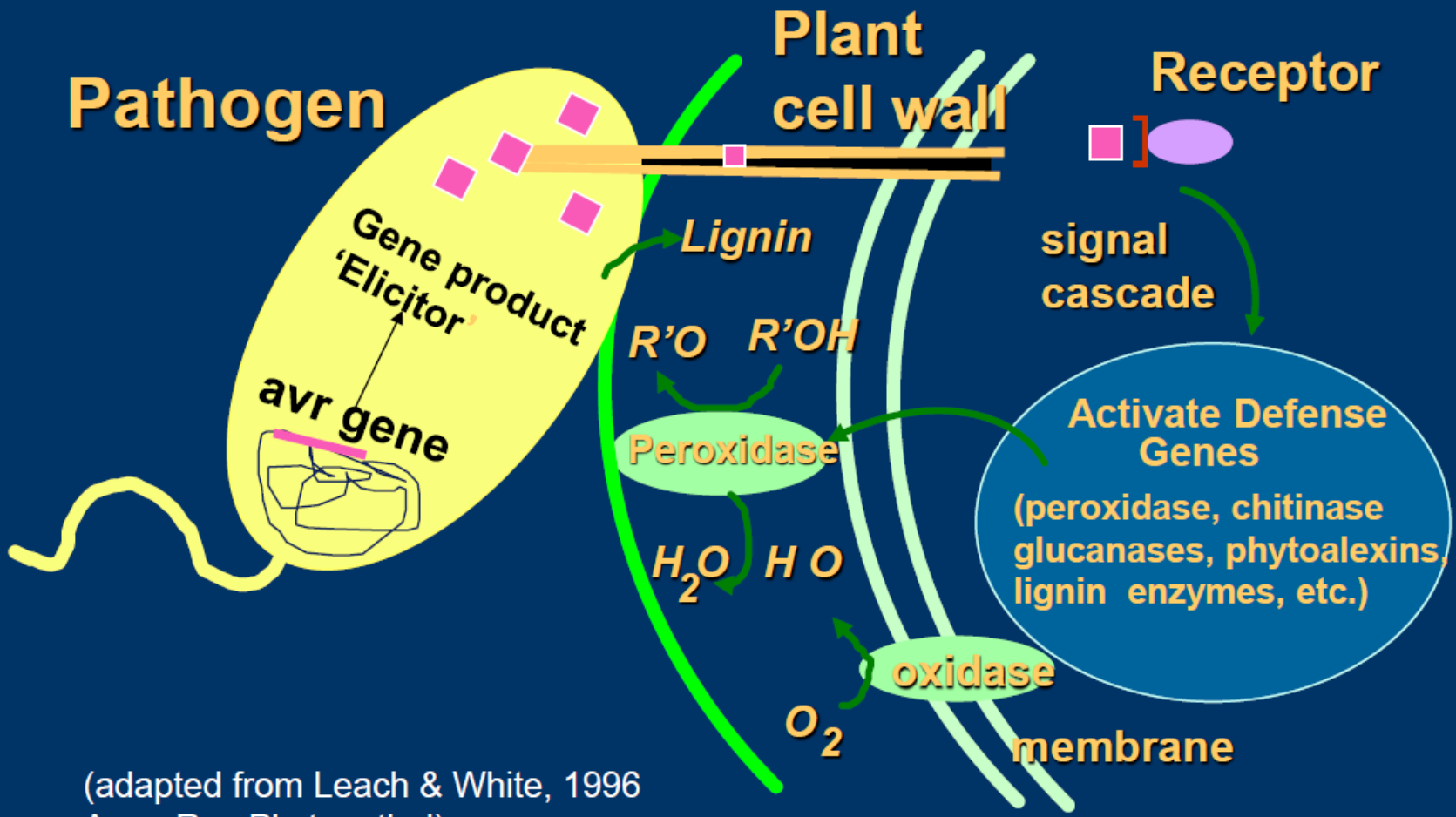
	X. oryzae pv. *oryzae* MAFF 311018	*X. axonopodis* pv. *citri* 306	*X. campestris* pv. *campestris* ATCC 33913
Length (bp)	4,940,217	5,175,554	5,076,187
G+C content (%)	63.7	64.7	65.0
Total number of predicted genes	4,372	4,313	4,182
Plasmid	0	2	0
Insertion sequence elements (IS)	386(225)	87	109
Proposed HrpX regulons	37	20	17
Putative avirulence genes (family)			
avrBs1	0	0	2
avrBs2	1	1	1
avrBs3 / pth	16 (genome)	4 (plasmid)	0
avrPphE	0	3	1
avrXca	0	0	2
yopJ	0	0	1
avrC	0	0	1

Bacterial pathogenesis on plants
Plant derived molecules

Plant Strategies for Defence

1. *R* genes
2. PGIPs
3. Anti-microbial (antibacterial) proteins
4. Phytoalexins
5. Pathogenesis related proteins (PRs)

Host-Pathogen Interactions



(adapted from Leach & White, 1996 Annu Rev Phytopathol)

Plant derived molecules

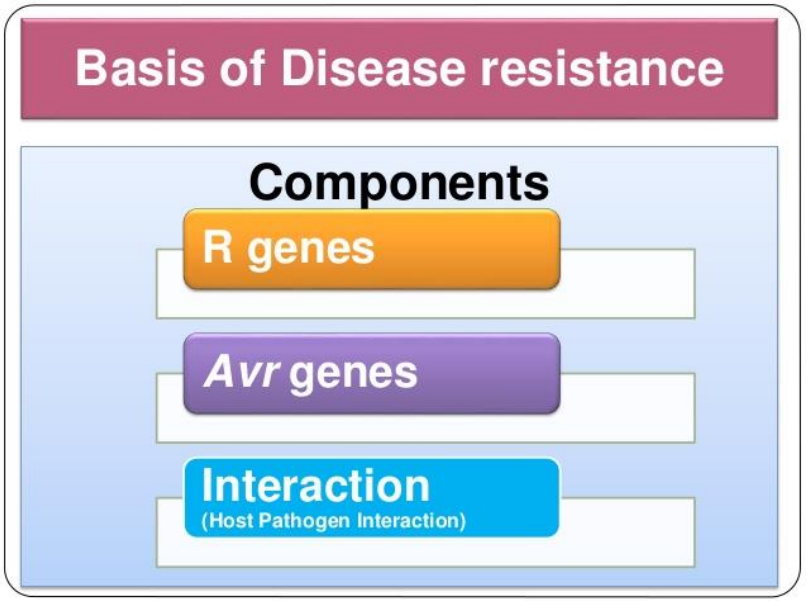
- Resistance (R) genes: Leucine rich repeats.
- PGIPs: Polygalactoronase - inhibiting proteins which protect cell walls.
- Anti-microbial proteins: Phytoalexins.
- Pathogenesis related proteins (PR): Increase after infections (phenols, lignins, tannins,…).
- LAR (local acquired resistance) or SAR (systematic acquired resistance).

Resistance(*R*) genes

Leucine rich repeat regions

Plant resistance (*R*) genes

- Plants need to defend themselves against attack from viruses, microbes, invertebrates, and even other plants.
- Because plants lack a circulatory system, each plant cell must possess a preformed and/or inducible defense capability, so distinguishing plant defense from the vertebrate immune system.



Plant resistance (*R*) genes

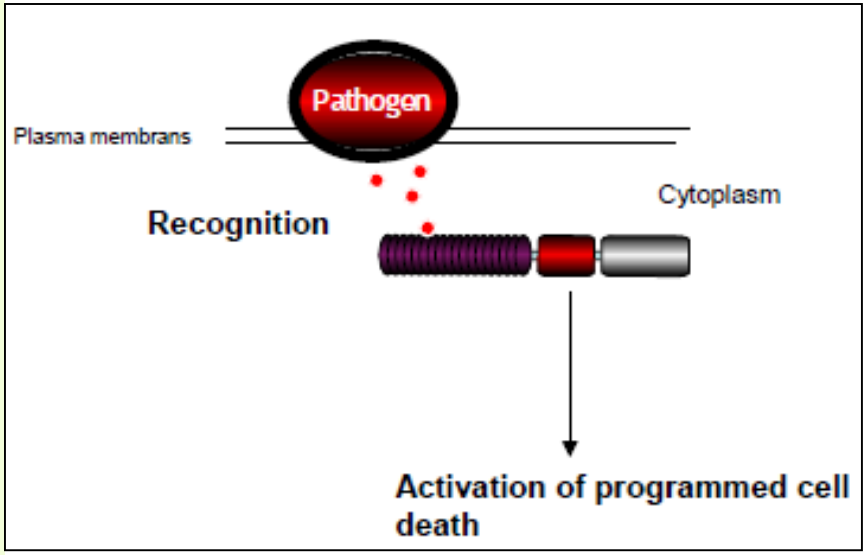
General features of disease resistance genes

- The plant hosts, are not passive recipients of pathogens and have evolved complicated defense mechanisms that respond to effectors.
- Plants apparently express a large array of *R* genes in all living cells to detect the presence of pathogens.
- There will be many copies of each receptor on the surface of each plant cell.
- In compatible interactions, the plant sensors do not appear to be receptors for effectors but rather seem to monitor the cellular targets of the pathogen effectors.

Plant resistance (*R*) genes

General features of disease resistance genes

- Confer resistance to diverse pathogens such as virus, bacteria, fungus and insects.
- Induce a programmed cell death.
- Expression is constitutive.
- Belong to a large gene family.
- Arabidopsis genome sequence (125 Mb) encodes for ~150 NBS-LRR genes.
- Evolve in gene clusters.



James *et al.*, 2007

Plant resistance (*R*) genes

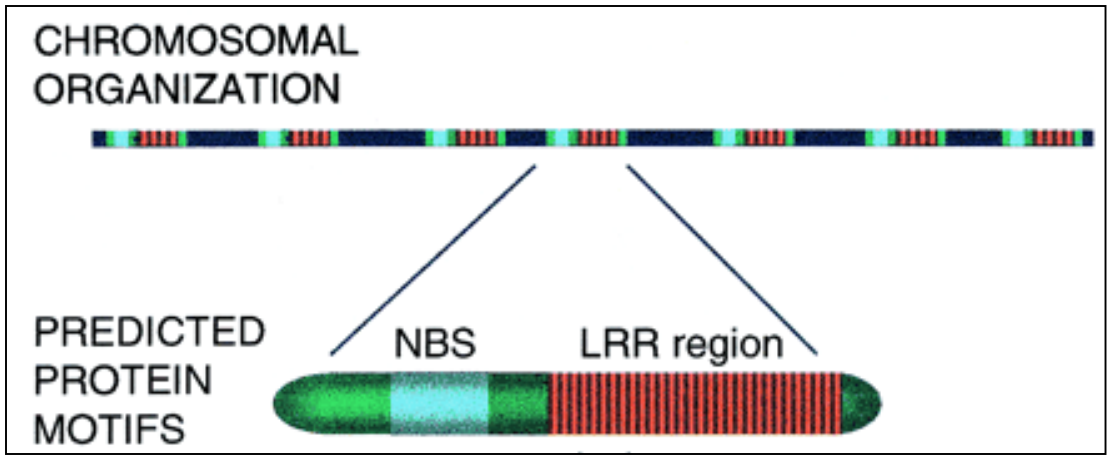
General features of disease resistance genes

- Basically, all these genes seem to encode components of receptor systems and form part of a signal transduction pathway, which triggers general defense reactions such as:

1. Reinforcement of the cell wall (cell wall thickening);
2. Synthesis of phytoalexins and oxidation of phenolic compounds;
3. Activation of defense-related genes, and
4. The HR.

R genes in plant genome

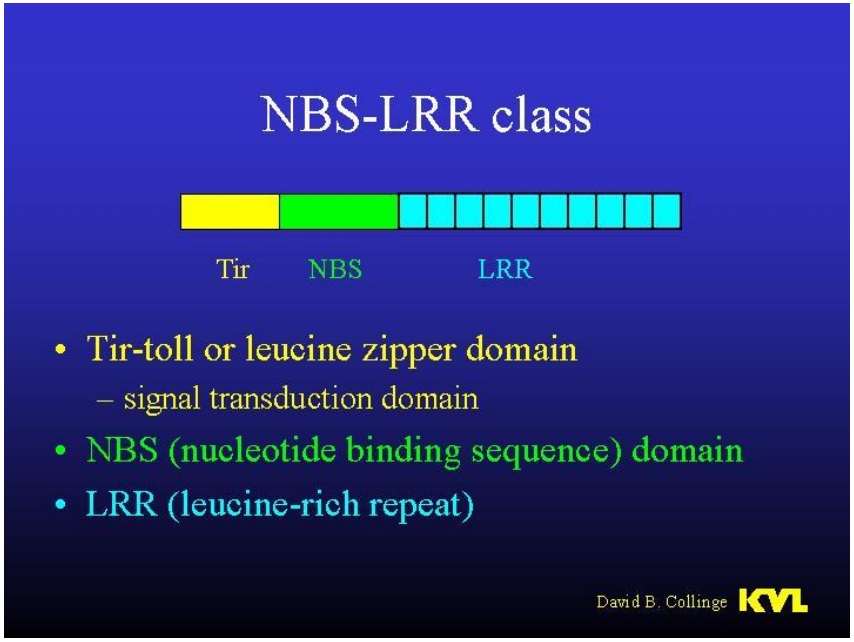
- Resistance gene are often present as gene clusters of different specificities in the plant genome.
- Majority of these *R* genes show conserved DNA sequences and amino acid domains irrespective of whether they confer resistance to bacterial, fungal, viral, or nematode pathogens.



R genes common structure

- Though different classes of resistance genes exist, the *R* genes products have common structural features. i.e.
- Bipartite structure of:
- Receptor domain, and
- Catalytic domain.
- Most *R* proteins contain:

1. Leucine-rich repeats (LRRs),
2. A central nucleotide-binding site (NBS), and
3. A variable amino-terminal domain.



TIR (*Drosophila* Toll/Interleukin-1 a cytoplasmic receptor) domain

The Tir toll, leucine zipper domain can also be called coil-coil depending on origin. It has been suggested that TIR-NBS domain actually represents a histidine-asparatic acid transfer domain.

Identification of resistance(R) genes through genetics

- Plant breeders have successfully introduced disease resistance through introgression of foreign *R*-genes.
- However, pathogen races quickly evolve that lack the cognate *avr* genes.
- The adaptive ability of pathogens has limited the durability of most *R*-gene-based resistance.
- Sources of new *R*-genes are sought.
- These may be introduced into commercial crop lines through:

1. Introgression (breeding), or by
2. Genetic engineering.

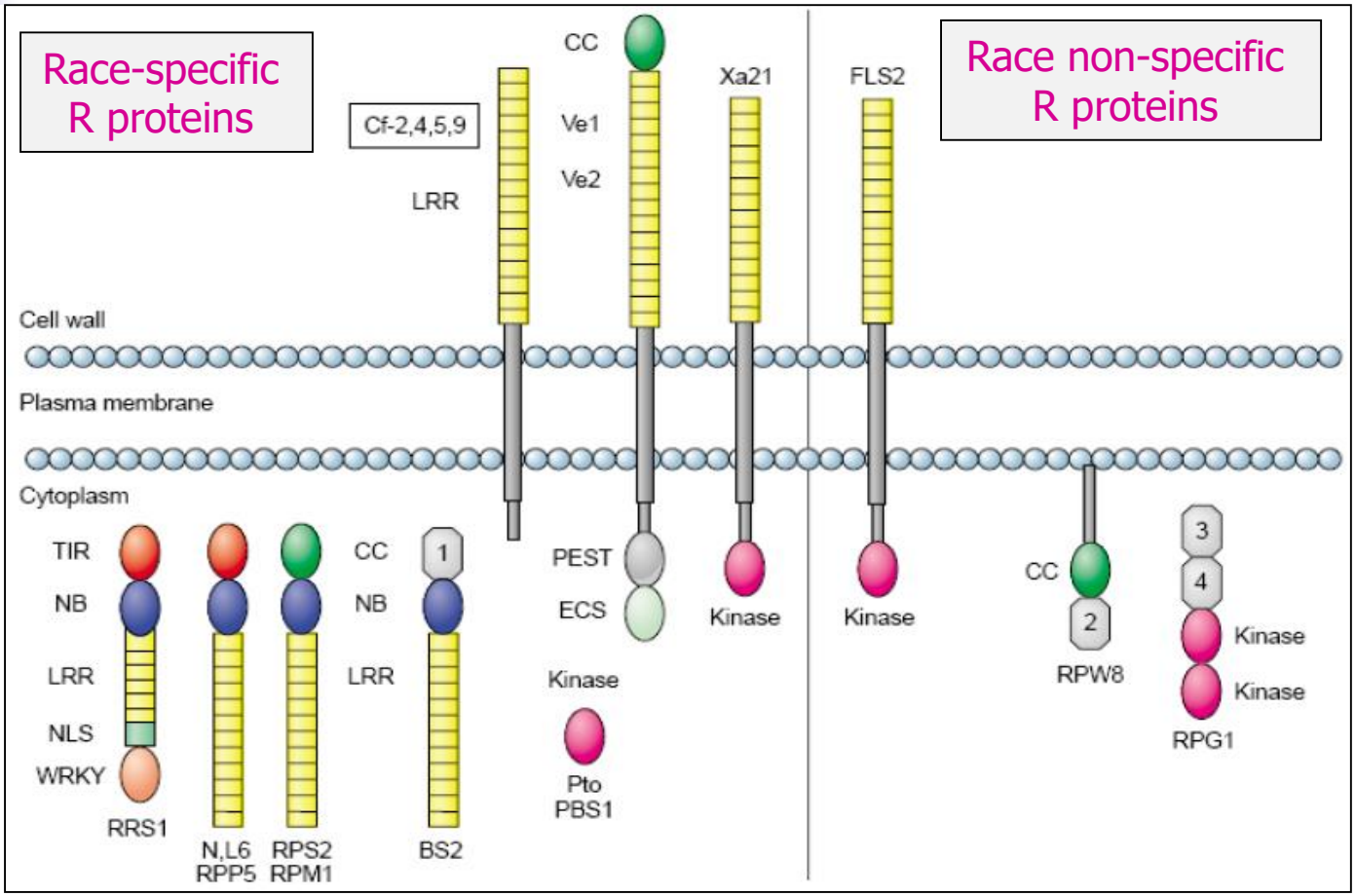
- The latter requires molecular cloning of *R* genes (e.g. in a wide host range cosmid cloning vector,pLARF1).

R genes
Pathogen specific genes

- Resistance genes which are pathogen specific are generally ineffective against other pathogens.
- Plants behave as a non-host for a bacterial pathogen, because it recognizes many of that bacteria's gene products as avirulent determinants.
- Therefore, many mutations are necessary to extend host range.

Avirulent= if the agent is avirulent it is a variant of a pathogen that does not cause severe disease. Nonvirulent= not virulent, harmless.

R genes
Pathogen specific genes



RRS1 resistance to *R. solanacearum*; RS2 resistance to bacterial speck 2 (Xcv).

R genes
Against necrotrophs

- Why are plant resistance genes that work against necrotrophs so rare?
- It has been suggested that necrotrophs are less dependent on suppression of plant defenses and may even benefit from induction of some defense pathways (Glazebrook,2005).
- This hypothesis remains to be broadly tested.

Allen *et al.*,2009

R loci encoding for several specificities

- *R* genes are often found clustered on chromosomal loci.
- Any locus conferring race-specific resistance on a plant cultivar is called 'specificity'.
- These specificities have been shown to exhibit a particular distribution within the plant genome.
- Many clusters of *R* loci encoding for several specificities have been found, especially in the case of resistances to biotrophic fungi.

R loci encoding for several specificities

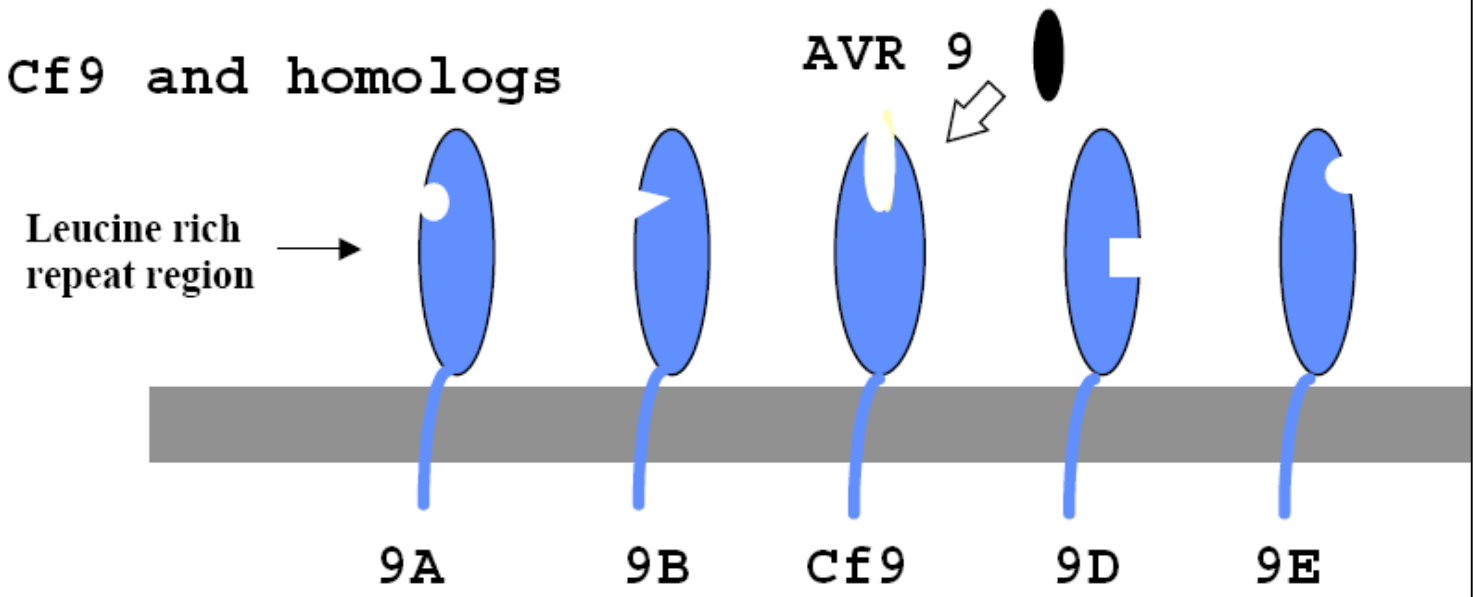
- The non-random distribution of *R* loci in the plant genome is due to two types of genomic organization:

1. Allelic variation
2. Chromosomal tandems and clusters of tightly linked genes.

An allele is a variant form of a given gene. Sometimes, different alleles can result in different observable phenotypic traits, such as different pigmentation.

R loci encoding for several specificities

Cf9 region of tomato plant and its different binding sites to perceive Avr9 of fungus *Cladosporium fulvum*



Above: Simplistic illustration of the Cf9 region showing different potential binding sites on each homologue with AVR9 about to bind to *Cf9*. Each of the other homologs has the potential to perceive a different pathogen gene product (i.e., an Avr gene product).

Kinds of *R*-genes

1. **An enzymatic *R*-gene**

   e.g. *Hm* gene from maize and the HC-toxin from *Cochliobolus* (*Helminthosporium*) *carbonum*.

2. **An antiviral *R* gene**

   e.g. *N*-gene (TMV/tobacco).

3. ***R*-genes active against fungi**

   a. Cf9 (*Cladosporium fulvum* /tomato).

   b. *Pi-ta* (*Magnaporthe grisea* /rice).

4. ***R* genes against bacterial pathogens**

   a. *Pto* (*Pseudomonas syringae* /tomato).

   b. *Rps2, Rpm1* (*Pseudomonas syringae* /*Arabidopsis*).

   c. *Xa21* (*Xanthomonas oryzae* /rice).

Classes of resistance genes

R-genes

- Some are composed predominantly of:

1. An LRR region, and
2. Transmembrane (TM) domain.

- Another is comprised of:

1. A LRR region, and
2. A protein kinase domain.

Five major classes of *R* gene products

Classes of R proteins

Based upon their structural domains

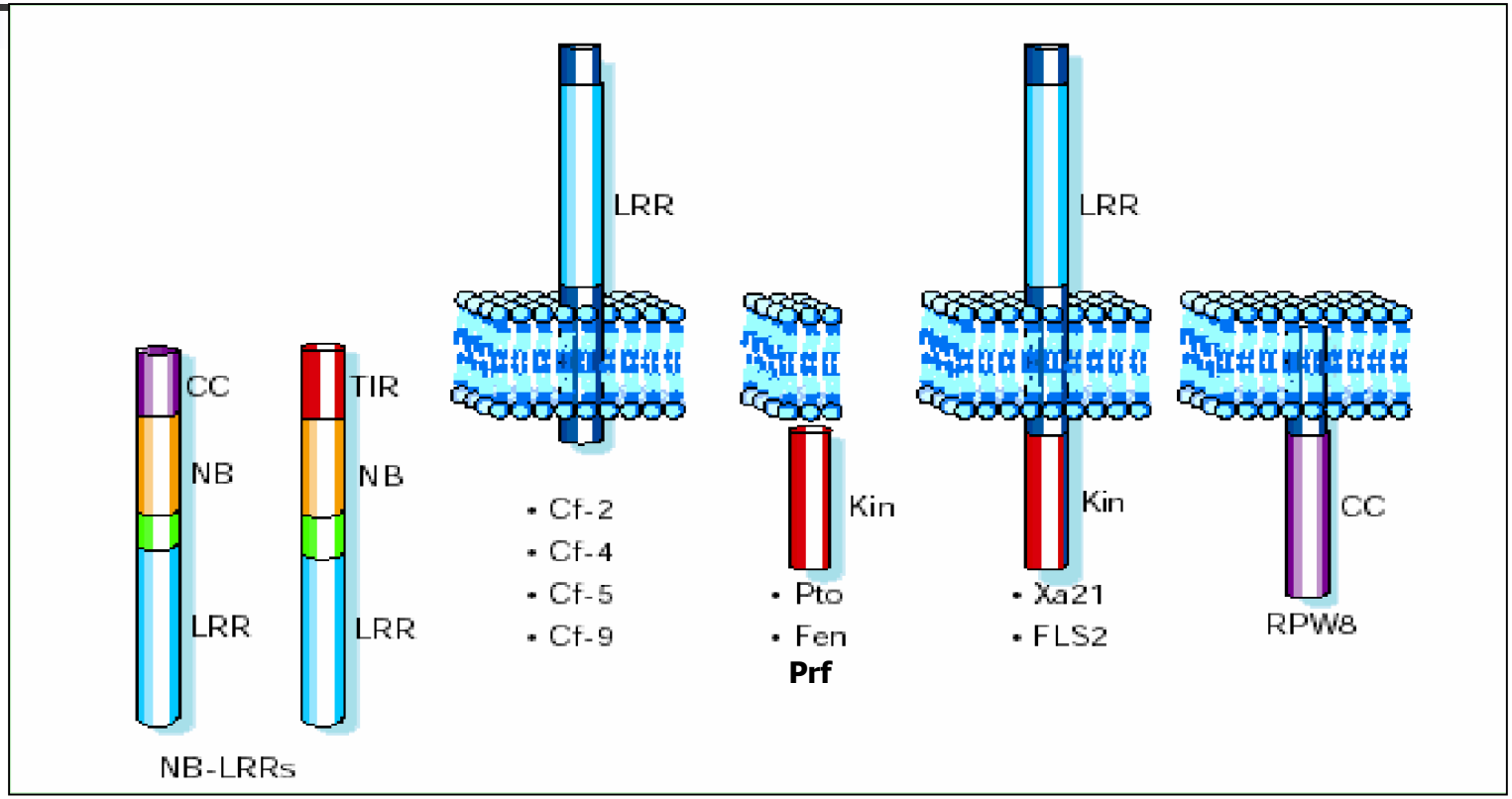
- **Class 1:** Pto-Serine/Threonine Kinase
- **Class 2:** LRR-NBS-LZ/CC
- **Class 3:** LRR-NBS-TIR
- **Class 4:** Extracellular LRR (Cf genes)
- **Class 5:** Extracellular LRR-Serine thronine kinase

Class 1 differs from the other classes since it lacks leucine-rich repeats (LRR).

Martin *et al.*,2003

5 major classes of R proteins

Localization of most R proteins

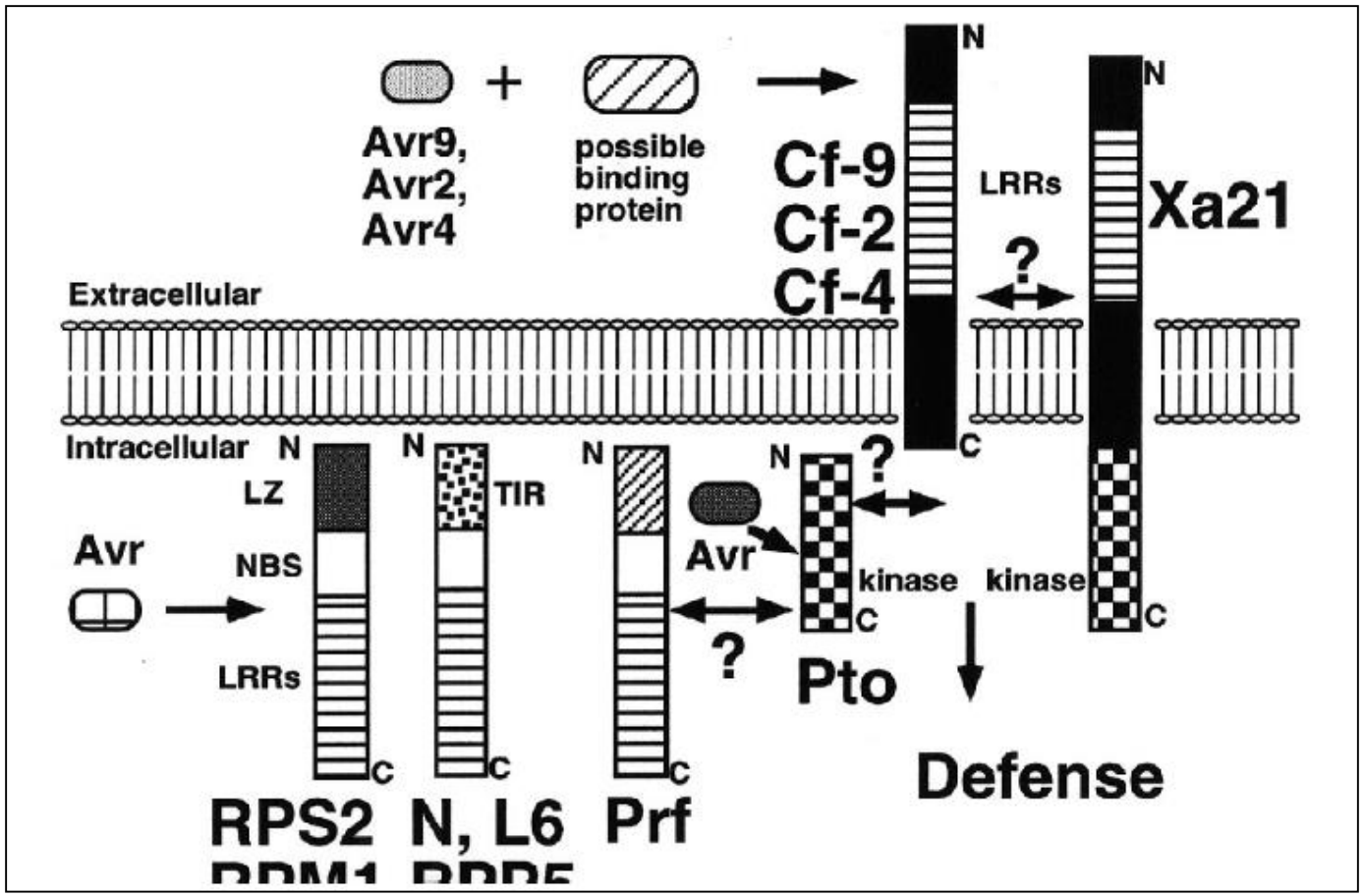


NB-LRR proteins are likely localized in the cytoplasm

Dangl and Jones,2001

5 major classes of R proteins

Localization of most R proteins



Hammond-Kosack and Jones,1997

Five major classes of *R* gene products Based upon their structural domains

Presence or absence of TM domain

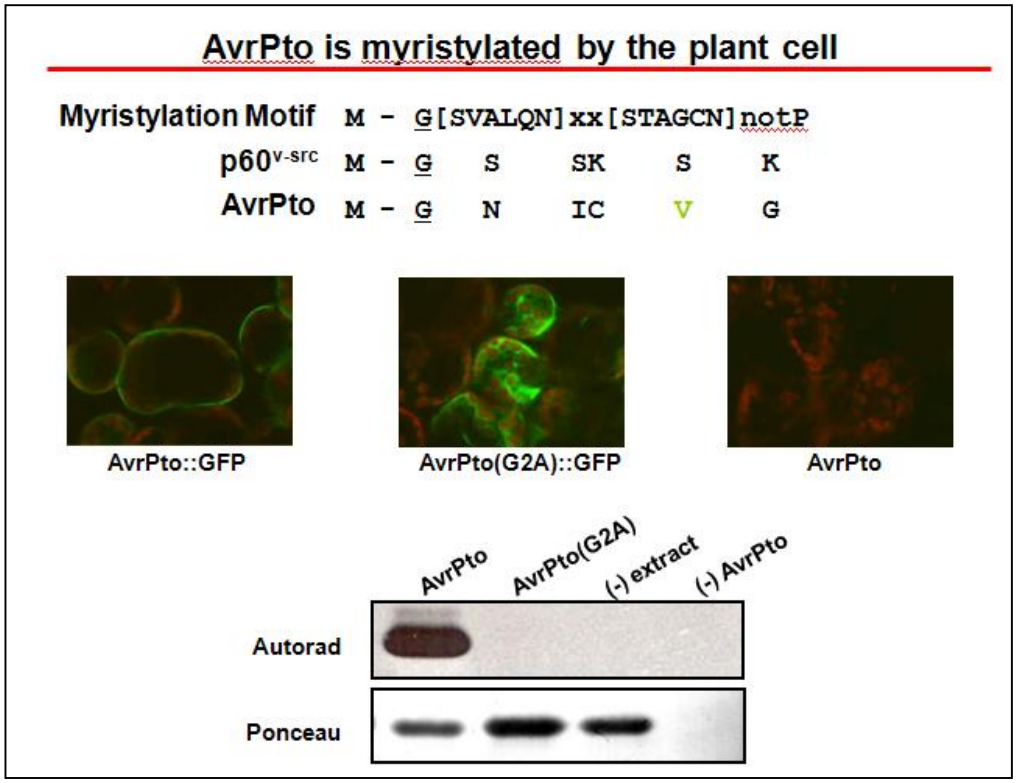
- Class I: Consists of just one member, *Pto* from tomato.
- It has a possible membrane anchor site (myristoylation site) at its N-terminus and a serine-threonine kinase domain.
- No transmembrane (TM) domain for attachment to membranes and all are thought to be localized intracellularly.

- Class II: A large class of R proteins that have LRR-leucine rich repeats, nucleotide binding site(NBS), LZ- leucine zipper or CC-coiled coil region and a signal peptide.
- e.g. flax-rust resistance gene L6.
- No transmembrane (TM) domain and serine-threonine kinase.

Pto gene from tomato

AvrPto is myristylated by the plant cell

- Protein myristoylation is a means by which cells anchor proteins into membranes.



Bogdanove,2011

Five classes of *R* gene products

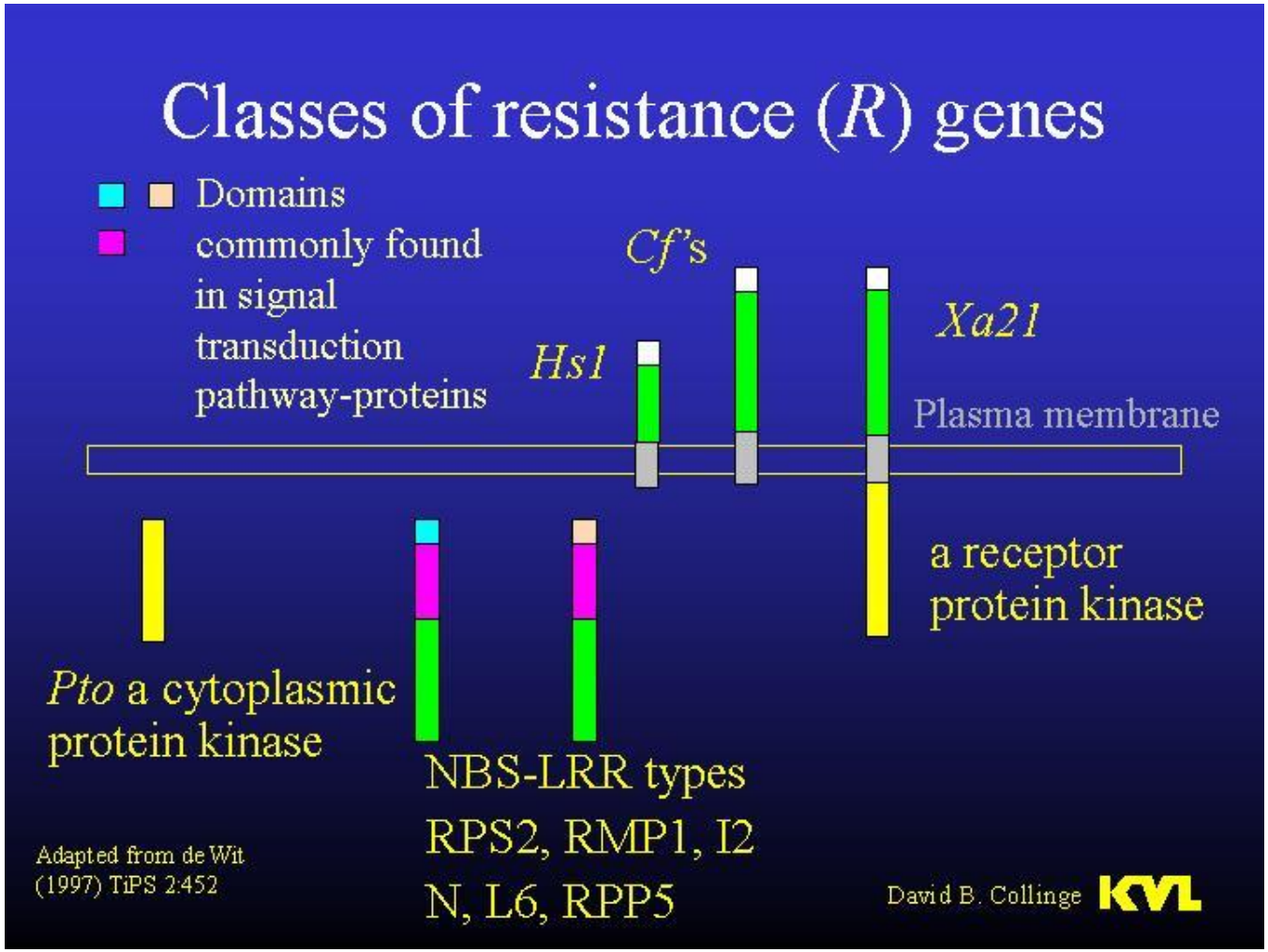
Based upon their structural domains

Presence or absence of TM domain

- Class III: Class 3 is similar to class 2 but instead of TZ or CC region it has a TIR domain at its N terminus, similar to Drosophila Toll protein and the mammalian interleukin-1 receptor (IL-R) protein. e.g. *RPS2* from *Arabidopsis*.
- Also no TM.
- Class IV: Have transmembrane (TM), LRR and signal peptide domains. e.g. tomato *Cf* genes. It lacks the serine-threonine kinase domain.
- Class V: Xa21 of rice - Consists of just the Xa21 protein from rice. In addition to an extracellular LRR and a TM, it has a cytoplasmic serine/threonine kinase region and a signal peptide.
- Note the R proteins belonging to the first three classes lack transmembrane (TM) domains.

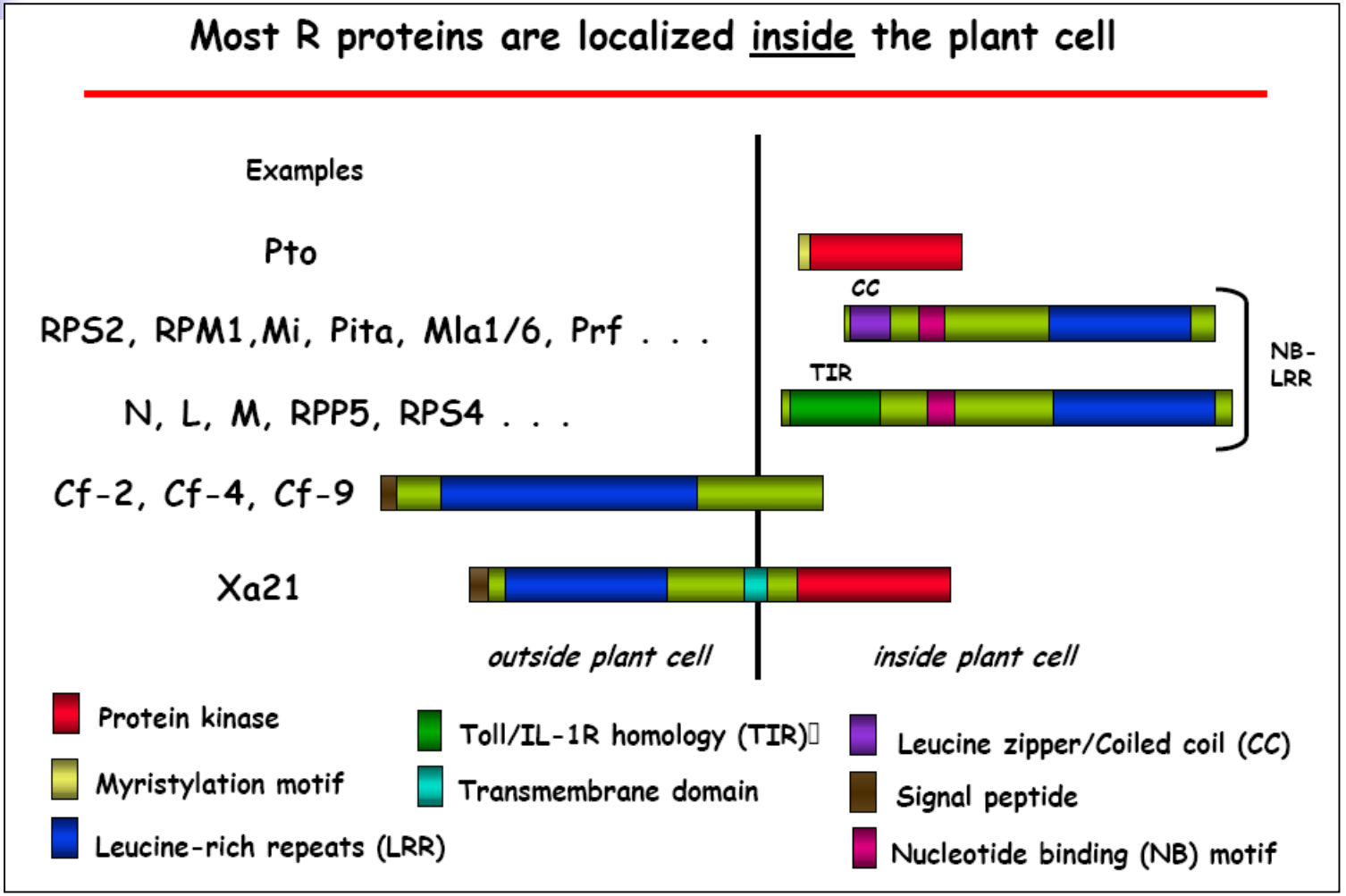
Five major classes of *R* genes

Localization of most R proteins



Features of the 5 major classes of R proteins

Localization of most R proteins



The five major classes of plant disease resistance genes

Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)
1	Pto	Tomato	*Pseudomonas syringae* (B)	AvrPto, AvrPtoB
2	Bs2	Pepper	*Xanthomonas campestris* (B)	AvrBs2
	Dm3	Lettuce	*Bremia lactucae* (F)	
	Gpa2[a]	Potato	*Globodera pallida* (N)	
	Hero	Potato	*G. rostochiensis*, *G. pallida* (N)	
	HRT[b]	*Arabidopsis*	Turnip Crinkle Virus	Coat Protein
	I2	Tomato	*Fusarium oxysporum* (F)	
	Mi	Tomato	*Meloidogyne incognita* (N)	
	Mi	Tomato	*Macrosiphum euphorbiae* (I)	
	Mla	Barley	*Blumeria graminis* (F)	
	Pib	Rice	*Magnaporthe grisea* (F)	
	Pi-ta	Rice	*M. grisea* (F)	AVR-Pita
	R1	Potato	*Phytophthora infestans* (O)	
	Rp1	Maize	*Puccinia sorghi* (F)	
	RPM1	*Arabidopsis*	*P. syringae* (B)	AvrRpm1, AvrB
	RPP8[b]	*Arabidopsis*	*Peronospora parasitica* (O)	
	RPP13	*Arabidopsis*	*P. parasitica* (O)	
	RPS2	*Arabidopsis*	*P. syringae* (B)	AvrRpt2
	RPS5	*Arabidopsis*	*P. syringae* (B)	AvrPphB
	Rx1[a]	Potato	Potato Virus X	Coat Protein
	Rx2	Potato	Potato Virus X	Coat Protein
	Sw-5	Tomato	Tomato Spotted Wilt Virus	
	Xa1	Rice	*X. oryzae* (B)	
3	L	Flax	*Melampsora lini* (F)	
	M	Flax	*M. lini* (F)	
	N	Tobacco	Tobacco Mosaic Virus	Helicase
	P	Flax	*M. lini* (F)	
	RPP1	*Arabidopsis*	*P. parasitica* (O)	
	RPP4	*Arabidopsis*	*P. parasitica* (O)	
	RPP5	*Arabidopsis*	*P. parasitica* (O)	
	RPS4	*Arabidopsis*	*P. syringae* (B)	AvrRps4
4	Cf-2[c]	Tomato	*Cladosporium fulvum* (F)	Avr2
	Cf-4[d]	Tomato	*C. fulvum* (F)	Avr4
	Cf-5[c]	Tomato	*C. fulvum* (F)	
	Cf-9[d]	Tomato	*C. fulvum* (F)	Avr9
5	Xa21	Rice	*Xanthomonas oryzae* (B)	

Martin *et al.*,2003

The five classes of cloned plant disease resistance genes

Hammond-Kosack and Jones,1997

TABLE 1 THE FIVE CLASSES OF CLONED PLANT DISEASE RESISTANCE GENES

Class	Gene	Plant	Pathogen	Infection type/ organ attacked	Predicted Features of R protein	Reference
1.	*Hm1*	Maize	*Helminthosporium maydis* (race 1)	Fungal necrotroph / leaf	Detoxifying enzyme HC-toxin reductase	[35]
2.	*Pto*	Tomato	*Pseudomonas syringae* p.v. *tomato* (*avrPto*)	Extracellular bacteria / leaf	Intracellular serine/ threonine protein kinase	[59]
3a	*RPS2*	Arabidopsis	*Pseudomonas syringae* p.v. *tomato* (*avrRpt2*)	Extracellular bacteria / leaf	L. Zip / NBS / LRR	[5] [65]
	RPM1	Arabidopsis	*Pseudomonas syringae* p.v. *maculicola* (*avrRpm1/ avrB*)	Extracellular bacteria / leaf	Intracellular protein with amino terminal leucine zipper domain , and nucleotide binding site (NBS) and leucine rich repeat (LRR) domains	[23]
	I_2 [B]	Tomato	*Fusarium oxysporium* f.sp. *lycopersicon*	Necrotrophic fungus/root and vascular tissue		[A]
3b	*N*	Tobacco	Mosaic virus	Intracellular virus / leaf and phloem	Toll / NBS / LRR	[105]
	L6 *M*	Flax	*Melampsora lini* (*AL6, AM*)	Biotrophic fungal rust with haustoria / leaf	Intracellular protein with amino terminal domain homology with Drosophila Toll protein, and NBS and LRR domains	[53] [C]
	RPP5	Arabidopsis	*Peronospora parasitica*	Biotrophic downy mildew fungus with haustoria / leaf		[D]
4	*Cf-9,* *Cf-2,* *Cf-4* *Cf-5*	Tomato	*Cladosporium fulvum* (*Avr9, Avr2, Avr4* *Avr5*)	Biotrophic extracellular fungus without haustoria / leaf	Extracellular LRR protein with single membrane spanning region and short cytoplasmic carboxyl terminus	[38] [14] [41] [E]
5.	*Xa-21*	Rice	*Xanthomonas oryzae* pv. *oryzae* (all races)	Extracellular bacteria / leaf	Extracellular LRR protein with single membrane spanning region and cytoplasmic kinase domain	[86]

[A] G. Simons and R. Fluhr, pers. comm.; [B] a very tightly linked marker to the wheat *Cre3* gene that confers resistance to the root invading cereal cyst nematode *Heterodera avenae* is highly homologous to this *R* gene class, E. Lagudah and S. Anderson, pers. comm.; [C] P. Anderson, G. Lawrence and J. Ellis, pers. comm.; [D] J. Parker, M. Coleman, V. Szabo, M. Daniels and J. Jones, unpublished; [E] M. Dixon, K. Hatzixanthis and J. Jones, unpublished.

Classes of resistance genes

Based upon their structural domains

Plant species	*R* gene	Localisation	Structure	Pathogen	Matching gene
Tomato	*Prf*	I	LZ–NBS–LRR	*Pseudomonas syringae* pv. *tomato*	*AvrPto*
Arabidopsis	*RPS2*	I	LZ–NBS–LRR	*Pseudomonas. syringae* pv. *tomato*	*AvrRpt2*
Arabidopsis	*RPM1*	I	LZ–NBS–LRR	*Pseudomonas syringae* pv. *maculicola*	*AvrRpm1, avrB*
Arabidopsis	*RPS5*	I	LZ–NBS–LRR	*Pseudomonas syringae* DC3000	*AvrPphB*
Arabidopsis	*RPP8*	I	LZ–NBS–LRR	*Peronospora parasitica*	*AvrRpp8*
Tomato	*Mi*	I	LZ–NBS–LRR	*Meloidogyne incognita* and *Macrosiphum euphorbia*	
Tomato	*I2c*	I	NBS–LRR	*Fusarium oxysporum*	
Tomato	*I2*	I	NBS–LRR	*Fusarium oxysporum*	
Rice	*Xa1*	I	NBS–LRR	*Xanthomonas oryzae* pv. *oryzae*	
Rice	*Pib*	I	NBS–LRR	*Magnaporthe grisea*	
Potato	*Rx*	I	NBS–LRR	Potato virus X	Coat protein
Potato	*Gpa2*	I	NBS–LRR	*Globodera rostochiensis*	
Wheat	*Cre3*	I	NBS–LRR	*Heterodera avenae*	
Pepper	*Bs2*	I	NBS–LRR	*Xanthomonas campestris*	*AvrBs2*
Corn	*Rp1-D*	I	NBS–LRR	*Puccinia sorghi*	
Rice	*Pi-ta*	I	NBS–LRR	*Magnaporthe grisea*	*AvrPITA*
Barley	*Mla*	I	NBS–LRR	*Erysiphe graminis*	
Tobacco	*N*	I	TIR–NBS–LRR	Tobacco mosaic virus	Replicase
Arabidopsis	*RPP1, 10, 14*	I	TIR–NBS–LRR	*Peronospora parasitica*	
Flax	L^6 L^{1-12}	I	TIR–NBS–LRR	*Melampsora lini*	
Flax	*M*	I	TIR–NBS–LRR	*Melampsora lini*	
Arabidopsis	*RPP5*	I	TIR–NBS–LRR	*Peronospora parasitica*	
Arabidopsis	*RPS4*	I	TIR–NBS–LRR	*Pseudomonas syringae* pv. *pisi*	*AvrRps4*
Rice	*Xa21*	E	LRR-TM-PK	*Xanthomonas oryzae* pv. *oryzae*	
Tomato	*Cf-2*	E	LRR–TM	*Cladosporium fulvum*	*Avr2*
Tomato	*Cf-4*	E	LRR–TM	*Cladosporium fulvum*	*Avr4*
Tomato	*Hcr9-4E*	E	LRR–TM	*Cladosporium fulvum*	*Avr4E*
Tomato	*Cf-5*	E	LRR–TM	*Cladosporium fulvum*	*Avr5*
Tomato	*Cf-9*	E	LRR–TM	*Cladosporium fulvum*	*Avr9*
Sugar beet	$HS1^{pro-1}$	E	LRR–TM	*Heterodera schachtii*	

Takken and Joosten,2000

Class 6

Some resistance genes do not fit into the five established classes of R gene products

- These (group 6) include several other proteins whose functions are not known
- In addition, the Hm1 toxin resistance gene is in this group:

Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)
6	Hm1	Maize	*Cochliobolus carbonum* (F)	
	$HS1^{pro-1}$	Beet	*Heterodera schachtii* (N)	
	mlo	Barley	*B. graminis* (F)	
	Rpg1	Barley	*Puccinia graminis* (F)	
	RPW8	*Arabidopsis*	*Erisyphe chicoracearum* (F)	
	RRS1-R	*Arabidopsis*	*Ralstonia solanacearum* (B)	
	RTM1	*Arabidopsis*	Tobacco Etch Virus	
	RTM2	*Arabidopsis*	Tobacco Etch Virus	
	$Ve1^{e}$, $Ve2^{e}$	Tomato	*Verticillium alboatrum* (F)	

Martin *et al.*,2003

Eight Classes of resistance genes

Class	Gene	Interaction (Host/pathogen)	Predicted protein structure	Complex locus[a]	Introgressed from wild species
1	*L*	Flax/*Melampsora lini*	TIR-NBS-LRR	No	No
	M	Flax/*Melampsora lini*	TIR-NBS-LRR	Yes	No
	N	Tobacco/TMV	TIR-NBS-LRR	Yes	Yes
	P	Flax/*Melampsora lini*	TIR-NBS-LRR	Yes	No
	RPP1	*Arabidopsis*/*Peronospora*	TIR-NBS-LRR	Yes	No
	RPP5	*Arabidopsis*/*Peronospora*	TIR-NBS-LRR	Yes	No
	RPS4	*Arabidopsis*/*Pseudomonas*	TIR-NBS-LRR	No	No
	Bs2	Pepper/*Xanthomonas*	NBS-LRR	Yes	Yes
	Dm3	*Lettuce*/*Bremia*	NBS-LRR	Yes	No
	Gpa2/Rx1	Potato/*Globodera* Potato/PVX (*Rx1*)	NBS-LRR	Yes	Yes
	I2	Tomato/*Fusarium*	NBS-LRR	Yes	Yes
	Mi	Tomato/*Meloidogyne*/ *Macrosiphum*	NBS-LRR NBS-LRR	Yes Yes	Yes Yes
	Mla	Barley/*Blumeria*	NBS-LRR	Yes	No
	Pib	Rice/*Magnaporthe*	NBS-LRR	Yes	No
	Pi-ta	Rice/*Magnaporthe*	NBS-LRR	No	No
	Prf[b]	Tomato/*Pseudomonas*	NBS-LRR	Yes	Yes
	Rp1	Maize/*Puccinia*	NBS-LRR	Yes	No
	RPM1	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No
	RPP8/HRT	*Arabidopsis*/*Peronospora* *Arabidopsis*/TCV (*HRT*)	NBS-LRR	Yes	No
	RPP13	*Arabidopsis*/*Peronospora*	NBS-LRR	No	No
	RPS2	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No
	RPS5	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No
	Rx2	Potato/PVX	NBS-LRR	Yes	Yes
	Sw-5	Tomato/*Tospovirus*	NBS-LRR	Yes	Yes
	Xa1	Rice/*Xanthomonas*	NBS-LRR	No	No
2	*Cf-2/5*	Tomato/*Cladosporium*	LRR-TM	Yes	Yes
	Cf-4/9	Tomato/*Cladosporium*	LRR-TM	Yes	Yes
3	*Pto*	Tomato/*Pseudomonas*	Protein Kinase	Yes	Yes
4	*Xa21*	Rice/*Xanthomonas*	LRR-TM-Kinase	Yes	Yes
5	*HS1*[pro-1]	Beet/*Heterodera*	Unique[c]	No	Yes
6	*Rpw8*	Arabidopsis/*Erisyphe*	Unique	Yes	No
7	*mlo*	Barley/*Blumeria*	Membrane Prot.[d]	No	No
8	*Hm1*	Maize/*Cochliobolus*	Toxin reductase	No	No

Hulbert *et al.*,2001

A complete list of isolated plant disease resistance genes

With indication of donor species, related disease and pathogen

Gene Name	Donor Species	Disease	Pathogen
Asc1	*Solanum lycopersicum*	Alternaria stem canker	*Alternaria alternata*
At1	*Cucumis melo*	Cucurbit downy mildew	*Pseudoperonospora cubensis*
At2	*Cucumis melo*	Cucurbit downy mildew	*Pseudoperonospora cubensis*
Bs2	*Capsicum chacoense*	Bacterial spot	*Xanthomonas campestris pv. vesicatoria str. 85-10*
Bs3	*Capsicum annuum*	Bacterial spot	*Xanthomonas campestris pv. vesicatoria str. 85-10*
Bs3-E	*Capsicum annuum*	Bacterial spot	*Xanthomonas campestris pv. vesicatoria str. 85-10*
Bs4	*Solanum lycopersicum*	Bacterial spot	*Xanthomonas campestris*
Cf2	*Solanum pimpinellifolium*	Leaf mould	*Passalora fulva*
Cf4	*Solanum habrochaites*	Leaf mould	*Passalora fulva*
Cf4A	*Solanum habrochaites*	Leaf mould	*Passalora fulva*
Cf5	*Solanum lycopersicum var. cerasiforme*	Leaf mould	*Passalora fulva*
Cf9	*Solanum pimpinellifolium*	Leaf mould	*Passalora fulva*
Cf9B	*Solanum pimpinellifolium*	Leaf mould	*Passalora fulva*
Dm-3	*Lactica sativa*	Downy mildew	*Bremia lactucae*
EFR	*Arabidopsis thaliana*	Eliciting bacteria	*Bacteria with flagellum*
ER-Erecta	*Arabidopsis thaliana*	Bacterial wilt (Arabidopsis)	*Ralstonia solanacearum*
FLS2	*Arabidopsis thaliana*	Eliciting bacteria	*Bacteria with flagellum*
Gpa2	*Solanum tuberosum*	Yellow potato cyst nematode	*Globodera*
Gro1.4	*Solanum tuberosum*	Late blight potato	*Phytophthora infestans*
Hero	*Solanum lycopersicum*	Yellow potato cyst nematode	*Globodera*
Hm1	*Zea mays*	Leaf spot	*Bipolaris zeicola*
Hm2	*Zea mays*	Leaf spot	*Bipolaris zeicola*
HRT	*Arabidopsis thaliana*	Turnip crinkle virus	*Turnip crinkle virus*
Hs1	*Beta procumbens*	Beet cyst nematode	*Heterodera schachtii*
I2	*Solanum lycopersicum*	Fusarium wilt	*Fusarium oxysporum*
L6	*Linum usitatissimum*	Flax rust	*Melampsora lini*
LeEIX1	*Solanum lycopersicum*	Eliciting fungus	*Fungal ethylene-inducing xylanase*
LeEIX2	*Solanum lycopersicum*	Eliciting fungus	*Fungal ethylene-inducing xylanase*
M	*Linum usitatissimum*	Flax rust	*Melampsora lini*
Mi1.2	*Solanum lycopersicum*	Root-knot nematode	*Meloidogyne, Paratrichodorus minor*

Sanseverino *et al.*,2010

A complete list of isolated plant disease resistance genes

With indication of donor species, related disease and pathogen

MLA10	*Hordeum vulgare*	Powdery mildew (barley)	*Blumeria graminis*
Mlo	*Hordeum vulgare*	Powdery mildew (barley)	*Blumeria graminis*
N	*Nicotiana glutinosa*	Tobacco mosaic Virus	*Tobacco mosaic virus*
P2	*Linum usitatissimum*	Flax rust	*Melampsora lini*
PEPR1	*Arabidopsis thaliana*	Damping off	*Pythium*
PGIP	*Phaseolus vulgaris*	Eliciting fungus	*Fungus producing polygalacturonases*
Pi33	*Oryza sativa*	Rice blast disease	*Magnaporthe grisea*
Pi-ta	*Oryza sativa Japonica Group*	Rice blast disease	*Magnaporthe grisea*
Prf	*Solanum pimpinellifolium*	Bacterial speck	*Pseudomonas syringae*
Pto	*Solanum pimpinellifolium*	Bacterial speck	*Pseudomonas syringae*
R1	*Solanum demissum*	Late blight tomato	*Phytophthora infestans*
R3a	*Solanum tuberosum*	Late blight tomato	*Phytophthora infestans*
RCY1	*Arabidopsis thaliana*	Cucumber mosaic virus	*Cucumber mosaic virus*
RFO1	*Arabidopsis thaliana*	Fusarium wilt	*Fusarium oxysporum*
Rmd-c	*Glycine max*	Powdery mildew	*Microsphaera sparsa*
RPG1	*Hordeum vulgare*	Stem rust	*Puccinia Graminis*
Rpi-blb1	*Solanum bulbocastanum*	Late blight tomato	*Phytophthora infestans*
Rpi-blb2	*Solanum bulbocastanum*	Late blight tomato	*Phytophthora infestans*
RPM1	*Arabidopsis thaliana*	Bacterial blight	*Pseudomonas syringae*
RPP13nd	*Arabidopsis thaliana*	Downy mildew	*Hyaloperonospora parasitica*
RPP4	*Arabidopsis thaliana*	Downy mildew	*Peronospora parasitica*
RPP5	*Arabidopsis thaliana*	Downy mildew	*Hyaloperonospora parasitica*
RPP8	*Arabidopsis thaliana*	Downy mildew	*Hyaloperonospora parasitica*
Rps1-k-1	*Glycine max*	Phytophthora root	*Phytophthora sojae*
Rps1-k-2	*Glycine max*	Phytophthora root	*Phytophthora sojae*
Rps2	*Arabidopsis thaliana*	Bacterial blight	*Pseudomonas syringae*
Rps4	*Arabidopsis thaliana*	Bacterial blight	*Pseudomonas syringae*
RPS5	*Arabidopsis thaliana*	Bacterial blight	*Pseudomonas syringae*
RPW8.1	*Arabidopsis thaliana*	Powdery mildew	*Golovinomyces cichoracearum*
RPW8.2	*Arabidopsis thaliana*	Powdery mildew	*Golovinomyces cichoracearum*

Sanseverino *et al.*,2010

A complete list of isolated plant disease resistance genes

With indication of donor species, related disease and pathogen

RRS1	*Arabidopsis thaliana*	Bacterial wilt	*Ralstonia solanacearum*
RTM1	*Arabidopsis thaliana*	Synergistic disease syndromes	*Tobacco etch virus*
RTM2	*Arabidopsis thaliana*	Synergistic disease syndromes	*Tobacco etch virus*
Rx	*Solanum tuberosum*	Latent mosaic	*Potato virus X*
Rx2	*Solanum acaule*	Latent mosaic	*Potato virus X*
RY1	*Solanum tuberosum subsp andigena*	Potato virus Y	*Potato virus Y*
Sw5	*Solanum lycopersicum*	Tomato spotted wilt	*Tomato spotted wilt virus*
Tm2	*Solanum lycopersicum*	Tobacco mosaic virus	*Tobacco mosaic virus*
Tm2a	*Solanum lycopersicum*	Tobacco mosaic virus	*Tobacco mosaic virus*
Ve1	*Solanum lycopersicum*	Verticillium wilt potato	*Verticillium*
Ve2	*Solanum lycopersicum*	Verticillium wilt potato	*Verticillium*
Xa1	*Oryza sativa*	Bacterial blight	*Xanthomonas oryzae*
Xa21	*Oryza sativa Indica group*	Bacterial blight	*Xanthomonas oryzae*

PRGdb is a web accessible open-source (http://www.prgdb.org) database that represents the first bioinformatic resource providing a comprehensive overview of resistance genes (R-genes) in plants.

Sanseverino *et al.*,2010

Plant disease resistance genes

Plant resistance gene database (PRGdb)

- The plant resistance gene database (PRGdb), is the first comprehensive bioinformatics resource dedicated to known and predicted plant disease resistance genes.
- It is a web accessible open-source (http://www.prgdb.org) database providing a comprehensive overview of resistance genes (R-genes) in plants.
- PRGdb holds more than 16000 known and putative R-genes belonging to 192 plant species challenged by 115 different pathogens and linked with useful biological information.

Sanseverino *et al.*,2010

Plant disease resistance genes

Plant resistance gene database (PRGdb)

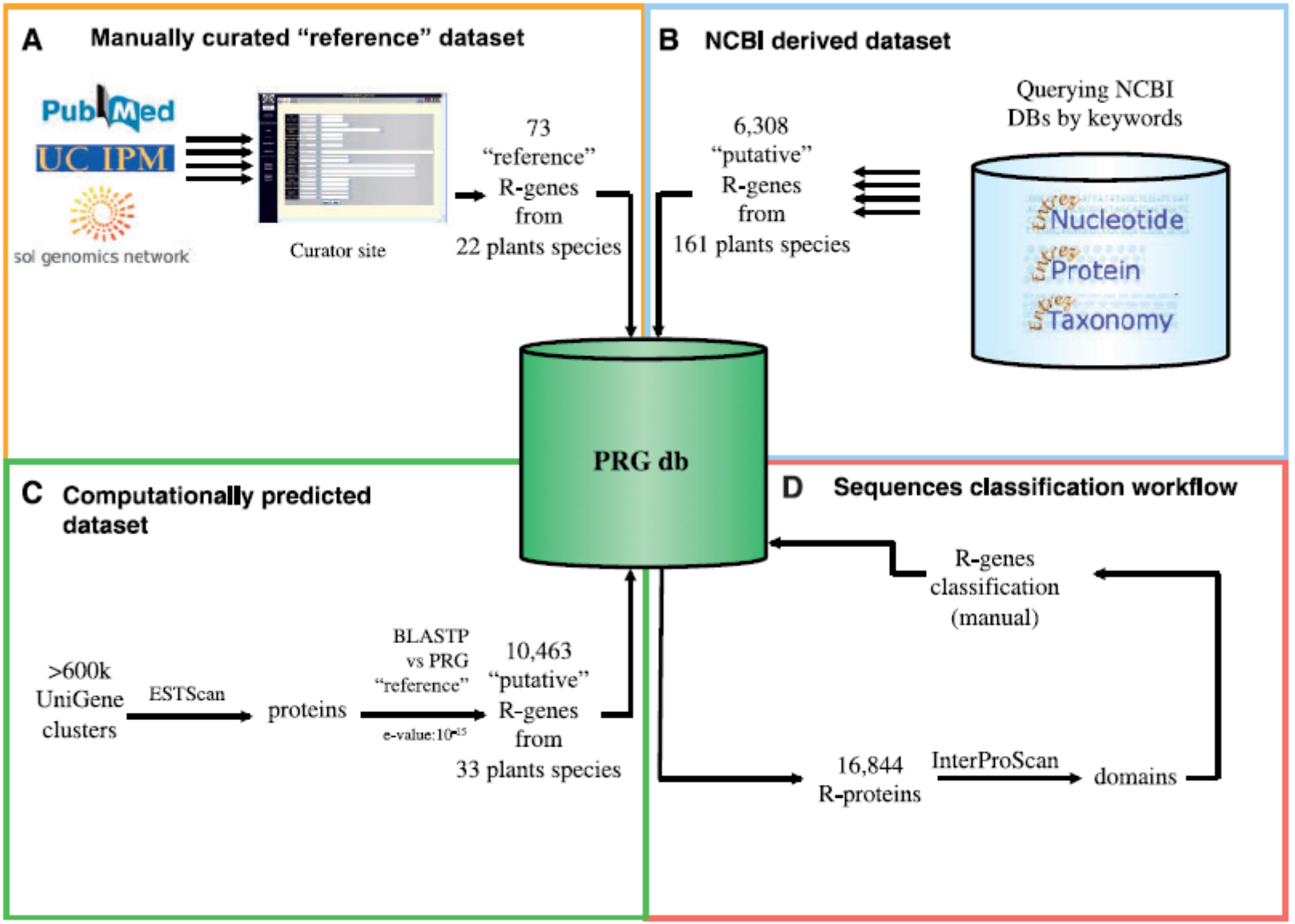
- The complete database includes:

1. A set of 73 manually curated reference R-genes,
2. 6308 putative R-genes collected from NCBI, and
3. 10463 computationally predicted putative R-genes.

Sanseverino *et al.*,2010

Plant disease resistance genes

Plant resistance gene database (PRGdb)



Sanseverino *et al.*,2010

Plant resistance gene database (PRGdb)

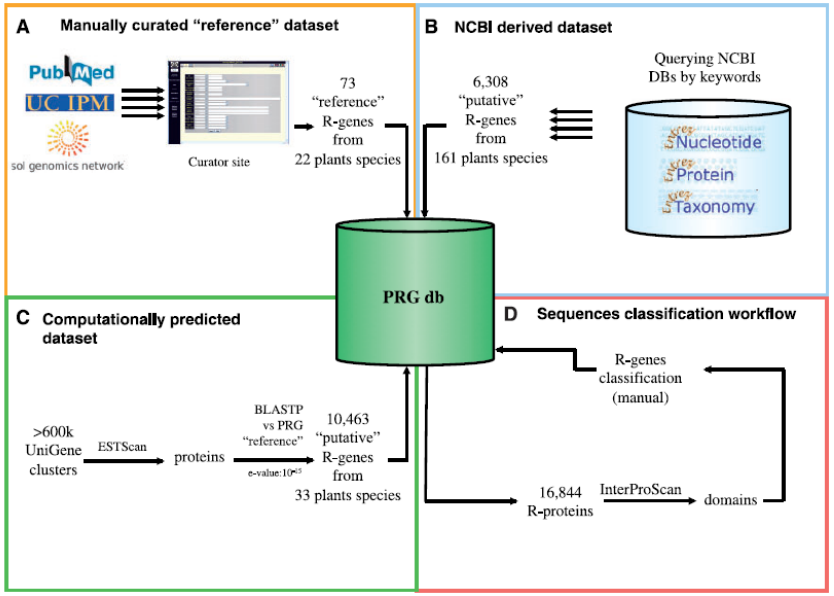
A schematic view of the PRG database showing the origin of dataset used and the sequences characterization

A. The manually curated dataset that contains 73 literature cited R-genes from 22 different plants.

B. The NCBI dataset containing 6308 sequences related to reference R-genes retrieved by the NCBI database.

C. The computationally predicted dataset using the DRAGO pipeline containing 10 463 putative R-genes.

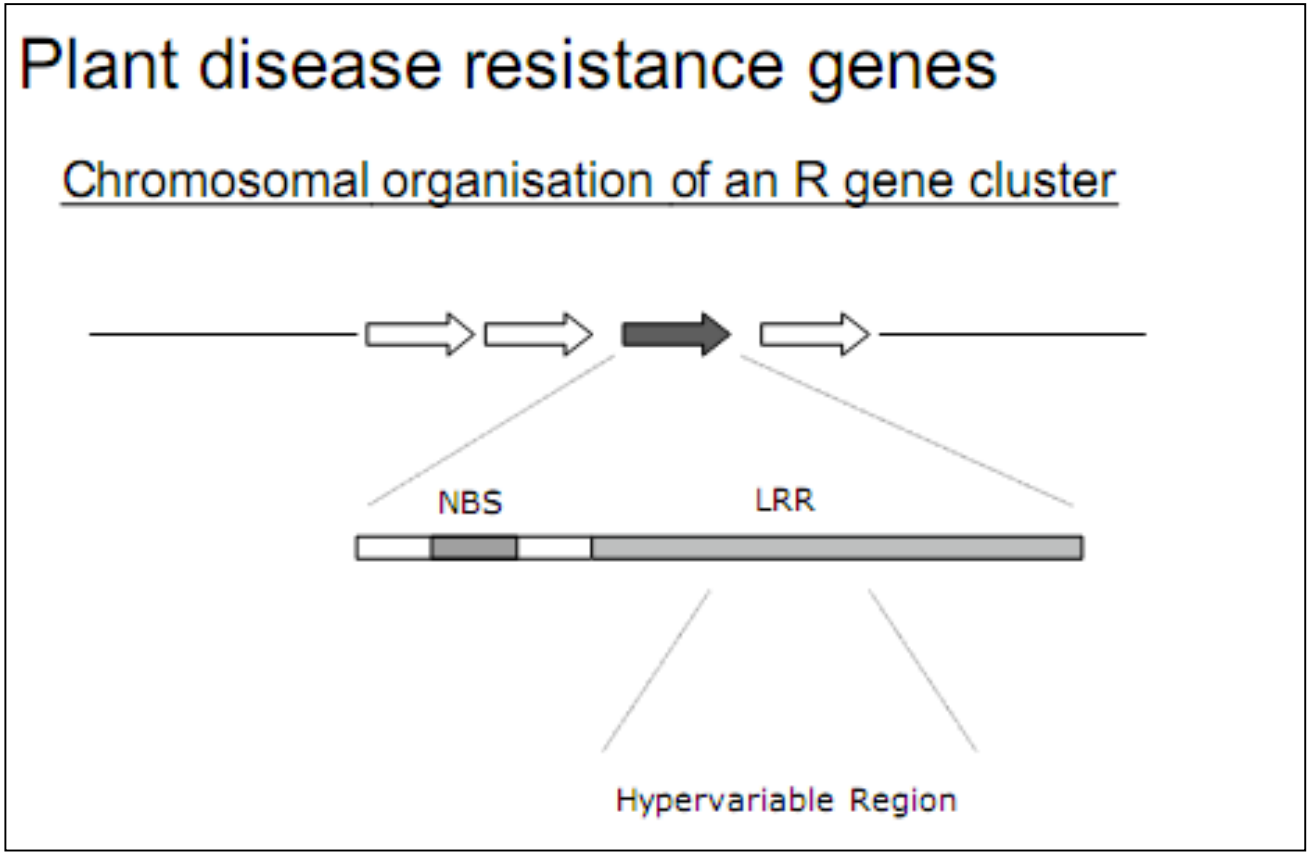
D. Workflow of conserved domain analysis and sequence classification.



Sanseverino *et al.*,2010

Plant disease resistance genes

Chromosomal organization of an R gene cluster

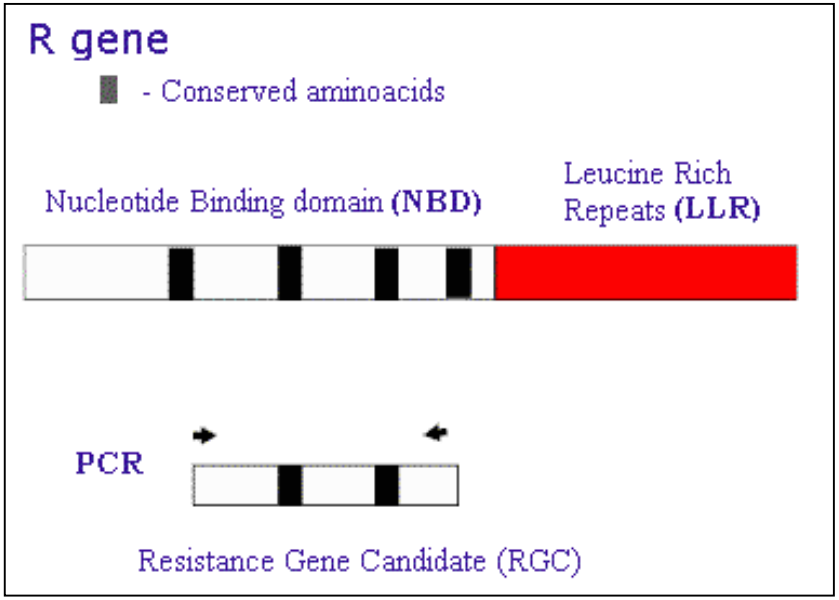


Sophien Kamoun

Plant disease resistance genes

Identification of resistance genes by the polymerase chain reaction

- There are a large number of sequences with similarity to *R* genes in plant genomes.
- PCR using degenerate primers designed to amplify sequences conserved between *R* genes has allowed identification of families of sequences from several plants.



Degenerate primers: These are actually mixtures of similar, but not identical primers. They may be convenient if the same gene is to be amplified from different organisms, as the genes themselves are probably similar but not identical.
Note: only a fraction of the degenerate 'primer' will actually work in the PCR
(See also Diagnosis-part 1 file)

Isolation and cloning of *Pto* homologue sequences from banana by degenerate PCR

Pto-like and receptor-like kinases in banana

- The main aims:

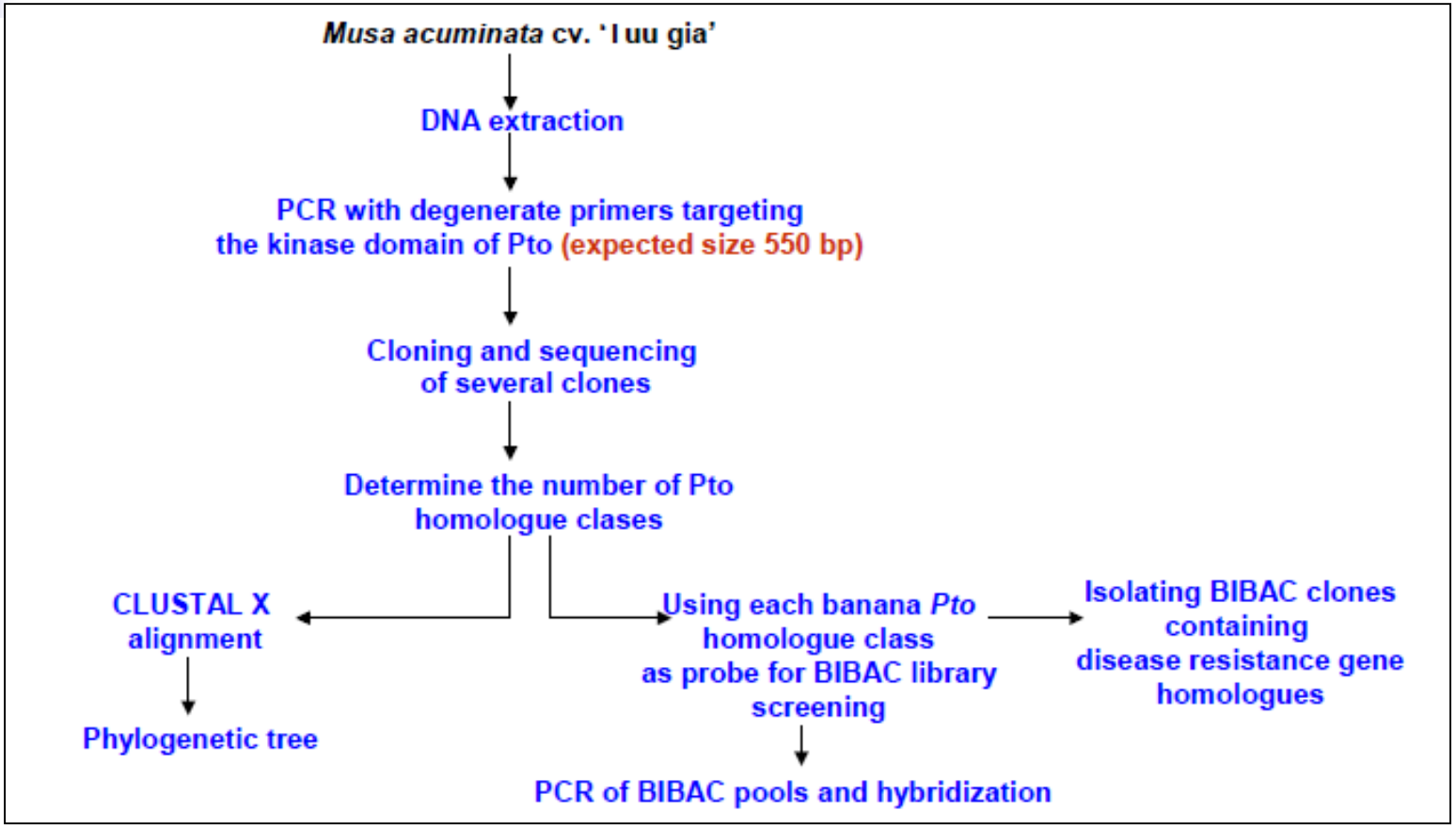
1. To isolate and characterize resistance gene homologues from banana.
2. To isolate and characterize BIBAC clones containing disease resistance gene homologues.
3. To generate a collection of BIBAC clones containing different classes of resistance gene homologues.

A plant transformation-component binary bacterial artificial chromosome (BIBAC) library was constructed from Musa cv. Tuu Gia (AA).

James *et al.*,2007

Isolation and cloning of *Pto* homologue sequences from banana by degenerate PCR

Pto-like and receptor-like kinases in banana

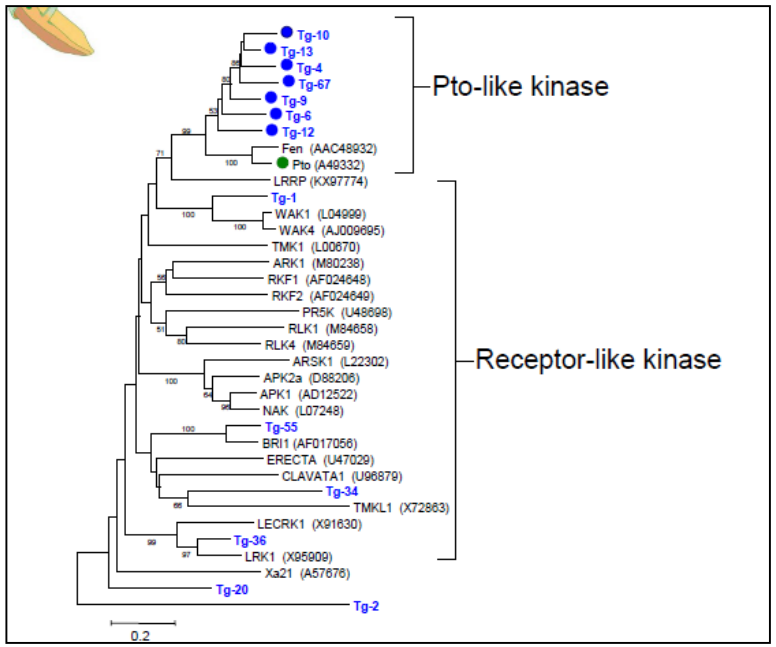


James *et al.*,2007

Isolation and cloning of *Pto* homologue sequences from banana by degenerate PCR

Pto-like and receptor-like kinases in banana

- Neighbour-joining tree based on the ClustalX alignment of the deduced amino acid sequence of 13 kinase-like sequences from banana, cv. 'Tuu Gia' (sequences in blue) and other plant protein kinases.
- The kinase catalytic domains (subdomains II to IX) were used to construct the phylogenetic tree.
- The number below the branches indicate the percentage of 1000 bootstrap replications supporting the particular nodes, and only those above 50% are shown.
- Sequences with blue circles correspond to banana Pto-like sequences and the sequence with a red circle corresponds to the tomato Pto resistance protein.

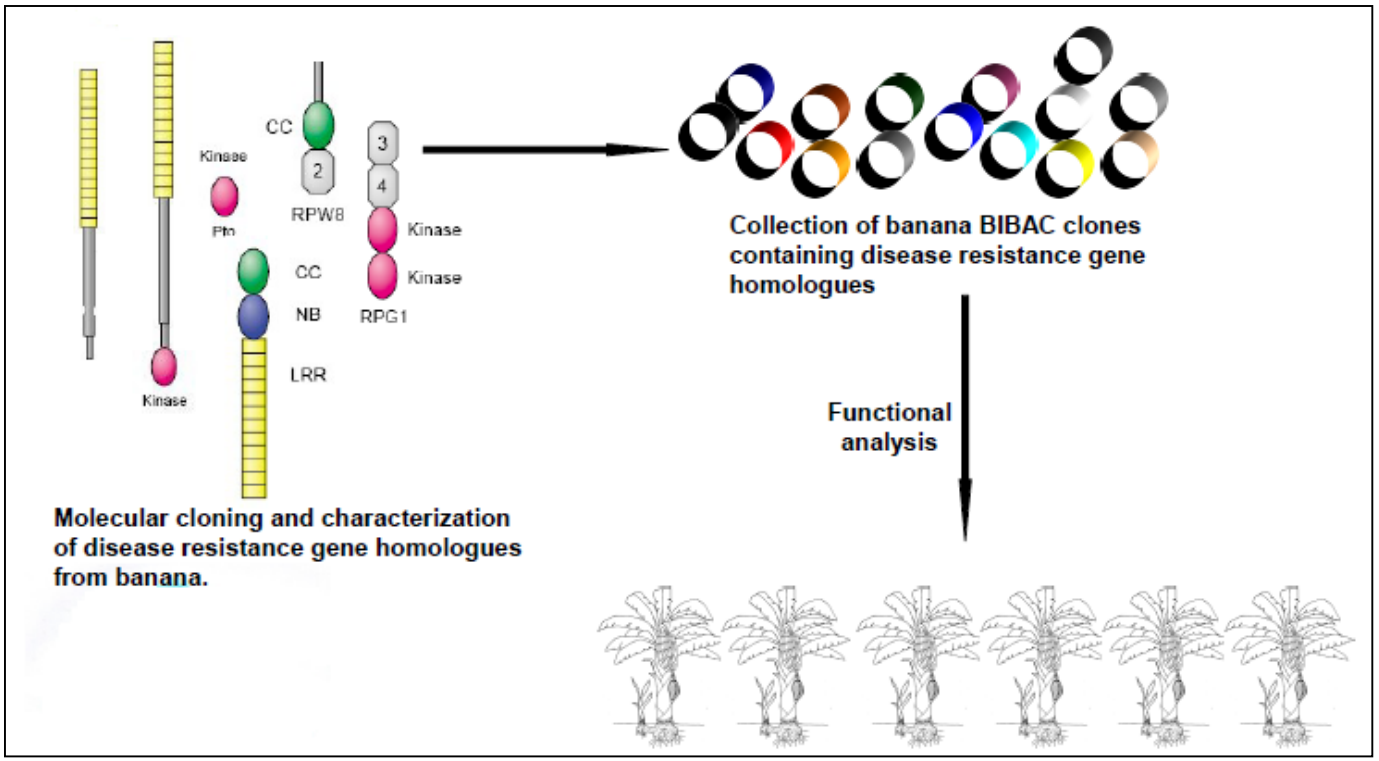


James *et al.*,2007

Isolation and cloning of *Pto* homologue sequences from banana by degenerate PCR

Pto-like and receptor-like kinases in banana

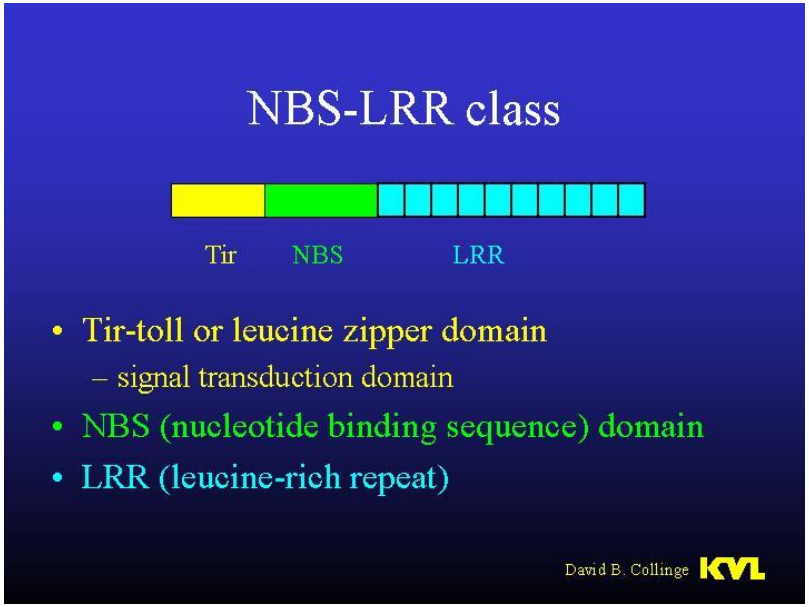
- Towards the development of a collection of banana BIBAC clones containing disease resistance gene homologues.

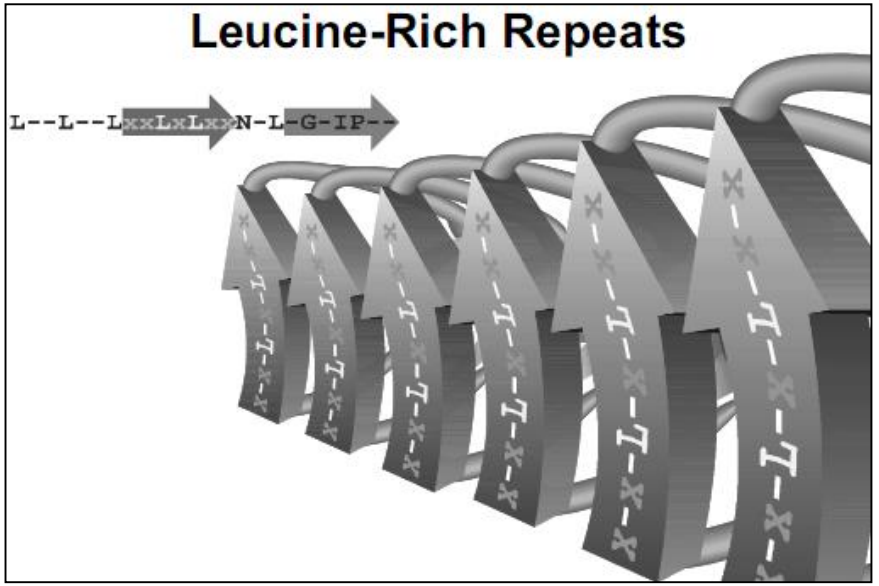


James *et al.*,2007

Common structural features of *R* genes products

The structure of a NBS-LRR protein





Leucine-rich repeats (LRR): Segments of amino acids containing multiple copies of leucine present repeatedly in a protein; these proteins are known as LRR proteins.

Major families of R proteins

- There are several different classes of R genes.
- The major classes are the NBS-LRR genes.
- Within the NBS-LRR class of R genes are two subclasses:

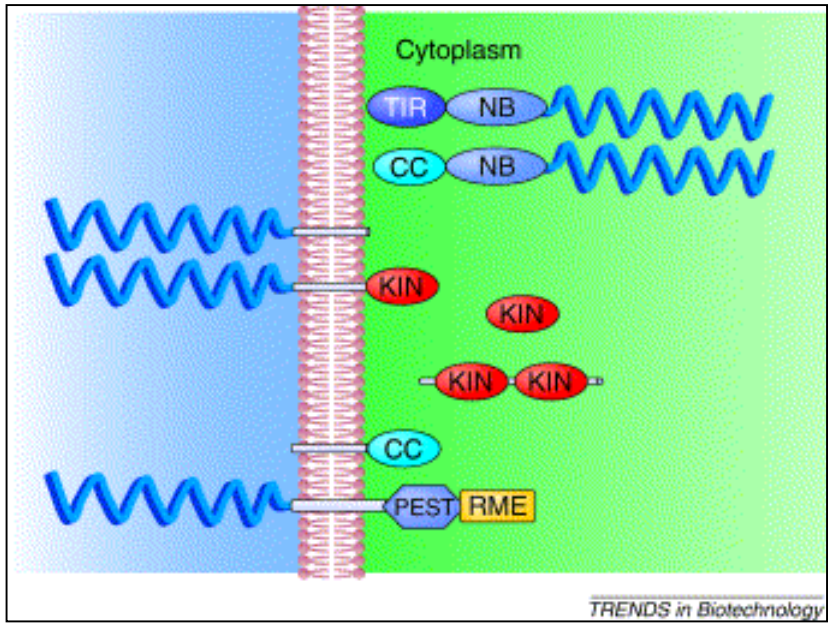
1. One subclass has an amino-terminal Toll/Interleukin 1 receptor homology region (TIR). This includes the *N* resistance gene tobacco against TMV.
2. The other subclass does not contain a TIR and instead has a leucine zipper(LZ)region at its amino terminal.

Major families of R proteins

- NBS-LRR genes carries:

1. Leucine-rich repeats (LRRs), and
2. Nucleotide-binding site (NBS) domains.

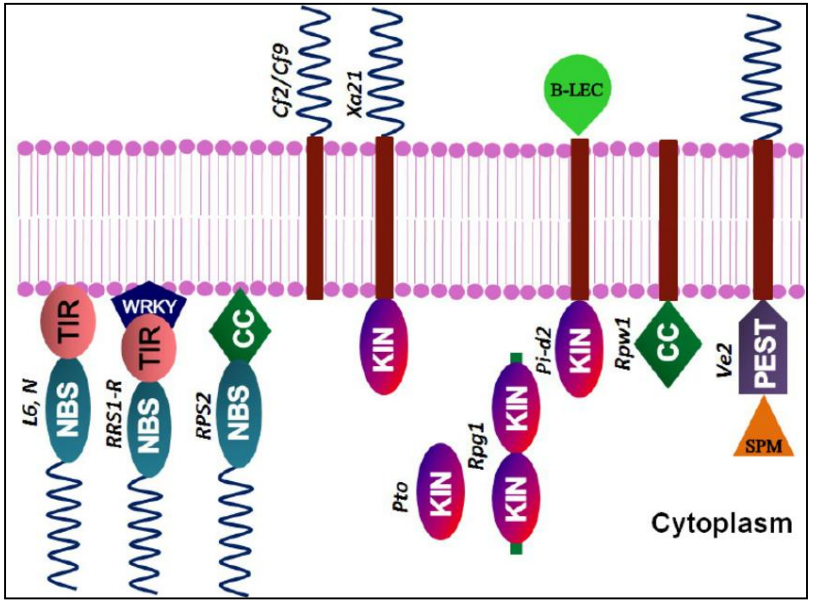
- Leucine-rich repeats (LRRs, depicted in blue), have a major role in recognition specificity.



McDowell and Woffenden,2003

Major classes of R proteins
Plant disease resistance proteins

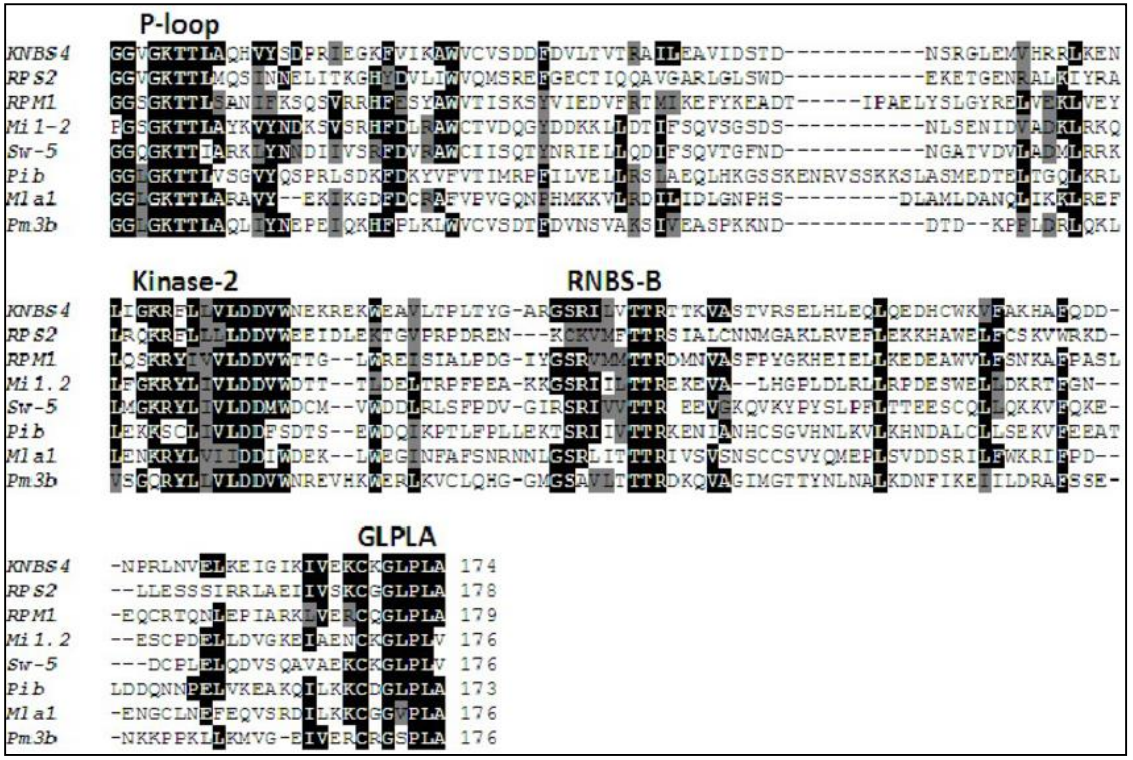
- Most *R* genes contain Leucine- rich repeat motifs (LRR region), suggesting that plants may share common mechanisms for disease resistance to diverse pathogens.



Major classes of R proteins. The widely represented R-protein family (NB-LRR) also contains a nucleotide-binding site (NBS). NB-LRRs are localized in cytoplasm as membrane proteins and contain either a TIR domain (L6 , N) homologous to metazoan Toll-interleukin receptor or a putative coiled-coil (RPS2) domain at the N-terminus. A recent modification identified in the TIR-NBS-LRR protein is an additional transcriptional factor WRKY.

Joshi and Nayak,2011

Major classes of R proteins
Plant disease resistance proteins

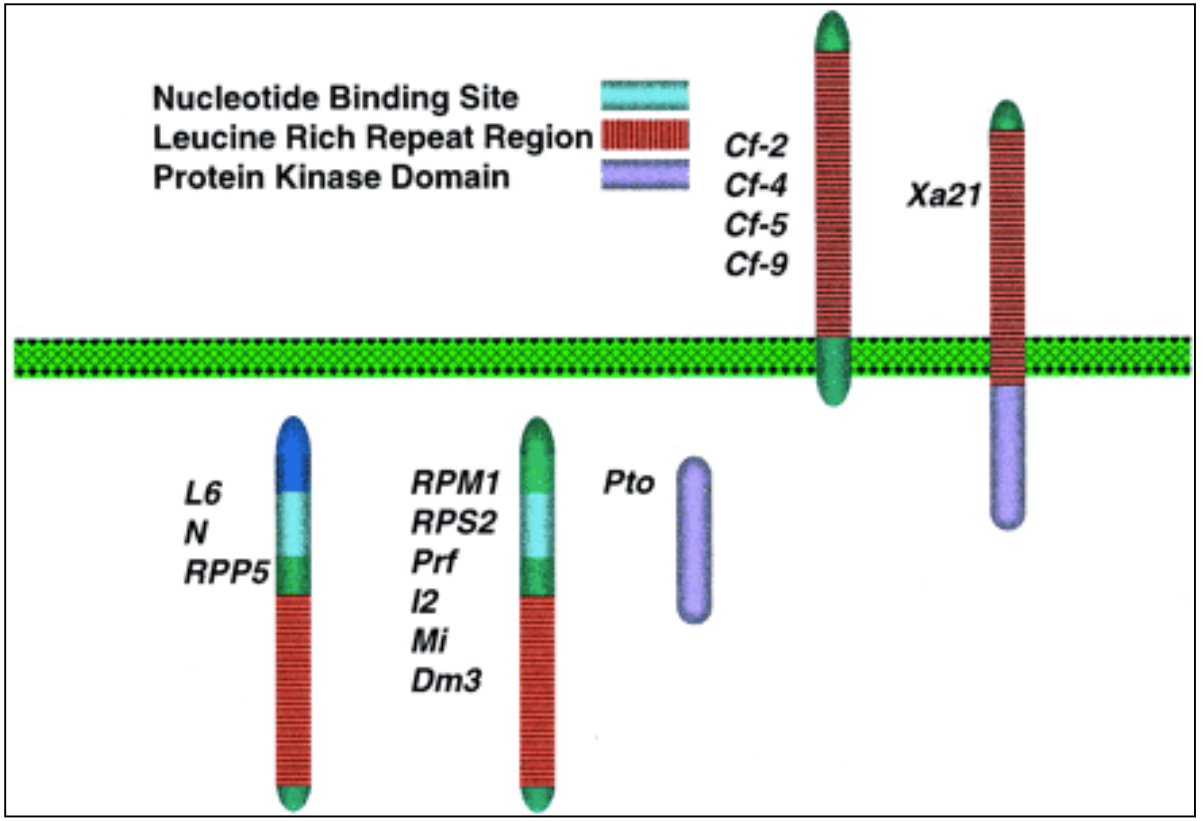


Alignment of the NBS domain of multiple NBS-LRR disease resistance proteins. The conserved NBS domains as determined by Meyers et al. (1999) are indicated. Identical amino acids are shaded in black and conservative substitutions are shaded in gray. NBS = nucleotide-binding site; LRR = leucine-rich repeat.

Joshi and Nayak,2011

Classes of resistance gene

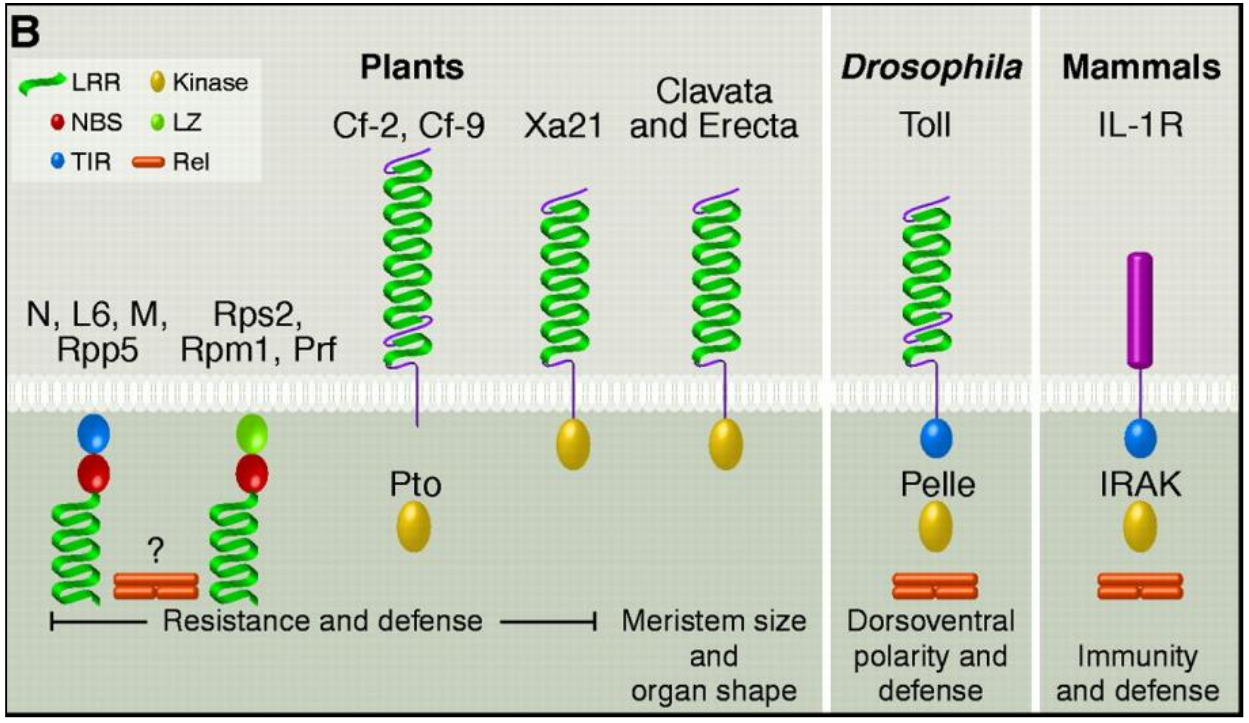
Common motifs



Protein motifs indicate that *R* genes are part of signal-transduction systems.

Classes of resistance gene

Shared structural domains among plant, insect, and mammalian defense and developmental pathway genes



TIR, Toll–IL-1R homology domain; kinase, serine-threonine kinase; LZ, leucine zipper; Rel, Rel-related transcription factors (Dorsal and Dif of *Drosophila* and NF-κB of mammals) and inhibitors (Cactus of *Drosophila* and IκB of mammals).

Baker *et al.*,1997

R gene functions

- *R*-gene-encoded proteins have to fulfill two tasks:

1. To recognize a pathogen-derived signal,
2. To initiate a coordinated plant defense reaction.

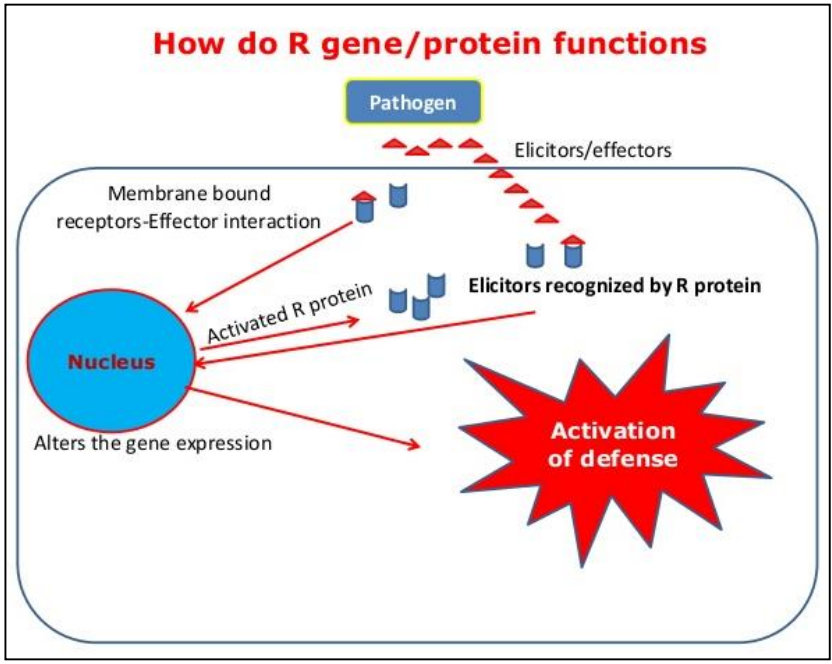
- In another word:

a. Effector recognition- Enable plants to detect *avr* gene specified pathogen molecules.
b. Initiate signal transduction to activate defenses.
c. Have the capacity to evolve new *R* gene specificities rapidly.

Recognition and signal transduction functions of *R* genes

- Resistance gene products do not act alone in controlling defense reactions.
- Different regions of an *R* gene product must function together to conduct both the:

1. Pathogen recognition, and
2. Signal transduction functions.



1. LRR domain

Structure and functions

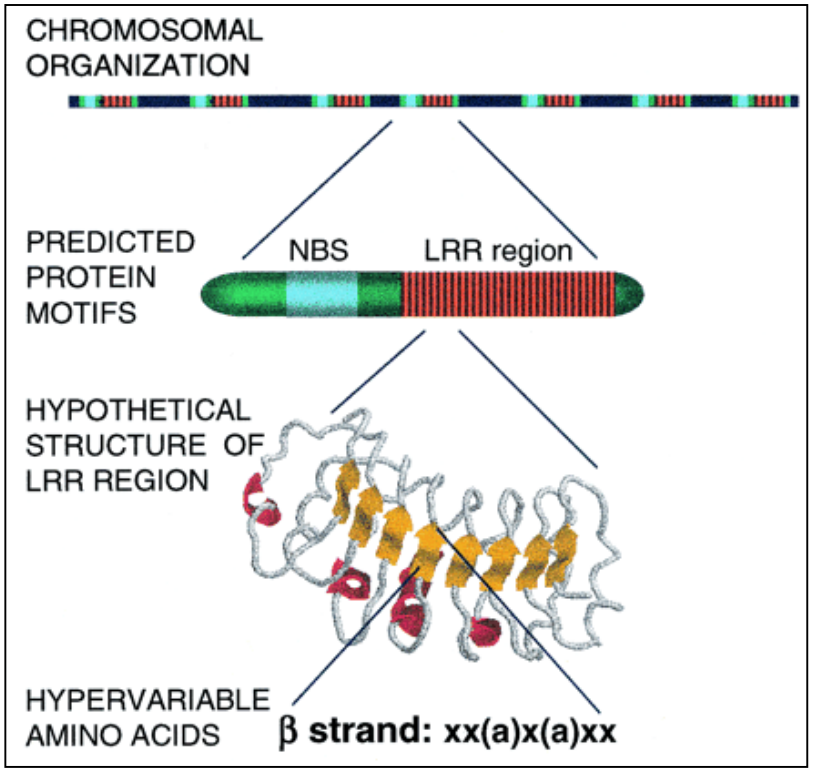
- **Structure:**
- LRR domains are typically thought to be the major determinant of specificity in *R* genes that carry them based on their known history in other proteins and the high levels of polymorphism between alleles in these domains.
- A short stretch of amino acid residues with leucine at every second or third position is repeated to form a flexible, solvent-exposed, parallel beta-sheet.
- **Function:**

1. The LRRs are mainly involved in recognition, whereas
2. the amino-terminal domain determines signalling specificity.

- Have a major role in recognition specificity.

R-gene products are postulated to have receptor and effector domains

- A variety of motifs suggest involvement in protein-protein interactions; these could be involved in ligand binding or effector functions.
- This hypothetical structure shows the thyrotropin receptor.
- In the consensus sequence for the β-strand, (x) any amino acid; (a) aliphatic residues.



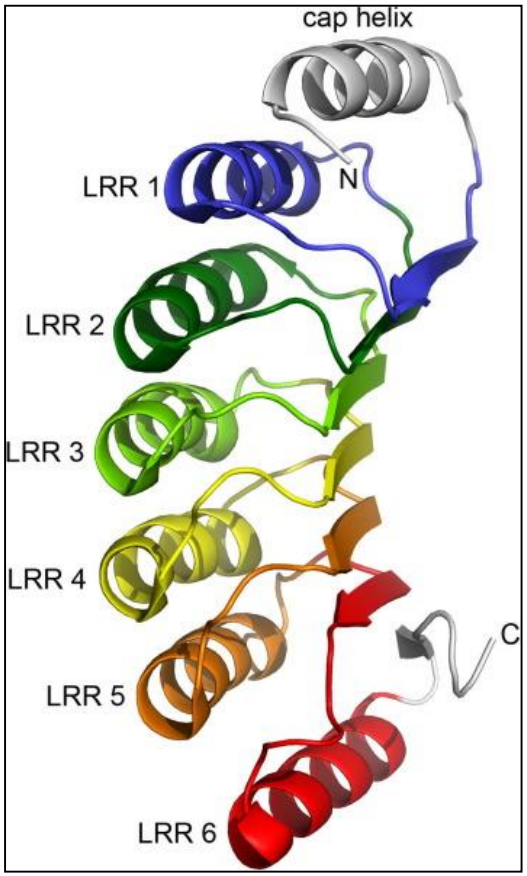
Thyrotropin: A tripeptide hormone. Aliphatic: A protein side chain contains only carbon or hydrogen atoms.

The crystal structure of a protein containing LRRs

- Ribbon diagram of the crystal structure of a protein(ribonuclease inhibitor) that contains leucine-rich repeats (LRRs).
- Each LRR is an iterative(repeat) unit composed of:

1. A roughly parallel α helix (coil structures) joined by a short loop, and
2. A β sheet (inner surface).

- Because the α helices pack less tightly, the molecule forms a horseshoe-shaped structure.



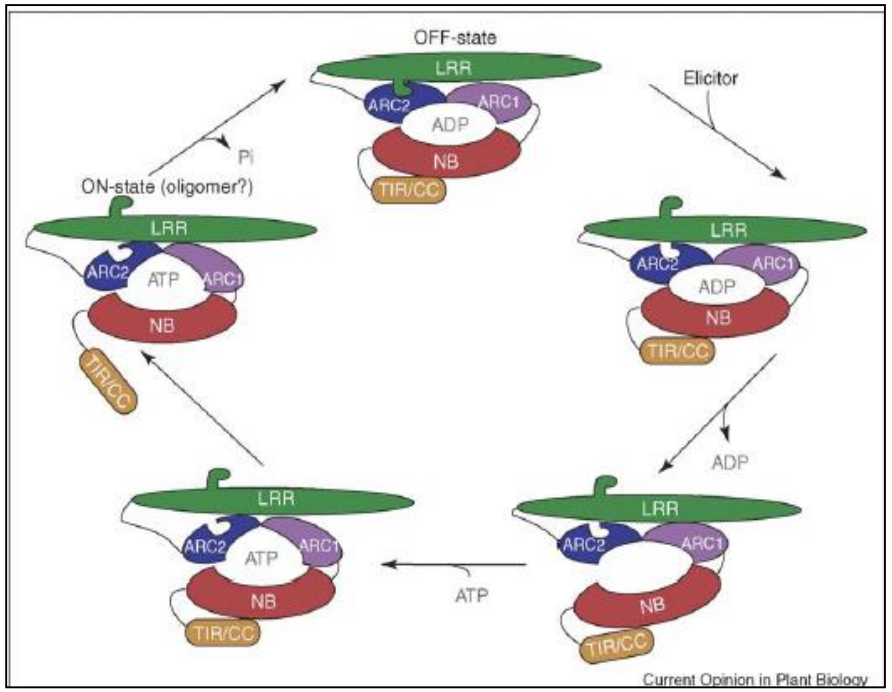
2. NB-ARC domain

Structure and functions

- The NBS forms part of NBARC-LRR domain that presumably functions as a molecular switches of plant defense.
- May affect R protein function through nucleotide binding or hydrolysis.
- In AvrPto/Pto interaction(*Pst*/tomato), the tomato NBARC-LRR protein Prf interacts with Pto Kinase to regulate specific plant immunity.

Model for the switch function of NBS-LRR proteins

- In the absence of a pathogen, an NBS-LRR protein is in the OFF-state (resting state), in which the LRR exerts its negative role by stabilizing the ADP-bound state.
- The presence of an elicitor (Avr) affects the LRR domain, which induces a conformational change in the NB-ARC domain that allows the release of ADP.
- ATP binding subsequently triggers a second conformational change in the N-terminal effector domain, releasing its signalling potential.
- The ATPase activity of the protein attenuates the signalling response and returns the protein to its resting state.



Takken *et al.*,2006

3. TIR domain

Structure and functions

- Some NB-LRR proteins contain a TIR domain at N terminus.

1. Can play a role in pathogen recognition.
2. Implicated in signaling.

The Tir toll, leucine zipper(LZ) domain can also be called coil-coil depending on origin.

4. CC domain

Structure and functions

- Other NB-LRR proteins contain a putative coiled-coil domain (CC) at the N-terminus.
- The CC structure is a repeated heptad sequence with interspersed hydrophobic amino acid residues, of which the leucine zipper is one example.
- This domain may be involved in signaling rather than recognition.

The Tir toll, leucine zipper(LZ) domain can also be called coil-coil depending on origin.

Pto

The first cloned *R* gene

Prf functions as an independent resistant gene

- ***Pto* and *Prf* with cooperative function**
- The *Pto* gene encodes a Ser/Thr kinase but has no apparent receptor domain i.e. LRR.
- It requires the presence of a linked NBS-LRR gene, *prf*, for activity.
- Tomato Prf is a member of the Leucine-Rich Repeat.
- Prf can also function as an independent host recognition determinant of bacterial infection.
- **Pto is a resistance gene which is an argument against the guard hypothesis.**

See also decoy model in this file

Tomato *R*-genes
Pto and *Prf* with cooperative function

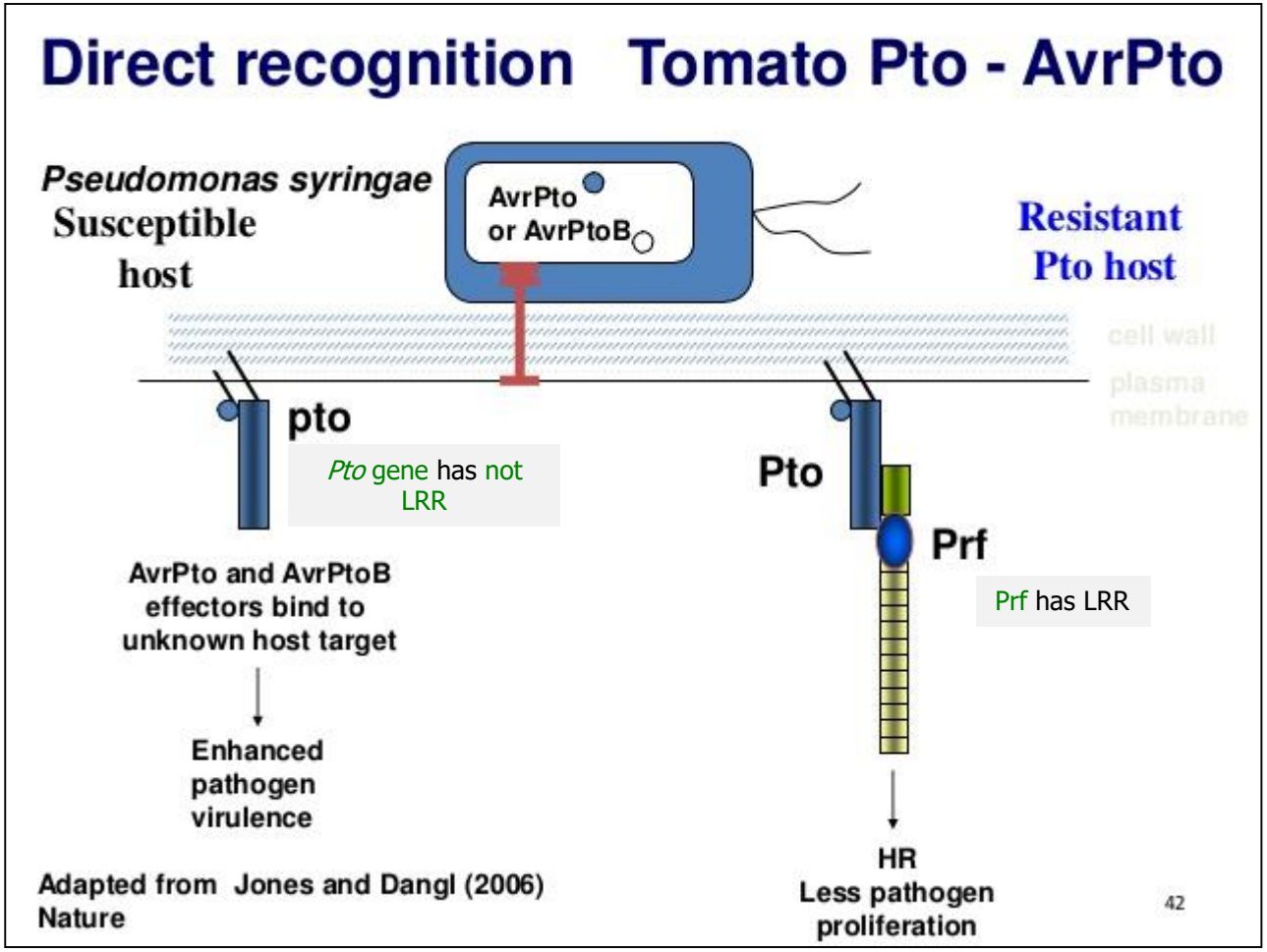
- *Pto* and *Prf* are clustered at a single genomic locus with four *Pto* homologs, which suggests cooperative function.
- Pto appears to signal by a conformational change rather than by phosphorylation of a downstream substrate(s).
- *Prf* on the other hand, is a signaling protein regulated by Pto.
- Pto and Prf act coincidentally in signal transduction.

Mucyn *et al.*,2006

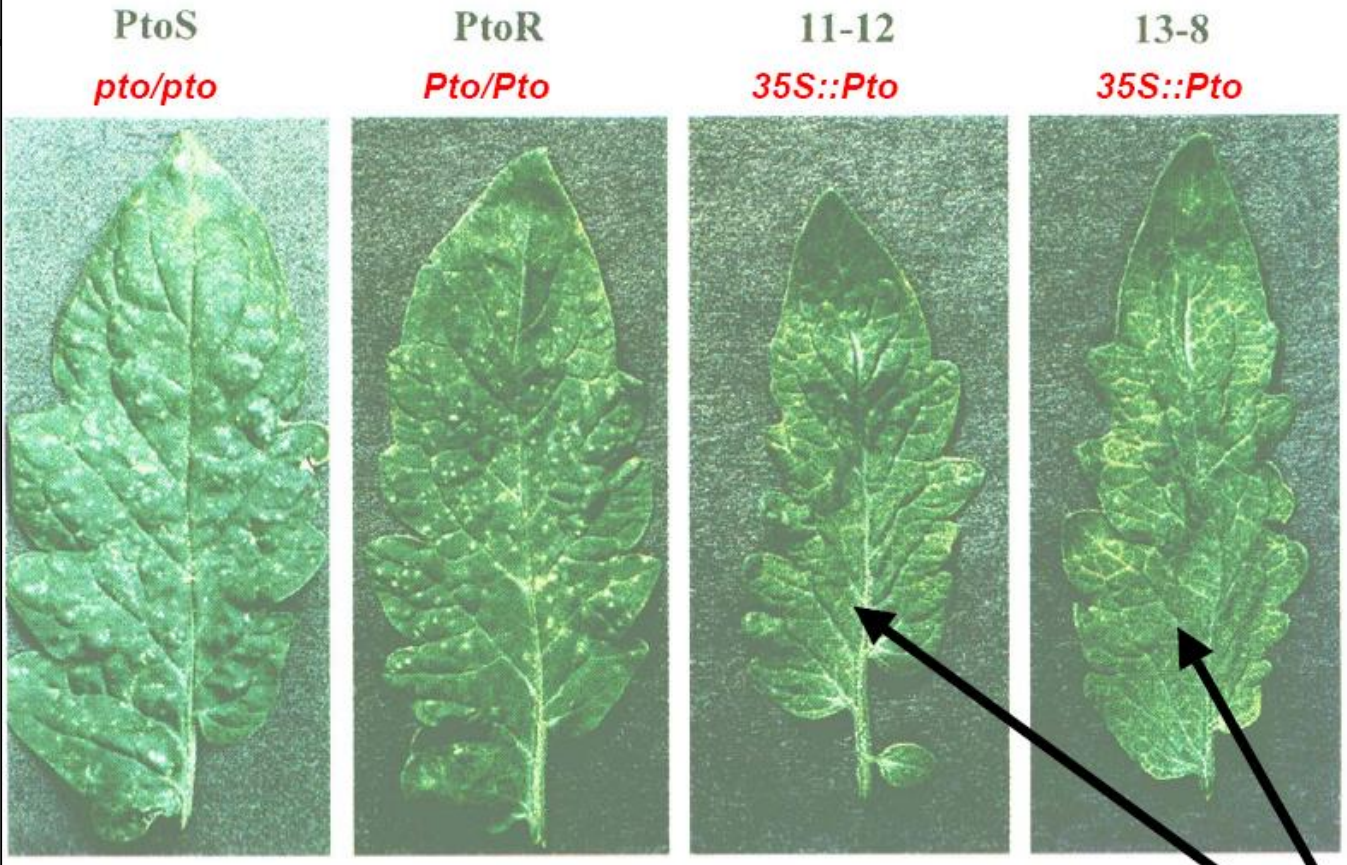
See also NBARC-LRR proteins

Tomato *R*-genes

Pto and *Prf* with cooperative function



Pto-overexpression increases resistance to *Xanthomonas campestris pv. vesicatoria*



Xiaoyan Tang et al., Plant Cell 11:15-29 (1999)

But, Pto overexpression stunts plants.

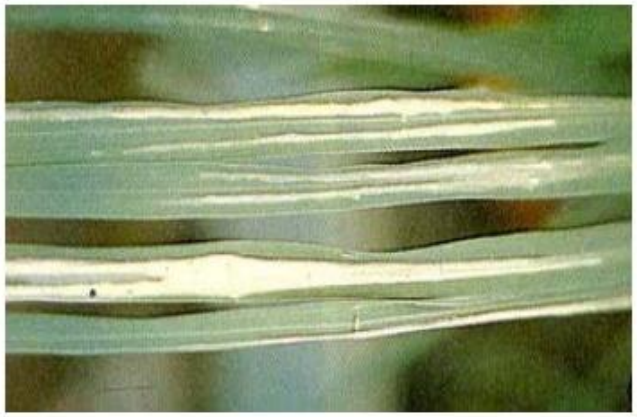
PtoB
The second *R* gene

- A second avirulence gene product, AvrPtoB, interacts with Pto in tomato to trigger a resistance response.
- This response is the classical hypersensitive response (HR) that involves rapid, localized cell death.
- This is an example of programmed cell death (PCD) in plants.
- There is an increasing amount of evidence that some bacterial avirulence products such as AvrPtoB can promote virulence by interfering with the plant defense response.

R genes diverse pathogens encode proteins with leucine-rich repeats (LRRs)

N gene / tobacco mosaic virus

Cf9 gene / fungal leaf mold



Xa21gene / bacterial blight

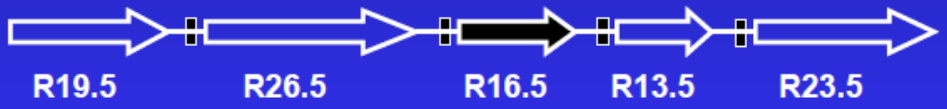
Mi gene / root-knot nematode

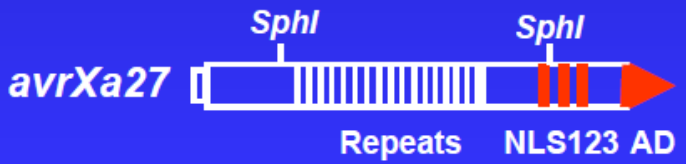
Xa27

Confers resistance to a variety of Xoo strains

***Xa27* confers resistance to a variety of Xoo strains in a gene-for-gene manner**

AvrXa27 resides in a cluster of gene family



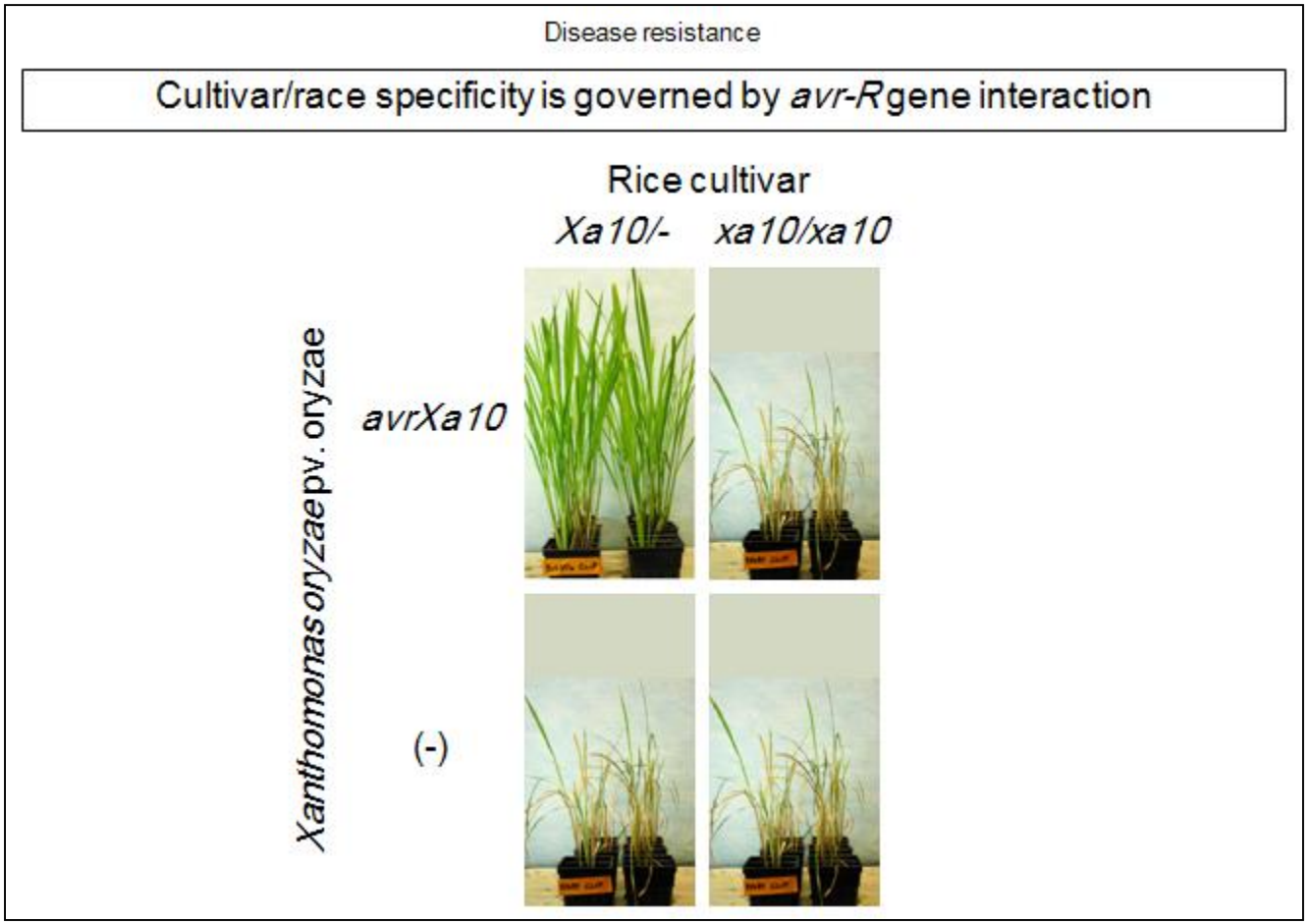


Gu et al, Nature 435: 1122-1125, 2005

Zhaokaijun

Xa10

Confers resistance to *X. oryzae*



Bogdanove,2011

Bacterial pathogenesis on plants
avr/*R* interactions

Plant-Microbe Interactions

1. **Elicitor recognition** (PAMPs/PRRs interactions)
2. **Gene-for-gene hypothesis** (Avr/R interactions)
3. **The guard hypothesis**
4. **Signal transduction**
5. **Mechanisms of general defences**

Pathogens proteins

PAMPs/MAMPs

General elicitors derived from structural components of the bacterial cell

- A relatively recent concept.
- Plants can recognize certain broadly-conserved molecules associated with pathogens- PAMPs (pathogen-associated molecular patterns).
- Also known as MAMPs (Microbe-associated molecular patterns)
- Examples include:

1. Flagellin;
2. Elongation factor-Tu;
3. LPS;
4. etc.

Roger Innes. Tom Ashfield Laura Ong

Pathogens proteins

PAMPs/MAMPs

General elicitors derived from structural components of the bacterial cell

- The bacterial cell wall is an important source of PAMPs. e.g.

1. Peptidoglycans(PGNs) from both gram-positive and gram negative bacteria.
2. Lipopolysaccharide (LPS) is the principal component of the outer membrane of gram-negative bacteria.
3. Flagella(Flagellin) from nearly all bacteria.
4. The elongation factor Tu (EF-Tu) is one of the most abundant and conserved bacterial proteins which acts as a very potent bacterial PAMP.
5. Chitin
6. The bacterial siderophore pseudobactin is also a potential PAMP.

Nicaise *et al.*,2009;..

Plant resistant genes

Kind of receptors

PAMPs/MAMPs vs. PRRs

- Receptor-like kinases (RLKs) and Receptor-like proteins (RLPs) play crucial roles in plant immunity, growth, and development.
- Plants deploy a large number of RLKs and RLPs as pattern recognition receptors (PRRs) that detect microbe- and host-derived molecular patterns as the first layer of inducible defense.

Plant resistant genes

Kind of receptors

PAMPs/MAMPs vs. PRRs

- Plants and animals recognize microbial invaders by detecting:

1. Pathogen genes(effectors), and
2. Pathogen associated molecular patterns (PAMPs) such as flagellin or LPS.

- There are several different classes of R genes.
- The major classes are:

1. The R genes, and
2. plant receptor-like kinases (RLKs) and Receptor-like proteins (RLPs) known as pattern recognition receptors or surface proteins (PRRs).

Known and Probable PRRs Involved in Plant Immunity.

Name	Family	Ligand	Plant
FLS2	LRR-RK	flg22	Arabidopsis
FLS3	LRR-RK	flgII-28	Tomato
EFR	LRR-RK	elf18	Arabidopsis
PEPR1/2	LRR-RK	Peps	Arabidopsis
XA21	LRR-RK	RaxX21-sY	Rice
DORN1	Lectin-RK	eATP	Arabidopsis
LORE	Lectin-RK	LPS	Arabidopsis
WAK1	EGF-Like-RLK	OGs	Arabidopsis
XPS1	LRR-RK	xup25	Arabidopsis
OsCERK1	LysM-RLK	Chitin	Rice
CEBiP	LysM-RP	Chitin	Rice
LYM1/3	LysM-RP	PGNs	Arabidopsis
LYP4/6	LysM-RLP	PGNs/chitin	Rice
RLP23	LRR-RP	nlp20	Arabidopsis
NbCSPR	LRR-RP	csp22	*N. benthamiana*
LeEix1	LRR RP	Eix	Tomato
LeEix2	LRR-RP	Eix	Tomato
ReMax/RLP1	LRR-RLP	eMax	Arabidopsis
Ve1	LRR-RLP	Ave1	Tomato
Cf-2	LRR-RLP	Avr2	Tomato
Cf-4	LRR-RLP	Avr4	Tomato
Cf-4E	LRR-RLP	Avr4E	Tomato
Cf-9	LRR-RLP	Avr9	Tomato
Cf-5	LRR-RLP	Unknown	Tomato
RLP30	LRR-RLP	SCFE1	Arabidopsis
ELR	LRR-RLP	Elicitin	Potato

Known PRRs refer to genetically confirmed receptors whose binding to patterns has been biochemically demonstrated, whereas probable PRRs refer to likely receptors with genetically confirmed roles in pattern recognition, but for which direct binding to patterns remains to be shown.

Breiden and Simon,2016

Pathogens proteins

Kind of receptors

PAMPs/MAMPs vs. PRRs

- While some RLKs and RLPs are known to act as PRRs that perceive danger signals,
- Others regulate
- plant growth and development,
- reproduction,
- symbiosis, and
- tolerance to abiotic stresses.

Breiden and Simon, 2016

Pathogens proteins

Kind of receptors

PAMPs/MAMPs vs. PRRs

- PAMPs carry out an essential function for fitness or survival.
- Plants recognize a wide range of bacterial PAMPs, most of which are derived from structural components of the bacterial cell(non specific).
- PAMPs are recognized by PRRs(pattern recognition receptors or surface proteins).

Nicaise *et al.*,2009

Pathogens proteins

1.PAMPs-PRRs interactions

General elicitors derived from structural components of the bacterial cell

- PAMPs and PRRs activate the plant basal defence responses.
- This detection leads to PAMP-triggered immunity (PTI), which effectively prevents colonization of plant tissues by non-pathogens.
- The most well studied system in PTI is the FLS2-dependent pathway.
- FLS2 recognizes the PAMP flg22 that is a component of bacterial flagellin.

See also Zigzag theory

Nicaise *et al.*,2009; Chakravarthy *et al.*,2009

Plant resistant genes

The receptors

Plant receptor-like kinases (RLKs) proteins

- The transmembrane kinases are collectively named the receptor-like kinases(RLKs).
- Receptor-like kinases (RLKs) are surface localized, transmembrane receptors comprising a large family of well-studied kinases.
- RLKs participate in a diverse range of processes, including:

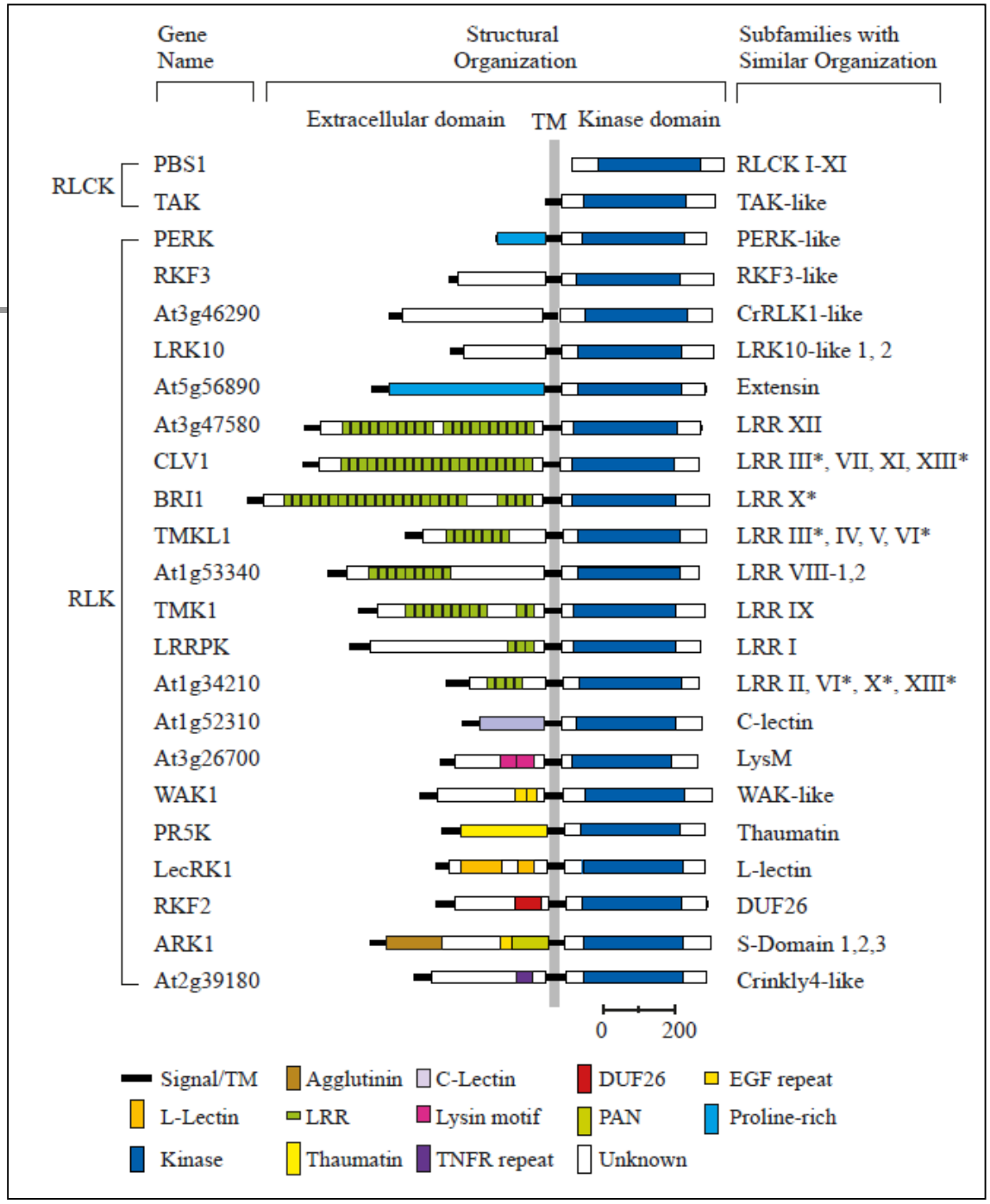
1. disease resistance,
2. regulation of development hormone perception, and
3. several interacting components upstream or downstream to the receptor protein kinases.

- **RLKs possess three domains:**

1. an N-terminal (extracellular) domain,
2. an intermediate transmembrane domain,
3. and a C-terminal kinase domain.

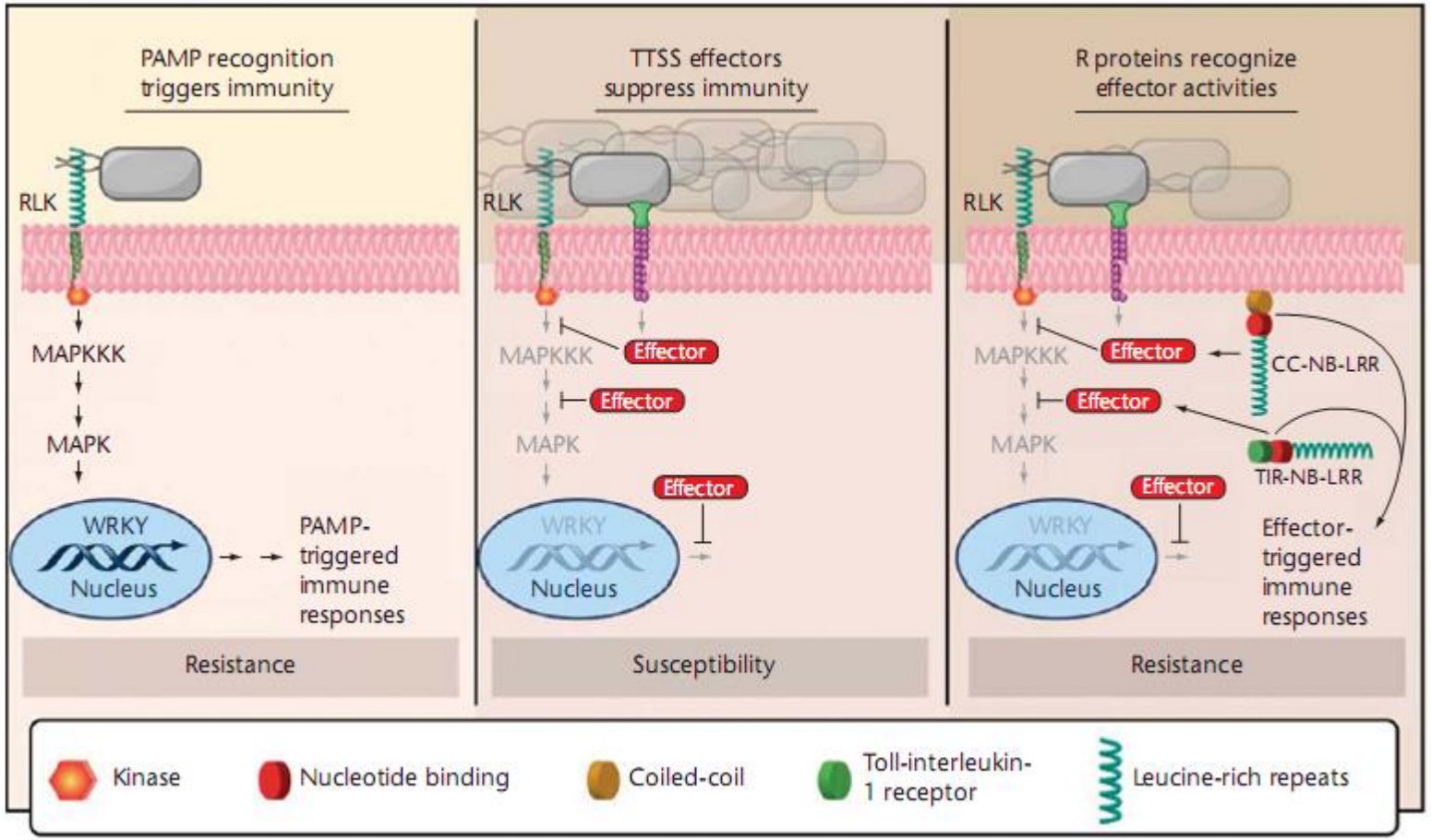
- The PRKs can be classified on the basis of their extracellular domains.

Shiu and Bleecker,2001



Plant R genes

The receptors



Rouxel and Balesdent,2010

Gene-for-gene hypothesis

2. Avr/R interactions

Flor concept

- The gene-for-gene hypothesis (Flor,1971; Keen,1990) explains host-pathogen recognition events.
- Plants with a particular resistance gene (*R* gene) recognize a pathogen-produced elicitor, a direct or indirect product of the corresponding avirulence (*avr*) gene.

Pioneer leaders in the field of molecular plant pathology

Harold Henry Flor, 1900-1991

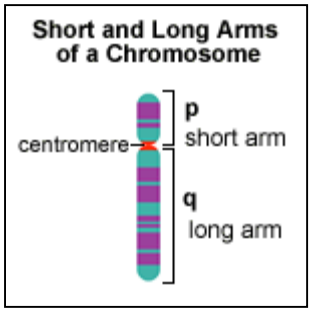
- In the 1940s, Flor developed the gene-for-gene concept to explain the genetic interactions between *Melampsora lini* and flax.
- This concept provided the underpinnings for research on the genetics of host-pathogen interactions for the next 70 years.

Noel Thomas Keen, 1940-2002

- He coined the term "elicitor" to describe the chemicals given off by invading organisms that elicited phytoalexin response in the host plant.
- This work led him to identify the single gene in *P. syringae* responsible for producing elicitors.

Flor concept

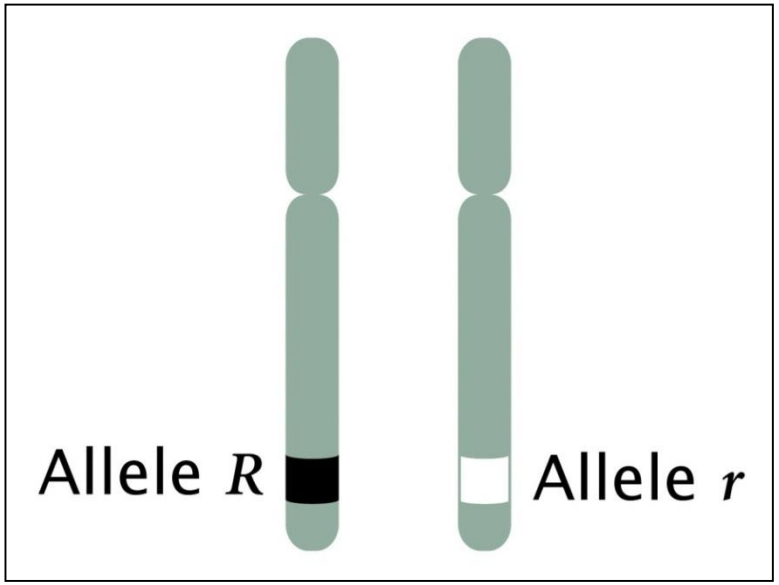
- Flor showed that a plant *R* gene and a pathogen avirulence gene (*avr* gene) are two dominant genes. i.e. many *R*-genes are dominant, as are their cognate pathogen avirulence (*avr*) genes.
- Only when both host *R* and the cognate pathogen *avr* gene are present, is resistance induced (HR and SAR).
- Interaction will be incompatible when the two genotypes possessed both genes corresponding to each other.
- In other situations(all other combinations), i.e. with the occurrence of *r* and/or *avr* recessive alleles, the interaction was compatible.



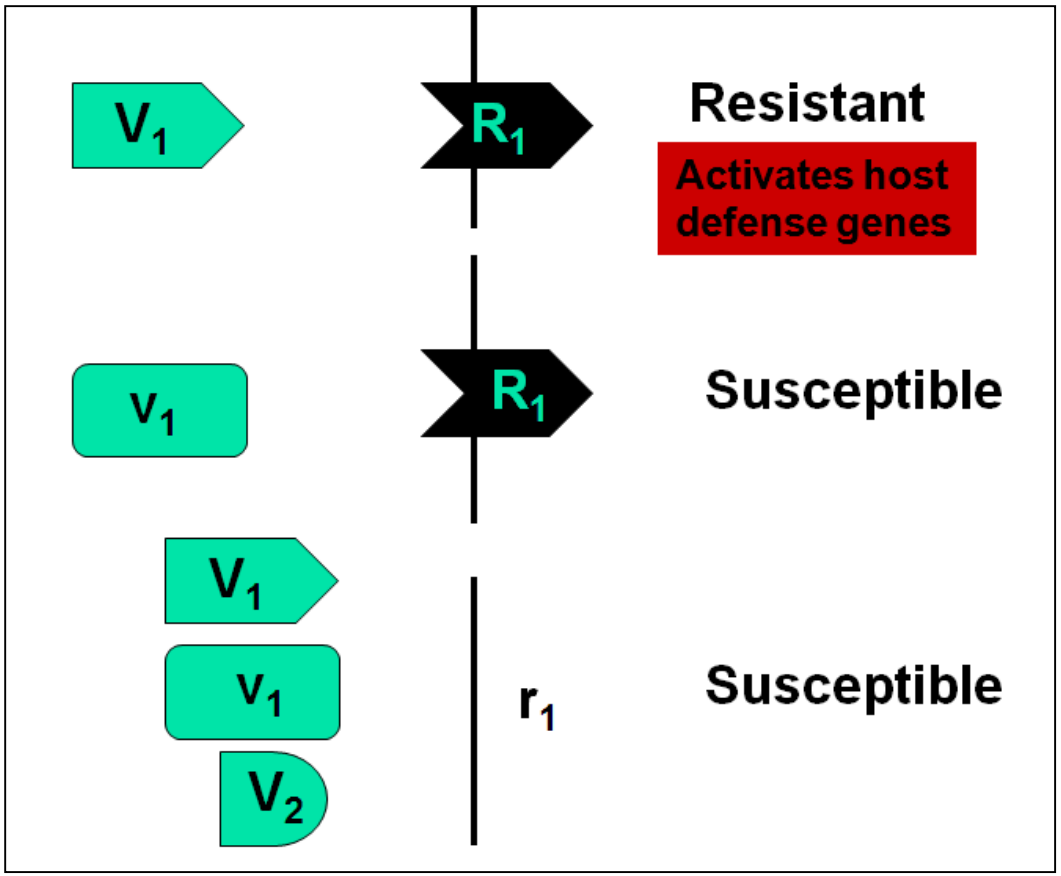
R genes

- At each locus, a diploid organism possesses two alleles located on homologous chromosomes, one inherited from each parent.
- These alleles may be

1. The same homozygous (two of the same alleles at a locus), or
2. Heterozygous (two of different alleles at a locus).

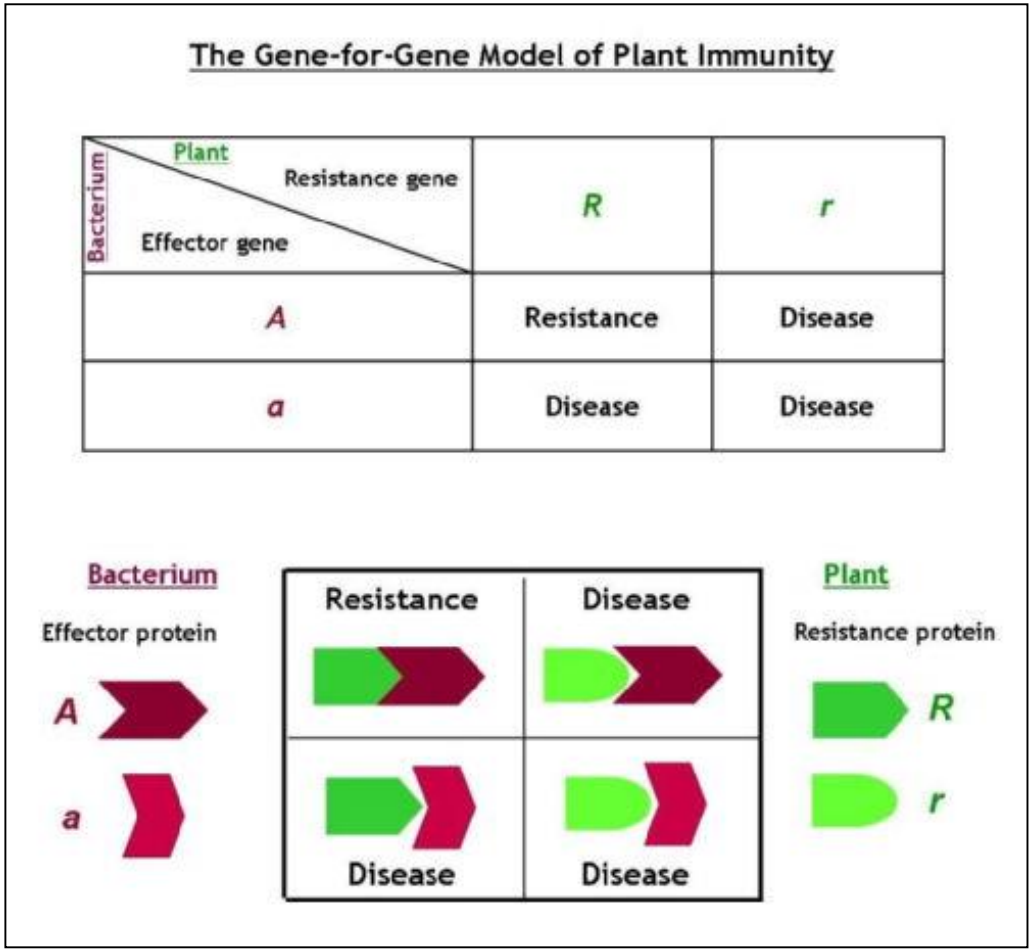


Gene-for-gene model



Virulence based on simple inheritance of a single recessive gene for virulence.

Gene-for-gene model



Warwick,2009

Gene-for-gene interaction between host and pathogen

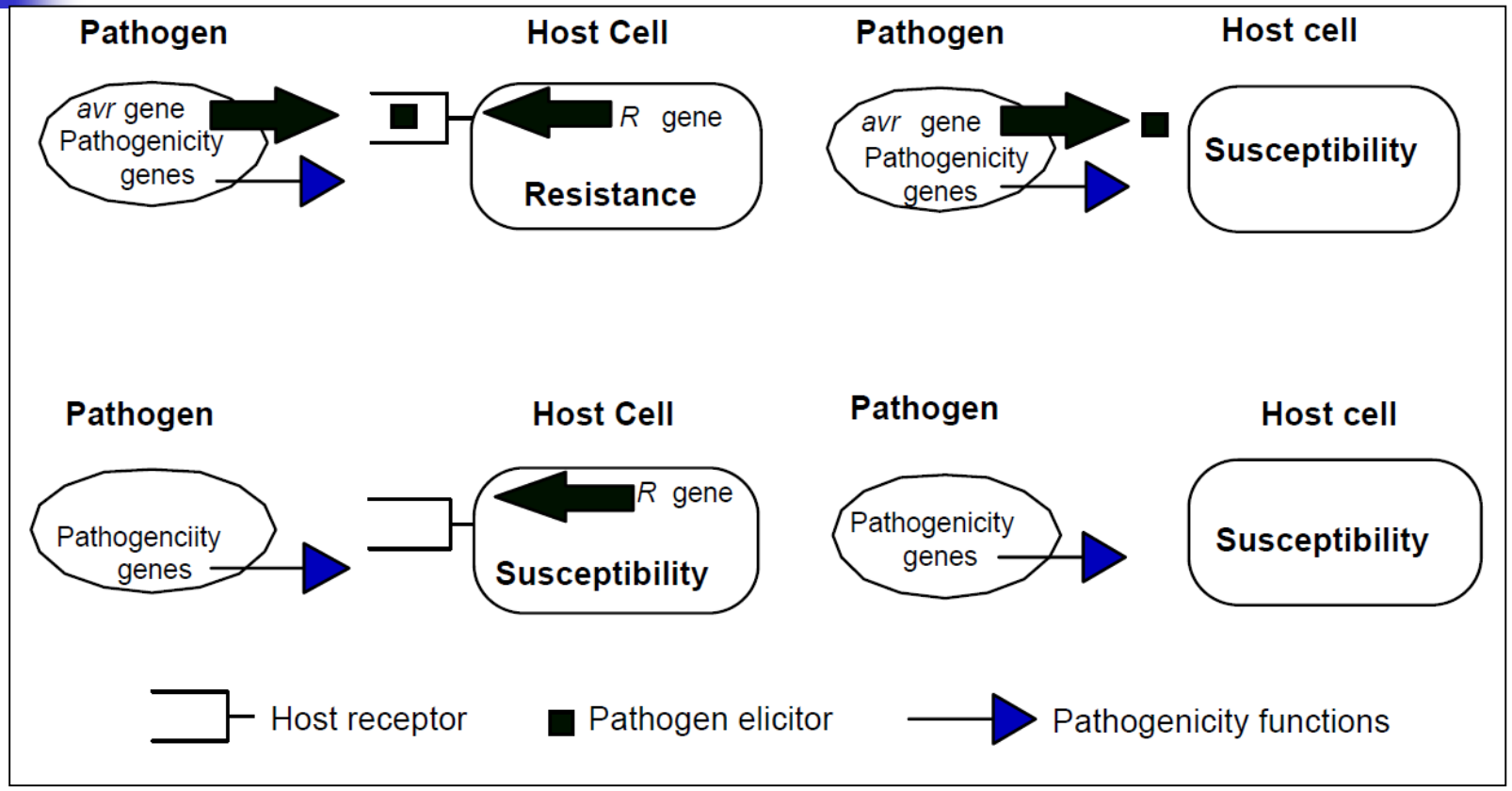
One pair of loci		Pathogen genotypes		
		AA	Aa	aa
Host genotypes	rr	+	+	+
	Rr	–	–	+
	RR	–	–	+

– = R (incompatible) + = S (compatible)

Virulence based on simple inheritance of a single recessive gene for virulence.

Gene-for-gene model

Host-pathogen gene-for-gene specificity model



Adapted from Lucas,1998

Gene-for-gene model

Gene specific interactions

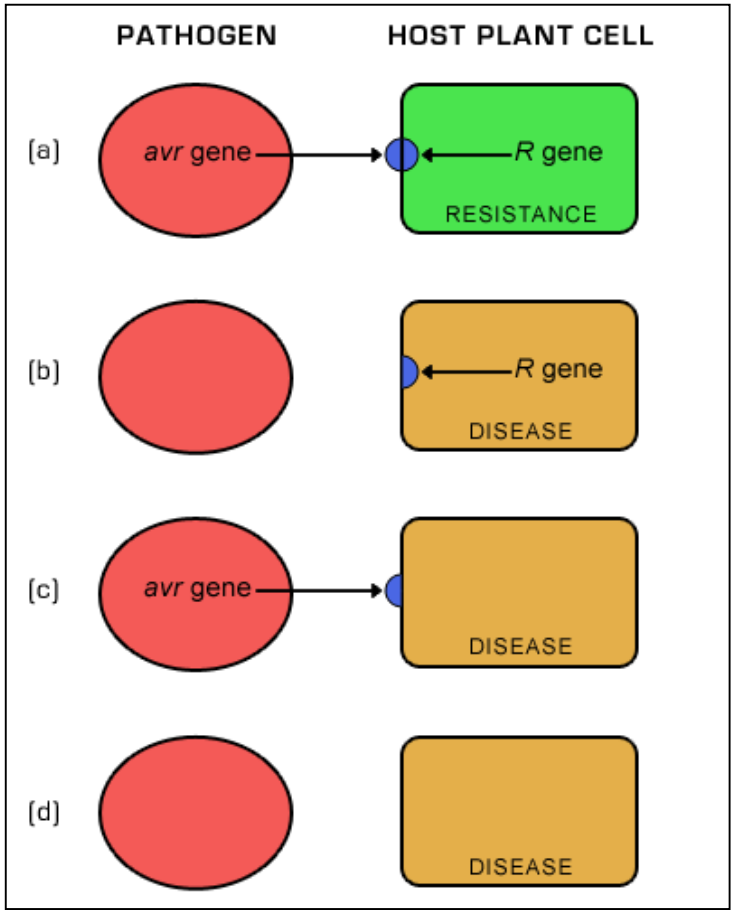
- Resistance is only induced when a plant cultivar in possession of a specific resistance (*R*) gene recognizes a pathogen race that contains:

a) The corresponding avirulence (*avr*) gene,

b) The absence of either the avirulence gene,

c) The resistance gene (*R*), or

d) absent in both interacting organisms which leads to lack of recognition by the host plant and the onset of disease.



Allele symbols

R = red *r* = white

The Red × Red mating must have been:

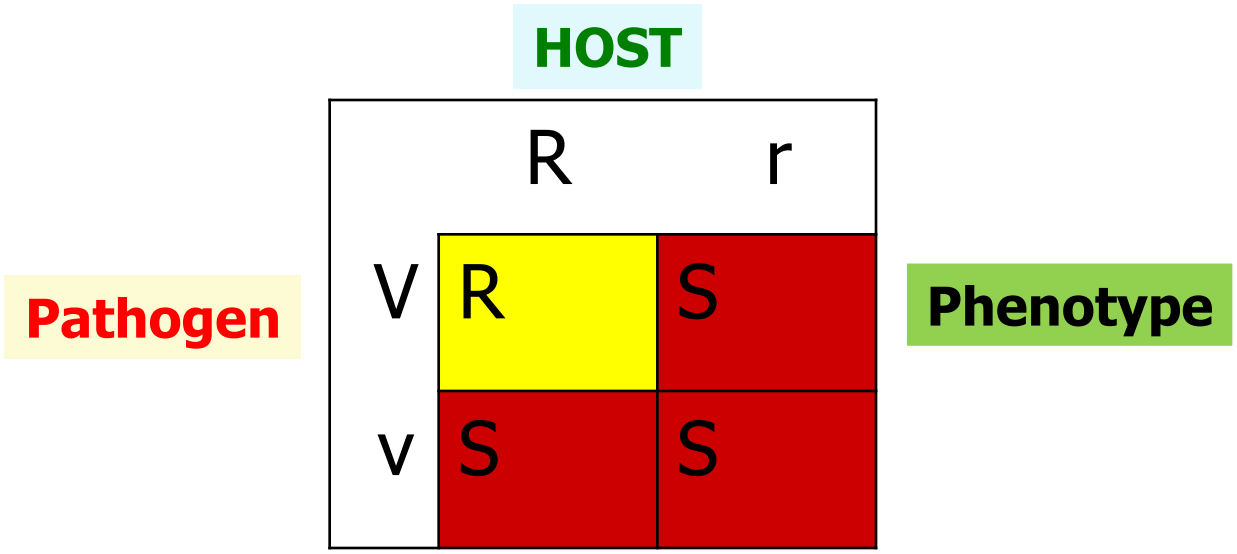
Rr* × *Rr

	R	***r***
R	***RR*** **Red**	***Rr*** **Red**
r	***Rr*** **Red**	***rr*** **White**

Resistance determined by single dominant gene

Pathogen	Plant R1	Plant R2
Avr1	Incompatible (Resistance)	Compatible (Susceptibility)
Avr2	Compatible (Susceptibility)	Incompatible (Resistance)

Virulence based on simple inheritance of a single recessive gene for virulence



Resistance determined by single dominant gene

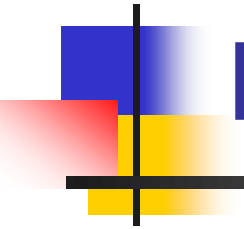
Virulence based on simple inheritance of a single recessive gene for virulence

HOST

Pathogen

	R_1R_2	R_1r_2	r_1R_2	r_1r_2
V_1V_2	R	R	R	S
V_1v_2	R	R	S	S
v_1V_2	R	S	R	S
v_1v_2	S	S	S	S

Phenotype



Elicitors

Introduction

Definition

The term elicitor

- A physical, chemical or biological stimulus that triggers defence responses in plants.
- Originally the term elicitor was used for molecules capable of inducing the production of phytoalexins, but it is now commonly used for compounds stimulating any type of plant defence.
- This broader definition of elicitors includes:
- Both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors).

Classification of elicitors

- According Radman *et al.,* 2003, elicitors are classified as:
- Physical or chemical;
- Biotic or abiotic;
- Complex or defined, depending on their origin and molecular structure.

Angelova *et al.*,2006

Classification of elicitors

- Elicitors may be also classified into two groups:

1. **General elicitors:** Including oligosaccharides, glycopeptides, and peptides (flagellins).
2. **Race specific elicitors:** While general elicitors are able to trigger defence both in host and non-host plants, race specific elicitors induce defence responses leading to disease resistance only in specific host cultivars.

General elicitors

Elicitors of plant defences

Elicitors of plants

Elicitors						Reported effects on
Physical Elicitors	Injury					P
	Abiotic		Metal ions (lanthanum, europium, calcium, silver, cadmium), oxalate			Pc
Chemical Elicitors	Biotic	Complex Composition	Yeast cell wall, Mycelia cell wall, Fungal spores			Pc ,F
		Defined Composition	Carbohydrates	Polysaccharides	Alginate	Pc, F, B
					LBG	F
					Pectin	Pc, F
					Chitosan	Pc
					Guar Gum	Pc
				Oligosaccharides	Mannuronate	F
					Guluronate	F
					Mannan	F
					Galacturonides	Pc
				Peptides	Glutathione	Pc
			Proteics	Proteins	Cellulase, Elic-itins, Oligandrin	Pc
			Lipids		Lipopolysaccharides	Pc
			Glycoproteins		Not characterized	Pc
			Volatiles		C_6-C_{10}	Pc

Abbreviations: P, plants; Pc, plant cell culture; B, bacterial cell culture; F, fungal cell culture.

Elicitors of plant defences

- Plants are able to recognize pathogen-derived molecules; elicitors that trigger a number of induced defences in plants.
- Microbial elicitors
- Constitute a bewildering array of compounds including different:

1. Oligosaccharides,
2. Lipids,
3. Peptides, and
4. Proteins.

Elicitor of pathogens

Signal compounds different form toxins

- Elicitors act as signal compounds at low concentrations, providing information for the plant to trigger defence, distinguishing elicitors from toxins, which may act:

1. Only at higher concentrations, and/or
2. Affect the plant detrimentally without active plant metabolism.

Elicitor of pathogens
Avr-R interactions

- Plant recognition of pathogens is mediated by large families of highly polymorphic *R* genes.
- The products of these genes function to recognize directly or indirectly the products of pathogen-encoded *avr* genes.

Pathogens genes
avr genes

- Many plant and animal pathogens are evolutionary cousins that have the ability and propensity to share genetic material.
- The greater evolutionary potential was seen in bacterial pathogens, due to their rapid generation time and ability to exchange genetic material horizontally.
- The pathogen *avr* genes are only present in certain strains of a given pathogen and show little similarity to each other.

Plant resistant genes

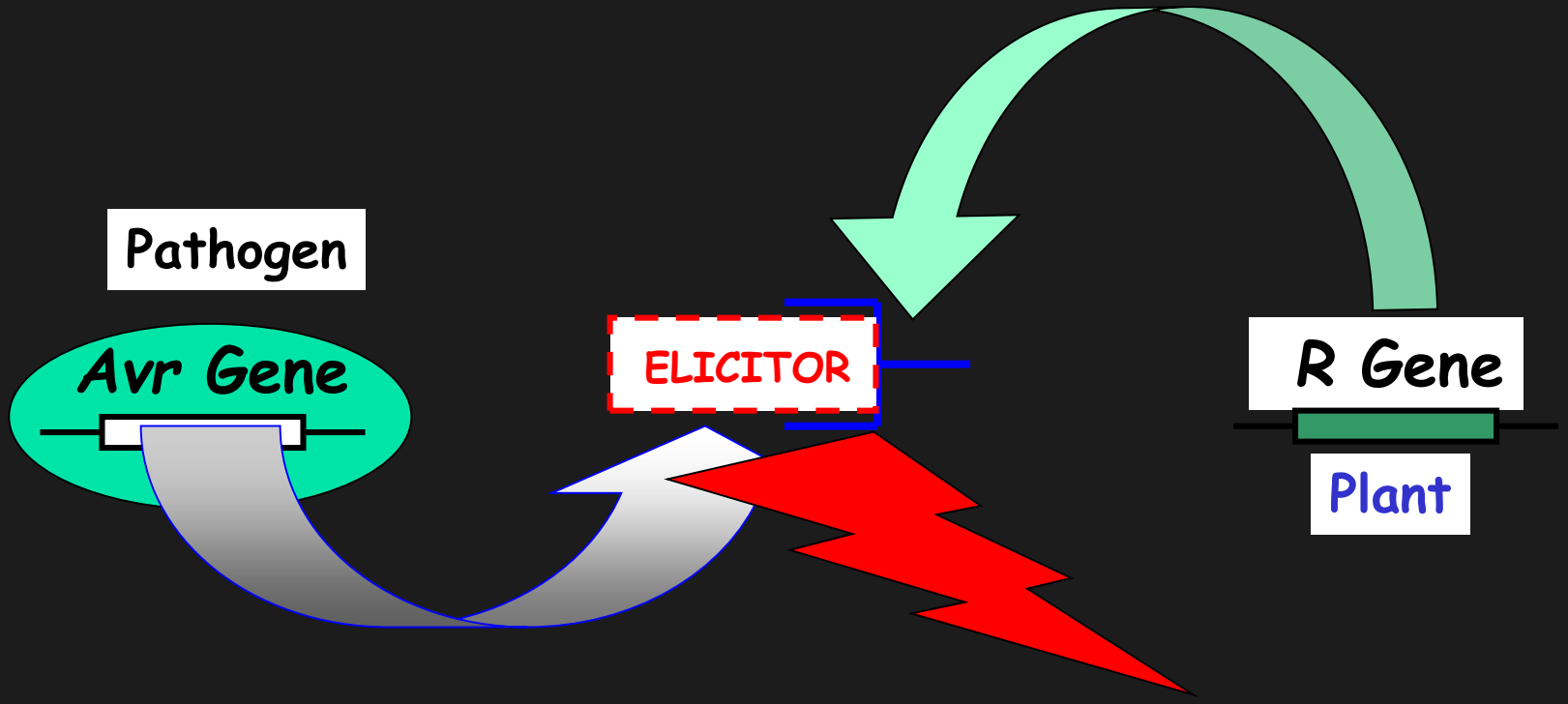
R genes

Interactions of bacterial avirulence products with *R* gene products

- **Major gene resistance:**
- Confers a high level of resistance.
- If pathogens exist as distinct races, avirulence genes in the pathogen are matched by corresponding resistance genes in the plant (gene-for-gene complementarity).
- **Minor gene resistance:**
- Confers a low level of resistance.
- In addition to race-specific resistance genes, plants also possess other, none race-specific, resistance genes which are equally effective against all races of the pathogen (minor gene resistance).

The *R* and *Avr* genes control pathogen recognition

Simple Model:
The *Avr* gene encodes a factor (a specific ELICITOR) that directly or indirectly interacts with the product of an R gene



Recognition of the elicitor triggers defense responses including the hypersensitive response

Elicitor recognition

Avr-R interactions

Proposed models

- Different models were proposed for R protein recognition of Avr proteins, mainly:

1. Ligand-Receptor model
2. Guard model
3. Affinity enhancement model

- These can be explained as direct or indirect interactions.

Elicitor recognition

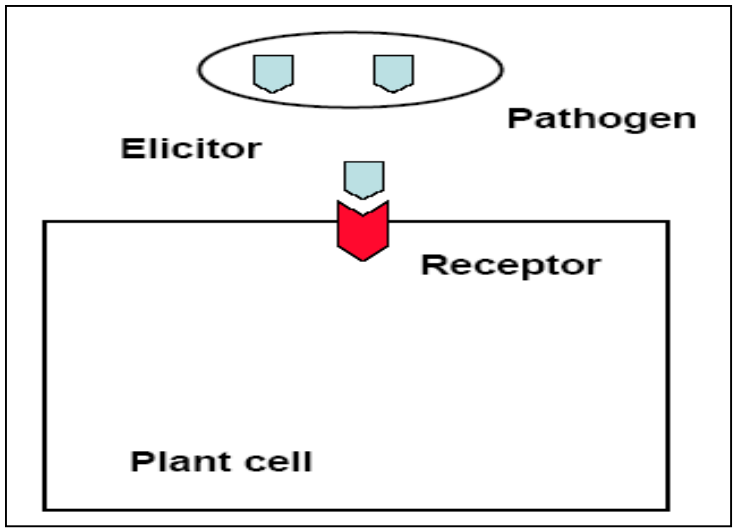
Direct elicitor – receptor interaction

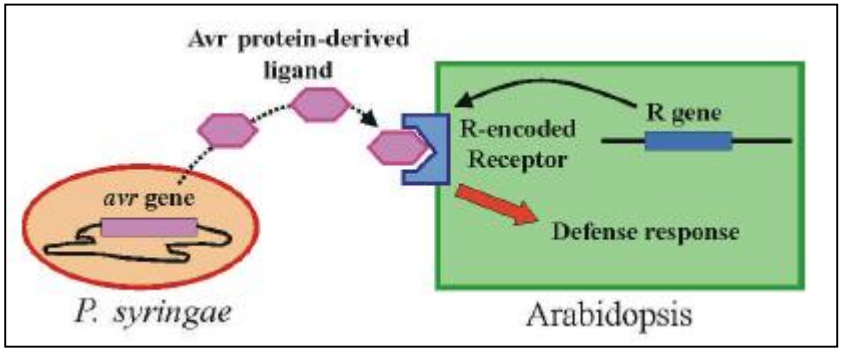
1. Ligand-receptor interactions

Direct physical interaction
Ligand-receptor interactions

1. In ligand/receptor interactions, or
2. Elicitor-receptor interactions, or
3. *avr* gene and pathogen or plant surface-derived elicitors

- Serve as ligands for receptors (*R* genes) located in the plasma membrane or in the cytosol (cytoplasm).





Direct physical interaction

Ligand-receptor interactions

AVR/R and PAMP/PRR interactions

1. AVR/R or effectors interactions:

- Effectors are pathogen-secreted proteins that manipulate host cell functions.
- Avr recognition by plants has been coined effector-triggered immunity.

2. PAMP/PRR interactions:

- PAMPs define molecular motifs common to many pathogens.
- Pathogen-associated molecular pattern (PAMP) triggered immunity (innate immunity/PTI).

Direct physical interaction
Ligand-receptor interactions

- Direct interaction between Avr proteins and R proteins was indeed demonstrated in a few cases by the yeast two-hybrid system and the immuno-precipitation.
- Examples:
- Fungal Pita(*Pi-ta*)-AvrPita (AVR/R interaction);
- Bacterial RRS-1R- popP2 interaction (AVR/R interaction);
- Bacterial FLS2 -flg22 interaction(PAMP/PRR interaction).

- Yeast two-hybrid system: An experimental technique of detecting the protein-protein interactions in yeast cells.
- Two-hybrid system: The yeast or bacterial system that is employed for detecting specific protein-protein interaction; the protein of interest is used as a "bait" to "fishout" proteins that may bind to it (referred to as "prey").

The direct physical interaction
Ligand-receptor interactions
AVR/R interactions

- Among different *R* genes, very few NB-LRR proteins (part of R proteins) have been found to directly interact with Avr proteins.
- Indeed, direct binding of a few Avr-R combinations was found, consistent with a receptor-ligand mode of action.
- However, for a number of Avr-R combinations, physical interactions have not been observed, and perception is thought to be indirect.

van der Hoorna Kamounb,2008

Direct receptor-elicitor interaction
Ligand-receptor interactions
PAMP/PRRs interactions

- PRRs (pattern recognition receptors) watch over and raise the alarm.
- Perception of PAMPs (flagella, LPS,..) by PRRs is common to all multicellular organisms and leads to an array of defense responses and redeployment of cellular energy in a fast, efficient, and multi response manner, which prevents further pathogen ingress.
- PAMP recognition leads to a chain of signaling events, broadly referred to as general defense responses in plants.
- PAMP perception also results in plant systemic acquired resistance (Mishina and Zeier,2007b).

Nicaise *et al.*,2009

Direct receptor-elicitor interaction
Ligand-receptor interactions
PAMP/PRRs interactions

- The active peptides are present in a variety of pathogenic bacteria and perceived at the nanomolar level by a broad range of plant species.
- The peptide flg22 (corresponding to 22 amino acids localized in the conserved region of flagellin) elicits responses in most plant species and is as active as the full-length flagellin.
- The flg22 region is required for bacterial virulence and motility, consistent with the fact that PAMP mutation has a fitness cost for microbes.
- The conserved segment of bacterial flagellin was recently demonstrated to be a potent elicitor of defense gene expression.
- The recent finding that flg22, as well as flagellin, induces the hypersensitive response.

Nicaise *et al.*,2009

Direct receptor-elicitor interaction
Ligand-receptor interactions
PAMP/PRRs interactions

- Rice (*Oryza sativa*) was reported to be insensitive to flg22 (Felix *et al.*,1999).
- However, recent results showed that flg22 is recognized by rice but that this response is weaker than with full-length flagellin (Takai *et al.*,2008).

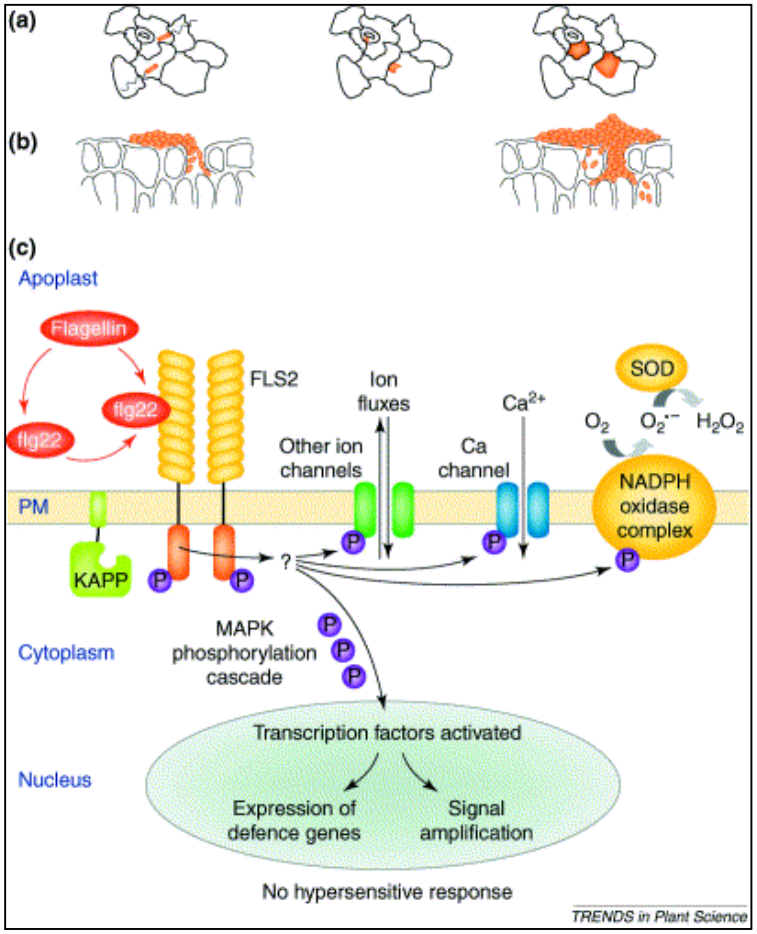
Nicaise *et al.*,2009

Direct receptor-elicitor interaction

Flagellin as potent elicitor(PAMP)

Model for flagellin signaling in *Arabidopsis*

1. Bacteria move on the plant surface and enter the plant via natural openings, wounds or hydathodes.
2. Bacteria multiply on the surface or in the intercellular space of the host plant.
3. Flagellin (released from bacterial flagellae) or flagellin fragments resembling flg22, are recognized directly or indirectly by the flagellin receptor at the plant plasma membrane (PM), a key part of which is FLS2.
4. Autophosphorylation of FLS2.



A sustained increase in cytosolic calcium which was specific in gene-for-gene interactions

Direct receptor-elicitor interaction

RRS1-PopP2 direct or indirect effector interactions

- The products of two groups of avirulence genes with virulence effects appear to be targeted to specific organelles within the host cell.
- Two effectors, AvrBs3 from *X. vesicatoria* and PopP2 from *R .solanacearum* have recently been shown to be localized in plant nuclei by immunolocalization and monitoring GFP fusions, respectively.
- In the case of PopP2, the corresponding RRS1 resistance gene was co-localized in the nucleus only in the presence of PopP2.

Direct receptor-elicitor interaction

RRS1-PopP2 direct or indirect effector interactions

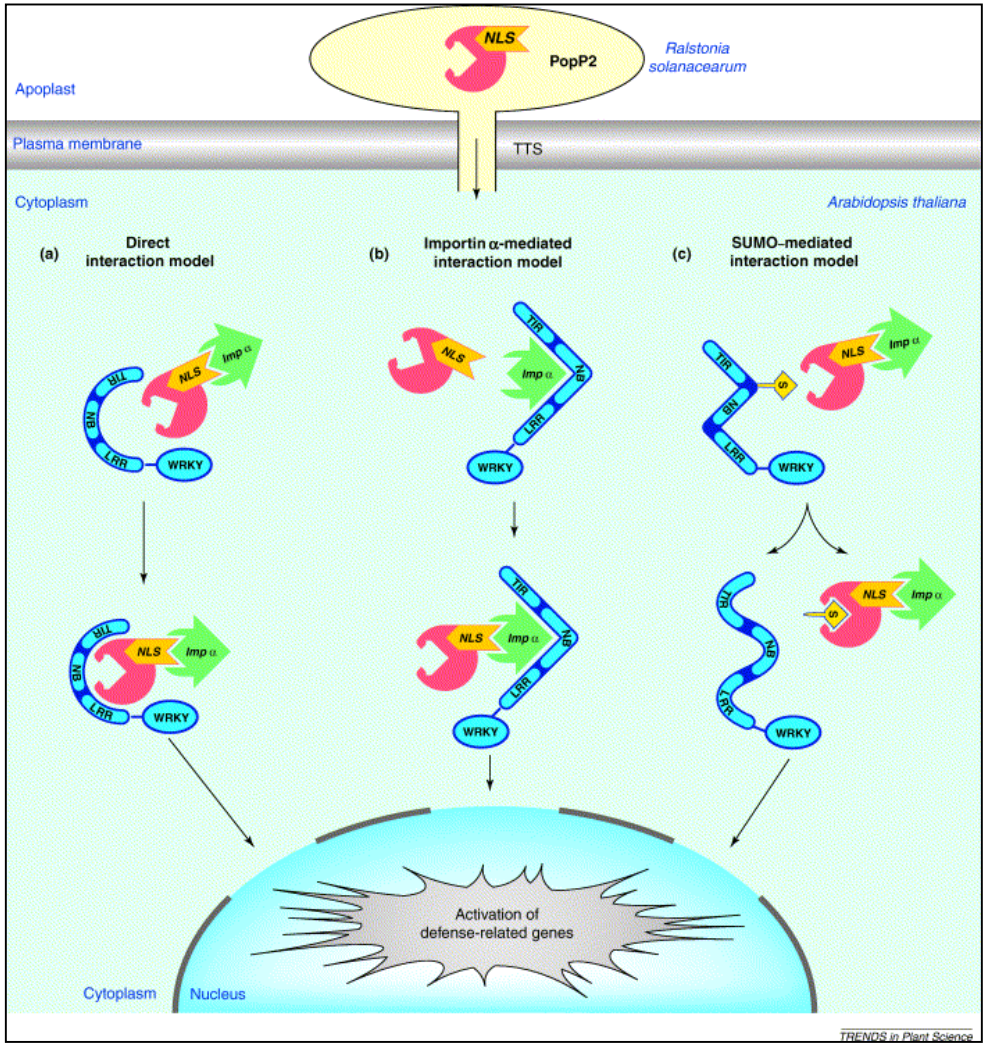
a) **Direct interaction.**

b) **Importin α-mediated interaction model: Leads to nuclear import of RRS1-R and activation of defense-related genes.**

c) **SUMO(a small ubiquitin-like modifier)-mediated interaction model: The interaction between RRS1-R and PopP2 is mediated by SUMO.**

- SUMO protease activity affects host transcription.

- Imp , importin α;
- LRR, leucine-rich repeat domain;
- NB, nucleotide-binding site;
- NLS, nuclear localization signal;
- S, SUMO, A small ubiquitin-like modifier;
- TIR, homologous to Toll/interleukin-1-receptor;
- TTS, type-III secretion system;
- WRKYs, DNA-binding proteins, regulating gene expression for plant defense.



Elicitor recognition

Indirect interactions of AVR-R proteins

Guard, Decoy and Integrated decoy models

Such guarding strategies are thought to afford the host more durable resistance to quick-evolving and diverse pathogens.

See Most recent slides in Bacterial Disease Management-Part II

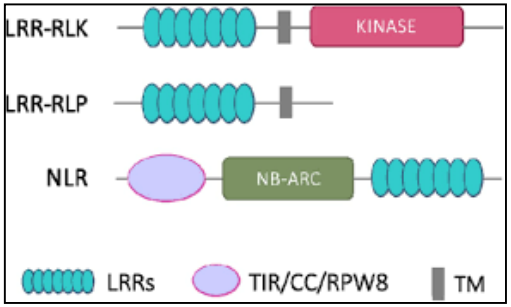
Classes of resistance genes

R genes classified by nine mechanisms

- To perceive pathogen threats, plants utilize both plasma membrane-localized and intracellular receptors.
- Nucleotide-binding domain leucine-rich repeat containing (NLR) proteins are key receptors that can recognize pathogen-derived intracellularly delivered effectors and activate downstream defense.

1. Some NLRs directly bind to effectors,
2. but others can perceive effector-induced changes on:

- targeted host proteins (guardees), or
- non-functional host protein mimics (decoys).

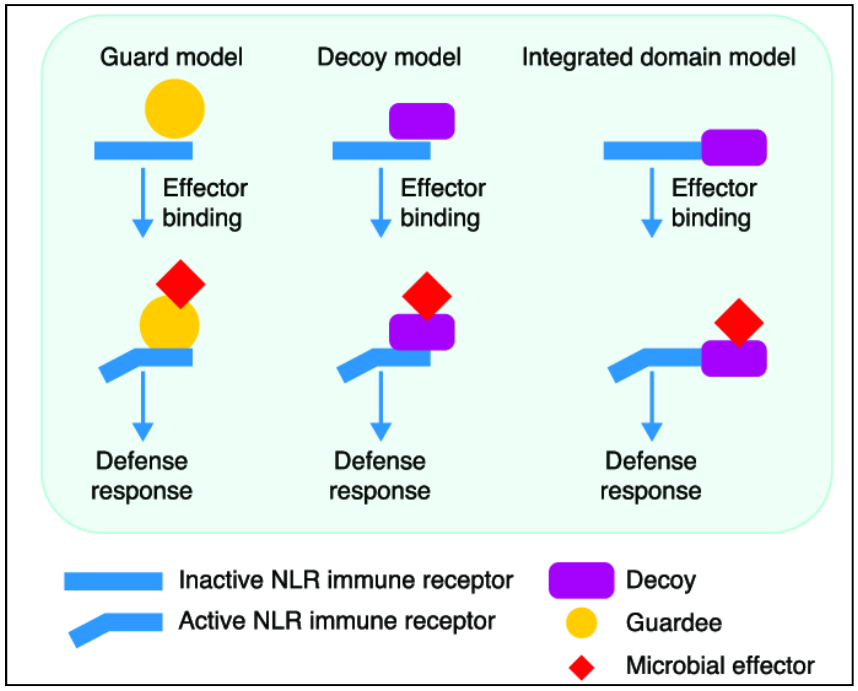


Ao and Li,2022

Indirect interactions of AVR-R proteins

Comparison of different Guard, Decoy and Integrated decoy models

1. In the guard model, NLR receptors perceive modifications in guardees that are introduced by microbial effector proteins.
2. Guardees often have a function in basal plant defense and they are therefore frequently targeted by effectors.
3. The decoy is a duplicated guardee without a function in plant immunity. Its sole role is to trap effectors, thereby activating the immune signaling cascade.



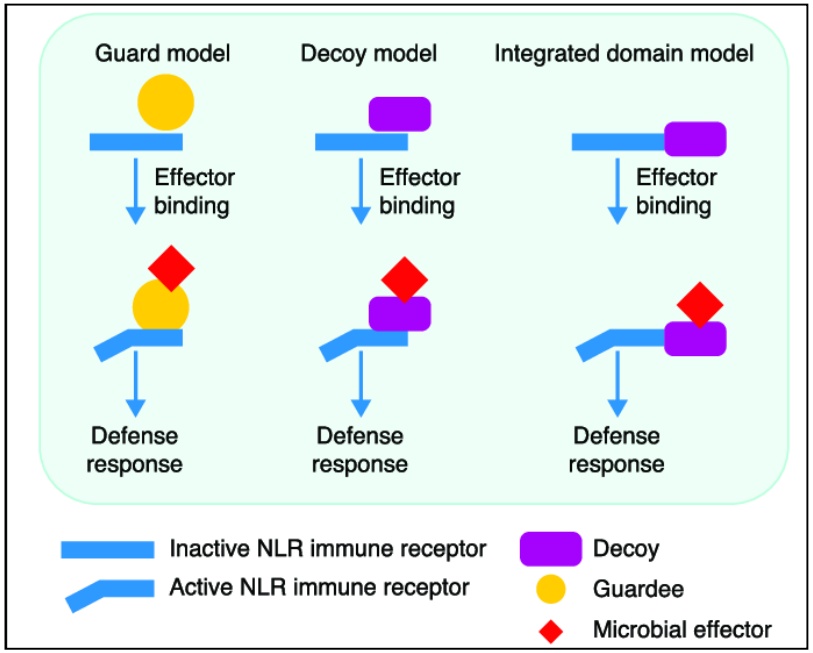
NLR (NBS-LRR of R proteins): A major class of plant immune receptors called nucleotide-binding domain and leucine-rich repeat containing proteins.

Krattinger and Jeller,2016

Indirect interactions of AVR-R proteins

Comparison of different Guard, Decoy and Integrated decoy models

- The guardee or decoy and the monitoring NLR are often encoded by different genes that likely bind to each other, but can dissociate.
- The integrated domain model involves a fusion between the decoy domain and the respective NLR, which together are encoded by one gene.



NLR: A major class of plant immune receptors called nucleotide-binding domain and leucine-rich repeat containing proteins

Krattinger and Jeller,2016

Genetic tests to discriminate between the Guard and Decoy Models

- Plants lacking both the R protein and the presumed operative target (s) should be challenged with pathogens in the absence or presence of the guardee/decoy.

1. A differential pathogen growth supports the Guard Model, whereas
2. An unaffected pathogen growth supports the Decoy Model.

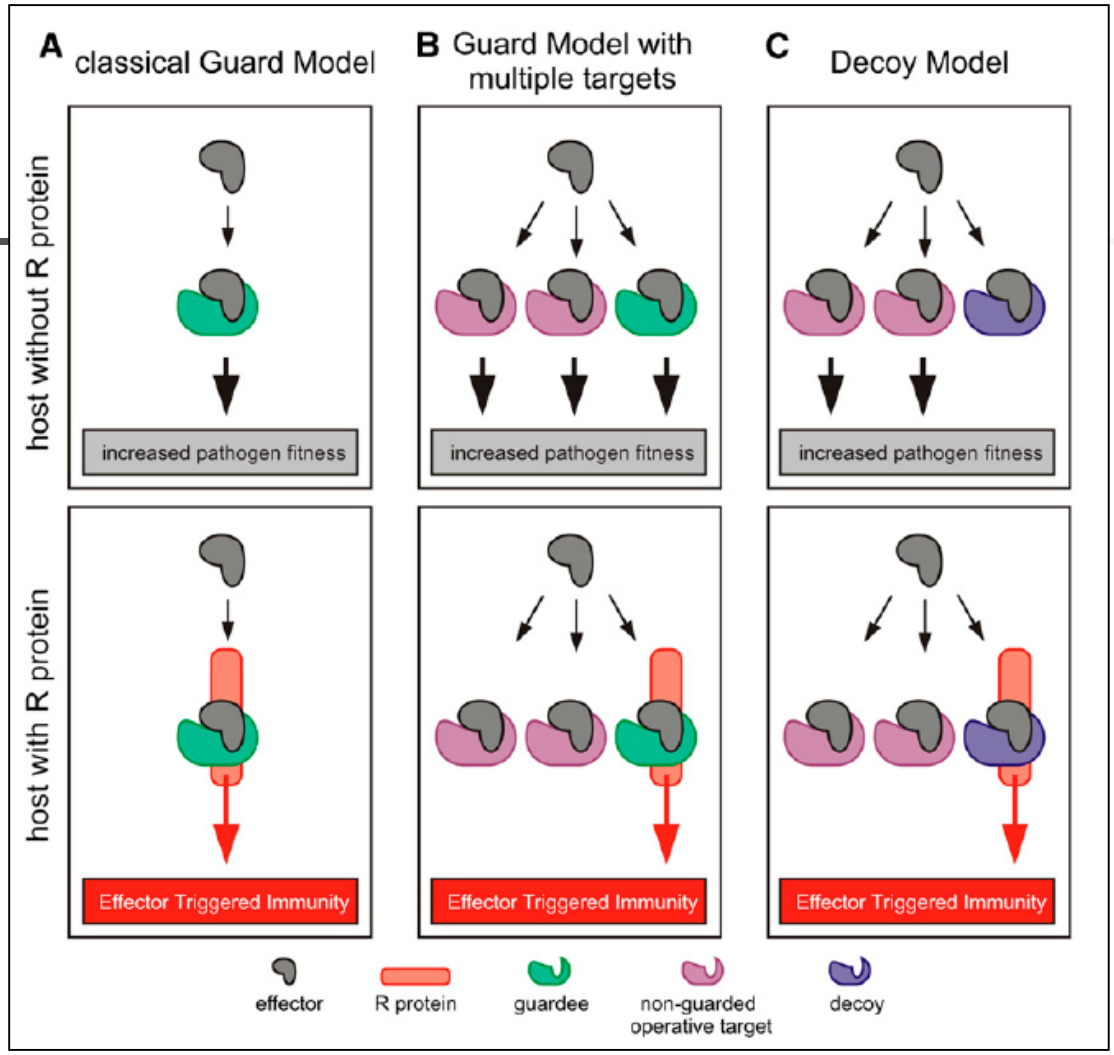
- The test of choice depends on the nature of the effector:

A. Effectors that promote positive effects on pathogen growth by manipulating their target should be present during the test to reveal target contributions.

B. Effectors that prevent negative effects on pathogen growth should be omitted to avoid them from suppressing a possible phenotype.

van der Hoorn and Kamounb,2008

Comparisons of the Guard and Decoy Models



A) The classical Guard Model is contrasted with a B) modified Guard Model in which the effector targets multiple plant proteins and c) the Decoy Model.
Effectors are depicted in gray, operative effector targets in purple, guardee in green, decoy in blue, and the R protein in orange. Operative target: Host target that when manipulated by a pathogen effector results in enhanced pathogen fitness (van der Hoorna Kamounb,2008).

Genetic tests to discriminate between the Guard and Decoy Models

- Plants lacking both the R protein and the presumed operative target(s) should be challenged with pathogens in the absence or presence of the guardee/decoy.
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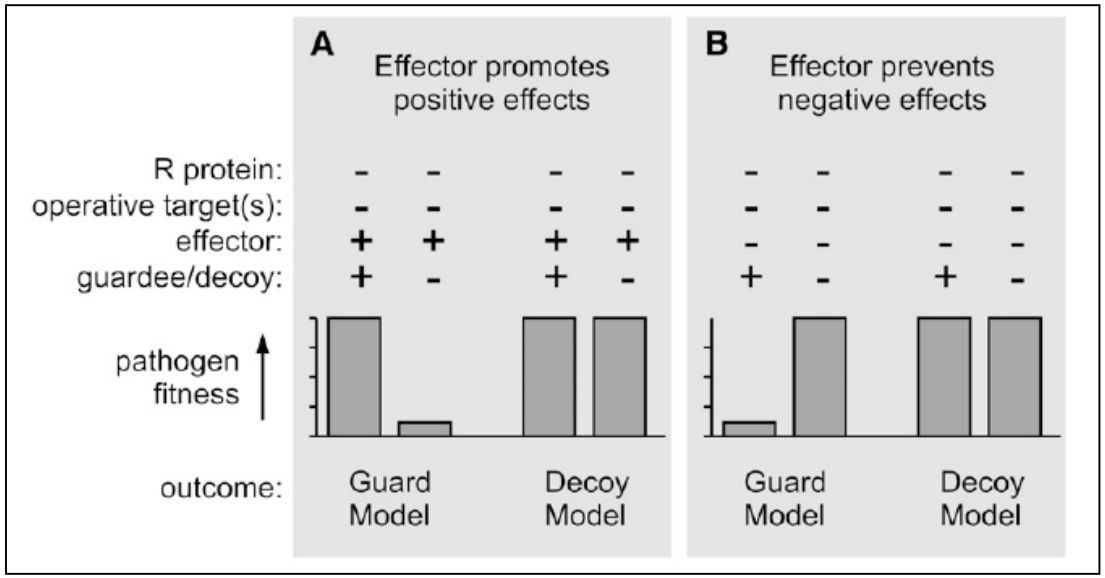


van der Hoorn and Kamounb,2008

Elicitor recognition

Indirect interactions of AVR-R proteins

1. Guard hypothesis

The Guard hypothesis
Evidence against direct interaction

- In many (most?) cases direct interaction between R-gene and Avr gene products can not be observed.
- If there are only a few hundred R-genes, this seems insufficient to deal with the huge number of pathogens around leads to hypotheses that some R-genes recognise Avr gene products indirectly.

The Guard hypothesis
Indirect interactions of AVR-R proteins

- This Guard Model predicts that R proteins act by monitoring (guarding) the effector target and that modification of this target by the effector results in the activation of the R protein, which triggers disease resistance in the host (Van der Biezen and Jones,1998; Dangl and Jones,2001).

Guardee: Effector target required for R protein function and with a function in host defense or susceptibility in the absence of its cognate R protein; effector alteration of the guardee results in enhanced pathogen fitness in plants that lack the R protein and triggers innate immunity in plants that carry the R protein.

van der Hoorna Kamounb,2008

The Guard hypothesis

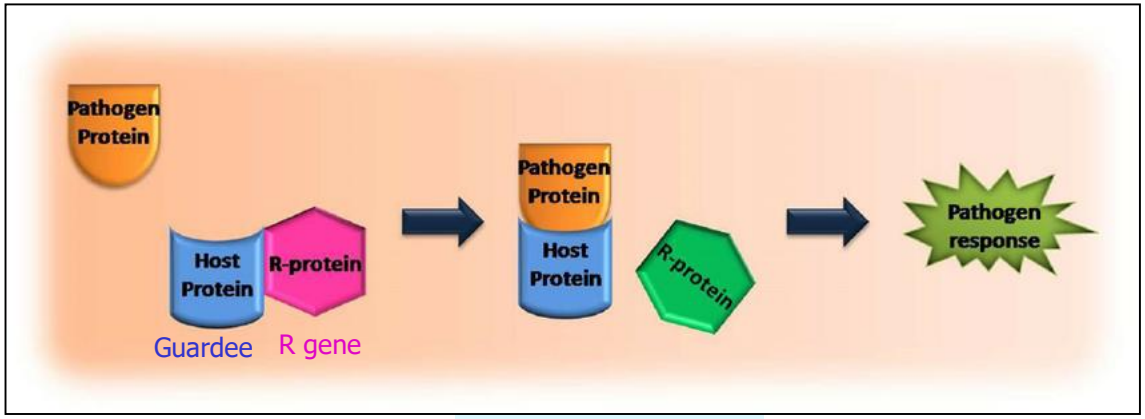
Indirect interactions of AVR-R proteins

- According to the 'guard model', Avr product does not directly interact with an R-protein, but at least three players are needed to trigger resistance.
- The third player is called as the guardee (pathogenicity target proteins/target host proteins/host plant proteins) which act as a molecular glue between complementary Avr and R product is required.

The Guard hypothesis

Indirect interactions of AVR-R proteins

- The plant R proteins (guard) are associated with the endogenous host protein (guardee) which are common target proteins for the pathogens.
- The interaction of effector pathogen proteins with the host proteins, causes a change in their structure which is then recognized by the guard proteins.
- As a result, a pathogen response signaling cascade is triggered against the microbial evasion.



Gururani,2012

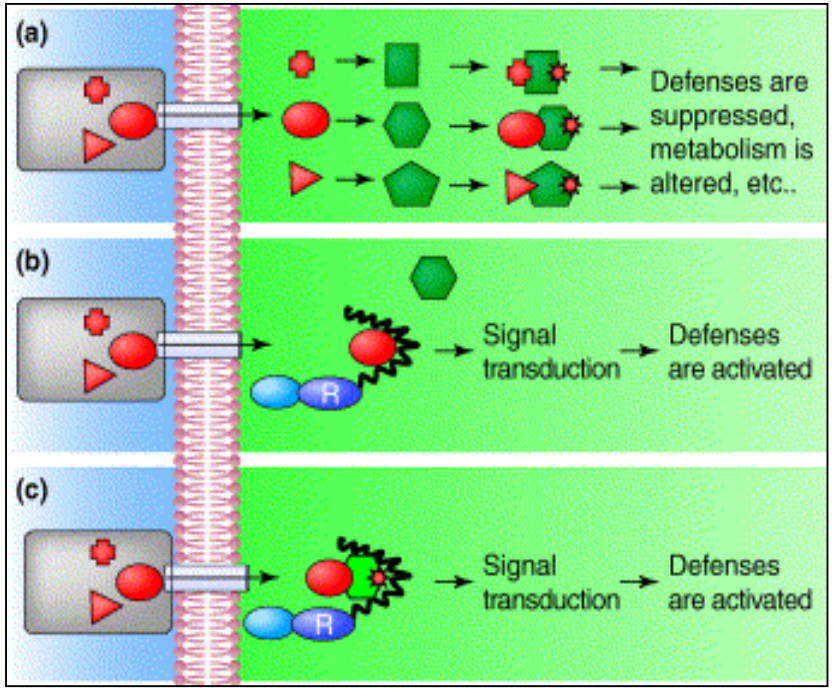
The Guard model and Ligand-Receptor model

Direct and indirect protein interactions

a) The plant cell does not express an R protein that is capable recognizing any virulence protein (disease).

b) R protein directly bind a virulence protein(Classic receptor-elicitor hypothesis).

c) The guard hypothesis, R protein(guard) detects a modified host protein (guardee, red star).



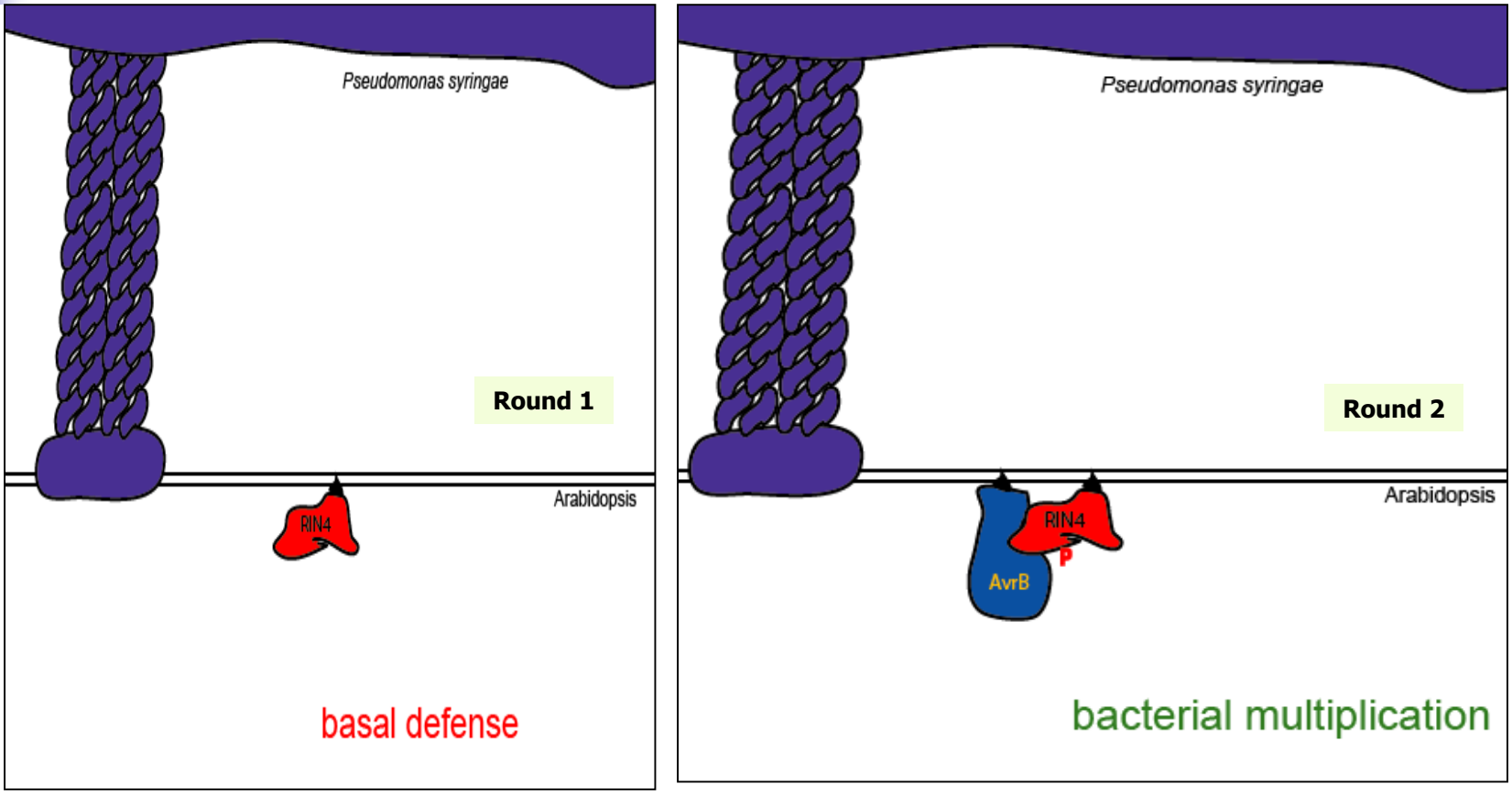
Virulence proteins (red) are translocated into plant clells via TTSS.

McDowell and Woffenden,2003

The Guard hypothesis

RIN4, the guardee protein in Arabidopsis

In 5 rounds

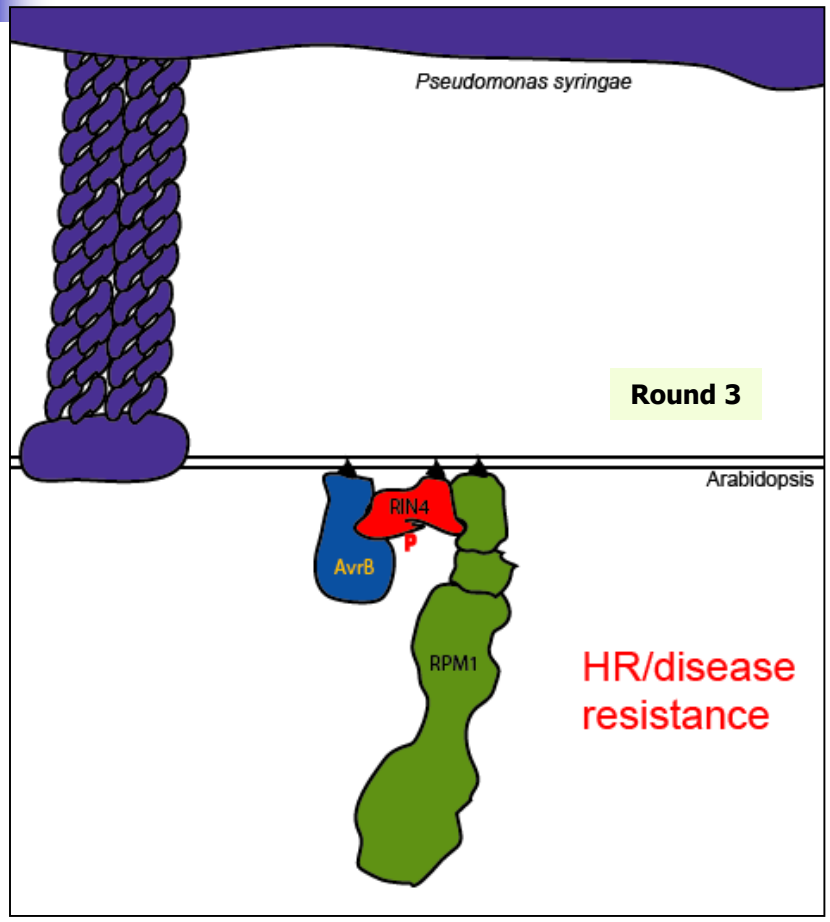


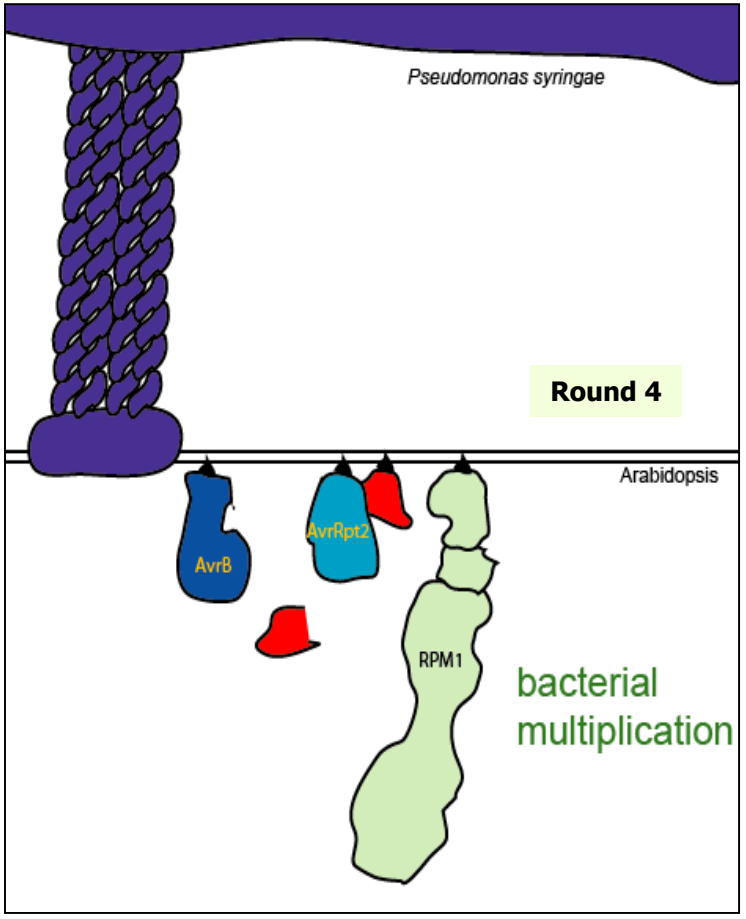
Roger Innes. Tom Ashfield Laura Ong

The Guard hypothesis

RIN4, the guardee protein in Arabidopsis

In 5 rounds





Roger Innes. Tom Ashfield Laura Ong

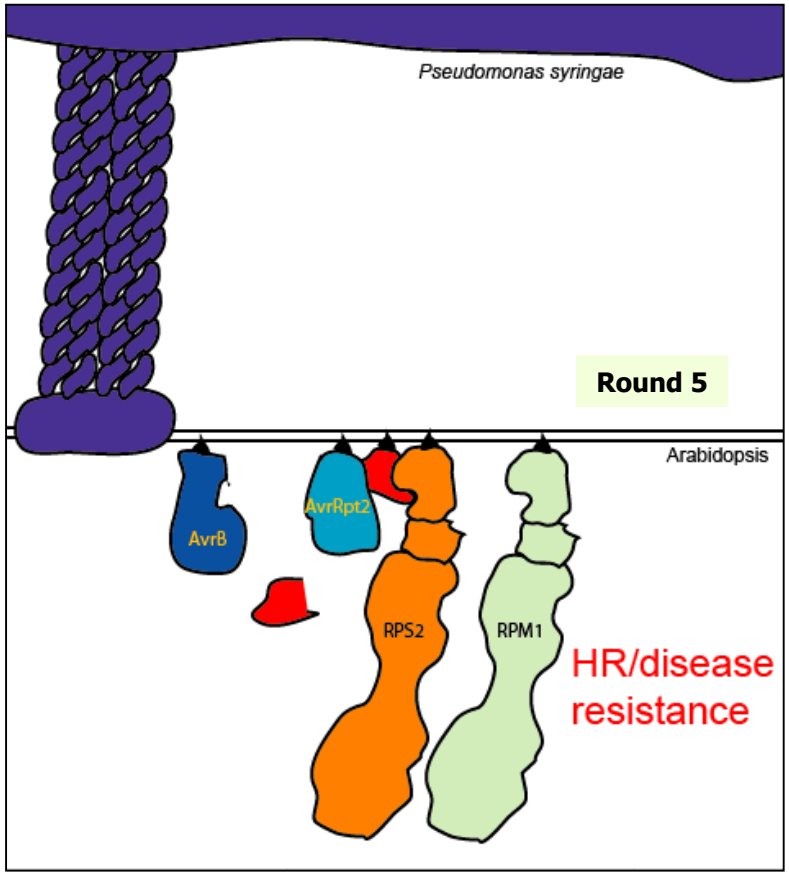
The Guard hypothesis

RIN4, the guardee protein in Arabidopsis

In 5 rounds

- So Rin4 is guarded by at least two R-genes in Arabidopsis:

1. RPM1(mediates AvrB recognition), and
2. RPS2 (mediated AvrRpt2 recognition).



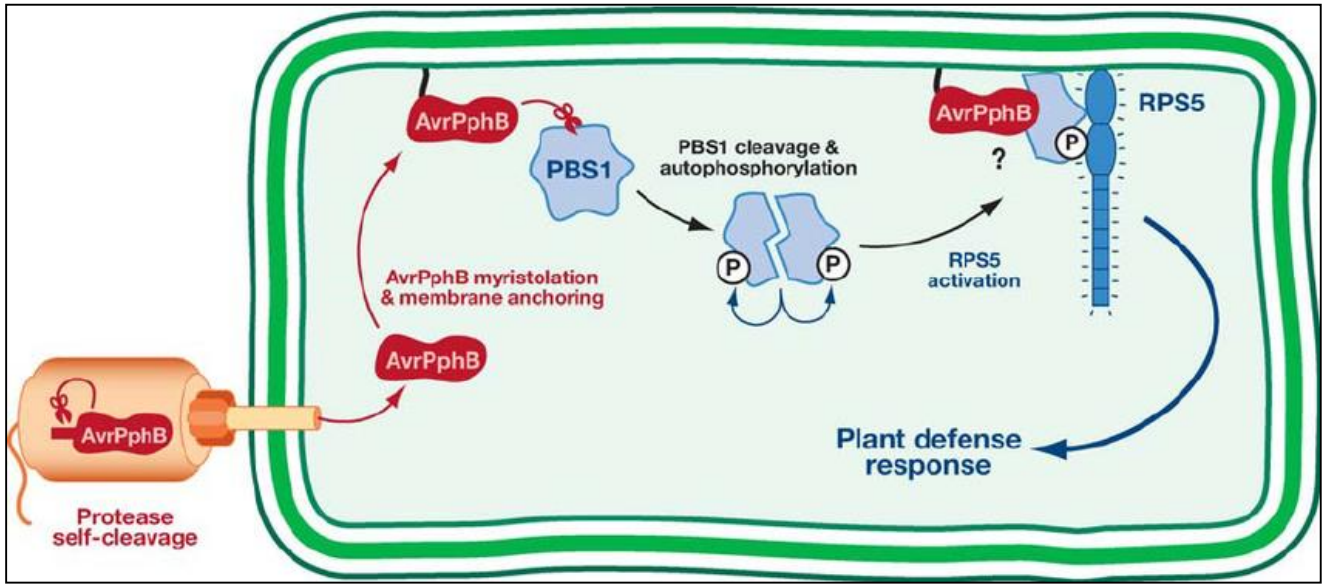
Roger Innes. Tom Ashfield Laura Ong

Evidence for the guard hypothesis
Indirect protein interactions
AvrPphB and RPS5+ PBS1 interactions

- RPS5 is a NBS-LRR protein from *Arabidopsis*.
- Recognizes *AvrPphB* from *P. syringae*.
- When RPS5 is expressed in tobacco it will only mediate HR in the presence of both AvrPphB and another Arabidopsis protein PBS1.
- RPS5 and PBS1 form complex in the cell.
- AvrPphB cleaves PBS1 triggering HR controlled by RPS5.

Evidence for the guard hypothesis
Indirect protein interactions
AvrPphB and RPS5+ PBS1 interactions

- Model of AvrPphB cysteine protease action in resistant RPS5 Arabidopsis plants infected with *Pseudomonas syringae* pv. *phaseolicola*.



Mudgett,2005

Elicitor recognition

Indirect interactions of AVR-R proteins

2. Decoy Model

Decoy Model

A new model for perception of plant pathogen effectors

- What do decoy means?

1. a type of bait used in fishing or hunting.
2. especially: an artificial bird used to attract live birds within shooting range.
3. a person used to lead another into a trap.

Decoy Model

A new model for perception of plant pathogen effectors

- A decoy protein whose sole purpose is to be an effector target and act as a "pathogen detector".

Roger Innes. Tom Ashfield Laura Ong

Decoy Model

Four cases supporting the decoy model

- Case 1: Pto
- *P. syringae* AvrPto/Tomato
- Case 2: pBS3
- *Xanthomonas campestris* pv.*vesicatoria* AvrBs3/Peper
- Case 3: RCR3
- The effector protein Avr2 of the fungus *C. fulvum*/Tomato
- Case 4: RIN4
- *P. syringae* effectors (AvrRpm1, AvrRpt2,and AvrB) and monitored by at least two R proteins (RPM1 and RPS2)/ *Arabidopsis*.

van der Hoorn and Kamounb,2008

Decoy Model

Four cases supporting the decoy model

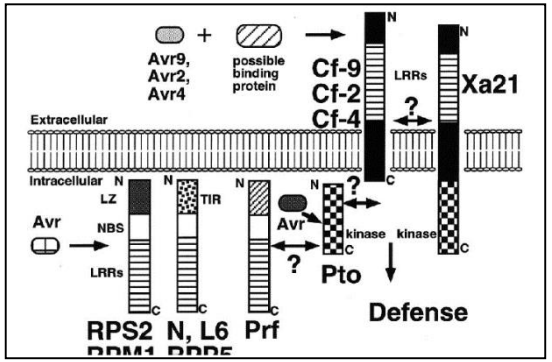
Case	1	2	3	4
Plant species	Tomato	Pepper	Tomato	*Arabidopsis*
Pathogen	*P. syringae* pv *tomato* (bacterium)	*X. campestris* pv *vesicatoria* (bacterium)	*C. fulvum* (fungus)	*P. syringae* (bacterium)
Site of perception	Cytoplasm	Nucleus	Apoplast	Cytoplasm
R protein	Prf	Bs3	Cf-2	RPS2
Biochemical function of R protein	NB-LRR	Flavin monooxygenase	Receptor-like protein	NB-LRR
Decoy	Pto	*pBs3*	RCR3	RIN4
Biochemical function of decoy	Kinase	*upa* box in promoter of *Bs3* gene	Cys protease	Negative regulator of basal defense (Kim et al., 2005)
Operative target	Le FLS2?	*pUpa20*	PIP1	Not yet identified
Structure and function of operative target	Receptor-like kinase required for basal resistance	*upa* box in promoter of cell size regulator Upa20 and other genes	Cys protease secreted abundantly during defense	Unknown
Effector	AvrPto	AvrBs3	Avr2	AvrRpt2

See original paper for a complete table

van der Hoorn and Kamounb,2008

Decoy Model

Case 1: Pto



- Tomato Pto encodes a Ser/Thr kinase that confers resistance to *P. syringae* strains carrying avrPto, an interaction that also requires the nucleotide binding (NB)-LRR R protein Prf.
- AvrPto contributes to virulence on tomato, even in the absence of Pto.
- Function of Pto is kinase, whereas AvrPto act as Kinase inhibitor.
- Pto inhibition by AvrPto activates Prf.
- FLS2, a receptor-like kinase in PAMP triggered immunity is an operative virulence target of AvrPto.
- If it so, Pto itself is a decoy.
- Pto then would restrict rather than promote pathogen fitness in the absence of Prf.

van der Hoorn and Kamounb,2008

Elicitor recognition

Indirect interactions of AVR-R proteins

3. Integrated decoy model

Gene-for-gene resistance

Indirect interactions of AVR-R proteins

Decoy model and integrated decoy model

- The decoy is a duplicated guardee without a function in plant immunity.

1. Its sole role is to trap effectors, thereby activating the immune signaling cascade.
2. Alternatively, the decoy may be integrated into the structure of the receptor component of an NLR(NBS-LRR of R proteins) pair, allowing AVR recognition by direct binding.

Indirect interactions of AVR-R proteins
Integrated decoy model

- Pathogen effectors target host proteins for manipulation in order to promote infection.
- Indirect recognition of these effectors occurs when:

1. these target proteins are guarded by host NLR(leucine-rich repeat receptors) proteins, or
2. if duplicated target genes evolve into decoy proteins monitored by host NLRs.
3. Alternatively the decoy may be integrated into the structure of the receptor component of an NLR pair, allowing AVR recognition by direct binding.

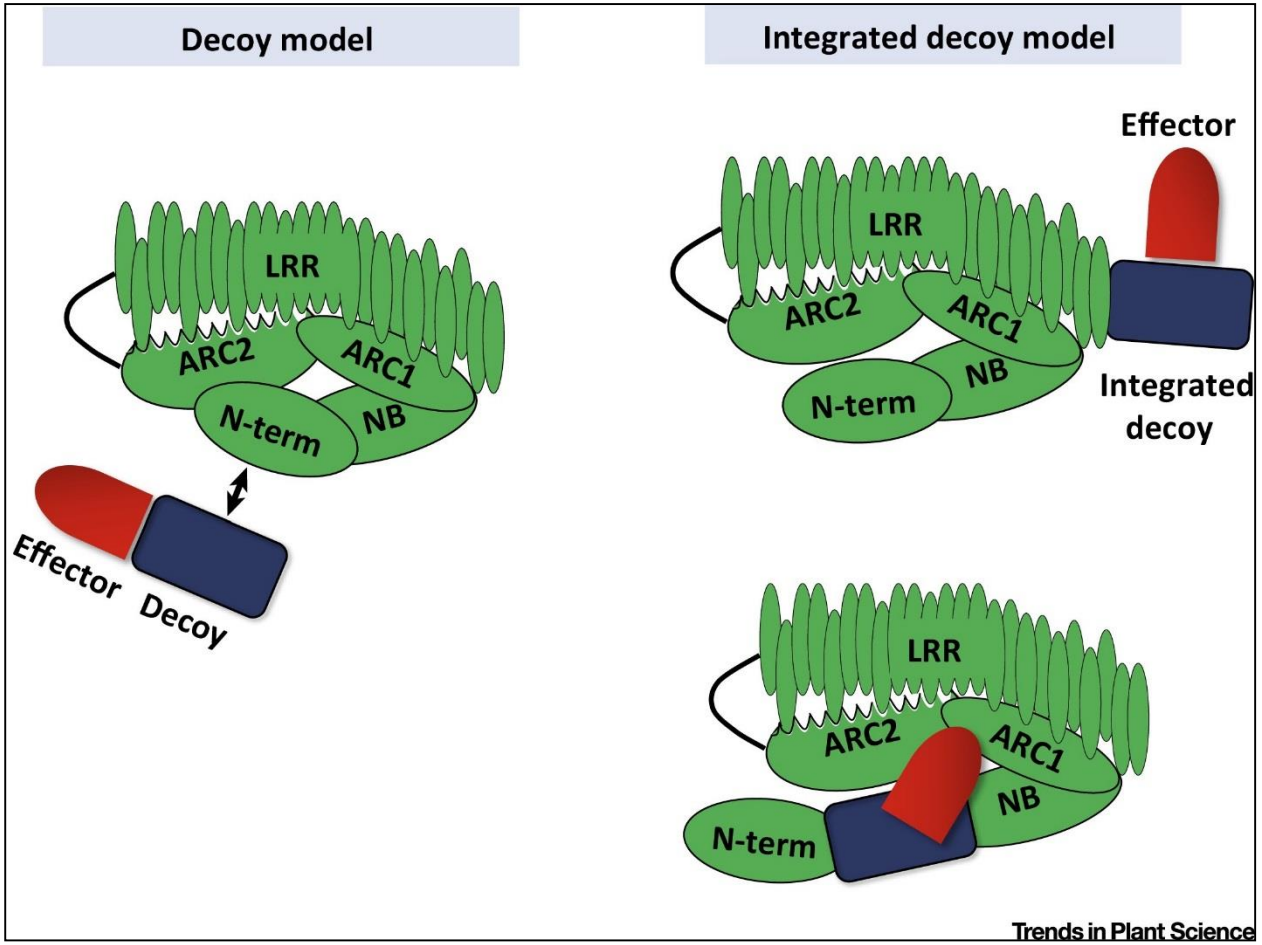
Cesari *et al.*,2014

Indirect interactions of AVR-R proteins
Integrated decoy model

- Recognition of pathogen effector proteins by plant NLR(leucine-rich repeat receptors) proteins often involves decoy proteins, which mimic effector targets.
- In certain examples such decoys are integrated as fusions into their cognate NLR.
- Two new studies have analyzed thousands of predicted NLRs to address how commonly plants use this 'integrated decoy' strategy.

Moffett,2016

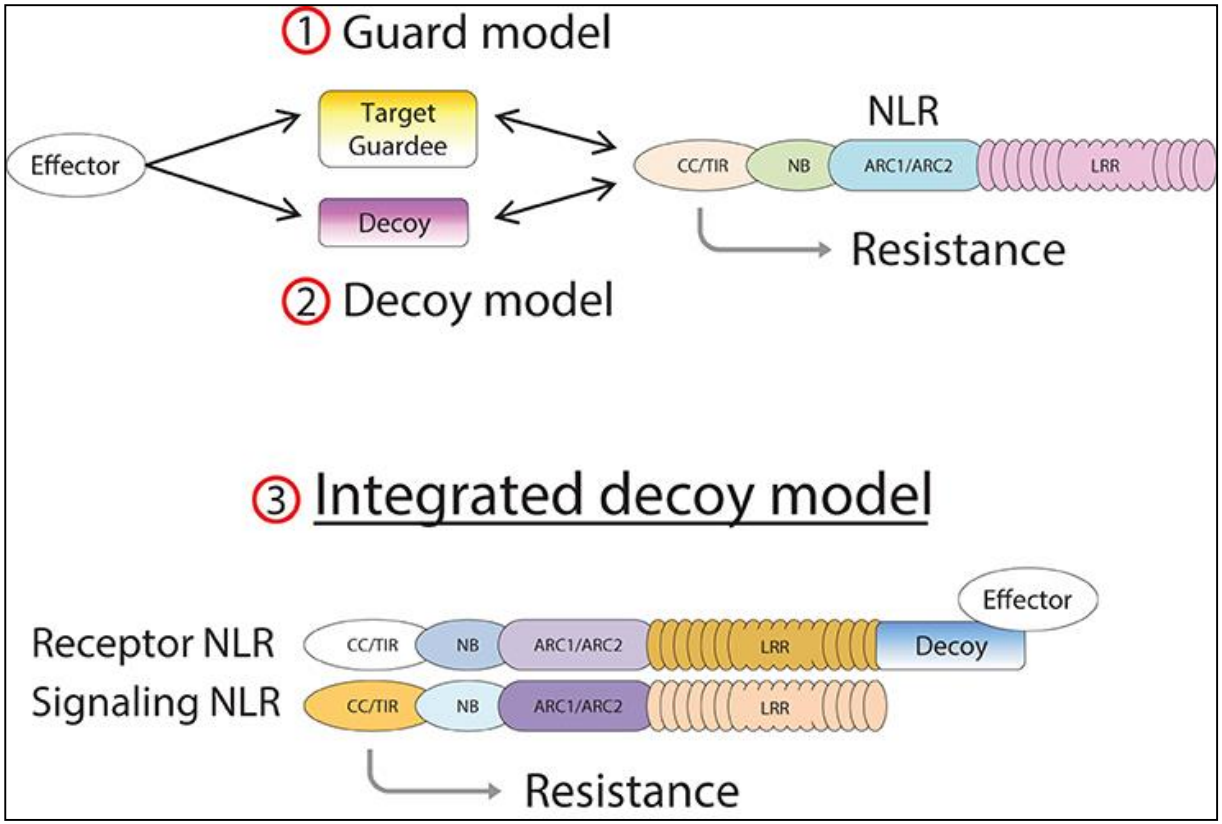
Indirect interactions of AVR-R proteins
Integrated decoy model



Moffett,2016

Gene-for-gene resistance

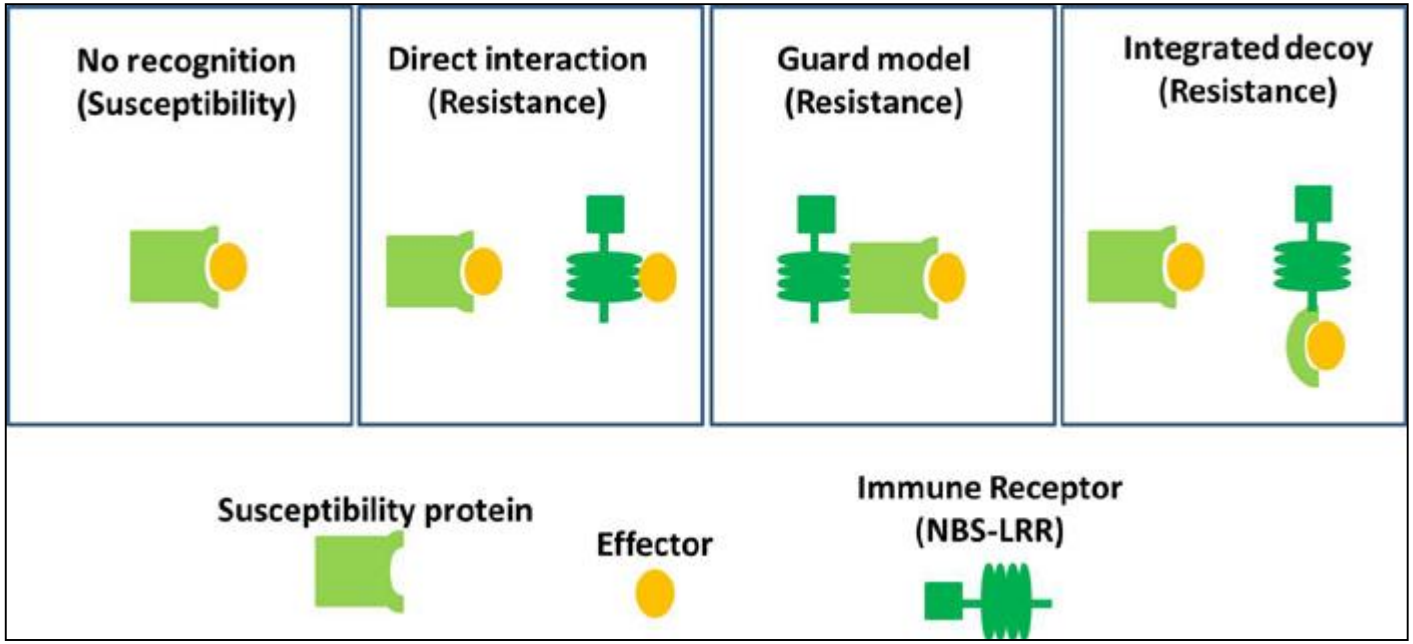
An emerging intermediary mechanism of effector perception called the 'integrated decoy hypothesis'



The NLRs or NBS-LRRs (nucleotide-binding, leucine-rich-repeat) form the largest resistance gene family in plants.

Cesari *et al.*,2014

Indirect interactions of AVR-R proteins
Integrated decoy model



The integrated decoy model proposes that domains of plant proteins (light green) normally targeted by pathogen effectors (orange circle) to perturbate the cell and trigger susceptibility can be integrated in NLR immune receptors (dark green).

Morel,2009

Plant defenses

The most common defences

Basal and R-gene-mediated defences in plants

- Plants lack mammalian-like adaptive immunity and therefore their various inducible defence responses are collectively referred to as 'innate immunity'.
- Plant responses to pathogen attack can be differentiated into:

1. Basal, and
2. Resistant (*R*)-gene-mediated defences.

- These two defence responses can be distinguished experimentally by several assays, but they:

1. Share some similar features, and might even
2. Share some common molecular mechanisms.

Abramovitch *et al.*,2006

Pathogen and nonpathogens

Plants are universally resistant to nonpathogens

Basal defence responses

1. **Non pathogenic bacteria:**

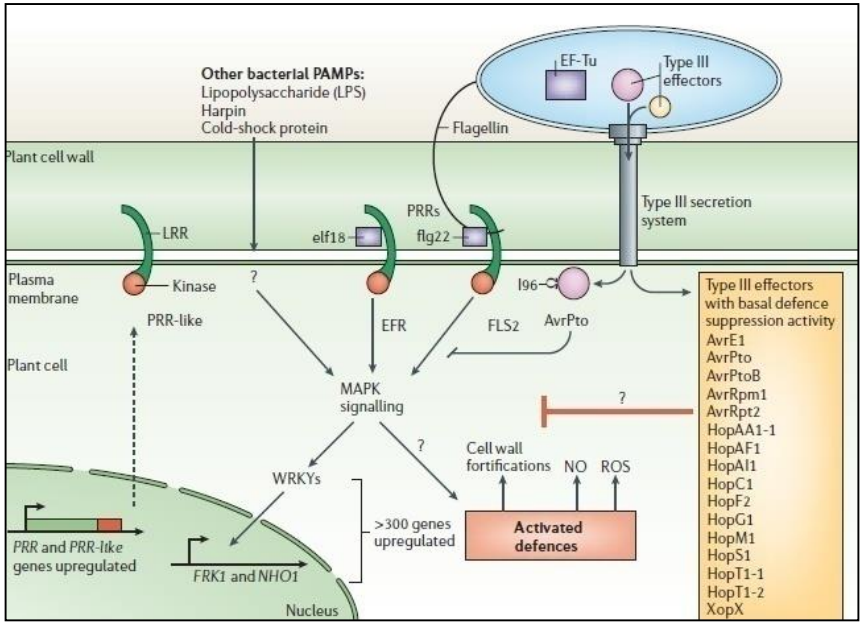
- Plants are universally resistant to nonpathogens, such as:
- *Escherichia coli*,
- *Pseudomonas fluorescens*,
- TTSS-deficient phytopathogen mutants.
- Nonpathogenic bacteria elicit a defense response not involving the HR, which is variously known as localized induced resistance, innate immunity, or basal resistance.

2. **Pathogenic bacteria:**

- The basal defenses must be suppressed by TTSS effectors before the interaction can progress to either:
- Nonhost resistance,
- Host resistance (race-specific resistance).
- Otherwise disease will occurs.

Basal defences and their suppression by type III effectors

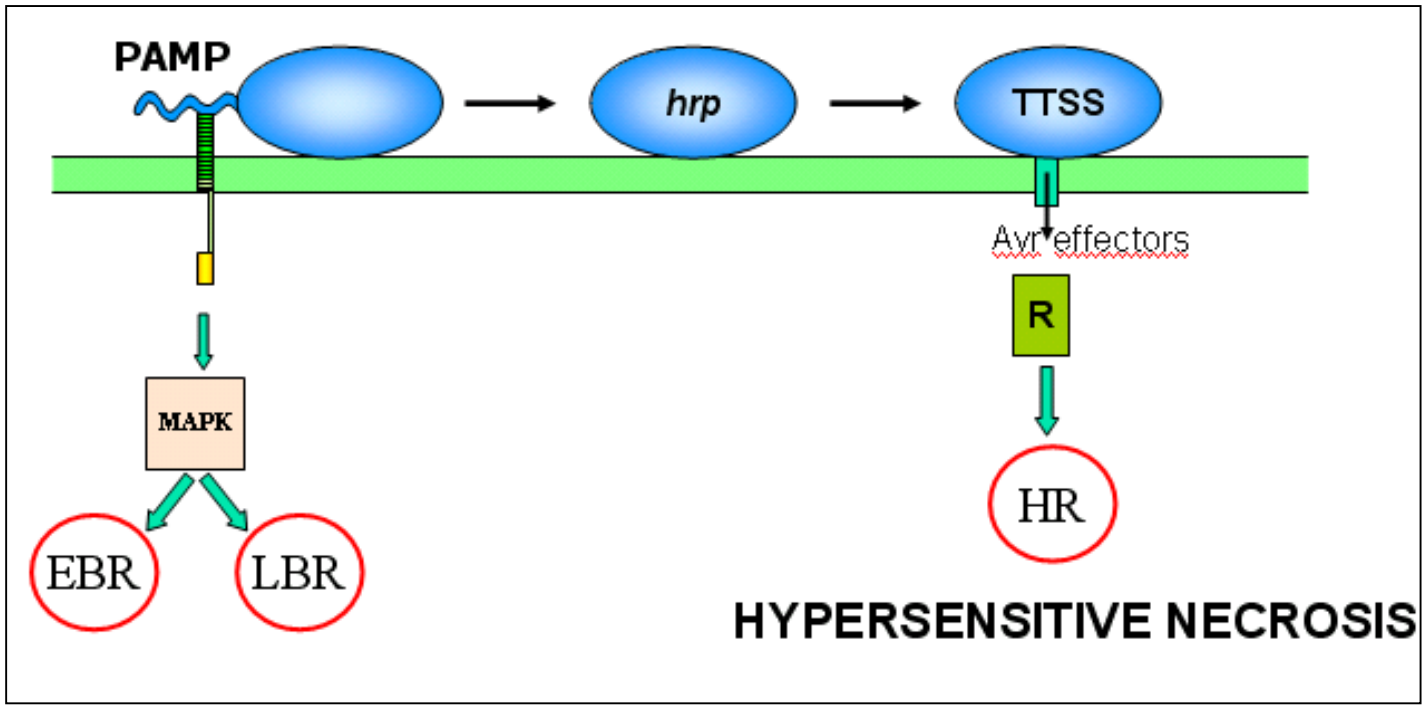
- Delivery of effector proteins through the type III secretion system (T3SS) into plant cells is one strategy that is used by bacteria to suppress PRR-mediated defences.
- As many as 16 effectors have been identified that suppress basal defences.



PRRs: Pattern recognition receptors or surface proteins

PAMPS

In incompatible interaction both EBR and HR are induced. When induction of HR precedes completion of EBR then EBR is masked by HR



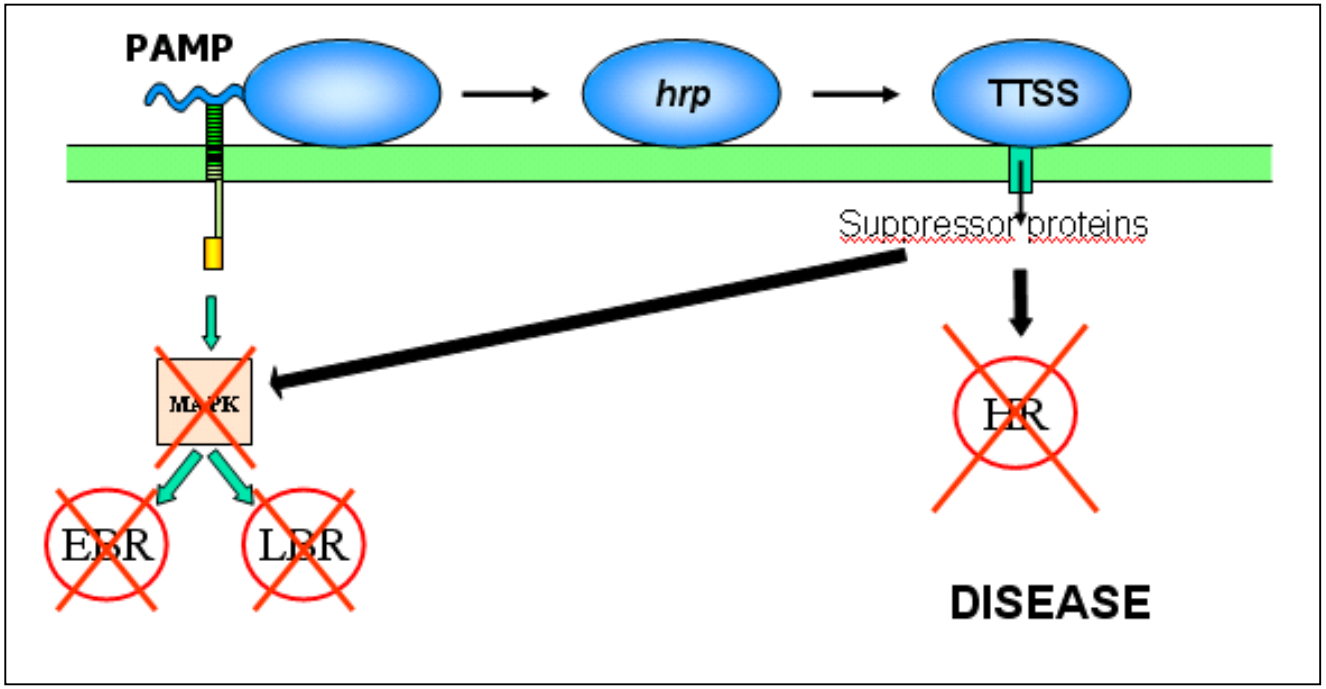
EBR: Early Basal (innate) resistance; LBR: Late Basal (innate) resistance

Courtesy to P.G. Ott

PAMPS

In compatible interaction the virulent pathogen induces EBR also, but it is blocked by the suppression of the EBR signal transduction pathway

- Because both EBR and HR are blocked the pathogen can multiply vigorously causing disease.



Courtesy to P.G. Ott

Plant disease resistance

- Plant disease resistance is a complex phenomenon that most commonly occurs in a non-specific manner, as a result of multiple genes conditioning the ability of the pathogen to cause disease and enabling the plant host to mount an effective defence response.
- However, plant disease resistance can also be induced in response to:

1. Particular pathogen races (race-specific resistance),
2. Particular host plant cultivars (cultivar-specific resistance), or
3. Both a specific pathogen race and plant cultivar interact (race-cultivar-specific or gene-for-gene resistance).

Angela Moncrieff,2003

Two levels of host defenses

- Non-specific plant disease resistance (general, non-host or basic resistance) is a response to all races of a particular pathogen, and occurs in all cultivars of a host plant species.
- In these basal defenses, HR is not elicited (basal defenses).
- Specific plant disease resistance is dependent upon the presence of:

1. A particular pathogen race
2. A particular host plant cultivar, or both.

- In this resistance, HR is elicited (HR defenses).

Specific plant disease resistance

- Specific plant disease resistance appears to be governed by a single gene or a small number of related genes.
- Genes conditioning host-pathogen specificity are found in particular subpopulations of the pathogen, plant host, or both interacting organisms.
- Specific plant disease resistance can be subdivided into three major categories on this basis.

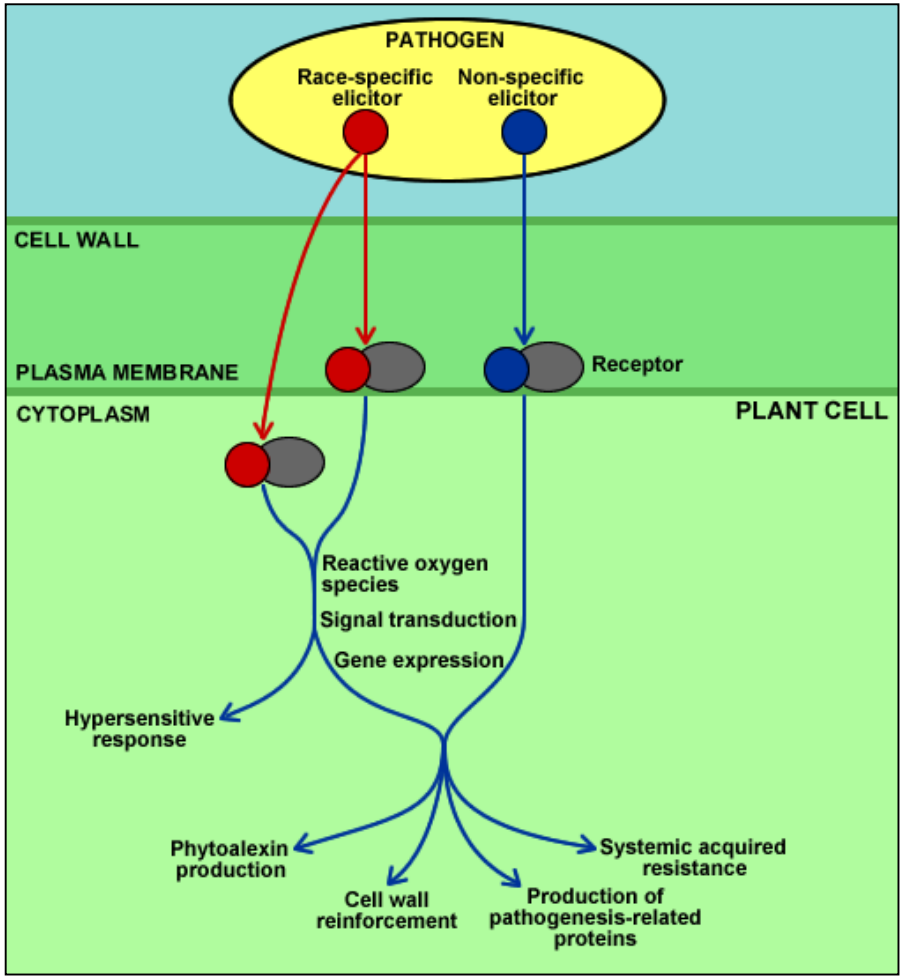
Specific plant disease resistance

- Race-specific resistance:
- Induced in response to only a particular race of pathogen, but occurs in all cultivars of the host plant.
- Cultivar-specific resistance:
- Activated only in a specific host plant cultivar, but in reaction to all races of a pathogen species.
- Race-cultivar-specific (gene-for-gene) resistance:
- This type of resistance is usually referred to as gene for gene resistance, because in most cases it requires the presence of both:
- *avr* gene in the pathogen, and
- One or more corresponding cultivar-specific *R* genes in the host plant.

Specific plant disease resistance
R-gene activated resistance

- Generally, *R*-gene activated resistance includes two related yet spatially and temporally distinct forms:
- **Local resistance:**
- Activated at the site of pathogen infection and often accompanied with HR.
- This primary defense reaction effectively contains the invading pathogen.
- **Systemic acquired resistance:**
- Shortly after or concomitant with local resistance, a secondary defense reaction, i.e. SAR is induced in the uninfected tissues, which leads to a more long-lasting resistance throughout the whole plant against infection by a broad range of pathogens.

Specific plant disease resistance
Race-specific elicitors



The most common defences

- Some of the most common types of resistance which can be observed in nature or mentioned in the literature:

1. An innate resistance:

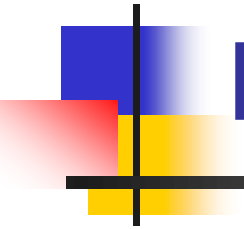
- (Distinct from innate immunity) can potentially be expressed by plants prior to any contact between them and their pathogens.

2. Preformed defences:

- Non-host resistance and incompatible reaction during a gene-for-gene relationship belong to the resistances that plants possess *per se*.

3. Acquired resistances:

- Expressed only by plants and are non-specific.
- In contrast, 'acquired immunity' in animals is highly specific.



Plant innate immune systems

Constitutive & Induced resistance

Innate immune systems

A comparison of animal and plant immune system

- Plants do not have mammalian-like adaptive immunity (circulatory system) and therefore cannot rely on a specialized, proliferative immune system.
- Instead they show various inducible defence responses which are collectively referred to as 'innate immunity'.
- Each plant cell has to be capable of defense, even though this defense is coordinated locally and systemically between cells.
- Despite this, a number of similarities have been found between the innate immune response of plants and humans.
- There are a variety of types of resistance genes and mechanisms, some induced and some constitutive.

Innate immune systems

A comparison of animal and plant immune system

Microbe-associated molecular patterns (MAMPs)

- Plants and animals have evolved sensitive perception systems to recognize microbe-derived products, such as small molecules and proteins.

Altenbach and Robatzek,2007

Innate immunity proteins

PAMPs/MAMPs act as general microbial elicitors

- Plants perceive several general elicitors from both host and non-host pathogens.
- These elicitors are essential structures for pathogen survival and are for that reason conserved among pathogens.
- These conserved microbe-specific molecules, also referred to as Pathogen-associated molecular pattern (PAMP) or microbe-associated molecular patterns (MAMPs).
- General bacterial elicitors, like
- Lipopolysaccharides (LPS),
- Flagellin (Flg),
- Elongation factor Tu (EF-Tu),
- Cold shock protein (CSP),
- Peptidoglycan (PGN), and
- The enzyme superoxide dismutase (SodM).
- These are known to act as MAMPs and induce immune responses in plants or plant cells.

Jackson,2009

Innate immunity proteins
PAMPs/MAMPs sensed by PRRs

- PAMPs/MAMPs are sensed by a broad spectrum of host specie(both plant and animals).
- The corresponding PRRs for some of these bacterial elicitors(PAMPS/MAMPs) have, in recent years, been identified.
- MAMPs are recognized by cognate pattern recognition receptors (PRRs) or surface proteins that trigger immediate defense responses leading to basal or nonhost resistance.

Altenbach and Robatzek,2007

Basal resistance occur early <10 minutes after contact, whereas *R*-gene-mediated defences typically detectable later (2-3 hours).

Innate immune systems

Reasons for failure of PRR proteins to detect microbial patterns

- Specialized pathogenic microbes are able to overcome host immunity by either:

1. Circumventing the detection of MAMPs (camouflage), or
2. Interfering with MAMP- or PAMP-triggered immunity (e.g., delaying, suppressing, or reprogramming host responses).

(See also bacterial sensing and zigzag theory).

- Te secretion of so-called effector molecules promotes pathogen virulence and leads to susceptibility and, ultimately, the expression of symptoms.

Altenbach and Robatzek,2007

Innate immunity proteins

Involvement of intracellular receptors to detect the effectors

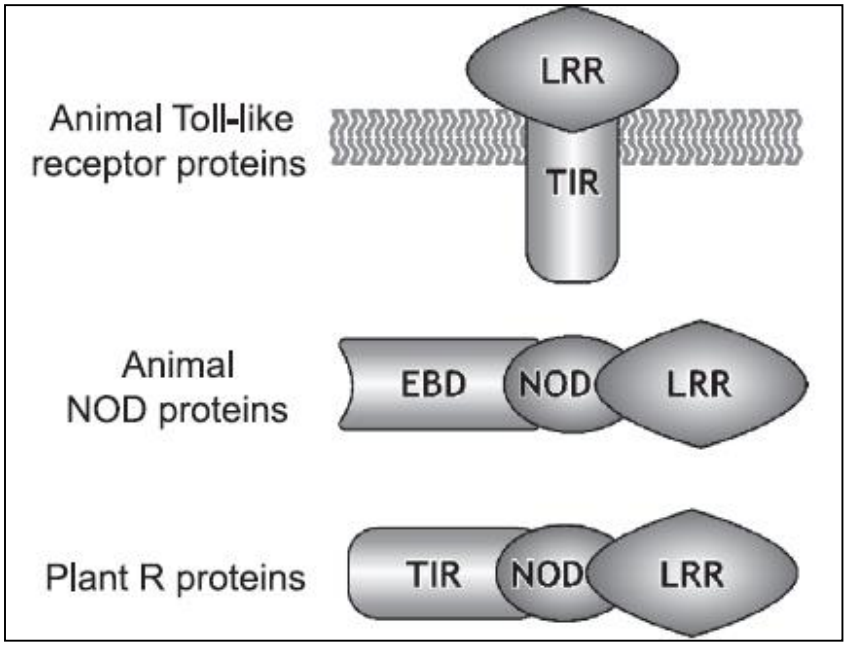
- Receptors detecting microbial patterns(PAMPs/MAMPs) can be divided into surface and intracellular receptors.
- Surface receptors:
- All known PRRs(pattern recognition receptors) in plants are plasma membrane-resident proteins, allowing the perception of MAMPs to occur at the cell surface.
- Intracellular receptors:
- These are the nucleotide-binding site (NB) leucine-rich repeat (LRR) class of receptors(NB-LRR).
- These are closely related to animal NOD-like receptor.
- In plants, they appear to function exclusively in effector recognition.

Altenbach and Robatzek,2007

Innate immune systems

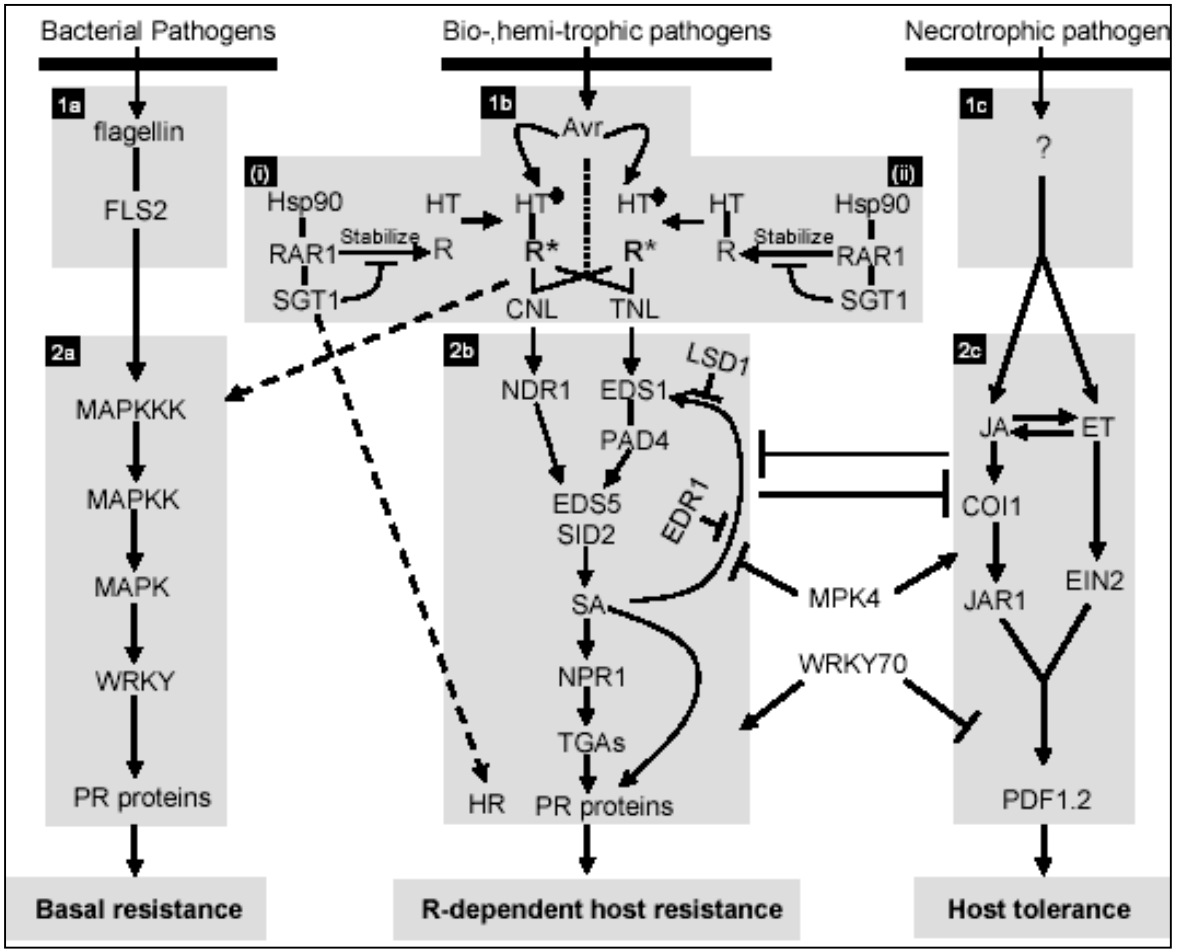
A comparison of animal and plant innate immunity proteins

- In animals an important family of PRRs is the cell-surface Toll-like receptors, which are characterized by an extracellular leucine-rich-repeat (LRR) domain and a cytoplasmic TIR (*Drosophila* Toll/Interleukin-1 receptor) domain.
- In pants the Leu-rich repeat receptor kinase (LRR-RK) FLS2 is the PRR for flagellin.
- It also intracellular resistance proteins have a C-terminal LRR domain, a NOD domain, and an N-terminal TIR domain.



Innate immune systems

Schematic overview of plant innate immunity



Xiao,2006

Innate immune systems

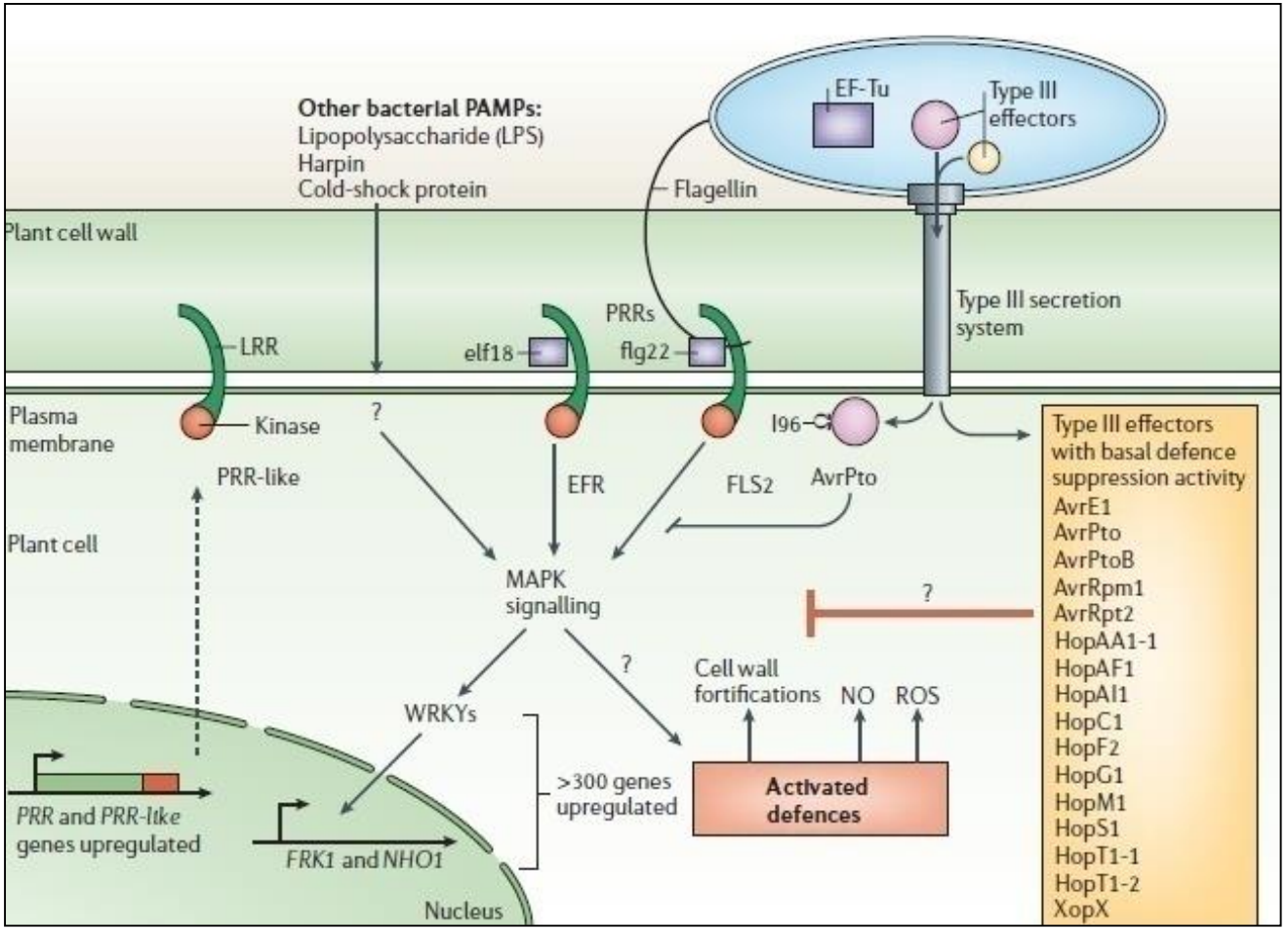
Model depicting the activation of PRR-mediated basal defences and their suppression by type III effectors

- PAMP-induced transcriptional reprogramming showed rapid induction of genes that encode transcription factors, proteins that are associated with:

1. Protein degradation,
2. Hormone-related proteins,
3. Phosphatases, and
4. Diverse protein kinases including a large number of LRR-receptor-like kinases (RLKs).

Innate immune systems

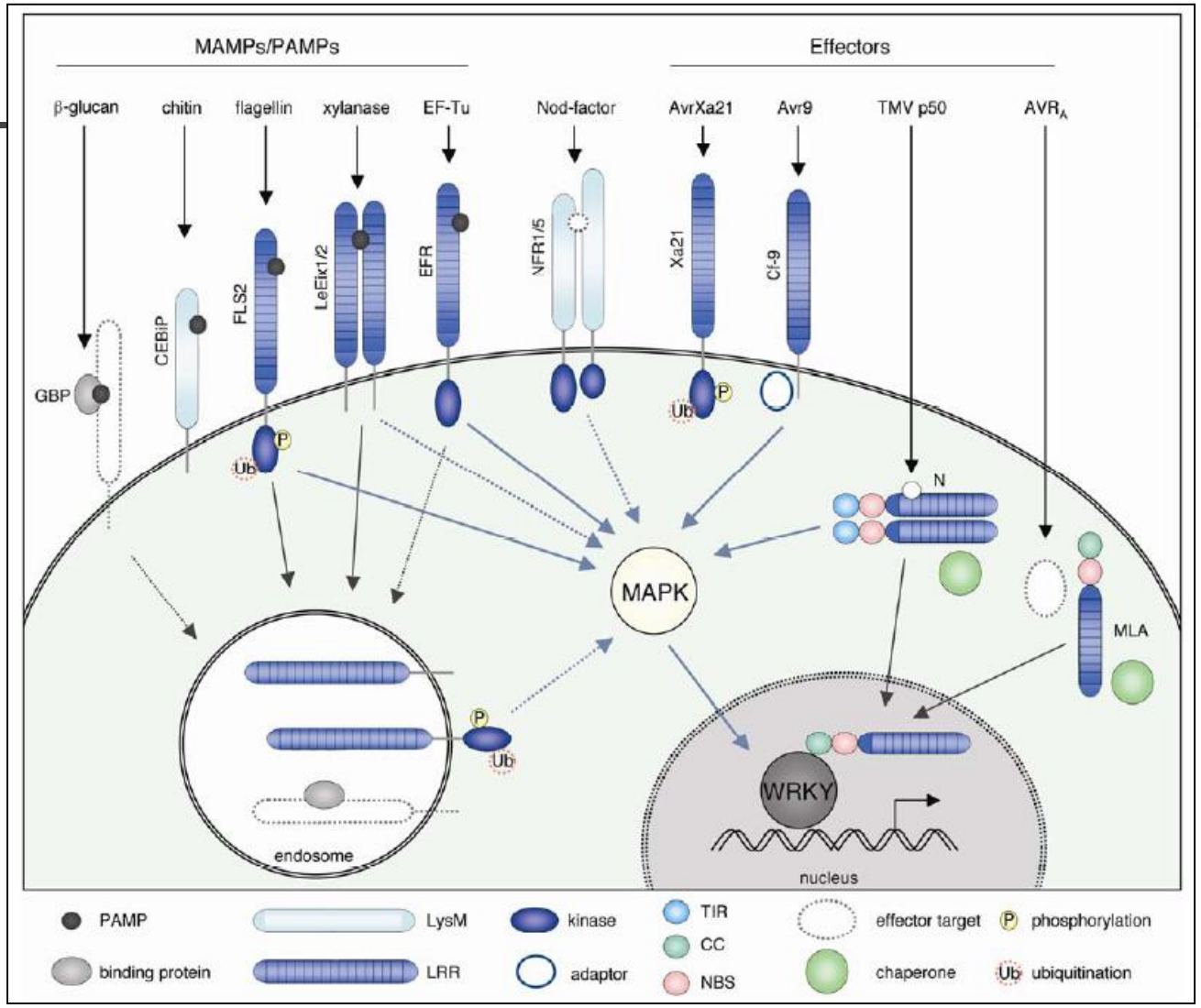
Model depicting the activation of PRR-mediated basal defences and their suppression by type III effectors



Abramovitch *et al.*,2006

Innate immune systems

Subcellular redistribution and signaling of receptors that mediate plant immunity



Altenbach and Robatzek,2007

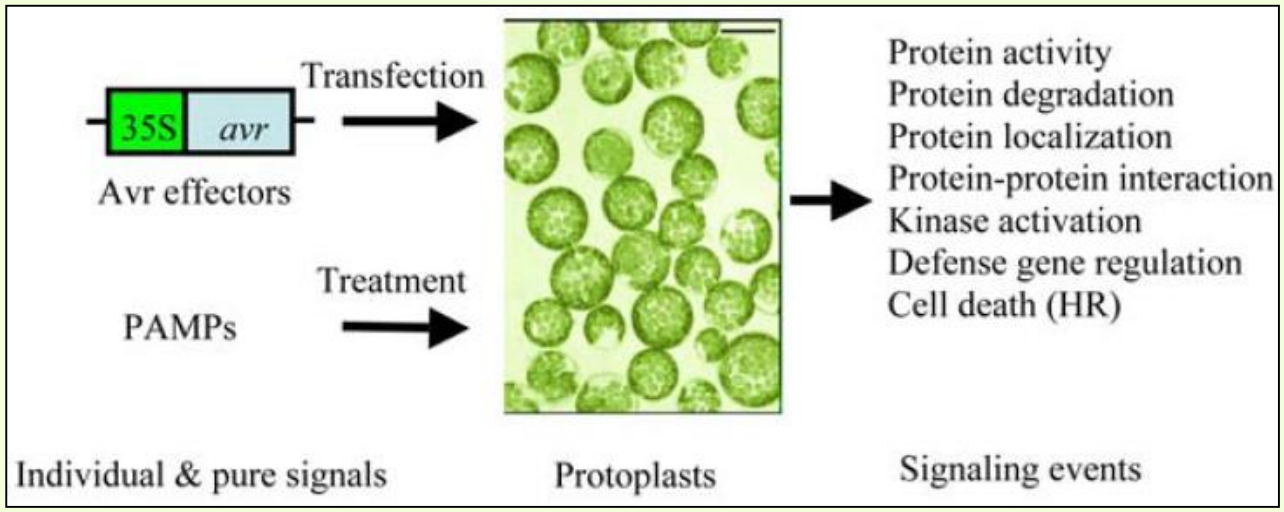
Innate immune systems

The use of protoplasts to study innate immune responses

- Recently, *Arabidopsis* protoplasts have been used successfully to study plant innate immune responses triggered by pathogen-derived elicitors.
- Also use of plant protoplast transient expression system has facilitated the discovery and dissection of many signal transduction pathways in response to hormones, metabolites, and stresses.
- Signal transduction is defined as Reception, conversion and transmission of chemical message by a cell.

The use of protoplasts to study signal transduction pathways

- The use of protoplast transient assays to study early signaling events mediated by Avr and pathogen-associated molecular pattern.
- 35S is the constitutive promoter derived from cauliflower mosaic virus. HR, hypersensitive response.



Ronald,2007

Host and non-host resistance

Plant defence mechanisms

Host and non-host resistance

- Two types of plant resistance response can be distinguished:

1. **Host or race/cultivar specific resistance:**

- Gene recognition mediated by *R* and *avr* genes.

2. **Nonhost/nonspecific resistance:**

- Recognition of specific pathogen or plant cell wall derived signal molecules.

Odjakova and Hadjiivanova,2001

Host and non-host resistance

Similar biochemical processes involved in pathogen resistance

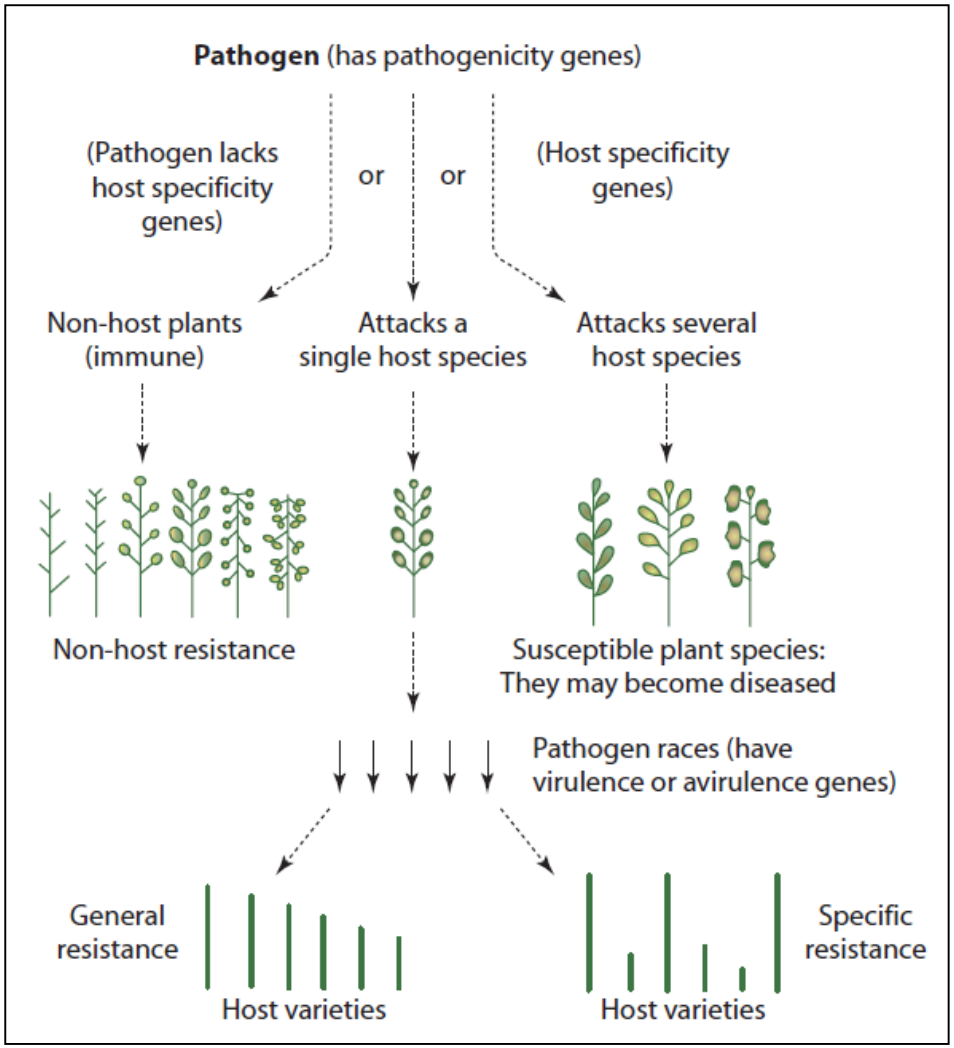
- **Host resistance** acts under the species level with some intraspecific biotypes being resistant and others susceptible.
- **Nonhost resistance** acts at the species level, with all intraspecific biotypes (cultivars) being resistant to the pathogen.
- Nonhost resistance exhibited against bacteria, fungi and oomycetes can be of two types:
- Type I nonhost resistance does not produce any visible symptoms whereas type II nonhost resistance results in a rapid hypersensitive response with cell death.
- Strong similarities exist between nonhost and gene-for-gene resistance responses but it is still not clear if the same mechanism is involved in producing these resistance responses.

Mysore and Ryu,2004

Non-host resistance

- Where the plant is never a host for a pathogen.
- Non-host concerns the resistance observed when a microorganism attacks a plant species which is never its host.
- The microorganism is called a "non-pathogen".
- The plant species is called a "non-host".
- The interaction is called a "non-host interaction".

General interactions of a pathogen with its host and nonhost plants



Agrios,2005

Non-host resistance

The most common form of disease resistance

- Nonhost resistance represents one of the most promising defence mechanisms in developing durable resistance against plant pathogens, namely due to its effectivemenss against a broad range of pathogen species and its durability in nature.

Non-host resistance in plants

- Most plants are resistant to most pathogens, so disease is the exception.
- A pathogen that can not cause disease on a non-host plant is referred to as non-host pathogen.
- Plants that cannot be successfully attacked by a given pathogen are called non-hosts for that pathogen, and we say that they display non-host resistance.

Non-host resistance

Most durable form of plant disease resistance

- Resistance of an entire plant species to all isolates of a microbial species is referred to as non-host or species resistance.
- Nonhost resistance is achieved through the recognition of specific pathogen (exogenous elicitors) or plant cell wall derived signal molecules (endogenous elicitors).
- These elicitors are often low-molecular-weight compounds that are either synthesized as such or are liberated from polymeric precursors during infection.

Exogenous/endogenous elicitors

- **Exogenous elicitors:**
- Pathogen-associated molecular patterns (PAMPs e.g. flagellin), conserved among several microbial species.
- **Endogenous elicitors:**
- Bacterial type III effectors in *Arabidopsis* protoplasts (avirulence [Avr] or type III effectors) specific to some races of a pathogen species.

Non-host disease resistance

- Nonhost disease resistance is the most common form of disease resistance exhibited by plants against the majority of potentially pathogenic microorganisms.
- Recently, several components of nonhost disease resistance have been identified.
- Nonhost resistance exhibited against bacteria, fungi and oomycetes.
- Two types of nonhost resistance have been distinguished on the basis of interaction phenotypes:

1. Type I nonhost resistance
2. Type II nonhost resistance

Mysore and Ryu,2003&04

Non-host disease resistance

1. Type I nonhost resistance occurs without apparent host cell death i.e. does not produce any visible symptoms.
2. Type II nonhost resistance is accompanied by HR, results in a rapid hypersensitive response with cell death.

- Strong similarities exist between nonhost and gene-for-gene resistance responses but it is still not clear if the same mechanism is involved in producing these resistance responses.

Mysore and Ryu,2003&04

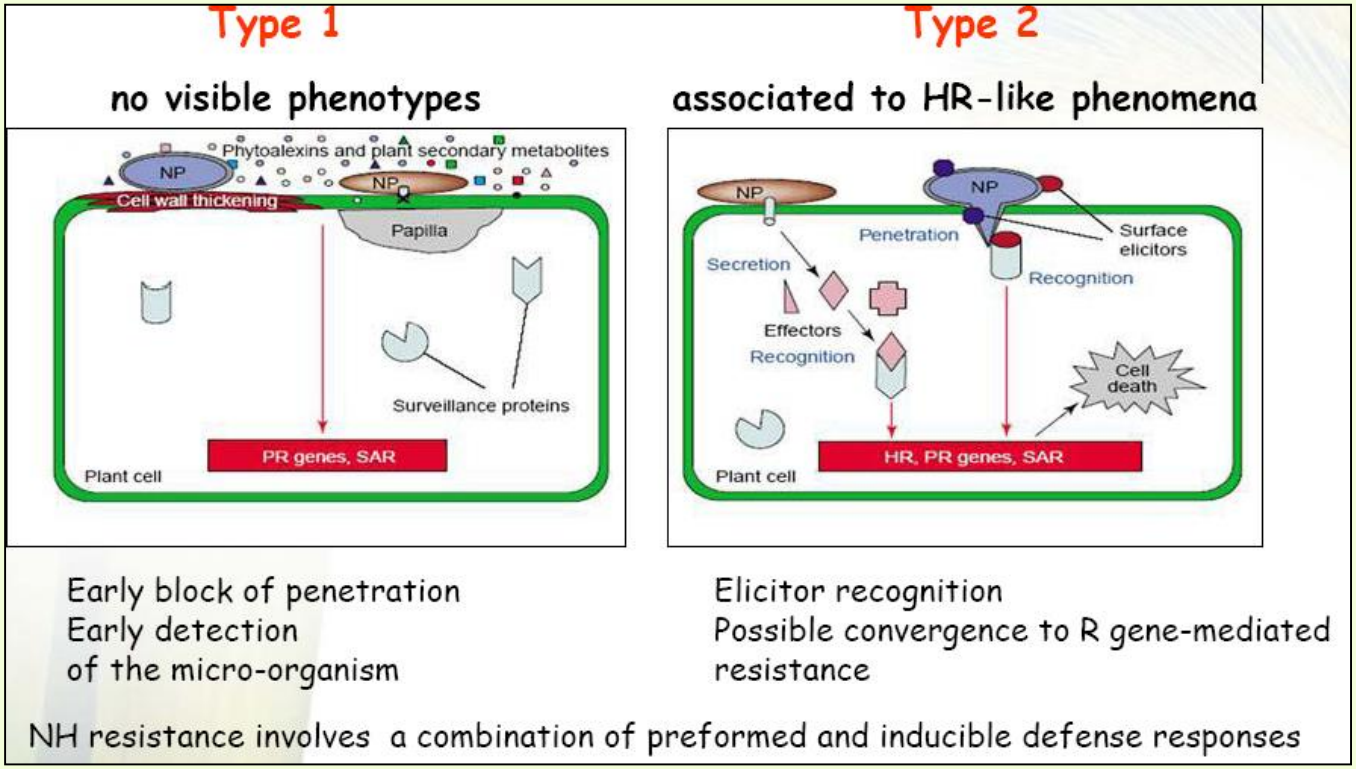
A model for type I and type II non-host resistance

- During type I nonhost resistance the nonhost pathogen **is not able to overcome** the preformed and general elicitor-induced **plant defense responses** such as cell wall thickening, phytoalexin accumulation, other plant secondary metabolites and papilla formation.
- Pathogenesis-related (PR) gene expression as a component of systemic acquired resistance (SAR) can be induced by general elicitors of the nonhost pathogen.

- During type II nonhost resistance the nonhost pathogen is **able to overcome** preformed and general elicitor-induced **plant defense responses**, probably by producing detoxifying enzymes.
- Specific pathogen elicitors are then recognized by the plant surveillance system and this triggers plant defense leading to a hypersensitive response (HR).
- PR gene expression and SAR are also induced during type II nonhost resistance.

A model for type I and type II non-host resistance

- Blue-colored NPs (nonhost pathogens) represent fungi or oomycetes and brown-colored NPs represent bacteria.



Defence mechanisms
Passive and active defense mechanisms

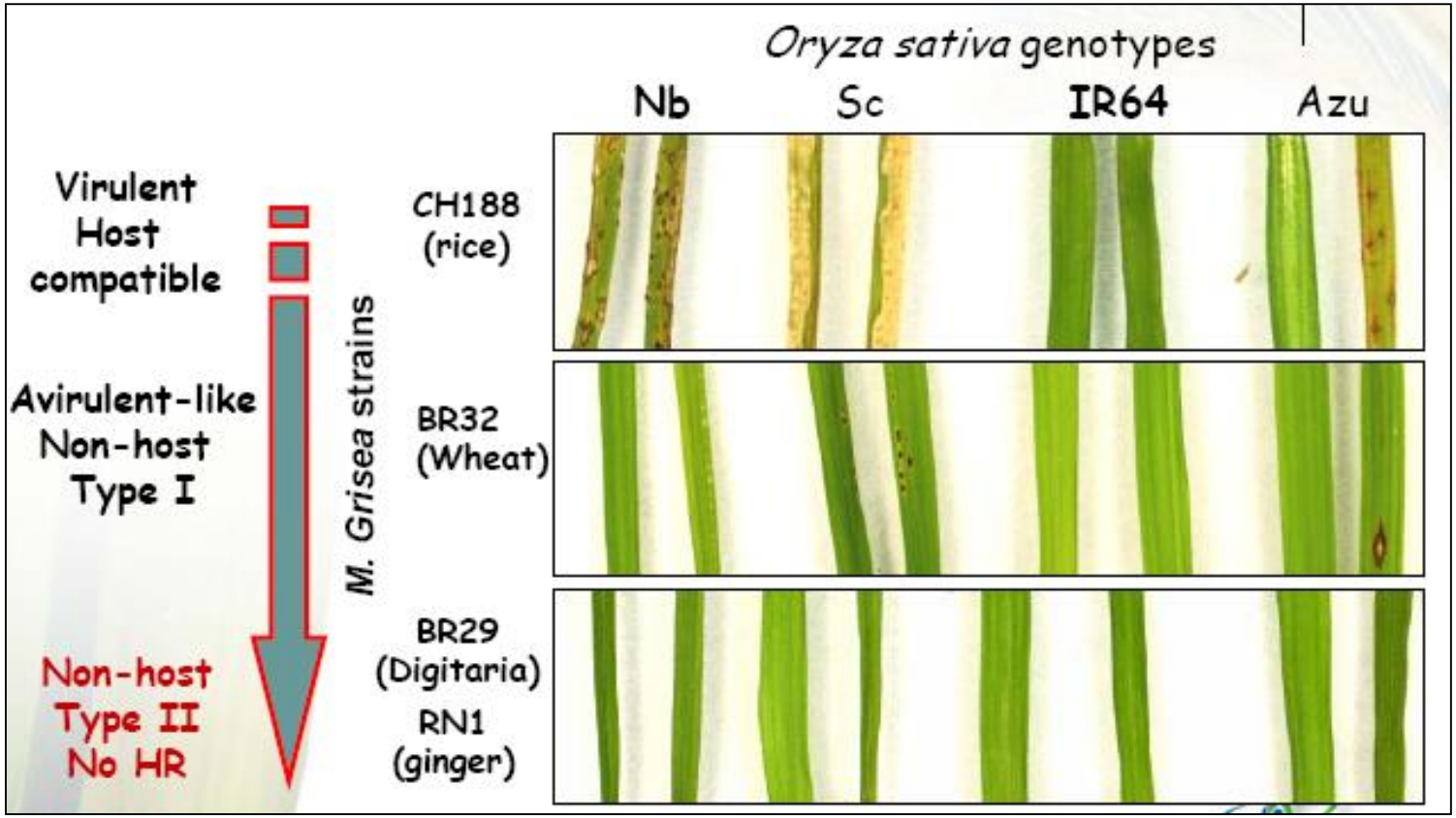
- Passive (preformed) defence lines such as cell walls, wax layers and chemical barriers confer broad resistance to a wide variety of pathogens.
- If a pathogen overcomes this first line of defence, there is a second line, which is mounted by proteins encoded by specific resistance (*R*) genes.
- Active defence mechanisms(defense-related genes, PR proteins) that render the plant resistant.

Arabidopsis:

Exhibiting both type I and type II non-host resistance

- Arabidopsis displays:
- Type I nonhost resistance against *P. syringae* pv. *phaseolicola*, and
- Type II nonhost resistance against *P. syringae* pv. *tomato*.

Characterization of non-host phenotypes in rice



Examples of type I and type II non-host resistance

Pathogen	Strain or isolate	Nonhost plant(s)	Visible symptoms
Type I nonhost resistance			
Pseudomonas syringae pv. phaseolicola	NPS3121	*Arabidopsis*	None
P. s. pv. phaseolicola (at 30 °C)	S2	*Nicotiana tabacum*	None
P. s. pv. syringae	B76	*Arabidopsis*	None
P. s. pv. savastanoi	213-3 (IAA^-)	*Arabidopsis*	None
P. s. pv. delphinii	PDDCC529	*Arabidopsis*	None
P. s. pv. morsprunorum	B60-1	*Arabidopsis*	None
P. s. pv. atrofaciens	B143	*Arabidopsis*	None
P. s. pv. coronafaciens	B142	*Arabidopsis*	None
Xanthomas campestris pv. campestris	8004	*Nicotiana benthamiana*	None
Gaeumannomyces graminis var.*tritici*	T5	*Avena strigosa*	None
Puccinia recondita f. sp.*tritici*	WBR I	Oat	None
Puccinia graminis f. sp.*tritici*	ANZ	Oat	None
Phytophthora infestans	88069	*N. alata* cv. lime green	None
P. infestans	88069	*N. clevelandii*	None
P. infestans	88069	*N. tabacum* cv. *xanthi*	None
Type II nonhost resistance			
Pseudomonas syringae pv.*maculicola*	m2	*Nicotiana benthamiana*	HR
P. s. pv. tomato	DC3000	*N. tabacum*	HR
P. s. pv. phaseolicola	NPS3121	*N. tabacum*	HR
P. s. pv. glycinea	PG4180	*N. tabacum*	HR
P. s. pv. pisi	ATCC # 11055	*N. tabacum*	HR
P. s. pv. syringae	61	*N. tabacum*	HR
P. cichorii	83-1	*Arabidopsis*	HR
Xanthomonas axinopodis pv. vesicatoria	82-8	*N. benthamiana*	HR
X. campestris pv. glycines	8ra	Pepper, tomato	HR
X. citri	3213	Cotton, bean	HR
Erwinia rubrifaciens		*N. tabacum*	HR
Alternaria brassicicola	MUCL20297	*Arabidopsis*	HR
Blumeria graminis f. sp. *tritici*	bgtA95	Barley	HR
Phytophthora infestans		*Arabidopsis*	HR
P. infestans	88069	*N. benthamiana*, *N. rustica*, parsley	HR
P. sojae		*Arabidopsis*	HR
Fusarium solani f. sp. *phaseoli*	W-8	Pea	HR

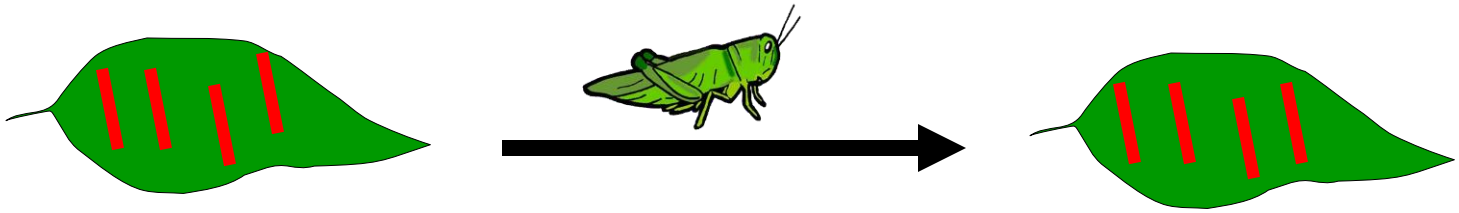
Mysore and Ryu,2004

Mechanisms of General Defenses

Defense Mechanisms in Plants

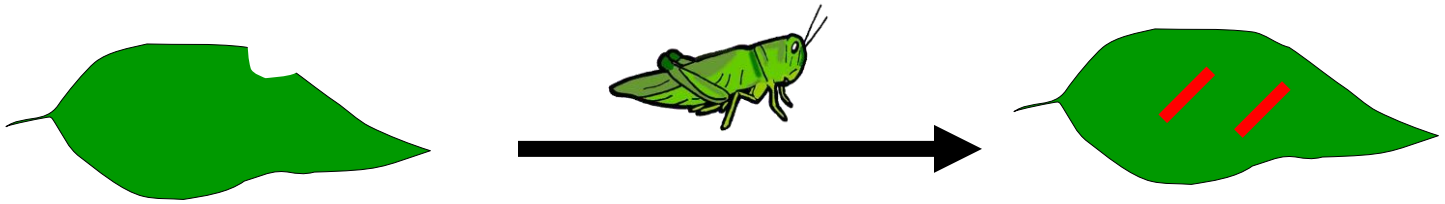
1. Passive (constitutive) defense (First line defense)
2. Active (inducible) defense (Second line defense)

Constitutive vs. Induced Defenses



Constitutive defense

always present



Induced defense

synthesized in response to challenge

Defense mechanisms
Passive and active defense lines

- Passive defence lines such as cell walls, wax layers and chemical barriers confer broad resistance to a wide variety of pathogens.
- If a pathogen overcomes this first line of defense, there is a second line, which is mounted by proteins encoded by specific resistance (*R*) genes.

Plant defense strategies
Passive and active defense lines

- Plants exhibit a wide array of defense strategies against pathogen attack.
- The resistance against pathogens is performed by both:

1. Constitutive (continuous) defense mechanisms
2. Inducible defense mechanisms

Defense mechanisms in plants

Passive defense lines

First line defense

- **A. Passive (preformed, preexisting, constitutive defense mechanisms).**
- First line defense includes:

1. Physical barriers such as cell walls, wax layers;
2. Chemical barriers barriers (secondary compounds) such as phenols, quinones, antimicrobial proteins and peptides which confer broad resistance to a wide variety of pathogens.

- Secondary compounds are so-called because they do not play a role in photosynthesis, growth, or respiration.

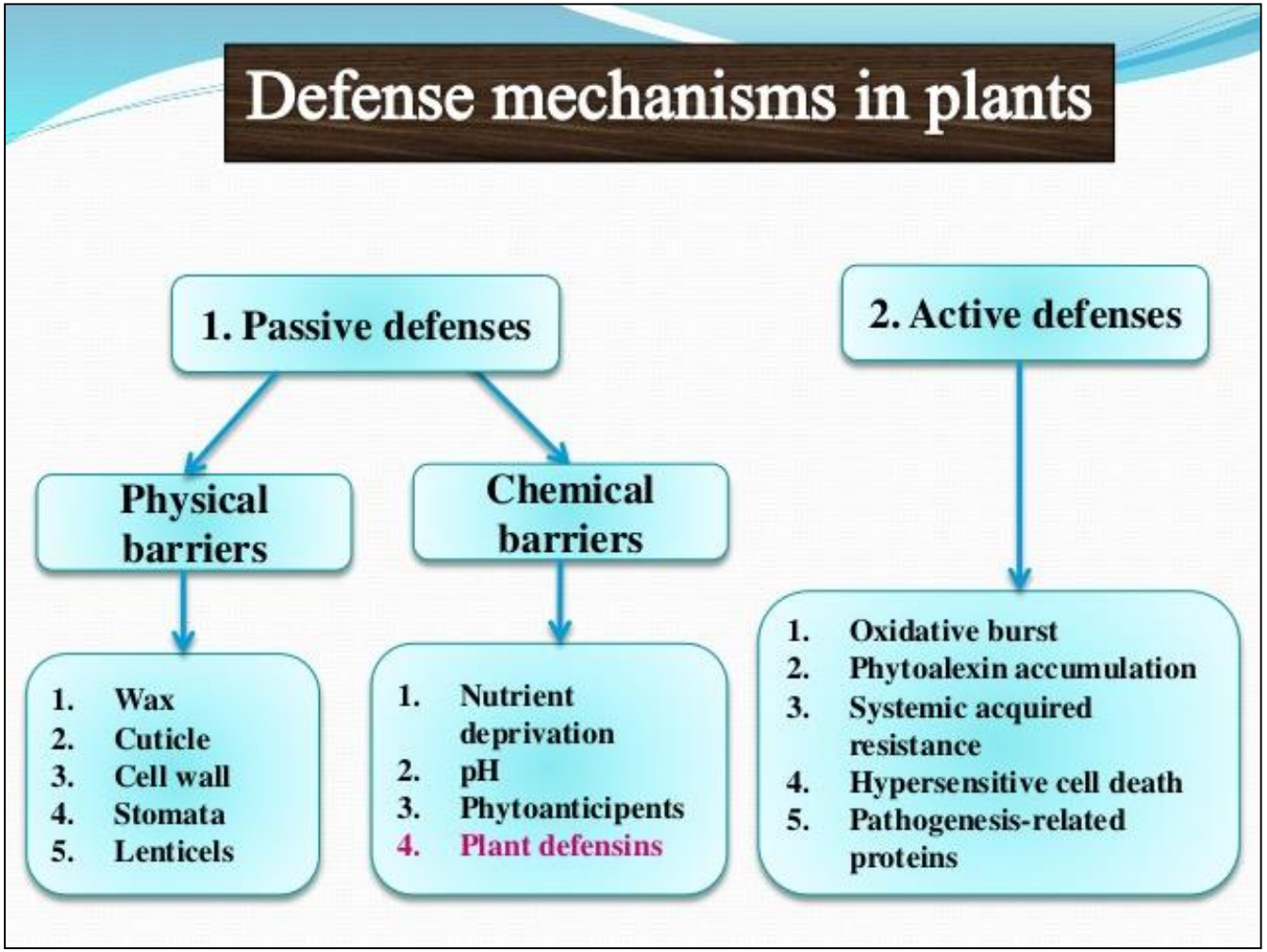
Defense mechanisms in plants

Active defense lines

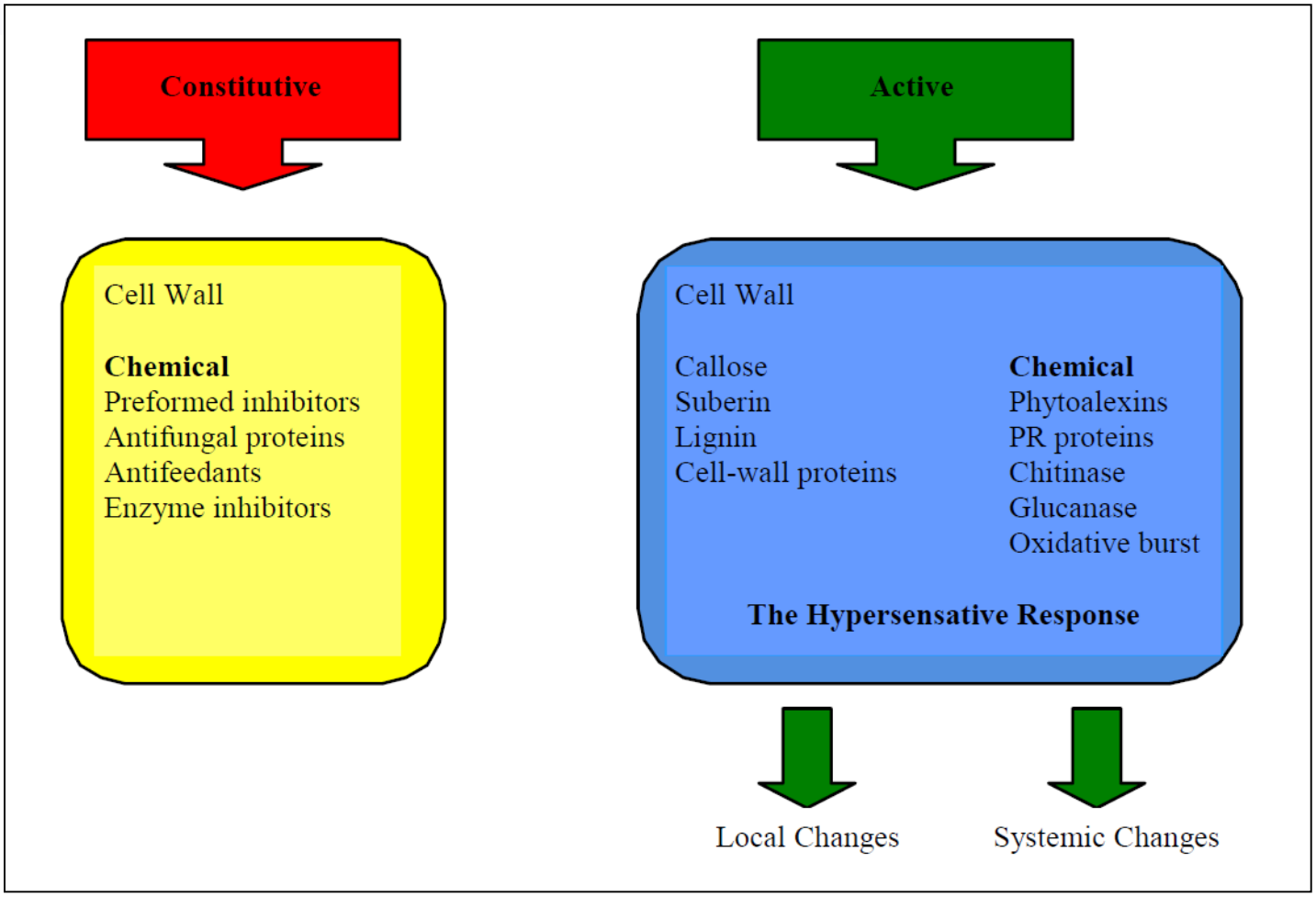
Second line defense

- **B. Active defence mechanisms**
- Second line defense includes:
- Hypersensitive response, phytoalexin synthesis, lignification, synthesis of pathogenesis-related proteins, Hydroxyproline-rich glycoproteins and systemic acquired resistance (SAR).
- If a pathogen overcomes this first line of defence, there is a second line, which is mounted by proteins encoded by specific resistance (*R*) genes.

Passive and active defense mechanisms
Plant defense response



Passive and active defense mechanisms
Plant defense response



Vaitkunas,2003

Constitutive Defence Mechanisms
The preexisting defense system

- **Passive defense mechanisms:**
  - **Physical barriers**
  - **Chemical barriers**

Defense mechanisms in plants

First line defense

Physical and chemical barriers

PHYSICAL DEFENSES

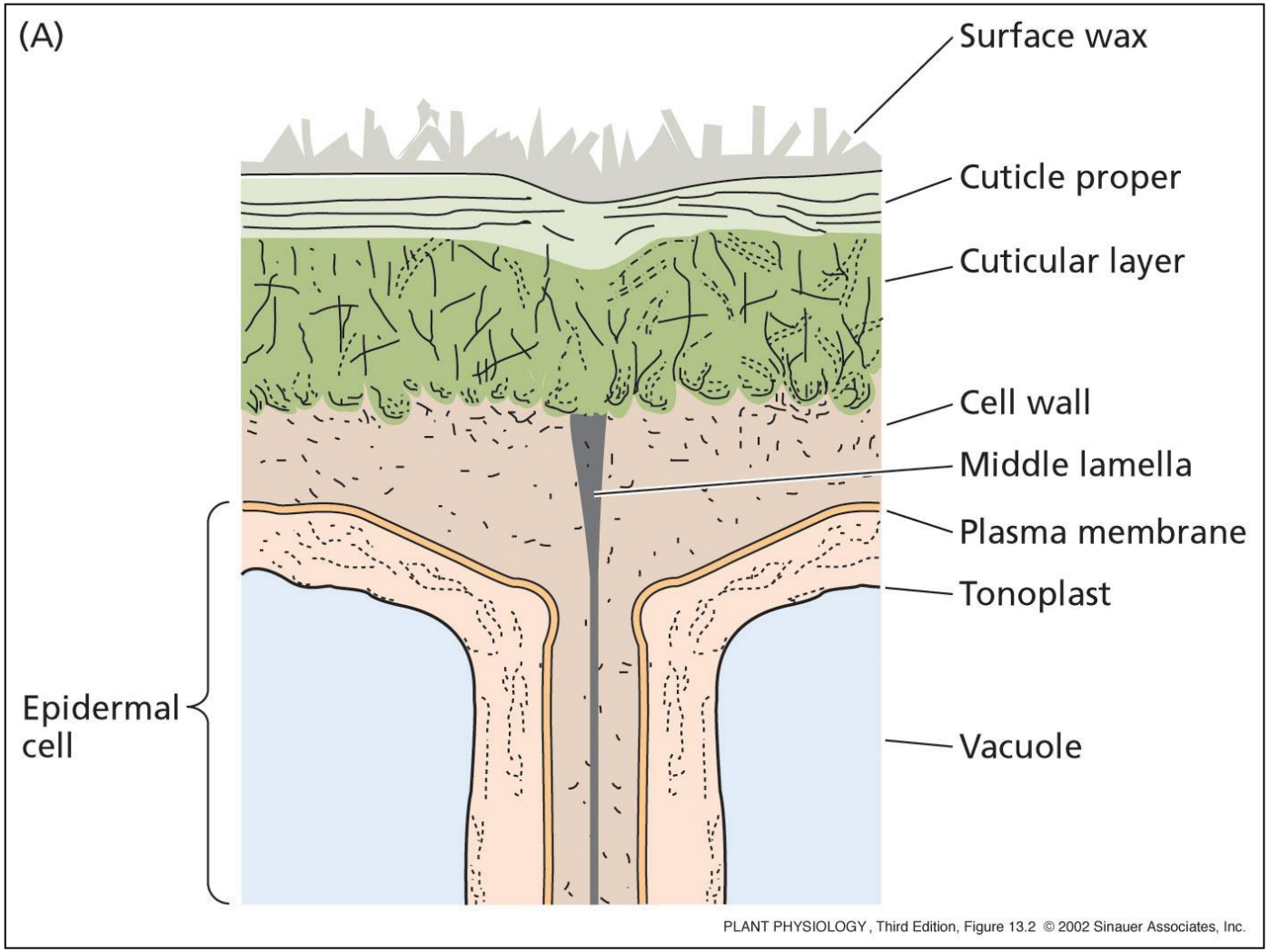
- Spines, thorns
- cutins
- waxes
- Suberins

SECONDARY METABOLITES

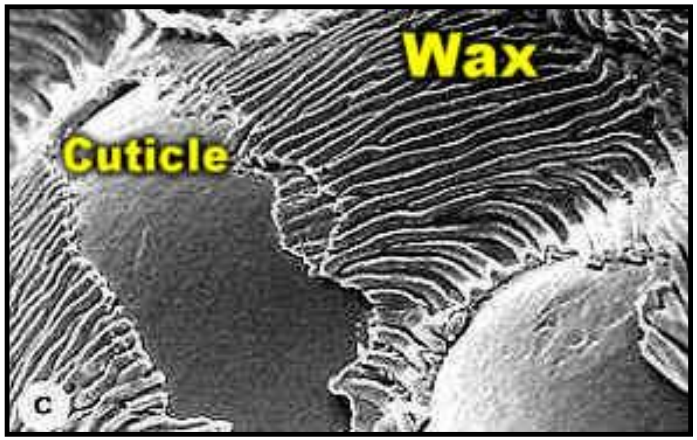
Phenolics	**Defense-related proteins**
phenolic glycosides	peroxidases
bound phenolics	polyphenol oxidase
lignin?	PAL
condensed tannins	hydrolysable tannins
Terpenes	**N-containing**
monoterpenes	Alkaloids
diterpene acids	Mustard oils

Constitutive defense mechanisms

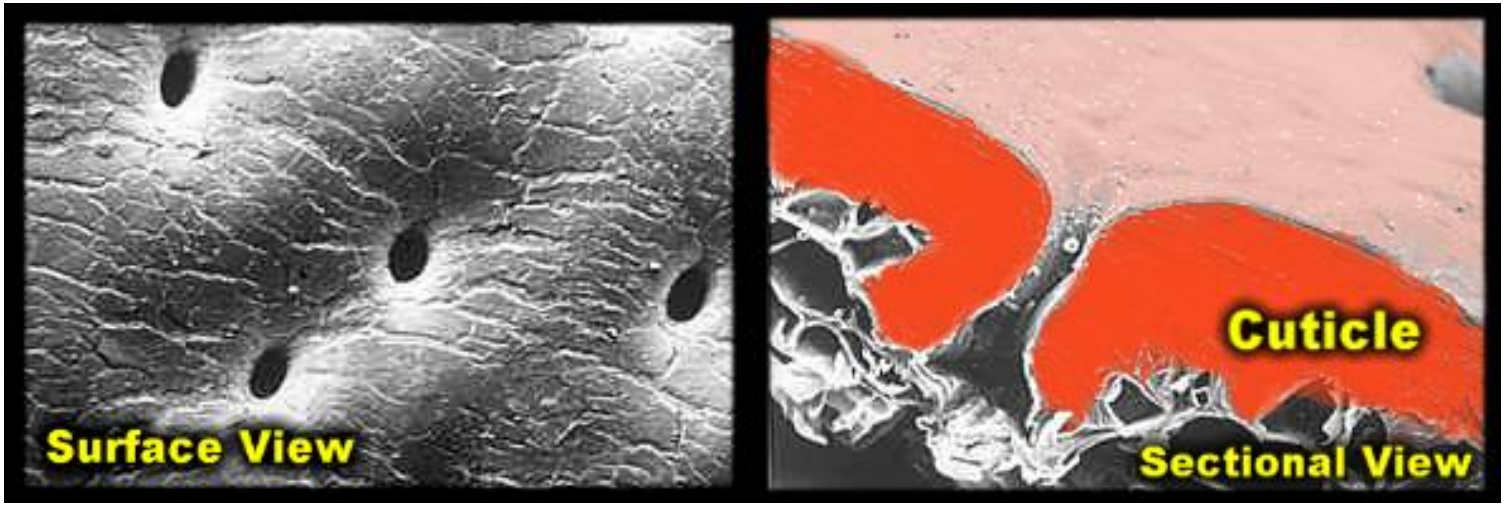
1. Physical barriers



Plants' cuticles often vary with the climate in which they live

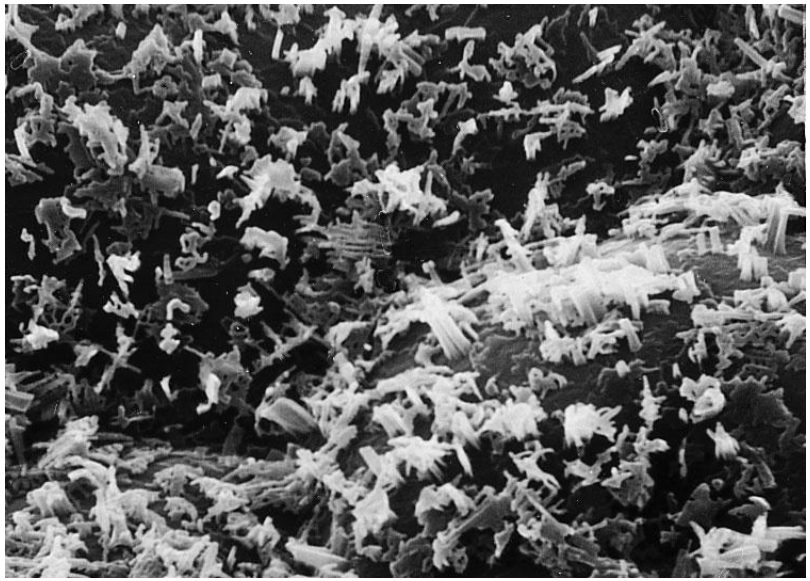


Cactus cuticle



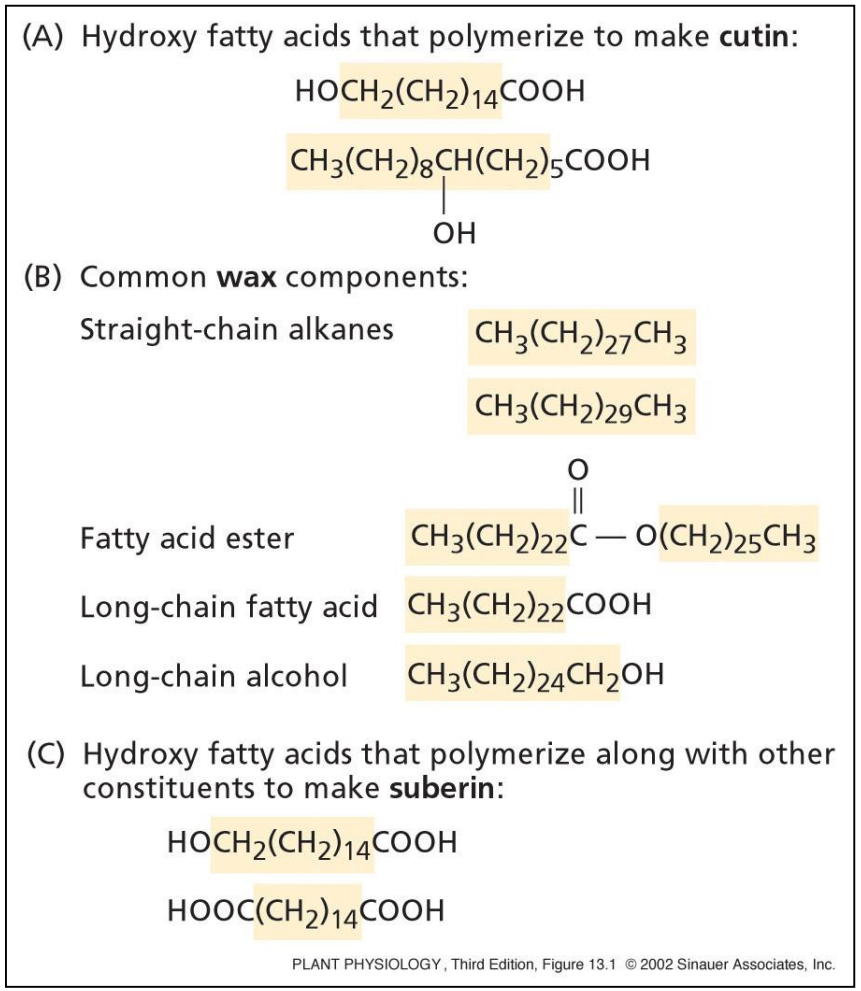
Physical barriers
Waxes

- Complex mixtures of long-chain lipids that are extremely hydrophobic.
- Waxes are synthesized by epidermal cells.
- Exuded through pores in the epidermal cell wall by an unknown mechanism.



Physical barriers
Wax and suberin

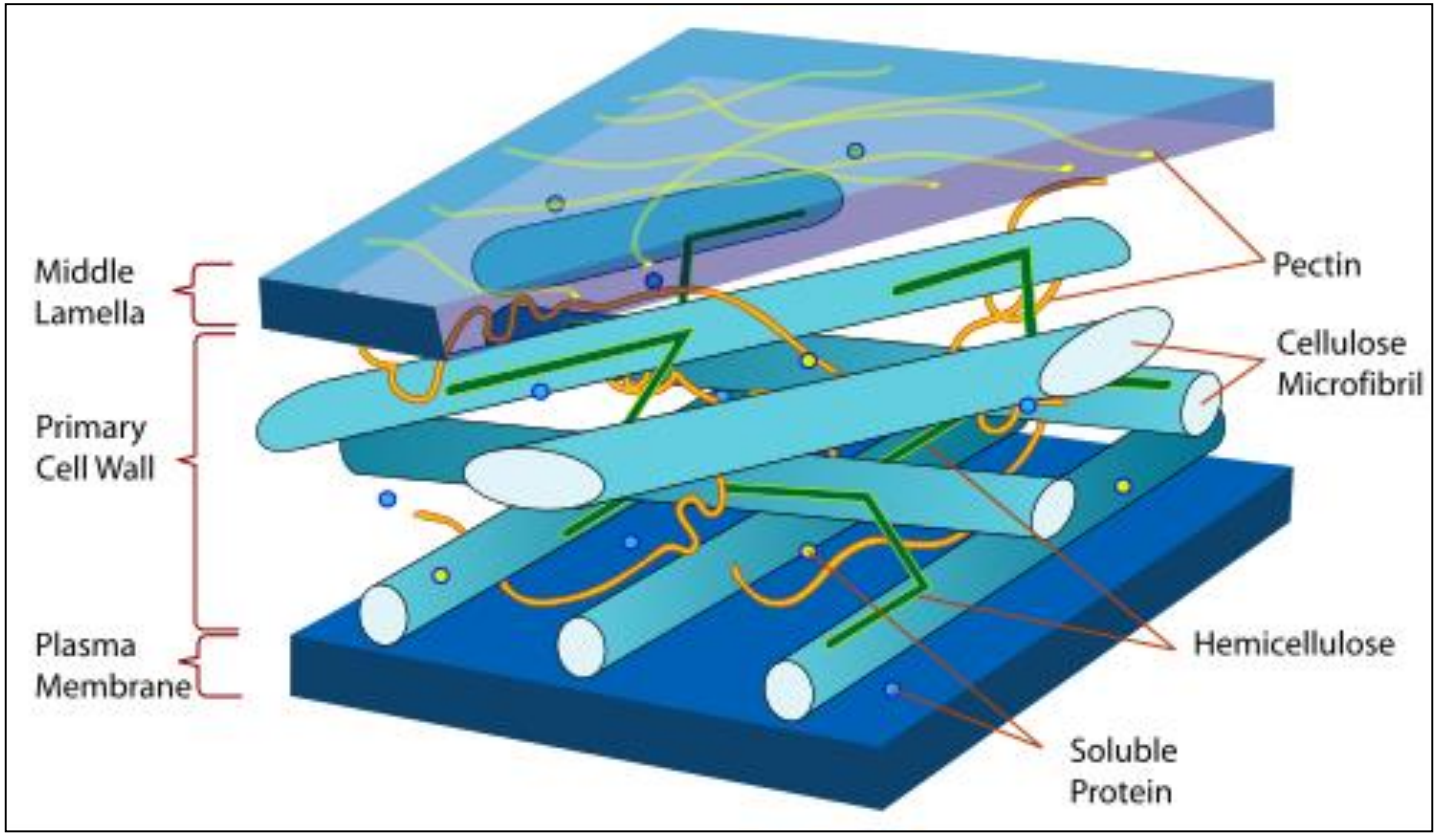
- Also formed from fatty acids but has a different structure from cutin.
- A cell wall constituent.
- Often within roots.
- Can protect against pathogens and other damage.
- Older parts of roots more suberized.
- Endodermis has suberin side walls, water must pass through plasma membrane to get to stele.



Passive defence mechanisms

Section of a cell wall

A model of the interconnections among components cellulose, hemicellulose and pectin



Wikipedia

Constitutive defence mechanisms
2. Chemical barriers

- **Low molecular weight compounds: e.g.**
  - Phenols and quinones
  - Cyanogenic glucosides
  - Saponins
  - Terpenoids
  - Stilbenes
  - Glucosinolates
  - Cyclic hydroxamic acids and related benzoxazolinone compounds
- **High molecular weight compounds: e.g.**
  - Tannins
  - Anti-microbial proteins and peptides
  - Defensins
  - Lysozyme
  - Proteinase inhibitors
  - Polygalacturonase-inhibiting proteins (PGIPs)

Major groups of antimicrobial compounds from plants

- Phenolics and Polyphenols;
- Quinones: Aromatic rings with two ketone substitutions;
- Flavones, flavonoids, and flavonols;
- Tannins: A group of polymeric phenolic substances;
- Coumarins: Phenolic substances made of fused benzene and a-pyrone rings;
- Alkaloids;
- Terpenoids and Essential Oils;
- Lectins and Polypeptides e.g. Thionins.

Secondary metabolites

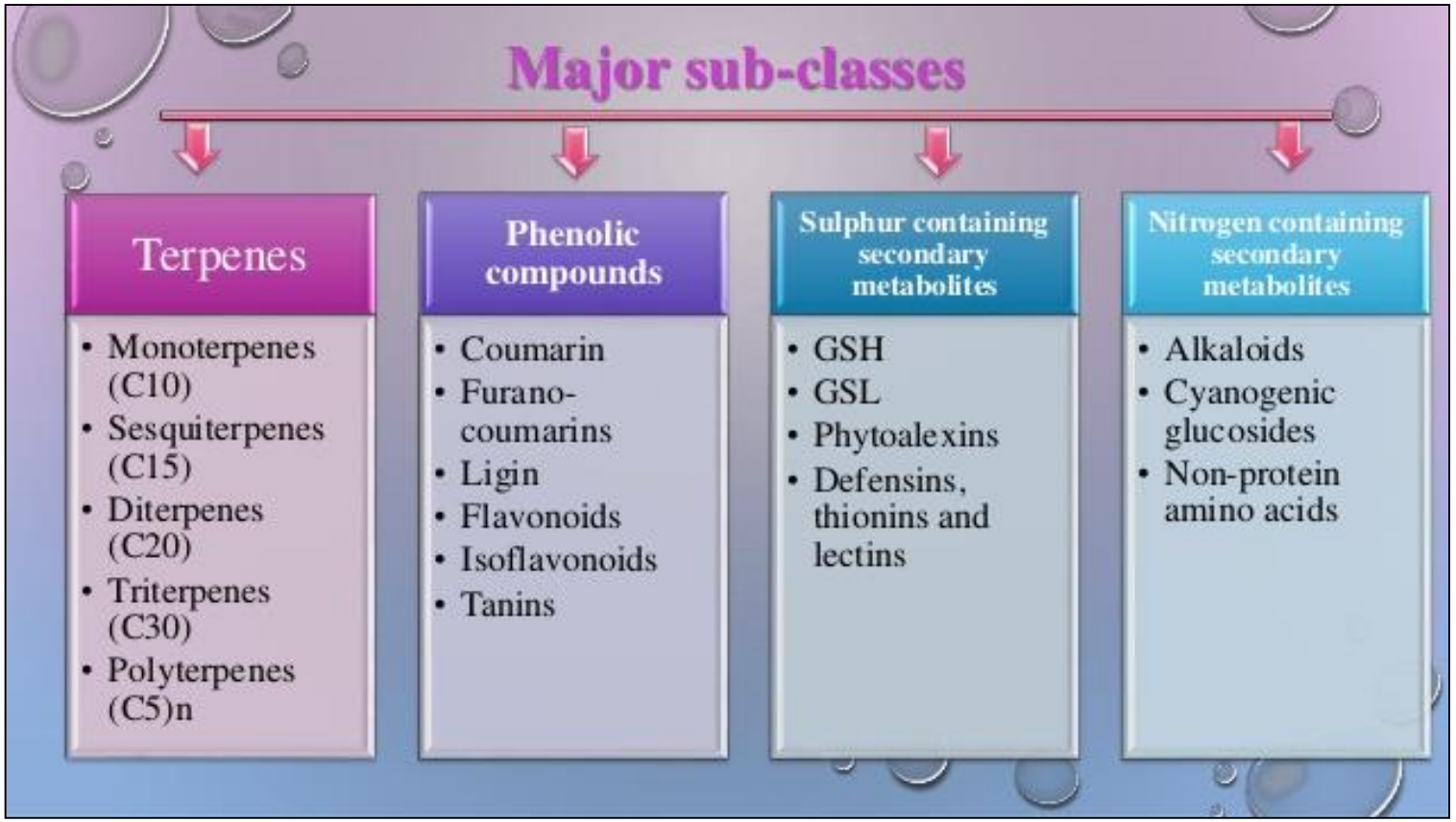
Do not play a role in photosynthesis, growth, or respiration, but protect the tissues from infections

- Secondary metabolites are not necessarily produced under all conditions, and in the vast majority of cases the function of these compounds and their benefit to the organism is not yet known.
- Some secondary metabolites are produced for easily appreciated reasons. e.g.

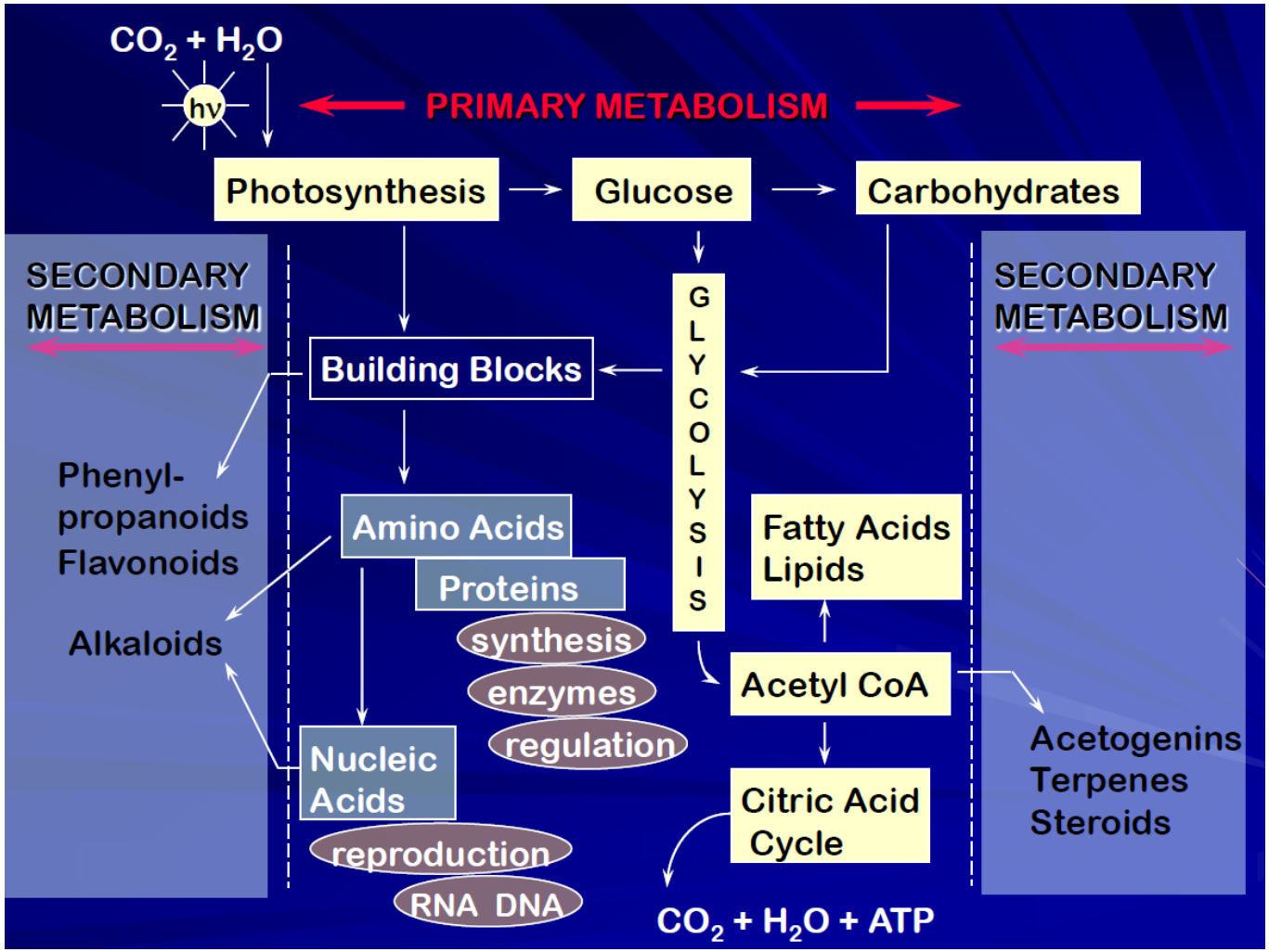
1. As toxic materials providing defense against predators.
2. As volatile attractants towards the same or other species.
3. As coloring agents to attract or warn other species.

Secondary metabolites

Do not play a role in photosynthesis, growth, or respiration, but protect the tissues from infections



Primary and secondary metabolite biosynthesis



Terpenes and Terpenoids

Isoprene-units

Constituents of essential oils

- Terpenes: Class of >20,000 natural organic compounds containing carbon atoms in multiples of five (5).
- The name 'terpene' is derived from the Greek word 'terebinth'.
- Terebinth is a type of pine tree from which terpene-containing resins are obtained.
- Terpenoids: Oxygen-containing terpenes (alcohols, ketones, aldehydes). These are derived from five-carbon isoprene units assembled and modified in thousands of ways.
- Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers.

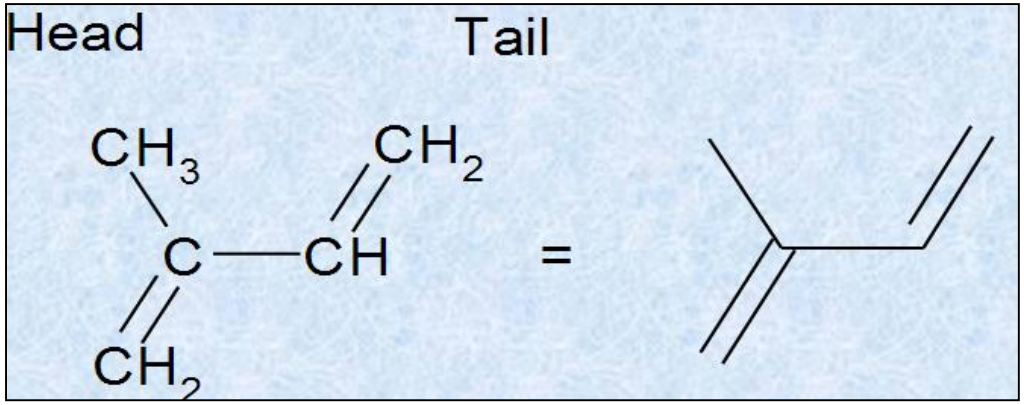
Terpenoids are terpenes that contain oxygen.

Terpenes and Terpenoids

Isoprene-units

Constituents of essential oils

- All terpenes are built up from units of isoprene-units linked in a "head-to-tail" fashion.
  - Head-branched end of isoprene;
  - Tail-unbranched end of isoprene.



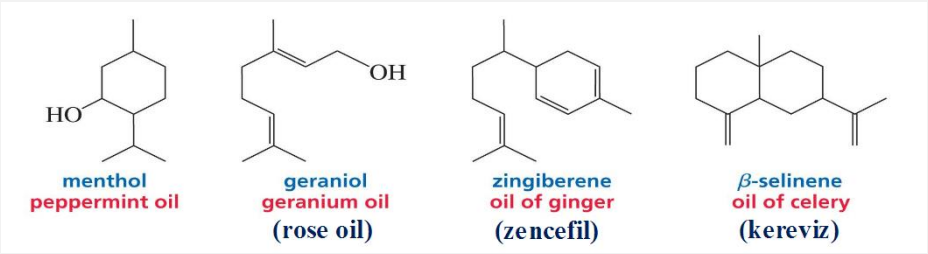
Terpenes and Terpenoids

Terpenoids are terpenes that contain oxygen

Classification of Terpenes

Type of terpenes	No. of carbon atoms	Isoprene units
hemiterpene	5	one(consist of 1 isoprene units i.e. 5 carbons)
monoterpenes	10	two
sesquiterpenes	15	three
diterpenes	20	four
sesterterpenes	25	Five (consist of 5 isoprene units i.e. 25 carbons)
triterpenes	30	six
tetraterpenes	40	eight

sesqui = one and a half
tetra = four
hemi = half
tri = three
di = two



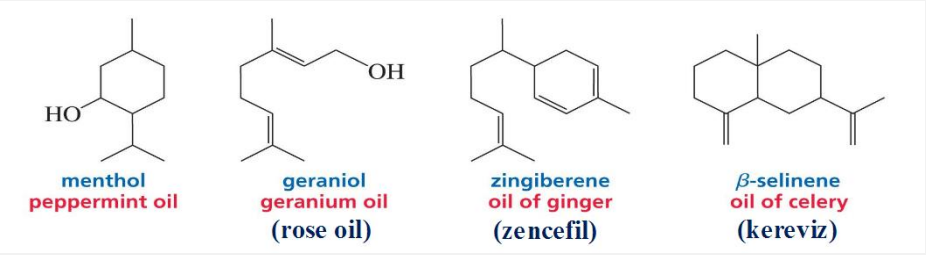
Terpenes and Terpenoids

Terpenoids are terpenes that contain oxygen

Classification of Terpenoids

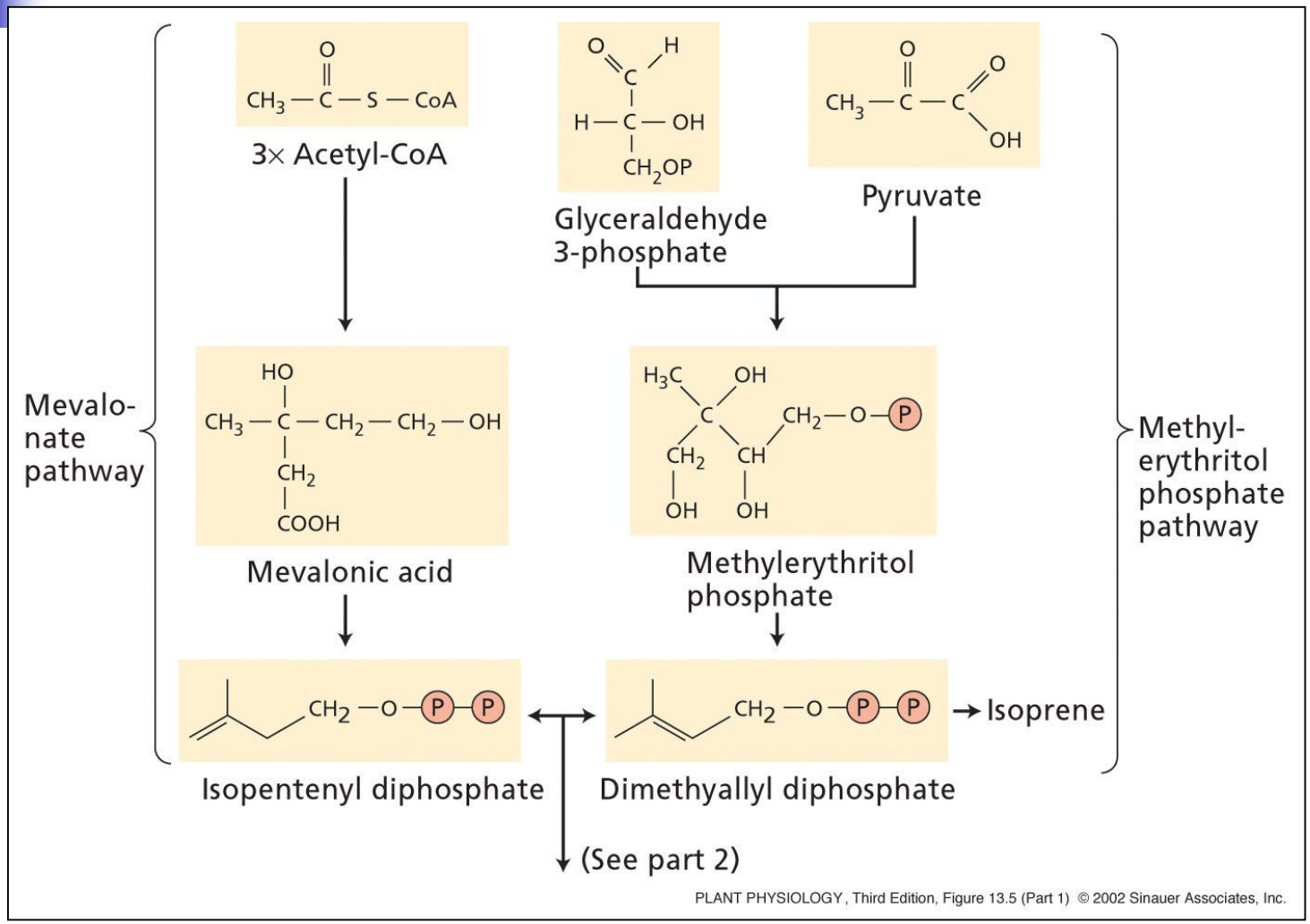
Type of terpenoids	No. of carbon atoms	Isoprene units
hemiterpene	C_5	oneone(consist of 1 isoprene units i.e. 5 carbons)
monoterpenoid	C_{10}	two
sesquiterpenoid	C_{15}	three
diterpenoid	C_{20}	four
sesterterpenoid	C_{25}	five
triterpenoid	C_{30}	six
tetraterpenoid	C_{40}	eight

sesqui = one and a half
tetra = four
hemi = half
tri = three
di = two



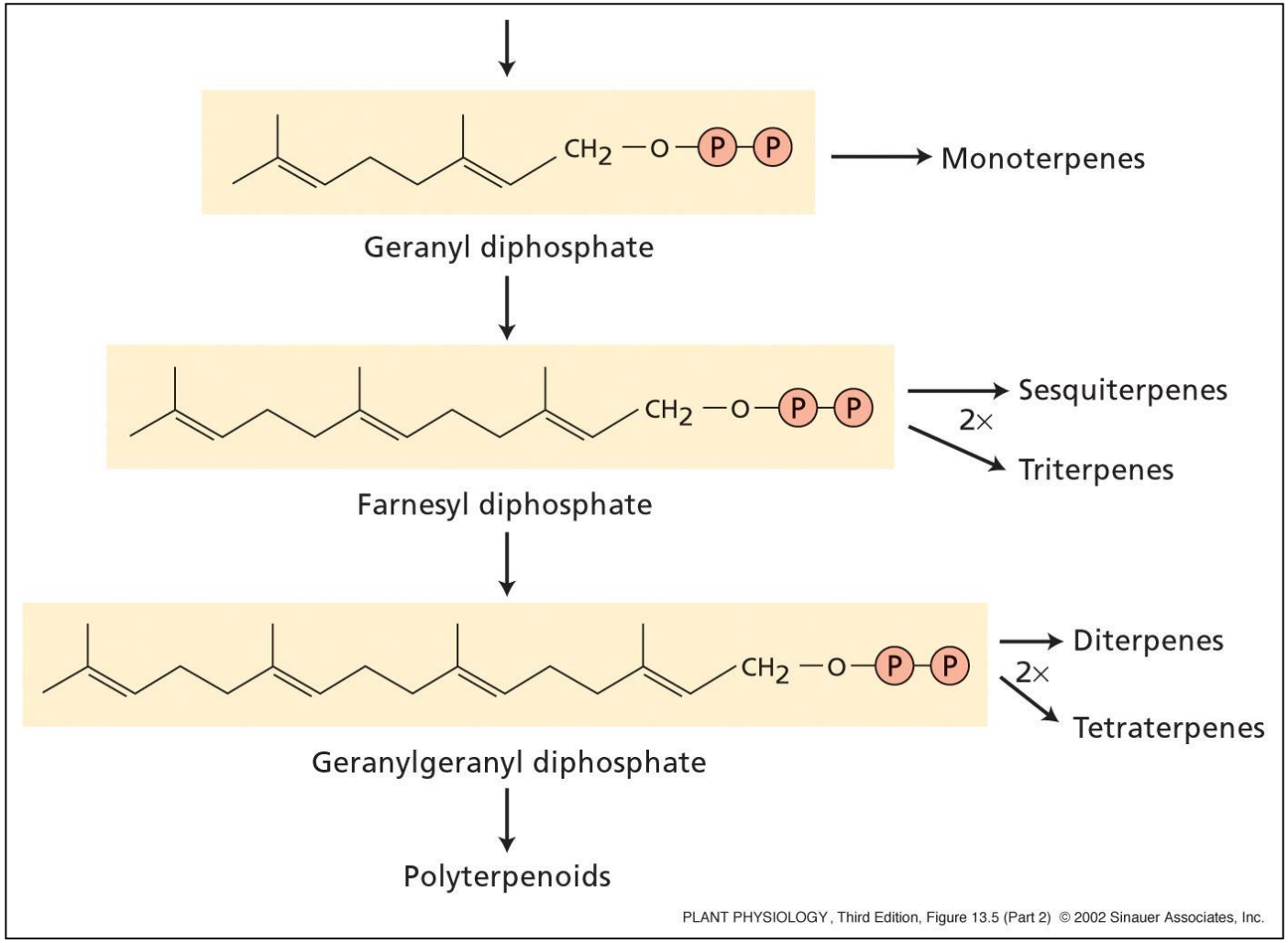
Terpenes

Produced from the mevalonic acid pathway



Terpenes

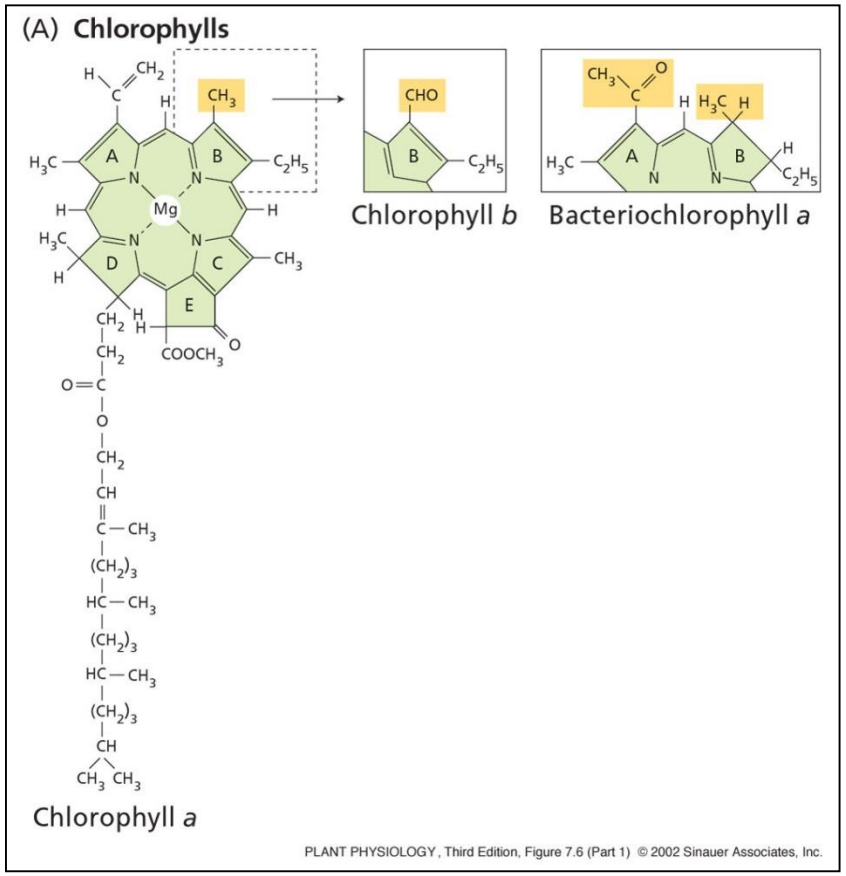
Produced from the mevalonic acid pathway



Terpene functions
1. Growth and development

- Growth and development

1. Carotenoid pigments are tetraterpenes.
2. Chlorophyll side chain is diterpene.
3. Giberellins (hormones) are diterpenes.
4. Abscissic acid (hormone) is a sesquiterpene C_{15}.
5. Sterols are triterpenes.



Terpene functions
2. As defensive compounds

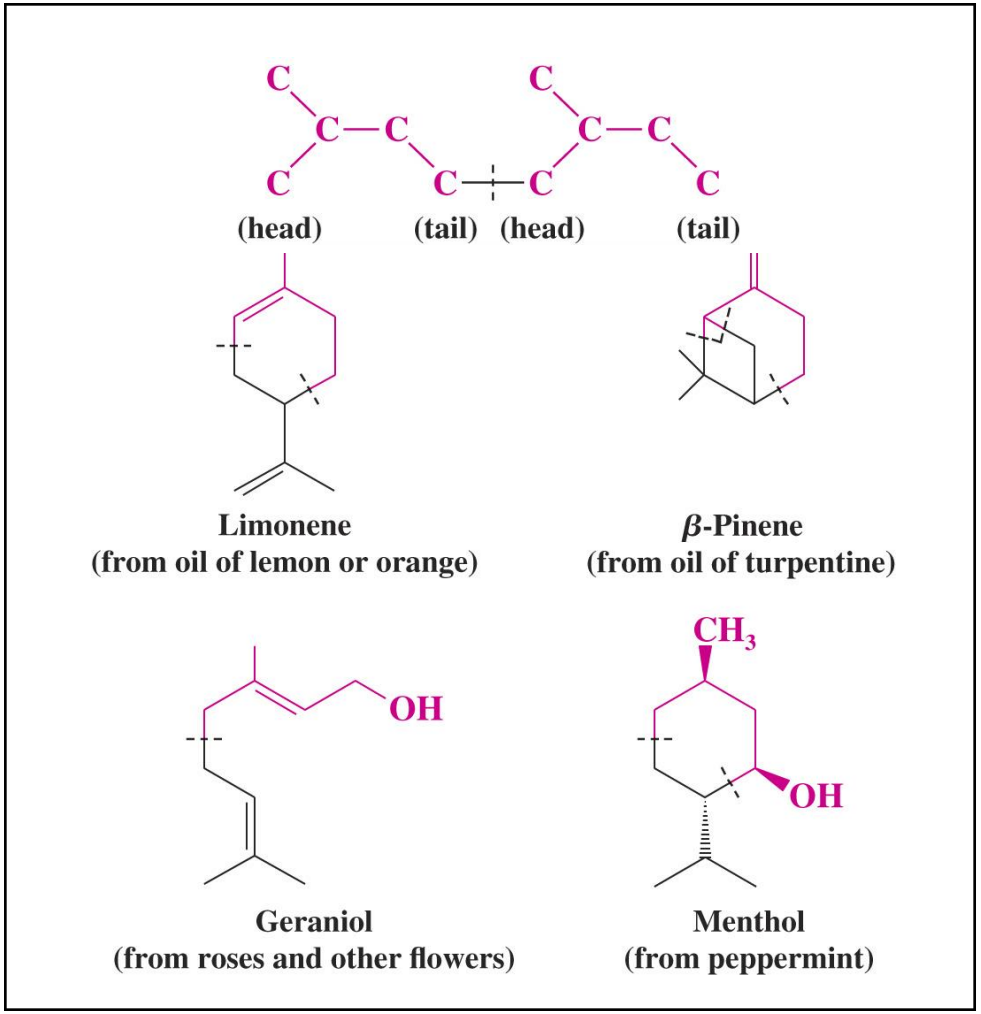
- As defensive compounds
- Toxins and feeding deterrents to pathogens, insects and mammals.
- Examples
- Resins of conifers are monoterpenes
- Essential oils - peppermint, limon, basil, sage, may be in glandular hairs on epidermis.

Non-volatile terpenes limonene.

Terpenes and Terpenoids

Isoprene units in terpenes are usually linked head to tail

Dashed lines can be used to delineate the isoprene units comprising a terpene



Antimicrobial peptides

Plant-derived molecules

High molecular weight compounds

- It is estimated that there are 250,000 to 500,000 species of plants on Earth.
- A relatively small percentage (1 to 10%) of these are used as foods by both humans and other animal species.
- It is possible that even more are used for medicinal purposes.

Antimicrobial peptides

Plant-derived molecules

High molecular weight compounds

- Many plants possess antimicrobial peptides.
- These peptides constitutively (continuously) expressed or induced after infection.
- These families of peptides include:
- Four-cysteine-type peptides
- Hevein and knottin-type peptides
- Lipid transfer proteins (LTPs)
- Plant defensins
- Ribosome-inactivating proteins (RIPs)
- Thionins.

Antimicrobial peptides

Plant defensins

- Defense-related, cysteine-rich, antimicrobial peptides present in the plasma membrane of host plant species.
- They constitute a group designated defensins capable of providing resistance against microbial plant pathogens.
- The first plant defensins that were demonstrated to possess antifungal activity were the two plant defensin isoforms Rs-AFP1 and Rs-AFP2 isolated from radish seed.

Plant defensins

- Some plant defensins as antibacterial peptides have also been reported to exert antibacterial activity such as:
- A *Clitoria ternatea* plant defensin (Ct-AMP1), which is active against *Bacillus subtilis*, and
- A potato tuber plant defensin, which inhibits:
- *Ralstonia solanacearum*, and
- *Clavibacter michiganensis*.

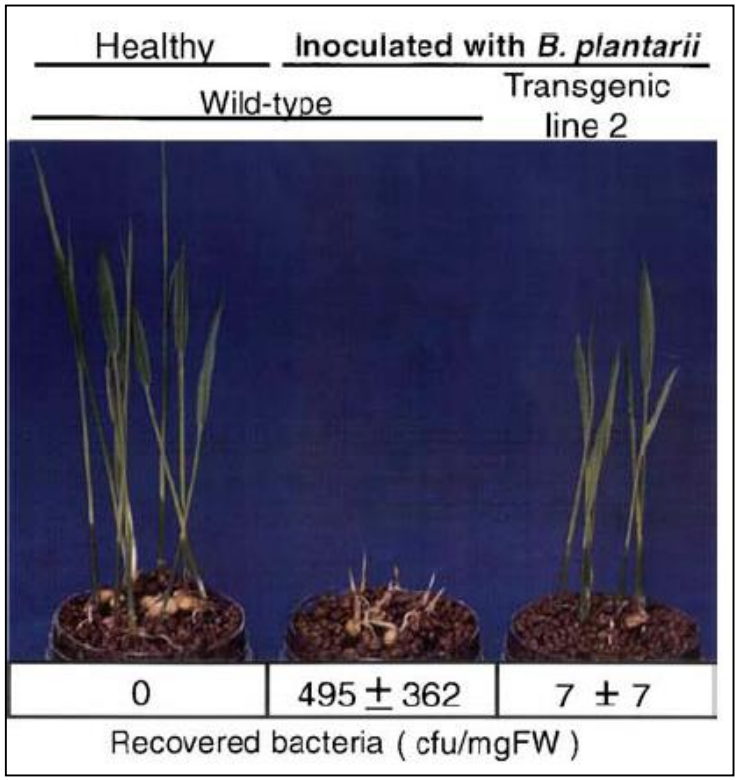
Antimicrobial peptides

Thionins

- The toxic activity of thionins was apparently first described by Jago and Jago in 1885.
- Toxic effects against yeast, bacteria and fungi and mammals were discovered.
- e.g.
- The Barley leaf thionins have repeatedly been shown to have antimicrobial activities *in vitro* against different phytopathogenic bacteria and fungi.
- During the past several years, strong evidence has been obtained for a resistance
- Overexpression of different thionins has resulted in enhanced resistance against bacterial and fungal pathogens.

Transforming rice with a thionin gene against *Burkholderia plantarii*

- The effectiveness of transforming rice with a thionin gene from oats in protecting the plant from infection by *Burkholderia plantarii*:
- left, uninoculated controls;
- centre, inoculated wild-type plants, and
- Right, inoculated transformants.
- Numbers below the pots are cfu of the bacterium/mg fresh weight of plant material recovered from the plants.



Iwai *et al.*,2002

Examples of primary amino acid sequences of natural antimicrobial peptides

Peptide	Structure
Gramicidin S	Cyclic (LOVPF^{d}LOVPF^{d})
Bacitracin	Cyclized I(C)LE^{d}I(KO^{d}IFHD)D^{d}-NH_2
Polymyxin B	Cyclized isooctanoyl BTBB(BF^{d}LBBT)
defensin (NP-1)	$VVC_1AC_2RRALC_3LPRERRAGFC_3RIRGRIHLC_2$ C_1RR
Defensin 1	$DHYNC_1VSSGQC_2LYSAC_3PIFTKIQGTC_2YRGKAKC_1C_3K$
Crab tachyplesin	$RRWC_1FRVC_2YRGFC_2YRKC_1R$
Cattle bactenecin	$RLC_1RIVVIRVC_1R$
Silk moth cecropin A	KWKFKKIEKMGRNIRDGIVKAGPAIEVIGSAKAI
Cattle indolicidin	ILPWKWPWWPWRR
Bacterial nisin	$IXA_1IULA_1Z_2PGA_2KZ_3GLAMGA_3NMKZ_4AZ_5A_4HA_5SIHVUK$

Hancock and Chapple,1999

Peptide antibiotics

Antimicrobial peptides

- Hundreds of peptide antibiotics have been described in the past half-century.
- These fall into two classes:

1. **Non-ribosomal peptides**, such as the gramicidins, polymyxins, bacitracins, glycopeptides, etc. These are largely produced by bacteria (also by fungi).
2. **Ribosomal (natural) peptides**, such as major component of the natural host defense molecules including AMPs which are produced by all species of life (including bacteria).

Anti-microbial peptides (AMP)

Natural peptides

- Anti-microbial peptides (AMP) make up a part of the innate immunity of most organisms and are often involved in the immune system's first line of defense when faced with an invader.
- AMPs can be found in prokaryotic and eukaryotic organisms and in vertebrates and invertebrates (Reddy,2004).
- Antimicrobial peptides are so widespread that they are likely to play an important protective role.

Induced Defense Mechanisms

Active defense mechanisms

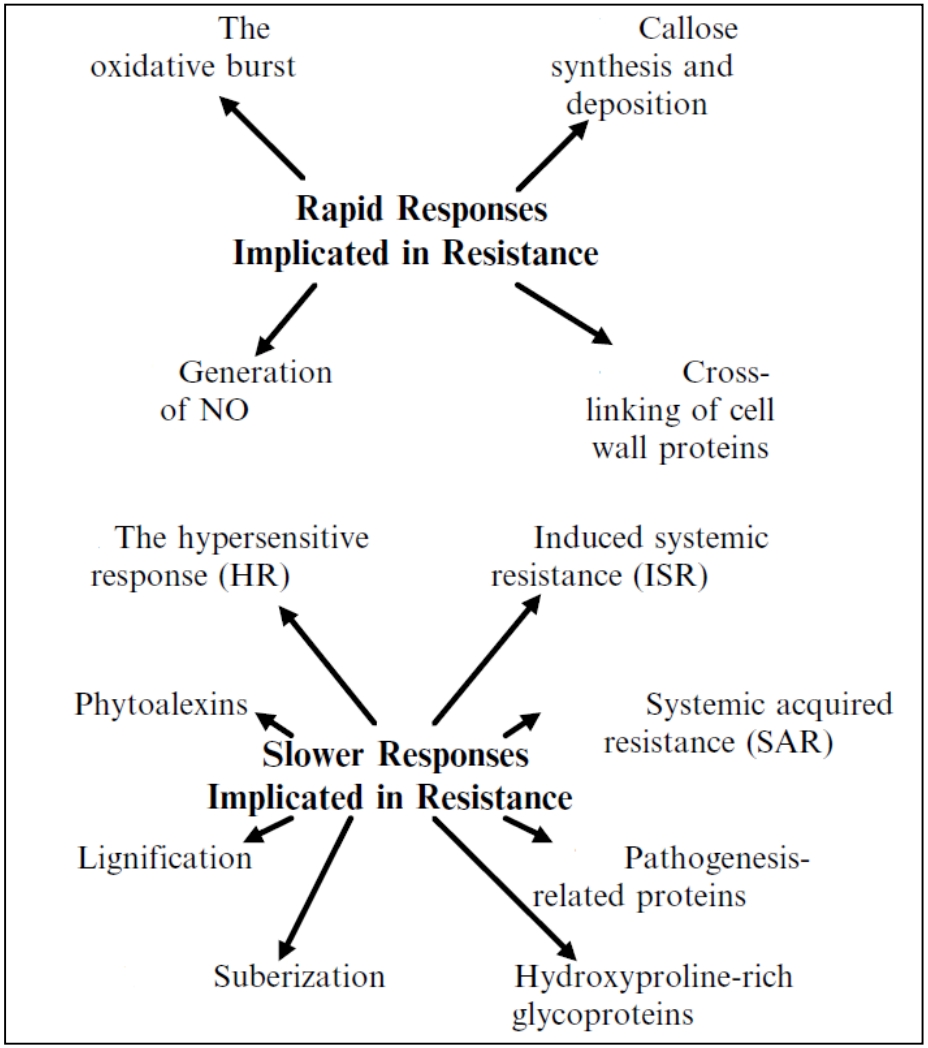
- Rapid responses implicated in resistance
- Slower responses implicated in resistance
- Oxidative burst, HR, SAR,..

Active defense mechanisms
The second defense line

- Plants synthesize a variety of compounds when exposed to biological stress viz., fungi, bacteria, viruses, insects and herbivores (Collinge and Slusarenko, 1987; Linthorst, 1991).
- The inducible defense responses include the production of:

1. reactive oxygen species (ROS),
2. phytoalexins,
3. cell wall components (callose, glycine or hydroxyproline-rich proteins), and
4. other specific group of proteins called pathogenesis-related (PR) proteins.

Active defence mechanisms of plants
Summarized diagrammatically



Strange,2003

Active defense mechanisms
The second defense line

1. Rapid responses implicated in resistance
- The oxidative burst (ROIs)
- The generation and role of nitric oxide (NO) in resistance
- Callose synthesis and deposition

2. Slower responses implicated in resistance
- **Hypersensitive response**
- Phytoalexins
- Lignification
- Suberization
- Synthesis of hydroxyproline-rich glycoproteins (HRGPs)
- Pathogenesis-related proteins (PRPs)
- Systemic acquired resistance (SAR)
- Induced systemic resistance (ISR)

3. Elicitors of defence responses

4. Elicitor perception

5. Signalling

Increases in cytosolic calcium
Mitogen-activated protein kinases (MAP kinases)
Salicylic acid (SA)
Jasmonates
Ethylene
Integration of signalling pathways

Active defence mechanisms

Abiotic or biotic elicitors

- The triggers for these active defence mechanisms are termed elicitors.
- Recognition of pathogens (exogenous elicitors or endogenous elicitors) by host plants led to many metabolic and structural modifications in the host.
- The elicitors are either abiotic or biotic:

1. Abiotic elicitors include physical insults such as ultraviolet irradiation and partial freezing as well as a multitude of chemicals such as the salts of heavy metals and DNA intercalating compounds.
2. Biotic elicitors may be specific and are, in some cases, the products of avirulence genes of pathogens.

Active defence mechanisms

Abiotic or biotic elicitors

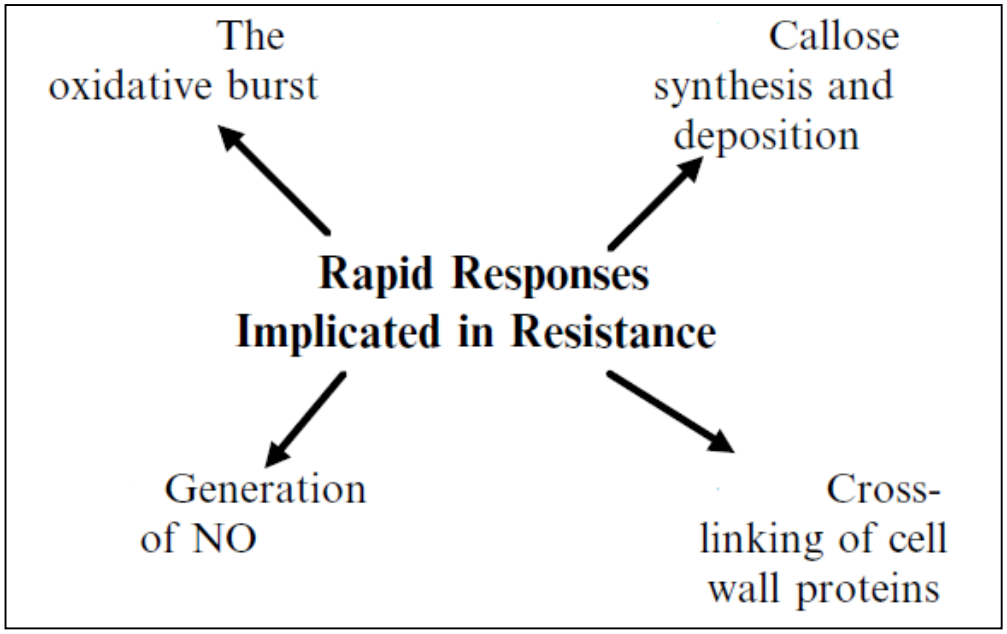
- Flagellin from bacteria, and
- Chitin and ergosterol from fungi are also known characterized microbial elicitors.
- Other miscellaneous elicitors are as:
- Polysaccharides,
- Enzymes,
- Fatty acids,
- Glycoproteins, and
- Proteins.

Disease resistance process

- Physiological features such as:
- K^+/H^+ exchange,
- Rapid oxidative burst,
- Hypersensitivity at the site of infection,
- Crosslinking of plant cell walls,
- Synthesis of antimicrobial compounds known as phytoalexins, and
- Induction of pathogen-related proteins such as chitinases and glucanases (Lamb *et al.*, 1989) represent some of the multiple metabolism turnover leading to disease resistance.

Active Defense Mechanisms

A. Rapid responses implicated in resistance



Active resistance consists of many defence responses that interact over time and space.

Strange,2003

Active Defence Mechanisms

Rapid responses implicated in resistance

Plant peroxidases

- Another important event associated with plant defense mechanisms is a rapid and transient release of different reactive oxygen species (ROS), such as the superoxide anion radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2).
- Of these ROS, production of hydrogen peroxide appears to occur early and is involved in:

1. A direct oxidative reaction with the pathogen;
2. Biosynthesis of phytoalexins;
3. Activating defense-related genes;
4. In the induction of acquired disease resistance.

Peroxidases
Two superfamilies

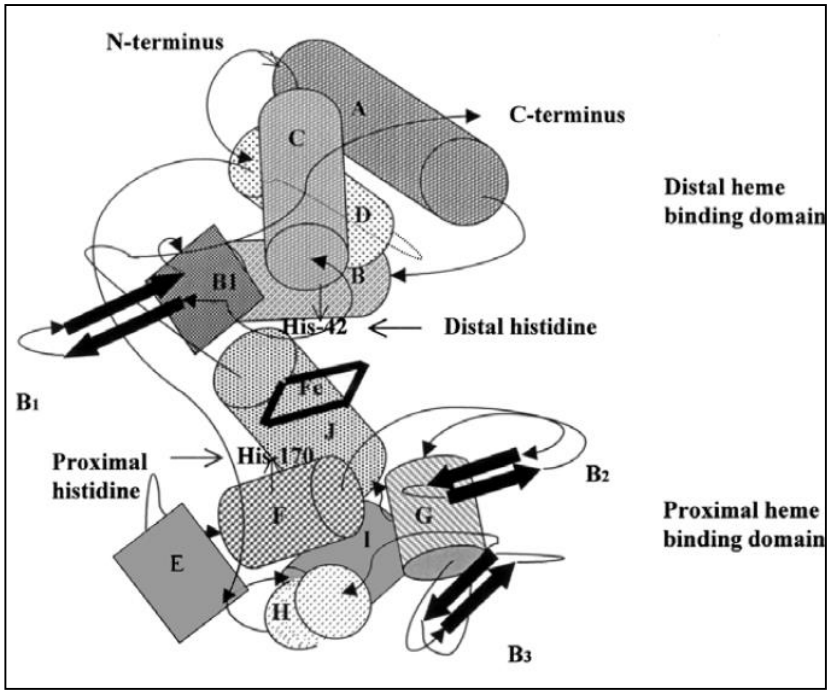
- Peroxidases have been classified into two large groups:

1. The plant peroxidase superfamily;
2. The animal peroxidase super-family.

- Peroxidases are also found in fungi and bacteria.

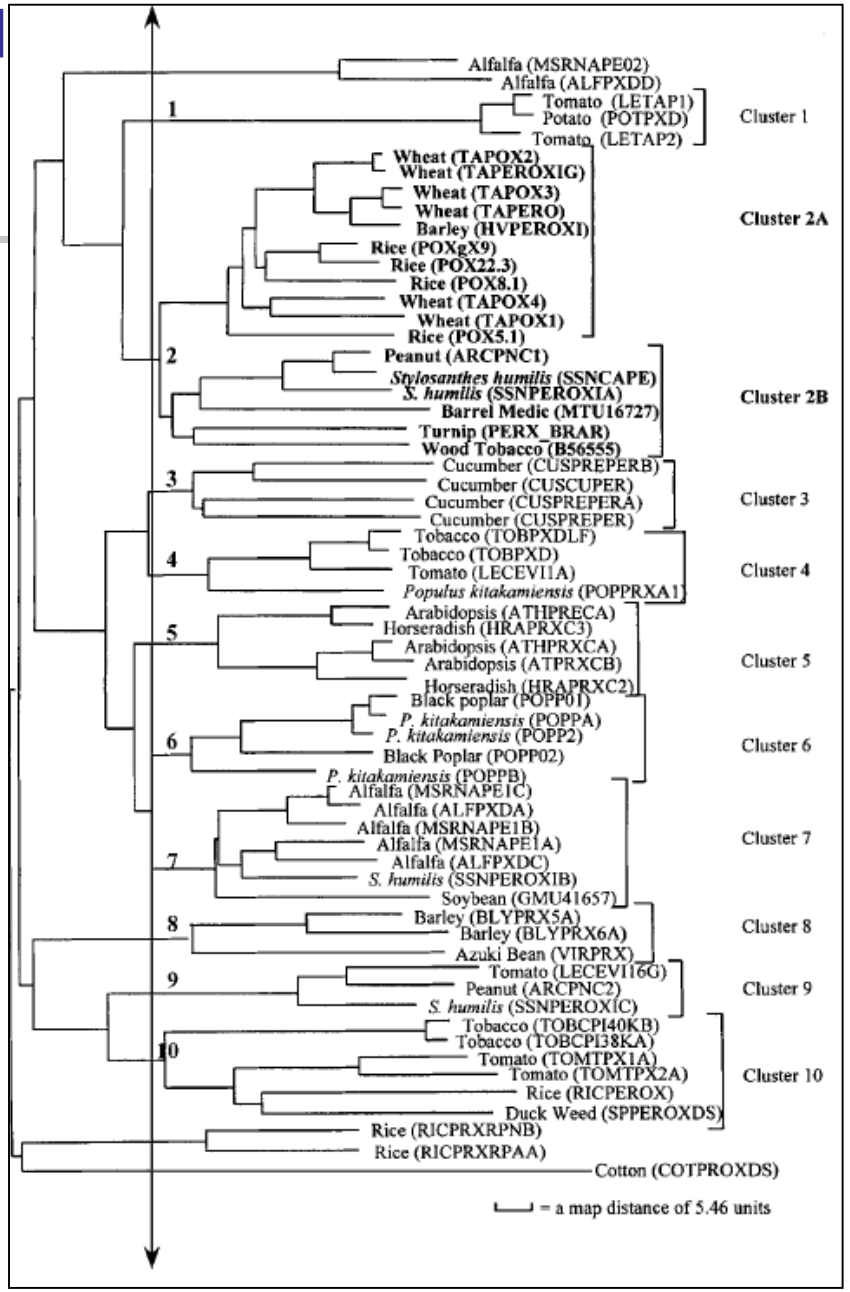
A diagrammatic representation of the structure of plant peroxidase

- Ten helices (A–J) and β-sheets (β1, β2, and β3) are represented by cylinders and parallel arrows, respectively.
- Reverse turns and coils that connect the helices and the β-sheets are shown as connecting lines.
- The proximal and distal heme-binding domains and the proximal and distal histidine residues (Hrp C numbering) are indicated.
- The rectangular box present between the proximal and distal heme-binding regions indicates the position of the central heme prosthetic group.



Phylogenetic analyses and comparisons of amino acid sequences from 62 plant peroxidases

10 peroxidase gene clusters were recognized.



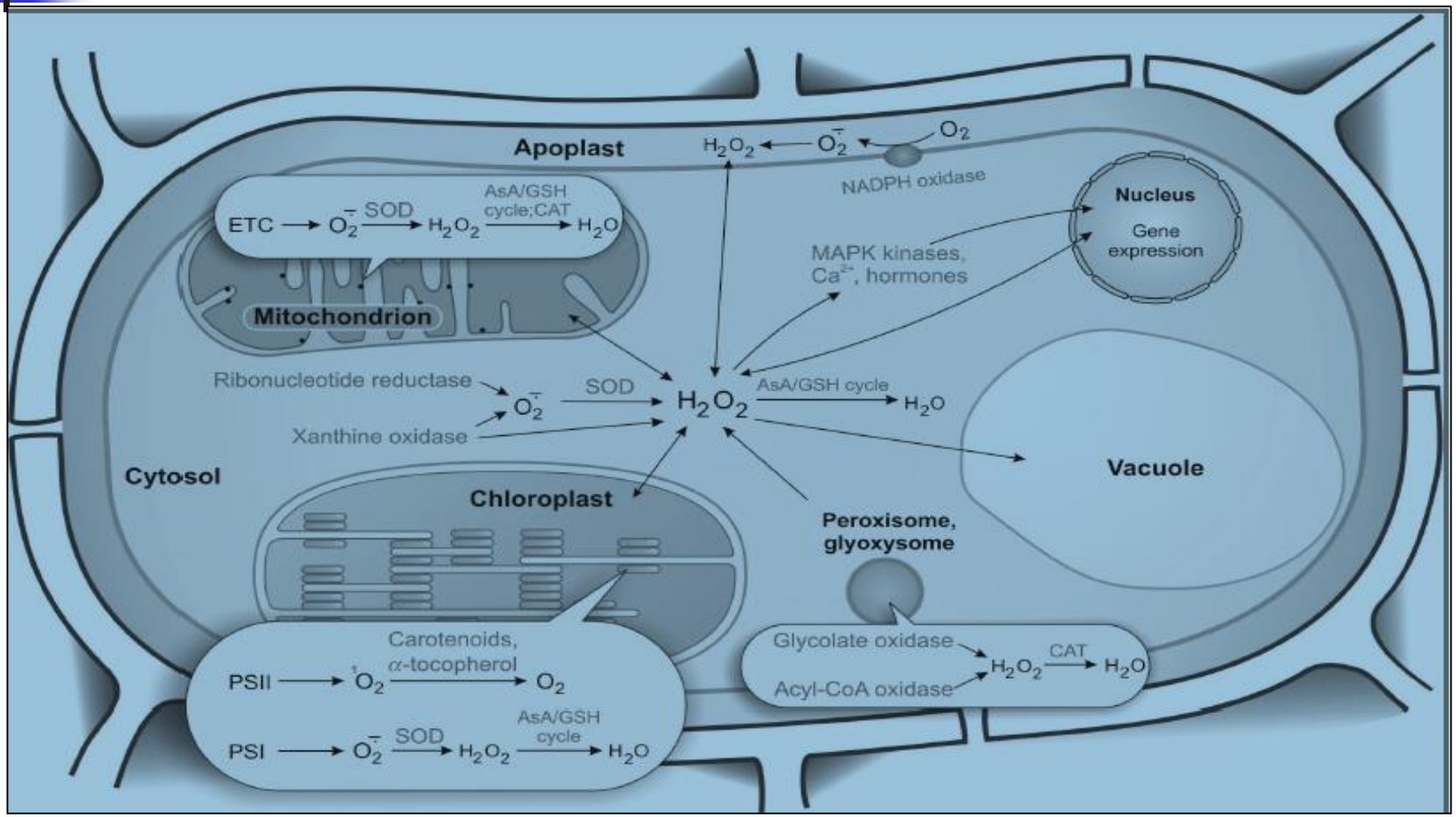
Oxidative burst
Toxic to pathogens

- H_2O_2 generated by wall-bound plant peroxidase or oxygenase during the oxidative burst, which occurs within minutes after pathogenic interaction, and free radicals, which are produced by extracellular peroxidase oxidative activity during cell wall fortification, are toxic to pathogens.

A free radical is an atom, molecule, or ion that has unpaired valence electrons. These unpaired electrons make free radicals highly chemically reactive towards other substances, or even towards themselves: their molecules will often spontaneously dimerize or polymerize if they come in contact with each other. E.g. the hydrocyl radical ($HO^{\bullet}$). Typically two free radicals combine to form a more stable species, for example: $2Cl^{\cdot} \rightarrow Cl_2$.

Plant peroxidases

Production and metabolic fate of various ROS (hydrogen peroxide, superoxide anion radical, singlet oxygen) in different plant cellular compartments



Oxidative burst

Toxic to pathogens and plant cells

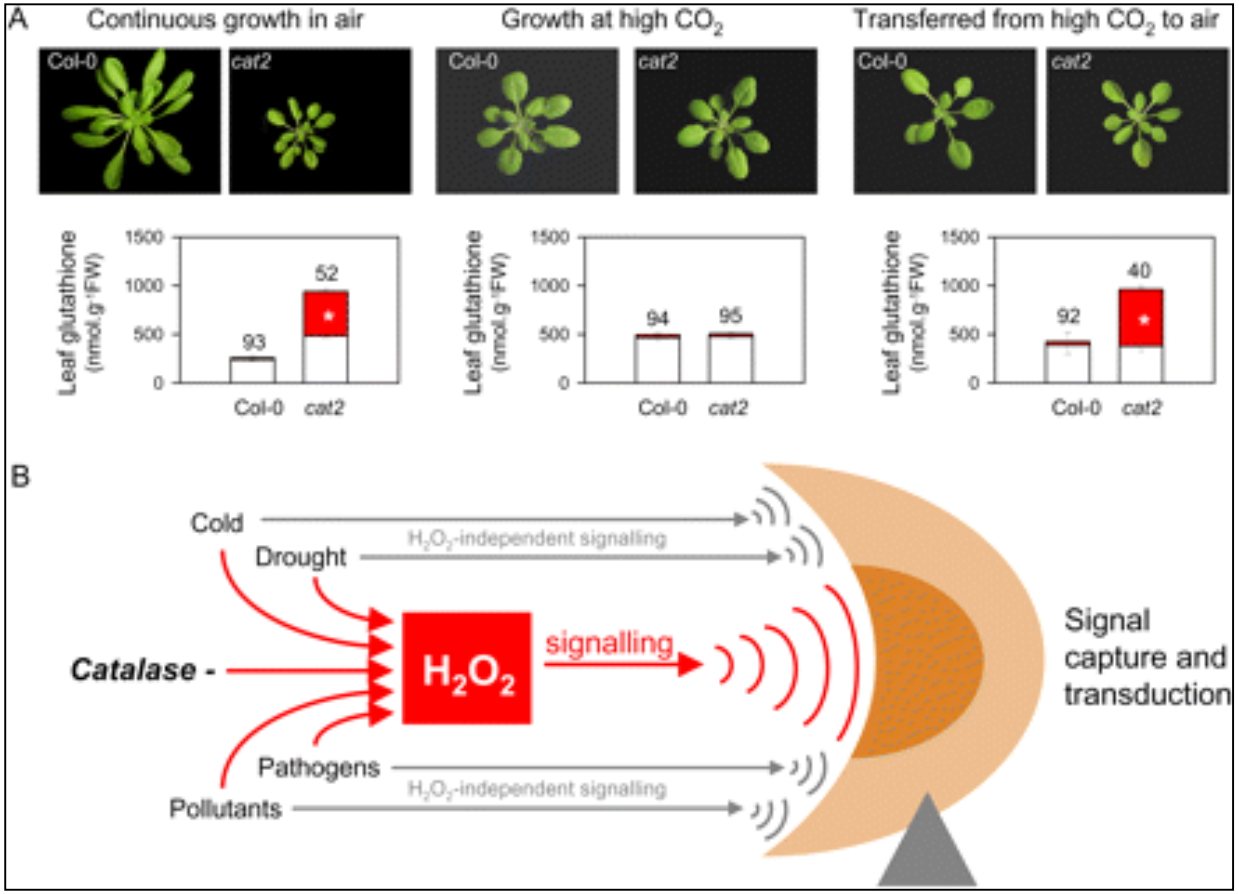
Enzymatic scavenging systems, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) scavenging ROS

- The production of reactive oxygen species (ROS) in plants occurs early in plant defense response to external stimuli.
- Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are the most important reactive oxygen species, and they rapidly accumulate.
- High levels of ROS may damage the bodies of living organisms.
- To prevent oxidation burst due to ROS production, plants have evolved complex protective mechanisms for scavenging ROS.
- These include the enzymatic scavenging systems (antioxidant enzymes), such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD).
- Catalase is a significant component of the cell protective mechanism against oxidative stress.

Zhao *et al.*,2016

Plant peroxidases

Catalase-deficient plants(catalase -ve) allow early and late events in oxidative signalling pathways



Mahamdi *et al.*,2010

Oxidative burst
Catalase (CAT) assay

- Catalase activity was assayed as described by Zhang et al.,2008.
- The reaction mixture consisted of 1 ml of Tris-HCl buffer (pH 7.0), 0.1 ml of partially purified enzyme extract and 0.2 ml of H_2O_2.
- Absorbance was read at 240 nm for 2 min and the enzyme activity was expressed as ΔOD min^{-1}.

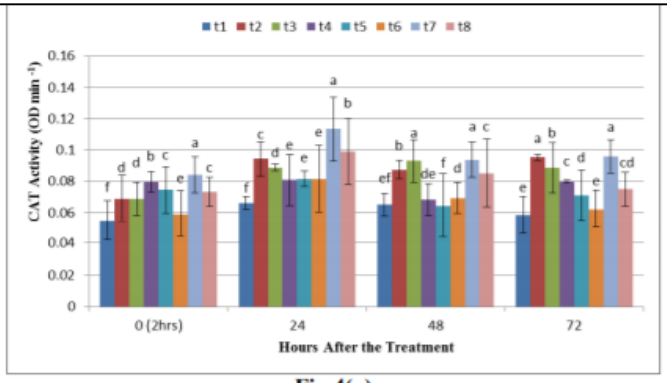


Fig 4(a)

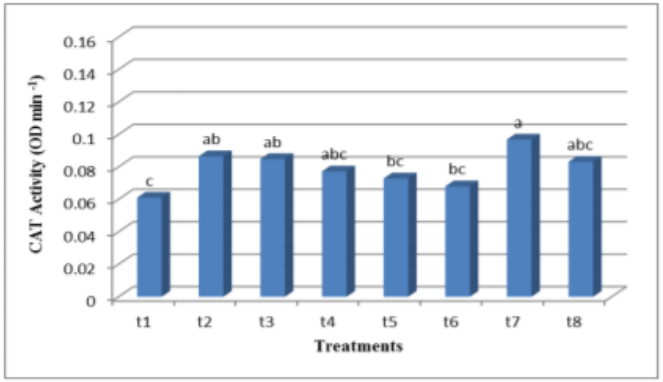


Fig 4(b)

Hegde *et al.*,2018

Oxidative burst

Reactive oxygen species

Plant pathogenic bacteria

- Increased level of hydrogen peroxide(H_2O_2) was observed which resulted in a simultaneously increased resistance to *P. carotovorum* ssp. *carotovorum*.
- Similarly, the same gene in transgenic cabbage conferred resistance to *X. campestris* pv. *campestris*, which correlated with glucose oxidase activity in leaves.
- Increased hydrogen peroxide may also account for resistance to the soft rot-causing bacterial pathogen *P. carotovorum* subsp. *atrosepticum* in potato tubers.

Oxidative burst

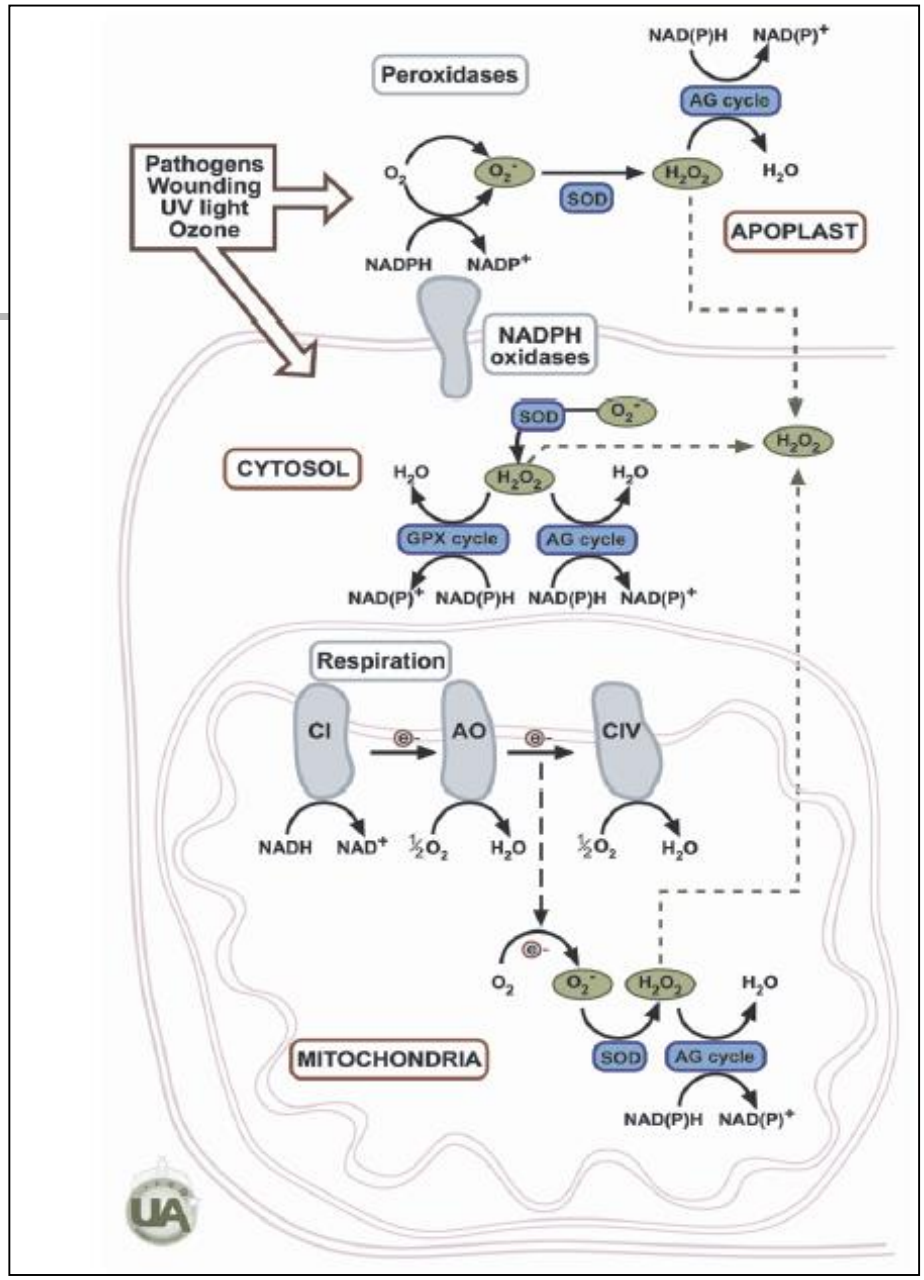
Reactive oxygen species

- Most of plant species generate ROIs by means of NADPH oxidase (NADPH-dependent).
- NADPH(Nicotinamide adenine dinucleotide phosphate) oxidase is a membrane-bound enzyme complex which produces ROSs.
- NADPH itself is the reduced form of NADP:

$$O_2 + NADPH \longrightarrow O_2^- + NADP + H^+$$

Oxidative burst

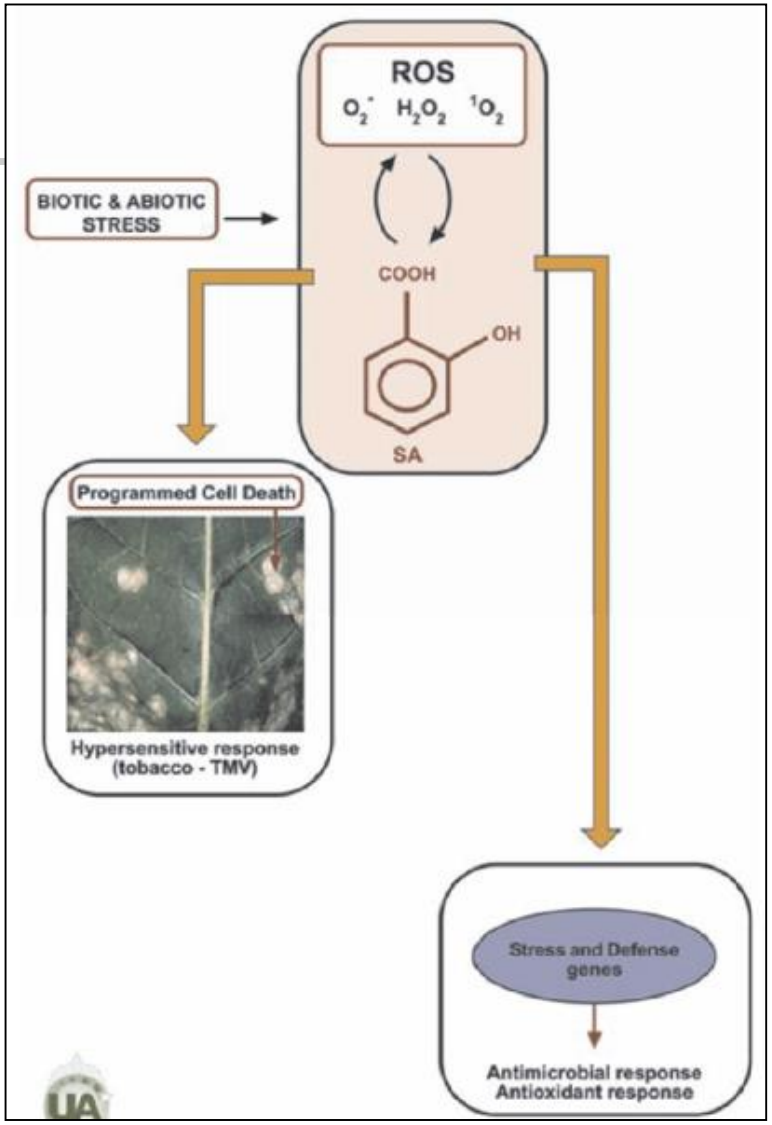
Pathways for ROS production and scavenging in plant cells



Hayat and Ahmad,2007

Oxidative burst

- Salicyclic acid has a pro-apoptotic effect during PCD by enhancing the effect of ROS.
- Also SA and ROS are signals for genetic-controlled defense reactions.



Nitric oxide (NO)

- Nitric oxide (NO) is an essential factor for the activation of innate immune responses in:

1. Insects,
2. Mammals (Nappi *et al.*,2000), and
3. A similar role has been suggested in plants (Delledonne *et al.*,1998).

Oxidative burst and nitric oxide

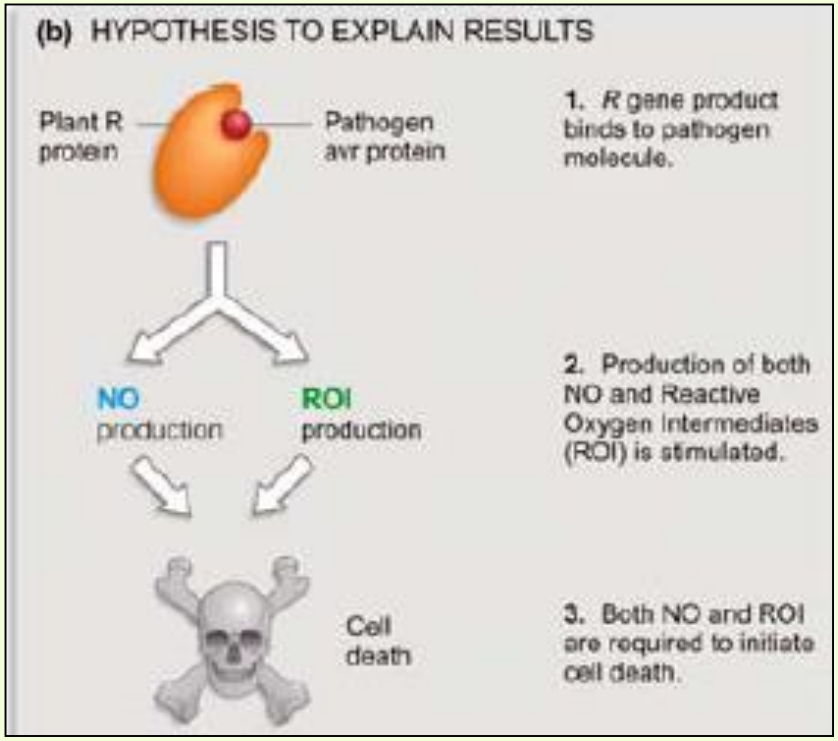
- During the oxidative burst, reactive oxygen intermediates (ROIs) such as O_2, H_2O_2 and OH are generated which protect the plant in several ways:

1. They are toxic to microorganisms.
2. This toxicity is thought to be potentiated by NO.
3. In fact ROIs with NO cause the hypersensitive death of host cells (HR).
4. Hydrogen peroxide acts as a substrate for lignifications, making cell walls more difficult to penetrate.
5. It is also involved in the induction of protective genes coding for pathogenesis-related proteins.

Reactive oxygen intermediates and nitric oxide

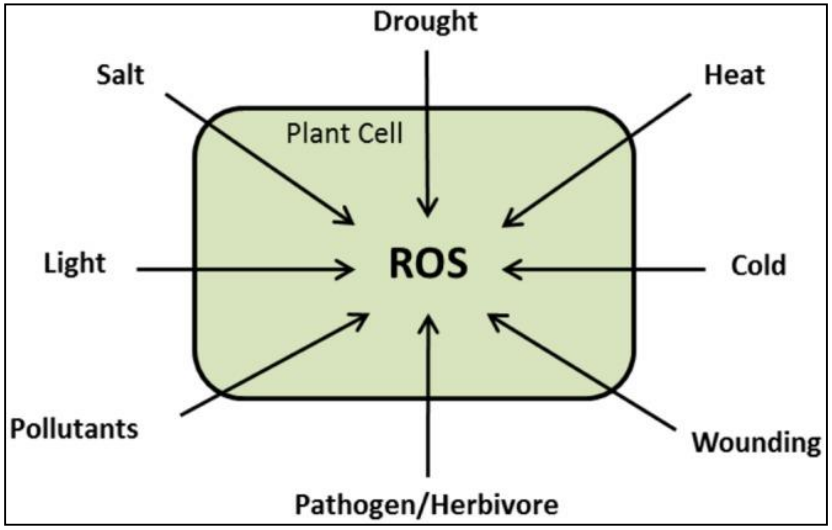
ROI and NO

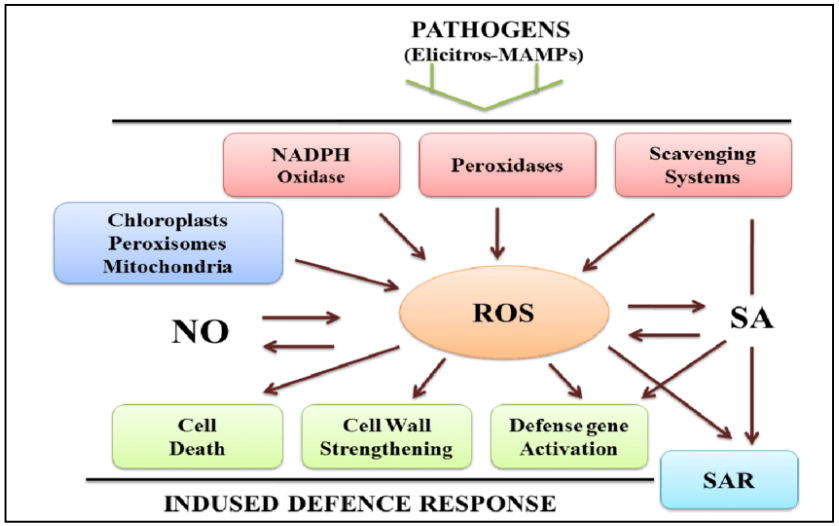
- ROS and NO are often produced during the early stages of a plant resistance response.



Reactive oxygen intermediates and nitric oxide

ROI and NO





ADPH is used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent.

Nitric oxide (NO)

An important signal molecule in plant–pathogen interactions

- **NO plays a major role in:**

1. Plant PCD elicited in response to certain types of pathogenic challenge.
2. The race-specific hypersensitive response (HR);
3. NO also acts in the regulation of non-specific, papilla-based resistance.
4. Possibly in systemic acquired resistance (SAR).
5. Equally, the potential roles of NO signalling/scavenging within the pathogen are being recognized.

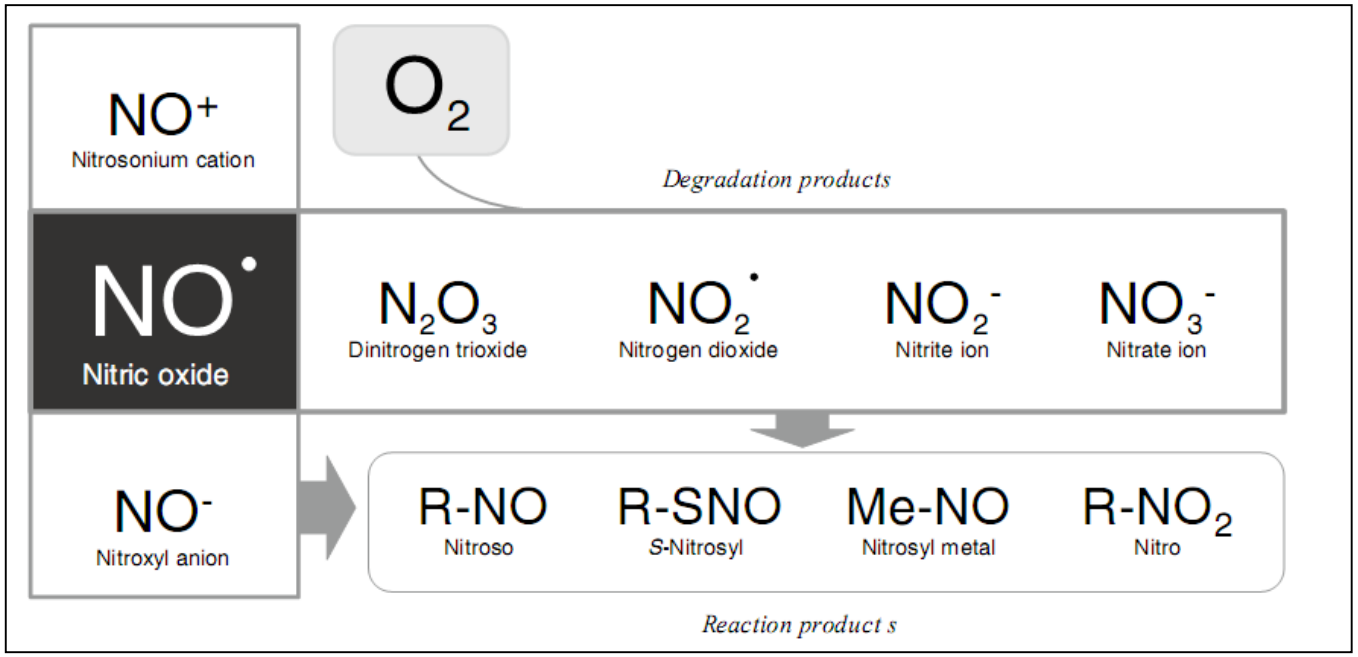
Nitric oxide (NO) production

- Nitric oxide (NO) and reactive oxygen species are two key components in the induction of the hypersensitive response (HR) during plant defense against pathogen infection.
- Isolation of the NOS protein and/or cloning of the corresponding gene will greatly facilitate the understanding of NO synthesis and its role in plant defense.
- NO generation may be a general response to biotic stress in plants.
- e.g.

1. The compatible bacteria (*Pseudomonas syringae* pv. *pisi*) induced NOS activity significantly, suggesting that NO generation may also be a general response to biotic stress in plants.
2. Also maximum NOS activity was detected three hours before the onset of HR in pea leaves infiltrated with incompatible bacteria (*Ralstonia solanacearum*).

Major target molecules that should be considered in NO measurements

NO and its derived nitrogen species

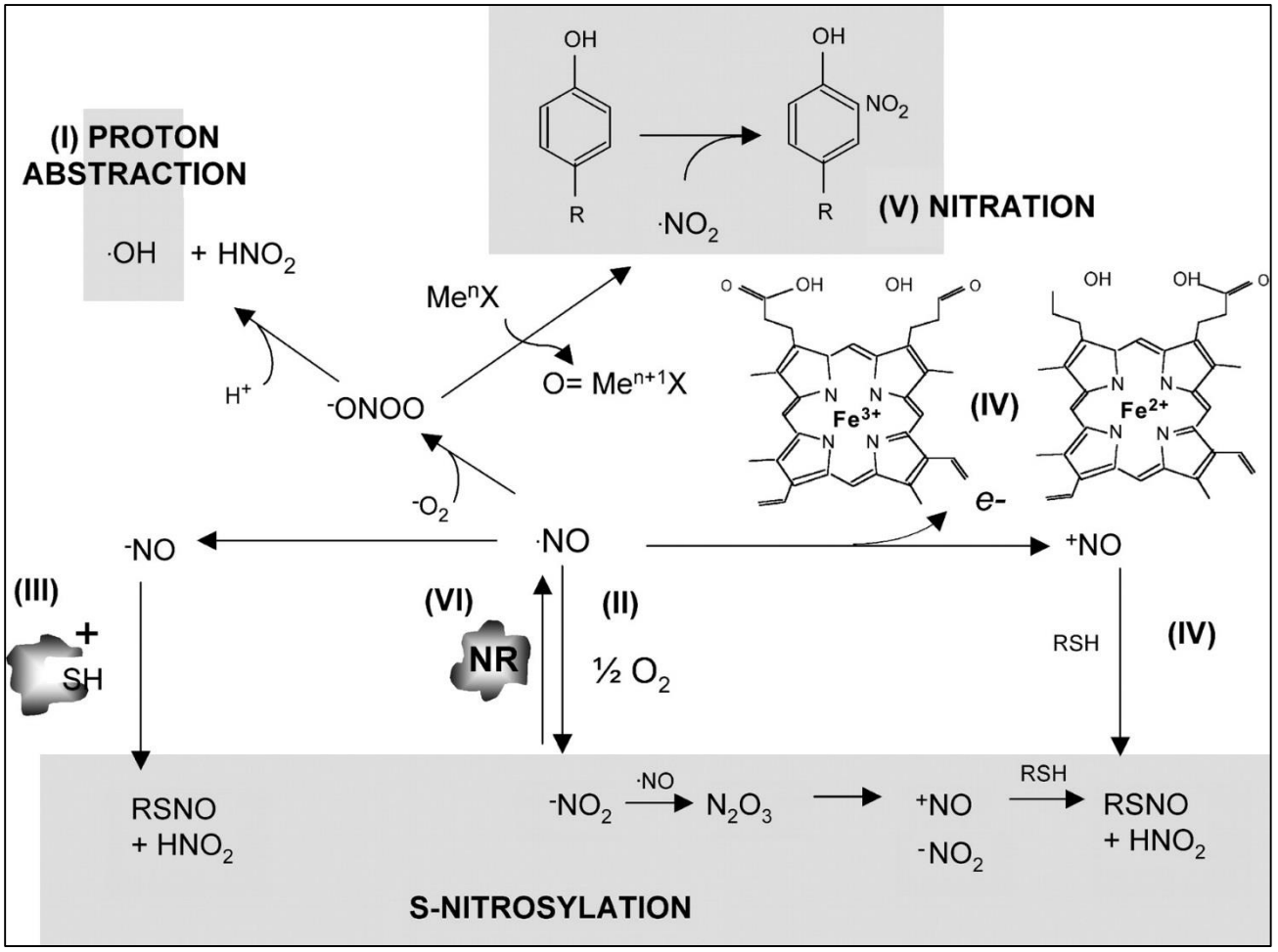


Major molecules that should be considered in NO measurement are presented.

Note that each name follows the widely accepted one rather than the IUPAC systematic nomenclature.

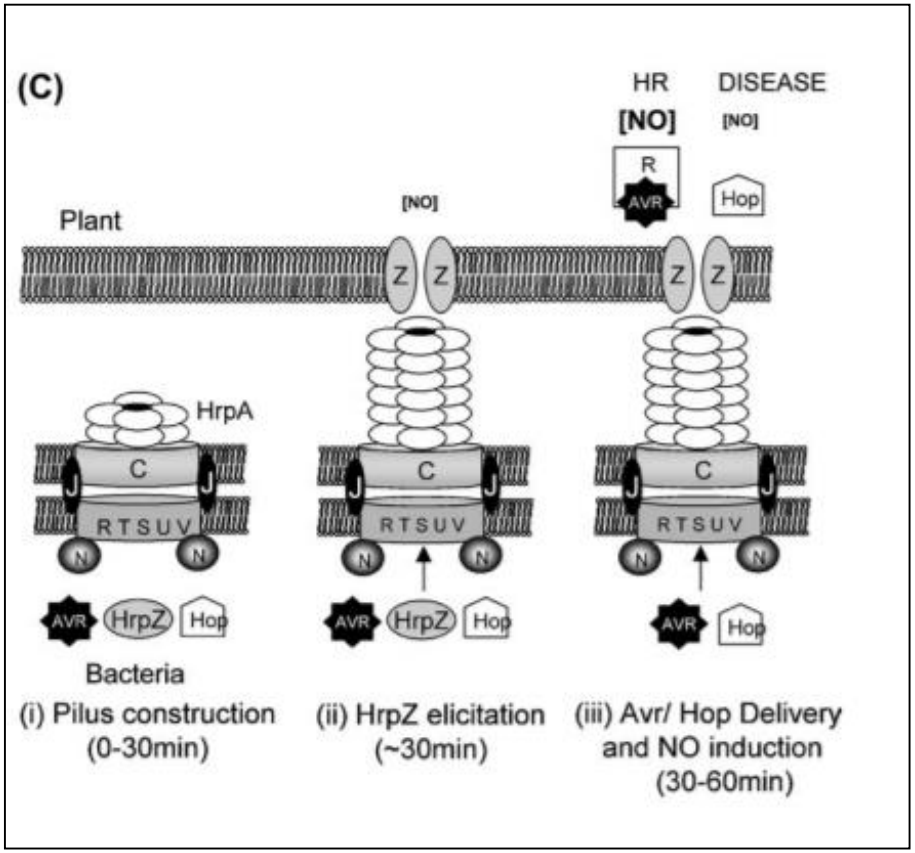
Yamasaki *et al.*,2016

An overview of nitric oxide (NO) chemistry
Four routes for NO generation



Mur *et al.*,2006

The elicitation of NO production by *Pseudomonas syringae* pathovars in tobacco and *Arabidopsis*



C) Proposed model for hrp-dependent elicitation of NO by *P. syringae* pathovars.

Mur *et al.*,2006

Active Defence Mechanisms

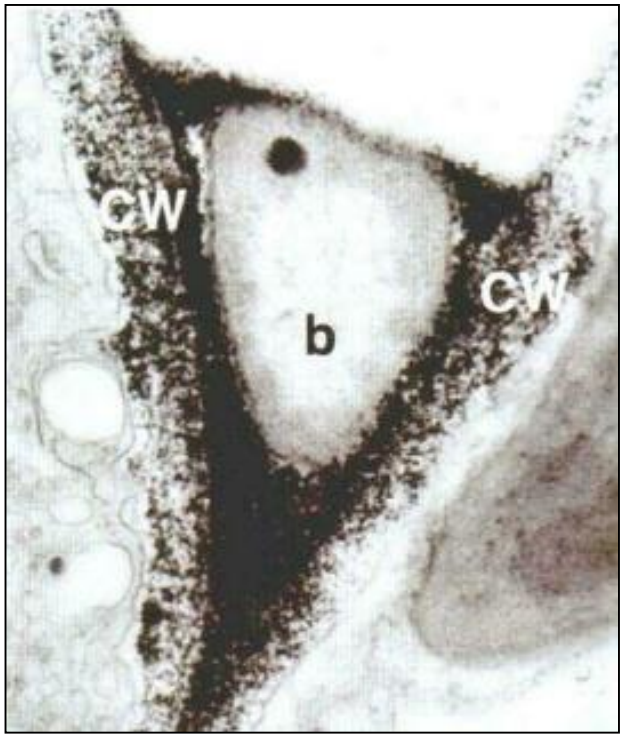
Rapid responses implicated in resistance

Callose synthesis and deposition

- Callose is a β-1,3-linked glucan which is often synthesized and deposited as papillae – localized wall appositions – as an early response to wounding or pathogen attack.
- Callose synthesis is catalyzed by plasma membrane bound ß-glucan synthase (callose synthase).
- The speed of callose deposition was emphasized in work with French bean (*Phaseolus vulgaris*) and *Xanthomonas campestris*.

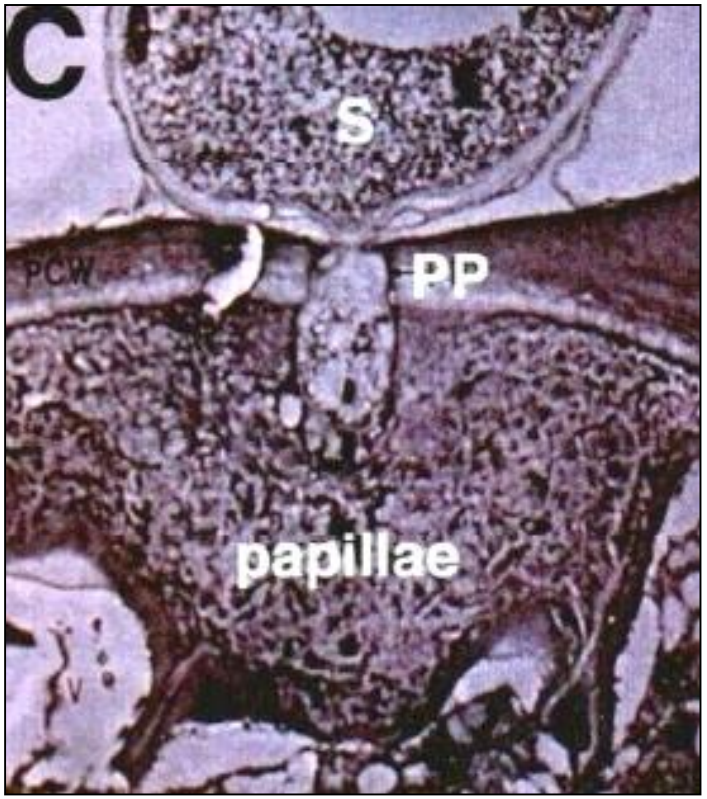
H_2O_2 generation in lettuce cell walls

- H_2O_2 generation in lettuce cell walls, in the vicinity of the incompatible bacterium *P. s.* pv. *phaseolicola*.
- H_2O_2 was detected in cell walls (CW) 5 hr after inoculation with bacteria (b) as black deposits in the transmission electron image by staining with cesium chloride.



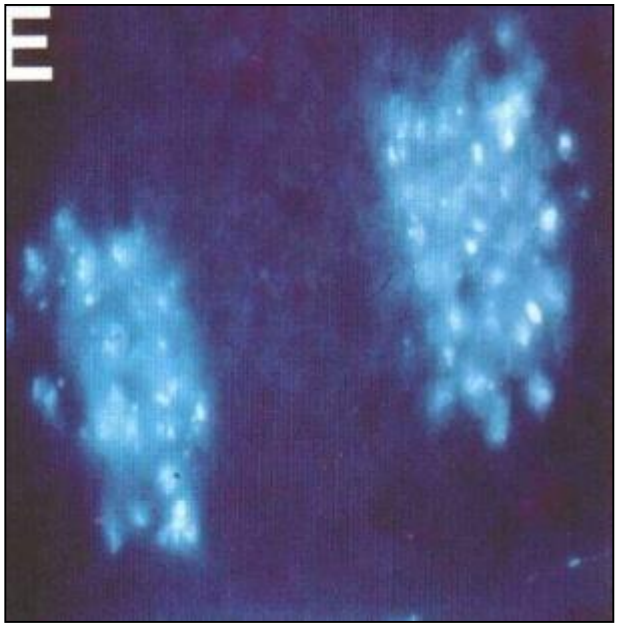
Papillae formation

- Papilla refers to the accumulation of material between the host plant's cell wall and cell membrane at the point of fungal penetration.
- A papilla is usually composed of silicon, lignin and proteins.
- Here papillae developed beneath the penetration peg (PP) and germinating spore (S) of an avirulent fungal isolate.



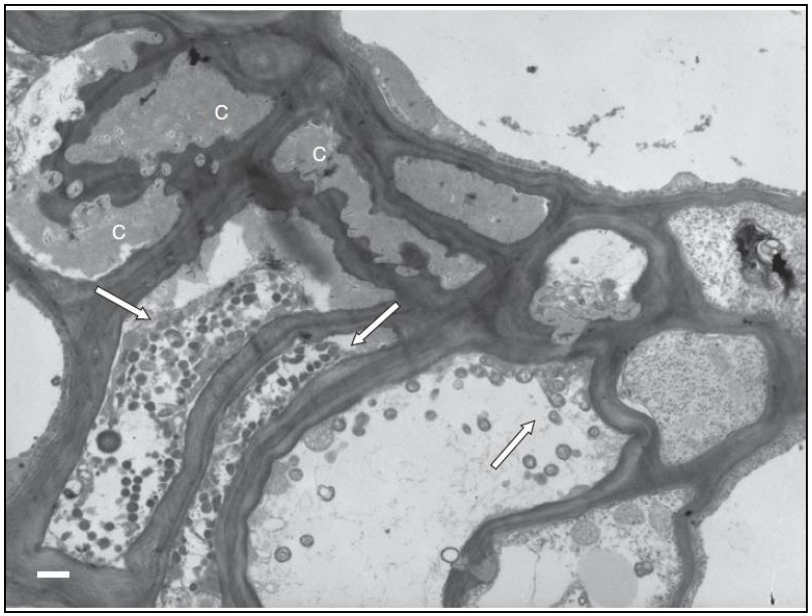
Callose deposition

- Callose deposition in Arabidopsis leaf mesophyll at the incompatible infection sites.
- Callose was detected by aniline blue staining and fluorescence under UV light (Parker *et al.*,1993).



Callose deposition

- Callose accumulation is a non-specific response of plants to pathogen attack and to wounds.
- Transmission electron micrograph of phytoplasmas (arrowed) in sieve tubes of the host plant.
- Sieve plates are filled by callose (C).
- Bar= 0.5 µm.



Weintraub and Jones,2010

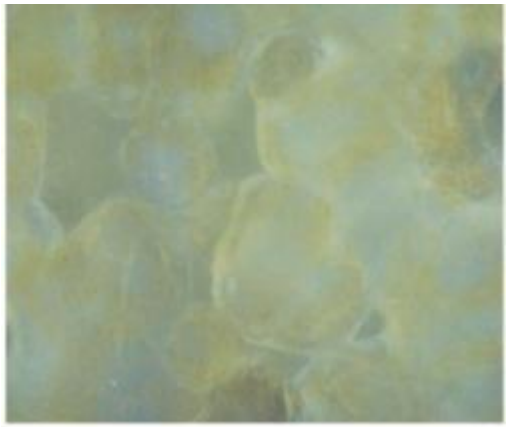
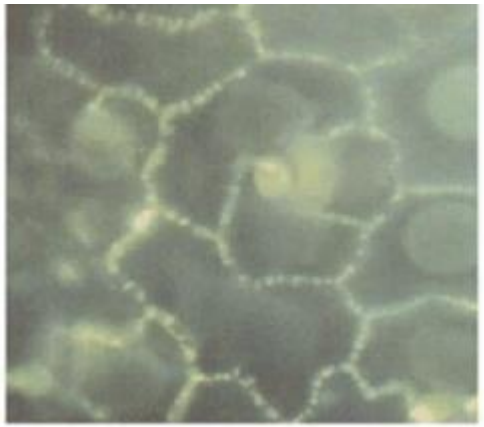
Salicylic acid and callose synthesis

- A rapidity in the activation of callose synthase after spraying the Arabidopsis plant with salicyclic acid implies a possibility that SA affects callose deposition on the cell surface.
- A significance of SA-dependent callose deposition in plant resistance was substantiated in experiments with *Pseudomonas syringae* harboring the mutated *CEL* gene (DebRoy *et al.*,2004).
- This gene encodes bacterial effectors suppressing plant SA-dependent defense responses.

Hayat and Ahmad,2007

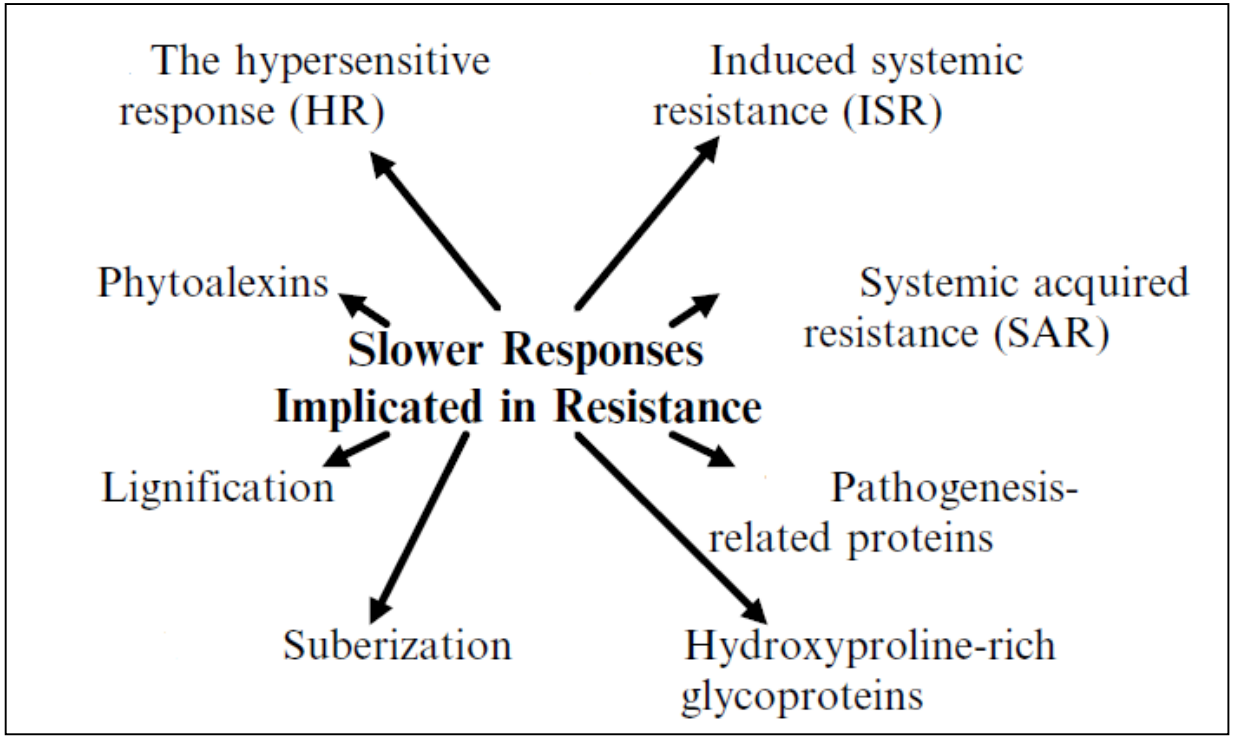
Salicylic acid and callose synthesis

- Callose deposition in the leaf epidermis of *Nicotiana glutinosa* is stimulated by SA.
- Callose was determined by its fluorescence after staining with aniline blue.

	
Untreated leaves	Leaves treated with 1mM SA, 1 hour ago.

Active defence mechanisms of plants

B. Slower responses implicated in resistance



Strange,2003

Active Defence Mechanisms

Slower responses implicated in resistance

- Slower responses implicated in resistance include:

1. The hypersensitive response,
2. Phytoalexin synthesis,
3. Lignification,
4. Suberization,
5. Cell wall components (callose, glycine or hydroxyproline-rich proteins),
6. The synthesis of pathogenesis-related proteins (PRPs), and
7. Systemic acquired resistance (SAR).

- All these require gene transcription and protein synthesis.

Active Defence Mechanisms

Slower responses implicated in resistance

1. The hypersensitive response

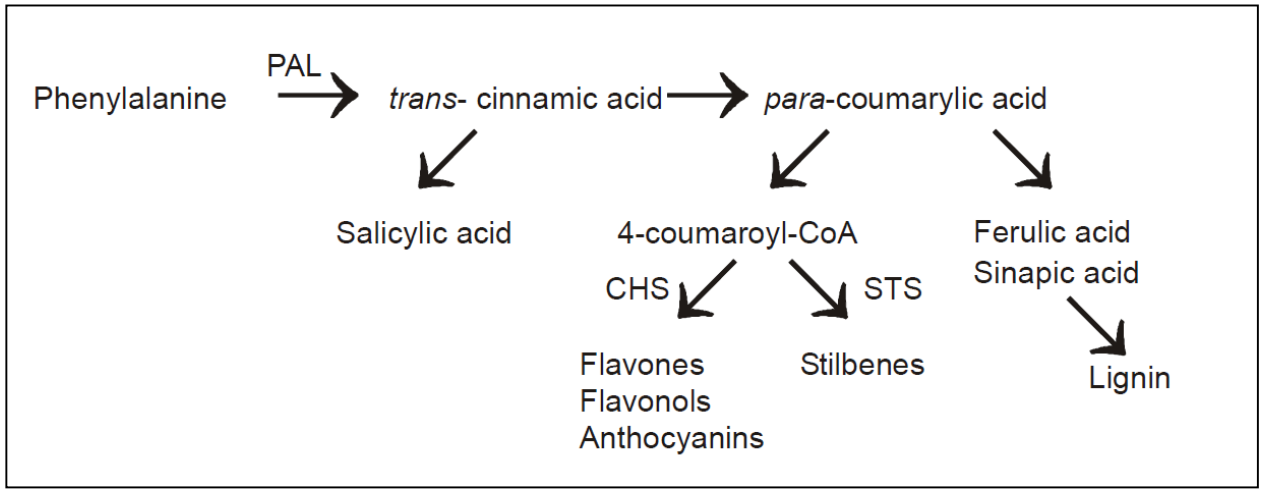
- Hypersensitive response is being developed in the place of pathogen penetration into the neighboring cells.
- Rapidly developing local process produces signal molecules spreading along vascular system of a plant.
- In dependence of local events, the set of signal molecules e.g. SA is being organized, which in turn, forms this or that generalized response.
- In the plant cells, the salicylic acid (SA) is produced in considerable amounts and causes activation of SA-induced genes.
- The integrity of these events is named as systemic acquired resistance (SAR).

The hypersensitive response

- The gene of one of the key enzymes, PAL (phenylalanine ammonia-lyase), is activated:

1. In the course of hypersensitive response, but also
2. In response to various external stimuli.

- PAL takes part in the synthesis of SA, phytoalexins, and lignin monomers.



Gene-for-gene interaction between host and pathogen

Combinations to remember

Combinations	disease develops	Hypersensitive reaction
Non-compatible host species and non-pathogen	-	-
Compatible species and compatible pathogen	+	-
Resistant cultivar of compatible host species and incompatible pathogen	-	+
Resistant cultivar of compatible host species and compatible pathogen	+	-

- Compatible (no recognition or defense response);
- Incompatible (recognition and defense response).

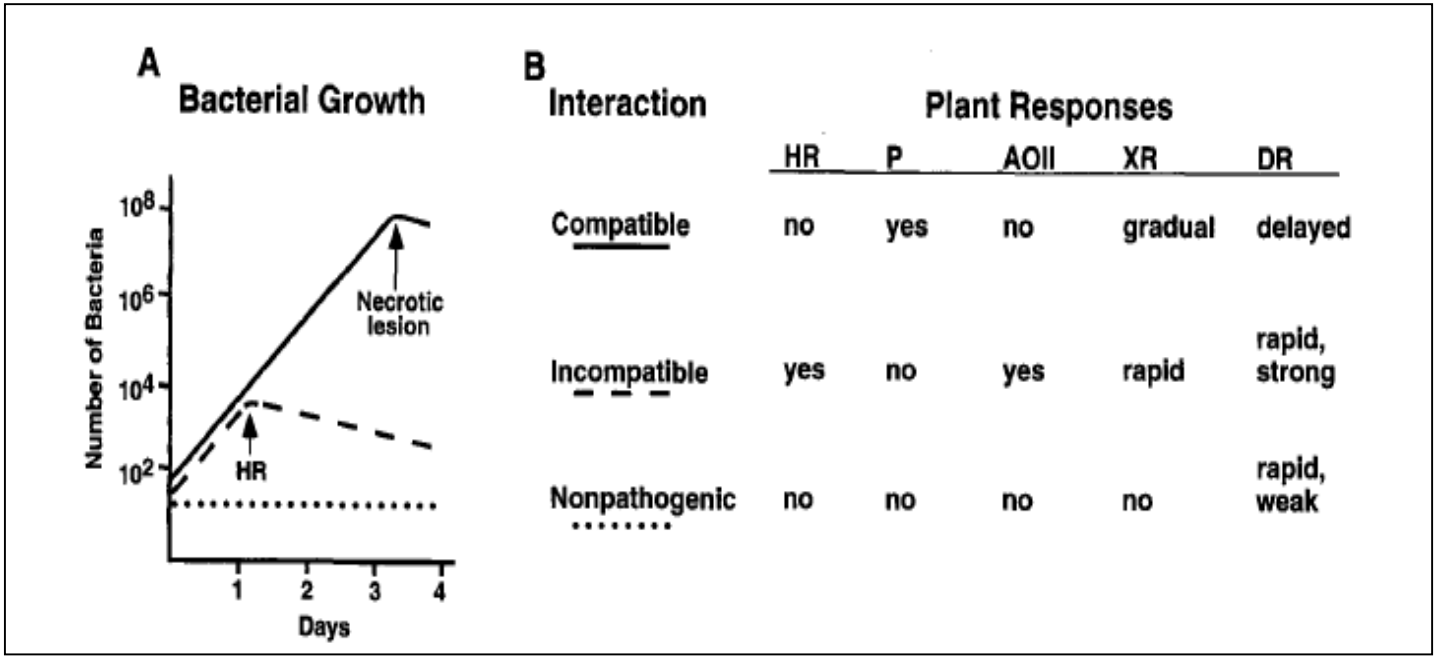
Gene-for-gene interaction between host and pathogen

Hypersensitivity vs. disease

One pair of loci		Pathogen genotypes		
		AA	Aa	aa
Host genotypes	rr	+	+	+
	Rr	—	—	+
	RR	—	—	+

— = R (incompatible) + = S (compatible)

Possible outcomes for the plant response to bacterial pathogens and non-pathogens



B

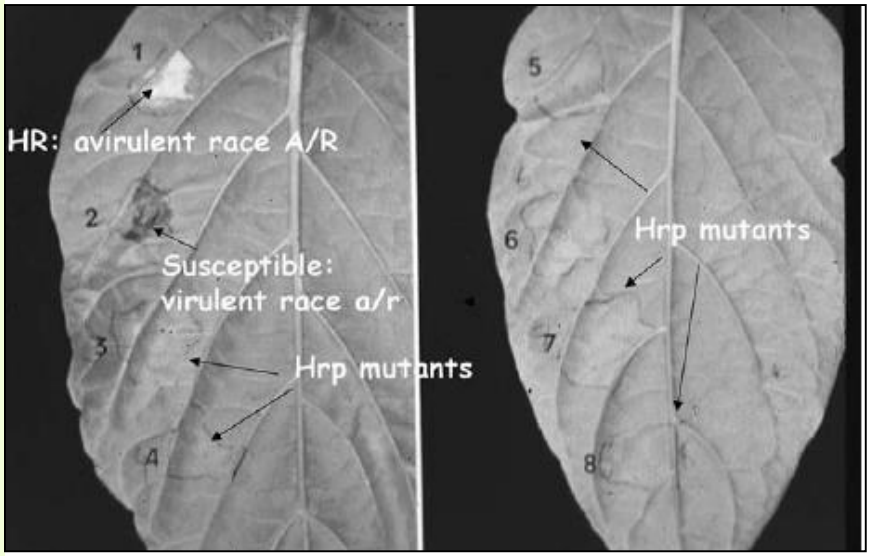
Interaction	Plant Responses				
	HR	P	AOII	XR	DR
Compatible	no	yes	no	gradual	delayed
Incompatible	yes	no	yes	rapid	rapid, strong
Nonpathogenic	no	no	no	no	rapid, weak

Figure right shows the reactions for compatible (no recognition or defense response), incompatible (recognition and defense response) and a nonpathogenic bacterium. AOII denotes a sustained generation of active oxygen that occurs 1.5 to 3 hr after inoculation. XR denotes a K^+ efflux/H^+ influx that occurs simultaneously with AOII in incompatible interactions. DR denotes the expression of a variety of defense-response genes.

Alfano and Collmer,1996

Reactions of pepper to *Xanthomonas campestris* pv. *vesicatoria* : virulence, avirulence and the null reaction to Hrp mutants

- The water-soaked lesions of the areas of the leaf inoculated with the virulent isolate and compare with the hypersensitive cell death caused by the avirulent race - the Hrp mutants are deficient in the type III secretory system and therefore cannot export effector molecules that cause either the susceptible reaction or the hypersensitive response.



Strange,2003

Hypersensitive vs. normosensitive reactions

- **Hypersensitive reaction:** Hypersensitive cell death is accompanied by the induction of multifaceted defense responses, including: production of active oxygen species and antimicrobial compounds (phytoalexins), rapid cross-linking of cell-wall proteins, local resistance and ultimately, resistance to pathogens.

- **Normosensitive reaction:**
- The slow, normosensitive plant cell death does not effectively prevent pathogen multiplication or spread and is therefore not associated with local resistance.

It is interesting that both hypersensitive and normosensitive cell death can lead to a systemic, broad-spectrum resistance response throughout the plant called SAR.

Hypersensitive vs. normosensitive reactions

1. A rapid, hypersensitive cell death localized at the site of infection during an incompatible interaction between a resistant plant and an avirulent pathogen, and
2. A slow, normosensitive plant cell death that spreads beyond the site of infection during some compatible interactions involving a susceptible plant and a virulent, necrogenic pathogen.

Phase	Response time (h) hypersensitive	Response time (h) normosensitive
Inoculation	0	0
Induction	0-4	Not comparable
Latent	2-12	0-144
Watersoaking	Not comparable	96-192
Collapse	8-14	Not comparable
Necrosis	10-48	144-216
Chlorosis	Not comparable	168-240
Systemic chlorosis	Not comparable	192-288

He,1996;..

Active Defence Mechanisms

Slower responses implicated in resistance

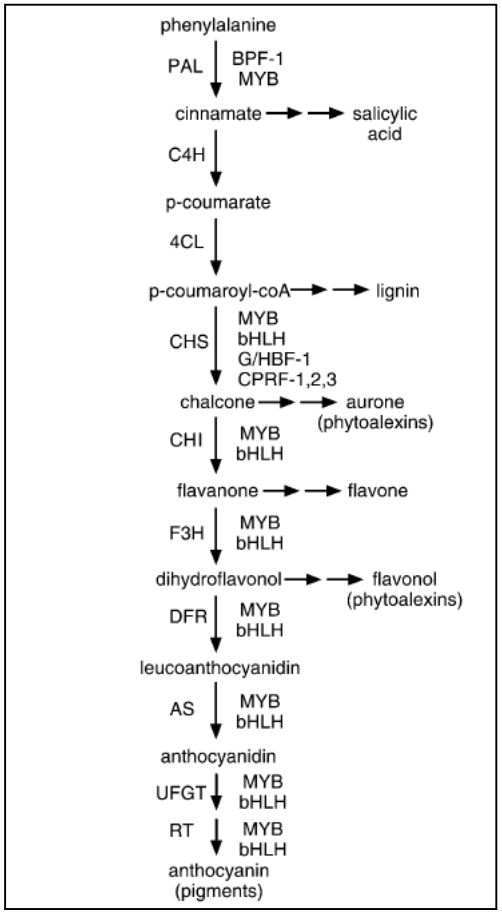
2. Phytoalexins synthesis

- Phytoalexins have been defined as low molecular weight antimicrobial compounds that are both synthesized by and accumulate in plants after exposure to microorganisms.
- Phytoalexins have been characterized from 31 plant families, mostly dicotyledonous, but they have also been isolated from such monocotyledonous plants as rice, sorghum, maize, wheat, barley, onions and lilies.
- Most phytoalexins are derived from one or other or a combination of three major biosynthetic pathways:

1. Shikimate
2. Acetate-malonate
3. Acetate-mevalonate

Phytoalexins, salicyclic acid and lignin synthesis

- The phenylpropanoid pathway is the source of a large number of compounds that are derived from the amino acid phenylalanine.
- Enzymes:
- PAL: Phenylalanine ammonia lyase;
- C4H: cinnamate 4-hydroxylase;
- CHS: chalcone synthase.

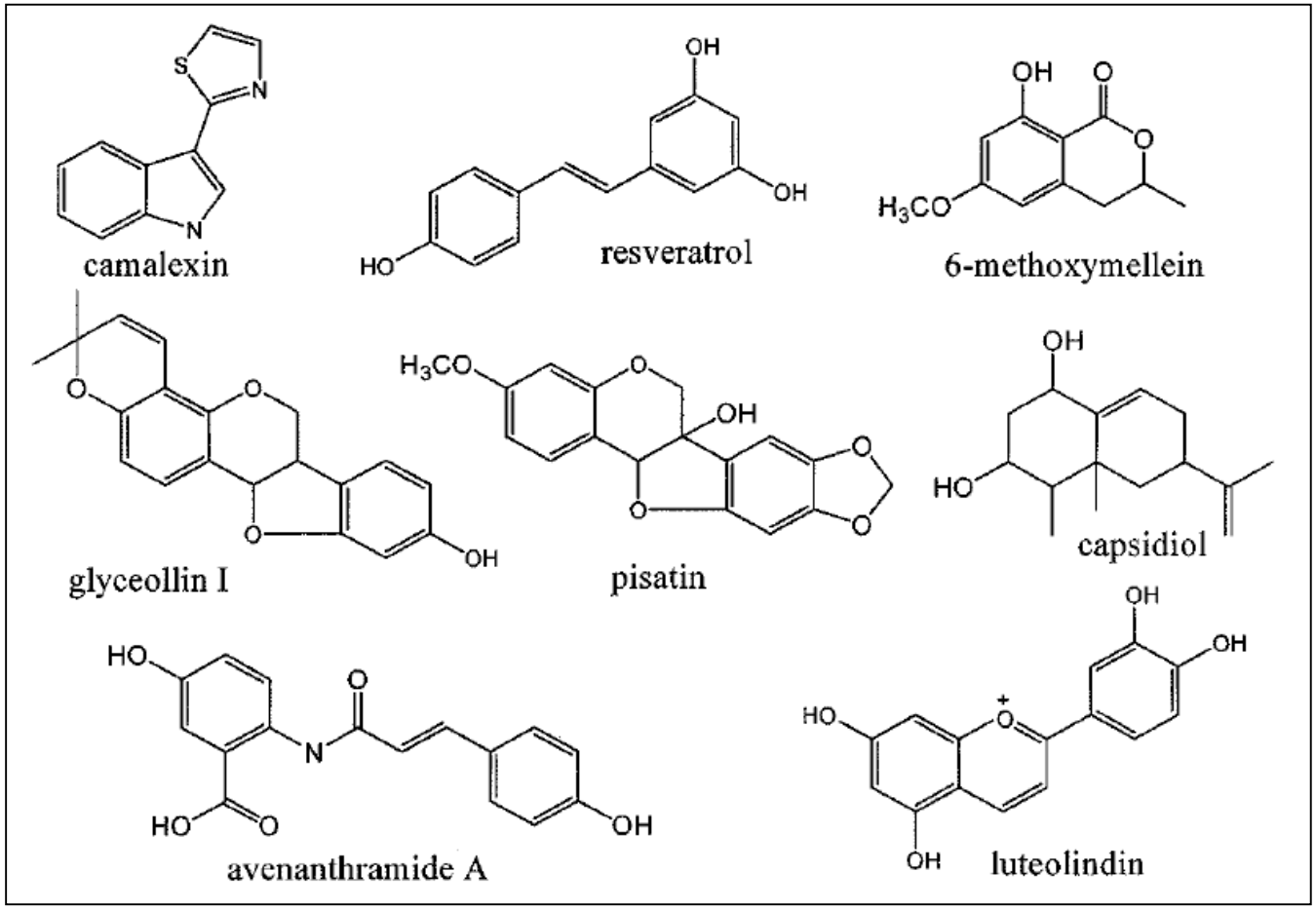


Phytoalexins are represented by members of the monoterpene, sesquiterpene, carboxylic.. Sesquiterpenoids are defined as the group of 15 carbon compounds.

Scheper & Zhong (eds.),2001

Phytoalexins

Representative phytoalexin structures



Hammerschmidt,1999

Direct evidence for the importance of phytoalexins in plant defence

- Studies with cotton and the pathogen, *Xanthomonas axonopodis* pv. *malvacearum*, have shown that the phytoalexins 2,7-dihydroxycadalene, lancilene C and lancilene C-7-methyl ether accumulated to concentrations significantly higher than those required to inhibit the bacterium *in vitro*.

Phytoalexins

Large differences in sensitivity of the pathogens

- Phytoalexins are non-specific biocides, affecting a wide range of organisms including bacteria, fungi, nematodes, higher animals and plants.
- Apart from Gram-positive bacteria being more sensitive than Gram-negative bacteria few generalizations can be made about the relative sensitivity of groups of organisms.
- Large differences in sensitivity, however, may be found among pathogens which attack plants that accumulate phytoalexins, virulent strains usually tolerating higher concentrations than avirulent ones.
- Such differences are usually attributable to the superior ability of virulent strains to degrade the phytoalexin.

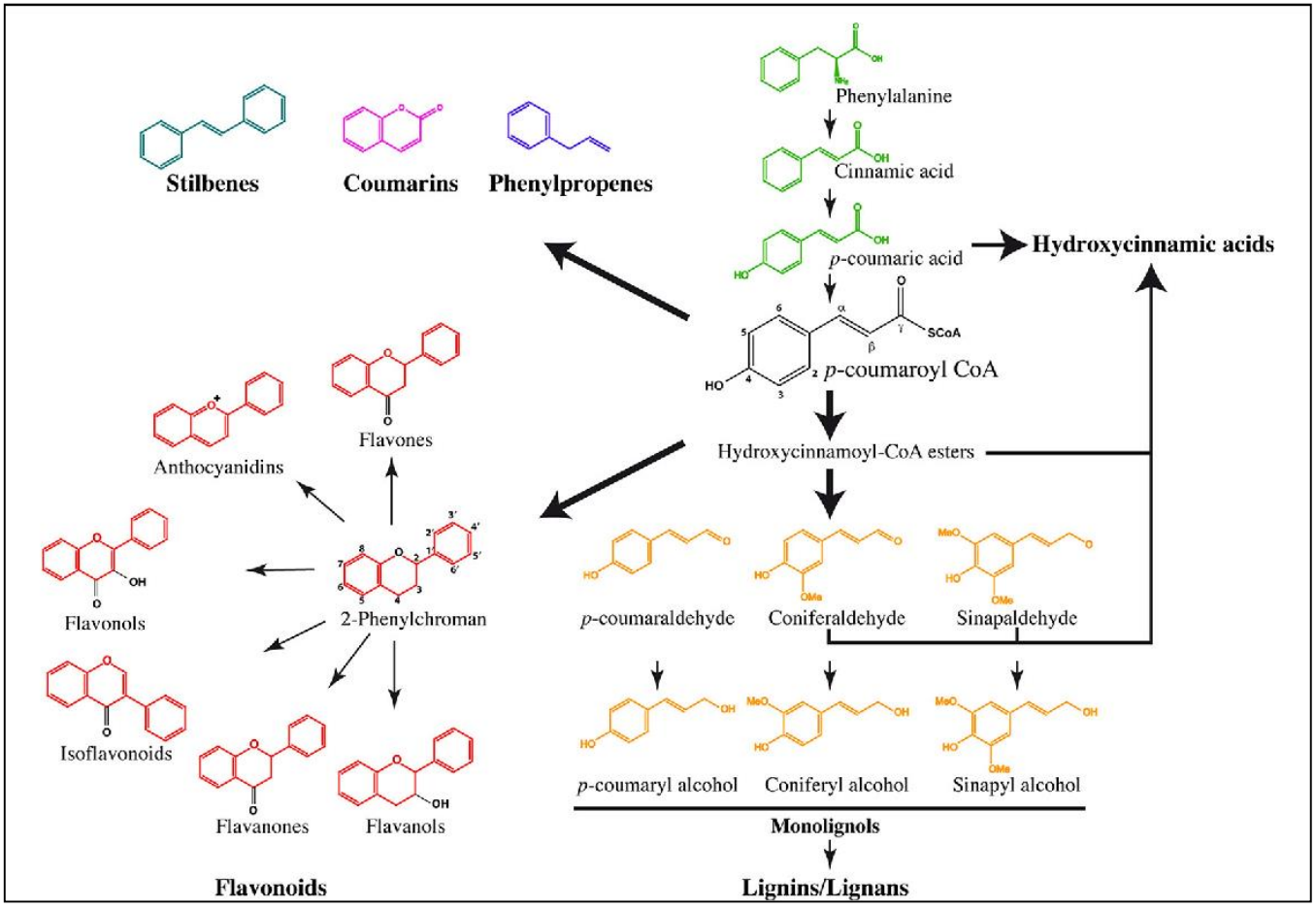
Active Defence Mechanisms

Slower responses implicated in resistance

3. Lignification

- The synthesis of lignin is similar to that of many phytoalexins since it involves the derepression of the phenylpropanoid pathway.
- Lignin is a very complex and resistant structure and lignification is thought to contribute to resistance by increasing the mechanical force required for penetration, increasing the resistance of cell walls to degradation by enzymes of the pathogen and setting up impermeability barriers to the flow of nutrients and toxins.
- In addition, lignin precursors such as coniferyl alcohol and free radicals may be toxic to the pathogen *per se.*

Lignin synthesis



Active Defence Mechanisms

Slower responses implicated in resistance

4. Suberization

- Like lignin, suberin is a constituent of healthy plant tissue and also like lignin, its synthesis may be enhanced by challenge with microorganisms.
- Suberization during wound-healing has long been associated with the resistance of potato tubers to infectious agents.
- Lulai and Corsini,1998 reported a study of the roles of the phenolic suberin in resistance to a bacterial and a fungal pathogen.
- They found that total resistance to infection by *P. carotovorum* subsp. *carotovorum* occurred 2-3 days after wounding when deposition of phenolics on the outer tangential wall of the first layer of cells was complete.

Active Defence Mechanisms

Slower responses implicated in resistance

5. hydroxyproline-rich glycoproteins (HRGPs)

- Hydroxyproline-rich glycoproteins (HRGPs) are important structural components of plant cell walls and also accumulate in response to infection as an apparent defense mechanism.

1. In response to pathogens and their signals, plant cell walls become highly enriched in HRGPs.
2. As well as being constitutive, they are also induced by wounding and infection.

- These are caused by a wide variety of fungi, bacteria (*Pseudomonas, Xanthomonas*), and other pathogens (viruses, nematodes).
- These are also increased during symbiosis upon interaction of plant roots with mycorrhizal fungi and in *Rhizobium* induced legume nodules.

Active Defence Mechanisms
hydroxyproline-rich glycoproteins (HRGPs)

- It participates in systemic acquired resistance (SAR) in that it is elicited by systemic signals and is involved in plant protection against diseases.
- HRGPs may confer resistance is by providing a template for lignin deposition in papillae.

Active Defence Mechanisms

6. Pathogenesis-related proteins (PRPs)

Background

- PRPs were discovered in 1970 in TMV-infected tobacco plants.
- Since the discovery of pathogenesis-related (PR) proteins nearly 40 years ago in tobacco plants, a great deal of research has focused on the isolation, characterization, and regulation of expression of this unique class of defense proteins in a variety of plants including rice.

Note: Most PRs is their antifungal effects. Plants are exposed to large number of pathogenic fungi. Although plants do not have an immune system, but they have a defense mechanism including antifungal activity. There are hundred's of antifungal peptides & proteins known, with more being discovered almost daily.

Datta and Muthukrishnan,1999;..

Active Defence Mechanisms
Pathogenesis-related proteins (PRs/PRPs)

1. PR proteins are proteins encoded but not expressed in host plant in the absence of interaction with a pathogen.
2. PR proteins are strongly induced in response to wounding or infection by pathogens. E.g. under conditions of nonpathogenic origin, such as stress.
3. accumulate abundantly at the site of infection, and
4. contribute to systemic acquired resistance (SAR).

Jiang *et al.*,2015

PRs vs. PR-like proteins

PRLs

- PR-proteins were defined as proteins coded by the host plant but induced only in pathological or related situations.
- The PR-proteins are identified easily in cell extracts of infected plants.
- However, related proteins were identified that accumulate in normal (uninfected) plants in certain tissues or developmental stages.
- These proteins are referred to as 'PR-like' proteins (Van Loon,1999).
- Thus, related proteins occurring in the absence of pathogen infection.

Van Loon *et al.*,2006;..

Active Defence Mechanisms

Slower responses implicated in resistance

Pathogenesis-related proteins (PRs/PRPs)

- Defense responses:

1. These protective reactions are known as "defense responses" of higher plants, and

- Defense-related proteins:

1. The proteins actively synthesized in accordance with this reaction are called "defense-related proteins".
2. PR proteins not only accumulate in various parts of normal tissues, but are induced by pathogen infection and improve the defensive capacity of plants.

Yagami,2005;..

PR proteins
Apoplast and vacuoles

- The plant apoplast plays a critical role in the:

1. transport of water and nutrients and
2. interactions between plants and pathogens.

- **Most of pathogenesis-related (PR) proteins are located in the apoplast.**
- In general,

1. acidic PR proteins are located in the apoplast, and
2. basic PR proteins, in the vacuole.

Naz *et al.*,2014; Enoki and Suzuki,2016

Apoplast
PR proteins functions

- These proteins have been implicated in active defense, potentially restricting pathogen development and spread.
- Some PR proteins possess antimicrobial activity.
- Also they can directly affect pathogen integrity, and/or generate signal molecules through their enzymatic activity that act as elicitors to induce other plant defense related pathways.
- One response of plants to attempted infection by pathogens is to accumulate apoplastic proteins, including pathogenesis related (PR) proteins.
- The expression of defense or PR proteins in apoplast of plant cells enhance plant resistance to pathogens.

Naz *et al.*,2014; Enoki and Suzuki,2016

Active Defence Mechanisms

Recognized and proposed families of pathogenesis-related proteins.

Family	Proteins/properties	Functions
PR-1	PR-1 a, PR-1 b, and PR-1 c 14-17kD	Antifungal
PR-2	β-1,3-Glucanases 25-35kD	Cleaves β-1,3-glucans
PR-3	Chitinase types I, II, IV, V, VI, and VII 30kD	Endochitinase
PR-4	Chitinase types I and II 13-19kD	Antifungal and chitinase
PR-5	Thaumatin-like proteins	Antifungal
PR-6	Tomato proteinase inhibitor I 6-13kD	Proteinase inhibitor
PR-7	Tomato endoproteinase P	Endoproteinase
PR-8	Cucumber chitinase	Chitinase III
PR-9	Tobacco lignin-forming peroxidase	Peroxidase
PR-10	Parsley "PR-1" Bet v 1, Mal d 1, Api g 1, and Dau c 1	Ribonuclease-like
PR-11	Tobacco chitinase type V	Chitinase
PR-12	Radish Rs-AFP3	Defensin
PR-13	*Arabidopsis* THI2.1	Thionin
PR-14	Lipid transfer proteins	Shuttling of phospholipids and fatty acids
PR-15	Barley OxOa	Oxalate oxidase
PR-16	Barley OxOLP	Oxalate-like oxidase
PR-17	Tobacco PRp27	Unknown

Yagami,2005; Sinha *et al.*,2014

Active Defence Mechanisms

Slower responses implicated in resistance

Pathogenesis-related proteins (PRs/PRPs)

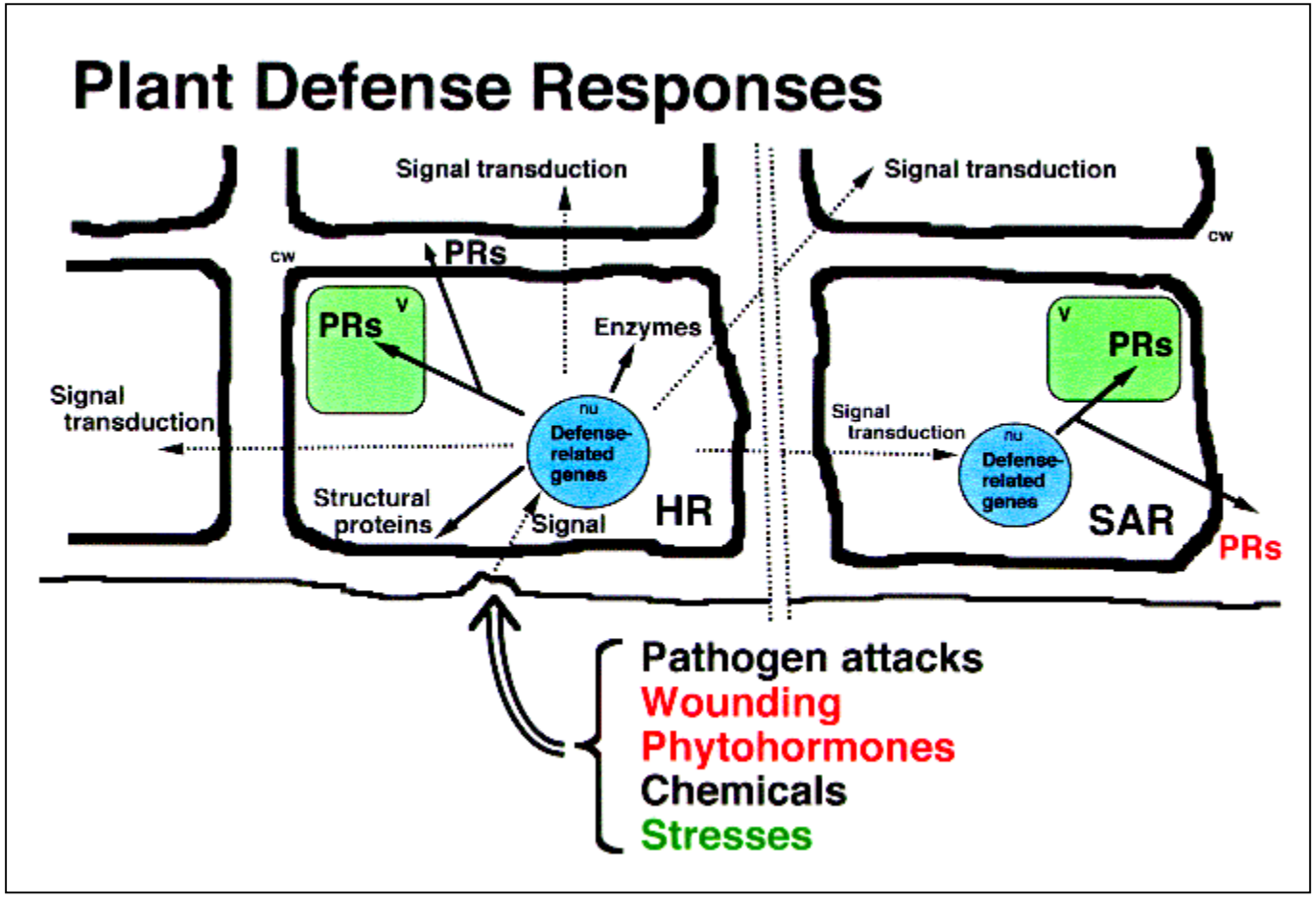
- *PR* genes have been shown to be induced by:
- Various abiotic stresses, such as:
- treatments with NaCl, heat, cold, PEG, UV irradiation, and ozone.
- Some PR proteins have also been reported to accumulate under specific physiological conditions, such as:
- pollen development, leaf senescence, fruit development and ripening.

Yagami,2005; Jiang *et al.*,2015

Active Defence Mechanisms

Slower responses implicated in resistance

Pathogenesis-related proteins (PRs/PRPs)

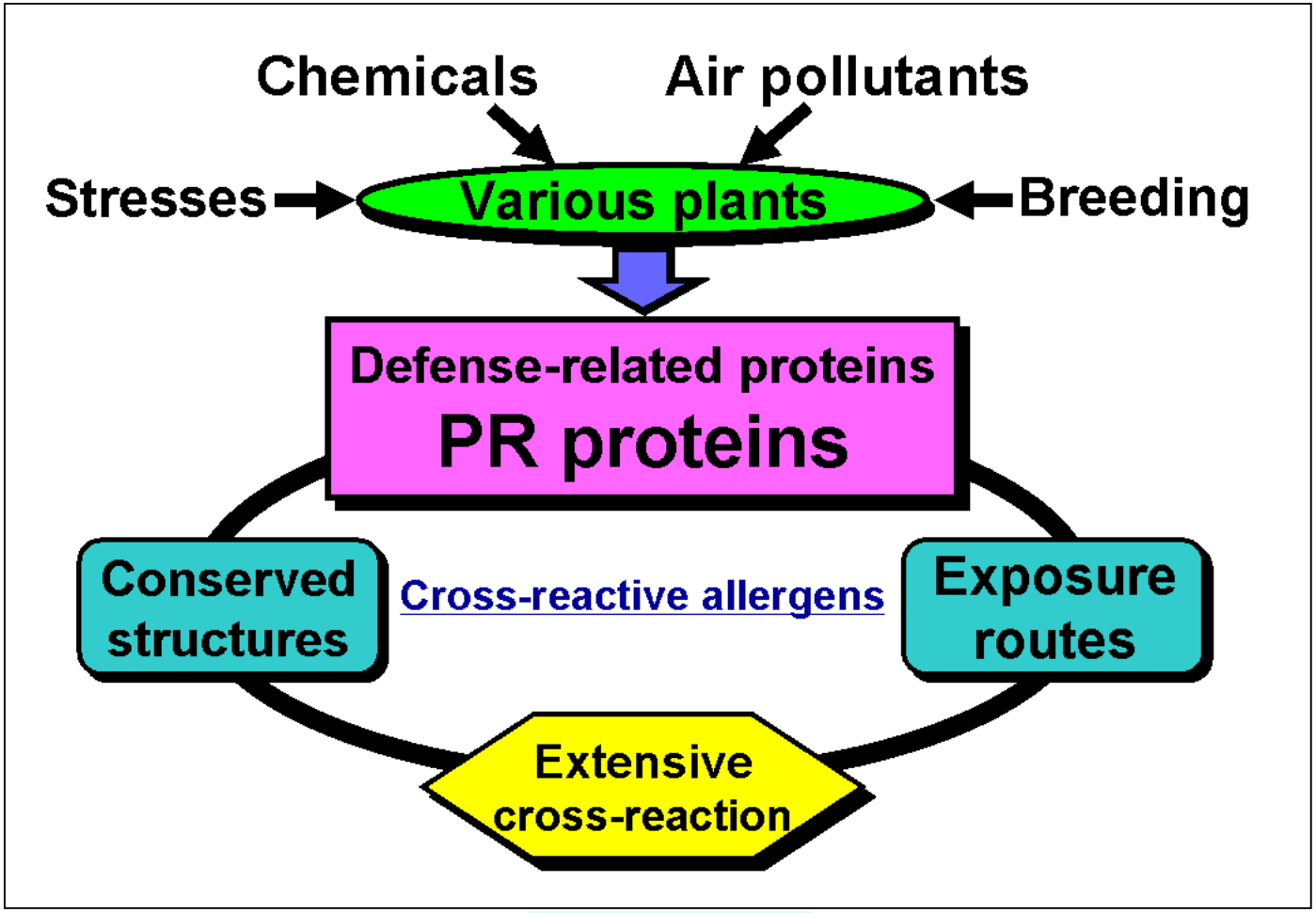


Yagami,2005

Active Defence Mechanisms

Slower responses implicated in resistance

Pathogenesis-related proteins (PRs/PRPs)



Yagami,2005

Active Defence Mechanisms

Slower responses implicated in resistance

Pathogenesis-related proteins (PRs/PRPs)

- These proteins are induced by the plants as a defense response system in stress conditions like microbial and insect infections, wounding, exposure to harsh chemicals, and atmospheric conditions.
- However, some plant tissues that are more exposed to environmental conditions like UV irradiation and insect or fungal attacks express these proteins constitutively.

Sinha *et al.*,2014;..

Active Defence Mechanisms

Pathogenesis-related proteins (PRs/PRPs)

Functions

1. An important common function of most PRs is their antifungal effects;
2. Some PRs also exhibit antibacterial, insecticidal or antiviral action.
3. Function as signals that spread "news" of the infection to nearby cells.
4. Infections also stimulate the cross-linking of molecules in the cell wall and the deposition of lignin, responses that set up a local barricade that slows spread of the pathogen to other parts of the plant.

See also the section "systematic resistance"

Active Defence Mechanisms

Pathogenesis-related proteins (PRs/PRPs)

Functions

4. Chitinase activity.
5. Peroxidase, ribonuclease and lysozyme activities.
6. Their hydrolytic, proteinase-inhibitory and membrane-permeabilizing ability.
6. They inactivate the proteins secreted by the parasites in the invaded plant tissues.

Wikipedia,2016

Active Defence Mechanisms

Pathogenesis-related proteins (PRs/PRPs)

Functions

- A special class of PRs inducers are hormones.
- PR protein expression also responded to defense/stress signaling molecules, including:
- salicylic acid (SA),
- ethylene (ET),
- abscisic acid (ABA), and
- jasmonic acid (JA).
- Jasmonic acid (JA), which might be an early signal establishing systemic immunity.

See also the section "systematic resistance"

Active Defence Mechanisms

Pathogenesis-related proteins (PRPs)

Classification of pathogenesis related proteins

- Pathogenesis-related (PR) proteins, classified into 17 families based on

1. amino acid sequences,
2. serological reaction,
3. enzymatic activity,
4. diverse structure, function and biological activity.

- PR proteins share many biochemical properties that render them easily distinguishable.
- They have relatively low molecular weights, are stably extractable at low pH, are highly resistant to proteases, and have extreme isoelectric points.

Sinha *et al.*,2014;. Enoki and Suzuki,2016

Biological role of PR-proteins

- An important common feature of most PRs is:

1. Their antifungal effect;
2. Some PRs exhibited also antibacterial (e.g. PR-8, PR-10, PR-14 proteins), insecticidal, nematicidal, and
3. As recently shown antiviral action.

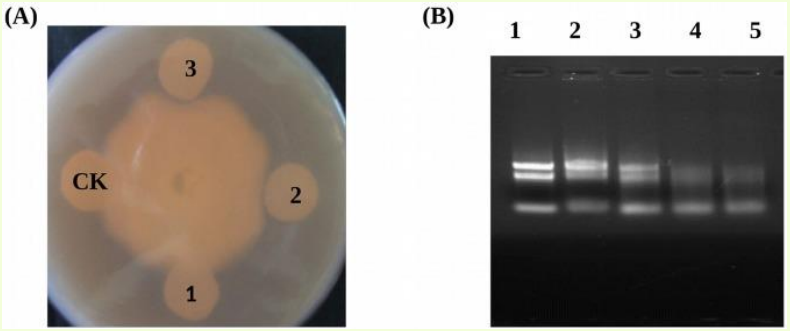
- Therefore:

1. PR proteins are generally used as ISR markers, but
2. no antiviral or antibacterial activity has yet been reported for any PR protein.

GmPRP protein

A novel PR protein/*GmPRP* gene was isolated from highly resistant soybean infected with *Phytophthora sojae*

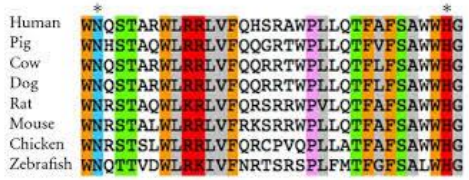
- Antimicrobial activity ribonuclease activity and assays for the recombinant GmPRP protein.
- *P. sojae* race 1 was grown on carrot agar plates at 28°C. The sterile filter paper discs were treated with 15 μg, 25 μg, or 35 μg of the renatured recombinant GmPRP protein. A control filter-paper disc containing boiled recombinant protein did not show an inhibitory effect.
- Two specific primers, *GmPRP* F: TTCAGCCTAAACGGAAGGAAGCCT and *GmPRP* R: TTGTCGTGAAGGCCTTATGGGATG), were used to determine the expression level of *GmPRP*.



Jiang *et al.*,2015

Multiple sequence alignment of β-1,3-glucanases

Amino acid sequence similarities



- Multiple sequence alignment of β-1,3-glucanases (PR-2 proteins)from tobacco, tomato, banana, and latex (Hev b 2).
- The identical residues are highlighted in grey.
- This protein shares 62.9% and 64.7% sequence identities with tobacco and tomato β-1,3-glucanases.

```
Tobacco  QSIGVCYGMLGNNLPNHWEVIQLYKSRNIGRLRLYDPNHGALQALKGSNIEVMLGLPNSD  60
Tomato   -QIGVCYGMMGNNLPSHSEVIQLYKSRNIRRLRLYDPNHGALNALRGSNIEVILGLPNVD  59
Hev b 2  AQVGVCYGMQGNNLPPVSEVIALYKKSNITRMRIYDPNQAVLEALRGSNIELILGVPNSD  60
Banana   ----------------------------ARMRLYDPNQAALQALRNSNIQVLLDVPRSD  31

Tobacco  VKHIASGMEHARWWVQKNVKDFWPDVKIKYIAVGNEISPVT-GTSYLTSFLTPAMVNIYK  119
Tomato   VKHISSGMEHARWWVQKNVRDFWPHVKIKYIAVGNEISPVT-GTSNLAPFQVPALVNIYK  118
Hev b 2  LQSLTN-PSNAKSWVQKNVRGFWSSVRFRYIAVGNEISPVNRGTAWLAQFVLPAMRNIHD  119
Banana   VQSLASNPSAAGDWIRRNVVAYWPSVSFRYIAVGNELIPGS----DLAQYILPAMRNIYN  87

Tobacco  AIGEAGLGNNIKVSTSVDMTLIGNSYPPSQGSFRNDARWFVDAIVGFLRDTRAPLLVNIY  179
Tomato   AIGEAGLGNDIKVSTSVDMTLIGNSYPPSQGSFRNDVRWFTDPIVGFLRDTRAPLLVNIY  178
Hev b 2  AIRSAGLQDQIKVSTAIDLTLVGNSYPPSAGAFRDDVRSYLNPIIRFLSSIRSPLLANIY  179
Banana   ALSSAGLQNQIKVSTAVDTGVLGTSYPPSAGAFSSAAQAYLSPIVQFLASNGAPLLVNVY  147

Tobacco  PYFSYSGNPGQISLPYSLFTAPNVVVQDGSRQYRNLFDAMLDSVYAALERSGGASVGIVV  239
Tomato   PYFSYSGNPGQISLPYALFTAPNVVVQDGSRQYRNLFDAMLDSVYAAMDRTGGGSVGIVV  238
Hev b 2  PYFTYAGNPRDISLPYALFTSPSVVVWDGQRGYKNLFDATLDALYSALERASGGSLEVVV  239
Banana   PYFSYTGNPGQISLPYALFTASGVVVQDGRFSYKNLFDAIVDAVFAALERVGGANVAVVV  207

Tobacco  SESGWPSAG-AFGATYDNAATYLRNLIQHAKEGSPRKP-GPIETYIFAMFDENNKNPELE  297
Tomato   SESGWPSAG-AFGATHENAQTYLRNLIQHAKEGSPRKP-GPIETYIFAMFDENNKNPELE  296
Hev b 2  SESGWPSAG-AFAATFDNGRTYLSNLIQHVKRGTPKRPKRAIETYLFAMFDENKKQPEVE  298
Banana   SESGWPSAGGGAEASTSNAQTYNQNLIRHVGGGTPRRPGKEIEAYIFEMFNENQKA----  263

Tobacco  KHFGLFSPNKQPKYNINFG 316
Tomato   KHFGMFSPNKQPKYNLNFG 315
Hev b 2  KHFGLFFPNKWQKYNLNFS 318
Banana   ------------------
```

Sinha *et al.*,2014

Pathogenesis-related proteins (PRPs)

Biochemical, structural characteristics, cellular and tissue localization of PRs

- The 5 classical groups of PR proteins(PR-1,-2,- 3,-4,-5) has 2 subclasses:

1. A basic subclass found in plant cell vacuole.
2. An acidic subclass found in the extracellular space.

- Thus PRs have dual cellular localization:

1. Vacuolar, and
2. Apoplastic space.

- The apoplast being the main site of their accumulation.

Sreeremaya,2014

PR-proteins and genes in wheat

Classes:
Expressed in:
inducted by:

Muthukrishnan *et al.*,2000

PR-protein family	Class/ subfamily/ enzyme activity	Name	Protein/ cDNA/gene	Expressed in	Induced by	Authors/reference*	GenPept accession no.
PR-1	Basic	PR-1.1	C	Leaves	Pathogen	Molina et al.	CAA07473
PR-1	Neutral	PR-1.2	C	Leaves	Pathogen	Molina et al.	CAA07474
PR-2	Basic glucanase		P	Grain	Developmental	Lai et al. (1993)	
PR-2	Subfamily B	LW2	C	Aleurone layer	Developmental	Lai et al. (1993)	CAA80493
PR-2	Subfamily D	Glc1	C	Roots	Aluminum toxicity	Cruz-Ortega et al.	AAA90953
PR-2	Glucanase		P	Leaves	Pathogen	Munch-Garthoff et al. (1997)	
PR-2	Glucanase	Clone SM289	C	Spikelet	Pathogen	Li et al. (1999)	AAD28732
PR-2	Glucanase	Clone SM638	C	Spikelet	Pathogen	Li et al. (1999)	AAD28734
PR-3	Class Ib chitinase		P	Germ	Developmental	Molano et al. (1979)	
PR-3	Chitinase		P	Leaves	Aphid infestation	Van der Westhuizen et al. (1998)	
PR-3	Class Ib chitinase	Wch1	G	Leaves	Pathogen	Liao et al.	CAA53726
PR-3	Chitinase		P	Leaves	Pathogen	Munch-Garthoff et al. (1997)	CAA53626
PR-3	Class IV chitinase	SM383	C	Spikelet	Pathogen	Li et al. (1999)	AAD28730
PR-3	Class VII chitinase	SM194	C	Spikelet	Pathogen	Li et al. (1999)	AAD28733
PR-4	Chitin-binding	wPR4-8	C	Seedling		Huh et al.	AAF02296
PR-5	TLP	Trimatin	P	Seeds	Developmental	Vigers et al. (1991)	
PR-5	TLP	pWIR232	C	Leaves	Pathogen	Rebmann et al. (1991)	CAA41283
PR-5	TLP	gbx3832	C			Mingeot et al.	CAA66278
PR-5	TLP	WAS-3	P	Suspension cells		Kuwabara et al. (1999)	
PR-6	Bowman–Birk	wali3	C	Roots	Al toxicity	Snowden et al. (1995)	AAA50848
PR-6	Bowman–Birk	wali5	C	Roots	Al toxicity	Snowden et al. (1995)	AAA50850
PR-6	Z-serpin	WZC1	C	Immature grain	Developmental	Rasmussen et al.	CAA90071
PR-6	Z-serpin	WZC1c	C	Immature grain	Developmental	Jensen & Rasmussen	CAB52709
PR-6	Z-serpin	WSZ2a	C	Immature grain	Developmental	Jensen & Rasmussen	CAB52710
PR-6	Z-serpin	WZS2	C	Immature grain	Developmental	Rasmussen	CAA72273
PR-6	Z-serpin	WZS3	C	Immature grain	Developmental	Rasmussen	CAA72274
PR-6	Trypsin-inhibitor		P	Seedlings	Pathogen, SA	Molodchenkova et al. (1998)	
PR-9	Peroxidase		P	Leaves	Aphid infestation	Van der Westhuizen et al. (1998)	
PR-9	Peroxidase		C	Roots	Development	Hertig et al.	CAA37713
PR-9	Peroxidase		C	Leaves	Pathogen	Rebmann et al. (1991)	CAA39486
PR-9	Peroxidase	Wir3	C	Seedlings	Pathogen	Schweizer et al.	CAA34211
PR-9	Peroxidase	pox1	C	Roots		Baga et al. (1995)	CAA59484
PR-9	Peroxidase	pox2	C	Roots, leaves	Pathogen (leaves)	Baga et al. (1995)	CAA59485
PR-9	Peroxidase	pox3	C	Leaves		Baga et al. (1995)	CAA59486
PR-9	Peroxidase	pox4	C	Roots		Baga et al. (1995)	CAA59487
PR-13	Alpha 1 & 2 thionin	pTTH14, 1	C	Seeds	Developmental	Castagnero et al.	CAA50003-4
PR-13	type V thionin		G			Castagnero et al.	CAA54191
PR-14	Lipid transfer protein		C			Dieryck et al.	CAA45210

* Authors/reference not followed by a year are not in the reference list. They are identified by the GenPept accession no. in the next column.

Pathogenesis-related proteins (PRPs)

Biochemical, structural characteristics, cellular and tissue localization of PRs

- PR proteins are mostly resistant to proteases and most of them show considerable stability at low pH.
- PR proteins are distinguished by specific biochemical properties:

1. Low-molecular proteins (6-43 kDa),
2. Acidic proteins (acid-soluble; extractable and stable at low pH (<3),
3. Thermostable, and
4. Highly resistant to proteases.

Pathogenesis-related proteins (PRPs)

The nomenclature of the PRs

Pathogenesis-related genes(*PR* genes)

- *PR* expression is known to be regulated by signaling compounds such as ABA, ET, JA, and SA.
- The current nomenclature of the PRs serves as an example that is being extended to other plant species.
- e.g., *Arabidopsis*, barley, bean, cucumber, and maize.
- The families are numbered and the different members within each family are assigned letters according to the order in which they are described.
- PR-genes(*PR* genes) are designated as *ypr* followed by the same suffix, in accordance with the recommendations of the Commission for Plant Gene Nomenclature. e.g. *ypr1*, *ypr2*, *ypr3*,etc.

Pathogenesis-related proteins (PRPs)

The nomenclature of the PRs

Pathogenesis-related genes(*PR* genes)

Family	Type member	Properties	Gene symbols
PR-1	Tobacco PR-1a	Unknown	*Ypr1*
PR-2	Tobacco PR-2	β-1,3-glucanase	*Ypr2*, [*Gns2* (‘*Glb*’)]
PR-3	Tobacco P, Q	Chitinase type I, II, IV, V, VI, VII	*Ypr3*, *Chia*
PR-4	Tobacco ‘R’	Chitinase type I, II	*Ypr4*, *Chid*
PR-5	Tobacco S	Thaumatin-like	*Ypr5*
PR-6	Tomato Inhibitor I	Proteinase-inhibitor	*Ypr6*, *Pis* (‘*Pin*’)
PR-7	Tomato P_{69}	Endoproteinase	*Ypr7*
PR-8	Cucumber chitinase	Chitinase type III	*Ypr8*, *Chib*
PR-9	Tobacco “lignin-forming peroxidase”	Peroxidase	*Ypr9*, *Prx*
PR-10	Parsley “PR1”	Ribonuclease-like	*Ypr10*
PR-11	Tobacco “class V” chitinase	Chitinase, type I	*Ypr11*, *Chic*
PR-12	Radish Rs-AFP3	Defensin	*Ypr12*
PR-13	Arabidopsis THI2.1	Thionin	*Ypr13*, *Thi*
PR-14	Barley LTP4	Lipid-transfer protein	*Ypr14*, *Ltp*
PR-15	Barley OxOa (germin)	Oxalate oxidase	*Ypr15*
PR-16	Barley OxOLP	Oxalate-oxidase-like	*Ypr16*
PR-17	Tobacco PRp27	Unknown	*Ypr17*

Gene *Ypr2* (*Gns2* or *GLb*) encodes for Glucanase, *Ypr14*(*Ltp*) encodes for Lipid-transfer protein (LTP4), etc. Among these PR proteins chitinases and β-1,3-glucanases are two important hydrolytic enzymes that are abundant in many plant species after infection by different type of pathogens.

Van Loon *et al.*,2006

Pathogenesis-related proteins (PRPs)
The nomenclature of the PRs
Pathogenesis-related genes(*PR* genes)

Families	Type member	Properties	Gene symbol
PR-1*	Tobacco PR-1a	Unknown	*Ypr1*
PR-2*	Tobacco PR-2	β-1,3-glucanase	*Ypr2, [Gns2 ('Glb')]*
PR-3*	Tobacco P, Q	Chitinase type I,II, IV,V,VI,VII	*Ypr3, Chia*
PR-4	Tobacco 'R'	Chitinase type I,II	*Ypr4, Chid*
PR-5*	Tobacco S	Thaumatin-like	*Ypr5*
PR-6	Tomato Inhibitor I	Proteinase-inhibitor	*Ypr6, Pis (´Pin´)*
PR-7	Tomato P_{69}	Endoproteinase	*Ypr7*
PR-8*	Cucumber chitinase	Chitinase type III	*Ypr8, Chib*
PR-9	Tobacco "lignin-forming peroxidise"	Peroxidase	*Ypr9, Prx*
PR-10*	Parsley "PR1"	Ribonuclease-like	*Y pr10*
PR-11	Tobacco "class V" chitinase	Chitinase, type I	*Ypr11, Chic*
PR-12	Radish Rs-AFP3	Defensin	*Ypr12*
PR-13	Arabidopsis THI2.1	Thionin	*Ypr13, Thi*
PR-14*	Barley LTP4	Lipid-transfer protein	*Ypr14, Ltp*
PR-15	Barley OxOa (germin)	Oxalate oxidase	*Ypr15*
PR-16	Barley OxOLP	Oxalate oxidase-like	*Yrp16*
PR-17	Tobacco PRp27	Unknown	*Yrp17*

*PRPs with allergenic potential. Fruit from some *Rosaceae* are reported to cause allergic reactions in certain individuals.

Maghuly *et al.*,2009

Characterization and functions of PR proteins

PR-1 antifungal with no known function

- PR-1 proteins were first found to be expressed in tobacco in response to tobacco mosaic virus (TMV) infection having 14 to 17 kDa molecular weights.
- These widely distributed proteins of plant kingdom have antifungal activity at the micromolar level against a number of plant pathogenic fungi, but their mechanism of action is not known.
- PR-1 proteins accumulates to high levels after pathogen infection and are antifungal both in plants *& in vitro*.
- PR-1 proteins have been found in wheat, rice, maize, tobacco.

Trunk injection of potassium phosphites (PH), acibenzolar-S-methyl (ASM), resulted in the significant induction of PR-1, PR-2, and PR-8 protein genes in apple leaves against the bacterium *E. amylovora* (A'cimovi'c *et al.*,2015).

Note: PR-1 (anti-oomycete activity), PR-2 (β-1,3-glucanase), and PR-8 (class III chitinase).

Characterization and functions of PR proteins

PR-1 antifungal with no known function

- PR-1 family contains the first discovered PRs and is also the most predominant.
- Despite extensive studies, no biochemical function is known for any of the PR-1 proteins (van Loon *et al.*, 2006).
- *PR-1* genes are induced by a plethora of different pathogens.
- It was reported that spraying tobacco plants with salicylic acid (SA) induced local resistance to TMV.
- Salicylate induces the expression of both acidic and basic *PR-1* genes.

Wheat cDNAs encode proteins PR-1.1 and PR-1.2.

Santén *et al.*,2007

Characterization and functions of PR proteins

PR-2 with glucanase activity

- The PR-2 proteins are divided into three classes based on amino acid sequence identity, primary structure, cellular localization, and mode of expression.
- The class I members with approximate size of 33 kDa are basic and localized in the cell vacuole and are found in tobacco, tomato, potato, and other plant species.
- Antifungal activity has been observed only in class I β-glucanases.
- The class II and class III proteins are acidic proteins with average molecular weights around 34 to 36 kDa secreted into the extracellular space.

Sinha *et al.*,2014

Characterization and functions of PR proteins

PR-3 with chitinase activity

- Proteins of the PR-3 family are endochitinases, which hydrolyze β-1,4-linkages between N-acetylglucosamines of chitin.
- Chitin is not a natural component of plant cells but is present in most fungal cell walls and the insect cuticula.
- Among the seven different classes of chitinases, chitinases of classes I, II, and IV are grouped under PR-3 family proteins.

Santén *et al.*,2007; Sinha *et al.*,2014

Characterization and functions of PR proteins

PR-4 with chitinase activity

- The less extensively studied proteins. Antifungal.
- The first PR-4 proteins to be described were from potato.
- PR-4 protein is known to be induced not only by pathogen attack, but by ethylene in *Arabidopsis* and peach plants.
- PR-4 proteins(chitin binding proteins) have been classified as endochitinases.
- It has conserved N-terminal cysteine-rich domain corresponding to the mature hevein, a small antifungal protein isolated from rubber plant (*Hevea brasiliensis*) latex.

Guevara-Morato *et al.*,2010

Characterization and functions of PR proteins

PR-5 (TL proteins) with antifungal activity

- Proteins that belong to the PR-5 family are also known as thaumatin-like (TL) proteins.
- TL proteins have been isolated from *A. thaliana*, pumpkin, wheat ,rice.
- The highly stabilized structure of PR-5 are resistant to protease degradation.
- Mechanism is not known.
- These proteins they are fungicidal against a wide no: of plant & human pathogens.
- Osmotins, proteins induced by salt stress, also belong to the PR-5 family.

Sreeremya,2014

Characterization and functions of PR proteins

PR-5 (TL proteins) with antifungal activity

- *PR5* genes expression:
- The PR-5 group proteins are not normally detected in leaves of young healthy plants, but they rapidly accumulate to high levels in response to biotic or abiotic stress.
- The PR-5 proteins (TLPs) exhibit antifungal action against many fungi, including *P. infestans*, a member of the class *Oomycete.*
- PR-5 family from maize seeds seemed consistent with a role in protection against phytophagous insects.

Characterization and functions of PR proteins

PR-6 with proteinase activity

- PR-6 are proteinase inhibitors implicated in defense against insects and other herbivores, micro-organisms, and nematodes.
- Pathogenic organisms colonizing plant tissues rely on a set of proteinases as part of their virulence factor.
- In parallel, plants have evolved genes encoding inhibitors (proteinase inhibitors) that inactivate some of these proteinases.
- Proteinase inhibitors (PIs) are highly stable defensive proteins of plant tissues that are induced in response to insect and pathogen attacks.

van Loon and Strien,1999

Characterization and functions of PR proteins

PR-7 with endoproteinase activity

- PR-7 has so far been characterized only in tomato, where it is a major PR and acts as an endoproteinase.
- Because lysis of fungal cell walls often requires degradation of cell wall proteins in addition to hydrolysis of chitin and glucan, it seems reasonable to assume that PR-7 serves as an accessory to antifungal action.

van Loon and Strien,1999

Characterization and functions of PR proteins

PRs 8, 9,10 and 11

- Chitinase activity was detected in PR-4, PR-8 and PR-11, PR-4 being referred to as chitin-binding proteins.
- PR-7(proteinase), PR-9(peroxidase), PR-10 (ribonuclease) and PR-8(with chitinase and lysozyme and activities) were among the other PR proteins.
- E.g. PR-8 can disrupt gram-positive bacteria due to lysozyme activity.
- PR-10 induced in hot pepper (*Capsicum annuum*) by incompatible interactions with TMVPo and *Xanthomonas vesicatoria*, was shown to function as a ribonuclease.

Characterization and functions of PR proteins

PRs 12, 13 and 14

- Additional families of PRs comprise the pathogen induced plant defensins (PR-12), thionins (PR-13) and Lipid transfer protein, LTPs (PR-14).
- All exhibit antifungal and antibacterial activity, exerting their effect at the level of the plasma membrane of the target micro-organism.
- PR-14 proteins are present in significant amounts in vascular tissue and in the outer cell layers of plants.
- PR-14 proteins are involved in plant defense against bacterial and fungal pathogeneses as well as under different environmental stresses such as drought, heat, cold, or salt. Plant lipid transfer proteins are responsible for the shuttling of phospholipids and other fatty acid groups between cell membranes.

van Loon and Strien,1999; Sinha *et al.*,2014

Characterization and functions of PR proteins

PRs 15 and 16

- PR-15 and -16 are typical of monocots and comprise families of:
- germin like oxalate oxidases, and
- oxalate oxidase-like proteins with superoxide dismutase activity, respectively.
- These proteins generate hydrogen peroxide that can be toxic to different types of attackers or could directly or indirectly stimulate plant-defense responses.
- Germins and germin-like proteins (GLPs) have been classified as PR-15 and PR-16; PR-16 has been isolated from hot pepper during the resistance response to bacterial and viral infection.

Characterization and functions of PR proteins

PR17

- PR-17 proteins have been found as an additional family of PRs in infected tobacco, wheat, and barley and contain sequences resembling the active site of zinc metalloproteinases.

A putative novel family (PR-18) comprises fungus- and SA-inducible carbohydrate oxidases, as exemplified by proteins with hydrogen peroxide-generating and antimicrobial properties from sunflower (but not yet confirmed).

van Loon *et al.*,2006

Assay of induced enzymes

Assay for peroxidase

Xanthomonas oryzae pv. *oryzae*

- Induced systemic resistance activates the multiple defense mechanisms which include increased pathogenesis related (PR) proteins (peroxidase, chitinase, etc).
- Phenylalanine ammonia lyase (PAL) and peroxidase are the main enzymes involved in the phenyl-propanoid metabolism (Xue *et al*.,1998).
- The Xoo inoculated leaf portions of rice plants sprayed with leaf extracts of *A. vasica* (Malabar nut) or streptomycin or water were collected at various time intervals (0, 24, 48, 72, 96 and 164 h) after pathogen inoculation and were quickly frozen in liquid nitrogen and stored at -20°C.

Govindappa *et al*.,2011

Assay of induced enzymes

Assay for peroxidase

Xanthomonas oryzae pv. *oryzae*

- Fresh plant leaves (1 g) were homogenized in 3 ml of 0.1 M sodium phosphate buffer (pH 7.0) with pre chilled mortar and pestle. The homogenate was centrifuged at 18,000 rpm at 5°C for 15 min and used within 2 to 4 h.
- Supernatant was served as an enzyme source.
- To a spectrophotometric sample cuvette, 3 ml of buffer solution, 0.05 ml guaiacol solution, 0.1 ml enzyme extract and 0.03 ml H_2O_2 solution were added and mixed well.
- Absorbance was recorded at 470 nm using spectrophotometer.
- The enzyme activity was expressed as changes in absorbance (min^{-1g-1}) of fresh weight (Hammerschmidt *et al.*, 1982). As such, three replicates were maintained for each treatment.

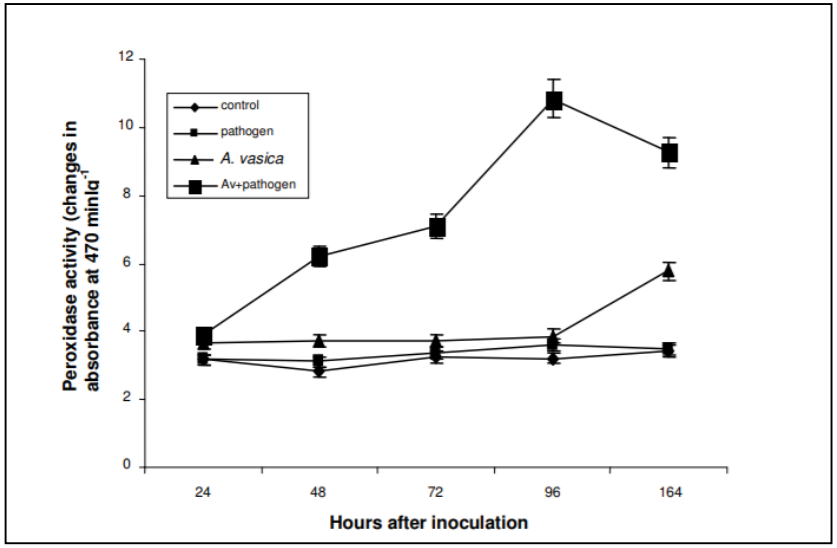
Govindappa *et al.*,2011

Assay of induced enzymes

Assay for peroxidase

Xanthomonas oryzae pv. *oryzae*

- Induction of peroxidase in rice leaves in response to *A. vasica* treatment against bacterial leaf blight.



Govindappa *et al.*,2011

Active Defence Mechanisms

Slower responses implicated in resistance

7. Systemic acquired resistance (SAR)

- The phrase systemic acquired resistance (SAR) describes the resistance which develops in plants at a distance from an initial local lesion.
- The phenomenon was first described by Chesters in the 1930s and by Ross (1966) in tobacco inoculated with tobacco mosaic virus.

Active Defence Mechanisms

Slower responses implicated in resistance

Systemic acquired resistance (SAR)

- Secondary response
- Systemic
- Broad-range resistance
- Leads to Pathogenesis-Related (PR) gene expression
- Signals: SA, JA, ethylene

Systemic acquired resistance
SAR

- Seven criteria have been suggested for distinguishing SAR from other mechanisms that reduce disease severity or incidence, and they are:

1. Absence of toxic effects of the inducing agent on the challenging pathogen;
2. suppression of the induced resistance by a previous application of specific inhibitors, such as actinomycin D which affect gene expression of the plant;
3. Necessity of a time interval between application of the inducer and the onset of protection;
4. Absence of a typical dose-response correlation known for toxic compounds;
5. Non-specificity of protection;
6. Local as well as systemic protection, and
7. Dependence on the plant genotype.

See also systematic resistance section

Active Defence Mechanisms

Slower responses implicated in resistance

8. Induced systemic resistance (ISR)

- In contrast to SAR, the induction of ISR in some systems at least requires ethylene and jasmonic acid.
- ISR is claimed to differ from SAR in that it is not accompanied by the accumulation of PRPs and is independent of the accumulation of salicylic acid (Knoester *et al.*,1999).
- However, DeMeyer and Hofte (1997) showed that salicylic acid production was essential for induction of resistance in bean to *Botrytis cinerea* by a strain of *Pseudomonas aeruginosa*.

See also systematic resistance section

Cell signalling
Signal transduction

Avr/R proteins recognition & signaling

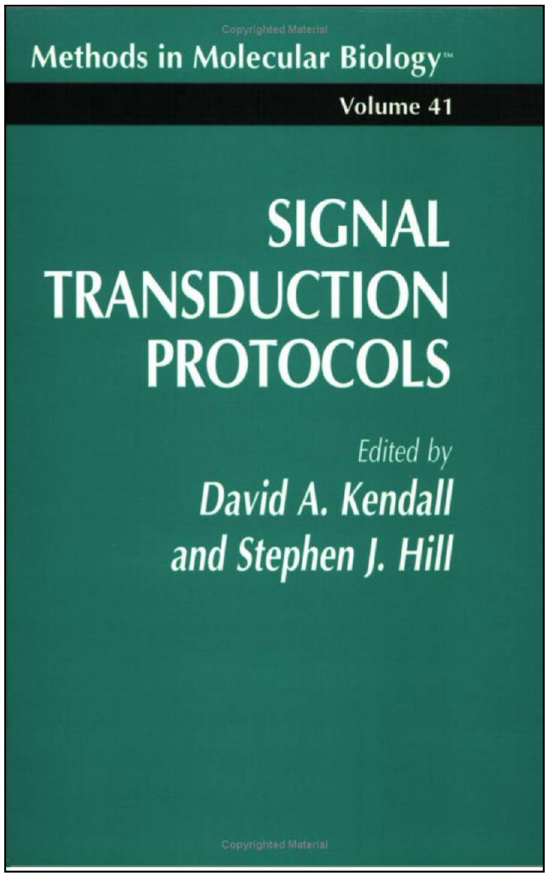
Science Signaling
Online Issues

- Science Signaling is a weekly journal, publishing 51 issues a year, as well as an online resource and information management tool that enables experts and novices in cell signaling to find, organize, and utilize information relevant to processes of cellular regulation.
- Science Signaling should be useful to:

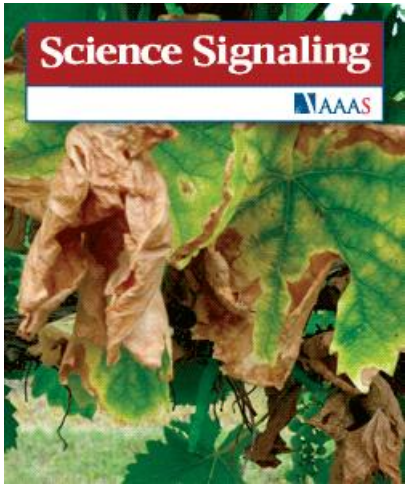
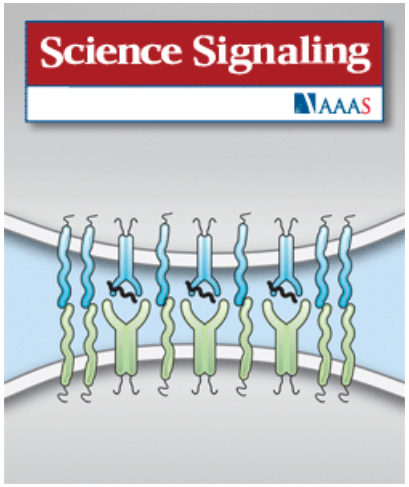
1. Scientists who specialize in signal transduction, as well as
2. The many scientists who need to follow and apply the current findings of this field even though their primary interest may not be the signal transduction mechanisms themselves.

Signal Transduction Protocols

- **Signal Transduction Protocols**
- Kendall and Hill (eds).
- 1995
- Humana Press Inc, Totowa, NJ.
- 299 pp.



Science Signaling
Online Issues

	
Interactions between *Xylella fastidiosa* and their hosts affect signaling pathways in the host.	Interactions between various molecules on the surfaces of antigen-presenting cells (APCs) and T lymphocytes cause T cell receptor (TCR) triggering.

A **T cell** is a type of **lymphocyte** which develops in the thymus gland and plays a central role in the immune response. **T cells** can be distinguished from other **lymphocytes** by the presence of a **T-cell** receptor on the **cell** surface.

Signal transduction
Definition

- In biology, signal transduction is a mechanism that converts a mechanical/chemical stimulus to a cell into a specific cellular response.
- Signal transduction starts with a signal to a receptor, and ends with a change in cell function.
- Since signal transduction mechanisms are the natural control circuits that regulate biological systems, they provide potent targets for:

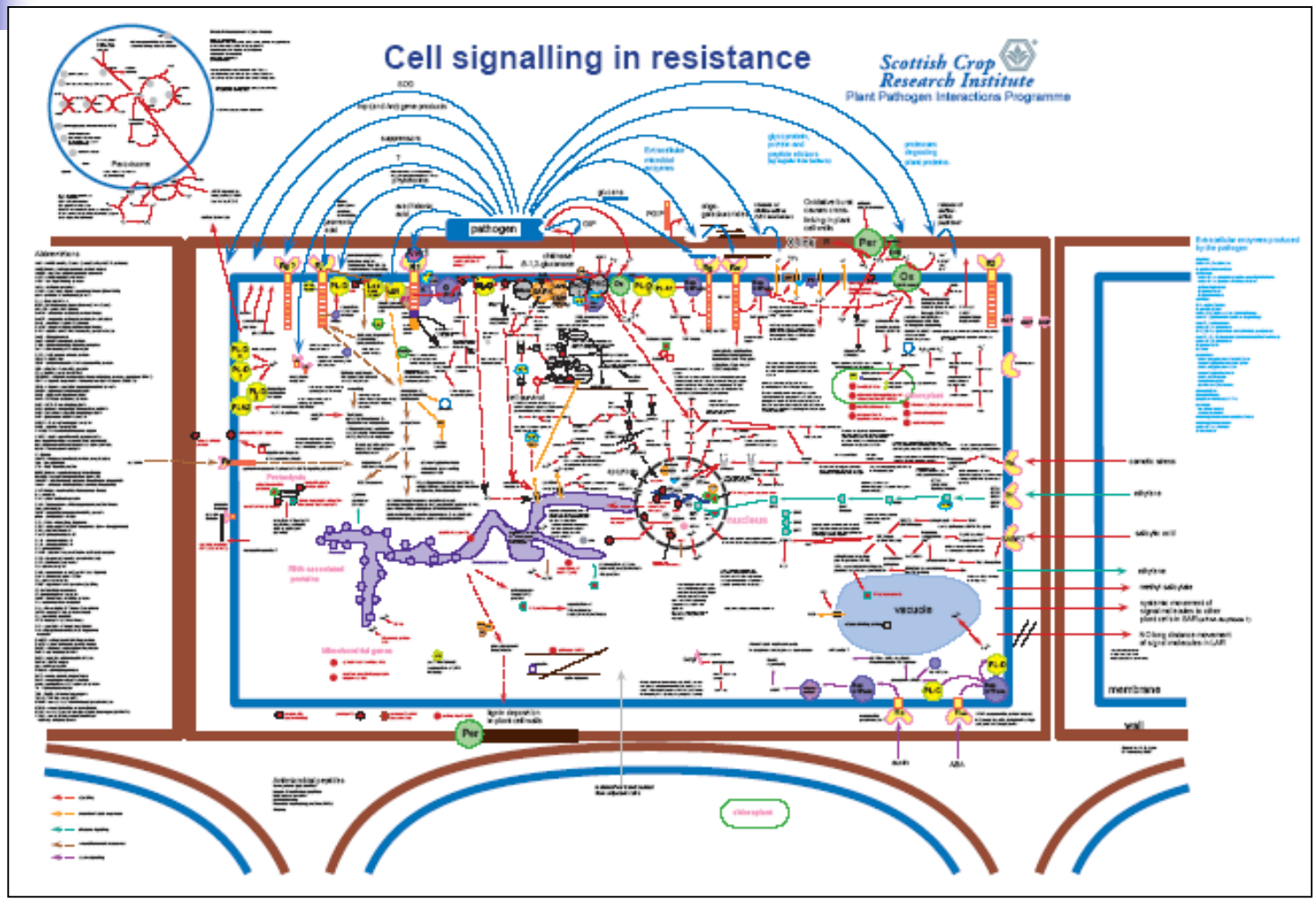
1. development of therapeutic agents to combat disease or otherwise
2. alter the behavior of biological systems.

Cell signaling
In bacteria

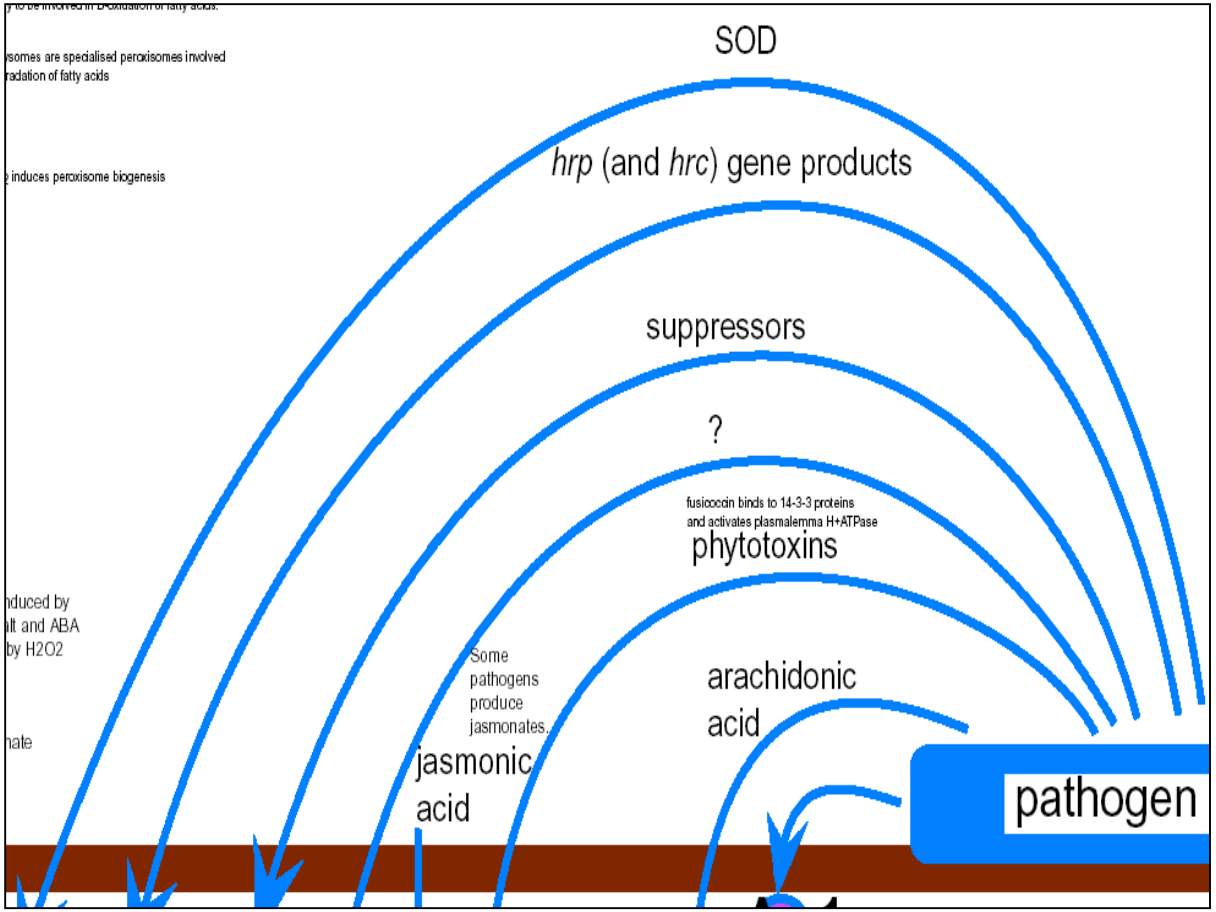
- In general, signal transduction systems in bacteria are very simple, consisting of only one or two proteins that regulate expression of various genes in response to changes in the environment.
- The navigation system, however, is significantly more complex and in some bacteria may involve dozens of different proteins.

Penland,2010

Cell signalling in resistance

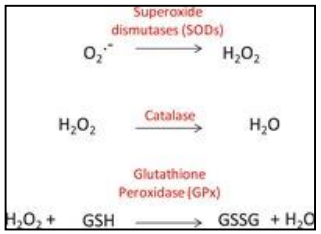


Cell signalling in resistance



The elicitor, superoxide dismutase (SOD).
Arachidonic acid (AA) is a fatty acid of the omega-6 class involved in cellular signaling.

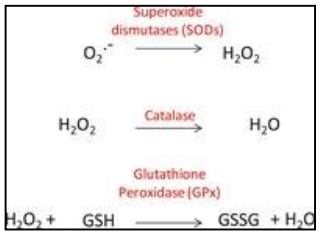
Assay of superoxide dismutase (SOD) activity



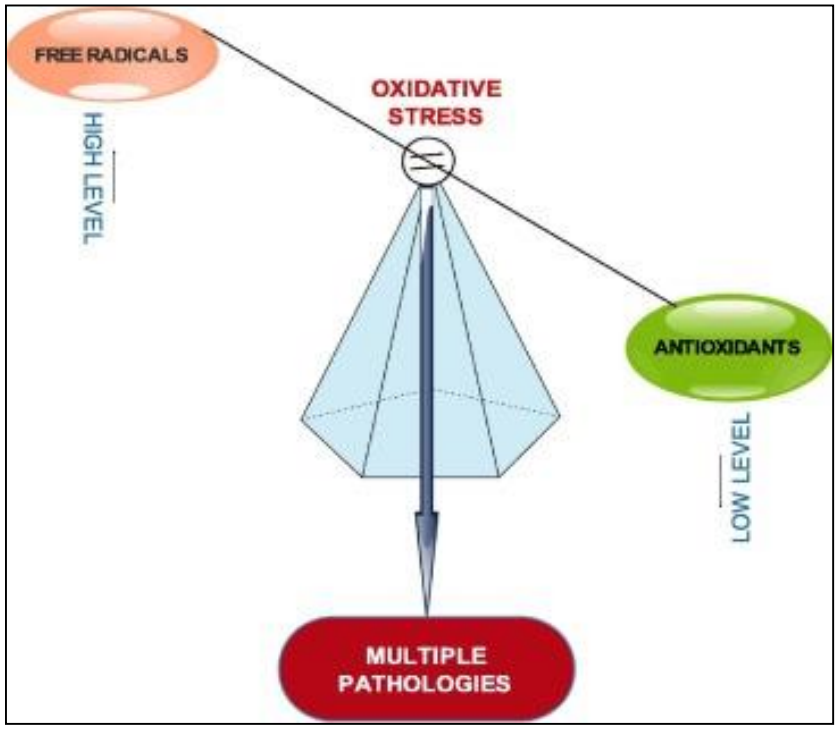
- These are a collection of antioxidants that act to suppress or prevent the formation of free radicals or reactive species in cells.
- They are very fast in neutralizing any molecule with the potential of developing into a free radical or any free radical with the ability to induce the production of other radicals.
- Three key enzymes:
- Superoxide dismutase, catalase and glutathione peroxidase are top on the list.
- These enzymes respectively dismutate superoxide radical, breakdown hydrogen peroxides and hydroperoxides to harmless molecules (H_2O_2/alcohol and O_2).

Ighodaroab and Akinloye,2018

Assay of superoxide dismutase (SOD) activity



- Reactive oxygen species such as superoxide (O2.-), peroxides (ROOR), singlet oxygen, peroxynitrite (ONOO-), and hydroxyl radical (OH.) are generated by cellular processes such as respiration.



John *et al.*,2007; Ighodaroab and Akinloye,2018

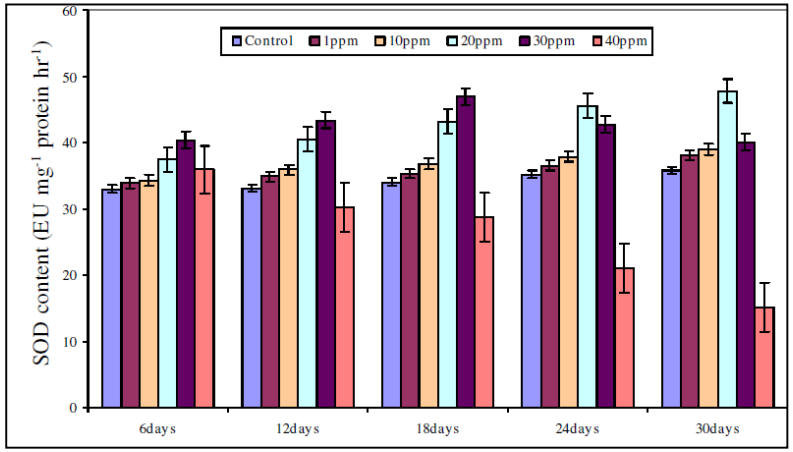
Assay of superoxide dismutase (SOD) activity

- SOD activity was measured by the method described by Dhindsa *et al.*, 1981.
- 0.2 g of fresh leaf material was homogenized in 2.0 ml of extraction buffer with the help of precooled mortar and pestle.
- The homogenate was centrifuged at 15,000 rpm at 4°C and supernatant was stored at 4°C.
- SOD activity in the supernatant was assayed by its ability to inhibit photochemical reduction of nitroblue tetrazolium.
- The test tubes containing assay mixture (1.5 ml reaction buffer, 0.2 ml of methionine, 0.1 ml enzyme extract with equal amount of NaCO, NBT solution, riboflavin, EDTA and 1.0 ml DDW) were incubated in light under 15 W inflorescent lamps for 15 min, illuminated and nonilluminated reactions without supernatant served as calibration standard.
- Absorbance of samples along with the blank was read at 560 nm wavelength.
- One unit of enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes (supernatant).

John *et al.*,2007

Assay of superoxide dismutase (SOD) activity

- Superoxide dismutase: SOD activity increased in all treatments except at the highest concentration of $CdSO_4.7H_2O$ (40 ppm), the activity declined even after 6 days.
- Mean enzymatic activity of SOD rose significantly ($p<0.05$) by 23% in 20 ppm treatment after four weeks.
- 30 ppm Cd treatment led to the increased enzymatic activity but after 3 weeks there was a decline of about 12% of mean activity, as compared to the control.



Effect of different Cd concentrations on SOD activity with time CD at $p>0.05$ between different cadmium sulfate(Cd) concentrations is 0.63. CD at $p<0.05$ between different time intervals (weeks) is 0.58.

John *et al.*,2007

The signal transduction of in both compatible and incompatible interactions
Qualitatively similar, quantitatively different

- Recent studies have shown that susceptible plants also mount active defense responses against virulent pathogens, as shown by the induction of pathogenesis-related (*PR*) genes in the compatible interaction at a later time after infection (Reuber *et al.*,1998).
- That plant responses in compatible and incompatible interactions are qualitatively similar although quantitatively different, suggesting that signal transduction mechanisms involved in compatible and incompatible interactions are largely shared (Tao *et al.*,2003).

Course of signal transduction

PAMPs/PRRs interactions

General elicitors derived from structural components of the bacterial cell

- The bacterial cell wall is an important source of PAMPs.
- Some bacterial PAMP signals:
- Peptidoglycans(PGNs): found in both gram-positive and gram negative bacteria.
- Lipopolysaccharide (LPS): is the principal component of the outer membrane of gram-negative bacteria.
- Flagella(Flagellin) from nearly all bacteria.
- The elongation factor Tu (EF-Tu): a molecular switch in protein biosynthesis. Elongation factors are part of the mechanism that synthesizes new proteins by translation at the ribosome.

Pattern recognition receptors (PRRs)
Pathogen-associated molecular pattern(PAMP)

Course of signal transduction
Avr/R interactions

- Specific recognition of the pathogen by the plant requires the presence of matching *avr* and *R* genes in the two species.
- As long as one pair of *avr* /*R* genes matches in pathogen and host, an HR results, while in the absence of this matching, disease is the outcome.
- Plant *R* genes encode proteins that both determine:

1. Recognition of specific Avr proteins
2. Initiate signal transduction pathways leading to complex defense responses.

Course of signal transduction

Secretion systems

TTS systems

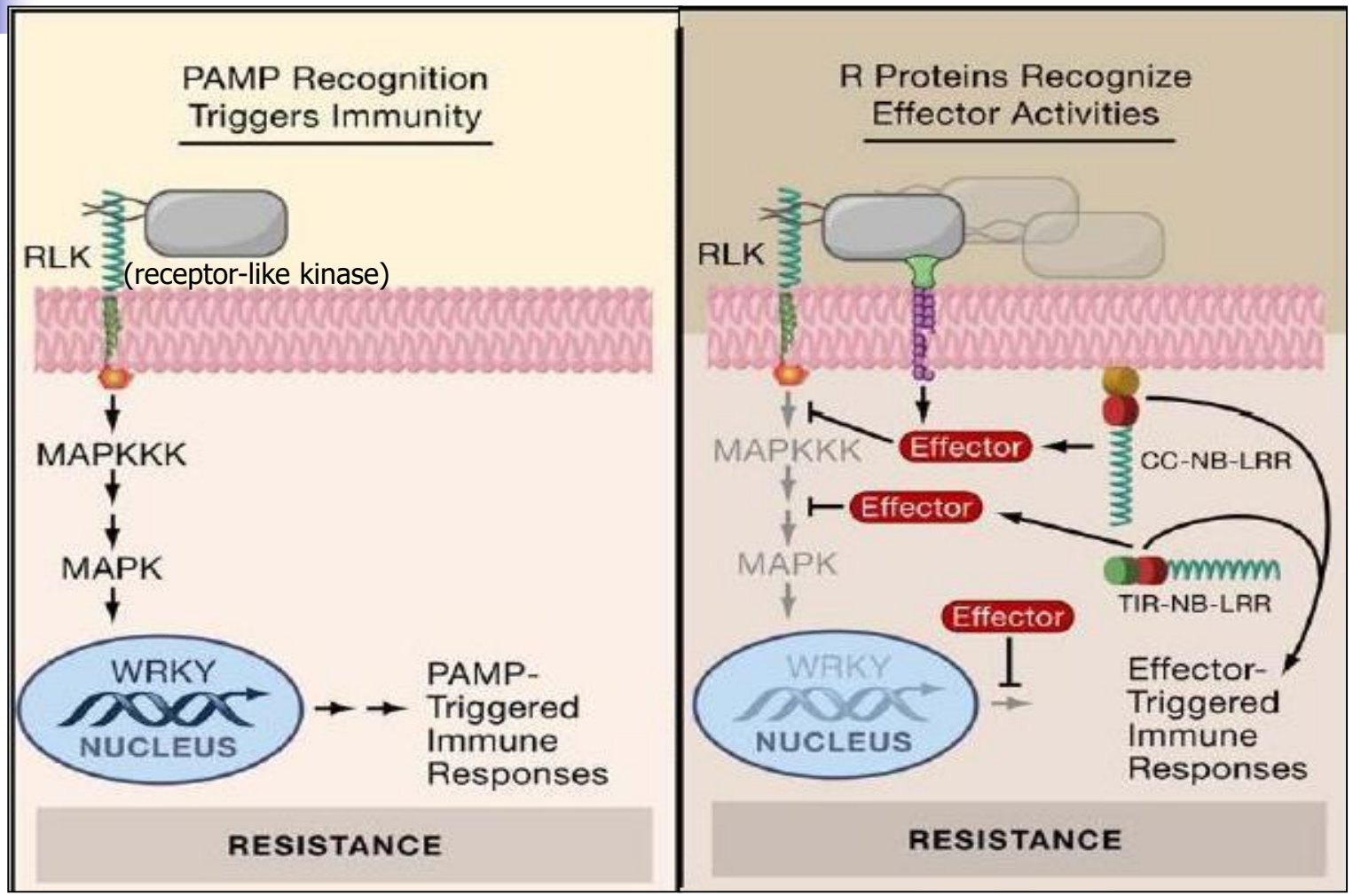
- TTSS effectors interact with host proteins both inside and outside the host cell to modulate the host response.
- This allows bacteria to precisely modulate host tissues and systems for the benefit of the pathogen.
- Some of these manipulations on host cell biological systems are:

1. Cytoskeletal structure,
2. Signal transduction,
3. Cell cycle progression,
4. Programmed cell death.

Course of signal transduction

PAMPs/PRRs vs. Avr/R interactions

Major components of the signal transduction



Course of signal transduction

R genes/R proteins

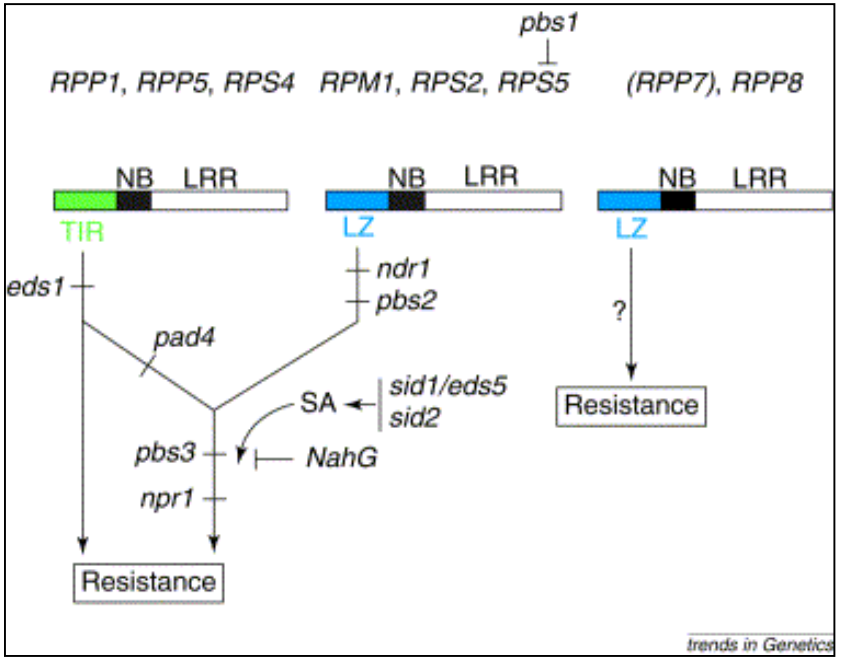
LRR domain and LZ and TIR domains

- The LRR domain is an obvious candidate for receptor function.
- LRRs can mediate protein-protein interactions.
- Also recognition specificity resides largely in the LRRs.
- Although the LZ and TIR domains could also have receptor role, it is more likely that these are involved in downstream signalling.
- Proteins containing LRR domains mediate interactions with other proteins playing a role in, for instance

1. signal transduction cascades, or
2. acting as receptors for peptide hormones.

R gene-mediated signaling pathways in *Arabidopsis*

- Genetic analysis of NB-LRR type *R* genes in *Arabidopsis* has shown that R proteins with an amino-terminal TIR domain predominantly signal through *EDS1*, whereas LZ-containing R proteins require *NDR1* and *PBS2* to initiate defense responses.



EDS=Enhanced disease susceptibility. The Arabidopsis *EDS1* and *PAD4* genes encode lipase-like proteins that function in resistance (R) gene-mediated and basal plant disease resistance. EDS1 is essential for elaboration of the plant hypersensitive response, whereas EDS1 and PAD4 are both required for accumulation of the plant defence-potentiating molecule, salicylic acid.

Course of signal transduction

The secondary signals

- Upon infection by pathogens, plants often exhibit increased production of:

1. Reactive oxygen species (ROS),
2. Salicylic acid (SA),
3. Ethylene, and
4. Jasmonates.

- These molecules can serve as secondary signals to activate plant defense.
- Recent results also indicate that nitric oxide is an important secondary signal during plant defense.
- Many of these secondary signals are well-known inducers for PR gene expression.
- For example, SA induces PR genes (encoding mostly acidic PR proteins) that are normally activated during SAR.

Course of signal transduction
Different pathways

- The plant defense responses triggered by many different *R* genes and via following pathways:

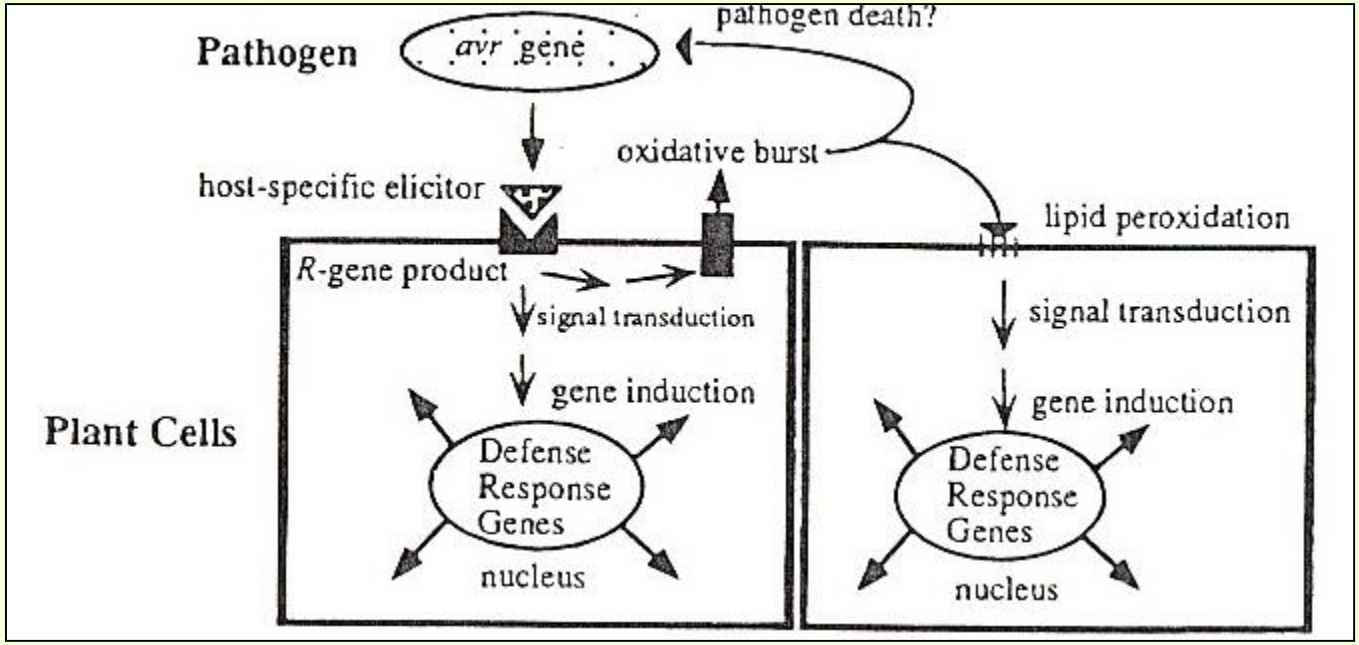
1. MAPK signaling cascades
2. The SA-dependent pathway
3. Cross-talk between SA-pathway and other pathways

Crosstalk or molecular dialogue can have different meanings. However, most biologists studying crosstalk would include a network of signal interactions in which functional outcomes can be positive, negative, or neutral.

Signalling pathways
A general scheme

- Binding of elicitor to the receptor activates a signal transduction pathway, which in turn activates two distinct responses:

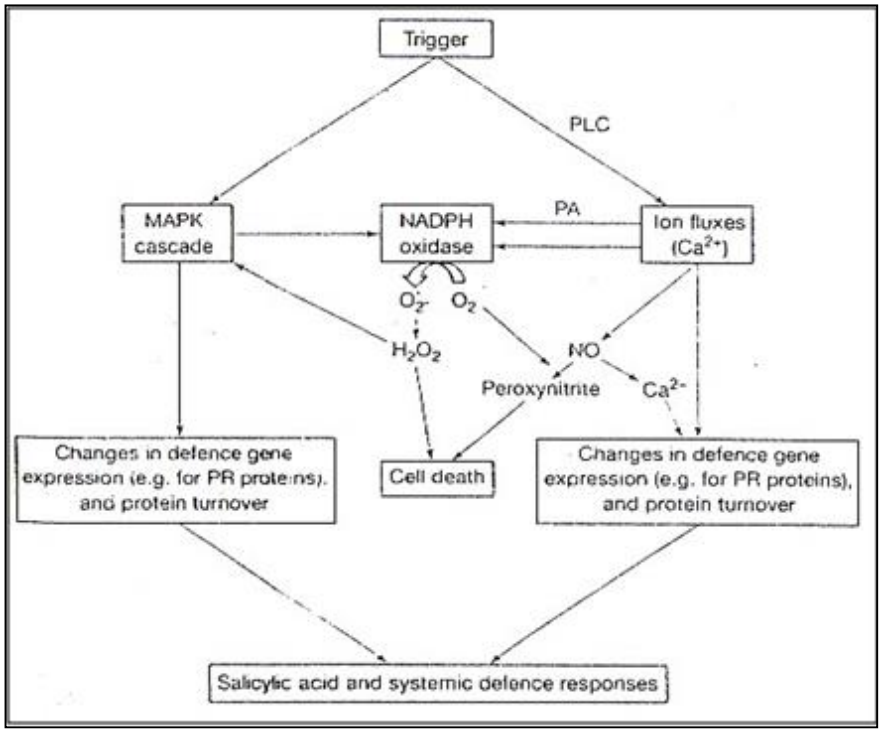
1. Production of an oxidative burst;
2. Induction of many different defense-response genes.



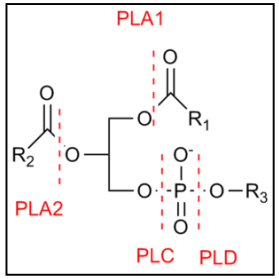
Signalling pathways

A general scheme indicating some of signalling pathways that are known to be triggered through resistance gene recognition of the pathogen elicitors

- MAPK cascade
- PLC= phospholipase C (a class of membrane-associated enzymes that cleave phospholipids just before the phosphate group);
- PA= phosphatidic acid(a part of common phospholipids, major constituents of cell membranes);
- NO= Nitric oxide (one of several oxides of nitrogen).
- The NADPH oxidase complex is a cluster of proteins that donate an electron from NADPH to molecular oxygen (O2) to produce superoxide ($O2^-$). NADPH is a reducing agent used as lipid synthesis.
- Peroxynitrite an unstable structural isomer of nitrate.



Cleavage sites of phospholipases.
Phospholipase C enzymes cut just before the phosphate attached to the R3 moiety

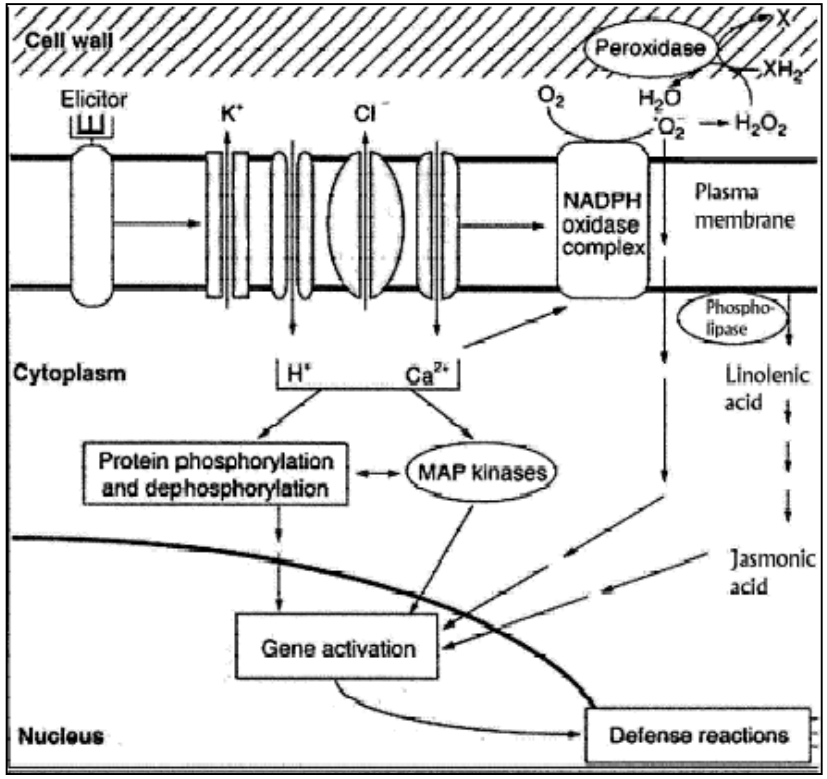


Signal pathways

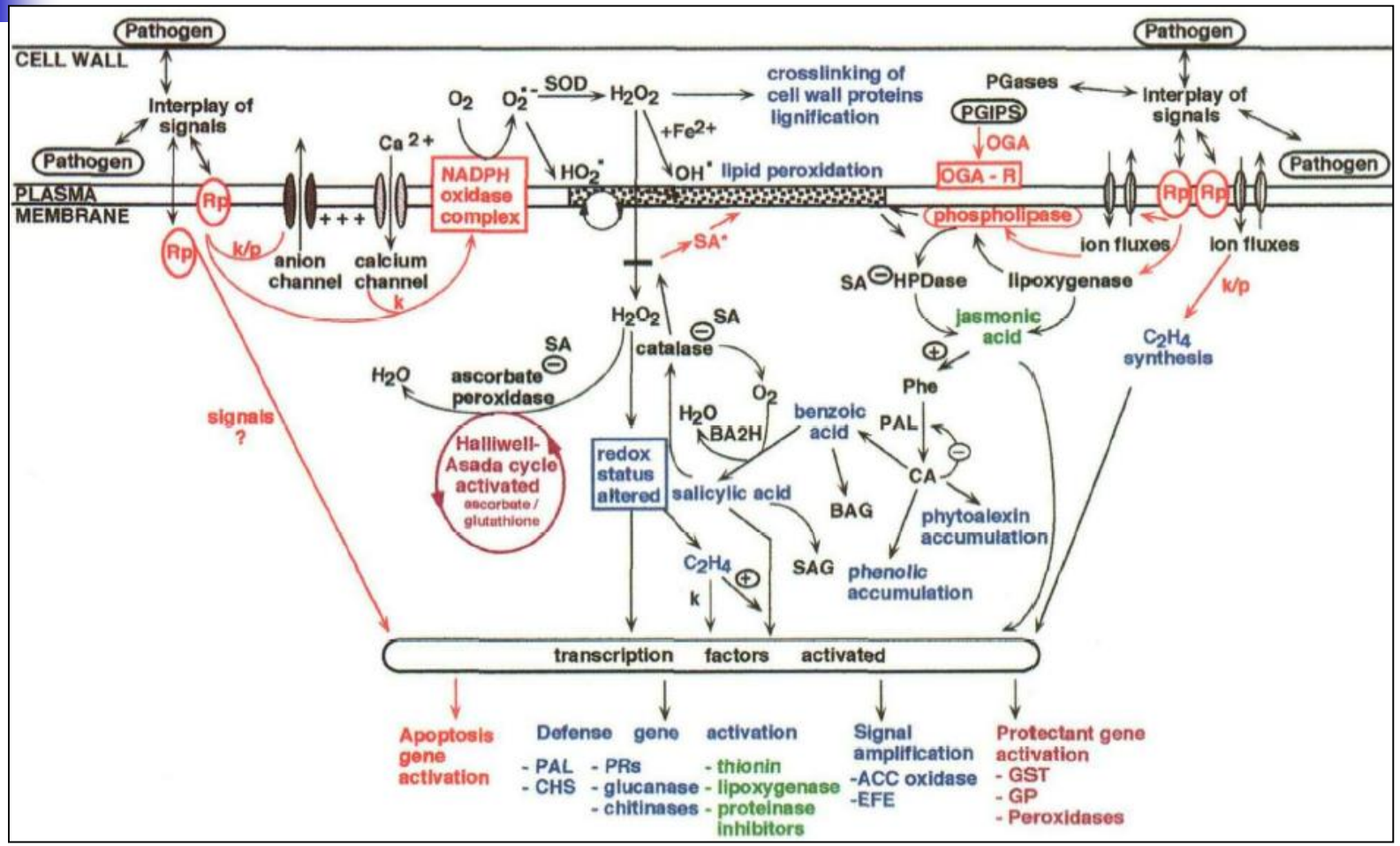
A general scheme

A mitogen is a chemical substance that encourages a cell to commence cell division, triggering mitosis. A MAPK cascade in Arabidopsis innate immunity

- Recognition of the elicitor by its plasma membrane receptor stimulates transient influxes of H^+ and Ca^+, and effluxes of K^+ and Cl^-.
- These ion fluxes are prerequisite for the activation of specific MAP (mitogen-activated protein) kinases and for the generation of reactive oxygen intermediates (the oxidative burst).
- Phosphorylation and dephosphorylation of some proteins is also observed.
- Binding of the elicitor stimulates also the generation of jasmonic acid via a membrane associated phospholipase.

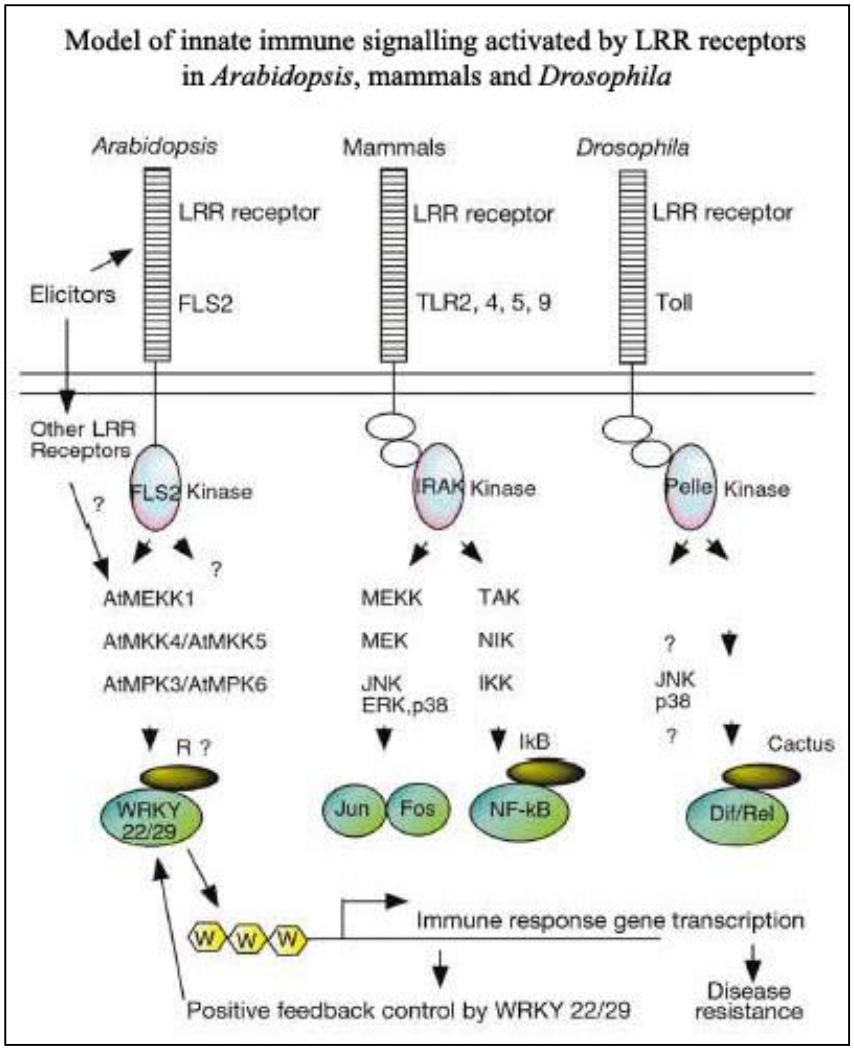


Signal pathways
A general scheme



Hammond-Kosack and Jones,1996

Signalling pathways
Activated by LRR receptor

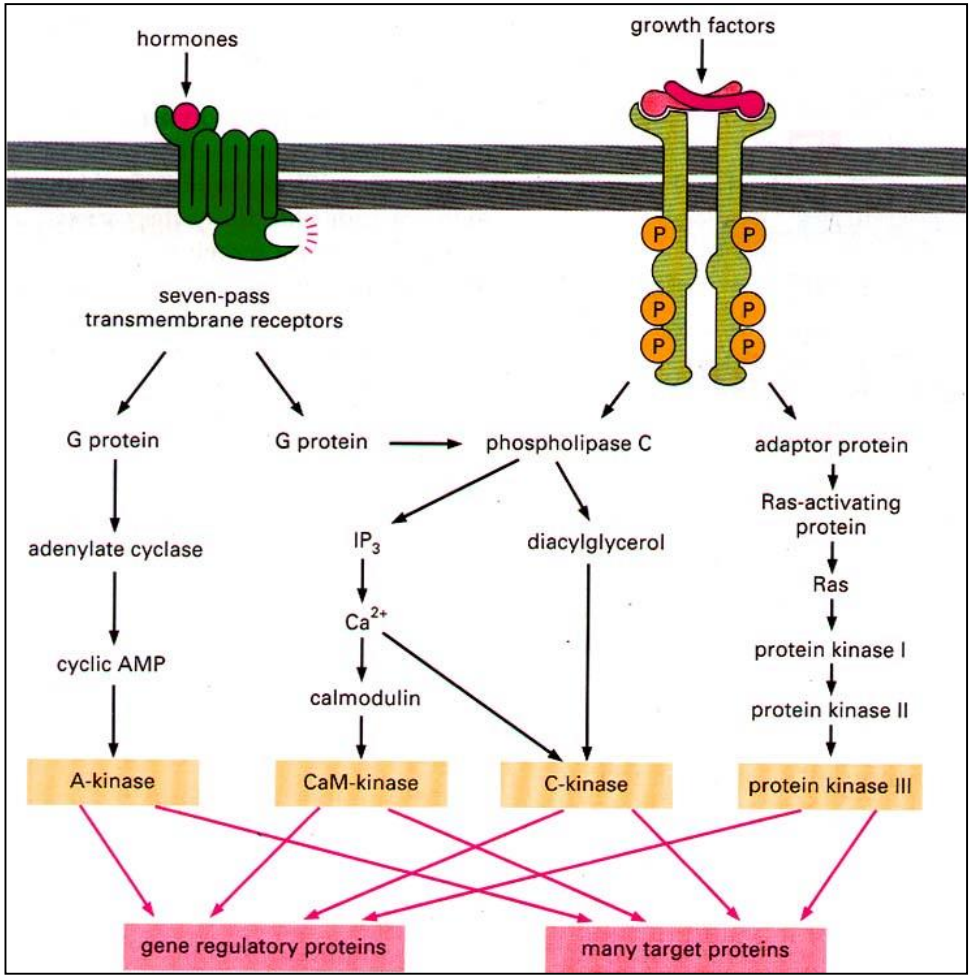


Crosstalk between SA-pathway and other pathways

- The plant defense network appears to be more complicated than originally envisioned.
- The known components which are implicated in regulating defense response and /or crosstalking with SA-dependent defense pathway:
- SA,
- H_2O_2, and
- Plant hormonal molecules, such as nitride oxide (NO), JA, ET, abscisic acid (ABA) and brassinosteroid (BR).

Crosstalk

Signals can be passed back and forth between different pathways



Calmodulin(CaM) is a calcium-binding protein expressed in all eukaryotic cells.

Brassinosteroid (BR)

The steroid hormone

The BRI1 signaling pathway

- Brassinosteroids (lipophilic hormones) are a new class of plant hormones with a polyoxygenated steroid structure showing pronounced plant growth regulatory activity.
- (A) BRI1 is the receptor for brassinosteroids (BRs), which are crucial growth-promoting hormones in plants.
- Brassinosteroid (BR) perception by BRI1 triggers its autophosphorylation (P) and phosphorylation-dependent release of BKI1 and BSK proteins from the receptor, allowing basal signal transduction by BSKs to downstream factors and association of BRI1 with BAK1.

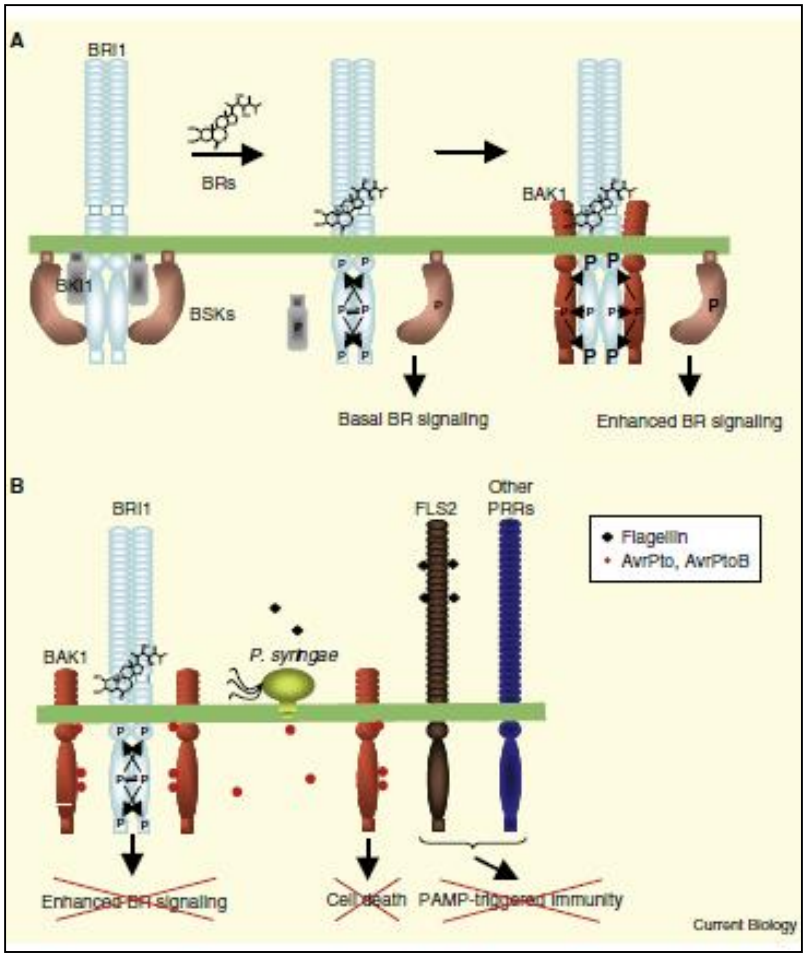
Vert,2008

Brassinosteroid (BR)

The steroid hormone

The BRI1 signaling pathway

- Transphosphorylation(A reaction involving transfer of a phosphoric group from one compound to another) of BAK1 leads, in turn, to full activation of BRI1 by further BRI1 phosphorylation and promotes enhanced BR responses.
- (B) *P. syringae* injects into the cell the AvrPto and AvrPtoB effectors, which bind to co-receptor BAK1 and prevent its flagellin-dependent association with FLS2 and other pattern recognition receptors (PRRs), leading to PAMP-triggered immunity (PTI) suppression.



Vert,2008

Signal Transduction
Cell signaling

Universal signal transduction mechanisms

Cell signaling
Two basic characteristics

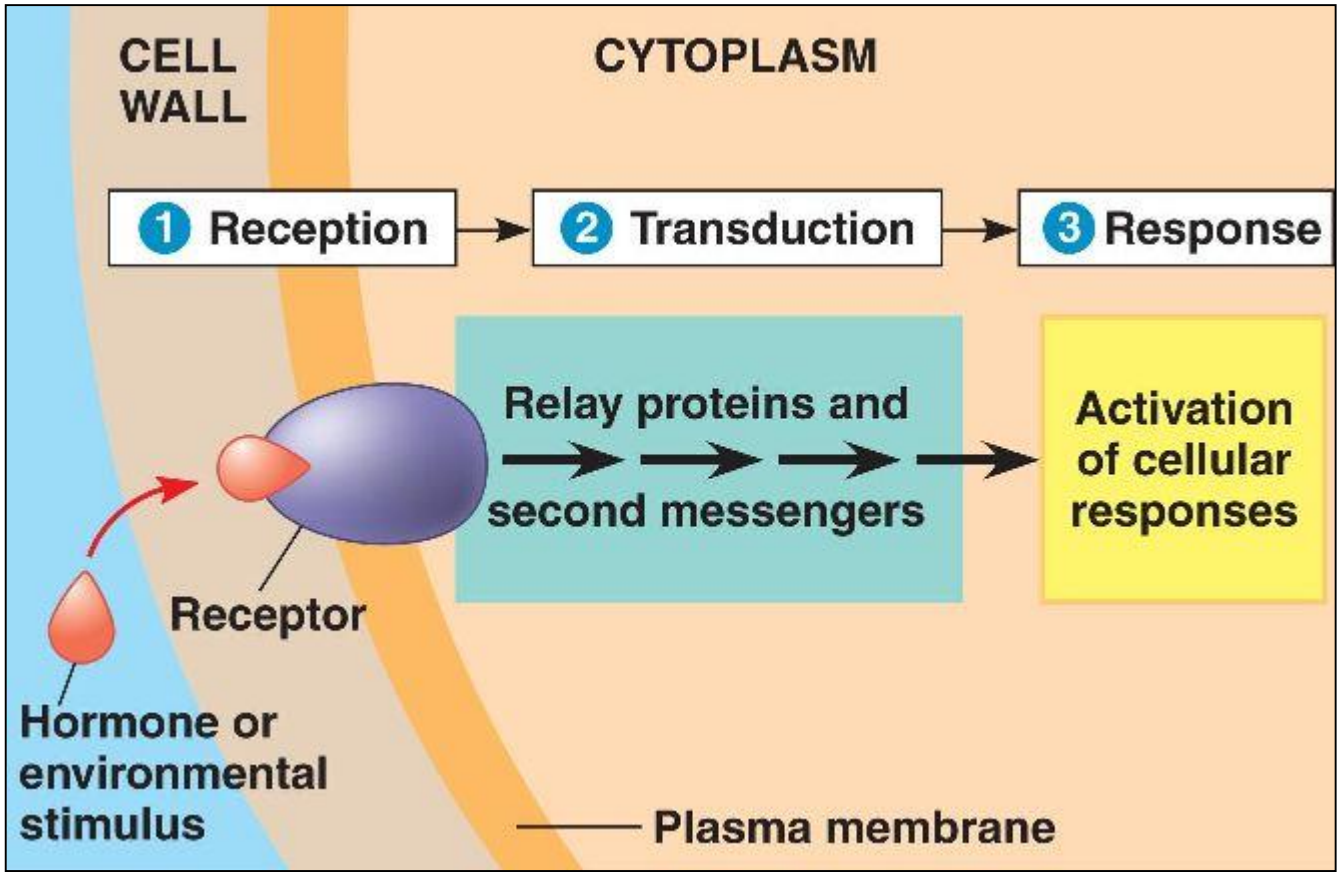
- Signal transduction (also known as cell signaling) is the transmission of molecular signals from a cell's exterior to its interior.
- Thus basic characteristics of cell signaling:

1. Cell must respond appropriately to external stimuli to survive.
2. Cells respond to stimuli via cell signaling.

Signals received by cells must be transmitted effectively into the cell to ensure an appropriate response. MAPKs are important signal transducing enzymes. Mitogen-activated protein kinases (MAPKs) comprise a family of serine/threonine kinases act on both serine and threonine and tyrosine-protein kinases act on tyrosine.

Cell signaling

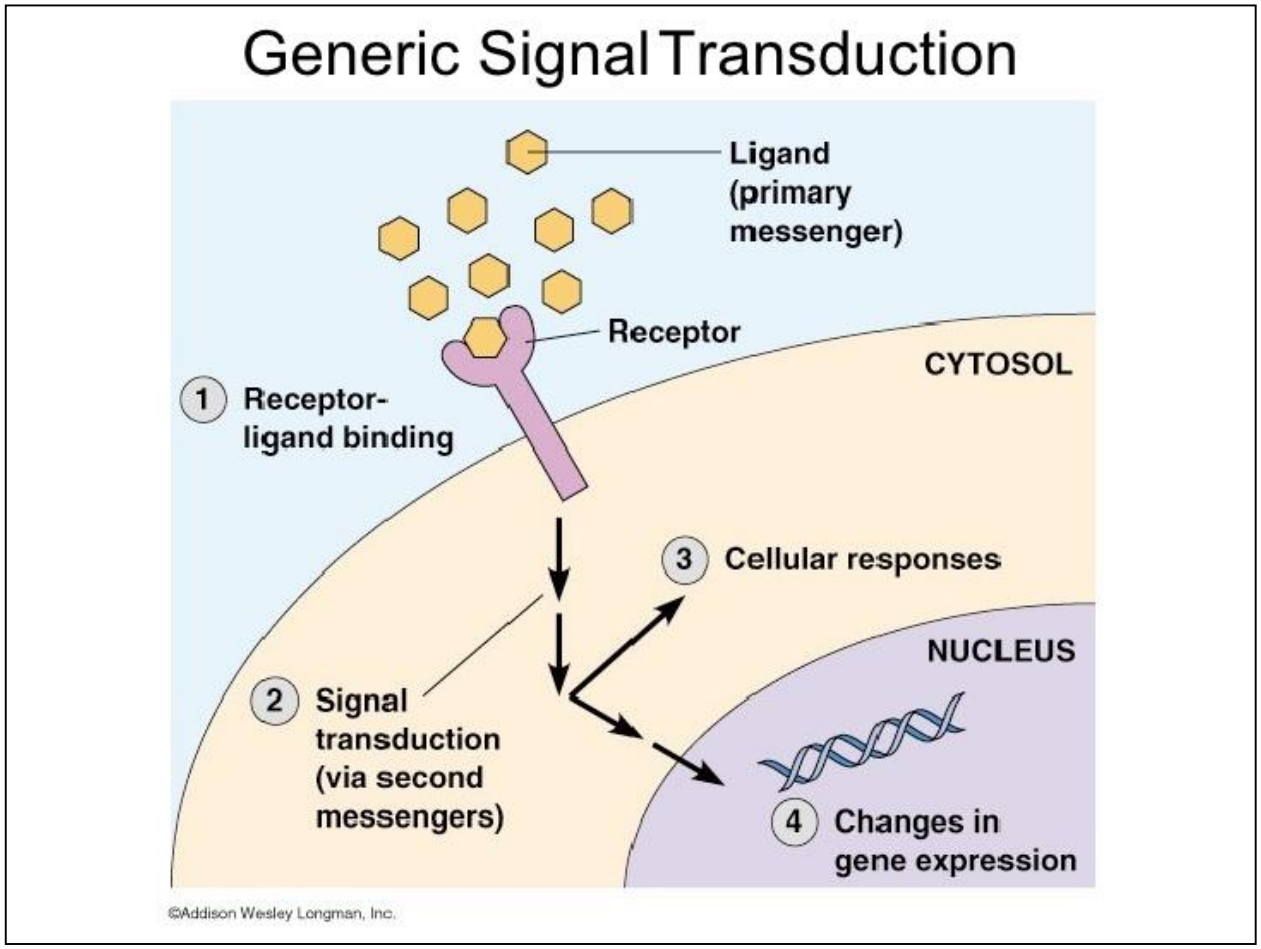
Three stages of signal transduction



Cell signaling

Three stages of signal transduction

Ligands are signals that bind cell surface receptors



Cell signaling
Protein kinases

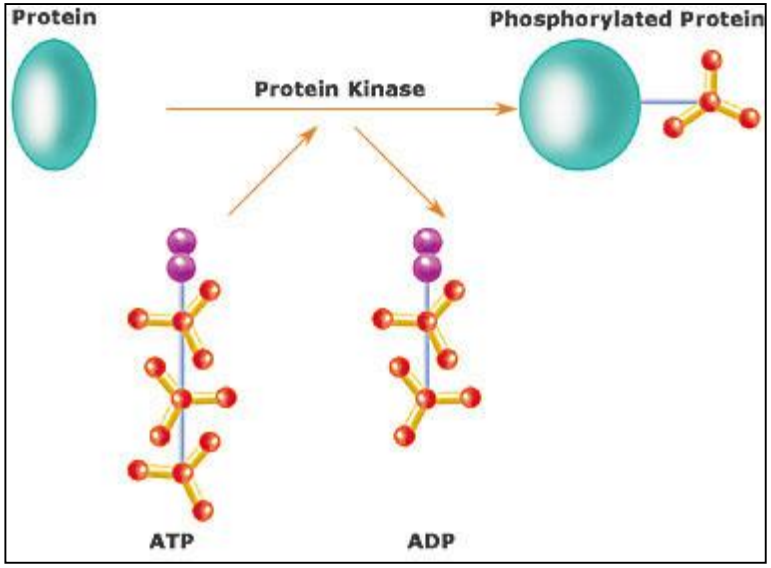
- MAPKs are important signal transducing enzymes that are involved in transmitting signals from a wide variety of extracellular stimuli including those of growth factors, hormones, cytokines, and neurotransmitter.
- A kinase is a type of intracellular protein, an enzyme that activates other proteins by giving them a high energy phosphate group.
- These MAPKs are essentially operated through three-tiered consecutive phosphorylation events catalyzed by a MAPK kinase kinase(MAPKK), a MAPK kinase(MAPKK), and a MAPK.
- MAPKs lie in protein kinase cascades.

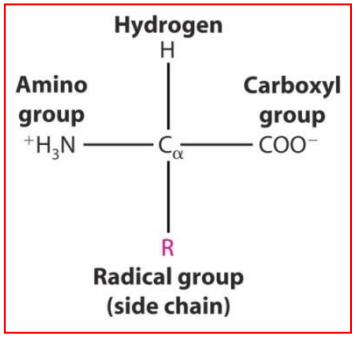
Cell signaling

Protein phosphorylation

Protein kinases are found in bacteria and plants

- Protein Kinases are enzymes that modify the function of other proteins by attaching phosphate groups to them (phosphorylation).
- The transfer of a phosphate group (usually ATP) to one or more amino acid residues in a protein substrate side chain.
- These changes can be reversed by a separate class of enzymes called phosphatases, that remove the phosphate groups.





The key elements of an amino acid

Cell signaling
Protein kinases

- In a kinase cascade,

1. one kinase can phosphorylate multiple kinases in the next step of the signaling pathway, and
2. each of them can phosphorylate many more kinases leading to the original signal of maybe one activated protein being turned into a huge, non-reversible chain reaction.

- Kinase cascades are used to amplify intracellular signals in many pathways, normally from a cell membrane receptor to the nucleus so a cell detects an external change and changes its gene expression in response.

Plant MAPK pathways
Function of MAP kinases

- Activation of specific MAPK proteins has been observed in plants in response to hormones or environmental stimuli such as:
- Touch, Cold, Heat, Reactive oxygen species (ROS), UV, Wounding, Drought, and pathogen attack(infection).
- MAPK pathways are involved in plant:

1. Regulation of development,
2. Growth,
3. Programmed cell death.

- The oxidative signaling pathways leading to activation of MAPK cascades and programmed cell death (PCD).

Pitzschke *et al.*,2009; Bent,2011;..

Cell signaling

Mitogen-activated protein kinase

Kinase cascades

- MAPKs are involved in:
- directing cellular responses to a diverse array of stimuli, such as mitogens, osmotic stress, heat shock and proinflammatory cytokines.
- They regulate cell functions including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis.

MAPK pathways are signalling cascades differentially regulated by growth factors, mitogens, hormones and stress which mediate cell growth, differentiation and survival.

Mitogen: an agent that can induce mitosis.

Cell signaling

Mitogen-activated protein kinases (MAPK)

Kinase cascades

- Mitogen-activated protein kinases (MAPK) are protein kinases that are specific to the amino acids serine, threonine, and tyrosine.
- MAP kinase cascades are a standard player in the signal transduction literature for diverse organisms including:

1. yeasts,
2. mammals, and
3. plants.

- Numerous studies have shown that plant MAPKs are activated by:

1. Abiotic stresses;
2. Pathogens and pathogen-derived elicitors;
3. Plant hormones.

Cell signaling
Four types of protein kinases

- Protein kinases fall into four broad classes, characterized with respect to substrate specificity/chemical activity (phosphorylation).

1. Serine/threonine-protein kinases act on both serine and threonine;
2. Tyrosine-protein kinases act on tyrosine;
3. Dual specificity protein kinases (e.g. MEK - phosphorylates both threonine (Thr) and tyrosine (Tyr).
4. Still there are also protein kinases that phosphorylate other amino acids, including histidine kinases that phosphorylate histidine residues(in bacteria).

Serine/threonine and tyrosine kinases were also detected in bacteria. Therefore, the bacteria also possess kinase cascades similar to those described in eukarya.

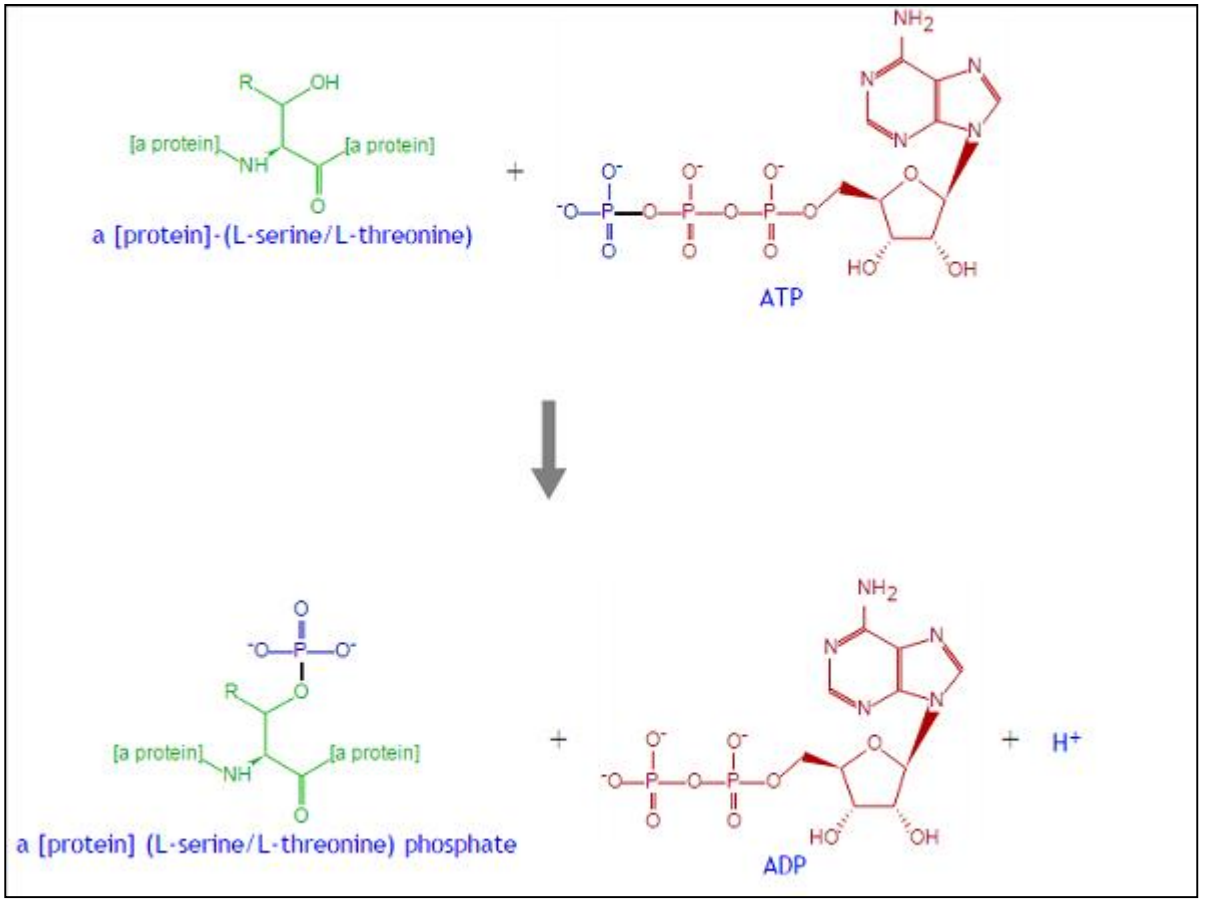
Cell signaling
Six types of protein kinases

- **Six classes of enzyme-linked receptors have thus far been identified:**

1. Receptor tyrosine kinases phosphorylate specific tyrosines on a small set of intracellular signaling proteins.
2. Tyrosine-kinase-associated receptors associate with intracellular proteins that have tyrosine kinase activity.
3. Receptor like tyrosine phosphatases remove phosphate groups from tyrosines of specific intracellular signaling proteins. (They are called "receptorlike" because the presumptive ligands have not yet been identified, and so their receptors function has not been directly demonstrated.)
4. Receptor serine/threonine kinases phosphorylate specific serines or threonines on associated latent gene regulatory proteins.
5. Receptor guanylyl cyclases directly catalyze the production of cyclic GMP in the cytosol.
6. Histidine-kinase-associated receptors activate a "two-component" signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

Cell signaling

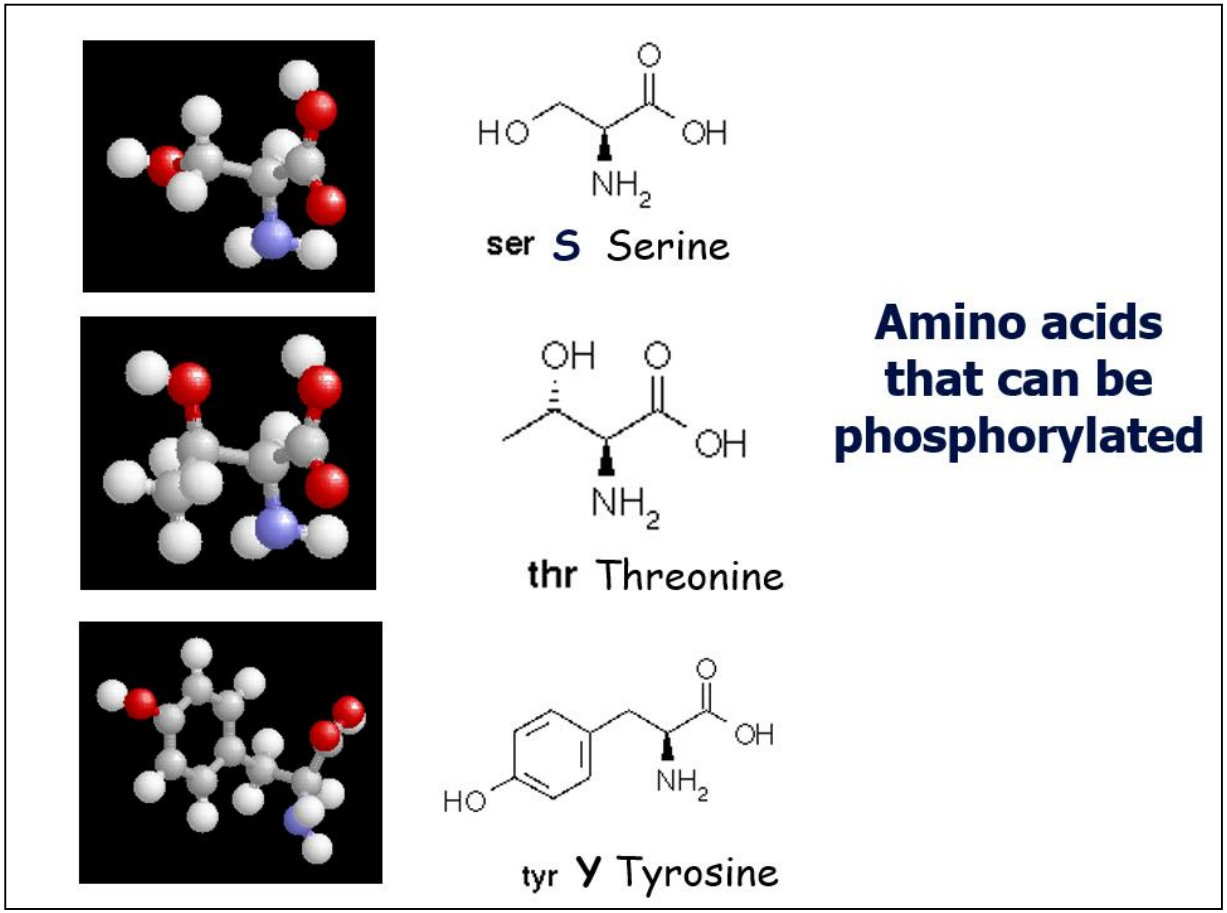
Serine/threonine-protein kinases act on both serine and threonine



SRI International,2016

Cell signaling

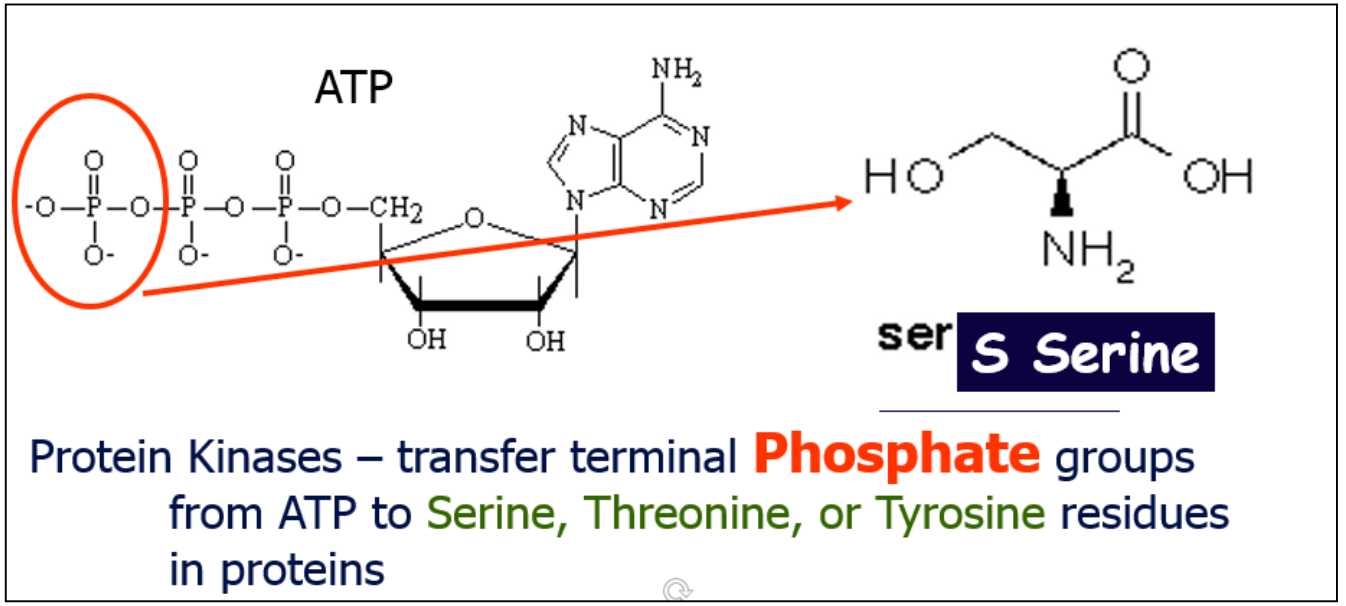
Amino acid phosphorylation is very important in intracellular signal transduction



Kopchick,2015

Cell signaling

Amino acid phosphorylation is very important in intracellular signal transduction

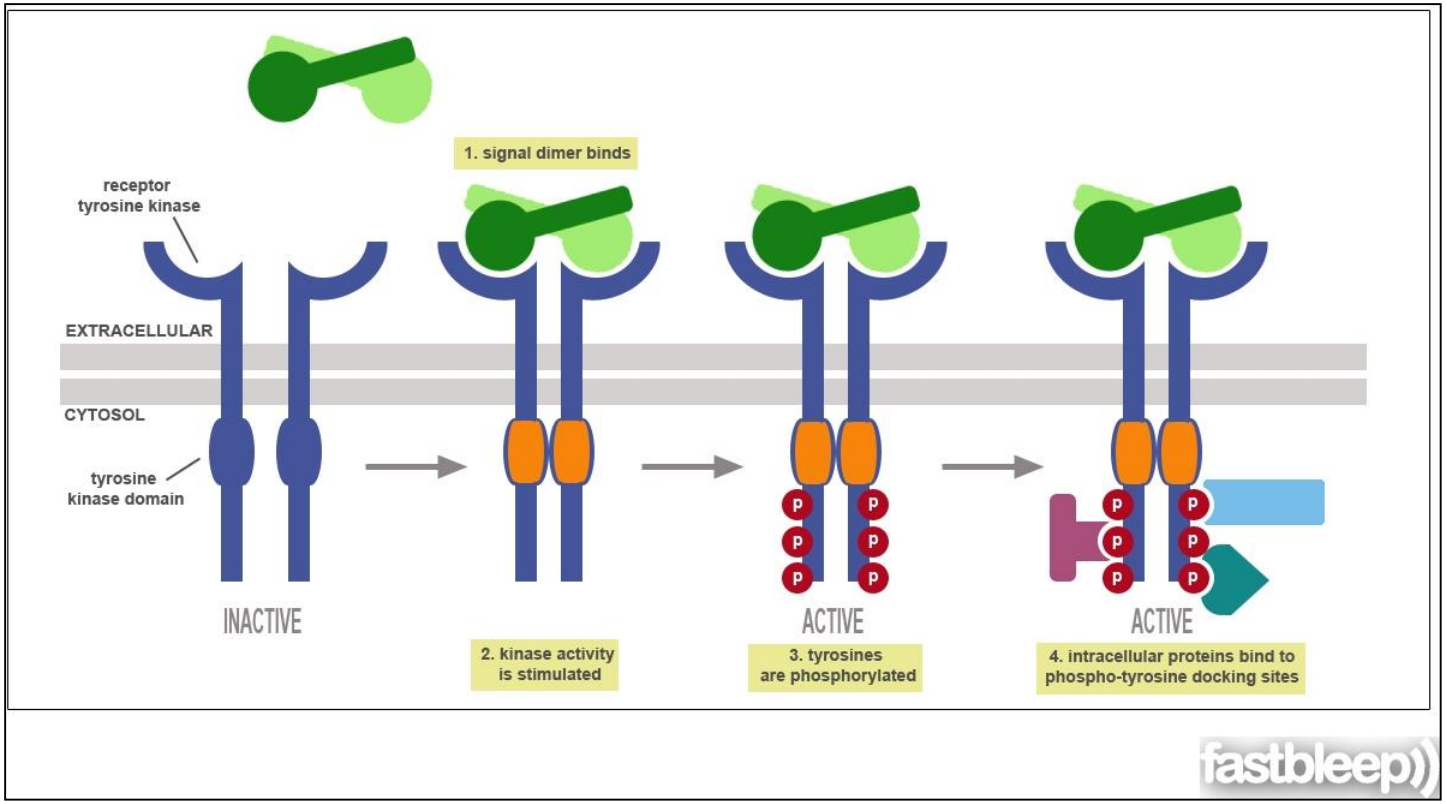


Result in activation or inactivation of the recipient protein!

Kopchick,2015

Cell signaling

Tyrosine-protein kinases act on tyrosine



Docking site= a place (or platform) for the loading or unloading of materials. The combining site of a molecular receptor.

Cell signaling
First and second messengers

- In order to respond to changes in their immediate environment, cells must be able to receive and process signals that originate outside their borders.
- Individual cells often receive many signals simultaneously, and they then integrate the information they receive into a unified action plan.
- **But cells aren't just targets.**
- **They also send out messages to other cells both near and far.**

www.nature.com

Cell signaling
First and second messengers

- As the result of binding of first signal to the receptor, other molecules or second messengers are produced within the target cell.
- Second messengers relay the signal from one location to another (such as from plasma membrane to nucleus).
- **Messenger molecules may be amino acids, peptides, proteins, fatty acids, lipids, nucleosides or nucleotides.**
- Often a cascade of changes occur within the cell which results in a change in the cell's function or identity.
- The signal transduction pathway can act to amplify the cellular response to an external signal.

Cell signaling
First and second messengers

- **First messenger:**
- An extracellular substance (as the hormone epinephrine or the neurotransmitter serotonin) that binds to a cell-surface receptor and initiates intracellular activity.
- **Second messenger:**
- An intracellular substance (as cyclic AMP) that mediates cell activity by relaying a signal from an extracellular molecule (as of a hormone or neurotransmitter) bound to the cell's surface.

Proteins responsible for detecting stimuli are generally termed receptors. cAMP is a derivative of adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms.

Cell signaling

Second messengers

Cyclic AMP (cAMP) is an important second messenger

- Cyclic adenosine monophosphate(Cyclic AMP) is a second messenger important in many biological processes.
- It was the first second messenger ever discovered.
- Activation of receptors can trigger the synthesis of small molecules called second messengers, which initiate and coordinate intracellular signaling pathways.
- cAMP is synthesized from ATP by the enzyme adenylyl cyclase, which resides in the cell membrane.
- The activation of adenylyl cyclase can result in the manufacture of hundreds or even thousands of cAMP molecules.

Cell signaling

The second messengers

cyclic AMP (cAMP)

- These cAMP molecules activate the enzyme protein kinase A (PKA), which then phosphorylates multiple protein substrates by attaching phosphate groups to them.
- Each step in the cascade further amplifies the initial signal, and the phosphorylation reactions mediate both short- and long-term responses in the cell
- How does cAMP stop signaling?
- It is degraded by the enzyme phosphodiesterase.

Cell signaling
First and second messengers

1. Most cell signals are chemical in nature.
2. Some are mechanical stimuli.

- For example, prokaryotic organisms have sensors that detect nutrients and help them navigate toward food sources.
- In multicellular organisms, growth factors, hormones, neurotransmitters, and extracellular matrix components are some of the many types of chemical signals cells use.
- These substances can exert their effects locally, or they might travel over long distances.

www.nature.com

Cell signaling

First and second messengers

In mammals

- For instance, neurotransmitters are a class of short-range signaling molecules that travel across the tiny spaces between adjacent neurons or between neurons and muscle cells.
- Other signaling molecules must move much farther to reach their targets.
- One example is follicle-stimulating hormone, which travels from the mammalian brain to the ovary, where it triggers egg release.

www.nature.com

Cell signaling

First and second messengers

In humans

1. Most cell signals are chemical in nature.
2. Some are mechanical stimuli.

- Some cells also respond to mechanical stimuli.
- For example, sensory cells in the skin respond to the pressure of touch, whereas similar cells in the ear react to the movement of sound waves.
- In addition, specialized cells in the human vascular system detect changes in blood pressure — information that the body uses to maintain a consistent cardiac load.

www.nature.com

Cell signaling

Internal signaling pathways

Three major classes of membrane receptors

- Receptors are generally transmembrane proteins, which bind to signaling molecules outside the cell and subsequently transmit the signal through a sequence of molecular switches to internal signaling pathways.
- Also amplifies the signals via signal transduction.
- Membrane receptors fall into three major classes:

1. G-protein-coupled receptors,
2. ion channel receptors, and
3. enzyme-linked receptors.

G Proteins are guanine-nucleotide binding proteins. Large numbers of G proteins provide diversity for signal transduction events. Some bind potassium or calcium ion channels in neurotransmitters. Some activate kinases (enzymes that phosphorylate).

Cell signaling

Three major classes of membrane receptors

1. Ion channels

- Ion channels: Their role in signal generation is mainly centred on the Ca^{2+} signaling pathway, which has a large number of Ca^{2+} entry channels and internal Ca^{2+} release channels, both of which contribute to the generation of Ca^{2+} signals.
- There are a large number of K^{+} channels and many of these function in different aspects of cell signaling.

Saludar,2011

Cell signaling

Three major classes of membrane receptors

2. G-protein-coupled receptors

- G-protein-coupled receptors: G-protein-coupled receptors (GPCRs) constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals.
- Coupling with G-proteins, they are called seven-transmembrane receptors because they pass through the cell membrane seven times.
- The alpha subunit of the G-protein being the primary signaling molecule.
- The general function of the G alpha-s subunit (Gs) is to activate adenylate cyclase which in turn produces cyclic-AMP (cAMP), leading to the activation of cAMP-dependent protein kinases (often referred to collectively as Protein Kinase A).

Cell signaling

Three major classes of membrane receptors

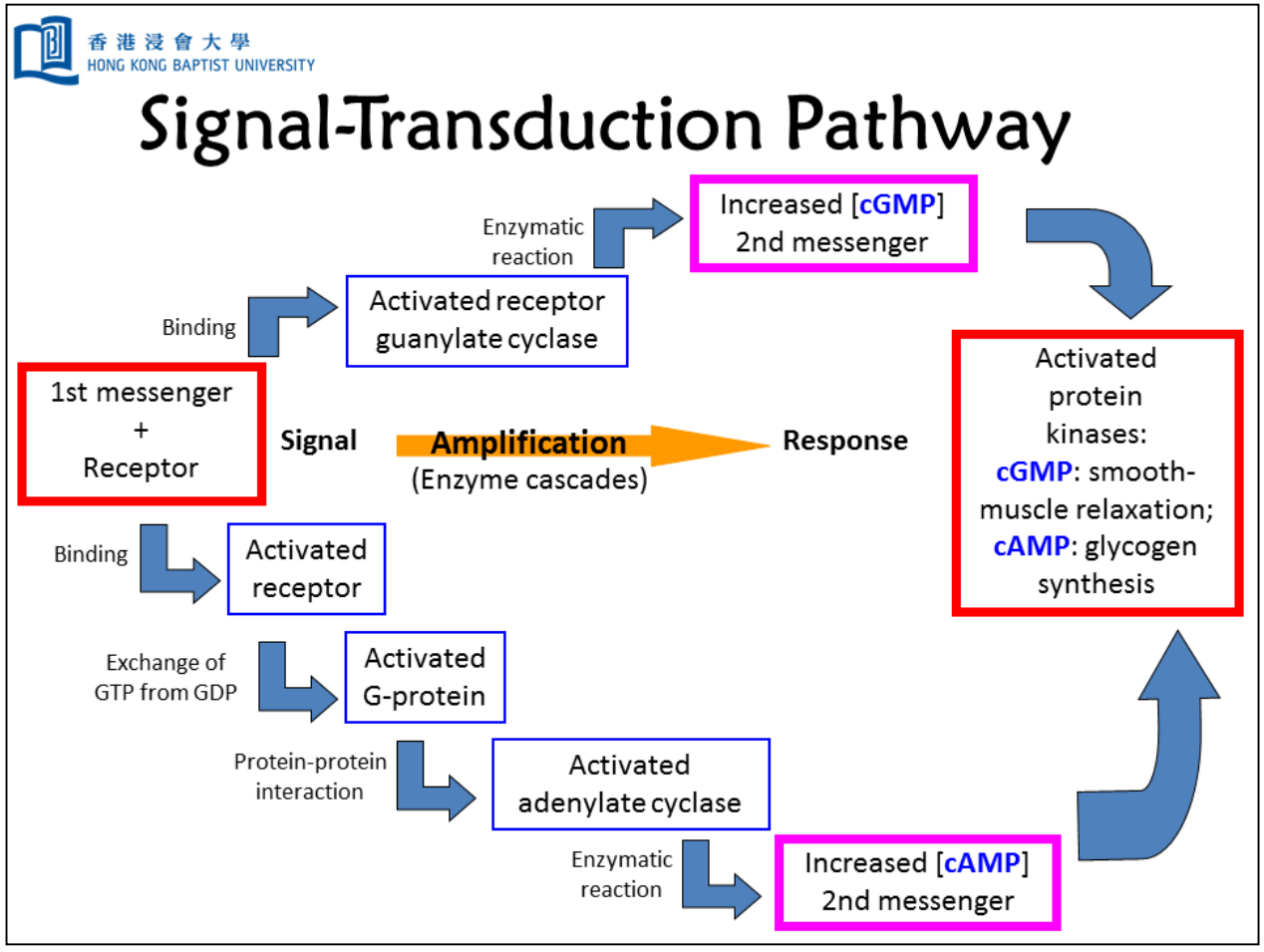
3. Enzyme-linked receptors

- Enzyme-linked receptors: Like G-protein-linked receptors, enzyme-linked receptors are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane.
- Enzyme-linked receptors also known as a catalytic receptors, where the binding of an extracellular ligand causes enzymatic activity on the intracellular side. They possessing both enzymatic catalytic and receptor functions.
- Six classes of enzyme-linked receptors have thus far been identified such as receptor tyrosine kinase, receptor serine/threonine kinases, etc.

Cell signaling

Internal signaling pathways

Three major classes of membrane receptors

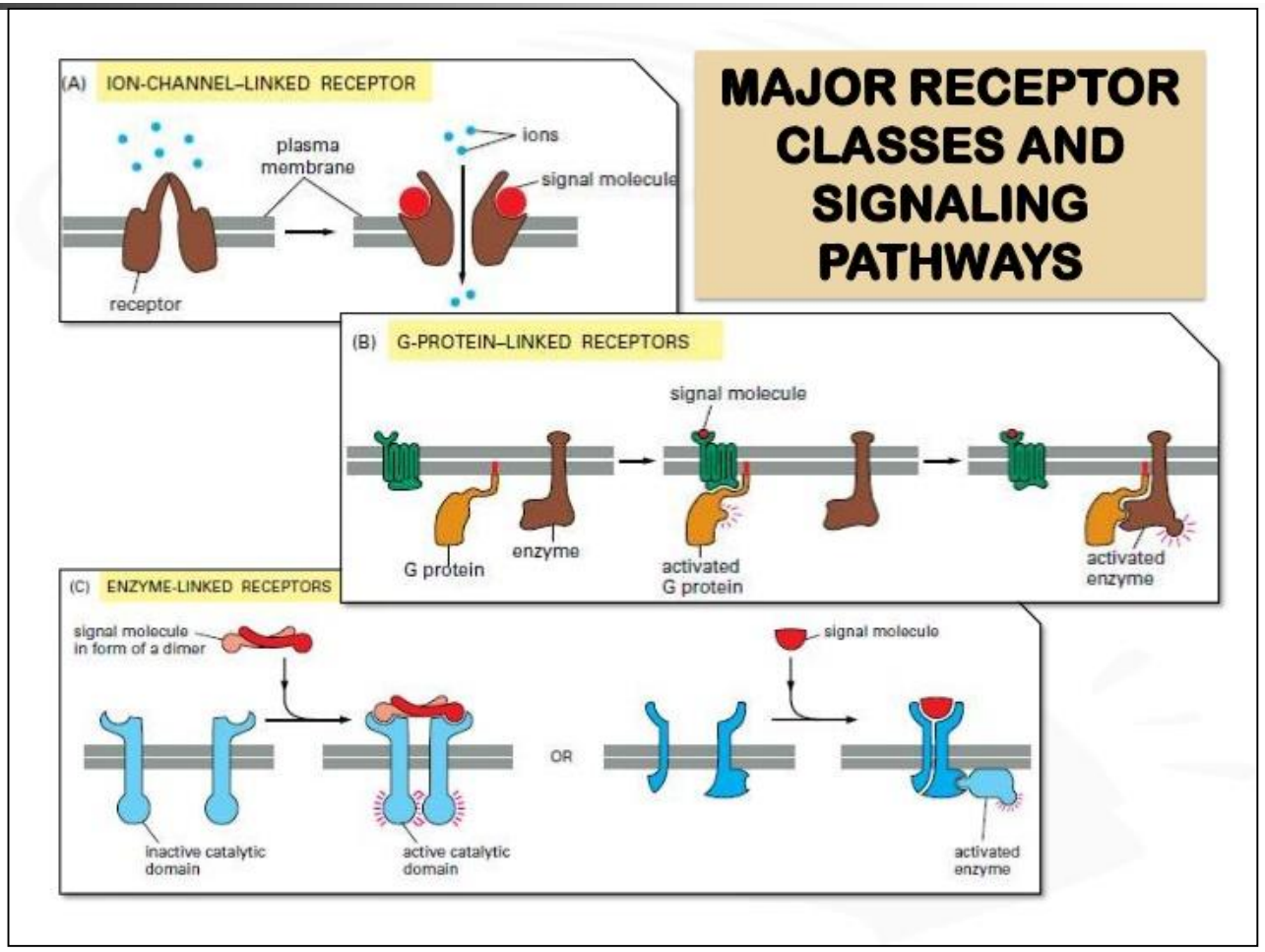


Saludhttps://pharmaceuticalintelligence.com

Cell signaling

Internal signaling pathways

Three major classes of membrane receptors



Saludar,2011

Cell signaling

Internal signaling pathways

Four classes of membrane receptors

	Type 1: ligand-gated ion channels	Type 2: G-protein-coupled receptors	Type 3: receptor kinases	Type 4: nuclear receptors
Location	Membrane	**Membrane**	Membrane	Intracellular
Effector	Ion channel	**Channel or enzyme**	Protein kinases	Gene transcription
Time frame	Milliseconds	**Seconds**	Hours	Hours
Examples	Nicotinic acetylcholine receptor, $GABA_A$ receptor	**Muscarinic acetylcholine receptor, adrenoceptors**	Insulin, growth factors, cytokine receptors	Steroid receptors
Structure	Oligomeric assembly of subunits surrounding central pore	**Monomeric or oligomeric assembly of subunits comprising seven transmembrane helices with intracellular G-protein-coupling domain**	Single transmembrane helix linking extracellular receptor domain to intracellular kinase domain	Monomeric structure with separate receptor- and DNA-binding domains

Cell signaling

Internal signaling pathways

Four classes of membrane receptors

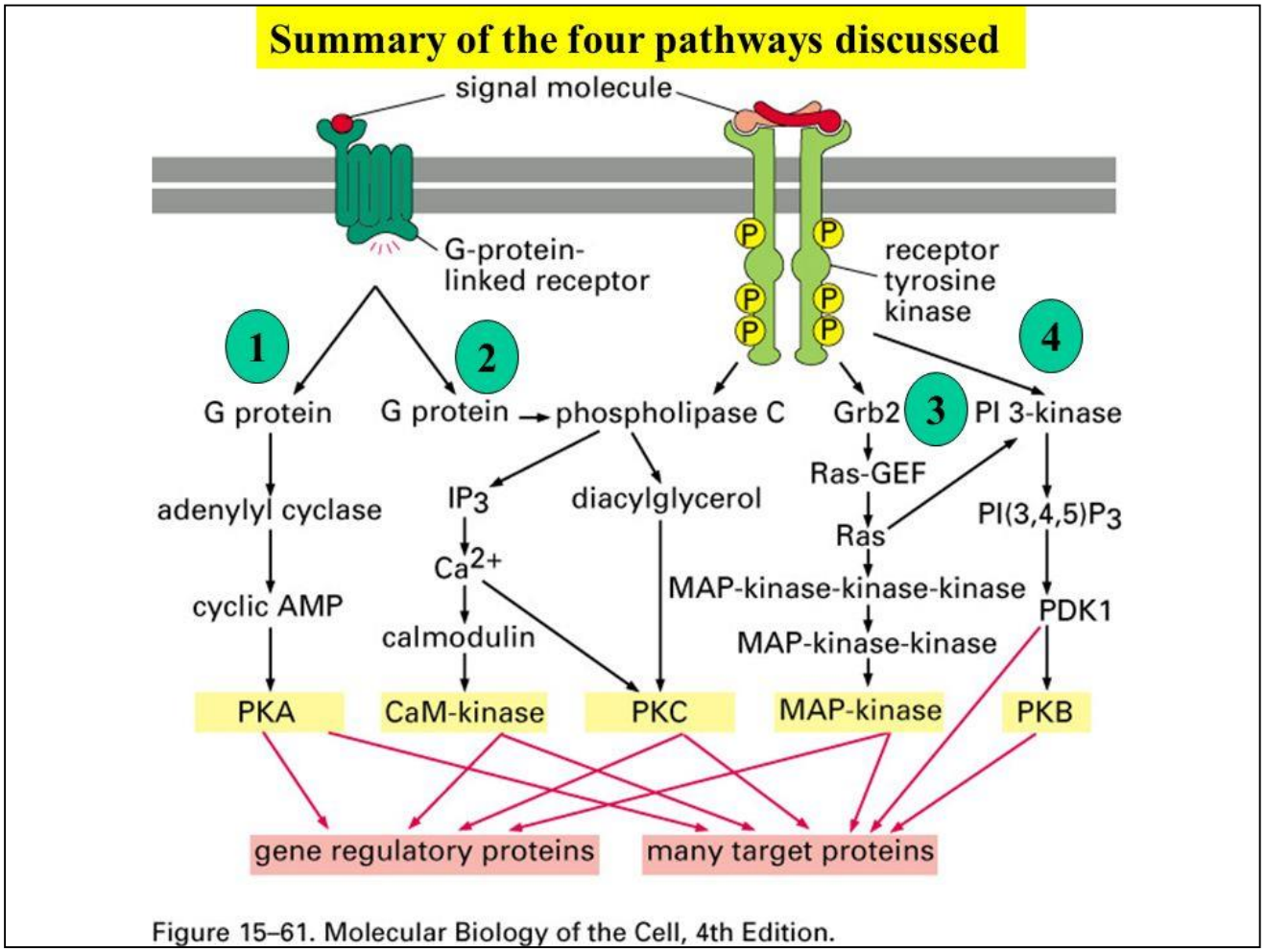
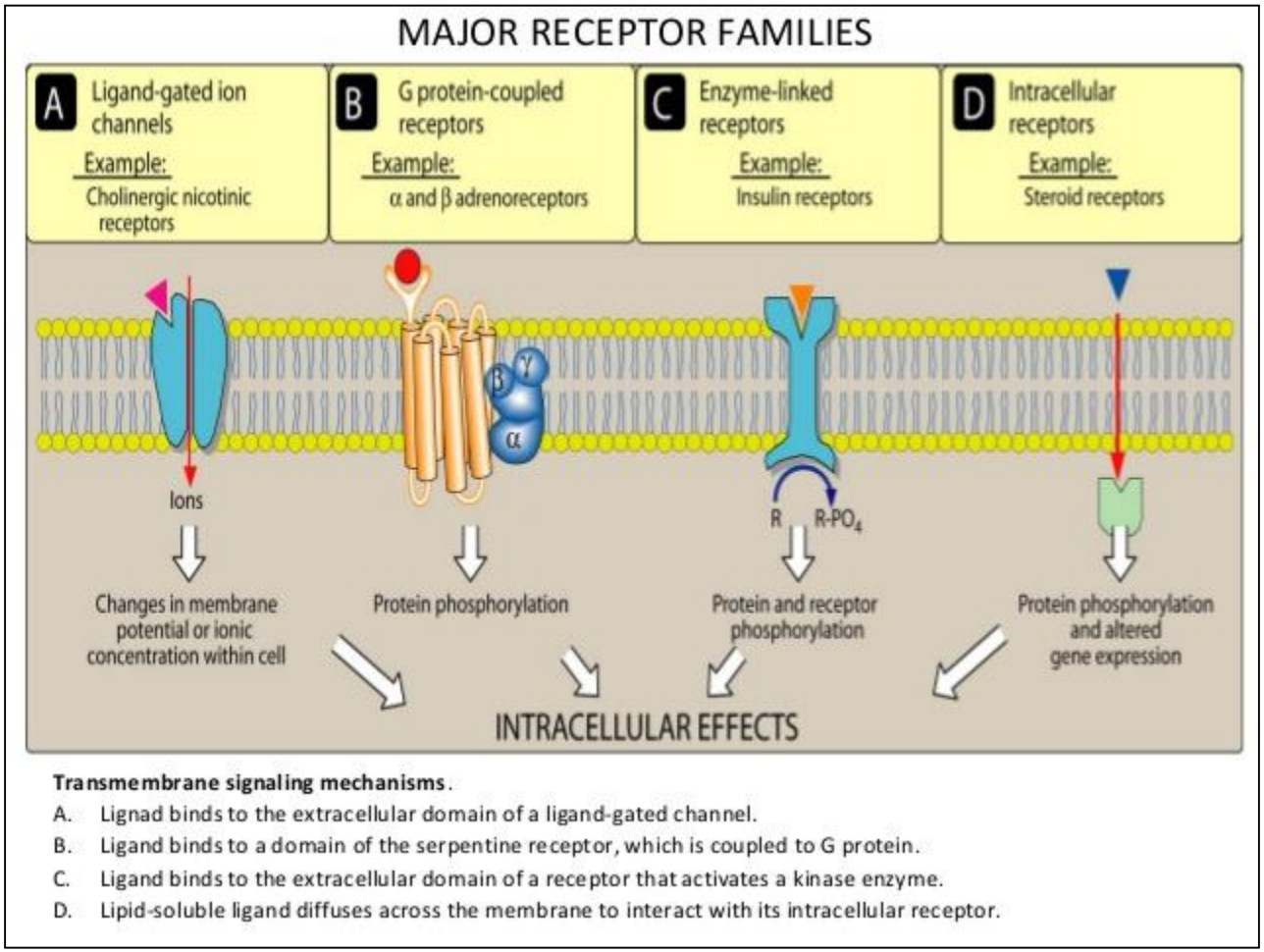


Figure 15–61. Molecular Biology of the Cell, 4th Edition.

Cell signaling

Internal signaling pathways

Four classes of membrane receptors



Transmembrane signaling mechanisms.

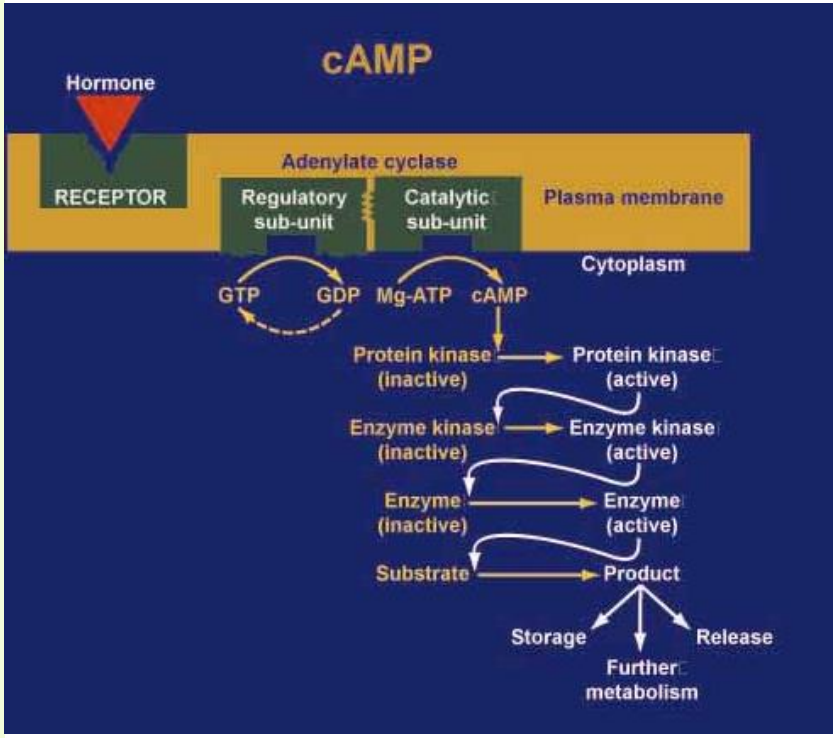
A. Lignad binds to the extracellular domain of a ligand-gated channel.
B. Ligand binds to a domain of the serpentine receptor, which is coupled to G protein.
C. Ligand binds to the extracellular domain of a receptor that activates a kinase enzyme.
D. Lipid-soluble ligand diffuses across the membrane to interact with its intracellular receptor.

Cell signaling

Signal cascade steps

Amino acid phosphorylation and dephosphorylation is very important in intracellular signal transduction

- Within proteins, the amino acids serine, threonine, and tyrosine are especially common sites for phosphorylation.
- These phosphorylation reactions control the activity of many enzymes involved in intracellular signaling pathways.
- Specifically, the addition of phosphate groups causes a conformational change in the enzymes, which can either activate or inhibit the enzyme activity. Then, when appropriate, protein phosphatases remove the phosphate groups from the enzymes, thereby reversing the effect on enzymatic activity.



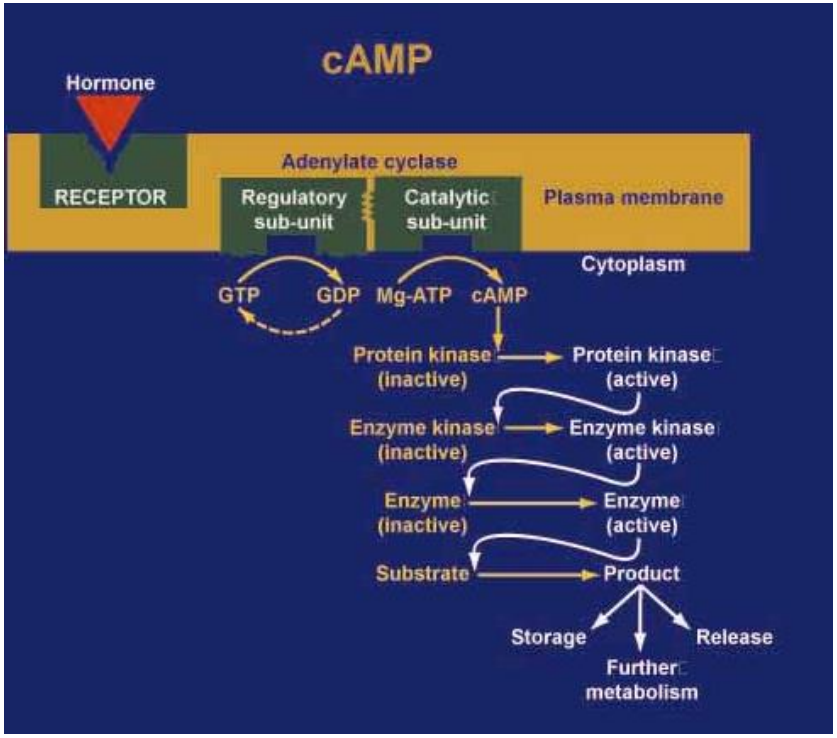
Wikipedia,2017; Kopchick,2015; www.nature.com

Cell signaling

Signal cascade

Amino acid phosphorylation and dephosphorylation is very important in intracellular signal transduction

- Cyclic adenosine monophosphate (cAMP, cyclic AMP, or 3',5'-cyclic adenosine monophosphate) is a second messenger important in many biological processes.
- cAMP is a derivative of adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway.
- How does cAMP stop signaling? It is degraded by the enzyme phosphodiesterase.

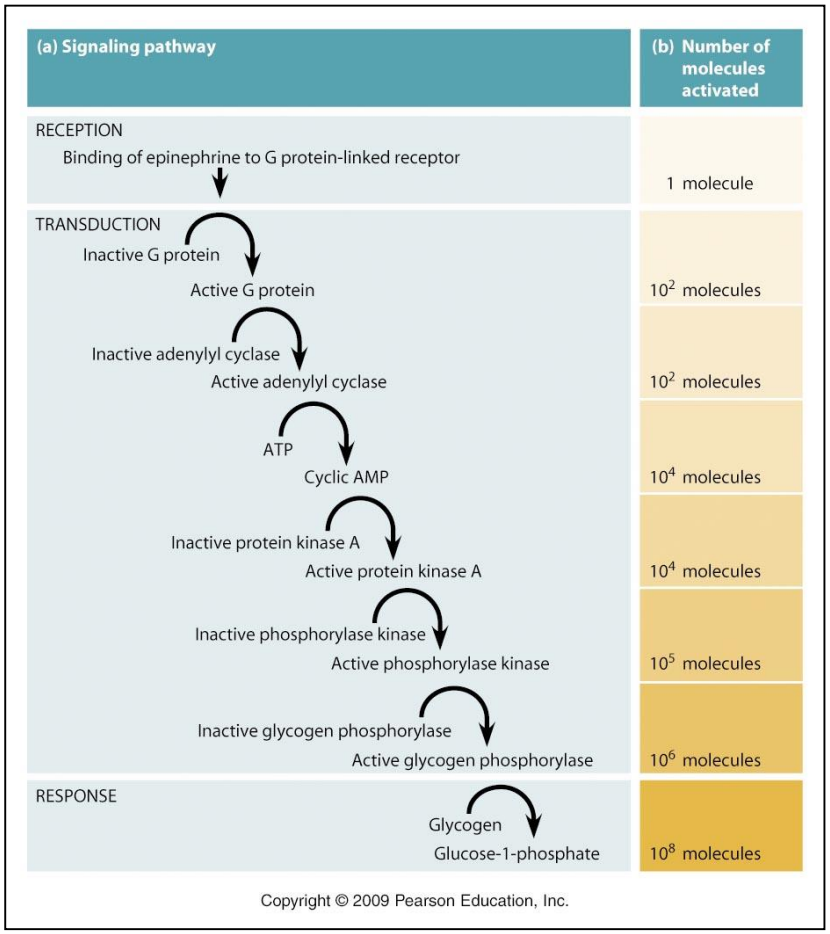


Wikipedia,2017; Kopchick,2015

Cell signaling

Signal cascade

Each step in the cascade further amplifies the initial signal



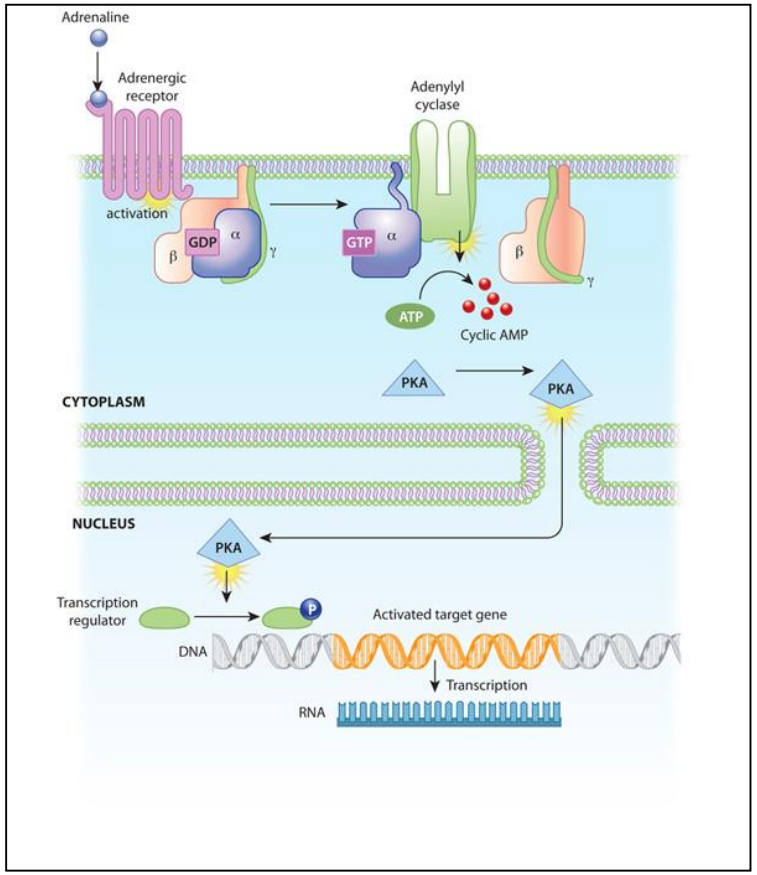
Pearson Education,2009

Cell signaling

Signal cascade

An example of a signal transduction cascade involving cyclic AMP

- The binding of adrenaline to an adrenergic receptor initiates a cascade of reactions inside the cell.
- The signal transduction cascade begins when adenylyl cyclase, a membrane- bound enzyme, is activated by G-protein molecules associated with the adrenergic receptor.
- Adenylyl cyclase creates multiple cyclic AMP molecules, which fan out and activate protein kinases (PKA, in this example).
- Protein kinases can enter the nucleus and affect transcription.



www.nature.com

Cell signaling

Protein kinase function is evolutionarily conserved from *Escherichia coli* to human

Eukaryotes vs. prokaryotes

- The eukaryotic protein kinases that directly phosphorylate proteins are divided into two major classes:
  1. those that phosphorylate tyrosine; and
  2. those that phosphorylate serine and threonine.

- Bacteria, whose cellular makeup is considered to be simplified and optimized for rapid bursts of growth, were usually thought not to possess such complicated kinase networks.
- The main sensing and signal transduction devices in bacteria are the so-called two-component systems, based on histidine/aspartate kinases (See QS in file1).

Serine/threonine and tyrosine kinases were also detected in bacteria. Therefore, the bacteria also possess kinase cascades similar to those described in eukarya.

Cell signaling

Kinase cascades

Definition

- Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used.
- The changes elicited by ligand binding (or signal sensing) in a receptor give rise to a signaling cascade, which is a chain of biochemical events along a signaling pathway.

Cell signaling

Kinase cascades

MAP kinase

- **Microtubule-associated protein (MAP):**
- Any of the high molecular weight proteins that bind to microtubules, enhancing polymerization.
- **Microtubule-associated protein (MAP) kinase:**
- A protein kinase that is activated in response to cell stimulation by many different growth factors and that mediates:

1. cellular responses by phosphorylating specific transcription factors and
2. other target proteins.

A MAPK cascade in Arabidopsis innate immunity.

Cell signaling

Serine/threonine and tyrosine kinases in bacteria

- Very recently it was reported that some bacteria such as *Bacillus subtilis* also possess protein serine/threonine and tyrosine kinases which resemble eukaryal kinases in their capacity to phosphorylate multiple substrates.
- There is good evidence that most of the prokaryotic Ser/Thr kinases carry out phosphorylation of their target proteins within the bacterial-cell cytoplasm.
- E.g.
- The *Streptomyces* Ser/Thr protein kinase AfsK phosphorylates the global regulator AfsR, which controls the production of certain antibiotics.

Signaling processes

Signal perception and defense activation

- The first process in signal transduction is:

1. Perception of an extracellular signal, and
2. Its transmission via the plasma membrane, resulting in accumulation of intracellular signaling molecules and
3. Induction of a phosphorylation/dephosphorylation cascade, a cue system for the activation of *R* gene expression.

Phosphorylation is the addition of a phosphate (PO_4) group to a protein or other organic molecule.

Signal Transduction

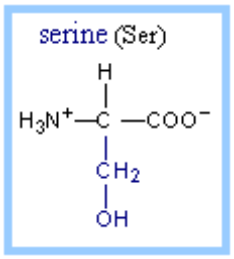
Phosphatases and phosphodiesterase

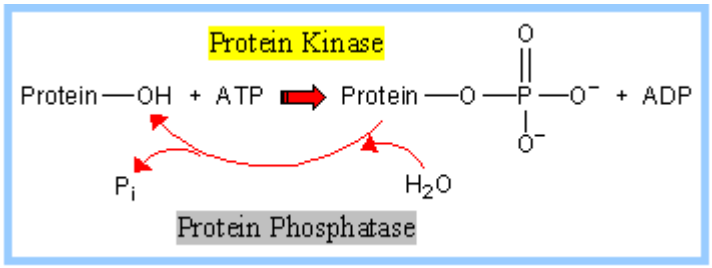
Inactivation of MAP kinases

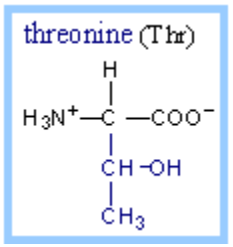
- How does cAMP stop signaling?
- It is degraded by the enzyme phosphodiesterase.
- Activity of MAP kinases is restricted by a number of protein phosphatases, which remove the phosphate groups that are added to specific serine or threonine residues of the kinase and are required to maintain the kinase in an active conformation.

Signal Transduction
Kinases and Phosphatases

- Many enzymes are regulated by covalent attachment of phosphate, in ester linkage, to the side-chain hydroxyl group of a particular amino acid residue (serine, threonine or tyrosine).
- Kinases: Enzymes which phosphorylate (add a phosphate group).
- Phosphatases: Enzymes which dephosphorylate (remove a phosphate group).







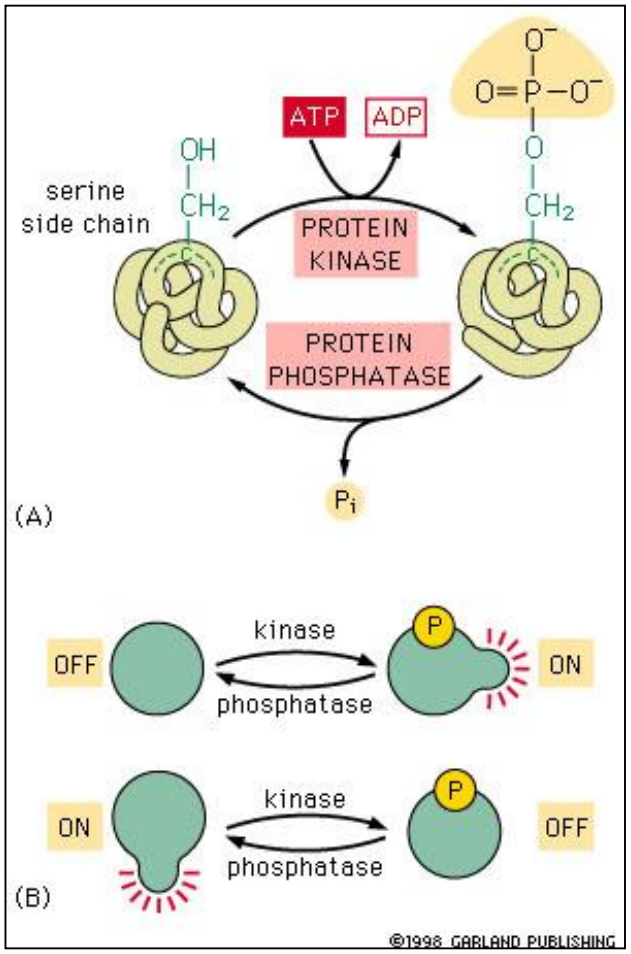
Diwan,2008

Signaling processes

Plant MAPK pathways

Phosphorylations and dephosphorylations

- Plant defense also appears to be under negative control of various MAPK cascades (negative regulation).
- Effectors that enhance the protein's activity are referred to as positive regulation, and those that decrease the protein's activity are called negative regulation.
- Note that negative regulatory roles turn out positive.

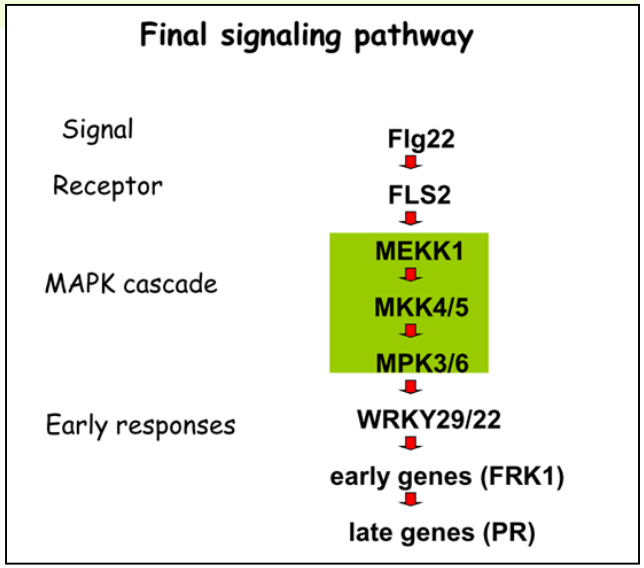


The prototypical MAPK cascade

WRKY transcription factors

1. **Early defense genes (FRK1);**
2. **Late defense genes(PR)**

FRK1:Cell surface plant receptor-like protein kinase



Signal

Sensor

MAPKKK

MAPKK

MAPK

Primary Response Genes

Secondary Response Genes

Input

Filter Amplify

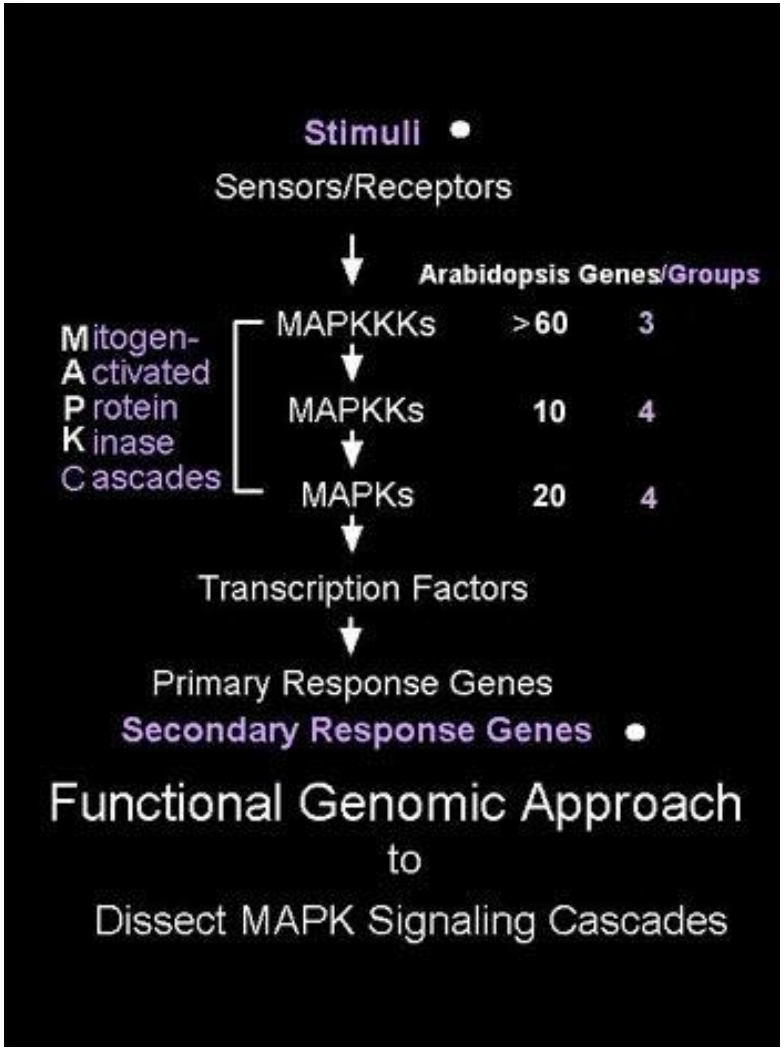
Output

Tena,2002

Plant MAPK pathways

Cascades in stress and hormonal signaling

NPR1 regulates PR gene expression by interacting with TGA TFs. Thus, TGA factors affect the transcription of PR genes.



Plant MAPK pathways

MAPKs in *Arabidopsis*

- The Arabidopsis genome has revealed large gene families encoding MAPKs and their immediate upstream regulators, MAPKKs and MAPKKKs.
- There are many MAPK-encoding genes in plant genomes.
- For example, the Arabidopsis genome contains about 110 genes coding for putative MAPK pathway components:

1. 20 MAPK genes,
2. 10 MAPKKs, and
3. More than 80 MAPKKKs.

Complexity of MAPK networks
20 x 10 x 80 = a lot of virtual combinations

Plant MAPK pathways

Complexity of MAPK networks

Model systems

- Mitogen-activated protein kinase (MAP kinases cascades) represent important and universal signal transduction mechanisms in diverse cellular responses in eukaryotic organisms.
- How to work with such a high number of possibilities?

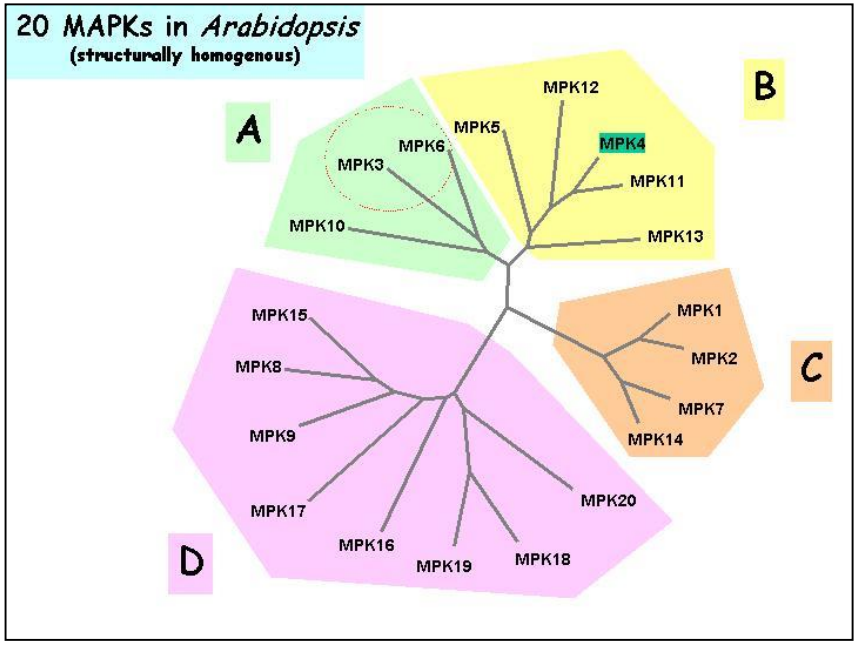
1. a simplified biological system;
2. possibility to do hundreds of functional analyses quickly.

Tena,2002

Plant MAPK pathways
Relatedness of Arabidopsis MAPKs

- A large number of MAPKs act as positive players in defense signaling.

1. MPK4 negatively regulates biotic stress signalling,
2. MPK3 and MPK6 act as positive mediators of defence responses.



Tena,2002

Plant MAPK pathways

Characterization and functions of MAP kinases

- The best-characterized MAPKs are MPK3, MPK4 and MPK6, all of which are activated by a diversity of stimuli including:

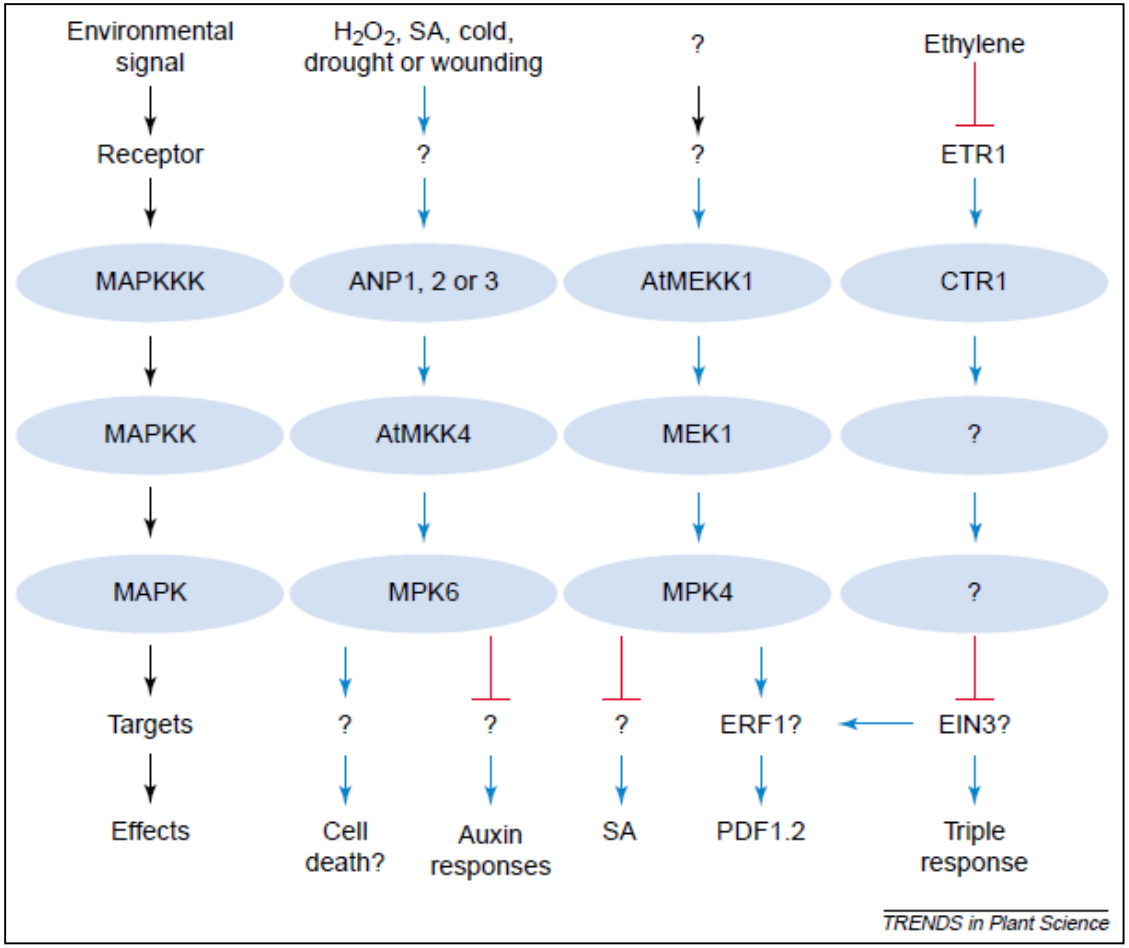
1. Abiotic stresses
2. Flg22 peptide-mediated defense responses(PAMP)
3. The ethylene pathway
4. Pathogens
5. Auxin
6. H_2O_2
7. Oxidative stress.

Pitzschke *et al.*,2009;…

Plant MAPK pathways

MAPK cascades in *Arabidopsis thaliana*

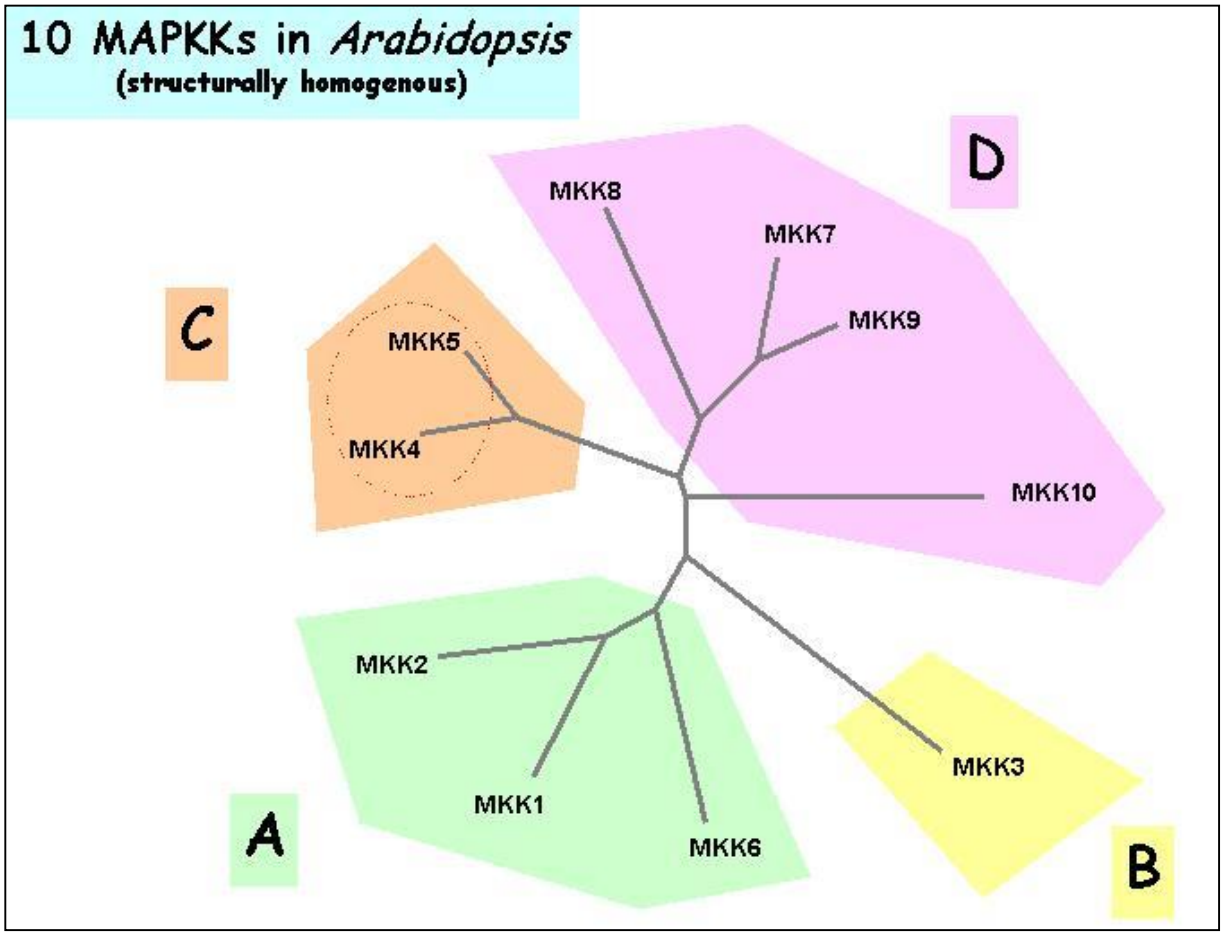
In response to abiotic, biotic stresses, hormones & H_2O_2



Innes,2001

Plant MAPK pathways

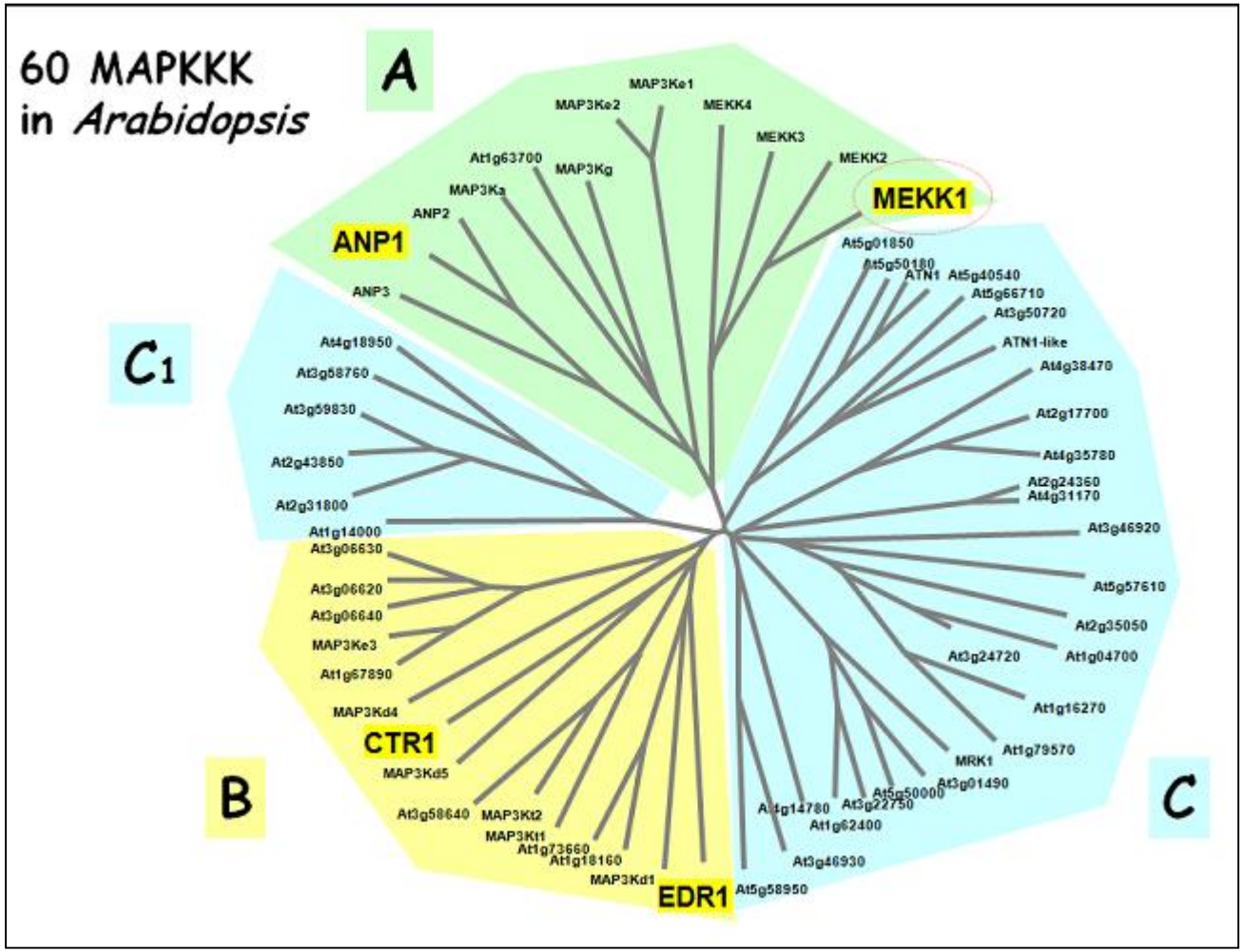
Relatedness of Arabidopsis MAPKKs



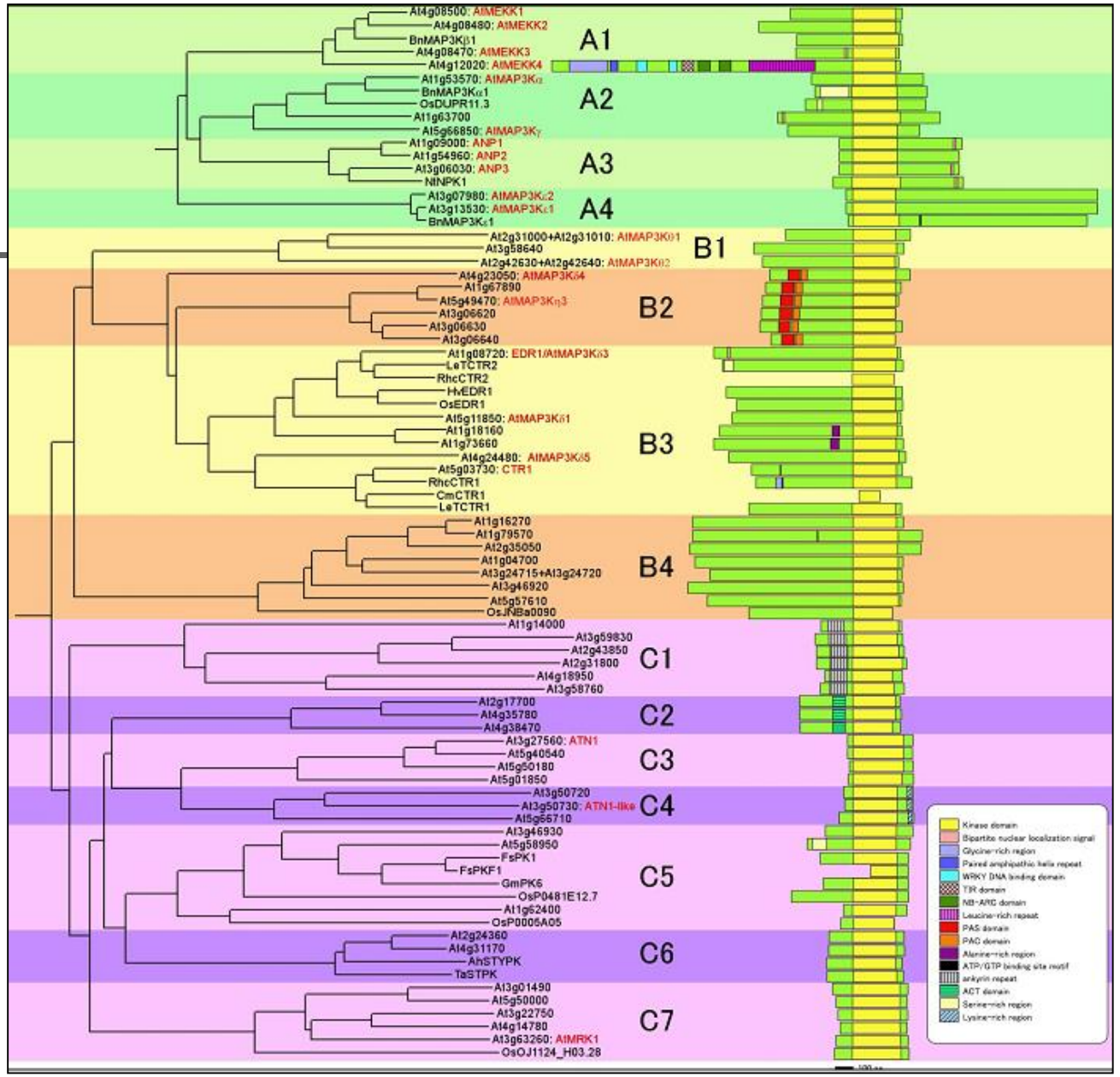
Tena,2002

Plant MAPK pathways

Relatedness of Arabidopsis MAPKKKs



Structural heterogeneity of plant MAPKKKs.



Tena,2002

Plant MAPK pathways

SIPK family(salicylic acid-induced protein kinase)

- SIPK has emerged as a universal key component of the MAPK cascade.
- SIPKs involve in fine-tuned regulation of the plant responses to:
- Ozone, wounding, SA, and jasmonic acid (JA).
- SIPKs respond to multiple stresses with different functions under different biotic and abiotic stresses.
- For example,

1. AtMKK2–AtMPK6 is involved in cold and salt stresses;
2. AtMKK4/AtMKK5–AtMPK6 cascade is involved in ET(ethylene) signaling.

Cho *et al.*,2008

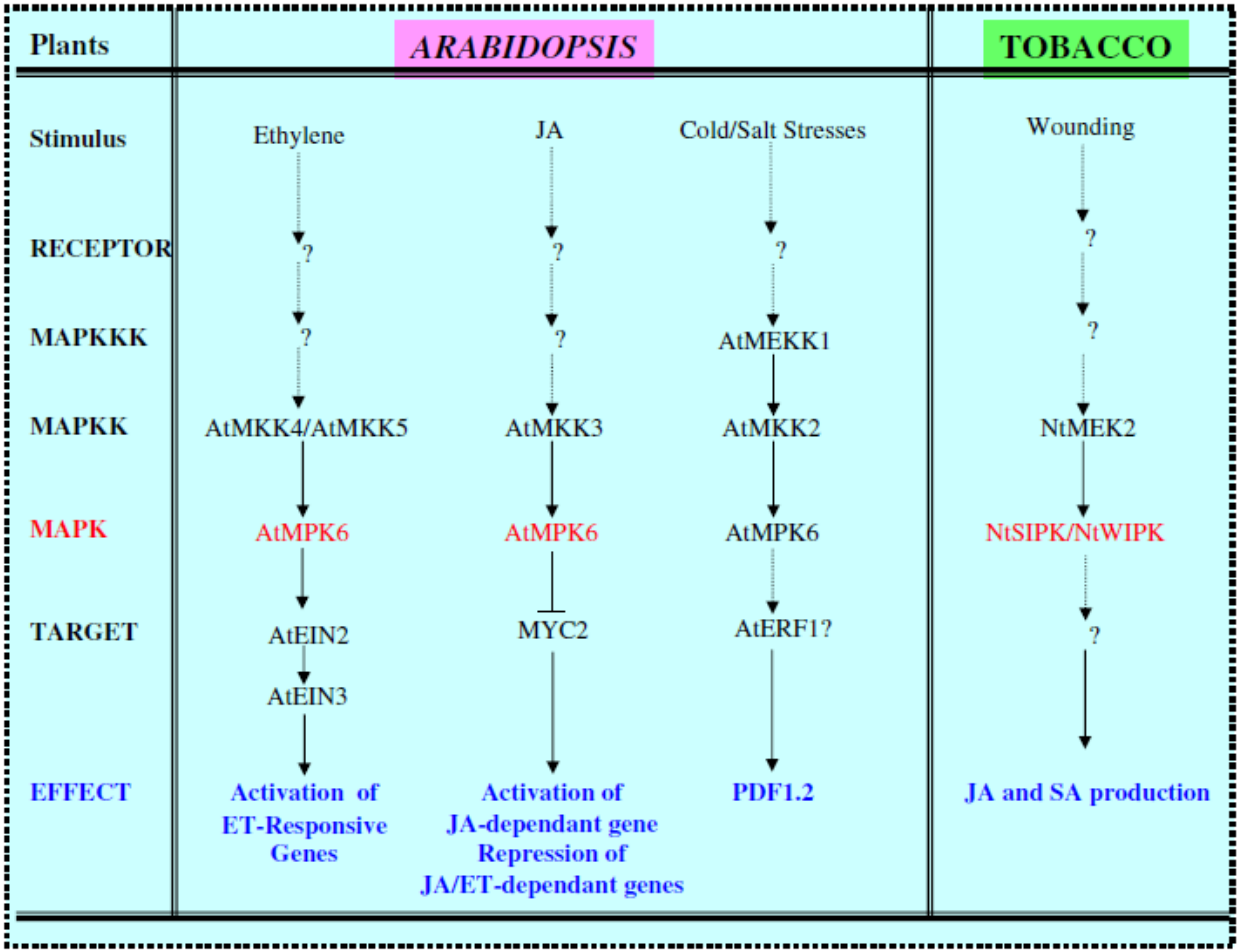
Plant MAPK pathways
SIPK family

- Over the past years, several plants MAPKs have been identified and characterized.
- Of these, rice SIPK (Salicylic acid (SA)-Induced Protein Kinase) and its orthologs in other plants are of particular interest.
- SIPK family includes:
- AtMPK6 in Arabidopsis,
- MsSIMK in alfalfa,
- NtSIPK in tobacco, and
- OsSIPK in rice.

Cho *et al.*,2008

Signal pathways of orthologs of OsSIPK in Arabidopsis and in tobacco

SIPK (Salicylic acid (SA)-Induced Protein Kinase)



Cho *et al.*,2008

Systemic Acquired Resistance and Induced Systemic Resistance
PR proteins

- **Systemic acquired resistance(SAR):**
- Induced by the exposure of root or foliar tissues to abiotic or biotic elicitors, is dependent of:

1. the phytohormone salicylate (salicylic acid), and
2. associated with the accumulation of pathogenesis-related (PR) proteins.

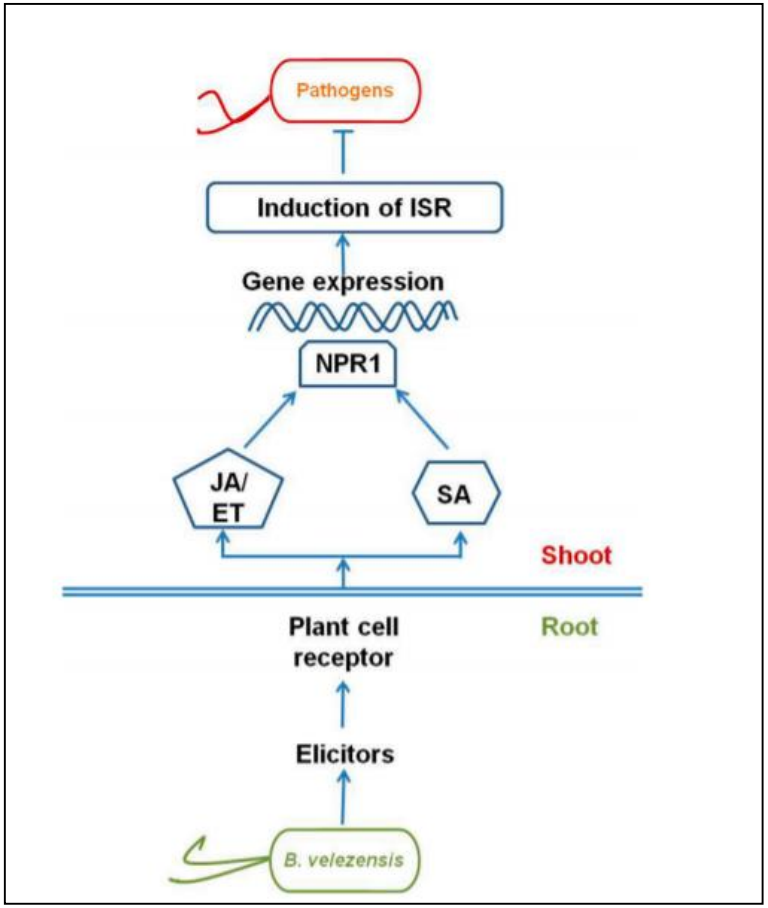
- **Induced systemic resistance(ISR):**
- Induced by the exposure of roots to specific strains of plant growth-promoting rhizobacteria, is dependent of

1. the phytohormones ethylene and jasmonate (jasmonic acid),
2. independent of salicylate, and
3. is not associated with the accumulation of PR proteins.

Vallad and Goodman,2004

Signal transduction pathway
SAR vs ISR

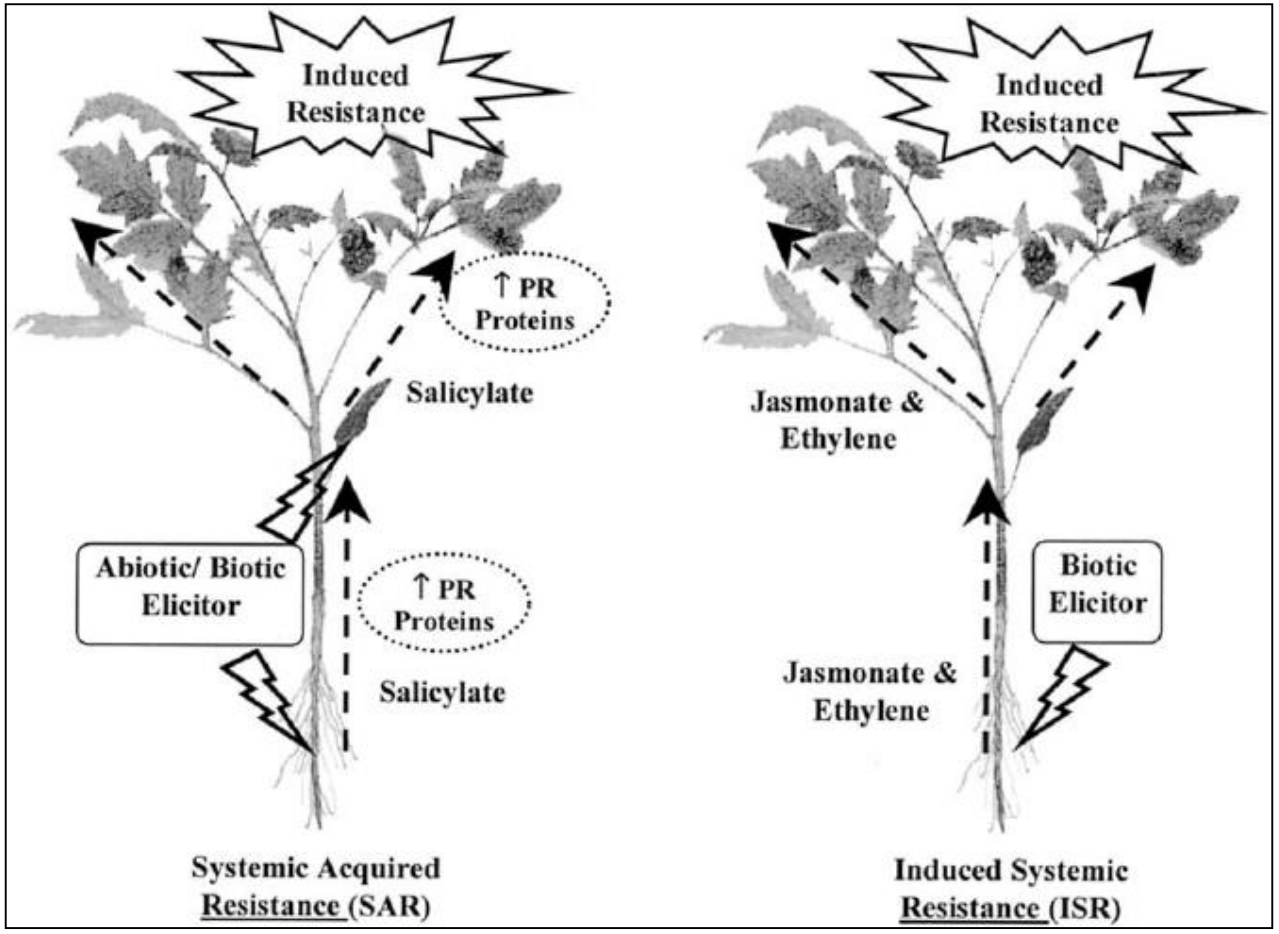
- Signal transduction pathway of induced systemic resistance stimulated by *Bacillus velezensis*.
- NPR1: non-expressor of PR1;
- JA/ET: the jasmonic acid/ethylene signaling pathways;
- SA: Salicylic Acid.



Rabbee *et al.*,2019

SAR/ISR

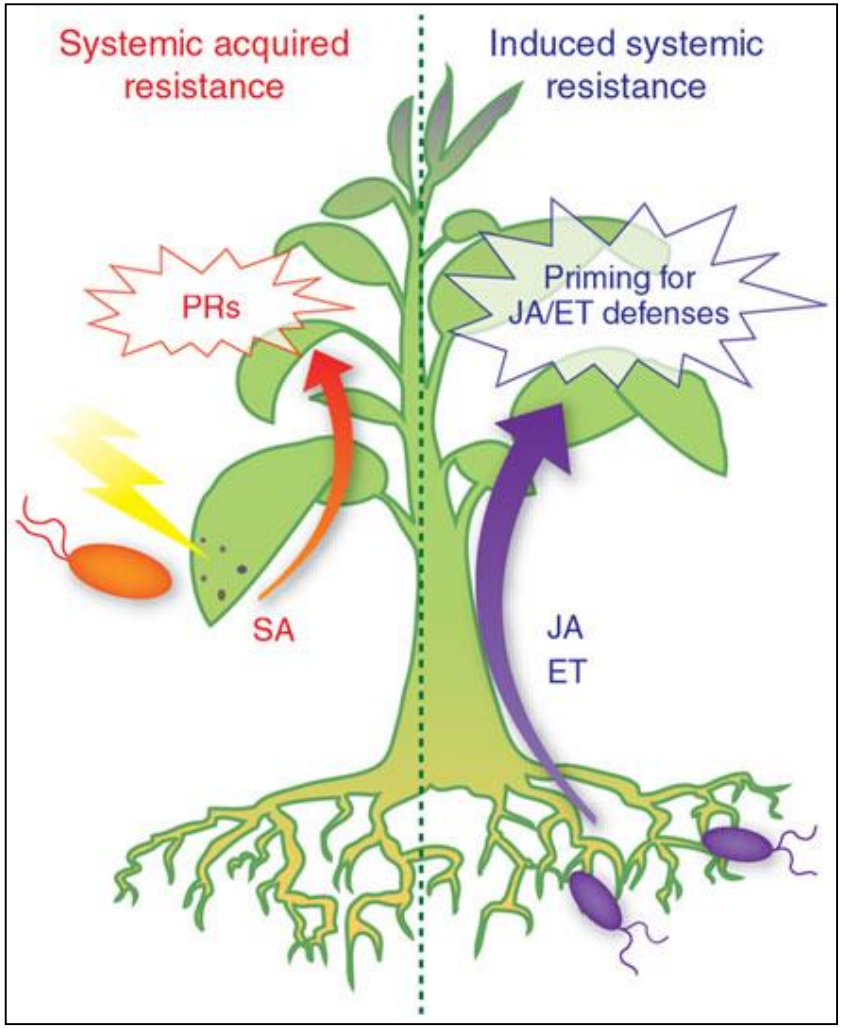
A pictorial comparison of the two best characterized forms of induced resistance in plants, both which lead to similar phenotypic responses



Vallad and Goodman,2004

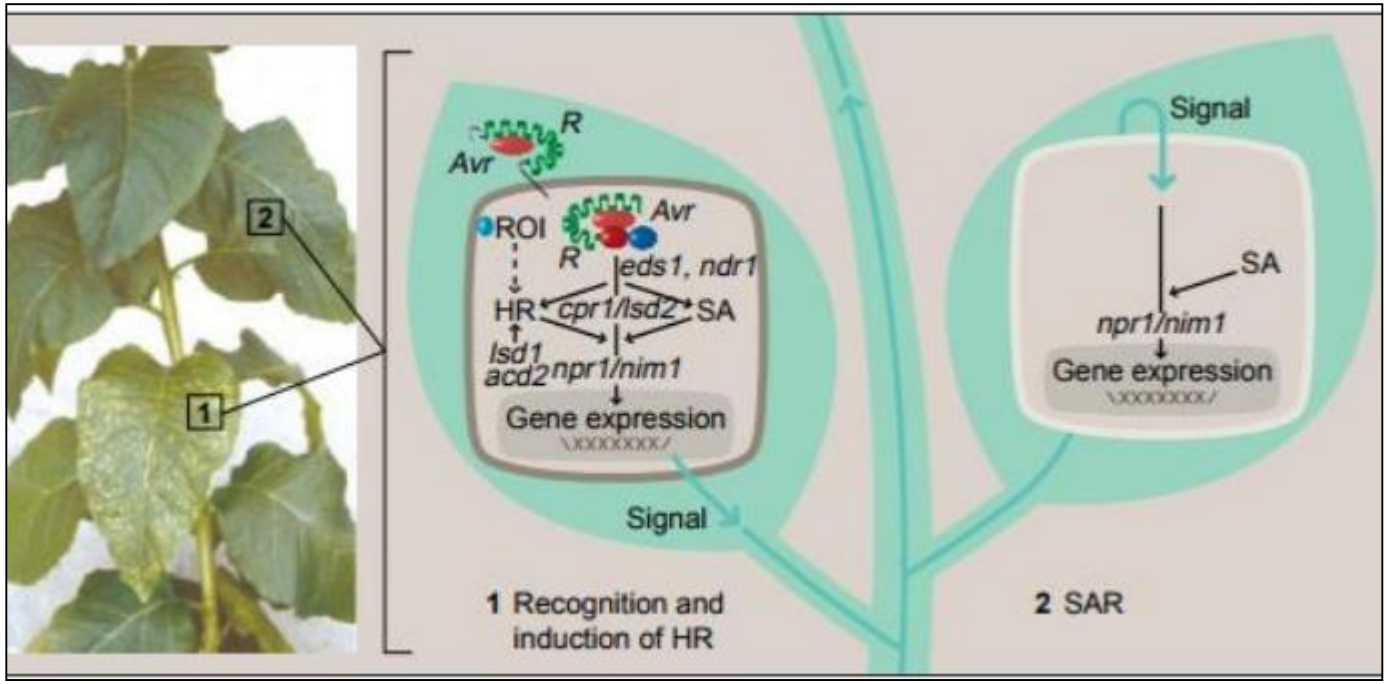
SAR/ISR

A pictorial comparison of the two best characterized forms of induced resistance in plants, both which lead to similar phenotypic responses



The plant *R-avr* gene interaction triggers a signal transduction pathway leading to the HR (1) and SAR (2)

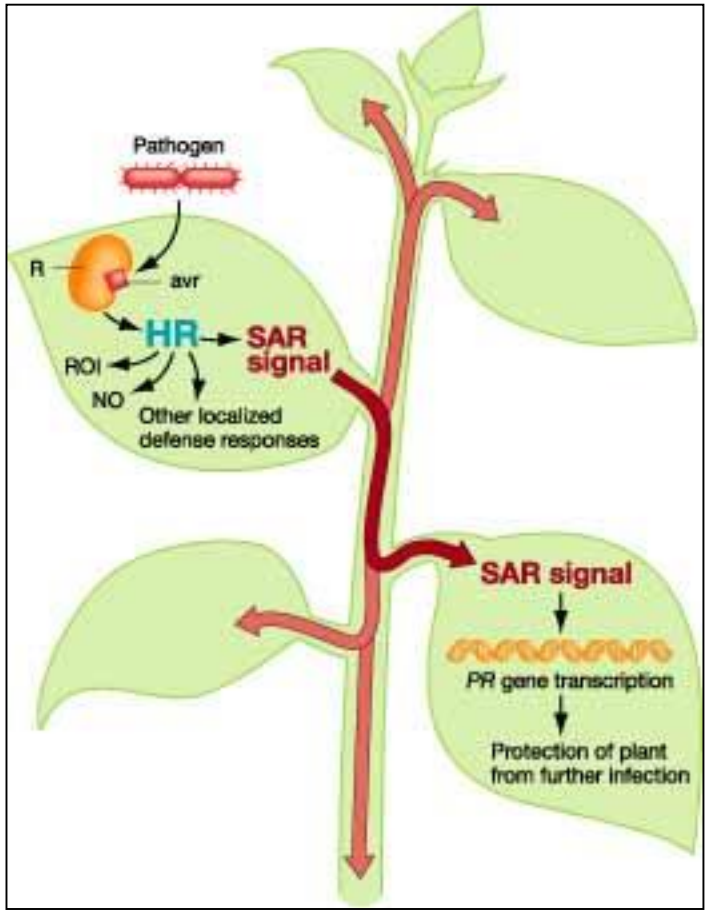
- SA was shown to move from the infected leaf into the other parts of the plant (up to 70% of labelled SA was found in the upper leaves).



Baker,1997

Systemic Acquired Resistance
SAR

- The graphic describe these defensive reactions in more detail than is possible here.



Elmore, 2015

Transcription factors
TGAs and WRKY

TGACG motif-binding protein (TGA) and WRKY transcription factors activate many PR-genes.

Transcription factors(TxF's)

Transcription factors involved in plant defense

TGA and WRKY transcription factors

- Some major families of transcription factors involved in plant defense:

1. TGA factors and TGA boxes belong basic leucine zipper containing domain proteins (bZIP),
2. amino-acid sequence WRKYGQK (WRKY) pronounced 'worky',

- TGACG motif-binding protein (TGA) and WRKY transcription factors for the activation of many PR-genes.

Transcription factors(TxF's)
TGA and WRKY transcription factors

- For a plant this may be:

1. abiotic (non-living) stress such as the rising or setting sun, drought, or heat,
2. biotic (living) stress such as insects, viral or bacterial infection, or
3. any of a limitless number of other events.

- The job of coordinating the function of groups of genes falls to proteins called transcription factors (TxF's).

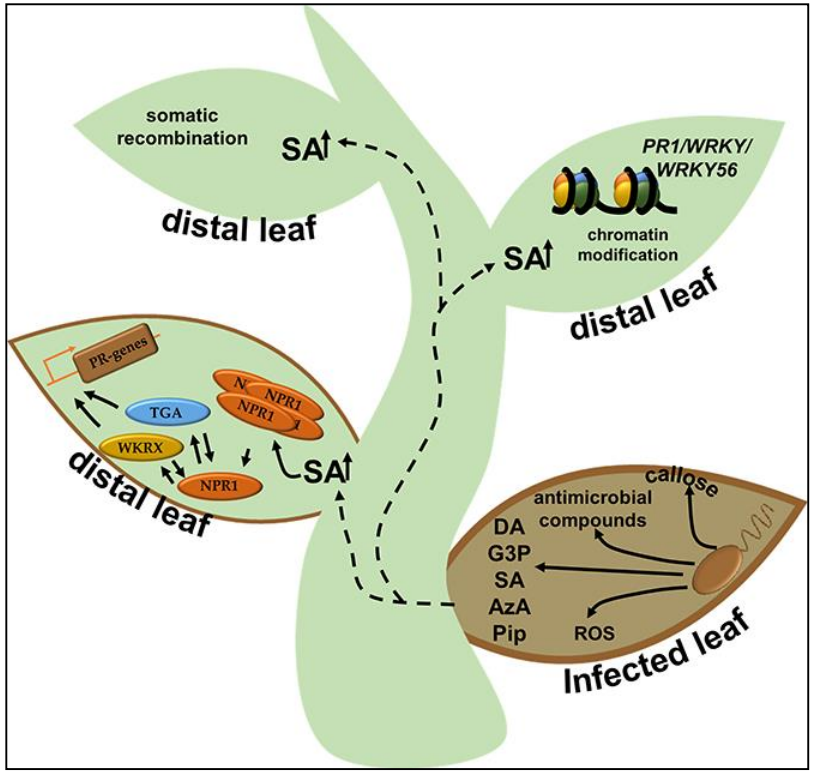
Monsanto Co.,2010

Transcription factors

SAR and priming

TGA and WRKY transcription factors

- The attack of a pathogen leads to signals, which give to the plant an immediately protection and a long-lasting immunity.
- The infected leaf generates callose for the fortification of cell walls, ROS and antimicrobial compounds to kill the bacteria, and defense-related metabolism.
- These processes are part of the innate immunity.
- Principally, the metabolite SA is transport through the phloem to the other part of the plants activating the SAR, which is a preventive defense mechanism.
- This includes the monomerization of NPR1 that in turn activates PR-genes, chromatin modification such as methylation and acetylation of histones and somatic recombination.



Naranjo-Arcos and Bauer,2016

Eukaryotic transcription machinery

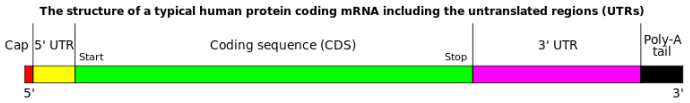
Transcription factors(TxF's)

Difference between Prokaryotic vs Eukaryotic transcription

- The eukaryotic transcription machinery is complex.
- The main differences are:

1. Number and functions of RNA polymerase(s);
2. Number and functions of promoter elements;
3. Transcription and translation occurs simultaneously in prokaryotes and in eukaryotes the RNA is first transcribed in the nucleus and then translated in the cytoplasm.
4. RNAs from eukaryotes undergo post-transcriptional modifications including: capping, polyadenylation, and splicing. These events do not occur in prokaryotes.

Polyadenylation: addition of about 250 adenine residues to form a poly(A) tail to a messenger RNA. It is part of the process that produces mature messenger RNA (mRNA) for translation.



Eukaryotic transcription machinery

Transcription factors(TxF's)

Difference between Prokaryotic vs Eukaryotic transcription

- The eukaryotic transcription machinery is complex.
- In prokaryotic cells there is only a single RNA polymerase that synthesizes with the help of sigma factor all classes of RNA:

1. mRNA,
2. rRNA,
3. tRNA.

- In eukaryotic cells, there are three different RNA polymerases that synthesize different classes of RNAs:

1. PolI transcribes rRNA (ribosomal RNA),
2. PolII mRNA (messenger RNA), and
3. PolIII tRNA (transfer RNA) and other small RNAs.

Eukaryotic transcription machinery

Transcription factors(TxF's)

Difference between Prokaryotic vs Eukaryotic transcription

Prokaryotic		Eukaryotic		
Bacterial	**Archaeal**	**RNAP I**	**RNAP II**	**RNAP III**
Core	**Core**	**(Pol I)**	**(Pol II)**	**(Pol III)**
β'	A′/A″	RPA1	RPB1	RPC1
β	B	RPA2	RPB2	RPC2
α^{I}	D	RPC5	RPB3	RPC5
α^{II}	L	RPC9	RPB11	RPC9
ω	K	RPB6	RPB6	RPB6
	[+6 others]	[+9 others]	[+7 others]	[+11 others]

Note: The subunits in each column are listed in order of decreasing molecular weight.

Source: Data adapted from Ebright R.H. 2000 *J. Mol. Biol.* **304:** 687–698, Fig. 1, p. 688. © 2000 Academic Press.

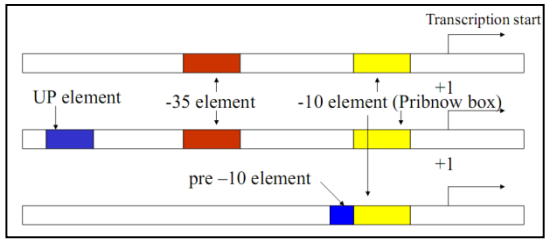
Eukaryotic transcription machinery

Transcription factors(TxF's)

Difference between Prokaryotic vs Eukaryotic transcription

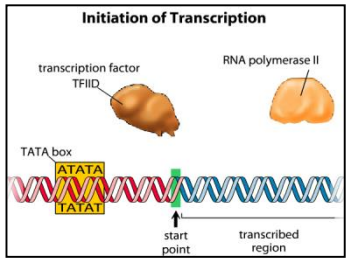
- Prokaryotes only contain three different promoter elements:

1. -10,
2. -35 promoters, and
3. upstream element.



- Eukaryotes contain many different promoter elements:

1. TATA box,
2. initiator elements,
3. downstream core promoter element,
4. CAAT box (start points of eukaryotic transcription units),
5. GC box(known for binding general transcription factors).



Eukaryotic transcription machinery

Transcription factors(TxF's)

Difference between Prokaryotic vs Eukaryotic transcription

- mRNAs in prokaryotes tend to contain many different genes on a single mRNA meaning they are polycystronic.
- Eukaryotes contain mRNAs that are monocystronic. i.e. it contains the genetic information to translate only a single protein chain (polypeptide).
- Termination in prokaryotes is done by either *rho*-dependent or *rho*-independent mechanisms.
- In eukaryotes transcription is terminated by two elements: a poly(A) signal and a downstream terminator sequence.

Transcription factors(TxF's)

TxF's are proteins that regulate gene expression

Numbers in each cell

- Transcription factors are essential for the regulation of gene expression and are, as a consequence, found in all living organisms.
- The number of transcription factors found within an organism increases with genome size:
- The larger genomes tend to have more transcription factors per gene.
- Approximately 10% of genes in the genome code for transcription factors, which makes this family the single largest family of human proteins.

Wikipedia,2016

Transcription factors(TxF's)

TxF's are proteins that regulate gene expression

How do transcription factors work?

- A transcription factor (sometimes called a sequence-specific DNA-binding factor) is a protein that binds to specific DNA sequences, thereby controlling the rate of transcription of genetic information from DNA to messenger RNA.
- Transcription factors perform this function alone or with other proteins in a complex, by:

1. promoting (as an activator), or
2. blocking (as a repressor) the recruitment (hiring) of RNA polymerase to specific genes.

Wikipedia,2016

Transcription factors(TxF's)

How do transcription factors work?

Genes turned on and off

- In order to allow coordinated gene function, a particular TxF may bind to multiple genes, and each gene may be controlled by multiple TxF's.
- Further– recall that each TxF is itself a protein, and TxF's often regulate other TxF's.
- TxF's form complex networks that may control from one to many thousands of genes (multiple protein complexes) in response to conditions inside or outside of the cell.

Monsanto Co.,2010

Transcription factors(TxF's)

How do transcription factors work?

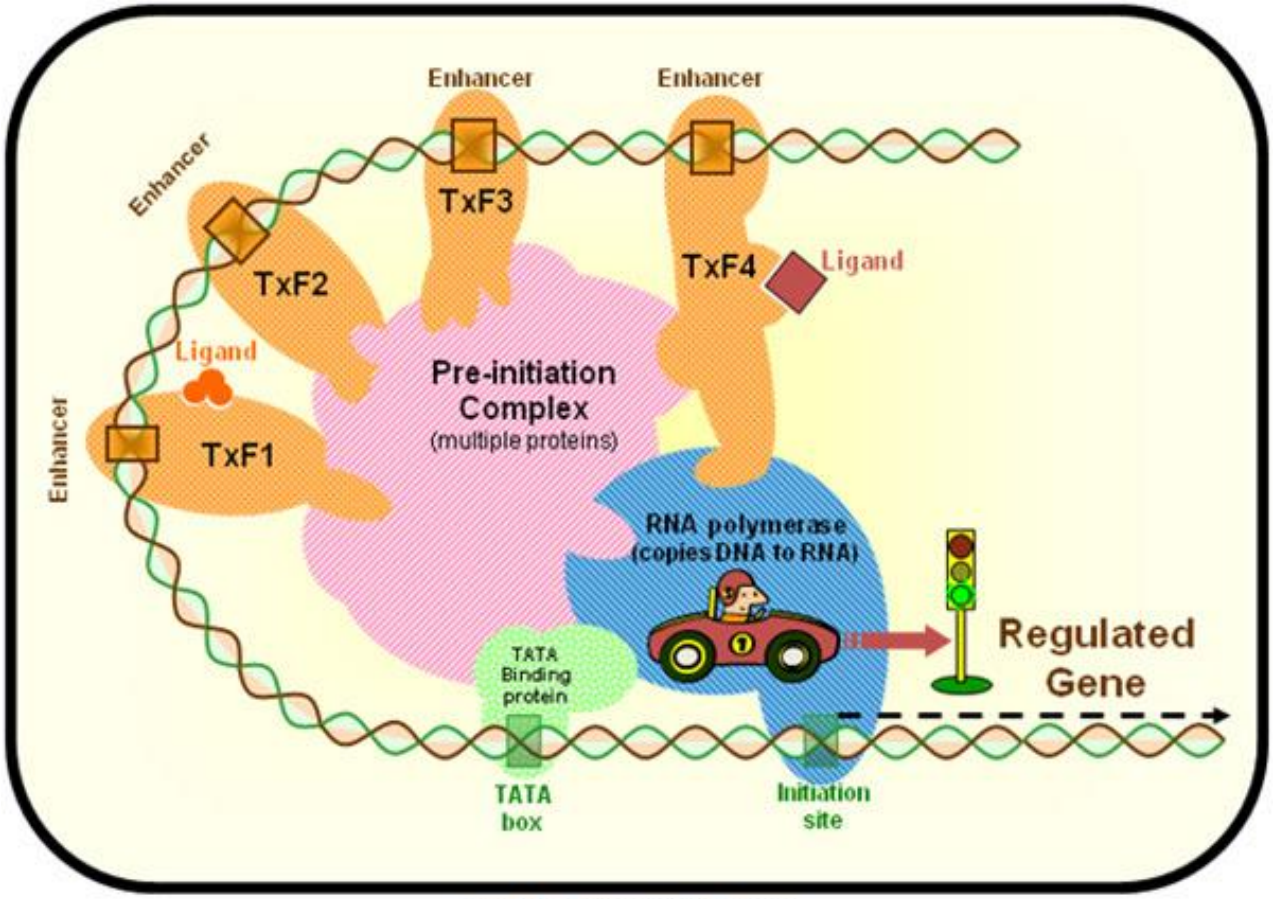


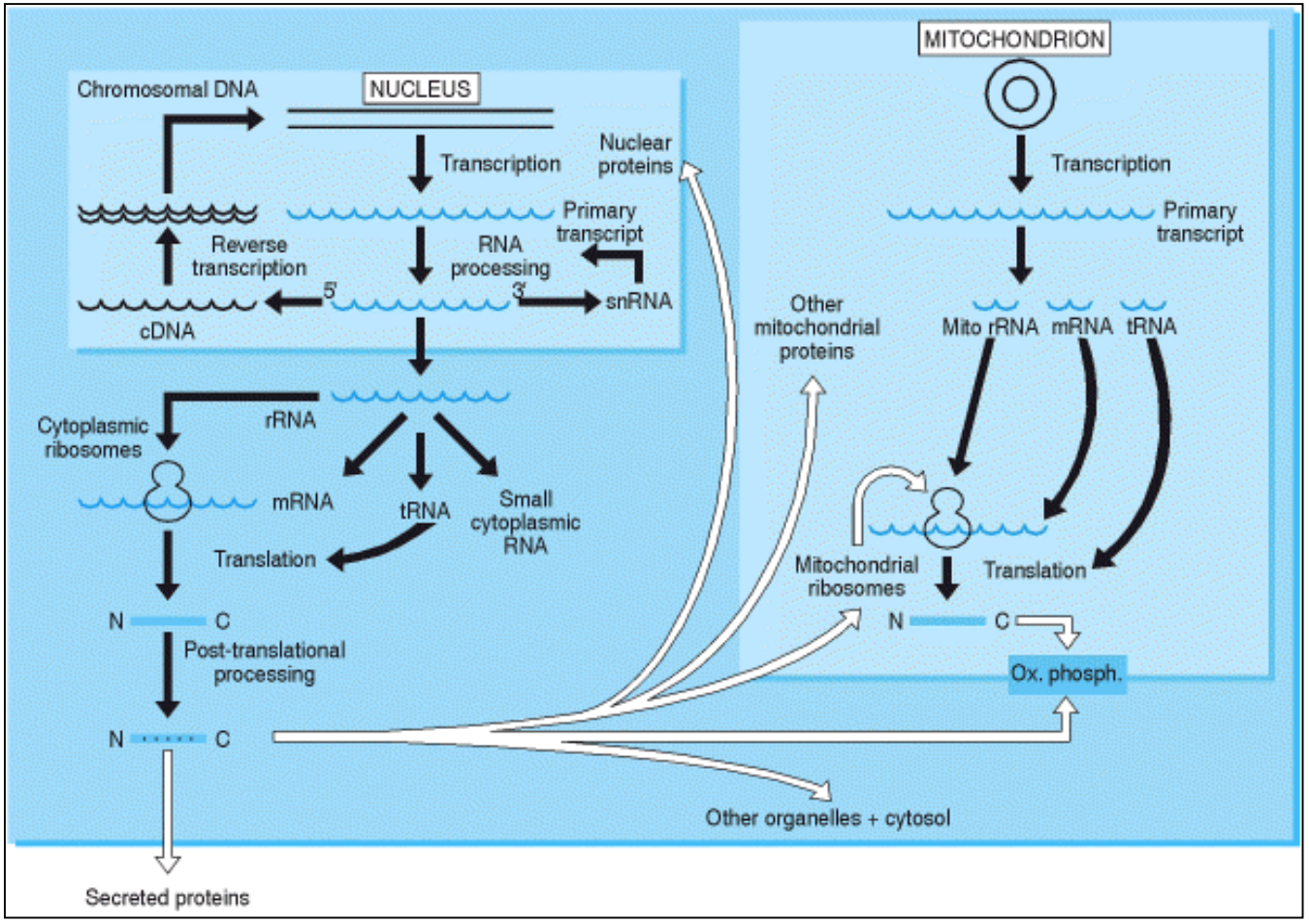
Figure 2 - Transcription Factors

Monsanto Co.,2010

Transcription factors(TxF's)

Control of gene/mRNA/protein activity

Nucleus vs. mitochondria in Eukaryotes



Transcription factors(TxF's)

TxF's are proteins that regulate gene expression

1. General transcription factors in eukaryotes

- In eukaryotes, an important class of transcription factors called general transcription factors (GTFs) are necessary for transcription to occur.
- Many of these GTFs don't actually bind DNA but are part of the large transcription pre-initiation complex that interacts with RNA polymerase directly.
- The most common GTFs are:
- TFIIA, TFIIB, TFIID (see also TATA binding protein), TFIIE, TFIIF, and TFIIH.

Wikipedia,2016

Transcription and Gene Expression

Protein-protein interactions

2. Transcription factors involved in plant defense

- Some major families of transcription factors involved in plant defense:

1. TGA factors and TGA boxes belong basic leucine zipper containing domain proteins (bZIP),
2. amino-acid sequence WRKYGQK (WRKY) pronounced 'worky',
3. myelocytomatosis related proteins (MYC),
4. myeloblastosis related proteins (MYB),
5. The AP2/EREBP family of plant transcription factors,
6. no apical meristem (NAM),
7. Arabidopsis transcription activation factor (ATAF),
8. cup-shaped cotyledon (CUC).

Alves *et al.*,2014

Transcription and Gene Expression

Protein-protein interactions

Transcription factors involved in plant defense

- The two major transcription factors involved in plant defense:

1. TGAs, the basic leucine zipper containing domain proteins (bZIP),
2. WRKYs, amino-acid sequence WRKYGQK (WRKY) pronounced 'worky'.

Leucine zipper motifs are a dimerization domain of the bZIP class of eukaryotic transcription factors. The two leucine zippers in effect form a Y-shaped structure, in which the zippers comprise the stem, and the two basic regions bifurcate symmetrically to form the arms that bind to DNA. This is known as the bZIP structural motif.

Transcription and Gene Expression

Protein-protein interactions

Transcription factors involved in plant defense

- In the nucleus, NPR1 can interact with different transcription factors (TFs), such as:

1. TGAs, and
2. WRKYs,

- to regulate the expression of downstream genes, like *PR-1*.

Transcription and Gene Expression

Plant transcription factors

1. TGA transcription factors and TGA boxes

- Salicylic acid (SA) is a simple phenolic compound distributed in a wide range of plant taxa.
- In response to pathogen attack, salicylic acid induces the expression of pathogenesis-related (PR) genes throughout the plant.
- TGA factors are believed to regulate this systemic induction because they bind to the *as-1 cis* element present in the promoters of PR genes, and because different TGA factors interact with the NPR protein, which is necessary for PR gene induction but does not bind to DNA by itself.

Jackoby *et al.*,2002

Transcription and Gene Expression

Plant transcription factors

TGA transcription factors and TGA boxes

- Salicylic acid (SA) is a simple phenolic compound distributed in a wide range of plant taxa.
- Defense pathways in plants are strongly associated with four compounds:

1. SA,
2. ethylene (ET),
3. jasmonic acid (JA), and
4. abscisic acid(ABA).

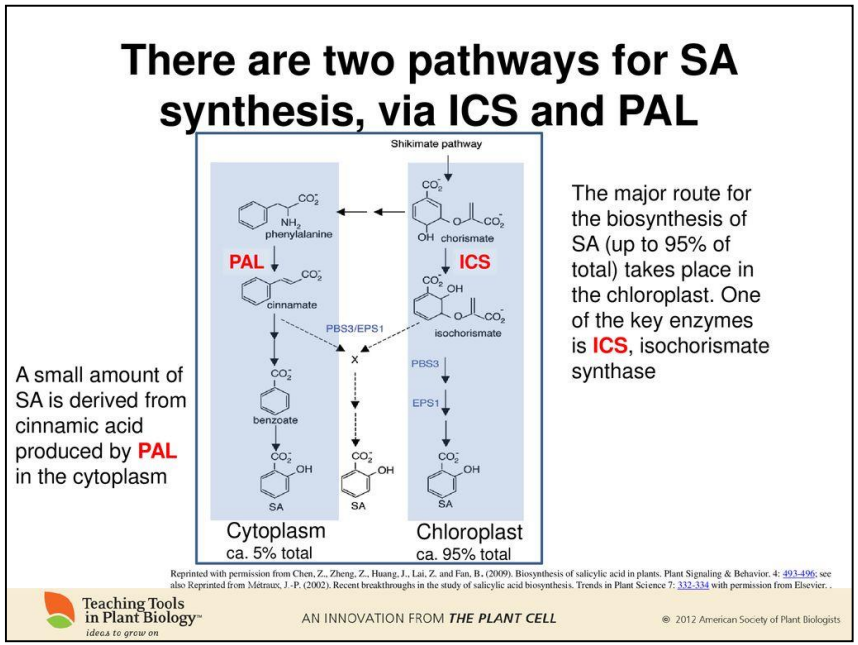
Kumar and Almomin,2018

Transcription and Gene Expression

Plant transcription factors

TGA transcription factors and TGA boxes

- SA is synthesized in plants from cinnamate and isochorismate and its involvement in the regulation of plant defense against specific types of pathogens.



Kumar and Almomin,2018

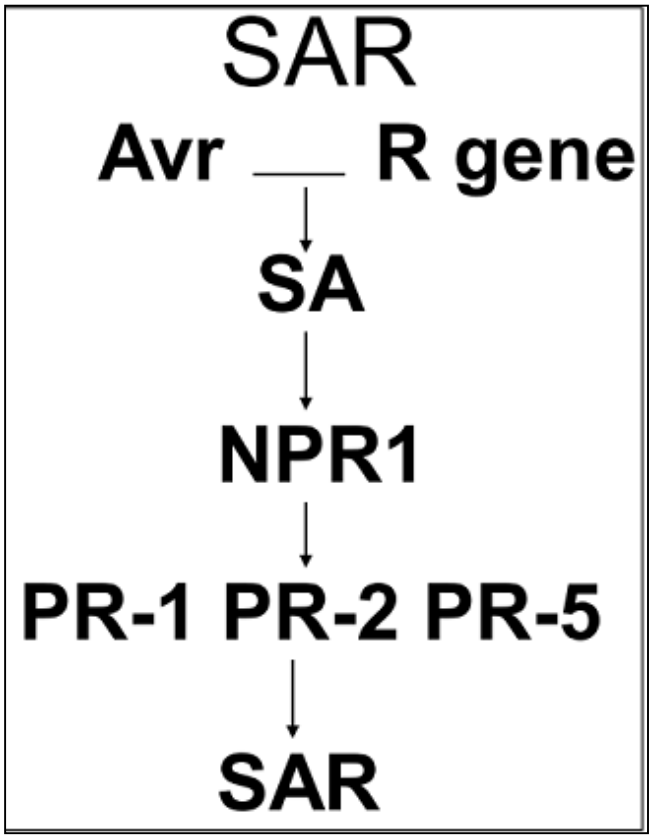
Transcription and Gene Expression

TGA transcription factors and TGA boxes

Regulation of defense gene expression by NPR1

- NPR1 protein is a receptor for the plant defense hormone salicylic acid (SA).
- SA controls NPR1 function by regulating its protein level in the nucleus.
- Downstream of SA, upstream of PR genes.

1. *npr1* mutants are susceptible to various pathogens.
2. Overexpression of NPR1 generates broad-spectrum resistance.

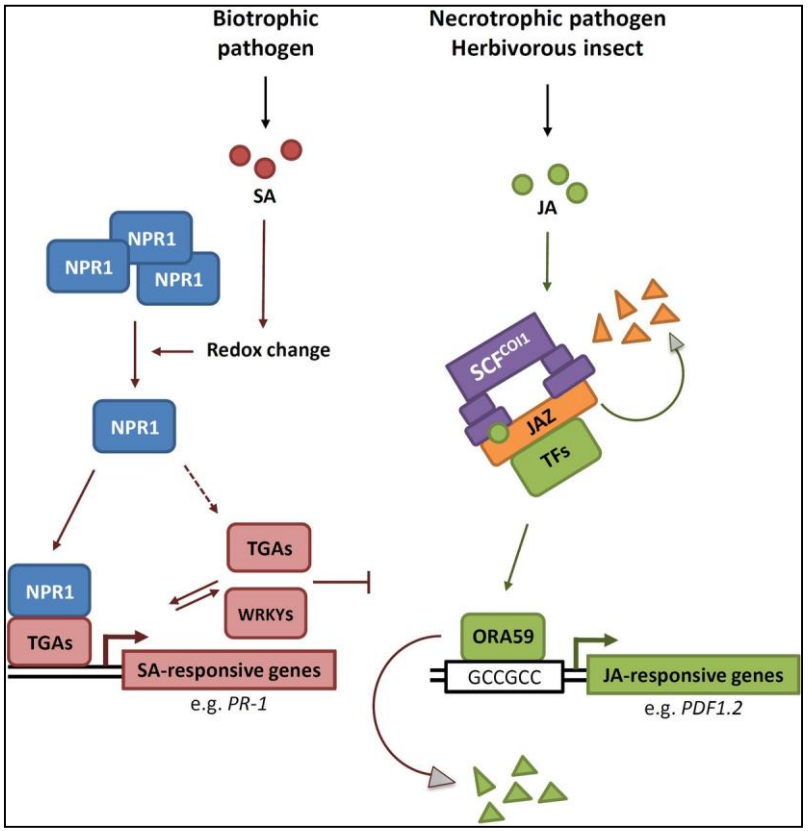


Transcription and Gene Expression

Plant transcription factors

TGA transcription factors and TGA boxes

- Of the other compounds, jasmonic acid (JA) plays a vital role in defense against insects and herbivores.
- Further complexity results from the significant overlap and crosstalk between the defense responses mediated by SA and those mediated by JA.



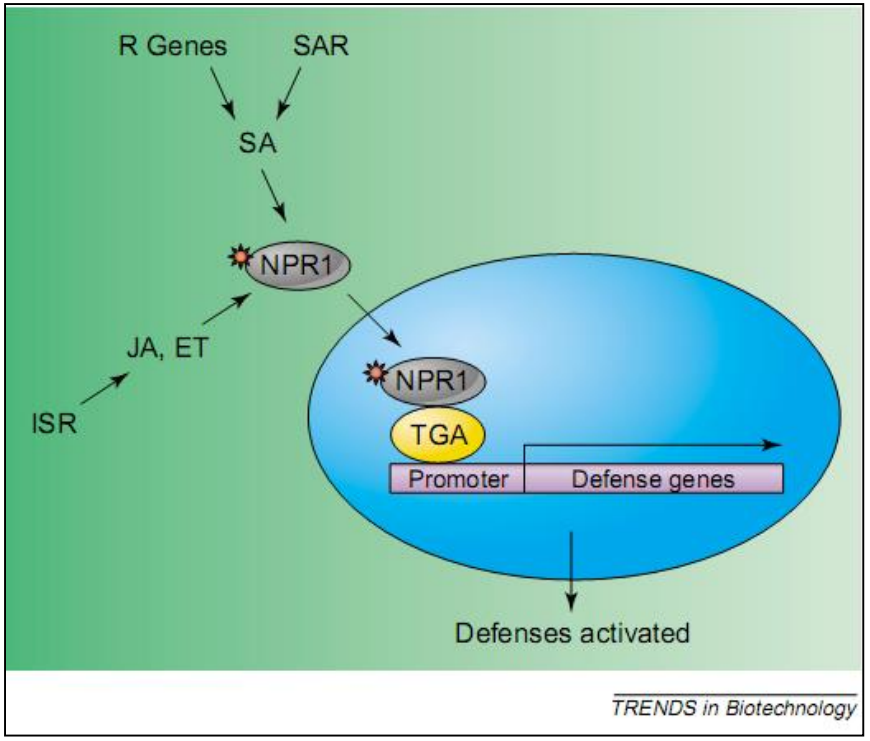
Kumar and Almomin,2018; Van der Does *et al.*,2013

Transcription and Gene Expression

TGA transcription factors (TFs) and TGA boxes

Regulation of defense gene expression by NPR1

- NPR1 gene encodes a transcription coactivator (NPR1) that plays a major role in the mechanisms regulating plant defense response.
- NPR1 plays a role in other defense-signaling pathways.
- NPR1 protein is a receptor for the plant defense hormone salicylic acid (SA).

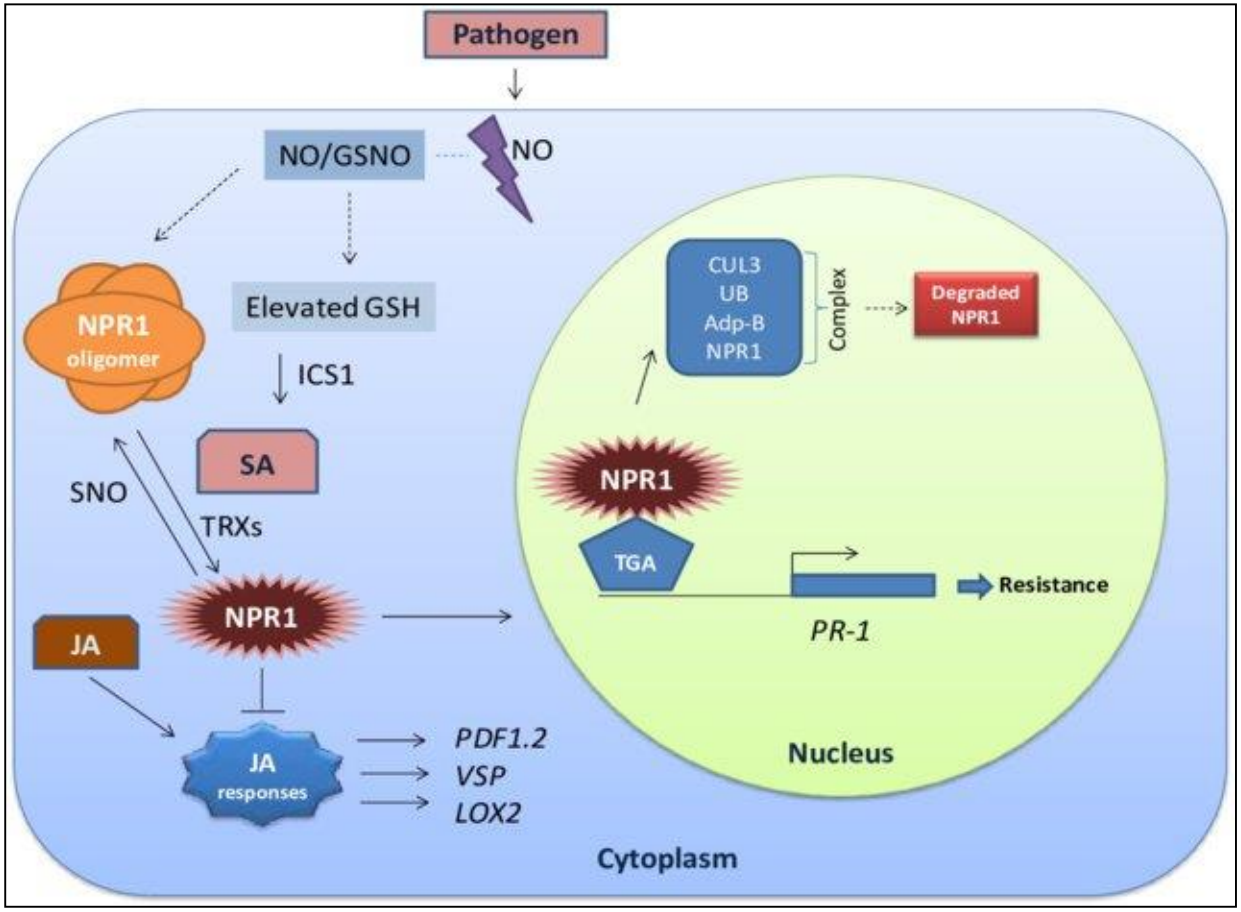


McDowell and Woffenden,2003

Transcription and Gene Expression

Plant transcription factors

TGA transcription factors and TGA boxes

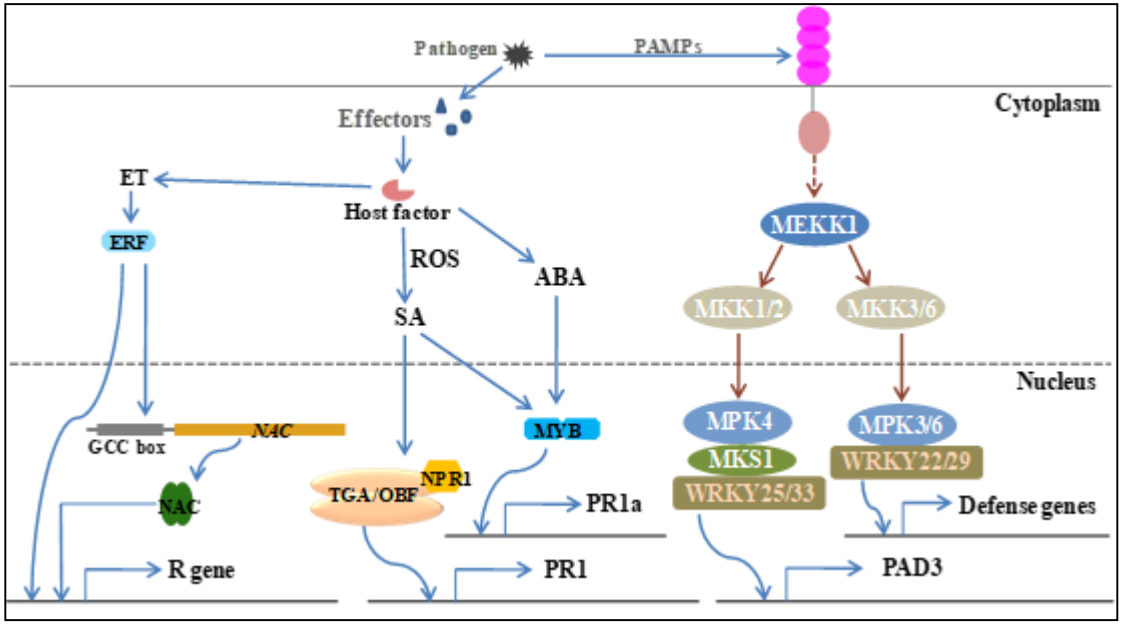


Kumar and Almomin,2018

Transcription and Gene Expression

bZip plant transcription factors

TGA factors and TGA boxes



Schematic illustrations of transcription factor in regulating defense gene expression. PAMP perception stimulates the induction of MKK, and then MPK forms a complex with WRKY. Phosphorylation of MKS1 by MPK4 and dissociation of the MKS1-WRKY25/33 complex from MPK4. This leads to the latter complex binds the promoter region of PAD3, which is required for the synthesis of antimicrobial camalexin. Effectors perception stimulates the induction of hormone signal and manipulated ERF, TGA, and MYB TFs to regulate R genes expression directly or indirectly. Some transcription factors could regulate reciprocally by binding to the promoter correspondingly.

Zhang *et al.*,2018

Transcription and Gene Expression

Plant transcription factors

TGA factors and TGA boxes

- TGA factors and TGA boxes:

1. belong to the family of bZip plant transcription factors, and
2. involved in regulation of defense gene expression by NPR1.

- The NPR1 protein is a receptor for the plant defense hormone salicylic acid.
- Salicylic acid (SA) is a plant immune signal produced after pathogen challenge to induce systemic acquired resistance.

In plants, basic region/leucine zipper motif (bZIP) transcription factors regulate processes including pathogen defence, light and stress signalling, seed maturation and flower development.

Transcription and Gene Expression

bZIP transcription factors in Arabidopsis

TGA factors and TGA boxes

- There are 10 TGA transcription factors in Arabidopsis of which seven (TGA1–TGA7) have been characterized with respect to their interaction with NPR1.
- These seven TGAs can be further divided into three subgroups based on sequence homology:

1. group I: TGA1 and TGA4;
2. group 2: TGA2, TGA5, and TGA6; and
3. group III TGA3 and TGA7.

Kesarwani *et al.*,2007

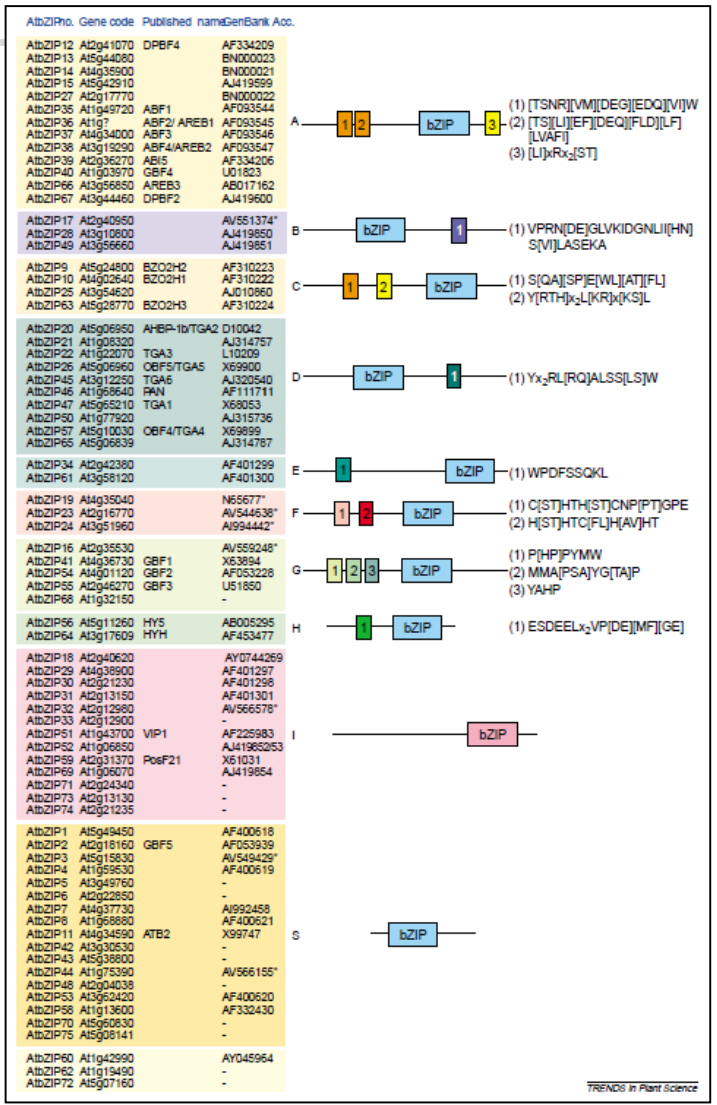
Transcription and Gene Expression

bZip plant transcription factors

TGA factors and TGA boxes

- Ten groups of bZIPs (A to S) with a similar basic region and additional conserved Motifs.
- Group D genes participate in defence against pathogens and development.
- Their involvement in defence mechanisms comes from work on the TGA factors (group D) in tobacco and *Arabidopsis*.

TGA factors (group D)



Jackoby *et al.*,2002

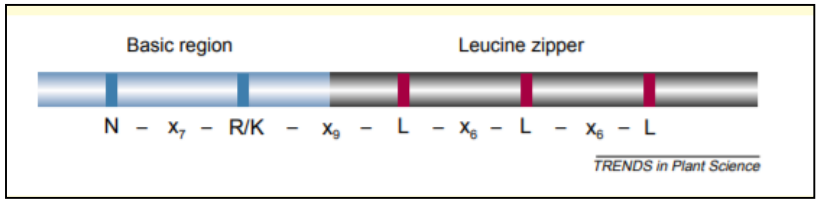
Transcription and Gene Expression

TGA factors and TGA boxes

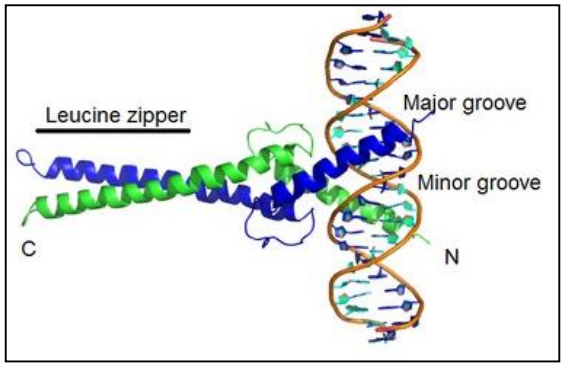
Involved in regulation of defense gene expression by NPR1

- The Arabidopsis genome sequence contains 75 distinct members of the bZIP family.
- basic region/leucine zipper (bZIP) TFs have:

1. a basic region that binds DNA, and
2. a leucine zipper dimerization motif.



bZIP dimer is bound to DNA fragment - each alpha helix represents a monomer.



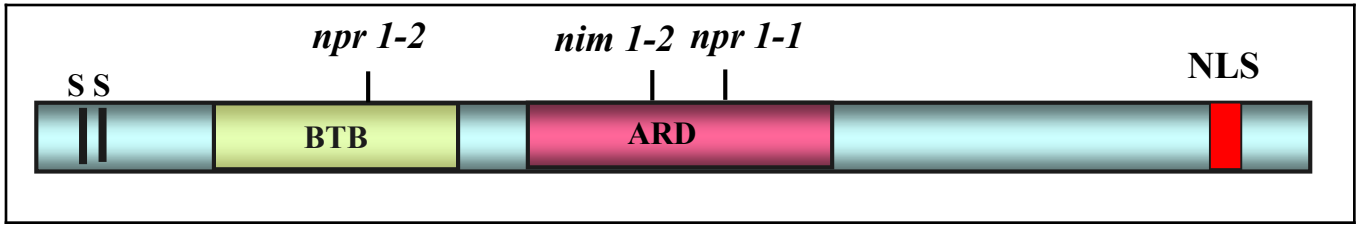
Jackoby *et al.*,2002;Wikipedia

Transcription and Gene Expression

Regulation of defense gene expression by NPR1

Structural and function of NPR1

- TGA transcription factors are implicated as regulators of pathogenesis-related (*PR*) genes.
- Induction of plant systemic acquired resistance (SAR) correlates with the expression of pathogenesis-related (*PR*) genes.



Transcription and Gene Expression

Plant transcription factors

2. WRKY factors and W box

- WRKY transcription factors(pronounced 'worky') are one of the largest families of transcriptional regulators found mostly **in plants** (green algae and land plants).
- The family has expanded during the evolution of plants and in turn, co-evolving of land plants with their adapted pathogens.
- The W box is a common binding site for WRKY transcription factors.

W box is a deoxyribonucleic acid (DNA) cis-regulatory element sequence, (T)TGAC(C/T), which is recognized by the family of WRKY transcription factors.

Rushton *et al.*,2010; Bakshi and Oelmüller,2014

Transcription and Gene Expression

Plant transcription factors

WRKY factors

- WRKY transcription factors have diverse biological functions in plants, but most notably are key players in plant responses to biotic and abiotic stresses.
- A (WKRY) gene that confers resistance or tolerance to multiple stresses would be highly useful for breeding.
- However, WRKY genes can also have opposite effects on abiotic and biotic stress tolerance since complex interactions among signaling networks can lead to both synergistic and antagonistic effects on regulation of plant responses to different stresses.

Bai *et al.*,2018

Transcription and Gene Expression

Role of WRKY proteins in both abiotic and biotic stresses

Stress type	Gene	Inducible factors	Function in stress
Abiotic stress	AtWRKY2	NaCl, mannitol	Negatively regulates ABA signaling
	AtWRKY18	ABA	ABA signaling and salt tolerance
	AtWRKY26	Heat	Heat tolerance
	AtWRKY39	Heat	Heat tolerance
	AtWRKY40	ABA	ABA signaling
	OsWRKY08	Drought, salinity, ABA, and oxidative stress	Tolerance towards oxidative stress
	OsWRKY11	Heat, drought	Xerothermic stress tolerance
	OsWRKY89	Salinity, ABA, and UV-B	UV-B radiation tolerance
	OsWRKY45	Salt, drought	Salt and drought tolerance
	OsWRKY72	Salt, drought	Salt and drought tolerance
	GmWRKY21	Salt, cold, and drought	Cold tolerance
	GmWRKY54	Salt, drought	Salt and drought tolerance
Biotic stress	AtWRKY38	Target of NPR1 during Systemic Acquired Resistance (SAR)	Increases Salicylic Acid- (SA-) mediated response
	AtWRKY53	Target of NPR1 during SAR	Increases SA- mediated response
	AtWRKY66	Target of NPR1 during SAR	Increases SA- mediated response
	AtWRKY70	Target of NPR1 during SAR	Node of convergence for SA-mediated and jasmonic acid- (JA-) mediated defence signaling
	OsWRKY31	*Magnaporthe oryzae*	Increased resistance
	OsWRKY45	*M. oryzae*	Increased resistance
	OsWRKY77	*Pseudomonas syringae*	Positively regulates plant basal resistance
	HvWRKY10	Effector Triggered Immunity (ETI)	ETI activator

In tomato there are 83 WRKY genes identified.

Banerjee and Roychoudhury,2015

Transcription and Gene Expression

Plant transcription factors

Three groups of WRKY transcription factors

- WRKY transcription factors (WRKYs) are a large family of transcriptional regulators, which are defined by the highly conserved WRKY domain (the WRKYGQK motif at the end of the N-terminal and a zinc-finger-like motif at the C-terminus.
- WRKYs are categorized into three groups:
- Group I (with two WRKY domains);
- Group II (with one WRKY domain) contain the zinc-finger-like motif C_2–H_2 (C–X_{4-5}–C–X_{22-23}–H–X_1–H);
- Group III contains one WRKY domain and a C_2–HC zinc-finger-like motif (C–X_7–C–X_{23}–H–X_1–C).

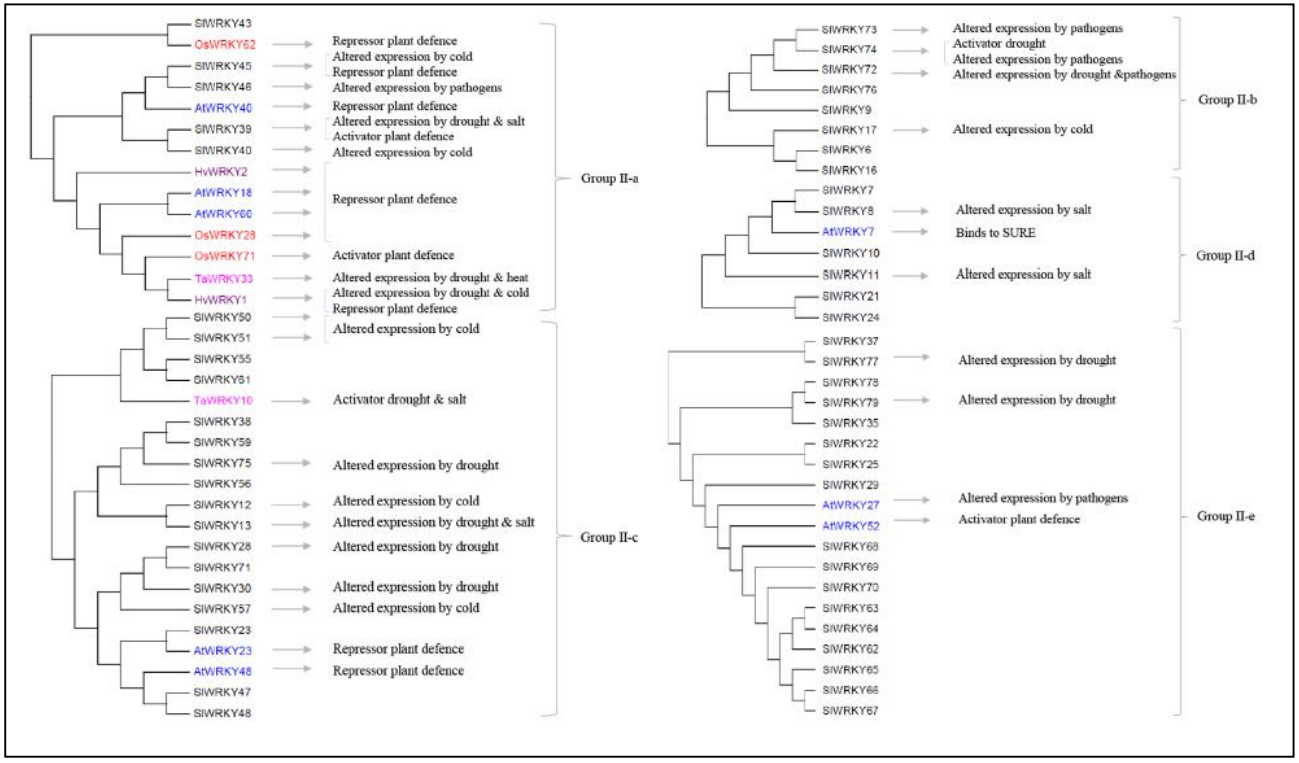
Bai *et al.*,2018

Transcription and Gene Expression

Plant transcription factors

Three subgroups of WRKY Group II

- Based on the primary amino acid sequences, Group II can be further divided into three subgroups (IIb, IId and IIe).



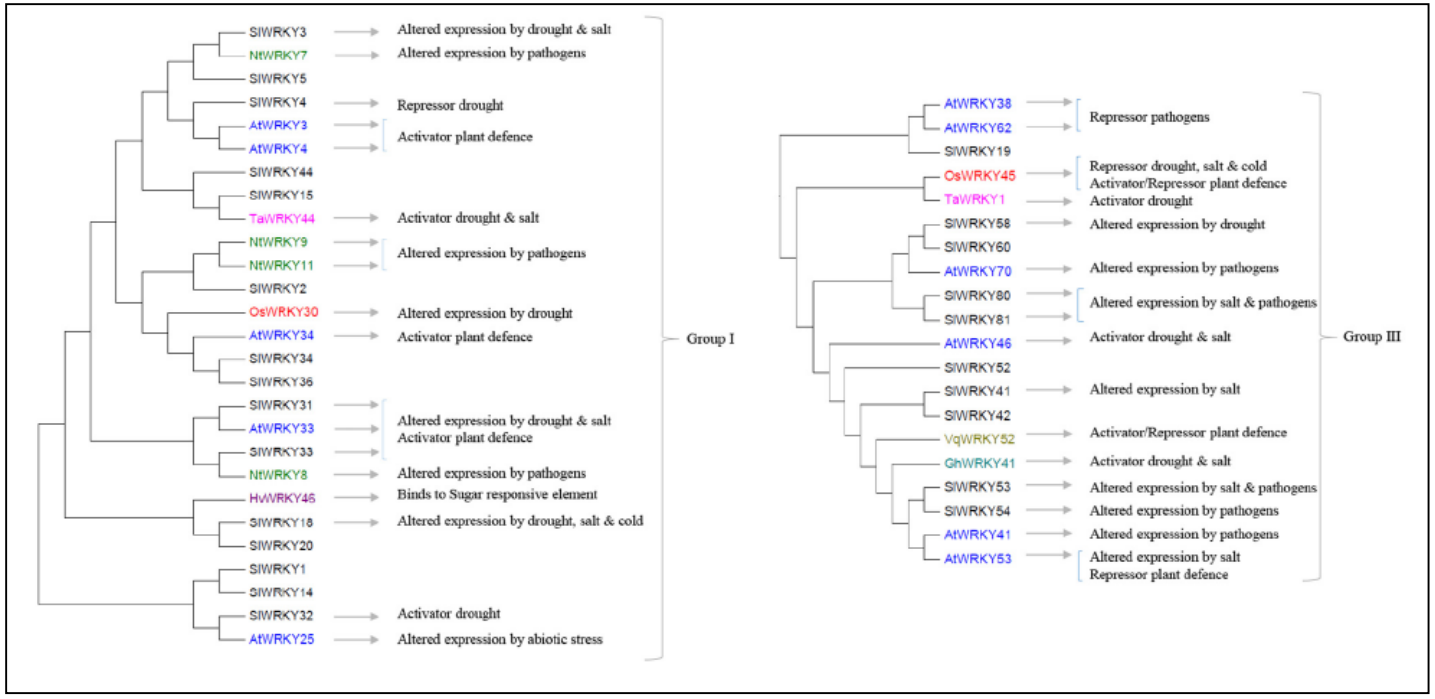
Bai *et al.*,2018

Transcription and Gene Expression

Plant transcription factors

WRKY factors and W box

- The involvements of Group I and III tomato SlWRKY genes and their homologs (highlighted in different colors) in plant responses to biotic and abiotic stresses.



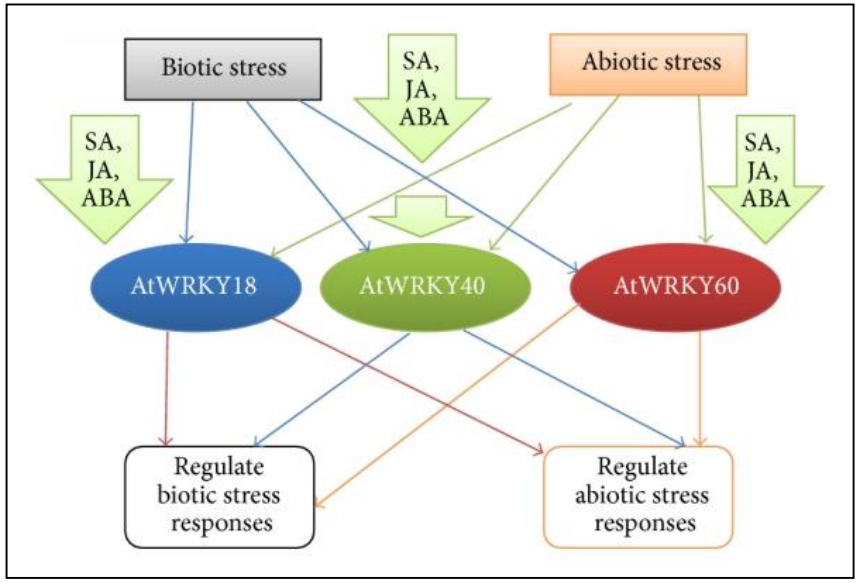
Bai *et al.*,2018

Transcription and Gene Expression

Plant transcription factors

WRKY factors and W box

- Specific WRKY proteins like AtWRKY18, AtWRKY40, and AtWRKY60 have been depicted as mediators of cross talk between plant responses against abiotic and biotic stresses.
- It has been reported that these proteins get accumulated in response to SA and JA during biotic stress as well as ABA during abiotic stress responses.

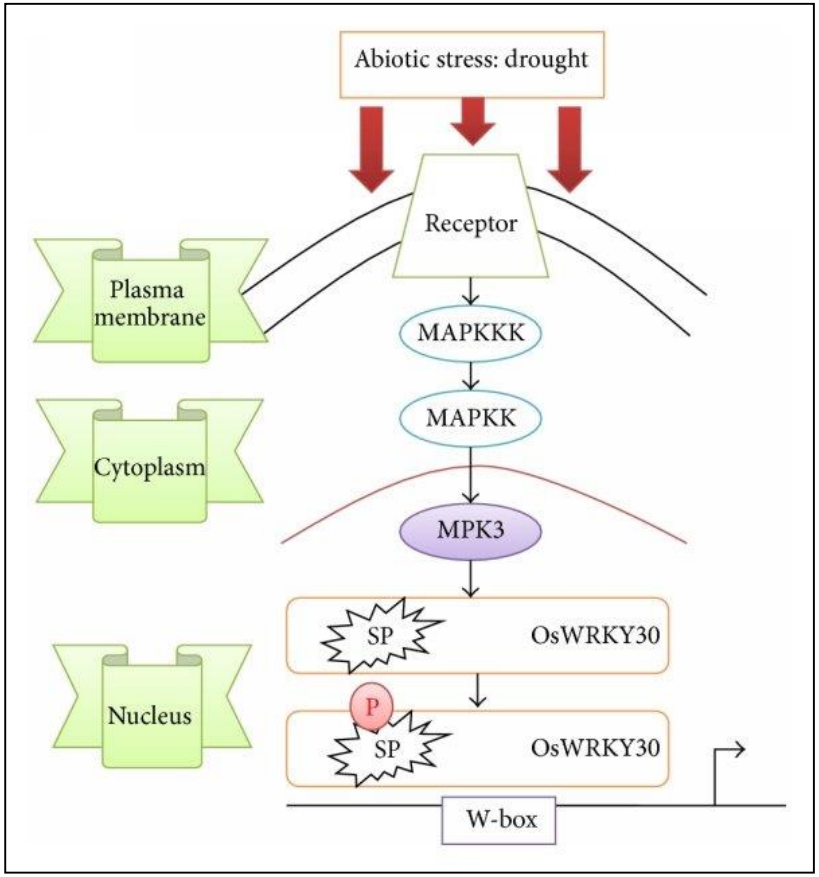


Banerjee and Roychoudhury,2015

Transcription and Gene Expression

The mitogen-activated protein kinase (MAPK) pathway induces the activity of OsWRKY30 during drought stress

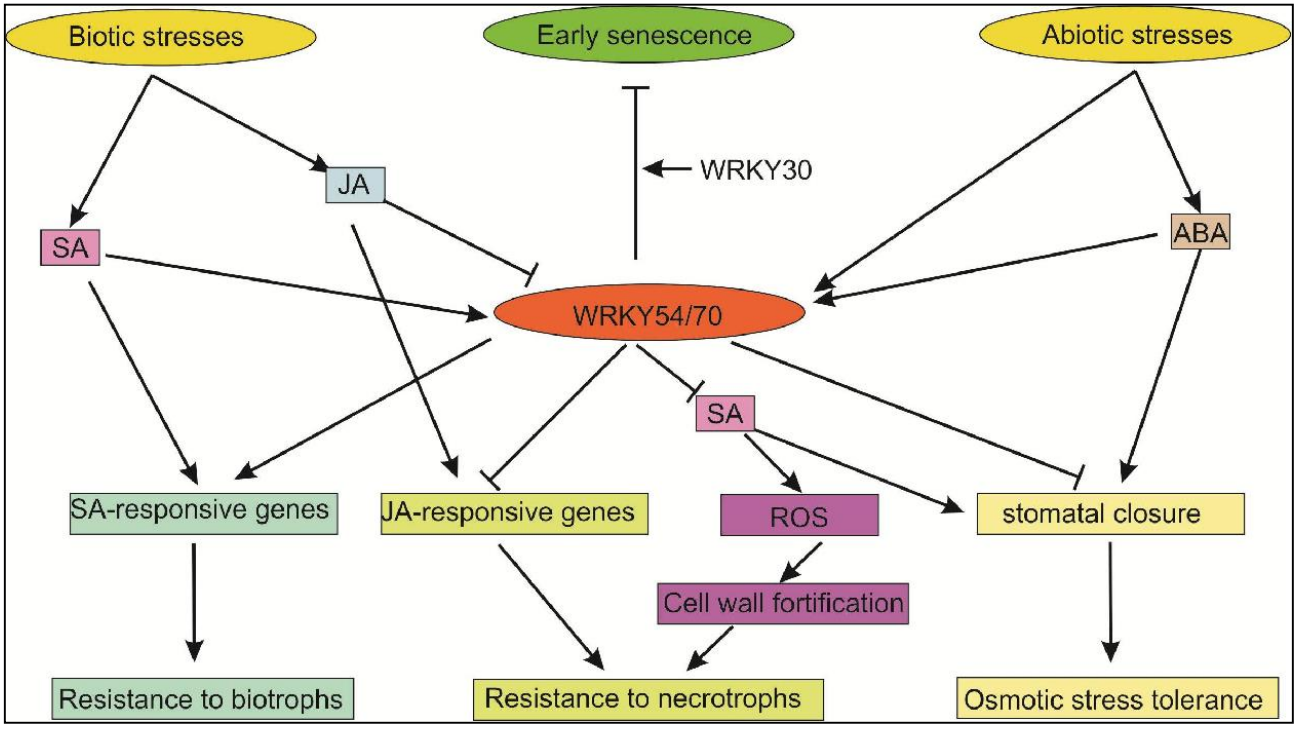
- The mitogen-activated protein kinase (MAPK) pathway induces the activity of OsWRKY30 during drought stress.
- Stress signals are sensed via a transmembrane receptor, which with the help of some unknown molecules and adaptor proteins activates the MPK/MAPK pathway.
- This leads to the phosphorylation and activation of the MPK3.
- MPK3 phosphorylates the target Ser residue in the SP motif of OsWRKY30 and activates the same.
- The activated WRKY protein then undergoes a conformational change which favourably allows it to bind to the W-box of its target gene to induce transcription.
- The protein product encoded by the target gene probably helps the plant system in combating the drought stress.



Banerjee and Roychoudhury,2015

Transcription and Gene Expression

The involvement of two transcription factors WRKY54 and WRKY70 in developmental senescence, osmotic stress as well as plant defense responses



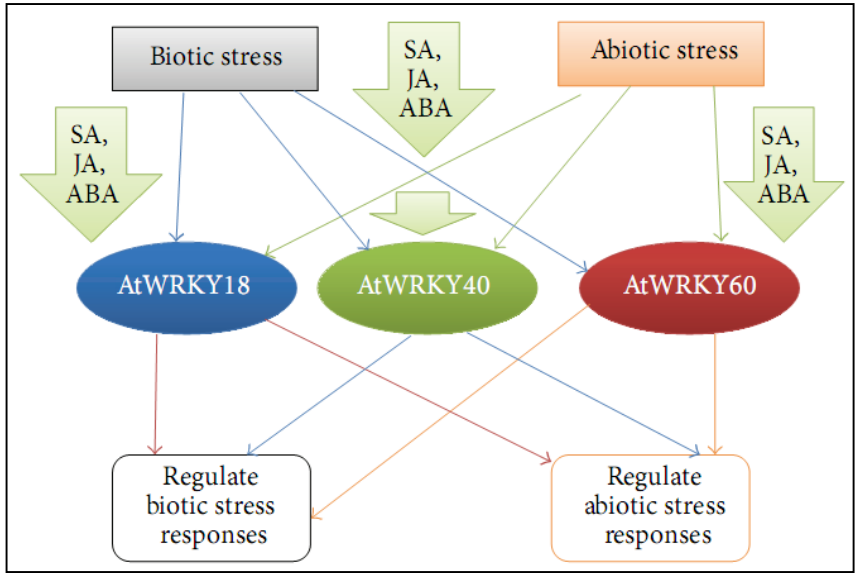
The involvement of two transcription factors WRKY54 and WRKY70 in developmental senescence, osmotic stress as well as plant defense responses. The arrows indicate induction or positive modulation; the blunt-end arrows indicate block or suppression. SA, salicylic acid; JA, jasmonic acid; ABA, abscisic acid; ROS, reactive oxygen species.

Li,2014

Transcription and Gene Expression

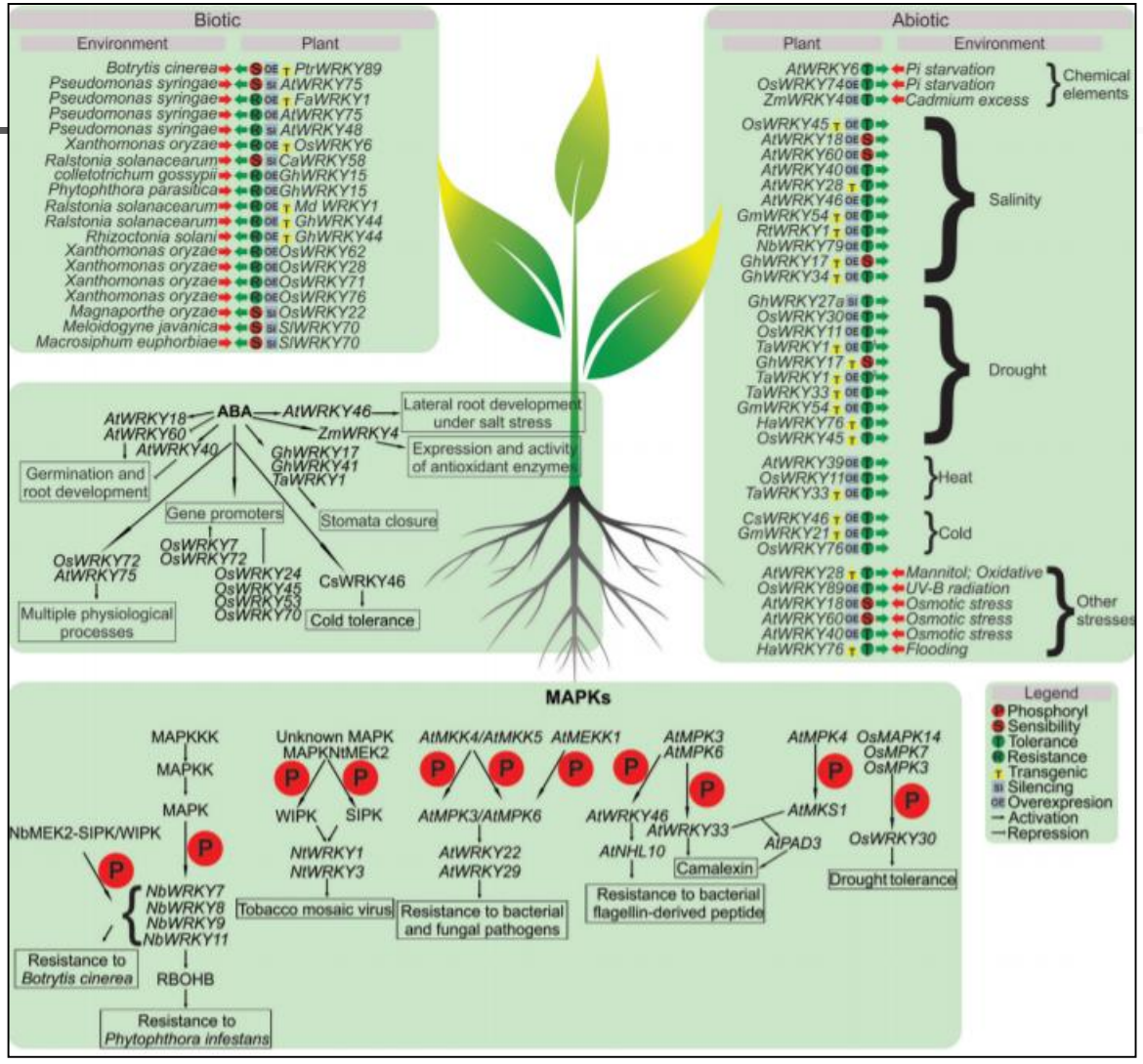
The structurally related proteins AtWRKY18, AtWRKY40, and AtWRKY60 have provided some hint towards the occurrence of such crosstalk

- Specific WRKY proteins like AtWRKY18, AtWRKY40, and AtWRKY60 have been depicted as mediators of crosstalk between plant responses against abiotic and biotic stresses.
- It has been reported that these proteins get accumulated in response to SA and JA during biotic stress as well as ABA during abiotic stress responses.



Banerjee and Roychoudhury,2015

Overview of the most recent reports concerning WRKY roles in plant defense, including the relationships with MAPKs, abscisic acid(ABA) signaling, and responses to biotic and abiotic stresses.



Finatto *et al.*,2018

WRKY proteins
WRKY domain /W box

- The WRKY domain(DNA binding domain) has almost invariant WRKY amino acid sequence at the N-terminus
- The invariant core of the W box is essential for function and WRKY binding.

a) The WRKY domain consensus for each WRKY subfamily in higher plants:

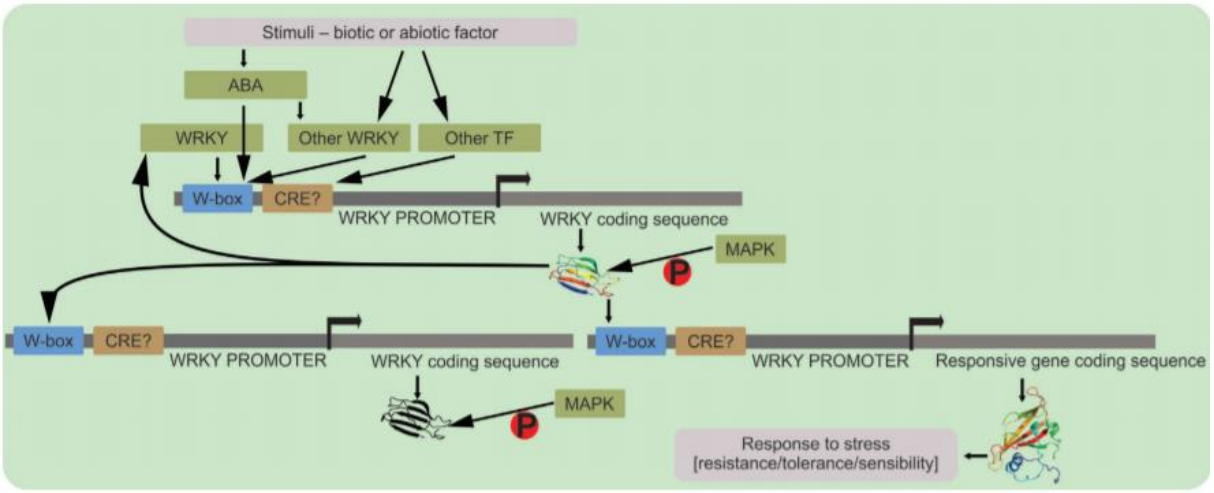
- The WRKY motif is highlighted in yellow and the cysteines and histidines that form the zinc finger are shown in blue.
- The four β-strands are shown in red.

b) Two views of a spacefill structural model of the C-terminal WRKY domain from AtWRKY4.

Transcription and Gene Expression

Plant transcription factors

WRKY factors and W box



Regulatory mechanism of WRKY transcription factors.
The signaling pathway starts with an environmental stimulus through ABA signaling or by *trans*-regulation, which comprise direct transcription factor activation. The *trans* regulation can occur with a WRKY member, in case of presence of a W-box, or with other TFs from different families. An auto-regulation mechanism can occur, or regulation by promoter binding of a different WRKY member. In some cases, the phosphorylation by an MAPK cascade is a determinant process for the correct WRKY protein function. When activated, WRKY TFs can regulate a different WRKY by binding its W-box sequence, or regulate some other responsive gene conferring tolerance, resistance, or sensibility toward the environmental stimuli.

Finatto *et al.*,2018

Transcription and Gene Expression

Plant transcription factors

WRKY factors and W box

- The W box is a common binding site for WRKY transcription factors, proteins that have been shown to be involved in regulation of early defense-response gene expression.
- WRKYs can act as:

1. transcriptional activators, or
2. repressors.

Transcription and Gene Expression

Plant transcription factors

WRKY factors and W box

- E.g.

1. WRKY25 and -33 are involved in biotic and abiotic stress responses; they function as negative regulators after *P. syringae* infection, but
2. As positive regulators in conferring resistance against NaCl, cold, and heat and oxygenic stress.

Transcription and Gene Expression

Plant transcription factors

Functions of WRKY TFs

- WRKY transcription factors have diverse biological functions in:

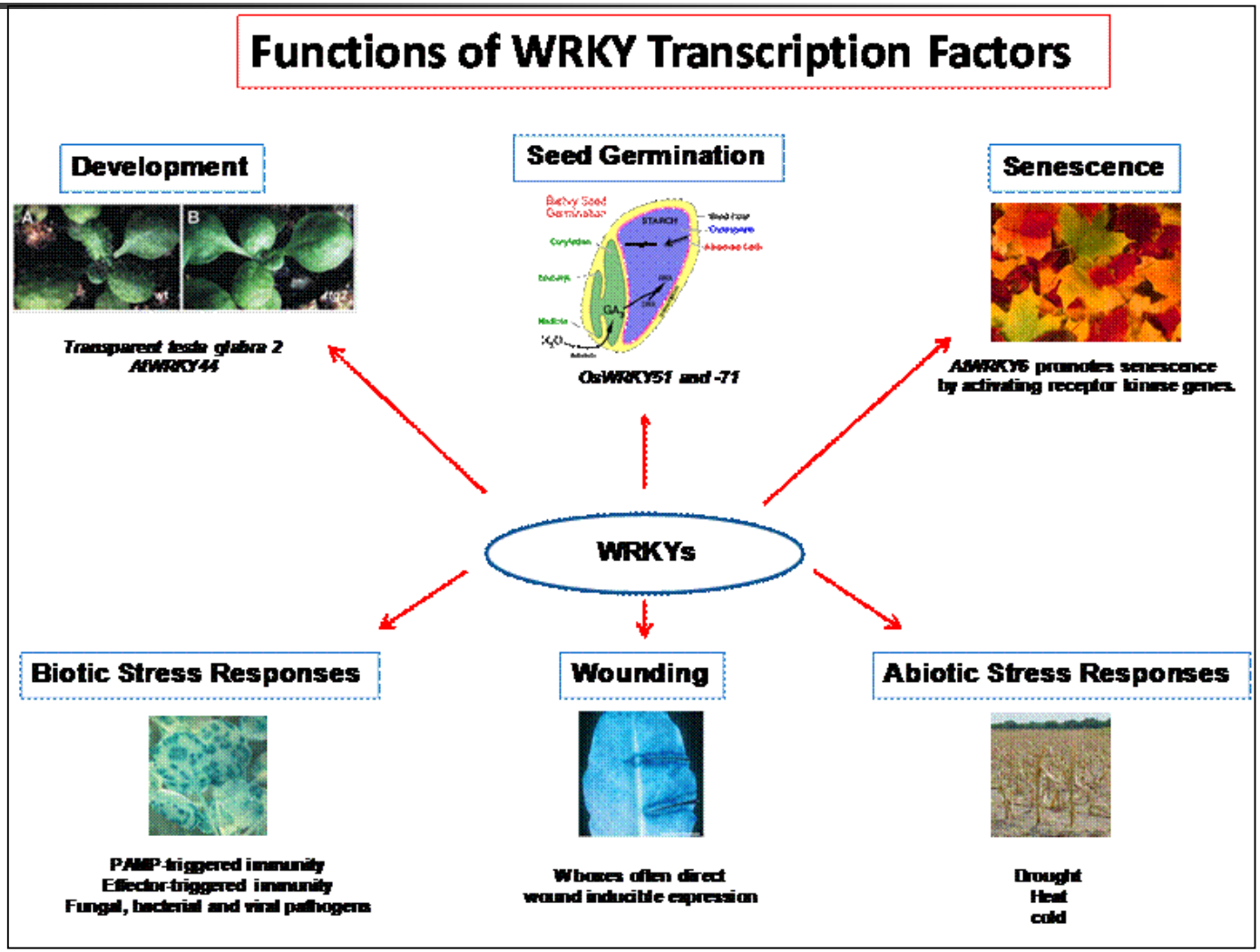
1. plant disease resistance,
2. abiotic stress responses,
3. nutrient deprivation,
4. senescence,
5. Seed, and
6. trichome development, embryogenesis, as well as additional developmental and hormone-controlled processes.

Bakshi and Oelmüller,2014

Transcription and Gene Expression

Plant transcription factors

Functions of WRKY TFs

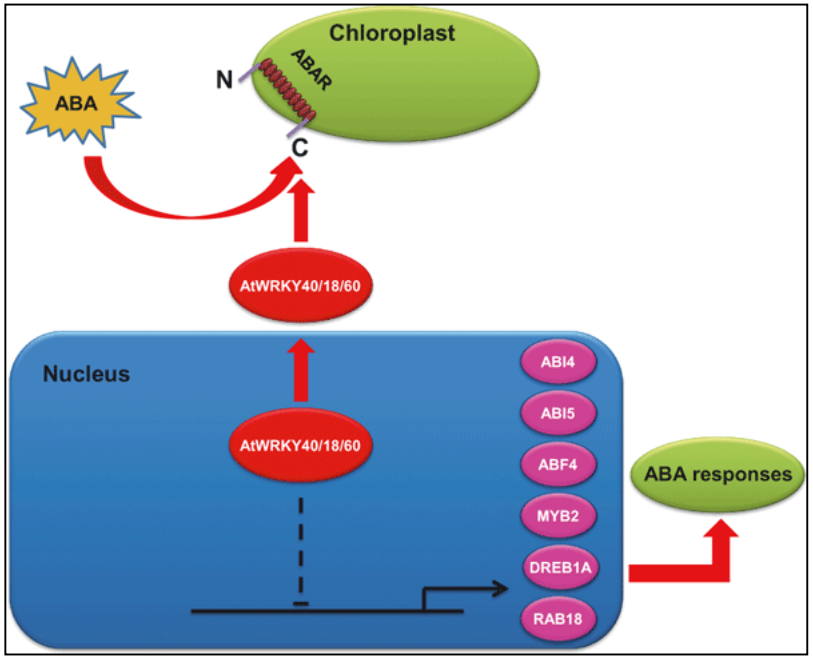


Transcription and Gene Expression

Functions of WRKY TFs

The role of WRKYs and their genes in abiotic stress (A)

- A schematic representation of the mechanism by which AtWRKY40, AtWRKY18 and AtWRKY60 regulate abscisic acid(ABA) responses.
- Eexpression of ABA-responsive genes such as *ABF4*, *ABI4*, *ABI5*, *DREB1A*, *MYB2* and *RAB18* is induced and ABA responses occur.
- The dotted line denotes de-repression of gene expression as the WRKY TFs are removed from the nucleus.



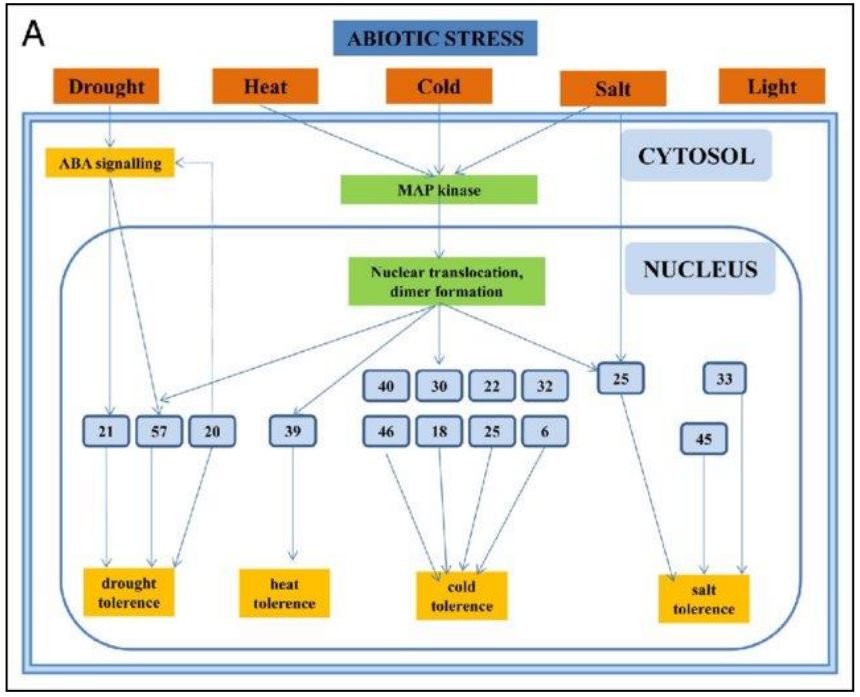
Rushton *et al.*,2012

Transcription and Gene Expression

Functions of WRKY TFs

The role of WRKYs and their genes in biotic and abiotic stresses

- **Abiotic stress:**
- The role of WRKYs and their genes in abiotic stress (**A**), biotic stress (**B**), the cross-talk between biotic and abiotic stress (**C**), metabolism (**D**), hormone signaling (**E**), and epigenetic control (**F**).
- The gray square boxes with the number refer to the individual WRKY proteins. *WRKY* genes are in green. The crosses demonstrate inhibition of expression. A P in a yellow star refers to phosphorylation. Ub, ubiquitin.



Ubiquitin is a small regulatory protein that exists (ubiquitously) in all eukaryotic cells.

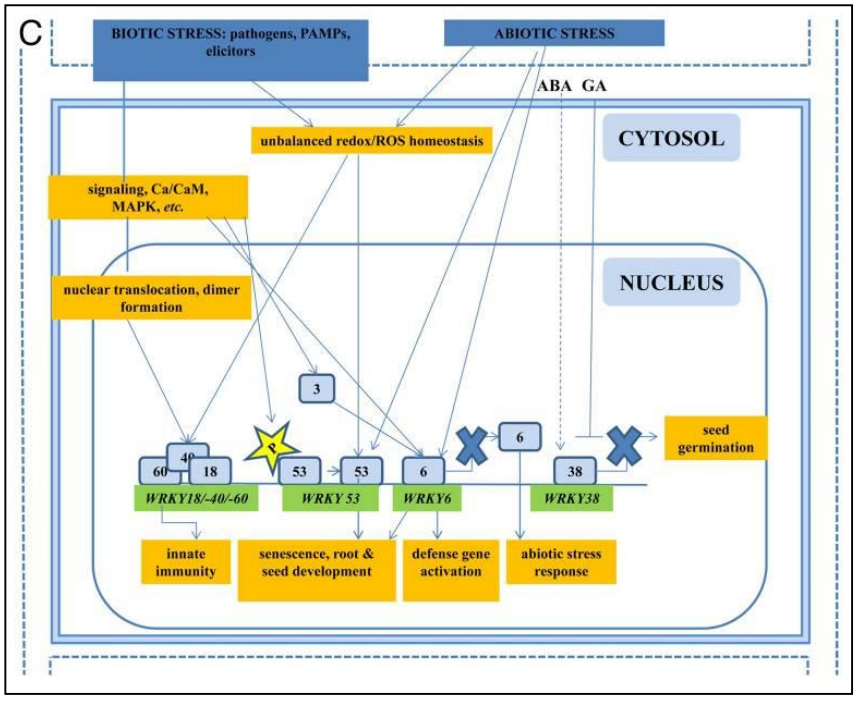
Bakshi and Oelmüller,2014

Transcription and Gene Expression

Functions of WRKY TFs

The role of WRKYs and their genes in biotic and abiotic stresses

- **Biotic stress: pathogens, PAMPs and elicitors:**
- The role of WRKYs and their genes in abiotic stress (**A**), biotic stress (**B**), the cross-talk between biotic and abiotic stress (**C**), metabolism (**D**), hormone signaling (**E**), and epigenetic control (**F**).
- The gray square boxes with the number refer to the individual WRKY proteins. *WRKY* genes are in green. The crosses demonstrate inhibiton of expression. A P in a yellow star refers to phosphorylation. Ub, ubiquitin.



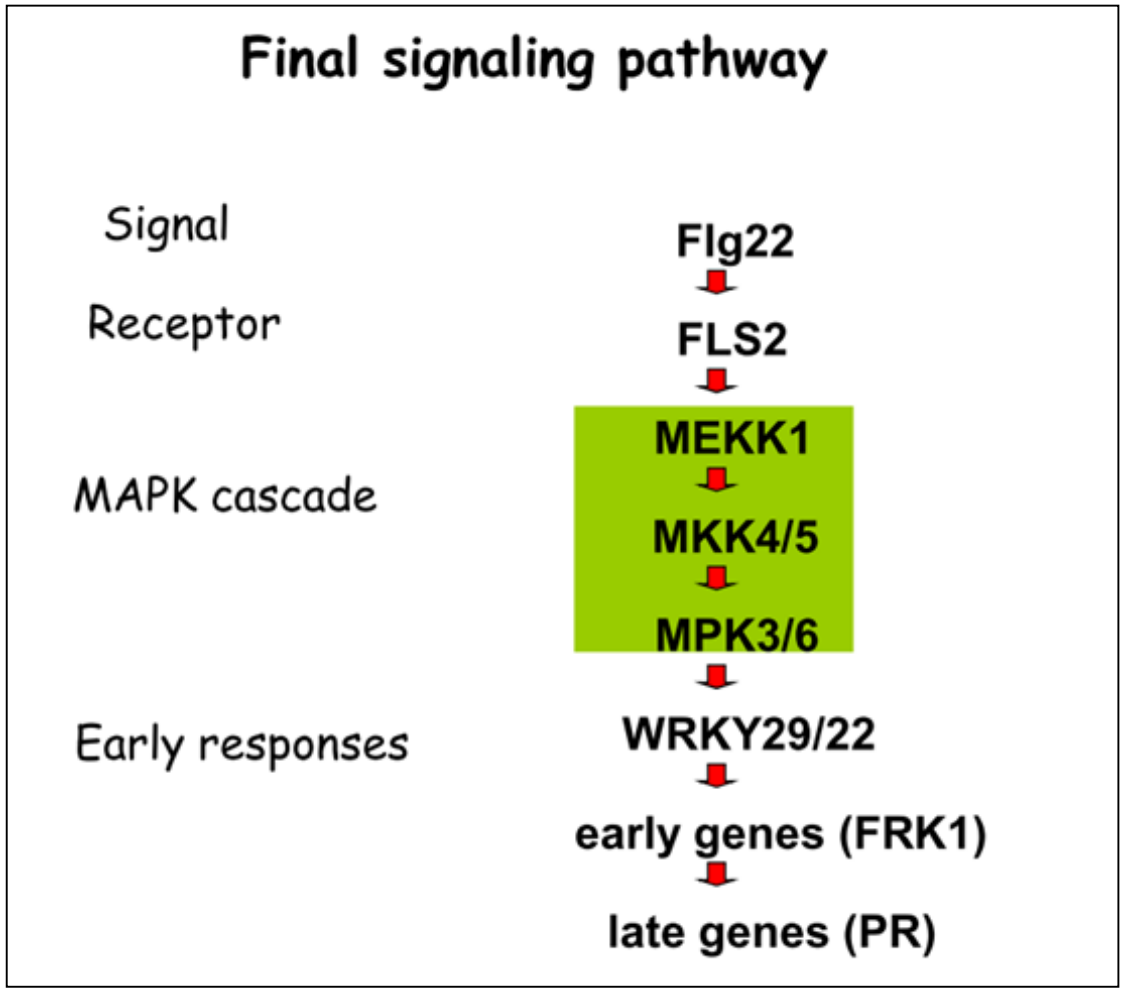
Ubiquitin is a small regulatory protein that exists (ubiquitously) in all eukaryotic cells.

Bakshi and Oelmüller,2014

WRKY transcription factors

MTI or PTI: MAMP or PAMP-triggered immunity

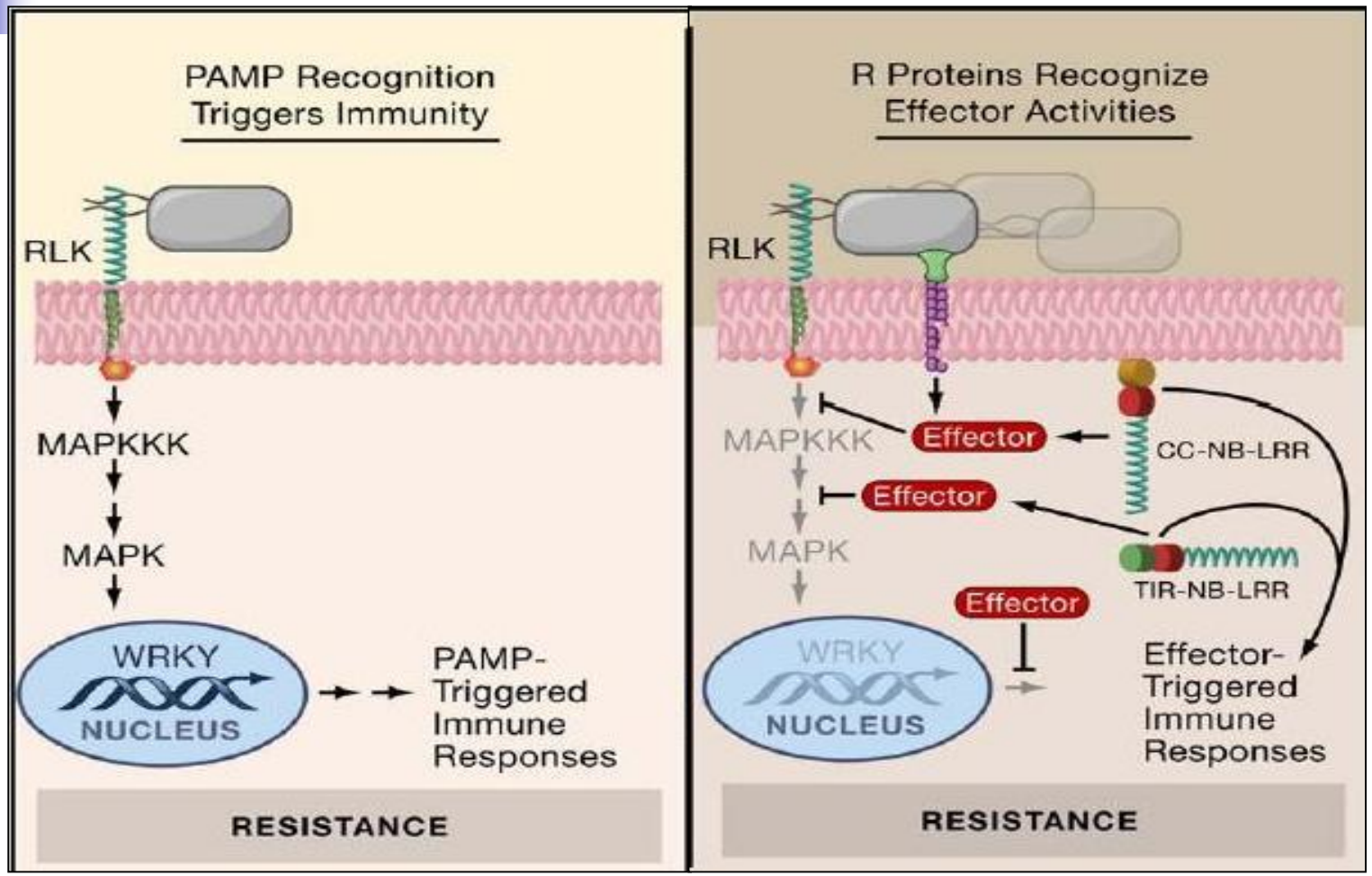
The effect of WRKY29



WRKY transcription factors

Involved in defense-related signal transduction pathways

PAMP or effector triggered immunity



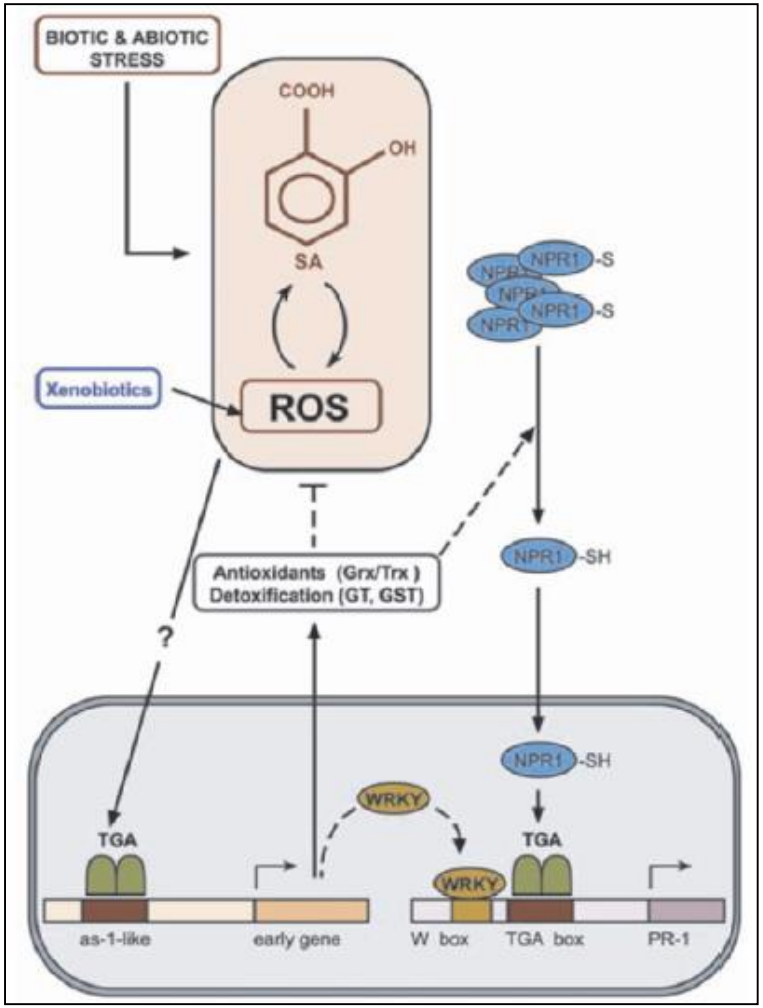
RLK: Plant receptor-like protein kinases. Plant receptor-like kinases (RLKs) are transmembrane proteins.

Transcription and Gene Expression

WRKY genes/W box in Arabidopsis genome+ TGA factors and TGA boxes

1. Early defense genes (FRK1);
2. Late defense genes(PR).

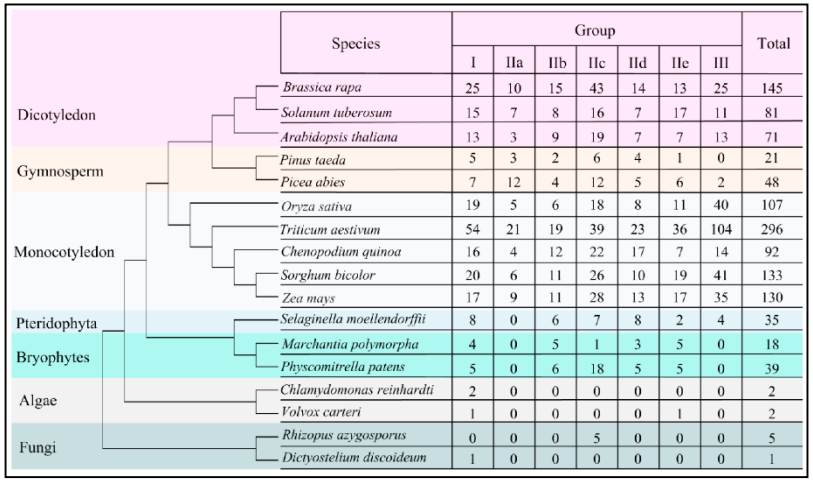
- Several genes coding for WRKY factors are early induced by SA and pathogens, and some of them do not require NPR1 for their induction.
- SA and ROS are signals for genetic-controlled defense reactions.
- Interestingly, WRKY factors are activated by MAPK and ROS.



WRKY proteins

Species phylogeny and numbers of WRKY genes in each species

- The WRKY gene family plays a unique role in plant stress tolerance.
- Quinoa is a cultivated crop worldwide that is known for its high stress tolerance.
- Using a genome-wide search method, we identified 1226 WRKY genes in:
- 15 plant species,
- seven animal species, and
- seven fungi species.



	Species	Group							Total
		I	IIa	IIb	IIc	IId	IIe	III	
Dicotyledon	*Brassica rapa*	25	10	15	43	14	13	25	145
	Solanum tuberosum	15	7	8	16	7	17	11	81
	Arabidopsis thaliana	13	3	9	19	7	7	13	71
Gymnosperm	*Pinus taeda*	5	3	2	6	4	1	0	21
	Picea abies	7	12	4	12	5	6	2	48
Monocotyledon	*Oryza sativa*	19	5	6	18	8	11	40	107
	Triticum aestivum	54	21	19	39	23	36	104	296
	Chenopodium quinoa	16	4	12	22	17	7	14	92
	Sorghum bicolor	20	6	11	26	10	19	41	133
	Zea mays	17	9	11	28	13	17	35	130
Pteridophyta	*Selaginella moellendorffii*	8	0	6	7	8	2	4	35
Bryophytes	*Marchantia polymorpha*	4	0	5	1	3	5	0	18
	Physcomitrella patens	5	0	6	18	5	5	0	39
Algae	*Chlamydomonas reinhardti*	2	0	0	0	0	0	0	2
	Volvox carteri	1	0	0	0	0	1	0	2
Fungi	*Rhizopus azygosporus*	0	0	0	5	0	0	0	5
	Dictyostelium discoideum	1	0	0	0	0	0	0	1

Yue *et al.*,2019

Transcription and Gene Expression

WRKY factors and W box

The different groups of WRKY Domains in flowering plants

Wikipedia,2019

The major types of host-pathogen interactions

Pathogenicity/virulence mechanisms of some important plant bacteria

Pathogenicity of:

Gram-negative plant pathogenic bacteria

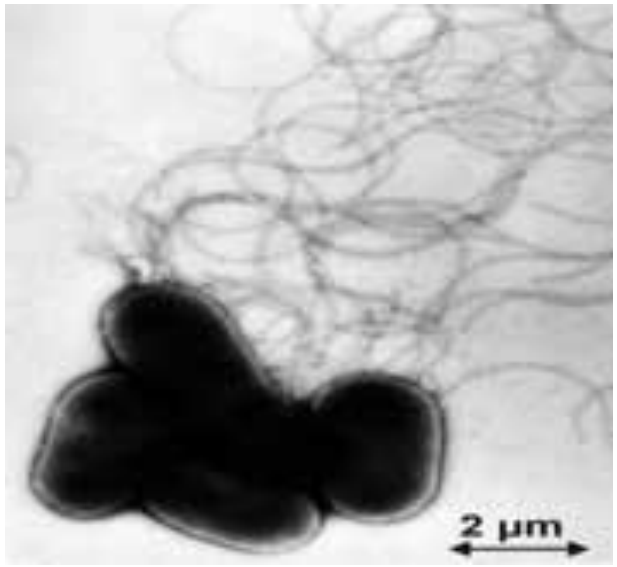
Virulence mechanisms of Gram-positive plant pathogenic bacteria

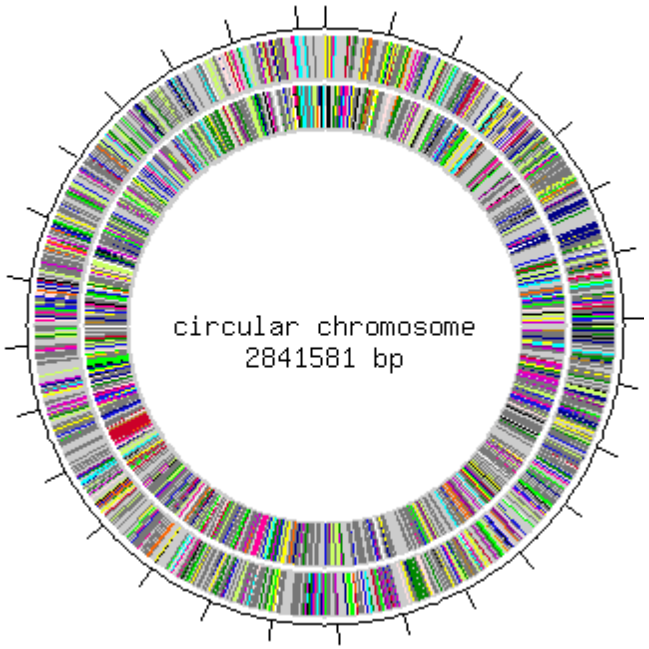
Pathogenicity of:
Agrobacterium tumefaciens

Crown Gall Disease

Cell and chromosome

Agrobacterium tumefaciens

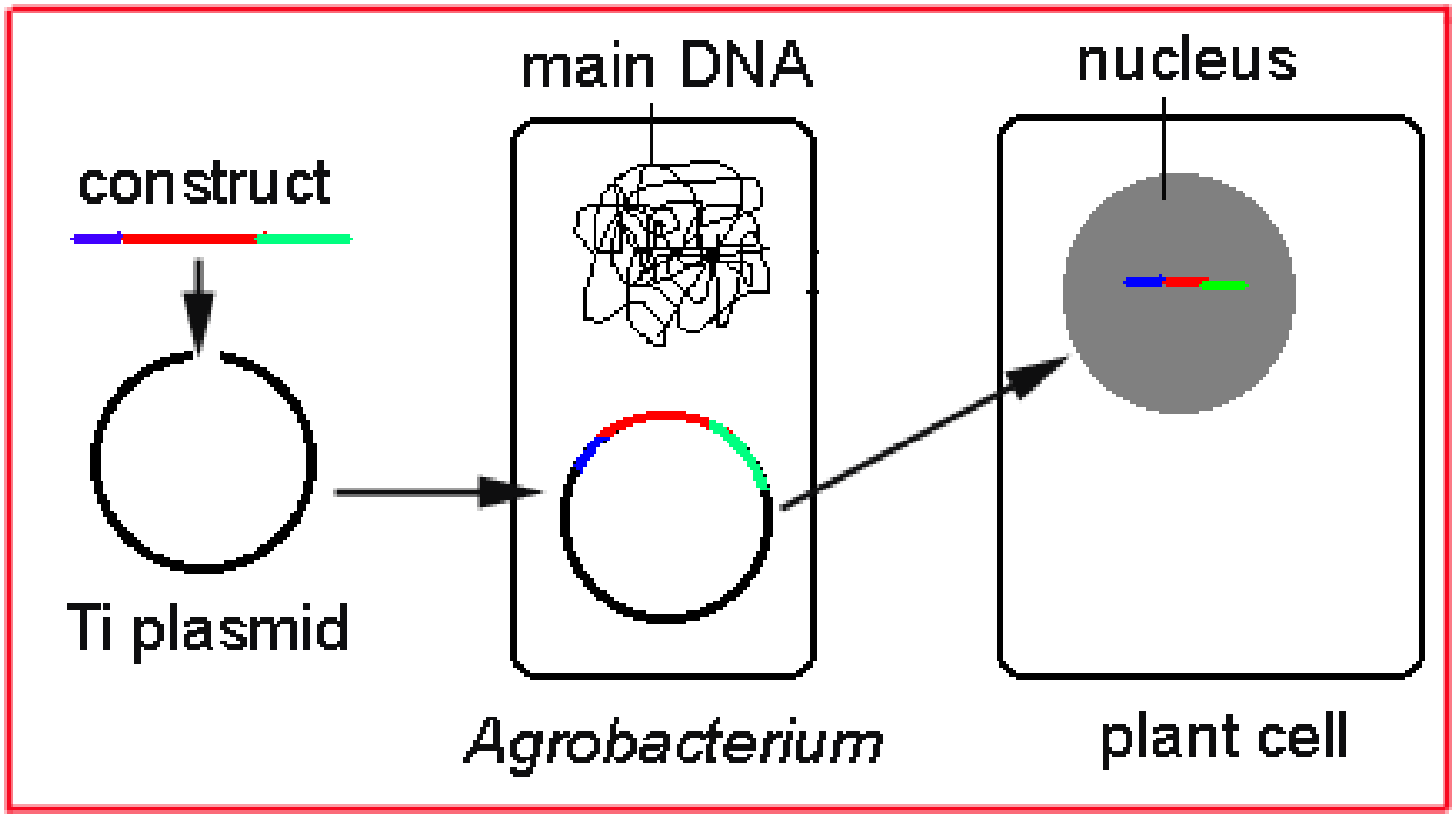




Collins Johnson

How does *Agrobacterium* cause galls on plants?

Chromosome or plasmid?



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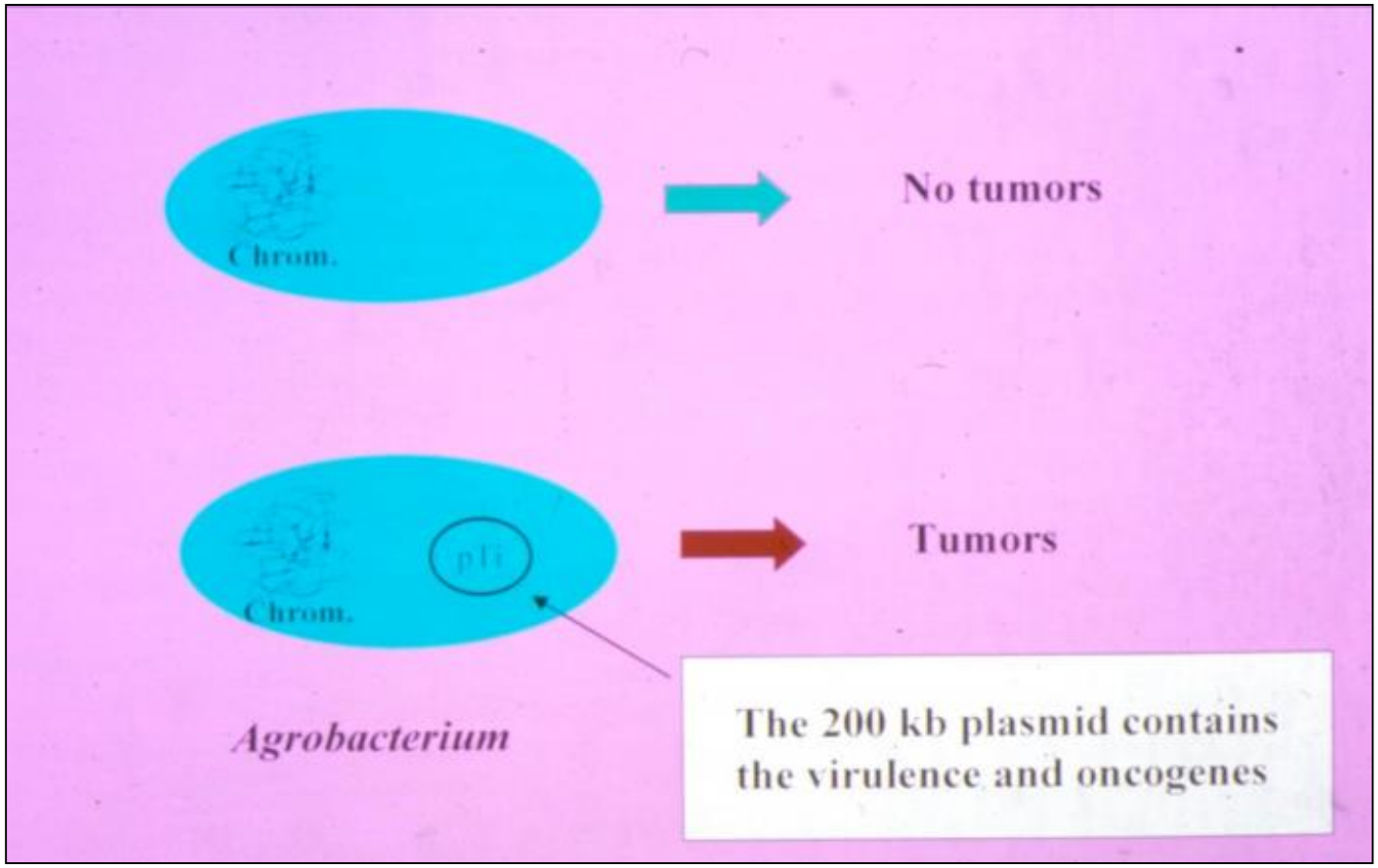


A. tumefaciens infection process

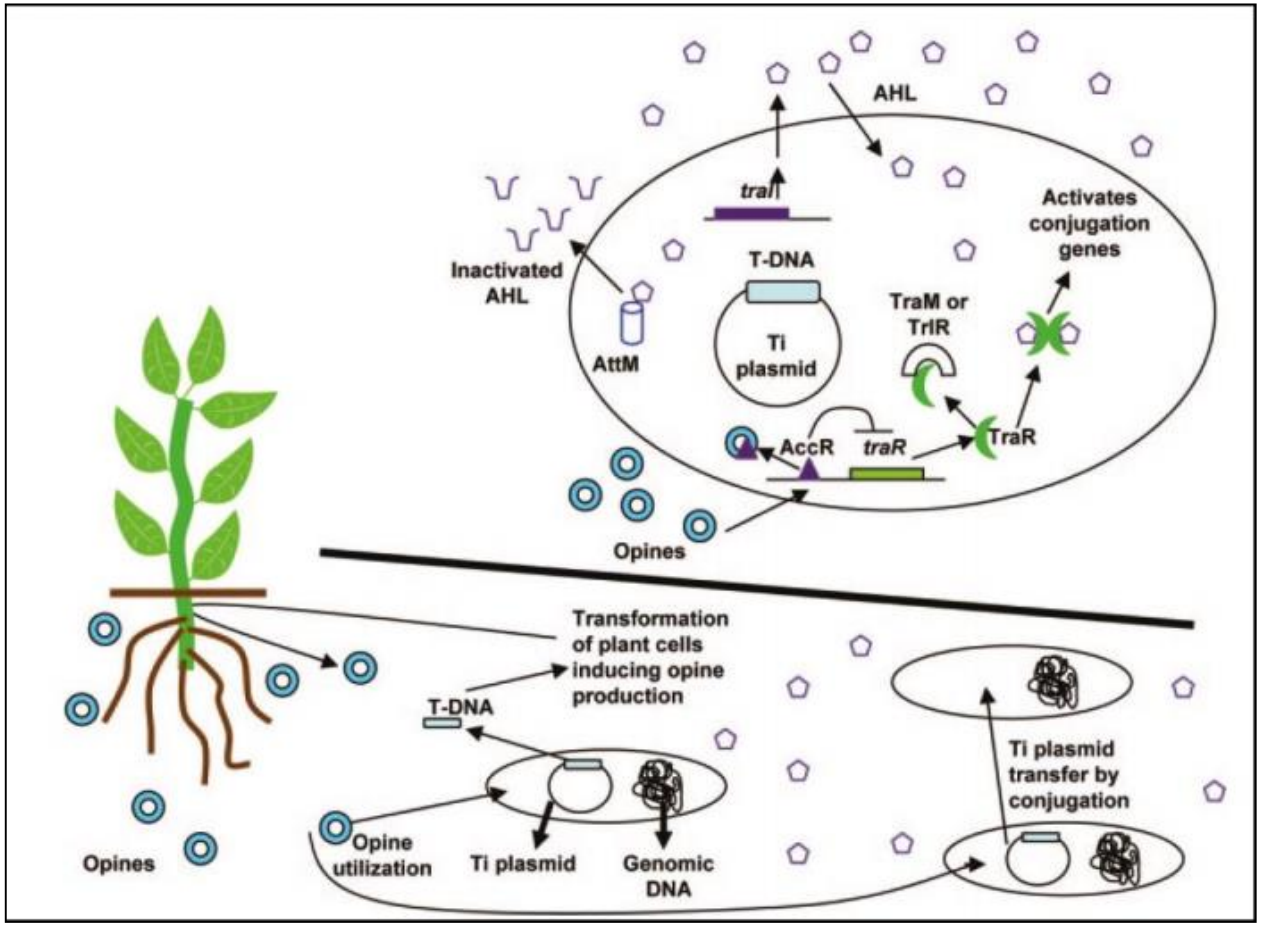
Quorum-sensing cross talk between *A. tumefaciens* and its host plant

- The transfer and integration of bacterial T-DNA into plant cells result in tumorogenesis, leading to crown gall disease in plants.
- The tumor cells directed by the bacterial DNA produce and release opines, which are metabolized by the *A. tumefaciens* present in the soil around the plant roots.
- The conjugal transfer of the Ti plasmid among the rapidly proliferating *A. tumefaciens* in soil is regulated by bacterial quorum sensing as well as by plant-produced opines.

González and Keshavan,2009

A. tumefaciens infection process

Quorum-sensing cross talk between *A. tumefaciens* and its host plant

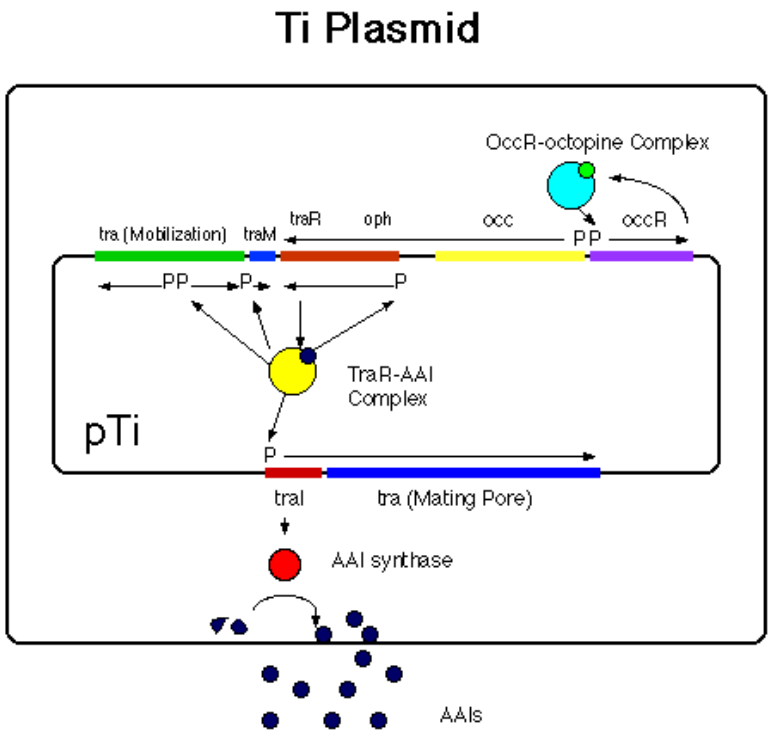


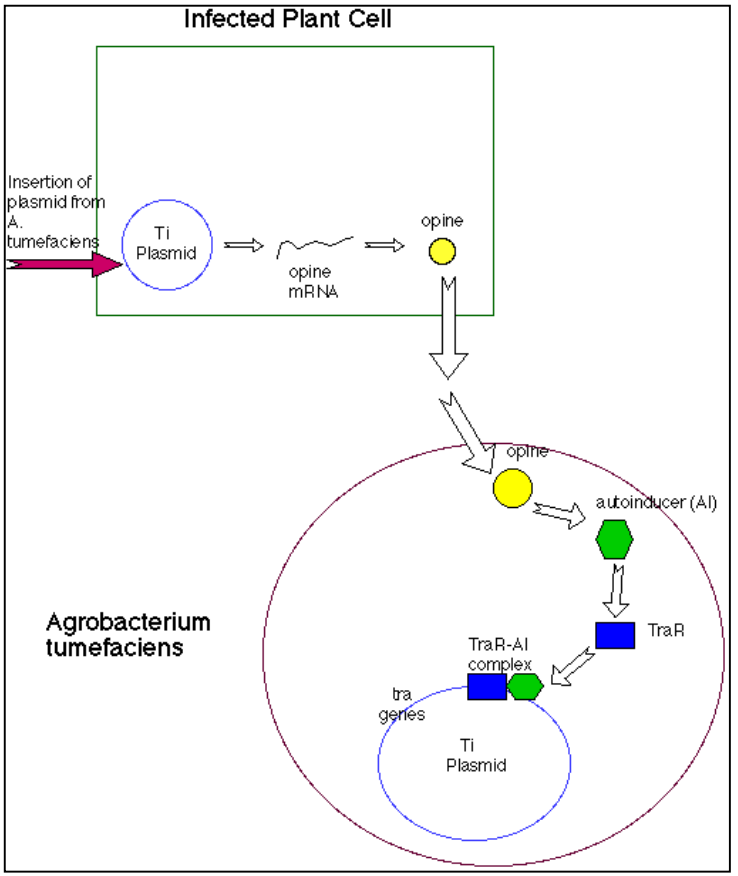
Virulence mechanism
Agrobacterium tumefaciens

- In the case of *Agrobacterium tumefaciens*, the QS system is closely associated with the pTi plasmid that harbors a single AHL synthase gene, traI.
- TraI is responsible for the synthesis of N-(3-oxo-octanoyl)-L-homoserine lactone (3-oxo-C8-HSL).

Virulence mechanism

Agrobacterium tumefaciens





Coval *et al.*,1999

A. tumefaciens infection process

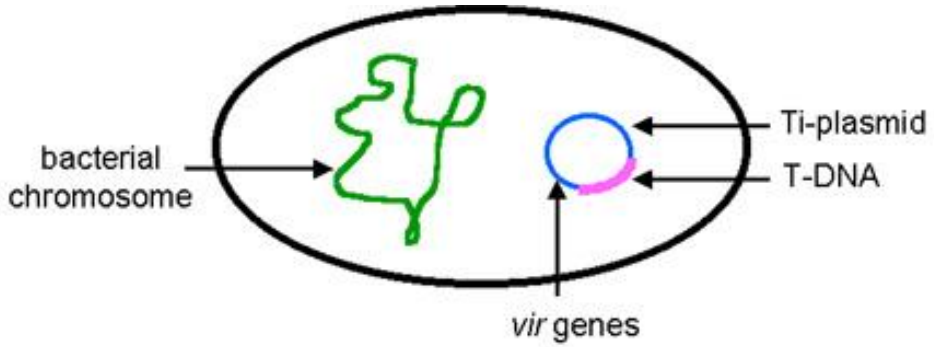
- At least 3 genetic components are required for tumorigenesis:

I. T-DNA;

II. A series of *vir* (virulence) genes that direct the infection process.

III. The virulence (*vir*) region located on the Ti plasmid (~20 kb long).

IV. A suite of chromosomal virulence (*chv*) genes, involved in bacterial chemotaxis toward-and attachment to the wounded plant cell wall. A variety of bacterial polysaccharides (e.g. cyclic glucans synthesized by chvA and chvB), which bind host polysaccharides.



Virulence mechanism

Agrobacterium tumefaciens

- During infection, a copy of the T-DNA is transferred into the host cell and is stably incorporated into the plant genome.
- The integrated DNA (T-DNA) contains genes for auxin and cytokinin synthesis and their presence explains the ability of explants of tumours to grow in tissue culture without auxin or cytokinin supplements.
- T-DNA also contains genes for the synthesis of arginine derivatives, known as opines, which may be catabolized by the bacterium but are unavailable to the plant.

Virulence mechanism

Agrobacterium tumefaciens

- Thus the first of these is the Vir region, which contains genes that are expressed in the bacterium.
- These genes are required for T-DNA transfer to plant cells, in addition to affecting the efficiency of this transfer.
- The second main region is the T region which is transferred to the plant cell, where it, then, becomes integrated into one of the host chromosomes as T-DNA.
- The T-DNA:

1. encodes for the production of the plant hormones auxin and cytokinin,
2. responsible for tumor formation, in addition to a specific amino-acid derivative called an opine.

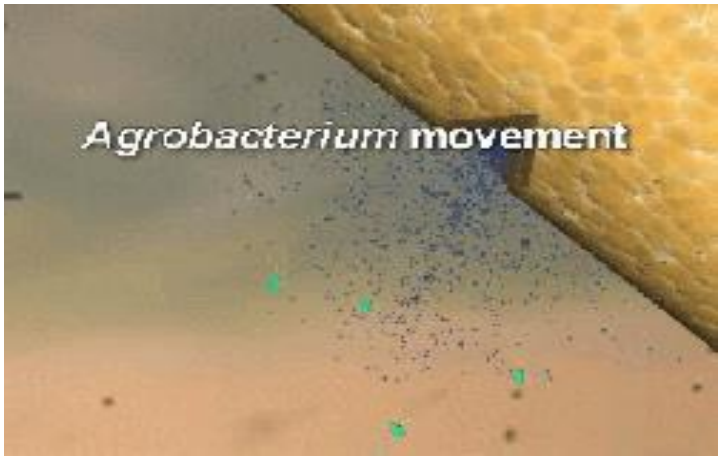
EPS
Agrobacterium tumefaciens

- *Agrobacterium tumefaciens* produces an acidic EPS succinoglycan, like *R. meliloti*.
- No particular phenotype could be correlated with *A. tumefaciens* mutants deficient in succinoglycan synthesis.
- Reuhs *et al*.,1997 have reported the attachment of *Agrobacterium tumefaciens* to carrot cells and arabidopsis wound sites is correlated with the presence of a cell-associated, acidic polysaccharide.

Vanneste,2000

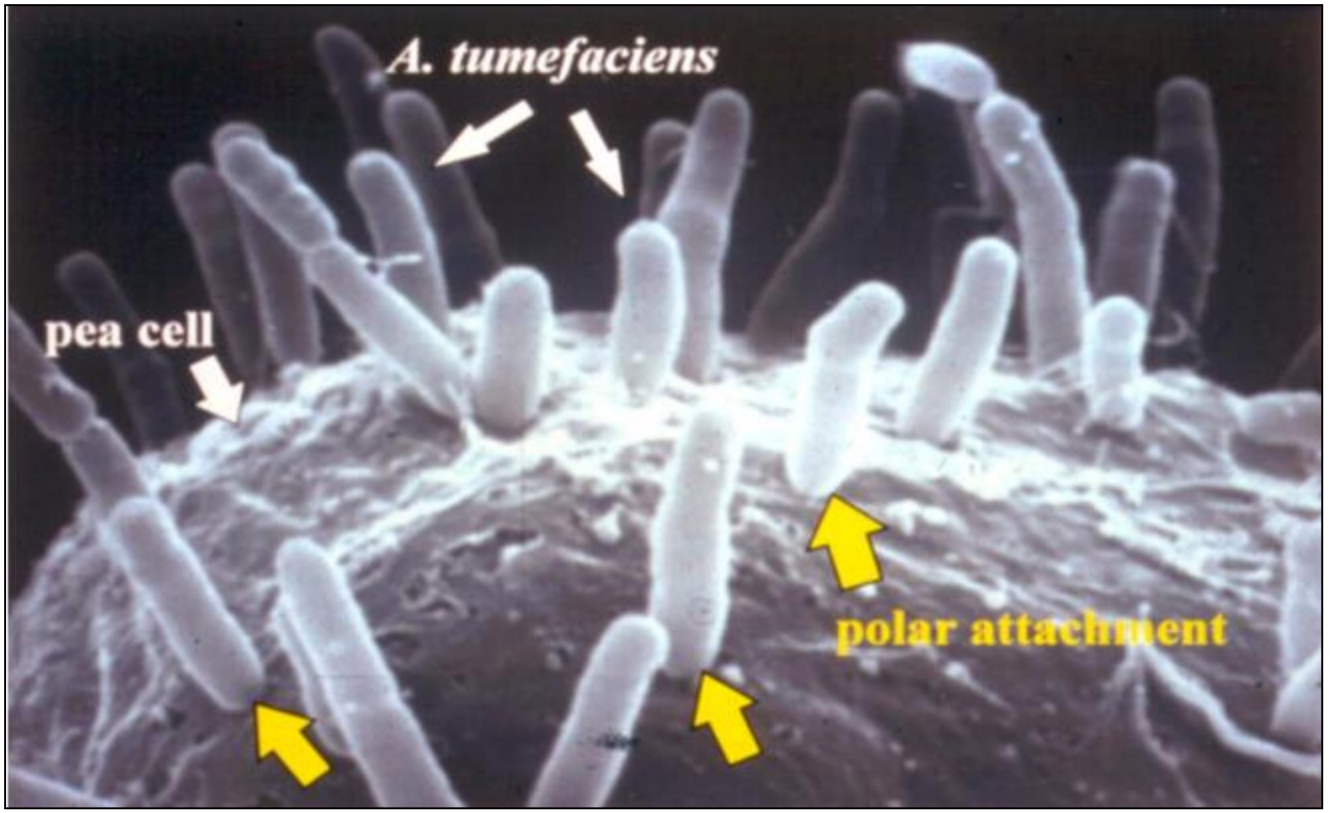
Chemo-taxies toward acetosyringone

Acetosyringone and its derivatives are specific phenolics



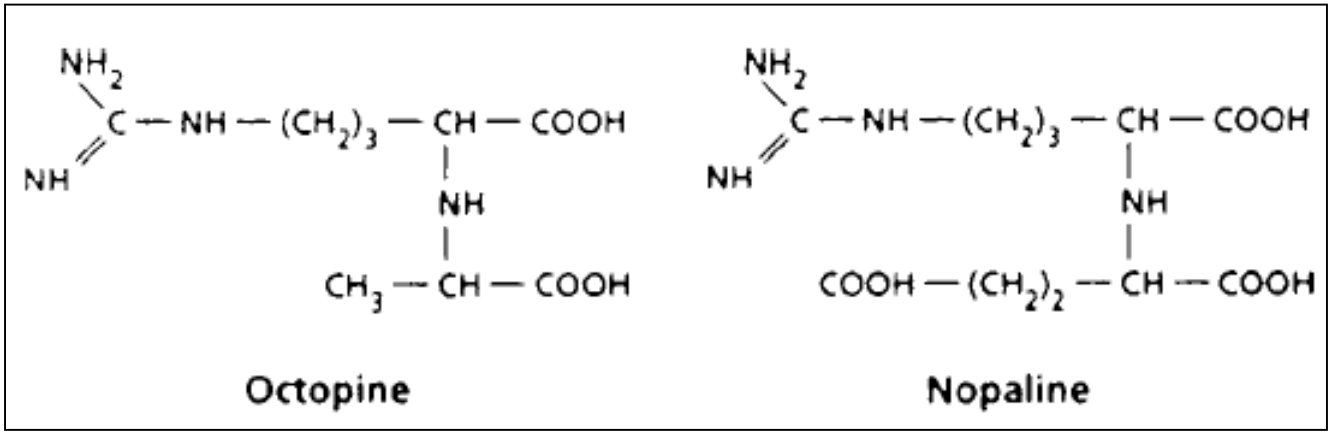
Cell-cell recognition

Polar attachment of *A. tumefaciens* to pea cells



Opines

- Octopine and nopaline, compounds that are found in crown gall tumours.
- Genes for their synthesis are present in the T-DNA that is transferred from *Agrobacterium tumefaciens* to the plant.

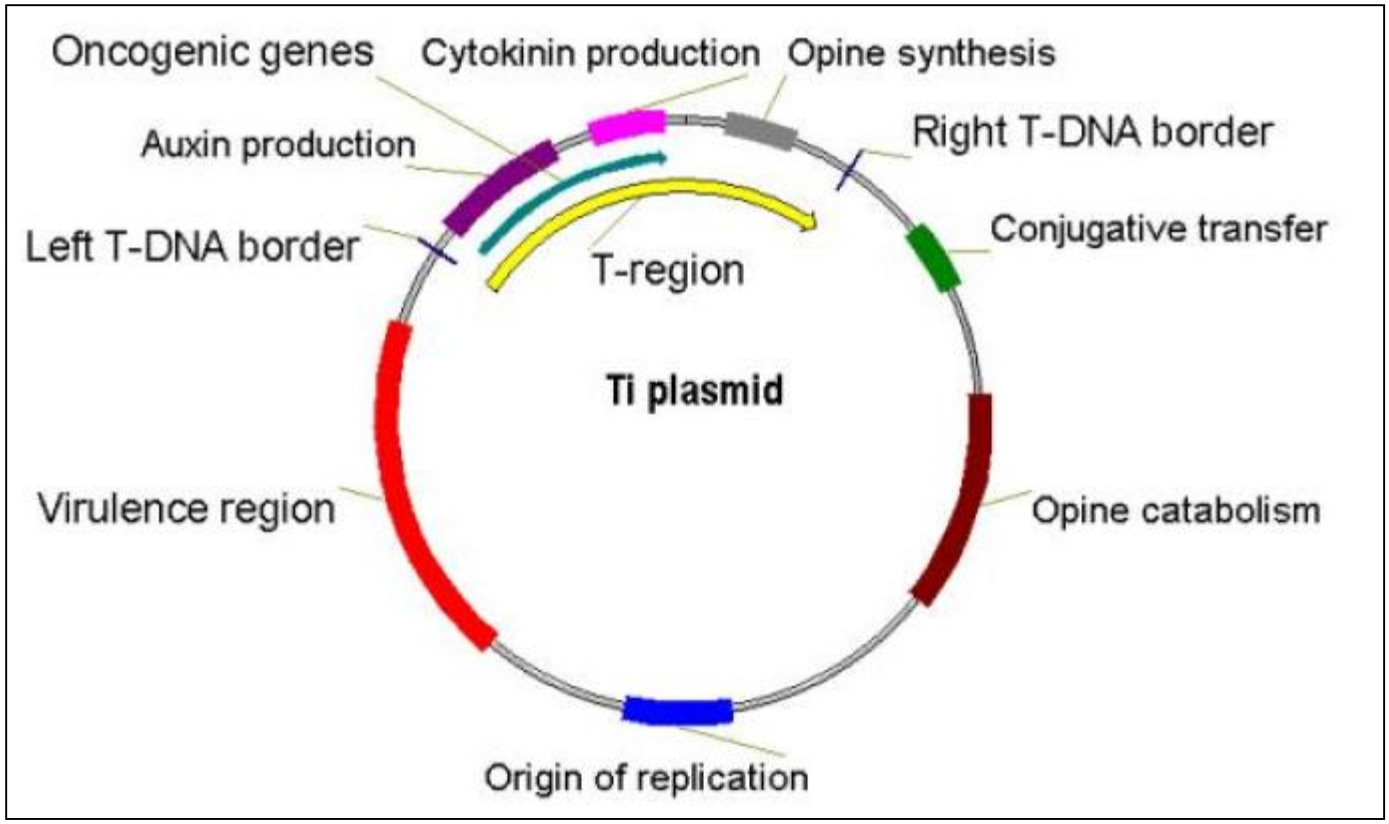


Strange,2003

Structure of tumor-inducing plasmid

Ti plamid

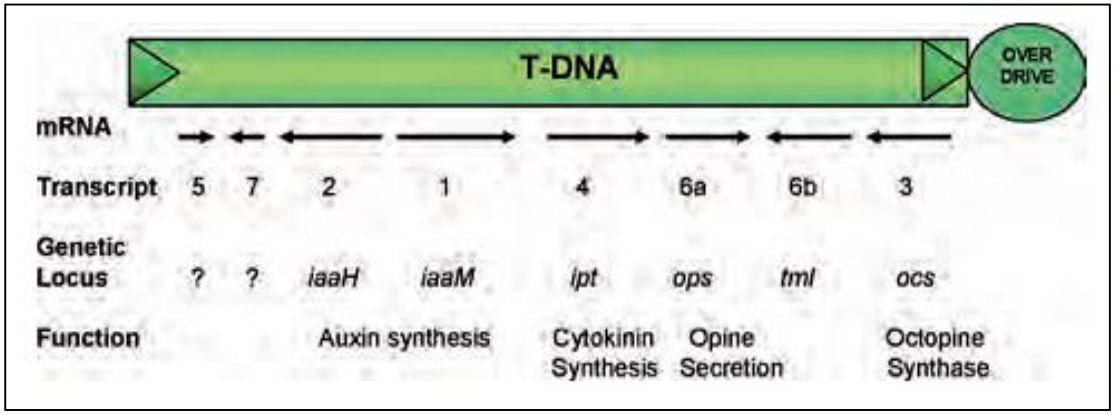
Delimited by short imperfect repeat border sequences



An example of Selfish Genes.
A. tumefaciens **genetically engineers plants to make specialized food for it.**

Genetic map of the TL transferred DNA (T-DNA) of an octopine type Ti plasmid *Agrobacterium tumefaciens*

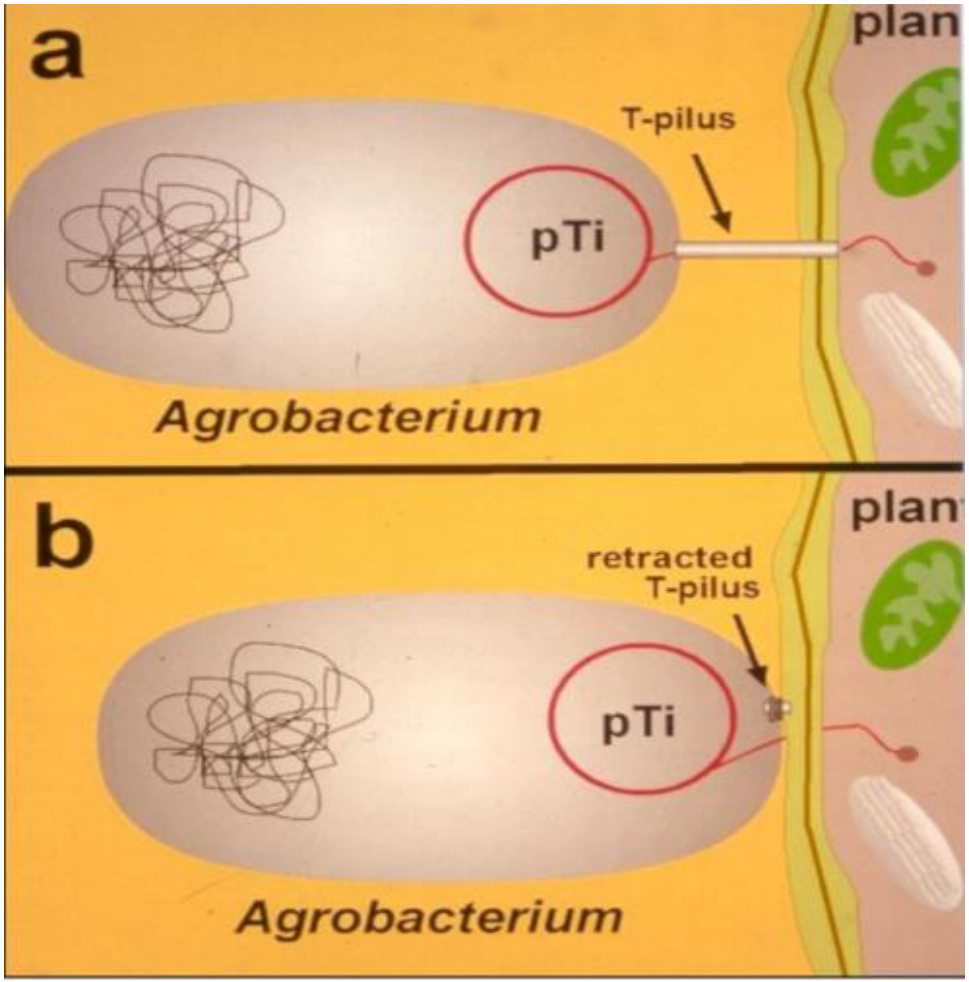
- The arrowheads in the box represent the left and right borders of the T-DNA, and they indicate the direction of T-DNA transfer, from right to left.
- *iaaH* (indoleacetamide hydrolase), *iaa M* (tryptophan monooxygenase), *ipt* (isopentenyl transferase), *ops* (octopine secretion), *tml* (tumor morphology large), *ocs* (octopine synthase).
- Arrows indicate the direction and length of T-DNA encoded mRNAs produced by transformed plant cells.



Tzfira and Citovsky,2008

Transfer of T-DNA into a plant cell

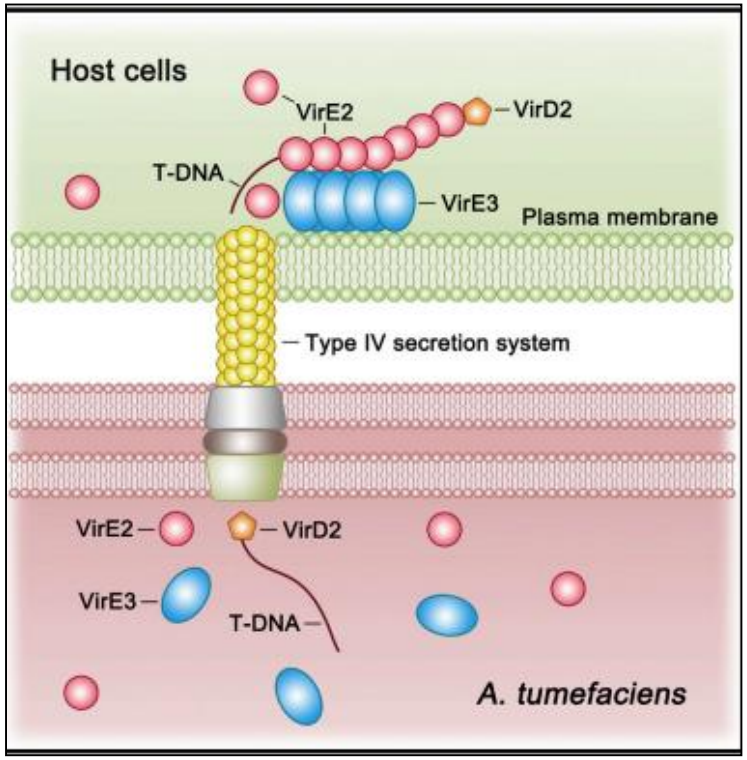
T-DNA transfer is mediated by the pilus T, belonging to the type IV secretion system



Transfer of T-DNA into a plant cell

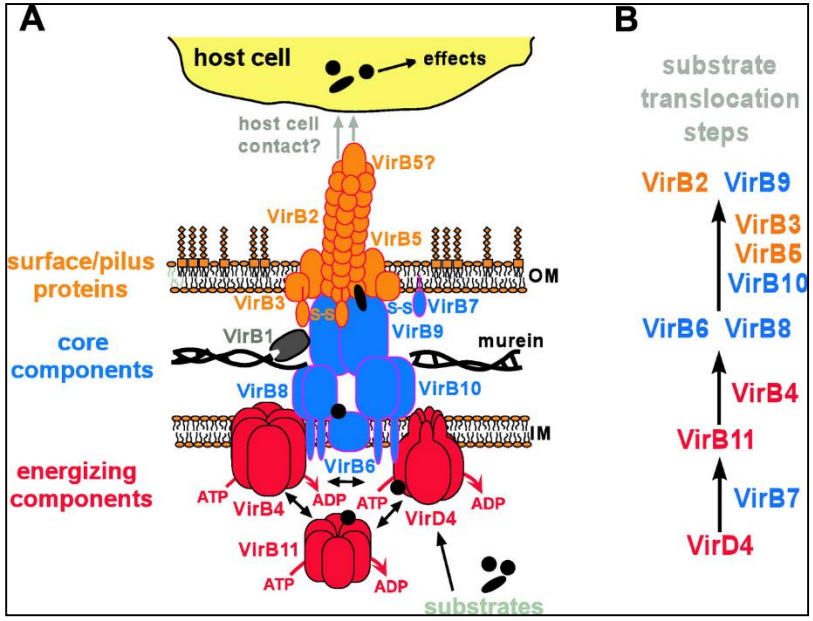
T-DNA transfer is mediated by the pilus T, belonging to the type IV secretion system

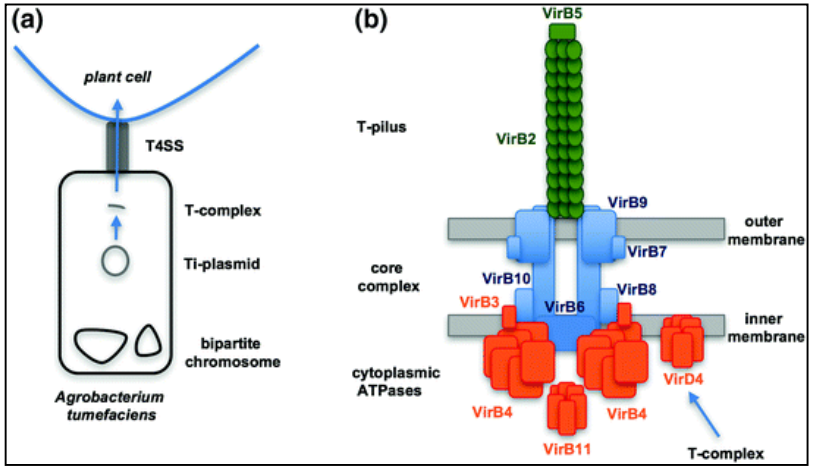
- *Agrobacterium* transfers T-DNA and virulence proteins into host cells.
- A virulence protein, VirE3, anchors its companion protein VirE2 on host membranes at the entrance to facilitate T-DNA protection.
- VirE3 represents a class of proteins, anchorage proteins, which are present only in agrobacteria and rhizobia.



Li *et al.*,2018

Pathogen-generated secreted proteins
Effectors
Type IV Secretion in *Agrobacterium tumefaciens*



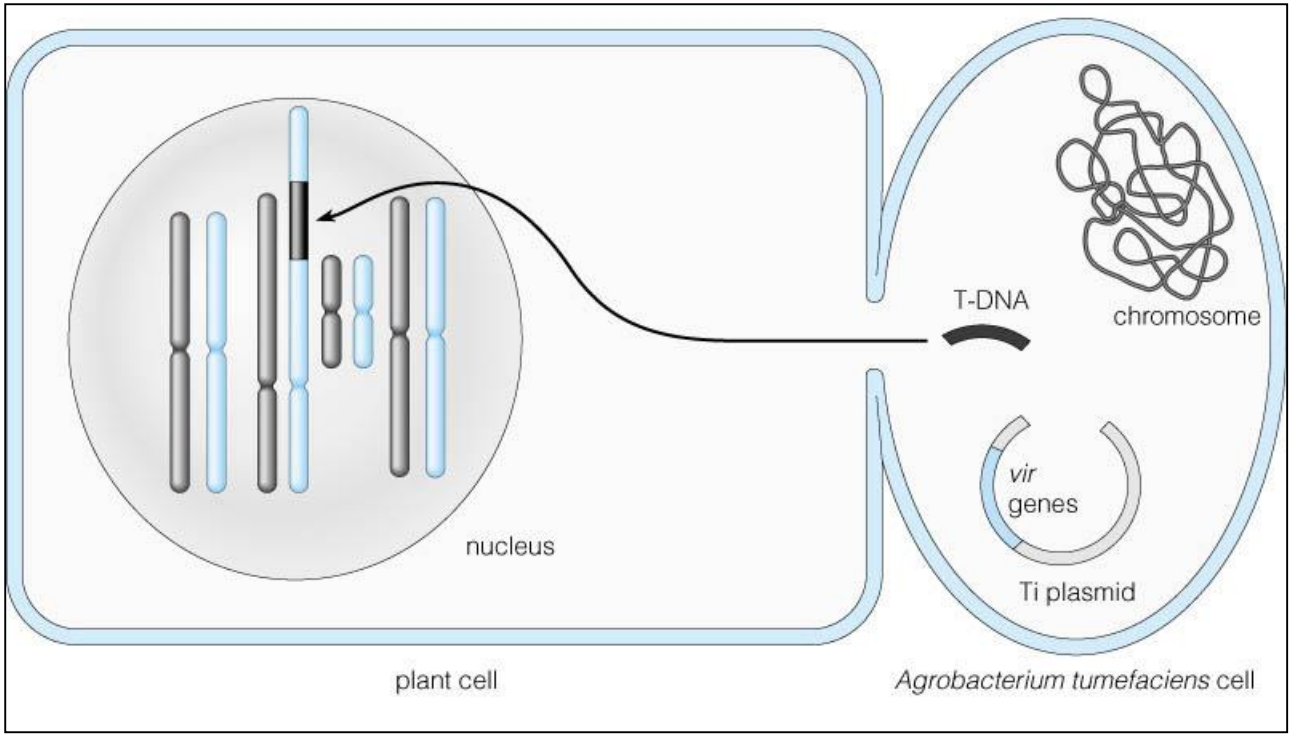


The *Agrobacterium tumefaciens* VirB/D4 type IV secretion system (T4SS) comprises 12 membrane-bound proteins, and it assembles a surface-exposed T-pilus.

Baron,2006;Sharifahmadian and Baron,2018

Agrobacterium infection process

T-DNA is transferred into the chromosomes of plant cells



Books/Cole,2005

Gene silencing
Turning off gene

- Gene silencing: Interrupting or suppressing the activity of desired gene (s), resulting in the loss of coordination for production of specific proteins.
- The term gene silencing is generally used to describe the "switching off" of a gene by a mechanism other than genetic modification.
- That is, a gene which would be expressed (turned on) under normal circumstances is switched off by machinery in the cell.

Gene silencing as a way of controlling few bacterial diseases

Candidate genes for plant transformation in order to enhance resistance

- Introduction of additional copies of genes in order to increase their expression often, paradoxically, results in decreased expression.
- One promising application of this technology is in the control of crown gall.
- Here it is proposed that transformation of plants with the genes that the pathogen uses for the production of IAA and cytokinins would give durable resistance since these genes are responsible for the unorganized growth of tumours in infected plants.

Control of crown gall by genes silencing

Transgenes with bacterial sequences suppress bacterial replication

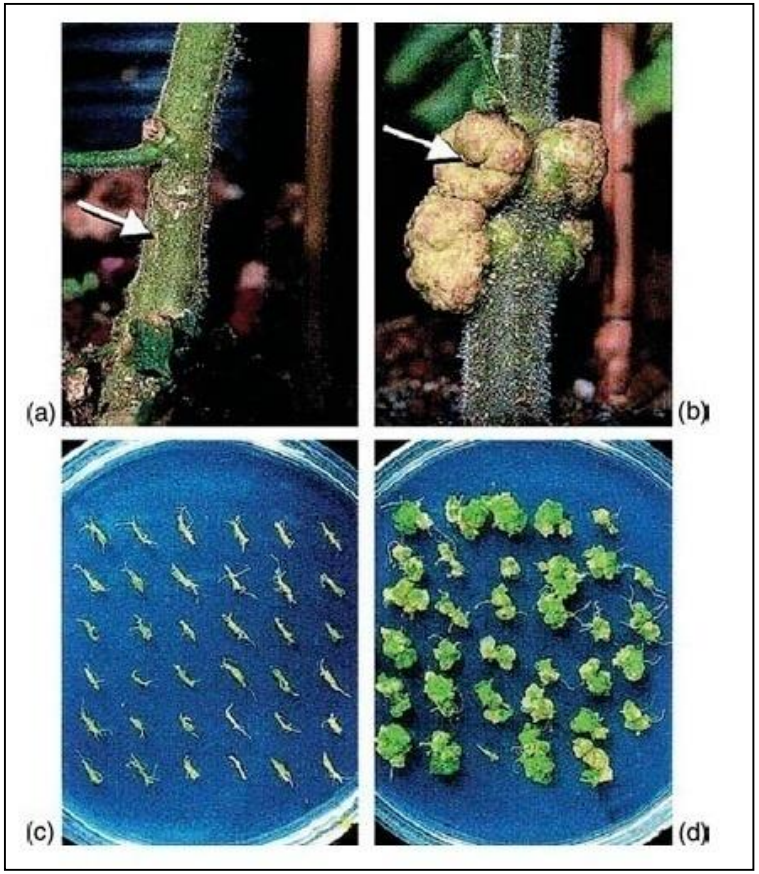
- Control of crown gall by silencing (turning off) of the genes used by the bacterium for the synthesis of IAA and cytokinins:

a) tomato transgene;

b) tomato control;

c) *Arabidopsis* transgene;

d) *Arabidopsis* control

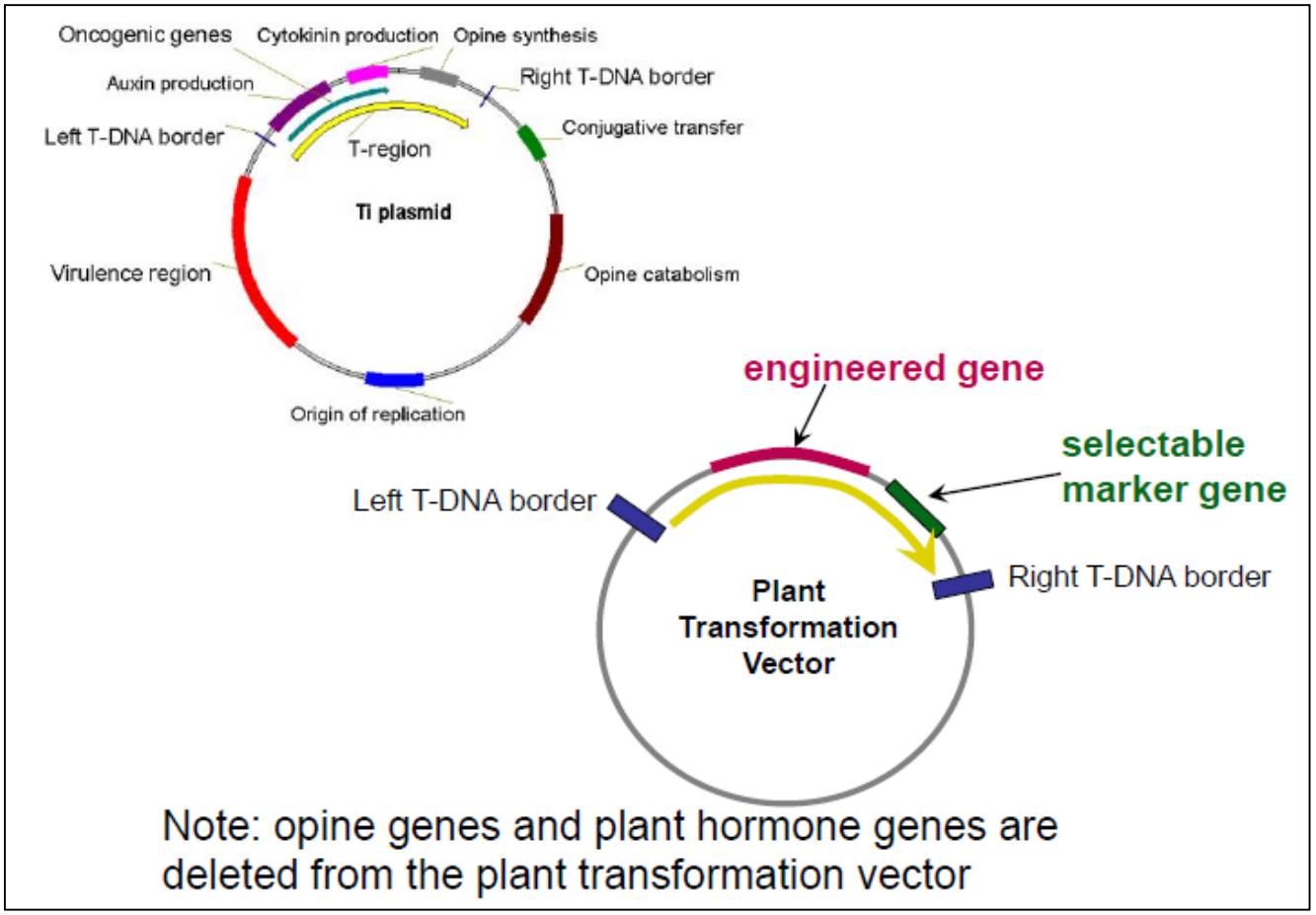


Strange,2003

Industrial use

Plant transformation, construction of genetically-modified plants

Bio-engineering plants with *Agrobacterium*



Pathogenicity of:

Erwinia amylovora

Fire blight Disease

Fire blight

Harpin (HrpN) originally isolated from this bacterium

E. amylovora
A unique and fascinating bacterium

- *E. amylovora* is a unique and fascinating bacterium which is closely related to many important human and animal pathogens such as *E. coli*, *Salmonella*, *Shigella*, and *Yersinia*.
- Genome sequence has also revealed that *E. amylovora* is an ideal pathogen to study both the evolution and molecular mechanisms of pathogenesis, as it has evolved specifically to facilitate disease and survival in different environmental conditions, as well as different hosts.
- Interestingly, *E. amylovora* not only shares similar type III effectors with animal and human pathogen *Yersinia* and *Salmonella*, but also has common effectors with plant pathogen *P. syringae*.

Zhao,2007

Major pathogenicity factors

- **Known virulence genes, including:**

1. *hrp/hrc* components of the type III secretion system;
2. The major effector gene *dspE* ;
3. Type II secretion;
4. Levansucrase (*lsc*).

- Regulators of levansucrase and amylovoran biosynthesis, were upregulated during pear tissue infection.
- Levan is not strictly necessary for the pathogenicity of *E. amylovora* (Kube *et al*., 2010).

Major pathogenicity factors

- Known virulence factors previously identified in *E. carotovora* and *Pseudomonas syringae* were identified for the first time in *E. amylovora* and included:

1. HecA hemagglutinin family adhesion,
2. Peh polygalacturonase,
3. New effector Hop PtoCEA, and
4. Membrane-bound lytic murein transglycosylase MltEEA.

- Therefore *E. amylovora* utilizes a variety of strategies during plant infection and to overcome the stressful and poor nutritional environment of its plant hosts.

Major pathogenicity factors
Sugar metabolism

- Sorbitol another factor that influences virulence in *E. amylovora* is the metabolism of sugars.
- Rosaceous plants contain sorbitol and sucrose as storage and transport carbohydrates, and the distribution of these carbohydrates is dependent on the environmental conditions, species, and plant tissue.
- An operon, *srl*, necessary for sorbitol metabolism, has been identified in *E. amylovora*.
- This operon consists of six genes: three are required for sorbitol uptake (*srlA*, *srlB*, and *srlE*), one encodes a protein that converts sorbitol to fructose (*srlD*), and two other genes are regulatory (*srlM* and *srlR*).

Palacio-Bielsa *et al.*,2012

Contribution of secreted and cytoplasmic control proteins from plant pathogenic bacteria to virulence

E. amylovora

Bacterial Species/Predicted Protein Function	Protein[a]	Localization[b]	Contribution to T3S and Virulence
E. amylovora:			
Pilus protein	HrpA	S	Essential for disease and T3S
Translocon proteins	HrpK	S	Homologous to HrpK1 from *P. syringae*, dispensable for virulence
Control protein	HrpN	S+T	Harpin, contributes to translocation of the effector DspA/E
	HrpJ	S	Required for virulence, contributes to secretion of harpins and translocation of the effector DspA/E

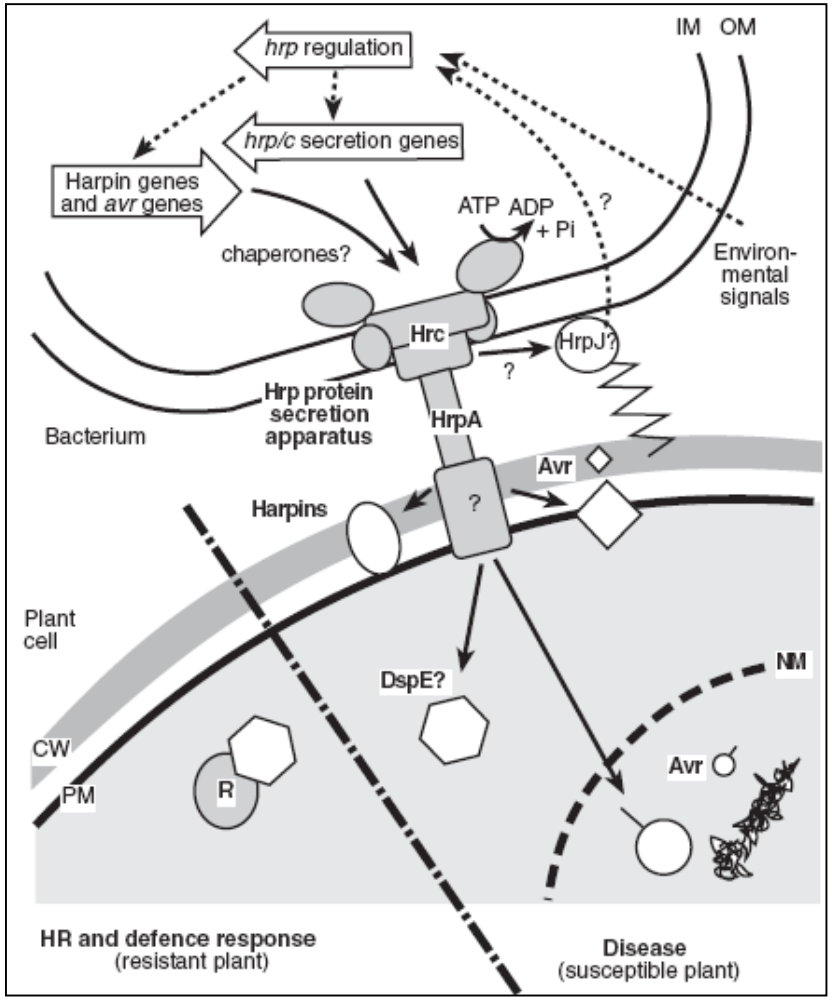
[b] Localization: C, cytoplasmic; n.a., not analyzed; NT, not translocated; S, secreted; T, translocated.

Büttner and He,2009

Hypothetical model of the Hrp protein secretion apparatus and possible destinations of Hrp-transported proteins of *Erwinia amylovora*

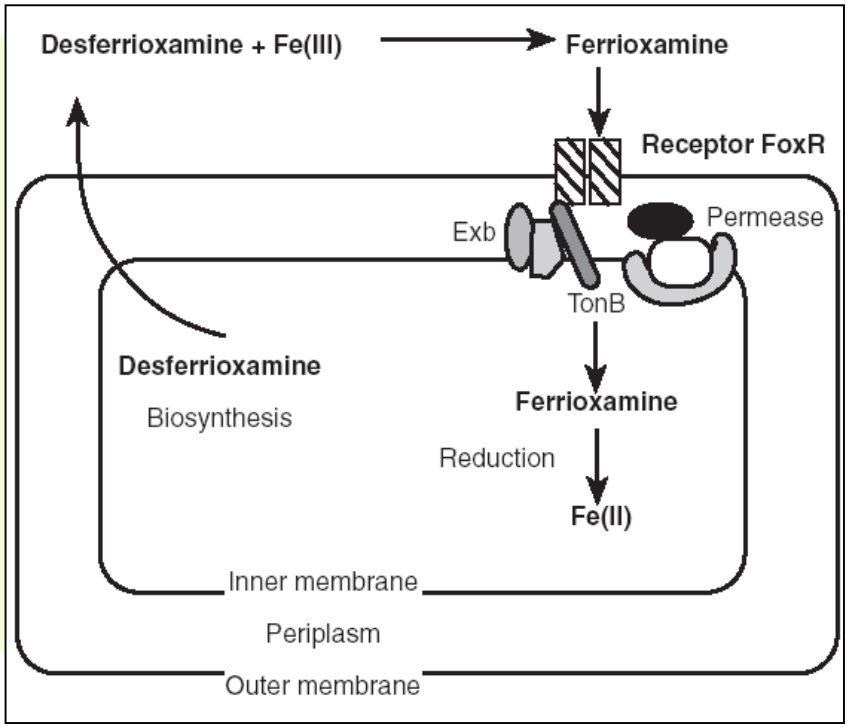
- Hrc proteins are thought to be core proteins that constitute the Hrp apparatus. HrpJ is a putative contact sensor that may be a part of the secretion complex. HrpA is a component of the Hrp pilus, which may form a conduit through which effector proteins are secreted or may make close contact between the bacterium and a plant cell.
- Harpins may function at the plant cell wall or may assist virulence effector proteins to get into plant cytoplasm or nucleus.
- Virulence proteins, including Ave-like proteins, may travel the secretion pathway to be destined for the plant targets.
- DspE is a Hrp-secreted pathogenicity protein of *E. amylovora* that is functionally homologous to AvrE of *Pseudomonas syringae*.
- IM, inner membrane; OM, outer membrane; CW, plant cell wall; PM, plant plasma membrane; NM, nuclear membrane; R, plant resistance gene.

Vanneste,2000



Role in pathogenicity of desferrioxamine E, the major siderophore of *E. amylovora*

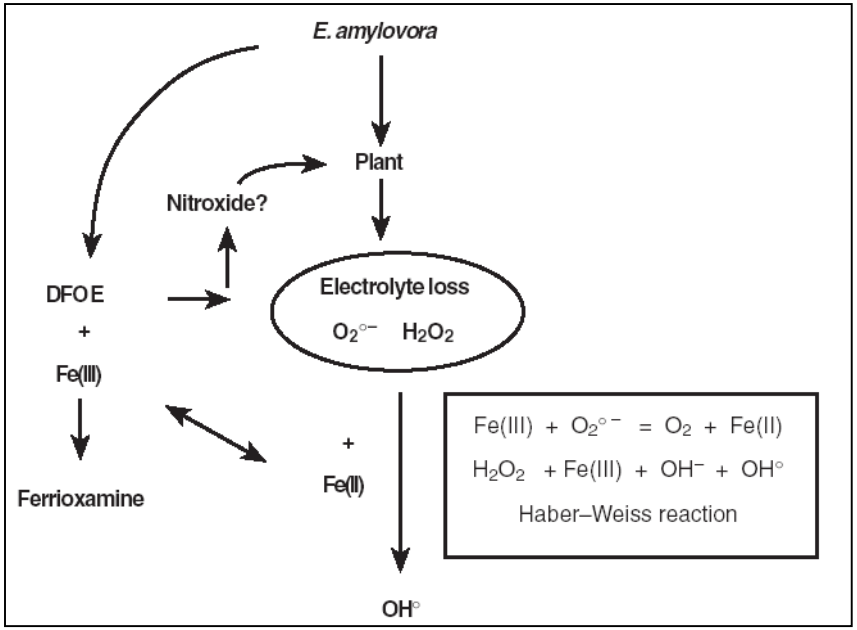
- Compounds of the desferrioxamines (DFO family), of which DFO E appeared to be predominantly produced, were identified in various strains of *E. amylovora*



Vanneste,2000

Model showing potential roles of DFO in plant host–*Erwinia amylovora* interactions

- Infection by *E. amylovora* of plant tissues causes electrolyte leakage and accumulation of active oxygen species, including the superoxide radical anion and hydrogen peroxide (O_2 and H_2O_2).
- They act as antimicrobial agents directly or indirectly through the production of hydroxyl radicals (OH) generated by the Fenton reaction during the Haber–Weiss cycle catalysed by iron.
- Iron complexation by DFO interrupts the chain radical reactions, thereby protecting bacterial cells and possibly plant cells from extensive oxidative damage.
- In addition, depending on the ratios of Fe(III) and O_2 to DFO, DFO could react with O_2 to generate the DFO nitroxide radical, which might activate the plant defence mechanism.



Vanneste,2000

Pathogenicity of:
Lonsdalea (*ex. Brenneria*) *populi*

Bark canker on and *Populi*

Poplar canker disease

Lonsdalea (*ex. Brenneria*) *populi*

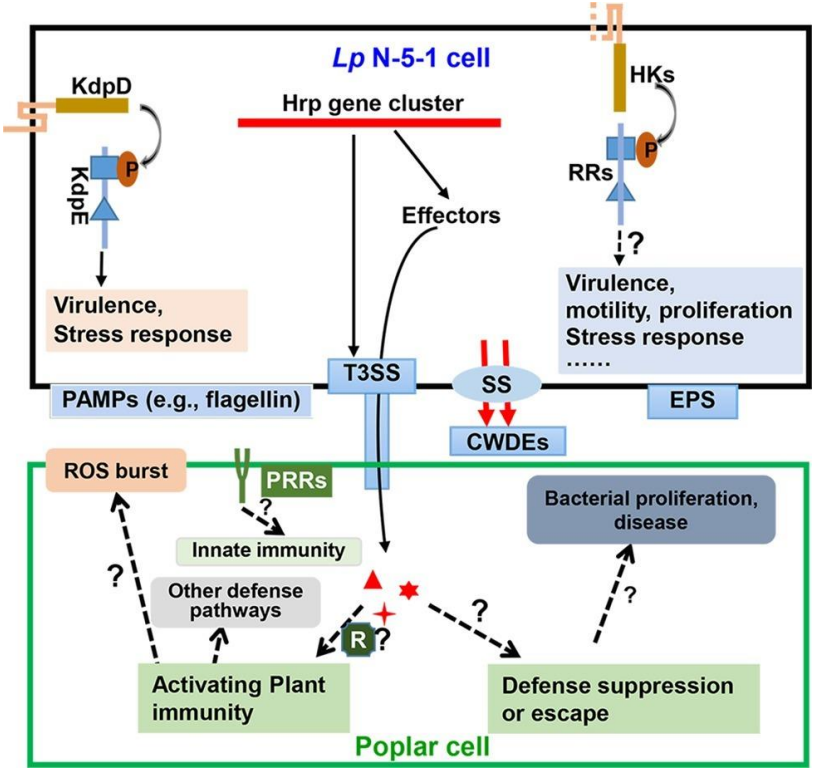
- Bacterial canker disease on Populus × euramericana clone "I-214" caused by *Lonsdalea populi* in Serbia.
- (a) Necrotic bark with foamy sap flowing from the infection site. (b) Canker with cracked bark and exudates emerging from the infected stem. (c) Softening and darkening of the vascular part of the trunk in the cankered area. (d) Dead tree with exudates staining the bark. (e) Colonies of *L. populi* after 24 h of incubation at 30°C on tryptone soya agar. (f) Water-soaked sunken canker formed on *P.* × *euramericana* rooted cutting one month after inoculation with *L. populi*. (g) Dark, soft, and watery wood beneath the bark of a canker formed on *P.* × *euramericana* rooted cutting one month after inoculation with *L. populi*.
- (h) Negative control showing absence of canker development.

Zlatkovice *et al.*,2020

Poplar canker disease

Lonsdalea (*ex. Brenneria*) *populi*

- A diagram of the two-component signaling and secretion systems during interaction of *Lonsdalea populi* and *Populus* × *euramericana*.
- The bacterium *L. populi* N-5-1 mainly perceives external signals *via* two-component systems (TCS) comprising:
  1. histine kinases (HKs), and
  2. response regulators (RRs).
- This leads to activation of TCS signaling that modulates expression of genes associated with virulence, motility, proliferation, stress response, etc.
- A well-characterized TCS of *L. populi* N-5-1, KdpD-KdpE, has been demonstrated to control virulence and stress response (Yang et al.,2018).



Li and He,2019

Pathogenicity of:
Pectobacterium carotovorum

Soft rot, blackleg, or stem rot diseases

Bacterial soft rot of vegetables

Major pathogenicity factors
Degradative enzymes

- *P. carotovorum* subsp. *carotovorum* is a phytopathogenic enterobacterium responsible for the soft rot, blackleg, or stem rot of a number of economically important crops.
- The disease is characterized by extensive maceration of the affected tissue caused by a variety of plant cell wall-degrading enzymes secreted by the pathogen.
- The major pathogenicity determinants are an arsenal of extracellular pectinases, including several pectate lyase isozymes, pectin lyase, pectin methylesterase, and pectin polygalacturonase.
- In addition, a range of other degradative enzymes, such as cellulase and protease, are secreted through few secretion systems.

Major pathogenicity factors

Other virulence factors

- The main virulence determinants of *Pectobacterium* i.e. pectolytic enzymes are secreted through the type II secretion system.
- Although these enzymes are required for development of symptoms, many other virulence genes have been shown to contribute to *Pectobacterium* pathogenicity, including:
- The type III secretion system (T3SS) genes,
- The *cfa* gene cluster, and the
- Type IV secretion system genes.
- Some *Pectobacterium* strains lacking the T3SS (isolated from diseased potato tubers) are still virulent in potato tubers and stems.

Kim *et al.*,2009

Major pathogenicity factors
Pectobacterium spp.

- Many Gram-negative pathogenic bacteria secrete virulence proteins, known as effectors, through the T3SS into host cells.
- Once inside host cells, the effectors manipulate host defenses and promote bacterial growth.
- Unlike many other gram negative plant pathogens, *Pectobacterium* does not require the T3SS for pathogenicity.
- Rather, this secretion system makes a small, but measurable, contribution to the early stages of *P. carotovorum* growth in leaves of the model plant *Arabidopsis thaliana* and contributes to the virulence of *P. atrosepticum* on potato.

Kim *et al.*,2009

Major pathogenicity factors

Pectobacterium spp.

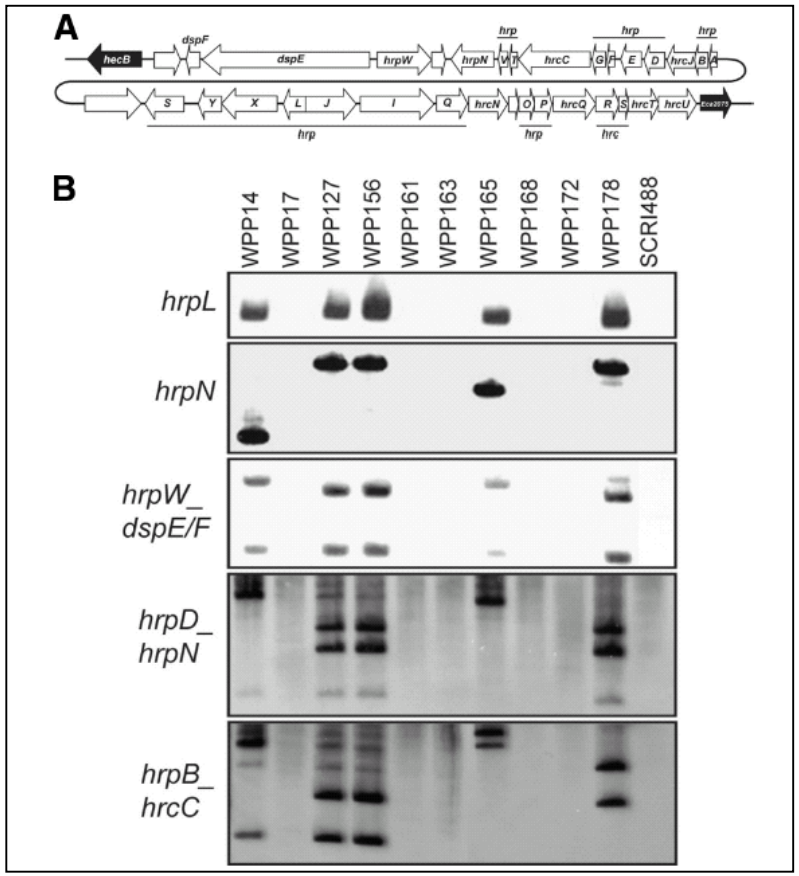
T3SS gene cluster

- *Pectobacterium* isolates unable to elicit an HR did not contain the genes for the *Pectobacterium* T3SS.

A. The *P. carotovorum* WPP14 T3SS is indicated by open arrows, and the border genes, *hecB*, and ECA2075 are indicated by **filled arrows**.

B. Genomic DNA from isolates unable to elicit an HR did not hybridize to DNA amplified from the *P. carotovorum* WPP14 T3SS gene cluster, including:

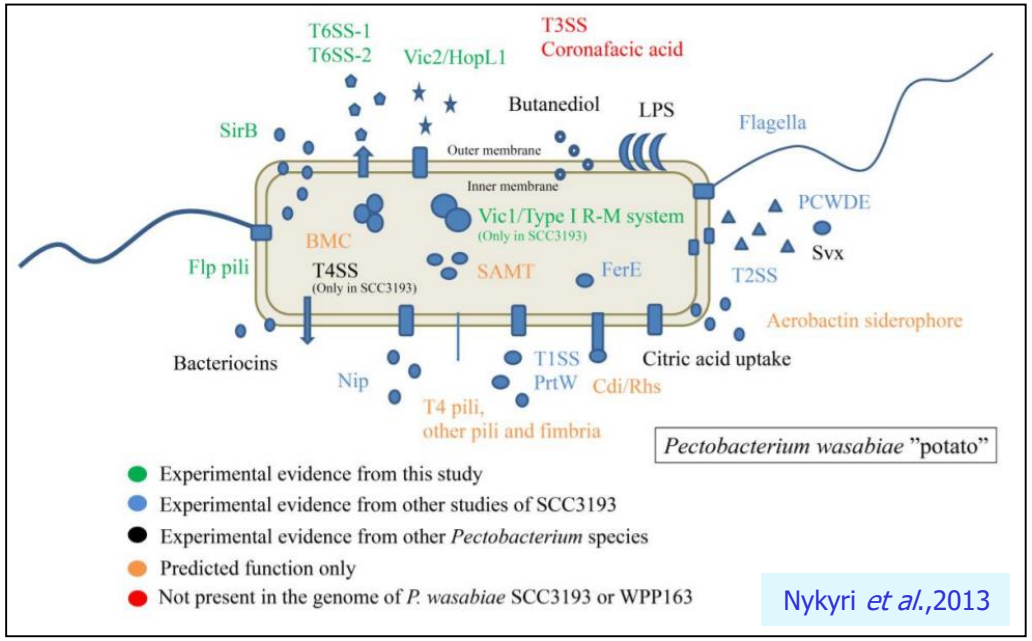
- *hrpL, hrpN, hrpW, dspE, dspF, hrpD, hrpE, hrpF, hrpG, hrcC, hrpT, hrpV, hrpB,* and *hrcJ.*



Kim *et al.*,2009

Major pathogenicity factors
Pectobacterium wasabiae

- Novel and known virulence and fitness related traits in the potato pathogenic *Pectobacterium wasabiae* strains SCC3193 and WPP163 (II).
- These strains share many traits that are rare or absent from other *Pectobacterium* strains and *P. wasabiae* type strain CFBP 3304T.
- Such traits are for example SAMT, FerE, Cdi, LPS loci composition, aerobactin and T4 pili.



Pathogenicity of:

Pantoea agglomerans* pv. *gypsophilae
Pantoea agglomerans* pv. *betae
Pantoea stewartii* subsp. *stewartii

Gall and wilt diseases

Major pathogenicity factors

Pathogenicity associated plasmid pATH

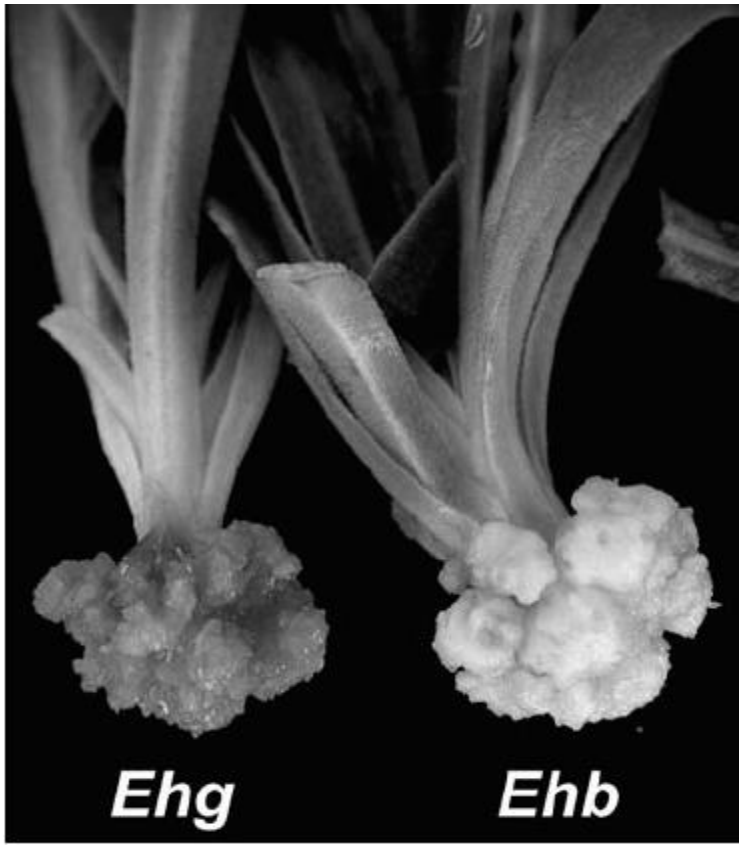
IAA and cytokinin

- Gall formation by the bacterium *P. agglomerans* (ex. *Erwinia herbicola*) pv. *gypsophilae* on *Gypsophila paniculata* is conferred by the pathogenicity associated plasmid pATH and a similar plasmid is found in *P. agglomerans* (ex. *Erwinia herbicola*) pv. *betae* which confers pathogenicity for beet (*Beta vulgaris*) as well as *Gypsophila paniculata*.
- Many virulence genes are harboured by the plasmids including a cluster that specify IAA and cytokinin production.
- One of these, *etz*, is homologous to other cytokinin biosynthesis genes that have been described.
- The mutant did not produce cytokinins, induced only small galls in *Gypsophila* cuttings and was almost completely symptomless in a whole plant assay.

Gall formation on *Gypsophila paniculata*

***Pantoea agglomerans* pv. *gypsophilae* (left labelled Ehg) and by pv. *betae* (right labelled Ehg)**

- More recently, the importance of both IAA and cytokinins in gall formation was demonstrated by PCR using primer pairs that were based on IAA or cytokinin biosynthetic genes.
- The primers were specific to gall-forming strains of *E. herbicola* and distinguished them from other gall-forming bacteria.



Strange,2003

Stewart's wilt of corn

An endoglucanase is required for full virulence in sweet corn

- In silico analysis of the *P. stewartii* subsp. *stewartii* genome revealed several open reading frames (ORF) encoding putative host cell-wall-degrading enzymes (CWDE), including:
- Two cellulases,
- Two xylanases,
- One polygalacturonase, and
- One endoglucanase (EGase) (ASAP database) (Glasner *et al.*,2006), indicating that *P. stewartii* subsp. *stewartii* may be capable of host cell wall degradation.

Photo by Melodie Putnam,2000

Mohammadi *et al.*,2012

Stewart's wilt of corn

An endoglucanase is required for full virulence in sweet corn

- *Pantoea stewartii* subsp. *stewartii* produces an Endoglucanase that is required for full virulence in sweet corn.
- The gene encodes an endoglucanase (EGase) was designated *engY*.
- EngY contributes to movement in the xylem and disease severity during the wilting phase of Stewart's wilt but is not required for water-soaked lesion formation.

Mohammadi *et al.*,2012

Pathogenicity of:
Dickeya (ex. *Erwinia*) *chrysanthemi*

Soft rot diseases in a wide range of plant species

Main virulence-determinants

Pectinases

Pectate lyases and pectin methylestrase

- Symptoms result from the disorganization of the plant cell wall caused by a set of extracellular enzymes such as pectinases(mainly pectate lyases), cellulase, and proteases.
- Among these degradative enzymes, pectate lyases play a predominant role in plant tissue maceration.
- The production of pectate lyases, the main virulence determinant, is modulated by a complex network involving several regulatory proteins.
- One of these regulators, PecS, also controls the synthesis of a blue pigment identified as indigoidine.

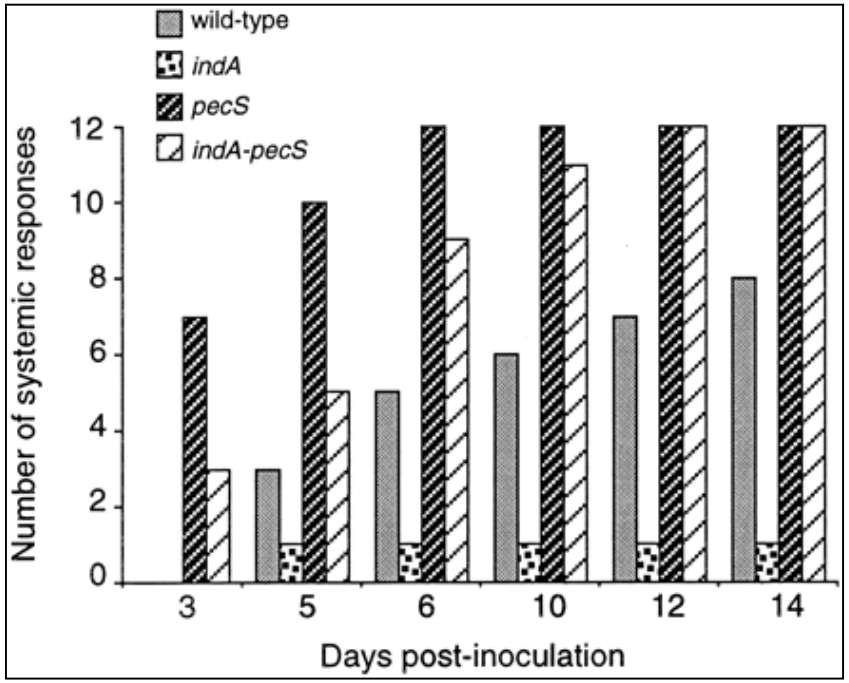
Reverchon *et al.*,2002

Indigoidine production
Protect the bacteria from oxidative burst

- Mutants impaired in indigoidine production were unable to cause systemic invasion of potted *Saintpaulia ionantha*.
- Moreover, indigoidine production conferred an increased resistance to oxidative stress, indicating that indigoidine may protect the bacteria against the reactive oxygen species generated during the plant defense response.

Indigoidine production
Contributes to aggressiveness

- The wild type produced systemic lesions in 8 of 12 plants inoculated after 14 days, whereas only 1 systemic response was observed with the *indA* mutant.
- These results indicate that indigoidine contributes to aggressiveness.



Development of symptoms caused by *D . chrysanthemi* 3937 (wild type) and its *indA*, *pecS*, and *indA-pecS* derivatives on *Saintpaulia ionantha*.

Reverchon *et al.*,2002

Pathogenicity of:
Pseudomonas syringae

Epiphytic, pathogenic and ice nucleus bacterium

The evolution of *Pseudomonas syringae* host specificity and type III effector repertoires

- The host specificity of the c. 50 pathovars of *P. syringae* for different plant species is a particularly striking aspect of this variability that has been investigated for more than 45 years but is still poorly understood.

Lindeberg *et al.*,2009

The evolution of *Pseudomonas syringae* host specificity and type III effector repertoires

- *Pseudomonas syringae* and many other Proteobacteria causing diseases in plants and animals inject effector proteins into host cells via the type III secretion system (T3SS).
- The diversity of effector proteins (highly polymorphic nature of effector repertoires) is one of the main factors in limiting host range among *Pseudomonas syringae* pathovars.
- Over 30 effectors are likely to be delivered by *Pseudomonas syringae* pv.*tomato*.

Contribution of secreted and cytoplasmic control proteins from plant pathogenic bacteria to virulence

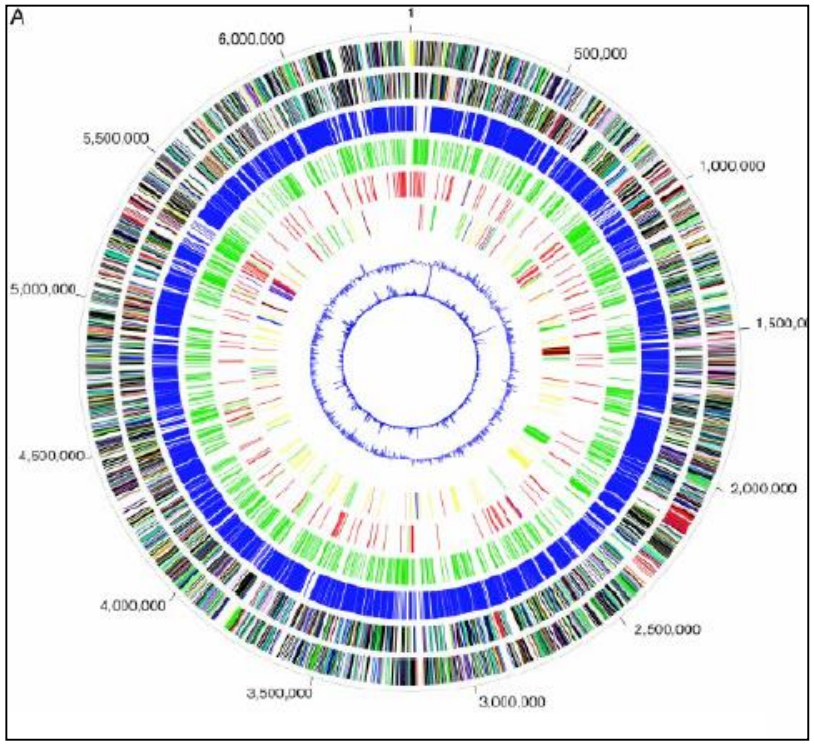
P. syringae pv.*tomato*

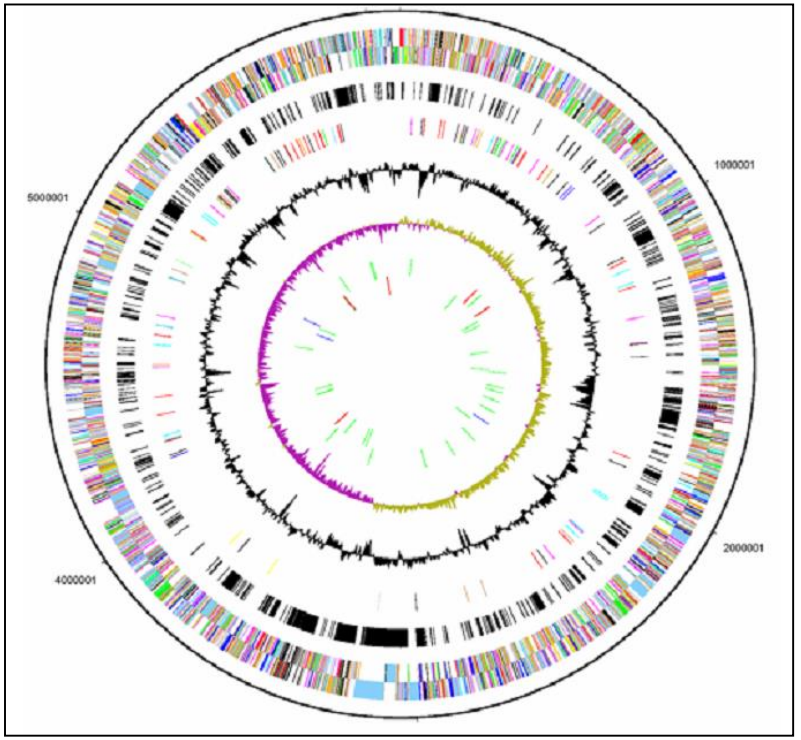
Bacterial Species/Predicted Protein Function	Protein[a]	Localization[b]	Contribution to T3S and Virulence
P. syringae pv *tomato*:			
Pilus protein	HrpA	S	Essential for disease and T3S
Translocon protein	HrpK1	S+T	Contributes to disease and effector protein translocation
Control proteins	HrpZ1	S+T	Harpin, forms ion channels
	HrpW1	S+T	Harpin, C-terminal pectate lyase domain
	HopAK1	S+T	Harpin, C-terminal pectate lyase domain
			HrpZ1, HrpW, and HopAK1 contribute to effector protein translocation and disease
	HrpH	T	HrpH, HopP1, and HopAJ1 are predicted lytic transglycosylases and contribute to effector protein translocation and disease
	HopP1	T	
	HopAJ1	NT	
	HrpJ	S+T	Required for disease, contributes to T3S
	HrpP	T	Predicted T3S4 protein, essential for disease

[b] Localization: C, cytoplasmic; n.a., not analyzed; NT, not translocated; S, secreted; T, translocated.

Büttner and He,2009

Comparison of the complete genome sequences of *P. s.* pv. *syringae* B728a(right) and *P. s.* pv. tomato DC3000(left)





Feil *et al.*,2005;..

Comparison of the complete genome sequences of *P. s.* pv. *syringae* B728a(right) and *P. s.* pv. tomato DC3000(left)

- The *Pss* B728a genome is composed of one circular chromosome of 6,093,698 bp.
- *Pss* B728a shares 4273 genes with *Pst* DC3000 and has 976 genes with no counterparts in *Pst* DC3000.
- The vast majority of genes on the two plasmids of *Pst* DC3000 are not present in the *Pss* B728a genome.
- Among the 976 genes of *Pss* B728a with no counterpart in *Pst* DC3000 are those encoding for syringopeptin (SP), syringomycin (SR), indole acetic acid biosynthesis, arginine degradation, and production of ice nuclei.

Feil *et al.*,2005

Important virulence-associated systems

- *P. syringae* uses an impressive variety of virulence-associated systems during the course of its host interactions (host colonization and pathogenesis).
- These systems produce:

1. Toxins,
2. Ice nucleation proteins,
3. Antimicrobial resistance, and
4. Secreted effectors.

- The best-studied virulence-associated factors are the effector proteins secreted through the type III secretion system.

Guttman,2005

Specific ice genes

The bacterial phenotype is due to a protein product of a single gene

- In spite of many studies being done, the location of ice nucleation gene whether in chromosomal or plasmid DNA has not been identified.
- **Plasmids** have been introduced into host *Escherichia coli*, converting the *E. coli* phenotypically from Ina^- (no ice-nucleating activity) to Ina^+.
- The ice-nucleation gene for *P. viridiflava* KUIN-2 is in the plasmid DNA.

Ohgama *et al.*,2014;…

Ice nucleation and anti-freeze proteins
P. syringae

- As the most ice nucleation active bacterial species, *P. syringae* is responsible for inciting frost injury to frost sensitive plants that can supercool and avoid damaging ice formation if not colonized by ice nucleation active bacteria.
- We present here a genomic comparison between strains:

1. *Pseudomonas syringae* pv. *syringae* (*Pss* B728a), and
2. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) as well as between
3. these strains and *P. aeruginosa* and *P. putida*, two additional Pseudomonads recently sequenced.

Feil *et al.*,2005

Ice nucleation and anti-freeze proteins

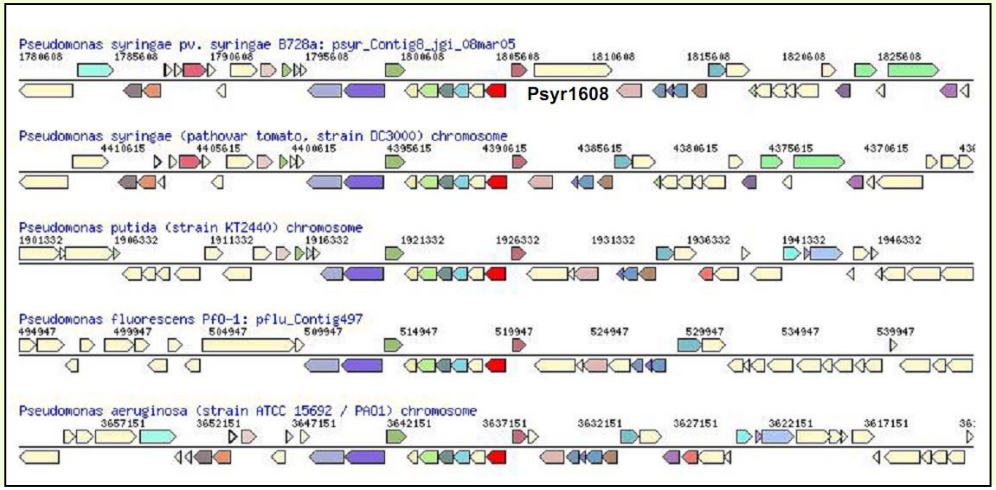
Pss B728a exhibits several traits such as ice nucleation activity and that are lacking in many other strains of *P. syringae* including *Pst* DC3000

- *Pss* B728a (produce both INPs and AFPs) and *Pst* DC3000(unable to produce either INPs and AFPs) are two economically important species of plant pathogenic bacteria differ in host range and apparent patterns of interaction with plants.
- *Pss* B728a also has an unlinked gene encoding for an anti-freeze protein (Psyr0931).
- Anti-freeze proteins are secreted into the medium, where they inhibit the growth of external ice by adsorbing onto the ice surface and lowering the temperature at which it can grow.
- The putative antifreeze gene is found in an operon with 2 glycosyltransferase genes (Psyr0929-0930).

Feil *et al.*,2005;..

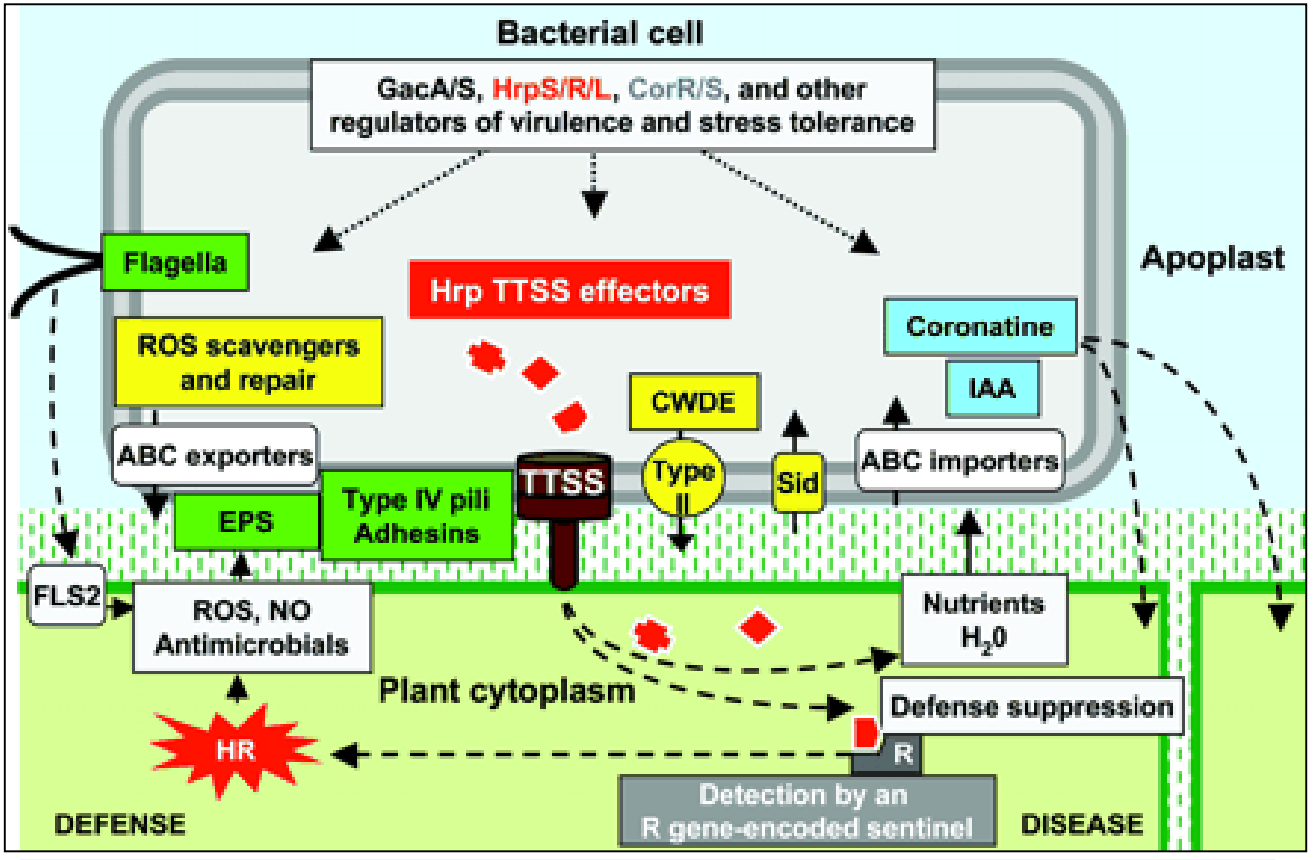
Ice nucleation and anti-freeze proteins
P. syringae

- Neighboring region of the *Pss* ice nucleating gene (Psyr1608, Bacterial ice-nucleation proteins octamer repeat) compared to conserved neighborhood regions in other Pseudomonads.
- Regions are aligned to Psyr1606 (ABC transporter, periplasmic substrate-binding protein, marked in red) of *Pss*.
- Notice the differences occurring in the region between the Bacterial regulatory protein (GntR, marked in brown) and the coding region for Aspartyl aminopeptidase (marked in pink).



Feil *et al.*,2005

Overview of *P. syringae* plant interactions with virulence factors



Motility favors bacterial survival and entry into leaves but flagellin is also recoqnized by means of FLS2 receptor-like kinase as an elicitor of general defenses.

Overview of *P. syringae* plant interactions with virulence factors

- Host-pathogen factors associated with defense and disease are separately grouped on the left and right of the diagram, respectively.
- The central pathogenic process is the injection of multiple effector proteins into plant cells by the TTSS, which is depicted as the brown structure traversing the bacterial inner and outer membranes, plant cell wall, and plasma membrane.
- The effectors may suppress defenses and promote nutrient and water accumulation in the apoplast unless any one of them is detected by a resistance (R) gene-encoded sentinel, in which case strong defenses associated with the hypersensitive response (HR) are triggered.
- Motility favors bacterial epiphytic survival and entry into leaves, but flagellin is also recognized by means of the FLS2 receptor-like kinase as an elicitor of general defenses, which are not as strong as HR-associated defenses and may be suppressed by Hrp effectors.
- Exclusion or tolerance of reactive oxygen species (ROS) and other antimicrobials is likely promoted by extracellular polysaccharides (EPS), scavengers, and ABC exporters.
- Favoring virulence are:
- type IV pili (Tfp) and possibly adhesins, coronatine synthesis and regulation, plant cell-wall-degrading enzymes (CWDE) and other variously secreted proteins, iron-scavenging siderophores (Sid), indoleacetic acid (IAA), and probably numerous ABC transporters and other nutrient uptake systems.

Buell *et al.*,2003

ATP-binding casssette transporters
Subfamilies

- **There are many known ABC transporters present in prokaryotes:**
- **Prokaryotic subfamilies**
- **Importers** (e.g.):
- Carbohydrate Uptake Transporter-1 (CUT1)
- Polar Amino Acid Uptake Transporter (PAAT)
- Peptide/Opine/Nickel Uptake Transporter (PepT)
- Thiamin Uptake Transporter (ThiT)
- Siderophore-Fe^{3+} Uptake Transporter (SIUT)
- Lipid Exporter (LipidE)
- Molybdate Uptake Transporter (MolT)
- **Exporters** (e.g.):
- Capsular Polysaccharide Exporter (CPSE)
- Teichoic Acid Exporter (TAE): An enzyme found in Gram-positive bacteria that exports teichoic acid.
- β-Glucan Exporter (GlucanE)
- Protein-1 Exporter (Prot1E)
- Protein-2 Exporter (Prot2E)
- Peptide-1 Exporter (Pep1E)
- Peptide-2 Exporter (Pep2E)
- Peptide-3 Exporter (Pep3E)
- Drug/Siderophore Exporter-3 (DrugE3)

Wikipedia,2008

Major pathogenicity factors of the bacterium 5,763 genes (orfs)

- 298 genes thought to encode functions involved in virulence.
- This explains why mutations that block virulence are usually found to only occur in the *hrp* secretion genes.
- Catagories:

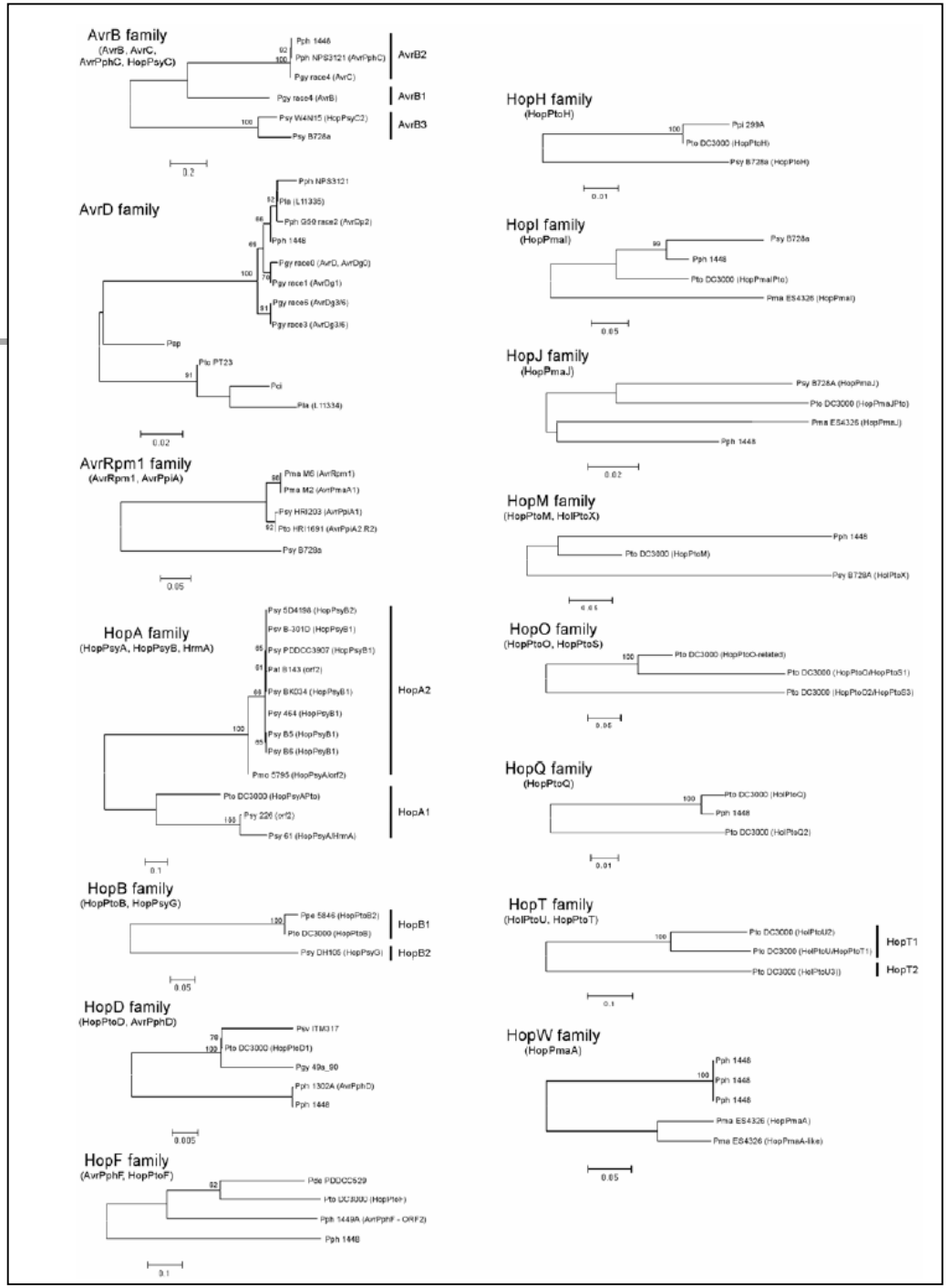
1. 31 protein secreted by the type III hrp system; these are called HOP proteins for hrp outer protein and avirulence proteins.
2. Siderophore biosynthetic genes (sid.) – iron acquisition.
3. Coronatine (COR) toxin biosynthesis;
4. Indole-3-acetic acid (IAA) biosynthesis genes.
5. Adhesins for adhesion to plant cells during colonization.
6. Extracellular polysaccharide(EPS) synthesis.
7. Tolerance to reactive oxygen species and heavy metals.
8. Degradative enzymes - e.g. pectin enzyme and cellulase.

Nomenclature & Phylogeny of Hops in *P. syringae*

- The term "Hop" applies generically to expressed proteins that are secreted or translocated by the TTSS of *P. syringae* and related plant pathogens.
- Lack of a systematic nomenclature has resulted in multiple names being assigned to the same Hop.
- Guidelines are provided for phylogenetic characterization and name selection for Hops that are novel.
- e.g.
- If >60% of a new protein's sequence can be significantly aligned ($e < 10^{-5}$) with one or more members of a Hop family previously characterized using criteria B, C, or D, the new protein can be given a Hop name reflecting this relationship.

Phylogenetic analysis of Hops

Phylogenetic trees for some Hrp outer protein (Hop) families with three or more members.



Lindeberg *et al.*,2005

Type IV secretion system

Mediate attachment of various *P. syringae* pathovars to the leaves

- Type IV pili(TFP) have been shown to mediate attachment of various *P. syringae* pathovars to the leaves of both susceptible and non-susceptible plants.
- *P. syringae* tfp mutants show significantly reduced adhesion to plant tissues and the ability to generate symptoms in host plants when spray inoculated also correlates with the presence of tfp.
- Taken together these results indicate that tfp-mediated adhesion of *P. syringae* phytopathogens to plant tissues is an important factor in the disease process.

Whitchurch,2006

Other genes involved in symptom development

GacS/GacA:The two-component regulatory system

- Genes in the *hrp* regulon are not directly required for symptom development by *P. syringae*.
- Unlike *hrp* genes, the requirement for *gacS* (global activator sensor kinase) in *P. syringae* appears to be pathovar specific.
- Evidence that GacS (sensor kinase) and GacA (response regulator) are partners of a two-component system was first obtained genetically in *P. syringae* pv. *syringae* and subsequently confirmed for in a wide variety of Gram-negative bacteria such as enteric bacteria and fluorescent pseudomonads.

GacS/GacA
The two-component regulatory system

- The two-component regulatory system comprised of:
1. the sensor kinase, GacS(formerly lemA), and
2. its response regulator, GacA.
- GacS/GacA is involved in regulation of secondary metabolism and many other aspects of bacterial physiology.
- The sensor kinase GacS, initially called LemA, was first described in *Pseudomonas syringae* pv. *syringae* strain B728a.

Heeb and Hass,2001

GacS/GacA

The two-component regulatory system

Small regulatory RNAs (RsmY and RsmZ)

- The GacS/GacA two-component system, a global regulator of the expression of bacterial products by turning on the transcription of small RNAs.
- These small RNAs act as antagonists of the unique RNA-binding protein RsmA, which negatively controls the expression of QS-related genes and several extracellular products.
- GacA was first described in *P. fluorescens* biocontrol strain CHA0 as a global activator of antibiotic and cyanide production.

Zaho *et al.*,2014; Heeb and Hass,2001

GacS/GacA

The two-component regulatory system

Small regulatory RNAs (RsmY and RsmZ)

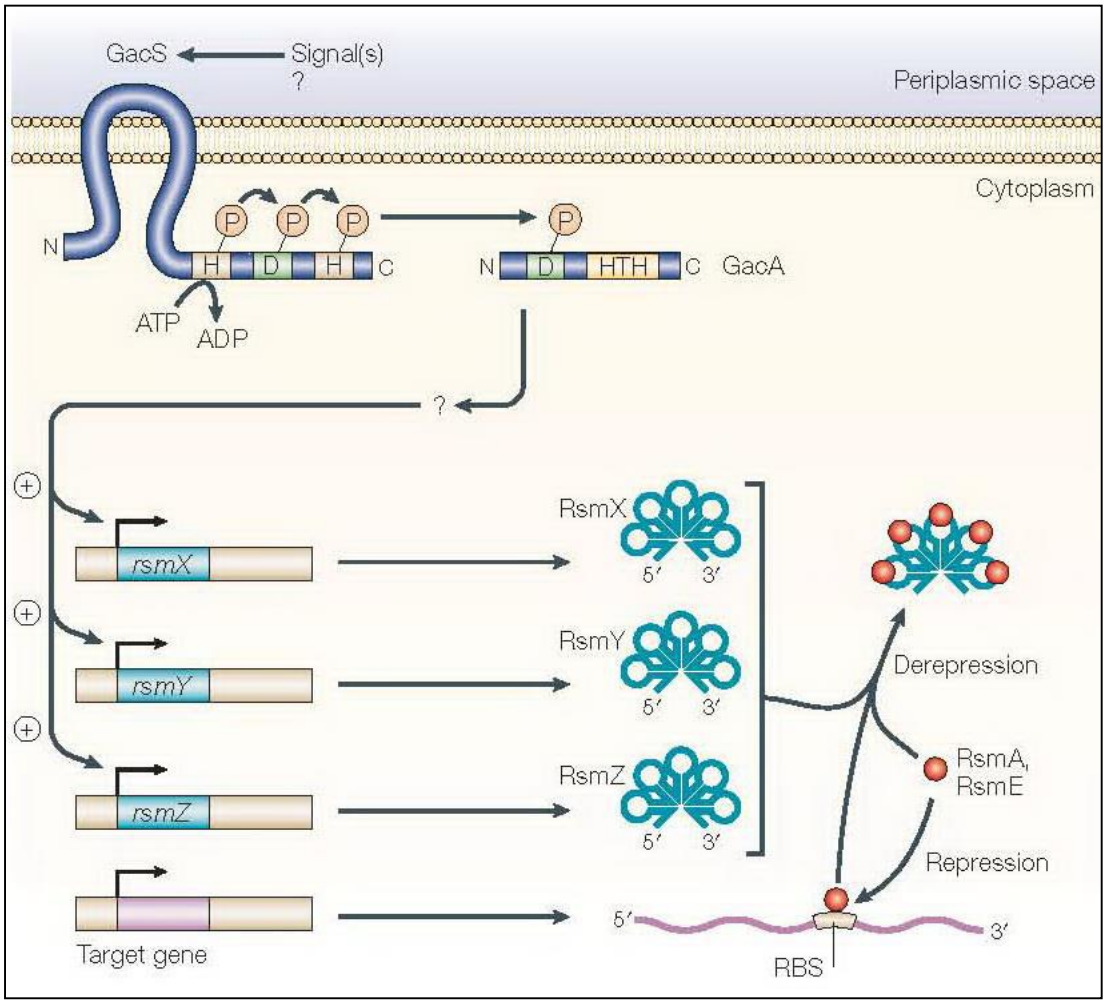
- Small regulatory RNAs (RsmY and RsmZ) serving as early responders, can promote the expression of dependent genes (e.g. *lasR*) to boost the synthesis of intracellular enzymes and coordinate instant cooperative behavior in bacterial cells (QS).
- These early responders, acting as a rheostat to finely modulate bacterial cooperation, which may be quickly activated under environment threats, but peter off when critical QS dependent genes are fully functional for cooperation

Zaho *et al.*,2014

GacS/GacA

The two-component regulatory system

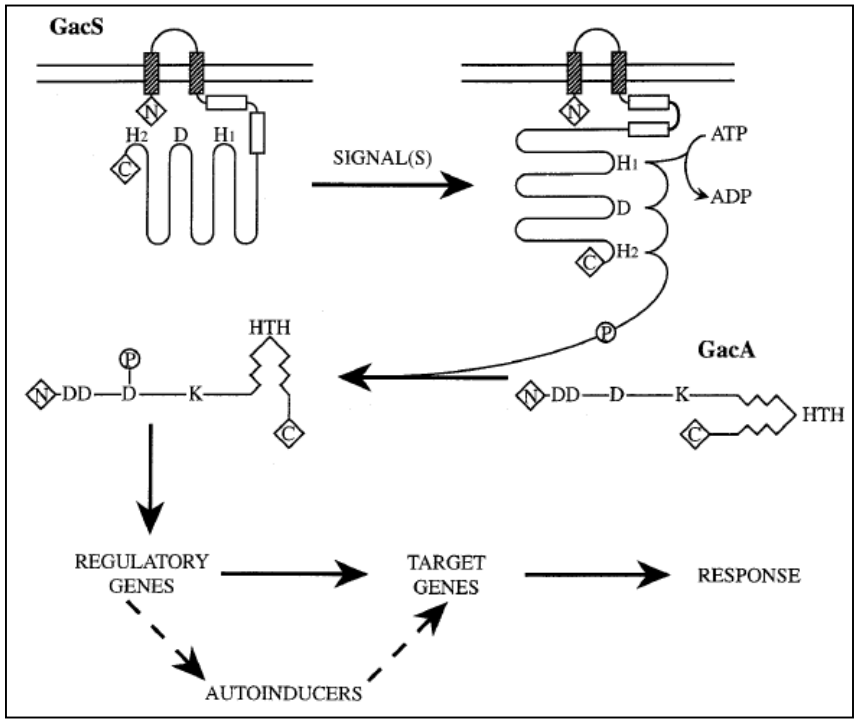
Small regulatory RNAs (RsmY and RsmZ)



GacS/GacA two-component system

Model of the signal transduction pathway mediated by the GacS/GacA two-component system

1. GacS protein senses abiotic (e.g., pH, temperature, or osmolarity) or biotic (bacteria) signals and activates, via a phosphorelay mechanism.
2. The GacA transcription regulator, in turn triggers the expression of regulatory genes such as QS-related genes and other target genes.



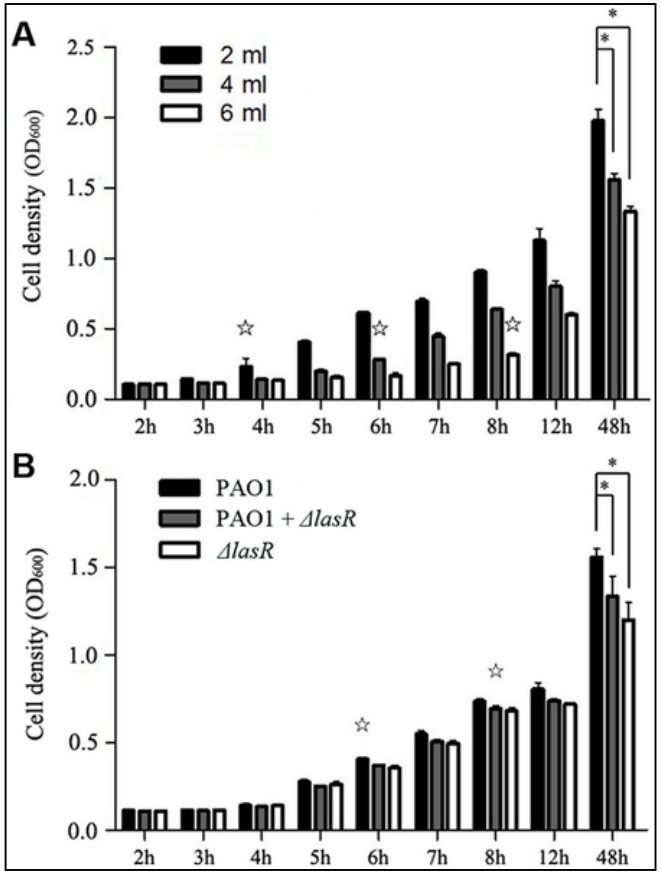
N and C terminus; HTH, helix-turn-helix motif

Heeb and Hass,2001

GacS/GacA two-component system
Cooperation activation was delayed due to the presence of lasR mutant

A. Genes as early responders regulate quorum-sensing and control bacterial cooperation in *Pseudomonas aeruginosa* strain PAO1.

B. In the absence of the central regulatory QS gene *lasR*, the expression of dependent genes was impaired along with the dramatically increased expression of *rsmY* and *rsmZ* compared with the WT PAO1.

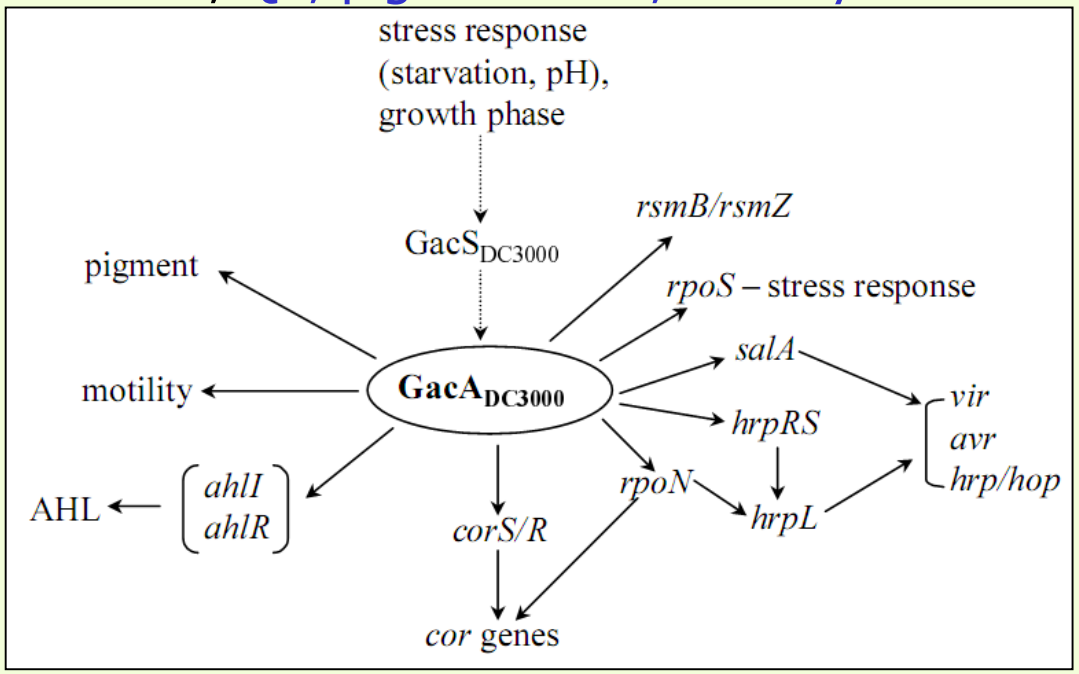


Zaho *et al.*,2014

GacS/GacA

GacS/GacA two-component system controls regulation of secondary metabolism and many other aspects of bacterial physiology

- The GacS/GacA system controls the production of secondary metabolites and extracellular enzymes involved in pathogenicity to plants and animals, biocontrol of soilborne plant diseases, ecological fitness, QS, pigmentation, motility and tolerance to stress.

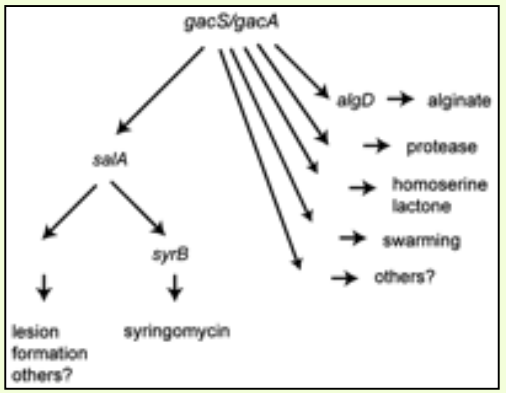


Chatterjee *et al.*,2003; Heeb and Hass,2001

GacS/GacA

GacS/GacA two-component system controls regulation of secondary metabolism and many other aspects of bacterial physiology

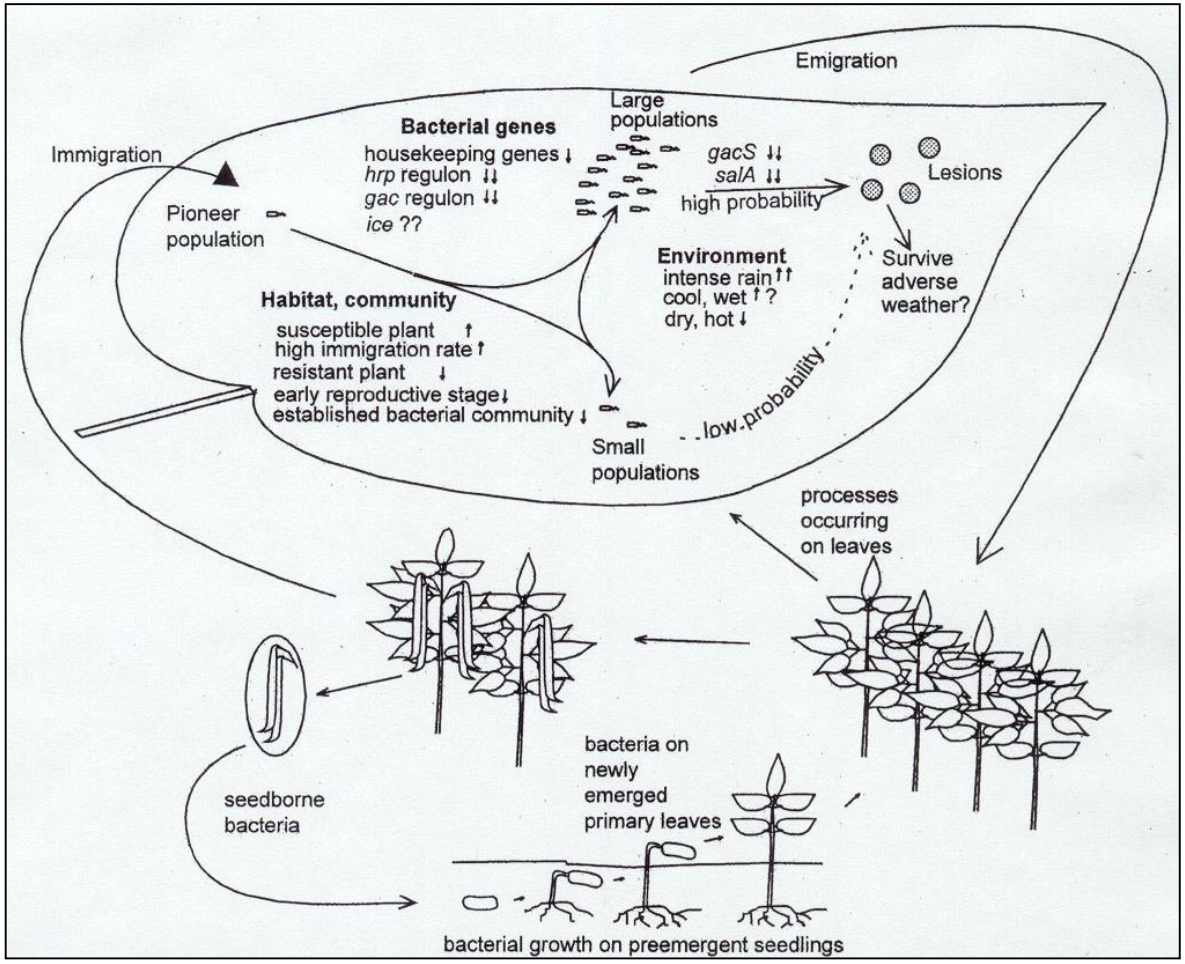
- *gacS* and *gacA* mutants of strain B728a are affected in production of:



1. Extracellular proteases
2. Acyl homoserine lactone(QS)
3. Alginate
4. Toxins, and
5. Swarming behavior on soft agar medium.

Bacteria in the leaf ecosystem

With emphasis on *Pseudomonas s. phaseolicola*- A pathogen, ice Nucleus, and epiphyte



Hirano & Upper,2000

The role of ethylene in the pathogenicity process of *P. syringae* pathovars

- Ethylene production is important in the pathogenicity process of *P. syringae* pathovars.
- Ethylene may play a role in symptom development, because it can cause chlorosis, abscission, and senescence and thus may be important in the predisposition of plant tissue to disease.

Determination of ethylene and bacterial growth *in vitro*
By gas chromatography

- For the routine determination of ethylene production by *P. syringae* pathovars, 1 ml of an overnight culture in synthetic 5a medium was transferred in a sterile 5-ml syringe sealed with a rubber cap and incubated on a rotary shaker at 90 rpm and room temperature (22 to 248C) for 2 h.
- After incubation, gas samples (1 ml) were withdrawn with a gas-tight syringe (Hamilton) and ethylene was determined with a gas chromatograph equipped with an active alumina column and a flame ionization detector.

Weingart and Völksch,1997

Determination of ethylene and bacterial growth *in vitro*
By spectrophotometery

- Ethylene production *in vitro* and bacterial growth curves were obtained from 60-ml shaking cultures of 5a medium in 250-ml flasks (140 rpm, 288C).
- Ethylene was determined every 3 h as described above, and production was expressed in nanoliters per hour per cell.
- Bacterial growth was estimated with a spectrophotometer (578 nm).

Weingart and Völksch,1997

Determination of ethylene and bacterial growth *in planta*
On Petri plates

- Fifteen discs (7 mm in diameter) were excised with a cork borer from infected leaves at designated intervals, transferred into 3-ml syringes (five discs per syringe) sealed with rubber caps, and incubated at room temperature (22 to 248C) for 6 h.
- A 1-ml gas sample was taken from each syringe, and ethylene production was determined as described earlier.
- After we determined the ethylene concentrations, the bacterial populations in the leaf discs were monitored.
- The five discs of a syringe were macerated together in 5-ml isotonic NaCl. Appropriate serial 10-fold dilutions were plated onto King's medium B.
- Plates were incubated at 28° C, and colonies from three replicate plates were counted after 3 to 4 days. The amount of ethylene produced was expressed in nanoliters per hour per square centimeter.

Weingart and Völksch,1997

P. syringae pathovars

Pathovars tested for their ability to produce ethylene

- Of six *P. syringae* pv. *pisi* strains, five produced ethylene.
- All strains of *P. s.* pv. *glycinea* isolated from soybean plants of various regions and all strains of *P. syringae* pv. *phaseolicola* isolated from kudzu produced ethylene
- However, strains of *P. s .* pv. *phaseolicola* isolated from beans and all other tested pathovars failed to produce detectable amounts of ethylene.

TABLE 2. *P. syringae* pathovars tested for their ability to produce ethylene

Pathovar	No. of tested strains	Ethylene production (10^{-8} nl h^{-1} $cell^{-1}$)
aptata	4	0
atrofaciens	3	0
atropurpurea	4	0
cannabina	1	0
coronafaciens	3	0
glycinea	50	5–100
lachrymans	3	0
maculicola	3	0
mori	1	0
morsprunorum	4	0
phaseolicola (from kudzu)	4	30–70
phaseolicola (from bean)	8	0
persicae	1	0
pisi	5	1–3
pisi	1	0
primulae	1	0
savastanoi	3	0
striafaciens	1	0
syringae	5	0
tabaci	4	0
tagetis	1	0
tomato	7	0

Weingart and Völksch,1997

Pathogenicity of:
Pseudomonas savastanoi pv. *savastanoi*

Olive knot Disease

Major pathogenicity factors of the bacterium

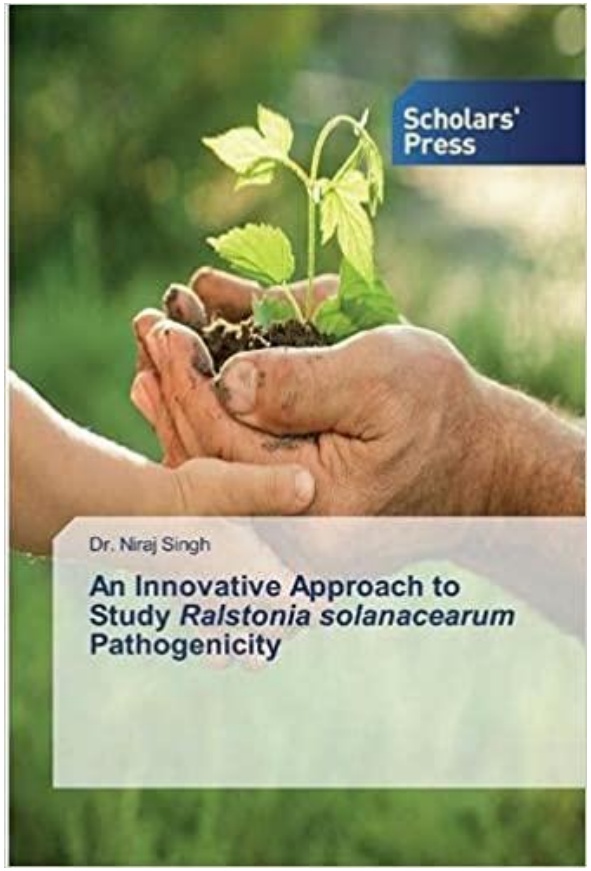
- Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis.
- Disease symptoms consist of tumorous outgrowths induced in the plant by bacterial production of indole-3-acetic acid (IAA).
- Synthesis of IAA occurs by the following reactions:
- L-tryptophan leads to indoleacetamide leads to indoleacetic acid, catalyzed by tryptophan 2-monooxygenase and indole acetamide hydrolase, respectively.
- Whereas the enzymology of IAA synthesis is well characterized, nothing is known about the genetics of the system.

Pathogenicity of:
Ralstonia solanaceraum

Bacterial wilt disease

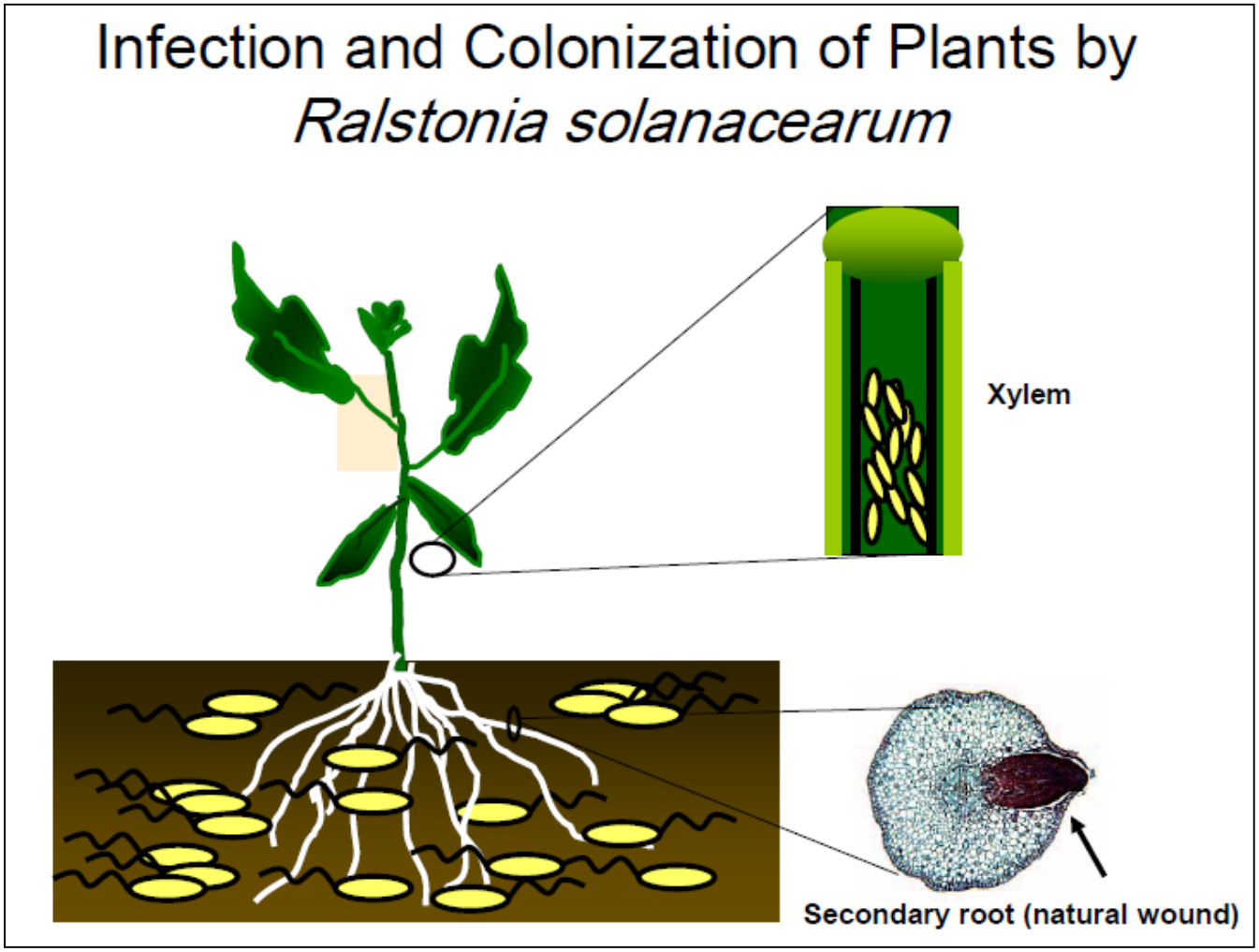
An Innovative Approach to Study *R. solanacearum* Pathogenicity

- **An Innovative Approach to Study *Ralstonia solanacearum* Pathogenicity**
- Niraj Singh
- Scholars' Press
- March 27, 2020
- 68 pages.



Infection and colonization of plants

Ralstonia solanacearum



EPS I: An important pathogenicity determinant in *Ralstonia solanacearum*

- An important pathogenicity determinant produced by *R. solanacearum* is the exopolysaccharide (EPS I), a high-molecular-mass acidic extracellular polysaccharide.
- It is a heterogeneous polymer containing a trimeric repeat unit of:

1. N acetyl galactosamine,
2. 2-N-acetyl-2-deoxy-L-galacturonic acid, and
3. 2- N-acetyl-4-N- (3-hydroxybutanoyl)-2-4-6-trideoxy-D-glucose (Orgambide *et al.*,1991).

- Genes coding for the biosynthetic pathway for EPSI are encoded by the 20kb *eps* operon (Schell,2000).

Gonzalez,2010

Protein secretion systems
R. solanacearum

- The pathogenicity of *R. solanacearum* is the result of the cooperation and coordination of various pathogenic factors, mainly including extracellular polysaccharide (EPS), type II secretion system (T2SS), and type III secretion system (T3SS).
- After *R. solanacearum* enters the vascular system of plants, it will secrete a large number of EPS to block the vascular bundle and eventually cause the wilting of plants.
- T2SS can secrete cell wall degradation enzymes, cellulose, and pectin enzymes and produce motion and attachment elements and chemotaxis, which play key roles in the pathogenicity of *R. solanacearum*.

Tan *et al.*,2022

Protein secretion systems

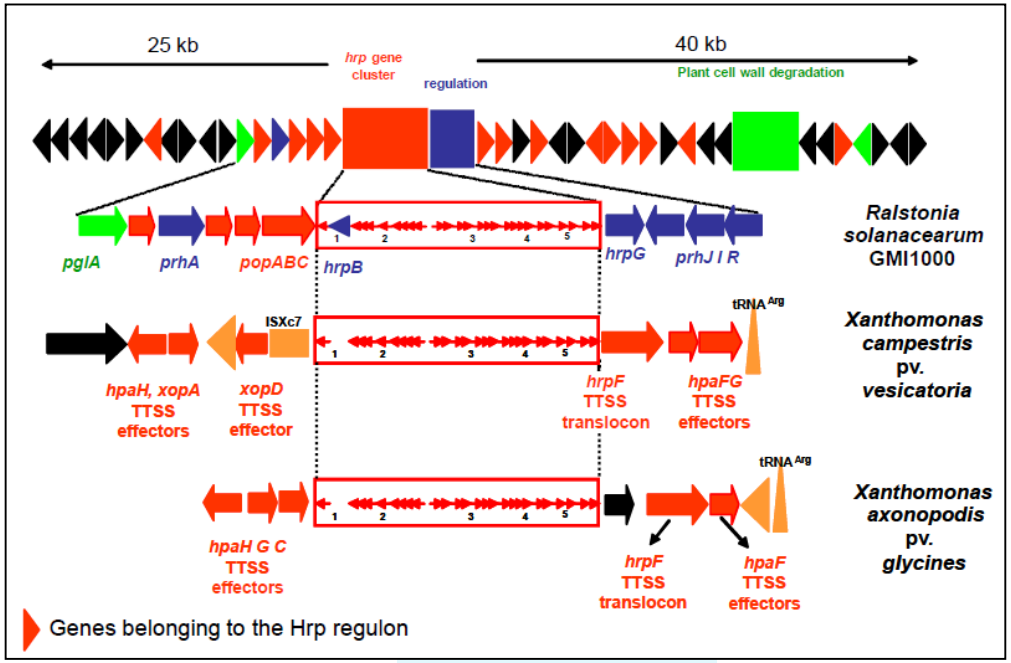
Protein secretion pathways in *R. solanacearum* GMI1000

Secretion pathways	Genes	Substrates	Impact on pathogenicity
Type I	Several predicted	Unknown/not characterized	Unknown
Type II	RSc3105–RSc3116 gene cluster	Several plant cell wall degrading enzymes and other uncharacterized proteins	Essential for pathogenicity
Type III	hrp gene cluster (RSp0849–RSp0874)	74 predicted Type III effectors	Essential for pathogenicity
Type IV	RSc2574–RSc2622 gene cluster	Conjugative transposon export machinery	Unknown
Type V	Two predicted autotransporter adhesin-like proteins (RSc0115 and RSc3162)		Unknown
Type VI	RSp0739–RSp0753 gene cluster	Unknown	Unknown
Two-partner secretion	Eight predicted systems	Adhesin-like proteins	Unknown
Tat export pathway	RSc2940–RSc2942	70 putative Tat-exported proteins	Reduced virulence

Poueymiro and Genin,2009

Genetic organization of the Hrp gene cluster of 3 plant pathogenic bacteria

- Red box indicates the *hrp* gene cluster.
- The head arrows with numbers indicate the hrp transcription units and the complete arrows indicate the different genes.
- Inside the red box of *R. solanacearum* Hrp gene cluster there is *hrpB* transcription regulator which is homologous to *hrpX* in *Xanthomonas* sp.



Gonzalez,2010

Regulation network of virulence functions in *Ralstonia solanacearum*

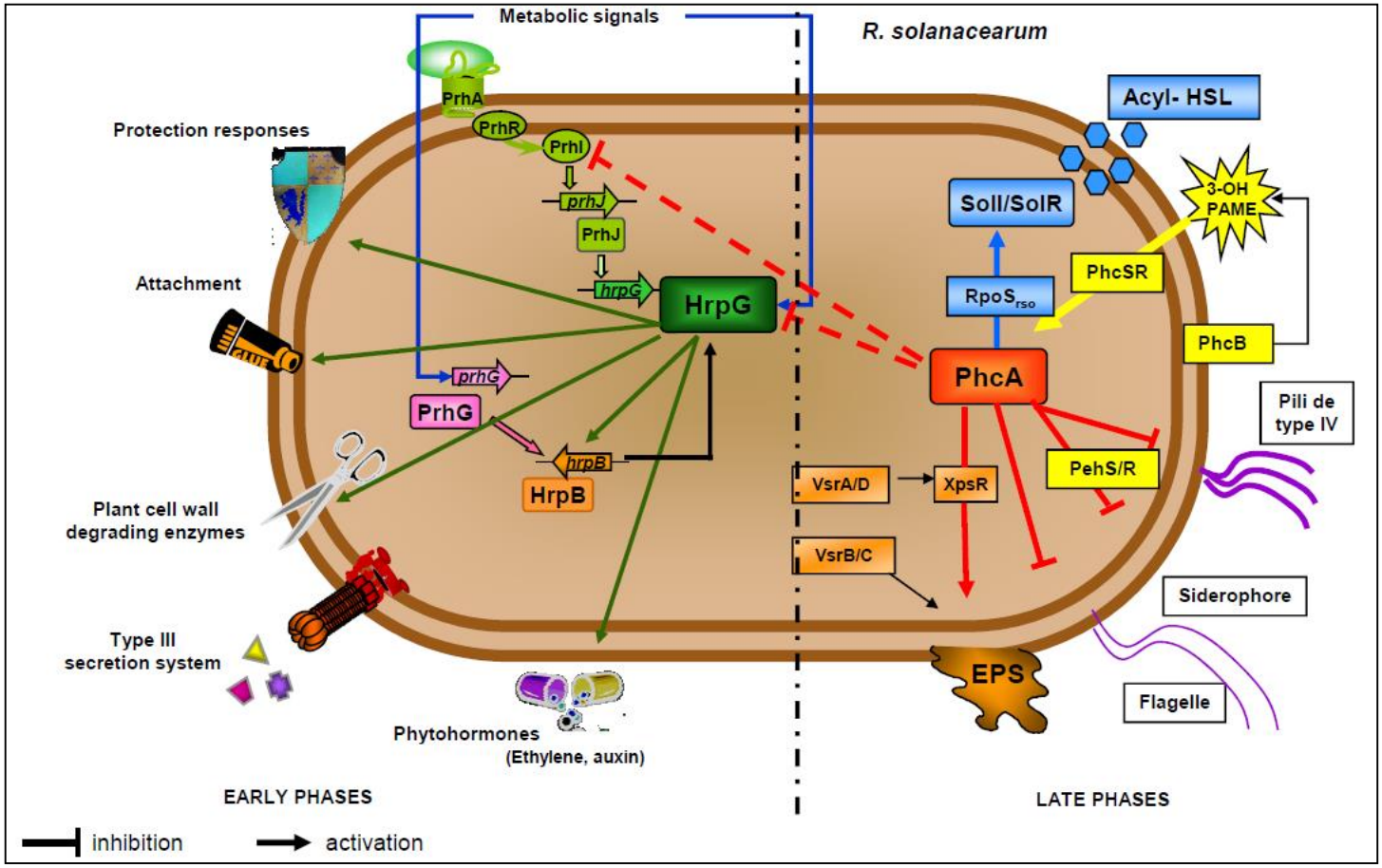
PhcA, a global regulator controlling phenotypic conversion

- At the center of the regulation network is PhcA (Phc, phenotype conversion), a LysR family transcriptional regulator which directly or through intermediary regulatory genes, coordinates the expression of several processes.
- It simultaneously activates diverse virulence genes such as those of:
- EPS biosynthesis, plant cell wall degrading enzymes as Pme and Egl exoproteins, swimming motility or Type IV pili(TFP), and represses others such as *hrp* genes and those related to production of polygalacturonases, siderophores, and motility.

Gonzalez,2010

Regulation network of virulence functions in *Ralstonia solanacearum*

PhcA, a global regulator controlling phenotypic conversion



Gonzalez,2010

Pathogenicity of:

Xanthomonas spp.

Xac, Xcc, Xam, Xcv,Xoo and Xtt

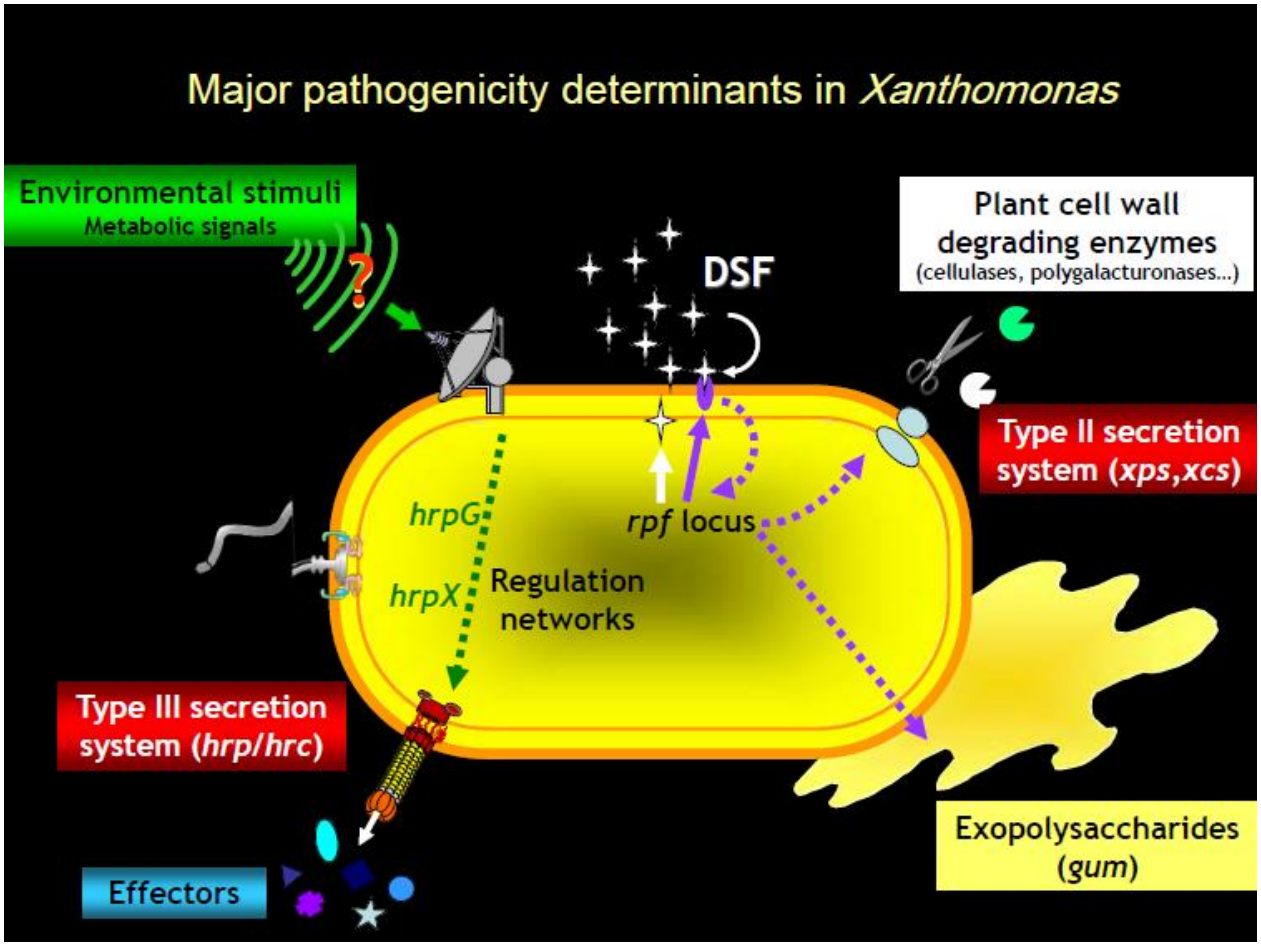
Blight, rot, streak and canker diseases

Major pathogenicity determinants in *Xanthomonas*

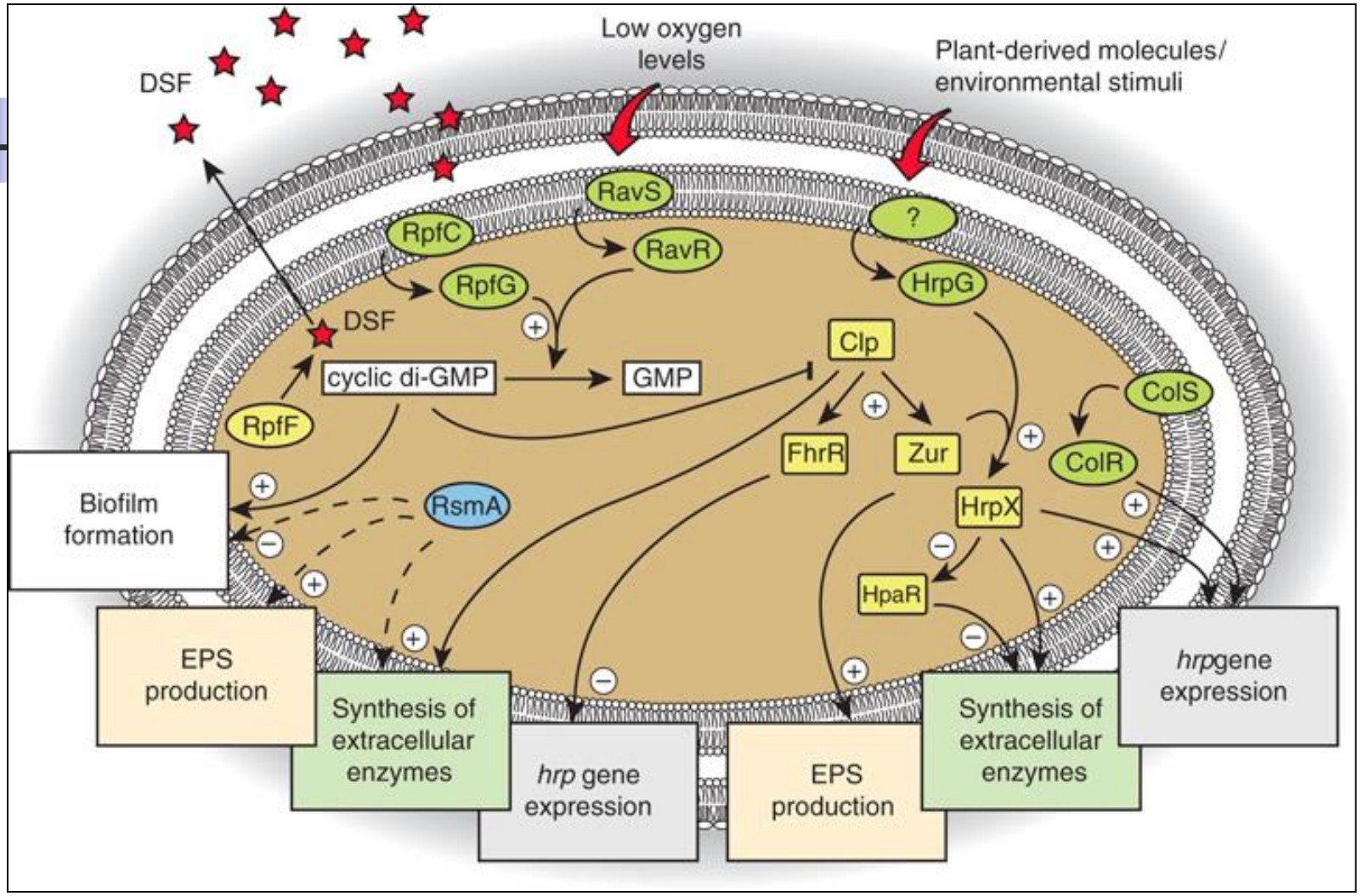
- Molecular biology studies have characterized several key pathogenicity determinants of Xcc.
  - Motility and attachment to plant,
  - EPS and plant cell wall degrading enzymes.
- The synthesis of these 2 last is regulated by a quorum sensing system via *rpf* genes.
  - Type III effectors (T3Es). These are delivered into plant cells via the type III secretion system (T3SS), which is encoded by the *hrp* [hypersensitive response (HR) and pathogenicity] regulon.
- Type III secretion system (T3SS), is regulated by 2 master regulatory genes hrpG and hrpX.

Bogdanove,2011;..

Major pathogenicity determinants in *Xanthomonas*



Bogdanove,2011



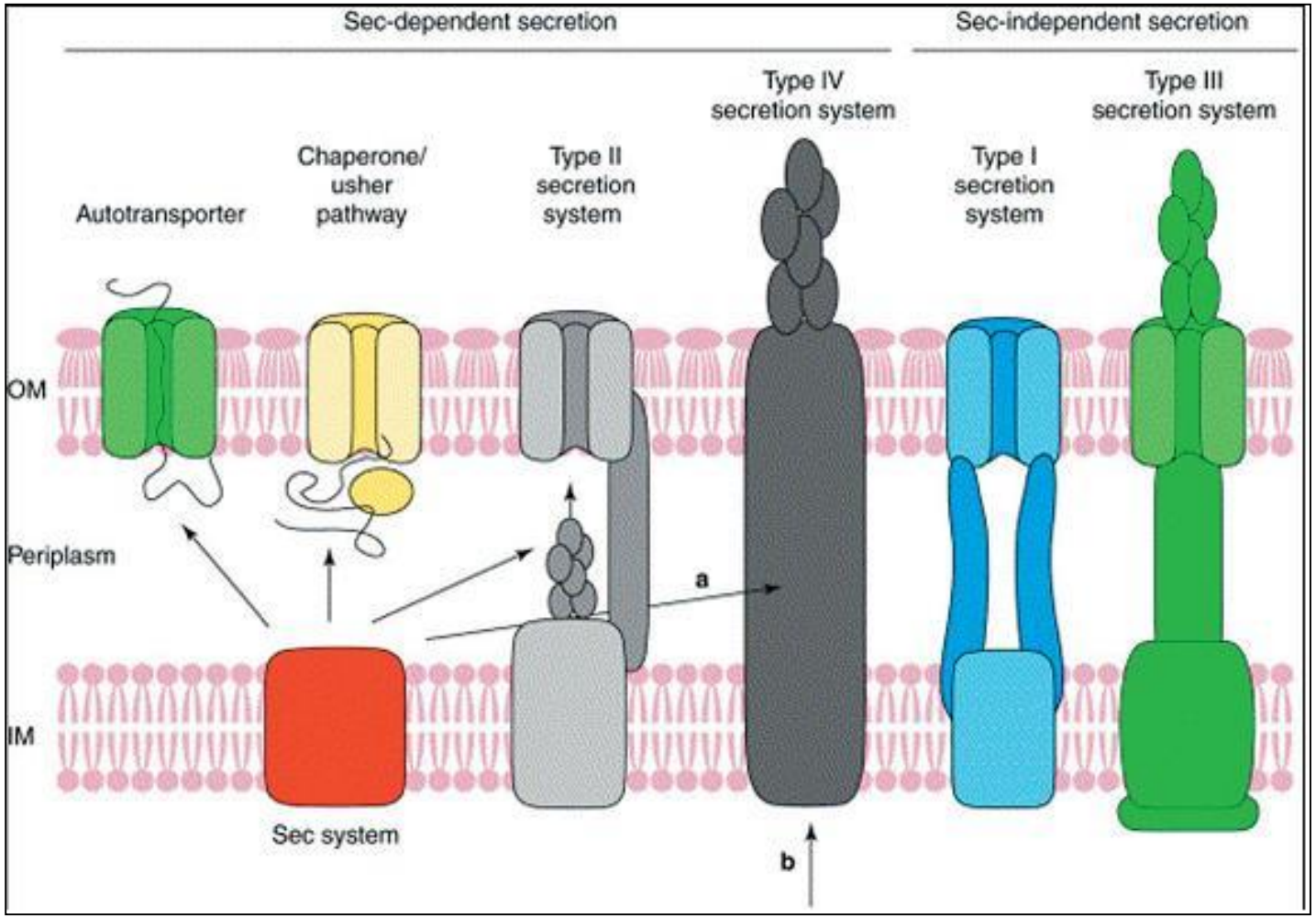
Buttner and Bonas,2010

Secretion systems
Xanthomonas spp.

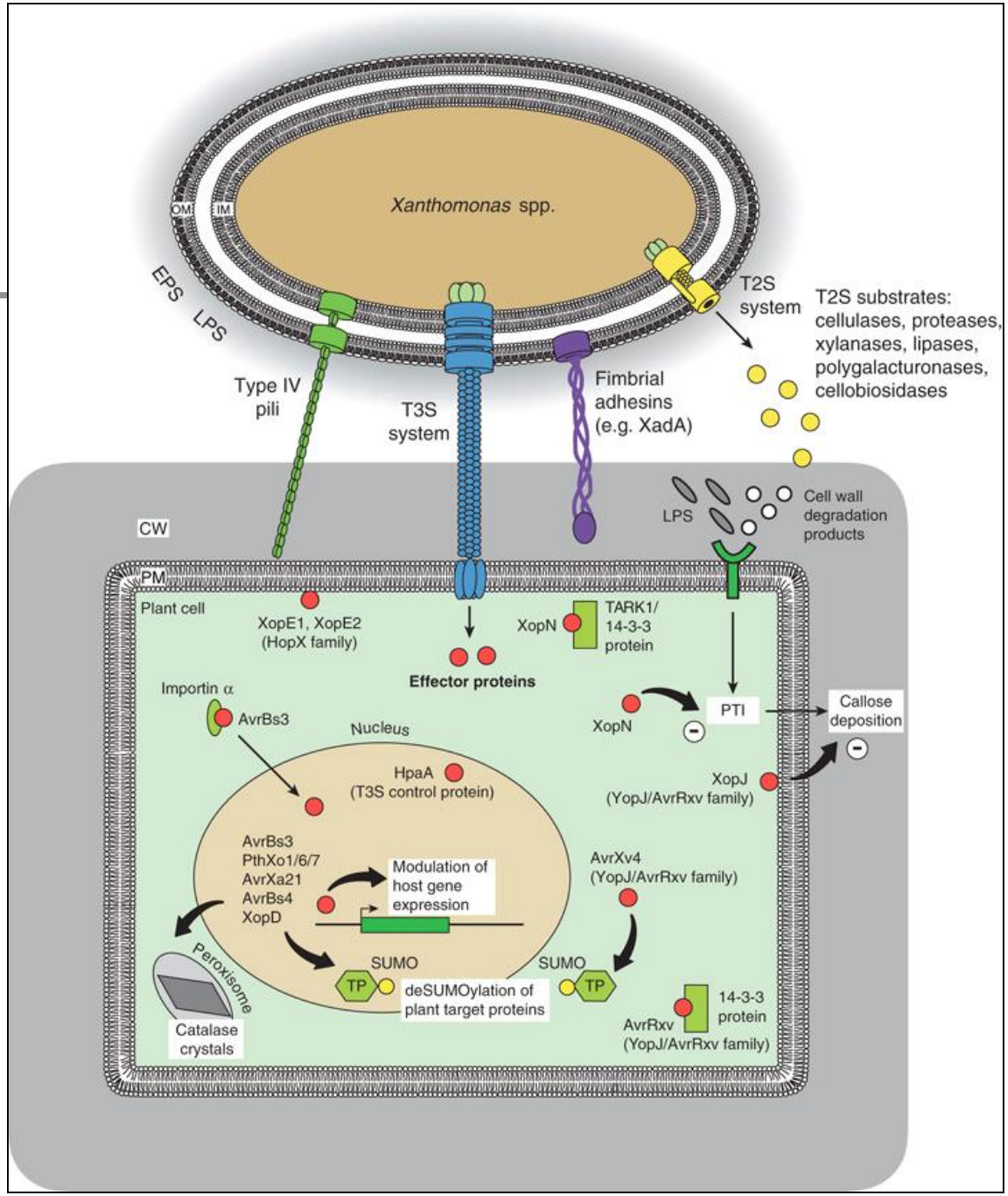
- Protein secretion systems play an important role in the interaction of pathogens with their host.
- *Xanthomonas* spp. contain genes for all known protein transport systems in Gram-negative bacteria, i.e.
- The Sec,
- Signal recognition particle, and
- TAT pathways;
- Type I, type II, type III, and type IV secretion systems of different types,
- Type V autotransporters,
- Two-partner secretion systems, and
- A type VI secretion system.
- However, for most *Xanthomonas* secretion systems the substrates and their importance for bacterial virulence are unknown.

Kay and Bonas,2009

Type I-V secretion systems in Gram-negative bacteria



Type II-IV secretion systems in *Xanthomonas* spp.



Buttner and Bonas,2010

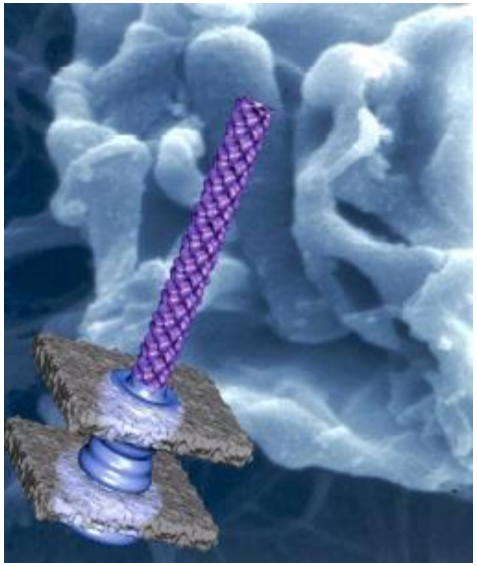
TTSS
The Hrp pathway

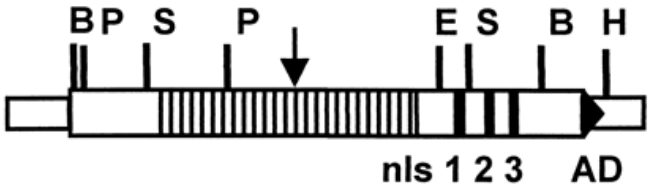
- Pathogenicity of *Xanthomonas* and most other Gram-negative phytopathogenic bacteria depends on a conserved type III secretion (T3S) system which injects more than 25 different effector proteins into the plant cell.
- Extensive studies in the last years on the molecular mechanisms of type III effector function revealed that effector proteins with enzymatic functions seem to play important roles in the interaction of Xanthomonas with its host plants, for example, the SUMO protease XopD.
- In addition, xanthomonads express a unique class of type III effectors to pursue another strategy.

Kay and Bonas,2009

TTSS
Type III secretion system

- *Xanthomonas* species can infect most types of plants.
- Inject proteins into plant cells with the T3SS.
- Proteins travel to plant nucleus, bind to DNA, and turn on specific plant genes that make the plant susceptible to infection.





Secretion systems

Known type III effectors of Xanthomonas and their (putative) function

Protein(s)	(Predicted) function	Homologs[a] in								Reference[b]
		Xac	*Xcc*	*Xcv*	*Xoc*	*Xoo*	*Ps*	*Rs*	apb	
AvrBs1	Unknown function	–	+	+	–	–	+	–	–	[9]
AvrBs2	Putative glycerophosphoryl-diester phosphodiesterase	+	+	+	+	+	–	–	–	[9]
AvrBs3, Avrb6, AvrXa7, PthA, and others	AvrBs3 family, TAL activity	+	–	+	+	+	–	+	–	[9,28••,29••,41]
AvrRxo1	Unknown function	–	–	+	+	–	–	–	–	[47]
AvrRxv, AvrBsT, AvrXv4, XopJ	YopJ/AvrRxv-family, putative cysteine proteases (C55 family) or acetyltransferases	–	(+)	+	–	–	+	+	+	[9,16]
AvrXccC	AvrB family, unknown function	–	+	–	–	–	+	–	–	[10]
AvrXv3	Unknown function	–	–	+	(+)	–	+	–	–	[48]
Ecf XopX	HopAE1 family, unknown function	+	+	+	+	+	+	–	–	[9]
HpaA	Unknown function	+	+	+	+	+	–	–	–	[49]
XopB	Unknown function	–	–	+	–	–	+	+	–	[9]
XopC	Unknown function	–	–	+	–	–	–	(+)	–	[9]
XopD	SUMO cysteine protease (C48 family), DNA-binding (HLH motif), EAR motifs	–	+	+	–	–	(+)	–	–	[9,18••]
XopE1, XopE2	HopX family, putative transglutaminases	+	+	+	–	–	+	–	–	[16]
XopF1, XopF2	Unknown function	(+)	(+)	+	+	+	–	–	–	[9]
XopN	Unknown function	+	+	+	+	+	+	–	–	[9,11]
XopO	Unknown function	–	–	+	+	–	+	–	–	[9]
XopP	Unknown function	+	+	+	+	+	–	+	–	[9]
XopQ	HopQ1-1 family, putative inosine-uridine nucleoside *N*-ribohydrolase	+	+	+	+	+	+	+	–	[9]

[a] Homologs of effectors from *X. campestris* pv. *vesicatoria* and, in case of AvrXccC, *X. campestris* pv. *campestris* were identified using BLAST algorithms [50]. (+) indicates partial homology or disrupted homologs. *Xac*, *X. axonopodis* pv. *citri*; *Xcc*, *X. campestris* pv. *campestris*; *Xcv*, *X. campestris* pv. *vesicatoria*; *Xoc*, *X. oryzae* pv. *oryzicola*; *Xoo*, *X. oryzae* pv. *oryzae*; *Ps*, *P. syringae*; *Rs*, *R. solanacearum*; apb, Gram-negative animal–pathogenic bacteria.

[b] *Xanthomonas* effectors are reviewed in [9]. Additional results are cited separately.

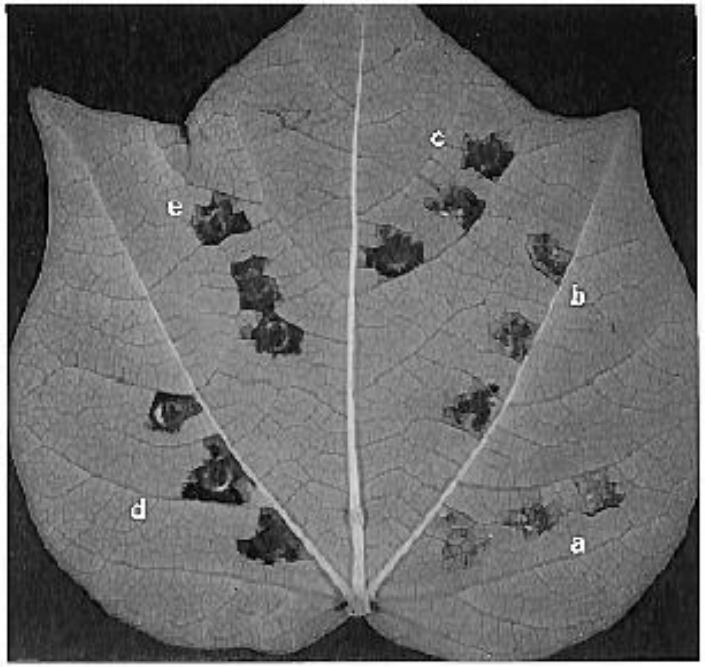
Kay and Bonas,2009

avr gene families in xanthomonads
Xanthomonas axonopodis pv. *malvacearum*

- There are at least 19 well-described races of the pathogen *Xanthomonas axonopodis* pv. *malvacearum*.
- 10 *avr* genes were cloned and characterized from a single North American strain of *X. axonopodis* pv. *malvacearum*, XcmH.
- These ten *avr* genes belong to a large family of *Xanthomonas avr/pth* (avirulence (*avr*)/pathogenicity (*pth*) gene family.
- Most of these *avr* genes genetically "recognize" multiple *R* gene loci in an *avr* gene-for-*R* genes (plural *R* genes) manner.

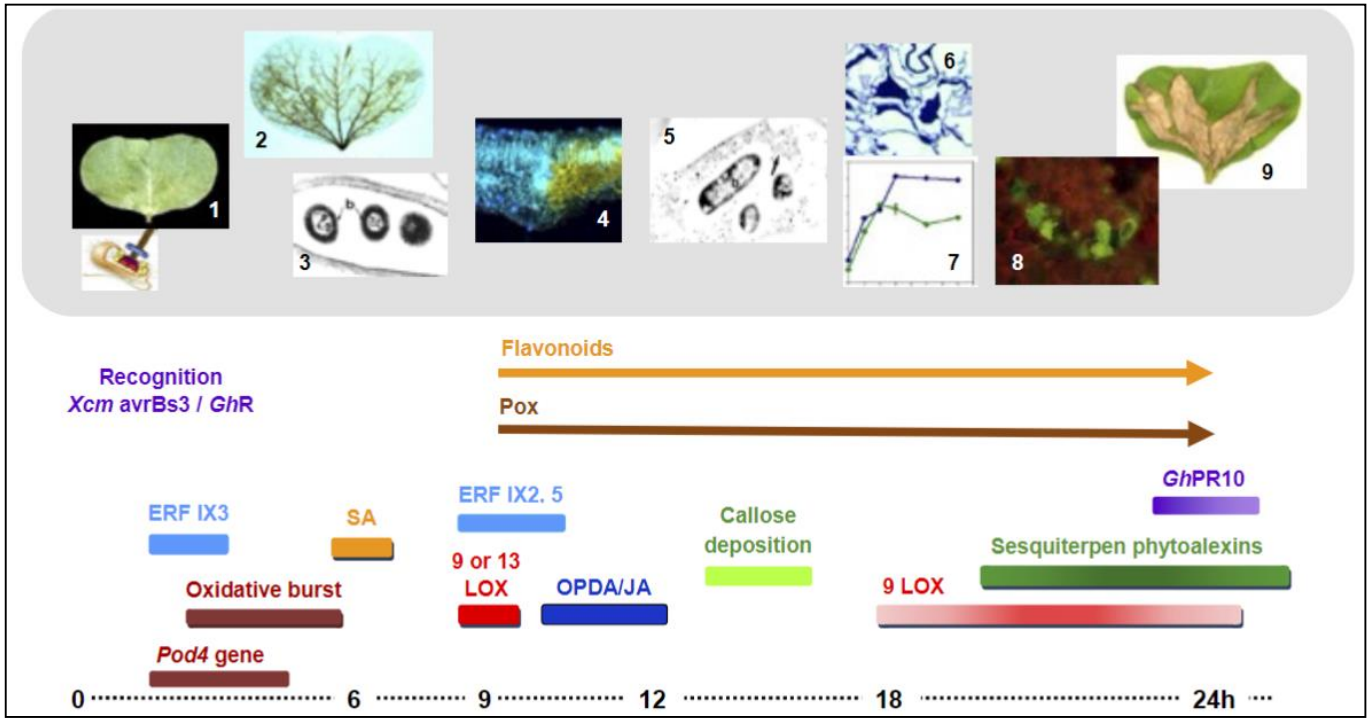
Large family of *Xanthomonas avr/pth* genes *Xanthomonas axonopodis* pv. *malvacearum* *pthN* and *pthN2*

- 10 genes belong to a large family of *Xanthomonas avr/pth* (avirulence/pathogenicity genes) were found in *X. a.* pv. *malvacearum*.
- Water-soaking symptoms caused by *pthN* and *pthN2*.
- Photo taken 5 days after inoculation.



Chakrabarty *et al.*,1997

Time sequence of physiological events involved in the cotton HR to *Xanthomonas citri* pv. *malvacearum*



Following penetration of leaves by the pathogen (figs. 1 and 3), bacterial cells injected the effectors within the host nuclei. When recognized by host R proteins, several specific mechanisms were activated through signaling pathways. Appearance of HR symptoms in infected areas from t= 24h (9)

Jalloul *et al.*,2015

Time sequence of physiological events involved in the cotton HR to *Xanthomonas citri* pv. *malvacearum*

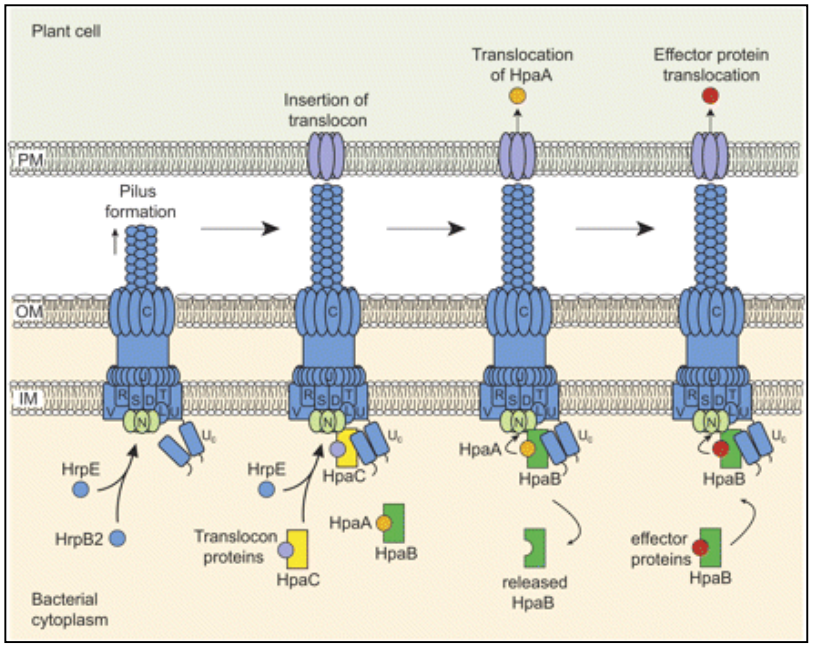
- Genes of the ERF IX3 group are transcribed in parallel to the production of ROS (t = 3h; 2: localization of H_2O_2 in resistant leaves).
- Accumulation of SA culminated after the burst (t= 6h) and before activation of a 9-or 13-Loxgene (t= 9h), transcription of ERF genes and synthesis of OPDA/JA (t= 12h).
- Production of flavonoids (orange stained in infected areas; 4) and total peroxidase activity (5: electron microscopic immunolocalization of peroxidase close to the bacteria) were also detected at the same time.
- Callose deposition contributed to stop the bacterial growth (7: green line) as compared to growth in susceptible plants (7: blue line), and preceded collapse of cells (6: condensation of the cytoplasm of HR cells).
- Appearance of HR symptoms in infected areas from t= 24h (9) occurred following dramatic increase in 9-Lox activity and strong accumulation of sesquiterpene phytoalexins (8: green fluorescence).

Jalloul *et al.*,2015

Model of T3S in the plant pathogenic bacterium *X. campestris* pv.*vesicatoria*

T3S chaperones

- In *X. campestris* pv. *vesicatoria*, translocation of effector proteins is differentially regulated by the global T3S chaperone HpaB, which specifically promotes the translocation of a certain class of effector proteins (Büttner *et al.*, 2006).
- The activity of HpaB is presumably controlled by the secreted regulator HpaA that binds to HpaB in the bacterial cytoplasm and allows secretion of extracellular components of the T3SS.



Büttner and He,2009

Model of the molecular function of the type III effectors XopD and AvrBs3 *Xanthomonas*

- In the next slide the protein structures of:

a) XopD and

b) AvrBs3 are shown, and

c) The proposed mode of action of the effectors is illustrated.

- **XopD:**
- After translocation by the T3S system XopD is transported into the nucleus of the plant cell where it localizes to nuclear foci.
- Here, it binds to DNA unspecifically via a helix-loop-helix (HLH) domain.
- Via two EAR motifs, XopD might inhibit yet unidentified plant transcription factors (TFs).
- Furthermore, XopD possesses SUMO protease activity.
- The latter XopD activities lead to the suppression of:
- Defense- and senescence-associated genes resulting in delayed disease symptoms and increased bacterial multiplication.

Kay and Bonas,2009

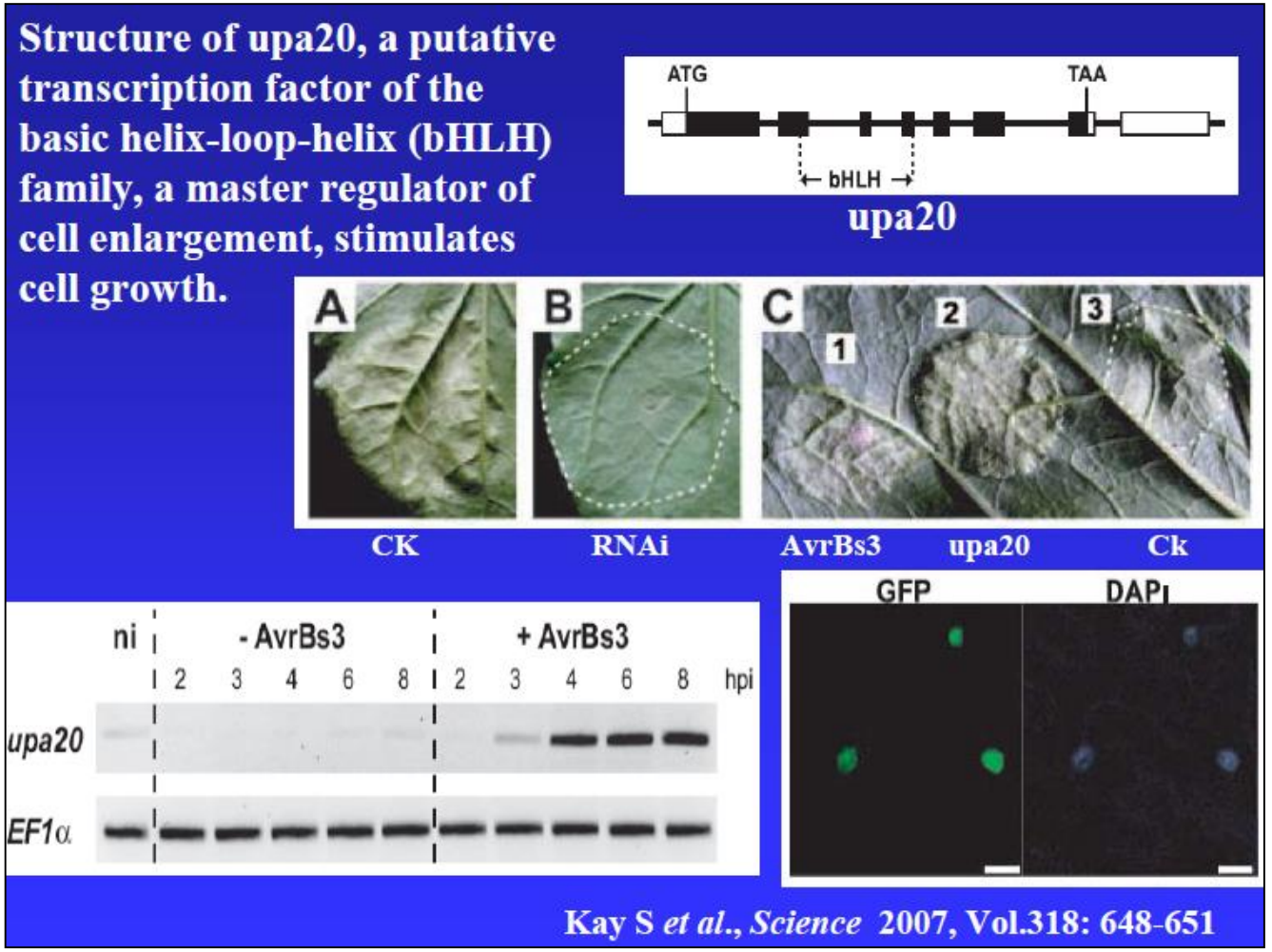
Model of the molecular function of the type III effectors XopD and AvrBs3 *Xanthomonas*

- **AvrBs3:**
- AvrBs3 dimerizes in the plant cell cytoplasm and interacts with importin α via its nuclear localization signals (NLSs).
- The protein complex is bound by importin β mediating nuclear import.
- Here, AvrBs3 binds to a specific DNA sequence, the UPA box, and activates transcription of more than 10 UPA genes.
- UPA20, one of the induced genes, is the key regulator of plant cell hypertrophy.
- In resistant pepper plants, activation of Bs3 leads to the HR.

Kay and Bonas,2009

Structure of upa20

An transcription factor and master regulator of cell enlargement, stimulates cell growth



The AvrBs3 family

Structure and functions of avrBs3 proteins

- The activities of several type III effector proteins ultimately result in a change of plant gene expression.
- One of these effectors is AvrBs3 family.
- So far AvrBs3 family only was identified in *Xanthomonas* spp. and *Ralstonia solanacearum*.
- This transcription factor mimic plant transcriptional activators and manipulate the plant transcriptome.

Kay and Bonas,2009

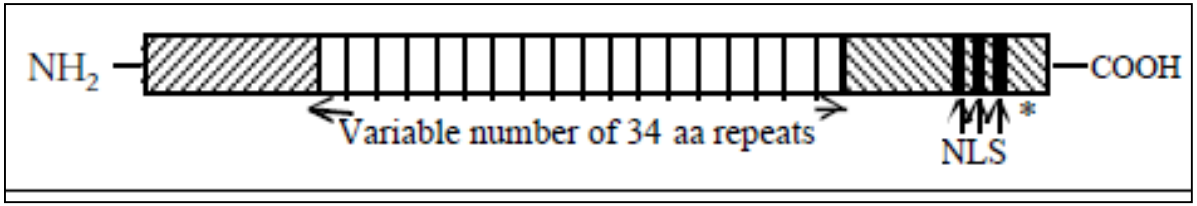
The AvrBs3 family

Structure and functions of avrBs3 proteins

- Three key structures of the gene products are:

1. A central repeat region;
2. Three nuclear localization signal (NLS) sequences; and
3. An acidic activation domain (AAD) have roles in avirulence and/or virulence.

- The central repeat region, which is composed of several nearly identical 34 aa-directly repeated sequences, is essential for virulence and host cultivar specificity.
- The repeat region is leucine rich, and at the end of the repeats there is an imperfect leucine zipper suggesting a potential role in protein-protein interactions.
- The NLS are necessary for both avirulence and virulence functions, and to localize the Avr protein into the plant cell nucleus.



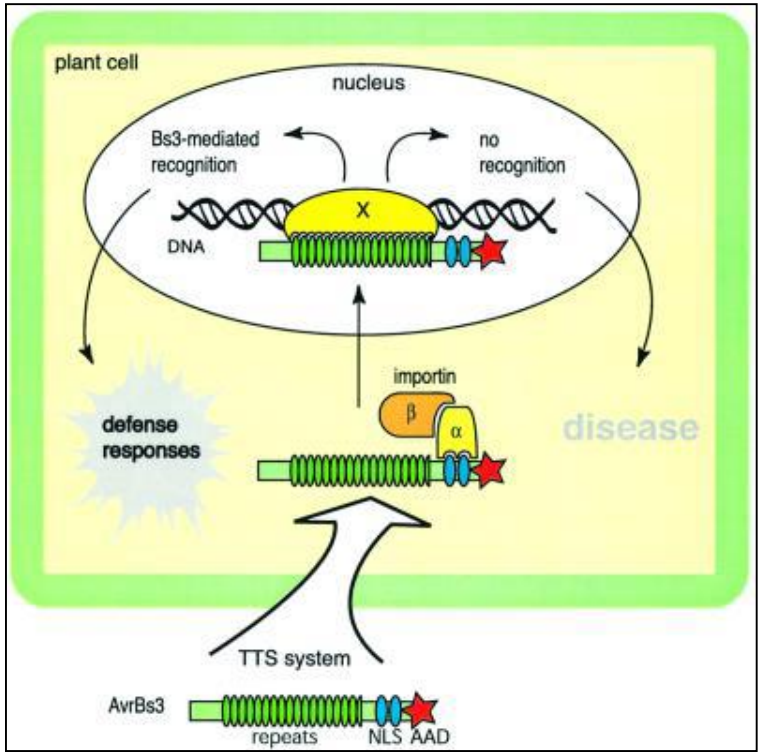
NLS: nuclear localisation signals;* AD or ADD: transcription activation domain

Vivian and Arnold,2000

AvrBs3

Arrival-AvrBs3 localizes to the plant cell nucleus

- The exact mechanism of nuclear transport of the Avr proteins is not known, however, AvrBs3 from *Xanthomonas vesicatoria*, the bacterial spot pathogen of pepper, binds to importin α from pepper in yeast two-hybrid assays via the second NLS.
- Importin α is a subunit of a heterodimeric NLS receptor complex that binds to the NLS of a substrate in the cytoplasm and then is associated with importin β, which mediates transfer of the substrate into the nucleus.
- Thus, it is possible that AvrBs3 and other family members localize to the nucleus via importin α/β nuclear transport.



AvrBs3 interacts with pepper host cell protein α-importin for the nuclear localization.
Importin α which, together with importin β, mediates nuclear protein import.

The AvrBs3 family
known as TAL effectors (TALEs) in *X. vesicatoria*

- *Xanthomonas* spp. produce type III effectors (T3Es) that can modulate virulence in host plants; T3Es are delivered into plant cells via the type III secretion system (T3SS).
- These T3Es are categorized into:

1. transcription activator-like effectors (TALEs), and
2. non-TALEs, which are commonly known as *Xanthomonas* outer proteins or Xops.

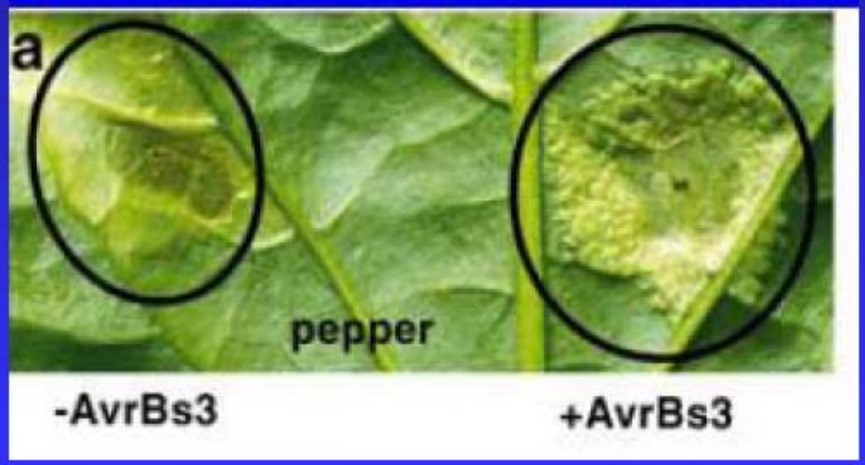
The number of *tal* genes in *Xanthomonas* spp. is highly variable; for example, strains of *X. translucens* pv. *translucens*, pv. *undulosa*, and pv. *cerealis* were shown to harbor eight, seven and two *tal* genes, respectively.

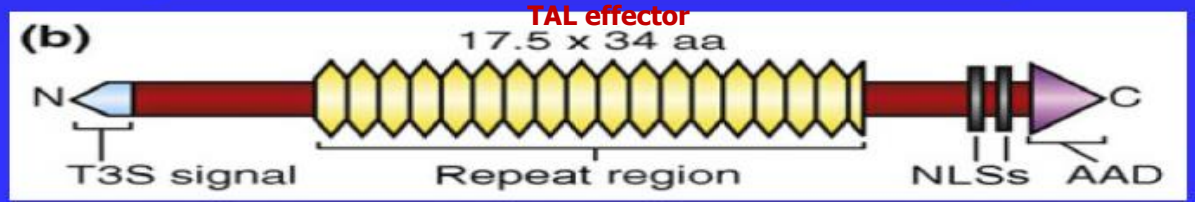
Ali Shah *et al.*,2019

Pathogenicity of AvrBs3

known as TAL effectors (TALEs) in *X. vesicatoria*

AvrBs3 specifically induces a hypertrophy in susceptible pepper plants and other solanaceous species. In field studies, it has indeed been shown that *AvrBs3* promotes bacterial spreading.





Zhaokaijun

The TALE family

Discovery of TALE effectors

First found in *Xanthomonas vesicatoria*

- A new paradigm for protein-DNA interaction has been discovered in bacterial pathogens of plants (Boch *et al.*,2009; Moscou and Bogdanove,2009).
- TALEs (transcription activator like effectors) has been shaped by 20 years of research, beginning with the discovery of the AvrBs3 protein of the pepper and tomato pathogen *X. campestris* pv.*vesicatoria* (*Xcv*) (Bonas *et al.*,1989)

Bogdanove *et al.*,2010; Christian*et al.*,2010

The TALE family

Discovery of TALE effectors

Now found in many Xanthomonads

- TALEs has now be found in several but not all pathogenic members of the genus *Xanthomonas*.
- Individual *Xanthomonas* strains may have as few as one or as many as several dozen TALEs.
- *Xanthomonas* uses TAL effectors to manipulate gene function in plants in ways that benefit the pathogen.

Closely related proteins have been found in several biovars of *Ralstonia solanacearum*; some of these are delivered into host cells, they act as but whether transcriptional activators is not yet known.

Bogdanove *et al.*,2010

The TALE family
Possible applications

- Xanthomonsds utilize these proteins that function as eukaryotic transcription activators to effect the host environment.
- Transcription activator like (TAL) effectors use a novel protein motif with an extremely simple DNA recognition code.
- The understanding of this code enables a number of highly useful tools, including targeted gene activation or repression, engineering of genomes to add, remove, or alter genes, and site-specific gene insertion.

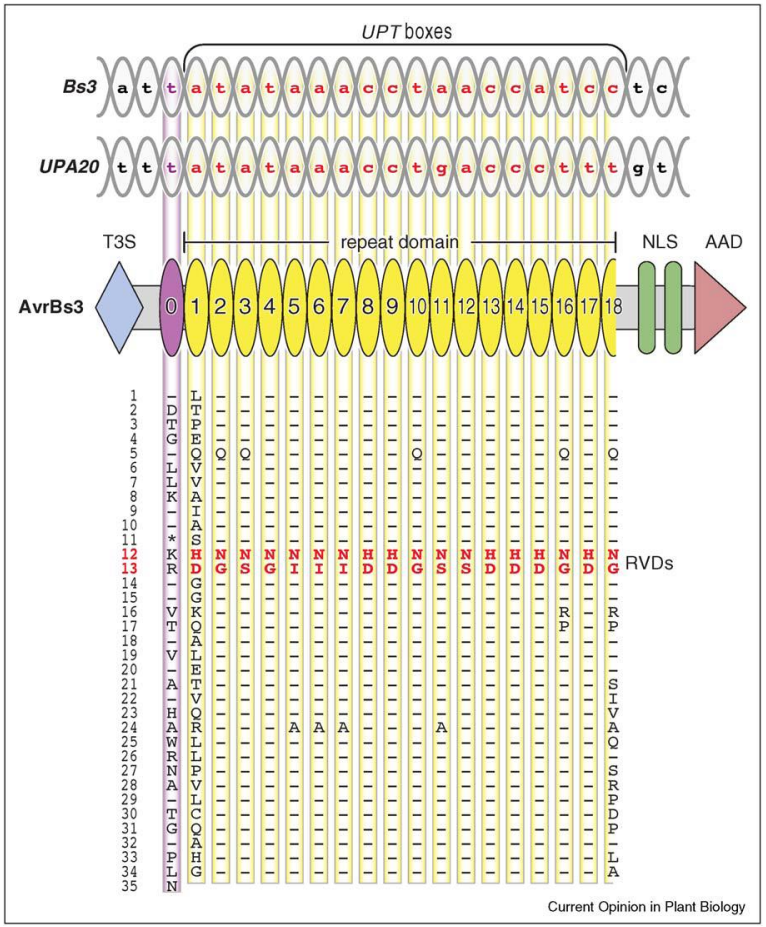
Bogdanove *et al.*,2010; Horvath,2011

AvrBs3

The founder of the TALE family

Structural features of TALEs

- Structural features of TALEs and matching binding sites (UPT boxes) exemplified by AvrBs3, the founder of the TALE family, and the corresponding pepper Bs3 and UPA20 promoters.
- TALEs recognize plant DNA sequences through a central repeat domain.



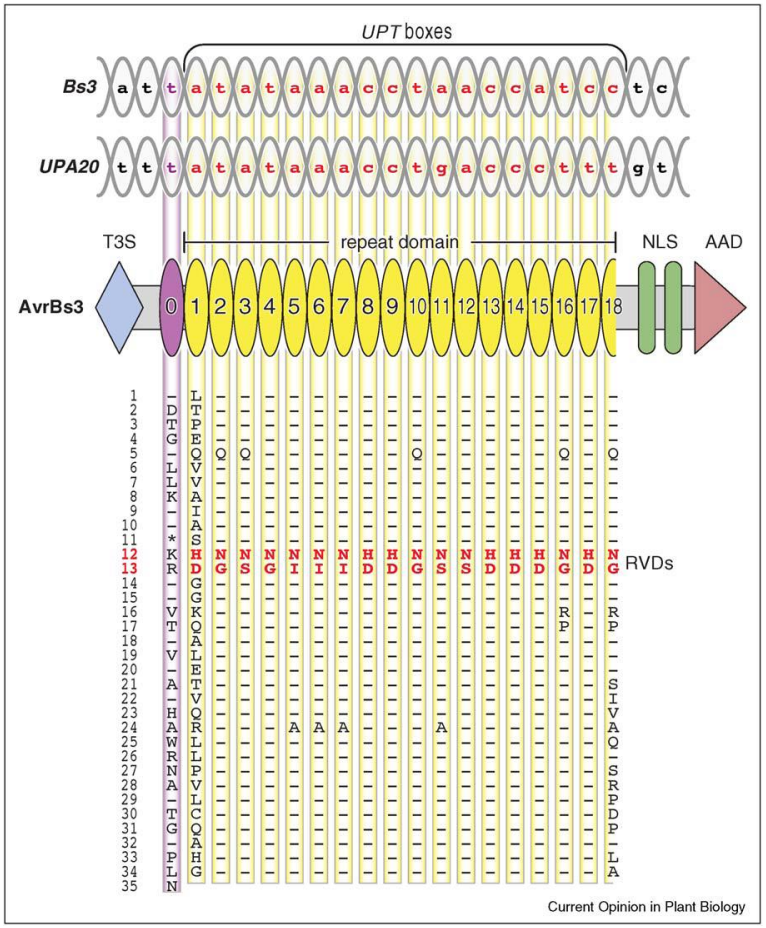
Bogdanove *et al.*,2010

AvrBs3

The founder of the TALE family

Structural features of TALEs

- AvrBs3 contains a N-terminal type III secretion and translocation signal (T3S), a repeat domain consisting of 17.5 repeat units (yellow ovals), two nuclear localization signals (NLS) and a C-terminal acidic activation domain (AAD/AD).
- Repeat polymorphism occurs largely at residues 12 and 13, the repeat-variable diresidues (RVDs, uppercase boldface red letters).



Bogdanove *et al.*,2010

The TALE family
TALEs function

- TALEs function as:

1. Virulence factors (led to plant disease),
2. Plant-recognized 'avirulence' factors (led to resistance), or
3. Both.

- Interaction with plants has selected TALEs that activate host genes that facilitate bacterial colonization and spread.

Bogdanove *et al.*,2010

The TALE family
TALEs function

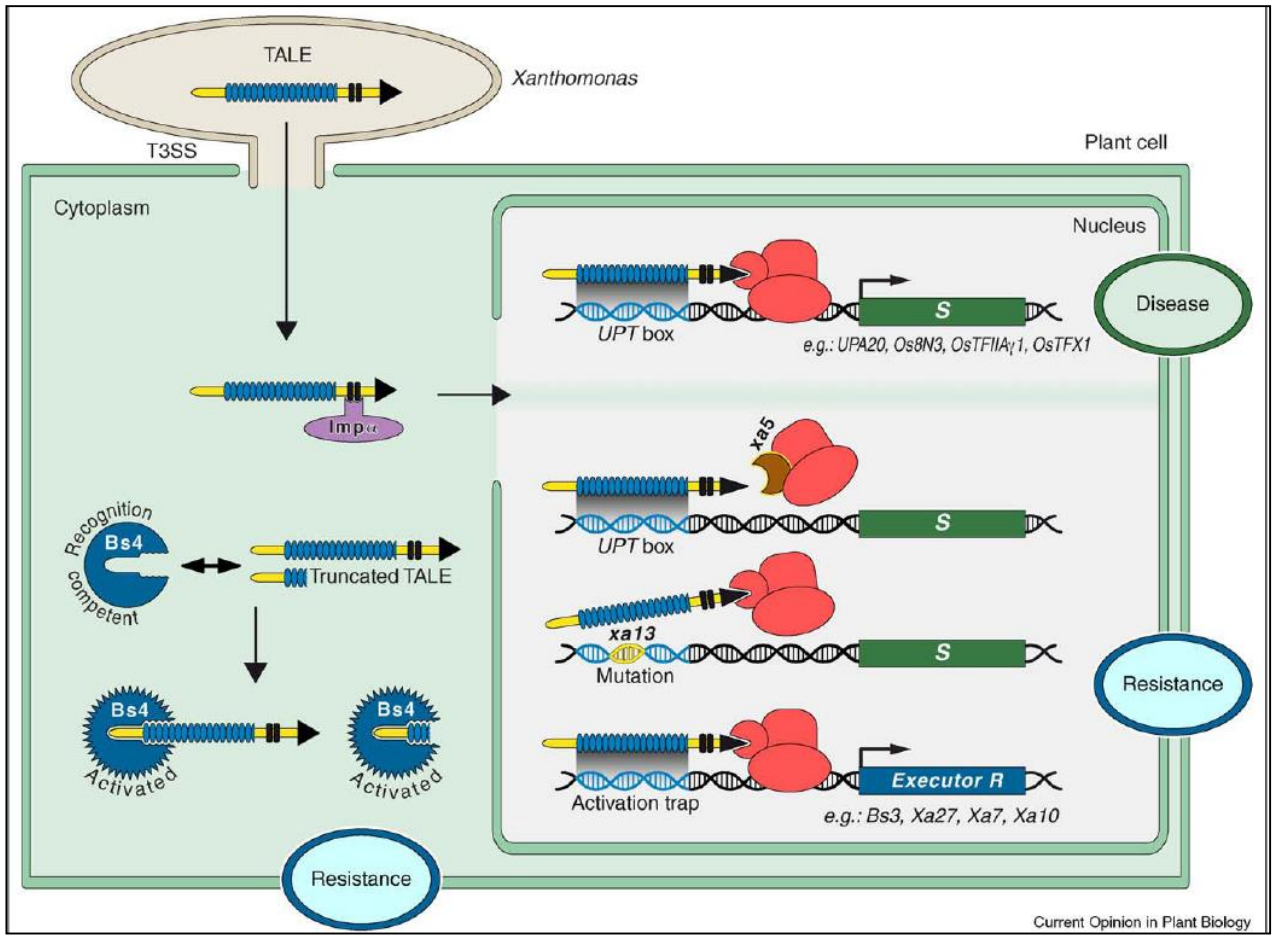
- TALEs enter the nucleus, bind effector-specific DNA sequences, and transcriptionally activate gene expression.
- Typically, activation of target genes increases plant susceptibility to pathogen colonization, but in some cases, it triggers plant defense.
- TALE binding to DNA is mediated by a central region of these proteins that contains as many as 30 tandem repeats of a 33- to 35-amino-acid-sequence motif.

Christian *et al.*,2010

Pathogenicity or resistance

The TALE family

TAL (transcription activator-like) effectors



Bogdanove *et al.*,2010

Pathogenicity or resistance

The TALE family

TALE roles in plant disease and resistance

- **1. Disease incidence:**
- Upon injection via the type III secretion system (T3SS) TALEs can be detected in the host cytoplasm as exemplified by the tomato NB-LRR type resistance (R) protein Bs4, which is capable of detecting the full-length TALE AvrBs4 as well as deletion derivatives that lack nuclear localization signals.
- Upon interaction with importin α (impα) TALEs translocate to the nucleus, bind to matching *UPT* boxes, and transcriptionally activate matching host susceptibility (S) genes such as:
- *UPA20*, *Os8N3*, *OsTFIIAγ1*, or *OsTFX1*, resulting in disease.

Bogdanove *et al.*,2010

Pathogenicity or resistance

The TALE family

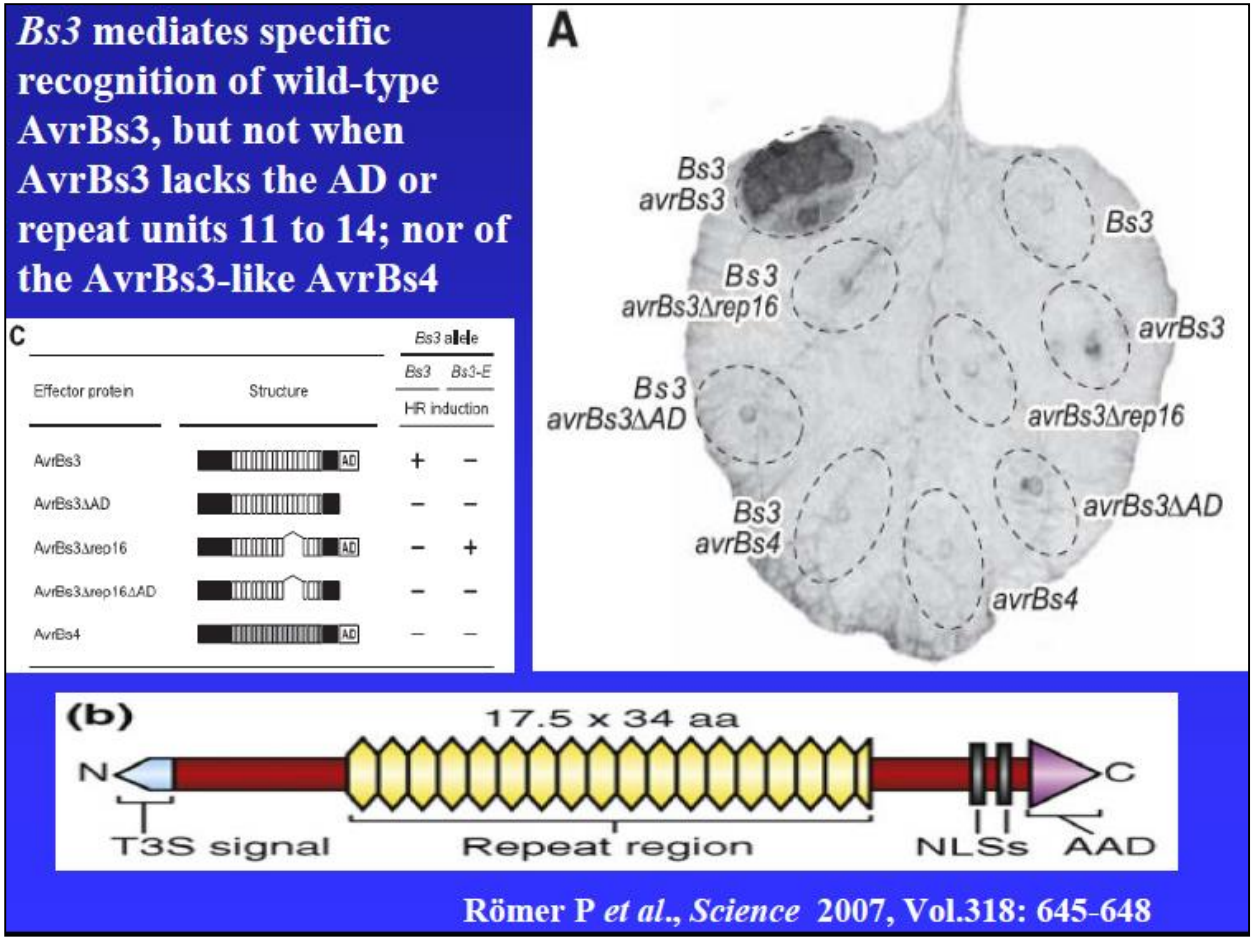
TALE roles in plant disease and resistance

- **2. Plant resistance:**
- Activation of *S* genes can be suppressed either by:
- A variation in the RNA II polymerase complex (red shapes) such as *xa5* (brown shape), or
- By mutations in the individual *UPT* boxes (*xa13* mutation).
- Plants have also evolved activation traps for resistance in which executor *R* genes are under transcriptional control of *UPT* boxes that would normally be present in *S* gene promoters.

Bogdanove *et al.*,2010

Pathogenicity or resistance

TALE roles in plant disease and resistance



Zhaokaijun

XopD
SUMOylation and deSUMOylation

- In plants, SUMOylation and deSUMOylation regulate a number of processes, for example, in abiotic stress responses, pathogen defense, and flower induction.
- SUMOylation itself stabilizes proteins.
- XopD SUMO protease affects host transcription.
- In *Xanthomonas*, the XopD protein has a modular structure and shows different biochemical activities (Figure 1a).
- XopD possesses SUMO protease activity mediating deSUMOylation of yet unknown target proteins.
- The XopD activities lead to the suppression of:
- Defense- and senescence-associated genes resulting in:

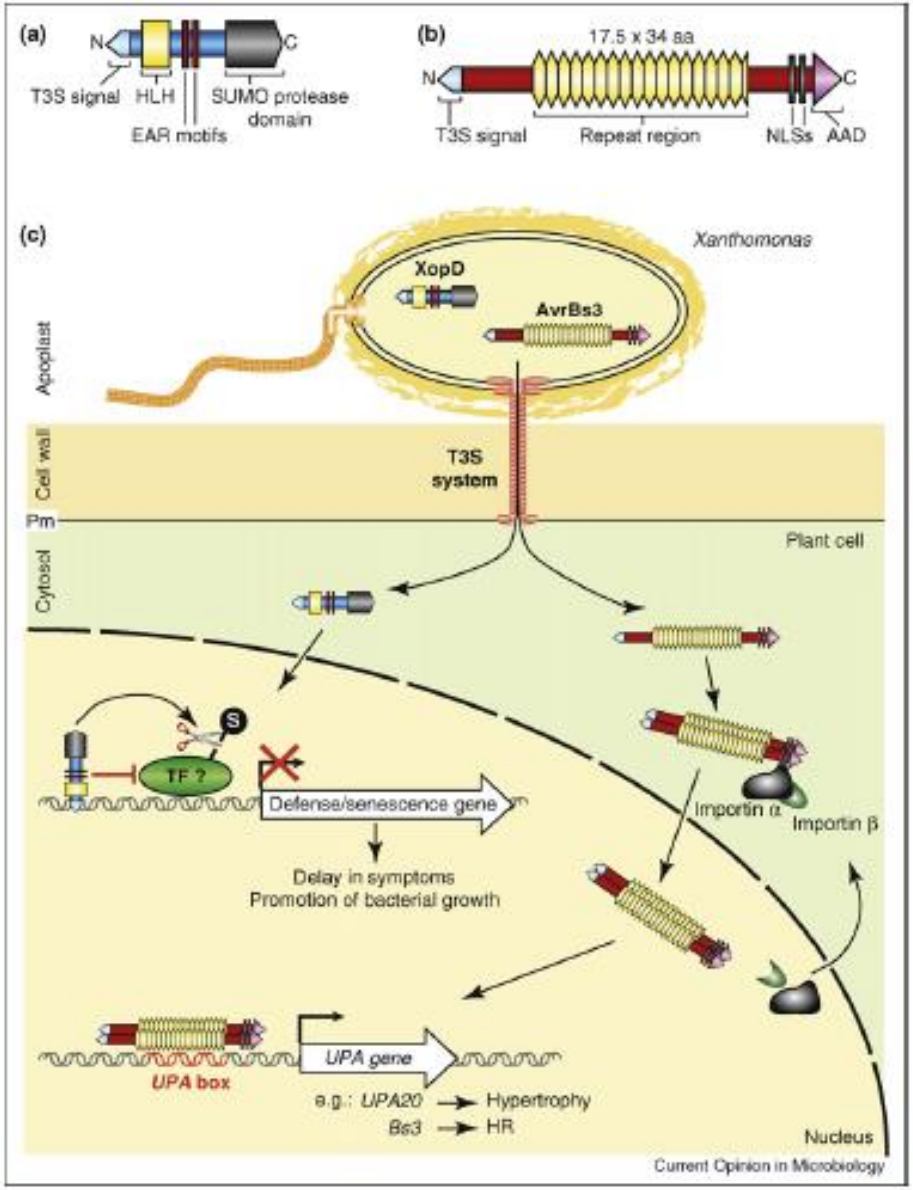
1. Delayed disease symptoms, and
2. Increased bacterial multiplication.

Model of the molecular function of the type III effectors XopD and AvrBs3 in *Xanthomonas*

The C terminus of XopD contains a small ubiquitin-like modifier (SUMO) protease
Structuraly similar to yeast ubiquitin-like protease 1.

Ubiquitin is a small regulatory protein that exists (ubiquitously) in all eukaryotic cells.

EAR motif act as a transcriptional repressor and inhibit plant transcription factors (TFs).
Helix-loop-helix (HLH) domain essential for maximal DNA-binding.



AAD, acidic activation domain; Pm, plasma membrane

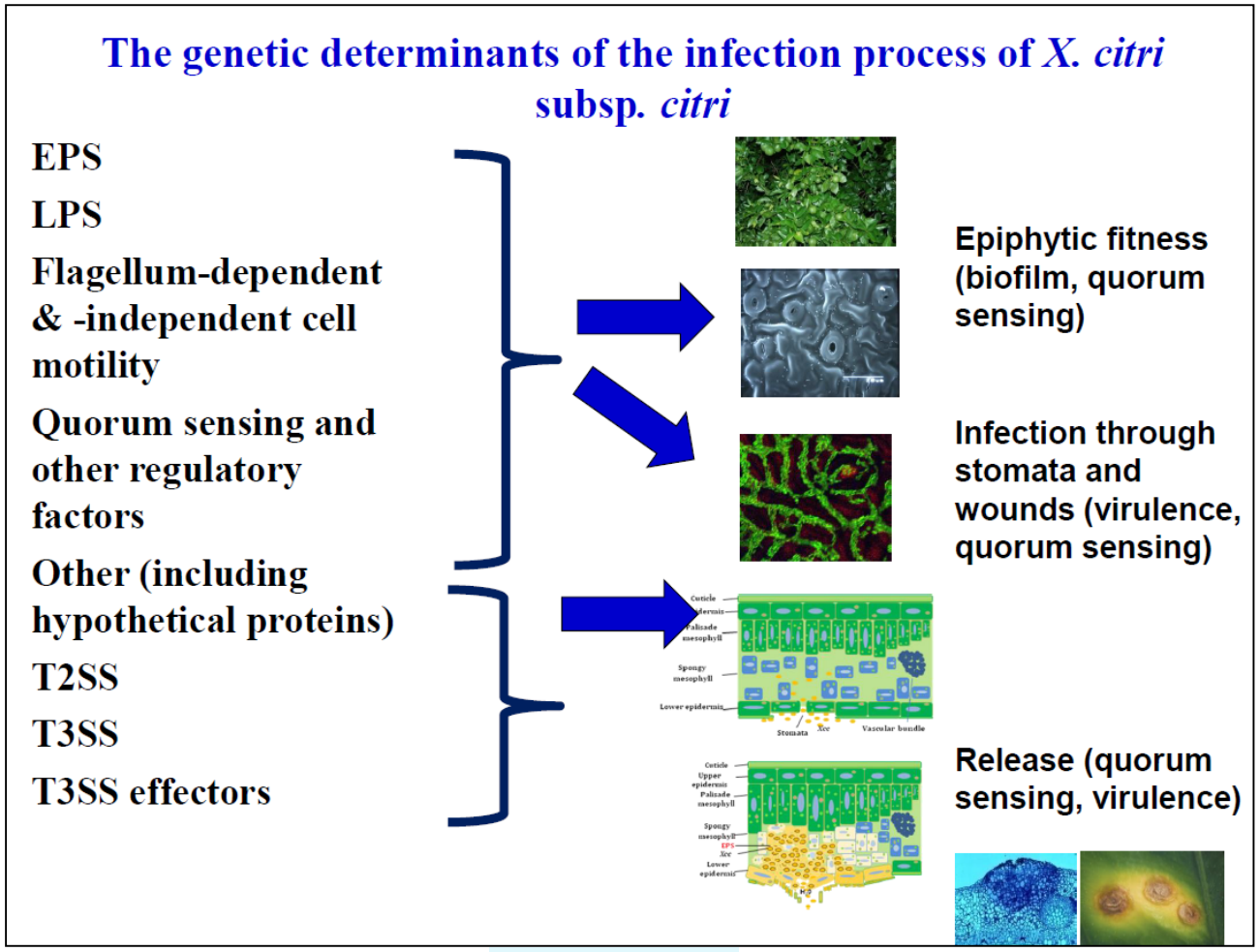
Kay and Bonas,2009

Secretion systems

Type II secretion systems

- In addition to TTSS, pvs. *campestris* and *citri* encode type II secretion systems too.
- e.g. *X. campestris* pv. *campestris* is equipped with two sets of type II secretion systems (*xps* and *xcs*).
- From 11 annotated genes related to the *xps* system, 6 genes i.e. *xpsD*, *xpsE*, *xpsF*, *xpsK*, *xpsL*, and *xpsM* plus gene (*eng Xca* ,encoding exocellular cellulase) were identified in pathogenicity-deficient mutants.
- None the genes related to the *xcs* system were found in pathogenicity-deficient mutants.
- Therefore it was concluded *xcs* system may not play an essential role in *Xcc* pathogenesis.

The genetic determinants of the infection process of *X. citri* subsp. *citri*



Wang,2012

Pathogenicity

Citrus canker strains containing *pthA* pathogenicity genes

- Citrus canker disease (*Xanthomonas axonopodis* pv. *citri*) would be considered a host specific disease caused by strains containing *pthA* pathogenicity genes that are differentially adapted to *Citrus* spp.
- Citrus bacterial spot disease (*X. axonopodis* pv. *citri* strain type E which later the bacterium reclassified as *X. axonopodis* pv. *citrumelo*) is a disease caused by a diversity of strains with differential aggressiveness that represent a heterogeneous group, while the citrus canker group displays a higher homology among its members (Egel *et al.*,1991).

Pathogenicity
pthA -C

- Gene *pthA* is essential for *Xac* to elicit hyperplasia (cell divisions or cankers) on citrus, and *pthA* confers this ability to various *X. axonopodis* strains (for example, pathovars *alfalfae* and *citrumelo*).
- Functionally homologous genes (*pthB* and *pthC*) have also been identified and cloned from *X. axonopodis* pv. *aurantifolii* pathotype B and pathotype C, respectively.
- Mutations of genes encoding either type III secretion system encoded by *hrp* genes or the effector molecule, pth A/B/C, abolish pathogenicity of canker bacteria.

Pathogenicity

Difference in virulence mechanisms between *X. citri* spp. *citri* and *X. axonopodis* pv. *citrumelo*

- ***X. citri* spp. *citri***
- 22 T3SS effectors
- PthA and its homologs are missing. Expression of PthA in citrumelo strain causes typical canker symptoms.
- Deficient in pectate lyase
- LPS 22 genes

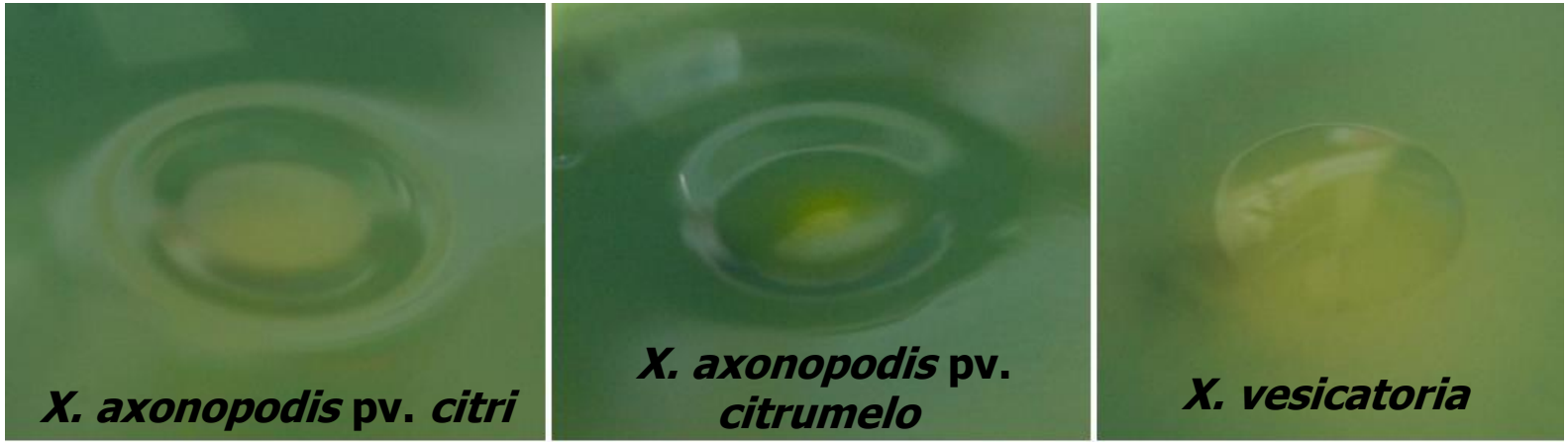
- ***X. axonopodis* pv. *citrumelo***
- 25 T3SS effectors
- PthA and its homologs (PthA1, PthA2, PthA3, and PthA4)
- Pectate lyase
- LPS 17 genes

Wang,2012

Pathogenicity

Comparison of the pecate lyase production by *X. axonopodis* pv. *citri* str. 306, *X. axonopodis* pv. *citrumelo* str. F1 and *X. campestris* pv. *vesicatoria* str. 85-10

- Pecate lyase production: pitting can be seen in the Hildebrand's agar medium.



Wang,2012

Pathogenicity of:
Xanthomonas oryzae pv.*oryzae*

Leaf blight of rice

Genome Sequence of *Xanthomonas oryzae* pv.*oryzae*

- The bacterium is a model organism for the analysis of plant-pathogen interaction, because more than 30 races differing in virulence and 25 resistance genes in rice have been reported to date.
- The size of the genome was 4,940,217 bp, in a single circular chromosome.
- The genome structure of *Xoo* MAFF 311018 was characterized by large numbers of effector (avr) genes of the *avrBs3/pth* family and insertion sequences (ISs).
- RFLP analysis of diverse strains using IS*Xo1* as a probe suggests that the prevalence of mobile elements in this species, which can bring about genome inversions and rearrangement, may have played a major role in generating the high degree of genetic diversity and race differentiation characteristic of this pathogen.

General features of the *Xanthomonas oryzae* pv.*oryzae* genome

No plasmid was detected in the course of genome assembly

- The assembled sequence was consistent with a single, 4 941 439 bp, circular chromosome.
- No autonomous plasmids were apparent.

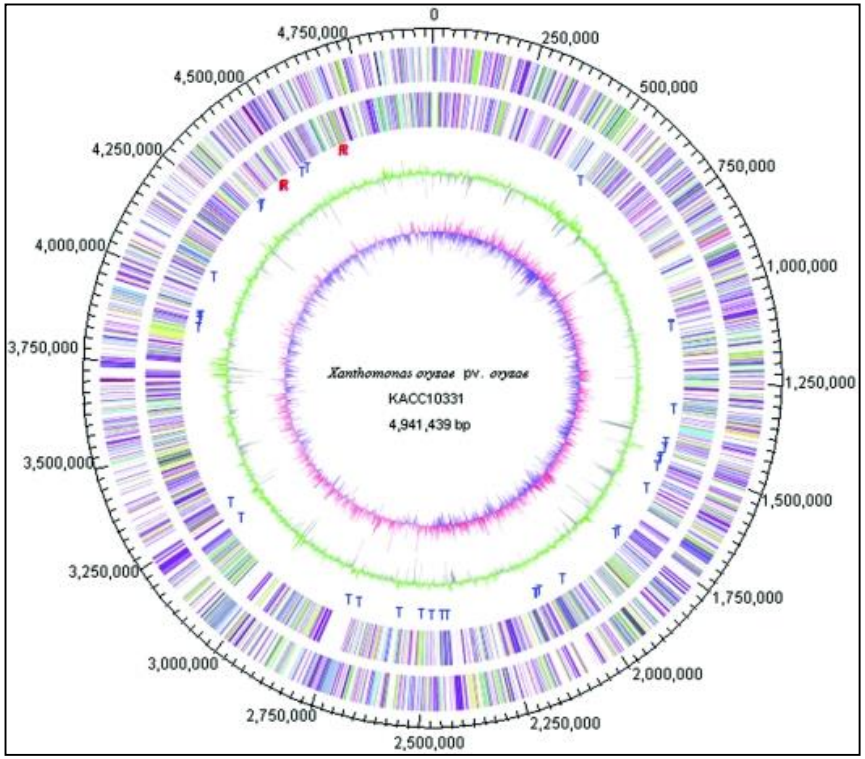
Length (bp)	4 941 439
G + C content (%)	63.7
Protein coding genes	
With function assigned	3340
Conserved hypothetical	1151
Hypothetical	146
Total	4637
Transfer RNA	54
Ribosomal RNA operons	2
Plasmids	0
Insertion sequence element (IS)	207

Lee *et al.*,2005

Graphic representation of the chromosome

Xanthomonas oryzae pv. *oryzae*

- Circular genome map of *X.oryzae* pv. *oryzae* str. KACC10331.
- Overall structure of the *X.oryzae* pv. *oryzae* genome. The putative origin of replication is at 0 kb.
- The outer scale indicates the coordinates (in base pair).
- Red symbols (character R) are positions of rRNA and blue symbols (character T) are tRNAs.
- The distribution of genes is shown on the first two rings within the scale.
- The next circle (green) shows G + C content and central circle (blue/red) shows GC-skew value.



Lee *et al.*,2005

The role of proteinase in Pathogenicity of XOO

- The role of an extracellular proteinase has been studied in *Xanthomonas campestris* pv. *oryzae* by transposon mutagenesis.
- The populations of a proteinase-defective mutant in rice plants were 10- to 100-fold smaller than those observed for the parental strain, suggesting an active role for proteinase in this disease.
- Similarly, a proteinase-deficient mutant of *Xanthomonas campestris* pv. *campestris*, the black rot pathogen, which lacked proteinase activity, showed considerable loss of virulence in pathogenicity tests when bacteria were introduced into mature turnip leaves through cut vein endings.

Pathogenicity of:
Xanthomonas translucens

- Three main clusters:

1. one consisting of pv. *cerealis*,
2. one consisting of pvs. *undulosa* and *translucens*, and
3. a third consisting of pvs. *arrhenatheri*, *graminis*, *phlei*, and *poae*.

Host range
Xanthomonads

- *Xanthomonas* spp. are subdivided into pathovars on the basis of:

1. host specificity and
2. can also be divided into two main phylogenetic groups based on sequence analysis of 16S rDNA, *gyrB, dnaK*, *rpoD*, and *fyuA*.

Ali Shah *et al*., 2019

Diseases caused by *Xanthomonas translucens* pathovars

1	*Xanthomonas translucens* pv. *arrhenatheri*	On *Arrhenatherum elatius*
2	*Xanthomonas translucens* pv. *cerealis*	On *Triticum* spp.; *Avena* spp.
3	*Xanthomonas translucens* pv. *graminis*	Leaf spot of Timoty grass; *Lolium* spp.
4	*Xanthomonas translucens* pv. *phlei*	On *Phleum praterse*
5	*Xanthomonas translucens* pv. *phleipratensis*	On *Phleum praterse* ; *Agropyron* sp.; *Bromus* sp.
6	*Xanthomonas translucens* pv. *pistaciae*	Dieback of pistachio
7	*Xanthomonas translucens* pv. *poae*	Bacterial wilt of Poa (*Poa trivialis*)
8	*Xanthomonas translucens* pv. *secalis*	Leaf & stem spotting of Secale (*Secale cereale*)
9	*Xanthomonas translucens* pv. *translucens*	Leaf streak and black chaff of small grains (barley, rye, wheat,..)
10	*Xanthomonas translucens* pv. *undulosa*	Leaf streaks of wheat & barley

Compiled from various references including ISPP LISTS, 2004; Bergey's Manuals, Janse,2006 and Euzeby,2010; Bull *et al.*,2010& 2012.

Virulence factors
Xanthomonads

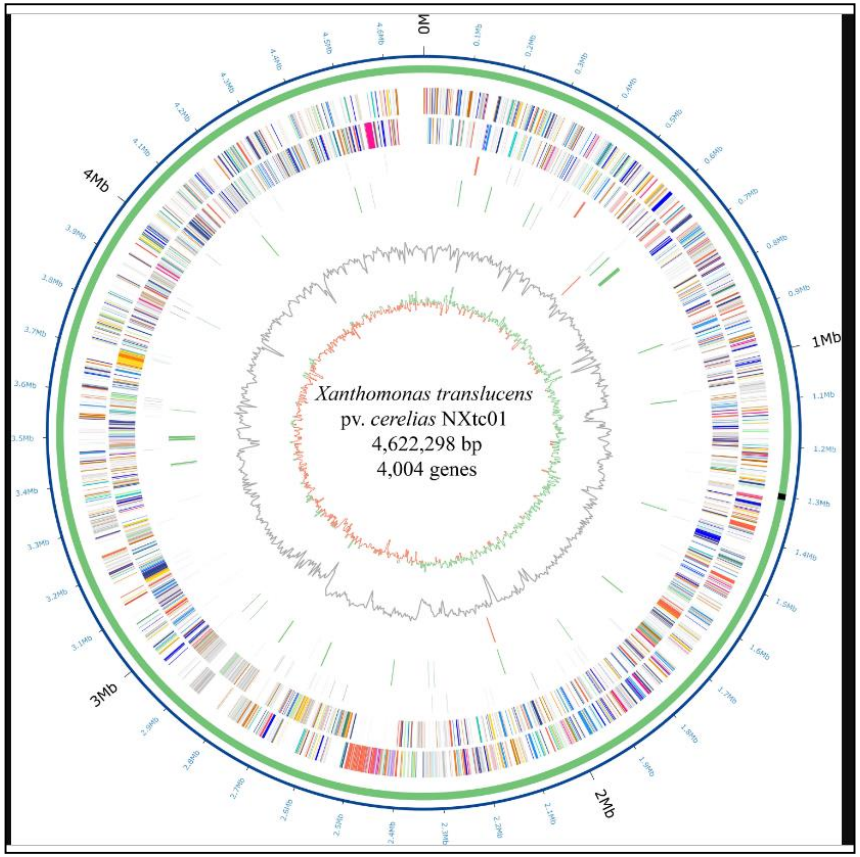
- The number of *tal* genes in strains of *X. translucens* pv. *translucens*, pv. *undulosa*, and pv. *cerealis* were shown to harbor eight, seven and two *tal* genes, respectively.

Ali Shah *et al.*, 2019

Graphic representation of the chromosome

Xanthomonas translucens pv. *cerealis*

- Circular representation of the *X. translucens* pv. *cerealis* strain NXtc01 genome starting from *gyrB*.
- Inner to outermost rings illustrate GC skew (G – C)/(G + C), GC contents, *tal* genes (orange), IS elements (green), tRNA (blue), rRNA (orange) and ncRNA (black) genes, reverse COGs (cluster of orthologous groups), forward COGs, chromosome (green), and coordinates (Mb).
- The T3SS genes cluster is indicated as black rectangle on chromosome.



Ali Shah *et al.*, 2019

Comparison of *X. translucens* pv. *cerealis* genomes

- Comparison of *X. translucens* pv. *cerealis* genomes from strains NXtc01 and CFBP 2541, which were isolated from *Triticum aestivum* in Xinjiang, China and *Bromus inermis* in the United States, respectively.

Contents	NXtc01	CFBP 2541
Genome size (bp)	4,622,298	4,518,140
GC%	67.23	67.34
Number of CDS	4,004	3,569
TAL effector genes	2	2
Non-TALE T3E genes	35	33
rRNA operons	2	2
tRNA genes	54	53
Insertion sequence elements (complete/partial)	80/56	88/58
CRISPR array	1	3

Ali Shah *et al.*, 2019

Pathogenicity of:
Gram-positive plant pathogenic bacteria

Virulence mechanisms of Gram-positive plant pathogenic bacteria

Virulence mechanisms of Gram-positive plant pathogenic bacteria

- Secreted proteins that act inside the plant cell are central to microbial pathogenesis.
- The lack of a type III protein secretion system (TTSS) in Firmicutes and Actinobacteria immediately raises questions about delivery of virulence proteins across the plant cell wall and membrane.
- In Plant pathogenic Actinobacteria:
- The genera *Streptomyces* and *Rhodococcus* have very wide host ranges.
- Whereas, the genera *Clavibacter* and *Leifsonia* are host-specific at the species or subspecies level.

Hogenhout and Loria,2008

Virulence mechanisms of Gram-positive plant pathogenic bacteria

Horizontal gene transfer: a short cut to virulence

- Actinobacteria and Firmicutes have evolved exclusive strategies for plant pathogenicity independent of Proteobacteria.
- There is also abundant evidence for acquisition of virulence genes through horizontal gene transfer and there are several examples of homologous virulence genes in multiple genera.

Hogenhout and Loria,2008

Virulence mechanisms of Gram-positive plant pathogenic bacteria

Horizontal gene transfer: a short cut to virulence

- Genome sequences reveal the importance of lateral gene transfer (HGT) in evolution of virulence in both Actinobacteria and Firmicutes.
- The *pat-1* gene, which is required for virulence in *Clavibacter michiganensis* is plasmid borne.
- The *tomA* gene in Cmm lies on a 129-kb region with a lower G+C content than the rest of the genome.
- This region also encodes several serine proteases that are required for virulence.

Hogenhout and Loria,2008

Virulence mechanisms of Gram-positive plant pathogenic bacteria

Horizontal gene transfer: a short cut to virulence

- Phytoplasmas can have two to four plasmids that vary in Size.
- Plasmids of spiroplasmas are also involved in insect transmission.
- These plasmids harbour adhesins and components of type IV translocation systems.

Hogenhout and Loria,2008

Pathogenicity of *Clavibacter michiganensis*

Canker of tomato

Three mechanisms explain the wilting disease caused by the *Clavibacter michiganensis*

- Pathogenicity determinant is plasmid- borne endo-b-1,4-glucanase.
- Three mechanisms have been proposed to explain the wilting disease caused by the vascular phytopathogen *C. michiganensis* subsp. *michiganensis*:

1. Mechanical plugging of xylem vessels
2. Toxic action of exopolysaccharides (EPS)
3. Enzymatic attack on plant tissue (e.g. polygalacturonase).

Three mechanisms explain the wilting disease caused by the *Clavibacter michiganensis*

- In several studies, plugging of xylem vessels by high titer colonization and production of high molecular weight EPS could be ruled out as the primary reason for the onset of plant wilting.
- In early stages of infection, *Cm* colonizes the xylem vessels, while colonization of the phloem, pith, and cortex of the upper parts of the plant is only observed at later stages.
- Wilting may therefore result from an enzymatic attack on xylem vessels and adjacent parenchymatic cells, allowing a lateral spread of *Cm* within the host plant, along with the release of nutrients necessary for bacterial proliferation and colonization.

Pathogenicity factors
Clavibacter michiganensis

- In the genus *Clavibacter*, production of extracellular enzymes such as endoglucanase, pectin methylesterase, polygalacturonase, and xylanase has been reported and endoglucanase activity has been implicated as a pathogenicity factor in recent reports.
- Endo-β-1,4-glucanases are members of the family A1 cellulases.

Pathogenicity factors of the *Clavibacter michiganensis*

- The phytopathogenic bacterium *Clavibacter michiganensis* NCPPB382, which causes bacterial wilt and canker of tomato, harbors two plasmids, pCM1 (27.35 kb) and pCM2 (72 kb), encoding genes involved in virulence.
- In the present study, we demonstrate that the *cel* A gene encoding an endoglucanase on pCM1 is required for the development of disease symptoms on infected tomato plants.

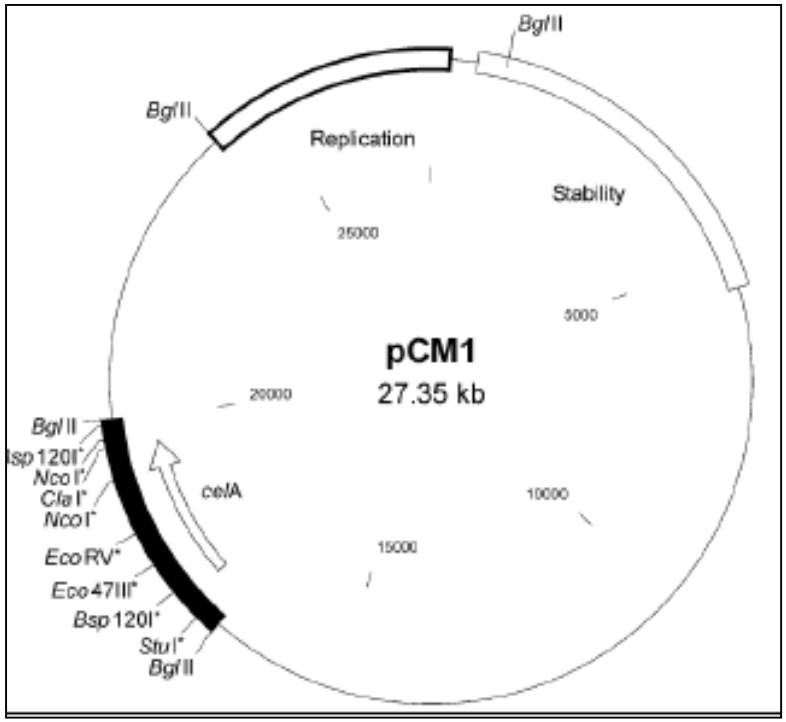
pCM1 function and mapping of the gene *celA*

Clavibacter michiganensis

- The region of pCM1 carrying the endoglucanase gene *celA* was mapped by deletion analysis and complementation.
- The *celA* gene encodes CelA, a protein of 78 kDa (746 amino acids).
- CelA plays a major role in pathogenicity.
- It was shown previously that in *Cm* the loss of plasmids pCM1 and pCM2 results in the avirulent endophytic, endoglucanase-negative Cm, lacking wilt-inducing ability.
- Reduced virulence was observed for the partially cured strain, carrying only pCM1 (not pCM2).
- The mutant strian also produced endoglucanase, but caused wilting of tomato plants with a delay of 6 days (compared to wild type).

Map of plasmid pCM1
Clavibacter michiganensis

- 3.2-kb *Bgl*II restriction fragment B1 of plasmid pCM1 carries an endoglucanase gene (indicated by black box and arrow).
- Regions essential for plasmid replication and stability, indicated as a:
- Bold-lined, or
- Thin-lined open box and labeled.



Restriction enzyme recognition sites not unique to pCM1, indicated by asterisks.

Plate assays for CMCase (endoglucanase^{+}) detection

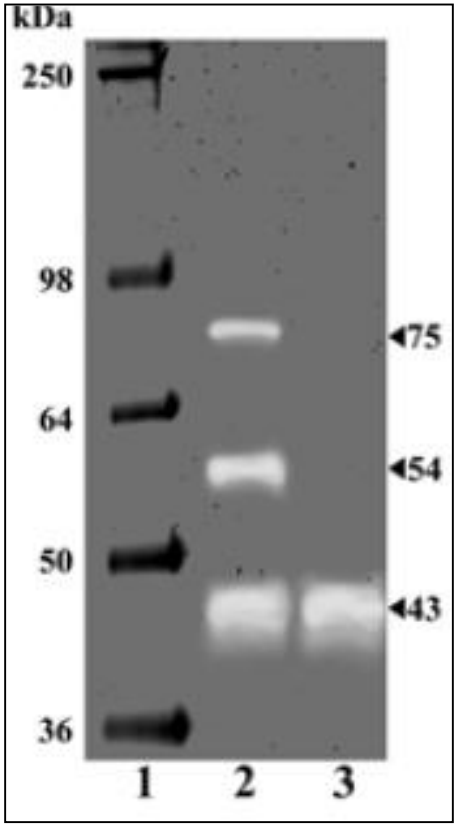
- Endoglucanase (CMCase) activity was detected on M9CMC agar plates containing carboxymethylcellulose (CMC) (Meletzus *et al.*, 1993).
- The plates were stained with 0.1% (wt/vol) congo red for 15 to 30 min and finally bleached with QR-buffer or NaCl solution (2 M NaCl, 50 mM Trizma base, pH 8.5).

The NaCl solution elutes the dye in the clearing zone where the cellulose has been degraded into simple sugars by the enzymatic activity.

SDS-PAGE analysis

Staining the CelA

- Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of CelA.
- Concentrated culture supernatant of Strain :
- CMM101(pCM1) (lane 2),
- Strain CMM100(pHJ1200) (lane 3), and
- A Pre-stained molecular weight marker (lane 1) were subjected to SDS- PAGE and stained with Congo red.
- The three active CMCase (endoglucanase+) species with 75, 54, and 43 kDa are indicated.



Pathogenicity of:
Rhodococcus fascians

Fasciation, leafy gall diseases

Rhodococcus fascians

- *R. fascians* can be a pathogen of plants, both angiosperm or gymnosperm.
- Infected plants show typical symptoms, such as:
- Leaf deformation,
- Witches' broom, and
- leaf gall.
- The development of these symptoms depends on:
- Plant's cultivar,
- Plant's age, and
- Bacterial strain.

Rhodococcus fascians
Leafy gall and fasciation diseases

- *Rhodococcus fascians* is the cause of malformations in a wide variety of host plants, the most severe being a leafy gall and shoot proliferations.
- *Agrobacterium* caused crown gall-like tumors and *R. fascians* produced leafy galls and shoot proliferations.
- Interestingly, we recovered *R. fascians* much less frequently than we did *Agrobacterium*.

Rhodococcus fascians
Leafy gall and fasciation diseases

- Both *Rhodococcus fascians* and *A. tumefaciens* are known to infect herbaceous and woody plants.
- Both bacteria have a wide host range (over 60 species for *R. fascians*, and hundreds for *A. tumefaciens*).
- In addition, *R. fascians* infects monocots as well as dicots, unlike *A. tumefaciens*, which infects only dicots.

Isolation methods

- On most of the plants we received for diagnosis we were making dual isolations, using one method for *Agrobacterium* and a different method for *Rhodococcus fascians*.

Rhodococcus fascians vs. *Agrobacteria* infections

- Leafy galls and shoot proliferations are quite different than the galls caused by the crown gall bacterium, *A. tumefaciens*.
- Infection with *A. tumefaciens* causes swelling of tissue into tumors or galls on stems or roots, but these galls do not differentiate into buds or stems.
- In contrast, leafy galls caused by *R. fascians* are well differentiated into easily recognized plant parts.

Shoot proliferation of *Iberis*

Crown gall at the base of a hibiscus

Genes that control virulence

Role of phytohormones during infection

- Virulence in *R. fascians* is controlled by genes on a plasmid (strains lacking that plasmid are not virulent) and on the chromosome.
- Strains that are cured of the plasmid are non-pathogenic.
- Using deletion mutations, it was possible to identify three loci the plasmid: *fas*, *att*, and *hyp*, and one locus on the chromosome, *vic*.
- *Rhodococcus fascians* carrying the fas-1 gene, which codes for an isopentyl transferase, the key enzyme in cytokinin biosynthesis, was found to be associated to stems and flower stalks symptoms.

R. fascians vs. *Agrobacteria* infections

- Unlike *A. tumefaciens* , presence of *R. fascians* on the infected plant is necessary, not only for the initiation of infection, but also for its maintenance.
- During the infection, *R. fascians* usually stays outside vegetal tissues, near a junction or cavity of a plant's cell walls, maybe to avoid environmental stresses.

Pathogenicity of:
Leifsonia xyli subsp. *xyli*

Ratoon stunt disease of sugarcane

Leifsonia xyli subsp.*xyli*
Ratoon stunting disease

- No general tissue disorganization results from this colonization, although the xylem may be blocked with a mucilaginous substance probably produced by the host.
- This blockage is reported to reduce sap flow up to 34% (Teakle *et al.*,1978).
- Infection with *L. xyli* subsp. *xyli* results in reduced cane diameter and plant stunting, symptoms that are often misinterpreted as general growth defects due to poor crop management.
- Ratoon stunting disease is regarded as the main disease of sugarcane worldwide.
- Yield losses of both cane and sugar per unit area may approach 50% in highly susceptible genotypes.
- Annual losses were estimated at US $11 million in Australia and US$36 million in Florida.

Monteiro-Vitorello *et al.*,2004

The circular genome of *Leifsonia xyli* subsp. *xyli*

- The genome sequence of *Leifsonia xyli* subsp. *xyli*, which causes ratoon stunting disease and affects sugarcane worldwide, was determined.
- The analysis also revealed 307 predicted pseudogenes or noncoding DNA sequence, which is more than any bacterial plant pathogen sequenced to date.
- Many of these pseudogenes, if functional, would likely be involved in the degradation of plant heteropolysaccharides, uptake of free sugars, and synthesis of amino acids.
- The large number of pseudogenes found in *Leifsonia xyli* subsp. *xyli* CTCB07 in the context of plant pathogenic bacteria suggests an ongoing process of genome decay.

Monteiro-Vitorello *et al.*,2004

Pseudogene counts in various organisms

Bacteria

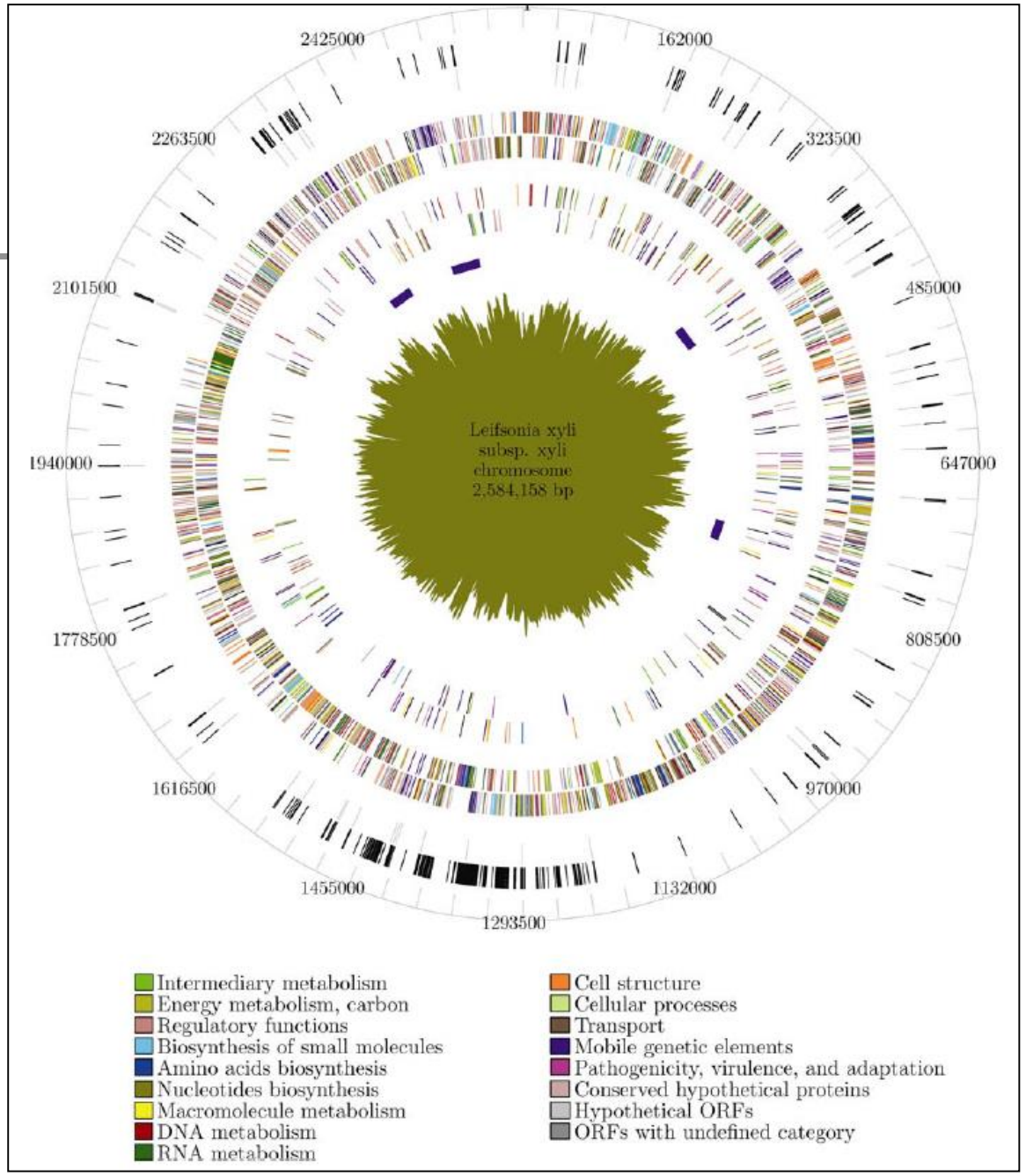
- *Leifsonia xyli* subsp. *xyli*
- *Xylella fastidiosa*
- *Xanthomonas axonopodis* pv. *citri*
- *Xanthomonas campestris* pv. *campestris*
- *Agrobacterium tumefaciens*
- *Ralstonia solanacearum*
- *Mycobacterium leprae*
- *Rickettsia prowasekii*
- *Sinorhizobium meliloti*
- *Streptomyces coelicolor*
- *Pseudomonas syringae*

Organism	Protein-coding genes	Pseudogenes (% of total number of genes)
Leifsonia xyli subsp. *xyli* CTC B07	2,326	307 (13.0%)
Xylella fastidiosa 9a5c	2,249	66[a] (2.9%)
Xanthomonas axonopodis pv. *citri*	4,427	85[a] (1.9%)
Xanthomonas campestris pv. *campestris*	4,181	73[a] (1.7%)
Agrobacterium tumefaciens C58	5,415	13[a] (0.2%)
Ralstonia solanacearum	5,036	38[b] (0.8%)
Mycobacterium leprae TN	2,653	905[b] (34.1%)
Rickettsia prowasekii Madrid E	797	40[b] (5.0%)
Sinorhizobium meliloti	6,138	57[b] (0.9%)
Streptomyces coelicolor	8,093	447[b] (5.5%)
Pseudomonas syringae	5,400	60[b] (1.2%)

[a] Source: GenBank.
[b] Source: Pseudogenes and Intergenic Analyses website.

Monteiro-Vitorello *et al.*,2004

The circular genome of *Leifsonia xyli* subsp.*xyli*



Monteiro-Vitorello *et al.*,2004

The circular genome of *Leifsonia xyli* subsp.*xyli*

- The outer scale corresponds to coordinates in base pairs.
- The first (outer) circle indicates Tnp insertions.
- The second indicates the positions of insertion sequences.
- The third and fourth circles represent predicted coding regions color-coded by gene category of the plus and minus strands, respectively.
- Genes on the fifth and sixth circles are pseudogenes (plus and minus strands).
- Seventh circle, genomic islands as defined by three criteria: codon bias, GC composition, and dinucleotide signatures and
- Eighth circle (innermost), G+C content.

Monteiro-Vitorello *et al.*,2004

The analysis of the genome sequence of *L. xyli* subsp. *Xyli*

Lateral gene transfer and virulence

- The limited number of pathogenicity genes appears to explain why *L. xyli* subsp. *Xyli* may be regarded as the near-perfect pathogen (Metzler *et al.* 1997), since it is able to reach high bacterial titers in the host without causing significant symptoms.
- Some of the predicted pathogenicity genes appear to have been acquired by lateral transfer and include genes for cellulase, pectinase, wilt-inducing protein, lysozyme, and desaturase.
- The presence of the latter may contribute to stunting, since it is likely involved in the synthesis of abscisic acid, a hormone that arrests growth.

Monteiro-Vitorello *et al.*,2004

Pathogenicity of:
Xylella fastidiosa

Scorch Disease

Genome of sequencing & pathogenicity factors

Lateral gene transfer and virulence

- The genome consists of a 52.7 per cent GC-rich 2 679 305-base-pair (bp) circular chromosome and two plasmids of 51 158 bp and 1285 bp.
- There are 2904 predicted coding regions and putative functions have been assigned to almost half of these.
- Those concerned with pathogenicity and virulence involve toxins, antibiotics and ion sequestration systems.
- Orthologues of genes encoding some of these proteins have been identified in animal and human pathogens, suggesting that they are constituents of pathogenicity islands.

Strange,2003

Genome of sequencing and pathogenicity factors

Lateral gene transfer and virulence

- These regions of the genome of pathogens are conserved and independent of the host and therefore imply horizontal gene transfer.
- This is corroborated by the finding of at least 83 genes in the genome of *X. fastidiosa* that are bacteriophage-derived and which include virulence-associated genes from other bacteria.

Strange,2003

Genome sequencing
CVC strain of *X. fastidiosa*

- The CVC strain of *X. fastidiosa* is the first plant pathogenic bacterium, the genome of which has been sequenced.
- 2,679,305 bp, 2782 ORFs, coding regions 88%.
- Two plasmid (51,158 bp and 1,285 bp).
- Do not have type III secretion system.
- Has a reduced genome.

Genomic comparison between *X. fastidiosa* (citrus stain) and *X. axonopodis* pv. *citri*

- *X. axonopodis* pv.*citri* (XAC) has two copies of a type II secretion system, a large number of cell wall–degrading enzymes and sugar transporters, a complete energy metabolism, a whole set of avirulence genes associated with a type III secretion system, and a complete flagellar and chemotatic system.
- By contrast, *X. fastidiosa* (citrus stain XF-9a5a) possesses more genes involved with type IV pili biosynthesis than does *X. axonopodis* pv. *citri* (XAC), contains genes encoding for production of colicins, and has 4 copies of Type I restriction/modification system while XAC has only one.
- XF-9a5c does not have a T3SS.

Moreira *et al.*,2004

General genome features of the three major citrus bacterial pathogens *X. fastidiosa*, *X. citri* pv. *citri* and *Ca.* Liberibacter asiaticus'

	X. citri	*X. fastidiosa*	*Ca.* Liberibacter *asiaticus*
Chromosome			
Length (bp)	5,175,554	2,679,305	1,227,204
G+C content (%)	64.7	52.7	36.5
Protein-coding genes	4,313	2,782	1,136
Hypothetical proteins	331	1,083	362
tRNA	54	49	44
Plasmids	2	2	0
Plasmid 1			
Length (bp)	64,920	51,158	–
G+C content (%)	61.4	49.6	–
Protein-coding genes	73	64	–
Hypothetical proteins	27	24	–
Plasmid 2			
Length (bp)	33,699	1,285	–
G+C content (%)	61.9	55.6	–
Protein-coding genes	42	2	–
Hypothetical proteins	14	1	–

Vojnov *et al.*,2010

Secretion system
Xylella fastidiosa

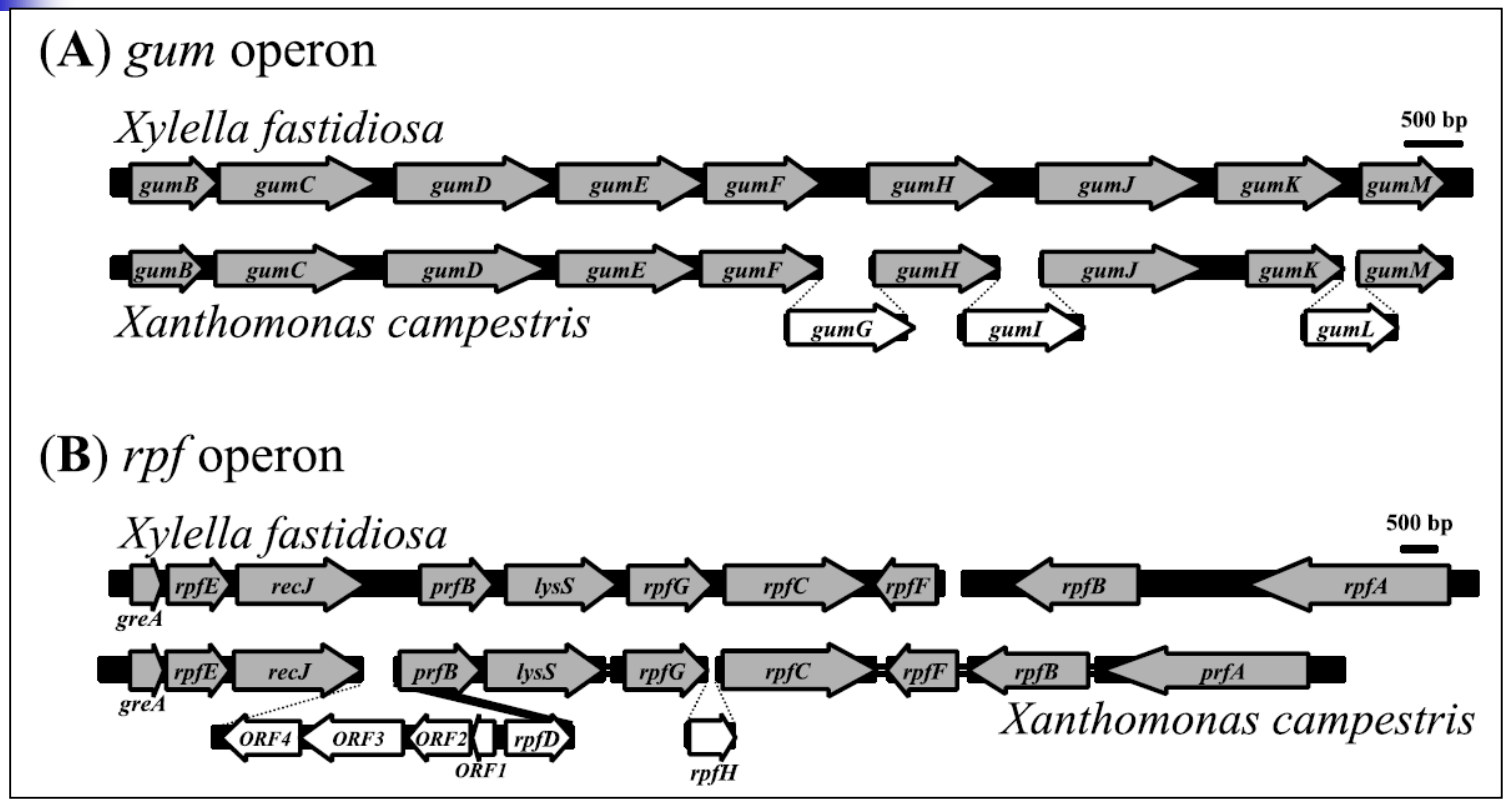
- *Xylella fastidiosa* does not carry a type III secretion system, and it is therefore assumed that this pathogen does not translocate effectors into plant cells for the elicitation of a host response.
- This hypothesis is supported by the fact that, in the xylem vessels, there is only fibre and dead cells, and the pathogen is introduced into this tissue by its vector, the sharpshooter leafhopper (Homoptera, Cicadellidae).
- However, *X. fastidiosa* has active type I and type II secretion systems, which could be associated with the efflux pump and the secretion of hydrolytic enzymes, respectively, allowing lateral movement of the bacterium through pit membranes and the digestion of plant cell walls.

Mansfield *et al.*,2012

Mechanism of disease development
Fastidian gum and twitching motility

- The fastidian gum(EPS) may be linked directly to the pathogenicity of this bacterium.
- The EPS could be involved in the formation of a biofilm required for the attachment and survival of the bacteria in the two hydrodynamically turbulent environments it is found: the xylem vessels and the sucking pumps of insect vectors.
- Terminal fimbriae (also called type IV pili) are important for biofilm formation.
- Terminal fimbriae aid in a type of incremental movement called "twitching motility" which enables bacterial cells to move against the xylem stream.

Mechanism of disease development
Fastidian gum and twitching motility



Genetic map of the *X. campestris* gum (A) and rpf (B) operons compared to genome.the region of the homologous genes in the *X. fastidiosa*.

De silva *et al.*,2001

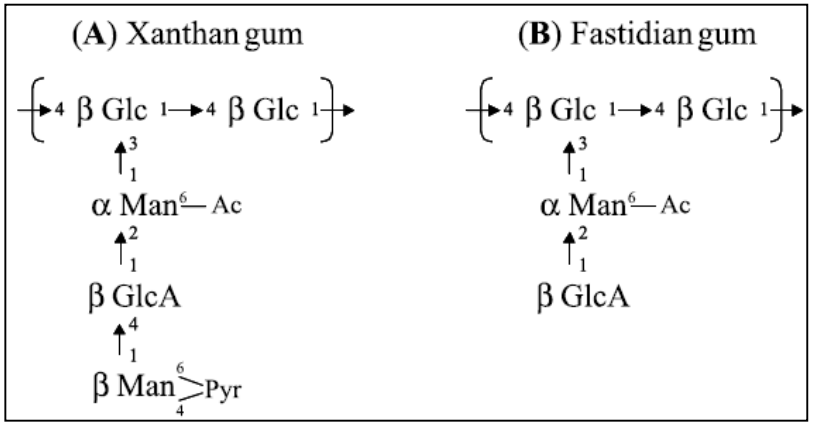
Mechanism of disease development
Fastidian gum and enzymes, toxins

- *X. fastidiosa* has 22 genes encoding regulatory proteins and enzymes involved in the synthesis of an EPS similar to xanthan gum, the EPS produced by *X. campestris*.
- The *X. fastidiosa* genome encodes for proteins involved in cell to cell interaction, degradation of plant cell walls, synthesis of toxins, and plant pathogenicity.

Mechanism of disease development

Structure of the repeating unit of xanthan gum (A) from *X. campestris*, and the proposed structure of fastidian gum (B) from *X. fastidiosa*

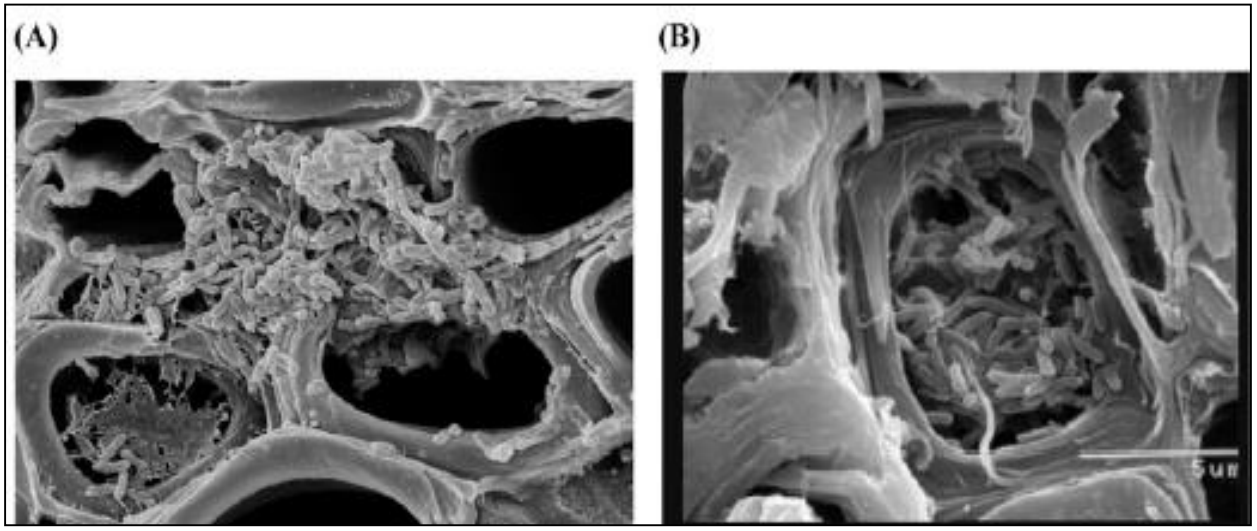
- The arrows point towards the reducing end of each repeat.
- Glc, Dglucose; GlcA, D-glucuronic acid; Man, D-mannose; Ac, acetyl ester; Pyr, acetyl-linked pyruvic acid.
- Some external mannoses of xanthan gum may contain an acetyl instead of a pyruvyl substituent.



De silva *et al.*,2001

Biofilm of *Xylella fastidiosa* blocking the xylem vessels of sweet orange tree

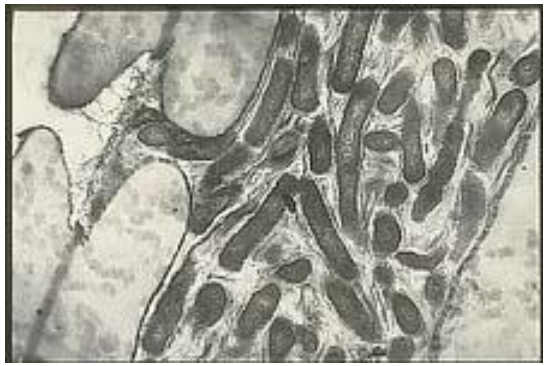
- Biofilms (A, B) are important for this pathogen to survive in environments with high turbulence, differential pressure and poor nutrient availability, such as xylem vessels and insect foreguts.



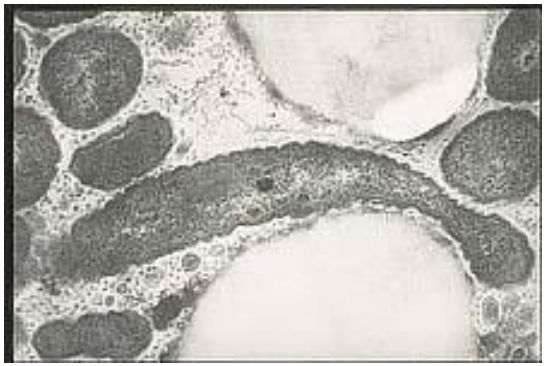
Photographs in (A) by E.W. Kitajima (Escola Superior de Agricultura Luis de Queiróz, USP, Piracicaba, SP, Brazil) and in (B) by J.O. Lima (Citrulima Viveiros, São João da Boa Vista, SP, Brazil) and Marcos A. Machado.

Mansfield *et al.*,2012

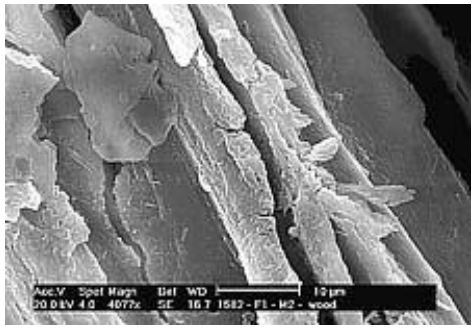
Mechanism of disease development



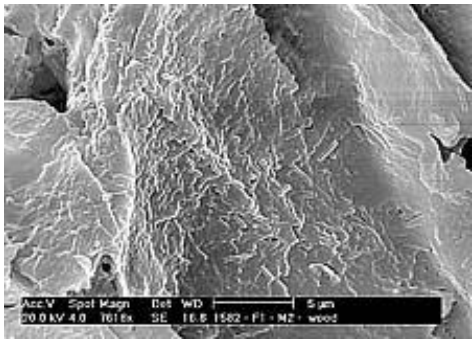
Cells of *Xylella fastidiosa* within the xylem tissue of a pin oak petiole



Close-up of a bacterial cell in the bordered pit of a vessel element



Biofilm of *Xylella fastidiosa* on Chard2 medium

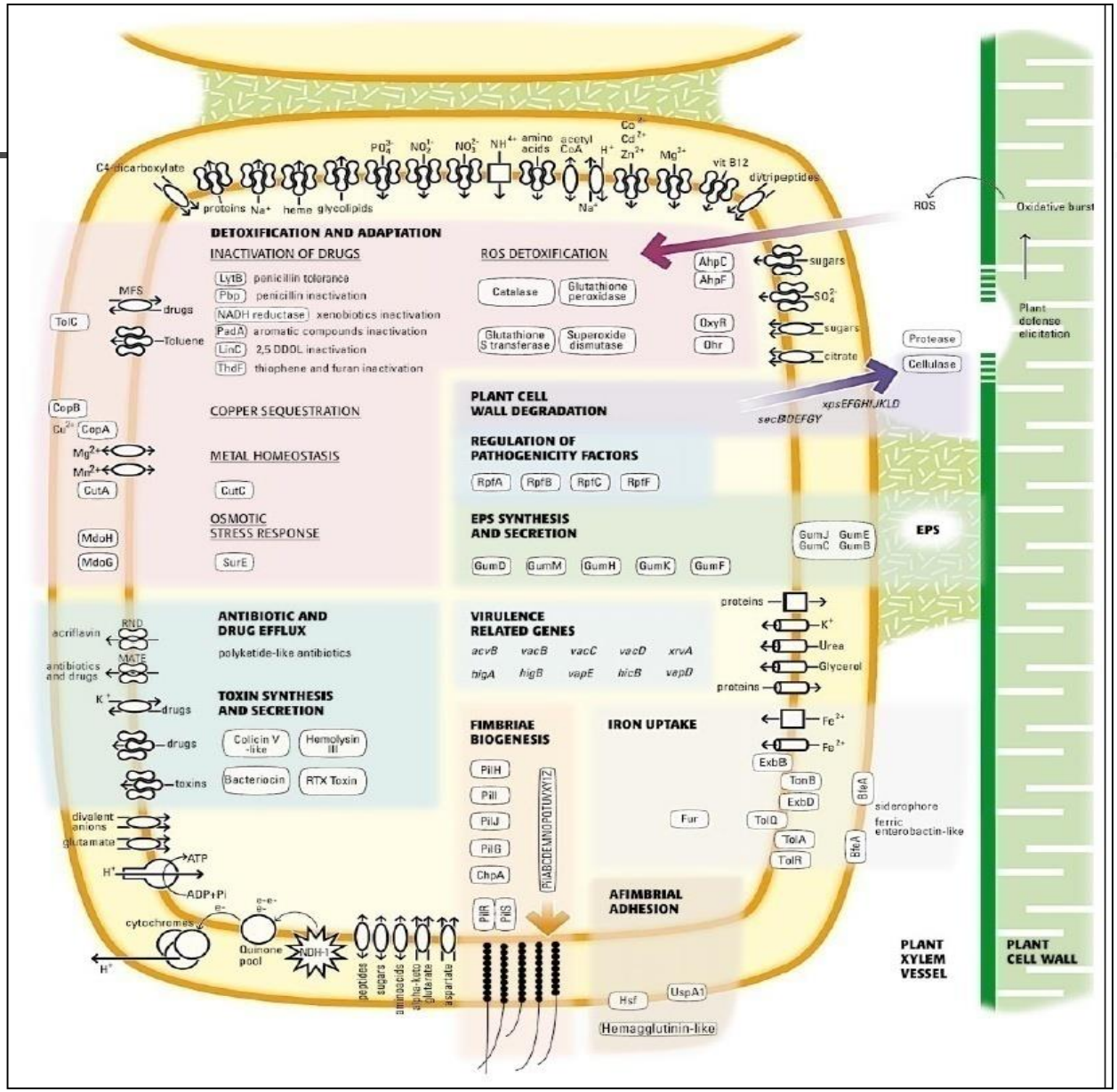


Close-up of individual bacterial cells

Biochemical processes involved in *Xylella fastidiosa* pathogenicity

- A comprehensive view of the biochemical processes involved in *Xylella fastidiosa* pathogenicity and survival in the host xylem.
- **Transporters are indicated as follows:**
- Cylinders, channels; ovals, secondary carriers, including the MFS family; paired dumbbells, secondary carriers for drug extrusion; triple dumbbells, ABC transporters(Type I secretion system); bulb-like icon, F-type ATP synthase; squares, other transporters.
- Icons with two arrows represent symporters and antiporters:
- (H+ or Na+ porters, unless noted otherwise). 2,5DDOL, 2,5-dichloro-2,5-cyclohexadiene-1,4-dol; EPS, exopolysaccharides; MATE, multi-antimicrobial extrusion family of transporters multidrug efflux gene (XF2686); MFS, major facilitator superfamily of transporters; Pbp, b-lactamase-like penicillin-binding protein (XF1621); RND, resistance-nodulation-cell division superfamily of transporters; ROS, reactive oxygen species.

Biochemical processes involved in *Xylella fastidiosa* pathogenicity and survival in the host xylem



Biological processes involved in the lifestyle of *X. fastidiosa*

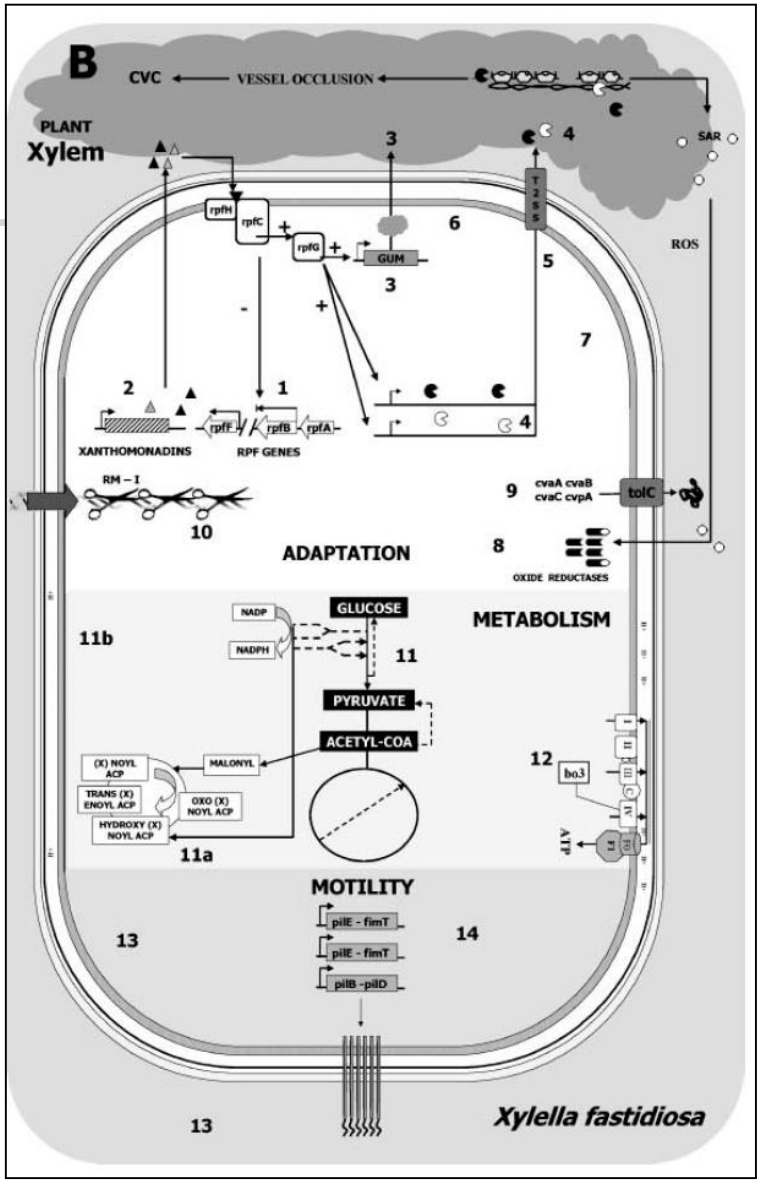
Xanthomonadins and xanthan gum

- Although there is no experimental evidence for xanthomonadins and DF production in *X. fastidiosa* (XF-9a5c), its genome has a cluster of genes that are similar to those present in the three species of *Xanthomonas*.
- DF is required for production of xanthan gum and xanthomonadins.
- The xanthan gum gene cluster in different strains of *Xanthomonas* is well conserved, with 98% identity.
- However, when compared with XF-9a5c genes, similarity decreases to values between 65% and 83%.
- The loss and degeneration of genes involved in xanthomonadin synthesis might be a consequence of *Xylella* adaptation to the xylem.

Biological processes involved in the lifestyle of *X. fastidiosa*

- Although the two bacteria have many genes in common, a series of XAC genes are absent or reduced in numbers in XF-9a5c.
- Among these overrepresented genes inXAC are: 12 groups of genes related to virulence, cellular growth, and adaptation.
- There are genes involved in the following:

(a) the type II secretion system,
(b) cell wall–degrading enzymes,
(c) sugar transporters,
(d) energy and general metabolism components,
(e) oxide-reduction enzymes,
(f) iron acquisition,
(g) type III secretion system components,
(*h*) flagella and the chemotactic system,
(i) regulators of pathogenicity factors genes,
(j) xanthomonadin biosynthesis,
(k) gum genes, and
(l) transcriptional factors.



See also *X. citri*

Endophytic bacterial populations

Their interaction with *Xylella fastidiosa* in occurrence and intensity of CVC disease symptoms

- *Methylobacterium* is the most frequently found genus associated to *Xfp*, and there is a positive association with the occurrence and intensity of symptoms of citrus variegated chlorosis, CVC.
- This interaction of *Methylobacterium* spp. with *Xfp* may occur by *Methylobacterium* spp. synthesis of pathological factors, such as siderophores and their utilization by *Xfp* (Simionato *et al.*,2006).
- The ability of *X. fastidiosa* to use siderophores produced by endophytic bacteria as source of iron was confirmed.

Lacava *et al.*,2008

Pathogenicity of:
Candidatus Liberibacter (BLO)

Citrus Huanglongbing (HLB, greening)

Candidatus Liberibacter (BLO)

The most serous disease of citrus

Greening disease

- Typical symptoms of greening disease on leaves of infected citrus trees include reduced plant height, pale yellowing of leaves, blotchy mottle and/or variegated chlorosis of leaves.
- Infected leaves can become upright, followed by leaf drop at the laminar abscission zone or petiole abscission and twig dieback at later stages (Bové 2006).


Wang,2012

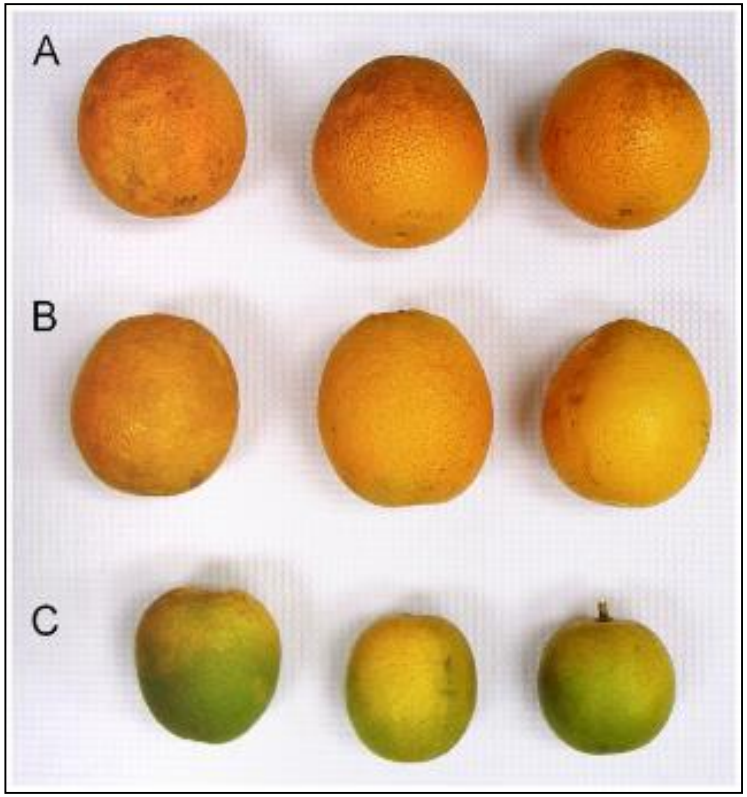
Candidatus Liberibacter (BLO)
Huanglongbing (HLB) or "citrus greening"
Stages of infection in the *Citrus* fruits

- The analysis of host responses in both leaves and fruits is important to understand the mechanisms of the fruit disorder and tree decline induced by the disease.

A. Fruits of apparently healthy plants;

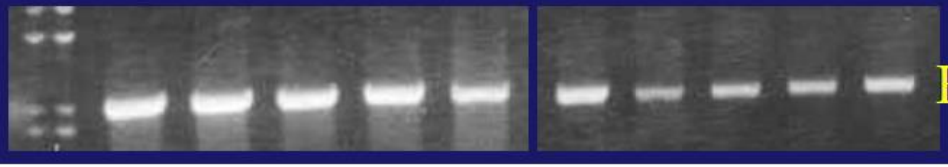
B. Asymtomatic fruits of infected plants;

C. Symtomatic fruits of infected.

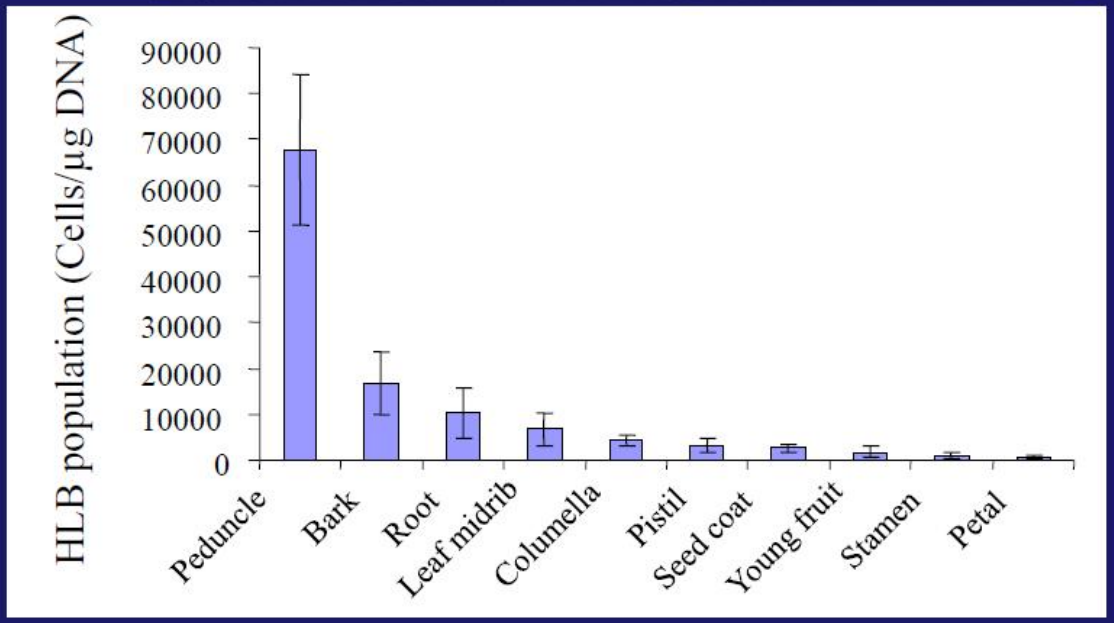


Martinelli *et al.*,2012

Distribution of the HLB bacterium *in planta*



HLB65/66



Tatineni et al. 2009
Phytopathology

Wang,2012

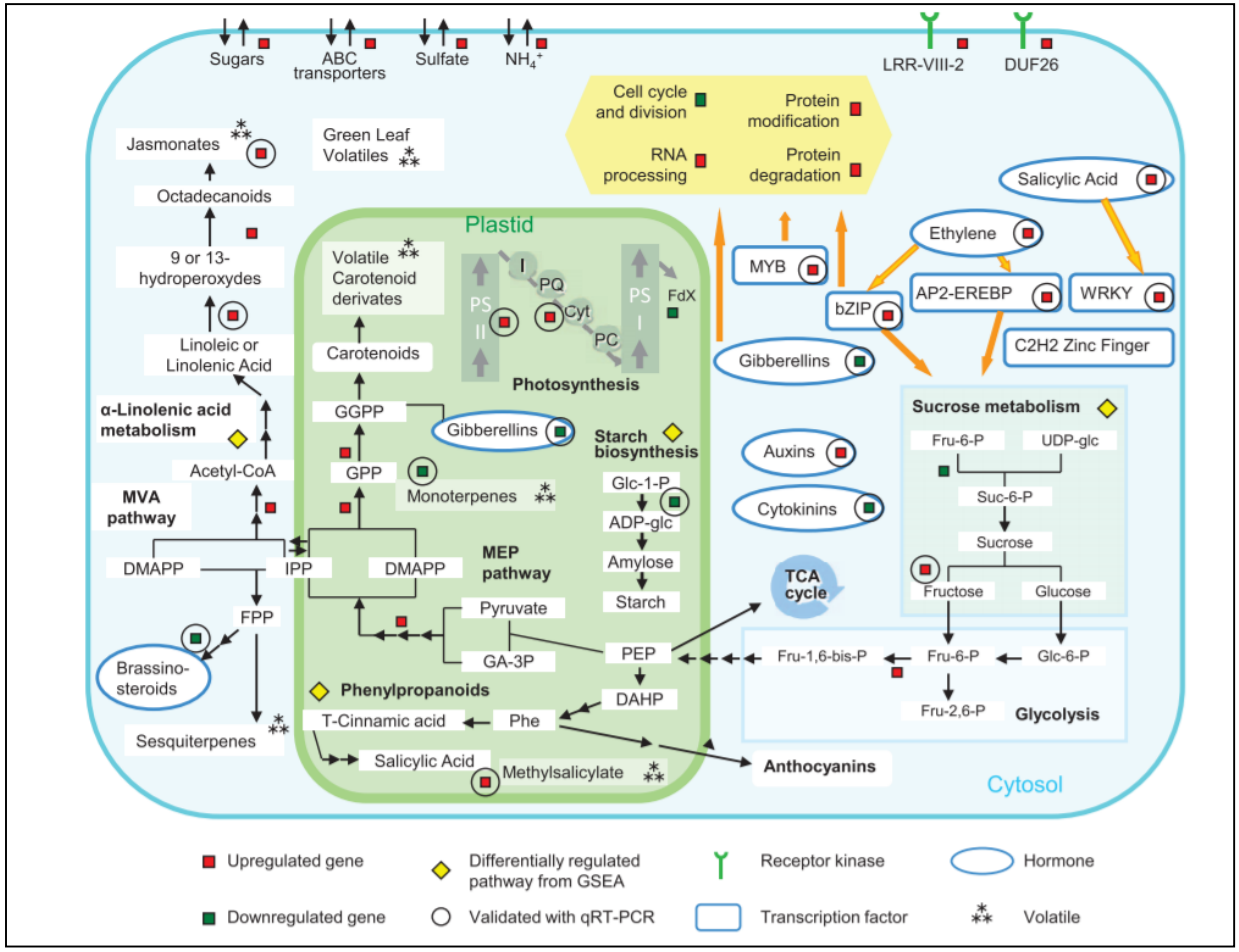
General feature of '*candidatus* liberibacter asiaticus' genome

Feature	Value
Size (bp)	1,258,278
G+C Content	35.24%
CDS (Protein-coding genes)	1,192
Hypothetical Proteins	405
tRNA genes	45
rRNA Operons	3
Putative pseudogenes (frameshifted ORFs)	35

doi:10.1371/journal.pone.0019135.t001

Lin *et al.*,2011

Fruit metabolism and regulatory pathways in CaLas-infected fruits. Genes, pathways, and cell functions that were differentially expressed are indicated with a square (red for up-regulated, green for down-regulated). Significantly differentially regulated pathways in gene set enrichment analysis are indicated in yellow



Martinelli *et al.*,2012

Differentially regulated pathways (up- and down regulated) involved in HLB response in the symptomatic fruit as determined using Pathexpress

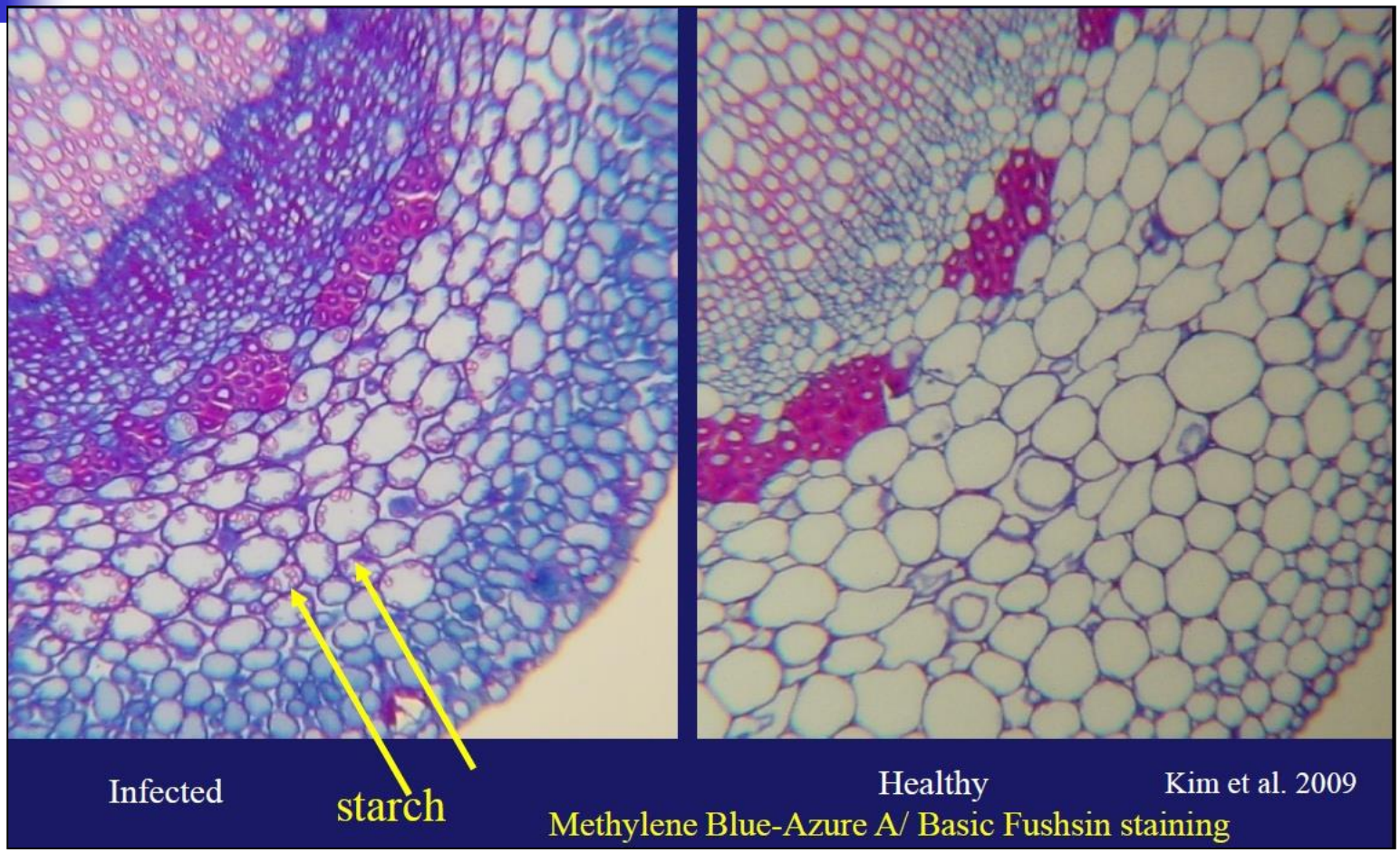
Regulated Pathways	Between Trees	Within Tree
Starch and sucrose	$5*10^{-4}$	0.03
Carbon fixation	$9*10^{-4}$	0.02
Ascorbate and aldarate	0.03	n.s.
Phenylpropanoid	0.03	0.02
Alpha-linolenic acid	0.04	$2*10^{-3}$
Pentose and glucoronate	0.06	n.s.
Pentose phosphate	0.07	n.s.
Fructose and mannose	0.08	n.s.
Cyanoamino acid	n.s.	0.06
Glycerophospholipids	n.s.	0.06
Flavonoids	n.s.	0.06

Analysis was performed on the list of genes differentially expressed at a significance level of $p<0.1$ comparing symptomatic to asymptomatic fruits within tree and between different trees "N.s." means not significant.

PathExpress: a web-based tool to identify relevant pathways in gene expression data.

Martinelli *et al.*,2012

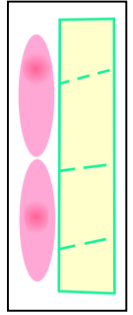
Citrus green disrupts phloem and cause starch accumulation



Wang,2012

Microbial strategies for attack

Sugar catabolism in *Ca.* Liberibacter *asiaticus*

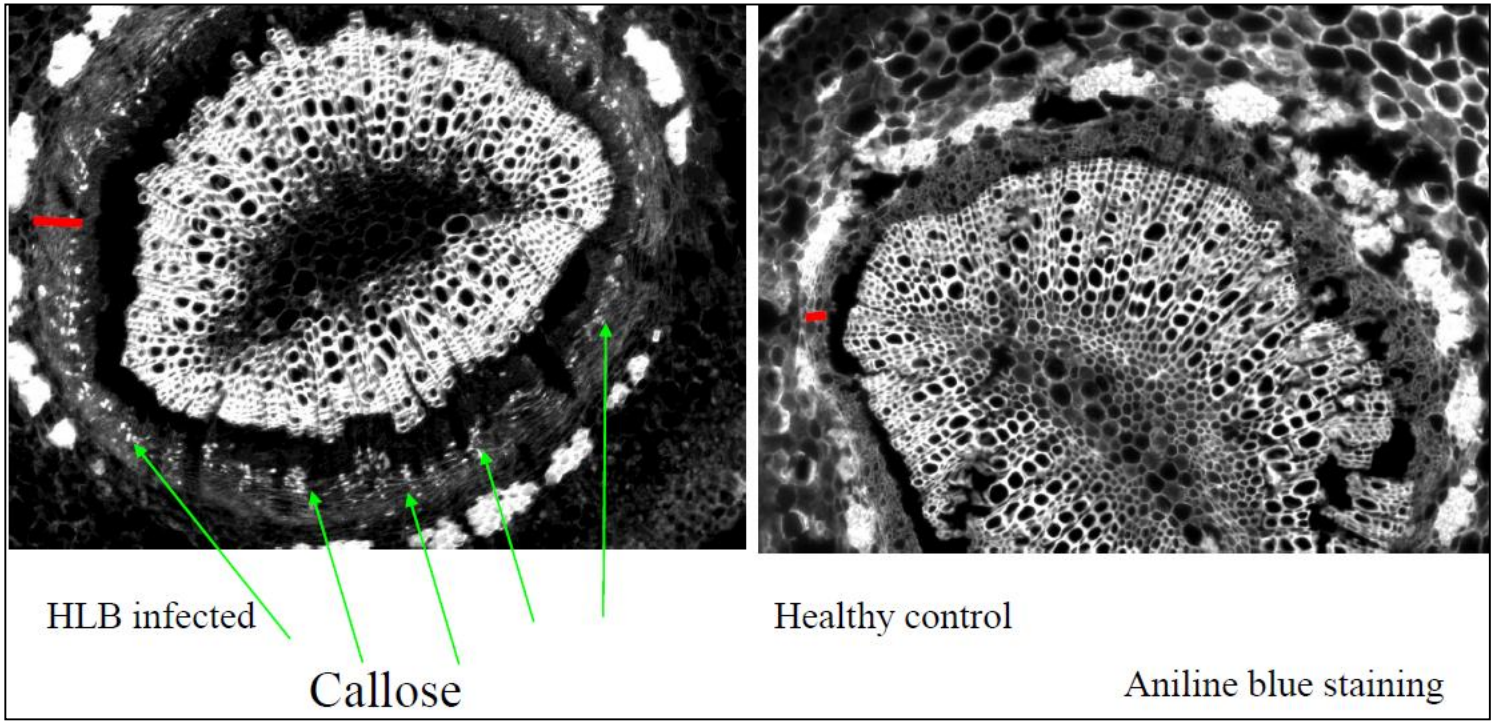


Solid green: sieve tube; dashed green: sieve tube plates; light pink: companion cell; dark pink: nucleus; yellow: nutrients

- HLB strongly affected pathways involved in source-sink communication, including sucrose and starch metabolism and hormone synthesis and signaling.
- It is important to note that elevated glucose and fructose has been demonstrated in citrus leaves infected with *Candidatus* Liberibacter *asiaticus* (CaLas).
- Increased glucose and fructose and decreased sucrose in fruit cells was demonstrated.

Martinelli *et al.*,2012

Callose accumulates in HLB infected leaves



Wang,2012

Pathogenesis-associated proteins

From the causal agent of citrus greening disease

- Of the 153 disease-state specific proteins identified, 74 were plant proteins, 24 were from Liberibacter, while the remaining 55 proteins were of bacterial origin other than Liberibacter.
- Of those proteins, 77%, 25%, and 29% of the plant, bacterial, and Liberibacter proteins are of unknown function.

Triplett,2012

Protein translocation pathways

In Gram-negative plant pathogenic bacteria *Candidatus* Liberibacter asiaticus

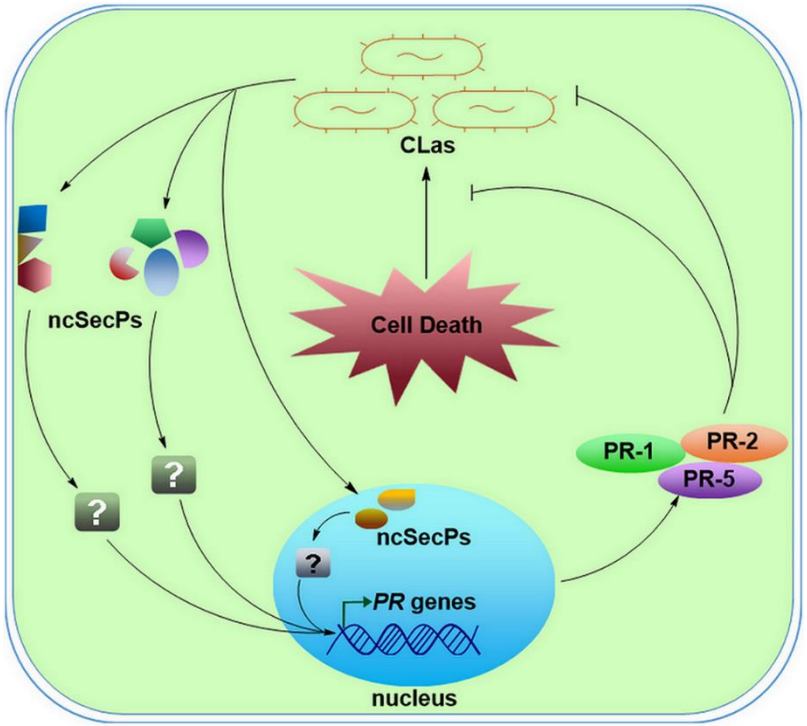
- "*Candidatus* Liberibacter asiaticus" (CLas), as a Gram-negative bacterium, lacks secretion systems T3SS, T4SS and T6SS, but it has the Sec secretion system.
- Several Sec-dependent secretory proteins have been identified.
- CLas does not secrete classical proteins (also termed effectors) but secretes nonclassically secreted proteins (ncSecPs).

Zhang,2019; Du *et al.*,2021

Protein translocation pathways

In Gram-negative plant pathogenic bacteria *Candidatus* Liberibacter asiaticus

- Model for CLas deploying nonclassically secreted proteins (ncSecP) to overwhelm hypersensitive responses in citrus plants.
- CLas is an intracellular bacterium and extracellularly exports no fewer than 10 ncSecPs that localize to different subcellular localizations but function cooperatively to upregulate gene expression of PR-1, PR-2, and PR-5 via unknown ways.
- The induced PR proteins subsequently lead to cell death suppression but otherwise contribute to defense against CLas.



Du *et al.*,2021

Pathogenicity of:
Spiroplasma citri

Stubborn disease

Virulence mechanisms of Gram-positive plant pathogenic bacteria

Mechanisms of spiroplasma pathogenicity

- Spiralin is the most abundant protein at the surface of the plant pathogenic mollicute *Spiroplasma citri* and hence might play a role in the interactions of the spiroplasma with its host plant and/or its insect vector.

Duret *et al.*,2003

Virulence mechanisms of Gram-positive plant pathogenic bacteria
Spiroplasma citri

- *S. citri* symptom development is linked to carbohydrate utilization preference in the phloem.
- Fructose uptake through the phosphoenolpyruvate:fructose phosphotransferase system (fructose PTS) by *S. citri* induces severe symptoms, whereas
- Glucose uptake through the glucose PTS does not.
- It is likely that in *S. citri* infected plants the activity of invertase, which converts sucrose to fructose and glucose in phloem companion cells, increases in response to low fructose concentration.
- This, in turn, leads to abnormally high glucose concentrations in the phloem, resulting in the chlorosis and internode shortening typical of *S. citri* infected plants.

Andre *et al.*,2005; Hogenhout and Loria,2008

Pathogenicity of:
Phytoplasmas

Yellow diseases

Membrane proteins and secreted proteins

- Since phytoplasmas are cell-wall-less bacteria and reside intracellularly within the host cell, the membrane proteins or secreted proteins of phytoplasmas seem to function directly in the cytoplasm of the host plant and insect cells.
- Therefore, in order to understand the phytoplasma-host interactions, it is important to identify the functions of membrane proteins or secreted proteins encoded in phytoplasma genomes.
- In the next slides, recent reports regarding the secretion system and the membrane protein of phytoplasmas will be discussed.

Weintraub and Jones,2010

Phytoplasmas
Candidate virulence factors

- It was hypothesized that some phytoplasmas secrete a variety of effector (virulence) proteins that interfere with plant development, leading to more phloem network for phytoplasma replication.
- There are several studies providing evidence that phytoplasma-infected plants are more attractive to insect vectors.
- It appears that phytoplasmas manipulate the production of plant volatiles and secondary metabolites to attract insect vectors.

Weintraub and Jones,2010

Pathogenicity and virulence
Phytoplasmas

- Very little is known about phytoplasma virulence.
- Energy metabolism is certainly a key topic for understanding phytoplasma biology and pathogenesis.
- It has been reported that altered levels of oxygen and carbon dioxide affect phytoplasma abundance in *Oenothera* leaf tip cultures.

Bertaccini and Duduk,2009

Phytoplasmas

Possible factors involved in disease development

- Several factors which may account for disease development include:
- Strain virulence, strain interference, phytoplasma concentration, toxins, plant hormone imbalance and attachment of phytoplasmas to host cell membrane.
- Also, a number of other putative pathogenicity factors are known from the complete genome sequences of the few phytoplasmas that have been determined to date.
- Sequence comparisons of the entire genomes of several phytoplasmas, including strains within a given taxon that differ greatly in aggressiveness, will provide insights into the largely unknown phytoplasma pathology.

Weintraub and Jones,2010

Pathogenicity and virulence

Phytoplasmas

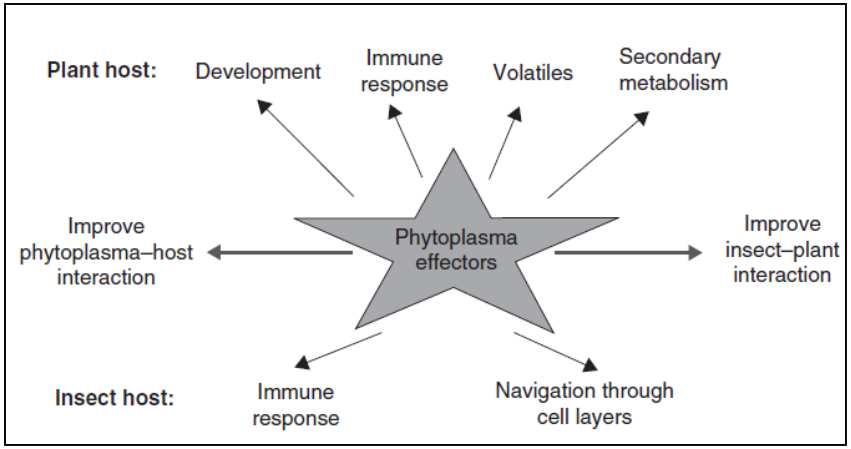
- Phytoplasmas do not possess characterized sugar PTSs but have ATP-binding cassette (ABC) transporters for uptake of maltose, trehalose, sucrose and palatinose.
- The utilization of one or more of these sugars in the phloem may be responsible for induction of symptoms in phytoplasma-infected plants.
- However, the phyllody, witches' broom, virescence and bolting are probably induced through other mechanisms.
- Since phytoplasma genomes lack identifiable plant hormone biosynthesis pathways, one or more of the various secreted virulence proteins are probably responsible for symptom induction.

Hogenhout and Loria,2008

Phytoplasma candidate virulence factors

Schematic illustration of the possible functions of phytoplasma effectors

- Effectors perturb the development, immune response, volatile production and secondary metabolism of the plant host and the immune response of insects.
- They can also aid phytoplasma navigation through the various cell layers of insect hosts.
- The overall effect is that phytoplasma fitness is enhanced through manipulation of plant and insect hosts and insect-plant interaction.



Phytoplasma candidate virulence factors
Secretion systems

- Phytoplasmas do not encode type-III or type-IV secretion systems (T4SS).
- These two secretion systems typically are found in Gram-negative bacteria but absent from Gram-positive bacteria and the mollicutes (see the next slide too).
- Furthermore, phytoplasmas do not have pili/pili-like structures.
- This is in contrast to spiroplasmas, which have pili-like structures (Ammar *et al.*,2004).

Weintraub and Jones,2010

Secretion systems
Pilus-like structures

- The bacterial type-IV secretion system (T4SS) is another important secretion system of plant and animal pathogens.
- T4SS comprises a large family of translocation systems as a pilus-like structure and mediates the transfer of DNA and protein substrates across the cell envelope to bacterial or eukaryotic cells, generally through a process requiring direct cell-to-cell contact.
- Although it was suggested that the T4SS is widely distributed among Gram-negative and -positive, yet phytoplasmas do not have either pili or T4SS.

Weintraub and Jones,2010

Phytoplasma candidate virulence factors
Secretion systems

- Phytoplasmas mostly depend on the SecA-dependent system for secretion of proteins.
- Sec system is essential for cell viability.
- In *Bacillus subtilis*, the Sec pathway is thought to be the most important transport pathways.
- However, there is another secretion system i.e. YidC which function separately from the Sec system.
- It seems YidC is specifically used for insertion of membrane proteins (Pf3 coat protein) and not for the translocation of exported proteins.
- Because YidC is an essential protein in *E. coli*, it also might have an important role in phytoplasmas.
- Based to many data it is assumed that effector proteins are probably secreted into the extracellular environment of phytoplasmas as they have to interact with host components.

Weintraub and Jones,2010

Phytoplasma candidate virulence factors
SecA-dependent system

- Proteins secreted via the SecA-dependent pathway in prokaryotes and eukaryotes typically have an N-terminal signal peptide (SP) that can be between 20 and 50 amino acids in length and consists of a consecutive stretch of positive, hydrophobic and polar amino acids.
- The SP is cleaved during the protein export process across the bacterial cell wall, leading to the presence of a mature protein without SP in the extracellular environment.
- Computer software, such as SignalP has been developed for recognition of the SP and cleavage sites of SPs in proteins.
- This software was successfully used to identify candidate effector proteins in the genome of '*Ca*. Phytoplasma asteris' AY-WB(aster yellows).

Weintraub and Jones,2010

Identification of phytoplasma virulence factors (effector proteins) using bioinformatics

- Bioinformatics pipeline used for the identification of '*Ca.* Phytoplasma asteris' AY-WB effectors.
- The SignalP software led to the detection of 20 secreted proteins that remain attach to the cell membrane after secretion and 56 proteins that appear to be released into the extracellular environment after secretion.
- The 20 proteins were characterized based on the presence of additional transmembrane (TM) regions in addition to the SP.
- Both antigenic membrane protein (AMP) and SAP11 have confirmed interactions with host components.

Weintraub and Jones,2010

Phytoplasma candidate virulence factors
SAP11 a candidate effector protein

- One of the AY-WB candidate effector proteins, named SAP11, also contains a nuclear localization signal (NLS).
- Since bacteria do not have nuclei, it is likely that SAP11 targets the nuclei of plant or insect cells.
- *Agrobacterium*-mediated transient expression assays in *Nicotiana benthamiana* leaves showed that SAP11 tagged at the N-terminus to green fluorescent protein (GFP) or yellow fluorescent protein (YFP) accumulates in plant cell nuclei and is dependent on an intact NLS and the host cell protein α-importin for the nuclear localization.
- The abundant presence of SAP11 in leafhopper salivary gland cells and canaliculi points to a possible role of effector proteins in improving plant-insect interactions.

Weintraub and Jones,2010

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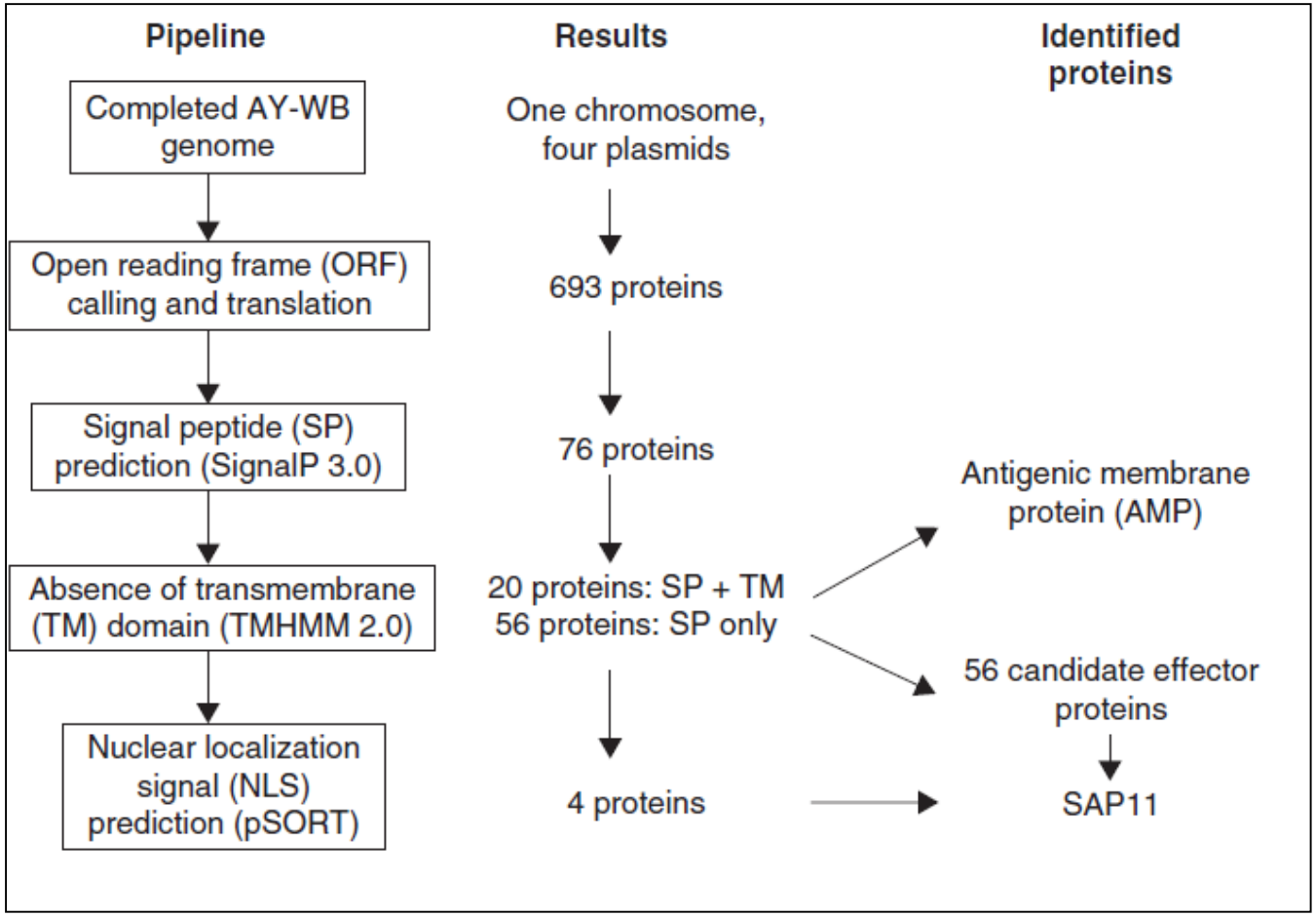

Weintraub and Jones,2010

General terms and abbreviations

- **Aggressiveness:** Virulent forms of pathogen that cause differing degrees of symptom severity.
- **Alleles:** An allele is a variant form of a given gene. A variant of the DNA sequence at a given locus is called an allele or variants of a gene found in the normal population.
- As individuals carry two copies of each gene, one on each pair of chromosomes, they may have identical(*homozygous*) or different (*heterozygous*) alleles.
- **Allosteric:** Change in the shape and biological function of a protein.
- **Binding site:** A region on a protein, DNA, or RNA to which specific other molecules such as ions or proteins form a chemical bond. A more specific type of binding site is the transcription factor binding site, present on DNA.
- **Biolistic:** This term has been coined from the words "biologic" and "ballistic"; refers to process involving the use of pellets coated with the desired genes that are fired from a gun into seeds or plant tissues in order to get plants expressing these transgenes.
- **cAMP or cyclic AMP:** Cyclic nucleotides which act as second messengers. Second messengers are molecules that relay signals received at receptors on the cell surface.
- **cDNA:** A duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector.

General terms and abbreviations

- **Cofactor:** A nonprotein component of enzymes is called cofactor.
- **Coenzyme:** If the cofactor is organic, then it is called a coenzyme.
- **Cytosol:** Unstructured aqueous phase of cytoplasm excluding organelles, membranes and insoluble cytoskeletal components. cytoskeleton offers structural support for the cell.
- **Downregulation of transcription:** Repression, or suppression decrease the rate of gene transcription.
- **Downstream:**
- **1.** Location of a motif or domain in a gene nearer the 3' end of the sequence than a reference site; gene sequences are read from the amino (NH2) terminal, also called the 5' end, to the carboxy (C) terminal or 3' end.
- **2.** Later reactions in a biochemical cascade or pathway.
- **Effectors:** These molecules can manipulate host cell structure and function, thereby facilitating infection (virulence factors/toxins) and/or triggering defense responses (avirulence factors/elicitors).
- **Flanking region**: The DNA sequences extending on either side of a specific locus or gene.

General terms and abbreviations

- **5' flanking region:** A region of DNA that is adjacent to the 5' end of the gene. The 5' flanking region contains the promoter, and may contain enhancers or other protein binding sites. It is the region of DNA that is not transcribed into RNA.
- **3' flanking region:** A region of DNA which is NOT copied into the mature mRNA, but which is present adjacent to 3' end of the gene.
- **Free radical:** A molecule that has one or more unpaired electrons.
- **G proteins or protein G:** A family of membrane-bound cell-signaling proteins regulated by guanine nucleotide binding. G proteins are so-called because they bind the guanine nucleotides GDP and GTP.
- **Helper Protein:** A term of convenience referring to extracellular accessory proteins (such as HrpA) plus other TTSS substrates (such as harpins) whose primary function is likely to be the translocation of true effectors through host barriers. Also called **translocators**.
- **Host and pathogen recognition:** chemical signals between plant and pathogen determine whether infection process proceeds.
- **Infection court:** physical location where inoculum and host come in contact.
- **Kinase:** An enzyme that catalyses phosphorylation.
- **MAP:** microtubule-associated protein; cf *MAPK.*

General terms and abbreviations

- **M_r:** relative molecular mass, no units; an M_r of 1000 is represented by 1K; M_r is numerically equivalent to daltons (1K = 1kDa).
- **Operon:** a unit of contiguous genes transcribed as a singletranscript under the control of a regulatory gene or genes; common in bacteria but rare in eukaryotes.
- **Open Reading frame (ORF):** A DNA or RNA sequence lying between a start codon and stop codons which is capable of transcription(can be translated into a polypeptide).
- **Orthologous/orthologue/orthology:** Genes in different species that are homologous (similar) because they are derived from a common ancestral gene (during speciation).
- **PKA:** cAMP-dependent protein *kinase* or protein kinase A.
- **Promoter:** A region of a DNA molecule to which an RNA polymerase binds and initiates transcription.
- **Regulon:** A system of more than one operon controlled by a single regulatory protein. e.g. genes in the *hrp* regulon.
- **Replicon:** DNA replicated under the control of a single replicator.
- **Signal transduction:** Reception, conversion and transmission of "chemical message" by a cell.

General terms and abbreviations

- **MAPK:** mitogen-activated protein kinase; originally known as microtubule-associated protein kinase (see MAP) because microtubule-associated protein had been used in the assay in which MAPK was discovered. Its name was changed when it was subsequently found to be activated by several messengers, many of them mitogens.
- **Mitogen:** an agent that can induce mitosis.
- **Signaling:** Communication established between and within cells of an organism.
- **Sessile bacteria:** Bacteria living within a biofilm.
- **Transphosphorylation:** The exchange of phosphate groups between organic phosphates, without their going through the stage of inorganic phosphates.
- **Transcription:** Copying of DNA by RNA polymerase into messenger RNA.
- **Tanscription factor:** A protein that participates in gene transcription often by binding to a specific DNA sequence.
- **Transposons:** are small segments of DNA that can move from one region of a chromosome to another region of the same chromosome or to a different chromosome or a plasmid.
- **Upregulation of transcription:** Activation, or promotion increase the rate of gene transcription.

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