Lecture Supplements

Plant Bacteriology

Bacterial Cell Structure

Compiled by N. Hassanzadeh

Version 4.25

January 1, 2025

Website Address:
http://www.phytobacteriology.com

Contact address

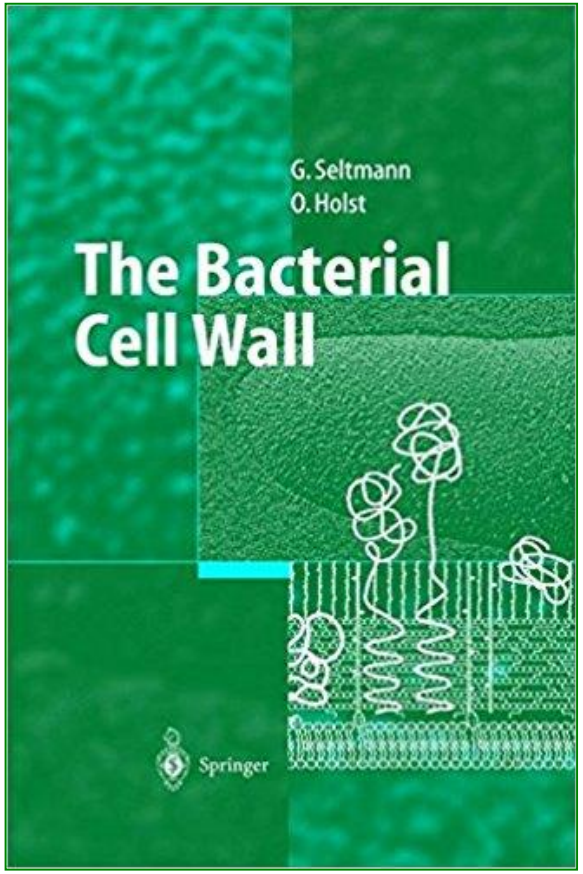
- Department of Plant Protection, Faculty of Agricultural Sciences and Food Industries, Science & Research Branch, Islamic Azad University, Tehran-Iran.
- P.O. Box: 14155/775, Postal Code: 1477893855
- Branch website: www.srbiau.ac.ir
- e-mail addresses:
- hasanzadehr@srbiau.ac.ir
- hasanzadehr@yahoo.com

Table of Contents

Table of Contents

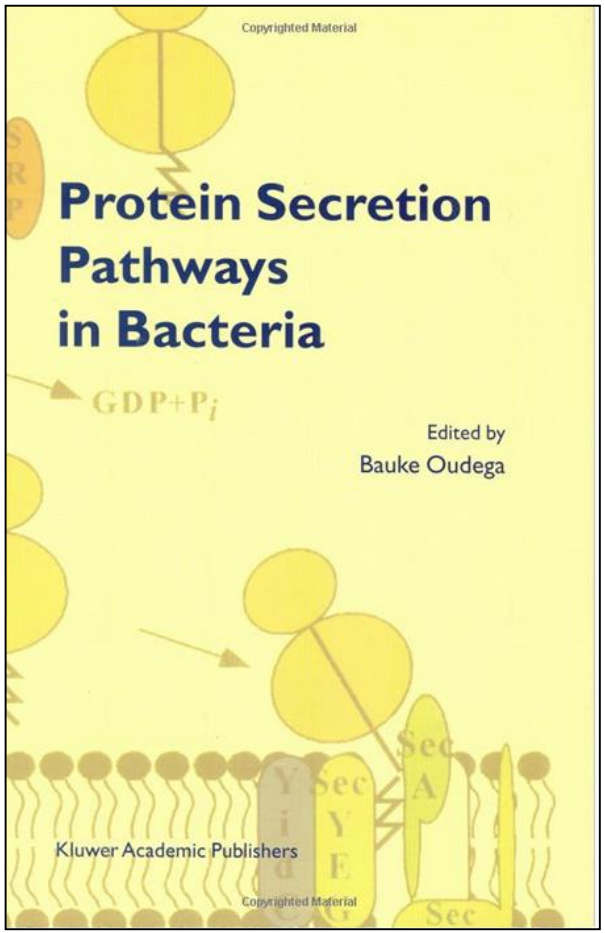
The Bacterial Cell Wall

- **The Bacterial Cell Wall**
- Gubtram Seltmann and Otto Holst
- Publisher Springer,
- 2002
- 280 pages



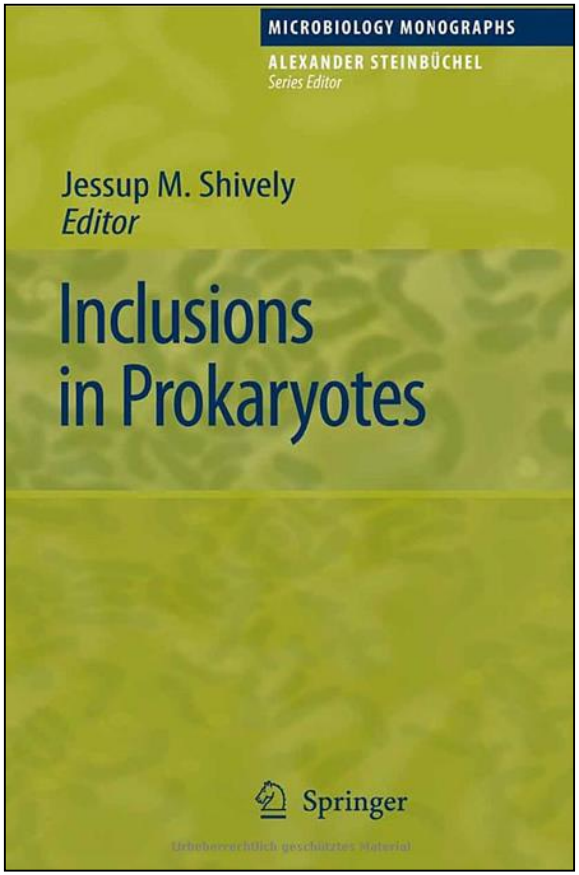
Protein Secretion Pathways in Bacteria

- **Protein Secretion Pathways in Bacteria**
- B. Oudega
- Springer Netherlands,
- 2003
- 296 pages



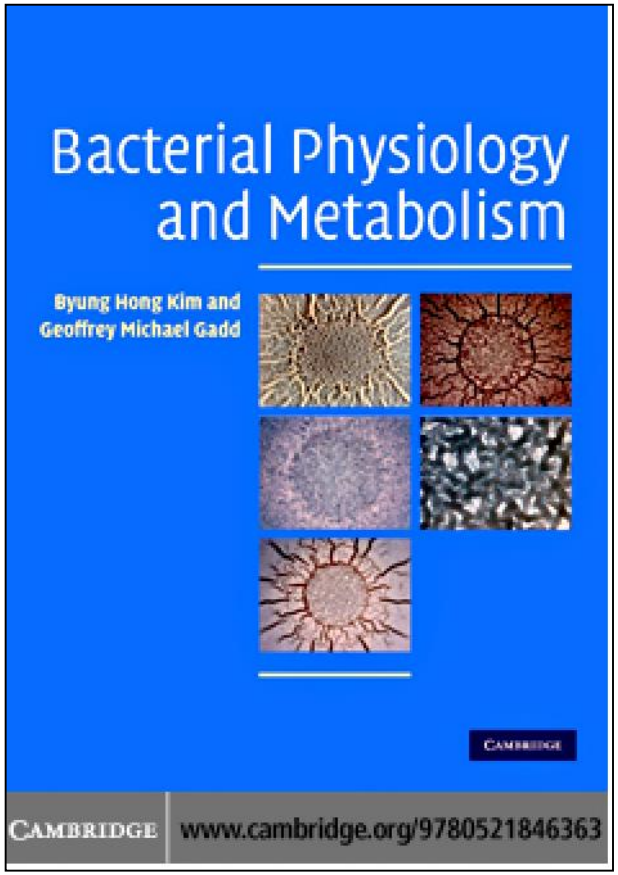
Inclusions in Prokaryotes

- **Inclusions in Prokaryotes**
- Volume Editor: Jessup M. Shively
- Springer-Verlag Berlin Heidelberg
- 2006
- 349 pp.



Bacterial Physiology and Metabolism

- **Bacterial Physiology and Metabolism**
- B. H. Kim and G. M. Gadd
- Cambridge University Press
- 2008
- 553 pp.

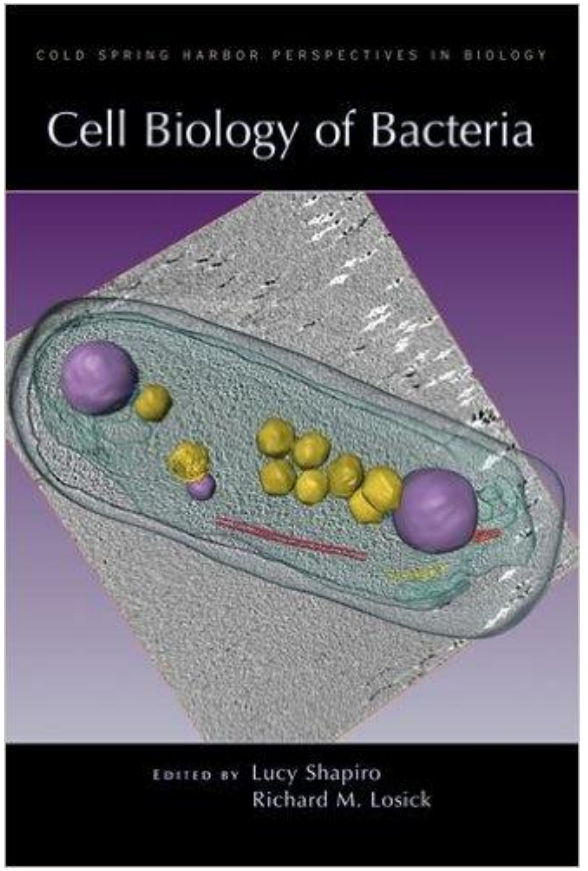


Pili and flagella

- **Pili and flagella**
- Edited by: Ken Jarrell
- Published by: Caister Academic Press
- 2009
- 238 pp.

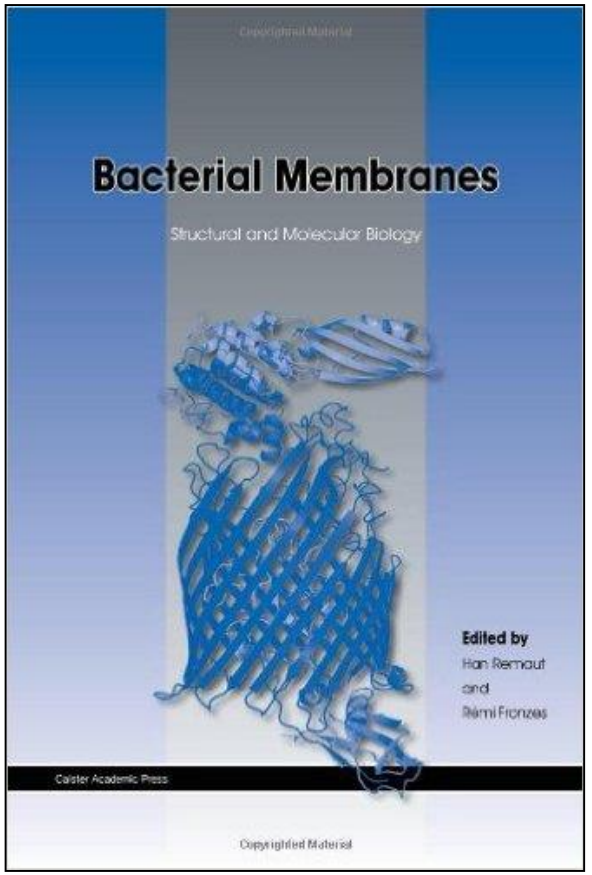
Cell Biology of Bacteria

- **Cell Biology of Bacteria**
- Lucy Shapiro (Editor) and Richard M. Losick (Editor)
- 2010
- 285 pages
- Cold Spring Harbor Laboratory Press
- 1 edition.



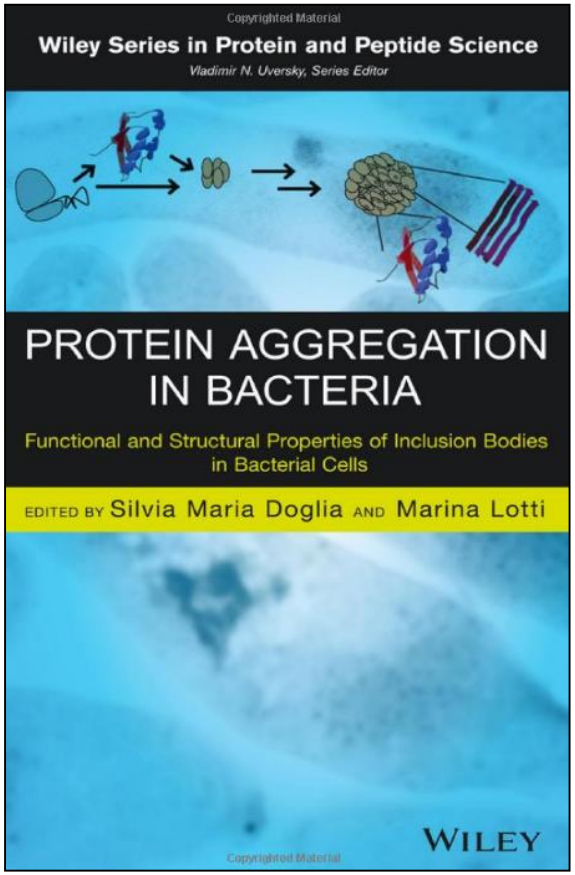
Bacterial Membranes: Structural and Molecular Biology

- **Bacterial Membranes: Structural and Molecular Biology**
- Han Remaut (Editor), Remi Fronzes (Editor)
- Caister Academic Press; 1 edition
- 2014
- Hardcover: 528 pages.



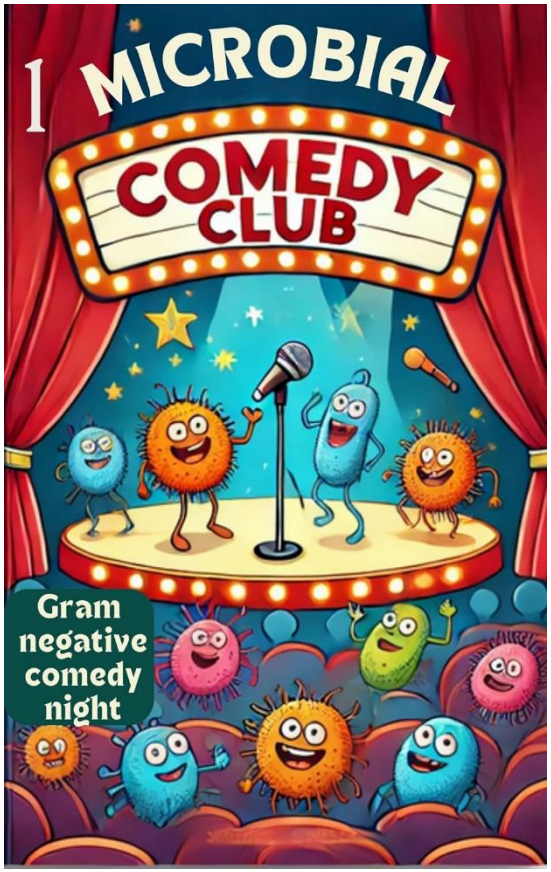
Protein Aggregation in Bacteria: Functional and Structural Properties of Inclusion Bodies in Bacterial Cells

- **Protein Aggregation in Bacteria: Functional and Structural Properties of Inclusion Bodies in Bacterial Cells.**
- Silvia Maria Doglia (Editor), Marina Lotti (Editor).
- Wiley Series in Protein and Peptide Science.
- 1st Edition
- 2014
- 288 pages.



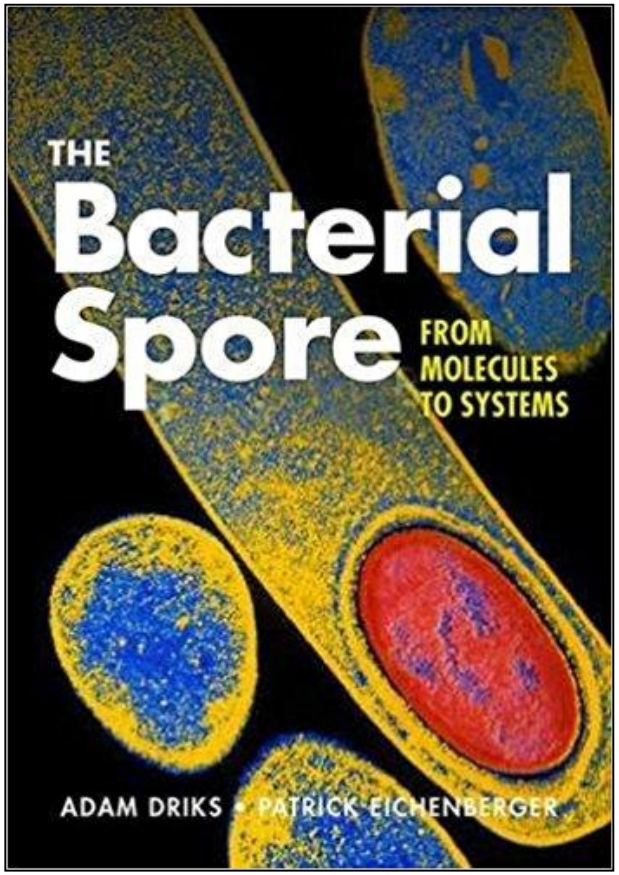
Sustainable Approaches to Controlling Plant Pathogenic Bacteria

- **Sustainable Approaches to Controlling Plant Pathogenic Bacteria**
- by V. Rajesh Kannan and Kubilay Kurtulus Bastas (Editors)
- CRC Press
- 2015
- 422 pages.



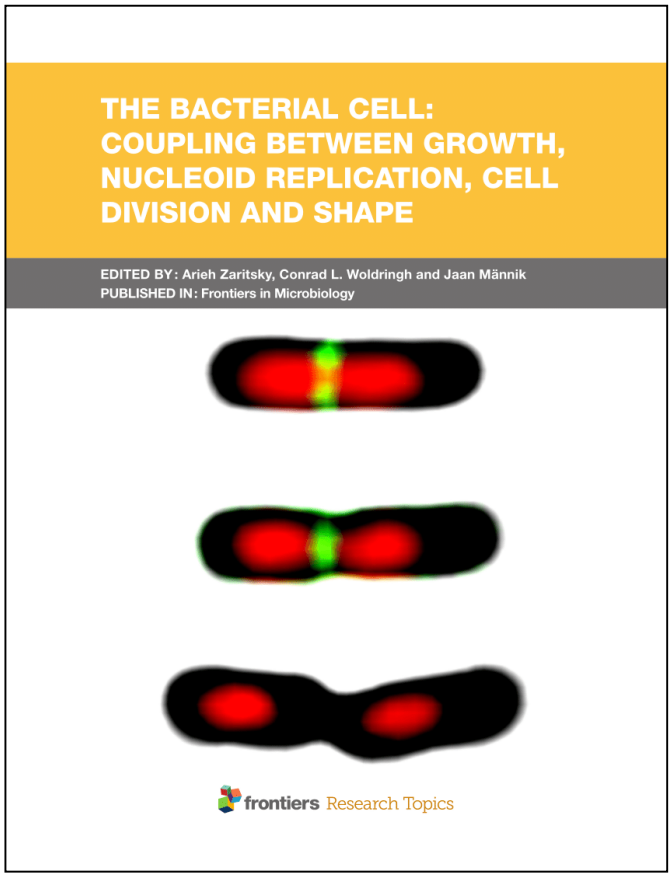
The Bacterial Spore: From Molecules to Systems

- **The Bacterial Spore: From Molecules to Systems**
- Adam Driks and Patrick Eichenberger (Editors).
- 1st Edition
- 2016
- 397 pages.



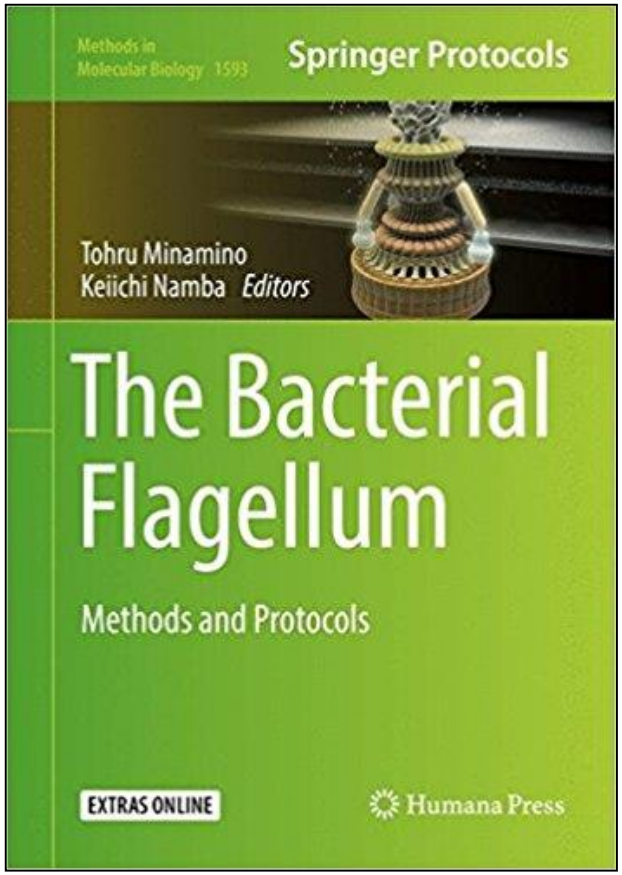
The Bacterial Cell: Coupling between Growth, Nucleoid Replication, Cell Division and Shape

- **The Bacterial Cell: Coupling between Growth, Nucleoid Replication, Cell Division and Shape**
- Book by Arieh Zaritsky, Conrad L. Woldringh, and Jaan Mannik
- 2016
- 324 pages.



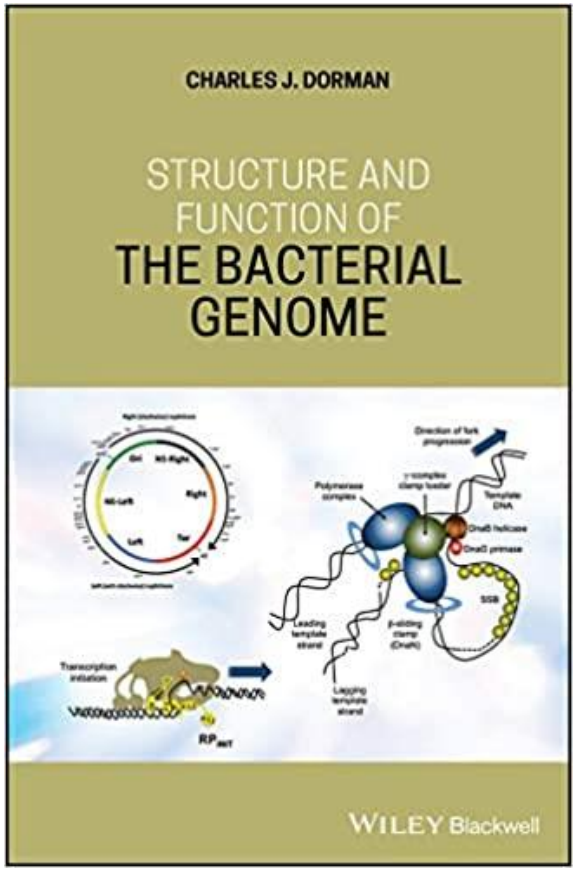
The Bacterial Flagellum-Methods and Protocols

- The Bacterial Flagellum-Methods and Protocols
- Tohru Minamino, and Keiichi Namba (Eds.)
- Publisher: Humana Press; 1st ed.
- 2017
- 326 pages.



Structure and Function of the Bacterial Genome

- Structure and Function of the Bacterial Genome.
- Charles J. Dorman
- Publisher: Wiley-Blackwell;
- 1 edition
- 2020
- 395 pages.



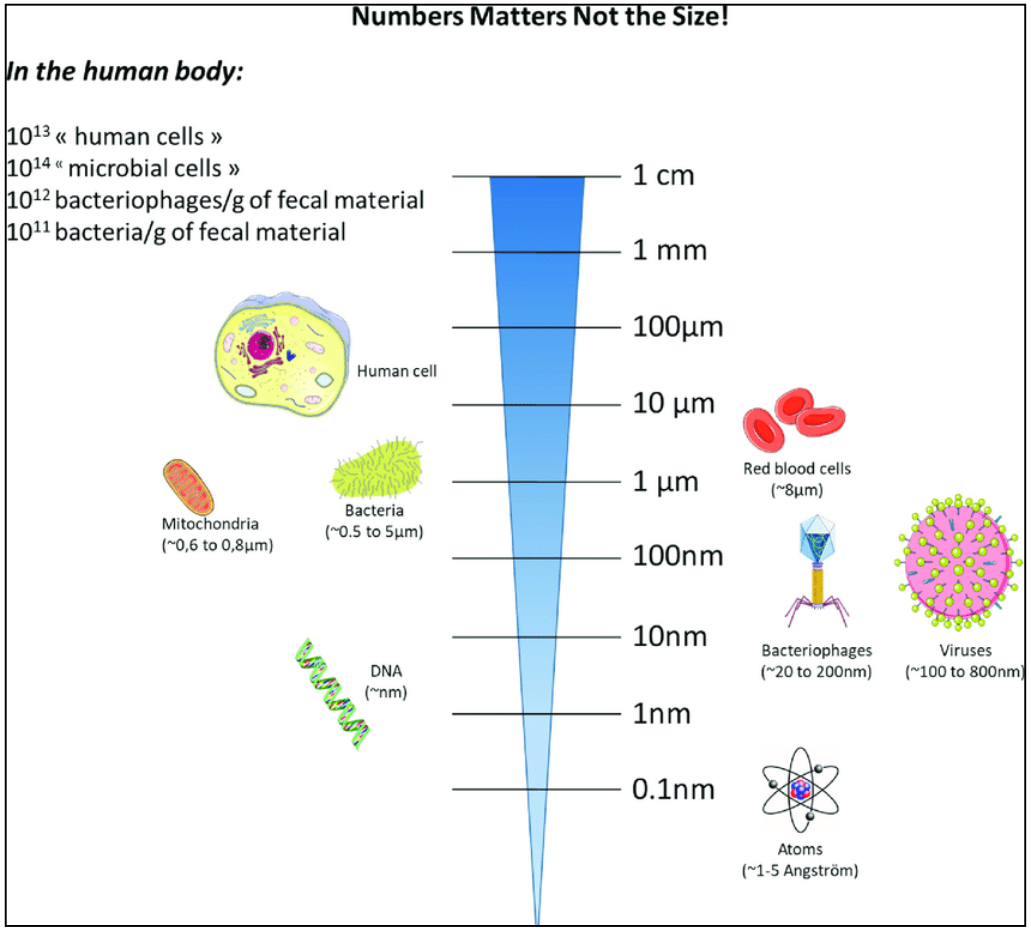
PowerPoints, Monograph, PDF files, Lecture Notes

- Bailey, R. 2017. Prokaryotes: Prokaryotic Cell Structure. Thought Co.
- Biljana Miljkovic-Selimovic *et al.*, 2007. Bacterial plasmids. Acta Medica Medianae 46(4):61-65.
- Chen, Gui-Qiang 2010 (ed.). Plastics From Bacteria: Natural Functions and Applications (microbiology monographs). Springer, 450 pages.
- El-Safey Mohmad El-Safey,2011. Introduction of bacterial taxonomy. 73 pp.
- Jones, J.B. 2006. Lecture 1 phytobacteriology. 19 pages.
- Rogers, A.2006. Chapter 4: Prokaryote cell biology, Chapter 6: Gram Stain. 78 slides.
- Seelke, R.W. 2010. Microbiology Lecture Notes.
- Schlink,2010. Cell shape/functions. 43 slides.
- Karki,G. 2017. Bacterial cell Structure and Function. Online pathology notes.
- Terrak, M. 2018. Research interest. Bacterial cell wall peptidoglycan synthesis and regulation. Inhibition of peptidoglycan synthesis. University of Liège.

Size of various objects in terms of nanometers

- A typical atom is anywhere from 0.1 to 0.5 nanometers in diameter.
- DNA molecules are about 2.5 nanometers wide.
- Most proteins are about 10 nanometers wide, and
- a typical virus is about 100 nanometers wide.
- A bacterium is about 1000 nanometers.
- Human cells, such as red blood cells, are about 10,000 nanometers across.
- At 100,000 nanometers, the width of a human hair seems gigantic.
- The head of a pin is about a million nanometers wide.
- An adult man who is 2 meters tall (6 feet 5 inches) is about 2 billion nanometers tall!

Relative sizes of major host cells and their components versus those of bacteria and viruses



Cani,2018

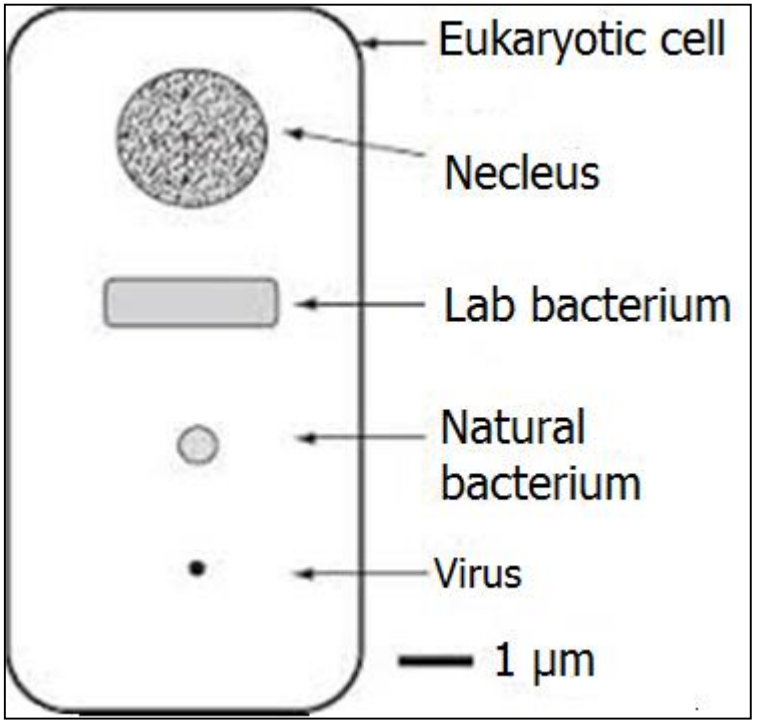
Scale of living forms

Prokaryotes

Bacteria

- Bacteria are prokaryotes.
- Pro: before
- Karyon: nucleus
- Prokaryotes are:

1. The simplest forms of life;
2. Earth's first cells;
3. Earth's most abundant life forms.

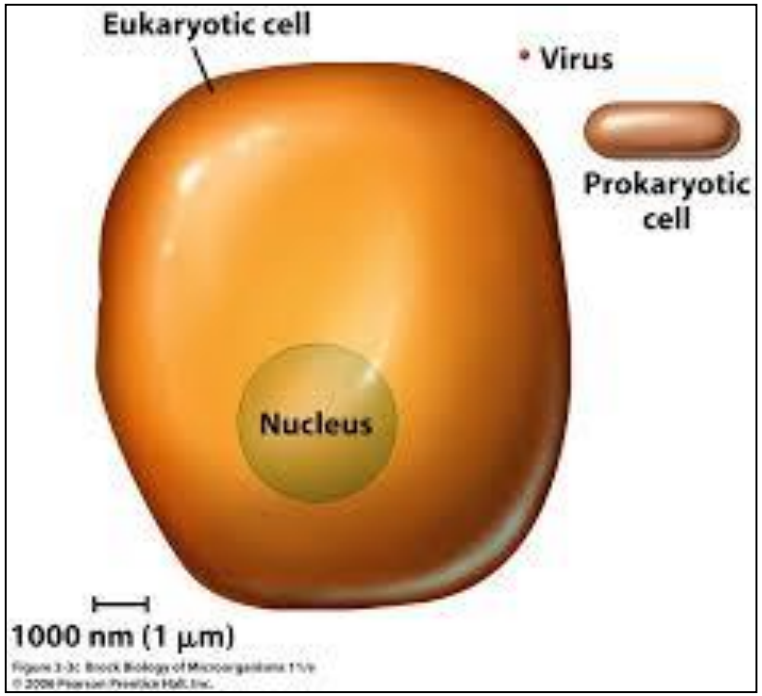


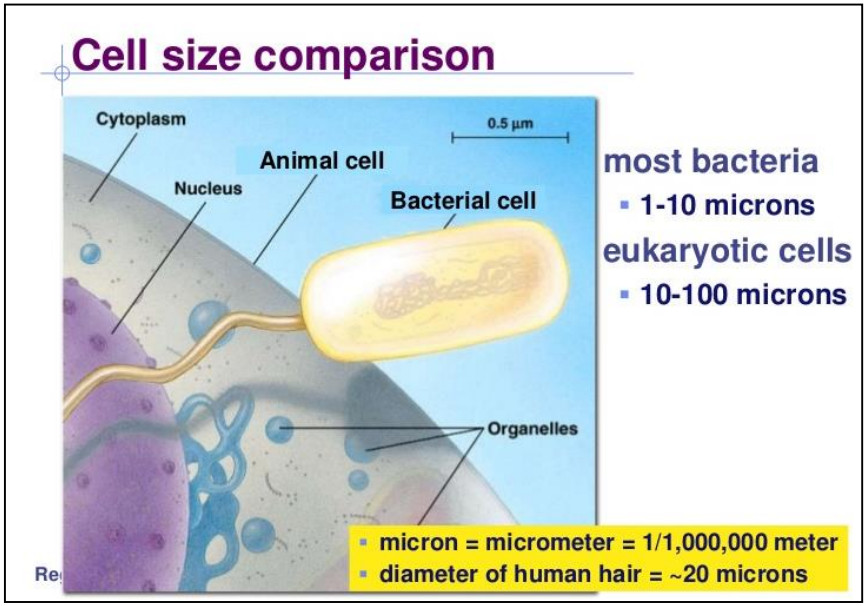
Relative sizes of biological molecules and cells:
Small virus: 30 nm;
Small bacteria such as Mycoplasma or Spiroplasma: 150-250 nm;
Typical bacteria: 1-10 μm;
Most human cells fall within a size range of 2-120 micrometers.

Scale of living forms

Cell size comparison

Bacteria vs. Virus; Bacteria vs. Eukaryotic cell



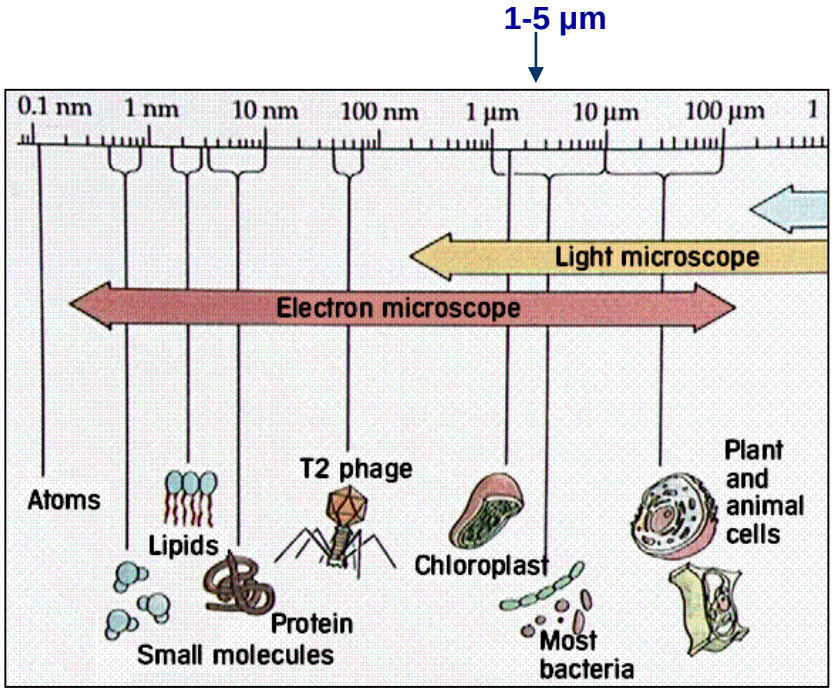


Scale of living forms

Cells range in size from small bacteria to large

- There is a huge difference in size of bacteria:
- Size ranges from 0.3 µm (the mycoplasmas) to 0.6 mm (*Epulopiscium fishelsoni*, which is genetically related to *Clostridium*).
- Average size of prokaryotic cells: 0.2-2.0 µm in diameter x 1-10 µm in length.

1. Typical eukaryote 10-500 µm in length (0.01-0.5 mm).



Farabee,2007;..

Among the smallest bacteria are members of the genus Mycoplasma, which measure only 0.3 µm, as small as the largest viruses. Bacterial cells are about one-tenth the size of eukaryotic cells.

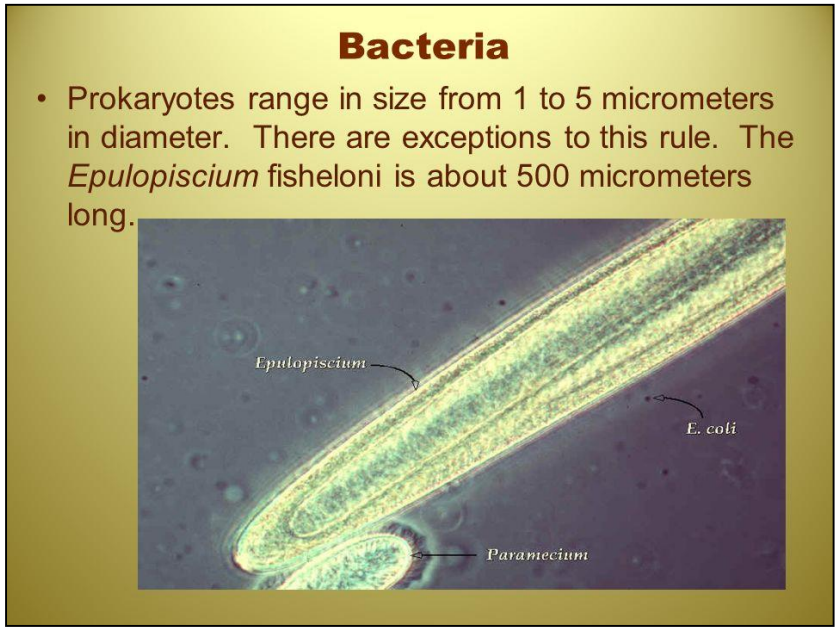
Diversity in bacterial cell size

Giant procaryote

Epulopiscium spp.

1. *Epulopiscium fishelsonii* can grow to a 0.6 mm, which is pretty impressive in the bacterial world.
2. *E. coli*, a traditional example of a bacterial cell, is about 0.001 mm, and

- The largest human cell is the ovum, or egg cell, which is about 0.12 mm in size (**0.2 mm smaller than** *E. fishelsonii*).



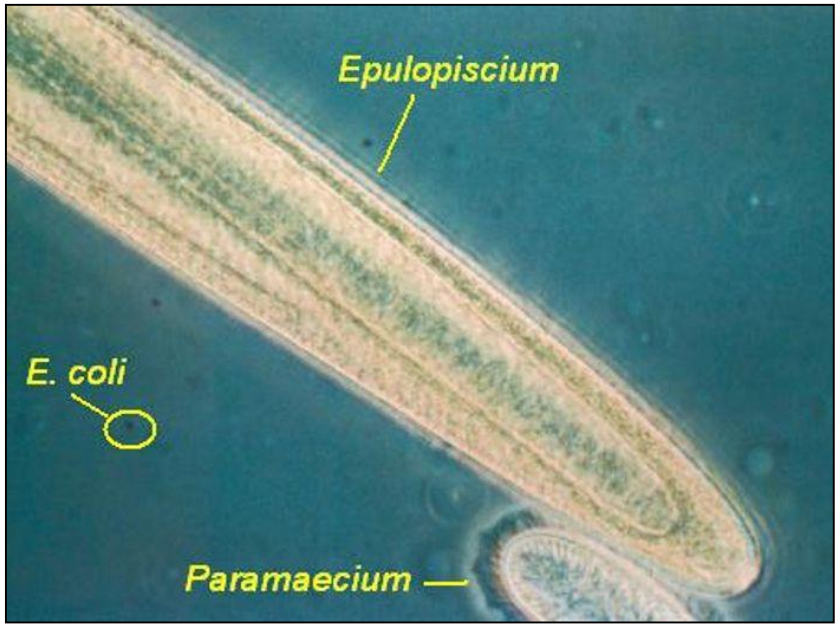
Piers Miles; Collens,2021

Diversity in cell size

Giant procaryote

Epulopiscium fishelsonii

- *Epulopiscium fishelsonii*, as a giant procaryote found in intestinal symbionts of certain species of surgeon fish in the Red Sea.
- Cigar shaped cells with a diameter as great as 80 µm and lengths up to 600 µm (0.6 mm).



The Prokaryotes (chapter 1.7),2006;..

Diversity in bacterial cell size

Giant procaryote

Epulopiscium has an enormous genome

- *Epulopiscium* are not only physical giants; they also have an enormous genome.
- While humans and most animals have only two sets of chromosomes (known as diploidy),
- *Epulopiscium* bacteria can have up to 400,000 sets of chromosomes (known as extreme polyploidy).

Collens,2021

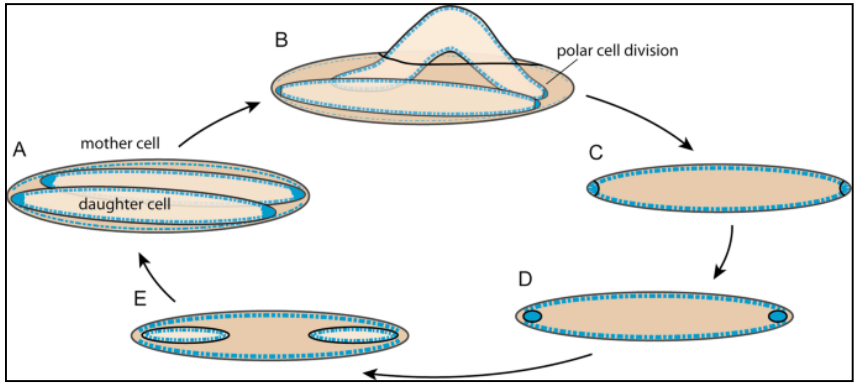
Diversity in bacterial cell size

Giant procaryote

Epulopiscium has an enormous genome

- *Epulopiscium* sp. type B daily life cycle.
- Binary fission has never been observed in *Epulopiscium* sp. type B.

A. Accumulation of DNA (in blue) at the poles of intracellular daughter cells marks the beginning of formation of the next generation (granddaughter cells).

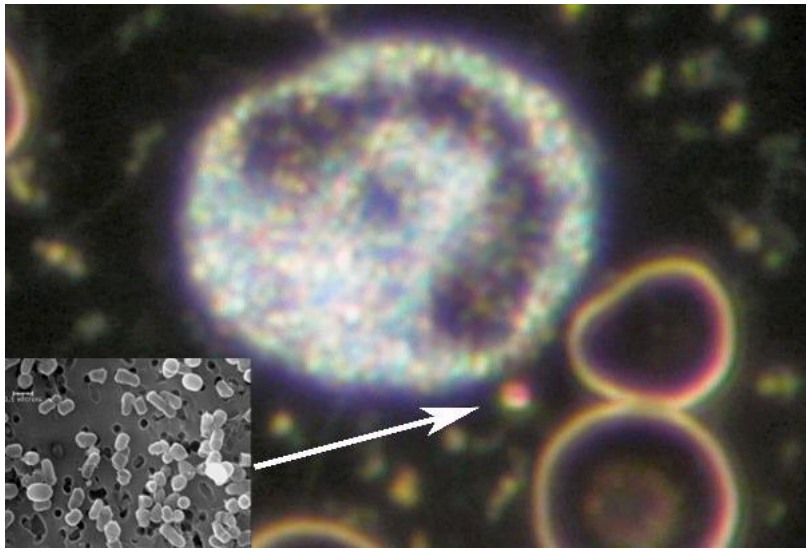


Arroyo *et al.*,2019

Diversity in bacterial cell size

Nanobacteria are the smallest cell-walled organisms on earth

- Nanobacteria (singular nanobacterium) or nanobes are nano-sized bacteria found in organisms (even human blood) and rocks.
- Smallest cell-walled organisms on earth, smaller than 300 nm (1/10 the size of bacteria).
- Nanobacteria self-replicate and form colonies even though they range from 50 to 200 nm in size.



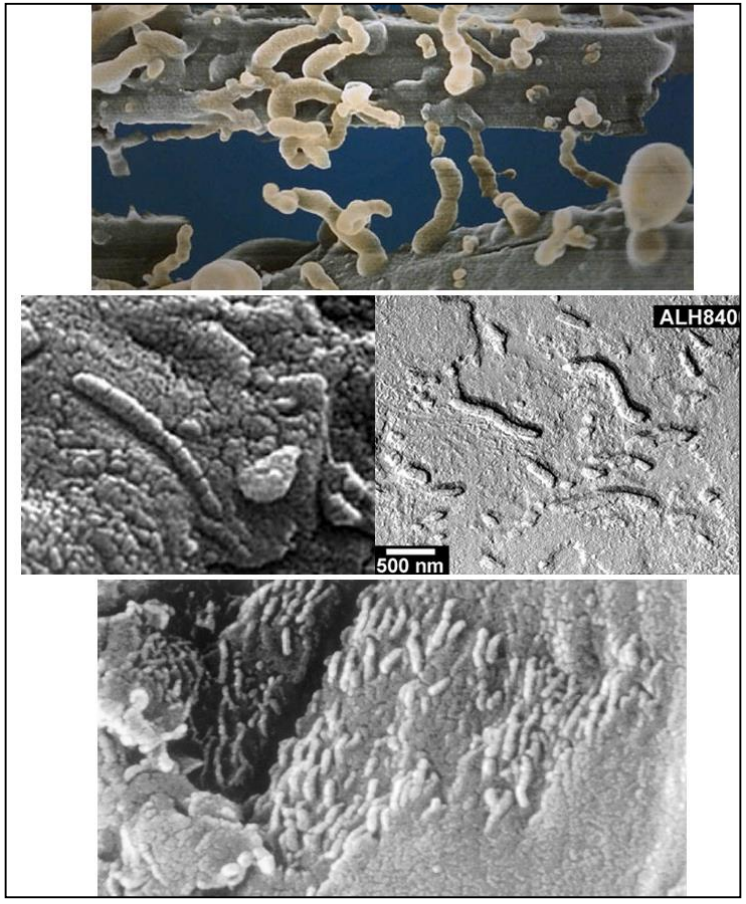
Nanobacteria compared to bacteria in size.
Potential role in forming kidney stones.

Joseph and Schild,2010

Diversity in cell size

Nanobacteria are the smallest cell-walled organisms on earth

- Some questioning whether or not an organism of this size has enough room to house necessary cell components such as DNA, RNA, and plasmids.
- Since they not only replicate but grow in size, it would be just one small step to cross the (arbitrary) threshold of 200 nm, to become large enough to incorporate DNA.

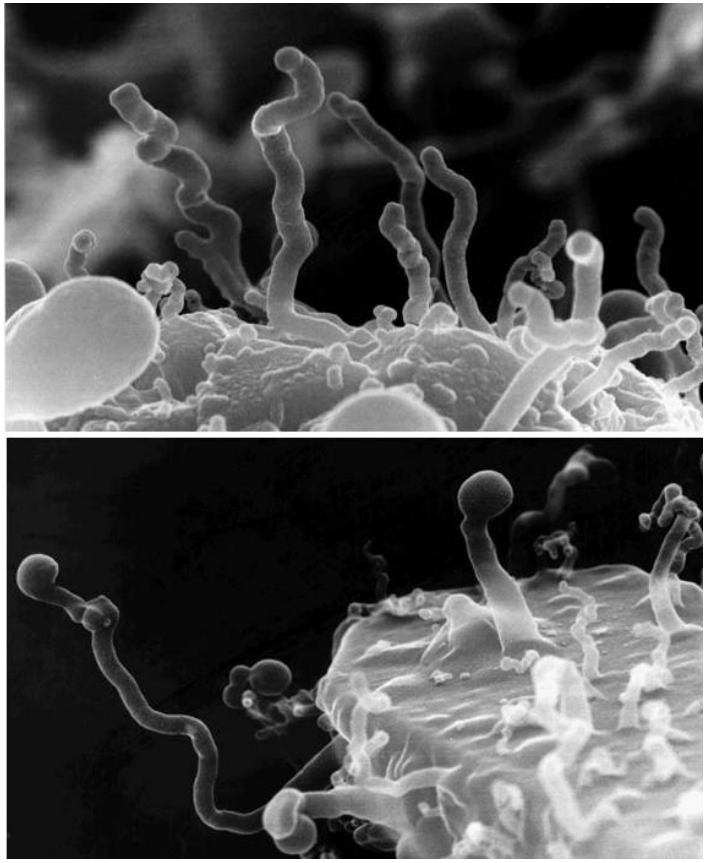


Wikipedia,2013; M.Bruckner; Joseph and Schild,2010

Diversity in cell size

Nanobacteria are the smallest cell-walled organisms on earth

- Nanobe studies challenge our perception of life.
- Microbes have already expanded our understanding of the harsh conditions that can support life.
- So, if nanobes do exist as living biota, they will broaden our perspective on the scale of life.
- Potential role in forming kidney stones.

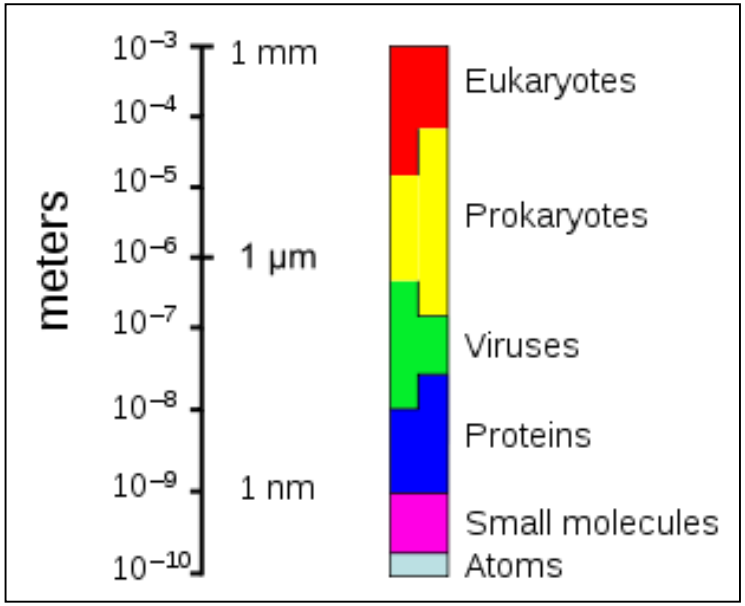


Wikipedia,2013; M.Bruckner; Joseph and Schild,2010

Prokaryotic cell size and contents

The range of sizes shown by prokaryotes, relative to those of other organisms and biomolecules

- Perhaps the most obvious structural characteristic of bacteria is (with some exceptions) their small size.
- For example:
- Mycoplasmas as small as 0.2 µm;
- *Bacillus* as large as 10 µm.
- *E. coli* cells, an average sized bacterium, are about:
- 2 µm long and
- 0.5 µm in diameter, with a
- Cell volume of 0.6-0.7 µm.



Wikipedia,2010;El Safey,2011

Prokaryotic cell size

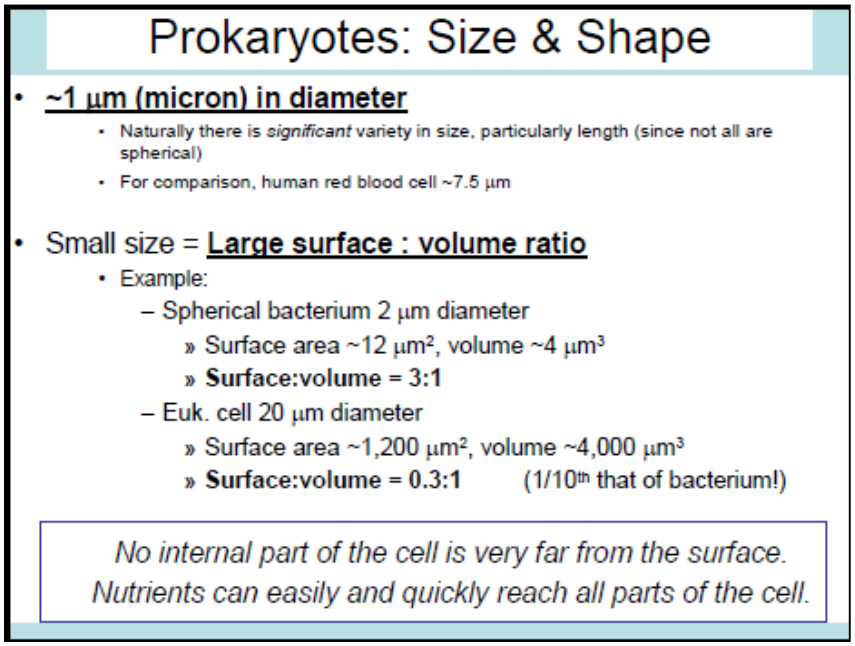
From smallest to extraordinary bacteria

Smallest bacteria	Cell size
Haemophilus influenzae	0.2-0.3 by 0.5- 2.0 µm
Escherichia coli	7 µm long and 1.8 µm in diameter
Clavibacter sepedonicus	0.5-1.0 µm
Phytoplasmas	0.2-0.8 µm in diameter
BLOs	0.25 to 0.5 by 0.8 to 4.0µm
Medium size bacteria	
Agrobacterium tumefaciens	1.5-3.0 x 0.6-1.0 µm
Xanthomonas	0.4-1.0 by 1.2-3.0 µm
Erwinia	0.5-1.0 µm by 1.0-3.0 µm
Bacilli	range between 3 to 5 µm
Largest bacteria	
Spirochaeta plicatilis	250 µm long by 0.75 µm thick
Extraordinary bacteria	
Epulopiscium fishelsoni	80 mm thick and >600 µm long

It is well established that cell size increases as growth rate increases (Chrzanowski *et al.*,2008).

Prokaryotic cell size and contents
Wet mass, surface area and volume

- Corresponds to a wet mass of ca. 1 pg, assuming that the cell consists mostly of water.
- The dry mass of a single cell can be estimated as 20% of the wet mass, amounting to 0.2 pg (picogram=10^{12} g).
- About half of the dry mass of a bacterial cell consists of carbon, and also about half of it can be attributed to proteins.



Rogers,2006

Eubacteria or Bacteria

- **Three categories of Eubacteria:**

1. **Gram-negative with cell walls**
2. **Gram-positive with cell walls**
3. **Unusual and well-ness bacteria**

Basic cell type
Prokaryotic vs. eukaryotic cells

- Bacteria are simple organisms.
- Genetic material not enclosed in a special nuclear membrane.
- Act as a Model system.
- We used bacteria to figure out how DNA is replicated, how genes are turned on and off, how cells respond to their environmental changes, etc.

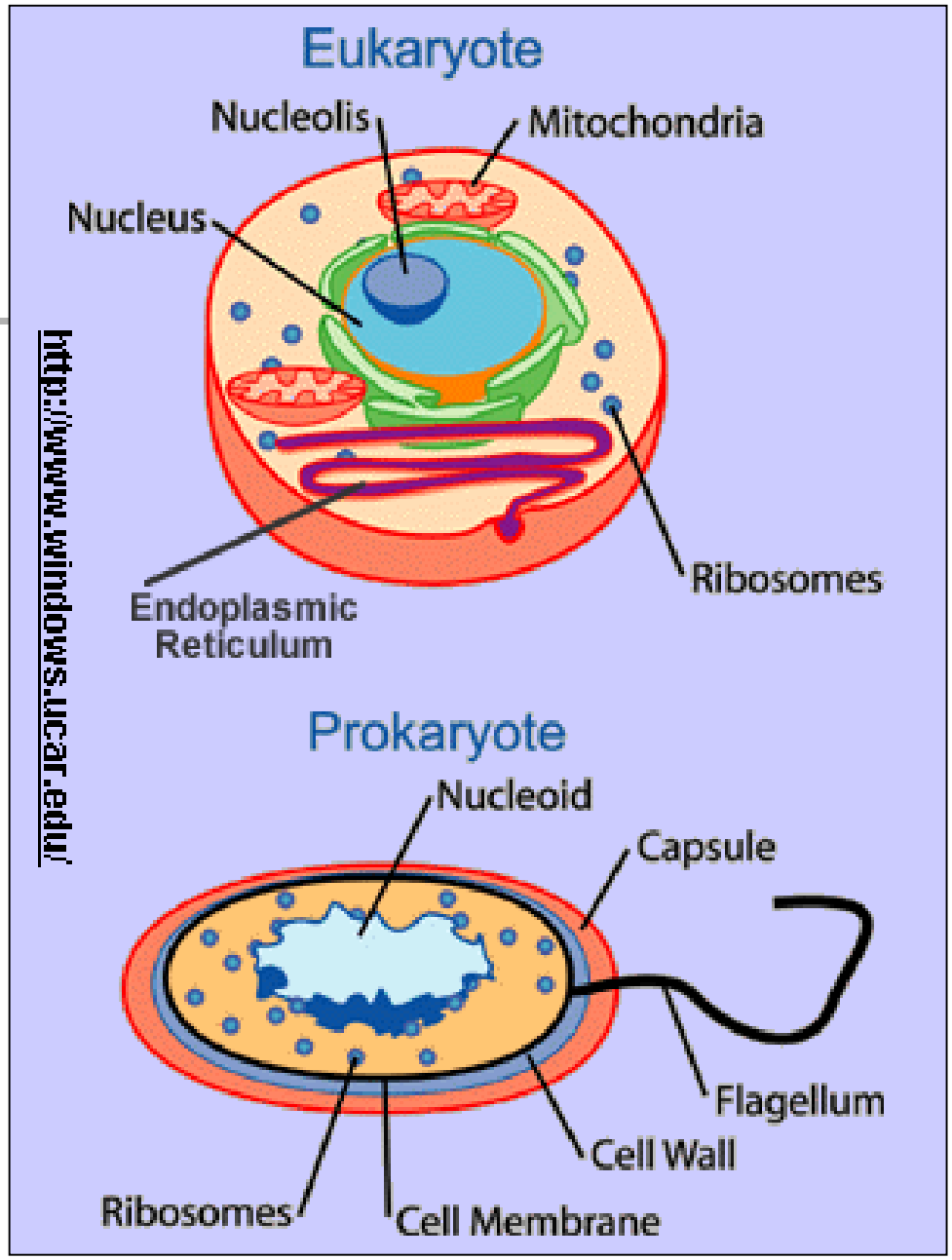
Prokaryotic cells

- Primitive cell
- No nucleus (Pre-nucleus). Only in bacteria.

Eukaryotic cells

- True cell
- True nucleus. All except bacteria.

Basic cell type
Prokaryotic
vs.
eukaryotic cells



Similarities of prokaryotic and eukaryotic cells

1. Contain all four biomolecules:
   (lipids, carbs, proteins, and nucleic acids)
2. Have ribosomes
3. Have DNA
4. Similar metabolism
5. Can be unicellular
6. Have cell/plasma membranes or cell wall.

Differences of prokaryotic and eukaryotic cells

	Achaea	Bacteria	Eukaryote
Cell type	**Prokaryote**	**Prokaryote**	**Eukaryote**
Cell wall	Varied, no peptidoglycan	Peptidoglycan	Varied, with CH_2O (formaldehyde)
Plasma membrane lipids	Branched	Straight chain	straight chain
Cytoskeleton	Rudimentary	Rudimentary (primary)	Yes
Flagella	Submicroscopic in size; composed of a fiber made from multiple different flagellin proteins.	Submicroscopic in size; composed of one protein fiber.	Microscopic in size; membrane bound; usually 20 microtubules in 9+2 pattern.

Bauman,2008

Differences of prokaryotic and eukaryotic cells

	Achaea	Bacteria	Eukaryote
True membrane-bound nucleus	no	no	yes
Chromosomes(DNA+ protein)	one circular chromosome	usually one circular, but some linear	chromosomes are more than one; linear
Plasmids	very common	very common. circular, but some linear	rare
Start to translation	methionine	Formylmethionine	methionine
Sensitivity to antibiotics	no	yes	no
Common arm on tRNA	lacking	present	present
Examples	Methanogens; extreme halophiles; hyperthermophiles	Gm + Gm - Cyanobacteria	Plants; Fungi; Animals

Bauman,2008;..

Differences and similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
Cell type	Prokaryotic	Prokaryotic
Cell morphology	Variable	Variable
Cell wall	Contains peptidoglycan	Does not contain peptidoglycan
Cell membrane type	Lipid bilayer	Lipid bilayer or lipid monolayer
Plasma membrane lipids	Fatty acids-glycerol ester	Phytanyl-glycerol ethers
Chromosome	Typically circular	Typically circular
Replication origins	Single	Multiple
RNA polymerase	Single	Multiple
Initiator tRNA	Formyl-methionine	Methionine
Streptomycin inhibition	Sensitive	Resistant
Calvin cycle	Yes	No

The Calvin cycle is the set of chemical reactions that take place in chloroplasts during photosynthesis. The cycle is light-independent because it takes place after the energy has been captured from sunlight.

Bacterial cellular anatomy

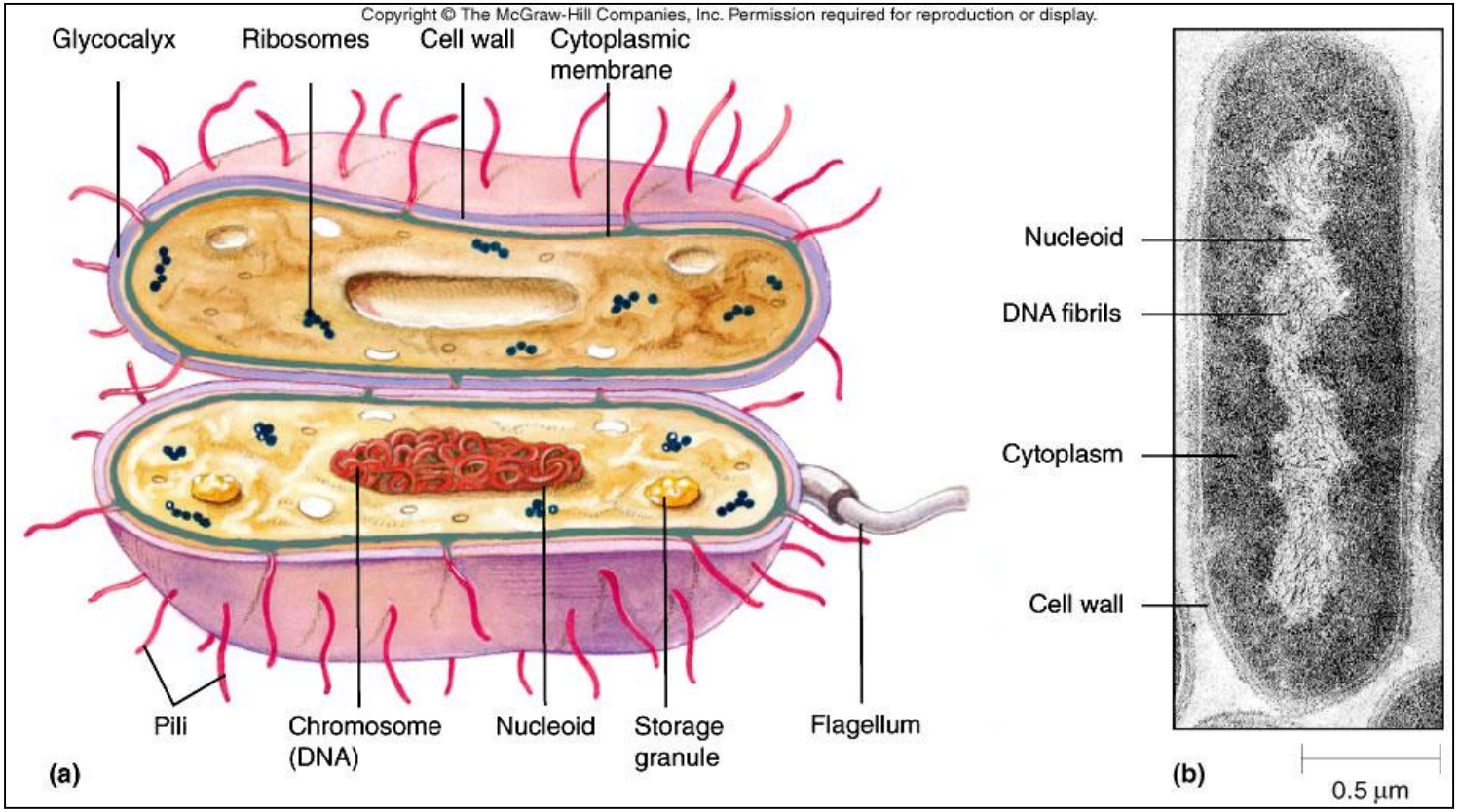
- **Structures external to the cell wall**

1. Flagella
2. Fimbriae and Pili
3. Glycocalyx/exopolysaccharide (EPS)
4. Lipopolysaccharide (LPS)

- **Structures inside cell membrane**

1. Cytoplasm (ribosomes, inclusions, gas vesicles)
2. Nucleoid (nucleus-like)
3. Spores.

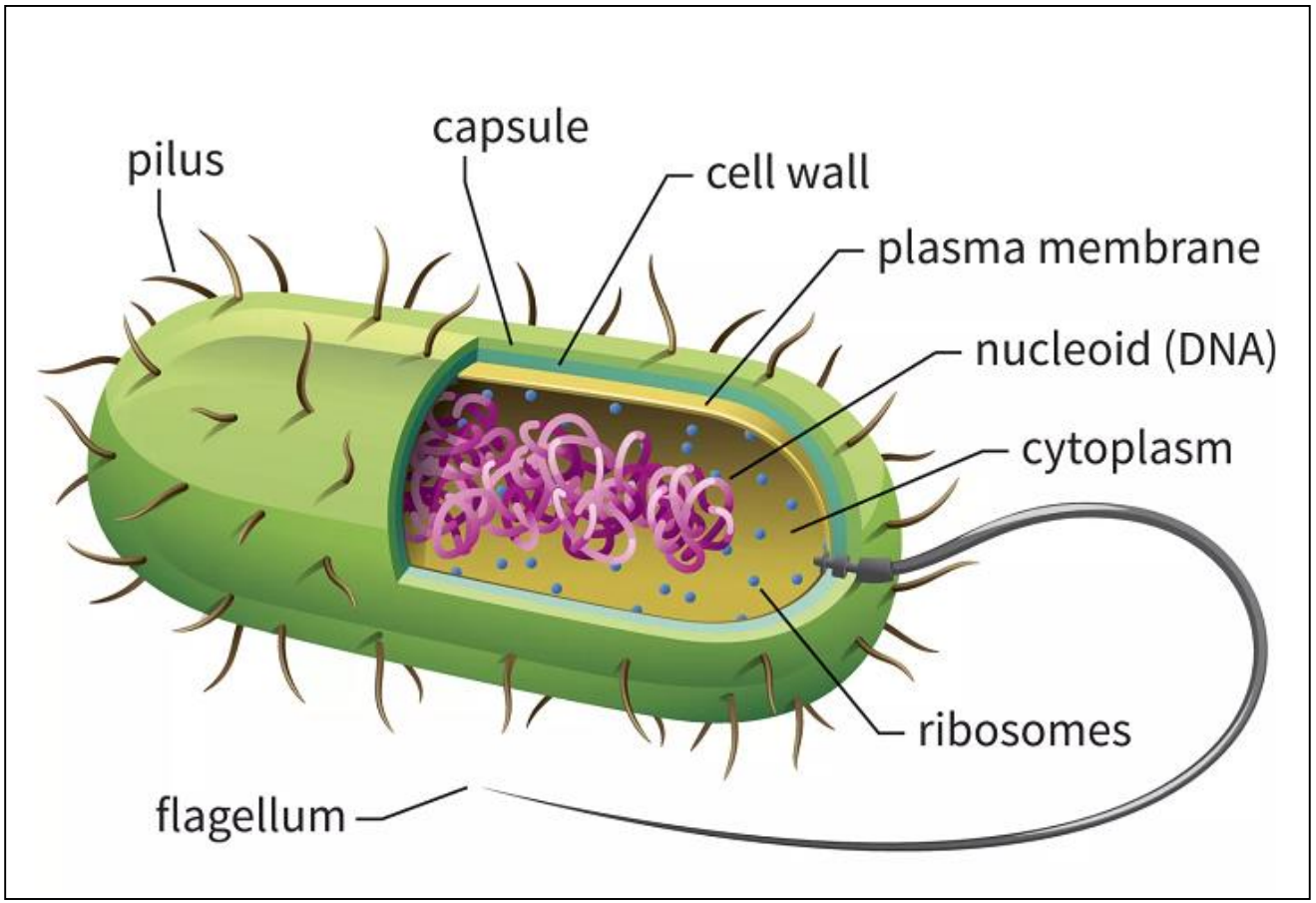
Morphology of a procaryotic cell



Seelke,2010

Morphology of a prokaryotic cell

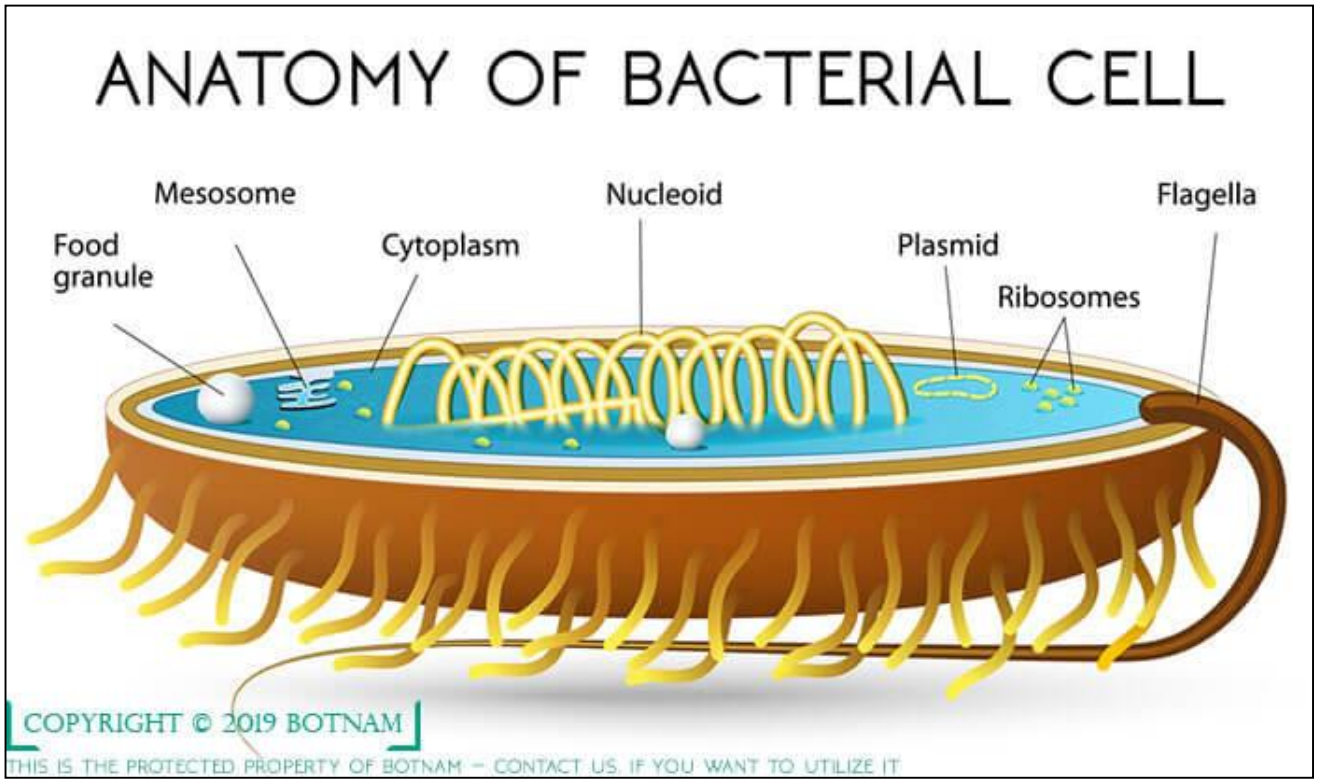
Bacterial cell anatomy and internal structure



Bailey,2019

Bacterial cell components

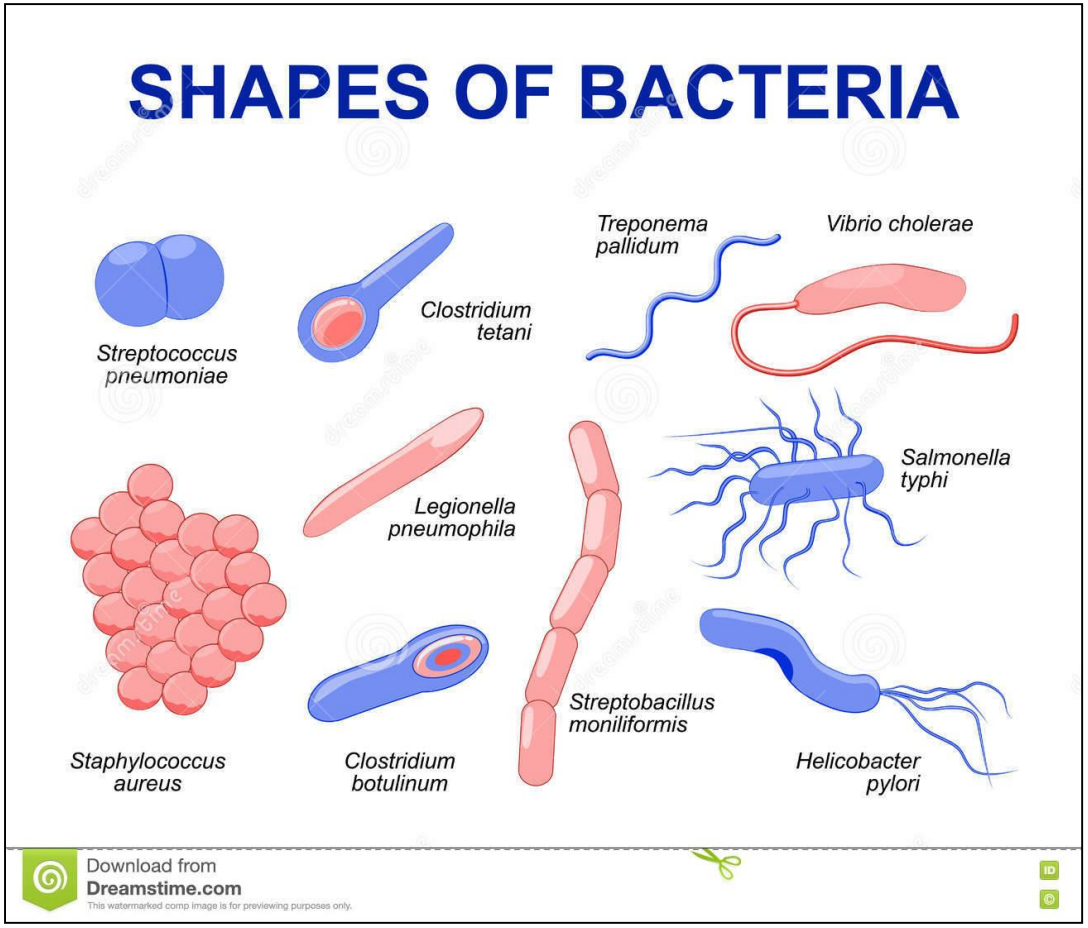
Gram negative



The membrane plasmalemma invaginates to become a complex structure known as mesosomes. It is believed that mesosomes have an active role in the cell division by wall synthesis and in the secretions of extra-cellular substances.

Bacterial morphological shapes

Variety of prokaryotic shapes

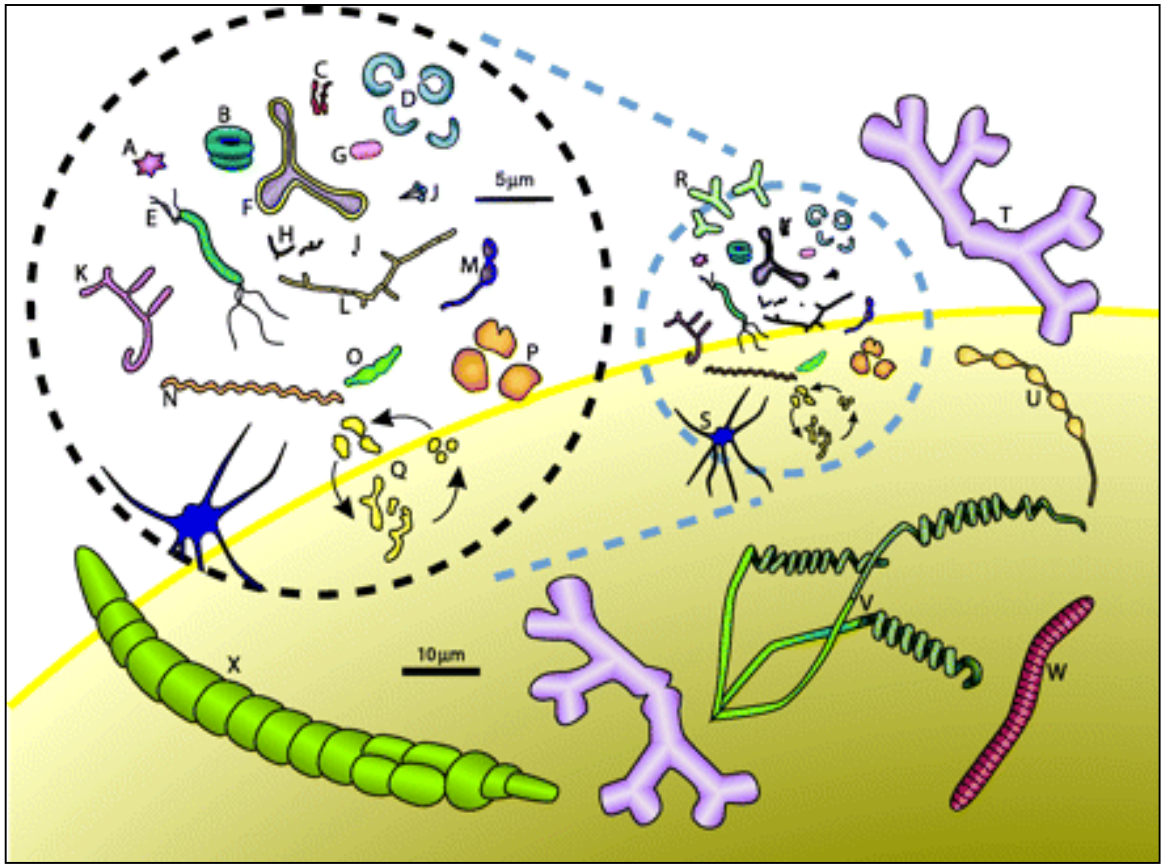


Dreamstime

Bacterial morphological shapes

Variety of prokaryotic shapes

There are cells that look like:
lemons, teardrops, or oblong spheroids; some are bent, curved, flat sided, triangular, bean shaped, or helical; others are rounded, squared, pointed, curved, or tapered.



Young,2006

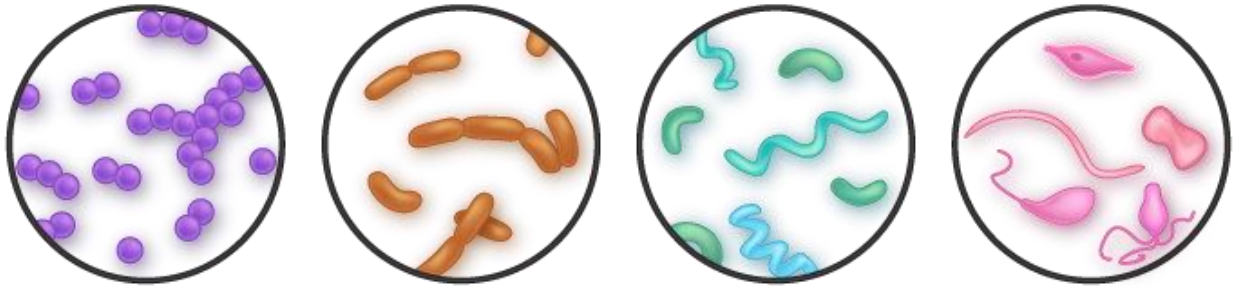
Bacterial morphological shapes

Variety of prokaryotic shapes

- Bacteria associated with plants have several morphological shapes:

1. Bacilli (rods),
2. Cocci (spherical),
3. Pleomorphic rods (tendency toward irregular shapes), and
4. Spiral shapes.

- The majority of plant-associated bacteria are rods.
- Most bacteria are monomorphic. They do not change shape unless environmental conditions change.

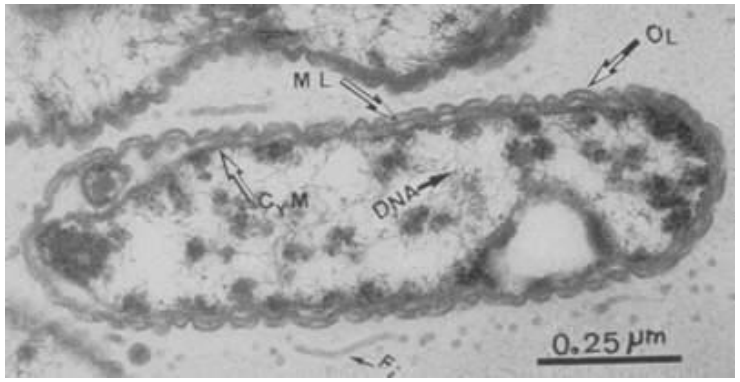


undulate

Cell shape

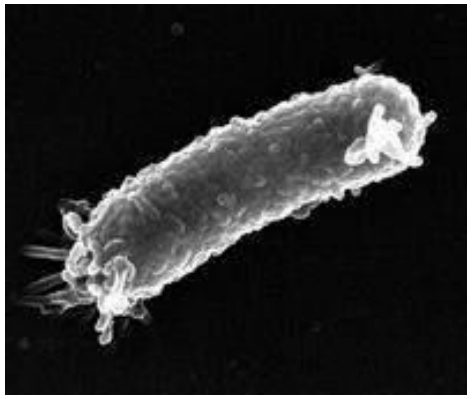
The ripple/undulating/wavy cell wall

Xylella fastidiosa



TEM micrograph

Note wavy outer wall layer.



Scanning electron micrograph

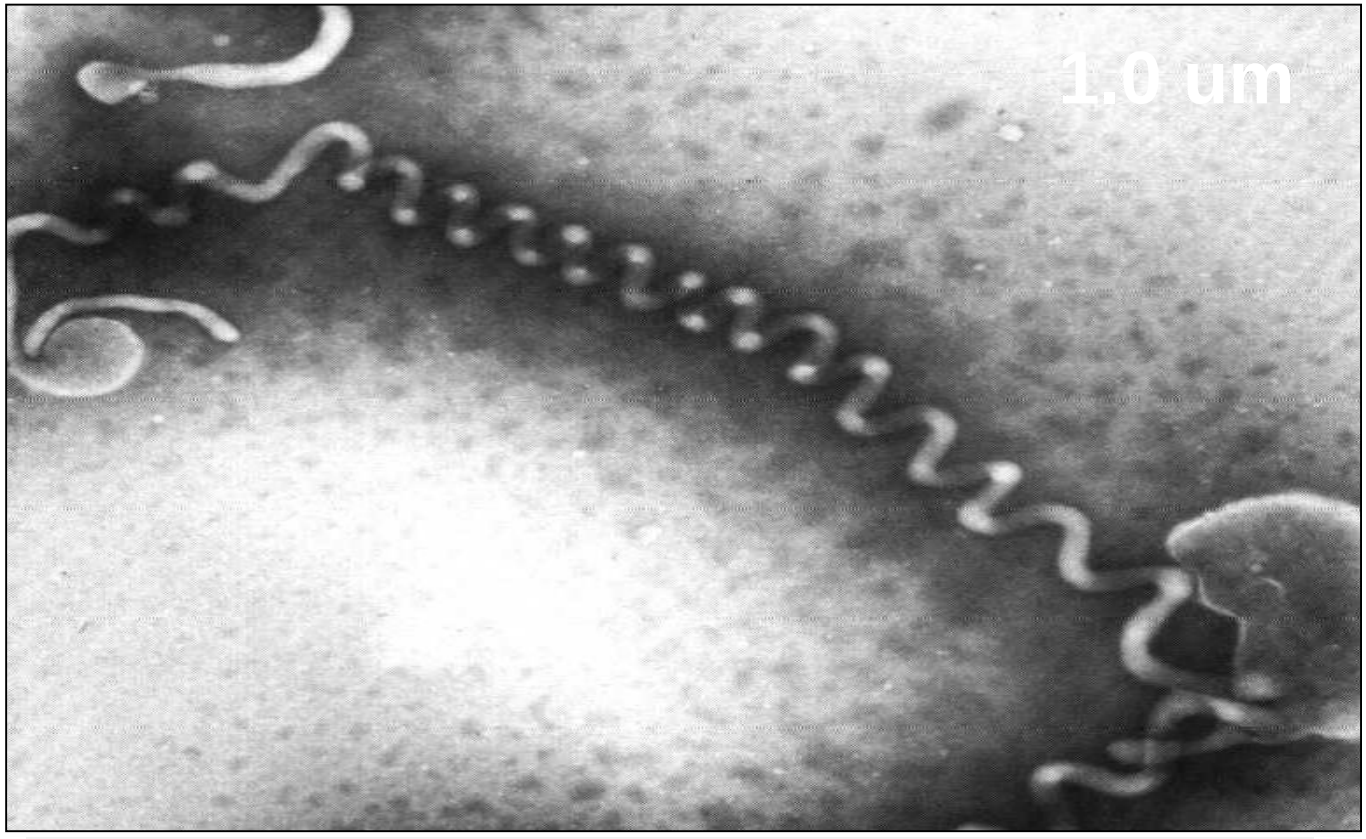
Xylella fastidiosa cells are small (0.25-0.5x1.0-4.0 µm), Gram negative, have no flagella. The outer layer of the cell wall is usually undulating or rippled.

The wall consists of:

1. an outer,
2. an inner layers (each comprised of 3-layered unit membrane structure), and
3. a middle peptidoglycan layer.

Cell shape

Filament detail of a 2 day old culture of *Spiroplasma citri*



Spiroplasmas form a helical cell shape and swim without flagella in viscous media. Cells of *Spiroplasma kunkelii* are just 0.15-0.2 µm in diameter while being 2.0-15 µm in length.

Bacterial morphological shapes

Bacterial shapes are not accidental but are biologically important

1. Why do bacteria have shape?
2. Is morphology valuable or just a trivial(insignificant) secondary characteristic?
3. Why should bacteria have one shape instead of another?

- Three broad considerations suggest that bacterial shapes are not accidental but are biologically important:

1. Cells adopt uniform morphologies from among a wide variety of possibilities,
2. Some cells modify their shape as conditions demand, and
3. Morphology can be tracked through evolutionary lineages.

- All of these imply that shape is a selectable feature that aids survival.

Young,2006

Bacterial morphological shapes

Bacterial shapes are not accidental but are biologically important

- Bacteria want what all other organisms want:
- to grow, they need to eat;
- to reproduce, they need to divide;
- if things are good where they are, they want to stay;
- if things are better somewhere else, they want to move;
- if threatened, they need to escape; and
- if the world around them changes, they must change.

Young,2006

Bacterial morphological shapes

Bacterial shapes are not accidental but are biologically important

- These are the basics of life:

1. accessing nutrients,
2. partitioning material to progeny,
3. attaching,
4. dispersing,
5. escaping predators, and
6. differentiating.

- Bacterial shape contributes at least some measure of survival value in response to the pressures imposed by these circumstances

Young,2006

Bacterial morphological shapes

Bacterial shapes are not accidental but are biologically important

- In recent years, cell shape has been shown to play a critical role in regulating the important bacterial functions of:

1. Attachment,
2. Dispersal,
3. Motility,
4. Polar differentiation,
5. Predation,
6. Cellular differentiation, and
7. Causing diseases.

Huang *et al.*,2008; Young,2006; Bamford,2007

Eukaryotic cytoskeletal proteins

A network of dynamic protein filaments

- A network of dynamic protein filaments, is an important tool to structurally organize the cells both in eukaryotes and prokaryotes.
- Eukaryotic cells contain three major cytoskeletal systems:

1. Microfilaments (assembled from actin proteins),
2. Microtubules (assembled from tubulin proteins), and
3. Intermediate filaments (In animal cells one of the major cytoskeletal systems is called the intermediate filaments (IF).

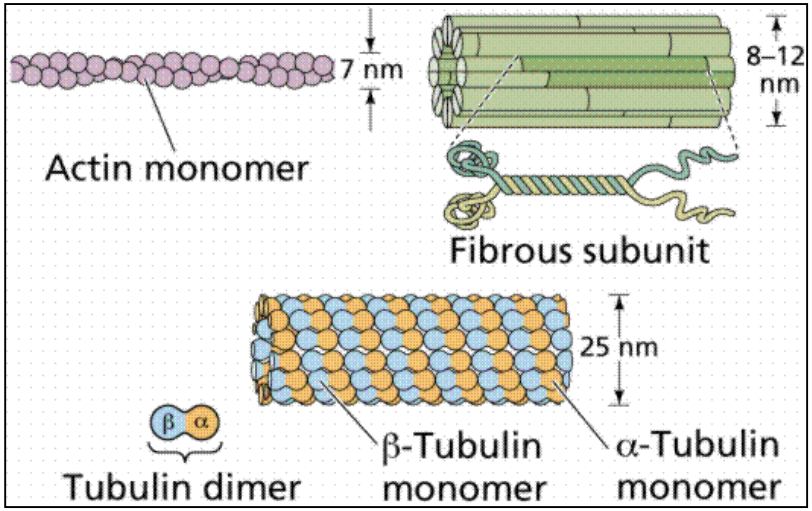
- Crescentin is the first bacterial protein that was recognized as an IF protein.
- These systems function to help maintain cell shape and integrity.

Tubulin: A family of globular cytoskeletal proteins that polymerize to form microtubules.

Eucaryotic cytoskeletal proteins
Functions of cytoskeletal elements

- These proteins have important roles in:

1. Maintains cell shape and integrity
2. Cell division
3. DNA segregation
4. Cell polarity
5. Sporulation



Cytoskeletal elements in Eukaryotic cells.

Actin filaments are thin threads that function in cell division and cell motility.

Procaryotic cytoskeletal proteins
Cytoskeletal elements and cell shape

1. **Protein FtsZ** (ancestor of tubulin):

- FtsZ was the first protein of the procaryotic cytoskeleton to be identified. It is encoded by the ftsZ gene that assembles into a ring at the future site of the septum of bacterial cell division (binary fission).
- FtsZ has been named after "Filamenting temperature-sensitive mutant Z".

2. **Proteins MreB and ParM** (ancestor of actin):

- Thus, one of the criteria often used by cell biologists to differentiate prokaryotic cells from eukaryotic cells can now be discarded, along with our naiveté about the simplicity of bacterial cells.

Procaryotic cytoskeletal proteins

Cytoskeletal elements and cell shape

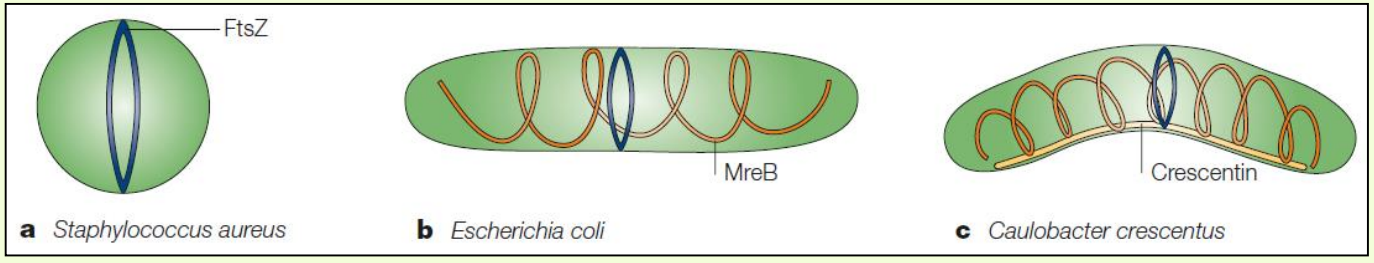
- These proteins have important roles in:

1. Cell growth-cell shape
2. Chromosome segregation.

a. FtsZ (ancestor of tubulin): forms a ring-shaped structure (blue) during cell division.

b. Actin-like MreB homologues (ancestor of actin): make the cells a rod shaped morphology like that seen in *Escherichia coli*.

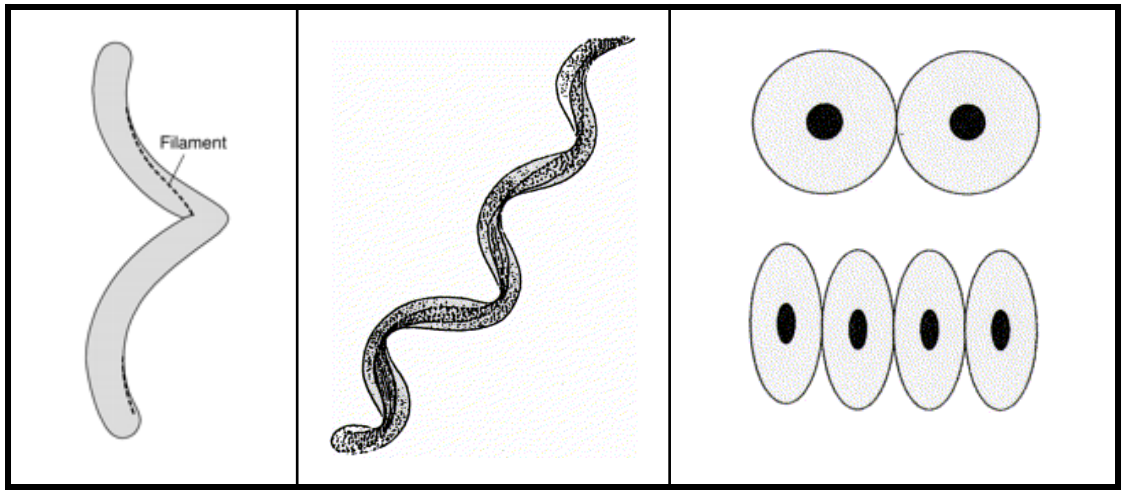
c. Crescentin (yellow) plus FtsZ and MreB makes a cell a crescent-shaped cell morphology. e.g. *Caulobacter crescentus*.



Cabeen and Jacobs-Wagner,2005

Procaryotic cytoskeletal proteins
Spiroplasma cytoskeleton

- Homologues of all three eukaryotic cytoskeletal elements i.e. actin, tubulin and intermediate filament proteins have now been found in bacteria (e.g. in *Spiroplasma* cells).
- Membrane-bound ribbon following the shortest helical line on the cellular coil, and composed of several well-ordered fibrils.



Bové and Garnier,2003

A. Structures External to the Cell Wall

- Flagella
- Fimbriae and Pili
- Glycocalyx (EPS)
- LPS

Flagella

Pili & Fimbriae

Motility
1. Flagella-mediated motility

1. Flagella-mediated motility:

- Many Gm-ve are characterized by swimming motility mediated by flagellation.
- Flagellation can be:

1. Polar (at one pole of cell):
2. Monotrichous, multitrichous (20-30 flagella)
3. Peritrichous (all around the cell) slower movement.

- Some +ve /-ve bacteria including cocci are generally non-motile. e.g. *Pantoea stewartii*.

Motility

2. Flagella, pili and growth-mediated motility

2. Other cellular movement:

1. Swimming movement: The most commonly studied mode of bacterial motility is swimming by rotation of thin helical appendages, called flagellar filaments. E.g. *P. aeruginosa*.
2. Gliding movement much slower than swimming movement, forms spreading and highly irregular structure colonies on solid media e.g. *Myxococcus xanthus*.
3. Twitching motility (surface motility) is mediated by the activity of hair-like filaments called type IV pili which extend from the cell's exterior. e.g. *Xylella fastidosa*.

- *P. aeruginosa* is capable of performing three types of motility, including swimming, swarming and twitching that is mediated either by its polar monotrichous flagellum or type IV pili (TFP).

Motility

Flagella, pili and growth-mediated motility

Planktonic	Bacteria growing as dispersed individuals in a liquid environment.
Flagella	The motor for swimming and swarming motility. Flagellar are complex molecular machines assembled from over 40 different proteins. Rotation of a membrane anchored basal body rotates a long, extracellular, corkscrew shaped filament that acts like a propeller to generate force.
Type IV pili	The motor for twitching motility. Proteinaceous pili that extend from one pole of the cell, attach to a surface, and retract. Retraction causes the cell body to move towards the anchor point of the pilus.
Focal Adhesion complex	A putative motor for gliding motility. A putative cell-surface associated complex that anchors a bacterium to a substrate. When coupled to an internal motor, the cell body moves relative to the focal adhesion complexes.
Hyperflagellate	An adjective describing a bacterium that has increased the number of flagella on the cell surface.
Surfactant	A secreted molecule that associates with a surface and acts like a lubricant to reduce surface tension.
Quorum sensing	A strategy by which bacteria regulate gene expression in a manner that is dependent on high population density.

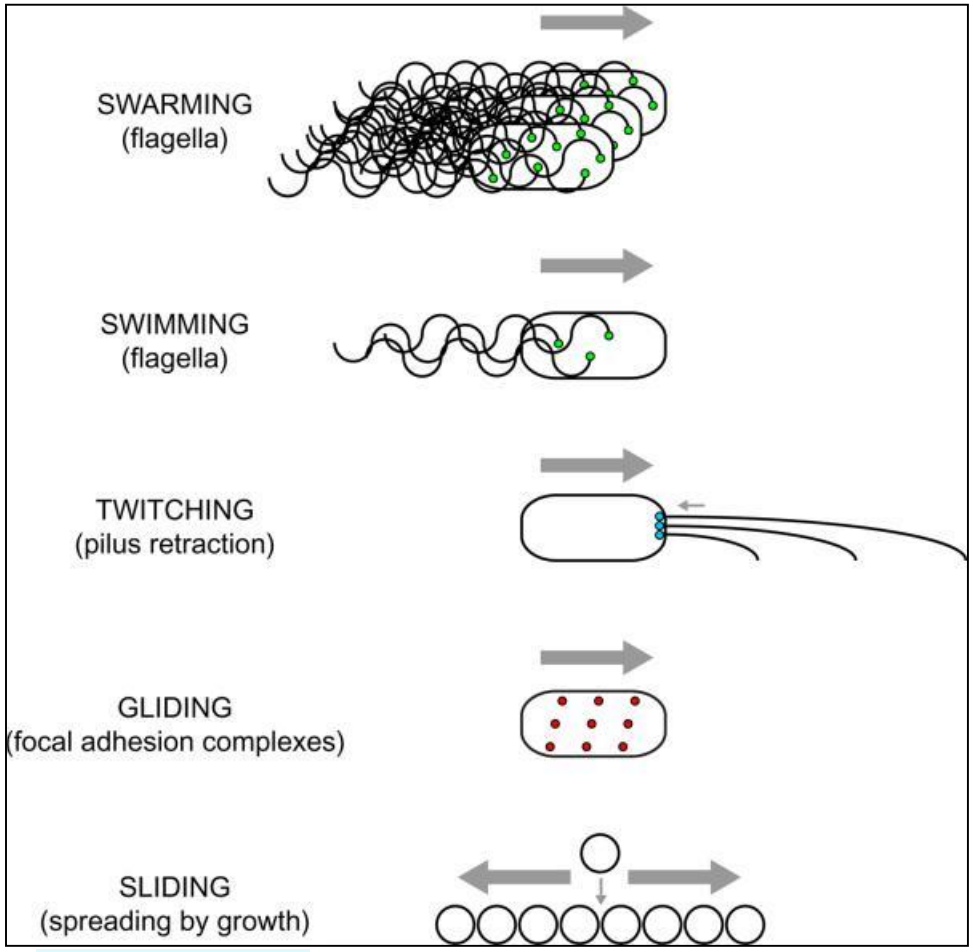
Kearns,2010

Motility

Flagella, pili and growth-mediated motility

1. Swarming is multicellular surface movement powered by rotating helical flagella.
2. Swimming is individual movement in liquid powered by rotating flagella.
3. Twitching is surface movement powered by the extension and retraction of pili.
4. Gliding is active surface movement that does not require flagella or pili and involves focal adhesion complexes.
5. Sliding is passive surface translocation powered by growth and facilitated by a surfactant.

- The direction of cell movement is indicated by a gray arrow and the motors that power the movement are indicated by colored circles.

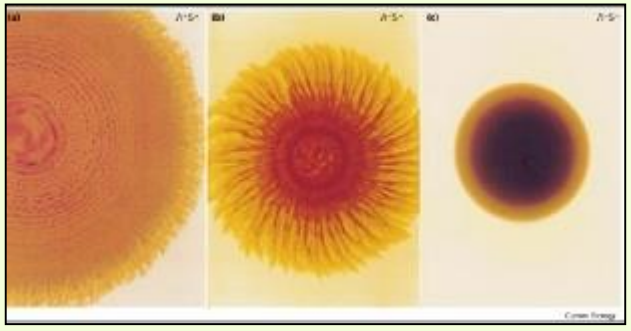


Kearns,2010

Gliding bacteria

Morphologies of colonies formed by *M. xanthus* cells on rich medium after growth for five days

- *Myxococcus xanthus* has two independent motility mechanisms: social motility and adventurous motility.
- Gliding motility is a catch-all definition for active surface movement that occurs along the long axis of the cell without the aid of either flagella or pili. Gliding generally involves the cell body moving through focal adhesion complexes that bind to the substrate.
- Sliding motility is a passive form of surface spreading that does not require an active motor, but instead relies on surfactants to reduce surface tension enabling the colony to spread away from the origin driven by the outward pressure of cell growth.



Youderian,1998; Kearns,2010

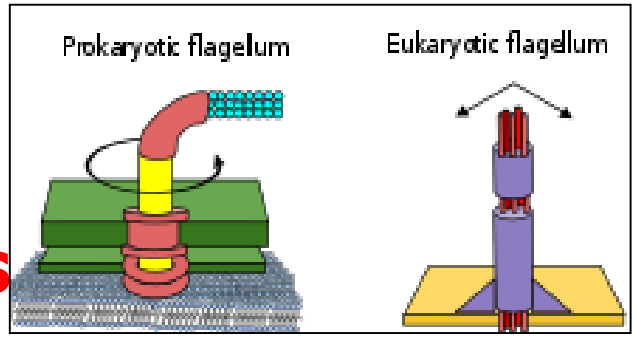
Flagella
Bacteria

- Flagella can be thought of as little semi-rigid whips that are free at one end and attached to a cell at the other.
- Bacterial flagella are polymers approximately 15 nm in diameter and up to 20 μm in length, composed of 53-kd subunits of a protein called flagellin.
- If a flagellum is cut off it will regenerate until reaches a maximum length.
- As this occurs the growth is not from base, but from tip.
- The filament is hollow and subunits travel through the filament and self-assemble at the end.

Paustain,2001;..

Flagella

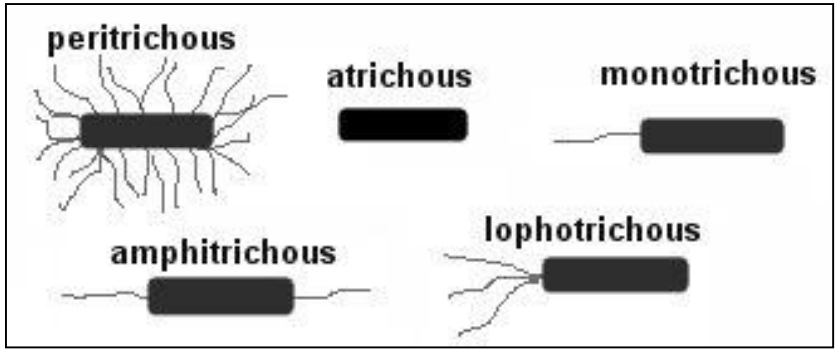
Prokaryotes vs. eukaryotes



- Possess simple flagella.
- ✓ Composed of single flagellin fiber.
- ✓ Lack 9+2 structure of eukaryotic flagella and cilia.
- ✓ Flagellar motion resembles spinning propeller.
- Flagella, usually at the poles of the cells (for movement) and fimbriae or pili, smaller thread-like appendages, usually at multiple locations (function in attachment or conjugation).
- There is some evidence that flagellated cells produce larger lesions than non-flagellated mutants.

Categories of flagellation

- Atrich/atrichous= No flagella.
- Monotrichous/Monotrich= single flagellum.
- Peritrichous/Peritrich= flagella all around.
- Amphitrichous/Amphitrich= flagella at both ends.
- Lophotrichous/Lophotrich= tuft of many flagella at one end or both ends.



In general, cells with single polar flagellum swim (>100 mms^{-1}) faster than cells with peritrichous flagella (<20 mms^{-1}). The cocci with two flagella bundles on one pole swim faster than 500 $\mu m \cdot s^{-1}$. The average swimming speed of cells with a single or two bundles is rather similar.
1 milimeter per second is 0.06 meters per minute.

Reynolds,2005;…

Bacterial motility
Speed of movement

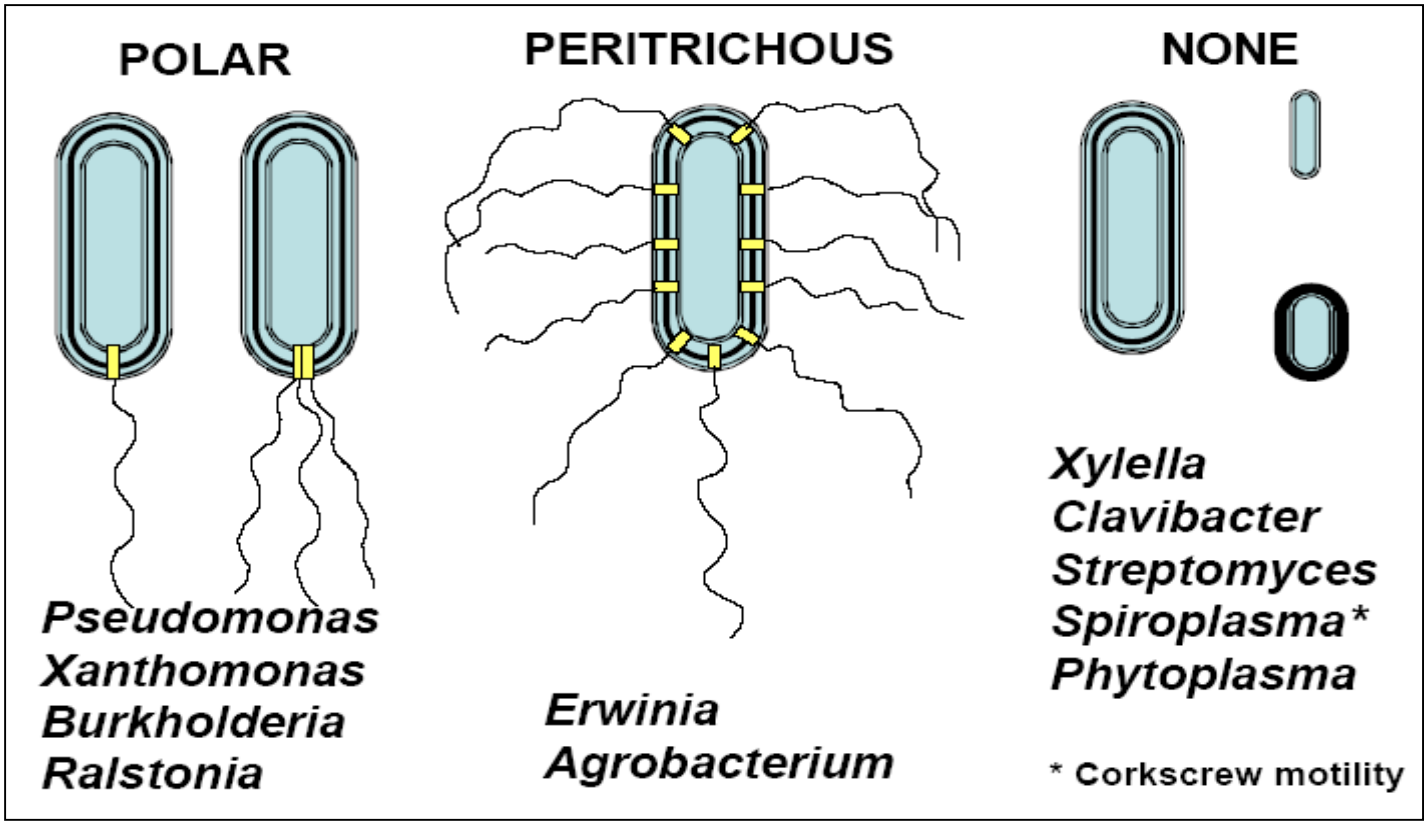
- How fast do bacterial cells move?
- They average 50 µm/sec, which is about 0.00015 kilometers/hr.
- This may seems slow but remember their tiny size.
- Table demonstrates a better comparison.

Organism	Kilometers per hour	Body lengths per second
Cheetah	111	25
Human	37.5	5.4
Bacteria	0.00015	10

Paustain,2001

Categories of flagellation

Swimming tails: help the bacteria swim towards plants. They can also swim around on the surface of plants until they find a way in!



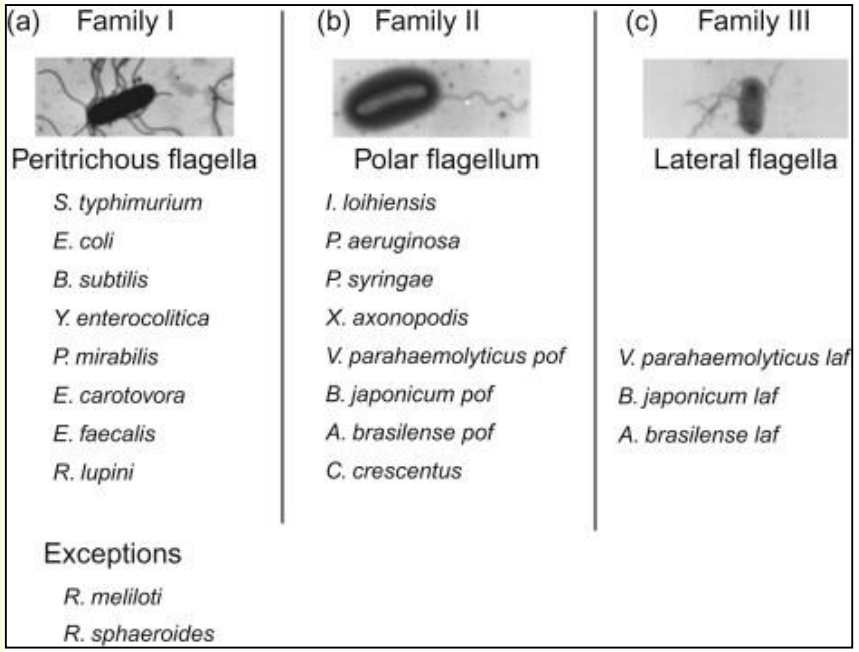
Grantham,2006; British Society for Plant Pathology,2013

Categories of flagellation

Peritrichous, polar and lateral flagella

Three families

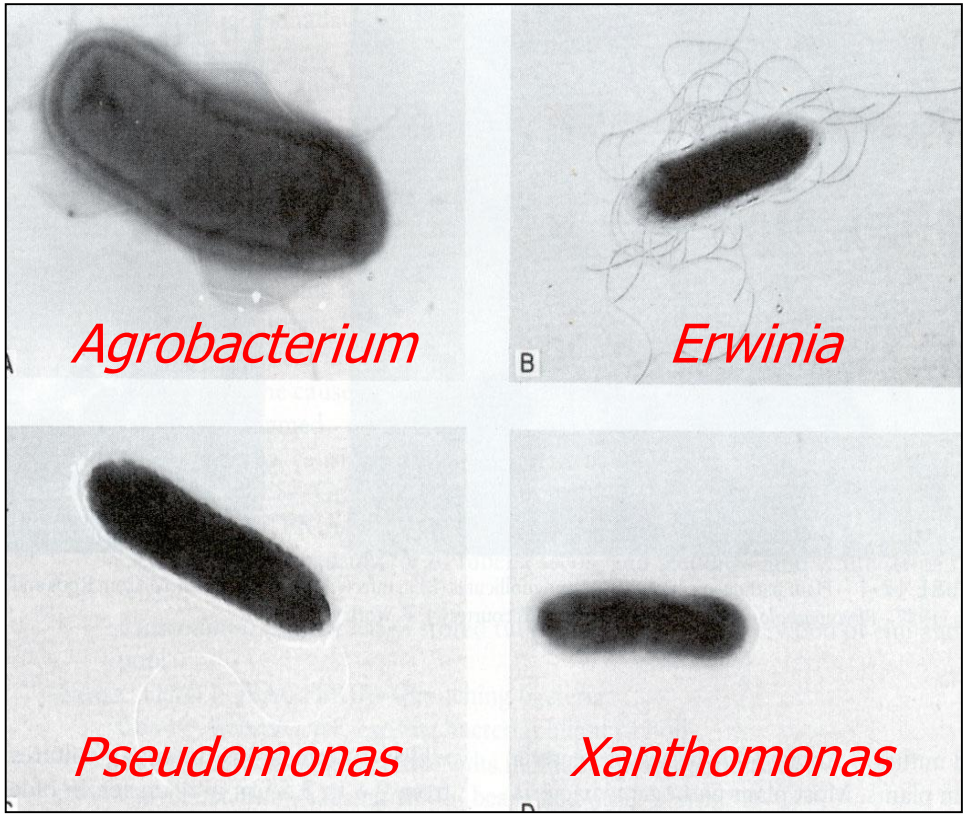
- Helical forms of peritrichous, polar and lateral flagella are independent from each other and belong to different flagellar families.



Shin-Ichi Aizawa,2015

Categories of flagellation

Plant pathogenic bacteria



Many plant pathogenic bacteria have flagella (polar or peritrichous). Cells of *R. solanacearum* are rod shaped and motile by 1-4 flagella motile (single to tuft polar flagella).

Flagellum structure

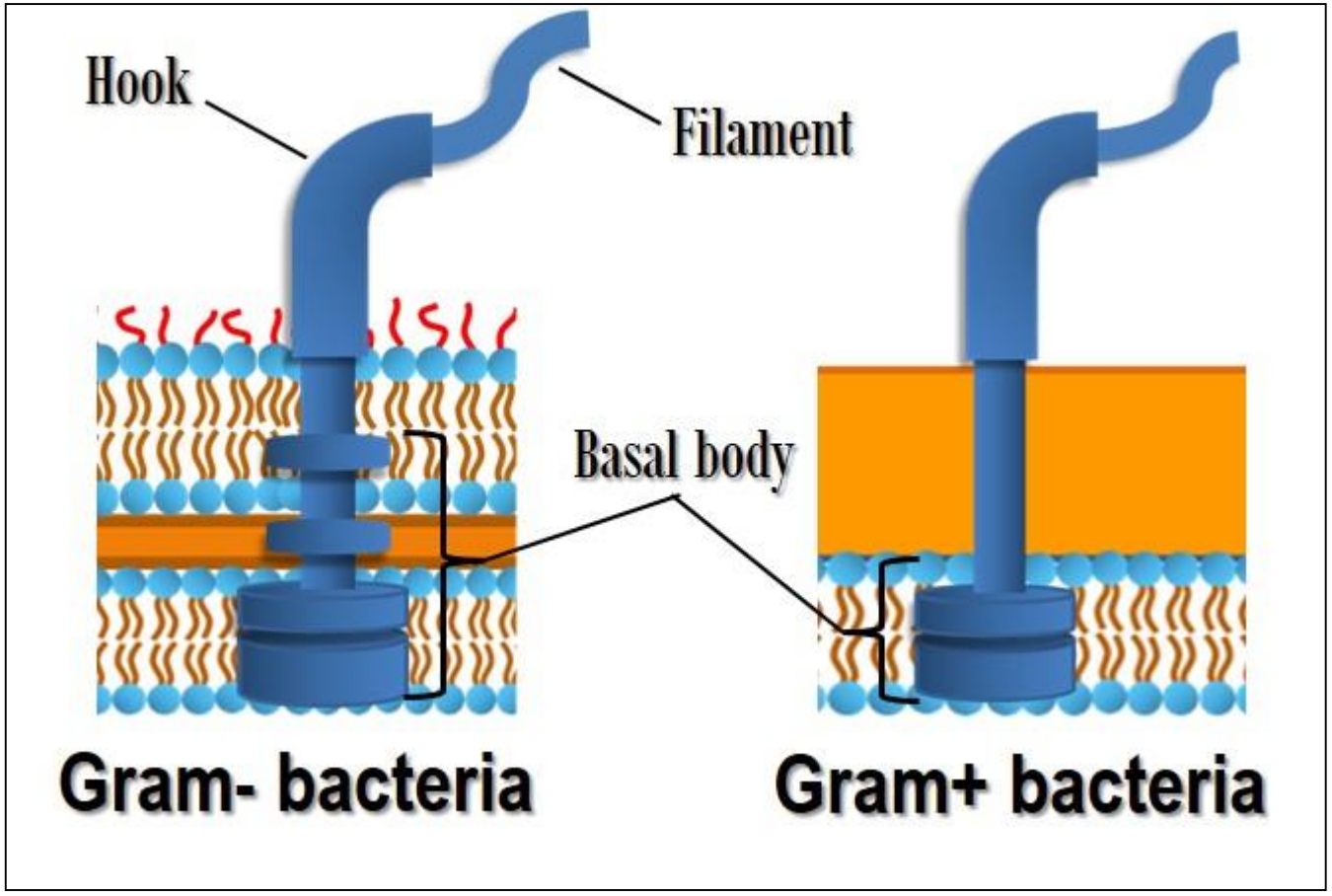
Composed of three parts

1. Filament: A filament (7-15 μm long) and thin (20 nm diam.);It is composed of primarily of a single, self-aggregating protein called flagellin.
2. Hook: Transition between filament and motor.
3. Basal body: Anchor in cell wall and motor.

The rod, is a major component of the basal flagellar body. The rods present in the basal body act as a reversible motor that propels the filament in a different orientation for specific functions.

Flagellum structure

Flagella of Gram-positive and Gram-negative bacteria



Laurent,2016

Flagellum structure

Flagella of Gram-positive and Gram-negative bacteria

- **Basal body:** The basal body is embedded in the cell (cytoplasmic membrane).

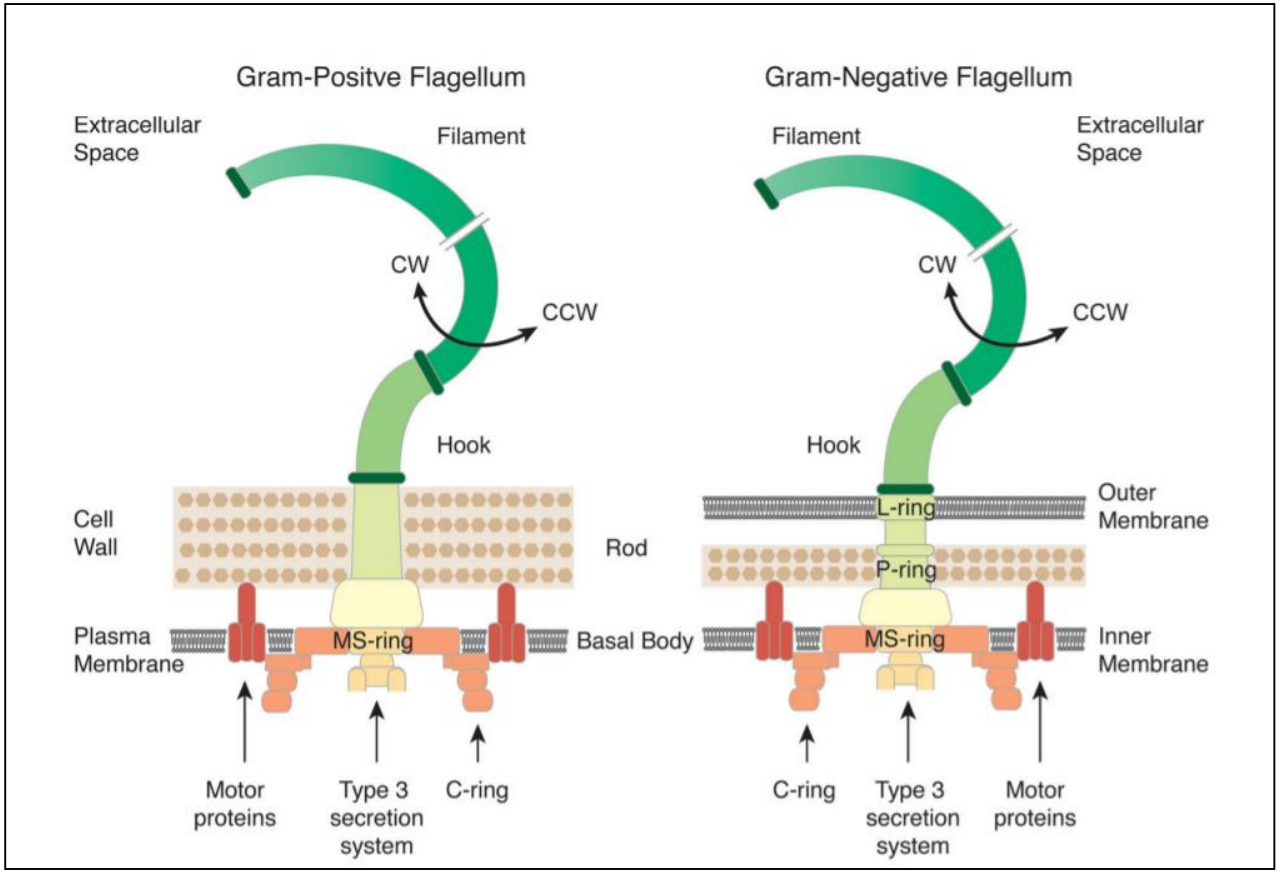
1. In the gram-negative bacteria, the basal body has four rings connected to a central rod (L, P, S and M).
2. Gram-positive bacteria have only three basal body rings (P, S and M).

- The C ring (switch) bound to MS ring. It is common for both bacteria.

Flagellum structure

Flagella of Gram-positive and Gram-negative bacteria

Gram-positive bacteria lack L-ring

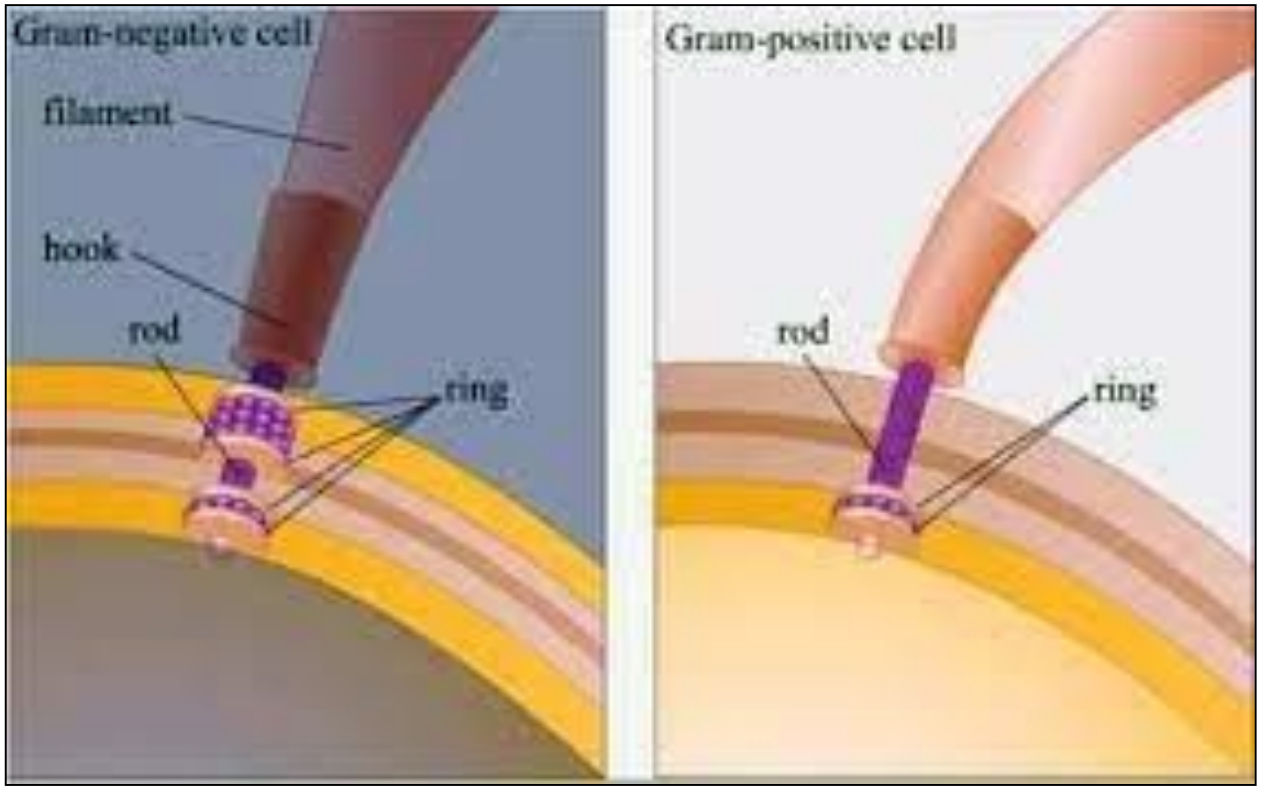


chuhmacher *et al.*,2015

Flagellum structure

Flagella of Gram-positive and Gram-negative bacteria

Gram-positive bacteria lack L-ring



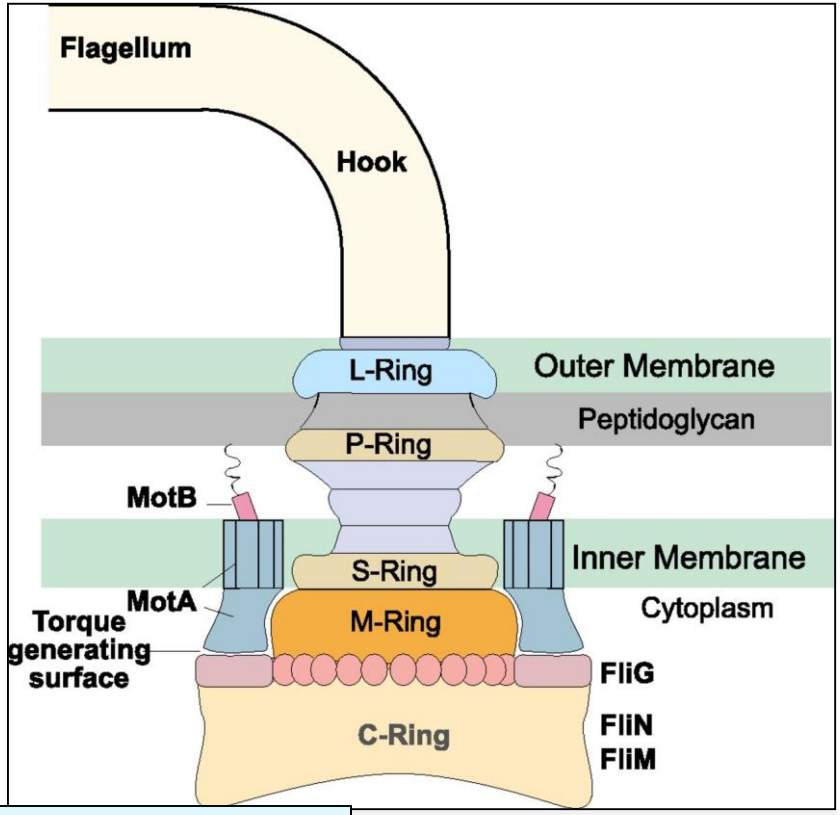
Flagellum structure
Basal body and ring systems

- The C ring (switch) bound to MS ring. The C ring is a cup-shaped structure attached to the cytoplasmic side of the basal body and works as the rotor of the motor and as a part of the secretion apparatus.
- Motor itself consist of stator and rotor known as Mot complex.
- Mot complex (a pair of proteins) is involved in driving rotatory motion of the basal body.
- A set of proteins present between MS rings known as Fli proteins those act as molecular switches and are responsible for changing direction of rotation.

Flagellum structure

The M, S, and C rings of the basal body are together called the rotor

- The C ring (switch) is bound to MS ring.
- The C ring is a cup-shaped structure attached to the cytoplasmic side of the basal body and works as the rotor of the motor and as a part of the secretion apparatus.
- Flagellar rotor is the only **circular rotor** found in nature, aside from human artifact.



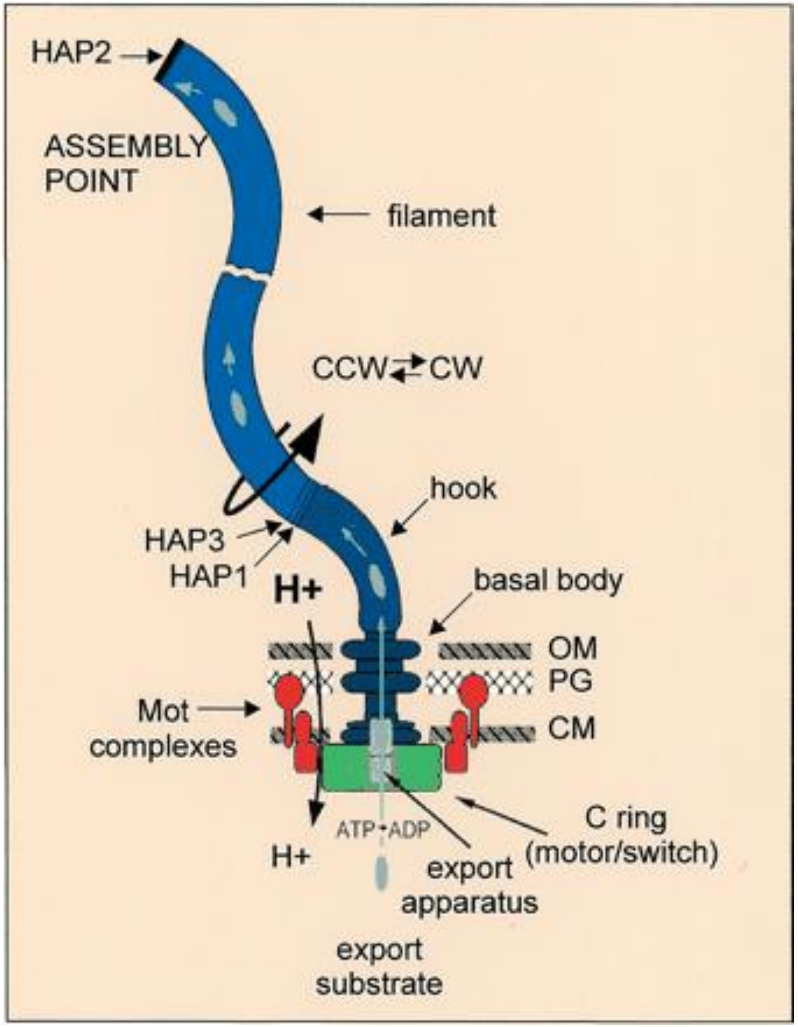
Mot complex: A pair of proteins driving rotatory motion of the basal body.

Fli proteins: Molecular switches responsible for changing direction of rotation.

Flagellum structure

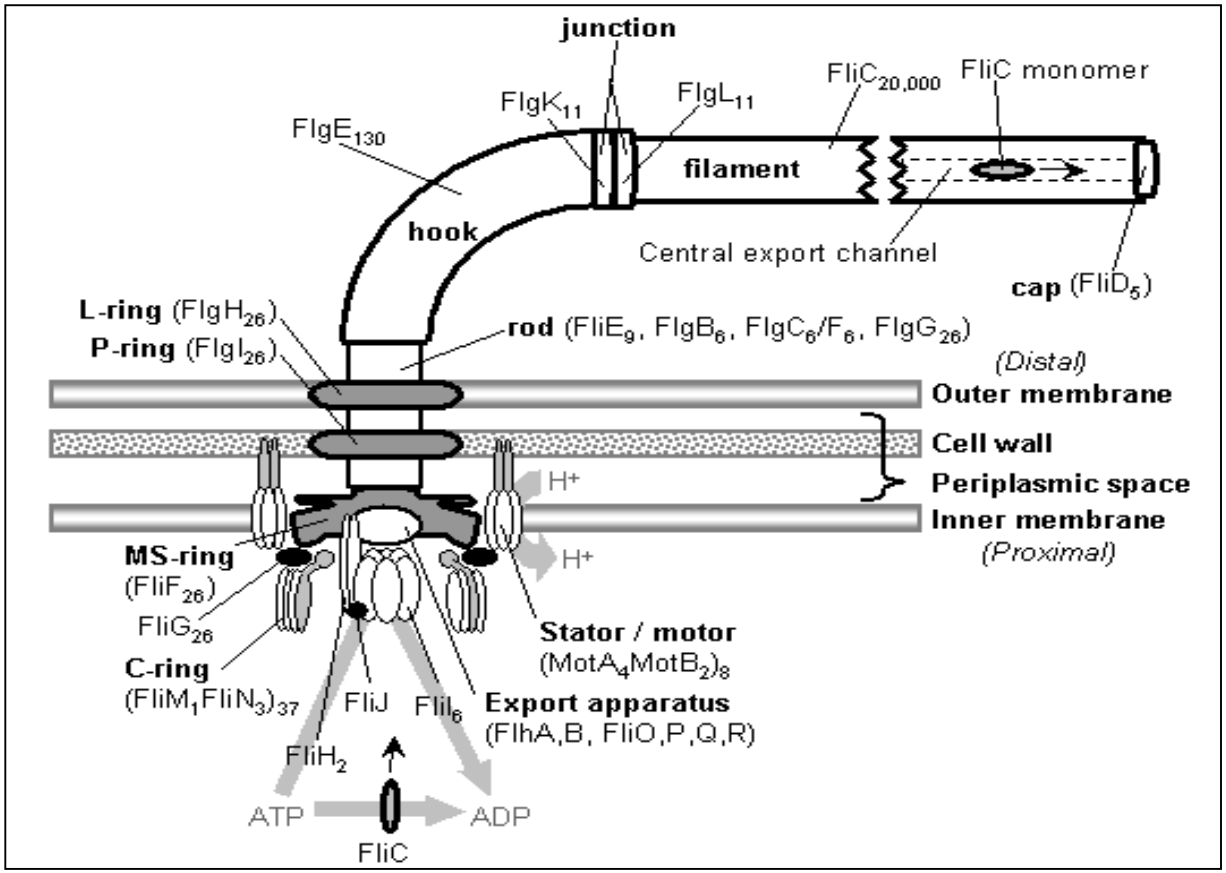
Basal body and ring systems

Gram-negative bacteria



Flagellum fine structure

About 40 genes required for the flagellar assembly are ordered in a hierarchical manner at the transcriptional level



Flagellins are immunogenic and constitute a group of protein antigens called the H antigens, which are characteristic of a given species, strain, or variant of an organism.

Some of the genes and gene products involves in the assembly of the flagellum.

- Non-motile mutants can be constructed by disrupting the:

1. fliC (encoding the subunit of the flagellar filament), and
2. fliM (encoding the flagellar motor switch protein) genes,

- reducing the virulence of mutants compared to the wild-type strain(Gonzalez,2010).

Gene (product)	*Function of gene product*
Class I genes	
flhC (FlhC)	Transcriptional activation of class II genes
flhD (FlhD)	Transcriptional activation of class II genes
Class II genes	
flgB (FlgB)	Rod (proximal)
flgC (FlgC)	Rod (proximal)
flgE (FlgE)	Hook
flgF (FlgF)	Rod (proximal)
flgG (FlgG)	Rod (distal)
flgH (FlgH)	L ring
flgI (Flgl)	P ring
flgK (FlgK)	Hook (distal end)
flgL (FlgL)	Hook (distal end)
flgM (FlgM)	Anti-sigma factor (delays FliA activity)
flhA (FlhA)	Export apparatus
flhB (FlhB)	Export apparatus
fliA (FliA)	Sigma factor (σ^{28}) for class III genes
fliD (FliD)	Filament cap
fliF (FliF)	MS ring
fliG (FliG)	Switch/C ring?/torque generation
fliH (FliH)	Export apparatus
fliI (FliI)	Export apparatus
fliJ (FliJ)	Export apparatus/chaperone
fliK (FliK)	Regulation of hook length
fliM (FliM)	C ring/ switch
fliN (FliN)	C ring/switch
fliO (FliO)	Export apparatus
fliP (FliP)	Export apparatus
fliQ (FliQ)	Export apparatus
fliR (FliR)	Export apparatus
Class III genes	
fliC (FliC)	Flagellin (protein subunit of filament)
motA (MotA)	Torque generation
motB (MotB)	Torque generation

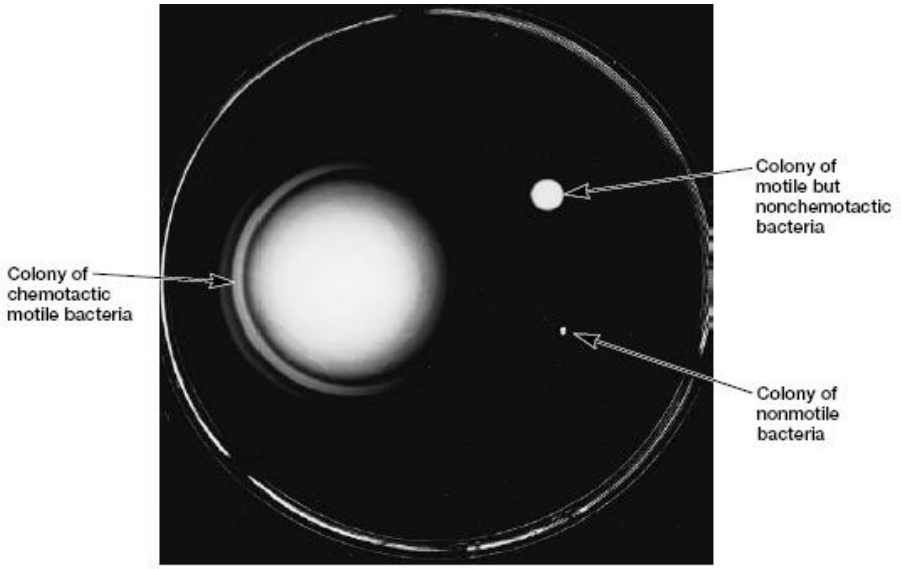
Bacterial movement
Chemotaxis

- Rotation propels bacterium through environment.
- Rotation can be clockwise or counterclockwise; reversible
- Bacteria move in response to stimuli (taxis).
  - Runs – movements of cell in single direction for some time due to counterclockwise flagellar rotation; increase with favorable stimuli (positive chemotaxis, positive phototaxis).
  - Tumbles – abrupt, random, changes in direction due to clockwise flagellar rotation; increase with unfavorable stimuli (negative chemotaxis, negative phototaxis).

Bacterial movement

Positive bacterial chemotaxis

- Bacteria do not always move aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products.
- Chemotaxis can be demonstrated on an agar plate that contains various nutrients.
- Positive chemotaxis by *E. coli* on the left.
- The outer ring is composed of bacteria consuming serine.
- The second ring was formed by *E. coli* consuming aspartate, a less powerful attractant.
- The upper right colony is composed of motile, but nonchemotactic mutants.
- The bottom right colony is formed by nonmotile bacteria.



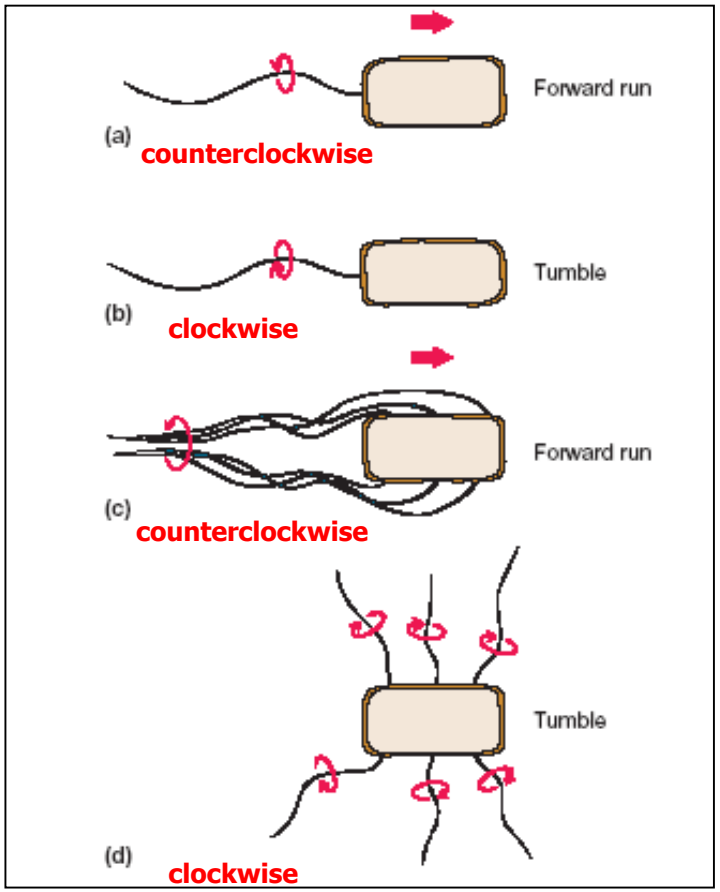
Prescott,2006

Bacterial movement
Chemotaxis

1. Movement involves runs and tumbles (twiddles).
2. When flagella rotate ccw this creates a force pushing on the bacteria this causes the bacteria to move in a new directions, called a run(or swim).
3. When flagella rotate cw, they all pull on the microbe. While all these forces pulling in different directions, it causes the bacteria to tumble (or twiddle, a little net displacement). In rotate clockwise, they cause a tumble.
4. When the twiddling is over, the bacteria will start out a new run in a completely random direction.

Flagellar motility
Direction of rotation

- In monotrichous, polar bacteria (Parts a and b): Reversible (can rotate CCW & CW) flagella can go forward or backward.
- In peritrichous bacteria(Parts c and d):
- Counterclockwise (CCW) rotation results in forward motion.
- Clockwise (CW) rotation causes dell to tumble.
- When the motors of peritrichous cells turn counterclockwise (CCW), their filaments form bundles that drive the cells forward.



Prescott,2006; Tans-Kersten *et al.*,2001;..

Bacterial motility

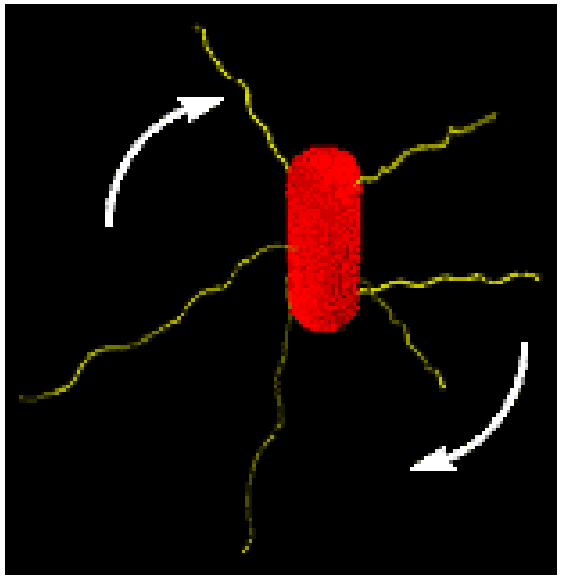
Filament capable of rotating 360º

- The flagellum is a rigid structure and rotates like a propeller.
- Rings in the basal body rotate relative to each other causing the flagella to turn.
- **Flagella can rotate:**

1. **Clockwise (CW):** In peritrichous cells, flagella then become limp, cell tumbles or twiddle.
2. **Counterclockwise (CCW):** Flagellar bundle then becomes rigid, cell runs.

- Rotor is always spinning one direction or other
- Some bacteria are motile w/o flagella.

3. **Gliding motility:** Depends upon contact with a solid surface, it moves slowly across surfaces, involves sulfur-containing lipids.



Bacterial movement

Chemotaxis

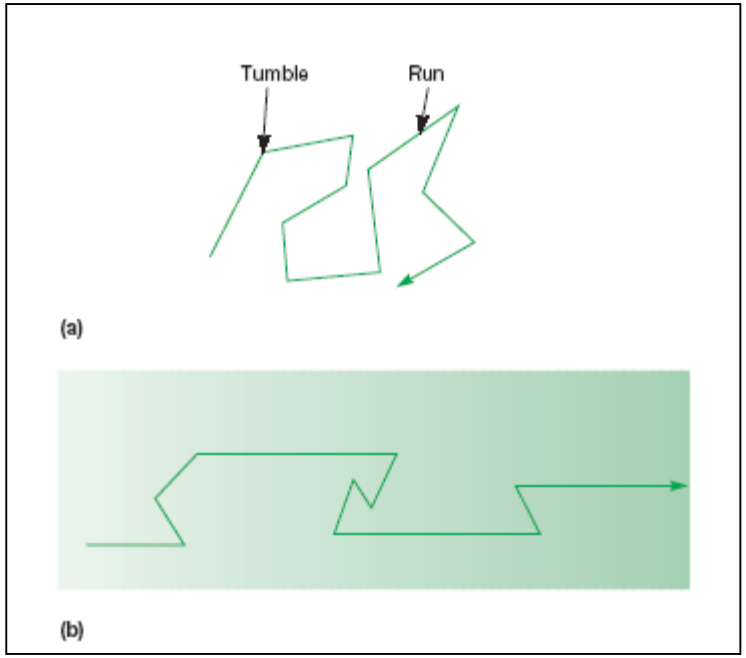
Tumble and run

a) Random movement of a bacterium in the absence of a concentration gradient.

- Tumbling frequency is fairly constant.

b) Movement in an attractant gradient.

- Tumbling frequency is reduced when the bacterium is moving up the gradient.
- Therefore, runs in the direction of increasing attractant are longer.

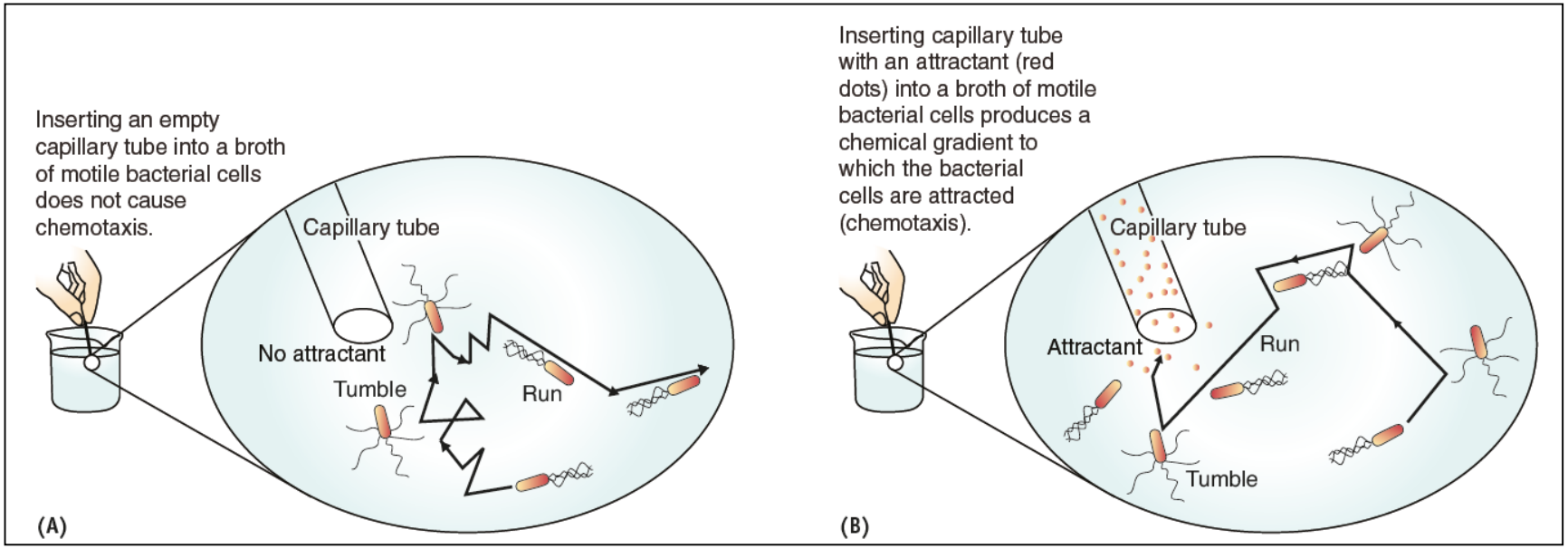


Prescott,2006

Bacterial movement

Chemotaxis

Tumble and run

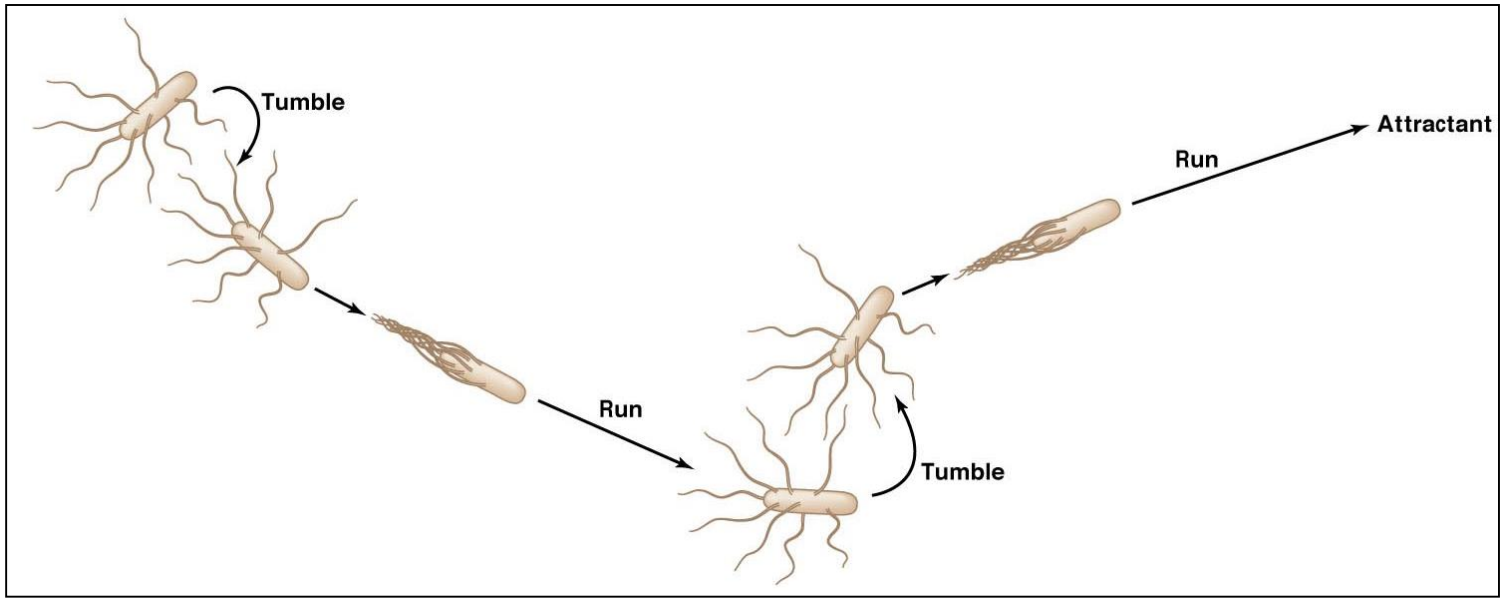


Jones and Bartlett publishers

Bacterial movement

Chemotaxis

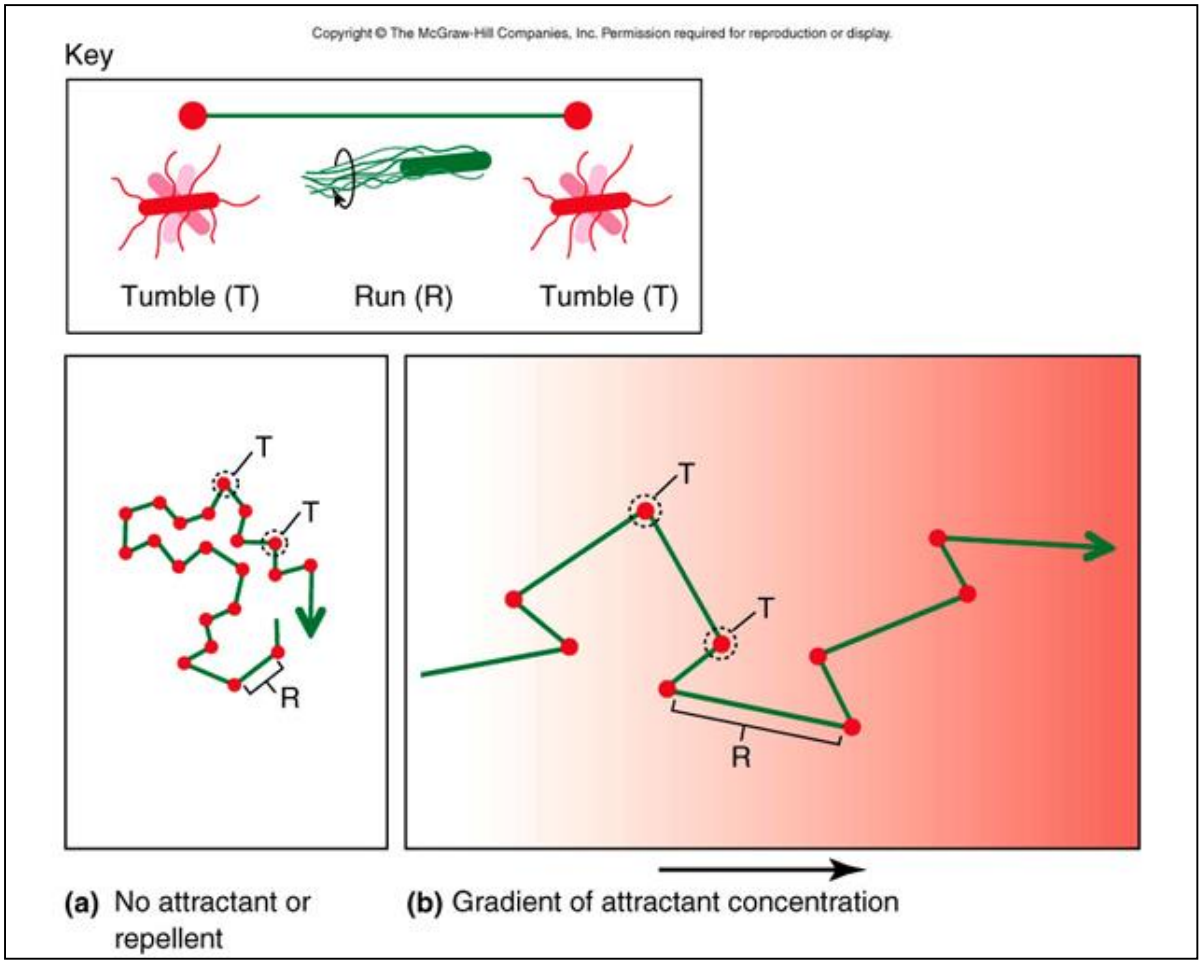
Tumble and run



Bacterial movement

Chemotaxis

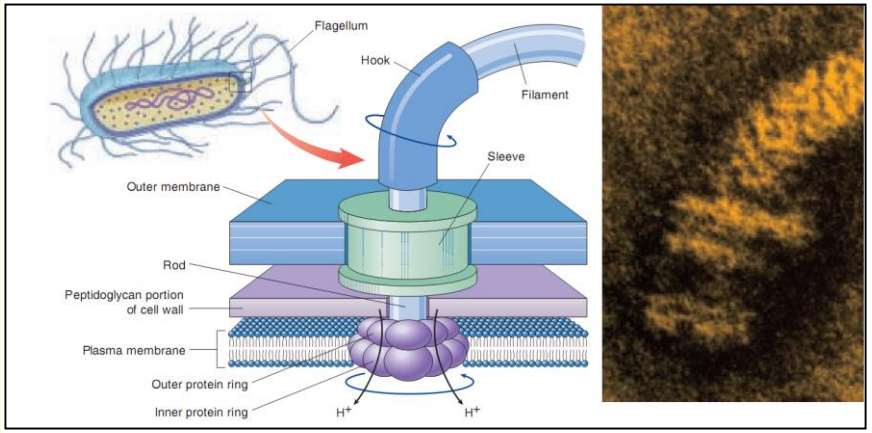
Tumble and run



Seelke,2010

Flagellar motility
Direction of rotation during run

- The shaft rotates when the inner protein ring attached to the shaft turns with respect to the outer ring fixed to the cell wall.
- The inner ring is an H ion channel, a proton pump that uses the passage of protons into the cell to power the movement of the inner ring past the outer one.



Flagellar motility

Direction of rotation during run

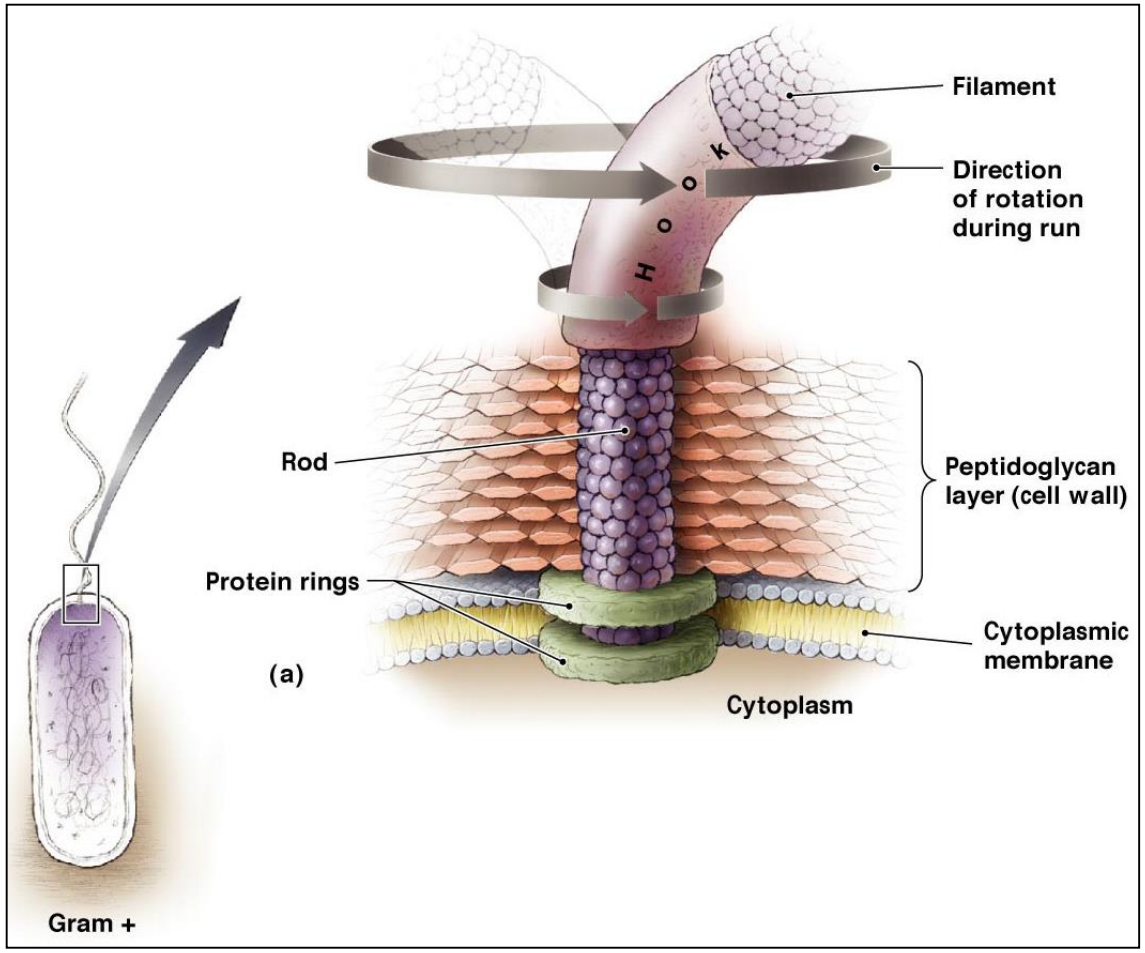
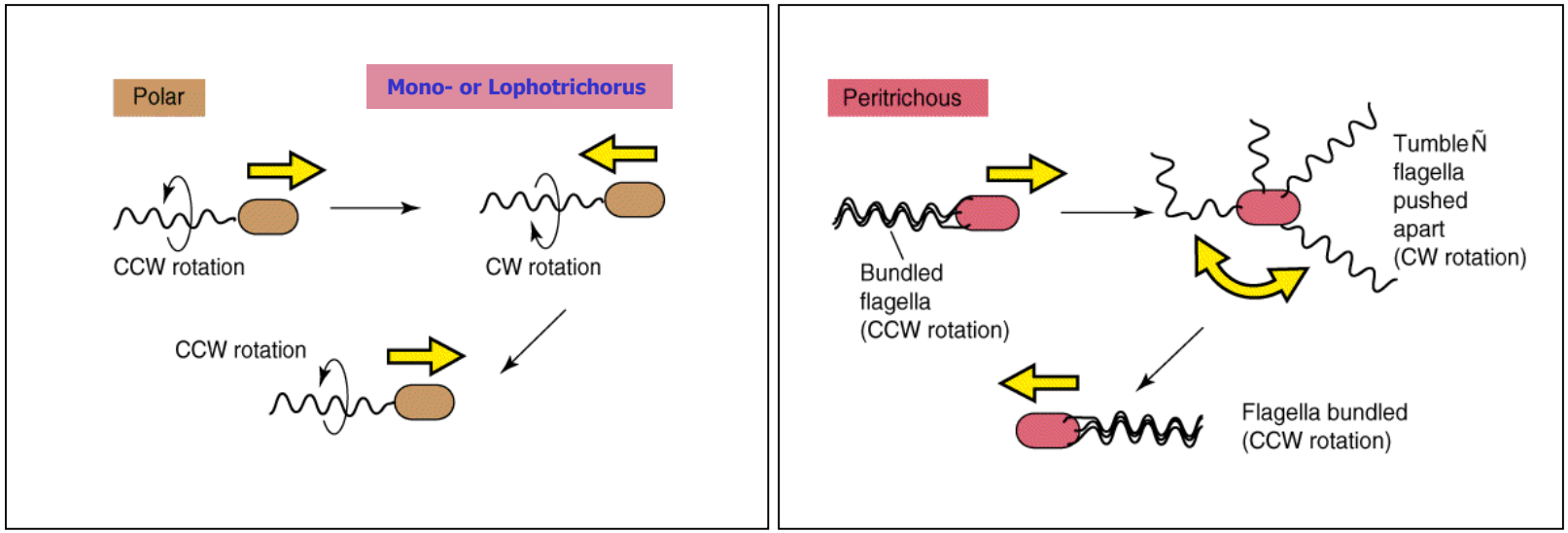


Figure 3.5a

Flagellar motility
Direction of rotation



Clockwise (CW): In peritrichous flagellate cells.
Counterclockwise (CCW): In polar flagellate cells.

Schlink,2010

Mechanism of flagellar rotatory motor and locomotion

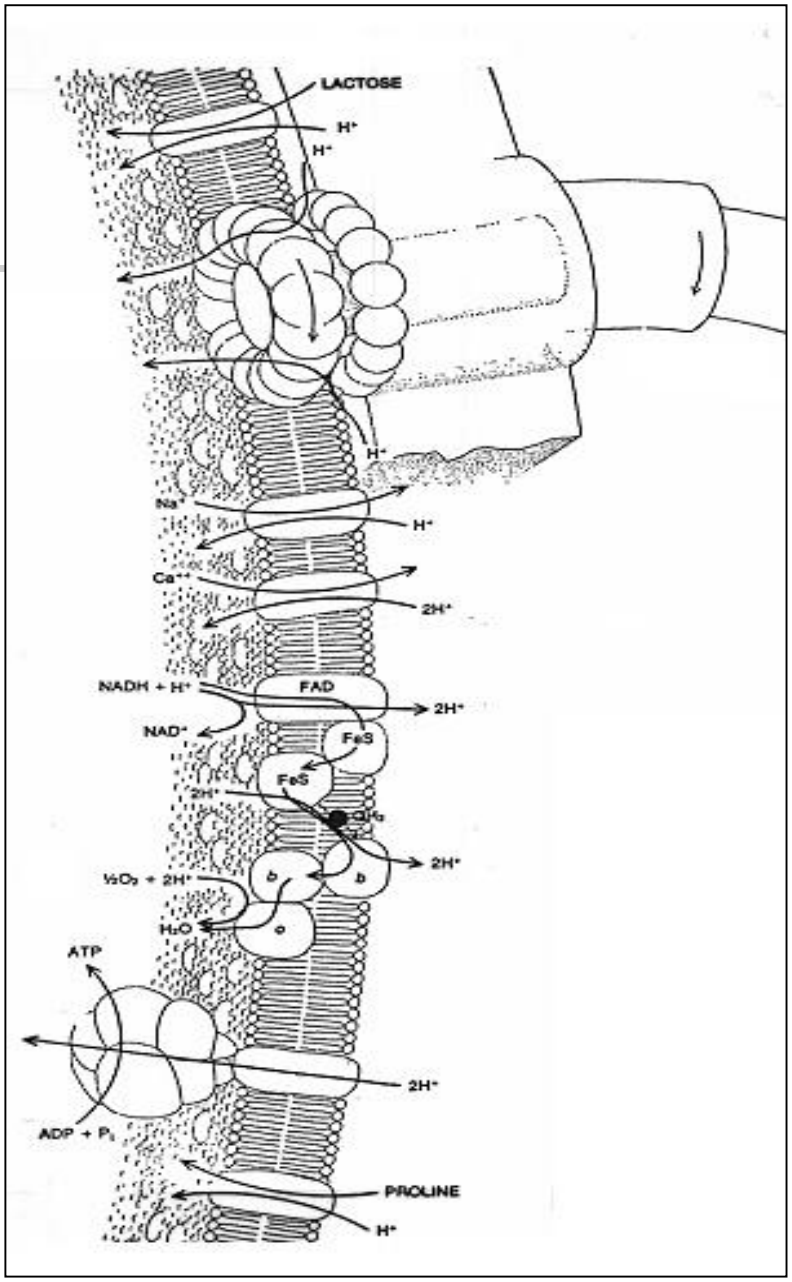
- Each flagellum rotates 360º around a central axis and affects the surrounding medium much as a ship's propeller would.
- The motor situated at the base of the flagellum can speed up, slow down, stop and go into reverse.
- The driving force comes from streams of protons-naked hydrogens stored near the motor and released in volleys by the chemical action of the sensory processing system(e.g. chemotaxis).
- The flagellum's motor is much like that of, say, an electric mixer, but the mixer's is driven by electrons instead of protons.

Mechanism of flagellar rotatory motor and locomotion

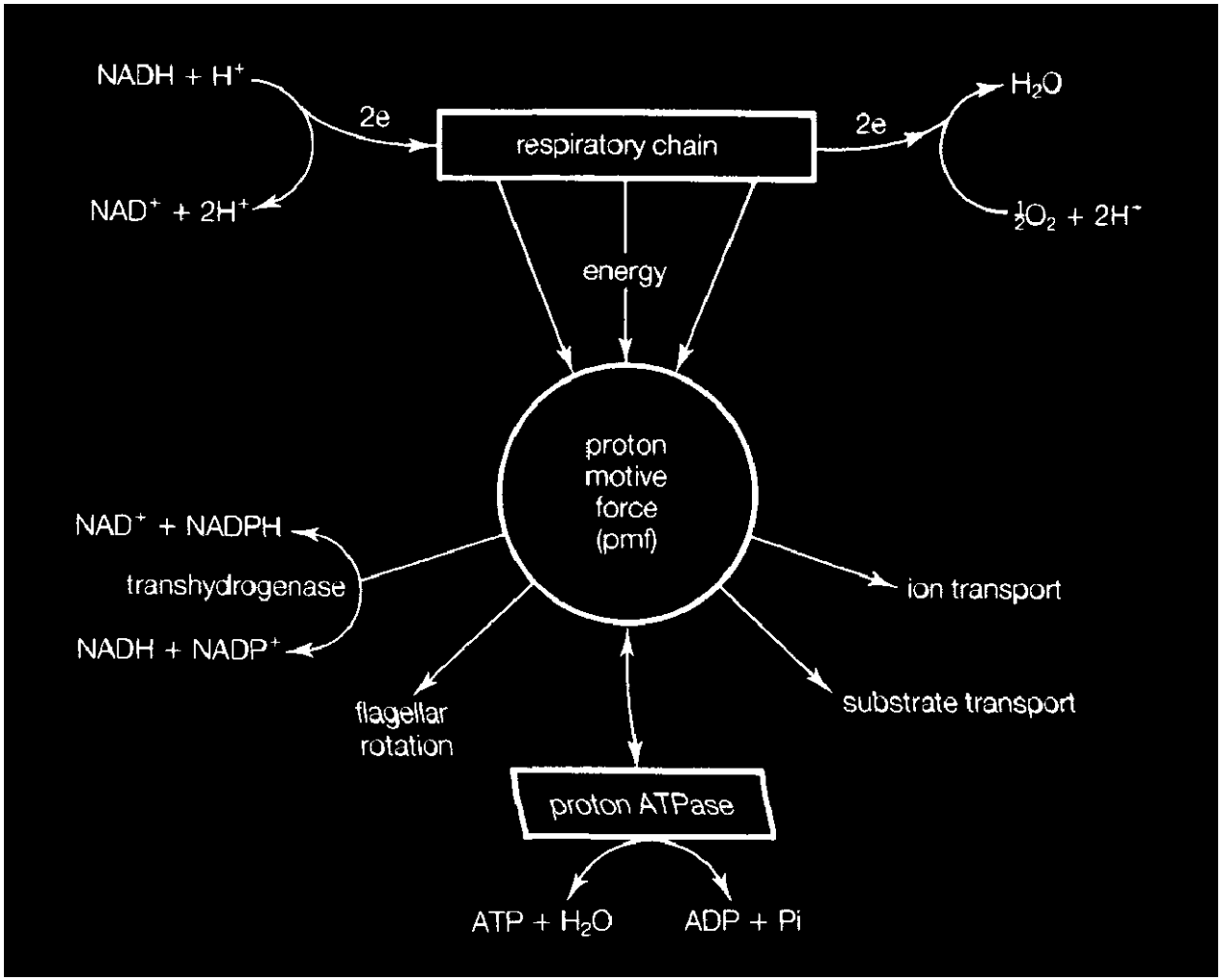
- Flagellum is propeller in action.
- The energy used to drive the flagellar rotation comes from the proton motive force.
- As a proton enters the cell through the mot complex, its energy is coupled to movement.
- In order to achieve a single rotation, 1000 protons must be translocated.
- The speed of rotation is directly proportional to the proton motive force.
- With flagellar activity a bacterium can attain a speed of 100 μ/second.
- The basal body contains the rotary motor, which is powered typically by a proton motive force.
- Basal body rotates typically at 20,000 RPM, but when detatched from the filament can go 100K RPM.

Flagella & Proton motive force

- Rotation of the filament is driven by the diffusion of protons into the cell through the basal apparatus after the protons have been actively transported by proton pumps in the plasma membrane.
- The electron transport system is shown oxidizing NAD by removal of a pair of electrons, passing them through its sequence of carriers eventually to O_2.
- ATPase is the transmembranous protein enzyme which is utilizing protons from the outside to synthesize ATP on the inside of the membrane.



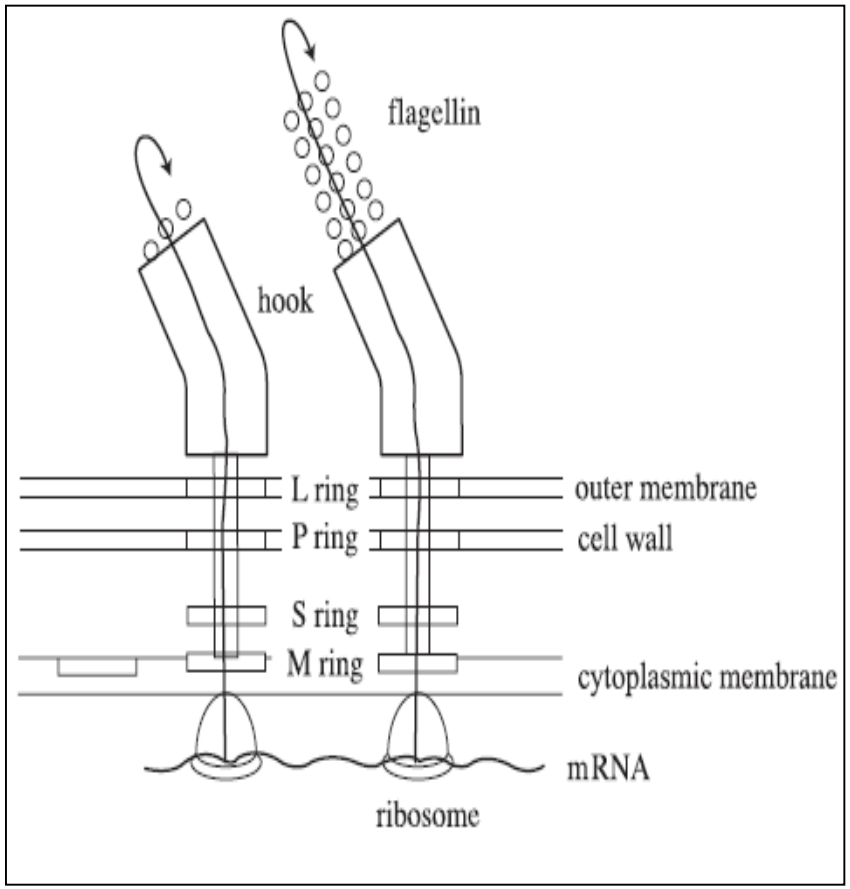
Some of the roles of proton motive force including flagellar rotation



Assembly of cellular structure

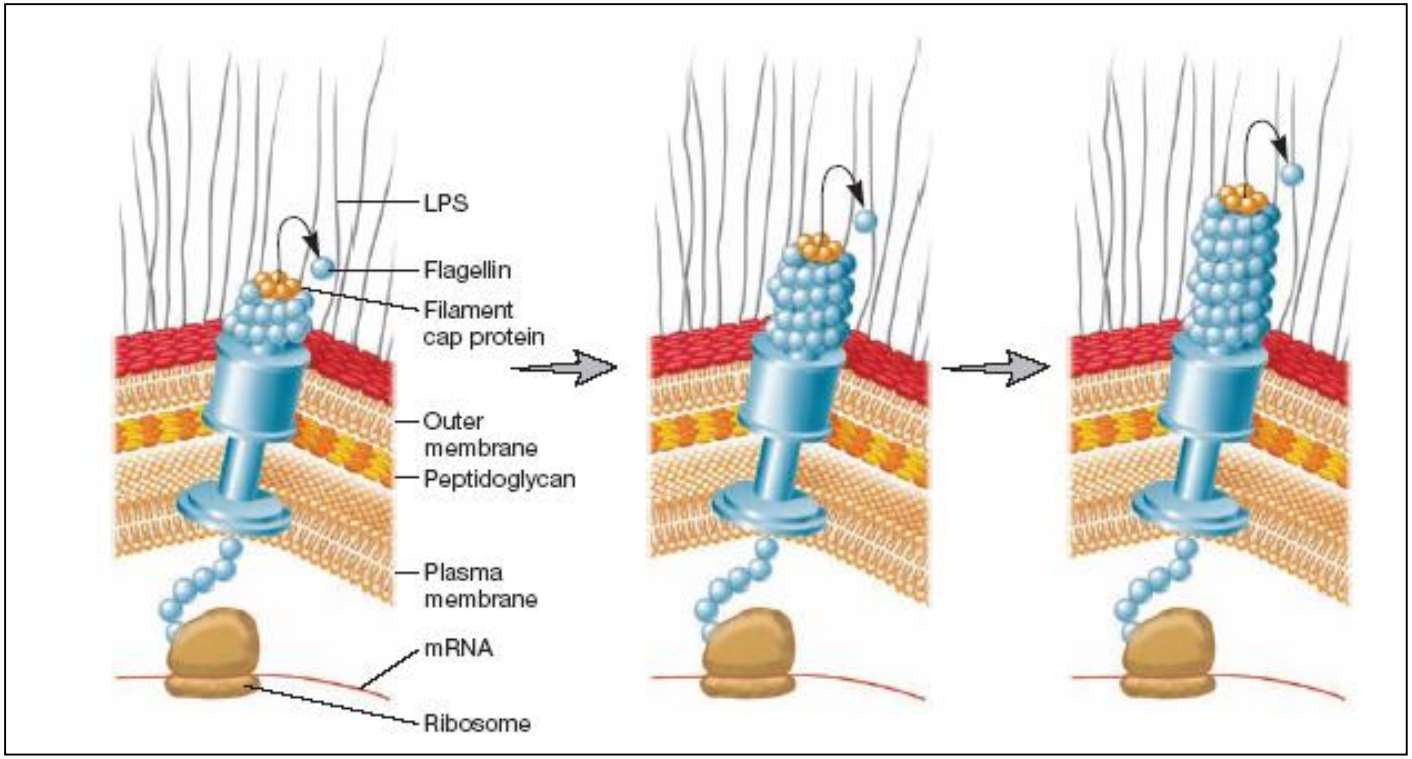
Flagellar filament formation in Gram-negative bacteria

- Proteins constituting the flagellum are not found in the cytoplasm, suggesting that they are exported as soon as they are synthesized.
- The proteins located beyond the cytoplasmic membrane are translocated through the type III flagellar export pathway.



Kim and Gadd,2008

Growth of flagellar filaments



Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

Prescott,2006

Pili (or Fimbriae)vs. flagella

- The terms pili and fimbriae are often used interchangeably.
- These are short, hair-like structures on the surfaces of bacterial cells.
- These are found mostly in male cells.
- Unlike flagella they grow from the inside of the cell outward, and not from the tip of the fiber.

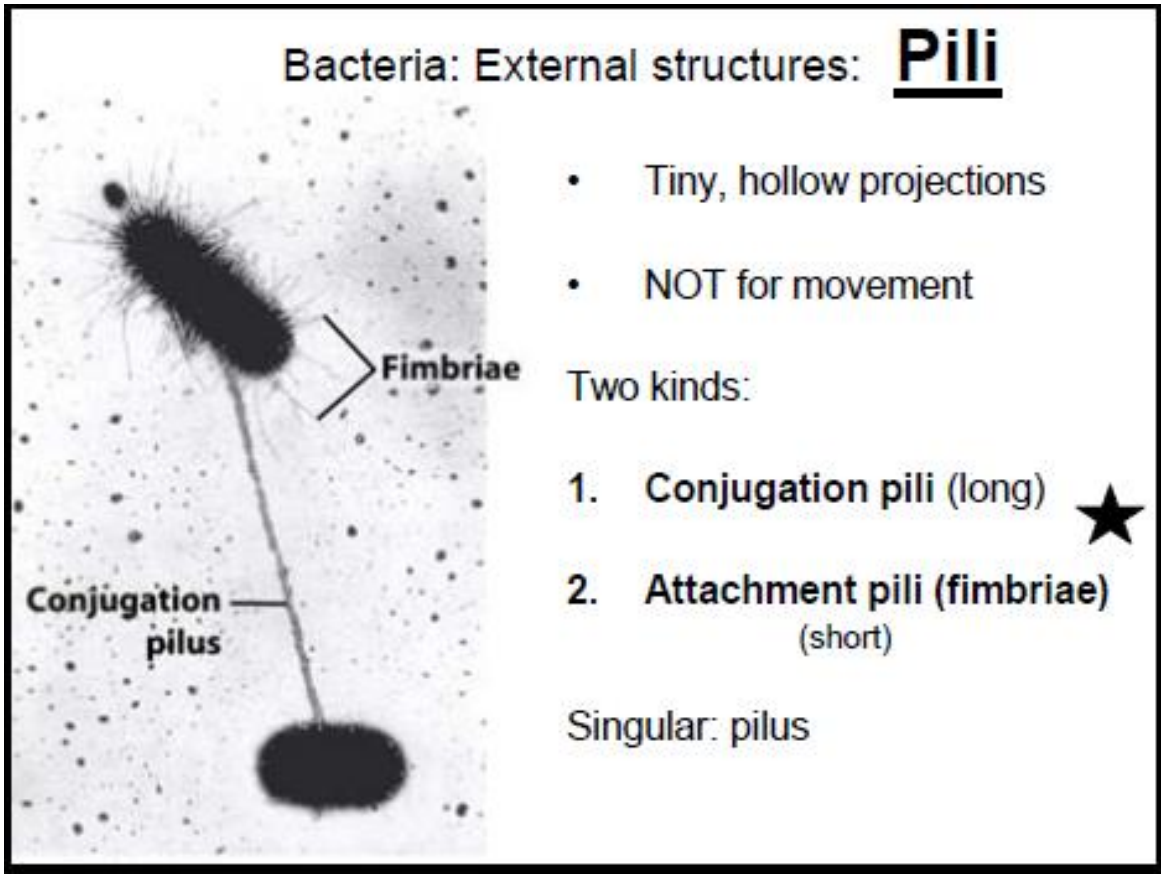
Fimbriae vs. Pili

Structural differences

Pili Made up of a protein called pilins	Fimbriae Made up of a protein called fimbrians
Longer, several μ long, 7.5 -10 nm thick	Shorter
Less in number	More in number. Several hundred pili can extend from the surface of a bacterial cell
Seen on Gram-negative rods.	Fimbriae are found in both Gram-negative and Gram-positive bacteria.
Act as a receptor site of bacteriophages and are encoated by viruses.	Mediate bacterial adhesion to host site.

Fimbriae vs. Pili

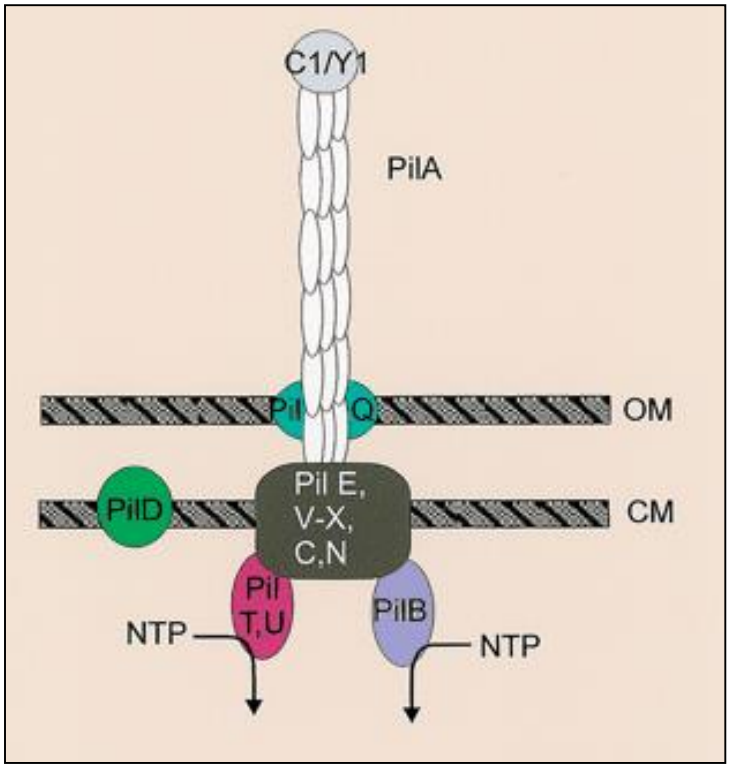
Structural differences



Rogers,2006

Pilin
pil gene cluster

- Long hollow tubules composed of pilin.
- A pilus is typically 6 to 7 nm in diameter.
- Pili can be encoded by chromosomal genes or plasmid genes.

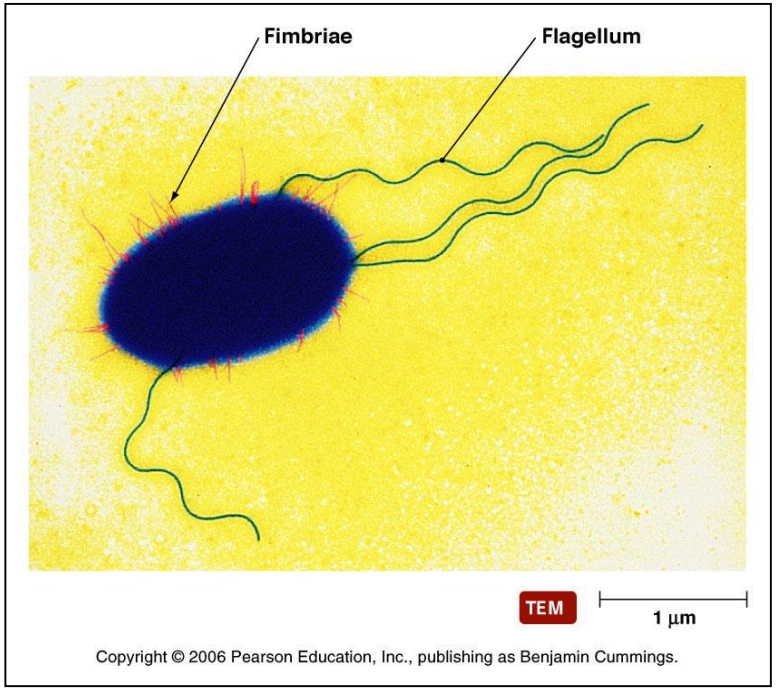


Fimbriae: A common pili

Non-motile extensions

Made up of a protein called fimbrians. Some fimbriae can contain lectin proteins generally at their tips

- Originate in the plasma membrane and protrude through the cell wall.
- Fimbriae are shorter than pili and serve to allow bacteria to attach to various surfaces.
- Sticky, proteinaceous, bristlelike projections.
  - Used by bacteria to adhere to one another, to hosts, and to substances in environment.
  - May be hundreds per cell and are shorter than flagella.
  - Serve an important function in biofilms.

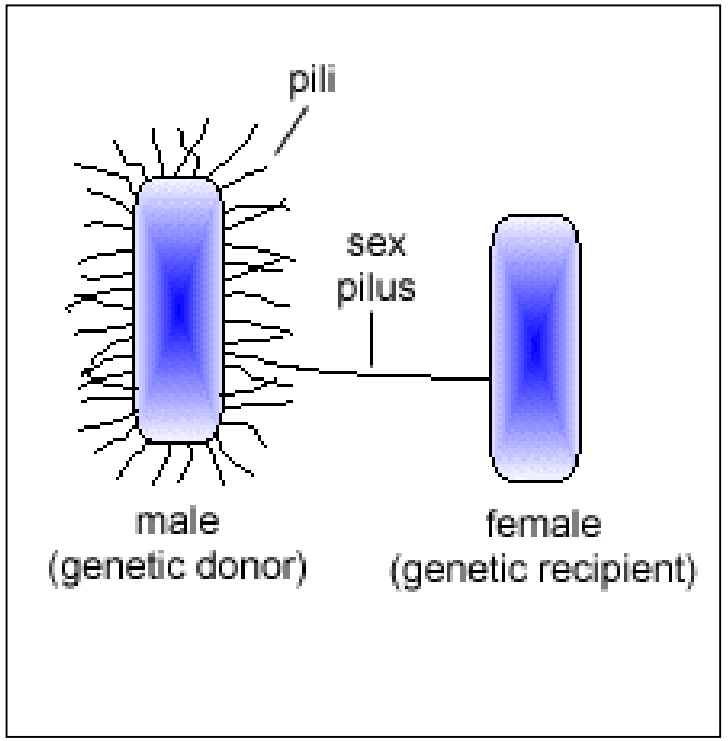


The pili

Made up of a protein called pilins

Found mostly in Gram negative bacteria

- Longer than fimbriae but shorter than flagella.
- Bacteria typically only have one or two per cell.
- There are a variety of different types of pili that differ in structure and function.
- Special pili (sex pili) involved in transfer of DNA from one cell to another (conjugation).
- The fertility factor(F^+) is required to produce sex pili.



Kaiser,1998

Types and functions of pili
Multifunctional organelles

- Numerous different types of pili have been characterized, and various forms of these appendages are involved in diverse activities of bacteria.
- These include:

1. Bacterial cell aggregation;
2. Adhesion to surfaces of host cells;
3. Adhesion to other microbial cells in biofilm;
4. Gene and protein injection into other cells;
5. DNA uptake by naturally transformable bacteria, and
6. Virulence attributes of pathogenic bacteria.

Ramey *et al.*,2008

Types and functions of pili

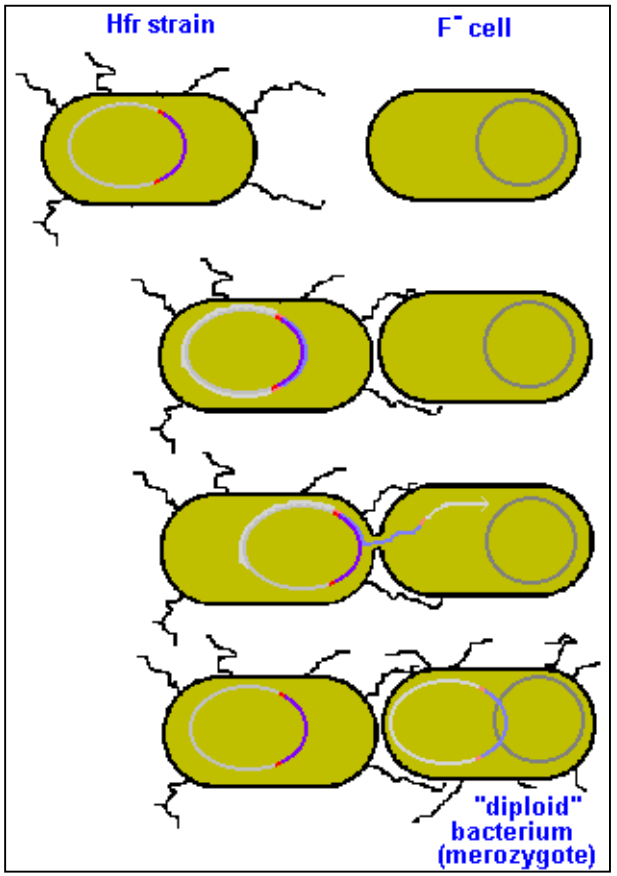
Conjugatively active pili

- There are two main types of such conjugatively active pili:

1. F-type sex pili- A long and flexible pili;
2. P-type sex pili- Shorter than the F-pili and rigid.

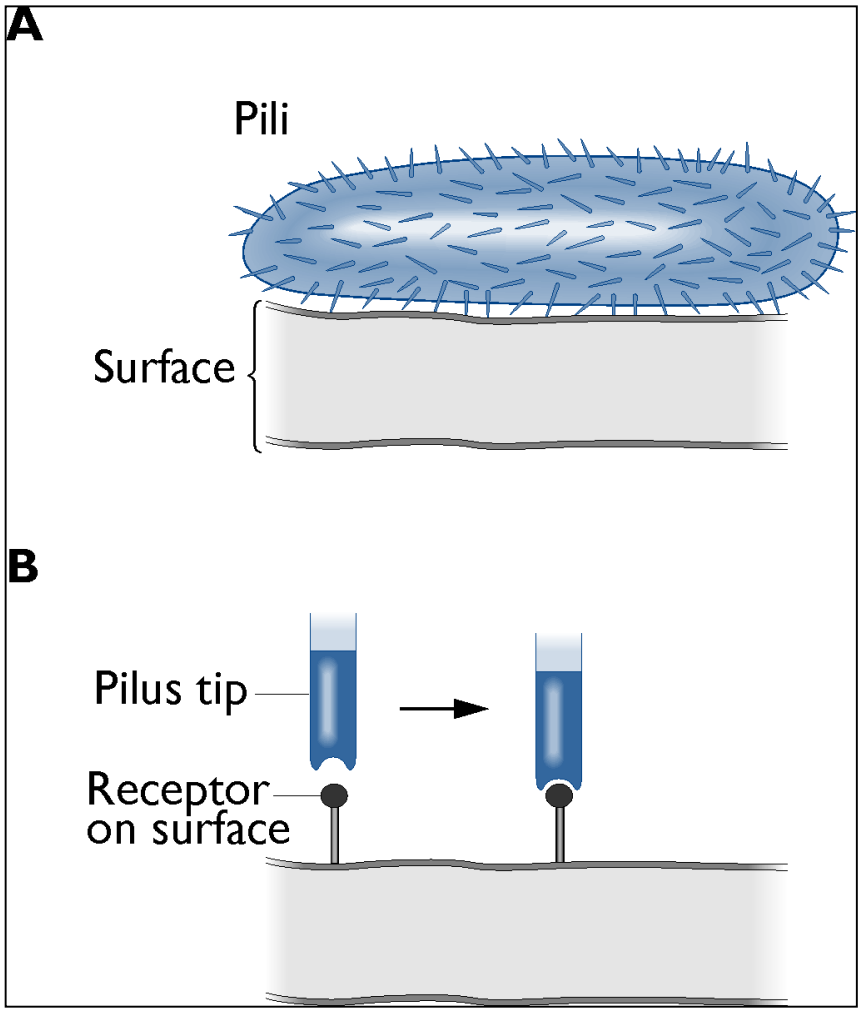
Sex pili or F^+ pili

- Conjugation is driven by a plasmid carrying the gene for fertility factor (F) also called sex factor.
- Cells carrying the F plasmid are called "F pilus" (F^+) while those without this plasmid are called "F minus" (F^-).
- The F^+cell uses its pili to attach to an F^- cell then replicates its F plasmid DNA.
- The F plasmid is not transferred from F^+ to F^+.



Attachment Pili

Can be an important virulence factor a feature of the organism that enhances its ability to cause disease



IV pili

Twitching motility and Biofilm formation

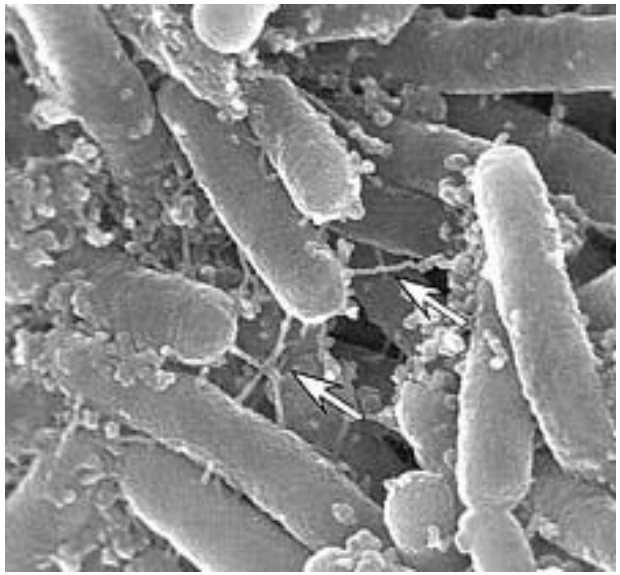
Bacterium crawling on a solid surface

- The Type IV pili are architectural marvels of biology.
- Type IV pili are remarkable multifunctional organelles expressed by diverse pathogenic bacteria.
- Twitching motility via type IV pili has been observed in a number of gram-negative bacteria.
- Type IV pili are essential for host colonization and virulence for many Gram negative bacteria, and may also play a role in pathogenesis for some Gram positive bacteria.
- Bacterial type IV pilins are similar in structure to the component flagellins of Archaeal flagella.

Ramey *et al.*,2008; Wikipedia,2010

Type I and type IV pili
Twitching motility and Biofilm formation
Xylella fastidiosa

- *Xylella fastidiosa* is a gram-negative, nonflagellated bacterium.
- Recently terminal fimbriae (or pili) was reported (arrows).
- Terminal fimbriae (also called type IV pili) are important for biofilm formation and a type of incremental movement called "twitching motility".
- According Fuente *et al.*,2007, *Xylella fastidiosa* possesses both type I and type IV pili at the same cell pole.
- Mutations in Type I and Type IV Pilus biosynthetic genes affect twitching motility rates in *Xylella fastidiosa*.



Gould and Lashomb,2005

Type I and type IV pili
Twitching motility and Biofilm formation
Xylella fastidiosa

- Particularly interesting is the fact that *X. fastidiosa* is the only bacterial species, to our knowledge, that possesses both types of pili at the same cell pole:

1. Type IV pili (1.0 to 5.8 μm in length), and
2. Type I pili (0.4 to 1.0 μm in length).

- This dual pilus configuration may confer advantages related to cell motility and biofilm development within the confines of xylem elements.
- Among these, the putative type IV pilus protein PilY1 is likely important for attachment to surfaces.

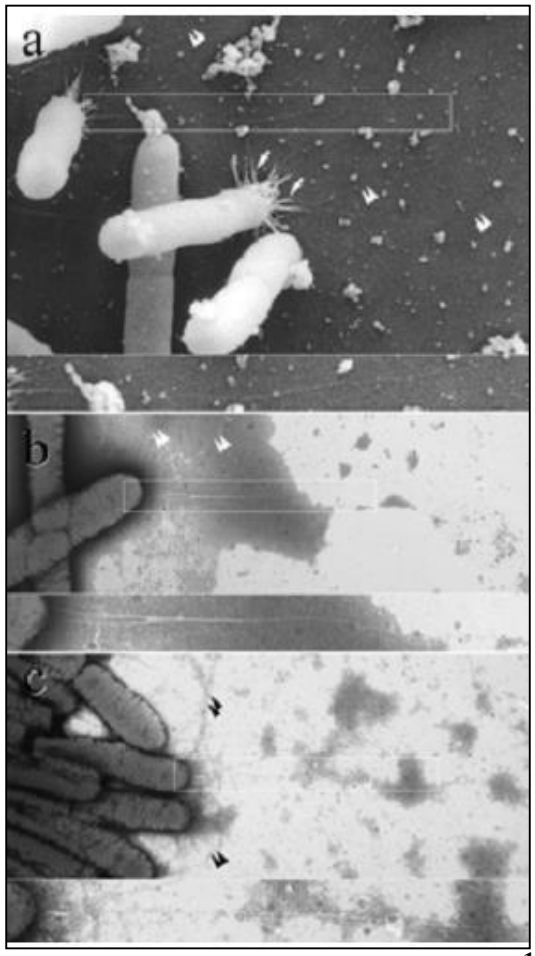
Fuente *et al.*,2007

Type I and type IV pili
Twitching motility and Biofilm formation
Xylella fastidiosa

a) Wild-type cells depicting an abundance of short type I pili at the cell pole in contact with the substratum and fewer long type IV pili.

b, c) Transmission electron microscopy micrographs of *fimA* (A type I pilus mutant (b) and *pilY1* (A type IV pilus mutant cells (c) negatively stained with phosphotungstic acid.

- Only type IV pili are present on the fimA (b) mutant cells, whereas both pilus types are present on the pilY1 (c) mutant cells.



Fuente *et al.*,2007

Pili-like structures
In plant-pathogenic Spiroplasmas

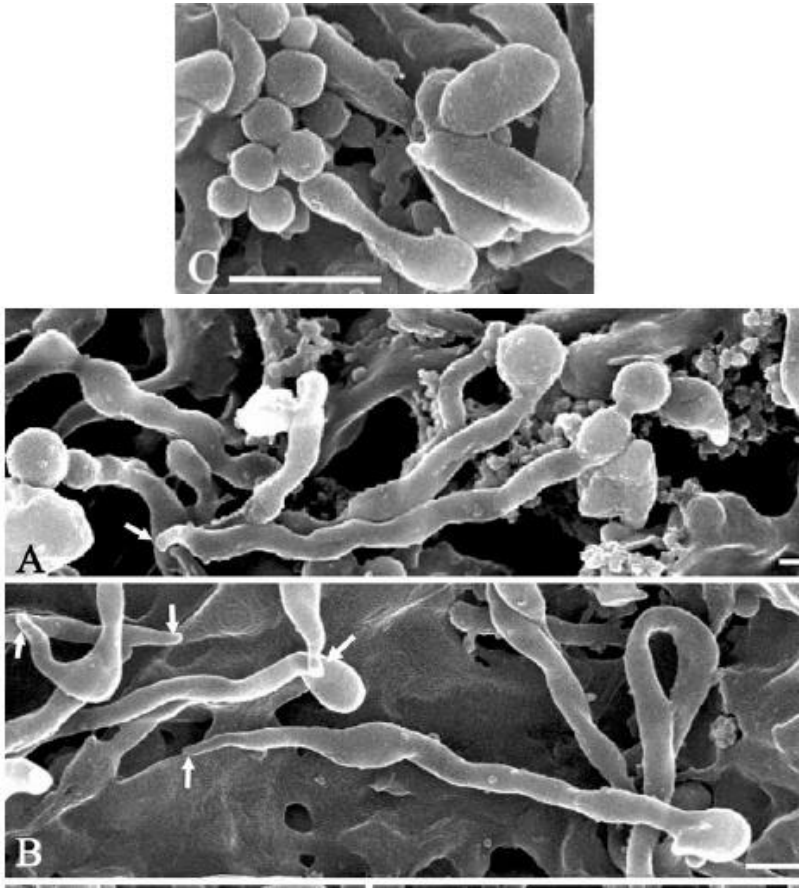
- In phytoplasmas no pili/pili-like structures were observes.
- This is in contrast to spiroplasmas, which have pili-like structures (Ammar *et al.*,2004).
- Scanning electron microscopy (SEM) preparations of cultured:

A. *Spiroplasma kunkelii*

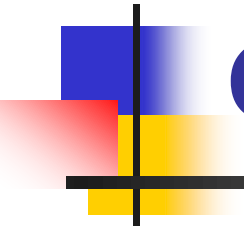
B. *Spiroplasma melliferum*

C. *Spiroplasma citri*

- Note the tip structures (arrows), globular parts of the helix (A, B), a bud-cluster(C).



Ammar *et al.*,2001



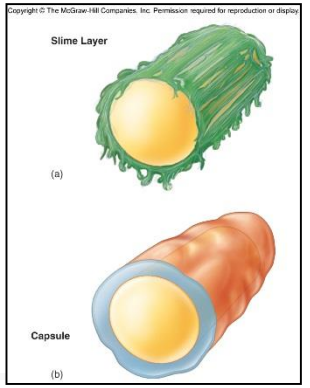
Glycocalyx

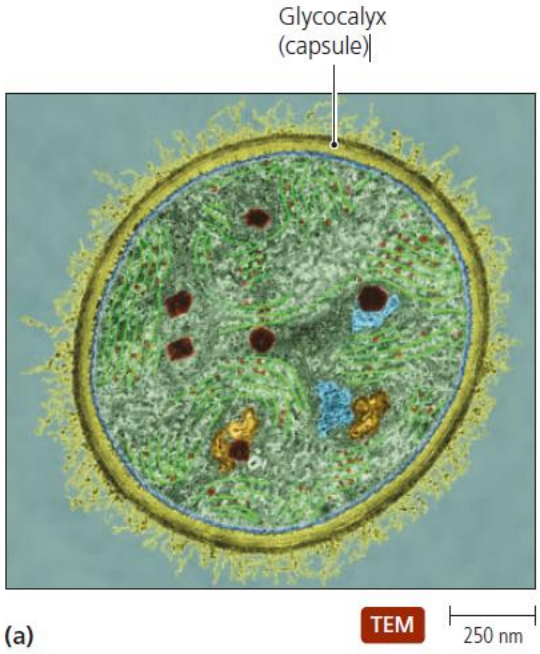
Capsule/S-Layers

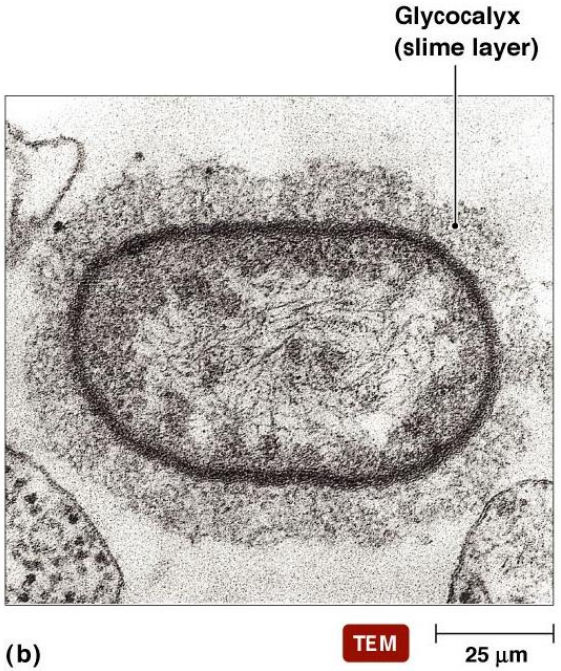
Glycocalyx
Capsule & S-Layers

- Glycocalyces are gelatinous, sticky substance surrounding the outside of most prokaryotic cells.
- These are composed of polysaccharides, polypeptides, or both.
- The term glycocalyx can be used to describe extracellular structures including the capsule and S-layer.
- Based on the strength of bonding between cell wall and glycocalyx it can be further classified as:
  - Capsule
  - Slime layer
- In some cases the polymers are tightly integrated with the cell while in others they are loosely associated.
- The former is called a capsule, and the latter a slime layer.

Glycocalyx
Capsule & S-Layers







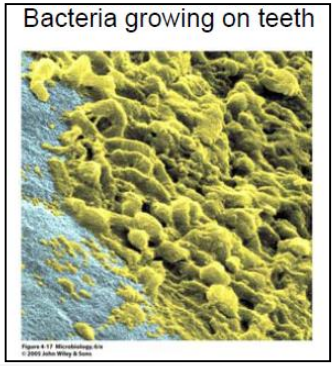
Firmly attached to cell surface.
Most bacteria including plant pathogenic bacteria have capsule.

Loosely attached to cell surface.
All bacteria do not have slime layer.
Bacteria like *Pseudomonas* and *Streptococcus* have slime layer.

Bio732 chapter 3;..

Function of glycocalyx

Capsule/S-Layers

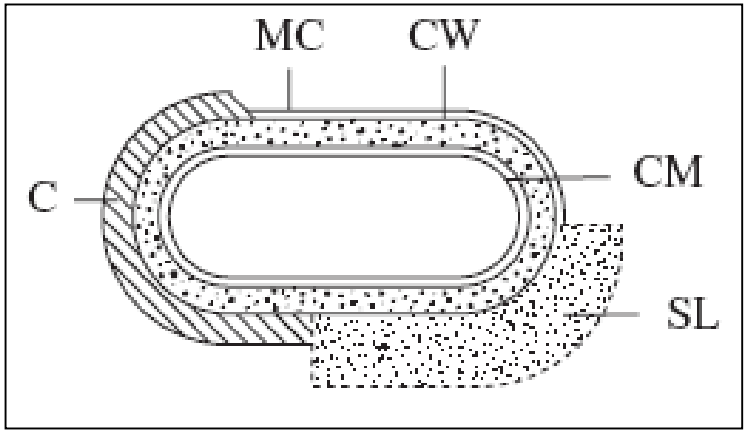


- It is involved in bacterial adhesion, a gelatinous layer called as "Biofilm".
- Glycocalyx is composed of 90% of water and protect bacteria from dessication or dehydration or drying up by acting as osmotic barrier and defensive buffer and may contain antigenic sites.
- Capsulated bacteria are more resistant to phagocytosis (antiphagocytic).
- *Pseudomonas* and *Streptococcus* have slime layer.
- *Streptococcus mutans* via capsule/slime attaches to cell surface protein of pellicle of teeth and produce lactic acid that causes decalcification of teeth.

Glycocalyx
Capsule and S-Layers

- S-layer is a surface protein layer found in many different bacteria and in some archaea where it serves as the cell wall.
- C, capsule;
- CM, cytoplasmic membrane;
- CW, cell wall;
- MC, micro-capsule;
- SL, slime layer.
- The s-layer is directly attached to the outer membrane, rather than the peptidoglycan.
- Capsule & slime layers help in:

1. Adhesion to host cells for invasion or to a solid surface,
2. To initiate and stabilize biofilm formation.



Kim and Gadd 2008

S-Layer

A surface protein layer

Glycoproteins

- The S-layer is directly attached to the outer membrane, rather than the peptidoglycan.
- S-layer is a surface protein layer found in many different bacteria and in some archaea where it serves as the cell wall.
- It is somewhat looser structures, more easily deformed layer.
- All S-layers are made up of a two-dimensional array of proteins and have a crystalline appearance, the symmetry of which differs between species.
- In certain bacteria the slime layer that surrounds the outermost components of cell walls are made up of glycoproteins of high molecular weight.
- Glycoprotein is a compound in which carbohydrate (sugar) is covalently linked to protein.

S-Layer

A surface protein layer

Glycoproteins

- Glycoproteins are ubiquitous in nature, although they are relatively rare in bacteria.
- In addition to forming these s-layers, glycoproteins also function as bacterial flagella.
- These are made up of bundles of glycoproteins protruding from the cell's surface.
- Their rotation provides propulsion.
- In plants, glycoproteins have roles in cell wall formation, tissue differentiation, embryogenesis, and sexual adhesion (certain algal species).

S-Layer
Functions

1. Slime layer as an adhesin is involved in attachment of bacteria(including pathogens) to other cells or environmental surfaces in order to colonize and form biofilms.
2. It may contribute to virulence by protecting the bacterium against complement attack and phagocytosis.
3. The S-layer may protect bacteria from harmful enzymes or changes in pH.
4. It protects bacteria from desiccation.
5. Slime layers can also be used as a food reserve for the cell.
6. It also allows dental caries to attach to teeth forming dental plaques.

Capsule
K-antigen

- Extracellular polymers are synthesized by both Gram-positive and Gram-negative bacteria.
- Most plant pathogenic bacteria produce protein capsules.
- Capsule also involved in disease, attachment.
- Capsules provide a protective function to bacterial cells.
- Two other major antigens of the bacterial cell are:

1. The lipopolysaccharide (O-antigen) and,
2. The flagellum (H-antigen).

Capsule
Functions

1. Attachment to host's cell;
2. Prevents ingestion by phagocytes (WBC) allowing bacteria to evade destruction;
3. Virulence – associated with pathogen ability to cause disease;
4. Increased virulence may occur in pathogen if they have structures that allow them to overcome host immune defenses such as having a capsule;
5. Prevents drying.

Capsules

Chemical composition of some bacterial capsules

- Capsule is of 3 types as made of:

1. Polysaccharide, or
2. Amino sugars, or
3. Poly alcohols

The amount of capsule produced by a cell depends on the culture conditions. Growth in high carbon, low nitrogen medium promote capsule formation.

Chemical composition of some bacterial capsules

Bacterium	Capsule composition	Structural subunits
Gram-positive Bacteria		
Bacillus anthracis	polypeptide	D-glutamic acid
Bacillus megaterium	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Gram-negative Bacteria		
Acetobacter xylinum	polysaccharide	(cellulose) glucose
Escherichia coli	polysaccharide (colonic acid)	glucose, galactose, fucose glucuronic acid
Pseudomonas aeruginosa	polysaccharide	mannuronic acid
Agrobacterium tumefaciens	polysaccharide	(glucan) glucose

Composed usually of polysaccharide (dextran and xanthan gums are derived from these), or sometimes simple amino acid repeats, often with D-amino acids.

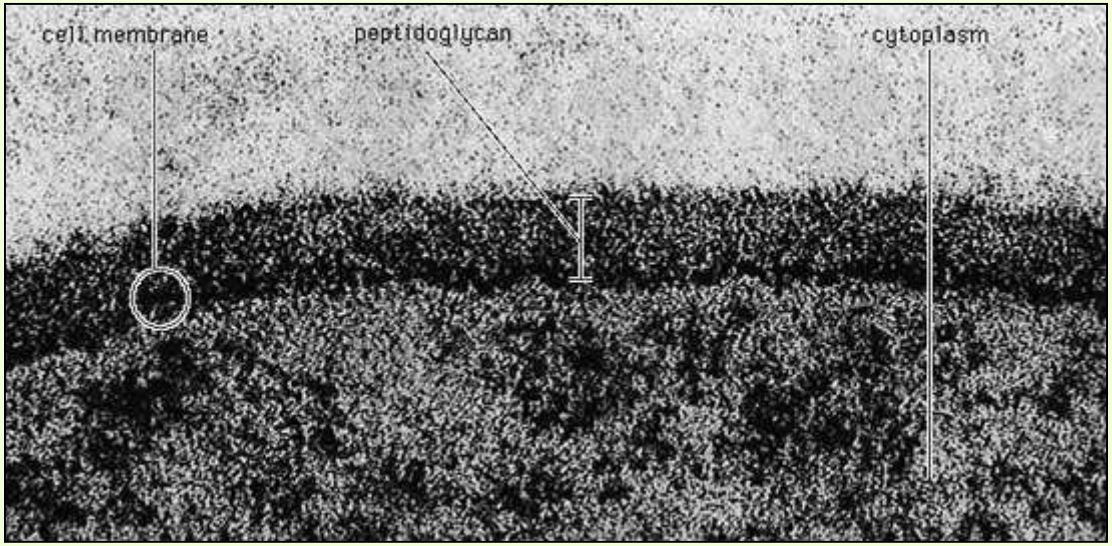
Bacterial cell envelope

There are three principal layers in the envelope; the outer membrane (OM), the peptidoglycan cell wall, and the cytoplasmic or inner membrane (IM).

Bacterial cell envelope

- Cell envelope is composed of:

1. Cell wall, and
2. Cytoplasmic (plasma) membrane.



A portion of the gram-positive bacterium *Bacillus coagulans* showing the cell wall's thick peptidoglycan layer that surrounds the cell membrane.

Britannica.com

Bacterial cell envelope

Significance of cell wall

1. Maintains cell shape, any cell that loses its cell wall, loses its shape as well.
2. Protects bacteria from osmotic lysis.
3. Acts as a barrier, protects cell contents from external environment.
4. Attachment site for flagella.
5. Site of action of certain antimicrobial agents (e.g. penicillins, cephalosporins).
6. Confer specific antigenicity to a strain/species that can be exploited to detect and identify an isolate.

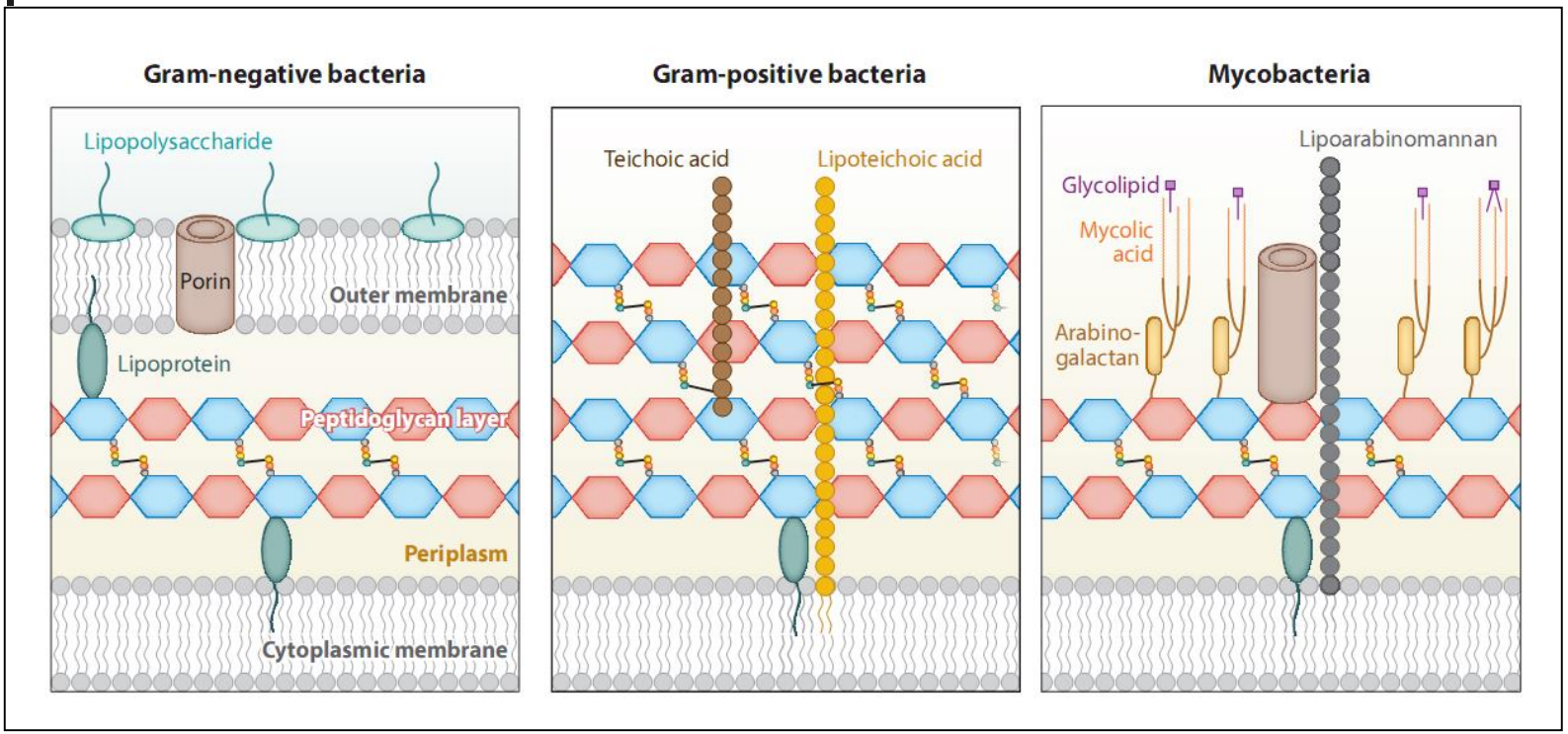
Bacterial cell envelope

- Based on major differences in cell surface properties of bacteria the following six major groups of bacteria were recognized:

1. Common Gram-positive bacteria
2. Common Gram-negative bacteria
3. Atypical Gram-positive bacteria with unique cell wall(arabinogalactan+mycolic acids) Mycobacteria
4. Fastidious G-ve *Xylella*
5. Fastidious G-ve BLO (*Liberibacter*)
6. Fastidious G+ve Mollicutes

Major types of bacterial cell envelope

Mycobacteria: A typical Gram-positive bacteria



Still there are a few bacteria such as fastidious bacteria do not possess cell walls and are contained only by the inner membrane. Not surprisingly, these bacteria are pleomorphic and thus lack a distinct shape.

Radkov *et al.*,2018

Bacterial cell envelope

Two major categories

- Most bacterial cell envelopes fall into two major categories:
- Gram positive and Gram negative.
- This is based on Gram staining characteristics that reflect major structural differences between the two groups.

1. Thick cell walls retain (= gram +);
2. Thin cell walls don't (= gram -).

- Other types of cell wall are found in a few bacterial species (neither Gram positive nor Gram negative).

Bacterial cell envelop components

Gram-ve (diderm) vs. Gram+ve (monoderm)

Gram negative bacterial cell envelop

1. Outer membrane
1.1. Lipopolysaccharide (LPS)
1.2. Lipoprotein
1.3. Phospholipids
2. Peptidoglycan
3. Thick periplasmic space
4. Plasma membrane

Gram positive bacterial cell envelop

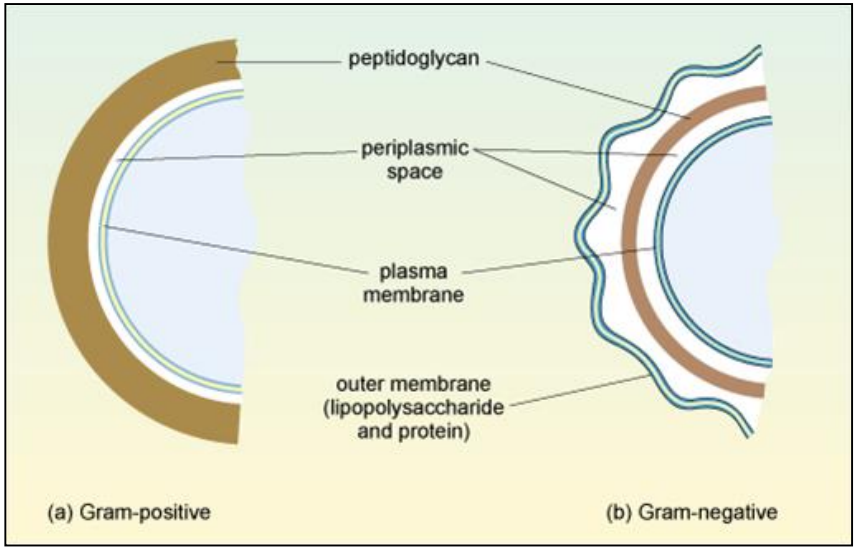
1. Peptidoglycan
2. Teichoic acid
3. Thin periplasmic space
4. Plasma membrane

The bacterial cell wall ranges from 20-80 nm thick for Gram positive and between 1.5-10 nm thick for Gram negative bacteria.

Bacterial cell envelop

Cell wall of Gram-positive vs. Gram-negative bacteria

- In Gram-positive bacteria, the cell wall has a thick peptidoglycan layer which is relatively porous, allowing substances to pass through it quite easily.
- In Gram-negative bacteria, this peptidoglycan layer is greatly reduced and is further protected by a second, outer membrane.

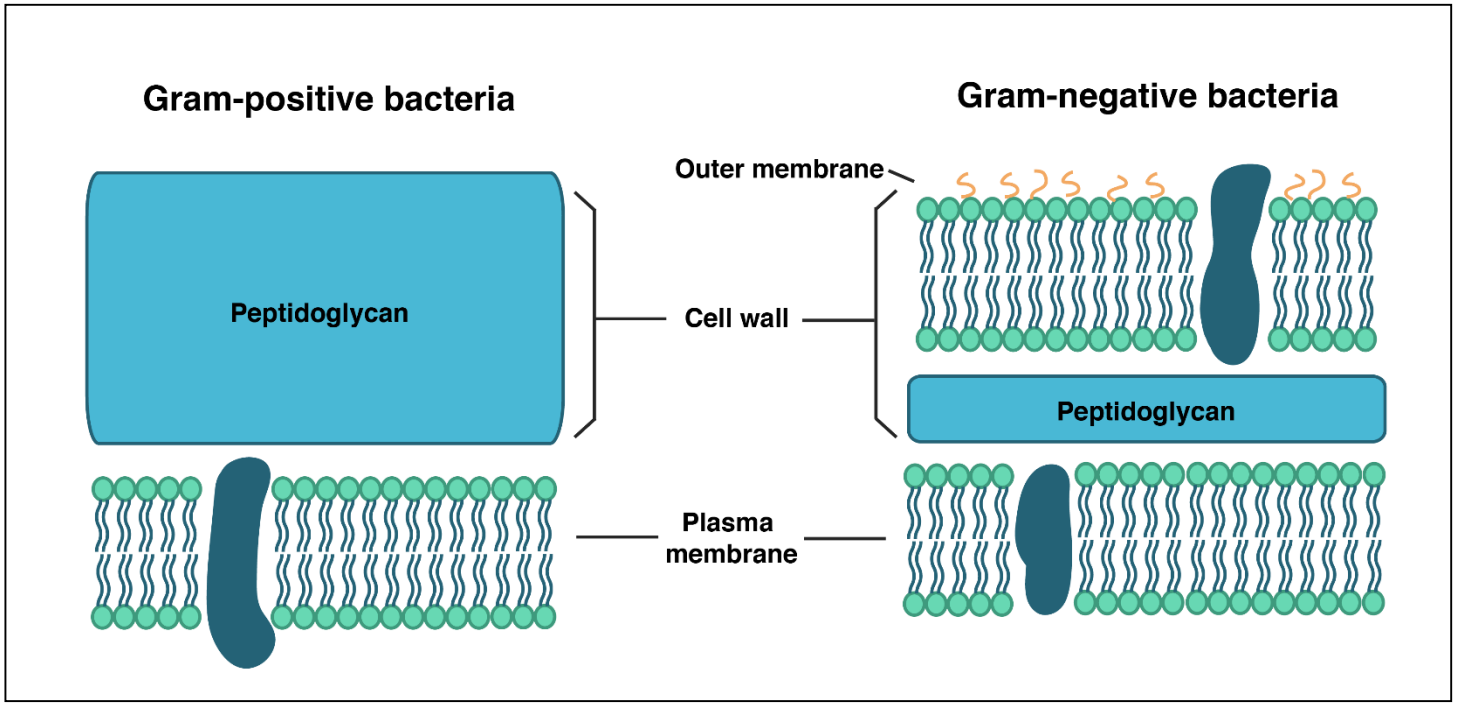


OpenLearn,2020

Bacterial cell envelope

Two major categories

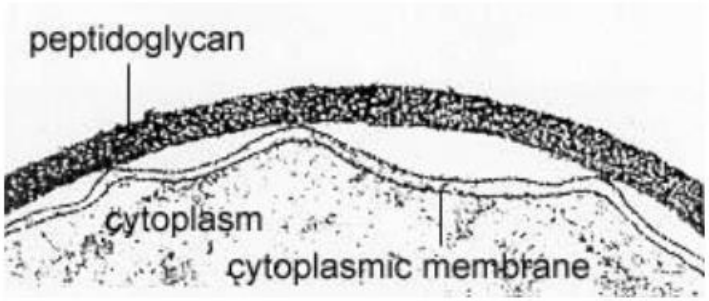
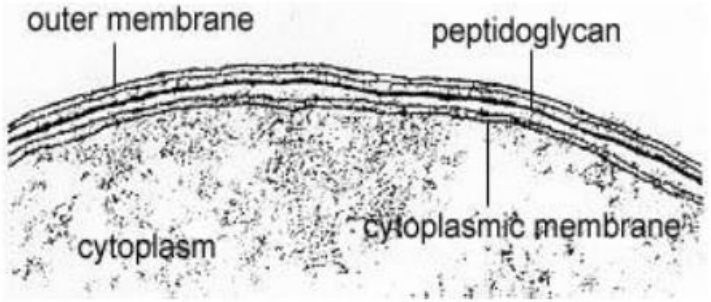
- OM is an additional layer present in gram negative bacteria.
- It is composed of lipid bilayer, protein and lipopolysaccharide (LPS) layer.



Karki,2019

Cell envelop components

Gram-ve vs. Gram+ve

Electron Micrograph of a Gram-Positive Cell Wall	Electron Micrograph of a Gram-Negative Cell Wall
	

Kaiser,2009

Bacterial cell envelop

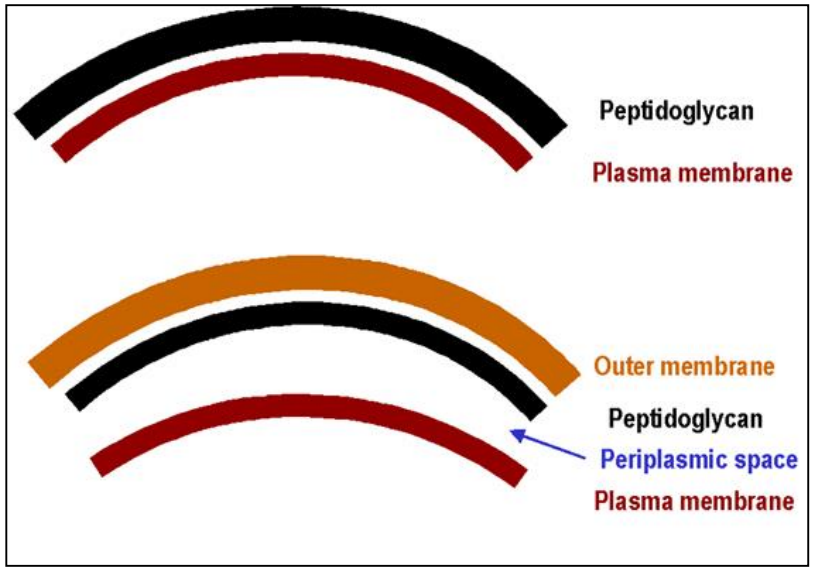
Differences between Gram+ve and Gram-ve

Property	Gram-positive	Gram-negative
Thickness of cell wall (95% of the cell wall is peptidoglycan)	thick (20-80 nm)	thin (10 nm)
Number of layers	1	2
Peptidoglycan (murein) content	>50%	10-20%
Teichoic acids in wall	present	absent
Lipid and lipoprotein content	0-3%	58%
Protein content	0	9%
Lipopolysaccharide content	0	13%
Sensitivity to lysozyme and penicillin	High	Low

Bacterial cell envelop

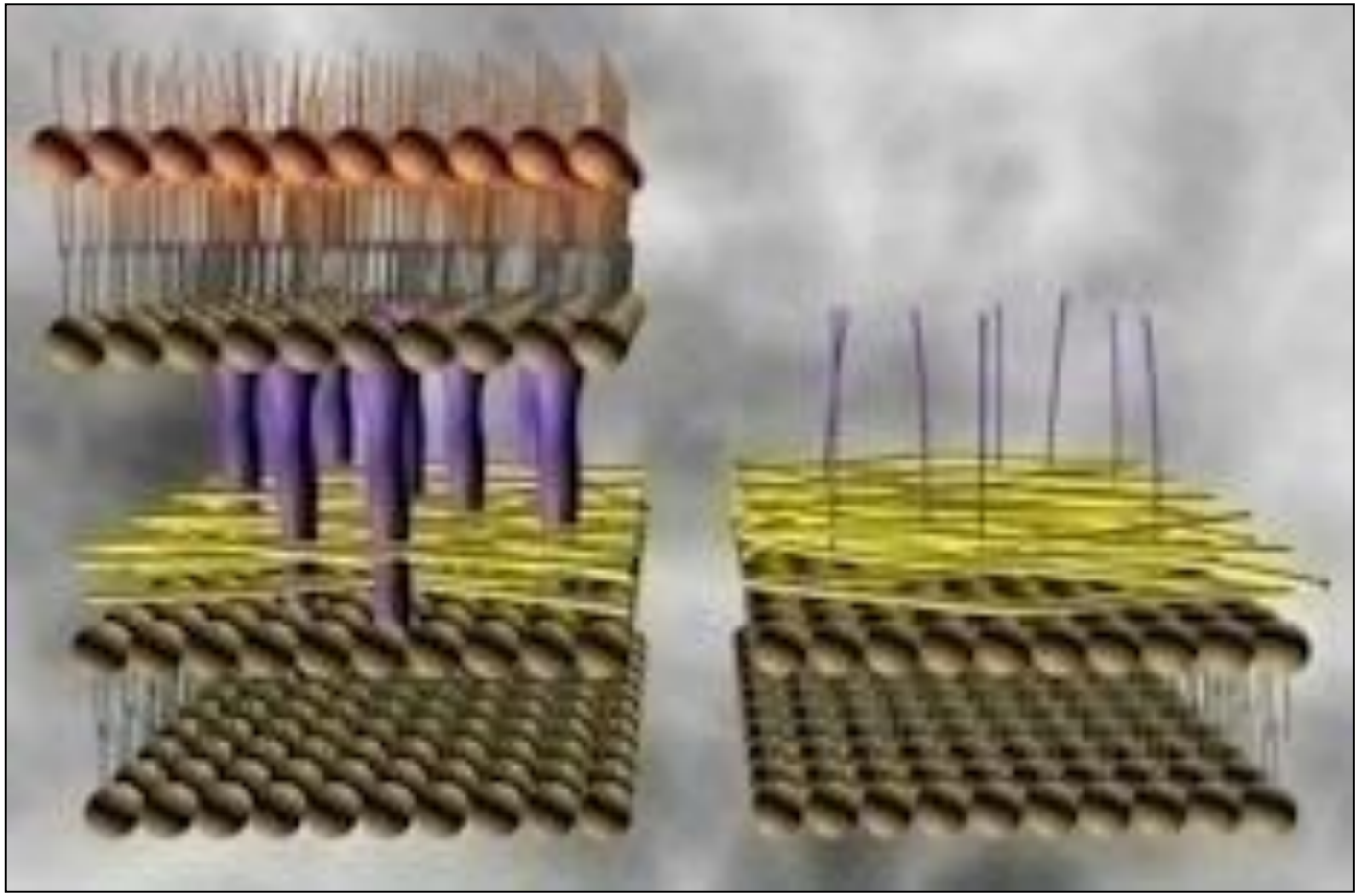
Differences between Gram+ve and Gram-ve

- The major differences lie in the thickness of the rigid peptidoglycan layer (95% of the cell wall) in Gram-positive and in the presence of an outer membrane in Gram negative cells.
- The peptidoglycan in Gram-negative cells is a much thinner and a looser structure than Gram-positive (5-10% of the cell wall).



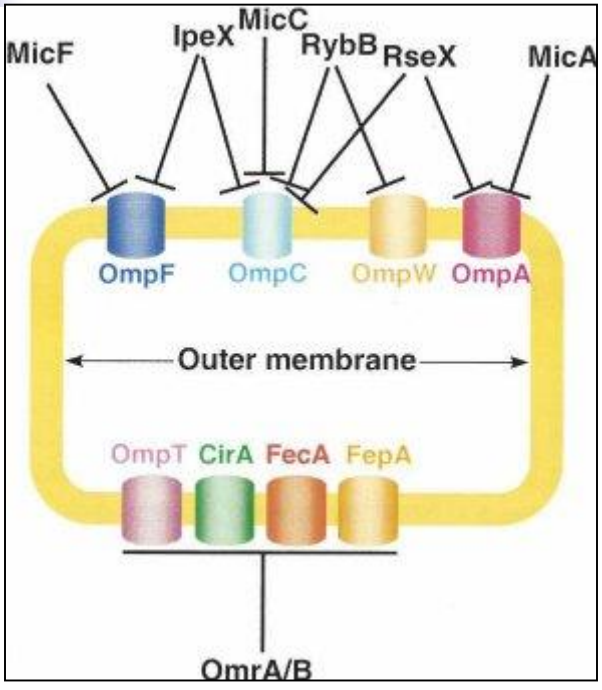
Bacterial cell envelop

Differences between Gram-negative (left) and Gram-positive (right) cell walls

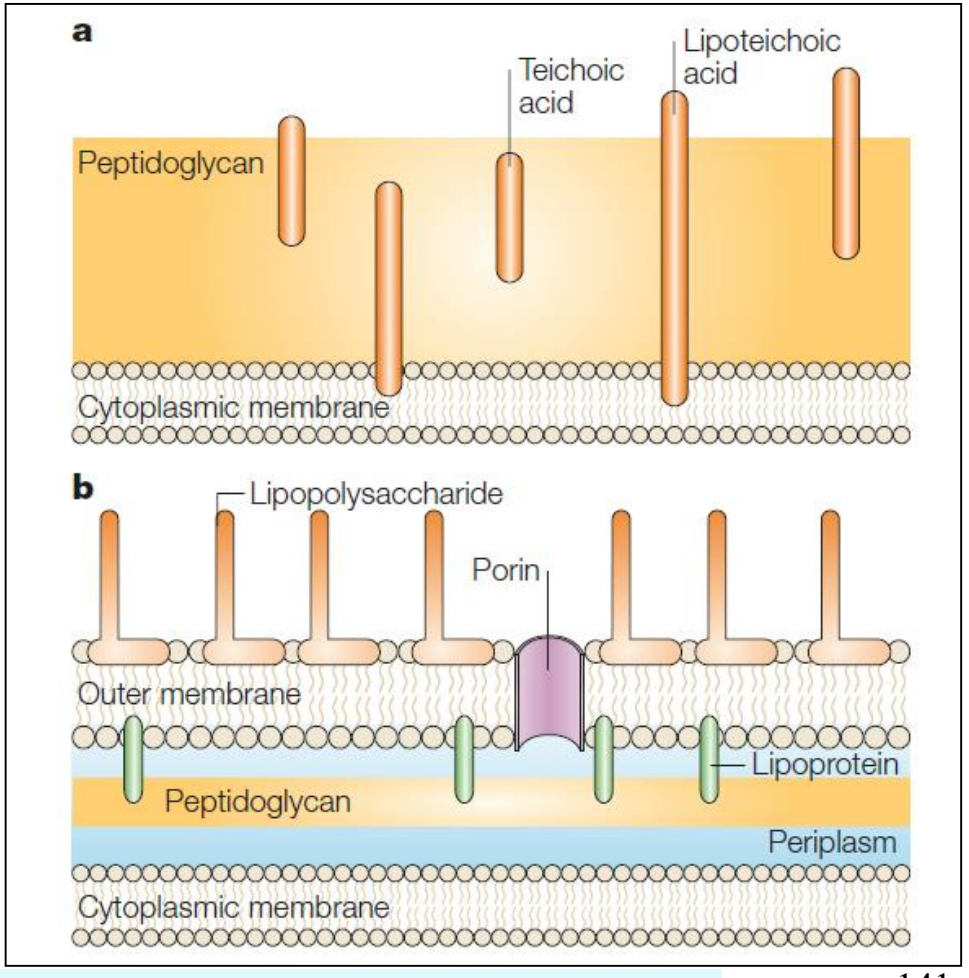


Bacterial cell envelop

Differences between Gram-positive (a) and Gram-negative (b) cell walls



Summary of small RNAs regulators of outer membrane proteins in G-ve bacteria. sRNAs are shown in black, with their identified targets depicted as outer membrane-barrel proteins. FepA is more strongly regulated by OmrA than by OmrB.

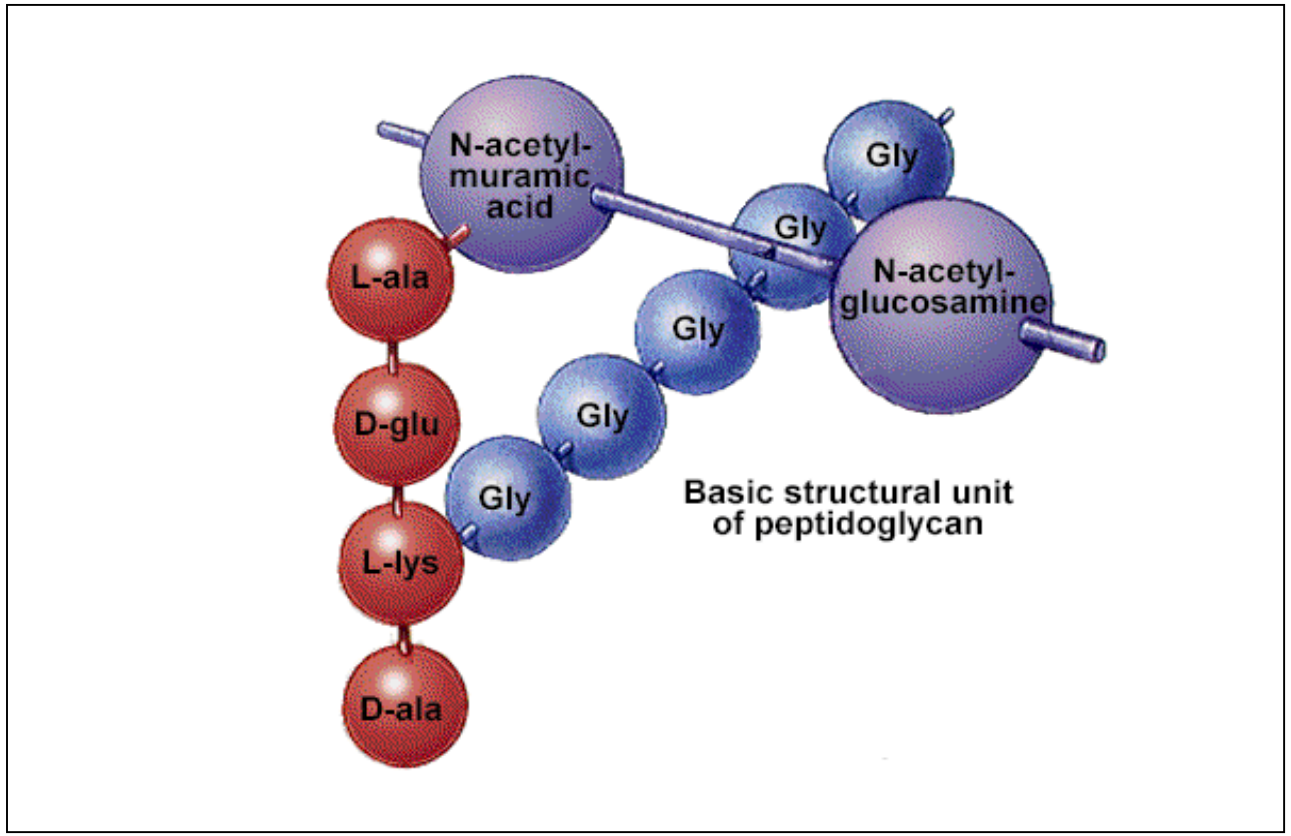


Cabeen and Jacobs-Wagner,2005; Guillier and Gottesman,2006

Bacterial cell wall

Peptido-glycan polymer (amino acids+sugars)

The peptidoglycan monomers

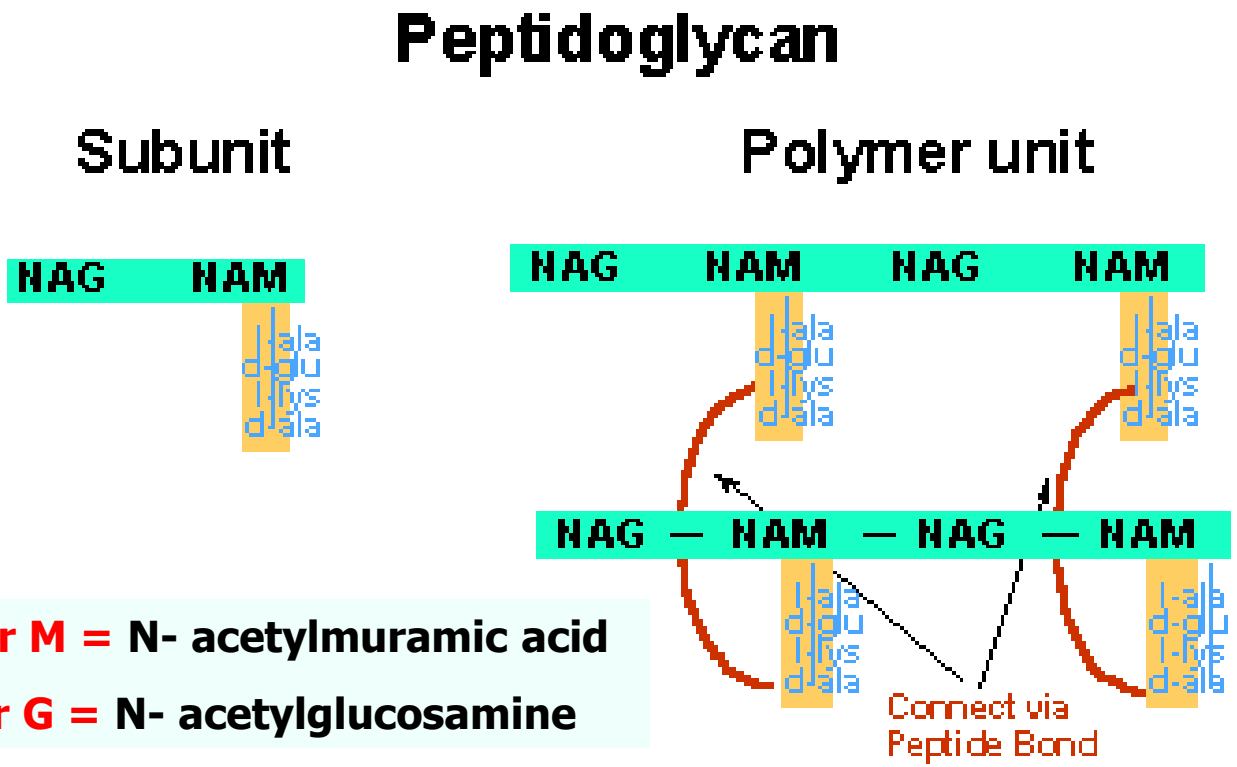


Schlink,2010

Peptidoglycan arrangement

Peptido-glycan polymer (amino acids+sugars)

The peptidoglycan monomers



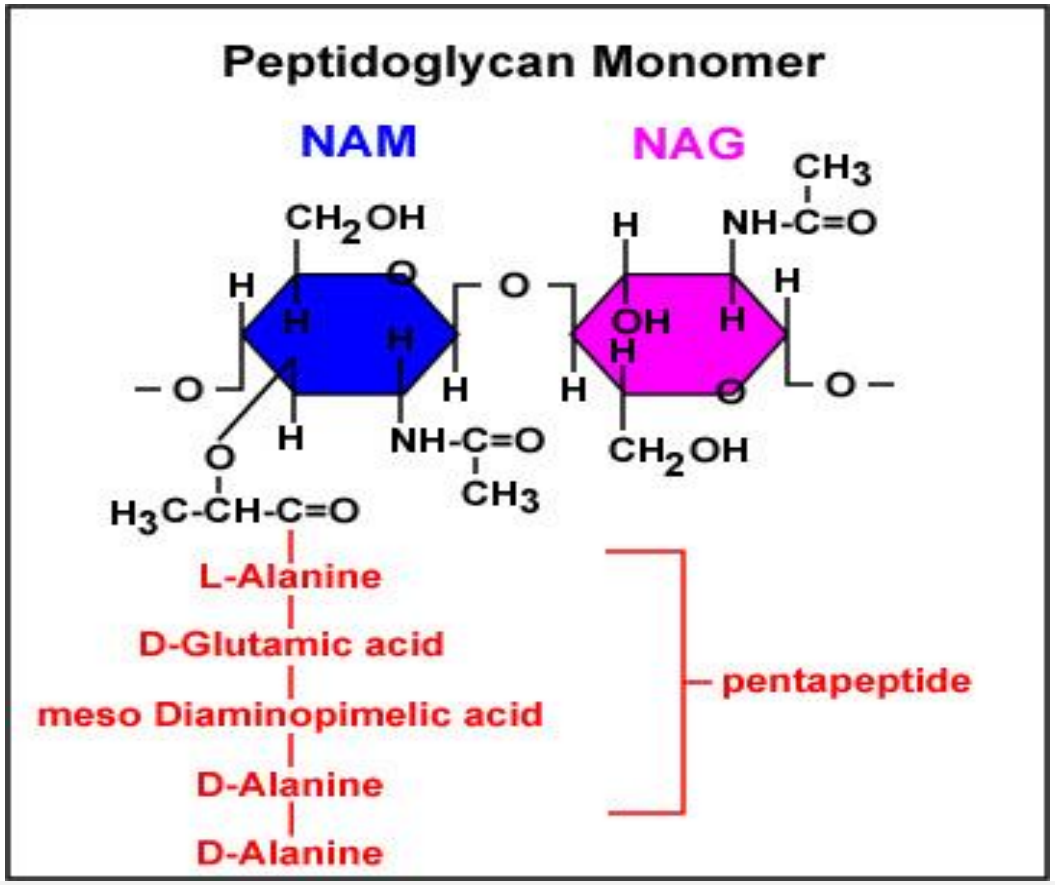
NAM or M = N- acetylmuramic acid

NAG or G = N- acetylglucosamine

Peptidoglycan is arranged in chains
M-G-M-G-M-G-M-G-M-G-M-G (10 to 65 sugars)

The peptidoglycan cell wall

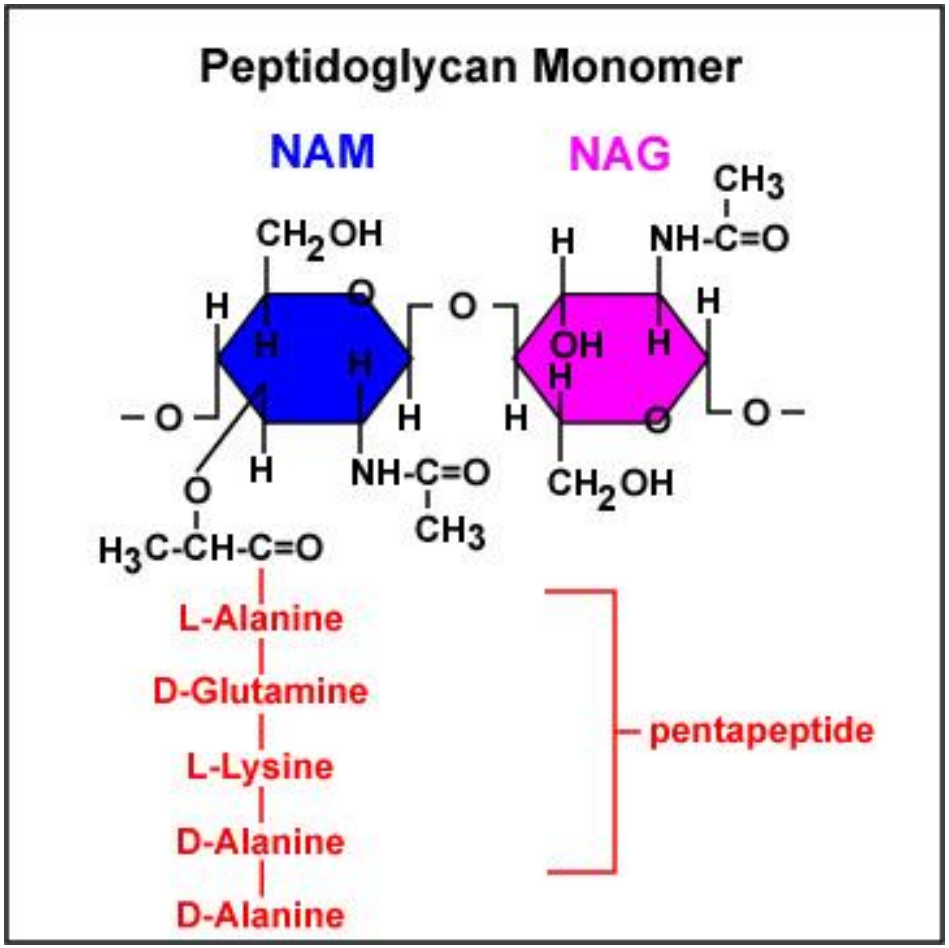
Peptidoglycan monomer in Gram-ve cell

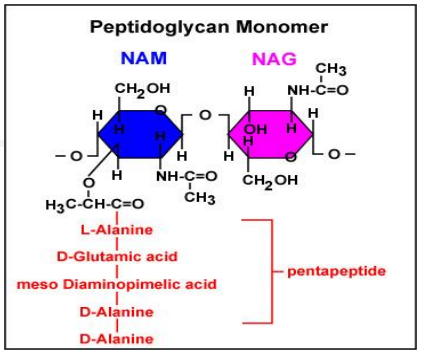


DAP

Diaminopimelic acid is an unusual amino acid found only in bacterial cell walls, more often in G-ve Bacteria.

Peptidoglycan monomer in Gram+ve Cell





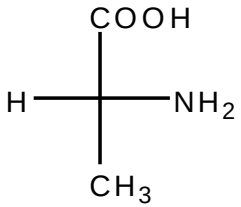
Gram-ve cell wall

The Peptidoglycan cell wall

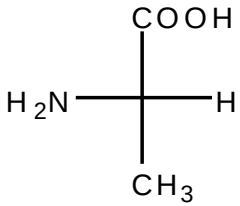
The structure of amino acids

D-and L-amino acids

- If the amine group (N-terminus) is on the right, it's a D-amino acid.
- If the amine group (N-terminus) is on the left, it's an L-amino acid.
- D form of Amino acids hard to break down.



D-Alanine



L-Alanine

Synthesis of peptidoglycan
Complete process

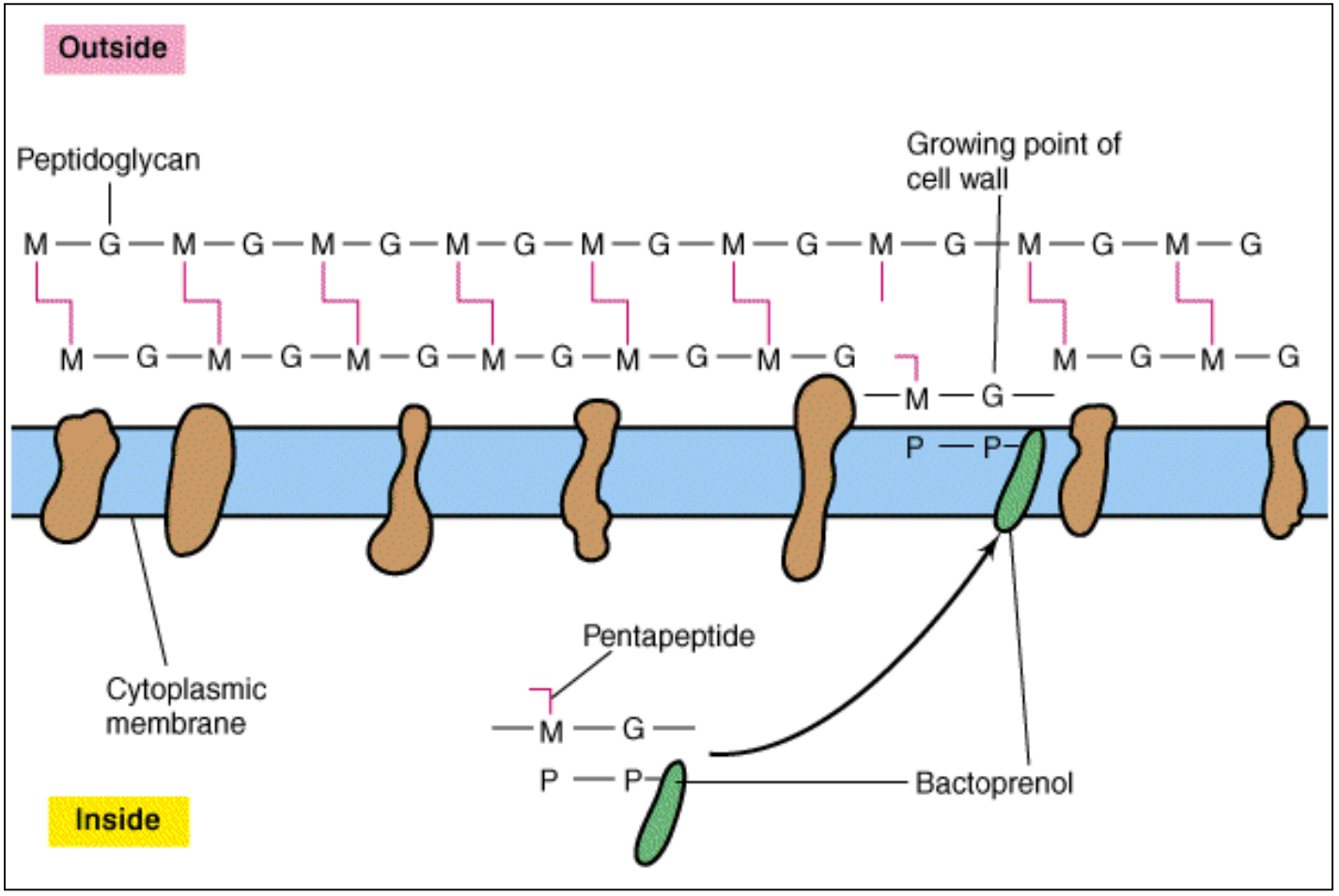
- The peptidoglycan monomers are synthesized in the cytosol of the bacterium and transport across the cytoplasmic membrane.
- The membrane carrier molecules called bactoprenols transport and inserted peptidoglycan monomers into the growing peptidoglycan chains through the following process:

1. Action of autolysins
2. Synthesis of peptidoglycan monomers
3. Action of transglycosidase
4. Action of transpeptidase.

Kaiser,2010

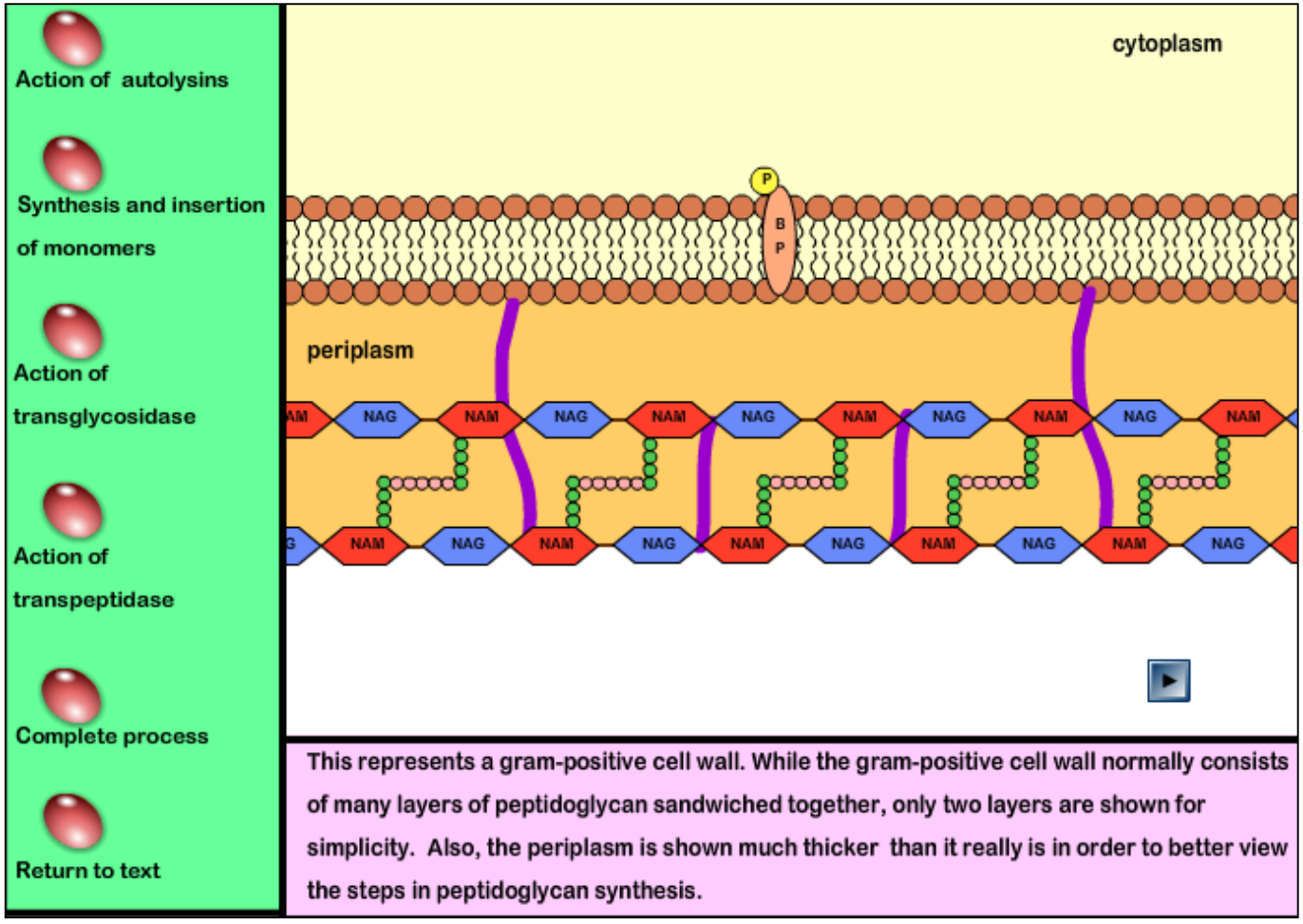
Synthesis of peptidoglycan

Complete process



Schlink,2010

Synthesis of peptidoglycan
Complete process



Kaiser,2010

Synthesis of peptidoglycan
Complete process

1. Action of Autolysins:

- To add new peptidoglycan monomers to the existing peptidoglycan, a group of bacterial enzymes called autolysins break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan.
- This will help bacterial cell division (binary fission).

2. Synthesis of Peptidoglycan monomers:

- Peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol.
- The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and helps insert them into the growing peptidoglycan chains.

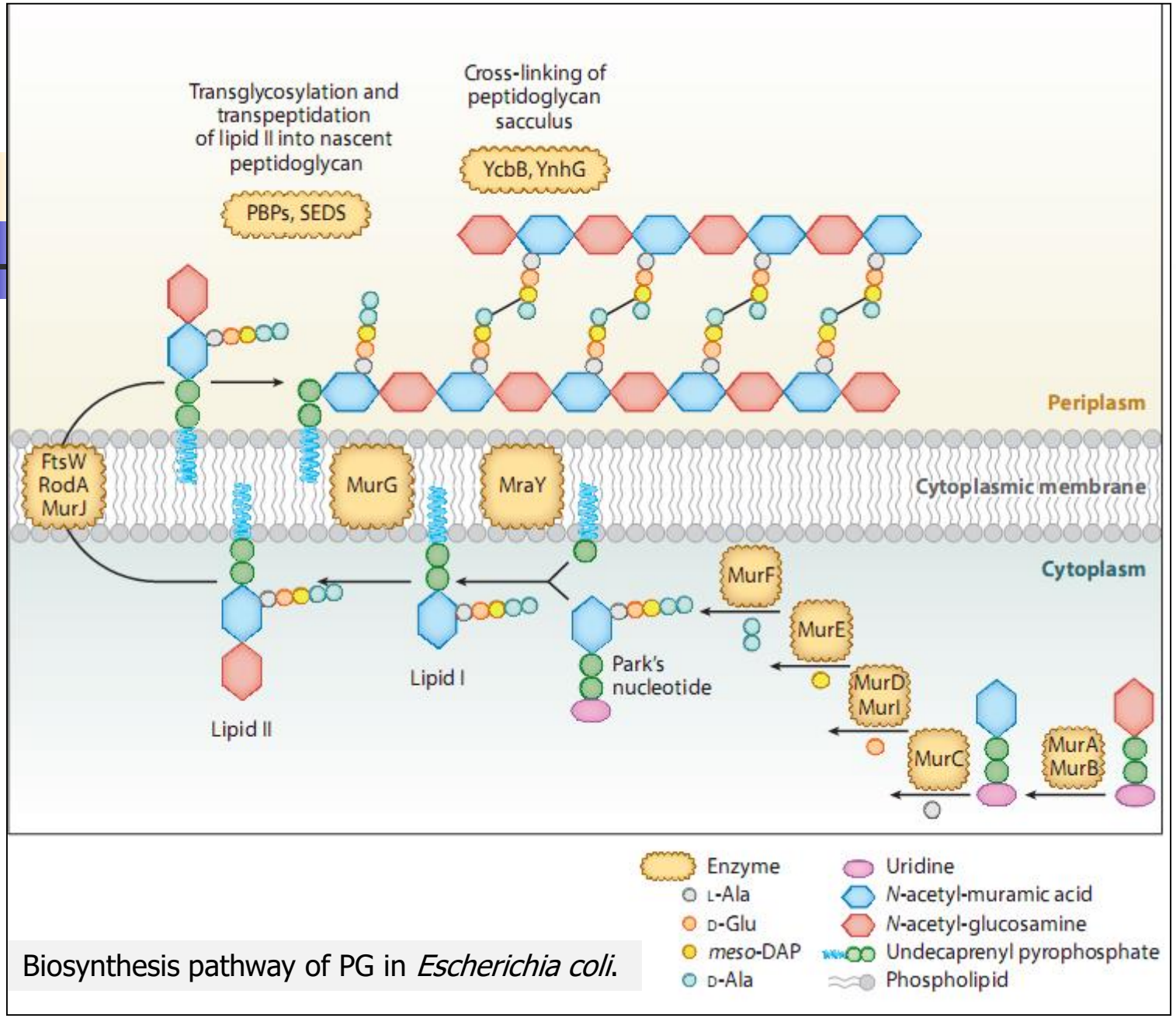
Synthesis of peptidoglycan
Complete process

3. Action of transglycosidase:

- The transglycosidase enzymes catalyze the formation of glycosidic bonds between the NAM and NAG of the peptidoglycan momomers and the NAG and NAM of the existing peptidoglycan.

4. Action of transpeptidase:

- Transpeptidase enzymes are responsible for attachment of long sugar chains (NAM-NAG…) to peptides coming off of the NAMs by means of peptide cross-links.
- The peptide cross-links provide tremendous strength to the cell wall.



Biosynthesis pathway of PG in *Escherichia coli*.

Radkov *et al.*,2018

Gram-negative cell walls

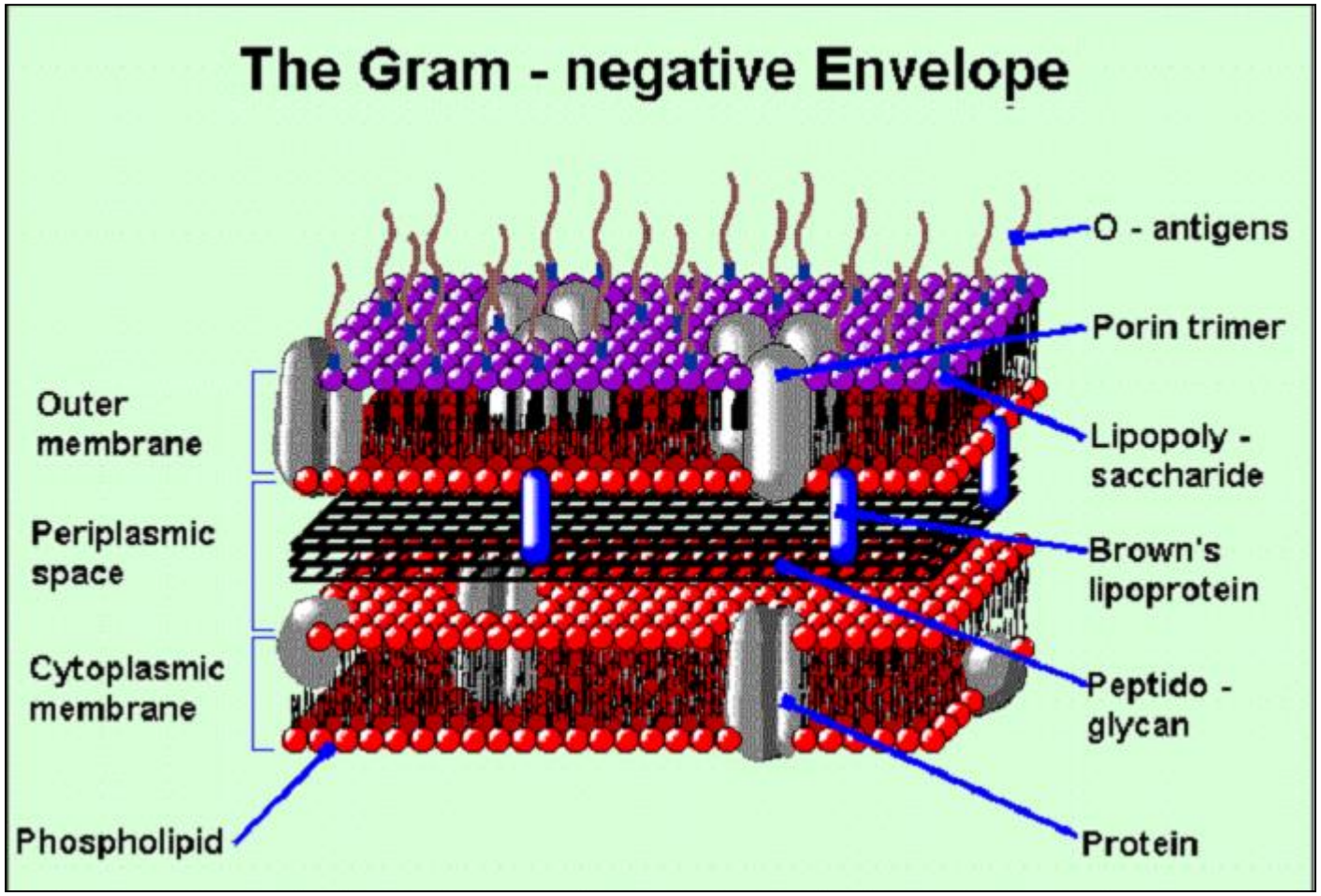
Outer membrane, periplasmic space,..

Gram-negative cell walls
Outer membrane

- The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane.
- Outer membrane acts as a coarse sieve, has only minor control over transport into & out of the cell.
- **Outer membrane contains:**
- Porin proteins
- Adhesion proteins
- Lipopolysaccharide a major virulence factor
- These molecules are also known as:

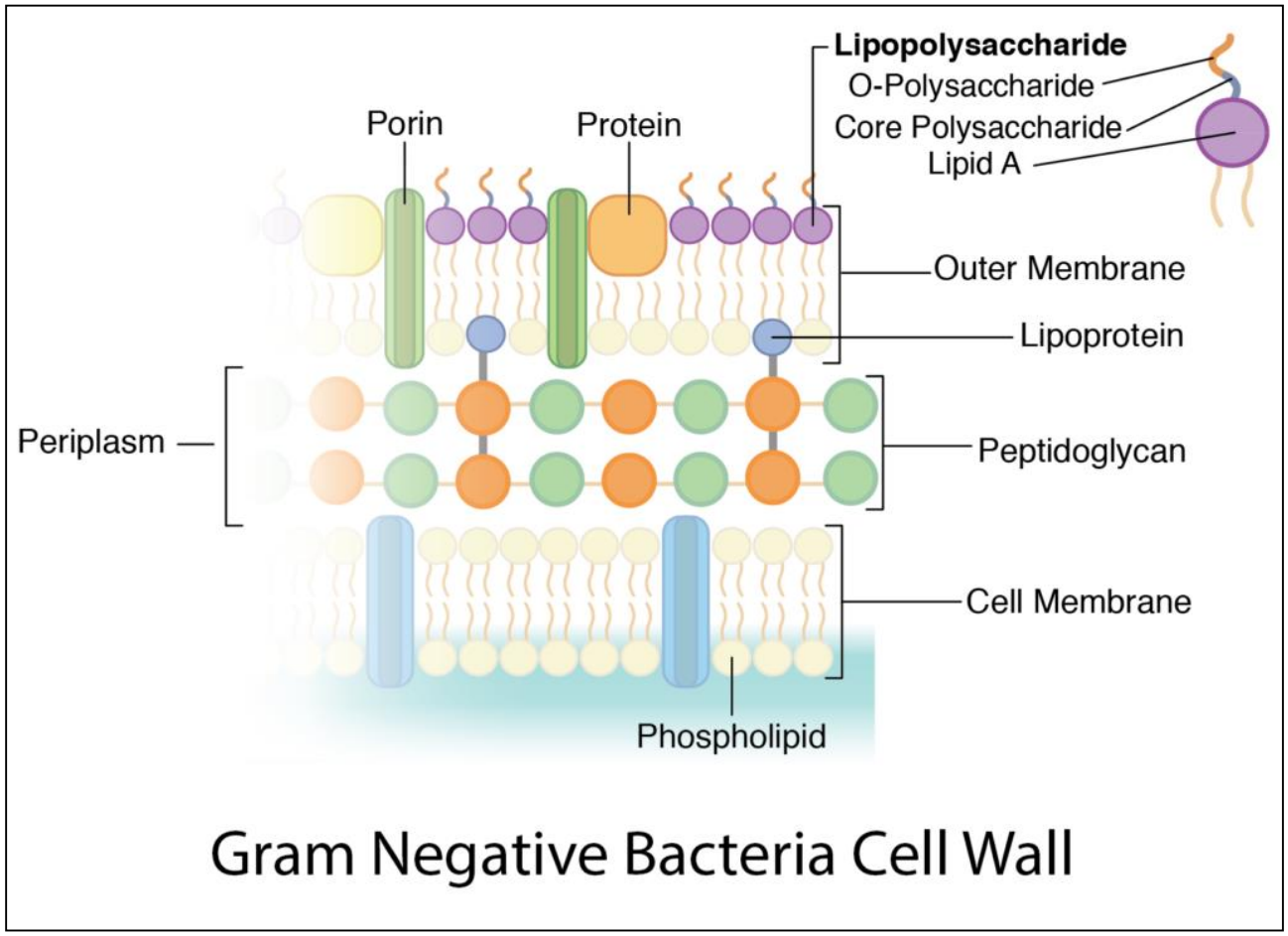
1. Phospholipids,
2. Lipopolysaccharides (LPS),
3. Lipoproteins,
4. Surface proteins.

Gram-negative cell envelop



Gram-negative cell wall

Outer membrane



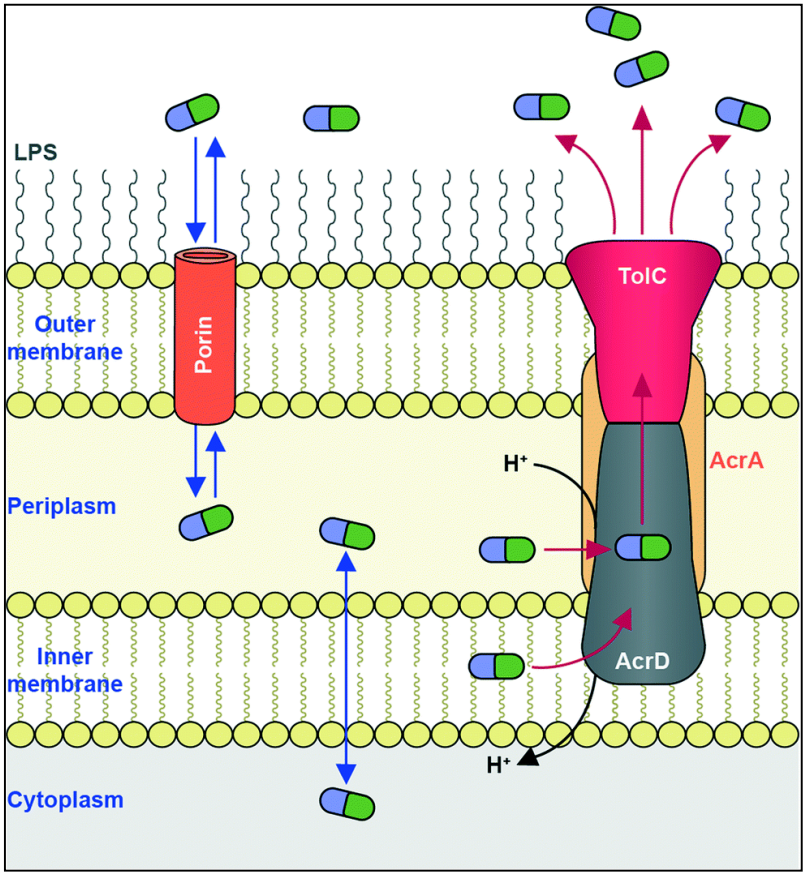
Karki,2019

Gram-negative cell wall

Outer membrane structure

Porin proteins in Gram-negative bacteria

- Porin proteins (trimeric protein pores) exist in the outer membrane and act as channels for low MW water soluble substances, phage receptors.
- Note that in gram-positive bacteria, molecules as large as 10^5 daltons can pass through the cell wall.



Todar,2008; El-Safey,2011; Garneau-Tsodikova and Labby,2016

Outer membrane

Functions of surface proteins

Gram-ve bacteria

Component	Function
Braun lipoprotein	Bacterial lipoproteins having a lipid-modified cysteine at the N-terminus are important components of the cell envelope and responsible for various cellular activities. They anchors the outer membrane to peptidoglycan (murein) sheet.
Omp C and Omp F porins	Proteins that form pores or channels through outer membrane (Gram-negative bacteria) for passage of useful molecules (nutrients) but not harmful substances from the environment.
Omp A protein	Provides receptor for some viruses and bacteriocins; stabilizes mating cells during conjugation.

Todar,2009

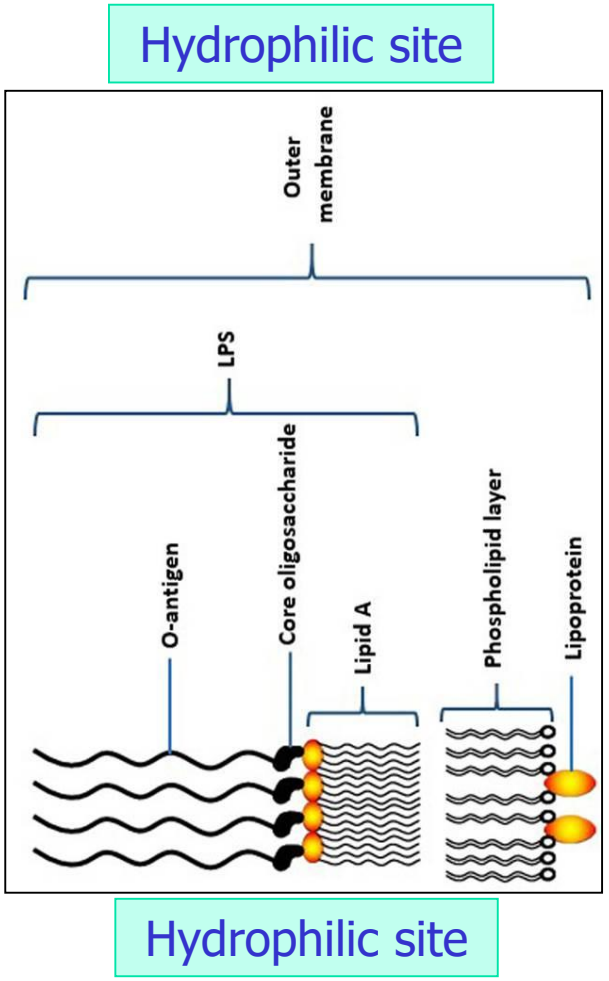
Outer membrane structure

The three major regions of lipopolysaccharides

Lipid A, core and O antigen

- Lipopolysaccharide (LPS), commonly known as endotoxin.
- Endotoxin is released when cell dies and causes pathogenicity of many Gram (-) diseases.

1. hydrophobic lipid A,
2. core polysaccharide, and
3. O-antigen (repeats of polysaccharide chain).

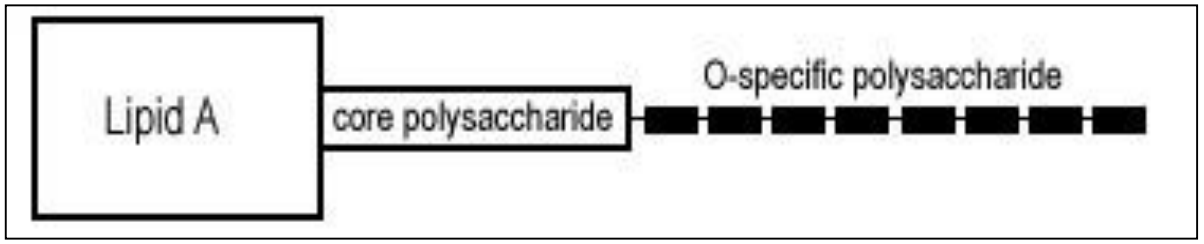


Outer membrane structure

The three major regions of lipopolysaccharides

Lipid A, core and O antigen

- O-antigens vary among bacterial strains and give bacteria a rough (R-type) or smooth (S-type) phenotype.
- O-antigens are responsible for bacteria evading the immune system, particularly the complement system of the host.



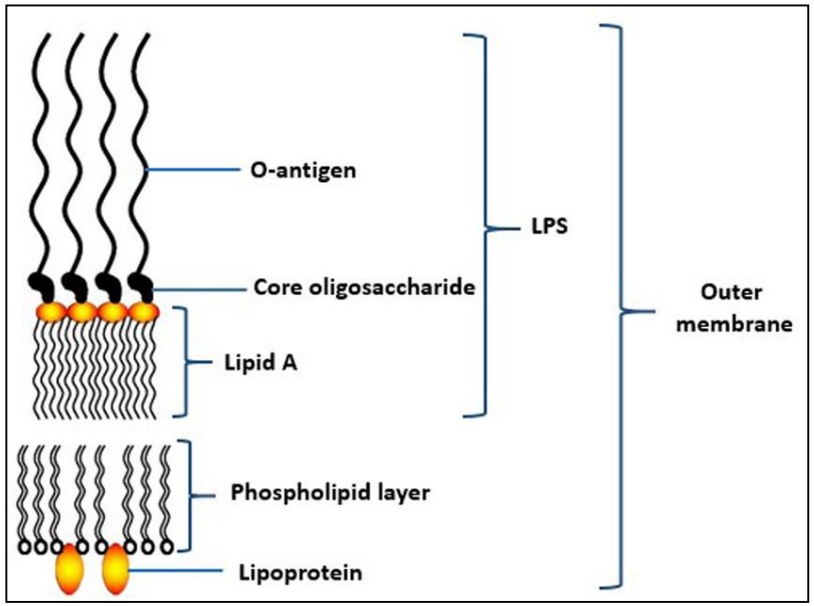
Mazgaeen and Gurung,2020

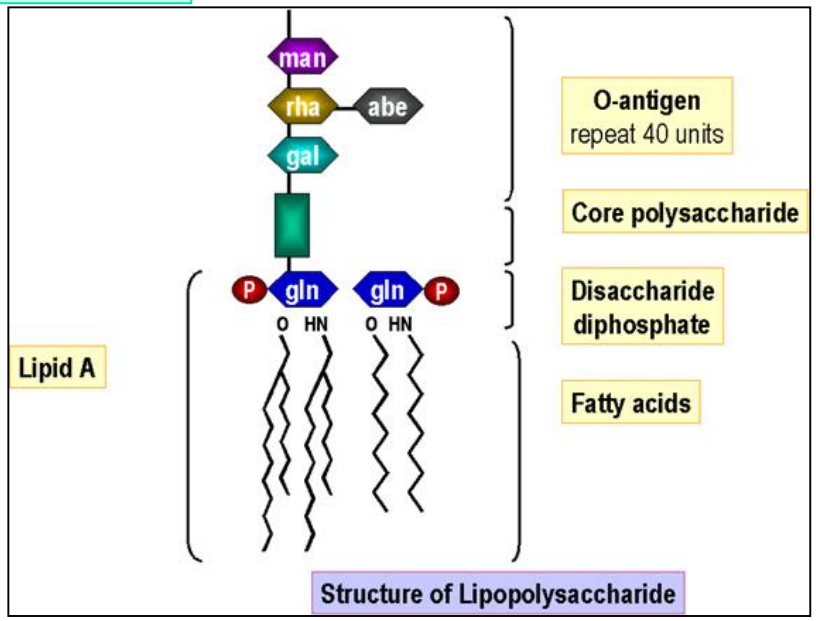
Outer membrane structure

The three major regions of lipopolysaccharides

Lipid A, core and O antigen

Hydrophilic site





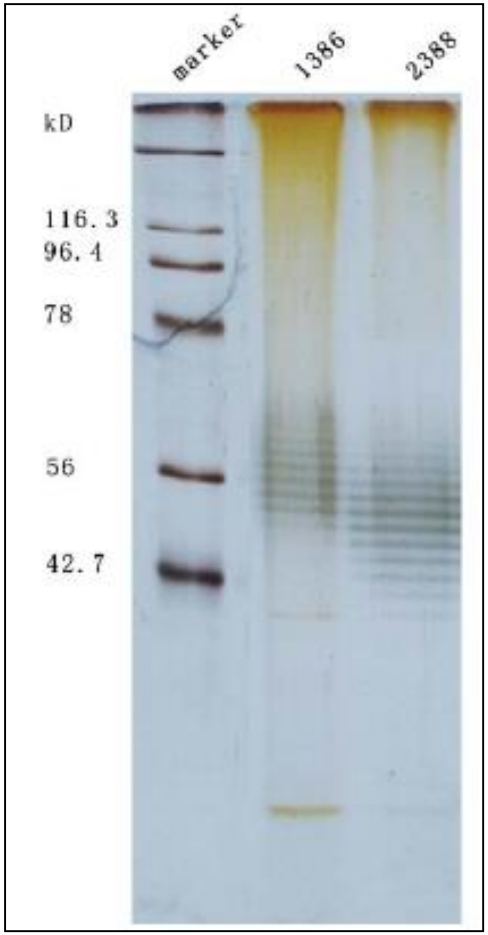
Hydrophilic site

O-antigen and core polysaccharides regions are hydrophilic, whereas Lipid A region is hydrophobic.

Extraction of bacterial lipopolysaccharides (LPS)

SDS-polyacrylamide gel electrophoresis

- SDS-polyacrylamide gel electrophoresis of lipopolysaccharide of two *X. a. malvacearum* strains (SPB 1386 and GSPB 2388) and silver-stained after electrophoresis.
- LPS was prepared as previously described (Senchenkova *et al.*,2002).
- The LPS samples were suspended in 0.1 M Tris-HCl (pH 6.8) containing 2% SDS, 10% glycerine and 20 ppm bromophenolblue, then heated for 5 min in a water bath (100°C) and loaded onto 3% stacking gel and 12% separating polyacrylamide gel.
- After electrophoresis, the gel was silver-stained.

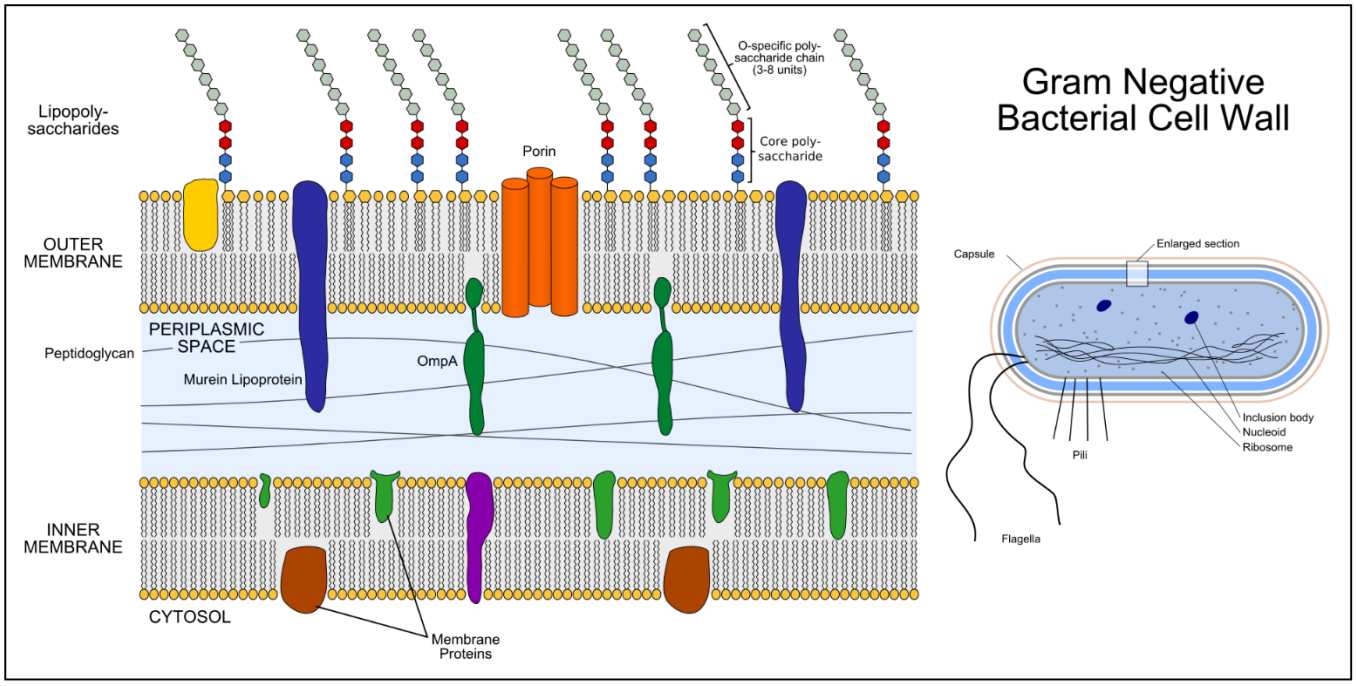


Huang *et al.*,2008

Periplasmic space

A concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane

- The periplasmic space is the region between outer membranes and plasma membrane. Also within the periplasmic space is peptidoglycan, which surrounds the cell.

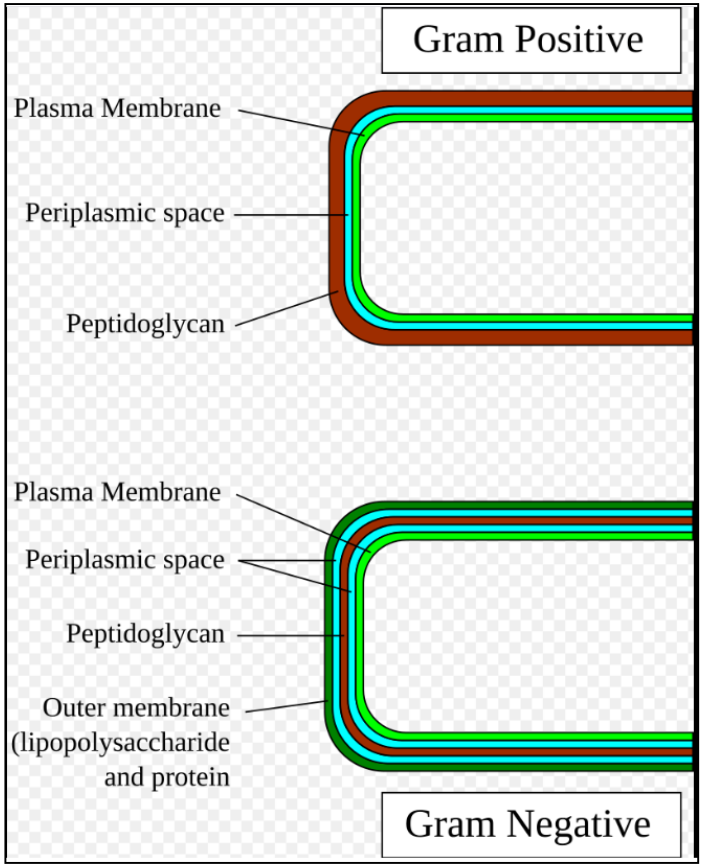


Wikipedia,2024

Periplasmic space

A concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane

- In Gram-negtive bacteria the periplasm is a thick concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane.
- In Gram-positive bacteria a thin periplasmic space is found between the inner cytoplasmic membrane and the peptidoglycan layer.

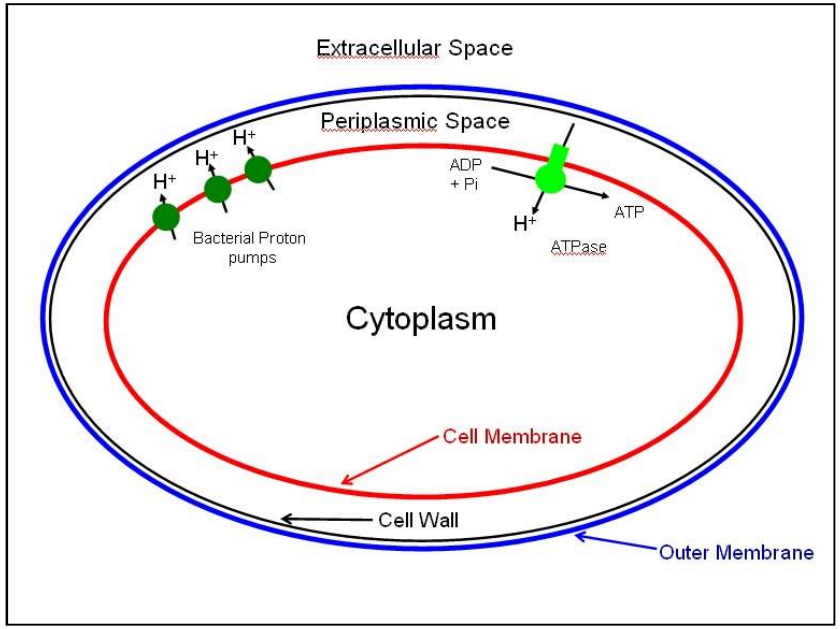


Wikipedia,2024

Periplasmic space

A concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane

- Gram negative bacteria have a periplasmic space which lies between the outer membrane and the plasma membrane.
- Very small thin layer otherwise nonexistent in gram-positive.



Periplasmic enzymes such as phosphatases, proteases, etc. are involved in breakdown of complex substances into simple substances.
Periplasmic binding proteins: involved in transport or substances.

Mack,2007;..

Periplasmic space

A concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane

- Gram negative bacteria have a periplasmic space which lies between the outer membrane and the plasma membrane.
- Very small or nonexistent in gram-positive.
- The periplasmic gel contains:

1. Water, nutrients, and
2. Substances secreted by the cell, such as:

i. Hydrolytic (digestive) enzymes, and
ii. Proteins such as alkaline phosphatase and beta-lactamase.

- There is a lot of activity in this area which has many soluble proteins that take part in transport, signaling in chemotaxis, and other processes.

Periplasmic space
In G-ve bacteria

- An aqueous space present between outer membrane and plasma membrane is called as periplasmic space.
- The peptidoglycan layer transverses the periplasmic space and partitions it into outer and inner periplasmic spaces.
- Periplasmic space is gel like because of the presence of abundant proteins called periplasmic proteins.
- **They are of several classes as:**
- **Hydrolytic enzymes**
  - They are involved in breakdown of complex substances into simple substances.
    - e.g. Phosphatases, Proteases etc.
- **Periplasmic binding proteins**
  - Involved in transport or substances.
    - pbp's for ions, amino acids, vitamins etc.

Periplasmic space
In G-ve bacteria

- **Biosynthetic enzymes**
  - Those involved in murine synthesis. e.g.
    - Transglycosylases
    - Transpeptidases
    - Carboxypeptidases
  - It also has enzymes for fimbrial synthesis & assembly.
  - It also has detoxifying or antibiotic degrading enzymes. e.g.
    - Beta-lactamase or pencillinase
    - Aminoglycoside posphorylating enzymes (almost equal to lysosomes).

Gram-positive cell walls
Peptidoglycan+Teichoic acids

The cell walls of gram positive bacteria are composed predominantly of peptidoglycan. In fact, peptidoglycan can represent up to 90% of the cell wall, with layer after layer forming around the cell membrane. Gram positive cell walls also contain chains of teichoic acids.

Gram-positive cell walls

Typical and atypical Gram-positive bacteria

1. **Typical Gram-positive bacteria, and**
2. **Atypical Gram-positive bacteria (acid fast bacteria and actinomycetes).**

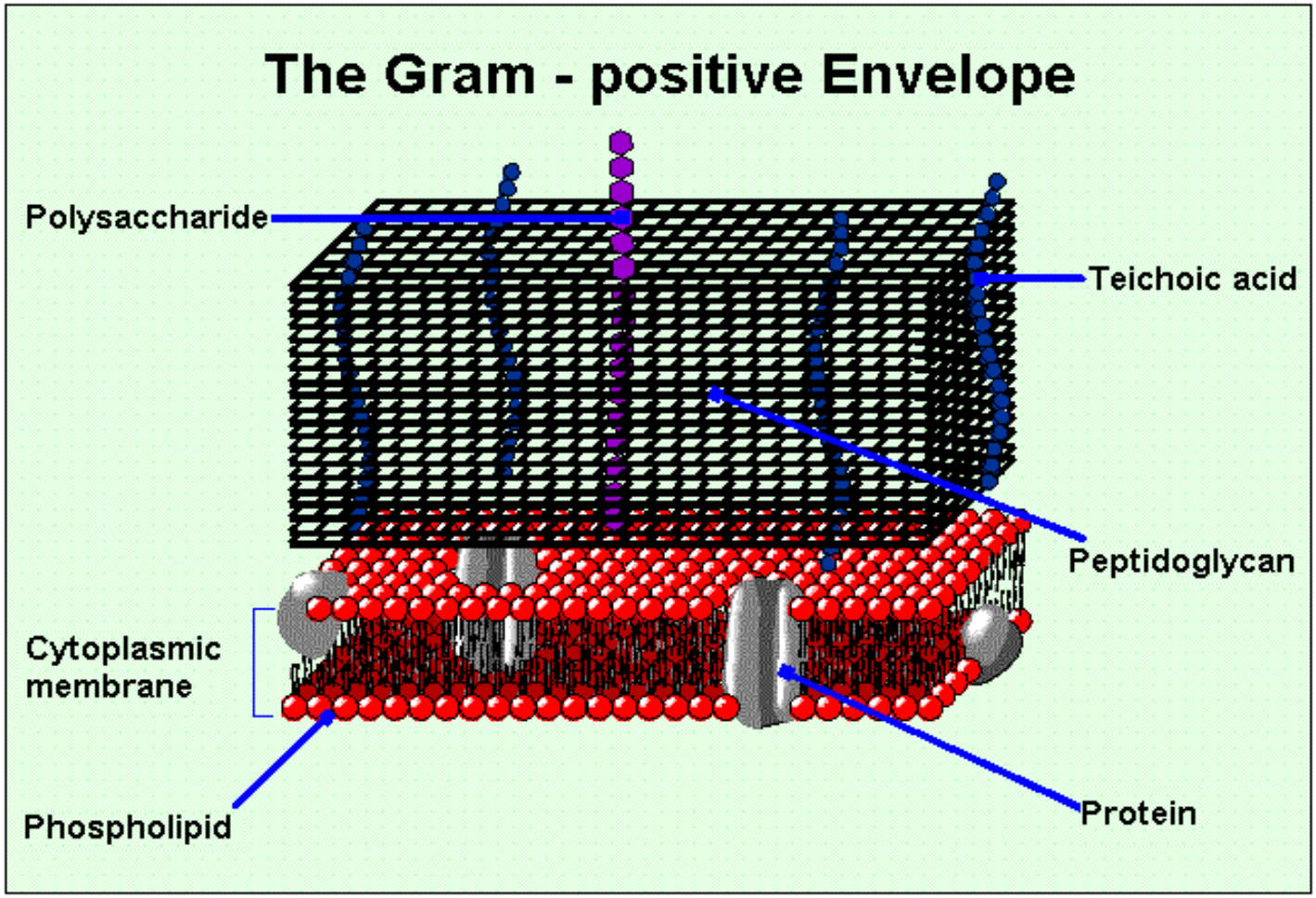
Gram-positive cell walls
Typical Gram-positive bacteria

- The Gram-positive cell wall appears as dense layer typically composed of:

1. Numerous rows of peptidoglycan, provides protection for bacteria and defines their shape,
2. Molecules of lipoteichoic acid, wall teichoic acid, and
3. Surface proteins. These proteins are required for: bacterial growth, cell wall maintenance, cell division, protection of bacteria from environmental challenges, biofilm formation, interaction with eukaryotic cells..

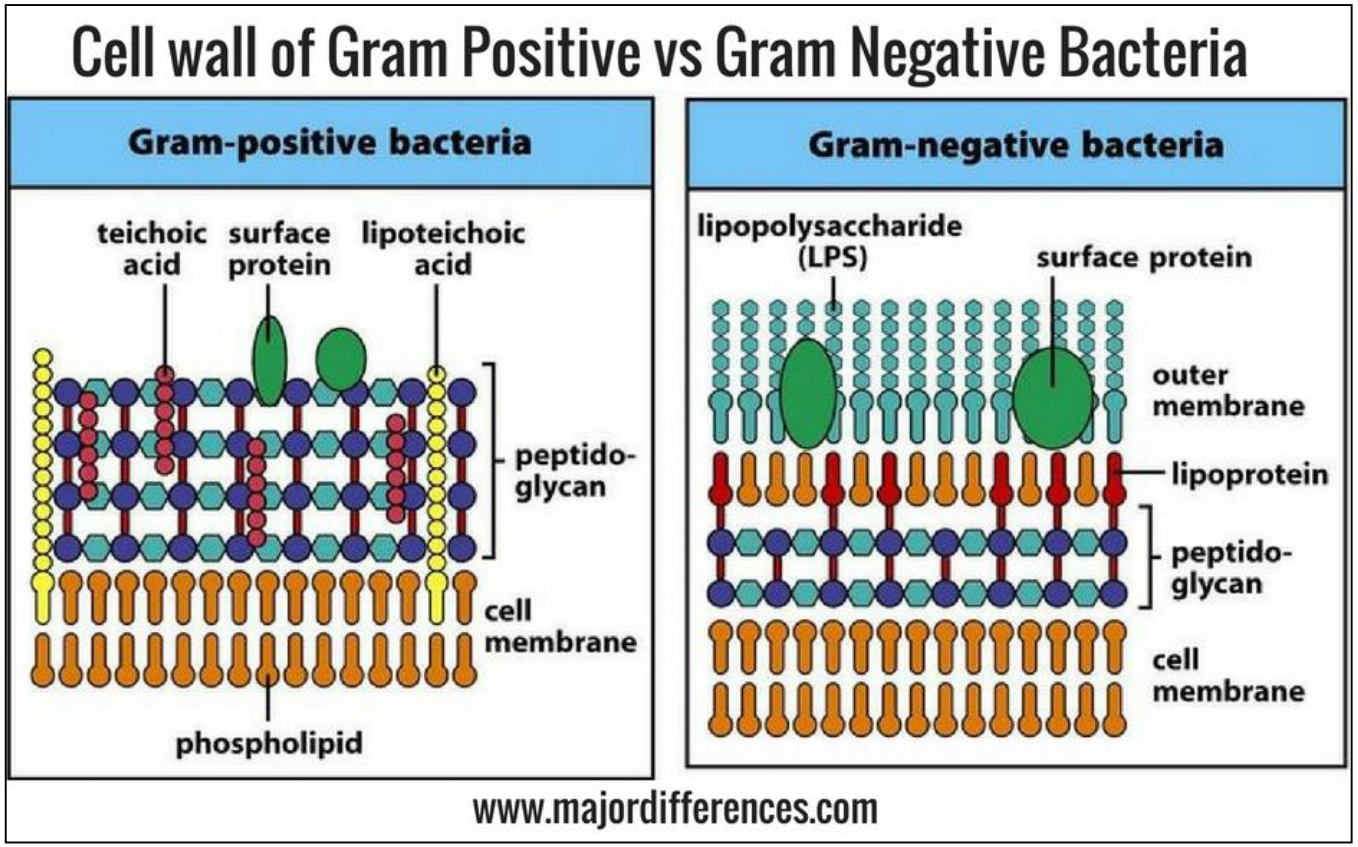
Gram-positive cell envelop

Typical Gram-positive bacteria



Bacterial cell envelop

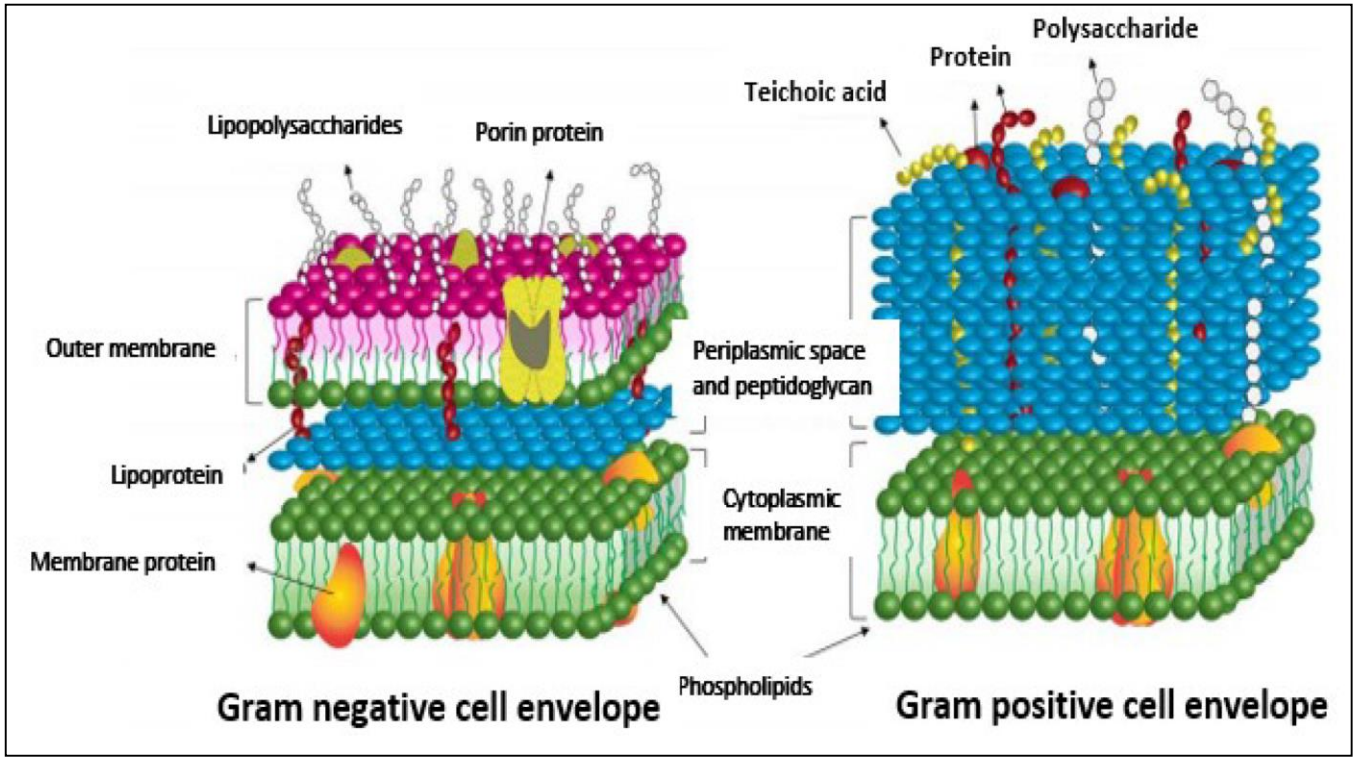
Cell wall of Gram-positive vs. Gram-negative bacteria



Gram-positive cell envelop

Cell wall of Gram-positive vs. Gram-negative bacteria

Typical Gram-positive bacteria



Gram-positive cell walls

Acid-fast bacteria

Atypical Gram-positive bacteria

- Some Gram positive bacteria have an additional component, mycolic acid, in their cell walls.
- Mycolic acids produce a waxy outer layer that provides additional protection for mycobacteria, such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis.
- Gram positive bacteria with mycolic acid are also called acid-fast bacteria because they require a special staining method, known as acid-fast staining, for microscope observation.
- They stain poorly with Gram stain procedure, appearing weakly Gram-positive.

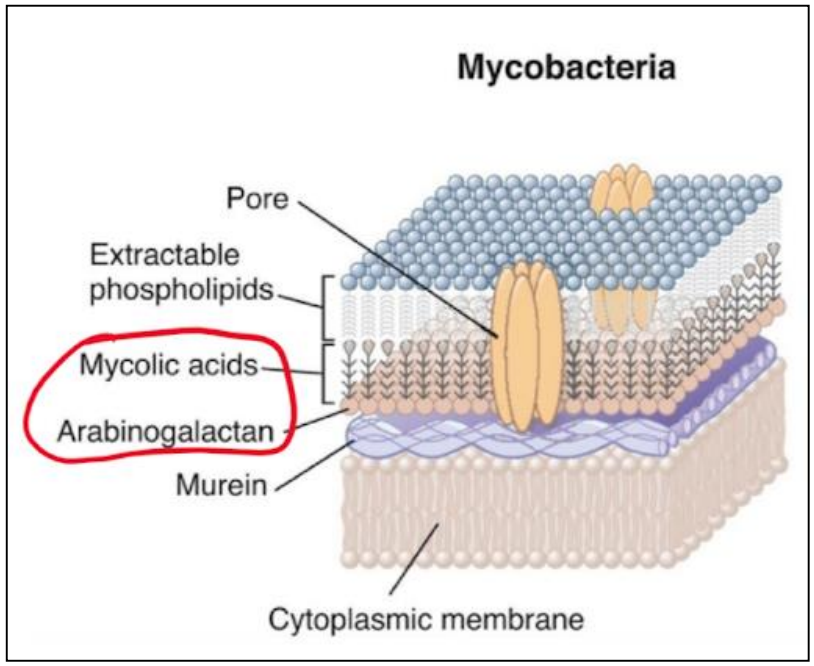
Gram-positive cell walls

Acid-fast bacteria

Atypical Gram-positive bacteria

- The petpidoglycan layer (murein) is surrounded by:

1. a layer of arabinoglycan, a polysaccharide which in turn is surrounded by
2. a layer of mycolic acid, a unique lipid.



Teichoic acids

Polymer of glycerol, or ribitol joined by phosphate groups

Found only in Gram positive bacteria

- Teichoic acids are short chain sugar-containing polymers (10-30 units long).
- There are two main types of teichoic acid:

1. Ribitol teichoic acids;
2. Glycerol teichoic acids (more widespread).

- There are two classes of teichoic acids:

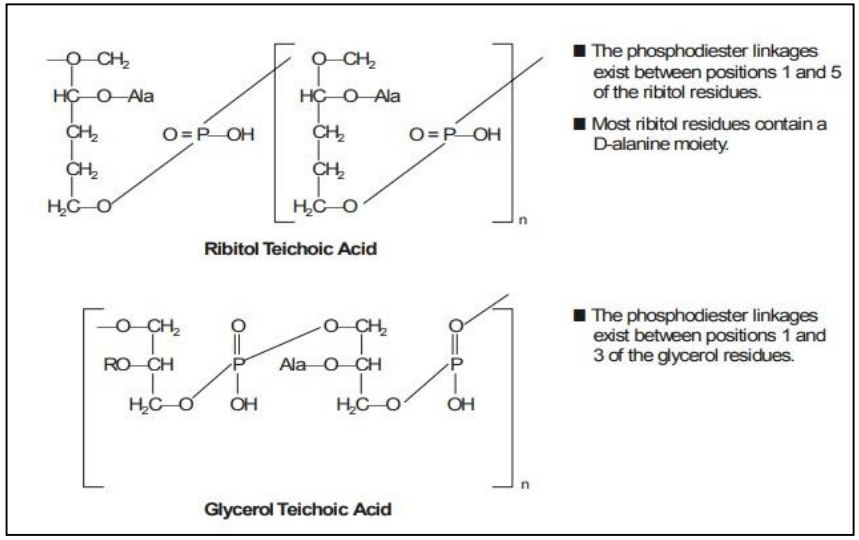
1. Wall teichoic acids (WTAs), are covalently linked to the peptidoglycan, whereas the
2. Lipoteichoic acids (LTAs), attach to the cytoplasmic membrane.

Teichoic acids

There are two types of teichoic acids:

Ribitol teichoic acids and Glycerol teichoic acids

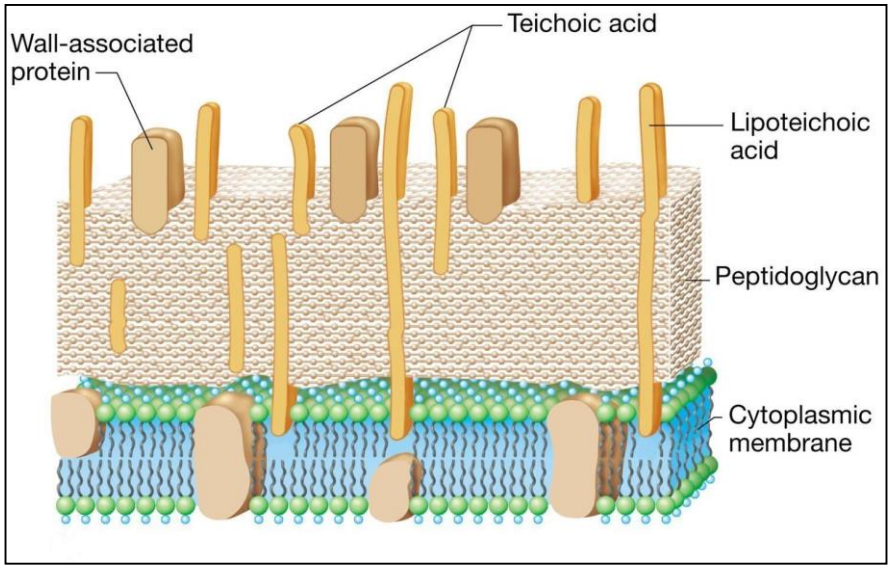
- Teichoic acids are sugar-containing polymers of:
  1. Glycerol, or
  2. ribitol joined by phosphate groups.
- Amino acids, such as D-alanine are attached.

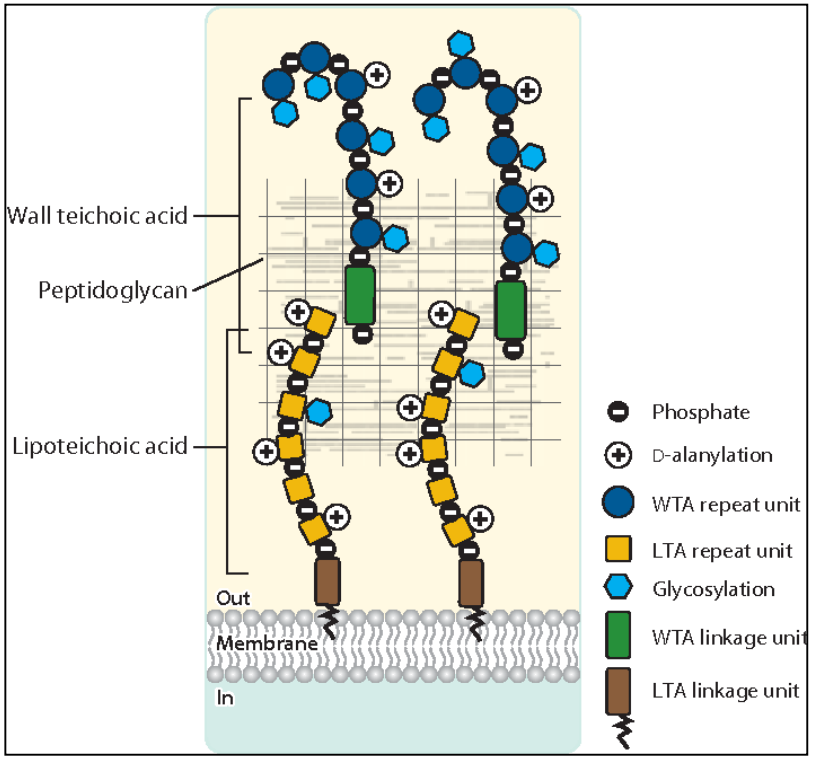


Teichoic acids

There are two classes of teichoic acids

Wall teichoic and Lipoteichoic acids





Tankeshwar,2013;..

Teichoic acids
Function

1. The main function of teichoic acids as sugar-containing polymers is to provide rigidity to the cell-wall by attracting cations such as magnesium (Mg^{2+}) and sodium (Na^{+}).
2. Teichoic acids serve as an attachment site for some parasites and bacteriophages.
3. Wall teichoic acids (WTAs) assist in maintaining cell shape and play a role in proper cell division.
4. Lipoteichoic acids (LTAs) helps some Gram positive bacteria to infect cells and cause disease. In fact, Lipoteichoic acids (LTAs), acts similar to the endotoxin of Gram-negative bacteria.

Gram-positive cell walls
Actinomycetes

Actinomycetes vs. Fungi

Atypical Gram-positive cell walls
Actinomycetes

- Because of their well developed morphological (hyphae) and cultural characteristics, actinomycetes have been considered as a group, well separated from other common bacteria.

Atypical Gram-positive cell walls
Actinomycetes

1. One distinctive feature of this group is the presence of several different peptidoglycans in the cell wall.
2. Teichuronic acid and polysaccharides.

- The peptidoglycan of actinomycetes consists of glycan (polysaccharides) chains of alternating N-acetyl-d-glucosamine (NAG) and N-acetyl-dmuramic acid (NAM) and diaminopimelic acid (DAP), which is unique in prokaryotic cell walls.
- Teichoic and teichuronic acid are chemically bonded to peptidoglycan.

Chamikara,2016

Gram-positive cell walls
Actinomycetes

- The chemical composition of their cell wall is similar to that of gram positive bacteria but because of their well developed morphological (hyphae) and cultural characteristics, actinomycetes have been considered as a group, well separated from other common bacteria.

Chamikara,2016

Gram-positive cell walls
Actinomycetes

- They do not have chitin and cellulose which are commonly found in the cell walls of fungi.
- The major components of the peptidoglycan (PG) of *Actinomyces viscosus* are N-acetylglucosamine, N-acetylmuramic acid, alanine, glutamic acid and lysine in the ratio 1:1:4:4:4, respectively.
- Cell wall teichoic acids was found in members of newly recognized genera of the order *Actinomycetales*.

Reid and Evans,1974; Streshinskaya *et al.*,2002;..

Cell wall structure
Actinomycetes vs. Fungi

Genera and species	Significant cell wall components		Acid products from fermentation[3]
	Amino acids[1]	Carbohydrates[2]	
Actinobaculum suis	Ala, Lys, D-Glu	Rham	Form, Ac
Actinomyces bovis	Ala, Lys, D-Asp	Rham, 6-Deoxyt, (Gluc)[4]	Ac, Lac, Suc
A. denticolens	Ala, Lys, Orn, D-Glu	Rham	Ac, Lac, (Suc)
A. georgiae	Not known	Not known	Ac, Lact, Suc
A. gerencseriae	Ala, Lys, Orn, D-Glu	Gal, Rham	Ac, Lac, Suc
A. hordeivulneris	Ala, Lys, Orn, D-Glu	Gluc, Gal	Ac, Lact, Suc
A. howellii	Ala, Lys, Orn, D-Glu	Gluc, Rham	Ac, Lac, (Suc)
A. israelii	Ala, Lys, Orn, D-Glu	Gluc, Gal	Ac, Lac, Suc
A. meyeri	Not known	Not known	Ac, Lac, Suc
A. naeslundii	Ala, Lys, Orn, D-Glu	Rham, 6-Deoxyt,[5] (Gluc)	Ac, Lac, Suc
A. neuii	Not known	Not known	Ac, Lac, Suc
A. odontolyticus	Ala, Lys, Orn, D-Glu Glu,	Man, Rham, 6-Deoxyt	Ac, Lac, Suc
A. radingae	Ala, Lys, Orn,[6] D-Glu	Not known	Lac, Suc
A. slackii	Ala, Lys, Orn, D-Glu	Gluc, Rham, (Gal)	Ac, Lac, (Suc)
A. turicensis	Ala, Lys, Orn, D-Glu	Not known	Lac, Suc
A. viscosus	Ala, Lys, Orn, D-Glu	Gluc, Rham, 6-Deoxyt, (Man, Gal)	Ac, Lac, Suc
Arcanobacterium	Ala, Lys, D-Glu	Not known	Ac, Lac, (Suc)
Propionibacterium	L-DAP, Gly (meso-DAP)	Gluc, Gal, Man, Rham	Ac, Prop, Suc,
Corynebacterium	(meso-DAP)	Gal, Arab	Ac, Prop, Lac, Suc
Rothia	Ala, Lys, D-Glu	Gal, Glu, Fruc	Ac, Lac, (Suc)

Reid and Evans,1974; Streshinskaya *et al.*,2002;..

A typical Gram-positive cell walls
Mycobacteria

Although most bacteria can be classified into one of these two classes, a notable exception are the mycobacteria whose cell wall has a unique organization, mainly due to the presence of arabinogalactan and mycolic acids.

A typical Gram-positive cell walls
Acid-fast bacteria

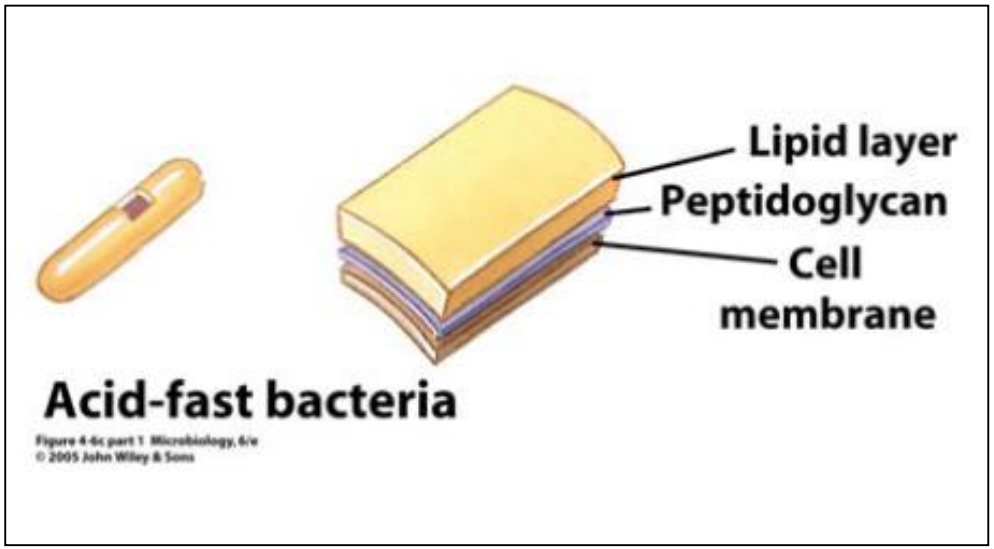
- The Gram-positive cell wall appears as dense layer typically composed of:

1. Numerous rows of peptidoglycan,
2. Molecules of lipoteichoic acid, wall teichoic acid, and
3. Surface proteins.

- Acid-fast bacteria contain up to 60% mycolic acid; helps cells survive desiccation.
- Acid fast bacteria grow very slowly.
- Most common Gram positive bacteria are acid fast negative.

Acid-fast bacteria

Cell wall is thick (not much peptidoglycan) but mainly composed of lipid



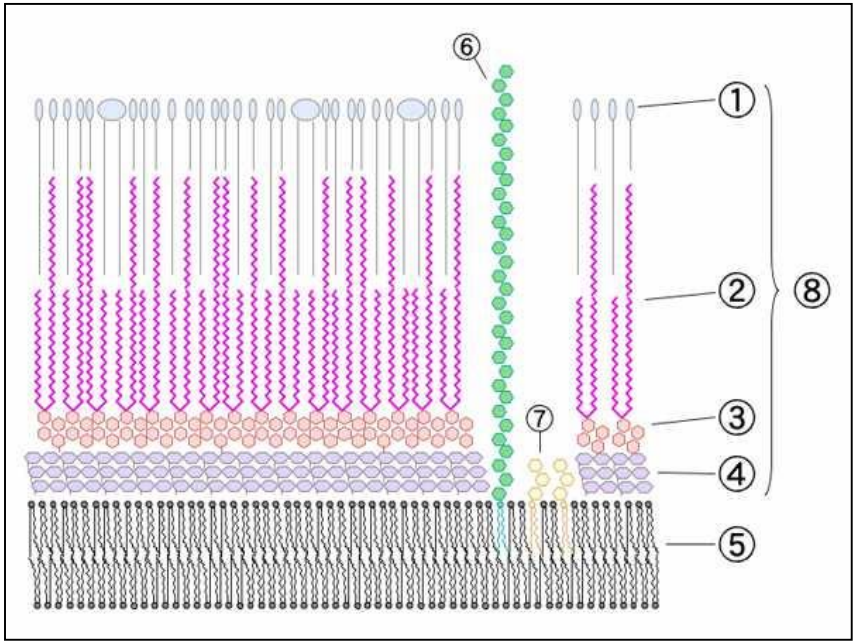
	acid-fast	nonacid-fast
carbolfuchsin	stained red	stained red -
acid alcohol	remain red	dye removed (colorless)
methylene blue	remain red	stained blue

Rogers,2006

Acid-fast bacteria

Mycobacterial cell wall

1. Outer lipids
2. Mycolic acid
3. Polysaccharides
4. Peptidoglycan
5. Plasma membrane
6. Molecules involved in evading host immune cells & function.
7. Molecules involved in evading host immune cells & function.
8. Cell wall.



Because of waxy cell wall, they can survive exposure to acids, alkalines, detergents, oxidative bursts, lysis by immune system, and many antibiotics.

Gram-positive/negative cell walls
Archaea

Pseudomurein

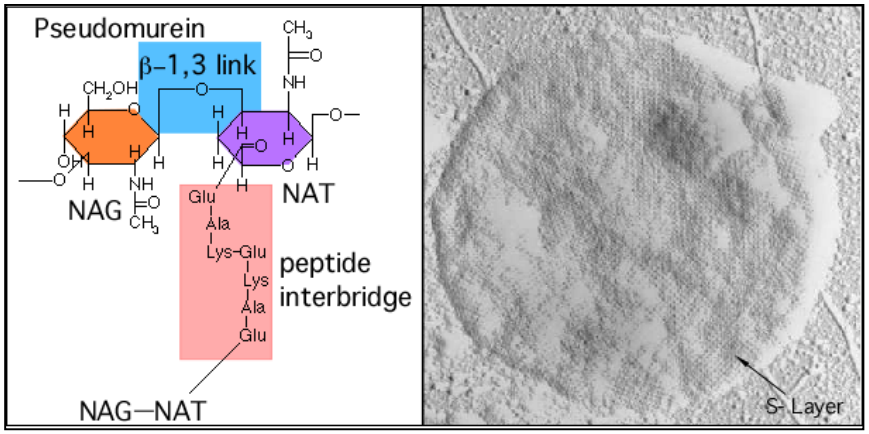
Gram-positive/negative cell walls
Archaea

- Gram staining is not used to classify archaea, since these microorganisms yield widely varying responses that do not follow their phylogenetic groups.
- Archaeal cell walls have a wide range of cell wall composition than eubacteria.
- Their cell walls are made up of pseudomurien, means they lack peptidoglycan but resembles the same chemistry and function.
- For example cell walls of *Methanococcus jannaschii* and *Sulfolobus acidocaldarius* are made up of single S-layer.
- Whereas, like in some sp. like *Methanospirillum hungatei* have multiple layers.

Gram-positive/negative cell walls
Archaea

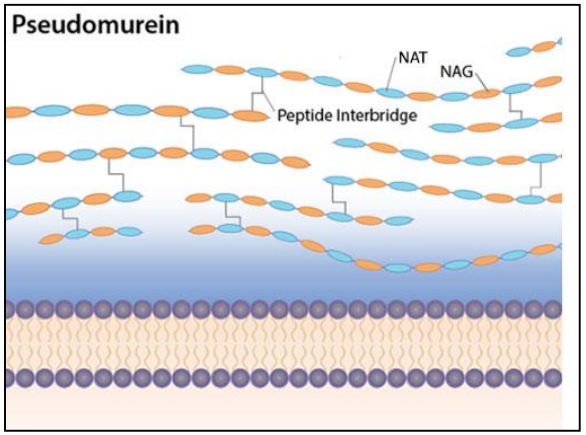
- Archaea have several different types of cell wall.

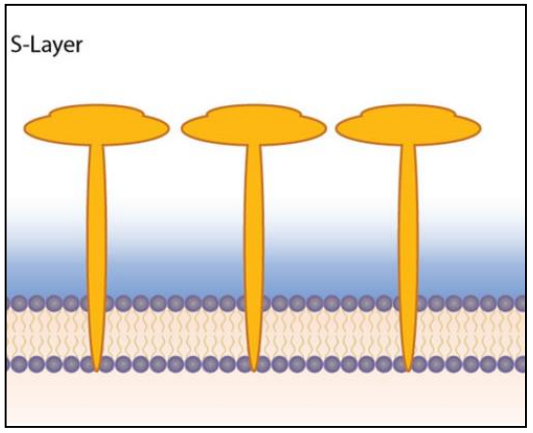
1. Some contain a structure reminiscent of peptidoglycan called pseudomurein.
2. Other will have a surface layer (S-layer) composed of repeating units of one or a few proteins, glycoproteins or sugar.



Gram-positive/negative cell walls
Archaea

- While archaea lack peptidoglycan, a few contain a substance with a similar chemical structure, known as pseudomurein.
- Instead of NAM, it contains N acetylalosaminuronic acid (NAT) linked to NAG, with peptide interbridges to increase strength.

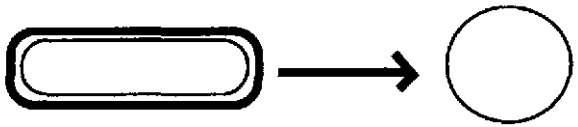




L-form bacteria

Gram+ve and Gram-ve L forms

Lister Institute where L-forms were discovered



Wall Deficient Bacteria

L-form bacteria

L-phase bacteria, L-phase variants or cell wall deficient (CWD) bacteria

- L-form bacteria also known as L-phase bacteria, L-phase variants or cell wall deficient (CWD) bacteria, are strains of bacteria that lack cell wall.
- Since they lack cell wall, they don't have a definite shape.
- They were first isolated in 1935 by by Emmy Klieneberger Nobel, who named them L-forms after the Lister Institute in London where she was working in cultures of *Streptobacillus monoliformis*.

Wall Deficient Bacteria

L-form bacteria

Gram staining

1. Bacterial L forms, which may arise when normal bacteria (mainly Gram negative bacteria) are subjected to an unfavorable environments.
2. Whereas in Gram positive bacteria with thick peptidoglycan layer, peptidoglycan synthesis was inactivated by action of lysozyme and penicillin, or its derivatives.

- Peptidoglycan layer in Gram positive bacteria was very sensitive to lysozyme and penicillin.
- Although L-forms can develop from Gram positive bacteria as well as from Gram negative bacteria, in a Gram stain test the L-forms always colour Gram-negative, due to the lack of a cell wall.

Wall Deficient Bacteria
L-form plant pathogenic bacteria

- There are only two plant pathogenic bacteria that are known to have an L-Phase:

1. *Agrobacterium tumefaciens* - crown gall;
2. *Erwinia carotovora* pv. *atroseptica* - black leg of potato.

- Only in *Agrobacterium tumefaciens* is the ability to cause disease retained in the L-Phase.

Bond,2011

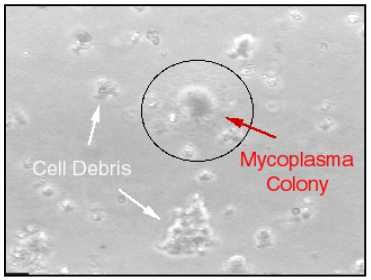
Wall Deficient Bacteria

L-form bacteria

Cell shape

- The cell wall is important for cell division which, in most bacteria, occurs by binary fission.
- The lack of cell wall in L-forms means that division is disorganized, giving rise to a variety of cell sizes, from very tiny to very big.

Wall Deficient Bacteria
L-shaped bacteria



- L-shaped bacteria look very similar to phytoplasma but are only formed under laboratory conditions and are very unstable.
- L forms colonies resemble the mycoplasmas: "fried-egg colony".
- L-forms are different from mycoplasma:

1. Mycoplasma have sterols in their membrane, the L forms may have reminiscent of cell wall but do not have sterols in their membrane.
2. DNA base composition of Mycoplasma species ranges from 23 to 36 mole per cent GC.

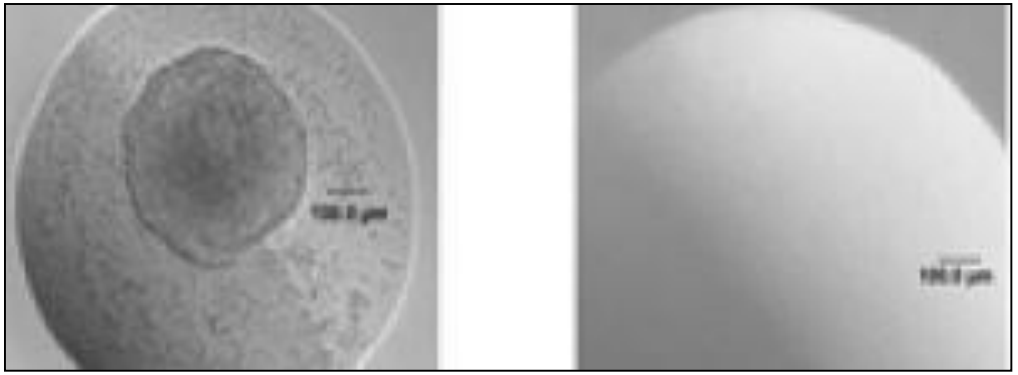
- Whereas most other bacteria and the L-forms usually have higher per cent GC content (63-64 mole per cent GC).

See also fastidious vascular bacteria (Mollicutes)

L-form bacteria

Comparison of L-form and classical colonies

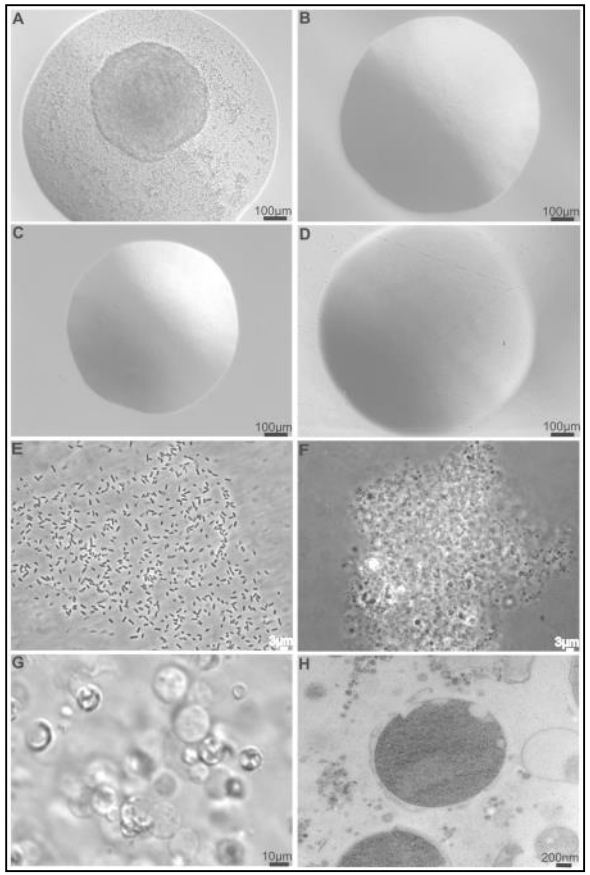
- Fried egg L-form bacteria (left) versus classic bacteria.
- L-form bacteria are capable of forming a typical "fried egg" colony, which resembles a fried egg rather than the smooth appearance of a classic bacteria colony.



L-form bacteria

Comparison of L-form *E. coli* and classical *E. coli* morphologies

A. *E. coli* colony on L-form induction media (LIM) exhibiting typical "fried egg" morphology.
B. Classical *E. coli* colony on Brain Heart Infusion (BHI) agar.
C. *E. coli* colony on BHI+10% Sucrose and 0.125% MgSO4 (BHI+sucrose control media).
D. *E. coli* penicillin G mutant colony on BHI+ Pen G.
E. Phase contrast of rod-shaped *E. coli* cells within a classical colony.
F. Phase contrast of coccoid cells within *E. coli* L-form colony agar squash.
G. Individual coccoid cells in soft agar LIM.
H. Transmission electron microscopy (TEM) photo of a coccoid cell within an L-form colony.



PLoS One,2009

Type of L-form bacteria

Class I: spheroplasts (with outer membrane can revert)
Class II protoplasts (without outer membrane cannot revert)

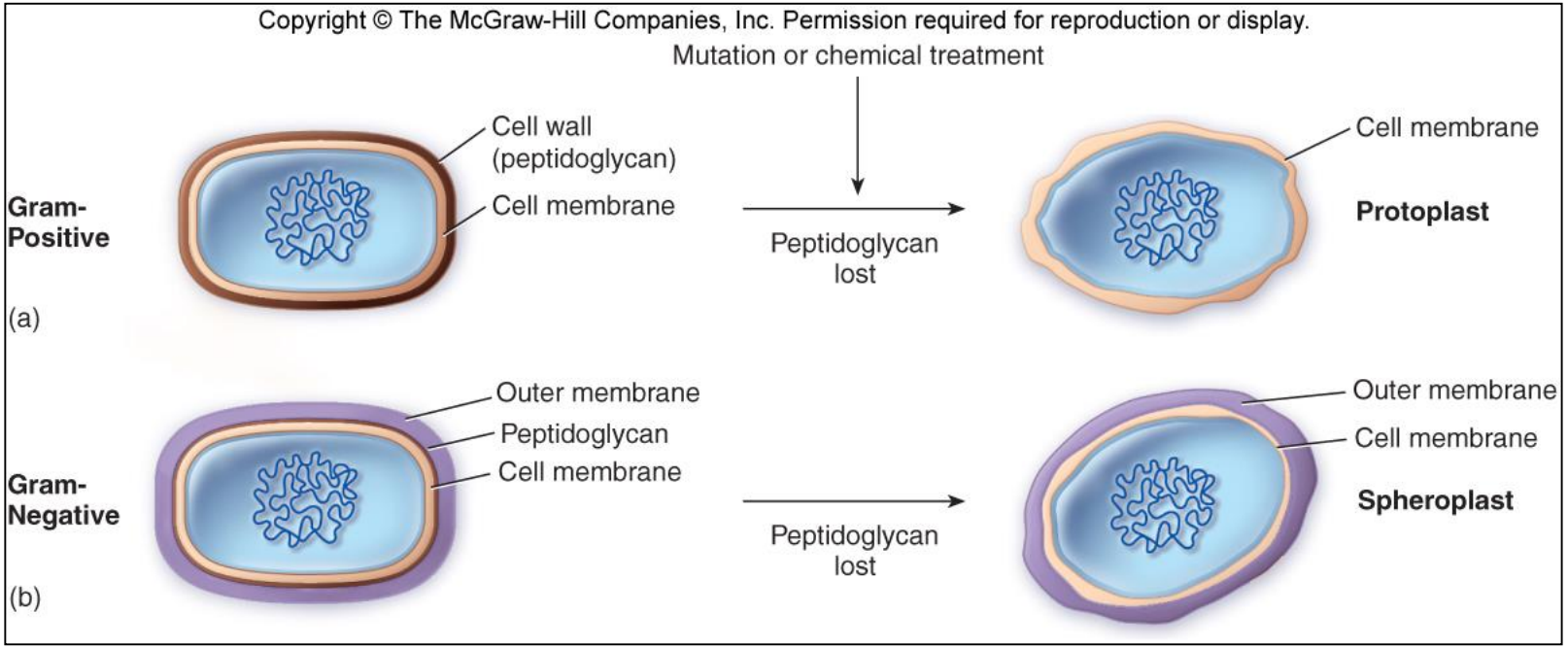
- L forms are difficult to cultivate and require medium that has right osmotic strength and low concentration of agar, inactivated serum and sucrose.
- Two types of L-forms are distinguished:

1. Class I (can revert): These are also known as unstable L-forms or spheroplasts.
   - Their cell wall only partially removed and in the absence of bactericidal compounds such as pencillin capable to revert to the original morphology, and
2. Class II (cannot revert): These are also known as stable L-forms or protoplasts.
   - Their cell wall entirely removed and unable to revert to the original bacteria.

Spheroplasts: The name stems from the fact that after a microbe's cell wall is digested, membrane tension causes the cell to acquire a characteristic spherical shape.

Type of L-form bacteria

Class I: spheroplasts (with outer membrane can revert)
Class II protoplasts (without outer membrane cannot revert)



Wall Deficient Bacteria

L- shaped bacteria

1. Spheroplasts (with outer membrane)

- G-ve+penicillin
- ✓ Inhibit peptidoglycan
- ✓ Damaged cell wall
- ✓ May multiply (capable to revert to the original morphology).

2. Protoplasts (no outer membrane)

- G-ve+lysozyme
- ✓ Removal of cell wall due to damaged peptidoglycan
- ✓ Can't multiply (unable to revert to the original morphology).

Lysozyme can't act on G-ve cell wall due to outer membrane unless treated with EDTA (ethylene diaminetetraacetic acetate) to disrupt it.

Wall Deficient Bacteria
L- shaped bacteria

- Other factors which can be induced L-form bacteria are:

1. Low light
2. Low doses of penicillin, to interfere with wall production during replication.
3. Inclusion of certain amino acids in growth medium.

Bond,2011

Cell wall lytic compounds

Making Wall-less forms

1. Lytic enzymes:

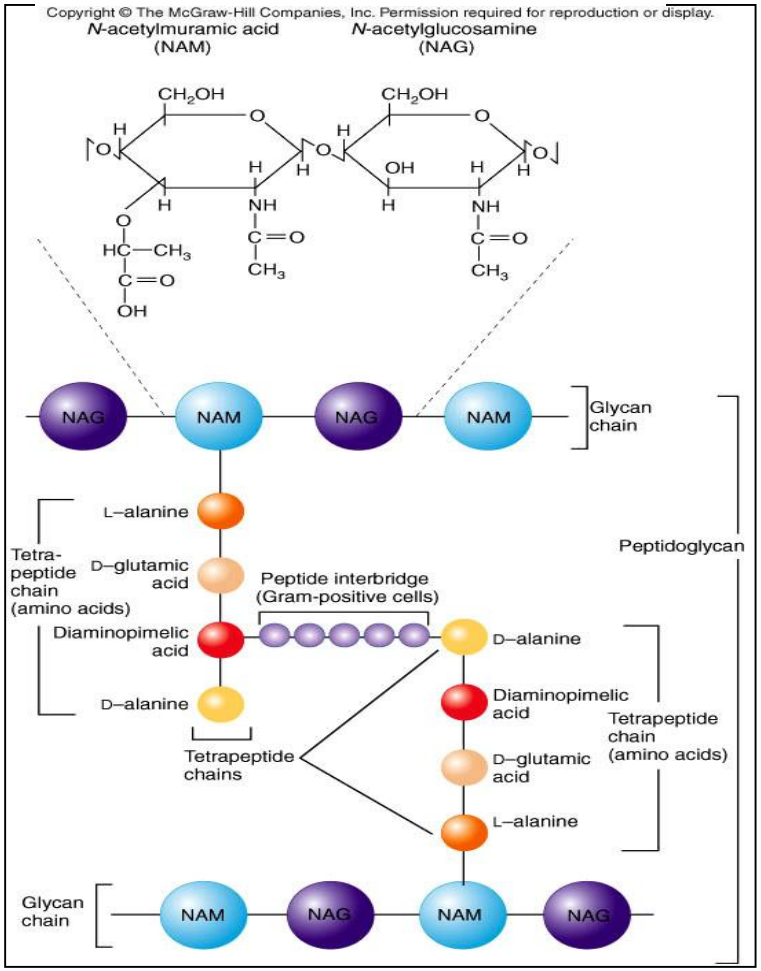
1. Lysozyme hydrolyses NAM-NAG bonds in glycan chains.
2. Endopeptidase cleaves peptide cross-links.
3. Amidases cleaves entire peptide from glycan.

2. Penicillin:

- Breaks the tetrapeptide bridge extending from NAM residues.

Peptidoglycan

NAM, NAG, amino acid bridges



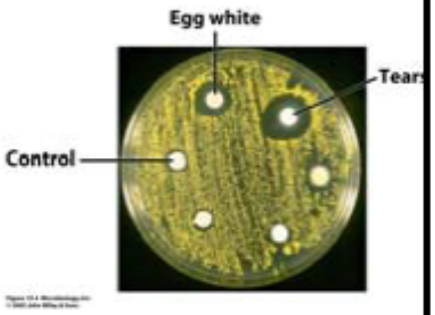
Lysozyme

Hydrolyses NAM-NAG bonds in glycan chains

- An enzyme occurring in tears, mucus, and saliva.

Cell walls & antibacterial agents

The bacterial cell wall is an excellent target for selective toxicity.

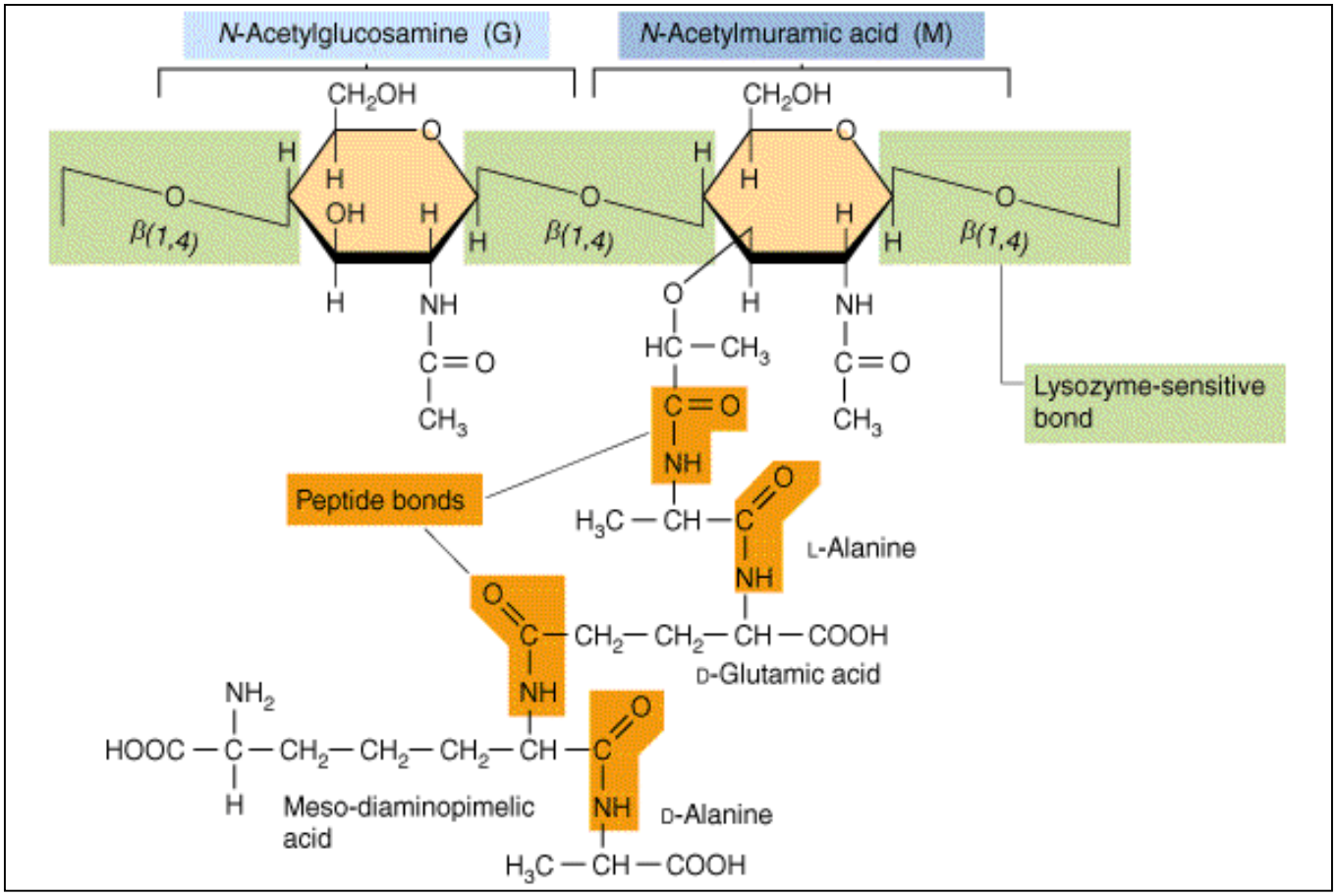


- **Lysozyme**:
  - An antibacterial **enzyme** found in tears, and a principle component of egg white
  - ★ ***Hydrolyzes the NAM-NAG glycosidic bonds of peptidoglycan***
  - Kills some Gram-positive bacteria

Rogers,2006

L-shaped bacteria

Lysozyme hydrolyses NAM-NAG bonds in glycan chains



Schlink,2010

Cell wall lytic compounds
Penicillin against transpeptidases

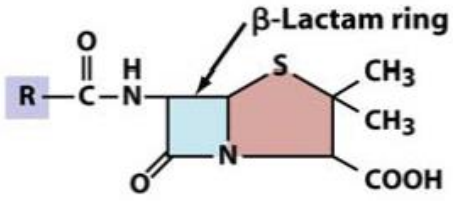
- One of the major peptidoglycan-synthesizing enzymes is transpeptidases.
- Transpeptidase reaction involves binding of the enzyme (Enzyme-OH) to the D-Ala-D-Ala end of the chain.
- Penicillin G, is one of many different types of β-lactam blocks cell wall formation and kill bacteria.
- β-lactam antibiotics resembles the D-Ala-D-Ala end of the peptide to which the transpeptidase enzyme binds.
- This structural similarity facilitates β-lactam antibiotics bind to transpeptidases and blocks the enzyme reaction.

Penicillin

β-lactam vs β-lactamases

β-Lactam ring

•Found in all natural & semisynthetic penicillins (and cephalosporins)
•Crucial to activity of these antibiotics

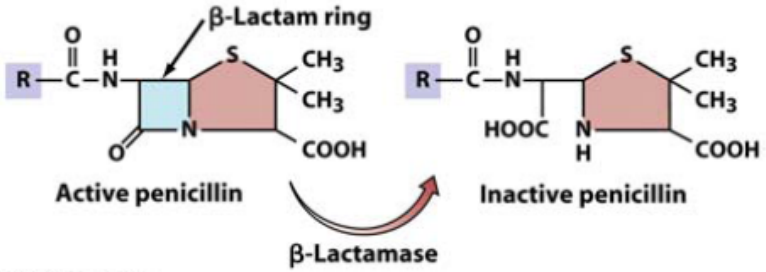


Active penicillin

Figure 13-7 Microbiology, 6/e
© 2005 John Wiley & Sons

★ β-Lactamases

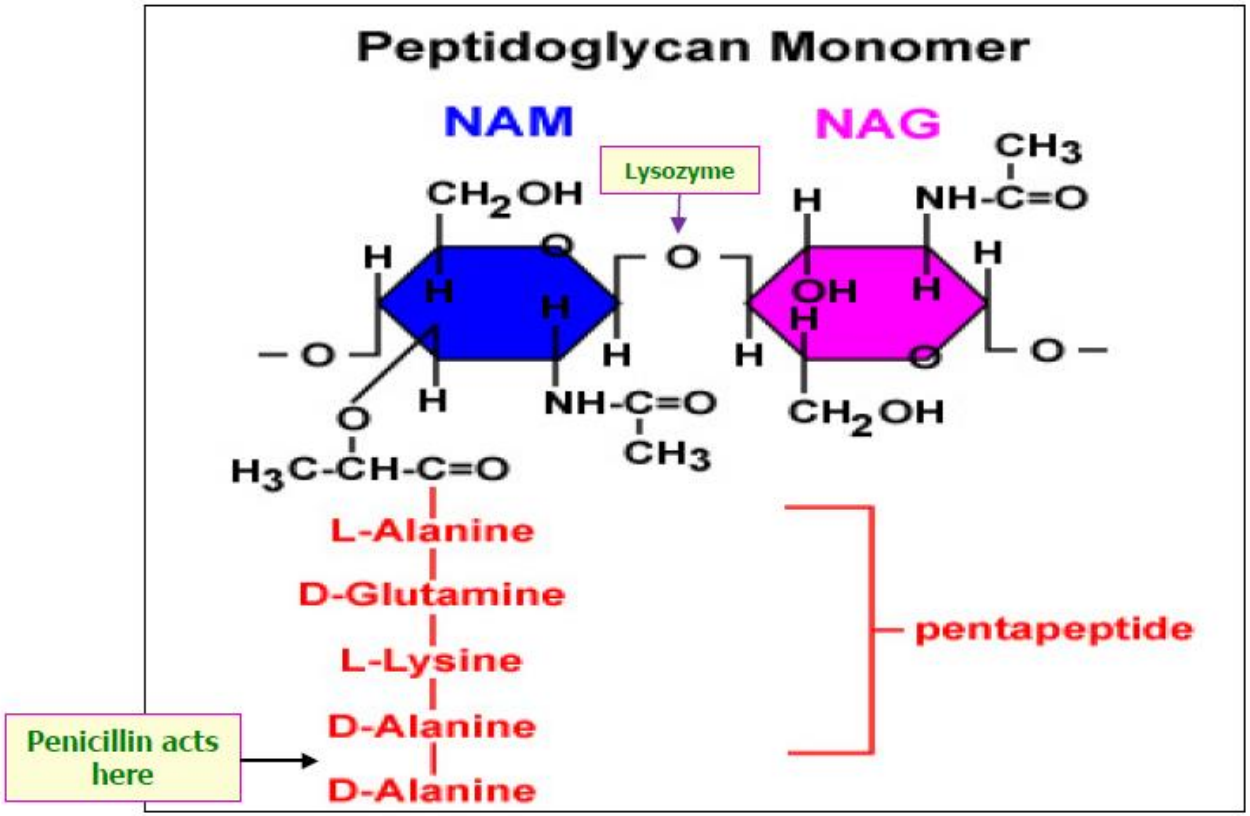
- Many mechanisms for resistance exist
- Resistance to penicillins often due to production of β-Lactamases
  - Enzymes which hydrolyze the crucial ring structure



Rogers,2006

L-shaped bacteria

Gram-positive bacteria are sensitive to Penicillin and lysozymes



Penicillin breaks the tetrapeptide bridge extending from NAM residues. A few Gram-negative bacteria are also sensitive to natural penicillins.

L-shaped bacteria

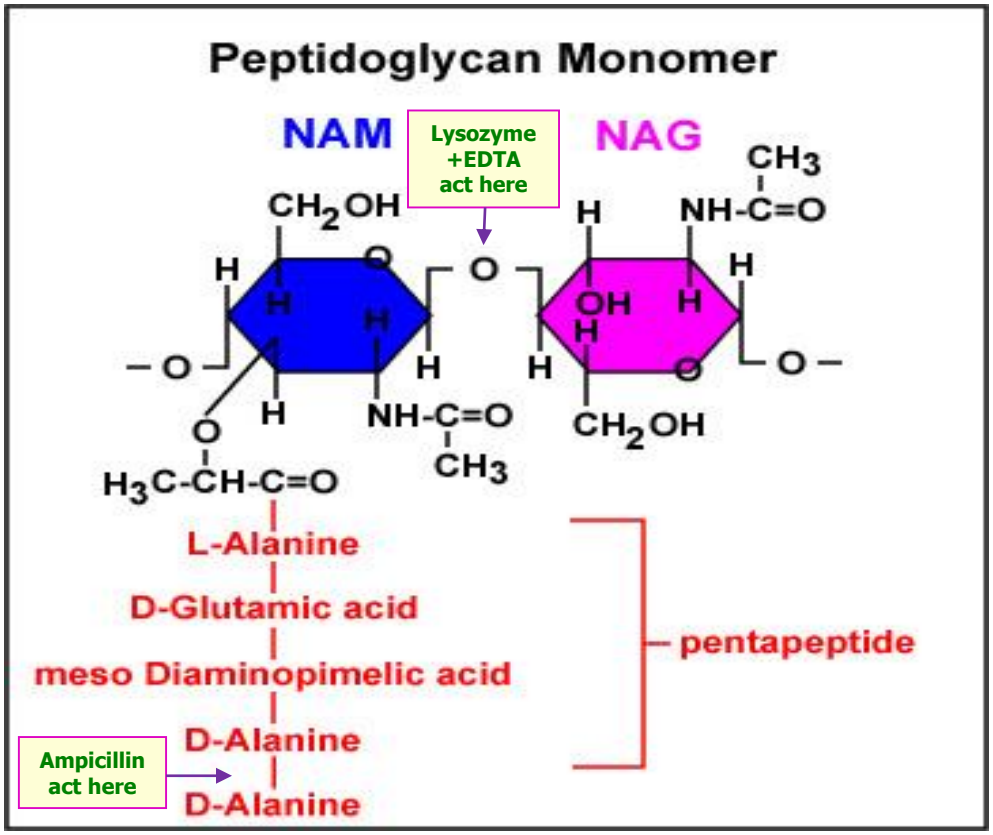
Gram-negative bacteria are sensitive to ampicillin and lysozyme with EDTA

- The outer membrane provides Gram negative bacteria with resistance to lysozyme and penicillin.
- But these are sensitive to lysozyme if pretreated by some procedure that removes the outer membrane and exposes the peptidoglycan directly to the enzyme.
- In the case of Gram negative bacteria, penicillins pass across the outer membrane using porins.
- However, alternative medicinal treatments such as lysozyme with EDTA and the antibiotic ampicillin have been developed to combat the protective outer membrane of some pathogenic Gram-negative bacteria.

Solvents such as NaOH or EDTA (ethylenediaminetetraacetic acetate) were used to removed lipids from cell wall.

L-shaped bacteria

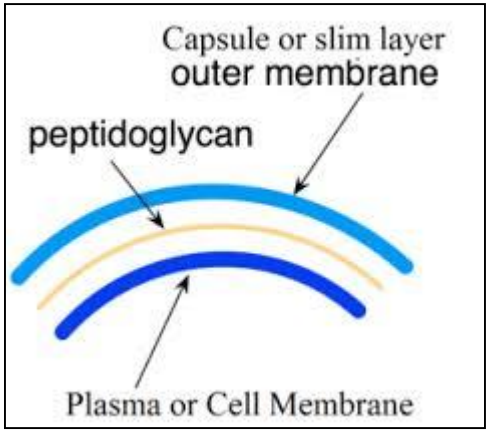
Gram-negative bacteria



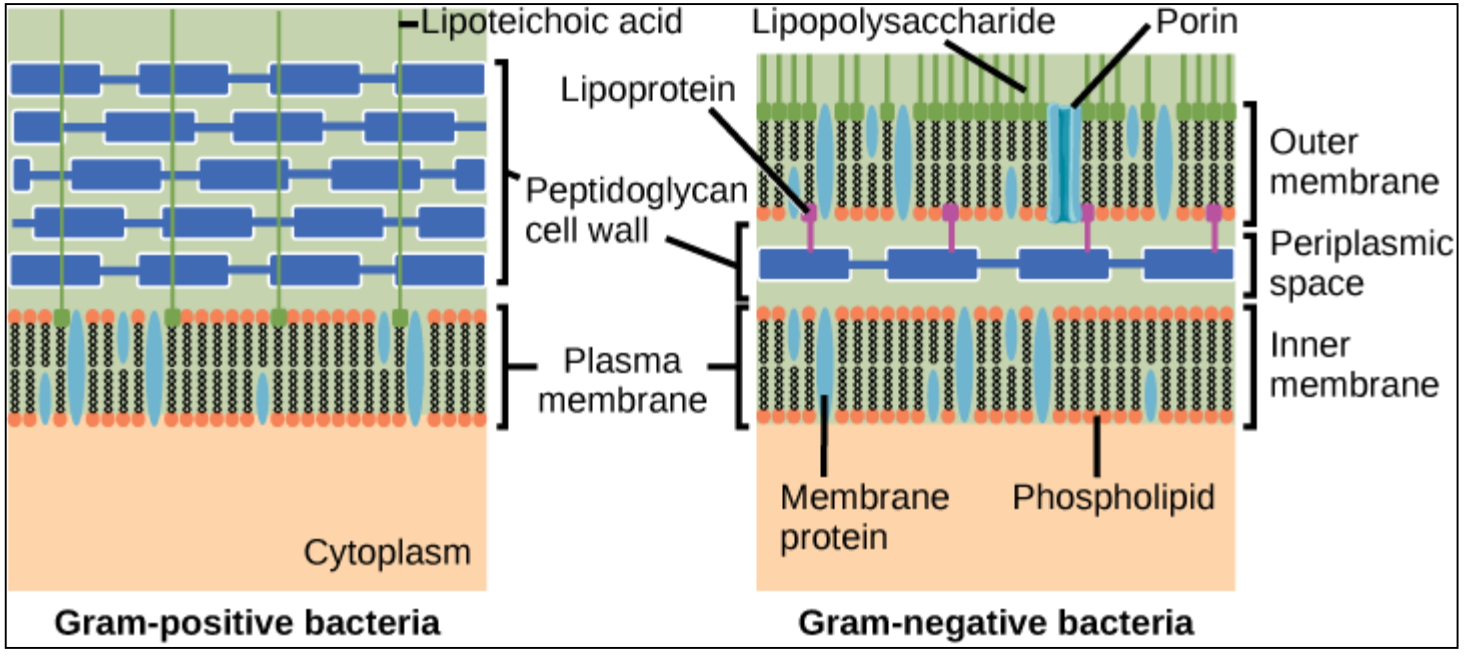
Lysozyme plus EDTA (ethylenediaminetetraacetic acetate) hydrolyses NAM-NAG bonds in glycan chains. Ampicillin breaks the tetrapeptide bridge extending from NAM residues.

Plasma membrane

Structure & Functions



Prokaryotic Cytoplasmic Membrane
Plasma membrane structure



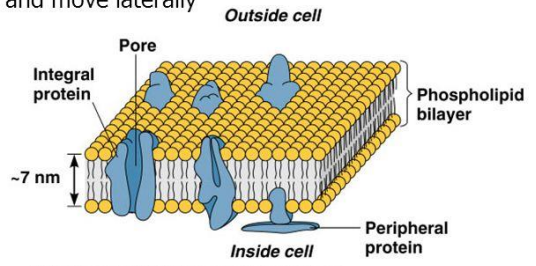
LibreTexts libraries,2019

Prokaryotic Cytoplasmic Membrane
Plasma membrane structure

- Cytoplasmic membrane (plasma membrane) surrounds the cytoplasm of the cell.
- 7.5 nm thick
- 40% lipid, 60% protein;
- Lipid: Phospholipid bilayer;
- Proteins:
  1. Integral proteins
  2. Peripheral proteins
  3. Glycoproteins
- Semipermeable.

Prokaryotic cell structures:
Plasma Membrane

- Phospholipid bilayer
- Proteins
- Fluid Mosaic Model
  - Membrane is as viscous as olive oil
  - Proteins move to function
  - Phospholipids rotate and move laterally



(b) Phospholipid bilayer of membrane Figure 4.14b

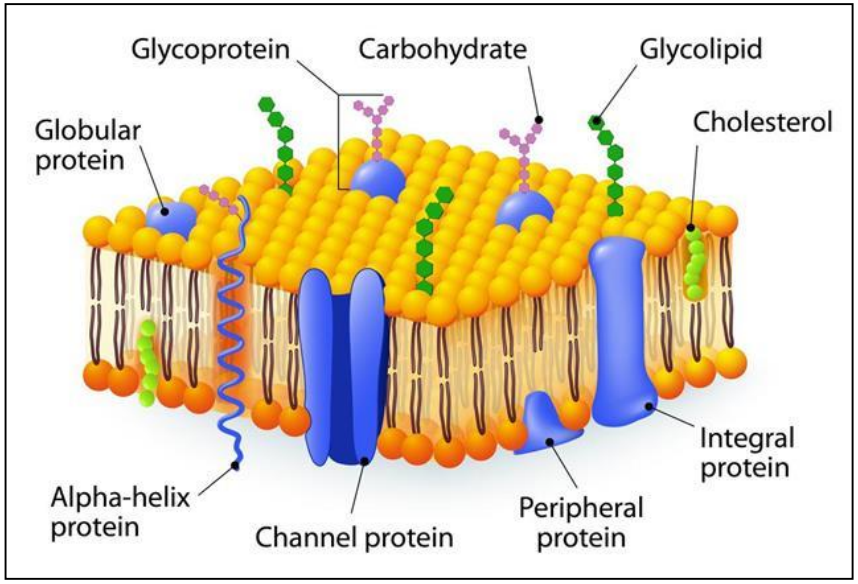
In bacteria plasma membrane act as respiratory apparatus.

Prokaryotic Cytoplasmic Membrane

Structure of a phospholipid

Integral and peripheral proteins

- Integral proteins: are trans-membrane proteins that span the lipid bilayer and have portions of the protein sticking out on both faces of the membrane.
- Peripheral proteins: are attached to the membrane indirectly, *via* protein-protein interactions.



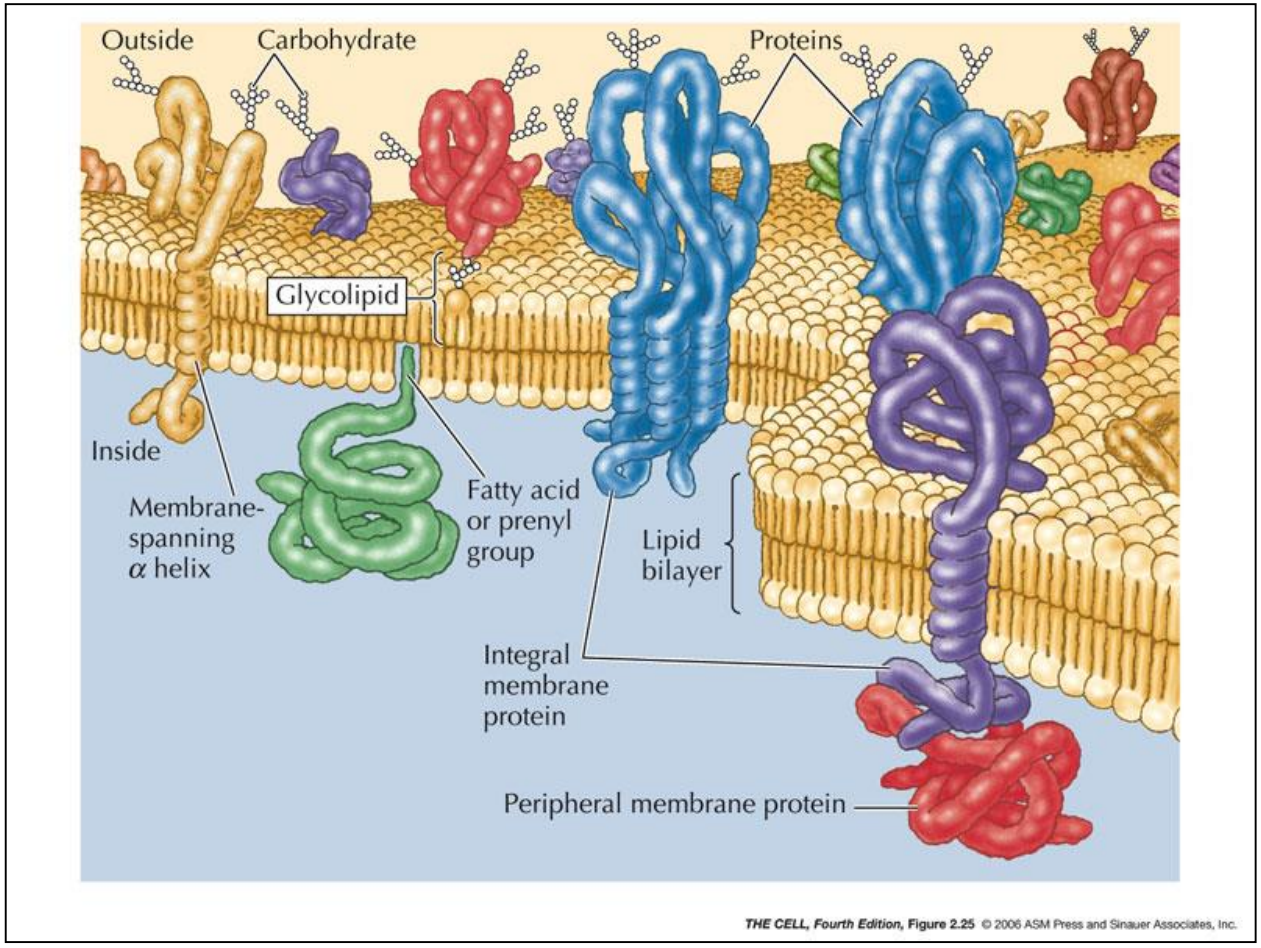
Peripheral proteins, along with integral proteins, may serve as enzymes, as structural attachments for the cytoskeleton's fibers, or as part of the cell's recognition sites.

BiologyWise; Indira Rajagopal,2009;..

Prokaryotic Cytoplasmic Membrane

Structure of a phospholipid

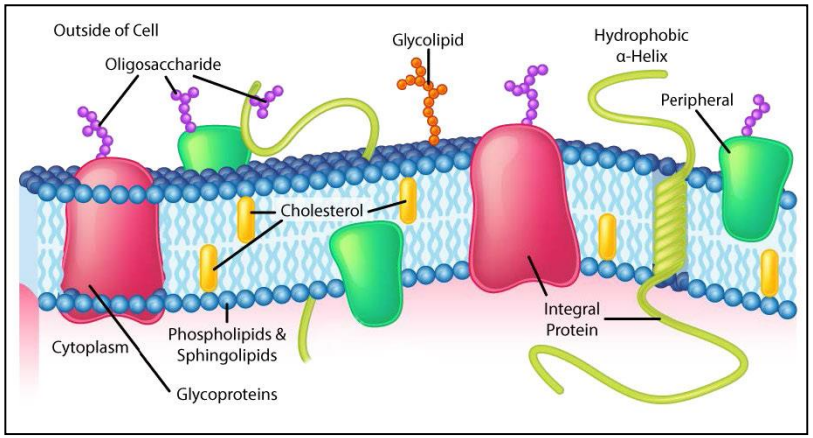
Integral and peripheral proteins



Indira Rajagopal,2009

Prokaryotic Cytoplasmic Membrane
Phospholipid structure

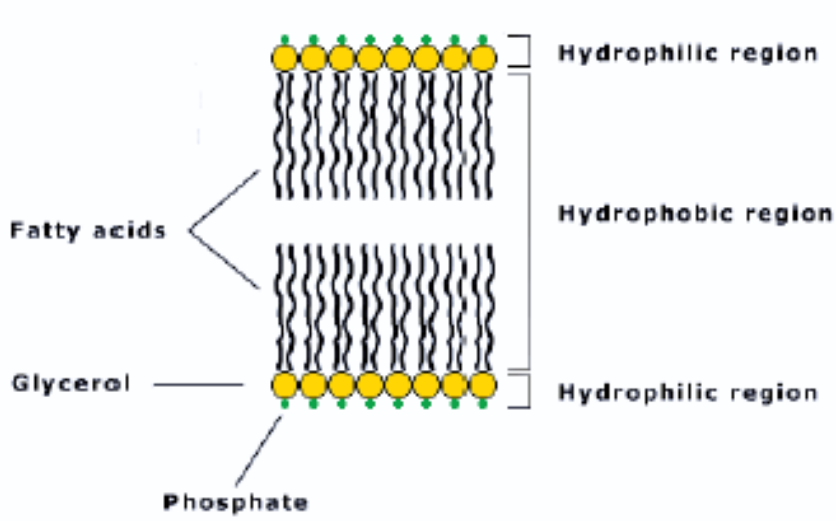
- All bacteria essentially have the same generalized cytoplasmic membrane structure.
- It consists of a lipid bilayer with associated proteins.
- There are no sterols in bacterial membranes (with the exception of mycoplasmas which acquire sterols from the host).



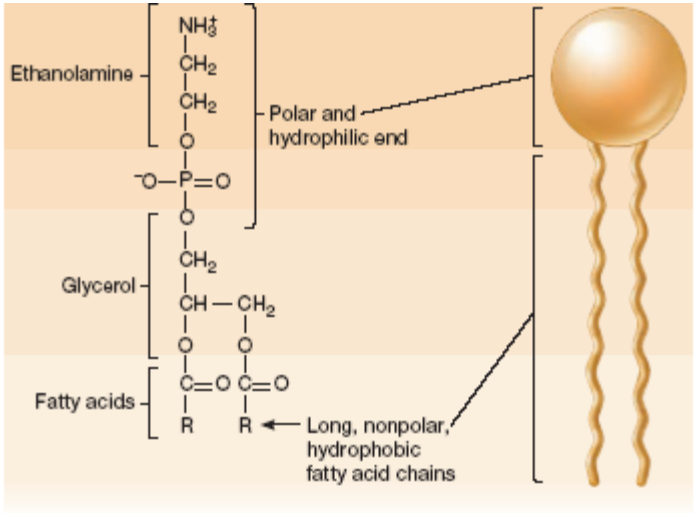
Prokaryotic Cytoplasmic Membrane
Prokaryotic plasma membrane proteins

- The proteins **inside** the plasma membrane involve in:
  - Cellular energy transformations
  - Transport of substances those involve group transfer protein (histidine containing mechanism);
  - Binding of DNA that includes DNA binding proteins;
  - Protein export which involves Docking proteins (predict orientation and position of two molecules forming a complex e.g. protein-protein or protein-ligand interactions).
- The proteins **outside** the plasma membrane involve in:
  - Cell wall biosynthesis e.g. Penicillin binding proteins
  - Flagellar motility e.g. M-proteins.
- The proteins **Within** the plasma membrane involve in:
  - Transport of substances e.g. Permeases or carriers;
  - Electron and proton transport e.g. Flavo proteins;
  - Chemotaxis e.g. methyl accepting chemotactic protein.

Structure of a phospholipid and a polar membrane lipid



Phospholipid & lipid biolayer

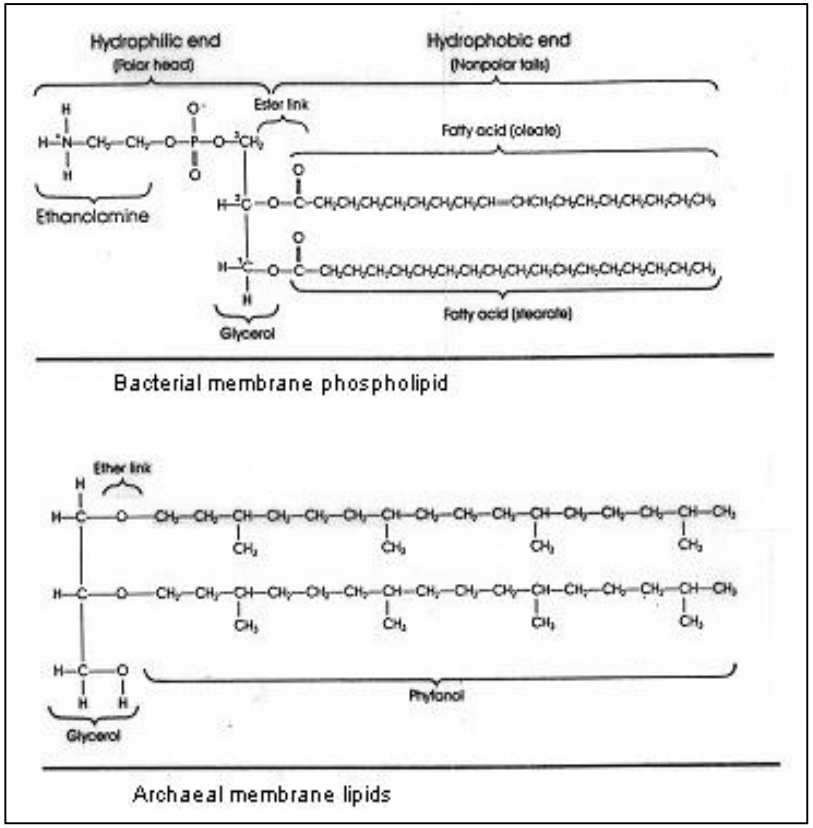


Structure of a polar membrane lipid

The R groups are long, nonpolar fatty acid chains.

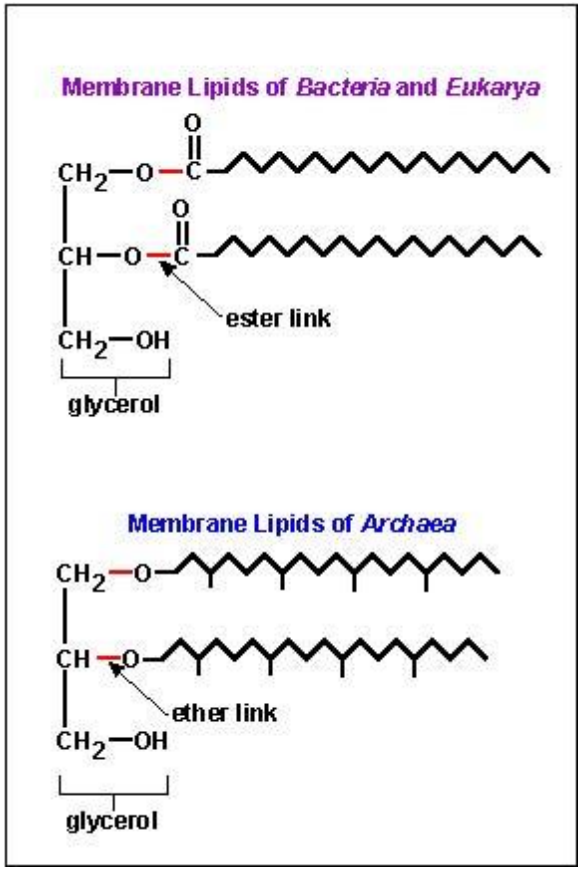
Cell membrane
Bacteria vs. archaea

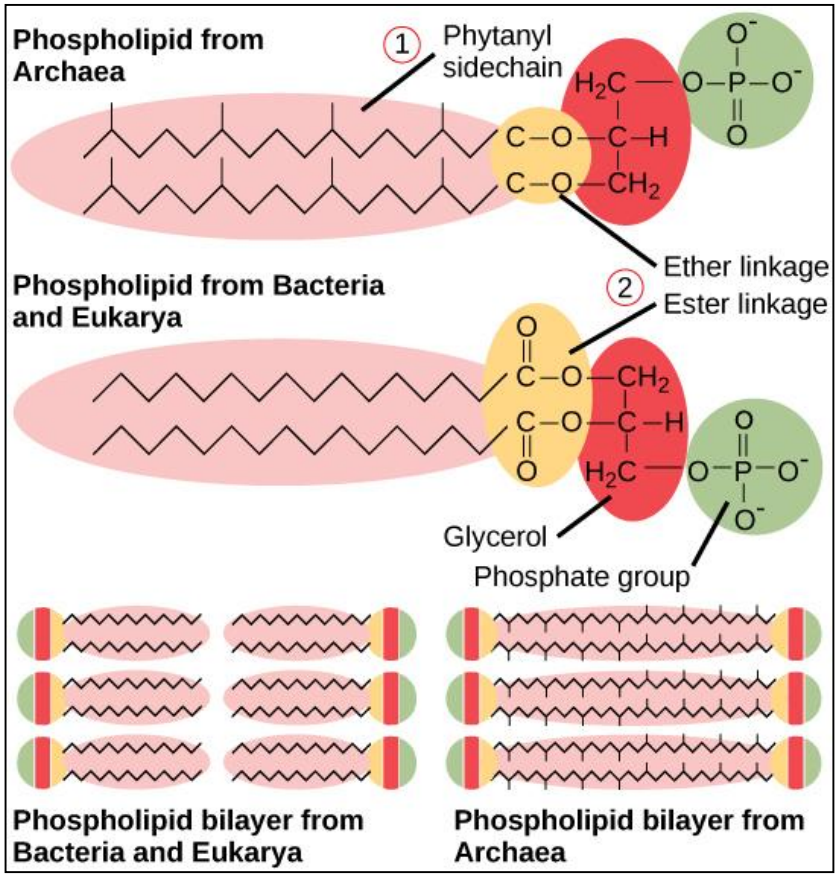
- **Bacterium**
- The R1 and R2 positions on glycerol are substituted with saturated or monounsaturated fatty acids, with ester linkages to the glyceride.
- **Archaea**
- The lipids in membranes of Archaea are diethers of glycerol and long-chain, branched, saturated hydrocarbons called isoprenoids e.g. phytanol.



Membrane lipids

In Archaea, Bacteria, and Eucarya

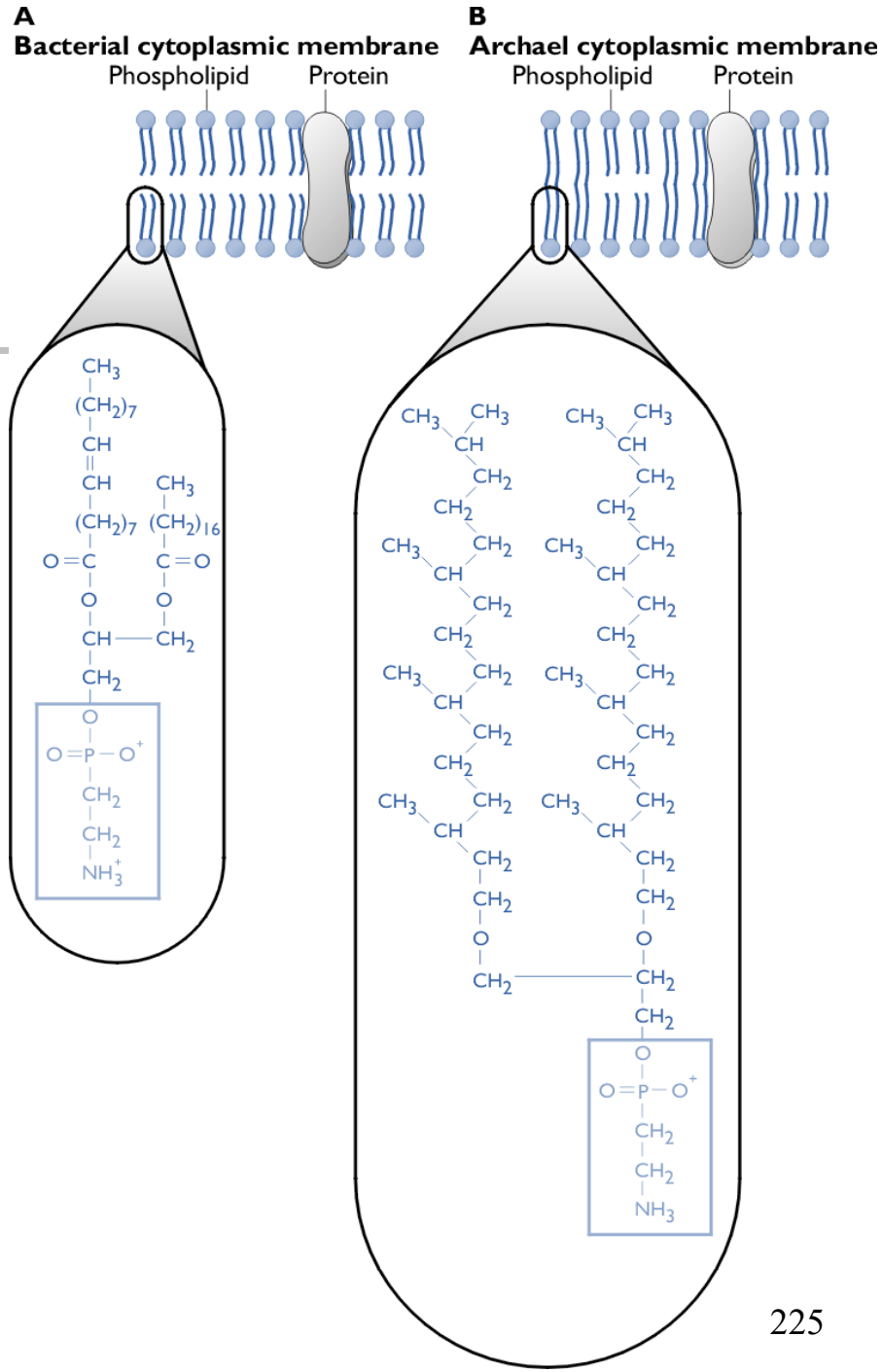




Bacterial

vs

Archaeal cell membrane



Prokaryotic Cytoplasmic Membrane

Functions of plasma membrane

Skills to develop

1. State the chemical composition and major function of the cytoplasmic membrane in bacteria.
2. Briefly describe the fluid phospholipid bilayer arrangement of biological membranes.
3. State the net flow of water when a cell is placed in an isotonic, hypertonic, or hypotonic environment and relate this to the solute concentration.
4. Define the following means of transport:
   - passive diffusion
   - osmosis
   - facilitated diffusion
   - transport through channel proteins
   - transport through uniporter
   - active transport
   - transport through antiporter
   - transport through symporter
   - the ABC transport system
   - group translocation
5. State how the antibiotic polymyxin and disinfectants such as orthophenylphenol, chlorhexidine, hexachlorophene, zephiran, and alcohol affect bacteria.
6. Define binary fission and geometric progression and relate this to bacteria being able to astronomically increase their numbers in a relatively short period of time.
7. Briefly describe the process of binary fission in bacteria, stating the functions of Par proteins, the divisome, and FtsZ proteins.

Functions of plasma membrane

- The cell membrane of bacteria is complex.
- Controls passage of substances into and out of the cell selectively permeable;
- Harvests light energy in photosynthetic prokaryotes;
- There are numerous proteins moving within or upon this layer that are primarily responsible for transport of ions, nutrients and waste across the membrane.
- **Cell membrane is the site for:**

1. Respiration (production of energy);
2. Synthesis of cell wall components;
3. DNA synthesis;
4. Transport and secretion of molecules;
5. Acting as an osmotic barrier.

Functions of plasma membrane

- It is also involved in energy transducing functions (ATP synthesis) by causing establishment of proton motive force.
- It is involved in chemotactic sensing and motility to taxis.
- It is involved in locomotion by having a part of flagellar apperatus.
- It is involved in cell wall biosynthesis.
- It is involved in attachment, replication, segregation and formation of septum and thus cell division.
- It is also involved in protein assembly and secretion.

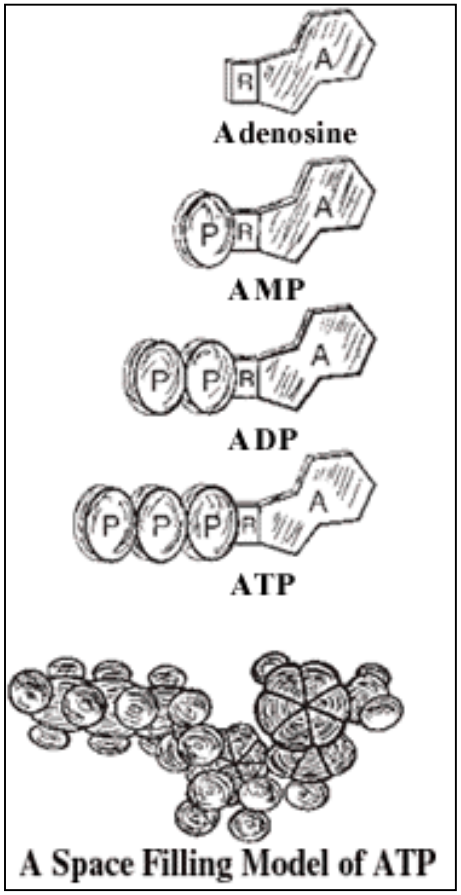
ATP (Adenosine Triphosphate)

The perfect energy currency for the cell

A simple structural formula and a space filled model of ATP

- ATP is an abbreviation for adenosine triphosphate, a complex molecule that contains the nucleoside adenosine and a tail consisting of three phosphates.
- As far as known, all organisms from the simplest bacteria to humans use ATP as their primary energy currency.
- Three ways energy can be obtained:

1. From organic chemicals;
2. From inorganic chemicals;
3. From light.

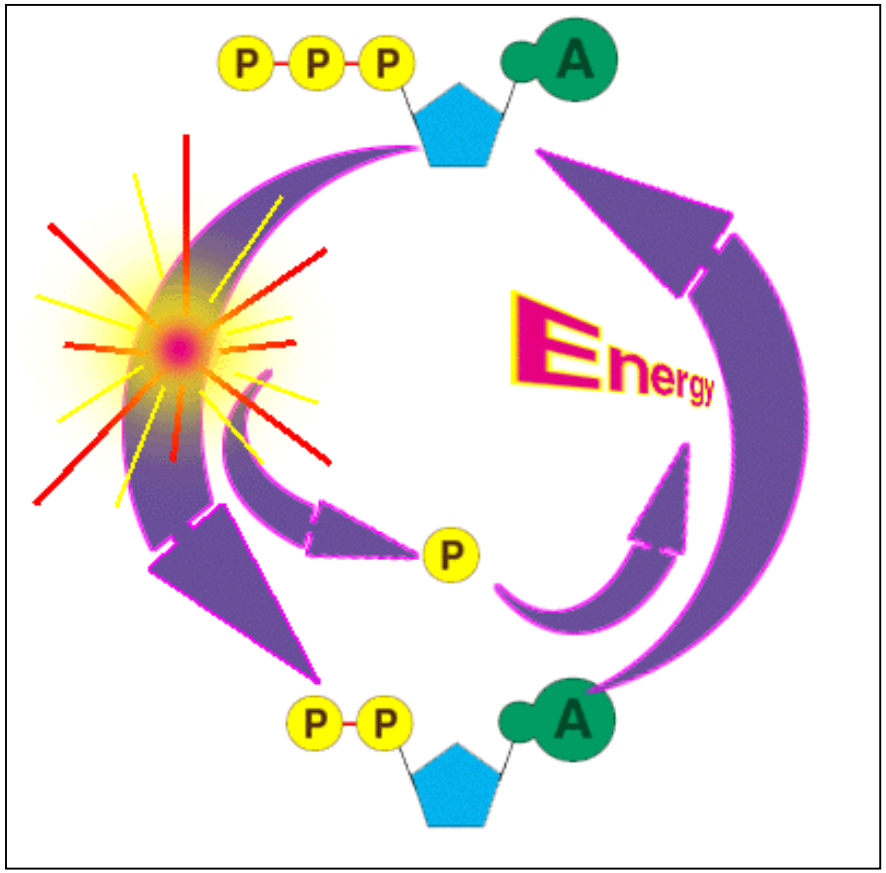


Bergman,1999

ATP

ATP production and energy storage

Energy storing nucleotide



Phosphorylation:
Organic phosphate is added to substrate.

Membrane transport

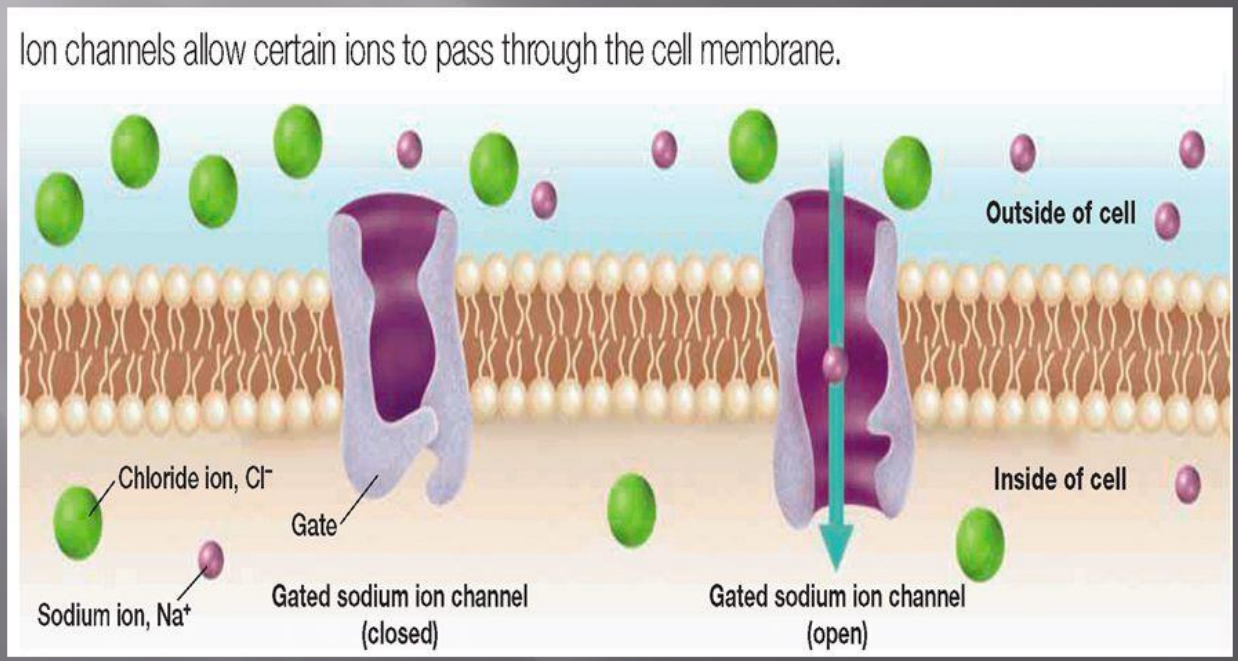
Transport systems

Section 4-1

Diffusion Through Ion Channels

Protein Ion Channel:

- protein channel through which ions can cross cell's membrane

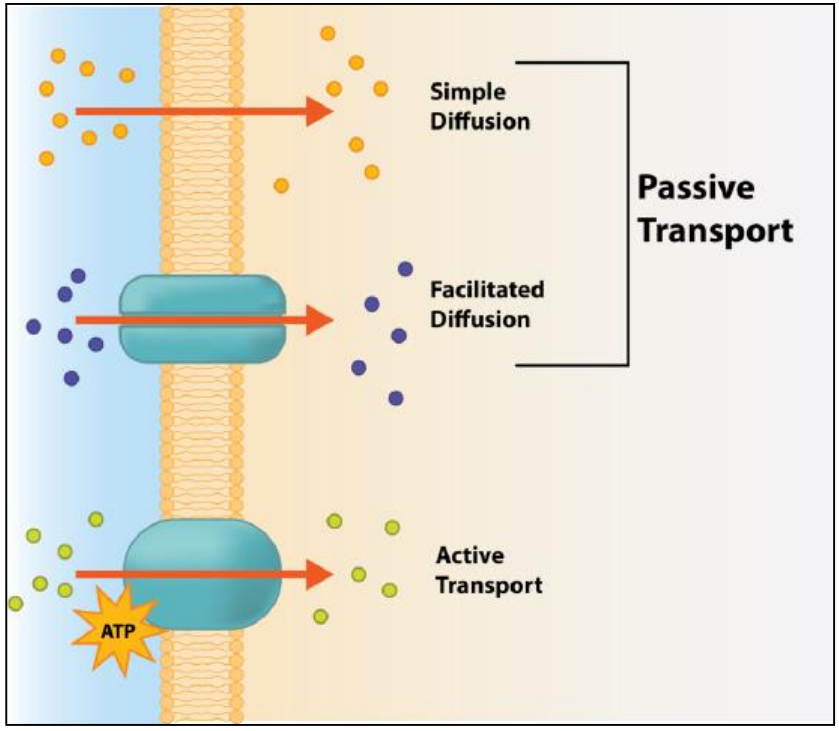


Membrane transport

Transport systems

- Molecules get through the membrane (Bacterial transport systems) through:

1. **Passive/Facilitated diffusion:** Does not require energy (ATP).
2. **Active transport:** required energy, ATP.
3. **Group translocation:** Required high-energy organic compound rather than ATP and modify the solute during its passage across the membrane.

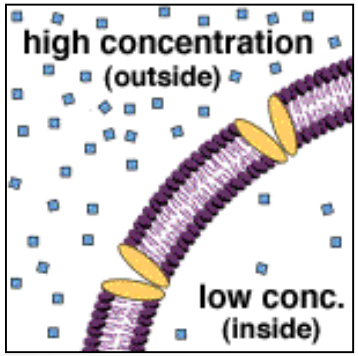


Todar,2008; LibreTexts libraries,2019

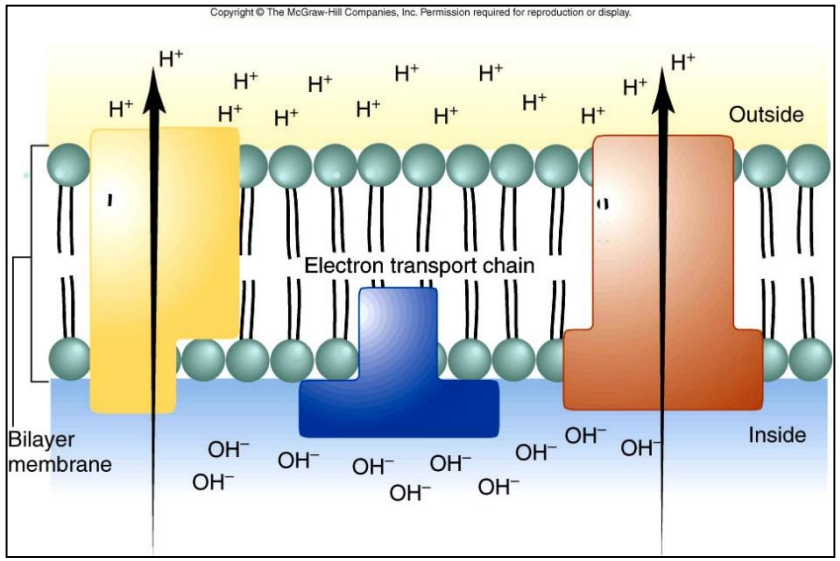
Membrane transport

Transport systems

Passive/Facilitated diffusion



- **Passive/Facilitated diffusion:** Does not require energy (ATP).
- Getting many molecules into the cell is simply a matter of opening up a protein channel of the proper size and shape.
- The molecules then move into the cell by diffusing down the concentration gradient.



Passive osmosis and diffusion: transports gases (such as O_2 and CO_2) and other small molecules and ions.

Facilitated diffusion: water, ions and molecules, specifically sugars, amino acids, fatty acids, glycerol, etc.

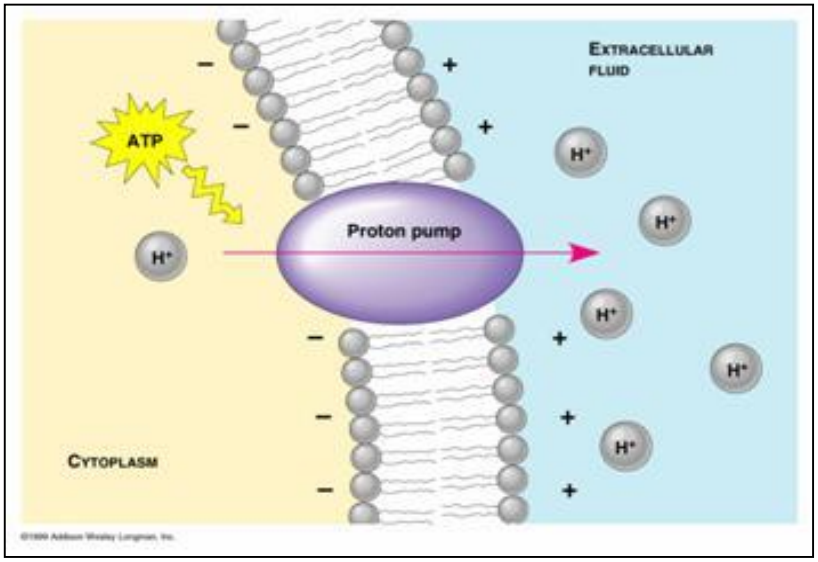
Todar,2008; LibreTexts libraries,2019

Membrane transport

Transport systems

Active transport

- **Active transport:**
- To get things to move from low to high (uphill), you need to add energy: the molecules must be pumped into the cell.
- Pumps are driven by ATP energy.



Examples include transport of large molecules (non-lipid soluble) and the sodium-potassium pump.

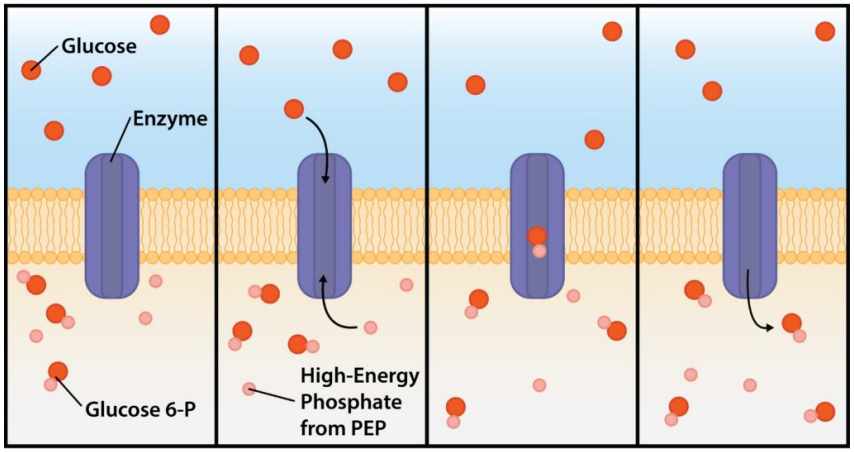
Rick Johns

Membrane transport

Transport systems

Group translocation

- **Group translocation:**
- Required high energy, REP and modify the solute during its passage across the membrane.
- **Phosphoenolpyruvate (PEP)** is a high-energy organic compound rather than ATP used by cells to transport sugars into the cell.



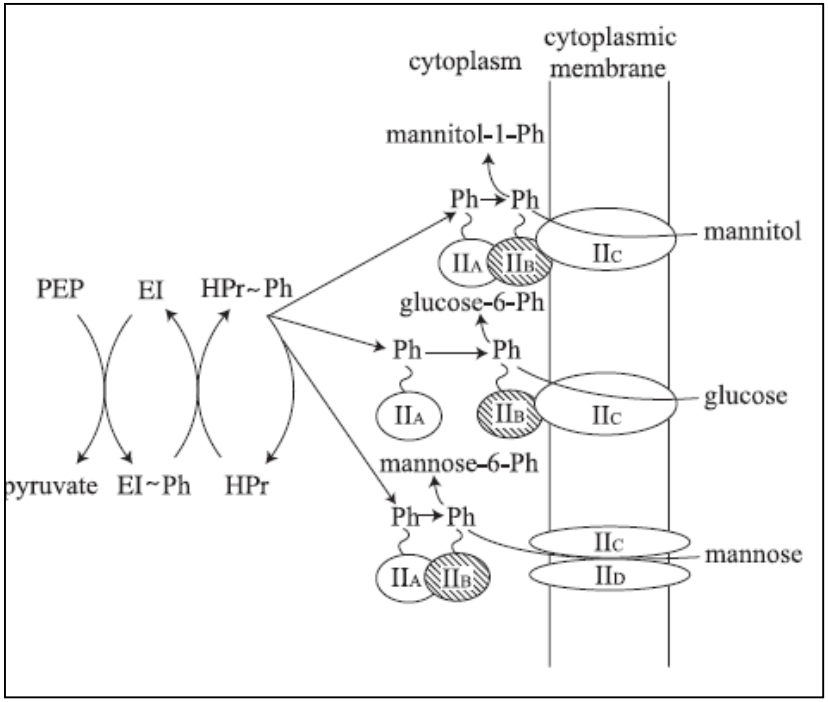
Todar,2008; LibreTexts libraries,2019

Membrane transport

Group translocation

Modification or alteration of sugars during their transport

- Sugars are transported into the cell as phosphorylated forms mediated by the phosphotransferase (PT) system.
- Phosphoenolpyruvate (PEP) serves as the phosphate donor.

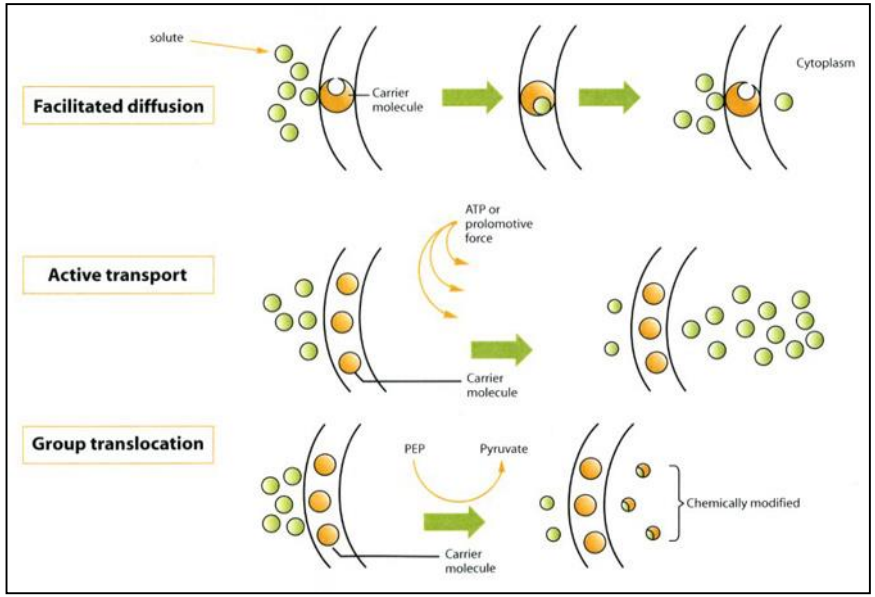


Kim and Gadd,2008

Membrane transport
Transport systems

- **Group translocation:**

1. It is a distinct type of active transport, using energy from an energy-rich organic compound that is not ATP.
2. Group translocation also differs from both simple transport and ABC transporters in that the substance being transported is chemically modified in the process.



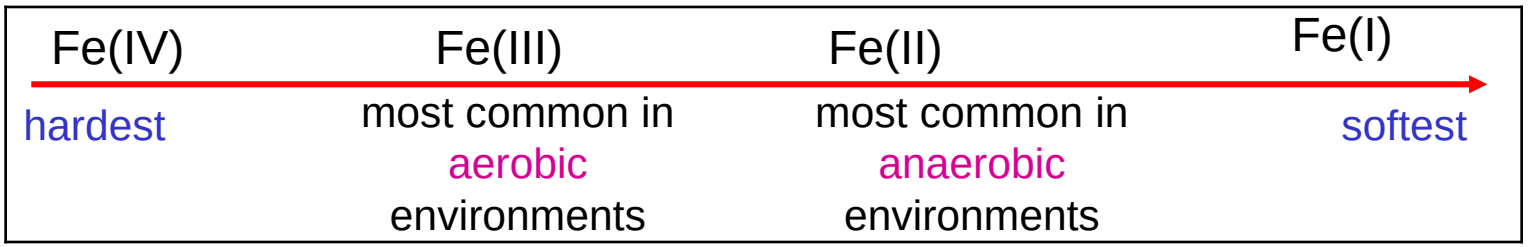
Todar,2008

Membrane transport
Ferric ion (Fe (III) uptake

- In fact iron exists in nature either as:

1. Ferrous (Fe++), or
2. Ferric (Fe+++) ions.

- Fe(III) is the predominant form of iron in aerobic and microaerobic environments and is highly insoluble.
- Ferric iron is virtually insoluble in water with a solubility of around 10^{-20} M and this is much lower than the 10^{-6} M necessary to supply adequate iron for most microbes.



DuBois,2011;AgriInfo, 2009

Membrane transport

Ferric ion (Fe (III) uptake

Siderophores

- Iron is required by microbes for the function of their cytochromes and enzymes, resulting in it being a growth-limiting micronutrient.
- However, little free iron is available in environments, due to its insolubility.
- Many bacteria have evolved siderophores, organic molecules that chelate or bind ferric iron with high affinity.

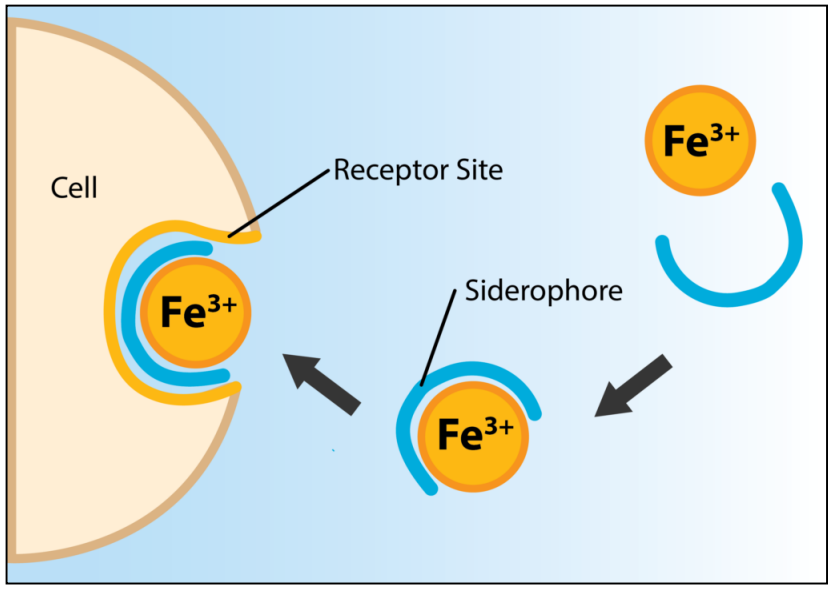
LibreTexts libraries,2019

Membrane transport

Ferric ion (Fe (III) uptake

Siderophores

- The iron-siderophore complex is then bound by a specific receptor on the outside of the cell, allowing the iron to be transported into the cell.



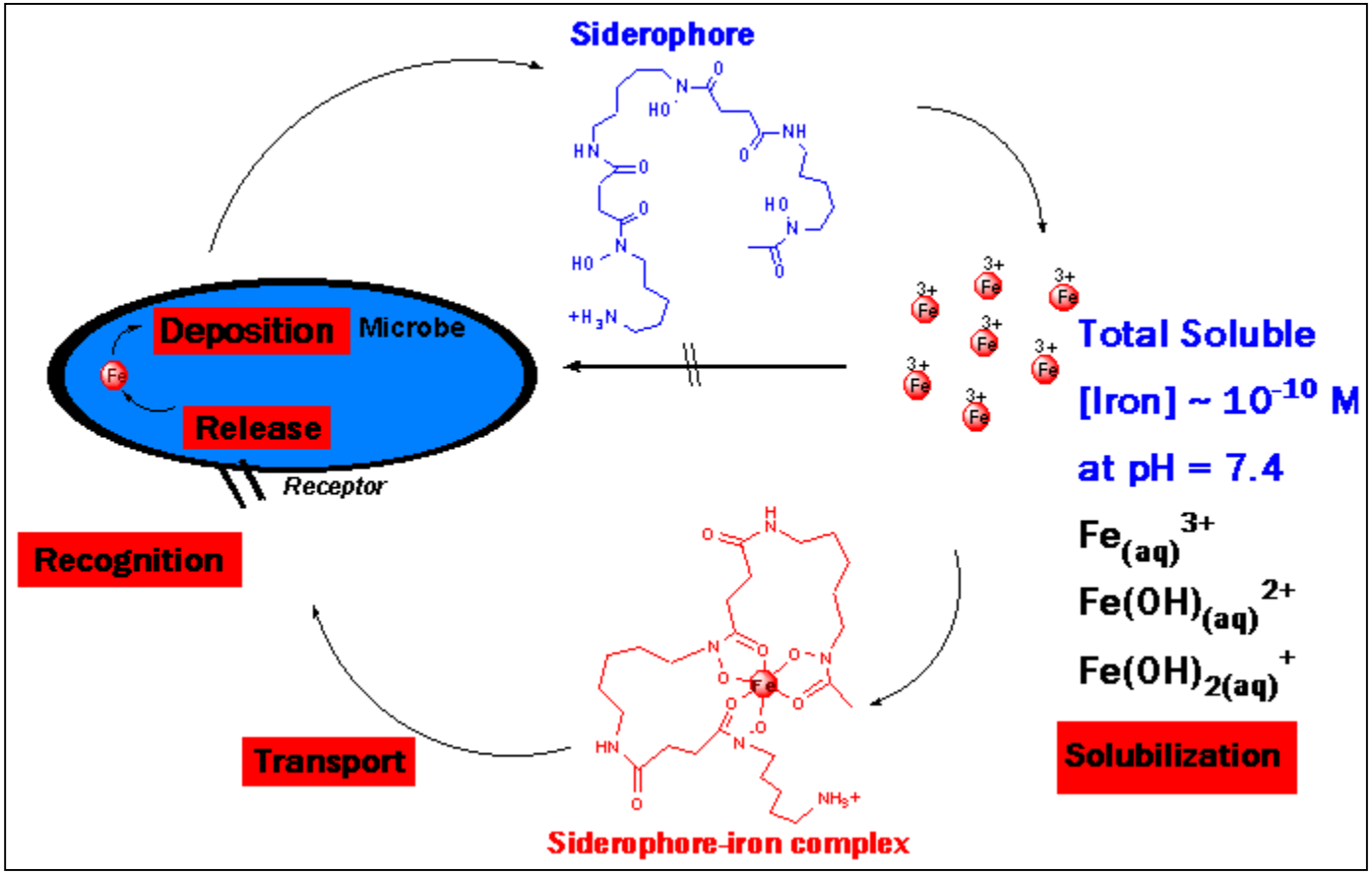
Imported the complex into the cell by an ABC transport system (ATP-binding cassette (ABC) general pathway).

LibreTexts libraries,2019;..

Membrane transport

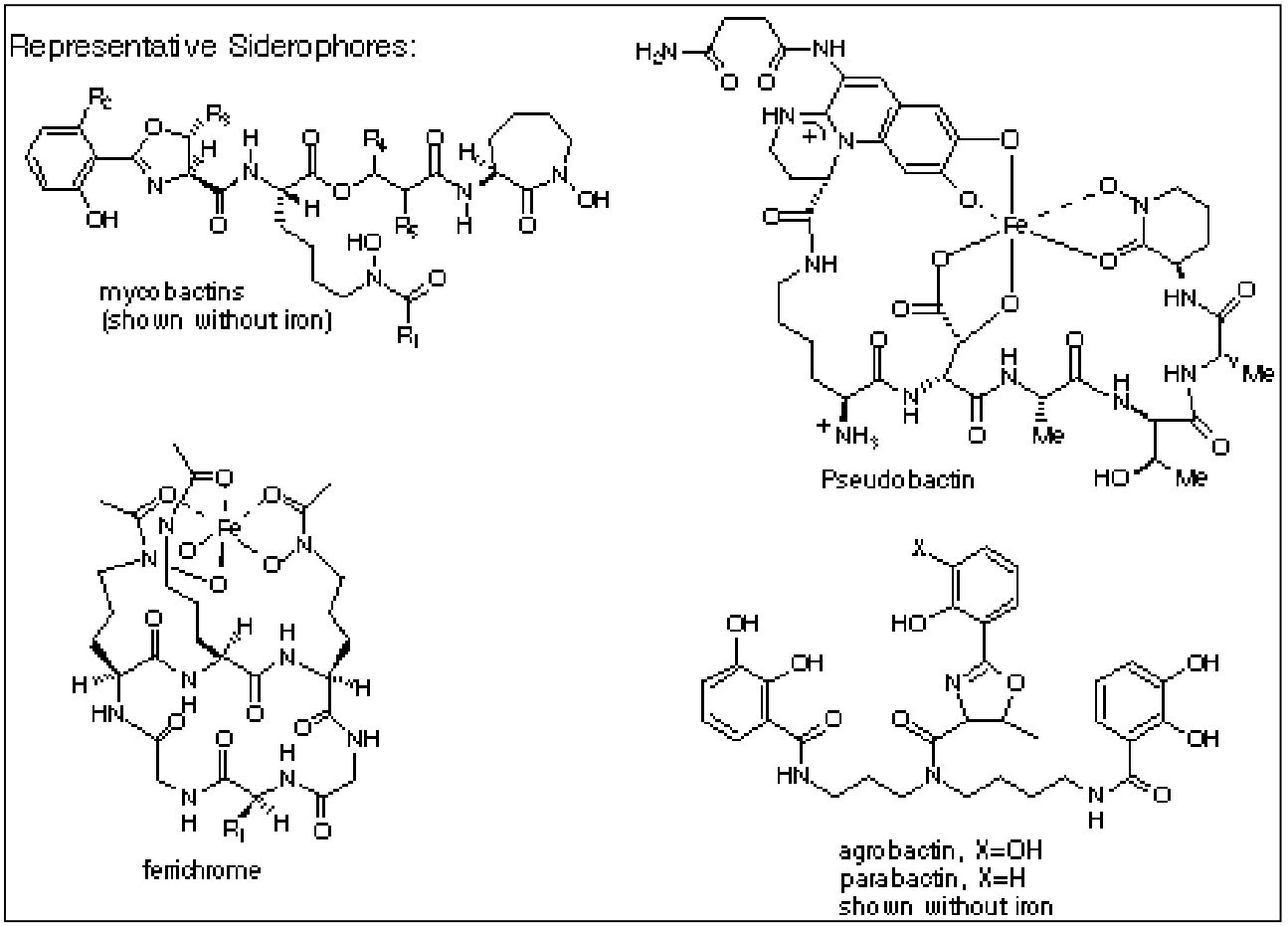
Ferric ion (Fe (III) uptake

Siderophores



Siderophore types

Structure of some microbial siderophores



DuBois,2011

Membrane transport
Cellular signaling

- Among the most sophisticated functions of the plasma membrane is its ability to transmit signals via complex proteins.
- These proteins can be receptors, which work as receivers of extracellular inputs and as activators of intracellular processes, or markers, which allow cells to recognize each other.
- Membrane receptors provide extracellular attachment sites for effectors like hormones and growth factors, which then trigger intracellular responses.
- Some viruses, such as Human Immunodeficiency Virus (HIV), can hijack these receptors to gain entry into the cells, causing infections.

LibreTexts libraries,2019

Membrane transport
Protein transport

- **Common pathways:**
- Translocation of proteins such as enzymes, toxins, etc. into and through the cytoplasmic membrane is referred to as protein transport.
- Proteins are transported through one of three mechanisms:

1. General secretory pathway (GSP),
2. ABC pathway (ATP-binding cassette (ABC) pathway),
3. Twin-arginine translocation (TAT) pathway.

- Since the outer membrane is another barrier for protein secretion in Gram-negative bacteria, several different mechanisms have been identified as protein secretion pathways.

Kim and Gadd,2008

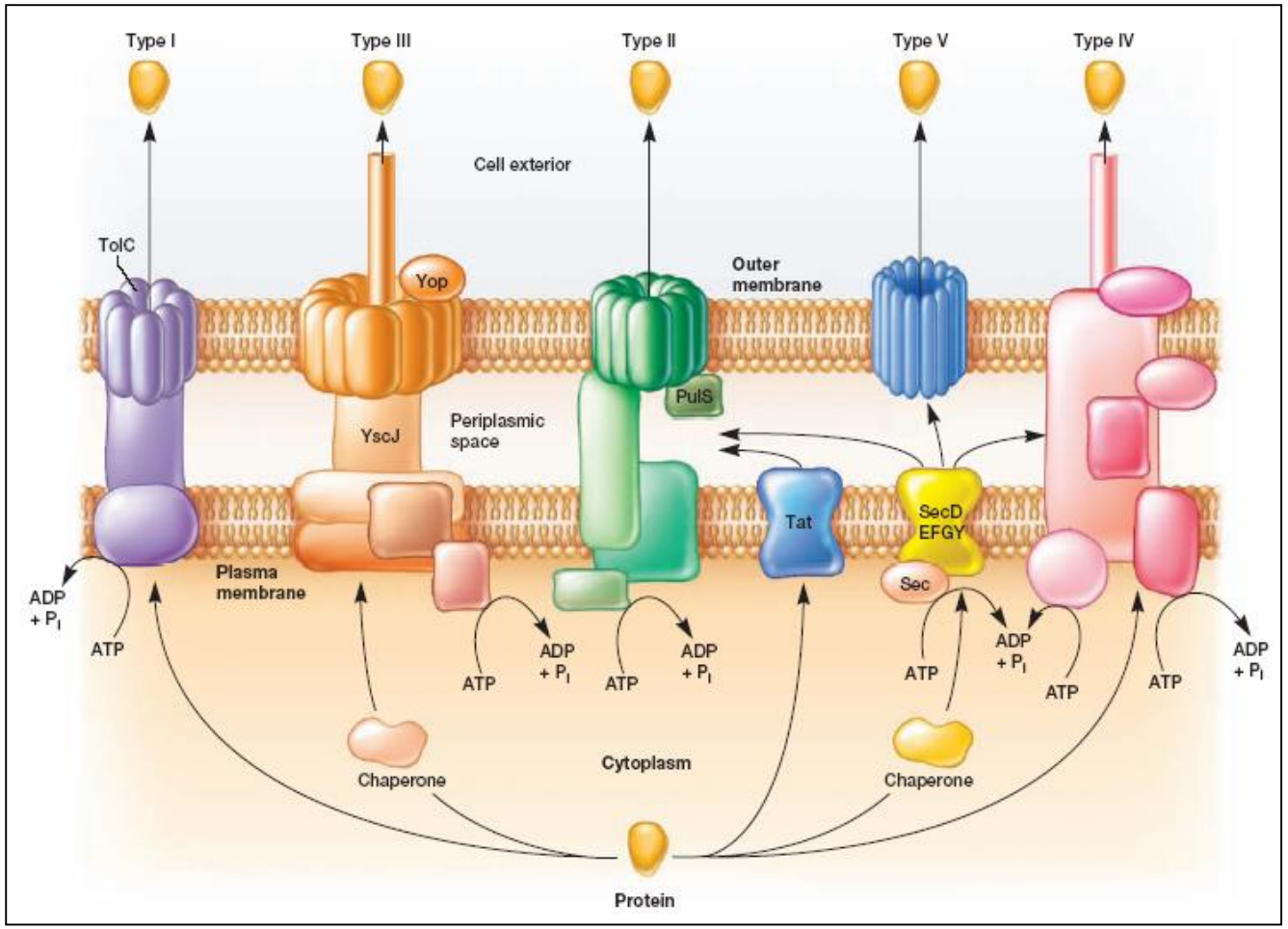
Membrane transport
Protein transport

- **Specific pathways:**
- In Gram-negative bacteria, some proteins have to be transported through the outer membrane after they cross the cytoplasmic membrane.
- Proteins are excreted through one of the following specific pathways:

1. Type I pathway
2. Type II pathway
3. Type III pathway
4. Type IV pathway
5. Type V pathway
6. Type V pathway

Kim and Gadd,2008

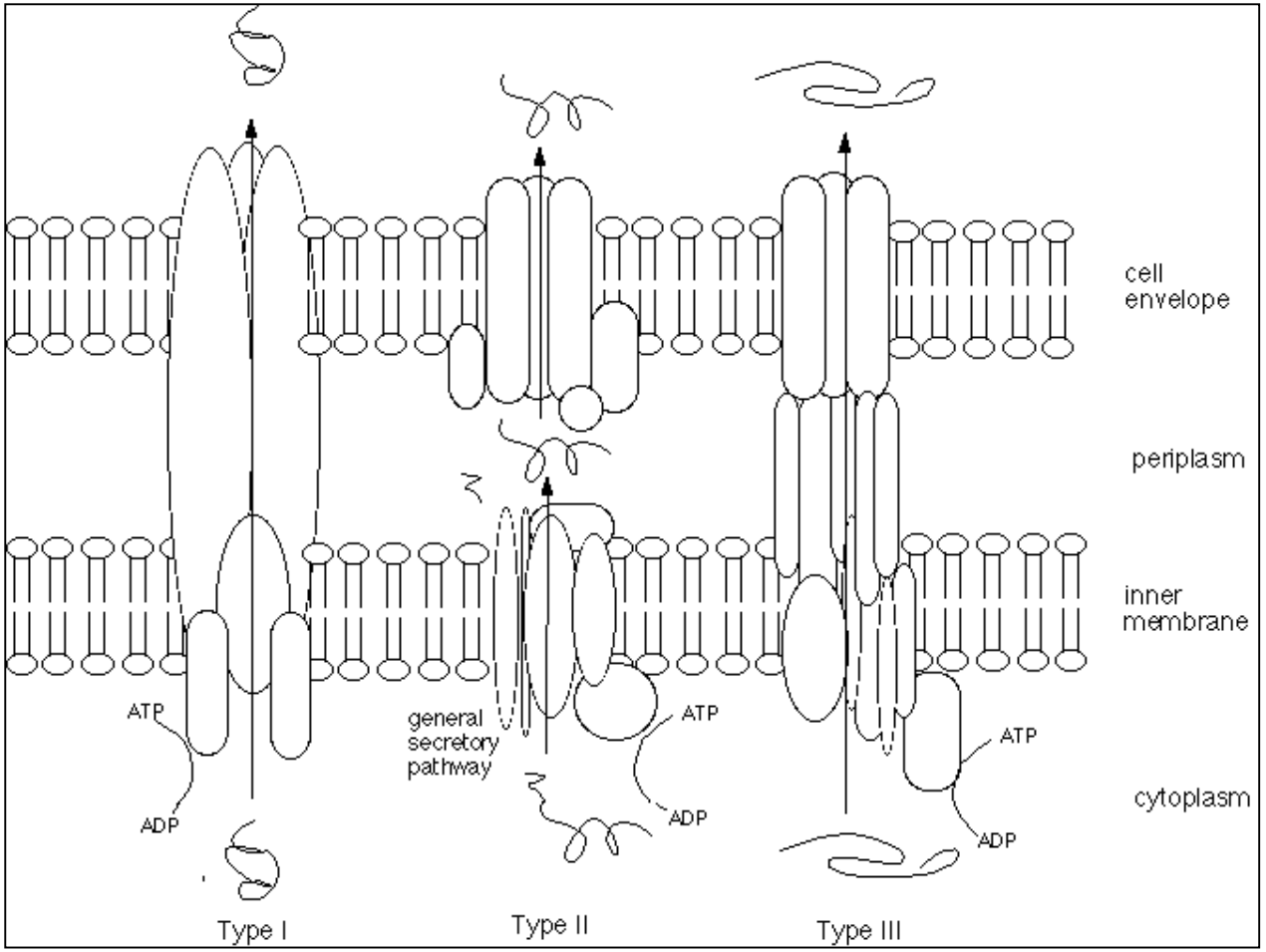
The Protein Secretion Systems of Gram-Negative Bacteria



Prescott,2006

Bacterial secretion systems

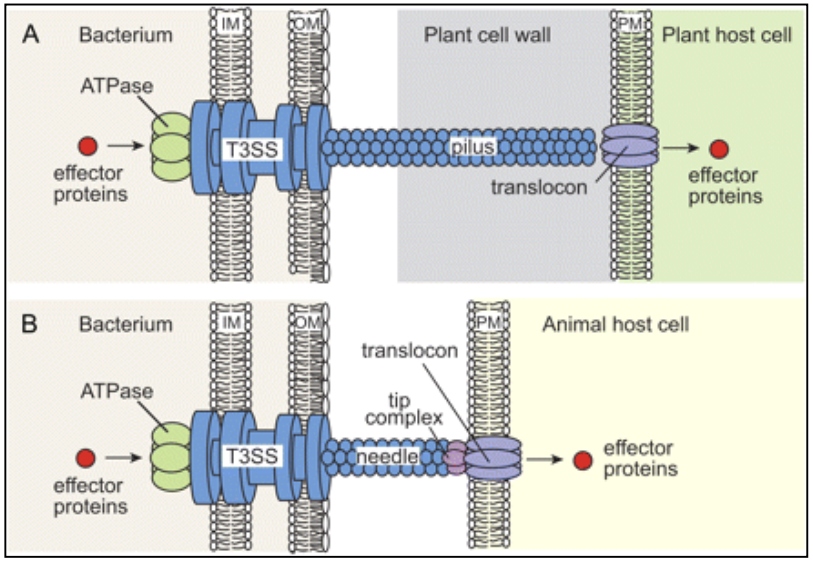
Cleavage or non cleavage of signal peptide



Type III secretion system

From plant (A) and animal (B) pathogenic bacteria

- The T3SS from plant pathogenic bacteria is connected to an extracellular pilus that presumably spans the plant cell wall.
- The T3SS system from animal pathogenic bacteria is associated with a short extracellular needle, which serves as a transport channel for secreted proteins.
- The needle is linked via the so-called tip complex.
- Evidence for the presence of a tip complex in plant pathogenic bacteria is still missing.



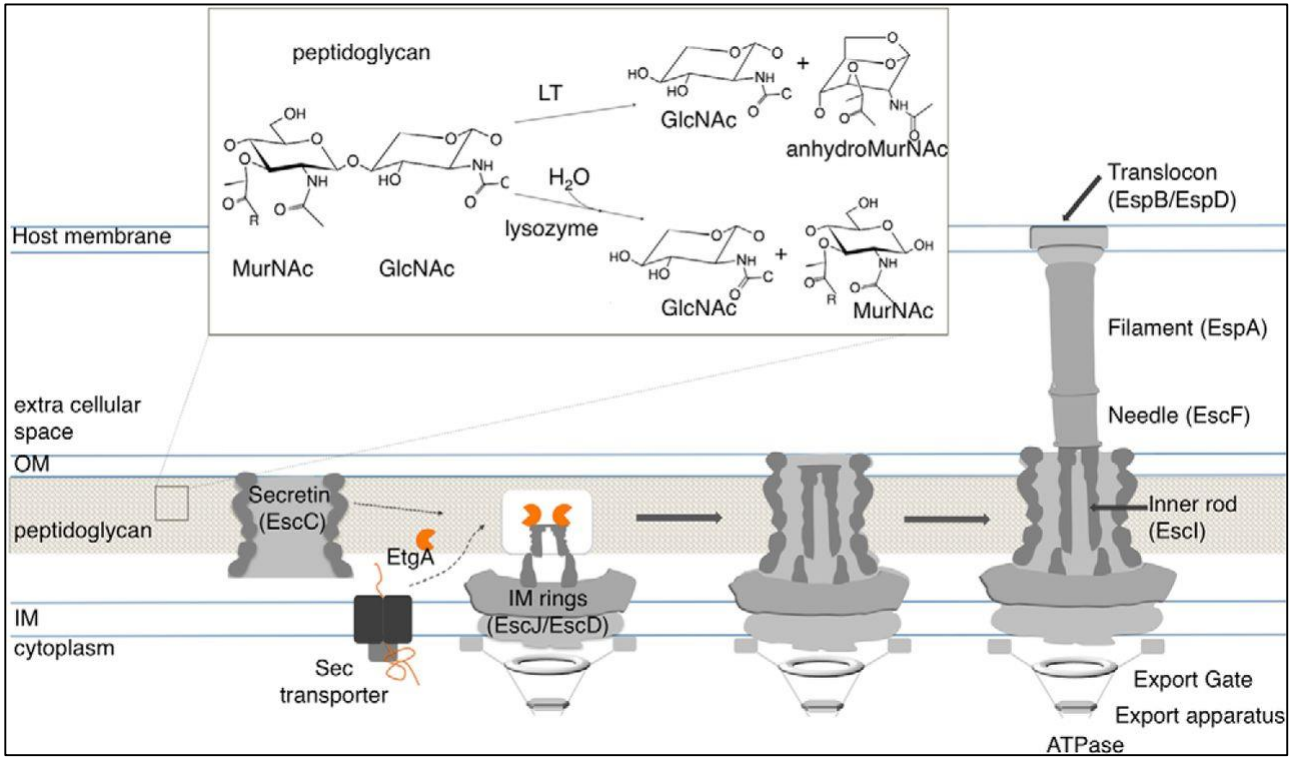
IM, Inner membrane; OM, outer membrane; PM, plasma membrane

Büttner and He,2009

Type III secretion system

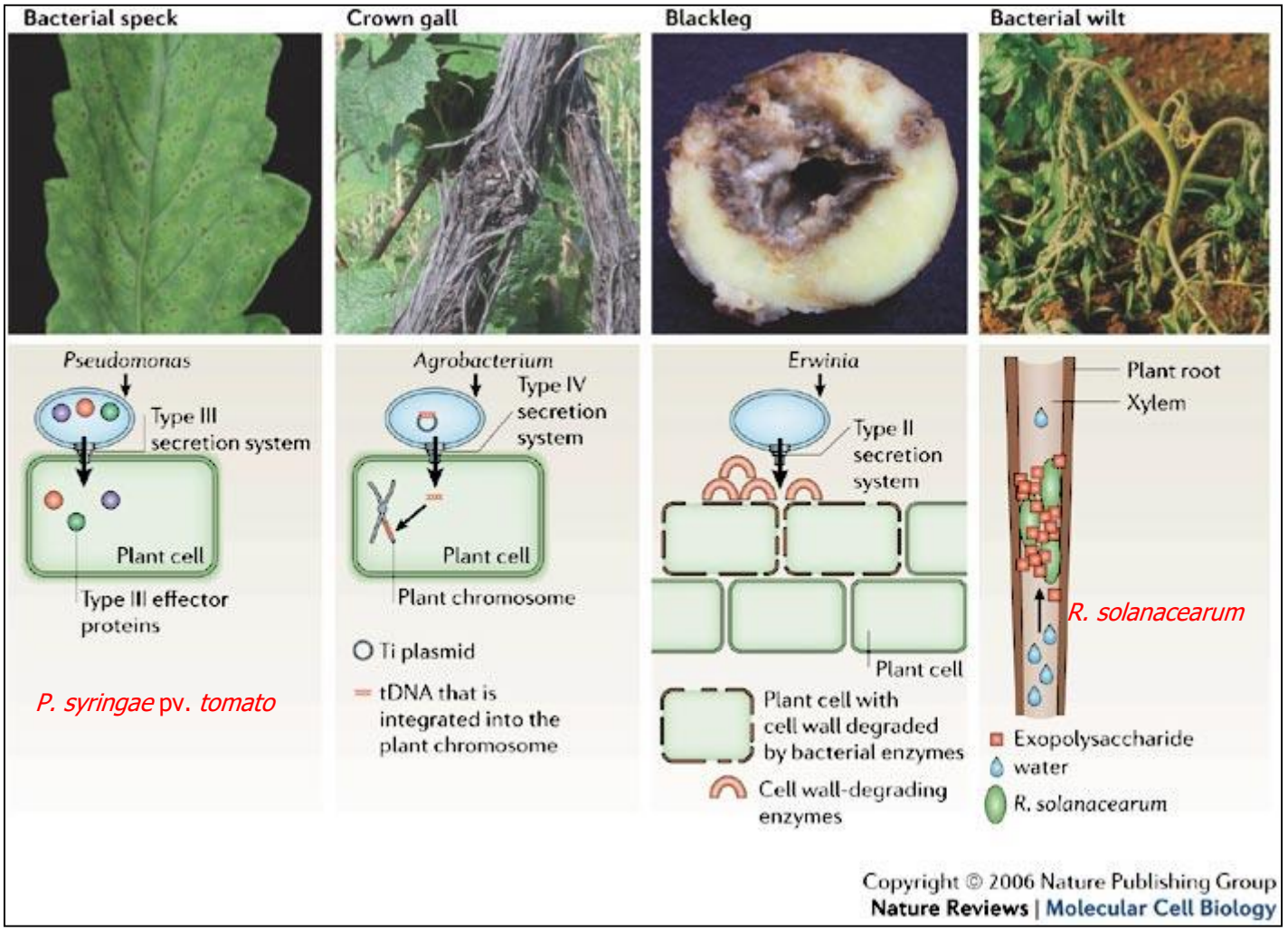
Schematic overview of T3SS apparatus components and role of EtgA during assembly

- EtgA, a lytic enzyme is transported to the periplasm by the Sec secretion system. It interacts with the inner rod, EscI, in the bacterial periplasm and locally clears peptidoglycan during assembly.



Burkinshaw *et al.*,2015

Disease symptoms caused by some bacterial pathogens of plants and representative virulence mechanisms used by these pathogens

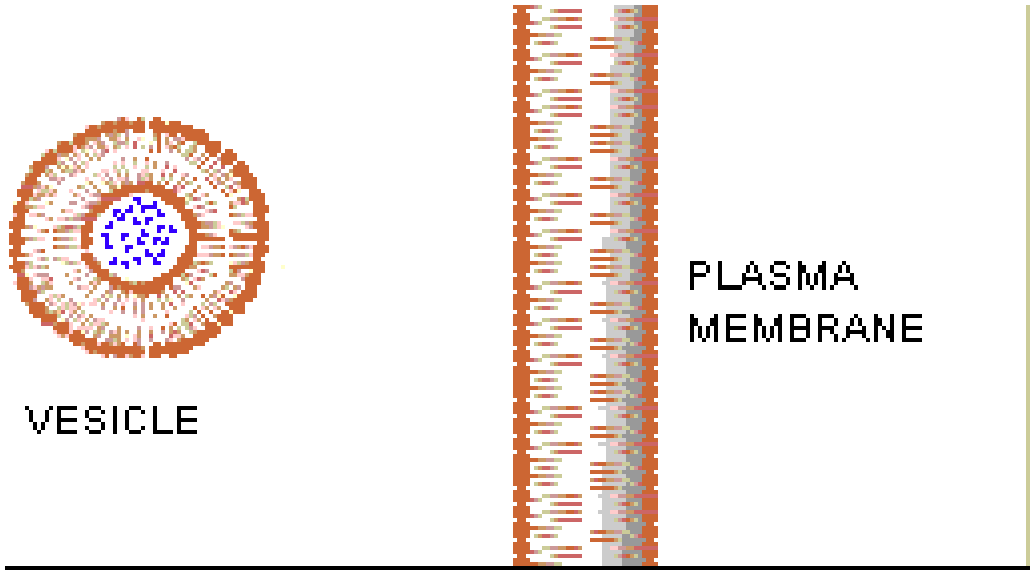


Protein transport
Release of outer membrane vesicles

- Gram-negative bacteria also possess another method for release of material:
- The formation of outer membrane vesicles (Kuehn and Kesty, 2005).
- Portions of the outer membrane pinch off, forming spherical structures made of a lipid bilayer enclosing periplasmic materials.
- Vesicles from a number of bacterial species have been found to contain virulence factors, some have immunomodulatory effects, and some can directly adhere to and intoxicate host cells.
- While release of vesicles has been demonstrated as a general response to stress conditions, the process of loading cargo proteins seems to be selective.

Membrane transport

Vesicles formation



Farabee,2001

Substances acting on cell membrane

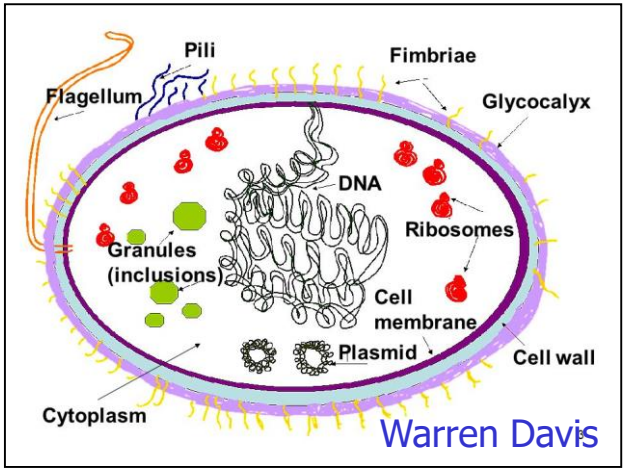
1. Detergents that contain lipophilic and hydrophilic groups disrupt cytoplasmic membranes.
2. Antibiotics such as Polymyxin B and Gramicidin selectively damages membrane.
3. Ionophores (e.g. Valinomycin) are compounds that permit rapid diffusion of cations through the membrane.
4. Chemical agents such as alcohols and quaternary ammonium compounds.

B. Structures Inside Cell Membrane

Cytoplasm

Cytosol– liquid portion of cytoplasm

Cytoplasm is a concentrated soup of organic salts, sugars, amino acids and other molecules.



Cytoplasm of Prokaryotes

Cell cytoplasm

- A bacterium consists of an outer wrapper called the cell membrane.
- Inside this membrane is a watery fluid called cytoplasm that is about 70% water.
- The portion of the cytoplasm surrounding organelles is called cytosol, which is the liquid part of the cytoplasm.
- Most of the cytosol is water, which makes up about 70% of the total volume of a typical cell.
- The other 30% is filled with enzymes (proteins the cell makes itself to use for energy).
- At the center of the cell is a ball of DNA.

Cell cytoplasm

Cytoplasm of prokaryotes vs humans

- The pH of the intracellular fluid in bacteria is 7.4.
- While human cytosolic pH ranges between 7.0-7.4, and is usually higher if a cell is growing.

Cytoplasm of Prokaryotes

Cell cytoplasm

- Cytoplasm is a concentrated soup of organic salts, sugars, amino acids and other molecules.
- The portion of the cytoplasm surrounding organelles is called cytosol, which is the liquid part of the cytoplasm.
- **This contains:**

1. Ribosomes for protein synthesis.
2. Enzymes that catalyze reactions during metabolism of specific compounds. e.g. biosynthesis of bacterial cell wall peptidoglycan.

- In fact, bacterial cell was sometimes considered to be a "bag of enzymes".

Cytoplasm of Prokaryotes

Cytoplasmic granules

Concentrated substances

- **Cytoplasmic granules:** Concentrated deposits of certain substances which are presented/located in the cytoplasm of certain bacteria are known as cytoplasmic granules or inclusion bodies.
- They serve as high energy of the cell.
- The number and nature of the inclusions vary depending on the:

1. bacterial species, and
2. the nutritional state of the organism's environment.

Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

- Three main reserve food granules:

1. **PHB** (store fats)
2. **Volutin granules** (polyphosphate, a polymerized phosphate)
3. **Glycogen** (a polysaccharide of glucose)

- **Gas vesicles/vacuoles** (regulate depth bacteria float in water)
- **Spore** (endo and exospore)
- **Ribosomes** (as many as 15,000 ribosomes)
- A **plasmid** is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently.
- **Nucleoid** (meaning nucleus-like) largely composed of about 60% DNA, plus a small amount of RNA and protein).

Cytoplasm of Prokaryotes

Cytoplasmic granules of bacteria

- The three most vital and important organic cellular reserve materials present in the prokaryotes are namely:

1. poly-β-hydroxybutyric acid;
2. Polysaccharide granules (glycogen), and
3. Starch.

S.No.	Organic Cellular Reserve Materials	Examples of Prokaryotes
1	Poly-β-hydroxybutyric acid + Glycogen	Purple bacteria ; certain blue-green bacteria ;
2	Poly-β-hydroxybutyric acid	Azotobacter ; Bacillus ; Beneckea ; Photobacterium ; Sprillium ;
3	Glycogen	Blue-green bacteria; Clostridia; Enteric bacteria;
4	Starch	Clostridia;

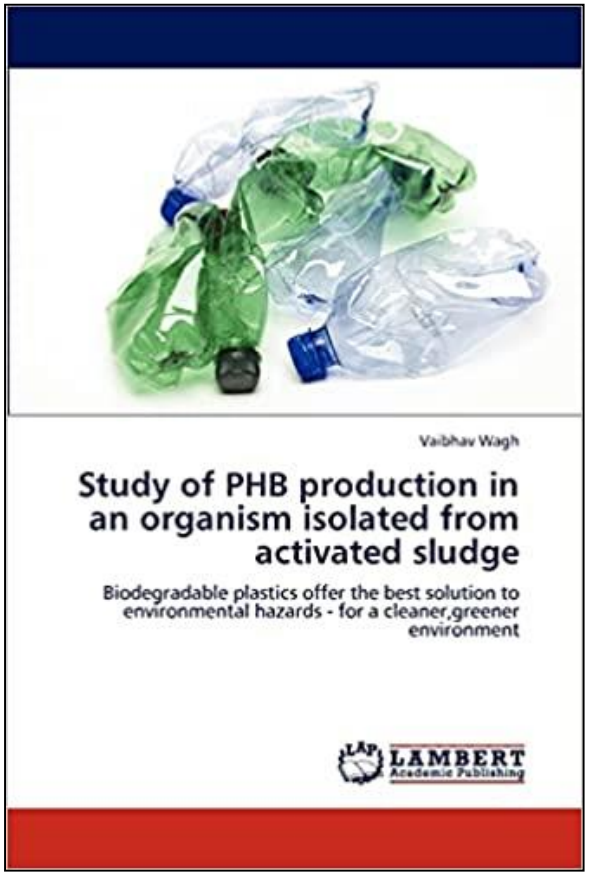
Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

PHAs (polyhydroxyalkanoate granules)

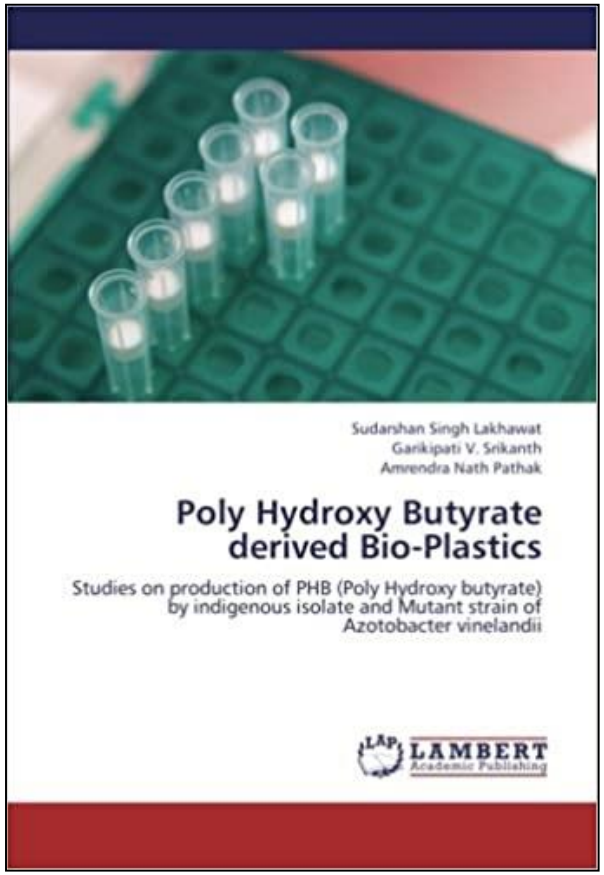
Study of PHB production in an organism isolated from activated sludge: Biodegradable plastics offer the best solution to environmental hazards - for a cleaner, greener environment

- by Vaibhav Wagh (Author)
- LAP LAMBERT Academic Publishing
- 2011
- 92 pages.



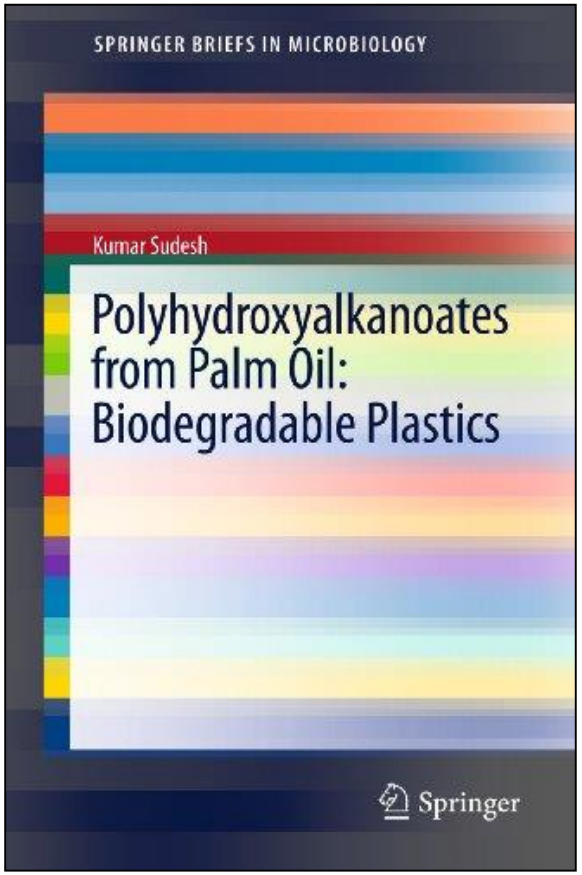
Poly Hydroxy Butyrate derived Bio-Plastics: Studies on production of PHB (Poly Hydroxy butyrate) by indigenous isolate and Mutant strain of *Azotobacter vinelandii*

- by Sudarshan Singh Lakhawat, Garikipati V. Srikanth and Amrendra Nath Pathak
- LAP LAMBERT Academic Publishing
- 2012
- 72 pages.



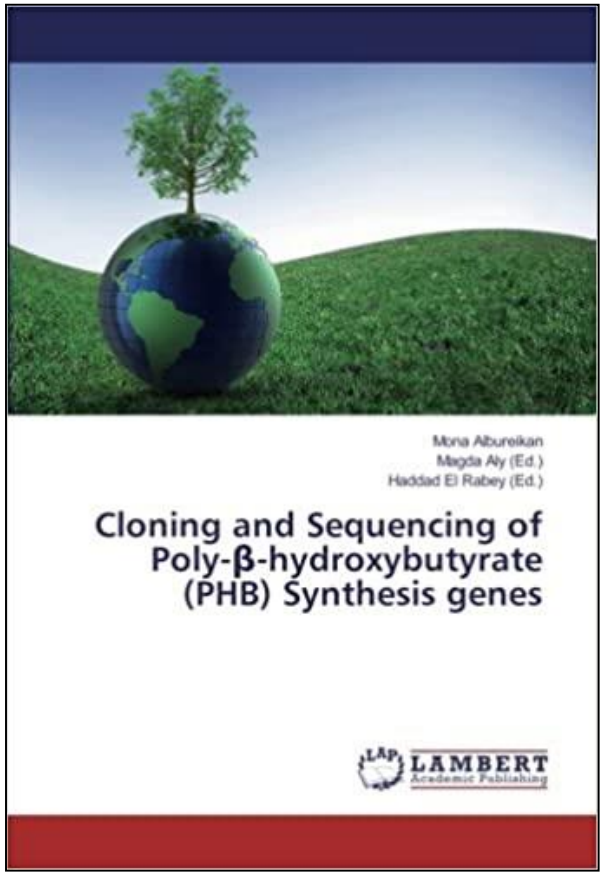
Poly Hydroxy Butyrate derived Bio-Plastics: Studies on production of PHB (Poly Hydroxy butyrate) by indigenous isolate and Mutant strain of *Azotobacter vinelandii*

- by Kumar Sudesh (Author)
- Springer
- 2012
- 138 pages.



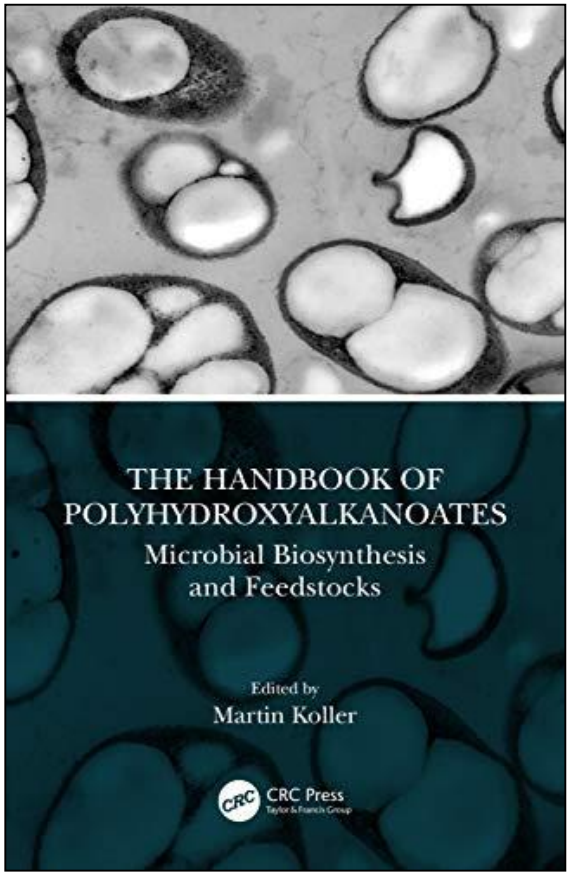
Cloning and Sequencing of Poly-β-hydroxybutyrate (PHB) Synthesis genes

- by Mona Albureikan (Author), Magda Aly (Editor), Haddad El Rabey (Editor)
- LAP LAMBERT Academic Publishing
- 2016
- 188 pages.



The Handbook of Polyhydroxyalkanoates: Microbial Biosynthesis and Feedstocks

- by Martin Koller (Editor)
- CRC Press; 1st edition
- 2020
- 452 pages.

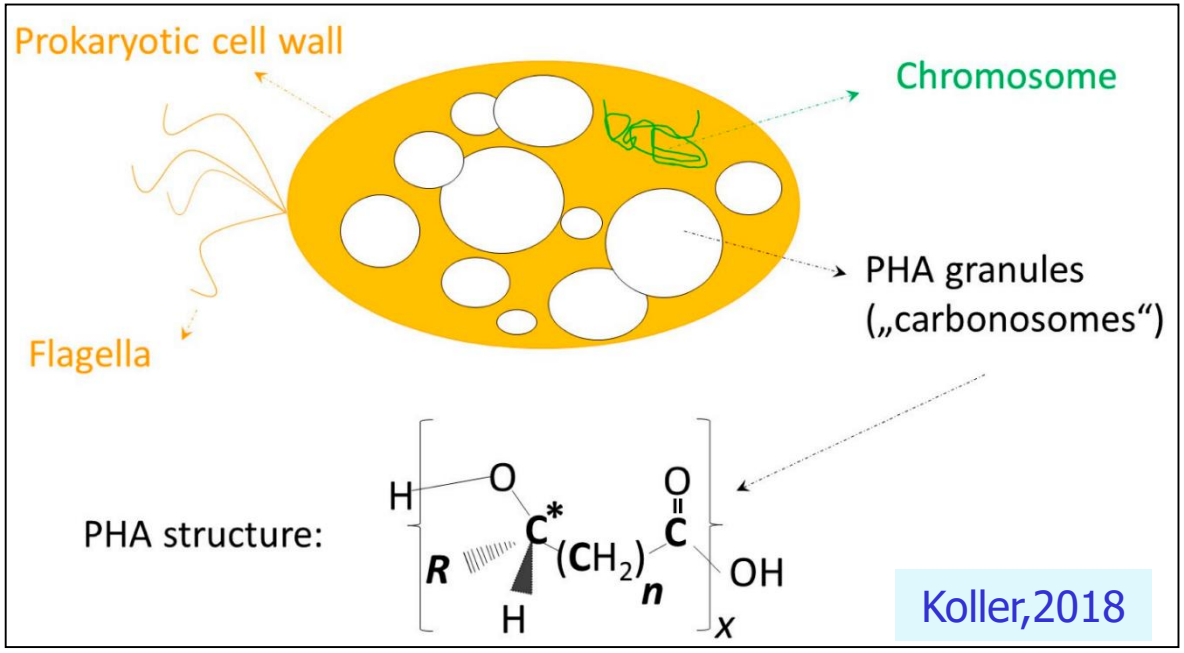


Cytoplasmic granules

PHAs such as PHB granules

Lipid inclusions/natural polyester/bioplastics

- PHAs (polyhydroxyalkanoate granules) are also proposed to be referred to as "carbonosomes" by some leading scientists in this field in order to underline their complex biological functions.



carbonosome (pl. carbonosomes): Any prokaryotic structure that stores carbon (and therefore energy).

Cytoplasmic granules

PHAs such as PHB granules

Lipid inclusions/thermoplastic polymer/bioplastics

- Considerable PHB synthesis is produced in the cells of microorganisms, as product of microbial secondary metabolism, when exposed to:

1. excess carbon source;
2. nutrient stress (when concentrations of nutrients such as nitrogen, phosphorous or oxygen are limiting growth).

- PHA accumulation ends either:

1. when the carbon feed is stopped, or
2. when the granule's surface gets shielded from polymer precursors by forming highly compact granule packages.

Shively,2006; Catone *et al.*,2014; Vadlja *et al.*,2016

Cytoplasmic granules

PHAs such as PHB granules

Lipid inclusions/thermoplastic polymer/bioplastics

- PHB is a natural polyester which is accumulated by many bacteria as an intracellular store of energy and carbon.
- PHAs such as PHB granules are store carbon and energy reserve in bacteria when essential nutrient supplies are imbalanced or depleted.
- PHAs allow survival of the cells in the absence of suitable carbon sources.
- PHAs (polyhydroxyalkanoates granules) are a class of bacterial storage compounds that are synthesized by many Gram-negative and Gram positive bacteria.

Shively,2006; Catone *et al.*,2014

Cytoplasmic granules

PHAs such as PHB granules

Lipid inclusions/thermoplastic polymer/bioplastics

- PHB is ecofriendly, biodegradable, biocompatible and is accumulated up to 90% of cell dry weight.
- Among the members of PHA family, polyhydroxybutyrate (PHB) is:

1. the most common biodegradable polymer, and
2. promising alternative to synthetic nondegradable plastics.

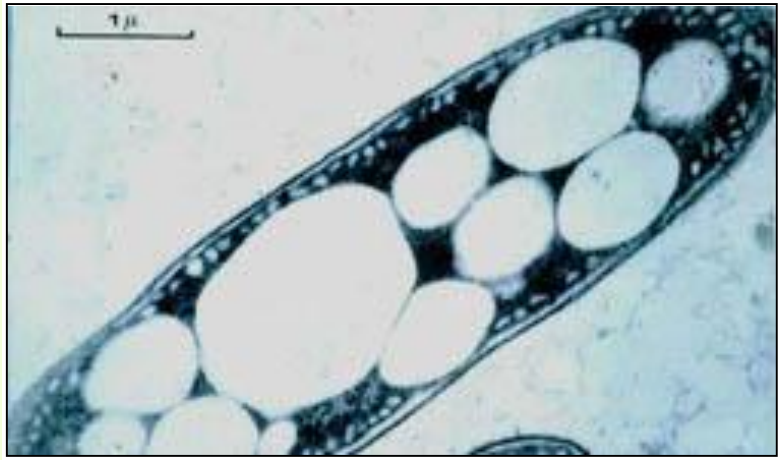
Shively,2006; Catone *et al.*,2014

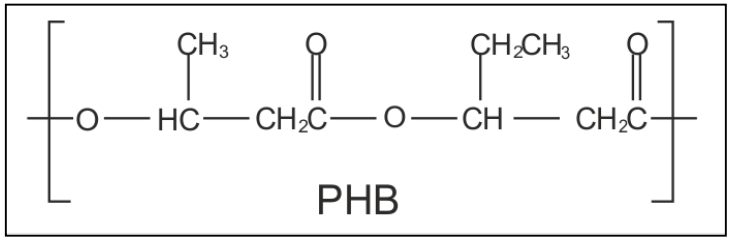
Cytoplasmic granules

PHB granules, short-chained fatty acids

Lipid inclusions/thermoplastic polymer/bioplastics

- Poly-3-hydroxybutyrate granules (PHB) is the most common PHA (polyhydroxyalkanoates granules) was first reported by the French scientist Maurice Lemoigne,1926 in *Bacillus megaterium*.
- These granules reserve carbon and energy source.
- Many bacteria store excess carbon in the form of PHB granules.





Poly-3-hydroxybutyrate (P3HB) and poly-4-hydroxybutyrate (P4HB) are short-chain length PHAs, contain 3-4 carbon atoms, respectively.

Short-chain PHAs, such as PHB

Lipid inclusions/thermoplastic polymer/bioplastics

What is the difference between PHA granules?

- According to the number of carbons in the monomer units, PHAs can be classified into three classes:

1. short-chain length PHA (scl-PHA; 3 to 5 carbon atoms). E.g. poly-3-hydroxybutyrate (P3HB) and poly-4-hydroxybutyrate (P4HB).
2. medium-chain length PHA (mcl-PHA; 6 to 14 carbon atoms). First identified in *Pseudomonas putida* GPo1, or
3. long-chain length PHA (lcl-PHA; 15 or more carbon atoms). Uncommon. Examples include Poly (3-hydroxypentadecanoate).

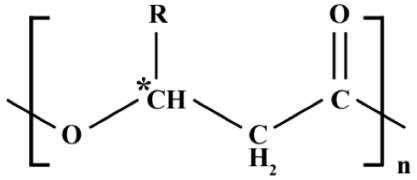
Amy Tan *et al.*,2014;..

Short-chain PHAs, such as PHB

Lipid inclusions/thermoplastic polymer/bioplastics

What is the difference between PHA granules?

- Polyhydroxyalkanoate (PHA) chemical structure.
- The nonmenclature and carbon number for PHA compounds is determined by the functional alkyl *R* group.
- Polyhydroxybutyrate (PHB) is a kind of PHA with R group being methyl.



Poly(3-hydroxyalkanoate)

R group	Carbon no.	PHA polymer
methyl	C_4	Poly(3-hydroxybutyrate)
ethyl	C_5	Poly(3-hydroxyvalerate)
propyl	C_6	Poly(3-hydroxyhexanoate)
butyl	C_7	Poly(3-hydroxyheptanoate)
pentyl	C_8	Poly(3-hydroxyoctanoate)
hexyl	C_9	Poly(3-hydroxynonanoate)
heptyl	C_{10}	Poly(3-hydroxydecanoate)
octyl	C_{11}	Poly(3-hydroxyundecanoate)
nonyl	C_{12}	Poly(3-hydroxydodecanoate)
decyl	C_{13}	Poly(3-hydroxytridecanoate)
undecyl	C_{14}	Poly(3-hydroxytetradecanoate)
dodecyl	C_{15}	Poly(3-hydroxypentadecanoate)
tridecyl	C_{16}	Poly(3-hydroxyhexadecanoate)

Amy Tan *et al.*,2014

Short-chain PHAs, such as PHB

Lipid inclusions in different bacteria

- More than 300 different bacterial strains are known PHB producers.
- To date, the number of known PHA monomers has increased to more than 140, including unsaturated and aromatic monomers (Kim *et al.*,2019).
- Nevertheless, there is only limited production of PHA worldwide.

Utsunomia *et al.*,2020

Short-chain PHAs, such as PHB

Lipid inclusions in different bacteria

- PHB are accumulated by different microorganisms such as

1. Bacteria,
2. Fungi (*Mucor racemosus Fresenius*),
3. Algae
4. Achaea, and
5. Yeast.

The biopolymers produced by fungi have not been adequately explored, and only some of them have been produced on an industrial scale (Rodrigues Araújo *et al.*,2016). Whereas, the bacteria-based biopolymer fabrication gain more attention due to it eases controlled, maximum biomass, simple downstream processing, more gene modification possibilities, etc. (Narayanan *et al.*,2020).

Short-chain PHAs, such as PHB
Lipid inclusions in different bacteria

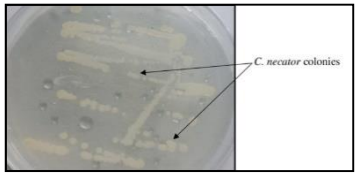
- These bacteria can use these intracellular PHAs as carbon and energy reserve.
- *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*
- *Pseudomonas oleovorans*, *P. putida*, *P. aeruginosa*, *P. mendocina*, *P. stutzeri*, *P. chlororaphis*, *P. citronellolis*,
- *Rhizobacter*
- *Acidovorax*
- *Burkholderia*
- *Ralstonia*
- *Azotobacter chroococcum*
- Recombinant *Escherichia coli* strains

Production of blue-colored polyhydroxybutyrate (PHB) by one-pot production and coextraction of indigo and PHB from recombinant *Escherichia coli*. Or the engineered *E. coli* K12 has been characterized for production of medical compounds and it is highly efficient, to the best of our knowledge, 50 g L^{-1} of polymer is produced in less than 48 h.

Short-chain PHAs, such as PHB

Lipid inclusions in different bacteria

- These bacteria can use these intracellular PHAs as carbon and energy reserve.
- *Ralstonia eutrophes*
- *Alcaligens eutrophus*
- *Rhodococcus*
- *Staphylococcus*
- *Micrococcus*
- *Archaebacteria*
- *Cupriavidus necator*
- *Methylobacterium rhodesianum*



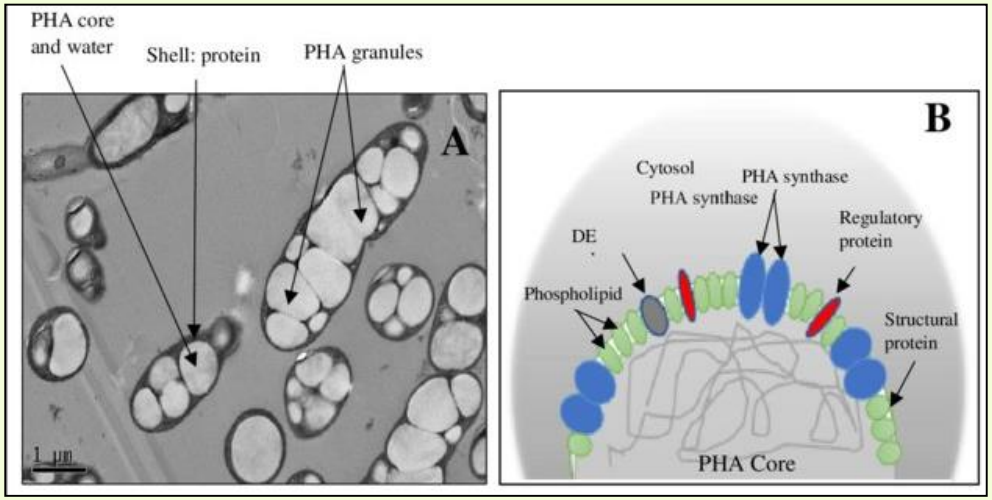
PHB granules

Under electron transmission microscope

Cupriavidus necator

A. Transmission electron microscopy (TEM) picture of *Cupriavidus necator* is a Gram-negative soil bacterium cells containing large amounts of PHB granules using waste date seed as a media; 1 µm;

B. Scheme of PHA granule updated from (Parlane *et al.*,2016).



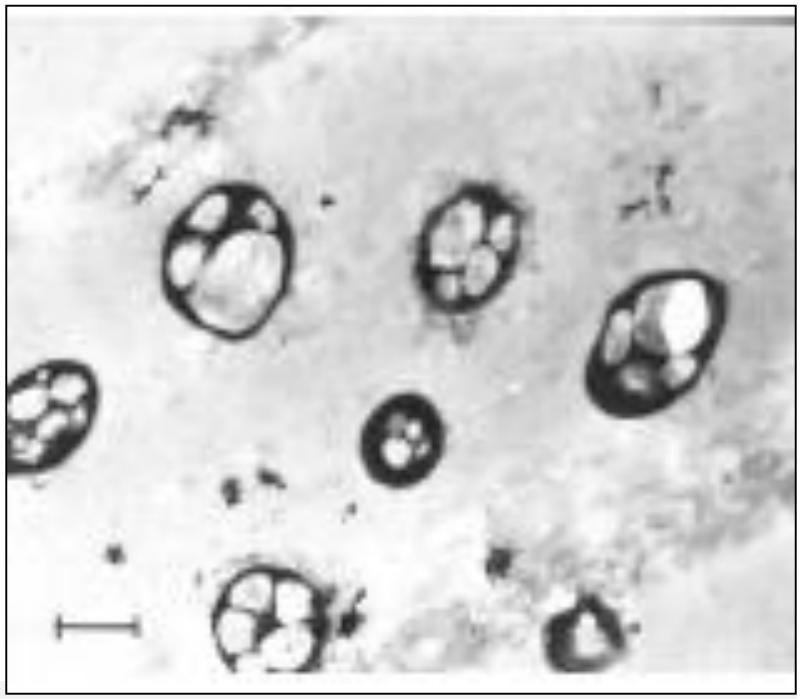
Yousuf,2018

PHB granules

Under electron transmission microscope

Azotobacter chroococcum

- Cells were harvested at mid exponential phase, treated with Osmium tetroxide (OsO4) which is a good fixative and excellent stain for lipids and then was fixed in uranyl acetate.
- Ultra-thin sections stained with lead acetate.



Transmission electron microscopy of ultra-thin section (700 Å) of cells of *Azotobacter chroococcum* showing P (3HB) granules.

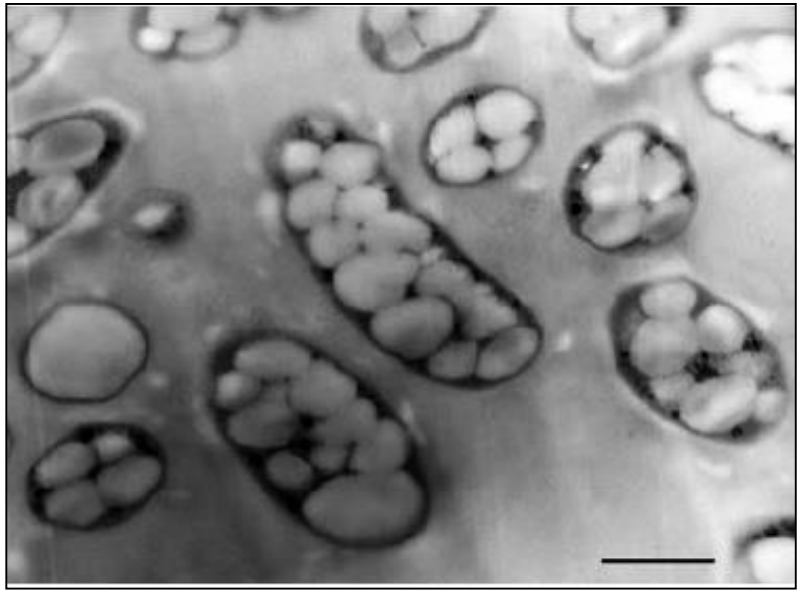
Pal and Paul,2001

PHB granules

Under electron transmission microscope

Ralstonia eutropha H16

- Electron micrograph of an ultrathin section of *Ralstonia eutropha* H16 cells.
- *R. eutropha* and many other bacteria produce short-chain length PHA (scl-PHA) contains 3 to 5 carbon atoms, with an alkyl side chain.
- Scale bar 1 μm).



Shively,2006;..

PHB granules

Under fluorescence microscope (FM)

Localization of PHB granules in *Ralstonia eutropha* H16 cells

- PHB-free cells from 24 h old seed cultures on NB were transferred to fresh NB medium supplemented with 0.2% gluconate and grown at 30°C.
- To provide a phase contrast-independent proof for the presence of PHB granules in the wild type, PHB granules were labelled by the expression of a fusion of the enhanced yellow fluorescent protein (eYFP) with an inactive PHB synthase (PhaC with C319A mutation) that specifically localizes at the surface of PHB granules.
- FM-images of samples taken at time points as indicated were generated after staining with Nile red in red channel (top rows) or without staining in green channel (bottom rows).

Pal and Paul,2001;Bresan *et al.*,2016

PHB granules

Under fluorescence microscope (FM)

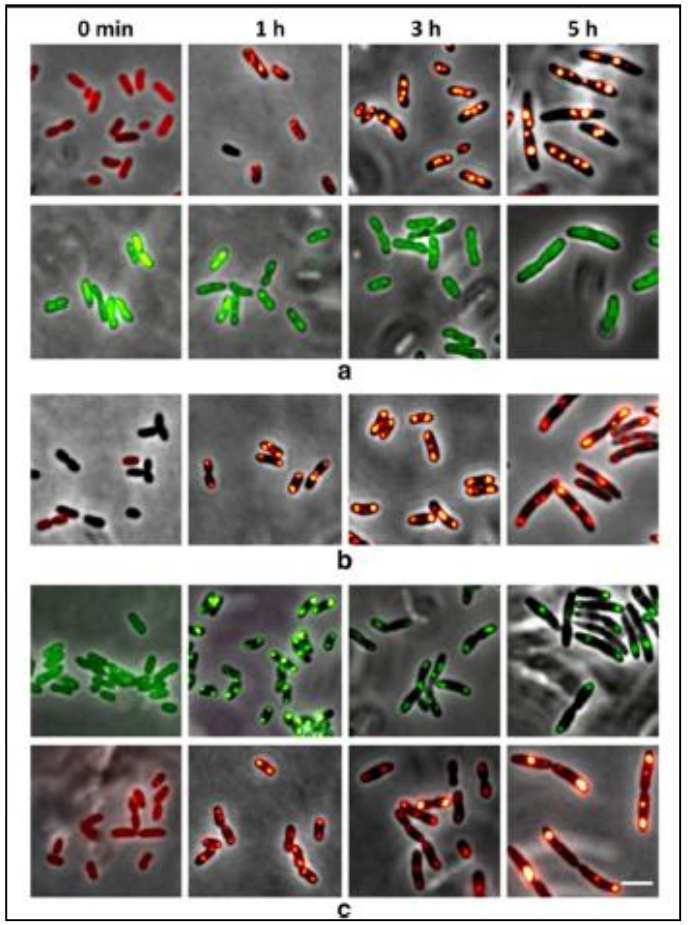
Localization of PHB granules in *Ralstonia eutropha* H16 cells

- Fluorescence microscopical (FM) investigation of:

a. *Ralstonia eutropha* H16 cloning plasmid vector (pBBR1MCS-2-PphaC-eyfp-c1) with over-expression of eYfp.

b. *R. eutropha* H16 (pBBR1MCS-2-PphaC-phaP5) with over-expression of PhaP5, and

c. *R. eutropha* H16 (pBBR1MCS-2-PphaC-eyfpphaP5) with over-expression of eYfp-PhaP5 fusion at various stages of PHB formation.



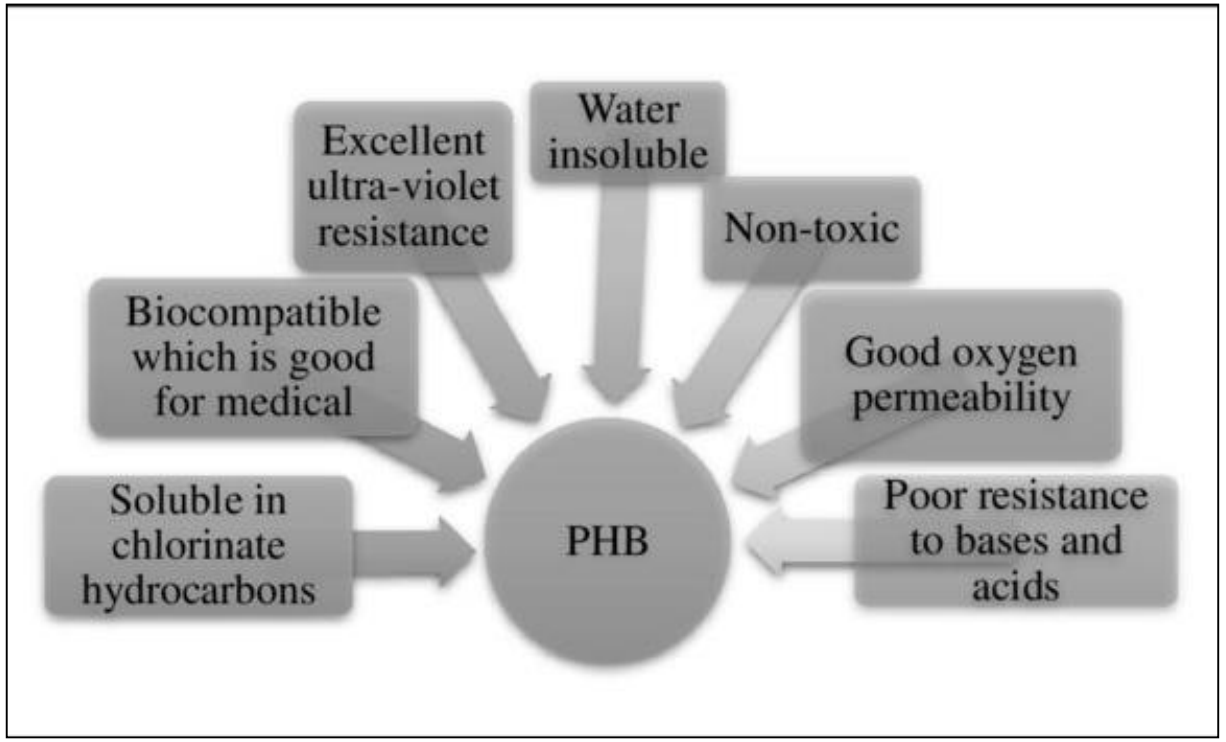
yellow fluorescent protein (eYFP)

Pal and Paul,2001

PHB granules

Some useful properties for PHB biopolymer

- Some useful properties for PHB biopolymer adapted from (Ojumu *et al.*,2004).



Yousuf,2018

P4HB

Applications of P4HB (Poly(4-Hydroxybutyrate) Tradename TephaFLEX®

- P4HB is a thermoplastic material that can be processed into various shapes and forms including fibers, films, tubes, foams, textiles, microspheres, diverse medical applications, and molded constructs using standard processing techniques.
- The engineered *E. coli* K12 with has been characterized for production of medical compounds is used for the production of P4HB, which is commercialized with the tradename TephaFLEX® (Cambrigde, MA, United States).

Utsunomia *et al.*,2020

P4HB

Applications of P4HB (Poly(4-Hydroxybutyrate)

- Out of the PHA family, poly (4-hydroxybutyric acid) (P4HB) has properties especially suitable for medical applications.
- P4HB is a thermoplastic material that can be processed into various shapes and forms including fibers, films, tubes, foams, textiles, microspheres, and molded constructs using standard processing techniques.

Publication number	Content	References
WO2015006737A1	Absorbable implants for plastic surgery	Felix et al., 2015
WO2012142100A1	Biodegradable coextruded multilayer films	Krishnaswamy, 2012
WO2008070428A2	Medical devices containing oriented films of poly-4-hydroxybutyrate and copolymers	Rizk et al., 2008
WO2007092418A3	Polymeric, degradable drug-eluting stents and coatings comprising copolymers or homopolymers of 4-hydroxybutyrate	Behrend et al., 2007
WO2006081517A2	Embolization of poly-4-hydroxybutyrate particles	Martin et al., 2006
WO2004101002A2	P(4HB) fiber useful in devices such as medical textile, tube, general surgical mesh, hernia mesh, pericardial patch, anti-adhesion patch	Martin et al., 2004
WO2001015671A2	Drug delivery devices or bandages	Williams, 2001
WO2001019422A1	Polyhydroxyalkanoate compositions for soft tissue repair, augmentation, and viscosupplementation	Williams and Martin, 2001

Utsunomia *et al.*,2020

PHB granules

Companies (Key players) in the commercialization of PHAs today

Company	Raw material	PHA	Capacity	References
Kaneka (Japan)	Plant oil	P(3HB-*co*-HHx) (PHBH™)	Estimated production capacity of 12'000 kilotons in 2020	1
Tepha Inc. (United States)	Not reported	P4HB (TephaFLEX®) and copolymers (TephaELAST®)	Not reported	2
Danimer Scientific (United States)	Canola oil	mcl-PHA (Nodax™)	Commercial plant (estimated to operate at 10'000 tons per year)	3
Tianjin Green Biosciences Co., Ltd., (China)	Not reported	P(3HB-*co*-4HB) (GreenBio)	10'000 tons per year	Greene, 2014;[4]
PHB Industrial S/A (Brazil)	Sugar from sugarcane	P3HB (BioCycle®)	50 tons per year	5
Bio-on (Italy)	Molasses and by-products of sugar beet production	P3HB (Minerv-PHA™)	Demonstration plant	
Mango Materials (United States)	Raw biogas (methane, carbon dioxide, and hydrogen sulfide)	P3HB	Pilot facility (250 kg per year). Short-term goal: 100 kg per week	

[1] *https://cen.acs.org/business/biobased-chemicals/PHA-biopolymer-whose-time-finally/97/i35;* [2] *https://www.tepha.com/platforms/overview/;* [3] *https://danimerscientific.com/2019/09/16/wincup-to-produce-biodegradable-straws-made-from-danimer-scientifics-nodax-pha-resin/;* [4] *http://www.tjgreenbio.com/en/about.aspx?title=Events&cid=26;* [5] *http://www.canaverde.com.br/produtos/.*

Utsunomia *et al.*,2020

PHB granules

Bioplastics from bacteria

Biopol – a trade name

- Biopol is a brand name for PHB (Polyhydroxybutyrate).
- It is an environmentally friendly, quality biodegradable plastic, produced through the fermentation of plant sugars and glucose, derived from sweet potatoes, pea starch, soya starch and vegetable oil.
- Biopol is a biopolymer and is classified as a polyester and has similar properties to polypropylene (PP).
- Biopol is compostable, degrading harmlessly in soil, after a few months.
- Currently, research is based on producing biopol directly from plants, a simpler process than biomass.

Ryan,2014

PHB granules

Bioplastics from bacteria

Biopol – a trade name

BIOPOL-advantages

1. Biopol is insoluble in water and will sink unlike the majority of 'plastics'.
2. Over time it will degrade harmlessly as it is non-toxic.
3. It has a similar tensile strength to that of polypropylene.
4. With a high melting point of 175 degrees centigrade, it can withstand most use, that requires resistance to hot liquids, such as beverages.

Ryan,2014

PHB granules

BIOPOL-advantages

PHA bottle biodegradation, type Biopol™

- PHA bottle biodegradation, type Biopol™, by incubation in mud for 80 days (Sudesh *et al.*,2000)

Yousuf,2018

PHB granules

Bioplastics from bacteria

Biopol – a trade name

BIOPOL- disadvantages

1. The main disadvantage, is that it more expensive to produce biopol than fossil fuel plastics.
2. Biopol has a low resistance to acids and bases, including bleach. This restricts its use as 'plastic' packaging.
3. Biopol does not resist impact as well as fossil fuel based plastics and cannot be used in situations such as containers, that could potentially be dropped or knocked.
4. The fermentation process is longer, when compared to plastics processed from fossil fuels such as oil.

Ryan,2014

PHB granules

Bioplastics from bacteria

Biopol – a trade name

BIOPOL- products

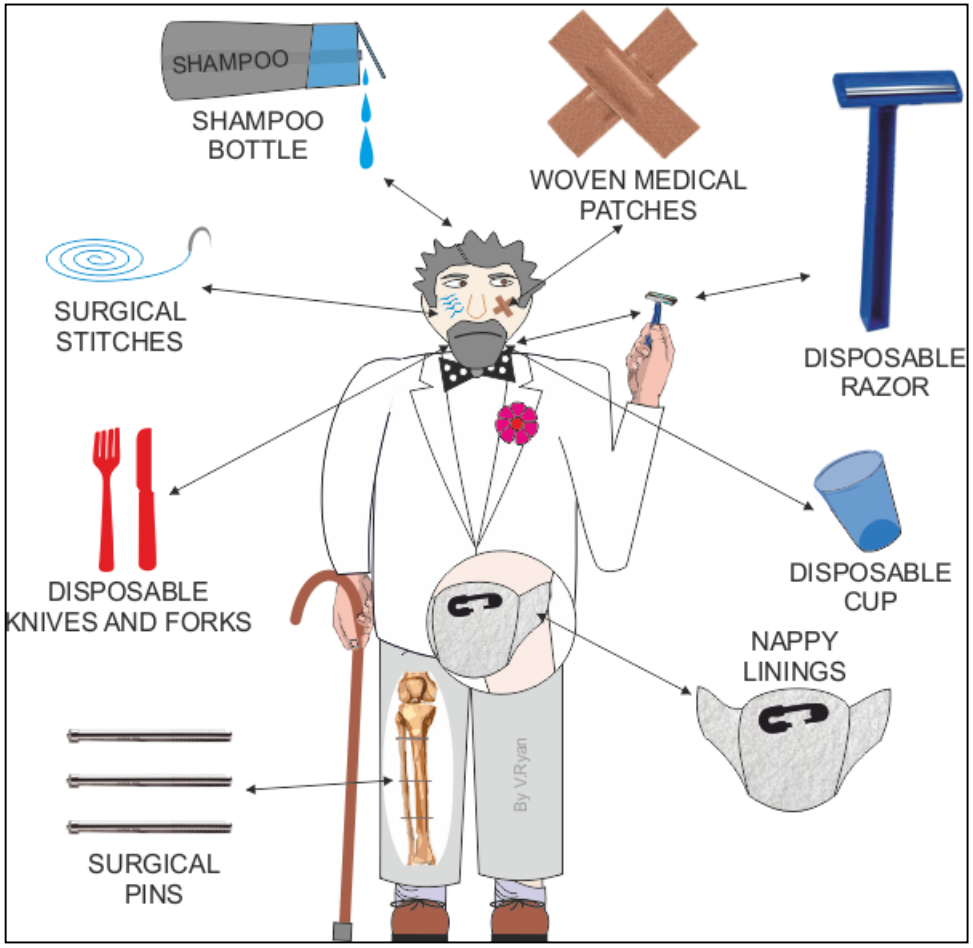
- Biopol PHB, can be injection moulded and vacuum formed.
- It has a range of uses such as, packaging, shampoo bottles, disposable razors, disposable cups, surgical stitches, surgical pins, disposable knives and forks, woven medical patches and nappy linings.

Ryan,2014

PHB granules

Bioplastics from bacteria

Biopol – a trade name



Ryan,2014

PHB granules

Bioplastics from bacteria

Biopol – a trade name

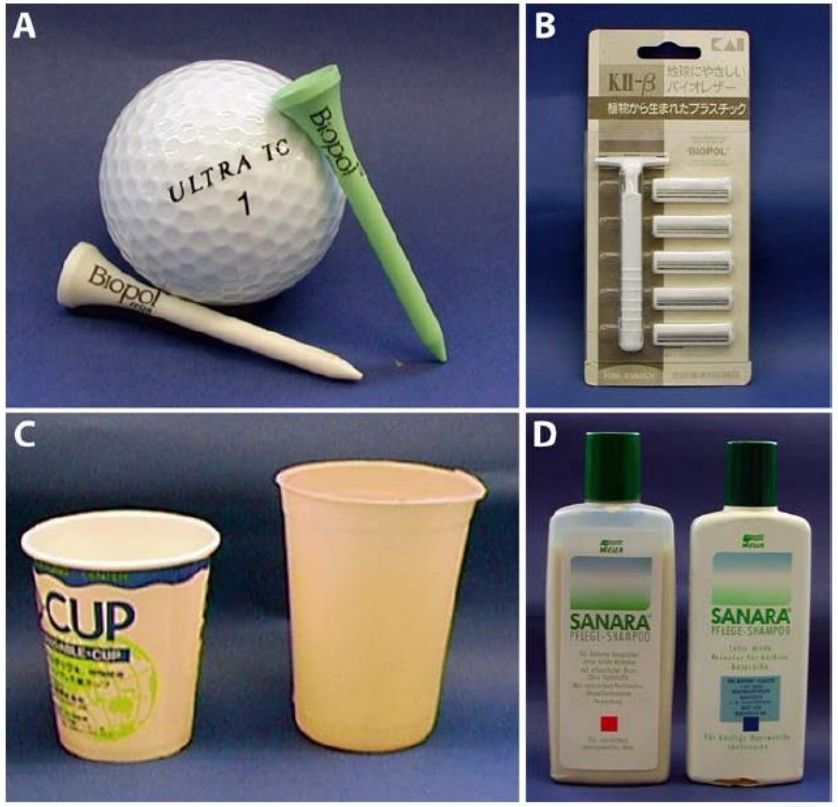
- Commercial products made from Biopol, a biodegradable polymer.

A. golf pins;

B. razor;

C. cups;

D. shampoo bottles.



Shively,2006

PHB producing *Artemia* spp.
Small brine shrimps

- Polyhydroxybutyrate (PHB) was also found in *Artemia* spp.(small brine shrimps)

PHB → hydroxybutyrate → butyrate

1. Protection of shrimps against pathogens (anitbacteria);
2. Increased survival;
3. Alternative to antibiotics in aquaculture.

Artemia is a genus of aquatic crustaceans known as brine shrimp.

PHB accumulating bacteria

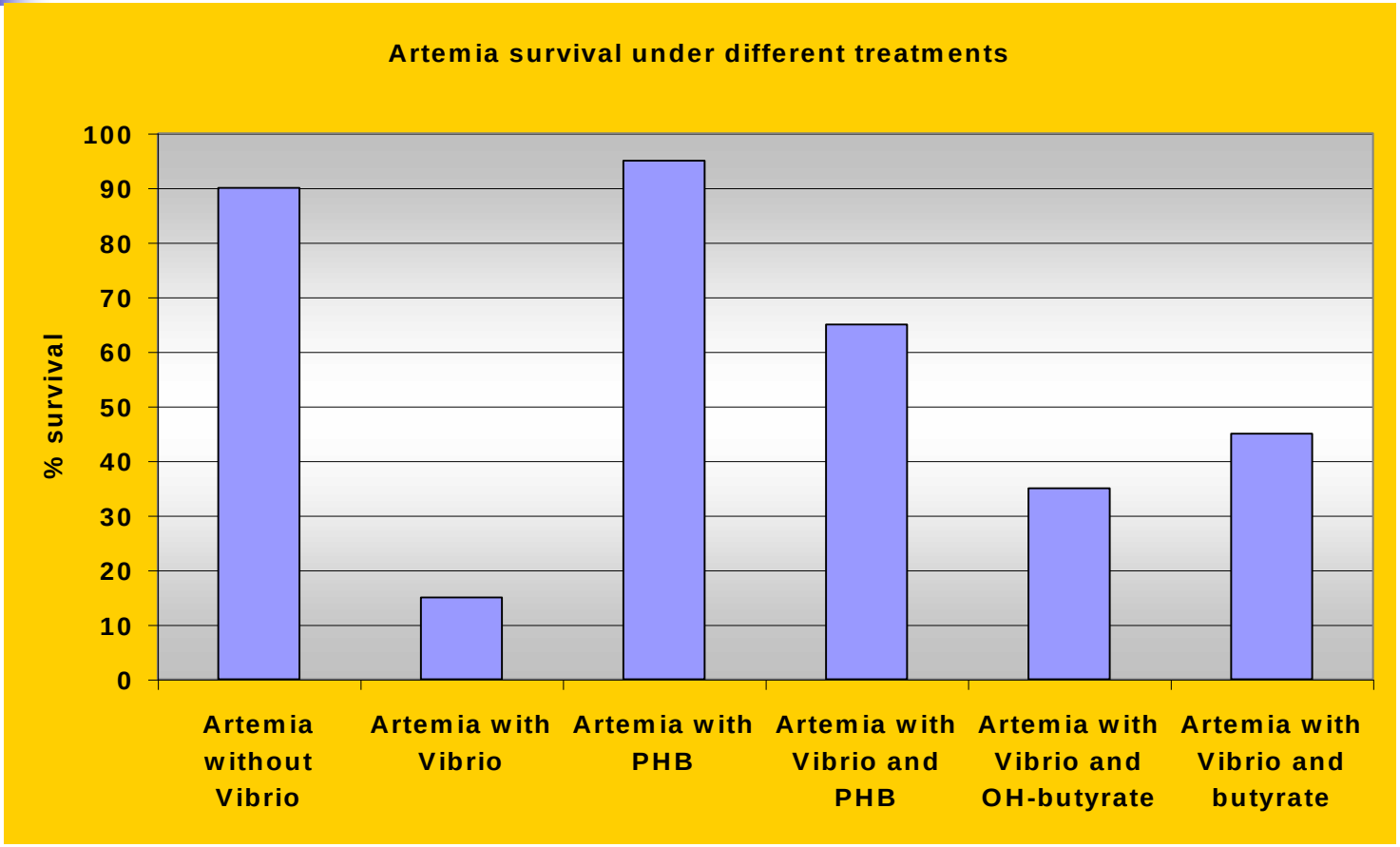
Small brine shrimp treated with different PHB producing bacteria gave good survival and protection against bacterial diseases of shrimps

- Nhan *et al*., 2010, *Artemia* enriched with PHB had better survival rate of shrimps and also could suppress the *Vibrio* spp.
- Defoirdt *et al*., 2007 stated that PHB could give better protection for *Artemia franciscana* against *Vibrio campbelli*, the causal of hepatopancreatic necrosis disease in shrimps.
- *In vitro* assays showed 14 bacterial PHB isolates produced a clear zone (inhibition zone).
- This ability is obtained from the short-chained fatty acids (butyric acid) in PHB compounds.

Susianingsih *et al*.,2020

PHB accumulating *Artemia* spp.

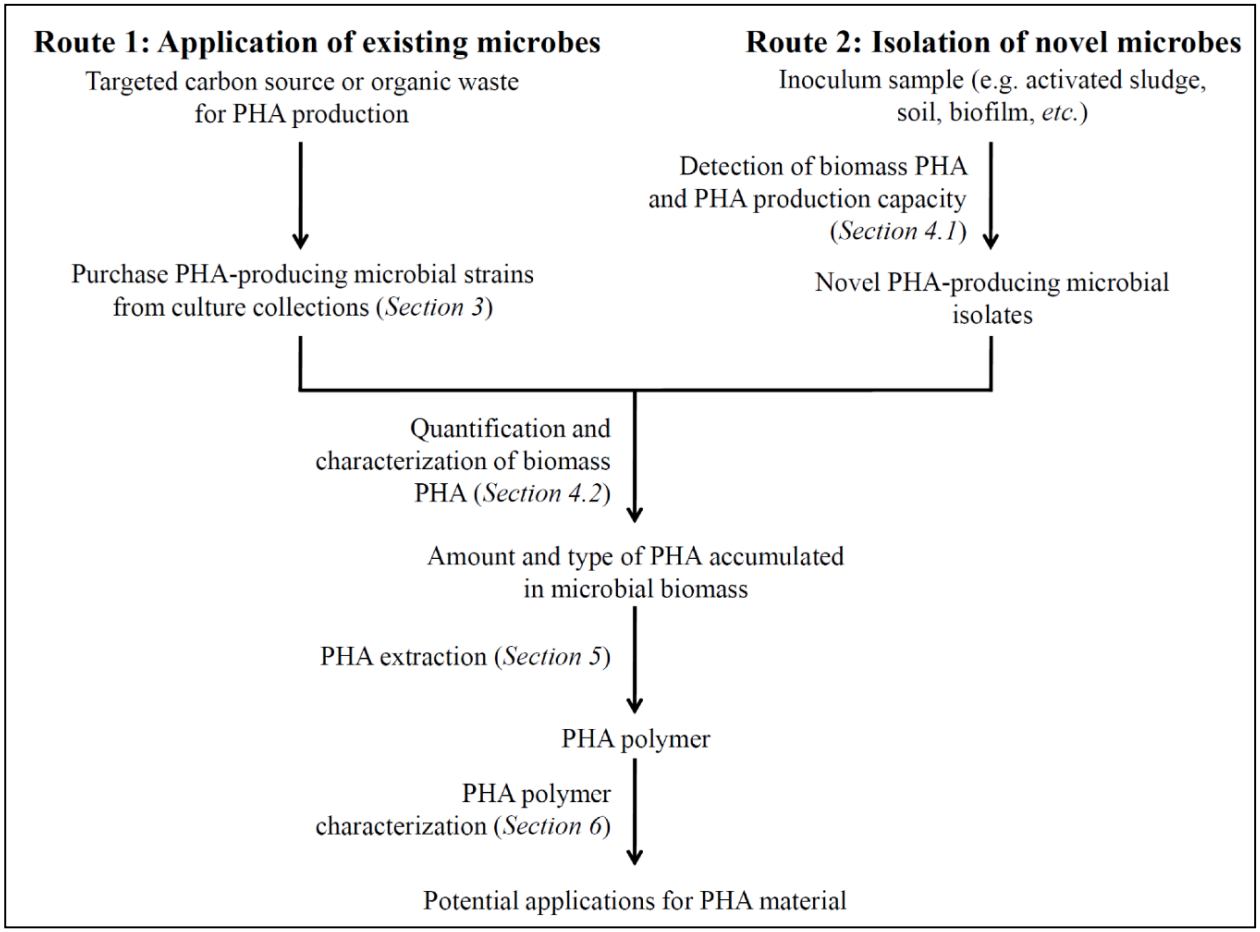
Small brine shrimp treated with different PHB producing bacteria for good survival of shrimps



Willy Verstraete

PHA granules

Schematic workflow processes for PHA research



Amy Tan *et al.*,2014

PHA granules

Screening and isolation bacterial from different samples

- The bacterial isolation from the samples was performed through a serial dilution using a sterile aquadest (sterile DH_2O).
- Each serial dilution on each sample was grown on the nutrient agar (NA) media in the Petri dish and incubated at 37°C for 48 hours.
- Plating on the nutrient agar medium with 1% glucose was recommended. Also the cultures were usually grown in phosphorus and nitrogen limited condition for 48 hours.
- All cultures were maintained on nutrient agar slants.

Sindhu *et al.*,2011; Bhuwal *et al.*,2013; Susianingsih *et al.*,2020

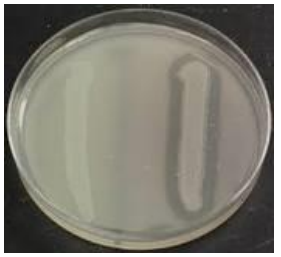
PHA granules

Intercellular PHA granules stained by Sudan Black stain

Screening and isolation bacterial from soil samples

- 1 gram of soil sample collected was inoculated in 100 ml Nitrogen-deficient medium having the following composition (g/L):
- Yeast extract (1.5), Ammonium nitrate (0.286), Potassium dihydrogen orthophosphate (0.75), Calcium chloride (0.4), Magnesium sulphate (0.4), Glucose (40) and pH was adjusted to 4.
- After 24 hour intervals, serial dilutions were made and the dilutions 10^{-5}, 10^{-6} and 10^{-7} were plated on Nitrogen-deficient medium containing Bacteriological agar (15 g/L).

Apparao and Krishnaswamy,2015



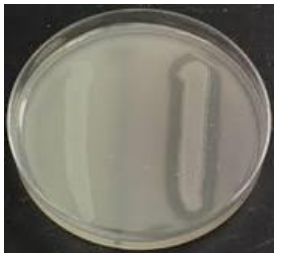
PHB granules

Lipase enzyme activity of bacteria

- To check the PHA production ability of purified strains we used some cheap carbon sources such as glucose, waste frying oil, diesel and cooking oil
- Strains were tested for their ability to utilize oils and the activity of lipase enzyme was checked by growing them on tributyrine agar.
- Strains were able to form clear halo after 24 h of incubation confirmed that have active lipase enzymes and hence are able to utilize oils.

Tributyrin agar is a differential medium that tests the ability of an organism to produce an exoenzyme, called lipase, that hydrolyzes tributyrin oil. Lipases break down lipids (fats). Tributyrin oil is a type of lipid called a triglyceride.

Tufail *et al.*,2017



PHB granules

Lipase enzyme activity of bacteria

- Tributyrine agar (g/L) :
- Peptone..... 5.0
- Yeast Extract..... 3.0
- Tributyrin (glyceryl tributyrate)... 10.0
- Agar.....15.0
- Final pH 7.5 ± 0.2
- **Preparation:**
- Melt the content of the bottle in a boiling water-bath at 100°C (loosing the caps partially unscrewed) until completely dissolved. Cool to 45-50°C, mix well avoiding the formation of bubbles and aseptically distribute into Petri dishes. Allow the medium to solidify. Store the plates in tightly closed containers.

PHB staining

Spot inoculation and staining with sudan black

- The growing bacterial colony was recultured on a spot of NA media through plating divided into 4 equal parts and incubated for 48 hours.
- Each colony was given an ethanolic 0.02% Sudan Black B solution and stood for 30 minutes, then rinsed with 96% ethanol to clean the color.
- The positive results of PHB-producing bacteria were shown in dark blue/bluish black color.
- Whereas, colonies unable to incorporate the Sudan black B appeared white.

Bhuwal *et al.*,2013; Susianingsih *et al.*,2020

PHB staining

Preparation of lipophilic Sudan black stain

- Sudan black is the specific stain to color various lipids, such as neutral lipids, phospholipids, and sterols, therefore can be used to select bacteria that have PHB compounds as a sudanophilic character (can be stained using a lipid staining).

The Sudan black stain was made by mixing 60 mg Sudan Black into 200 ml ethanol 70% (0.02% ethanolic solution), then incubated for 24 hours with a magnetic stirrer.

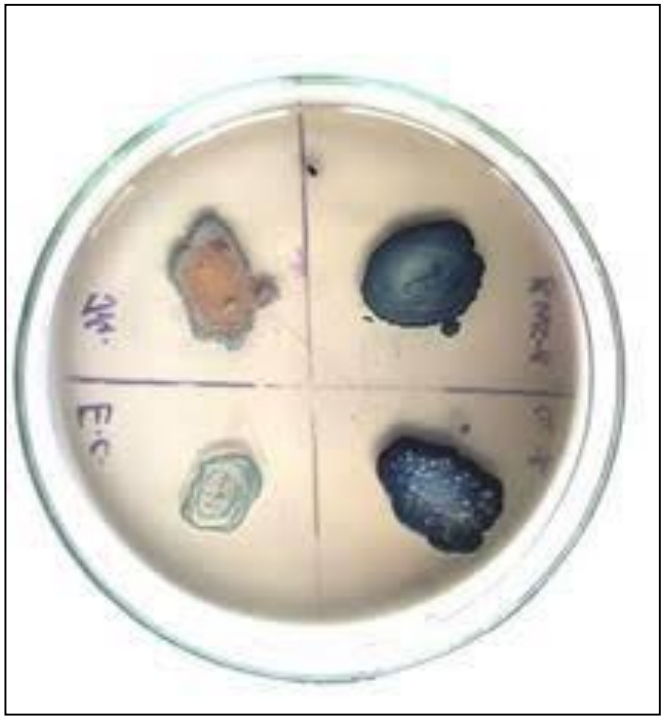
Ryan,2014;Susianingsih *et al.*,2020

PHB staining

The absorption of the dark blue color of Sudan black stain by PHB producing bacterial isolates

- Staining of the colonies on ager media:

1. Sudan Black B plate assay showing the presence of blue coloured colonies (lipid positive), and
2. white coloured colonies (lipid negative).

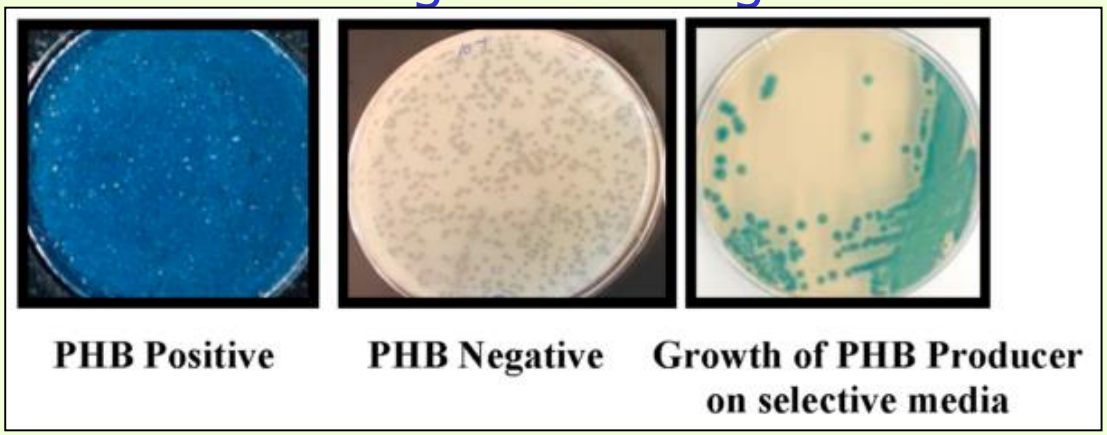


Mascarenhas and Aruna,2017

PHB staining

The absorption of the dark blue color of Sudan black stain by PHB producing bacterial isolates

- In the primary screening by Sudan Black B staining on test isolate grown petri dish results revealed that after the pouring of Sudan Black B stain into the petri dish, the color of colonies changed as dark greenish-blue color.



PHB selective media (containing 2.5 g, 25 g, 100 g, 20 g, 1 g, 100 g, 10 g, 1.2 g, 20 g L of H_2KO_4P, HNa_2O_4P, Mannitol, NaCl, $MgSO_4$, $C_3H_3NaO_3$, Peptone, Bromothymol blue, and agar, respectively).

Narayanan *et al.*,2020; Narayanan *et al.*,2020b

PHB staining

The absorption of the dark blue color of Sudan black stain by PHB producing bacterial isolates

- **Staining of bacterial cells on slide:**
- For more confirmation, the same of colony of bacteria was smeared onto clean slide, then stained with 0.3 g of Sudan black B in 70% (v/v) (ethanol) for 10 minutes.
- Subsequently, the smear was immersed with xylene to decolorize the cells, after that, 5% (w/v) Safranin water solution was used for 10 seconds as counter stain.
- Finally, the slide was washed with distilled water and dried before observation under an optical microscope:

1. Cytoplasm is pink,
2. Lipids (PHAs) are dark grey or black.

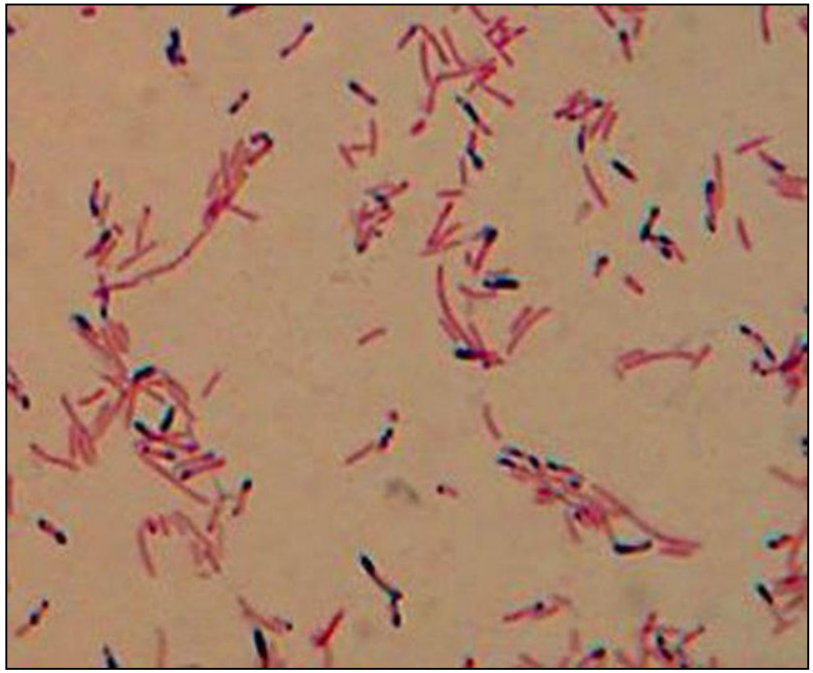
Modified method: Heat-fixed bacterial smears were stained with 0.3% (w/v) Sudan Black B in 60% Ethanol for 10 minutes, rinsed, and counter stained with Safranin. The slide was air dried and observed under 100 X oil immersion(Apparao and Krishnaswamy,2015).

Zargoun *et al.*,2015; Aragosa *et al.*,2021

PHB staining

The absorption of the dark blue color of Sudan black stain by PHB producing bacterial isolates

- **Staining of bacterial cells on slide:**
- A thin bacterial smear was made on the clean glass slide.
- The smear was air-dried and stained with a 3% Sudan Black B solution (3 gram w/100 ml in 70% ethanol) for 10 minutes.
- The smear was washed and air-dried.
- The sample was stained with Safranin (5% w/v in distilled water, Sigma) for 10 seconds, washed again with distilled water, and dried and observed under oil immersion lens.



1. Cytoplasm is pink,
2. Lipids (PHAs) are dark grey or black.

Jari *et al.*,2015

PHB staining

Alternative method

Nile Blue A Stain

- Sudan black B positive isolates were checked for PHA production by Nile blue A staining, a more specific stain for polyhydroxyalkanoic acids (PHAs) by a more rapid and sensitive, viable colony method.
- Therefore, all PHB-accumulating colonies were confirmed for PHB production by subjected to screening by Nile blue A staining.

Sindhu *et al.*,2011; Bhuwal *et al.*,2013; Gatia *et al.*,2017

PHB staining
Alternative method
Nile Blue A Stain


Coplin staining jar

- **Staining of bacterial cells on slide:**
- An aqueous solution of 1% (w/v) of Nile Blue A was prepared after dissolving at 50°C, and then filtered before use.
- Bacterial smear was fixed on the slide by heating, before adding the Nile Blue A.
- The slide was inserted into a coplin staining jar filled with aqueous solution at 55°C for 10 minutes.
- Afterwards, the slide was washed with tap water to remove excess stain and then with 8% (w/v) acetic acid for 1 minute.
- The smear was washed again with tap water and dried with bibulous paper.
- Finally, the smear was covered with a cover slip and then examined under a Nikon fluorescence microscope (Ostle and Holt,1982).

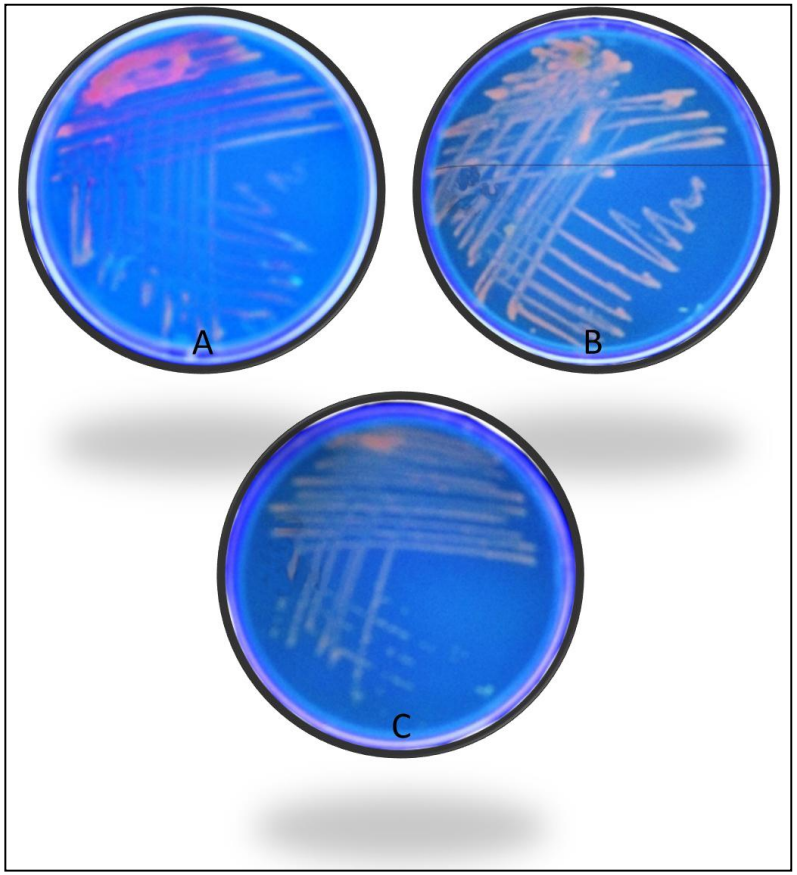
Zargoun *et al.*,2015

PHB staining

Nile blue A plate assay

- All isolates tested showed bright orange fluorescence on irradiation with UV trans-illuminator at a wavelength of 312 nm in Nile blue A as shown in this figure.
- pink- orange fluorescence under UV light by PHB producer:

A. High fluorescence,
B. Moderate fluorescence, and
C. Weak fluorescence.



Gatia *et al.*,2017

PHB degrading bacteria

Effective ecofriendly methods to convert bio-polymers to monomers

1. When some bacteria grown in increasing concentrations of carbon, a polymer called Poly β-hydroxyl butyrate (PHB) was produced and accumulated inside the bacterial cell up to 70% of the cell dry weight. This material can be used safely in different modern application to replace plastic which has negative effects on man, animals and environments.
2. Some other bacteria such as actinomycetes are able to degrade plastics and bioplastics (PHB), maintain the health of the environment. PHB depolymerases are responsible for extracellular PHB degradation.

Aburas *et al.*,2021

PHB degrading bacteria

Screening of the different bacteria for PHB degradation using two layer medium

- The homo- polymer of PHB was obtained as powder from Biomer Inc., Germany.
- The suspension of PHB (1.0 g/100 ml distilled water) was sonicated for 15 min (Ultrasonic Homogenizer 4710 series ColeParmer Instrument Co. Chicago, Illinois 60648).
- The sonicated solution was autoclaved separately and added aseptically as a carbon source to 900 ml of liquefied sterile agar medium.
- The obtained medium was mixed well and used.

Aburas *et al.*,2021

PHB degrading bacteria

Screening of the different bacteria for PHB degradation using two layer medium

- Agar plates were prepared from Mineral salts agar medium and each plate contained 10 ml of the previous medium as a bottom layer and 6 ml of the medium, contained 0.1% of the polymer suspension as the sole carbon source (the upper layer).
- At first all plates had an opaque appearance due to the presence of polymer in the top layer.
- The plate was inoculated with 1 ml of the bacterial suspension ($6x10^6$ CfU/ml), previously grown for one week in starch nitrate broth medium at 120 rpm for 4 days.
- The inoculum was spread over the surface of the medium. Incubation was then carried out at 25°C.
- Assessment of degradation activity was detected by measuring the mean value of the clear zone diameter and the mean diameter of the bacterial colony.

Aburas *et al.*,2021

PHB degrading bacteria

Screening of the different bacteria for PHB degradation using different media

- Images showing clear zone of diameter of microbial colonies in (A) and (B).
- Positive colonies showing PHB hydrolysis on PHB agar media.
- (C) *Enterobacter cloacae* CA655,
- (D) *Stenotrophomonas* sp. CB220, and
- (E) *Aeromonas caviae* Kuk1 sp.

Amir *et al.*,2021

Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

Volutin granules (complexed inorganic polyphosphate)

Volutin or metachromatic granules

Storage form of complexed inorganic polyphosphate

Disappear when the deficient nutrients are supplied

- Volutin granules are intracellular storages of complexed inorganic polyphosphate (poly P).
- Volutin, or metachromatic granules, contains large amounts of phosphorus, magnesium, potassium, and calcium.
- They have been considered to represent:
- A reserve of energy and phosphate for cell metabolism,
- but they are most frequent in cells grown under conditions of nutritional deficiency, and
- tend to disappear when the deficient nutrients are supplied.

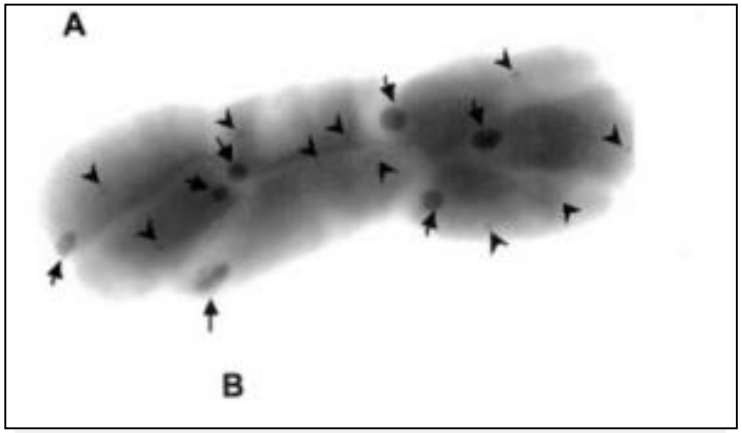
Wikipedia,2021

Volutin granules

Storage form of complexed inorganic polyphosphate

Disappear when the deficient nutrients are supplied

- Volutin granules of *A. tumefaciens* observed by transmission electron microscopy without fixation and staining.
- Fig. 1A, arrows: *A. tumefaciens* typically shows a large granule toward one of the cellular poles, and
- Fig. 1A, arrowheads: additional smaller granules in different regions of the cells.
- The large granules have a diameter of about 210±18 nm.
- Figs. G and H: volutin granule fractions.



One example is the Gram positive bacilli Corynebacterium, which stores phosphate in structures called "volutin" or metachromatic granules that are housed within the cell membrane.

Seufferheld *et al.*,2003;..

Cytoplasm of Prokaryotes

Cytoplasmic granules of bacteria

Original method

1. A heat-fixed bacterial smear is stained with Albert's stain for 3-5 minutes.
2. Rinse with water. Blot dry.
3. Stain with Lugol's iodine solution for 1 minute.
4. Rinse with water
5. Drain or blot to dry.

- Results:
- Cytoplasm appears light green, volutin granules blue-black.

- Albert's Stain Solution:

1. Malachite green 0.2 gm;
2. Toluidine Blue 0.15 gm;
3. 95% ethanol 2 ml;
4. glacial acetic acid 1 ml;
5. distilled water 100 ml.

- Dissolve the dyes in the ethanol. Mix the acid with the water and add to the dye solution. Let stand for 24 hours and then filter.

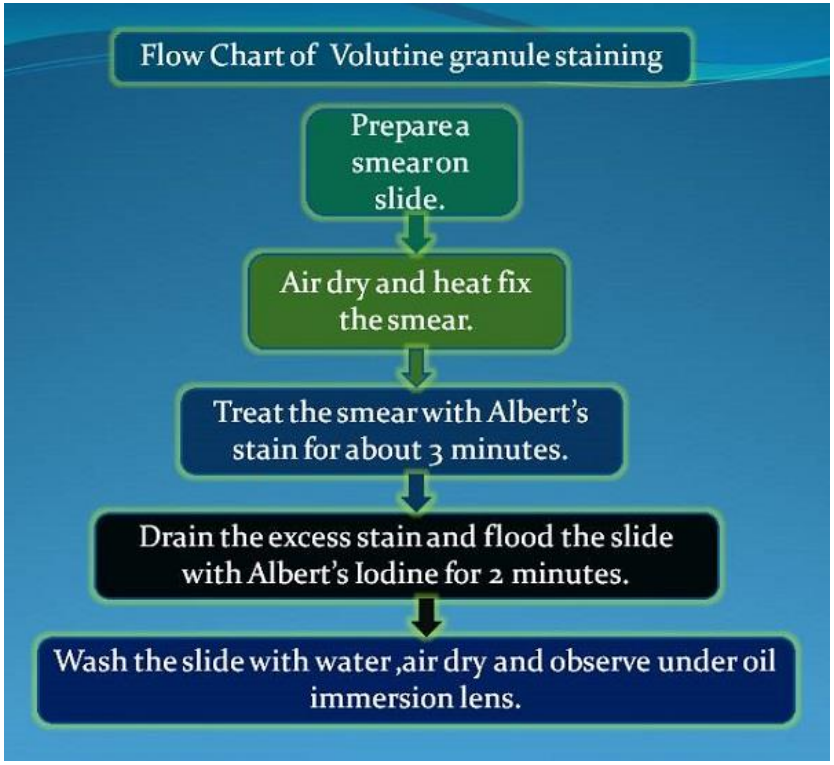
Albert,1921

Cytoplasm of Prokaryotes

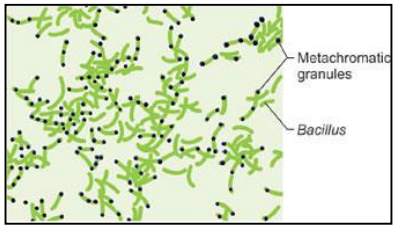
Cytoplasmic granules of bacteria

Alternative method

- Prepare a smear on clean grease free slide.
- Air dry and heat fix the smear.
- Treat the smear with Albert's stain (with toluidine blue or methylene blue dye) and allow it to react for about 3 mins.
- Drain of the excess stain do not water wash the slide.
- Flood the smear with Albert's iodine for 2 minutes.
- Wash the slide with water, air dry and observe under oil immersion lens.



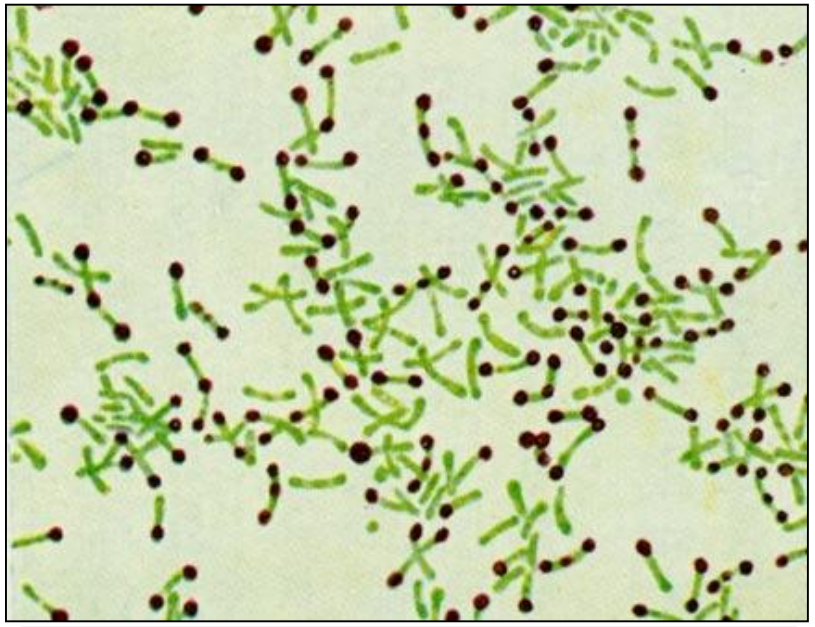
SumiGk,2013



Volutin granules

Storage form of complexed inorganic polyphosphate

- Volutin granules appear as metachromatic granules, stains intense reddish-purple color with methylene blue dye (instead of blue, as one would expect), and can be observed by light microscopy.



Metachromatic granules of *Corynebacterium* spp.

Tankeshwar,2013

Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

Gas vesicles or gas vacuoles

Gas vesicles or gas vacuoles
Aquatic bacteria

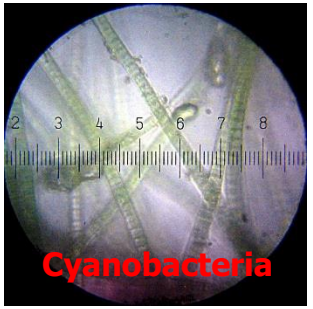
- Many aquatic bacteria produce gas vesicles/vacuoles.
- Gas vesicles, also known as gas vacuoles, are nanocompartments in certain prokaryotic organisms, which help in buoyancy.

1. Gas vesicles are composed entirely of proteins;

- No lipids or carbohydrates have been detected.

Wikipedia,2020;..

Gas vesicles/vacuoles



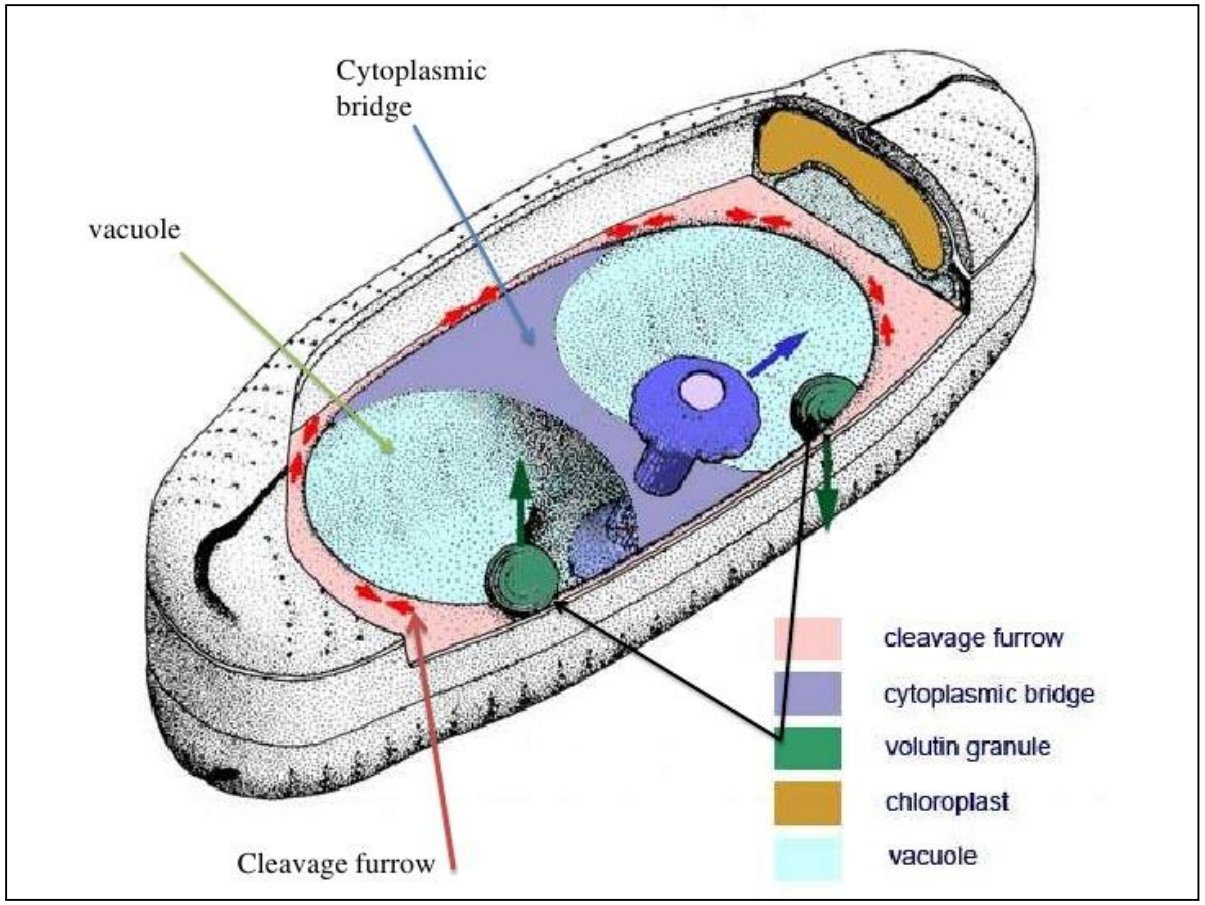
- Gas vesicles are spindle-shaped structures found in some ocean bacteria (e.g. phytoplankton and Gram negative cyanobacteria) that provides buoyancy to these cells by decreasing their overall cell density.
- Gas vesicles are made up of a protein coat that is very impermeable to solvents such as water but permeable to most gases.

Cyanobacteria, also known as blue-green bacteria, blue-green algae, and Cyanophyta. They are prokaryotic and represent the earliest known form of life on the earth. Cyanobacteria are aquatic and photosynthetic, that is, they live in the water, and can manufacture their own food through photosynthesis. These are true prokaryotes having no chloroplast but still perform photosynthesis.

Scanlan,2014;..

Gas vesicles or gas vacuoles

Aquatic bacteria

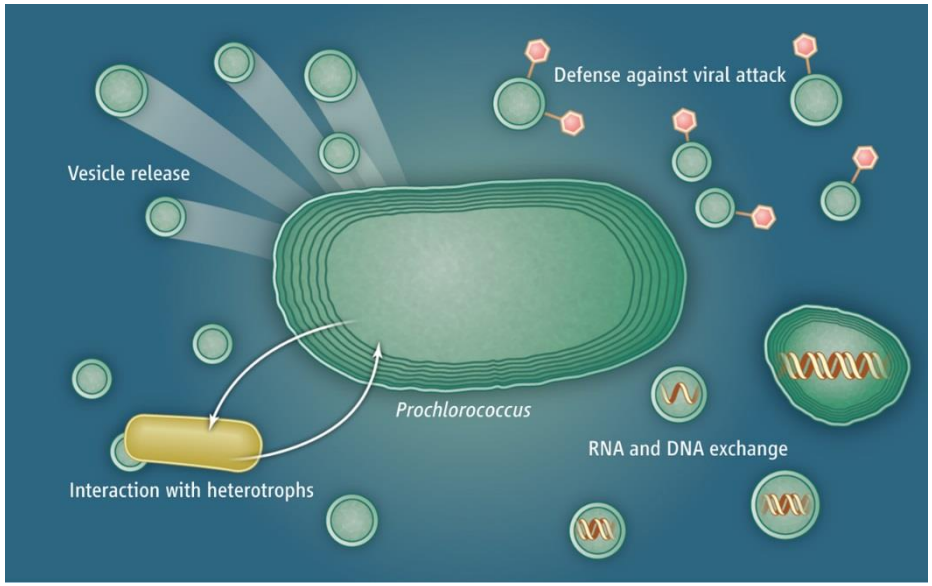


Jasin,2011

Gas vesicles or gas vacuoles

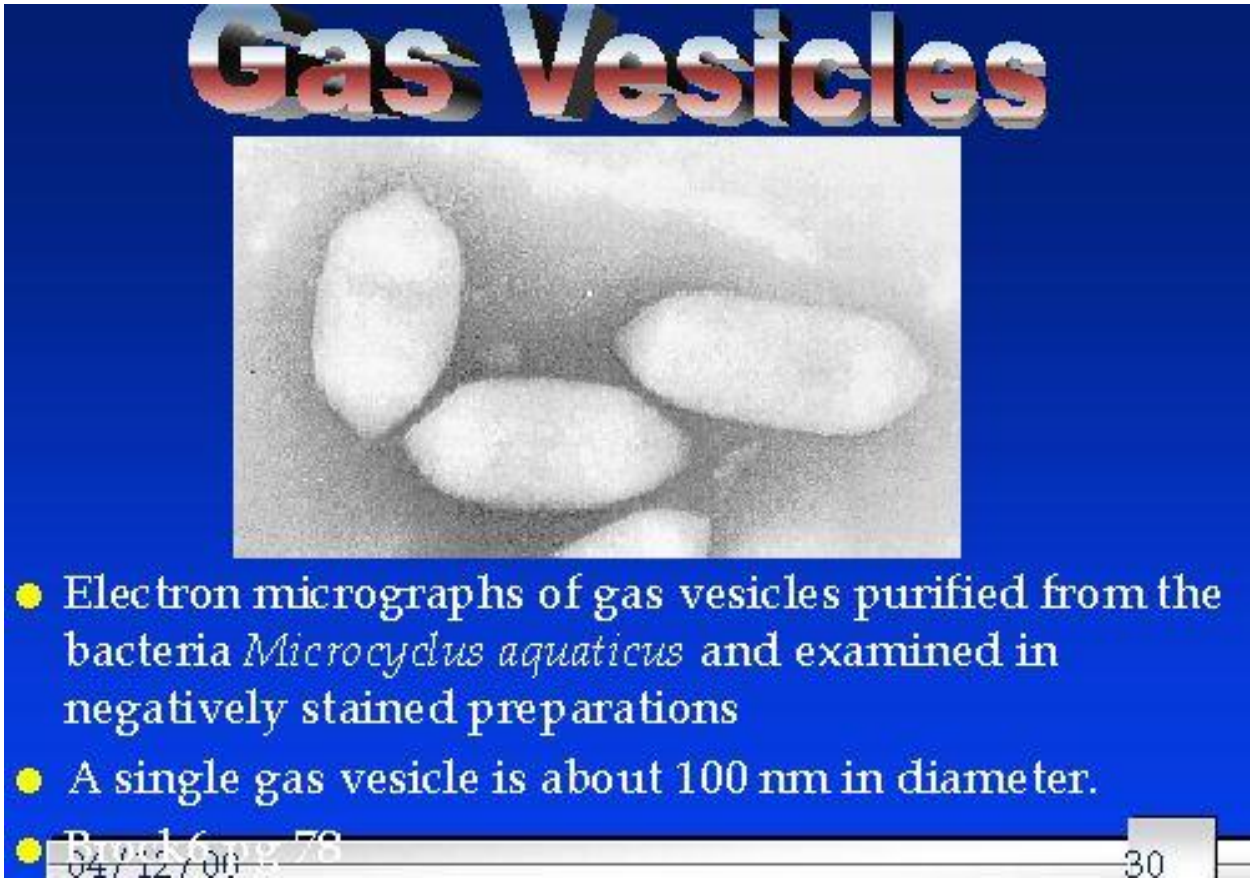
Defense against viral attack and DNA and RNA exchange

- By adjusting the amount of gas present in their gas vesicles, bacteria can increase or decrease their overall cell density and thereby move up or down within the water column to maintain their position in an environment optimal for growth.



Gas vesicles or gas vacuoles

Micrograph of gas vesicles



Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

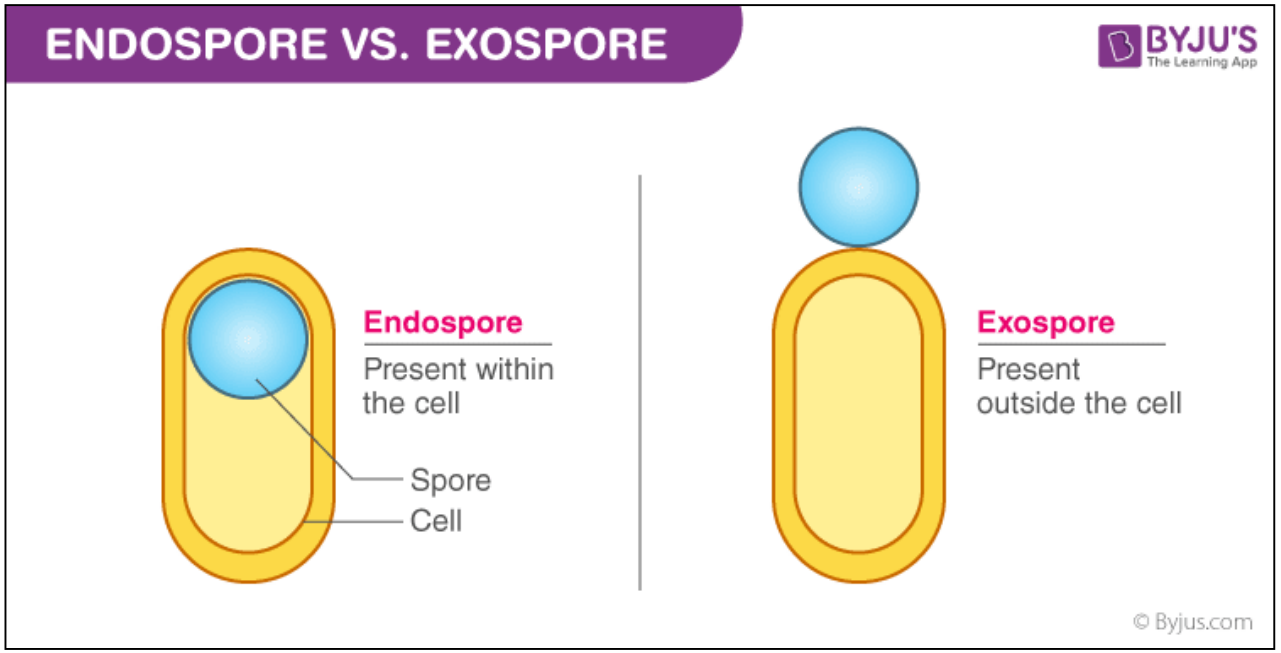
Spore-forming bacteria (endospores and exospores)

Gram positive bacteria

Spore-forming bacteria

Endospores and exospores

- Two types of reproductive structures or spores are Endospore and Exospore which are produced as stationary or resting systems.



Gram positive bacteria
Spore-forming bacteria
Endospores and exospores

1. Endospores are generated by the *Clostridium* *Bacillus* and *Sporosarcina* (closely related to the genus *Bacillus*) bacteria.
2. Exospores are produced by the members of the phylum Actinobacteria e.g. *Streptomyces* spp.

Unlike fungal spores, where one fungus can make many spores, bacterial endospores are a "one cell makes one endospore" affair.

Gram positive bacteria
Endospore-forming bacteria
In rods, cocci, filamentous bacteria

- The ability to form endospores is found among bacteria in a number of genera, predominantly gram-positive groups, including:

1. the aerobic rod *Bacillus*
2. the microaerophilic rod *Sporolactobacillus*
3. the anaerobic rods *Clostridium*
4. *Desulfotomaculum*, the coccus *Sporosarcina*, and
5. the filamentous *Thermoactinomyces*.

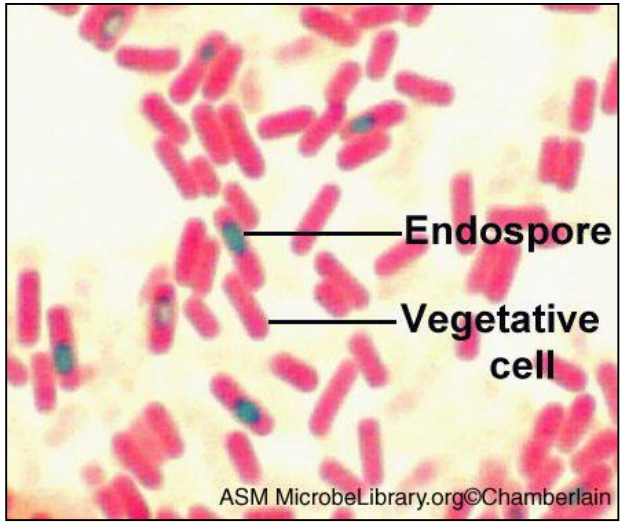
- Some *Bacillus* and *Clostridium* spp. are plant associated bacteria.

https://www.britannica.com

Gram positive bacteria

1. Endospores (spores)

- Dormant cell
- Produced when starved
- Resistant to adverse conditions:
  - High temperatures
  - Organic solvents
  - desiccation, and
  - ultraviolet radiation.
- Found in Gram+ve bacteria such as *Bacillus* and *Clostridium*.



The term "spore" comes from the Greek word for Seed.

Gram positive bacteria

Spore-forming bacteria

Endospores and exospores

Endospore	Exospore
Endospores are generated by the *Clostridium*, *Bacillus* and *Sporosarcina* bacteria.	Exospores are produced by the members of the phylum *Actinobacteria*.
Formed within or inside the vegetative cell.	Formation outside the vegetative cell.
It is a structure formed by bacteria.	It is an asexual spore that is separated from the mother cell.
Cell division is not involved in the formation of endospores.	Exospores are produced by the cell division.
Endospores are released by rupturing the mother cell.	Exospores are released by budding.
Only one endospore is produced by a single organism	Several exospores are produced by a single organism
Examples of endospores include *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Clostridium botulinum*, *Clostridium tetani*, etc.	Examples of exospores include Conidiospores, *streptomyces*, *actinobacteria* and diverse groups of fungi, algae and Cyanobacteria.

Gram positive bacteria

Endospores

Survival

- Endospores (*endo* means within), enable bacteria to lie dormant for extended periods, even centuries.
- There are many reports of spores remaining viable over 10,000 years, and revival of spores millions of years old has been claimed.
- There is one report of viable spores of *Bacillus marismortui* in salt crystals approximately 250 million years old.
- When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state.

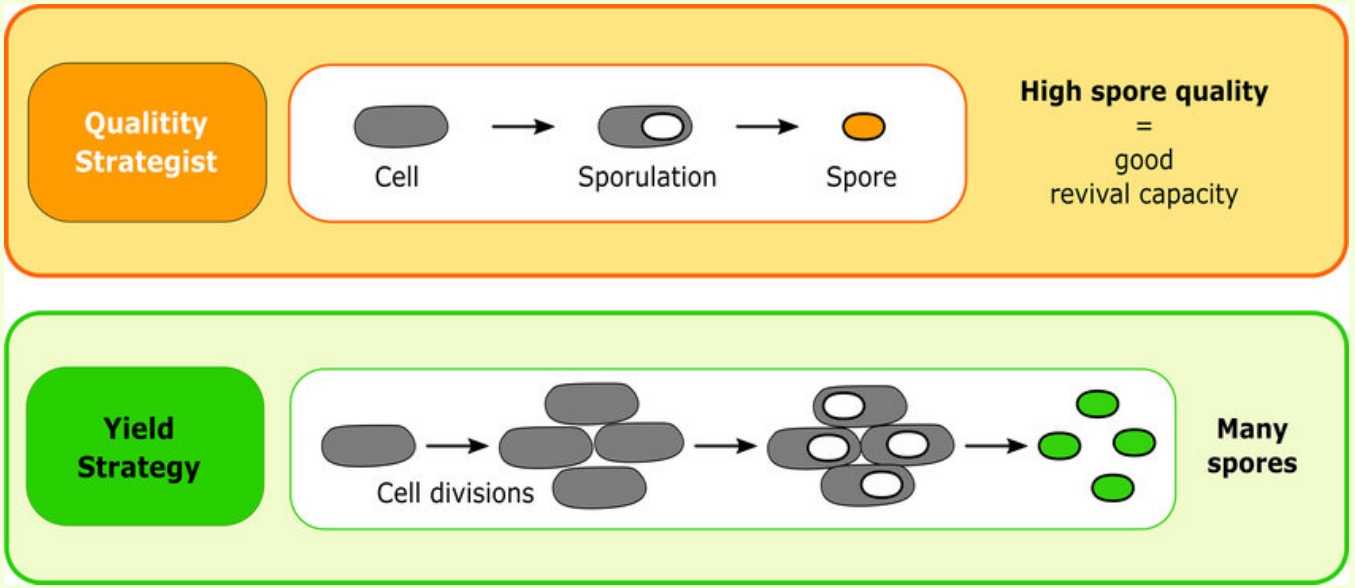
Wikipedia,2020

Gram positive bacteria

Spore-forming bacteria

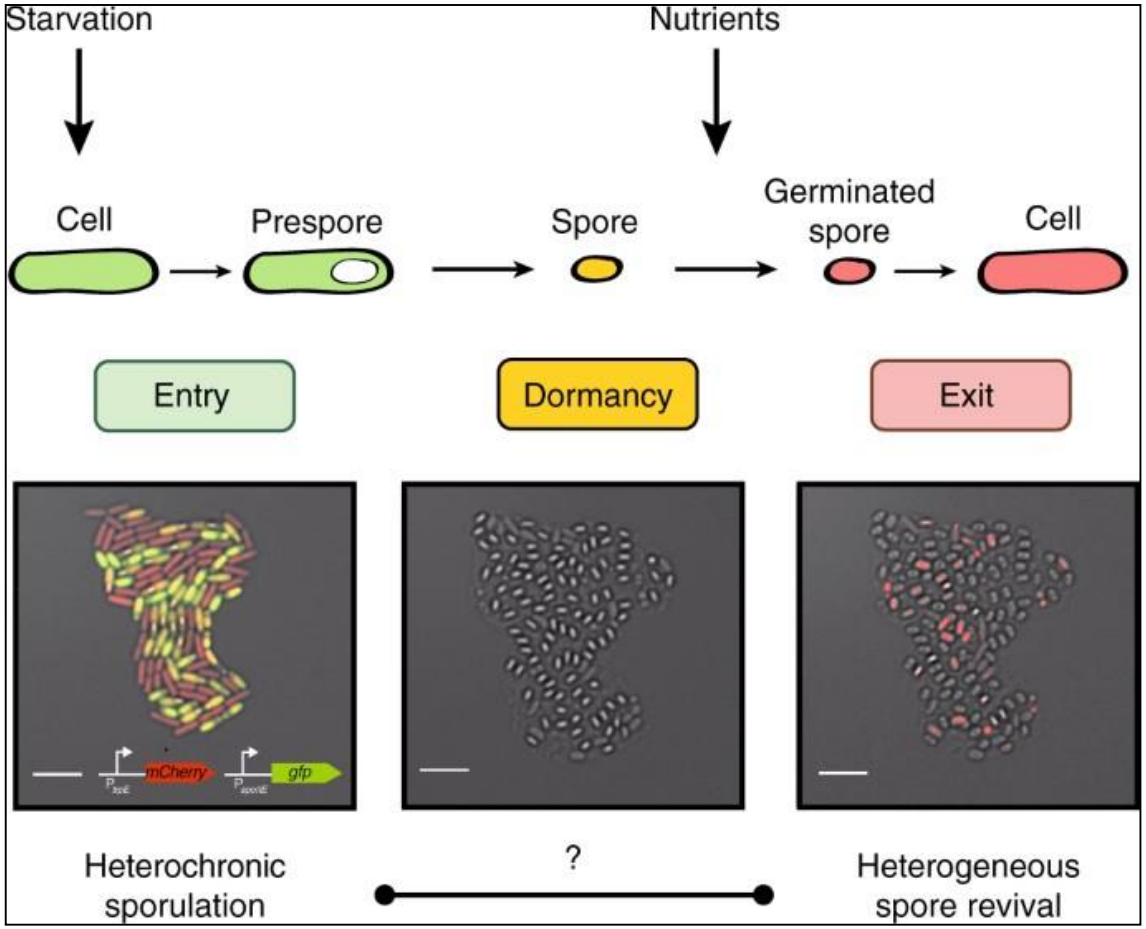
Only one spore is formed inside each bacterial cell during sporulation

- Spores of *Bacillus* and *Clostridium* species are metabolically dormant and extremely resistant to acute environmental stresses such as heat, desiccation, UV and γ-radiation, mechanical disruption, enzymatic digestion and toxic chemicals.



Endospore formation

After 4 days of starvation, both early and late spores had been released from the sporangia, which was taken as an indication that development was complete



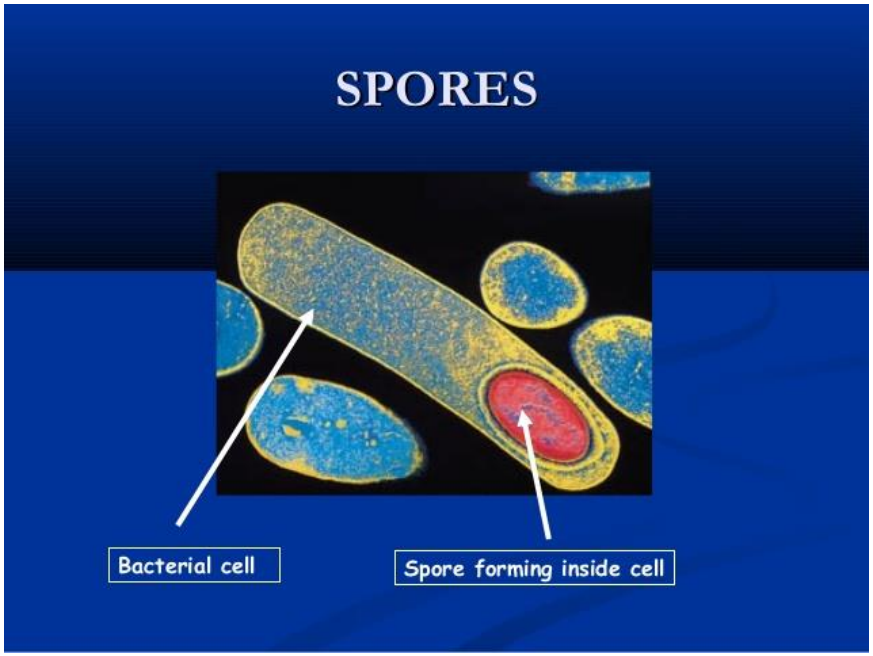
Mutlu *et al.*,2018

Gram positive bacteria

Endospores

Proposed mechanism of how sporulation gave rise to OM in bacteria

1. Sporulation in Gram-positive and Gram-negative bacteria.
2. Proposed mechanism of how sporulation gave rise to OM in bacteria.
3. Peptidoglycan remodeling during sporulation.



Tocheva *et al.*,2013;..

Gram positive bacteria

Endospores

Proposed mechanism of how sporulation gave rise to OM in bacteria

- Mechanistic clues about how endospore formation may have given rise to bacterial OMs came from comparing images of sporulating *B. subtilis* (monoderm) and a member of a lesser-known family of Clostridia Acetonema longum (diderm) cells.
- *B. subtilis* loses its outer spore membrane to become a monoderm, "Gram-positive" vegetative cell, whereas

1. *A. longum* retains both spore membranes and, amazingly, the outer spore membrane emerges as an OM.

Tocheva *et al.*,2013

Gram positive bacteria

Endospores

Proposed mechanism of how sporulation gave rise to OM in bacteria

- Here we review the images of sporulating monoderm and diderm cells which show how sporulation leads to diderm cells.
- Endospore formation offers a novel hypothesis for how the bacterial OM could have evolved:
- A primordial monoderm cell may have first developed the ability to form endospores, and then this process could have given rise to diderm vegetative cells.

Tocheva *et al.*,2013

Gram positive bacteria

Endospores

Proposed mechanism of how sporulation gave rise to OM in bacteria

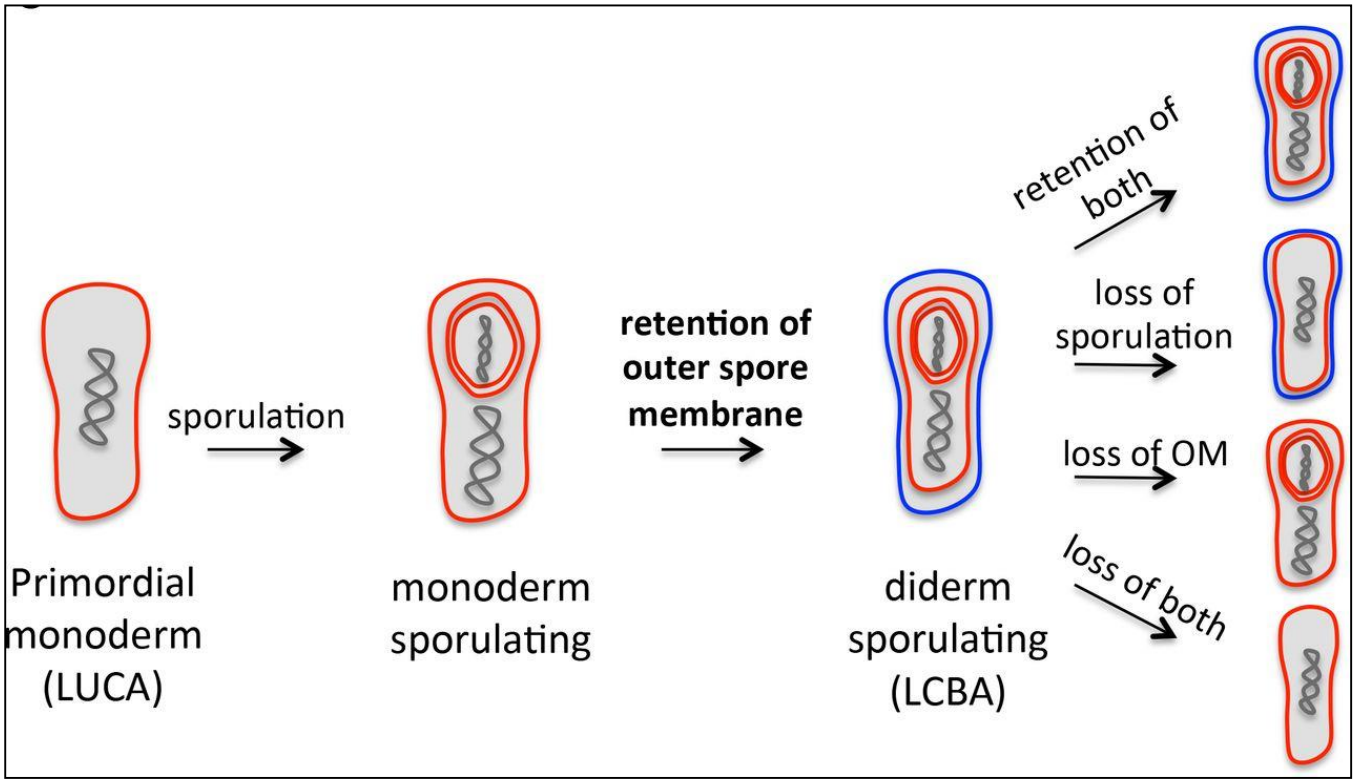
- At some early point in evolution, the cell division and nutrient-uptake processes of a primordial cell were combined into a sporulation-like process.
- Retention of the second spore membrane led to diderm sporulating species.
- Losses of the OM, the ability to sporulate or both in various lineages can explain the distribution of cell envelope architectures seen in modern bacteria.

Tocheva *et al.*,2013

Gram positive bacteria

Endospores

Proposed mechanism of how sporulation gave rise to OM in bacteria



Cellular evolution, where only spores survived (LUCA was a spore) and Last bacterial common ancestor (LBCA) was a diderm.

Tocheva *et al.*,2013

Endospore

Medical Importance of Bacterial Spores

Important features of Spores	Medical Implications
Spores are highly resistant to heating; spores are not killed by boiling (100˚C) but are killed at 121°C.	Medical supplies must be heated to 121°C for at least 15 minutes to be sterilized.
Spores are highly resistant to many chemicals, including most disinfectants.	Only solution designated as sporicidal will kill spores.
Spores can survive for many years in soil and other inanimate objects.	Wound contaminated with soils can be infected with spores and cause diseases such as tetanus (*Clostridium tetani*), gas gangrene (*Clostridium perfringens*).
Spores do not exhibit measurable metabolic activity.	Antibiotics are ineffective against spores.
Spores formed in adverse environmental conditions such as in nutrients insufficient.	Spores are not often found at the site of infection because nutrients are not limiting.

Tankeshwar,2013

Endospore formation

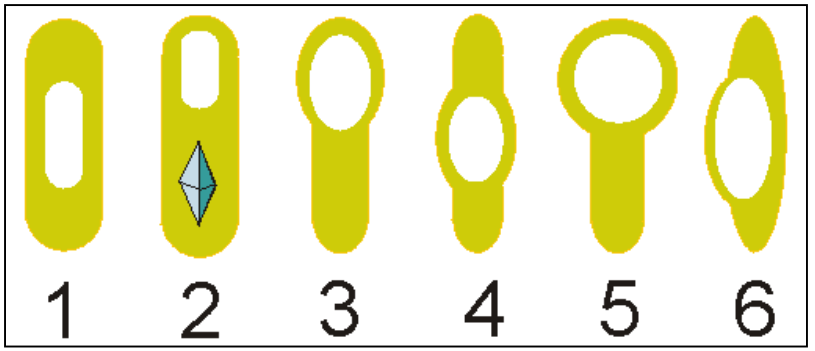
Life cycle of spore forming bacteria

One endospore is formed per bacterial cell

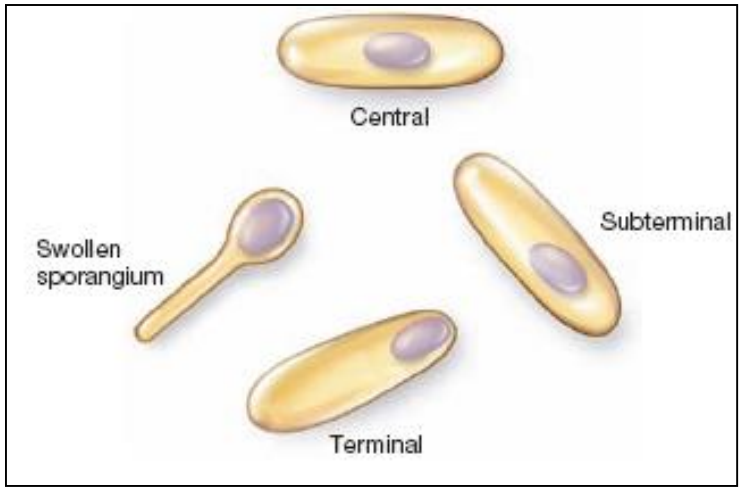
- The process of formation of endospore is called sporulation that occurs in an organized manner over a period of several hours (8hrs-19hrs).
- Sporulation is not a way of reproduction.

Endospore
Position of the spore

- Variations in endospore morphology:
- 1, 4: central endospore;
- 2, 3, 5: terminal endospore;
- 6: lateral endospore.



Metabacterium polyspora have the uncommon ability to produce as many as nine endospores per mother cell.



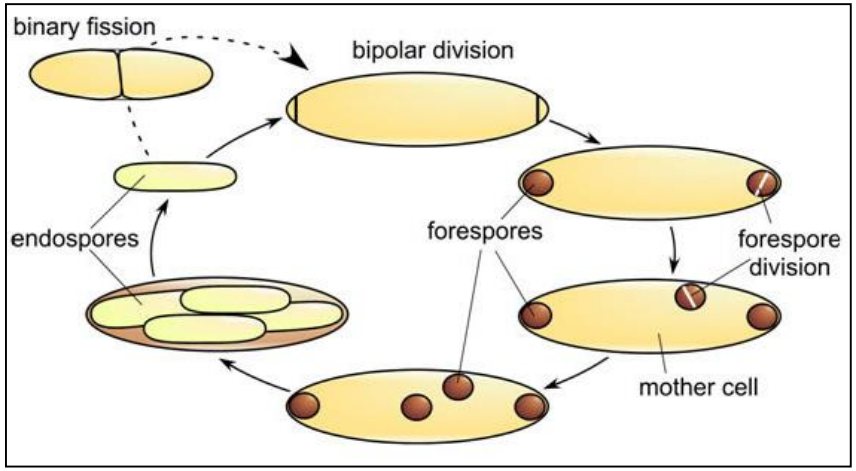
Schlink,2010;..

Endospore formation

Life cycle of spore forming bacteria

***Metabacterium polyspora* ncommon ability to produce as many as nine**

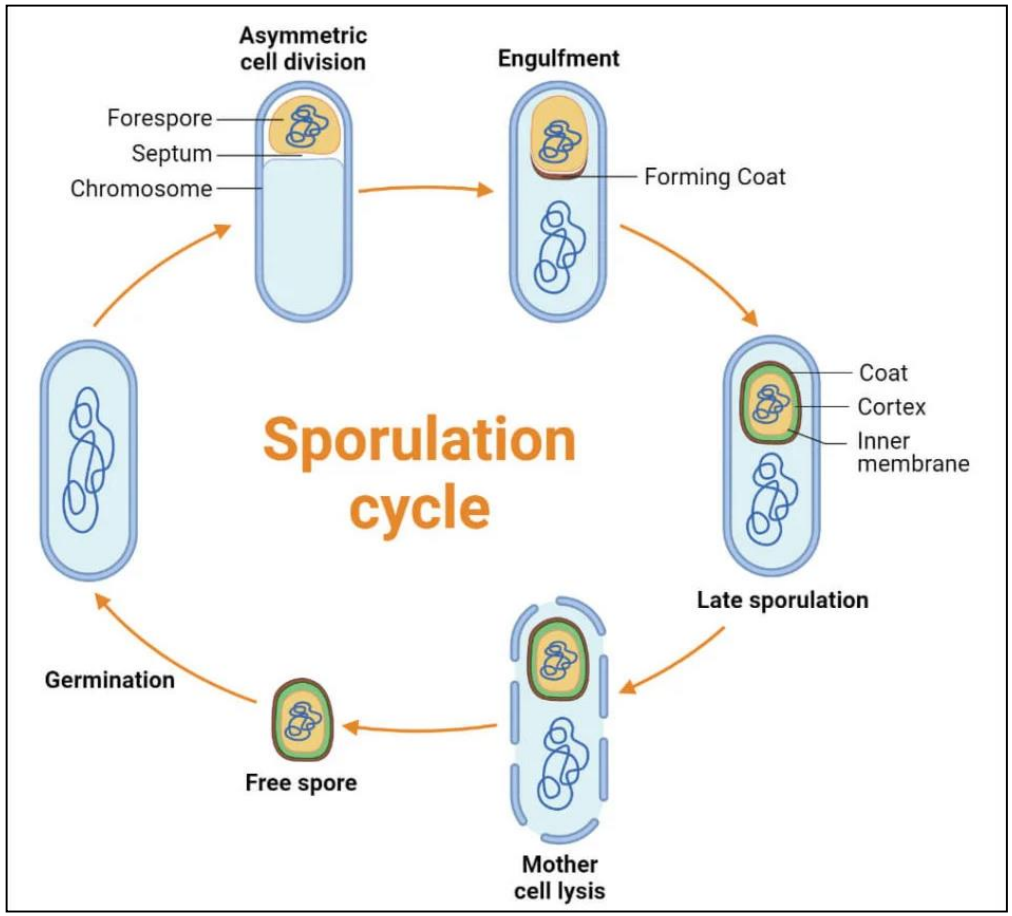
- *Metabacterium polyspora* is a gastrointestinal (GI) symbiont of the guinea pig.
- These large bacteria (12 to 35 µm long) have the uncommon ability to produce as many as nine, phase-bright endospores per mother cell.



Cornell University,2022

Endospore formation

Life cycle of spore forming bacteria



Aryal,2024

Endospore formation

Stages of spore development

Sporulation takes 8hrs-19hrs to complete

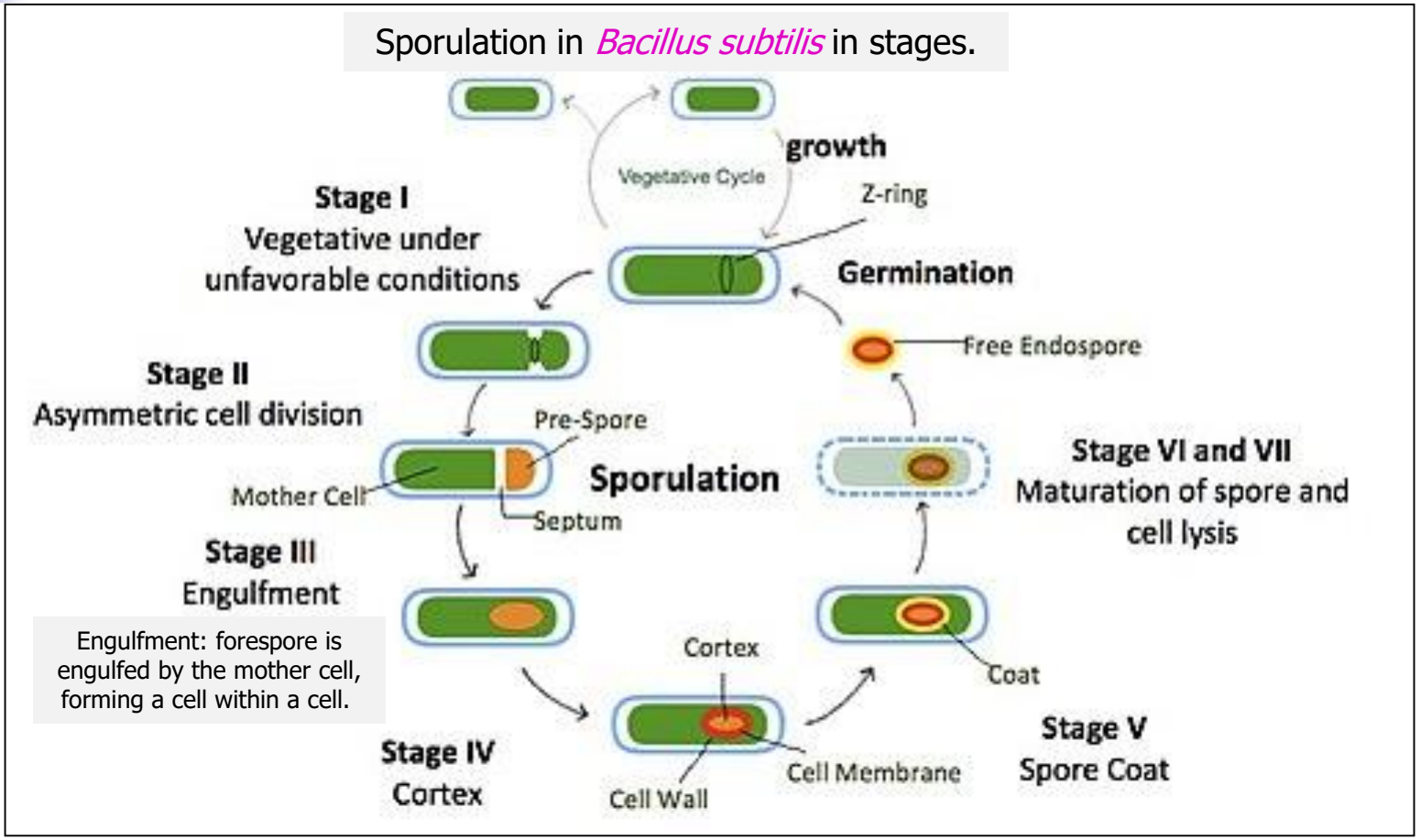
- The stages are indicated by Roman numerals.
- The circled numbers in the photographs refer to the hours from the end of the logarithmic phase of growth:
- 0.25 h: A typical vegetative cell
- 4 h: Stage II cell: septation
- 5.5 h: Stage III cell: engulfment
- 6.5 h: Stage IV cell: cortex formation
- 8 h: Stage V cell: coat formation
- 10.5 h: Stage VI-VII cell: spore mature in sporangium.
- Abbreviations used: C, cortex; IFM and OFM, inner and outer forespore membranes; M, mesosome; N, nucleoid; S, septum; SC, spore coats.

Engulfment: forespore is engulfed by the mother cell, forming a cell within a cell. Synthesis of the peptidoglycan cortex followed by formation of proteinaceous spore coat.

Endospore formation

Life cycle of spore forming bacteria

Seven stages of spore development

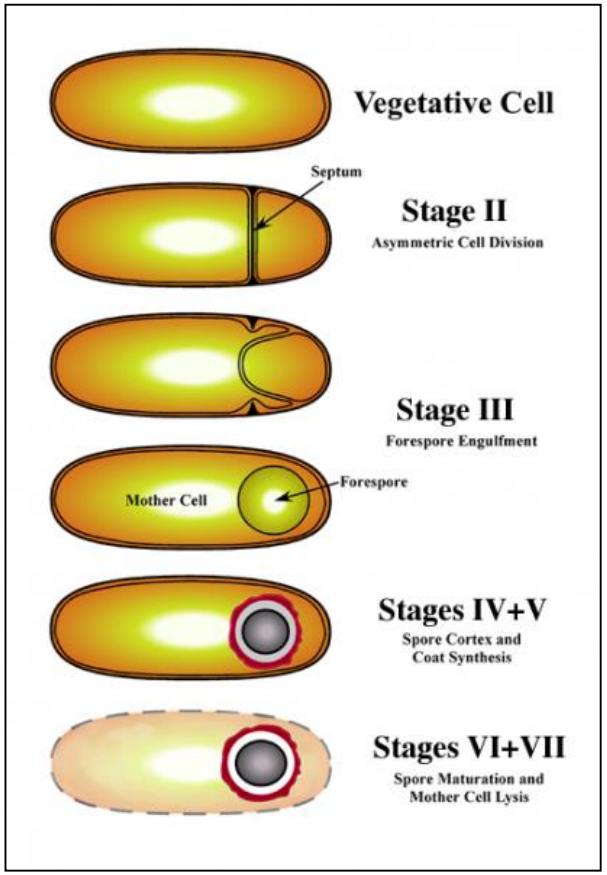


Formation of endospore

Sporogenesis

Sporulation takes 8hrs-19hrs to complete

- Develop inside the vegetative cell (actively growing cell) when environment becomes unfavorable.
- G+ve Bacterium gathers DNA and other essential goodies together.
- Mesosomes separate DNA, etc. form the rest of the cytoplasm.
- Spore walls forms around it.
- Outer cell disintegrates leaving spore.

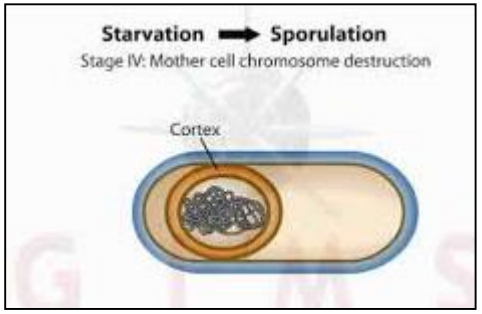


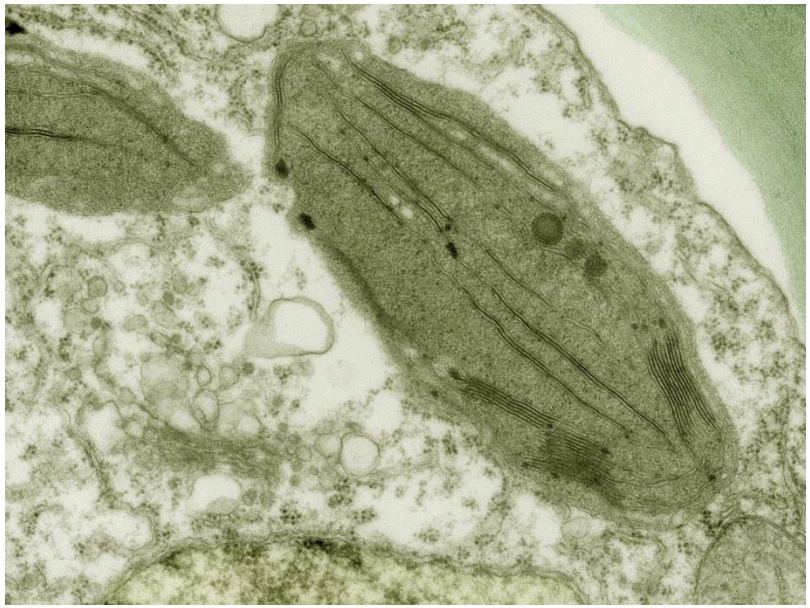
Mesosomes: cytoplasmic membrane-associated organelle (more easily seen in gram-positive bacteria).

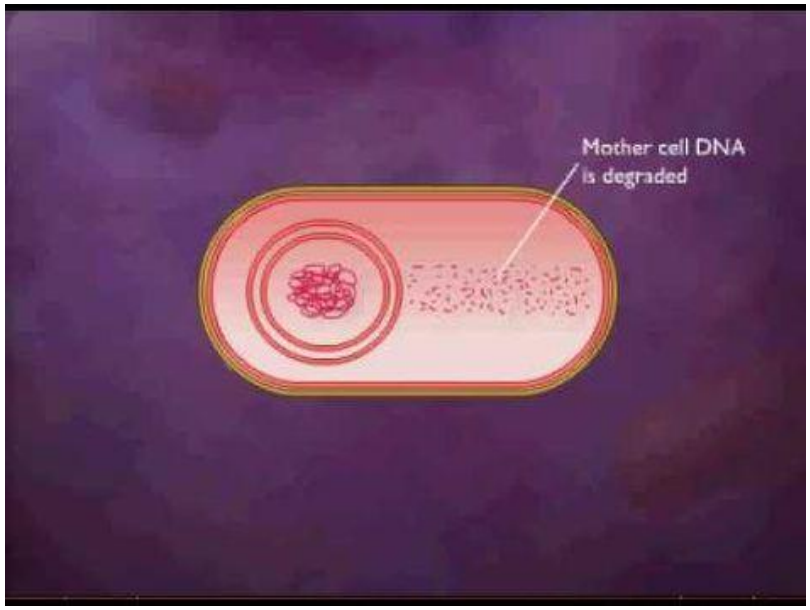
Spore mother cell

Endospore

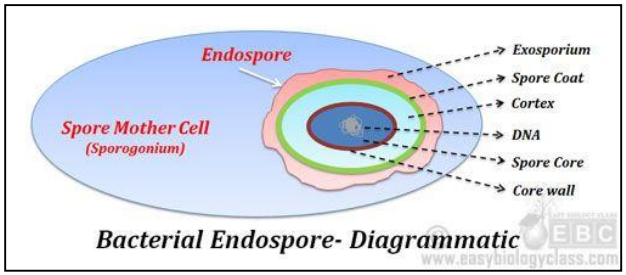
Starvation activate sporulation







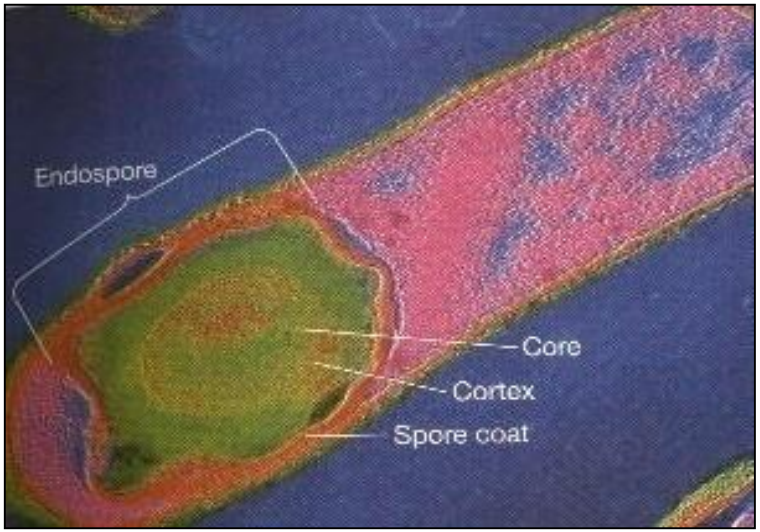
Pinterest.com; Wikipedia

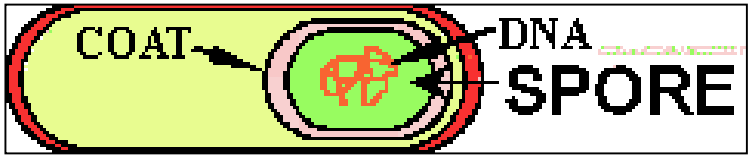


Endospore

Position and contents of the spore

- Bacteria produce a single endospore internally.
- The position of the spore in the mother cells sporangium is often used for identification purposes, because of the differences in different species.





Apart from genetic material, spores also contain some cytoplasm, specific acids, ribosome, and the appropriate enzymes among others that allow the spore to germinate during favorable environmental conditions.

Schlink,2010; MicroscopeMaster.com

Endospore

Spore coat and cortex

Spore coat

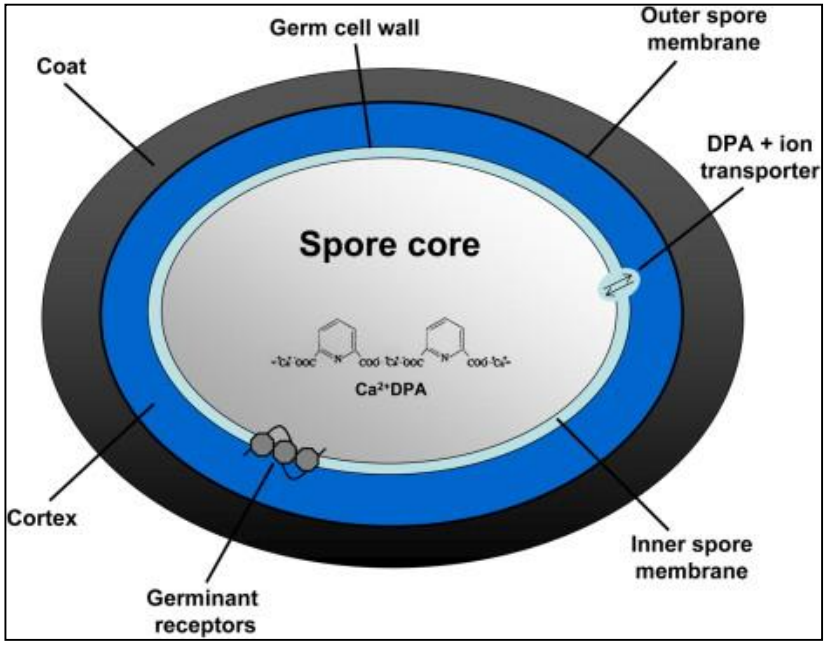
- The outer proteinaceous coat surrounding the spore provides much of the chemical and enzymatic resistance.

Cortex

- The cortex lies beneath the spore coat and consists of peptidoglycan.

Core

- The center of the endospore, the core, exists in a very dehydrated state and houses the cell's DNA, ribosomes and large amounts of dipicolinic acid+ Ca^{+2} ions which is know as calium dipicolinate.



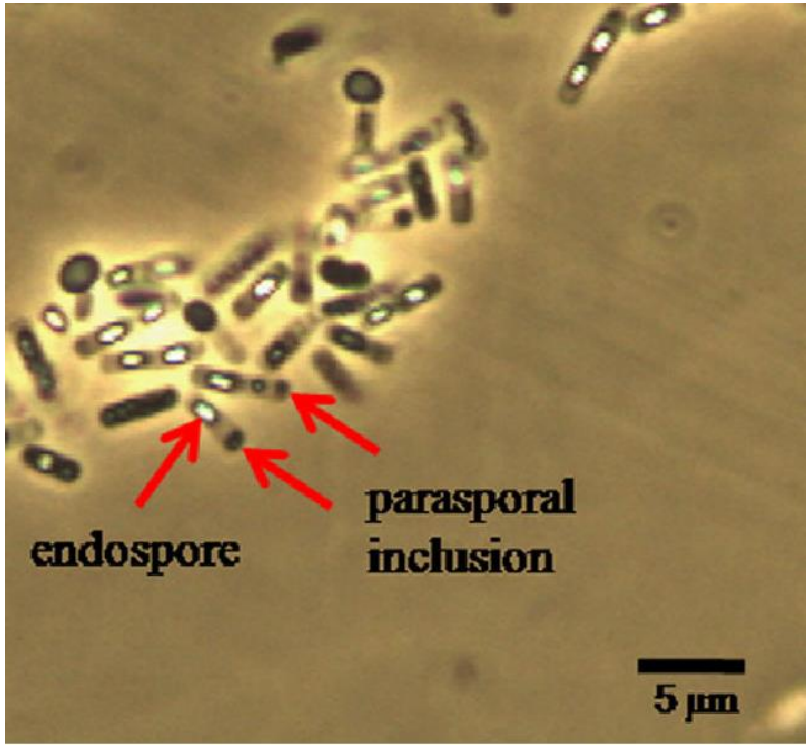
Dipicolinic acid could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents.

Wohlgemuth and Kämpfer,2014; Cornell university,2019;..

Endospore formation

Typical example of sporulated cells of *B. thuringiensis* and parasporal protein crystals

- Para (beside, side by side) + sporal= spore.
- Parasporal inclusion produced adjacent to endospore.
- **Parasporal:** Describing a crystalline protein that forms around a spore in some bacteria that acts as a toxin precursor when digested.
- These crystals are toxic to beetles.

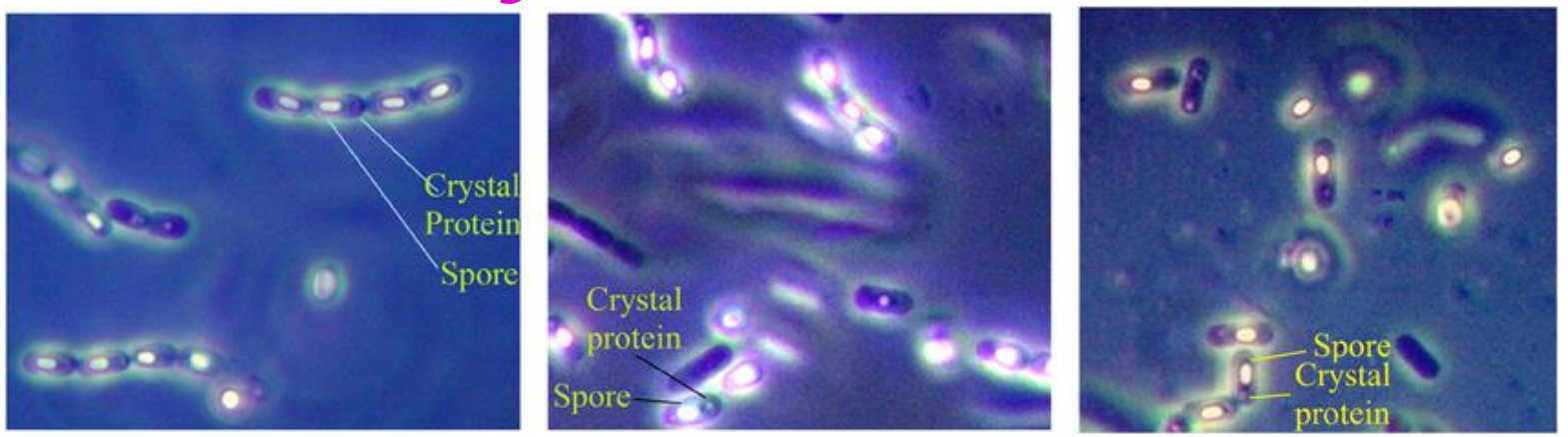


Phase contrast microscopic observation of Bt-S84-13a upon sporulation. Parasporal inclusion appeared as dark particles while endospore appeared bright.

Endospore formation

Typical example of sporulated cells of *B. thuringiensis* and parasporal protein crystals

- Examination of Bacterial isolates from the colonies incubated up to 72 h to allow sporulation by Phase contrast microscopy.
- Glowing spores and juxtaposed crystal protein as revealed under Phase Contrast microscope rendered them as *Bacillus thuringiensis* strains.

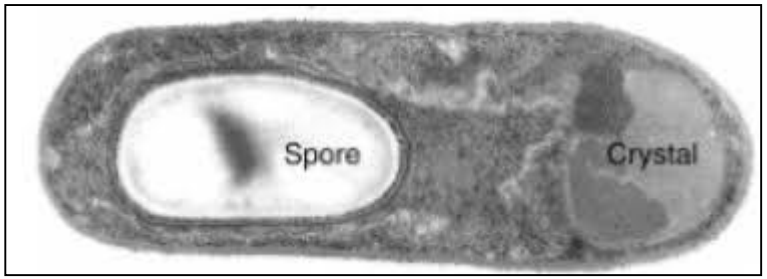


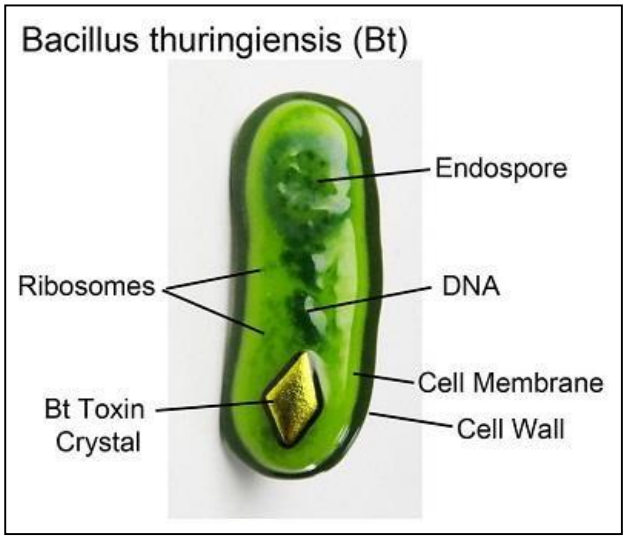
Shishir *et al.*,2014

Endospore formation

Typical example of sporulated cells of *B. thuringiensis* and parasporal protein crystals

- *Bacillus thuringiensis* is differentiated from other spore-forming bacilli by the presence of a parasporal body that is formed within the sporangium during sporogenesis.
- The parasporal body is a high-molecular-mass protein crystal, possesses some of the insecticidal properties of the bacterium.





ChemicalBook,2017

Endospore formation

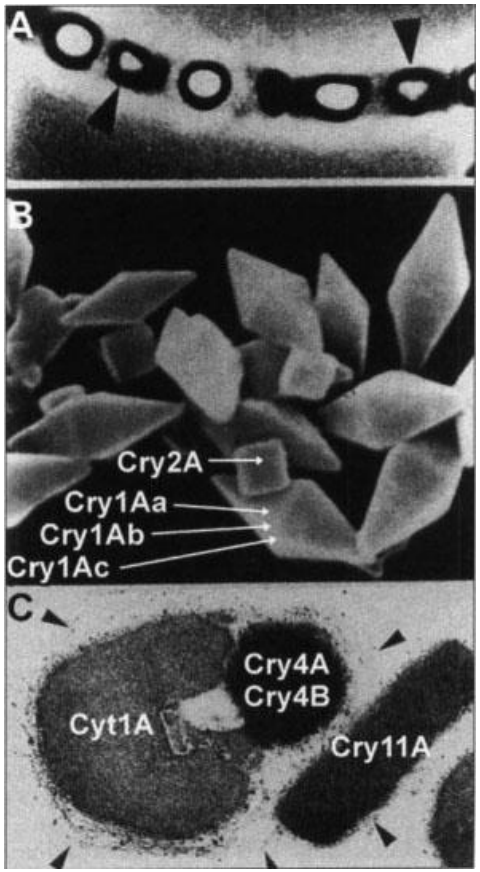
Typical example of sporulated cells of *B. thuringiensis* and parasporal protein crystals

- Sporulated culture and parasporal bodies of *Bacillus thuringiensis*.

A. Sporulated culture of *Bacillus thuringiensis* illustrating the spore and toxin-containing parasporal body.

B. Parasporal body protein inclusions containing Cry proteins produced by the HD1 isolate of *B.t. kurstaki*, the Bt isolate used most widely in products for control of lepidopterous pests.

C. Protein inclusion characteristic of *B.t. israelensis* used widely to control the larvae of mosquitoes and blackflies.



Shively,2006; Chai *et al.*,2014

Endospore formation

Classification of Cry toxins according to their insect host specificities proposed by Crickmore *et al.*,1998

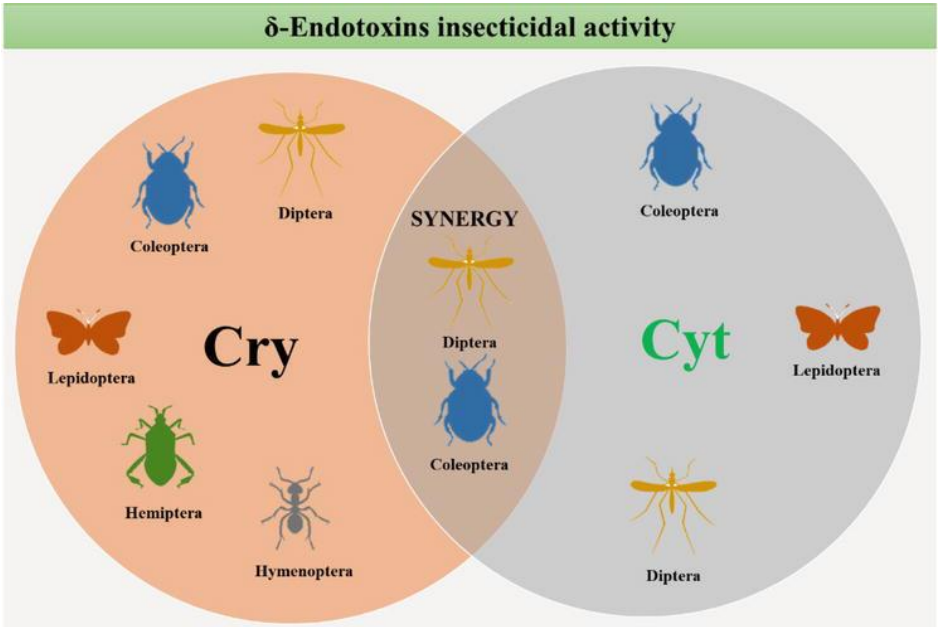
Main classes	Order	Cry toxins
Group 1	Lepidoptera	Cry1, Cry9, and Cry15
Group 2	Lepidopteran and dipterous	Cry2
Group 3	Coleoptera	Cry3, Cry7, and Cry8
Group 4	Diptera	Cry4, Cry10, Cry11, Cry16, Cry17, Cry19, and Cry20
Group 5	Lepidoptera and Coleoptera	Cry1I
Group 6	Nematodes	Cry6

Fernández-Chapa *et al.*,2018

Endospore formation

Typical example of sporulated cells of *B. thuringiensis* and parasporal protein crystals

Insecticidal activity of Cry and Cyt δ-endotoxins against the orders *Diptera*, *Coleoptera*, *Lepidoptera*, *Hemiptera*, and *Hymenoptera*.



Diptera	Cry1(A-C), Cry2(A-B), Cry4(A-C), Cry10(A), Cry11(A-B), Cry16(A), Cry17(A), Cry19(A-C), Cry20(A), Cry21(A-H), Cry24(A-C), Cry25(A), Cry27(A), Cry29(A-B), Cry30(A-G), Cry32(B-D), Cry39(A), Cry40(A-D), Cry44(A), Cry47(A), Cry48(A),Cry49(A), Cry52(A-B), Cyt1(A-D), Cyt2(A-D), Cyt3(A)
Coleoptera	Cry1(A-I), Cry2(A), Cry3(A-C), Cry7(A), Cry8(A-G), Cry9(D), Cry14(A), Cry18(A-C), Cry22(A-B), Cry23(A), Cry26(A), Cry28(A), Cry34(A-B), Cry35(A-B), Cry36(A), Cry37(A), Cry38(A), Cry43(A-C), Cry55(A), Cyt1(A), Cyt2(C)
Lepidoptera	Cry1(A-K), Cry2(A-B), Cry7(B), Cry8(D) Cry9(A-C,E), Cry15(A), Cry19(A-C), Cry20(A-B), Cry22(A), Cry32(A), Cry51(A), Cry54(A-B), Cry59(A-B), Cyt2(B)
Hemiptera	Cry3(A), Cry5(A), Cry22(A)
Hymenoptera	Cry2(A), Cry3(A), Cry11(A)

Fernández-Chapa *et al.*,2018

Endospore formation
Mechanism of Cry toxin action

- Although the mechanism of action of Cry toxins against various insects has been widely investigated, there are still many controversies.
- Two main models:

1. Mechanism of action of Cry proteins according to the sequential binding model;
2. Mechanism of action of Cry proteins according to the signaling pathway model.

Fernández-Chapa *et al.*,2018

Endospore formation

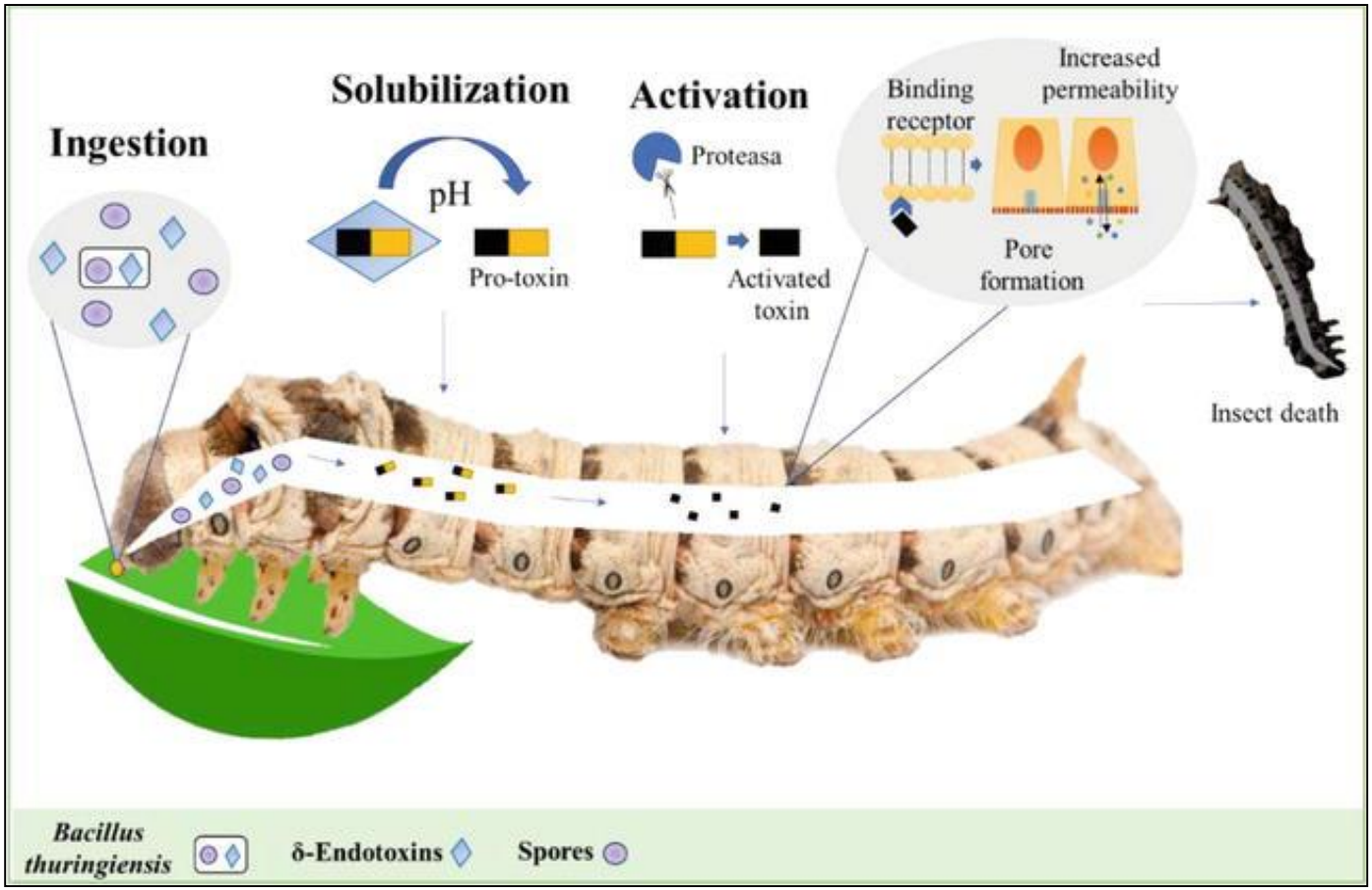
Mechanism of action of Cry proteins according to the sequential binding model

- The sequential union model is known as the classical mechanism.
- The crystals and their subunits are inert protoxins and are not biologically active.
- But after δ-endotoxins ingestion, the crystals are solubilized by the alkaline pH of the intestine, the inactive protoxins are digested by proteases of the midgut which produces an active toxin of about 60-70 kDa resistant to proteases, and then the Cry toxins come into contact with the surface of the membrane.
- This in turn, makes pre-pores in insect cell membrane.
- The pores cause an osmotic imbalance that causes cell death and lysis; the intestine is paralyzed, the insect stops feeding, and there is diarrhea, total paralysis, and finally death.

Fernández-Chapa *et al.*,2018

Endospore formation

Mechanism of action of Cry proteins according to the sequential binding model



Fernández-Chapa *et al.*,2018

Endospore formation

Mechanism of action of Cry proteins according to the signaling pathway model

- The signaling pathway model has similarities with the previous model; however, in this other causes for cell death are assigned.
- According to this theory, Cry proteins affect the cell in two ways:

1. first by the formation of pores in the membrane, as mentioned in the sequential binding model and,
2. second, by the production of successive reactions that alter the cellular metabolism.

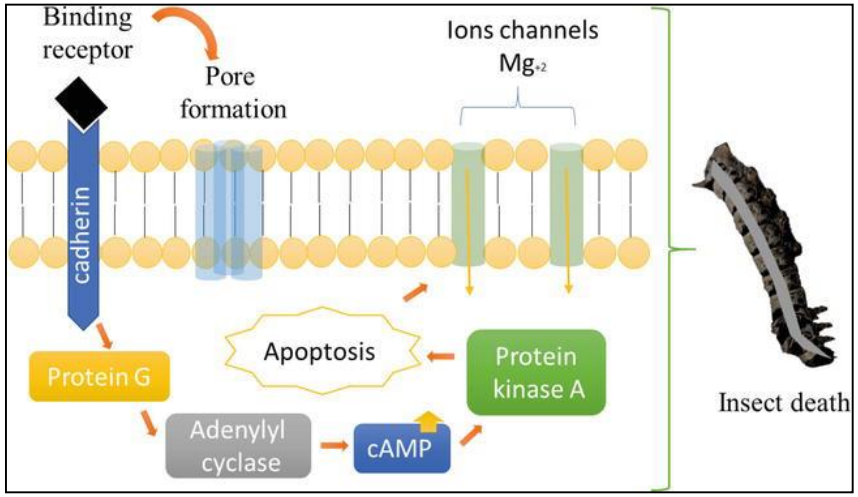
- The opening of these channels causes an abnormal movement of the ions in the cytosol, stimulating the process of apoptosis.

Fernández-Chapa *et al.*,2018

Endospore formation

Mechanism of action of Cry proteins according to the signaling pathway model

- Cry toxins bind to cadherin receptors, which stimulate heterotrimeric G protein and adenylyl cyclase with an increase in cAMP production.
- The cAMP activates the protein kinase A, which stimulates apoptosis with an activation of the Mg^{2+} channels in the plasma membrane.
- The opening of these channels causes an abnormal movement of the ions in the cytosol, stimulating the process of apoptosis.



Fernández-Chapa *et al.*,2018

Endospore formation

Varieties of Bt used as bioinsecticides, susceptible insects, expressing δ-endotoxin, and companies that produce it

Bt variety	Susceptible insects	δ-Endotoxin	Producer company
kurstaki	Lepidoptera	Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, and Cry2Ab	Abbott-Dupont and Certis
aizawai	Lepidoptera	Cry1Aa, Cry1Ab, Cry1Ba, Cry1Ca, and Cry1Da	Abbott-Dupont and Kenogard
san diego	Coleoptera	Cry3Aa	Mycogen
tenebrionis	Coleoptera	Cry3Aa	Thermo Trilogy, Columbia MD, Certis Mycogen, and Novo Nordisk
israelensis	Diptera	Cry4A, Cry4B, Cry11A, and Cyt1Aa	Abbott-Dupont, Novo Nordisk, and Certis
galleriae	Coleoptera	Cry8Da	Phyllom BioProducts

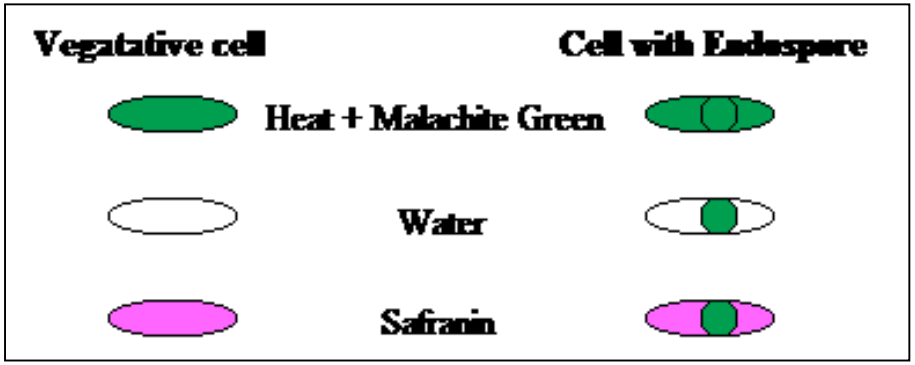
Delta= δ ro δ

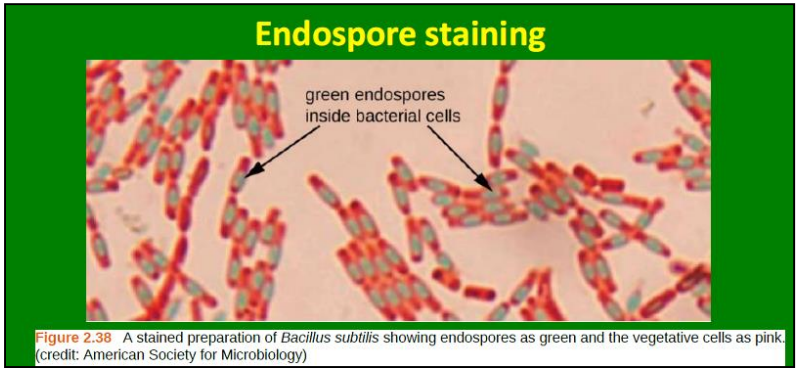
Fernández-Chapa *et al.*,2018

Gram positive bacteria
Endospore stain procedure

- Prepare smears of organisms to be tested for endospores.
- Heat fix the smears.
- Cover the smears with a piece of whatman absorbent paper cut to fit the slide.
- Saturate the paper with malachite green and holding the Bunsen burner in the hand heat the slide until steam can be seen rising from the surface.
- Remove the heat and reheat the slide as needed to keep the slide steaming for about three minutes.
- As the paper begins to dry add a drop or two of malachite green to keep it moist, but don't add so much at one time that the temperature is appreciably reduced. DO NOT OVERHEAT. The process is steaming and not baking.
- Remove the paper with tweezers and rinse the slide thoroughly with tap water.
- Drain the slide and counterstain 45 seconds with 0.5% safranin.
- Wash, blot, and examine.
- The vegetative cells will appear red and the spores will appear green.

Endospore staining
Procedures





Endospores will stain green, the rest of the cell pink.

Microbiology Lab Tutorial;..

Heat test for spores

Alternative method for endospore staining

- In order to test whether a sample of inoculum contains endospores, the sample is exposed to high heat (80°C) for 10 minutes.
- This treatment would kill all cells but endospores would survive and could grow when incubated at an appropriate temperature.

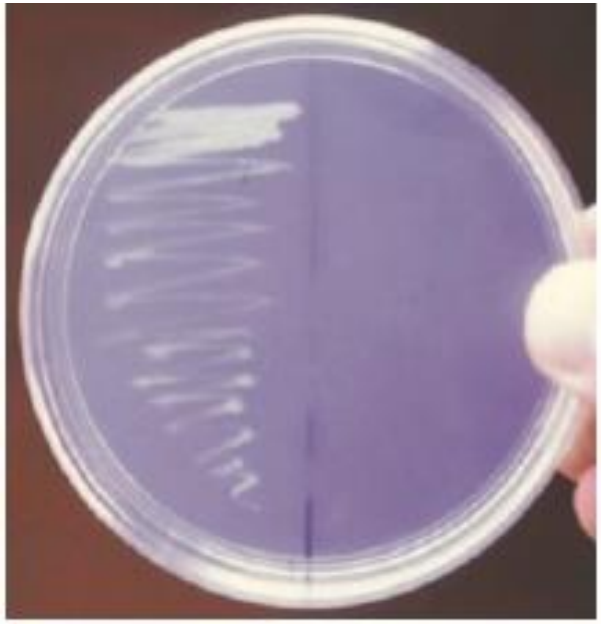
1. If endospores are present, the resultant inoculum in NB medium will give rise to a turbid culture (indicating bacterial growth);
2. If there are no endospores present, no growth is observed.

Christopher and Bruno,2003;..

Heat test for spores

Alternative method for endospore staining

- In this test, bacterial suspensions were exposed to 80°C for 10 minutes.
- One loopful was streaked on NA medium.
- The Growth on the agar plate is limited to the area streaked with bacterial inoculum containing endospore.



See also endospore staining method

Gram positive bacteria

2. Exospore formation

Streptomyces coelicolor as a model streptomycete

- Streptomycetes are filamentous soil bacteria belonging to the phylum Actinobacteria that are found throughout the world and produce a wide array of antibiotics and other secondary metabolites.
- Approximately

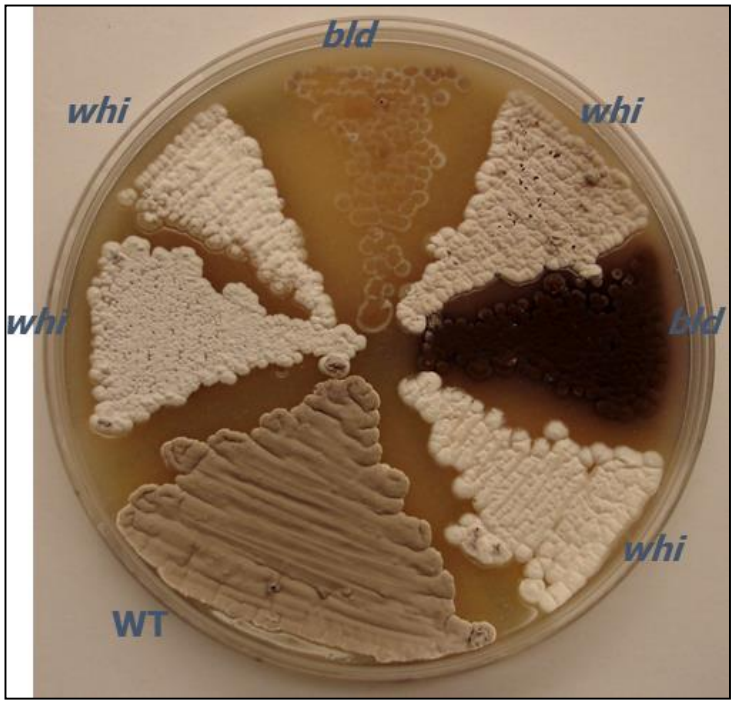
1. 75% of commercially used antibiotics, and
2. more than half of the naturally occurring antibiotics have been isolated from *Streptomyces*.

- *Streptomyces coelicolor* is a well-characterized, non-pathogenic species that is amenable to a variety of analyses in the lab.

Benneth *et al.*,2018;..

Spore formation
Streptomycetes

- On agar plates, they can easily be distinguished from true bacteria.
- Unlike slimy distinct colonies of true bacteria which grow quickly, actinomycete colonies appear slowly, show powdery consistency and stick firmly to agar surface.



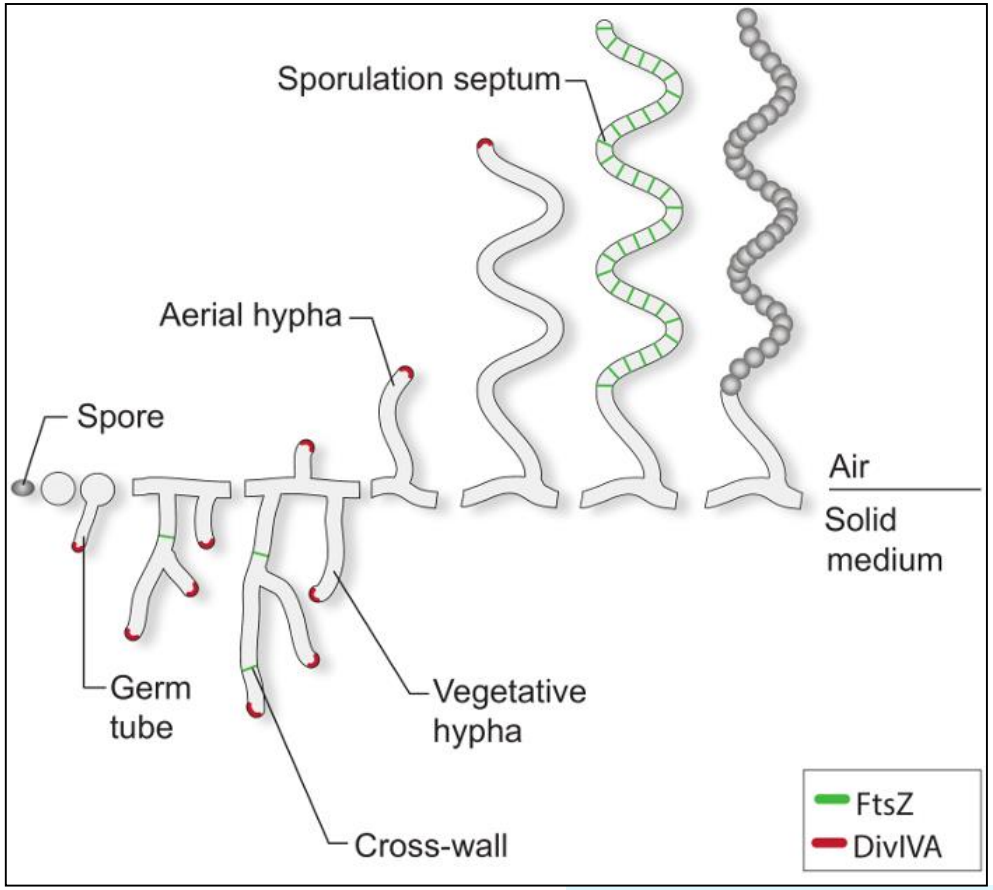
Benneth *et al.*,2018;..

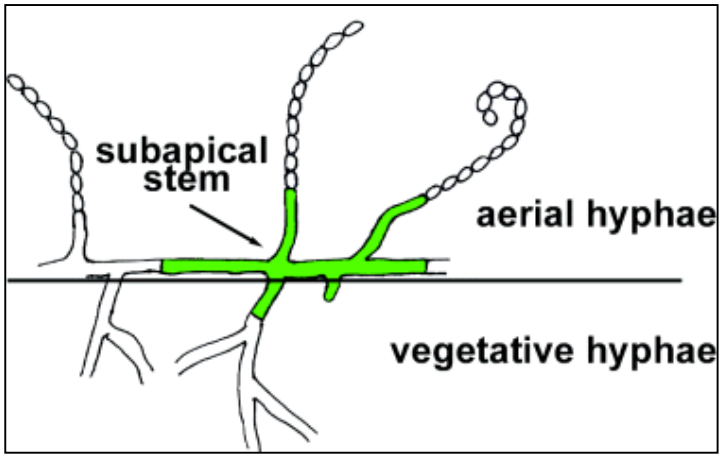
Spore formation
Streptomycetes

- As the growth of many *Streptomyces* species progress, sporulation begins in coordination with depletion of carbon source from the media.
- Figure shows fully sporulated *Streptomyces lividans* TK64 on solid oatmeal agar media.
- Spores are relatively more stable and can be stored for several years as a glycerol stock.
- **Exospores or arthrospores** (fungi-like uninucleoid spores) significantly differ from the endospores of *Bacilli* and *Clostridia* in morphology and function.

Shepherd *et al.*,2010

Spore formation
Streptomycetes





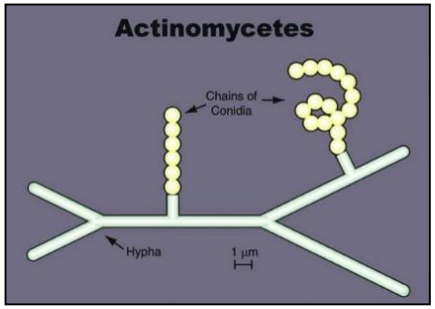
On solid medium
a spore germinates, grows vegetatively as a substrate mycelium and then develops into an aerial mycelium.
Long chains of spores form ed on aerial hyphae.

Schlimpert *et al.*,2016;..

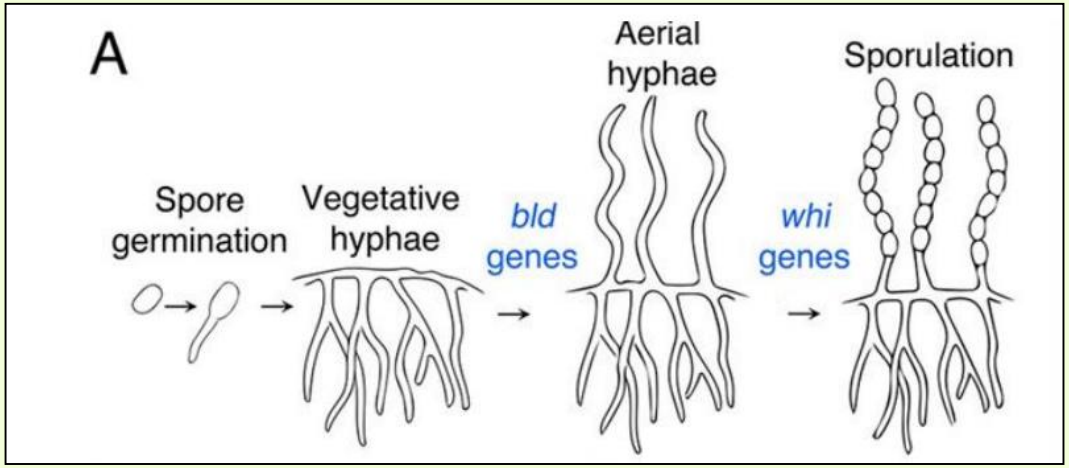
Gram positive bacteria

Exospore formation

Streptomycetes



- Spore arrangement in *Streptomyces griseus*.
- Grey spores arranged in straight chains.
- Aerial development requires the activity of the *bld* gene products, Whereas, *whi* gene requires for differentiation of aerial hyphae into spores.

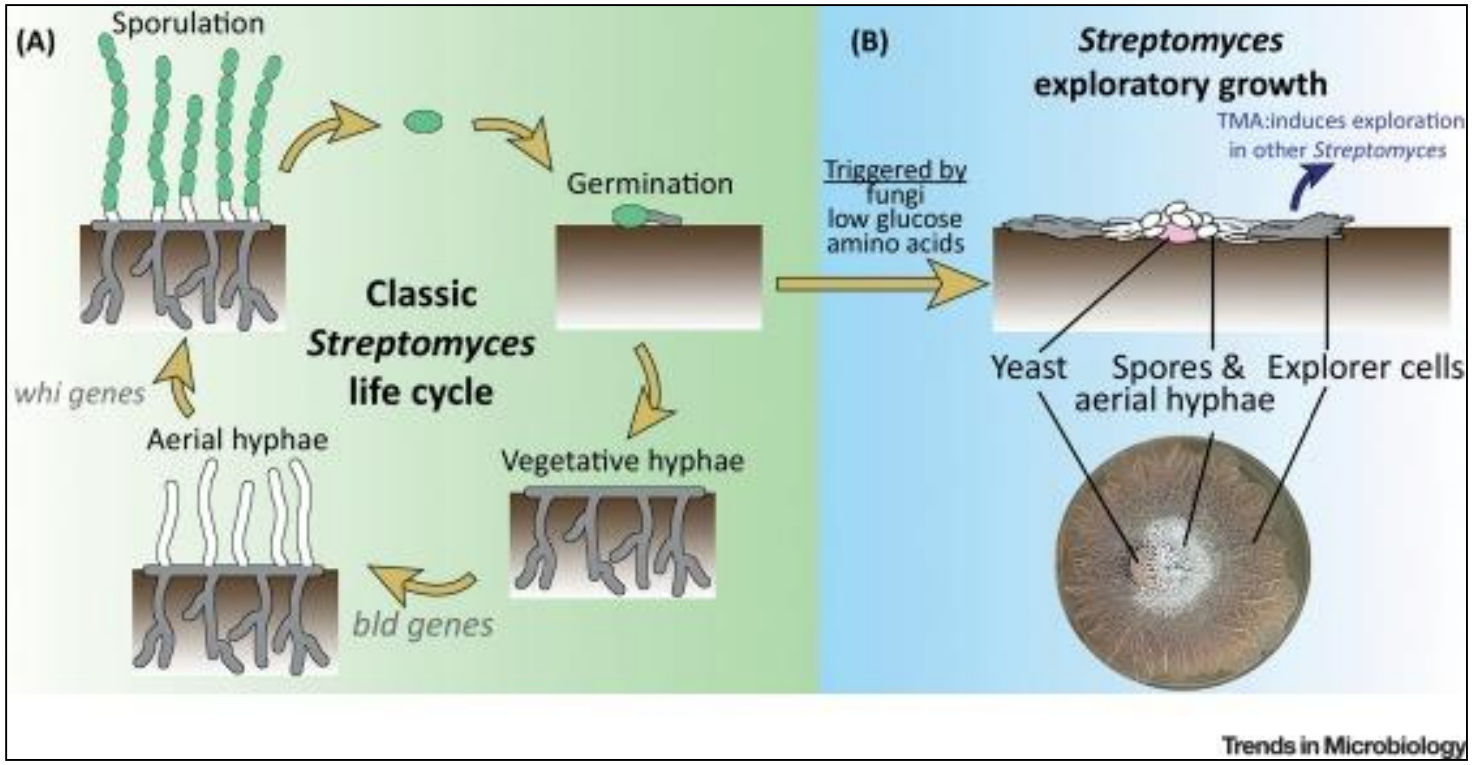


Bobek *et al.*,2017; Wikipedia,2019

Gram positive bacteria

Exospore formation

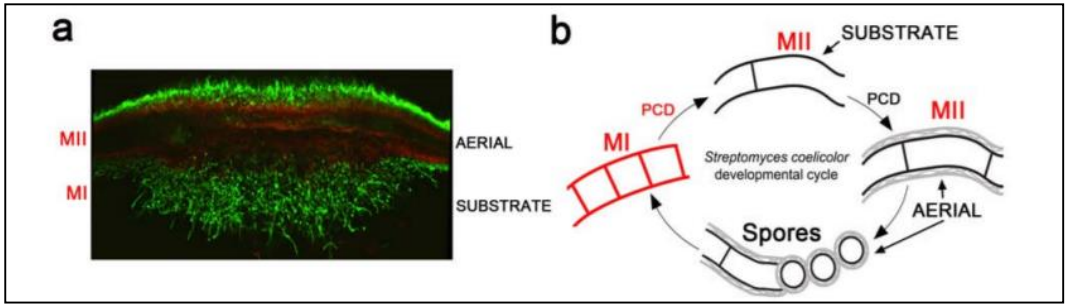
Streptomycetes



Aerial development requires the activity of the *bld* gene products, Whereas, *whi* gene requires for differentiation of aerial hyphae into spores.

Gram positive bacteria
Exospore formation

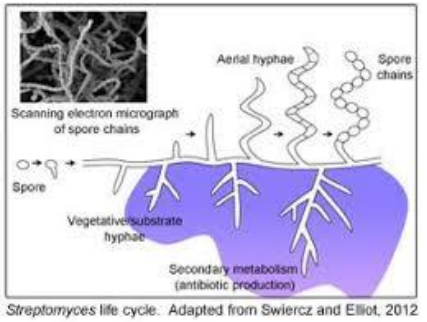
- Proposed developmental model for *Streptomyces* growing in natural soils. Mycelial structures (MI, first mycelium; MII, second mycelium), vegetative and reproductive phases, and PCD are indicated.
- The vegetative phase is the predominant one.
- Spore germination was a very slow, non-synchronous process that commenced at about 7 days and lasted for at least 21 days.



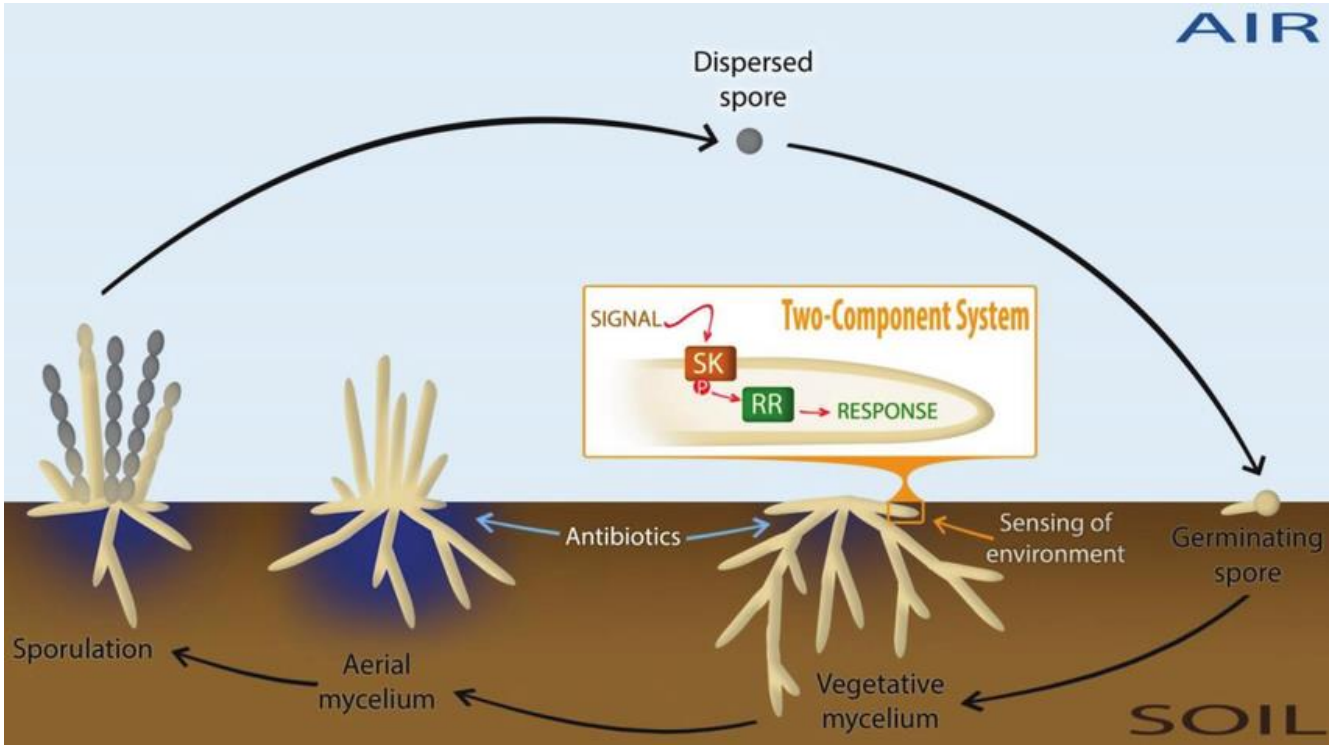
Yagüe *et al.*,2017

Gram positive bacteria

Exospore formation



Streptomyces life cycle. Adapted from Swiercz and Elliot, 2012



Spores of these organisms not only guard genetic information during unfavorable conditions, but are also adapted to wind dispersal and may remain airborne for long periods.

Urem *et al.*,2016

Gram positive bacteria
Exospore formation

- The streptomycete arthrospores (exospores) are more reminiscent of the spores of eukaryotic fungi e.g. molds (*Penicillum* spp.), possibly due to their convergent (close) evolution in the soil environment.

1. Both produce conidiophores (analogous to streptomycete aerial hyphae) that bear individually constricted conidiospores.
2. Both, streptomycetes and molds produce:

- large numbers of small hydrophobic spores,
- a relatively thick coat,
- protective small molecules including sugars (such as trehalose), and
- heat shock proteins.

Bobek *et al.*,2017

Microscopic observation wet mount
Streptomycetes

- On clean grease free glass slide, actinomycete colony was suspended in 1-2 drops of water and coverslip was placed then it was observed under microscope.
- It is used to study the shape, size spores, motility etc.

Jayalakshmi *et al.*,2011

Staining spores
Streptomyces and related genera

- Stain the bacterial preparation on a glass slide for 2 min with 2:2:1 mixture of 1% Bismarck brown, 0.1% toluidine blue, and a saturated solution of Ammonium sulfate $(NH_4)_2SO_4$.
- Wash with water, and mount under a microscope.

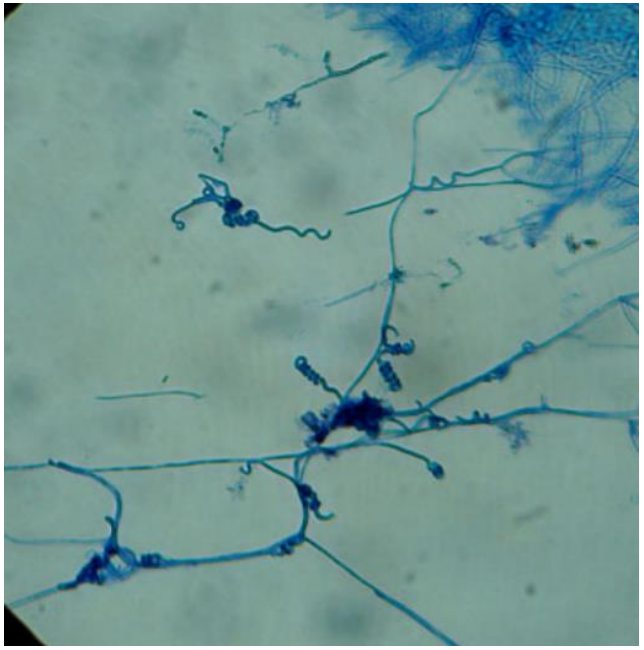
1. The hyphae stain bright yellow,
2. while the spores are blue.
3. Red brown granules can be seen in the hyphae.

- A blue stain may be picked up by some nonsporulating aerial hyphae.

Exospore staining

Streptomyces and related genera

- The cover slips were also withdrawn and mounted on the glass slide having one drop of methylene blue (0.3 g in 10 ml distilled water).
- The cover slips were fixed with feviquick (glue) and observed under light microscope.

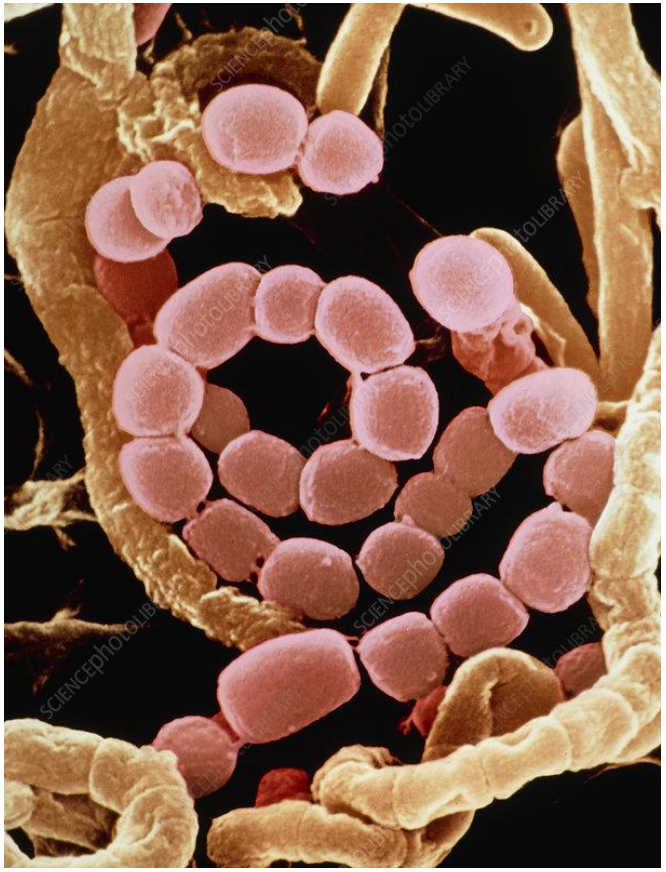


Spore chain morphology of *Streptomyces* sp. (Light microscopy, 1,000).

Spore chains of streptomycetes

Long chains frequently having up to 100 spores

- False-colour scanning electron micrograph (SEM) of the soil bacterium *Streptomcyes lividans*, showing the formation of a spiral spore chain (pink).
- *S. lividans* is a natural producer of the antibiotic Streptomycin.
- When spores of the kind shown here are mature, they rupture & are dispersed on the wind.
- Magnification: x 6500 at 6 x 4.5 cm size.

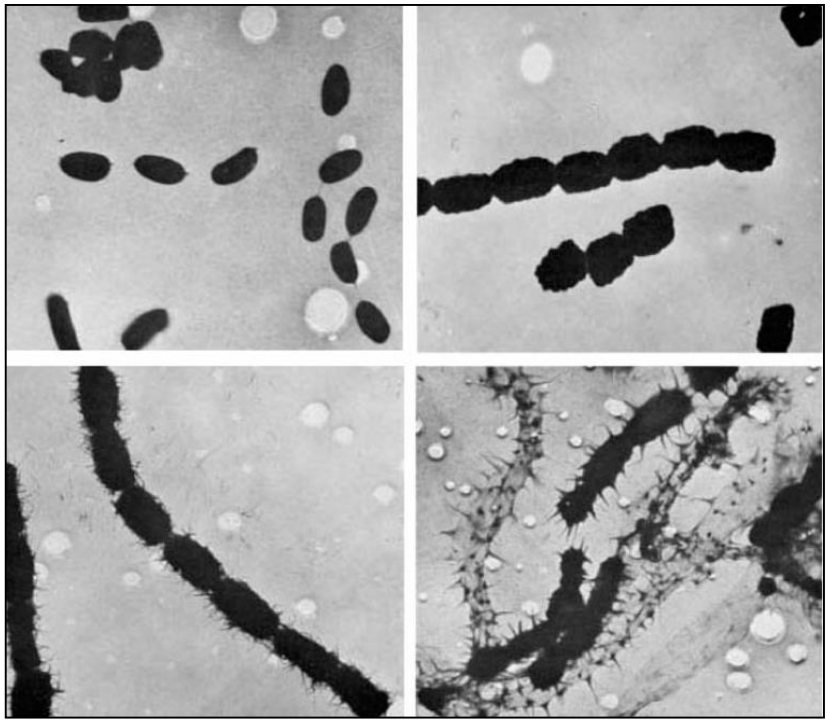


Science Photo Library (SPL)

Spore chains of streptomycetes

Long chains frequently having up to 100 spores

- Electron micrographs of four types of arthrospores (A body that resembles a spore but is not an endospore) of streptomycetes: smooth, warty, hairy and spiny.
- The spores are about 1 m long.



Kumar *et al.*,2011; Li *et al.*,2016

Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

The bacterial genomes

(chromosome, plasmids,..)

Cytoplasm of Prokaryotes

Cytoplasmic granules and inclusion bodies

The bacterial genomes

Chromosome(s)

The bacterial genomes

A genome is an organism's complete set of DNA, including all of its genes

1. The bacterial chromosome is one long, single (some bacteria have multiple circular chromosomes) molecule of double stranded, helical, supercoiled DNA.
2. Bacterial chromosome, located in the irregularly shaped region known as the nucleoid.
3. Chromosomes are made of DNA and protein.
4. The genome is made of a chemical called DNA. Genes are short sections of DNA.

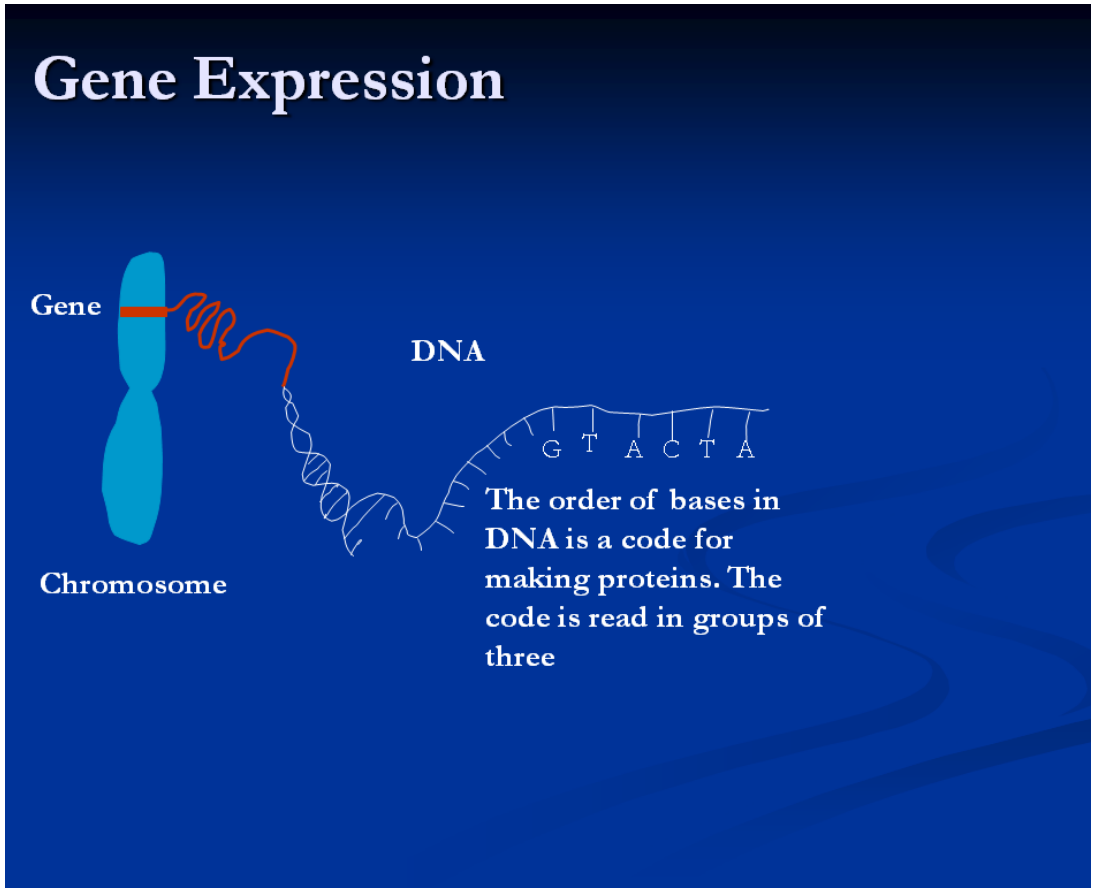
Deoxyribonucleic acid
Definition of a gene

- DNA is the basis of life because it contains the genetic code for all living organisms.
- In molecular terms, a gene can be defined as a segment of DNA that is expressed to yield a functional product, which may be either:

1. an RNA (e.g., ribosomal and transfer RNAs), or
2. a polypeptide.

- Genes are the fundamental units of inheritance in living organisms.
- Together, they hold all the information necessary to reproduce a given organism and to pass on genetic traits to its offspring.

Gene expression
For protein synthesis



DNA is made up of base pairs, proteins are made up of amino acids. The sequence of amino acids in a protein is defined by the sequence of a gene.

DNA structure
Bacteria vs. human

- The genome (genome is an organism's complete set of DNA, including all of its genes) of *E. coli* is approximately 4.6×10^6 base pairs long and contains 4288 genes, nearly 90% of the DNA used as protein-coding sequence.
- Bacteria have relatively small amounts of non-coding DNA.

- In human genome, we find some surprising things.
- The total length of the human genome is over 3 billion base pairs (3×10^9).
- Only about 1% of the three billion letters directly codes for proteins.
- Of the rest, about 25% make up genes and their regulatory elements.
- The function of the remaining letters is still unclear.
- Some of it may be redundant information left over from our evolutionary past.

DNA is dynamic and has high energy
But not stiff or static as first thought

- DNA appears a perfect spring that can be stretched and then spring back to its original conformation.
- It is dynamic with high energy existing naturally in a slightly underwound (supercoiled) state and its status changes in waves generated by normal cell functions such as:

1. DNA replication,
2. Transcription,
3. Repair and recombination.

Baylor College of Medicine,2009

DNA vs. RNA

- DNA and RNA share many similarities. However, a few key differences stand out.

1. DNA serves as the primary storage of genetic information for all cells, whereas RNA molecules assist to execute the instructions encoded by that DNA.
2. DNA always stays in the nucleus, whereas RNA travels from the nucleus to the cytoplasm.

1. RNA contains the base uracil (U) instead of the thymine (T) in DNA.
2. It is not uncommon, however, to find other types of base pairs in RNA: for example, G pairing with U occasionally.
3. Inosine (a precursor to adenosine) is a purine nucleoside containing the base hypoxanthine and the sugar ribose, which occurs in tRNAs.

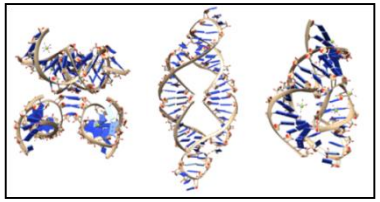
Comparison of DNA, RNA and proteins
The three essential macromolecules of life

	DNA	RNA	Proteins
Encodes genetic information	Yes	Yes	No
Catalyzes biological reactions	No	Yes	Yes
Building blocks (type)	Nucleotides	Nucleotides	Amino acids
Building blocks (number)	4	4	20
Strandedness (having a strand or strands)	Double	Single	Single
Structure	Double helix	Highly complex	Highly complex
Stability to degradation	Extremely high (more stable than RNA)	Variable (more susceptible to hydrolysis)	Variable
Repair systems	Yes	No	No

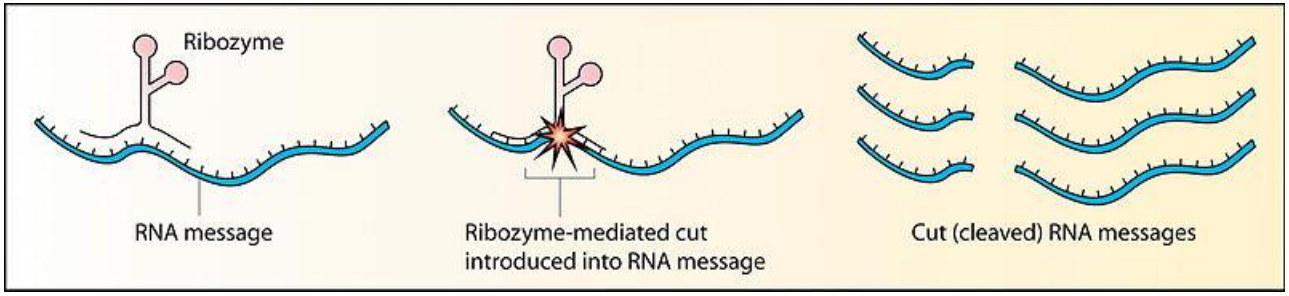
Ribozymes (ribonucleic acid enzymes) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes. These building blocks are four types of nitrogen bases and 20 amino acids in DNA/RNA and proteins, respectively.

Comparison of DNA, RNA and proteins
Ribozymes (ribonucleic acid enzymes)

- Ribozymes (ribonucleic acid enzymes) such as leadzyme, hammerhead ribozyme, twister ribozyme are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein.
- These enzymes are also participate in a variety of RNA processing reactions, including RNA splicing (removing introns).



Three types of ribozyme structures

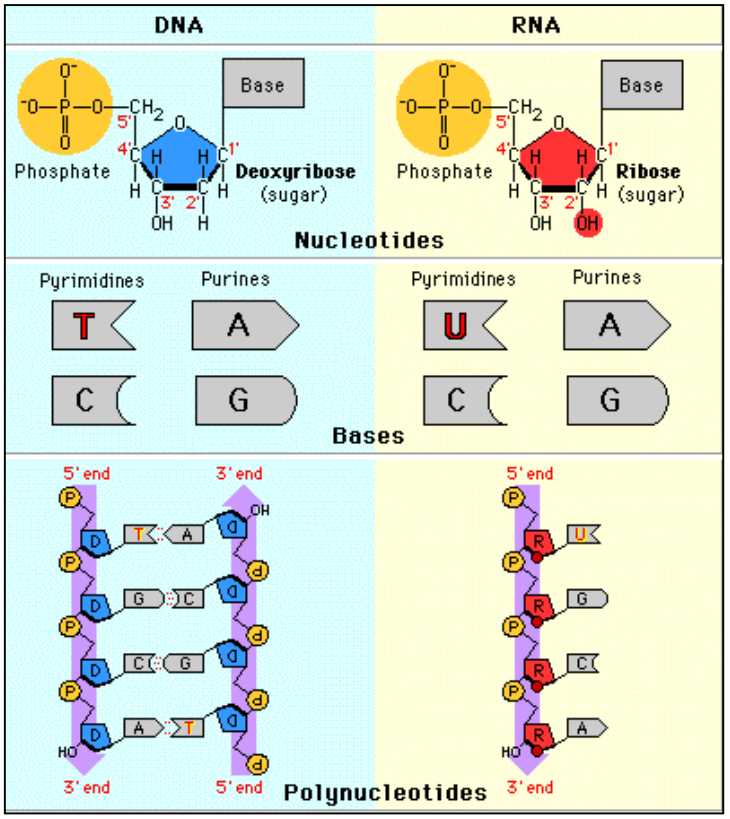


DNA vs. RNA

- The 2 DNA strands are antiparallel [5'--->3' pairs to 3'<---5'].
- Nucleotides consist of three parts:

1. A sugar;
2. A nitrogen base attached to the sugar 1' carbon;
3. A phosphate group or groups, usually attached to the sugar 5' carbon.

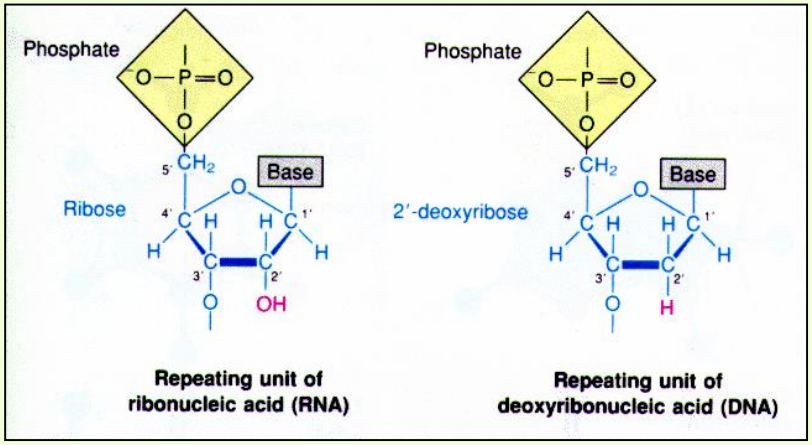
- The backbone of DNA is based on a repeated pattern of a sugar group and a phosphate group.



Watson & Crick Model,1953

A nucleotide
Units of bacterial DNA/RNA

1. **Nucleosides**: **Sugar + Base.**
2. **Nucleotides: Sugar + Base + phosphate group.**



Nucleotides carriers of chemical energy in the cell (e.g. ATP, GTP). Guanosine-5'-triphosphate (GTP) is a purine nucleoside.

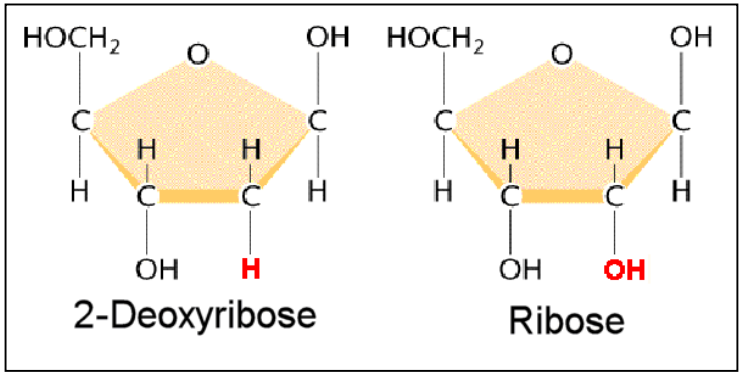
DNA vs. RNA
Pentose sugars

- Both ribose and deoxyribose are pentose sugars.

1. Ribose (in RNA only);
2. 2-deoxyribose (in DNA only).

- One oxygen distinguishing them:

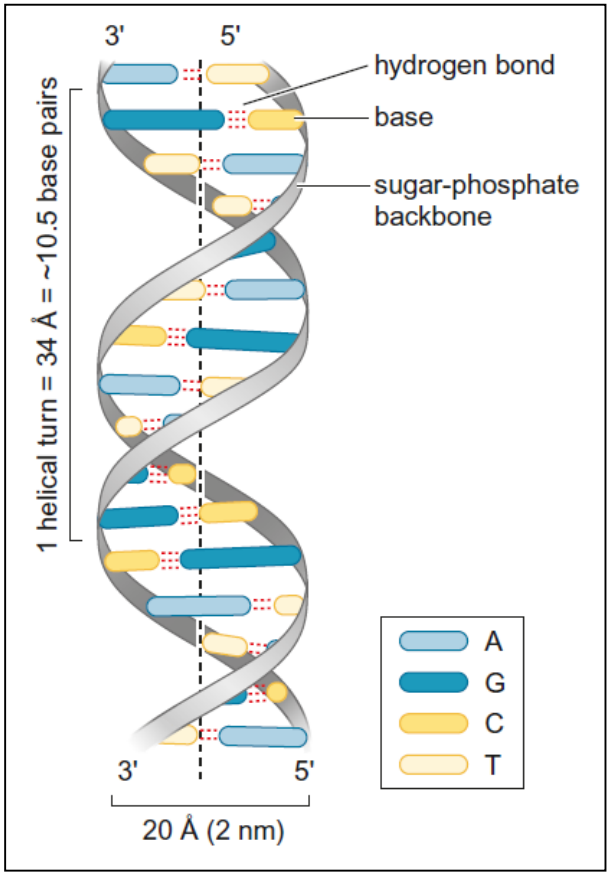
1. a hydrogen (H) bonds in deoxyribose, and
2. a hydroxyl (OH) bonds in ribose.



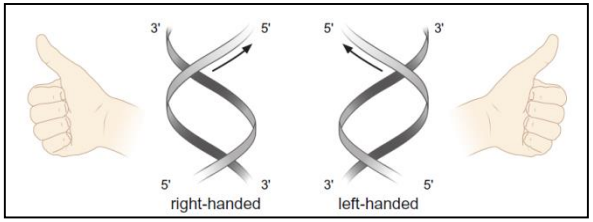
The nucleotides in RNA are ribonucleotides - that is, they contain the sugar ribose (hence the name ribonucleic acid).

DNA structure
DNA bases

- Each sugar molecule is covalently linked to one of 4 possible bases:
  1. Adenine,
  2. Guanine,
  3. Cytosine,
  4. Thymine.
- RNA also uses the pyrimidine cytosine (C), but instead of thymine, it uses the pyrimidine uracil (U).



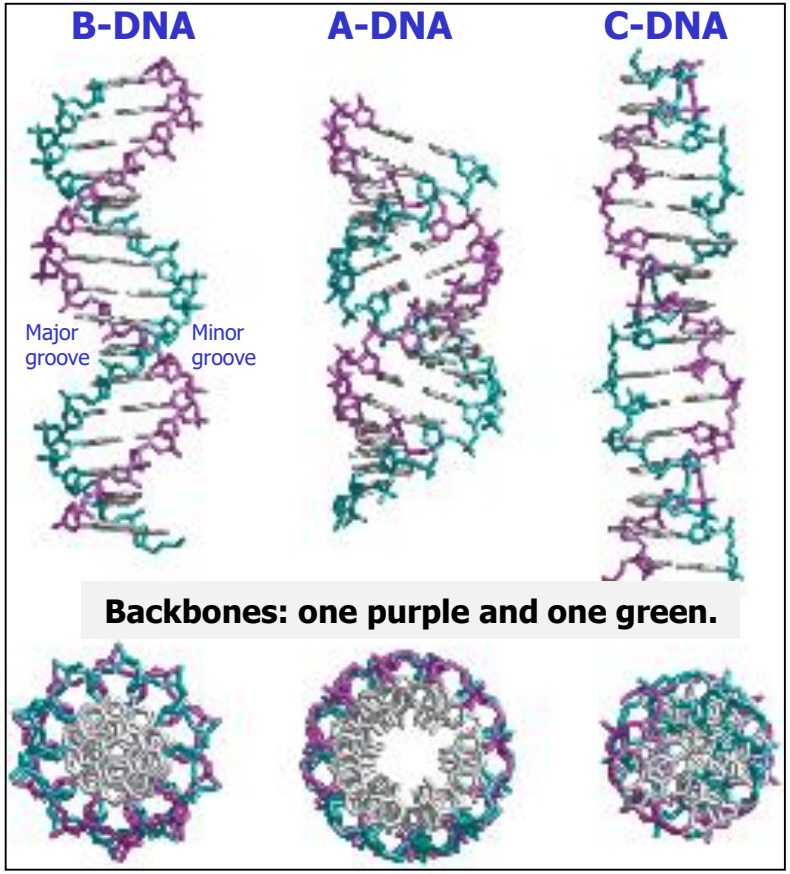
1 angstrom is a unit of length equal to 10^{-10} m or 0.1 nm.



DNA

Major and minor grooves of DNA

1. At least three DNA conformations are believed to be found in nature:
2. A-DNA (right-handed double helix).
3. B-DNA (right-handed double helix).
4. Z-DNA (left-handed helix; zigzag (Z) appearance, pronounced zig-zag-DNA).

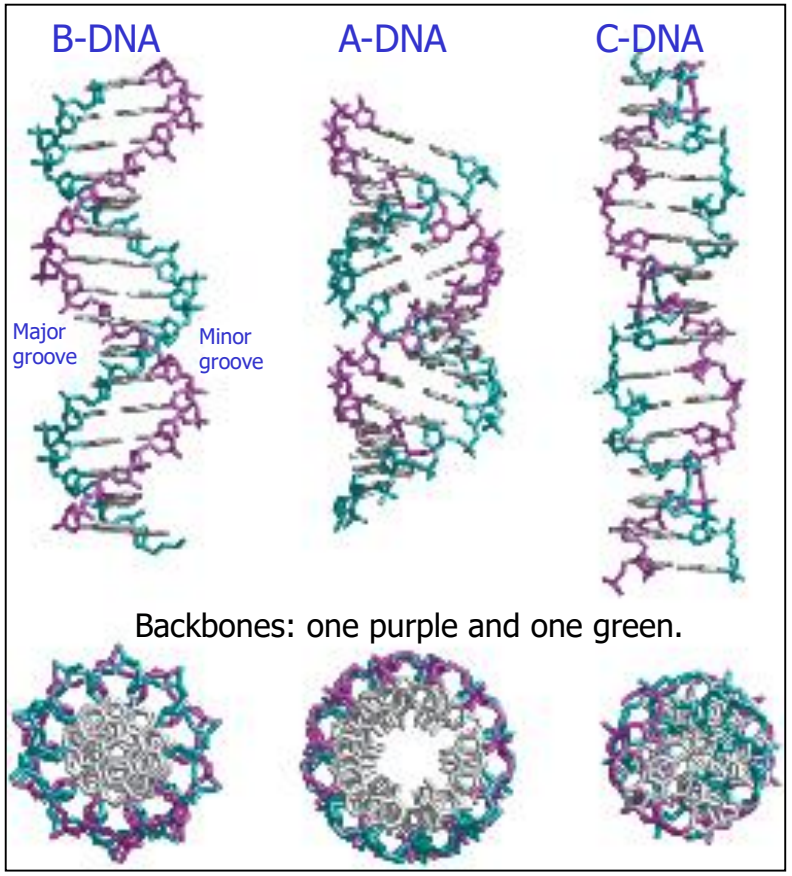


Z DNA is a variant of B DNA, with slightly different configuration.
B-DNA is driven into the A-DNA when under dehydrating conditions.

DNA

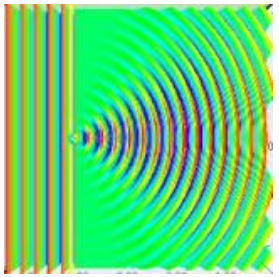
Major and minor grooves of DNA

1. A-DNA:
   - major groove is narrower and much deeper
   - minor groove is broader and shallower.
2. B-DNA:
   - major groove is wider than the minor groove.
3. Z-DNA:
   - The major and minor grooves, unlike A- and B-DNA, show little difference in width.



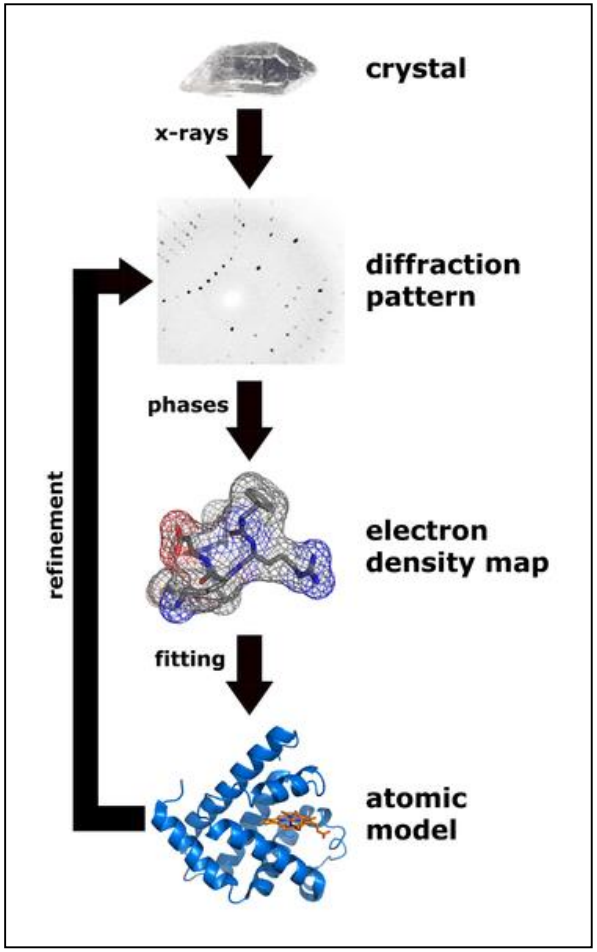
DNA conformations

X-ray Crystallography



Diffraction

- The major steps involved in DNA structure determination by X-ray crystallography showing the important role played by molecular models of DNA structure in this iterative process.
- Diffraction is the slight bending of light as it passes around the edge of an object.



Wikipedia,2016

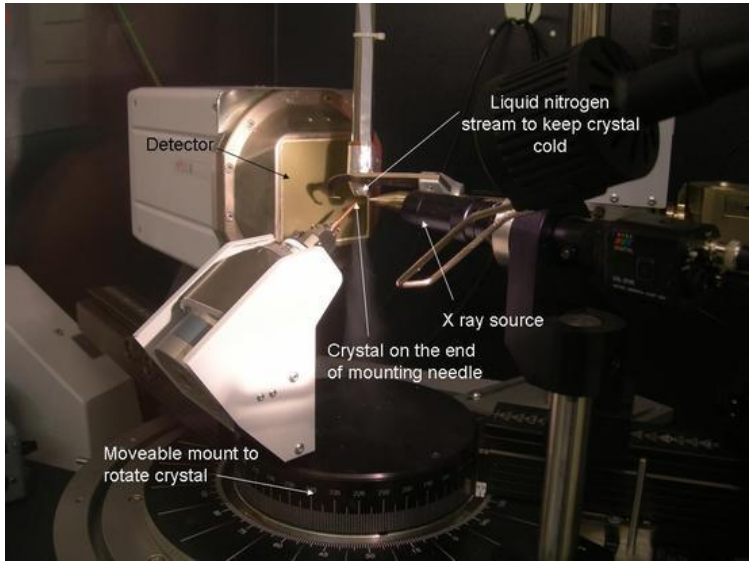
X-ray Crystallography
Crystallization

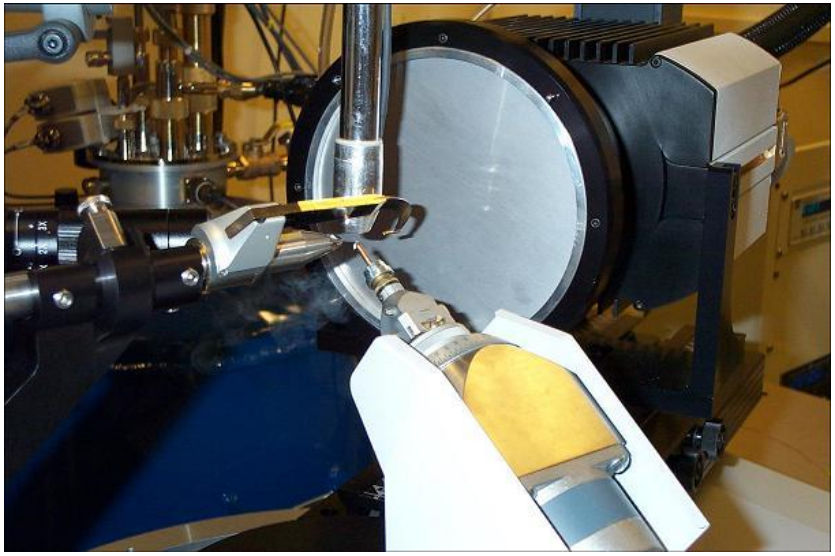
- In order to run an x-ray diffraction experiment, one must first obtain a crystal.
- Solutions are generally placed into a freezer (-78°C) in order to ensure all of the compound has crystallized.
- Cooling continues as a seed crystal forms.

Organometallic chromium crystals in a Schlenk under nitrogen.

X-ray Crystallography

View of the entire machine and (right) a crystal mounted on a goniometer shown with the x-ray generator and detector





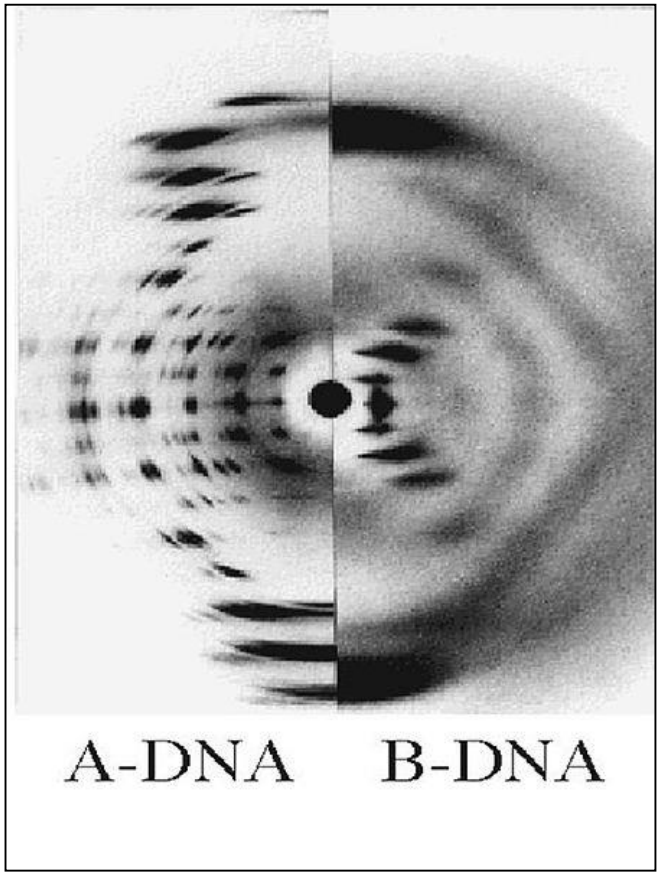
X-ray crystallography is a tool used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions.

ChemWiki

DNA conformations

X-ray Crystallography

- An image of actual A- and B-DNA X-ray patterns obtained from oriented and hydrated DNA fibers
- (courtesy of Dr. Herbert R. Wilson, FRS).



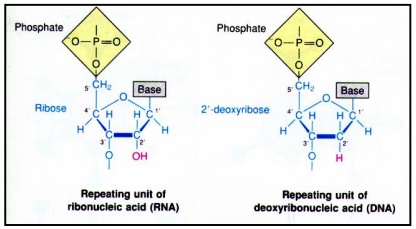
Wikipedia,2016

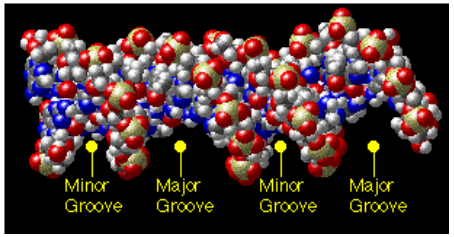
DNA conformations

A comparison of the structural properties of A, B, and Z DNAs as derived from crystal X-ray electron microscopy

Parameter	A-DNA	B-DNA	Z-DNA
Overall proportions	Short and broad	Longer and thinner	Elongated and slim
Helix rotation sense	Right-handed	Right-handed	Left-handed
Base pairs per turn	11	10	12
Diameter of helix (nm)	2.3	2.0	1.8
Stability	less stable	More stable	Unstable
Found in:	Bacteria to eukaryotes	Most organisms including bacteria	Bacteria to eukaryotes except *E. coli*

- A-DNA occurs when DNA is dehydrated.
- A-DNA helix being less stable than the B-DNA.
- The Z-helix is narrower than the A-and B.
- A small amount of the DNA in a cell exists in the Z form.
- Most human genes have been found to have Z-DNA-forming sequences near the transcription start site. It may provide supercoiling relief while DNA transcription occurs. Because this has left-handed coil. Negative supercoils can also relieved by local unwinding. Therefore, Z-DNA is important function in regulation of gene expression.



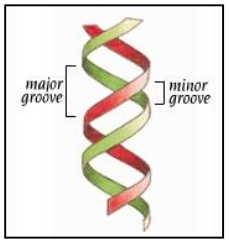


DNA

Major and minor grooves of DNA

- B-DNA or common DNA: The most common form of DNA found in most organisms including bacteria.
- B-DNA present at neutral pH and physiological salt concentrations.
- B-DNA, is right-handed double helix with about 10-10.5 base pairs per turn.
- This translates into about 20-21 nucleotides per turn.
- The major groove is approximately 50% wider than the minor.
- Thus, many proteins which bind to B-DNA do so through the wider major groove.

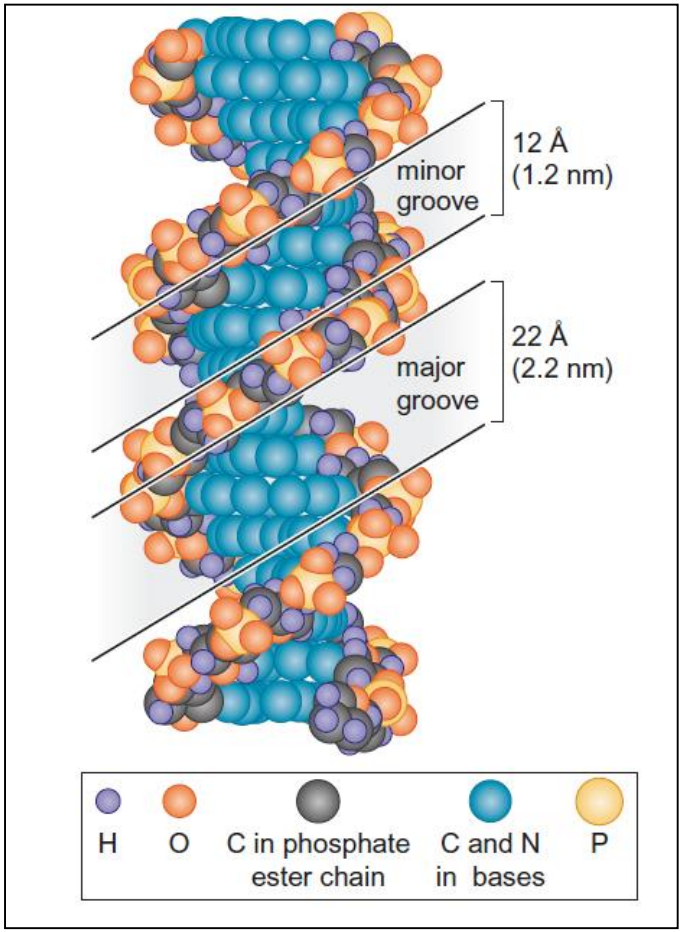
Watson_6;..



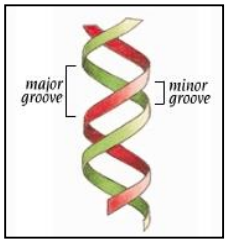
DNA

Major and minor grooves of DNA

- Twin helical strands form the DNA backbone.
- The major groove occurs where the backbones are far apart,
- The minor groove occurs where they are close together.
- The grooves twist around the molecule on opposite sides.
- The major groove is approximately 50% wider than the minor.
- The major groove, is 22 Å wide and the minor groove, is 12 Å wide.



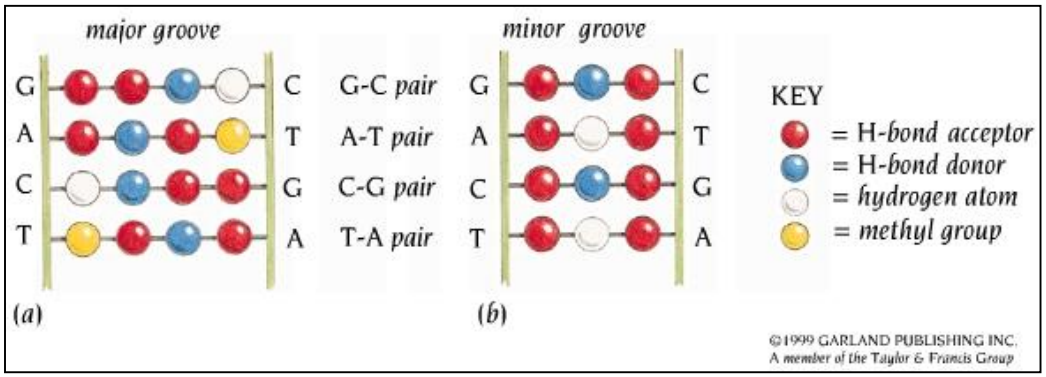
1 angstrom is a unit of length equal to 10^{-10} m or 0.1 nm.



DNA

Major and minor grooves of DNA

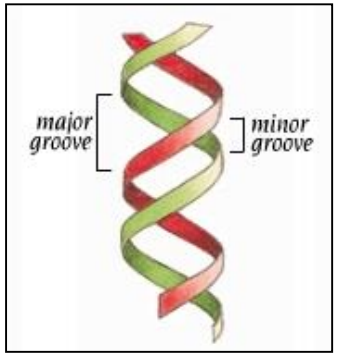
- The major groove has twice the information content of the minor groove.
- A-T base pairs can be differentiated from G-C pairs via either groove.
- But minor groove could not discriminate between A-T and T-A base pairs, or G-C and C-G.
- Hence the major groove has a four-symbol code, whereas the minor groove has only a two-symbol code, and only half information content.



DNA-binding proteins

DNA-binding domains

Structural DNA-binding motif



- Each structural motif contains features that are highly conserved among many organisms.

1. Some proteins bind DNA in its major groove (e.g. the zinc finger).
2. Some other in the minor groove (e.g. HMG-I(Y)–DNA complex), and
3. Some need to bind to both (e.g. the Leucine Zipper Motif).

The major groove, being wider than the minor groove, can accommodate larger structural motifs.

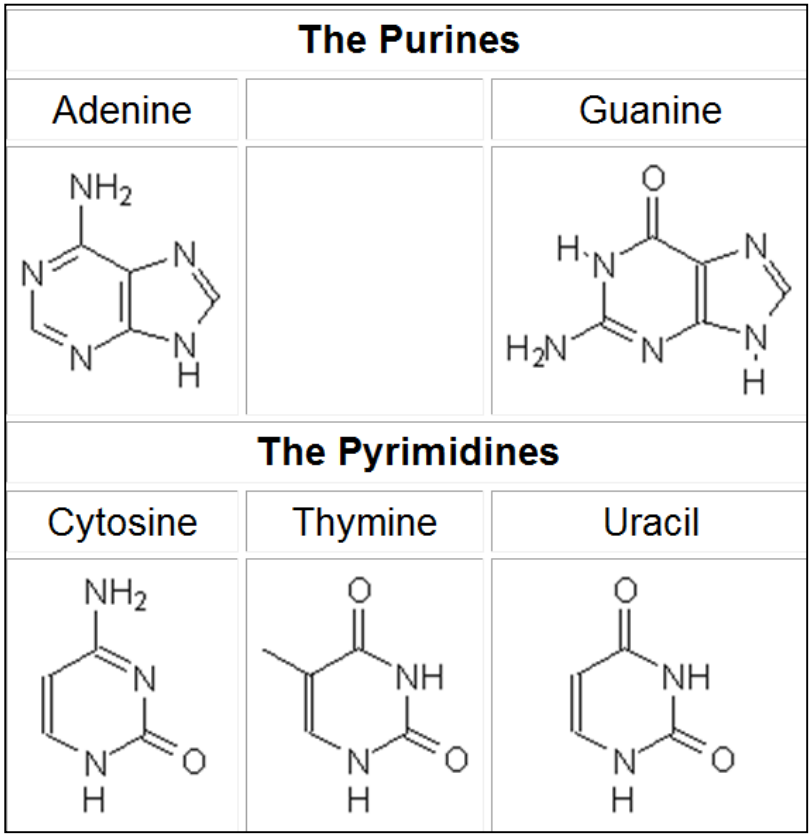
Atlas Genet Cytogenet Oncol Haematol, 2003

DNA vs. RNA

DNA bases

Purines 2 carbon rings, pyrimidines only have one

- A and G are double-ringed larger molecules (called purines);
- C and T are single-ringed smaller molecules (called pyrimidines).
- Only two purines and three pyrimidines occur widely in nucleic acids.



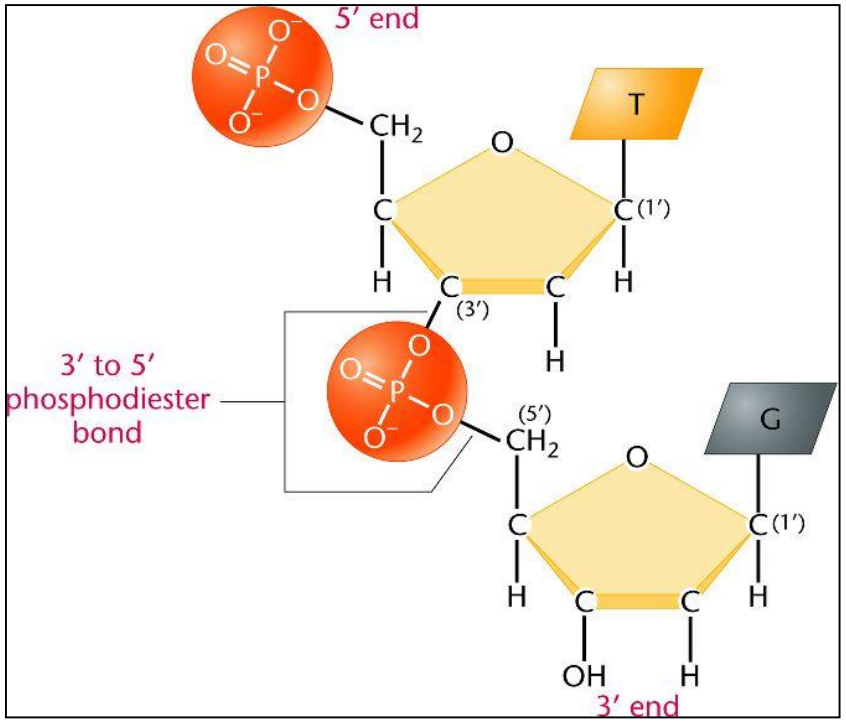
University of Maine,2008

A dinucleotide

Units of bacterial DNA/RNA

Dinucleotides form from a phosphodiester link between 2 mononucleotides

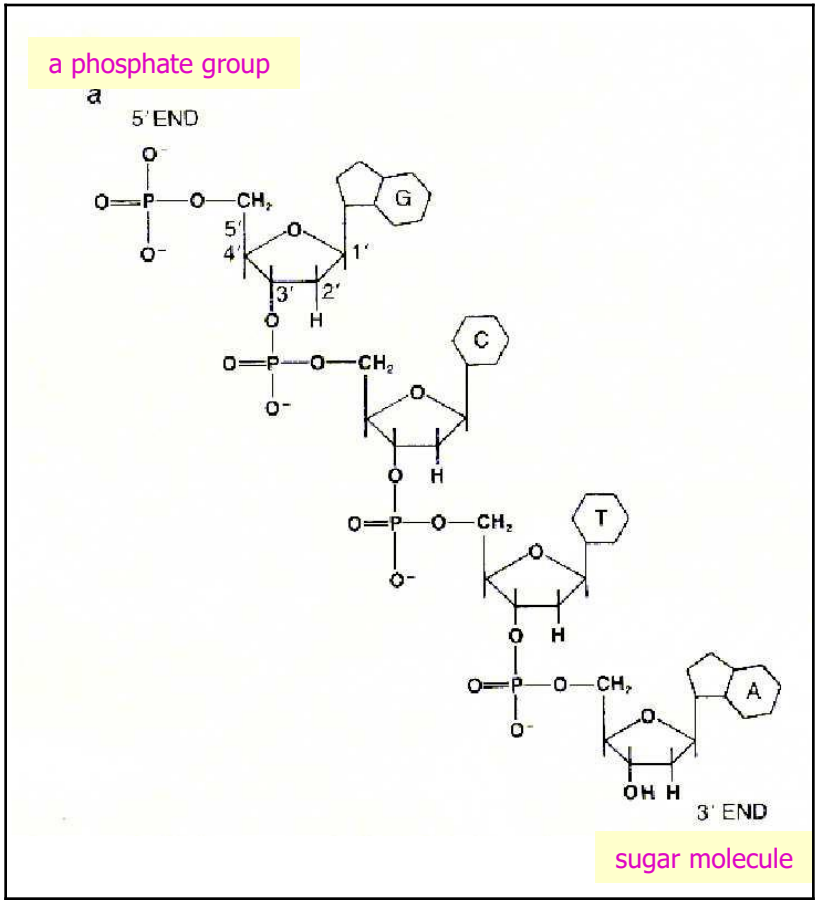
- Linkage of two nucleotides by the formation of a 3'-5' phosphodiester bond, producing a dinucleotide.
- Multiple phosphodiester bonds form a polynucleotide chain.



DNA structure

- All DNA strands are read from the 5' to the 3' end where:

1. the 5' end terminates in a phosphate group and
2. the 3' end terminates in a sugar molecule.



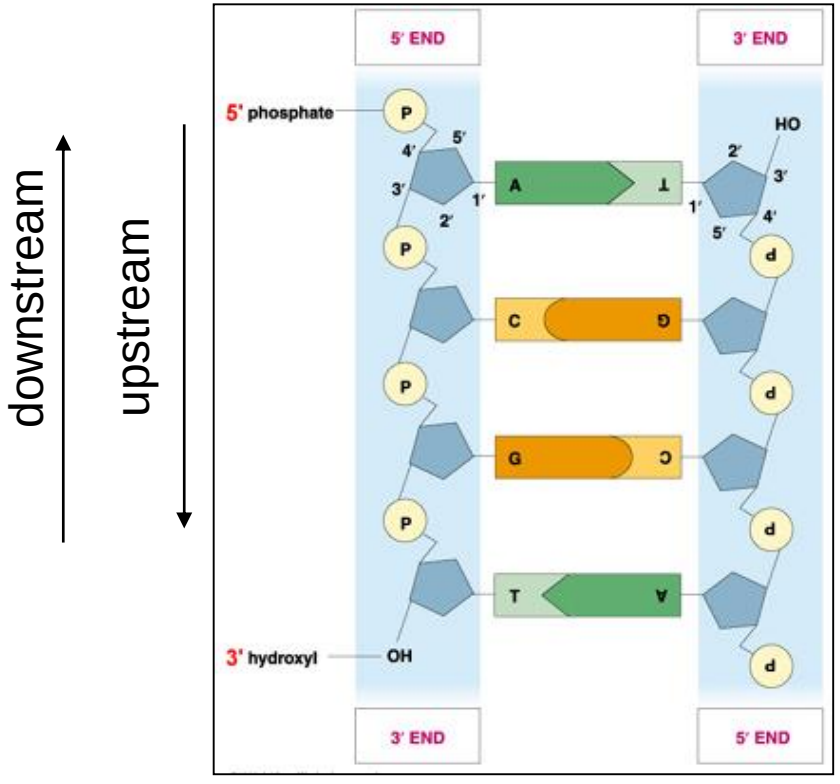
DNA/RNA structure
Upstream/Downstream

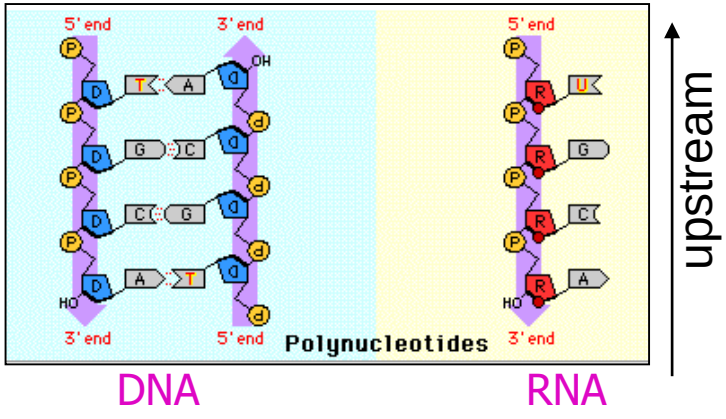
- DNA and RNA are synthesized (replicated) in the 5′ to 3′ direction.
- **In an RNA:**
- Anything towards the 5' end of a reference point is upstream of that point. Downstream is toward the 3' end.
- **In DNA:**
- The situation is a bit more complicated.
- Due to the anti-parallel nature of DNA, the 3' end of the template strand is upstream of the gene and the 5' end is downstream.

A ribosome moves along an mRNA from 5′ to 3′.
mRNA is also made in the 5' to 3' direction.

DNA Structure

In a DNA double helix, the strands run in opposite directions to permit base pairing between them, which is essential for replication or transcription of the encoded information





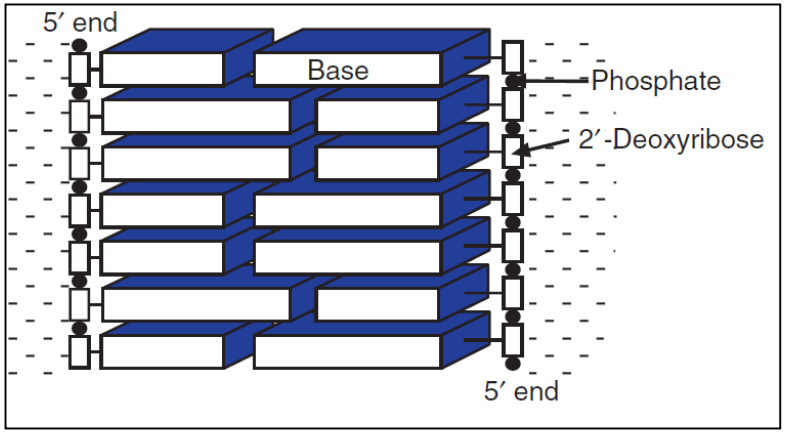
In DNA, due to the anti-parallel nature of DNA, the 3' end of the template strand is upstream of the gene and the 5' end is downstream. In RNA, Upstream is toward the 5' end of the RNA molecule and downstream is toward the 3' end.

Hydrophobic interactions of bases in DNA

Hydrogen bounds: bases pairing

1. Inside, the (hydrophobic) bases;
2. The outside (phosphate and sugar) is hydrophilic.

- The hydrophobic bases stack in the center of the helix, reducing their contact with water.

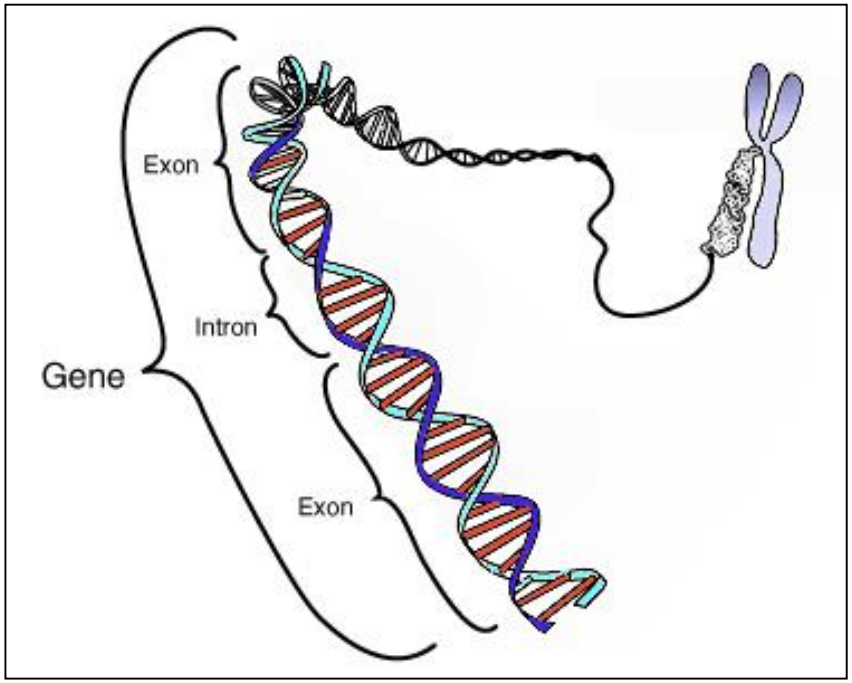


Dale and Park,2004;..

Bacterial DNA

Present exons (protein-coding regions) but not introns (non-coding sequences)

- Introns are regions often found in eukaryote but not prokaryote.
- In prokaryote only the exons encode the proteins.
- **Prokaryotes don't have introns - Genes in prokaryotes are continuous.**
- The genes of bacteria are tightly packed together; virtually all the DNA encodes proteins.



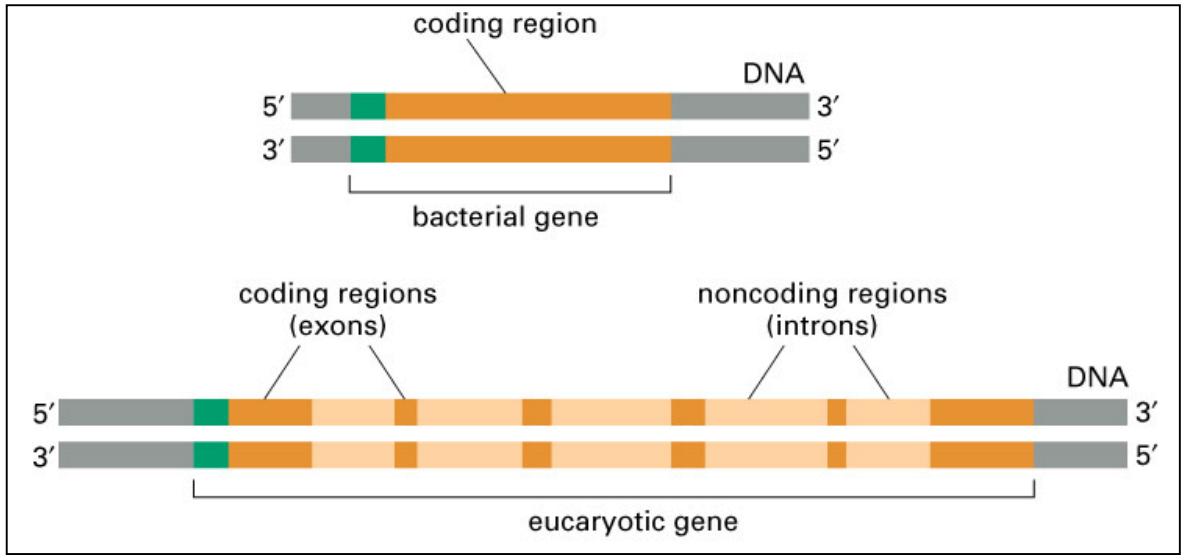
Representation of intron and exons within a simple gene containing a single intron.

Sequences that are joined together in the final mature RNA after RNA splicing are exons. Individual exons may contain coding DNA and/or noncoding DNA (untranslated sequences).

DNA structure

Bacterial genes don't have introns

- Intron (non-coding intervening sequence) and Exon (coding, or expressed, sequences) are found in eukaryotes.
- Exon (coding, or expressed, sequences) are found in bacteria.



Bjorkman,2011

RNA splicing

Splicing is the editing of the nascent pre-messenger RNA (pre-mRNA)

mRNA is spliced before leaving the nucleus to remove non-coding regions (introns)

- The process by which introns are removed and exons are joined together from an RNA transcript to produce an mRNA molecule.
- If RNA polymerase were to transcribe DNA from the start of an intron containing gene to the end, the RNA would be complementary to the introns as well as the exons.
- To get an mRNA molecule that yields a working protein, the cell needs to trim out the intron sections and then stitch (sewing or join) only the exon pieces together.

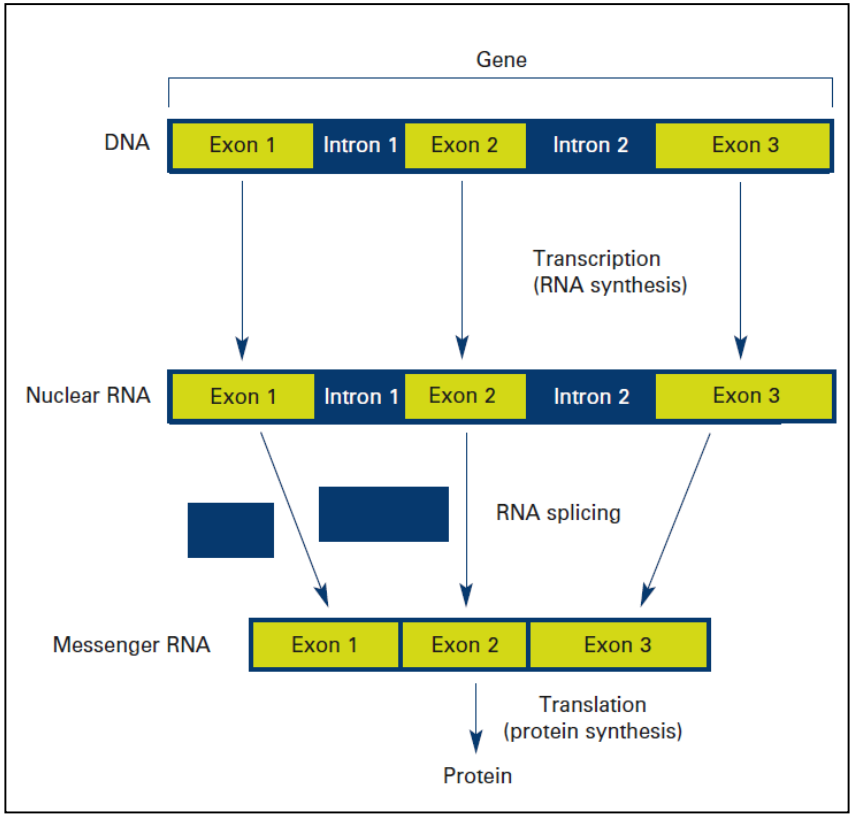
In bacteria, splice rarely and mostly non-coding RNAs.
This is because bacterial genes don't have introns.

The New Genetics,2010

RNA splicing

Excision of the intron is accompanied by the precise ligation of the coding regions (exons)

- Genes in eukaryotes are often interrupted by stretches of DNA (introns, blue) that do not contain instructions for making a protein.
- These intragenic regions (or introns), are short, non-coding regions that are found within genes.



The New Genetics,2010

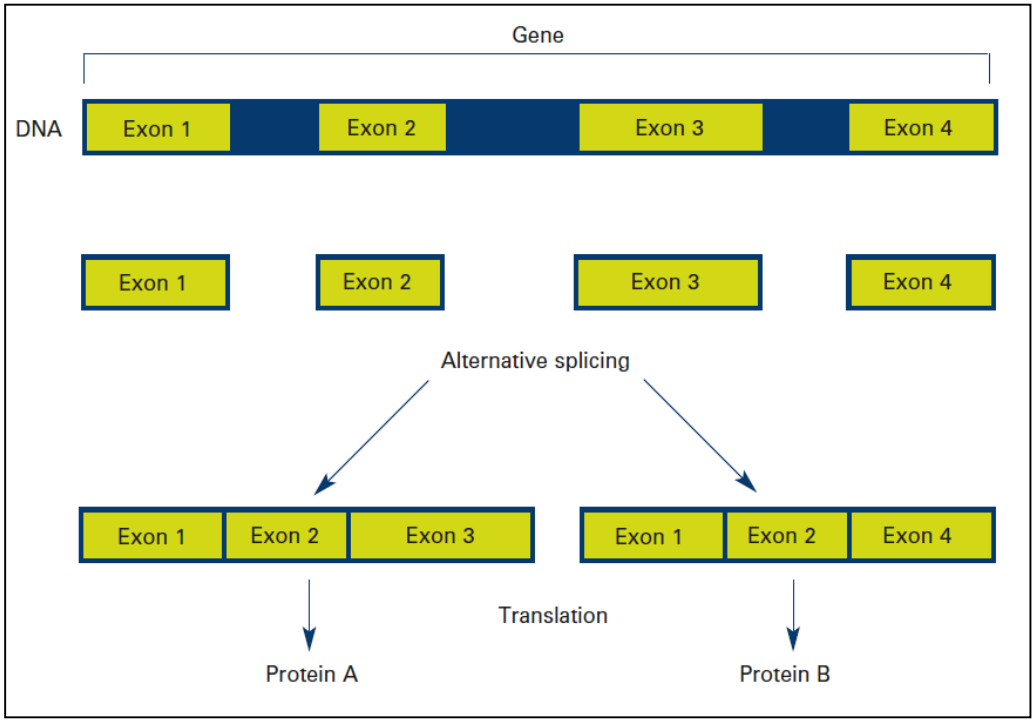
RNA splicing

mRNA is spliced by splicing machine, called the spliceosome (complex proteins including ribozymes)

- RNA splicing begins with assembly of helper proteins (spliceosome) at the intron/exon borders.
- The spliceosome then brings the exons on either side of the intron very close together, ready to be cut.
- One end of the intron is cut and folded back on itself to join and form a loop.
- The spliceosome then cuts the RNA to release the loop and join the two exons together.
- This process is repeated for every intron in the RNA.
- Numerous spliceosomes are involved.
- Each spliceosome removes one intron, releasing the loop before disassembling.

RNA splicing
Alternative splicing in Eukaryotes

- Arranging exons in different patterns, called alternative splicing, enables cells to make different proteins from a single gene.

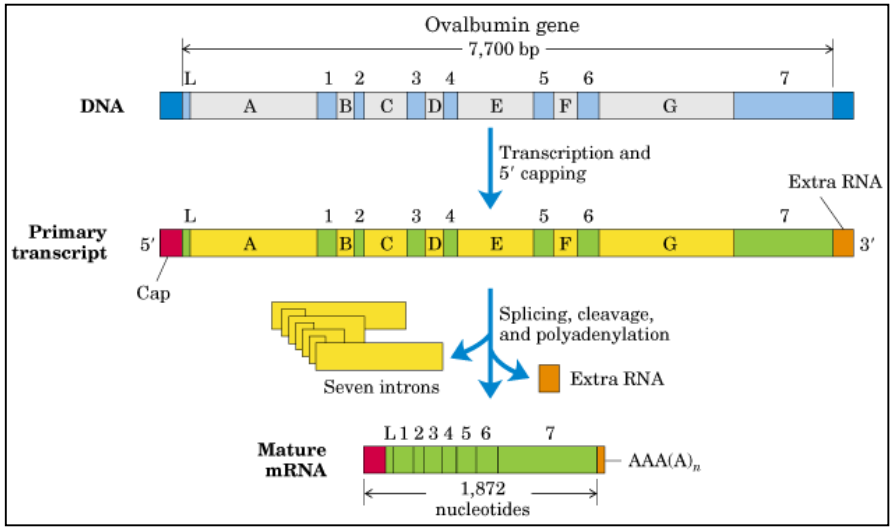


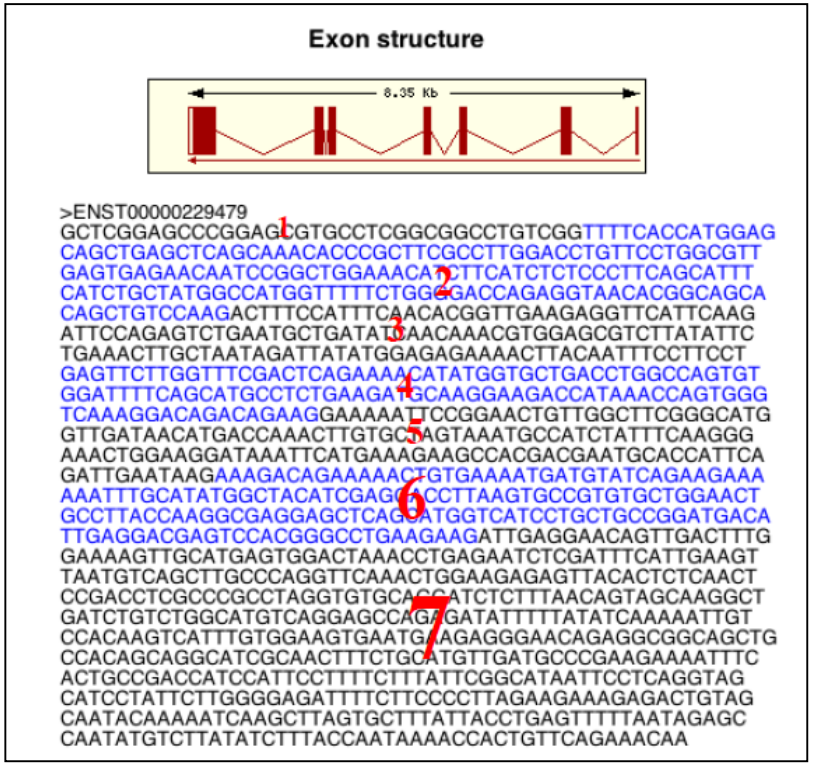
In bacteria, splice occurs rarely and mostly on non-coding RNAs.

The New Genetics,2010

Exon structure in eukaryotes

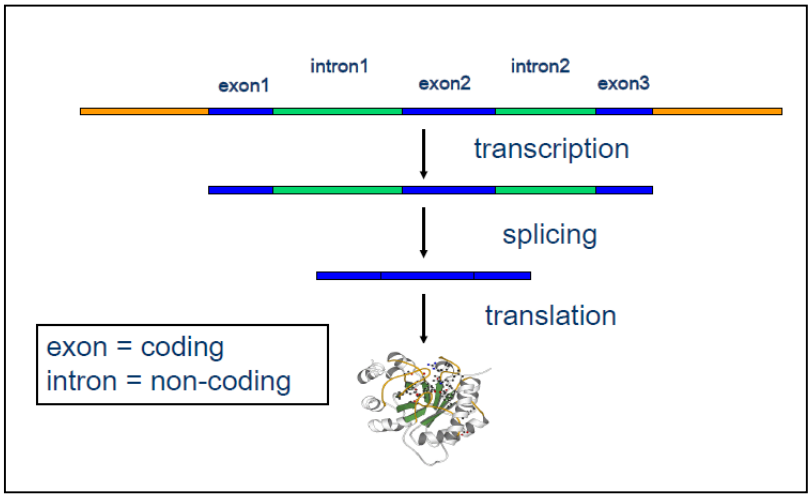
Exons 1-7

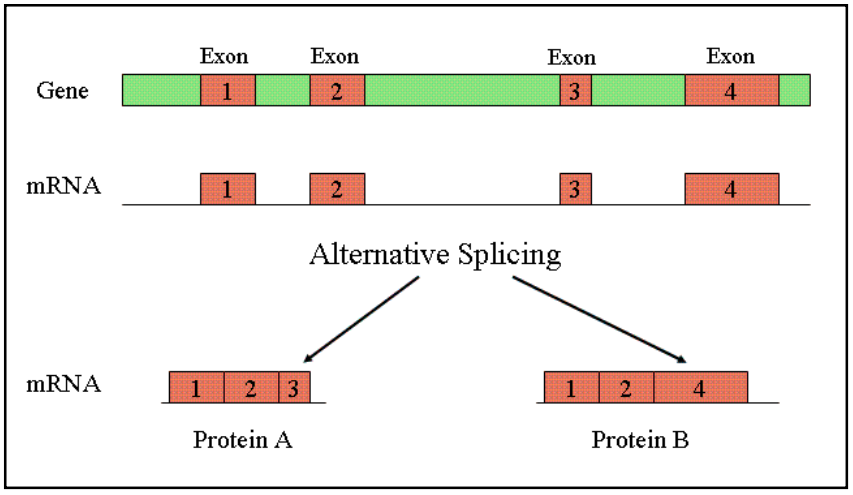




RNA splicing

Alternative splicing in eukaryotes





In bacteria, mRNA is polycistronic; in eukaryotes, mRNA is usually monocistronic. Most but not all eukaryotic mRNAs encode single gene product.

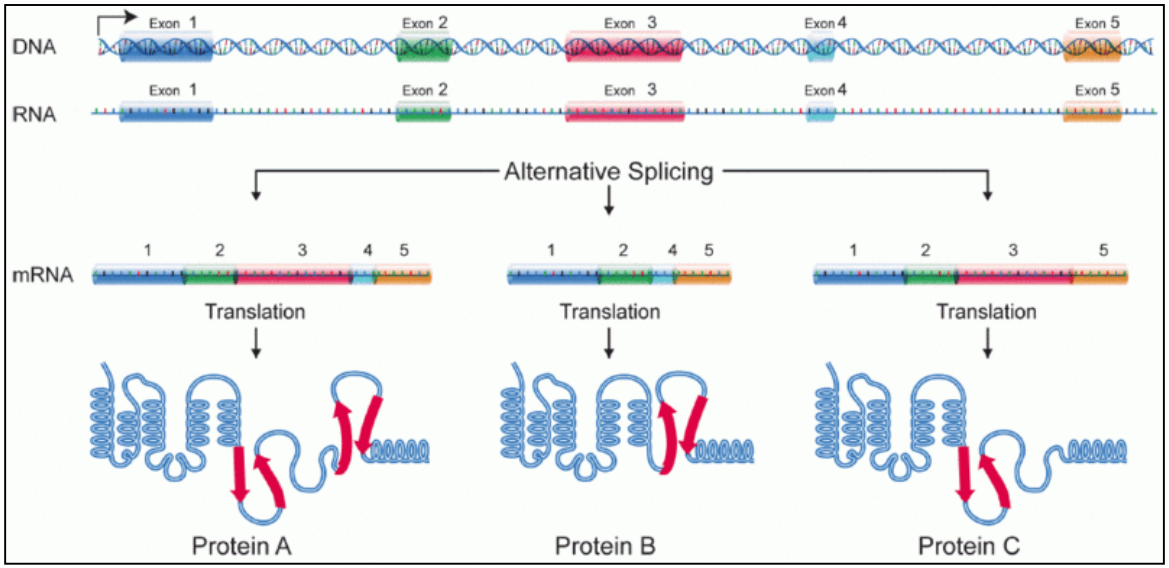
- Polycistronic mRNA: one mRNA codes for more than one polypeptide.
- moncistronic mRNA: one mRNA codes for only one polypeptide.

The New Genetics,2010

RNA splicing

Alternative splicing in Eukaryotes

- In humans, ~95% of multi-exonic genes are alternatively spliced.
- Alternative splicing produces three protein isoforms.



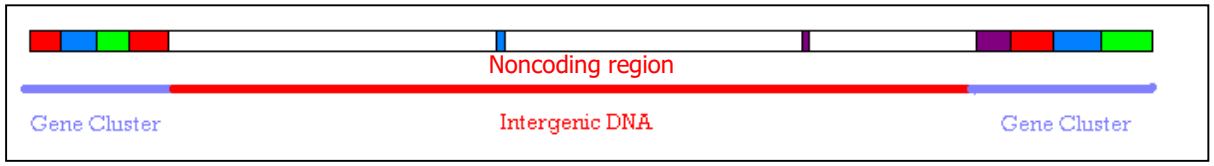
In bacteria, splice occurs rarely and mostly on non-coding RNAs.

The New Genetics,2010

DNA Structure

Intergenic region, IGR (or intergenic spacer, IGS) vs. Intragenic regions (or interons)

- An intergenic region (IGR) or intergenic spacer (IGS) is a stretch of DNA sequences located between genes.
- These regions are noncoding (non-functional or non-transcribed) region.
- Intergenic regions are different from intragenic regions (or introns), which are short, non-coding regions that are found within genes, especially within the genes of eukaryotic organisms.



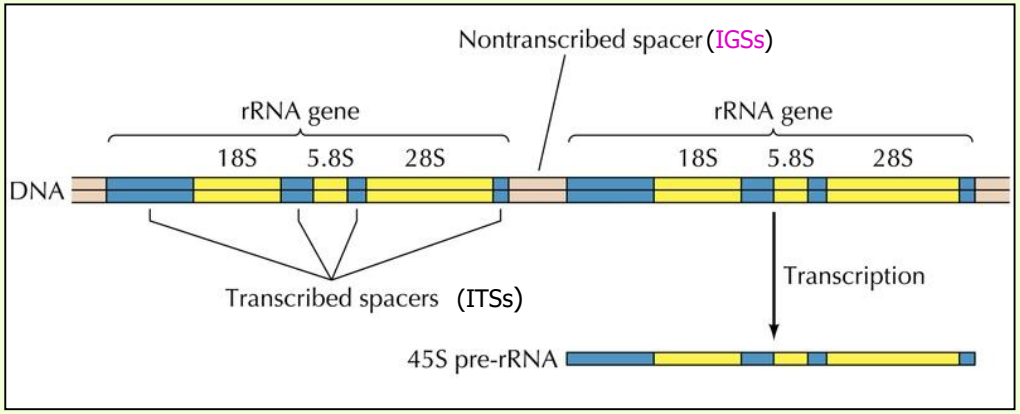
DNA Structure

Intergenic region, IGR (or intergenic spacer, IGS) and Intragenic regions (or interons)

The rRNA-ITS-IGS Sequence

- In eukaryotes:
- More than 98% of the human genome does not encode protein sequences, including:

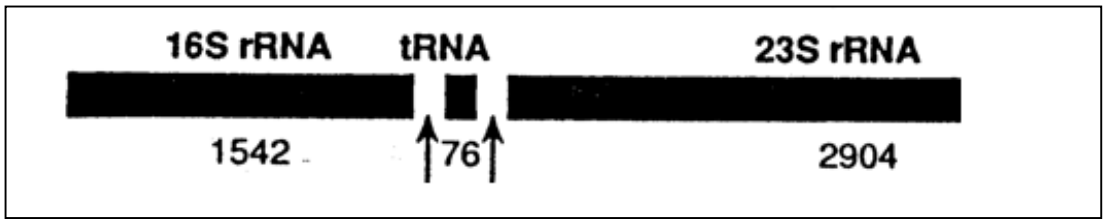
1. most sequences within introns, and
2. most intergenic DNA regions (IGR).



Bacterial DNA Structure

Intergenic spacer region (or ribosomal internal transcribed spacer, ITS)

1. The ITS region was presented between the 16S and 23S rDNA sequences.
2. The size of ITS was ranged between 500-1000 bp, and
3. It contains the number of tRNA encoding gene.
4. ITS sequences have been under less intensive selection pressure and are considered to be 10 times as variable as 16S rDNA.



Bacterial DNA Structure

Intergenic spacer region (or ribosomal internal transcribed spacer, ITS)

- The 16S-23S rDNA intergenic spacer (ITS) genes are considered as phylogenetic markers.
- The genes coding for ribosomal RNAs in prokaryotes are arranged in an operon in the following order:

5′-16S–23S-5S-3′

and are separated by two spacer regions known as the ITS1 and ITS2.

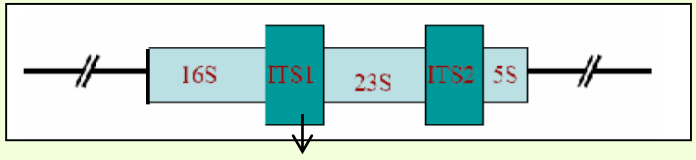
Tokajian *et al.*,2006

DNA Structure

Intergenic spacer region (or Internal transcribed spacer, ITS)

- In bacteria and archaea: ITS1 and ITS2 refer to the internal transcribed spacers between the 16S and 23S rRNA genes and between the 23S and 5S rRNA genes, respectively.

Bacterial rRNA operon

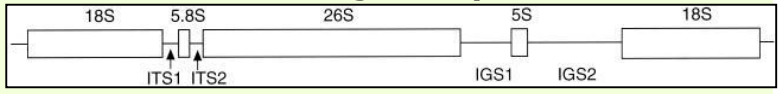


$tRNA^{Ala}$ and a $tRNA^{Ile}$

- In eukaryotes: There are two ITS's.

1. ITS1 is located between 18S and 5.8S rRNA genes, while ITS2 is between 5.8S and 25S (in plants, or 28S in animals) rRNA genes.

Ribosomal gene repeat



TS1 in eukaryotes corresponds to the ITS in bacteria and archaea, while ITS2 originated as an insertion that interrupted the ancestral 23S rRNA gene.

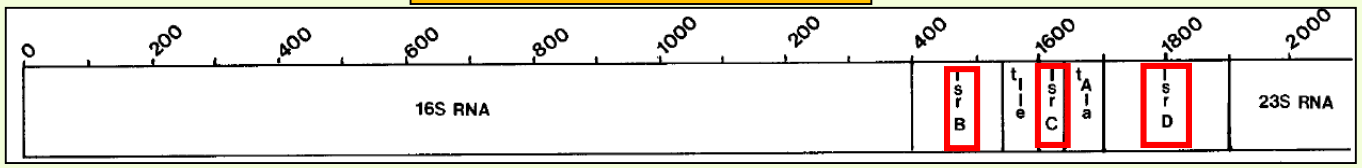
DNA Structure

Intergenic region (IGR or IGS or ISR)

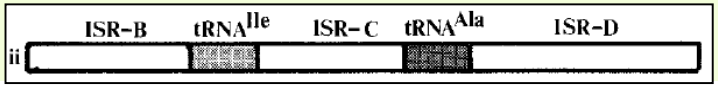
The rRNA-ITS-IGS Sequence

- Prokaryote genomes are considered compacted genomes and genes are continuous.
- **Only small fractions of their genomic DNA assigned to intergenic regions (less than 15%).**
- IGS regions located between the 16S and 23S rRNA genes on the bacterial chromosome. Intergenic spacers lying between tRNA genes.

16S-23S rRNA operon



Ribosomal RNA (rRNA) is a type of non-coding ribonucleic acid (RNA). An ITS region containing both tRNA genes. Noncoding intergenic spacer regions (ISR-B, ISR-C, and ISR-D) surround the tRNA genes code for isoleucine ($tRNA^{Ile}$) and alanine ($tRNA^{Ala}$). The internal transcribed spacer (ITS) is also known as ISR.



Boyer *et al.*,2001;..

Bacterial DNA Structure
16S ribosomal RNA

- 16S ribosomal RNA does not encode a protein.
- The 16S ribosomal RNA is a structural RNA that forms many critical stems and loops to create the structure that will be part of a scaffold holding many ribosomal proteins in position so that the ribosome can function.

DNA Structure

Intergenic spacer region (or ribosomal internal transcribed spacer, ITS)

- Internal transcribed spacer (ITS) refers to the spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes in the chromosome.
- Ribosomal RNA genes are commonly present in a single operon in the order of 16SrRNA-23SrRNA-5SrRNA each if which is separated by one ITS region.
- The two tRNA molecules/genes ($tRNA^{Ala}$ and a $tRNA^{Ile}$) are often present in 16S-23SrRNA ITS region.
- Thus, this section of the genome (ribosomal genes/ITSs) encodes for three ribosomal RNAs (16,23 and 5S rRNAs) as well as tRNAs, therefore a functional region.

$tRNA^{Ile}$: A transfer RNA which is specific for carrying isoleucine to sites on the ribosomes in preparation for protein synthesis.

Bacterial DNA Structure

Intergenic spacer region (or ribosomal internal transcribed spacer, ITS)

Vary from 1-15 copy numbers

- There is a great variation in the number of ITS regions.
- The arrangement of complete unit of ribosomal genes such as 16S-ITS-23S-ITS-5S are scattered in the genome of bacteria, which vary from 1-15 copy numbers.
- For example, in *E. coli* there are 7 copies of operons coding for the three rRNAs.
- Thus, this section of the genome codes for three ribosomal RNAs (rRNAs) as well as tRNAs.

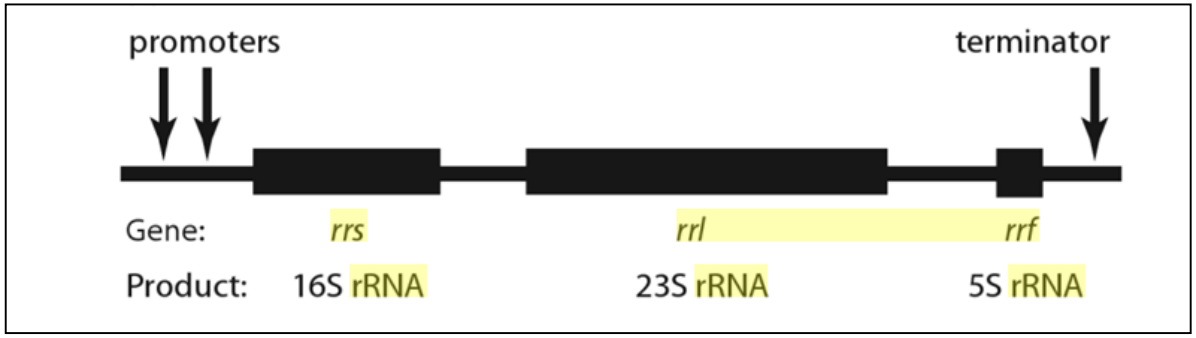
Eukaryotic cells may also contain anywhere from 50-5,000 copies of the rRNA gene.

Bacterial DNA Structure

A typical structure of an rRNA operon

Vary from 1-15 copy numbers

- As the name implies, ribosomal RNA (rRNA) is part of the ribosome.
- The number of rRNA operons encoded by a bacterium ranges from 1 to 15.



The function of the 30S subunit is primarily determined by the 16S RNA of which it is primarily comprised, while the 50S subunit's function is primarily determined by the bacterial 23S RNA.

The 16S rRNA is the RNA component of the small subunit (30S subunit) of the ribosome. The genes coding for it are referred to as 16S rRNA gene. 16S rRNA has been studied extensively. Its sequence and structure has been described, its relationship to ribosome assembly, structure, and function has been characterized.

Bacterial DNA Structure

A typical structure of an rRNA operon

E. coli

- There are seven ribosomal RNA (rRNA) operons in *E. coli*, called *rrsA*, *rrsB*, *rrsC*, *rrsD*, *rrsE*, *rrsG*, and *rrsH*.
- Each operon contains:

1. a 16S rRNA gene,
2. a 23S rRNA gene, and
3. a 5S rRNA gene (except the *rrsD* operon, which contains two 5S rRNA genes) interspersed with various tRNA genes.

- Regarding nomenclature,

1. "*rrs*" genes encode 16S rRNAs,
2. "*rrl*" genes encode 23S rRNAs, and
3. "*rrf*" genes encode 5S rRNAs.

Bacterial DNA Structure

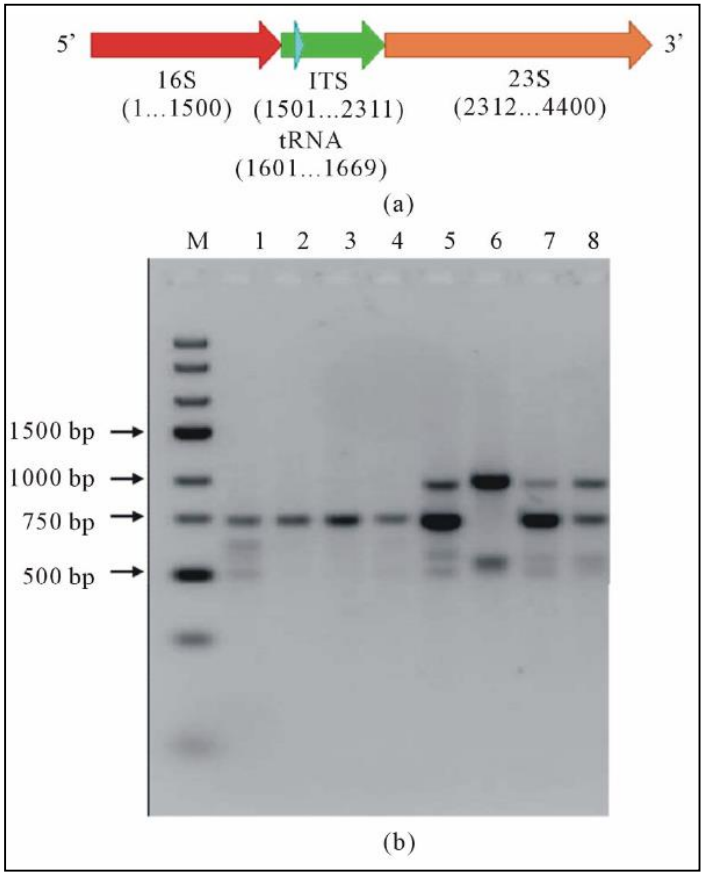
Intergenic spacer region (or ribosomal internal transcribed spacer, ITS)

Aeromonas hydrophila

a) Schematic representation of the position of ITS region in between 16S and 23S rDNA gene region.

b) PCR amplification of internal transcribed spacer (ITS) region from diverse isolates (1-8) of *Aeromonas hydrophila*.

- The size of ITS was ranged between 500-1000 bp.



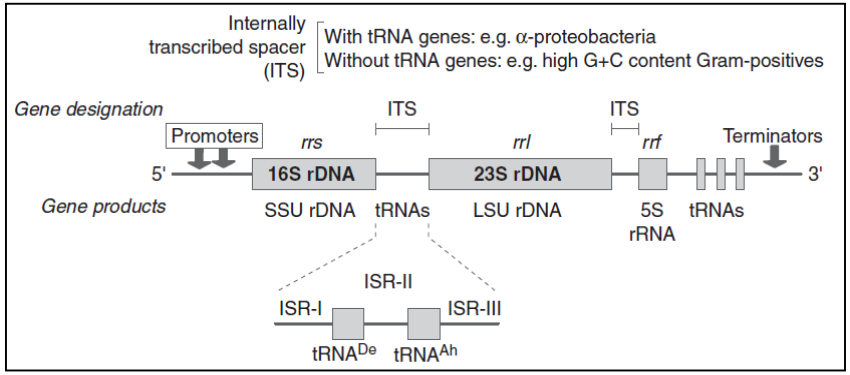
Singh *et al.*,2012

Bacterial DNA Structure

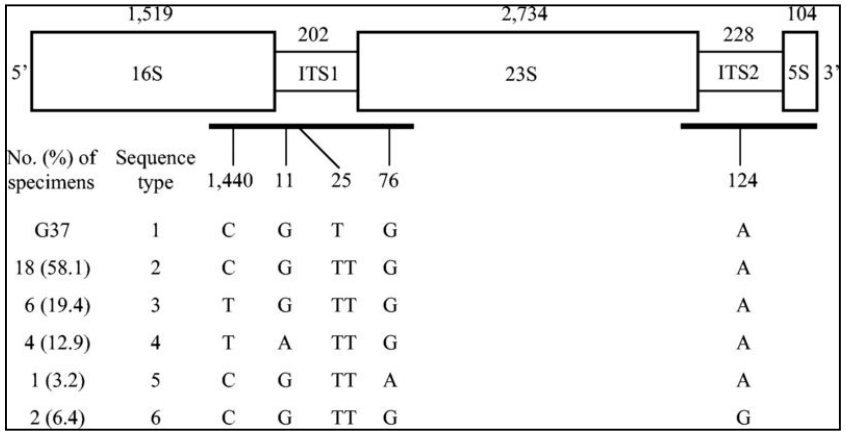
A typical structure of an rRNA operon

Intergenic spacer region (or Internal transcribed spacer, ITS) in bacteria which code sequences for tRNA genes

- In many bacterial species the ITS contains coding sequences for tRNA genes.
- The size (in base pairs) indicated ITS region was derived from the *Mycoplasma genitalium* genome sequence.
- ITS sequences varied in size from 492 to 578 nt within the genus *Xanthomonas* and the similarity among sequences ranged from 63 to 99%.
- The black bars represent the PCR amplified fragments used to detect polymorphic nucleotide.



A key feature of ITSs is the presence/absence of tRNA genes within them.



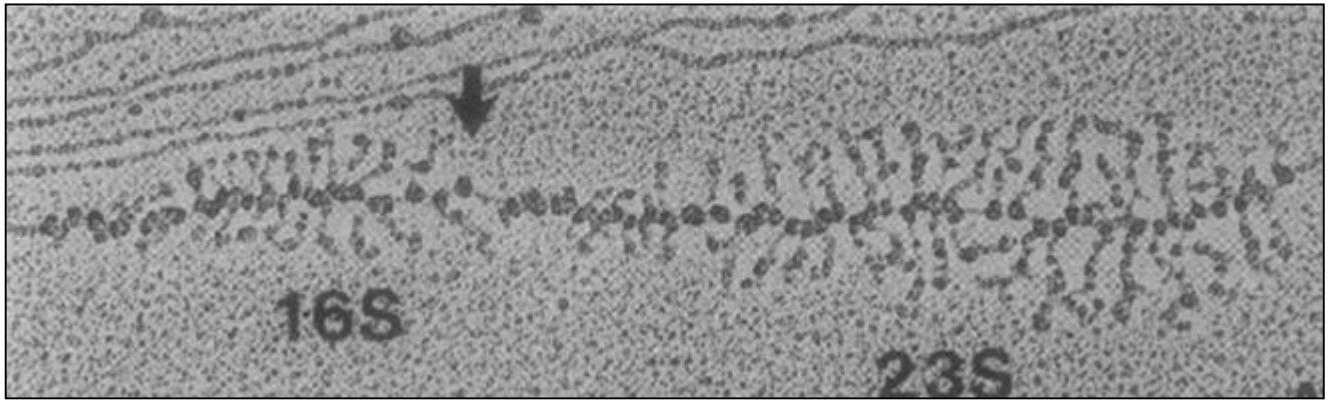
No. (%) of specimens	Sequence type	1,440	11	25	76	124
G37	1	C	G	T	G	A
18 (58.1)	2	C	G	TT	G	A
6 (19.4)	3	T	G	TT	G	A
4 (12.9)	4	T	A	TT	G	A
1 (3.2)	5	C	G	TT	A	A
2 (6.4)	6	C	G	TT	G	G

Genes Types

Patterns of gene expression

Co-transcription of 16S rRNA and 23S rRNA genes

- The 16S rRNA and 23S rRNA genes are co-transcribed.
- The arrow points to a region between these two genes.
- Intervening gene transcripts had been selectively removed by RNAse III.
- Direction of transcription is from left to right.



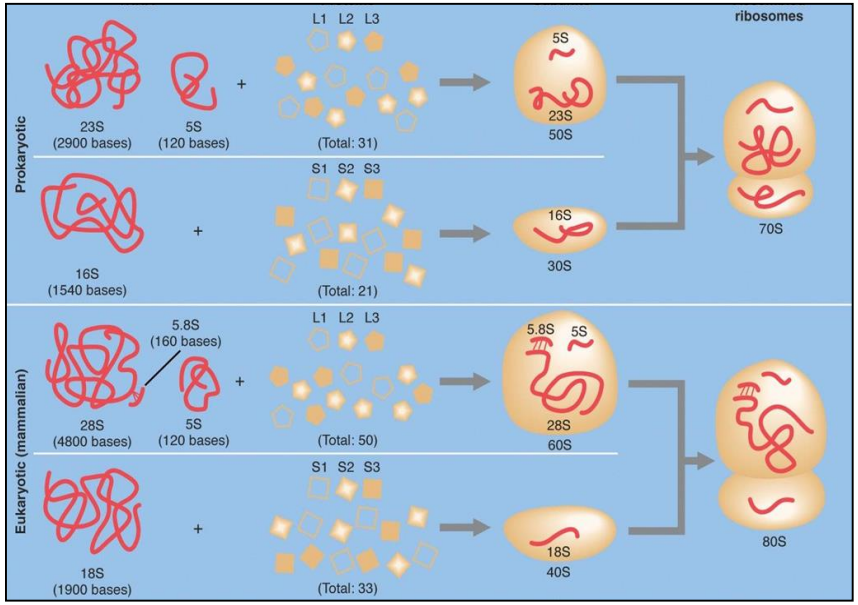
Elio,2013

Bacterial DNA Structure

A typical structure of an rRNA operon

Intergenic spacer region (or Internal transcribed spacer, ITS) in bacteria which code sequences for tRNA genes

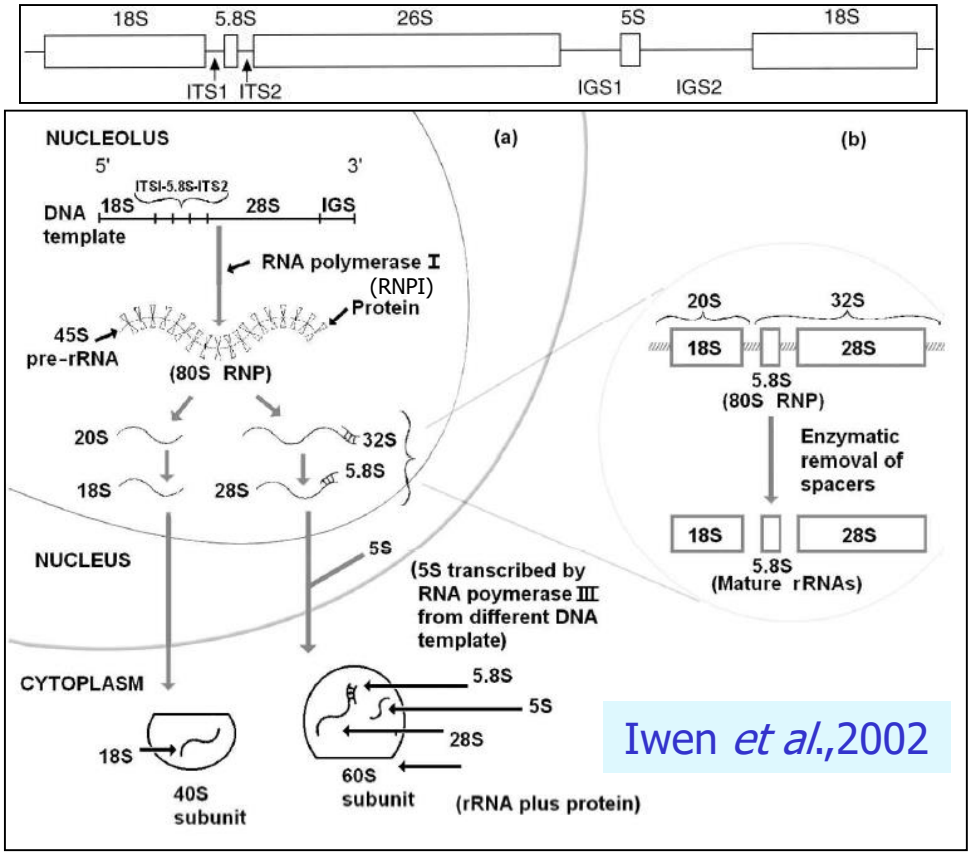
- There are two rRNA in each ribosome, one in the large subunit (LSU) and one in the small subunit (SSU).
- mRNA is sandwiched between the small and large subunits.
- *rrs*" genes encode structural 16S rRNA molecules,"*rrl*" genes encode 23S rRNAs, and "*rrf*" genes encode 5S rRNAs. The 16S (rrs), 23S (rrl) and 5S (*rrf*) *rRNA* genes are clustered and co-transcribed.
- The rrf, rrs and rrl rRNA genes encode for the structural rRNA molecules required for ribosome assembly and function (5S, 16S and 23S rRNAs, respectively).



DNA Structure

Intergenic spacer region (or Internal transcribed spacer, ITS) in eukaryotes code for ribosomal RNA (rRNA).Major steps in the assembly of ribosomes in the eukaryotic cell

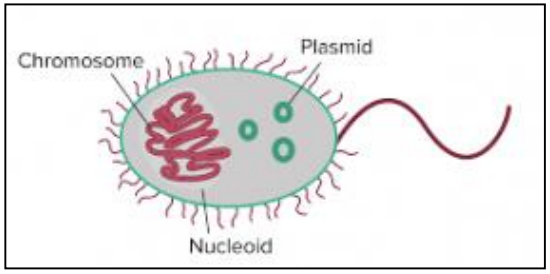
- This section of the genome includes the 18S, 5.8S and 28S genes which code for ribosomal RNA (rRNA).



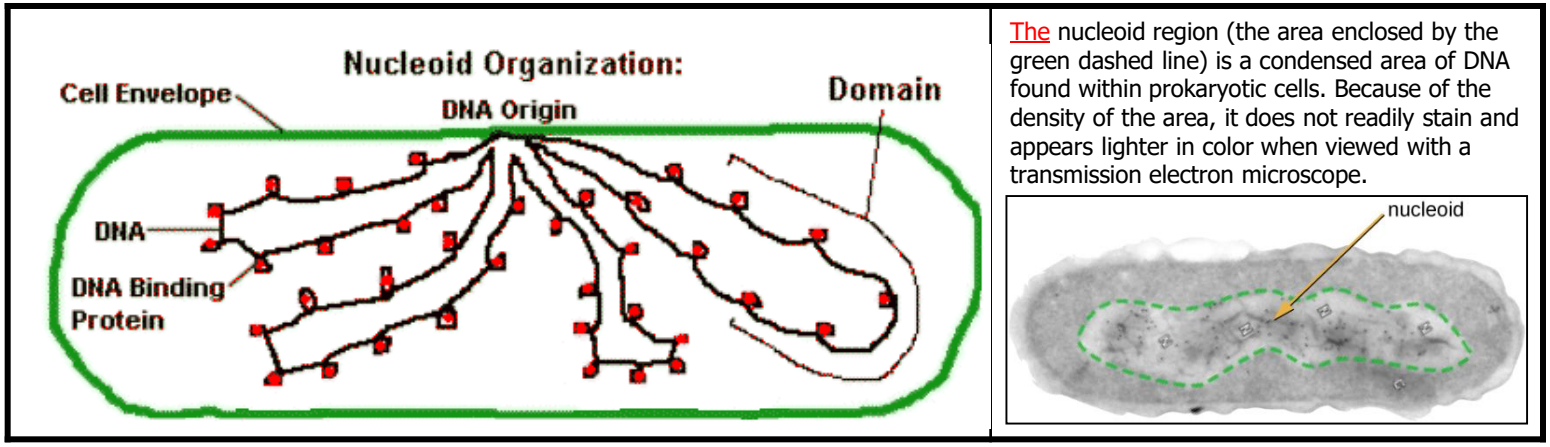
Bacterial DNA

Nuclear region (nucleoid)

Bacteria lack nucleus and histones



- Bacterial chromosome is not enclosed inside of a membrane-bound nucleus but confined to in an area referred to as the nucleoid.
- It is attached to the cell wall (plasma membrane).
- Though it isn't bounded by a membrane, it is visibly distinct (by transmission microscopy) from the rest of the cell interior.
- The DNA is not free but associated with many proteins.



Nucleoprotein complexes formed by bacterial nucleoid-associated proteins revealing novel types of DNA organization.

Bacterial DNA

Why do prokaryotic cells not have histones, while eukaryotic cells do?

- Prokaryotic cells do not have membrane bound organelles.
- The reason for the "histones" not being present in prokaryotic cells is because they do not consist of a nucleus or nuclei.
- Histones limits the modifications that can be done to the DNA because the DNA must "unwind" before it can be transcribed and translated by ribosomes.
- Prokaryotes do not contain these "DNA packing" histones.
- Therefore, the genetic material is readily open to modification (consequently, higher chances for mutation and speedier adaption.

Bacterial DNA

Nucleoid (nuclear region or chromatin body)

- Bacterial nucleoid contains:
  1. DNA (60%; 2-3% dry wt of cell);
  2. RNA (30%);
  3. Protein (10%).
- Does not have a nuclear membrane.
- No histones (A histone is a protein that provides structural support to a chromosome), but has histone-like proteins involved in determining chromosomal structure.

1. Bacterial DNA is usually associated with polyamines (organic compound having two or more primary amino groups-NH_2).
2. Eukaryotic DNA usually associates with histones (small basic proteins, occurring in the nucleus of eukaryotic cells) to form chromatin. Chromatin is a complex of macromolecules found in cells, consisting of DNA, protein and RNA.

Bacterial DNA

Nucleoid (nuclear region or chromatin body)

Chromatin body: The genetic material of bacteria

- Chromosome - Single (sometimes multiple), circular (or linear) and double-stranded DNA.

1. Up to 1 mm (1000 µm) in length.
2. Range in size from 600 to almost 5000 Kb.
3. Simplest bacterial genomes contain 500 to 600 genes.
4. Smaller chromosome, more dependent on host/environment.
5. 1 mm chromosome - supercoiled (underwound) to fit within the cell.
6. Occupies 10% of cell volume.
7. It fills an area of about 1 µm.

Chromosome up to 1 mm (1000µm) in length.
The ch. length 200 times more than the cell length (1000/5=200).

Genomes

DNA Molecules

The length of a single/double-stranded DNA molecules is measured in units of purine or pyrimidine base

DNA	Sequence	Length
Single-stranded	A-C-G-T-C	5 bases
Double-stranded	A-C-G-T-C T-G-C-A-G	5 base pairs

A kilobase (kb) is a unit of length of nucleic acids equal to 1000 base pairs of DNA or RNA.
kb (= kbp) = kilo base pairs = 1,000 bp
Mbp = mega base pairs = 1,000,000 bp or one million base pairs (=1000 kb)
Gb = giga base pairs = 1,000,000,000 bp.
Daltons: One hydrogen weighs 1 dalton (Da).
14,000,000=14×10^6 or 1.4×10^7

Genomes

Sizes and Numbers of Genes

Genome	Group	Size (kb)	Number of genes
Eukaryotic nucleus			
Saccharomyces cerevisiae	Yeast	13,500 (L)	6,000
Caenorhabditis elegans	Nematode	100,000 (L)	13,500
Arabidopsis thaliana	Plant	120,000 (L)	25,000
Homo sapiens	Human	3,000,000 (L)	100,000?
Prokaryote			
Escherichia coli (G-ve)	Bacterium	4,700 (C)	4,000
Hemophilus influenzae (G-ve)	Bacterium	1,830 (C)	1,703
Bacillus subtilis (G+ve)	Bacterium	4,214 (C)	4,100
Viruses			
T4	Bacterial virus	172 (L/C)	300
HCMV (herpes group)	Human virus	229 (L)	
200			
Eukaryotic organelles			
S. cerevisiae mitochondria	Yeast	78 (C)	34
H. sapiens mitochondria	Human	17 (C)	37
Marchantia polymorpha			
Chloroplast	Liverwort	121 (C)	136
Plasmids			
F plasmid	In *E. coli*	100 (C)	29
kalilo	In *Neurospora*, a fungus	9 (L)	2

NOTE: C = circular; L = linear; L/C = linear in free virus, circular in cell

Agrobacterium tumefaciens has one circular and one linear chromosome. Human cells have a linear chromosomes in the nucleus, and a circular chromosome in mitochondria.

Genomes

The numbers of genes in cellular genomes

Organism	Genome size (Mb)	Protein-coding sequence (percent)	Number of genes
Bacterium (*E. coli*)	4.6	90	4,288
Yeast (*Saccharomyces cerevisiae*)	12	70	5,885
Nematode (*Caenorhabditis elegans*)	97	25	19,099
Vinegar flies (*Drosophila*)	180	13	13,600
Human	3,000	3	100,000 (dropped now to 2000-25000 protein-coding genes)

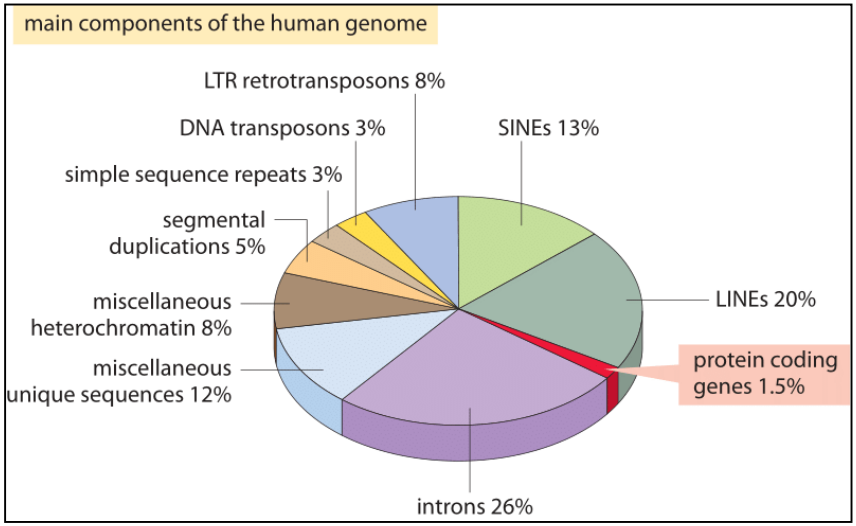
The genome size in red imported fire ant (*Solenopsis invicta*) is 480Mb (mega base pairs) with ca.16569 genes. The DNA of any two people on is 99.6 percent identical. But 0.4 percent variation represents about 12 million base pairs, which can explain many of the differences between individuals. It is also estimated that human body has the ability to generate 2 million different types of proteins, coded by only 20,000-25,000 of our genes.

Cooper,2000;Wikipedia,2018

Genomes

The numbers of genes in human genome

- About 1.5% of the genome consists of the ≈20,000 protein-coding sequences which are interspersed by the non coding introns, making up about 26%.
- Transposable elements are the largest fraction (40-50%) including for example long interspersed nuclear elements (LINEs), and short interspersed nuclear elements (SINEs).



Adapted from T. R. Gregory Nat Rev Genet. 9:699-708, 2005

Genome size

Number of genes in different organisms

- But why do lilies, butterflies, and lungfish (among others) need so much more DNA than we, self-described, complicated humans? This question remains unanswered.
- However, genome size is not a direct measure of genetic content over long phylogenetic distances.
- One needs to examine:

1. the fraction of the genome that codes for protein, or
2. contains other important information.

Organism	Genome size (Mbp)
Bacterium (*E. coli*)	4.6
Corn (*Zea mays*)	2,500
Human	3,000
Easter lily (*Lilium longiflorum*)	90,000
Lungfish (*Protopterus aethiopicus*)	139,000

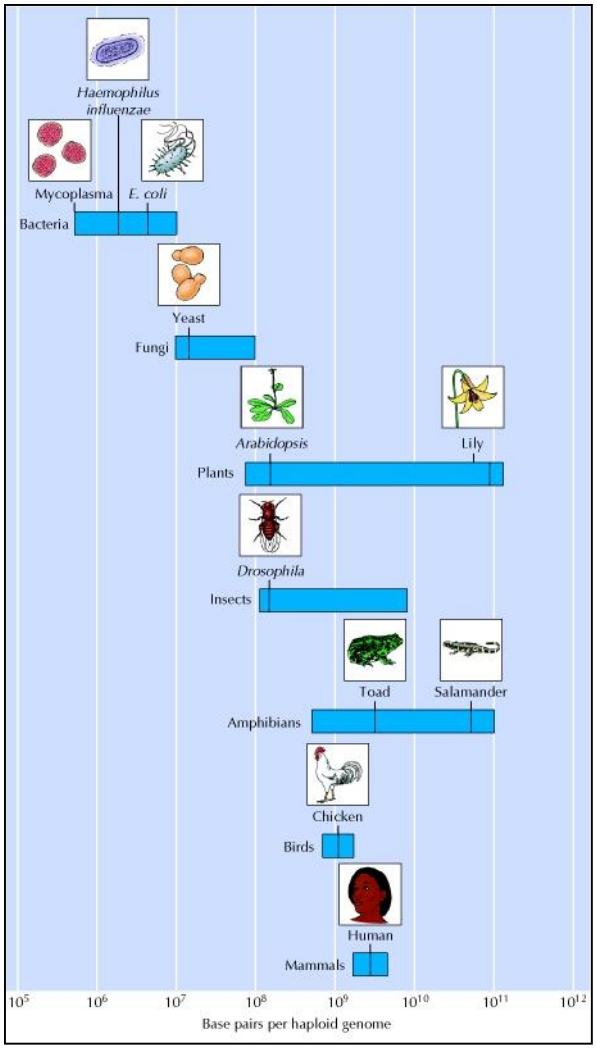
The genome of humans is almost 700 times larger than that of *E. coli*.

Bacterial chromosome

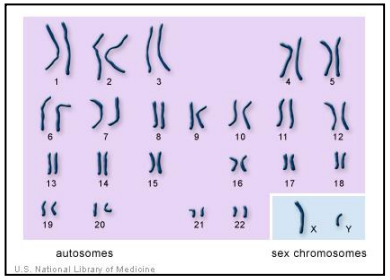
Number of genes in different organisms

Human genes were dropped to 20,000 to 25,000 when the sequence was finished

- Biologists once thought humans had 2 million genes. About 10 years ago we thought there would be about 100,000 because there were so many different proteins known.
- Over the years, the best estimate of the number of human genes has grown steadily smaller, but we still do not have an accurate count.
- Now it turns out we have fewer than nematode worms (closer to 19,000 than to 20,000)!



Chromosome



Bacterial cell/DNA sizes vs. Human cells/DNA sizes

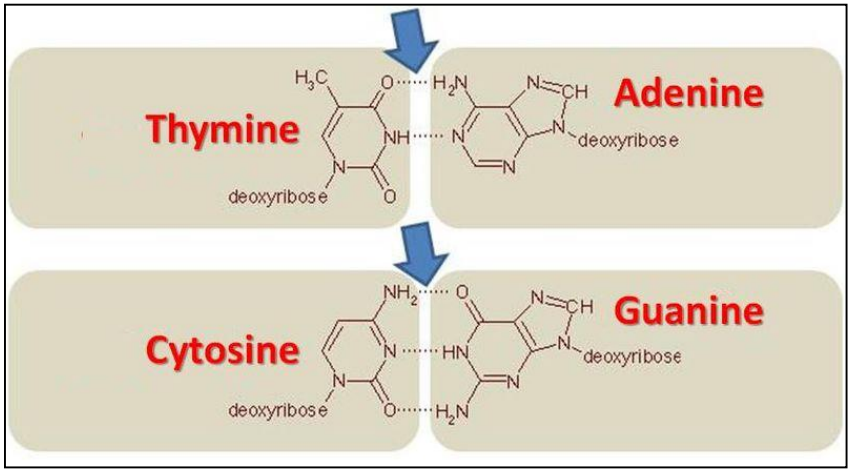
- Most bacteria are 0.2 μm in diameter and 2-8 μm in length.
- Bacterial genomes can range in size from 600 to almost 5000 Kb.
- Human cells are distributed amongst more than 200 different cell types.
- The size of human red blood cell is 9 μm and egg cells are about 130 μm.
- The average human cell is about 10-15 μm.
- Human cell comprised of 23 pairs of linear chromosomes, and the size of genome is approximately 3000 megabases (Kb) of DNA.

Bacterial genomes

DNA with high GC-content is more stable than DNA with low GC-content

- In terms of average genome size, we obtained the following ranking:

1. Gram-positive bacteria (2.53 Mb) with low G+C (average %49.4).
2. Gram-negative bacteria (2.67 Mb) with high G+C (average %51.71).



The GC pair is bound by three hydrogen bonds, while AT pairs are bound by two hydrogen bonds. The length of the coding sequence is directly proportional to higher G+C content. GC-rich means many protein coding genes. The ORF sequences in the gene-rich regions were generally more GC rich.

- The larger genomes e.g. >3 Mb, show relative greater G+C;
- The smaller genomes show relative less G+C.
- %GC in anaerobes 44.03 against 53.74% in aerobes.

XQ and Du,2014; Mann and Chen,2010;..

Average genome size and average genomic C+G content in different kingdoms and subkingdoms Gram+ve and Gram-ve bacteria

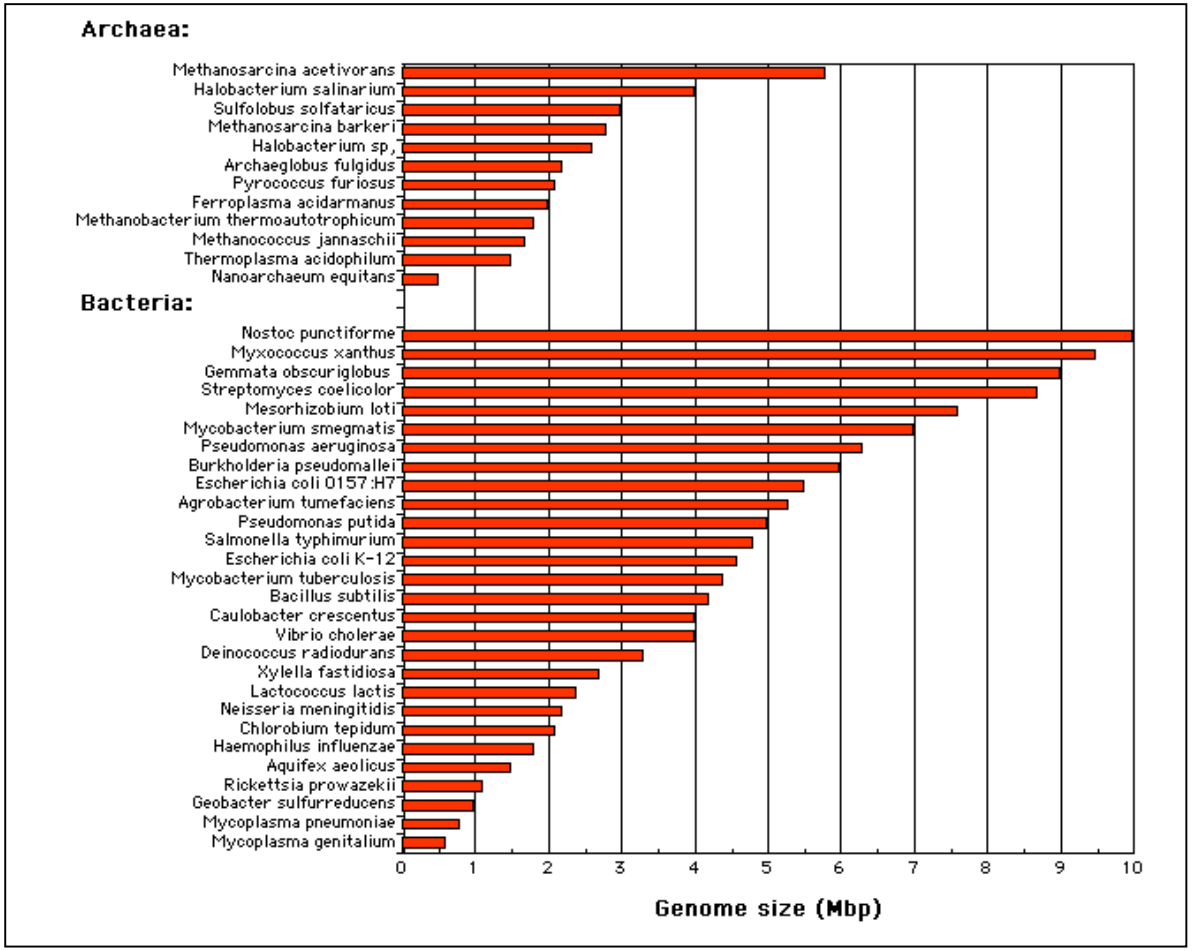
Kingdom or subkingdom	Number of genomes	Average genome size (Mb)	Average genome C+G (%)
Kingdoms:			
Archaeans	58	1.86	44.88
Bacteria	430	2.61	50.76
Fungi	11	18.44	47.96
Protists	21	3.16	26.42
Plants	10	538.84	41.10
Animals	18	1,837.44	41.20
Subkingdoms:			
Gram-positive bacteria	184	2.53	49.40
Gram-negative bacteria	246	2.67	51.71
Dicot plants	6	368.78	34.15
Monocot plants	4	793.93	45.93
Non-mammalian animals	6	674.06	39.44
Non-primate mammalian animals	7	2,190.40	41.97
Primate animals	5	2,739.35	40.85

doi:10.1371/journal.pone.0088339.t001

Li and Du,2014

Genomes

Sizes and numbers of gGenes



Microbial Genetics supplement

Bacterial chromosome

Environmental adaptation

High G+C and larger genomes in free-living and obligate bacteria

- Bacteria, unlike eukaryotes contain a wide range of genomic G+C.
- This wide variability may be viewed as a response to environmental adaptation.
- % G+C:

1. increase (HGT, aerobiosis), and
2. decrease (translesion, a damaged section of DNA, phage insertion, cytosine degradation).

- Note: %GC in anaerobes 44.03 against 53.74% in aerobes.
- On the other hand, nutrient limiting and nutrient poor environments dictate smaller genomes of low GC in attempts to conserve replication expense.

Mann and Chen,2010

Bacterial chromosome

Environmental adaptation

%GC in anaerobes 44.03 against 53.74% in aerobes

- There is a direct relationship between optimal growth temperature and G+C genome content.
- The environmental division between aerobic and anaerobic prokaryotes have shown increased G+C content for aerobes.

Measure (% G + C)	Anaerobic	Aerobic
Mean	44.03	53.74
Standard error	1.30	0.98
Median	43.10	58.65
Standard deviation	11.80	13.99
Sample variance	139.30	195.68
Skewness	0.28	-0.40
Range	46.30	47.40
Minimum	27.20	26.80
Maximum	73.50	74.20
Sum	3654.70	10963.90
Count	83	204
Largest (1)	73.50	74.20
Smallest (1)	27.20	26.80

- Nutrient limiting and nutrient poor environments dictate smaller genomes of low GC.
- Free-living bacteria(non-symbiotic) have average G+C content higher than obligatory pathogens and symbionts.
- Obligate and non-free-living organisms generally present shorter, A+T rich genomes. A/T synthesis is energetically less demanding.
- The GC content of human chromosomal DNA is very heterogeneous, rendering chromosome-wide statistics relatively meaningless. It has been shown that the human genome is a mosaic of GC-rich and GC-poor regions, of around 300kb in length, called isochores. The average GC content of the human genome is 41%.

Genome size and %GC in bacteria

Organism	Genome size (Mb)	Genome %GC
Cross environment		
Streptococcus suis 05ZYH33	2.1	41.1
Burkholderia ambifaria MC40-6	7.6	66.4
Yersinia pestis Antiqua	4.88	47.7
Delftia acidovorans SPH-1	6.8	66.5
Yersinia pestis Nepal516	4.61	47.6
Campylobacter jejuni subsp. jejuni 81–176	1.68	30.5
Campylobacter jejuni subsp. jejuni NCTC 11168	1.6	30.5
Burkholderia cenocepacia MC0-3	7.9	66.6
Pseudomonas putida KT2440	6.18	61.5
Campylobacter jejuni subsp. doylei 269.97	1.8	30.6
Pseudomonas fluorescens PfO-1	6.44	60.5
Ralstonia solanacearum GMI1000	5.8	67
Gluconobacter oxydans 621H	2.92	60.8
Escherichia coli SMS-3-5	5.25	50.5
Mesorhizobium loti MAFF303099	7.6	62.5
Sinorhizobium meliloti 1021	6.74	62.2
Listeria monocytogenes EGD-e	2.94	38
Methylococcus capsulatus str. Bath	3.3	63.6
Streptococcus pneumoniae TIGR4	2.2	39.7
Streptococcus pneumoniae R6	2.04	39.7
Agrobacterium tumefaciens str. C58	5.65	59
Oceanobacillus iheyensis HTE831	3.63	35.7
Klebsiella pneumoniae subsp. *pneumoniae* MGH 78578	5.69	57.1
Yersinia pestis KIM	4.7	47.7
Campylobacter jejuni subsp. jejuni 81116	1.6	30.5
Yersinia pestis CO92	4.88	47.6
Lactobacillus fermentum IFO 3956	2.1	51.5
Finegoldia magna ATCC 29328	1.99	32.1
Coxiella burnetii RSA 493	2.03	42.6
Roseobacter denitrificans OCh 114	4.3	58.9
Campylobacter jejuni RM1221	1.8	30.3
Shewanella woodyi ATCC 51908	5.9	43.7
Average values	4.21	49.07

Mann and Chen,2010

Cell starvation

(p)ppGpp production

A hyperphosphorylated guanosine nucleotide

- (p)ppGpp global regulator (ppGpp) and guanosine pentaphosphate (pppGpp) – collectively known as (p)ppGpp, are effector molecules, accumulated rapidly when bacterial cells encounter with nutritional stress (starvation) conditions.
- (p)ppGpp, is synthesized rapidly when bacterial cells starved for:

1. Amino acids;
2. Other cellular stresses, including deprivation of phosphorus, iron, carbon source or fatty acids.

- This is in order to reactivates translation.

Guanosine: A nucleoside consisting of guanine and ribose. It is a component of RNA.

(p)ppGpp

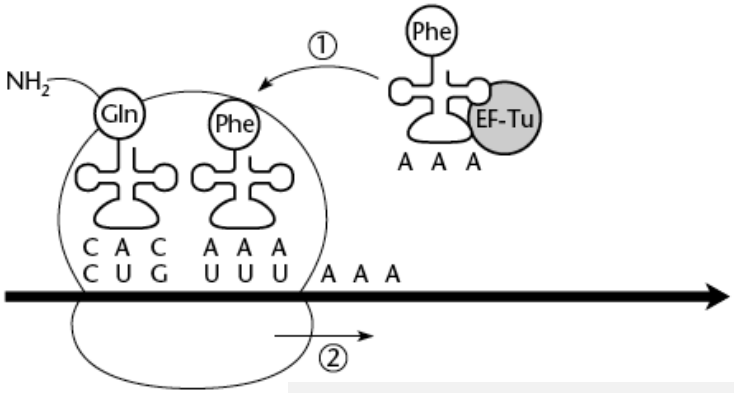
Reasons for synthesis and degradation of (p)ppGpp

- Transfer RNAs (tRNAs) are essential to the bacterial stringent response.

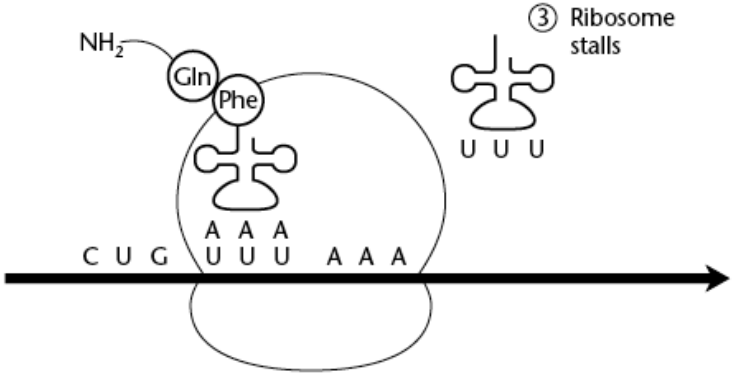
1. **Under normal conditions:** tRNAs are predominantly aminoacylated (charged), are involved in active protein synthesis, and are rapidly re-aminoacylated by their respective aminoacyl-tRNA synthetases upon release from the ribosome.
2. **Under amino acids limiting conditions:** the deacylated forms of the respective tRNAs ($tRNAs^{deacyl}$) rapidly accumulate in the ribosomal A-site.

Payoe and Fahlman,2016;..

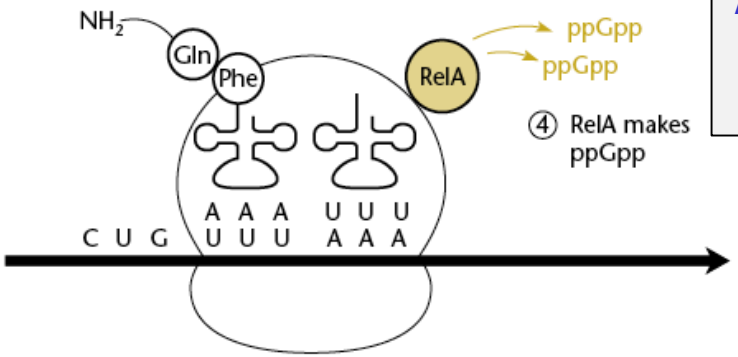
Under normal conditions:
Charged tRNA binds to the A-site in ribosome.



Under amino acids limiting conditions:
Amino acid starvation results in an uncharged tRNA.



-deacylated tRNA will bind to the A-site empty.



An uncharged tRNA is a signal for RelA binding to the ribosome and synthesizes ppGpp guanosine 3'5' bisphophate, also pppGpp.

ppGpp now interacts with RNA polymerase at key metabolic pathways.

(p)ppGpp

Reasons for synthesis and degradation of (p)ppGpp

1. Under normal conditions:

- Elongation of the polypeptide chain involves addition of amino acids to the carboxyl end of the growing chain.
- Elongation starts when the fMet-tRNA (in bacteria) enters the P site, causing a conformational change which opens the A site for the new aminoacyl-tRNA to bind.
- This binding is facilitated by elongation factor-Tu (EF-Tu), a small GTPase.

Wikipedia,2016;..

(p)ppGpp

Reasons for synthesis and degradation of (p)ppGpp

2. Under amino acids limiting conditions:

- The enzymatic activity of RelA does not affect the amount of $tRNA^{deacyl}$ bound to the A-site, suggesting that RelA does not remove $tRNA^{deacyl}$ from the ribosome.
- After the cell has overcome starvation, the $tRNA^{deacyl}$ may be chased out of the A-site by the cognate charged tRNA in complex with EF-Tu-GTP.

Castilho *et al.*,2014;..

(p)ppGpp

Reasons for synthesis and degradation of (p)ppGpp

Under amino acids limiting conditions:

- It has been proposed that RelA 'hops'(moves) from ribosome to ribosome to detect the presence of $tRNAs^{deacyl}$ at A sites, thereby the amount of synthesised (p)ppGpp reflects the amount of 'starved' ribosomes.
- RelA synthesises several (p)ppGpp molecules per 'hopping' event.
- Note, RelA is far less abundant in the cell than ribosomes (one RelA molecule per 200 ribosomes).

Castilho *et al.*,2014;..

(p)ppGpp

Reasons for synthesis and degradation of (p)ppGpp

Under amino acids limiting conditions:

- The synthesis of (p)ppGpp by RelA is stimulated by ribosomes with an uncharged or deacylated tRNA (deacyltRNA) bound in the A-Site.
- Ribosome idles on the mRNA waiting for a charged tRNA.
- RelA and ppGpp function to dislodge the uncharged tRNA.
- Activated RelA catalyzes (p)ppGpp formation until the deacylated tRNA (uncharged) passively dissociates from the ribosomal A-Site.

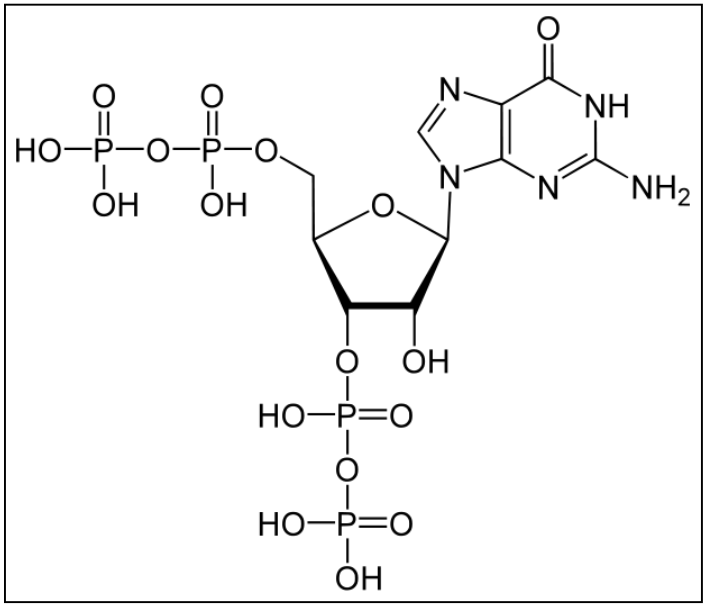
Payoe and Fahlman,2016;..

(p)ppGpp

Guanosine tetra- and pentaphosphate ((p)ppGpp)

Signal of amino acid or energy source depletion

- (p)ppGpp affects the expression of a wide range of genes such as the amino acid biosynthetic genes.
- Guanosine tetraphosphate (ppGpp)
- Guanosine pentaphosphate (pppGpp).
- ppGpp has about 8-fold greater efficiency than that of pppGpp.



Guanosine: A nucleoside consisting of guanine and ribose.
Adenosine: Composed of a molecule of adenine+ a ribose sugar molecule.
ATP (adenosine triphosphate): Consists of adenosine, ribose and three phosphate groups.

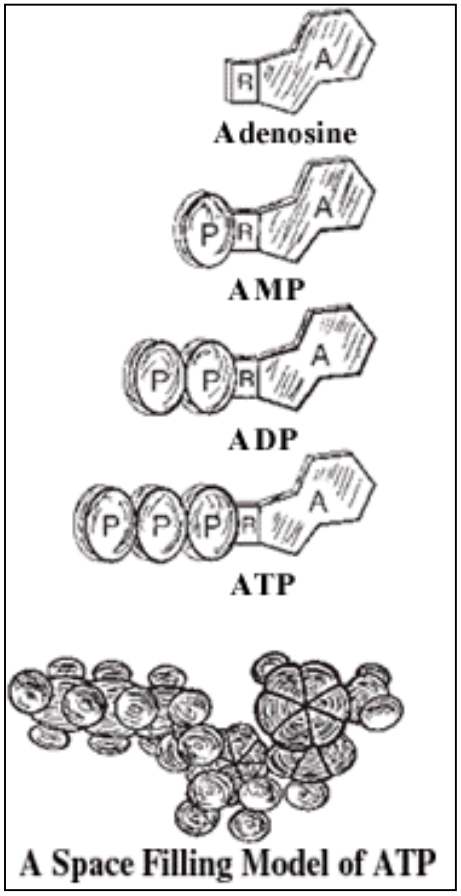
ATP (Adenosine Triphosphate)

The common and perfect energy currency for all cells

- As far as known, all organisms from the simplest bacteria to humans use ATP as their primary energy currency.
- Three ways energy can be obtained:

1. From organic chemicals;
2. From inorganic chemicals;
3. From light.

- ATP and AMP (adenosine monophosphate) is used as a way for a cell to sense how much energy is available.

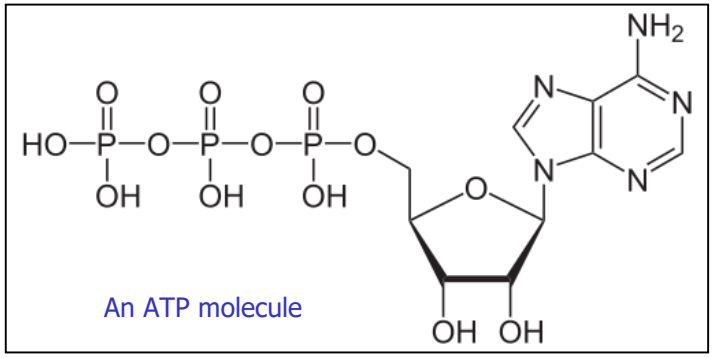


Simple structural formula and a space filled model of ATP.

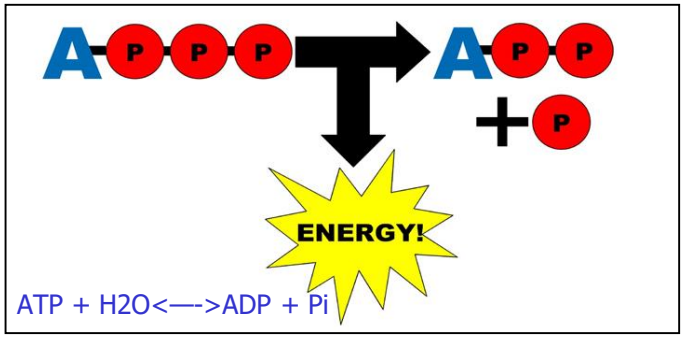
Bergman,1999;..

ATP (Adenosine Triphosphate)
Store energy like a rechargeable battery

- ATP, is the fuel for all the other molecular machines.
- ATP (adenosine triphosphate) can be broken down into ADP (adenosine diphosphate) and phosphate to release energy.
- Energy is released by hydrolysis of the third phosphate group.
- After this third phosphate group is released, the resulting ADP (adenosine diphosphate) can absorb energy and regain the group, thus regenerating an ATP molecule.
- This allows ATP to store energy like a rechargeable battery.



An ATP molecule

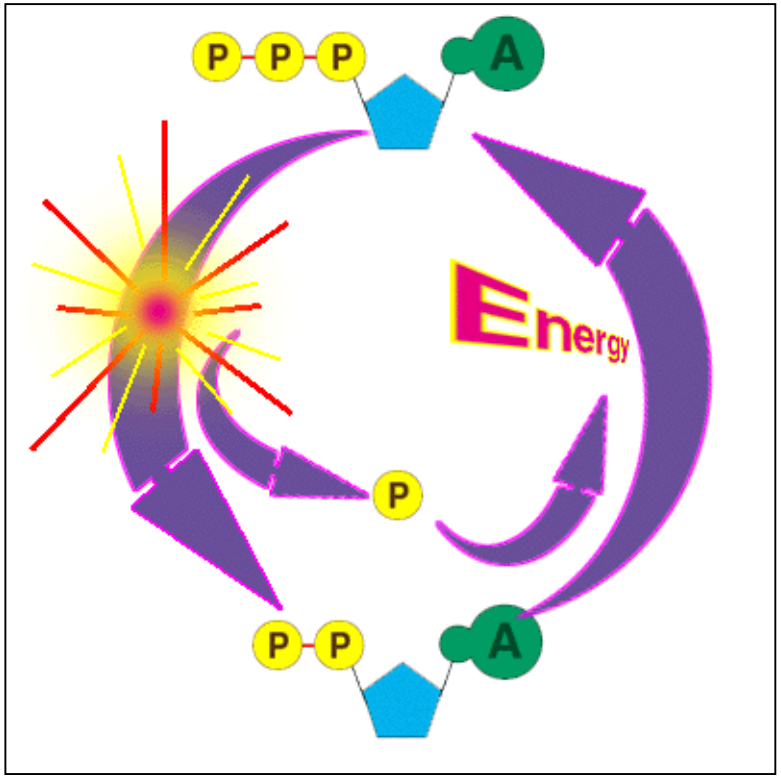


ATP + H2O<—->ADP + Pi

ATP (Adenosine Triphosphate)

Common energy storage

- ATP is an unstable molecule in water, in which it hydrolyses to ADP and phosphate.
- Hydrolysis of ATP in the cell releases a large amount of free energy.
- **Phosphorylation:** Organic phosphate is added to substrate.



ATP $\xrightarrow{\text{Hydrolysis}}$ ADP+P

ATP and other triphosphates

- The ATP molecule is composed of three components:

1. At the center is a sugar molecule, ribose (the same sugar that forms the basis of RNA);
2. Attached to one side of this is a base (adenine);
3. The other side of the sugar is attached to a string of phosphate groups. These phosphates are the key to the activity of ATP.

- Between ATP and other triphosphates, energy can be easily transferred.

In late stationary phase, cell faces acute shortage of energy sources, a cell has to conserve energy for survival. GTP is a key energy source required for diverse cellular processes.

Reaction of ADP with GTP:
ADP + GTP > ATP + GDP

ATP and other triphosphates

GTP (Guanosine-5'-triphosphate)

More specific energy source

- GTP is essential to signal transduction, in particular with G-proteins.
- GTP has the role of a source energy or an activator of substrates in metabolic reactions, like that of ATP, but more specific.

1. It is used as a source of energy for protein synthesis and gluconeogenesis.
2. It can also act as a substrate for the synthesis of RNA during the transcription process or DNA during DNA replication.
3. Many hormones have receptors that use G-proteins to transmit their signals to the rest of the cell.

G-proteins are a family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to its interior.

(p)ppGpp

Genes coding for synthesis of (p)ppGpp

- (p)ppGpp is synthesized by two stringent response genes:

1. *relA*, and
2. *spoT* in *E. coli* and different bacteria.

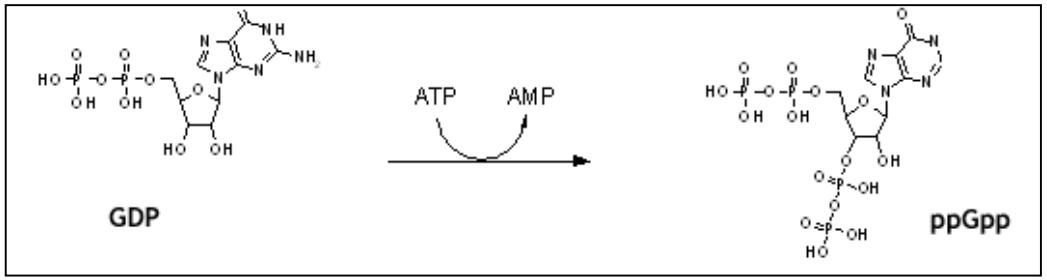
- Two proteins are central to the stringent response, RelA and SpoT, both catalyze the synthesis of (p)ppGpp.

Braeken *et al.*,2006

(p)ppGpp
Genes coding for synthesis of (p)ppGpp

- RelA is activated when an uncharged tRNA enters the A site.

1. RelA binds to 70S ribosomes, and upon recognition of A-site bound $tRNA^{deacyl}$, it converts ATP and (GTP) GDP into (p)ppGpp(and also AMP) i.e. RelA catalyzes the transfer of the γ,β-pyrophosphate from ATP to the 3'-hydroxyl of either GTP or GDP to form pppGpp and ppGpp, respectively.
2. Simultaneously (p)ppGpp provides the energy for RelA to dissociate off the ribosome.



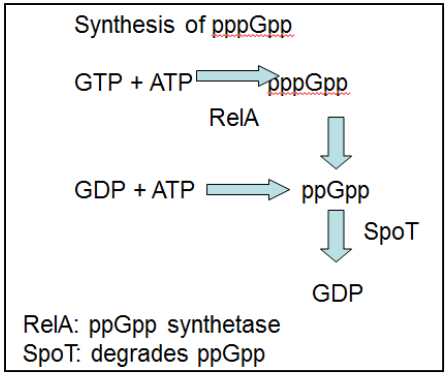
Synthesis of ppGpp

Payoe and Fahlman,2016;TriLink

(p)ppGpp
Genes coding for synthesis of (p)ppGpp

- In *Escherichia coli*,

1. RelA catalyzes the formation of the vast majority of (p)ppGpp synthesis during amino acid starvation.
2. SpoT is responsible for basal level (p)ppGpp formation and (p)ppGpp degradation upon cessation of the stringent response.



SpoT is a bifunctional enzyme that has both ppGpp synthetic and hydrolytic activities. When the amino acid balance in the cell is restored, (p)ppGpp is hydrolysed by SpoT.

Control of bacterial transcription, translation and replication

By (p)ppGpp, a hyperphosphorylated guanosine nucleotide

1. Mechanism of RelA-mediated (p)ppGpp synthesis:

- Under conditions of amino acid starvation, large pools of uncharged tRNAs are generated that can bind to the A-site of the ribosome with low affinity and block the ribosome as a result.

I. RelA detects a blocked ribosome.

i. RelA binds to the ribosome and mediates (p)ppGpp synthesis.

ii. RelA synthesizes pppGpp/ppGpp from GTP/GDP, respectively, using ATP.

II. Concomitantly with (p)ppGpp synthesis, RelA is released.

III. RelA hops to the next ribosome and the process is repeated, leading to levels of (p)ppGpp required to activate the stringent response.

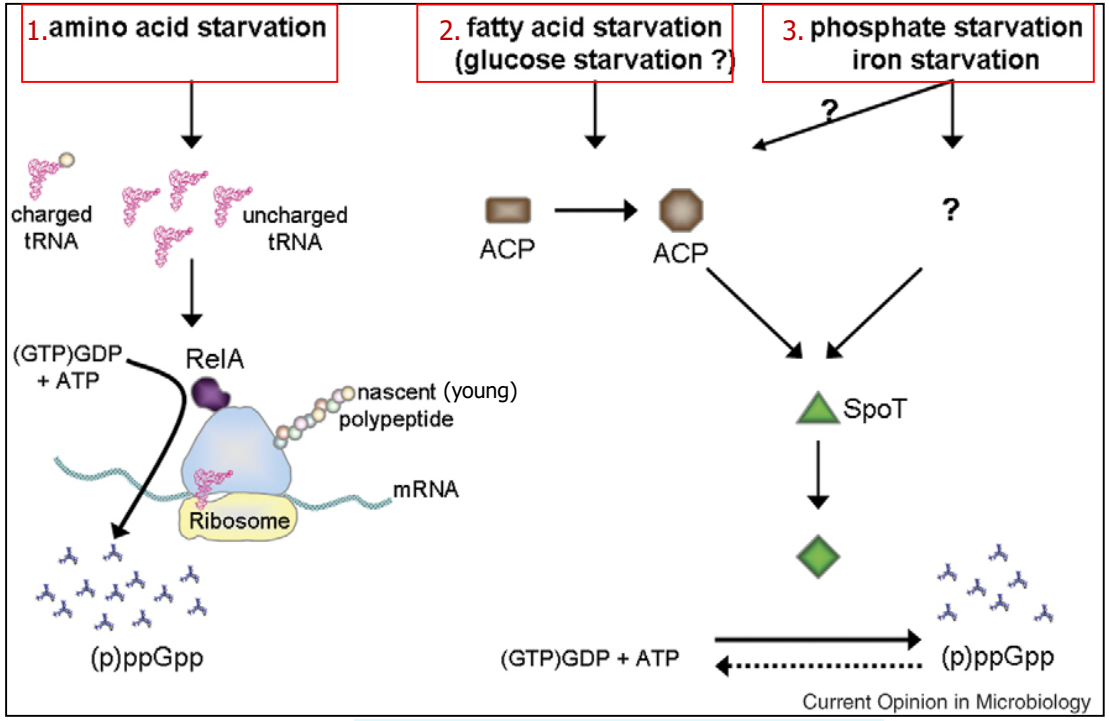
- Thus, a high levels of (p)ppGpp required to activate the stringent response and reactivates translation.

Braeken *et al*.,2006

Control of bacterial transcription, translation and replication

By (p)ppGpp, a hyperphosphorylated guanosine nucleotide

- The enzymes RelA and SpoT are triggered by different starvation signals (amino acid, fatty acid and phosphate starvations) to produce (p)ppGpp for reactivates translation.



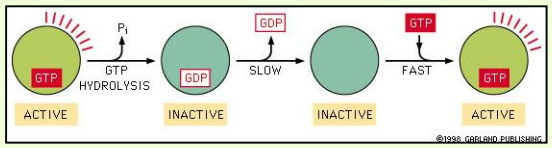
1. RelA synthesizes pppGpp/ppGpp from GTP/GDP.
2. RelA does not remove uncharged tRNA from the ribosome, but detects and binds to ribosome.
3. RelA and ppGpp function to dislodge the uncharged tRNA.

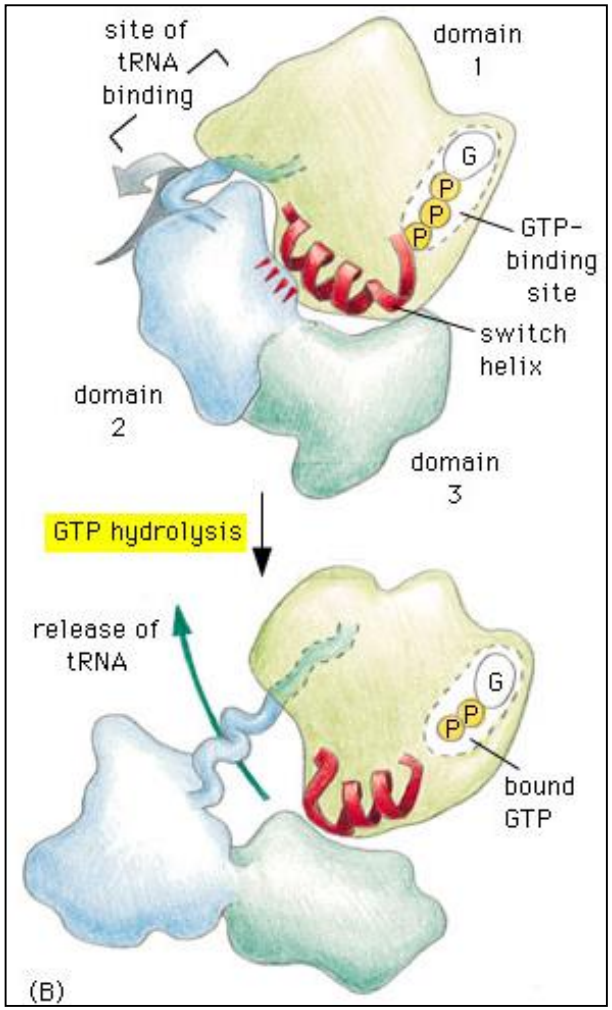
2. Acyl carrier protein (ACP) binds to SpoT and shifts the balance of its activity towards (p)ppGpp synthesis.

Srivatsan and Wang,2005

GTP/GDP-binding protein regulators
Regulate G proteins

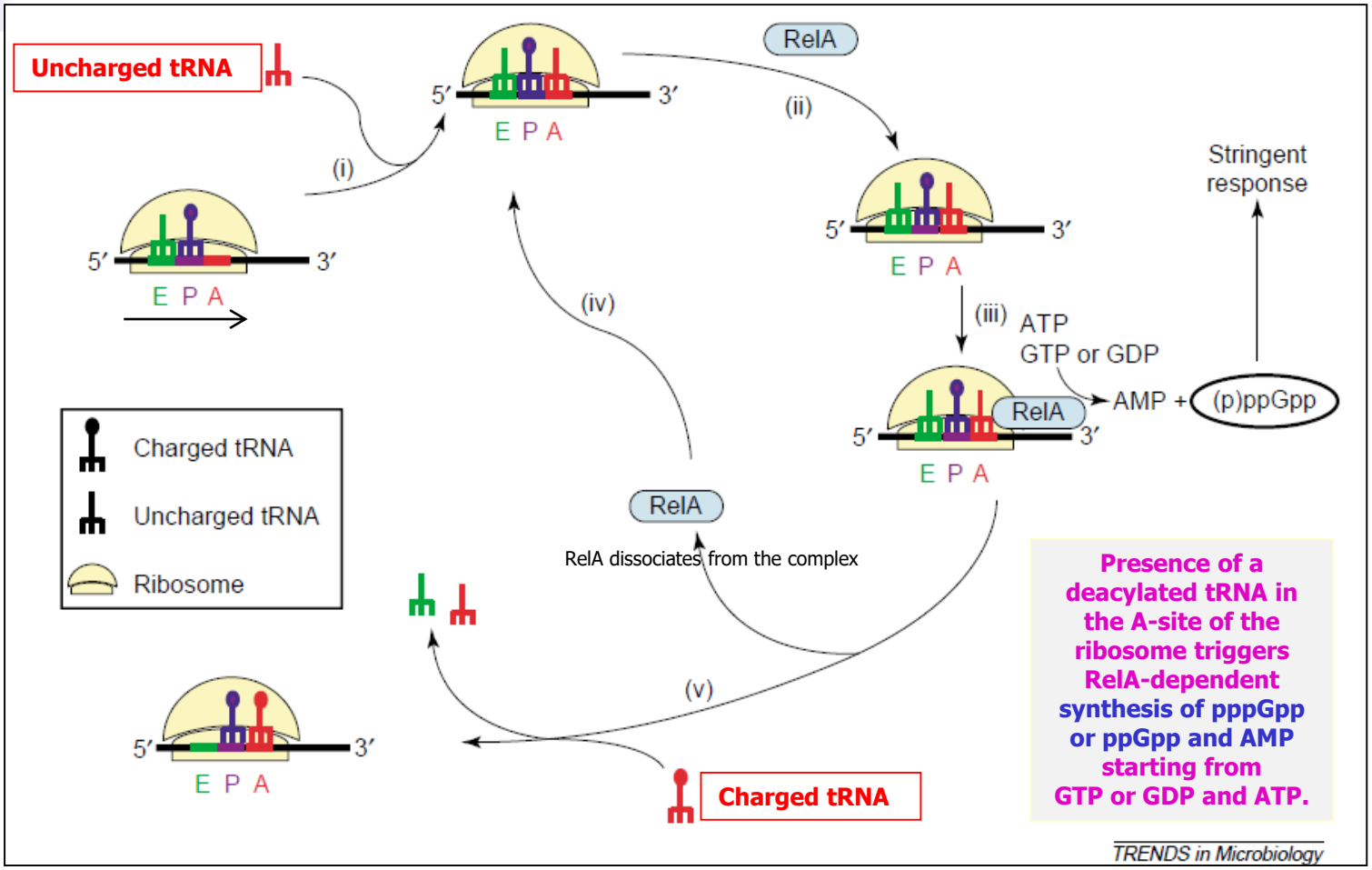
- During the elongation stage of translation, GTP is used as an energy source for the binding of a new amino-bound tRNA to the A site of the ribosome.
- GTP is also used as an energy source for the translocation of the ribosome towards the 3' end of the mRNA.
- GTP/GDP can act as a substrate for the synthesis of RNA during the transcription process.





Mechanism of RelA-mediated (p)ppGpp synthesis

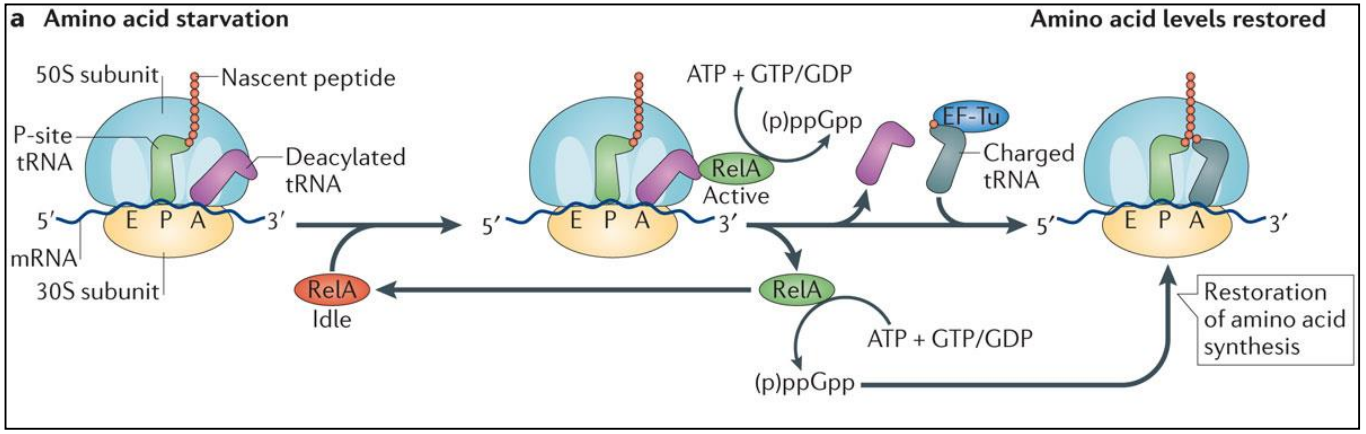
RelA dissociates from the complex. Then it can rebind or proceed to the next stalled ribosome. The cycle repeats until the deacylated tRNA passively dissociates from the ribosomal A-Site, preventing further pppGpp synthesis



Braeken *et al.*,2006

Control of bacterial transcription, translation and replication
Amino acid starvation

- Increased (p)ppGpp levels direct cellular metabolic resources to amino acid biosynthesis, which restores normal levels of tRNA aminoacylation.
- Aminoacylated (charged) tRNA is delivered to the ribosome by elongation factor Tu (EF-Tu) and inhibits the binding of RelA and deacylated tRNA.



Hauryliuk *et al.*,2015

Control of bacterial transcription, translation and replication

By (p)ppGpp, a hyperphosphorylated guanosine nucleotide

2. Mechanism of SpoT-mediated (p)ppGpp synthesis:

- SpoT synthesizes and hydrolyzes (p)ppGpp through distinct active sites.
- Fatty acid starvation or potentially, glucose starvation, triggers a conformational change in the acyl carrier protein (ACP), which binds to SpoT and shifts the balance of its activity towards (p)ppGpp synthesis.
- Phosphate or iron starvation also results in (p)ppGpp accumulation through modulation of SpoT activity.

Srivatsan and Wang,2005; Braeken *et al.*,2006

(p)ppGpp and starvation signaling
General functions

- (p)ppGpp facilitates cell survival by regulating a range of processes within the cell, such as:

1. up-regulation of many other genes involved in stress response including the genes for amino acid uptake (from surrounding media) and biosynthesis;
2. protein degradation;
3. DNA replication;
4. cell division;
5. Adaptation;
6. fatty acid biosynthesis, and
7. biofilm formation.
8. It has also been reported that (p)ppGpp signaling is key to the pathogenicity of some infectious bacteria.

(p)ppGpp and starvation signaling
General functions

- Furthermore, some other processes in bacteria are affected (regulated) by (p)ppGpp:

1. Transcription, replication and translation;
2. Regulation of virulence and pathogenesis;
3. Sporulation and morphological differentiation;
4. Persistence to adverse biotic and abiotic factors;
5. Bacterial social behaviour (Quorum sensing and biofilm formation);
6. Production of extracellular enzymes;
7. Production of an antibiotics e.g. in *Streptomyces* spp. and Bacilli.
8. Antibiotic resistance in bacteria.

Srivatsan and Wang,2005; Braeken *et al.*,2006

(p)ppGpp and starvation signaling
General functions

- **Presence of (p)ppGpp:**
- (p)ppGpp as a global regulator is important during starvation and stress signalling in many bacteria.
- (p)ppGpp pool increases with any types of nutrient limitation.
- **Absence of (p)ppGpp:**
- A complete absence of (p)ppGpp causes multiple amino acid requirements, poor survival of aged cultures, aberrant(abnormal) cell division, morphology, and immotility, as well as being locked in a growth mode during entry into starvation.

Stringent Control

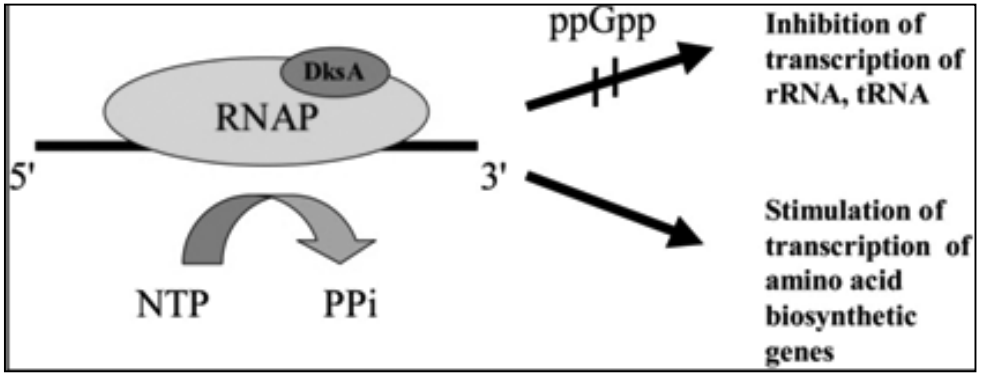
Redirection of transcription from growth-related genes to genes involved in stress resistance and starvation survival

1. The result of the ppGpp signal is a stop in protein synthesis, leading to adjustments in gene expression levels and causing cells to remain dormant until normal nutritional levels are restored.
2. This ppGpp-mediated mechanism prevents the outflow of unnecessary energy resources to help bacteria survive until adequate amino acid levels are reached.

Stringent Control

Redirection of transcription from growth-related genes to genes involved in stress resistance and starvation survival

- ppGpp strongly inhibits the synthesis of components of the translation machinery such as tRNA and rRNA, while simultaneously stimulating the transcription of genes involved in amino acid biosynthesis.



The stringent response to amino acid starvation as controlled by ppGpp in *E. coli*.

Stringent Control

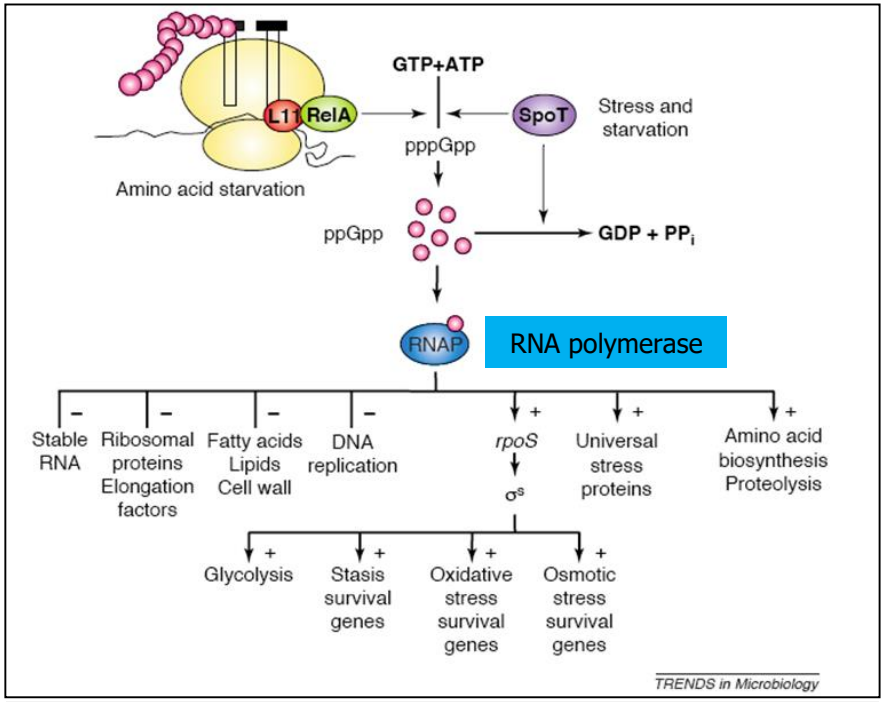
Redirection of transcription from growth-related genes to genes involved in stress resistance and starvation survival

1. Provides the cell with an efficient method to regulate the most abundant molecules in the cell;
2. Upregulates genes encoding metabolic enzymes, especially those needed for amino acid biosynthesis.
3. Shuts off synthesis of pathways utilized during growth phase.

Stringent Control

Redirection of transcription from growth-related genes to genes involved in stress resistance and starvation survival

- (p)ppGpp is produced from GTP and ATP by two parallel pathways in response to starvation and stress and is subsequently converted to ppGpp.
- ppGpp binds RNAP and redirects transcription from growth-related genes to genes involved in stress resistance and starvation survival.
- SpoT is also responsible for hydrolyzing ppGpp.



+, upregulation of gene expression such as *ropS*.
-, downegulation gene expression.

(p)ppGpp

Genes coding for synthesis of (p)ppGpp

In eubacteria and some archaea

- p)ppGpp is not restricted to prokaryotes but also:
- The RelA/SpoT genes have been identified in most eubacterial and some archaea species.
- Within the eubacterial kingdom there is an evolutionary dichotomy of RelA and SpoT genes.
- In eukaryotes, amino acid starvation is sensed by the general amino acid control (GAAC) system that is nonhomologous to the RSH system but is functionally analogous.

Braeken *et al.*,2006;..

(p)ppGpp

Genes coding for synthesis of (p)ppGpp

In eubacteria in including yeasts

- The protein kinase Gcn2 (general control nonderepressible 2) is present in virtually all eukaryotes and is of increasing interest due to its involvement in a large array of crucial biological processes. e.g. nutrient starvation and oxidative stress.
- GCN2 is a serine/threonine-protein kinase that senses amino acid deficiency through binding to uncharged transfer RNA (tRNA).
- In mammals, Gcn2 is important for e.g. long-term memory formation, feeding behaviour and immune system regulation.

Castilho *et al.*,2014;Wikipedia,2016

(p)ppGpp

Genes coding for synthesis of (p)ppGpp

In eubacteria in including yeasts

- The protein kinase Gcn2 plays a key role in modulating amino acid metabolism as a response to nutrient deprivation.
- The absence of essential amino acids causes a downregulation of key components of the lipid synthesis such as the fatty acid synthase.
- Following leucine-deprivation in mammals, GCN2 decreases the expression of lipogenic genes.

Dietary protein and amino acid requirements are normally highest in young, growing animals and decline with age during the approach to maturity. Recent studies also illustrate that modifying dietary essential amino acid (EAA) composition has profound effects on lipid metabolism and energy balance (Anthony *et al.*,2013). Because protein intake is monitored through nutrient-sensing mechanisms.

(p)ppGpp

Genes coding for synthesis of (p)ppGpp

In plants

- Homologues of RelA/SpoT have also been identified in plants.
- relA-spoT homologues, designated *RSH* genes are responsible for coding:
- At-RSH1 in *Arabidopsis thaliana*, and
- Nt-RSH2 of *Nicotiana tabacum* have been identified in plants.
- The long RSHs RelA and SpoT synthesize pppGpp from GTP and ppGpp from GDP, generating AMP as a by-product.

ATP and AMP (adenosine monophosphate) is used as a way for a cell to sense how much energy is available.

Temperature

Temperature difference between the nucleus and the cytoplasm in mammalian cell

Nucleus temperature

- Temperature is a fundamental physical parameter related to many cellular functions, including:
- Gene expression, protein stabilization, enzyme-ligand interactions and enzyme activity.
- FLIM(fluorescence lifetime imaging microscopy) analysis confirmed:

1. a temperature difference between the nucleus and the cytoplasm, and
2. heat production near the mitochondria.

The most common form of DNA(B-DNA), present at neutral pH and physiological salt concentrations.

Hayashi *et al.*,2015

Temperature

Temperature difference between the nucleus and the cytoplasm in mammalian cell

Nucleus temperature

- The temperature difference between the nucleus and the cytoplasm changed depending on the cell cycle status, suggesting a correlation between cellular function and temperature.

1. The temperature in the nucleus was approximately 1°C higher than that in the cytosol, and
2. The temperature near the mitochondria was higher than that of the rest of the cytoplasm.

- Novel cellular thermometers are needed to evaluate the temperature distribution inside of a cell in the near future.

The temperature difference (<ΔT>) was calculated by subtracting the average temperature of the cytoplasm from that of the nucleus. The temperature of the medium was maintained at 30°C.

Hayashi *et al.*,2015

DNA preservation
Storage condition of extracted DNA

- As a general rule isolated DNA can be stored:

1. at 4°C for several weeks,
2. at -20°C for several months, and
3. at -80°C for several years.

- The most common method of storage of DNA is as a suspension in ethanol at -80°C.
- DNA stored in buffer is much less stable if denatured prior to storage i.e. single stranded is less stable.
- An alternative is to add an antioxidant/scavenger such as 1% ethanol.

Bacterial chromosome

Variable form & numbers

Main and second chromosomes

- Essential core genes are very often carried by the main chromosome.
- However they can occasionally be found on secondary chromosomes, recently renamed chromids.
- Chromids have evolved from non-essential megaplasmids, and further acquired essential core genes and a genomic signature closed to that of the main chromosome.

Fournes *et al.*,2108

Bacterial chromosome

Variable form & numbers

Multiple chromosomes

- The number of chromosomes varies among different bacteria.
- Usually bacteria only have one copy of each gene (haploid).
- Smaller bacteria like *Rickettsia* and *Chlamydia* have chromosomes less than one third the size of that of *E. coli*.
- Bacterial DNA has been found usually in circle.
- But some have both:

1. circular (usually), and
2. linear forms.

- Linear chromosomes were observed in:

1. *Streptomyces* species,
2. *Rhodococcus fasciens*,
3. Spirochetes,
4. *Agrobacterium tumefaciens*.

Drlica and Bendich,2004;..

Bacterial chromosome

Variable form & numbers

Multiple chromosomes

- The existence of multiple chromosomes in bacteria has been known for some time.

1. The chromosomes is three for *Sinorhizobium* (formerly *Rhizobium*) *meliloti* (root nodules of leguminous plants);
2. Two to four among isolates of *Burkholderia cepacia*.
3. Some *Agrobacterium* species contain has two chromosomes, one linear and one circular.

- Thus, the idea that prokaryotes contain only one circular chromosome has been abandoned.

Drlica and Bendich,2004;..

Bacterial chromosome

Variable form & numbers

Multiple chromosomes

Bacteria	Chromosome Organization
Agrobacterium tumefaciens	One linear and one circular
Bacillus subtilis	Single and circular
Bacillus subtilis	Single and linear
Borrelia burgdorferi	Two circular
Brucella abortus	Two circular
Brucella melitensis	Two circular
Brucella ovis	Two circular
Brucella suis biovar 1	Two circular
Brucella suis biovar 2	Two circular
Brucella suis biovar 4	Two circular
Escherichia coli	Single and circular
Paracoccus denitrificans	Three circular
Pseudomonas aeruginosa	Single and circular
Rhodobacter sphaeroides	Two circular
Streptomyces griseus	Linear
Vibrio cholerae	Two circular
Vibrio fluvialis	Two circular
Vibrio parahaemolyticus	Two circular
Vibrio vulnificus	Two circular

MicrobeWiki,2012

Bacterial chromosome

Variable form & numbers

Multiple chromosomes/plasmids

Some examples of bacterial genome organization

Bacteria	Chromosome(s)	Plasmid(s)
Agrobacterium tumefaciens	one linear (2.1 Mb) + one circular (3.0 Mb)	two circular (450 + 200 Kb)
Bacillus subtilis	one circular (4.2 Mb)	
Bacillus thuringiensis	one circular (5.7 Mb)	six (each >50 Kb)
Borrella	one linear (0.91 Mb)	multiple circular + linear (5-200 Kb)
Bradyrhizobium japonicum	one circular (8.7 Mb)	
Brucella melitensis	two circular (2.1 + 1.2 Mb)	
Brucella suis biovars 1, 2, 4	two circular (1.0 + 2.0 Mb)	
Brucella suis biovar 3	one circular (3.1 Mb)	
Buchnera sp. strain APS	one circular (640 Kb)	two circular (< 7.8 Kb each)
Deinococcus radiodurans	two circular (2.6 + 0.4 Mb)	two circular (177 + 45 Kb)
Escherichia coli K-12	one circular (4.6 Mb)	
Leptospira interrogans	two circular (4.7 + 0.35 Mb)	
Paracoccus denitrificans	three circular (2.0 + 1.1 + 0.64 Mb)	
Pseudomonas aeruginosa	single circular (6.3 Mb)	
Rhizobacterium meliloti	two circular (3.4 + 1.7 Mb	one circular megaplasmid (1,400 Kb)
Rhodobacter sphaeroides	two circular (3.0 + 0.3 Mb)	
Ureaplasma urealyticum	one circular (0.75 Mb)	
Vibrio cholerae	two circular (2.9 + 1.1 Mb)	
Vibrio parahaemolyticus	two circular (3.2 + 1.9 Mb)	
Xylella fastidiosa	one circular (2.7 Mb)	two circular (51 + 1.3 Kb)

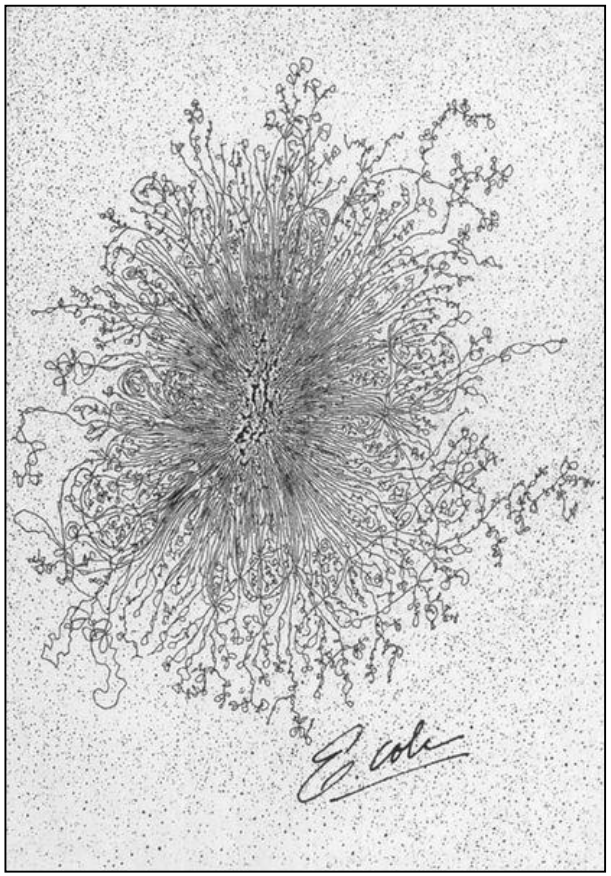
Microbial genetics,2002

Bacterial chromosome

Multiple chromosomes

Microscopic evidence

- In 1963, the structure of chromosomal DNA was shown by autoradiography, electron microscopy and moving pictures of DNA using fluorescence microscopy.
- The technique used was autoradiography where the chromosome for *E. coli* was labeled using tritium labeled thymidine, a radioactive isotope of hydrogen.
- However, the sizes of the chromosomes were variable and there was a low frequency of circular forms detected.



MicrobeWiki,2012

Bacterial chromosome

Multiple chromosomes

Pulse-field get electrophoresis (PFGE)

- The researchers were able to provide a complete physical map of the *R. sphaeroides* genome by obtaining chromosomal DNA fragments through restriction digest with *AseI*, *SpeI*, *DraI*, and *SnaBI* from the genomic DNA, which aided in proving the existence of multiple chromosomes.
- The pulse-field get electrophoresis (PFGE) is used to separate the DNA molecules by applying an electric field.
- Unlike the standard gel electrophoresis, the voltage is constantly switched in three different directions.

MicrobeWiki,2012

Bacterial chromosome

Multiple chromosomes

Pulse-field get electrophoresis (PFGE)

- The assumption here is that:
1. circular DNA is immobile while
2. most of the DNA is mobilized by breakage.
- The advantage of the PFGE is that it can separate DNAs from a few kilobases (kb) to over 10 megabase (Mb) pairs.
- The DNA fragments obtained from using endonucleases such as *SnaBI* produce a distinct pattern of bands useful for physical mapping of the chromosomes.

MicrobeWiki,2012

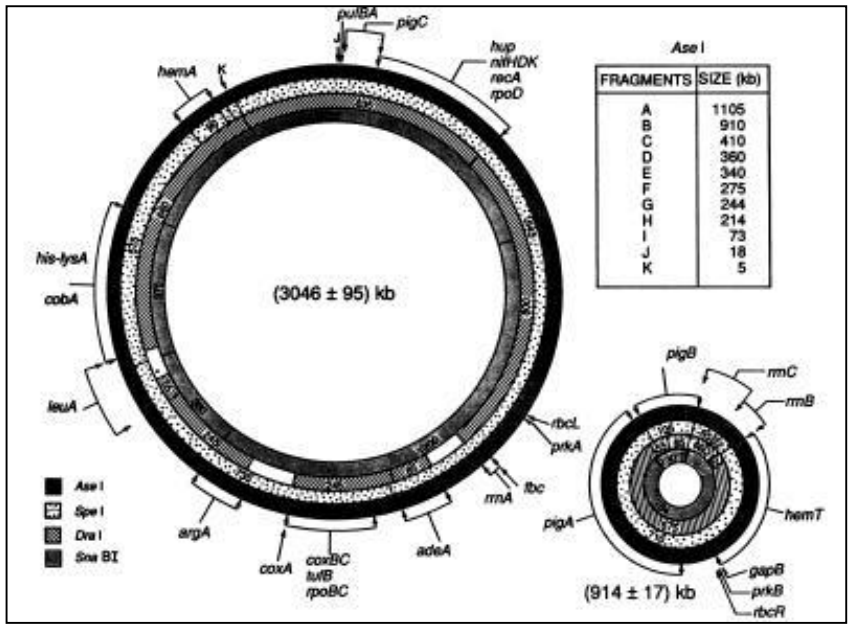
Bacterial chromosome

Multiple chromosomes

Pulse-field get electrophoresis (PFGE)

- Pulsed-field gel mapping indicated *Rhodobacter sphaeroides*, a facultative photosynthetic bacterium possess two circular chromosomes:

1. large (3 Mb CI), and
2. small (0.9 Mb CII) chromosomes.



PFGE: relatively similar to conventional gel electrophoresis except instead of constantly running the voltage in one direction, the voltage is periodically switched among three directions.

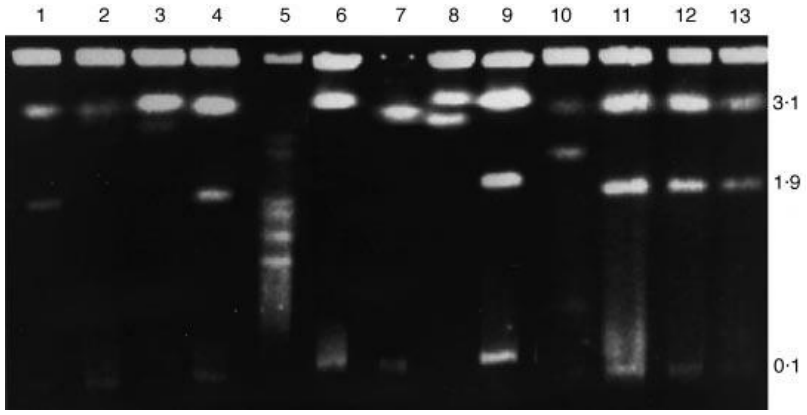
Drlica and Bendich,2004;..

Bacterial chromosome

Multiple chromosomes

Pulse-field get electrophoresis (PFGE)

- Pulsed field gel electrophoresis to show large replicons i.e. two, three or four large (>500 kbp) replicons.
- Lanes: 1, 4, 9-13, typical results for clinical strains of *Burkholderia cepacia*;
- 2 and 3, environmental strains;
- 5, *Hansenula wingei* size markers;
- 6, *Stenotrophomonas maltophilia*
- 7, *Pseudomonas aeruginosa*
- 8, *Ralstonia eutropha*.



Size of principal replicons shown in Mbp.

Wigley and Burton,2000

Bacterial chromosome

Multiple chromosomes

Reason behind their origins

- Multiple chromosomes may also represent a more ancient and less streamlined genomic organization.
- Multiple chromosomes may persist, not because such an organization confers a biological advantage, but rather because it does not confer a sufficient decrease in fitness to have been selected against and lost from the genome pool.

Drlica and Bendich,2004; Mackenzie *et al.*,1991

Bacterial chromosome

Multiple chromosomes

Reason behind their origins

- Some unknown questions:
- Why many bacteria have multiple chromosomes?
- Why some bacterial genomes are divided into multiple, large replicons and others comprised of only a single DNA molecule?
- A leading hypothesis is that secondary chromosomes evolved from plasmids and now serve as accessory genomes.
- In bacterial genomes composed of more than one chromosome:

1. One replicon (primary chromosomes) is typically larger, harbors more essential genes than
2. The others (secondary chromosomes).

Cooper *et al.*,2010

Bacterial chromosome

Multiple chromosomes

Advantage and disadvantages

- One advantage of a divided genome is the potential for faster replication and growth because of multiple origins of DNA replication. For example, *Vibrio* spp. with two chromosomes have among the fastest rates of cell division measured.
- On the other hand, genes on secondary chromosomes exhibited significantly weaker codon usage bias than those on primary chromosomes.
- In bacteria with multiple chromosomes, delayed replication of the smaller replicon could produce a similar effect on its expression and thus its evolution.

Cooper *et al.*,2010

Bacterial chromosome

Multiple chromosomes

Advantage and disadvantages

- The possession of two chromosomes surely increases for genetic lesions and decreased fitness of daughter cells.
- This is particularly noticeable in *Rhodobacter sphaeroides* (isolated from deep lakes and stagnate waters), which has both the large (3 Mb CI) and the small (0.9 Mb CII) chromosomes.
- Partial loss of CII could lead to:

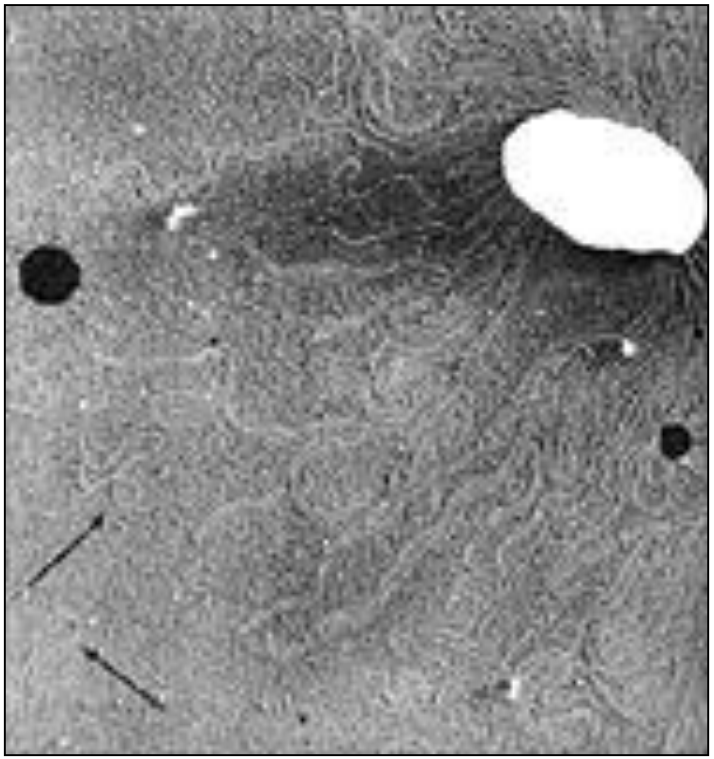
1. auxotrophy(requiring a specific growth substance);
2. inability to carry out translation or genome replication;
3. also increase in the complexity of coordinated gene expression.

Drlica and Bendich,2004; Mackenzie *et al.*,1991

Bacterial chromosome

Release of chromosomal (thread form) and circular plasmid (arrows)DNA

- When a bacterium such as *E. coli* is "gently lysed" the chromosomal DNA leaks out of the cell as a continuous molecule that is many times longer than the length of the cell.
- In KOH test, DNA of Gram-negative bacteria can be easily lyzed by KOH and visualized in the form of a thread.
- Gram-positive bacteria do not lyzed by KOH(resistant to KOH).

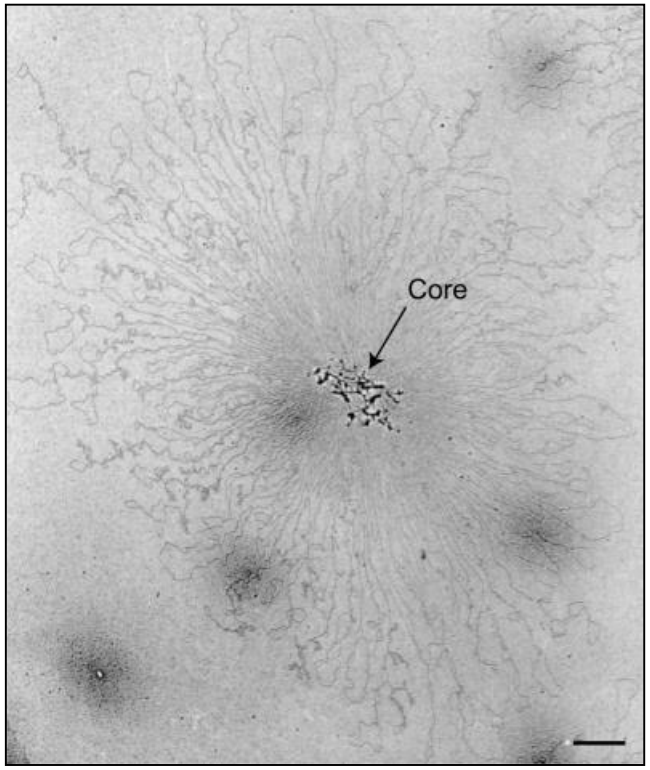


Ownley and Trigiano,2011

The bacterial chromosome

Bacterial chromosomes are generally 200-1000 times longer than the cells in which they reside

- Cell size up to 5μm.
- Chromosome up to 1 mm (1000μm) in length.
- The ch. length 200 times more than the cell length (1000/5=200).
- DNA occupies 10% of cell volume.
- The central core, from which several tens of loops (domains of supercoiling) radiate out from this core, is sensitive to RNAse.

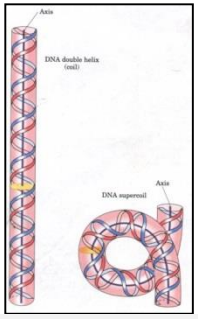


Electron micrograph of isolated membrane-free chromosomes from *E. coli*. Reprinted from Kavenoff and Bowen,1976.

Toro and Shapiro,2010

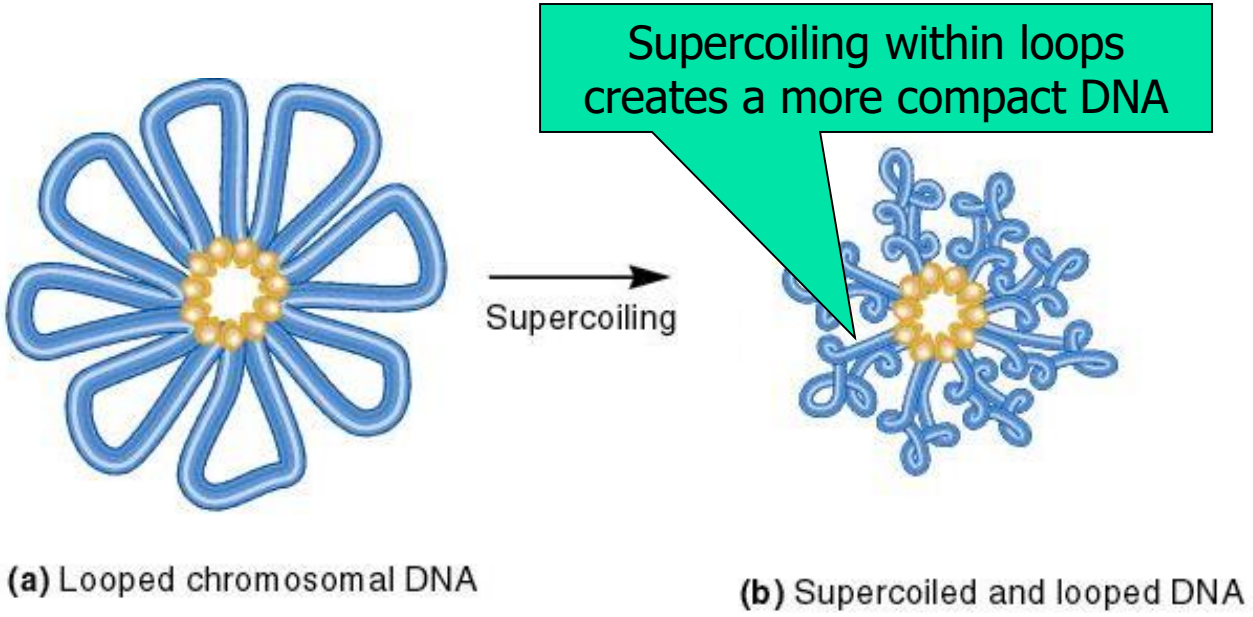
Bacterial chromosome

2. DNA supercoiling



Supercoiling is the twisting of the DNA axis upon itself.

- DNA supercoiling is a second important way to compact the bacterial chromosome. These domains supercoil independently.
- Supercoiling and other interactions causes further compaction, such that it fills an area of about 1 μm.

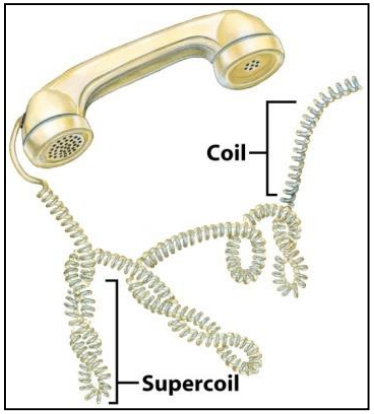


See also animation images in the bacterial genetics file.

DNA supercoil

What is supercoiling?

- Supercoiling induced by separating the strands of a helical structure.
- Twist two linear strands of rubber band into a right-handed double helix as shown. Fix the left end by having a friend hold onto it.
- If the two strands are pulled apart at the right end, the resulting strain will produce supercoiling as shown.



DNA supercoil

What is supercoiling?

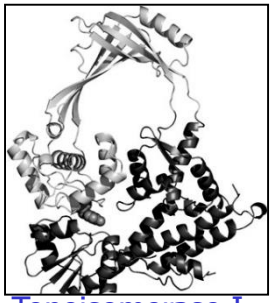
- **The helix of normal DNA is right-handed.**
- A right-handed coil is assigned a negative number (negative supercoiling), and
- A left-handed coil is assigned a positive number (positive supercoiling).

1. An excess of duplex turns would give rise to positive supercoiling (supertwist, overwinding).
2. The loosely coiling is referred to as negative supercoiling (underwinding or unwinding).

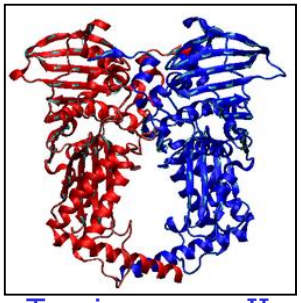
- Right handed supercoiling= negative supercoiling (underwinding)
- Left handed supercoiling= positive supercoiling (overwinding)
- Relaxed state is with no bends.

Bacterial topoisomerases

Classes of topoisomerases



Topoisomerase I



Topoisomerase II

Enzyme	Type	Subunit size (kDa)	Remarks
Topoisomerase I	IA	97 Monomer	Break single-strand DNA
Topoisomerase II (DNA gyrase)	IIA	97 and 90 tetramer structure	Break both strands of the DNA
Topoisomerase III[a]	IA	73 Monomer	Potent decatenating (separating the DNA strands) activity
Topoisomerase IV[a]	IIA	84 and 70 tetramer structure	Can relax, but not supercoil, DNA, potent decatenase (ATP-dependent)

[a]Note that topoisomerases III and IV do not represent 'types' of topoisomerase mechanism (cf. type I and type II). **DNA gyrase is not needed during eukaryotic DNA replication.**

global.oup.com;..

All DNA topoisomerases

Bacteria generally contain only 4 topoisomerases and lack the type IB enzymes

All cells (bacterial to human cells) contains DNA topoisomerases.
The types of topoisomerases are different in different cells.
Bacteria lack the type IB enzymes.

Enzyme	Type	Source	Subunit size (kDa) and composition	Remarks
Bacterial topoisomerase I (ω protein)	IA	Bacteria (e.g. *E. coli*)	97 Monomer	Cannot relax positive supercoils
Eukaryotic topoisomerase I	IB	Eukaryotes (e.g. human)	91 Monomer	Can relax both positive and negative supercoils
Vaccinia virus topoisomerase I	IB	Vaccinia virus	37 Monomer	ATP stimulates topoisomerase activity
Topoisomerase III[a]	IA	Bacteria (e.g. *E. coli*)	73 Monomer	Potent decatenating activity
Reverse gyrase	IA	Thermophilic Archaea (e.g. *Sulfolobus acidocaldarius*)	143 Monomer	Can introduce positive supercoils into DNA (ATP-dependent)
DNA gyrase	IIA	Bacteria (e.g. *E. coli*)	97 and 90 A_2B_2	Can introduce negative supercoils into DNA (ATP-dependent)
T4 topoisomerase	IIA	Bacteriophage T4	58, 51, and 18 2 copies of each subunit	Can relax, but not supercoil, DNA (ATP-dependent)
Eukaryotic topoisomerase II	IIA	Eukaryotes (e.g. human topoisomerase IIα)	174 Homodimer	Can relax, but not supercoil, DNA (ATP-dependent)
Topoisomerase IV[a]	IIA	Bacteria (e.g. *E. coli*)	84 and 70 C_2E_2	Can relax, but not supercoil, DNA, potent decatenase (ATP-dependent)
Topoisomerase VI	IIB	Archaea (e.g. *Sulfolobus shibatae*)	45 and 60 A_2B_2	Can relax, but not supercoil, DNA (ATP-dependent)

DNA supercoil

Functions of topoisomerase I and II

- The control of supercoiling in bacteria is accomplished by two main enzymes:

1. DNA topoisomerase I

- Break single-strand DNA. Removes supercoils from one strand.

2. DNA topoisomerase II (also termed DNA gyrase)

- Break both strands of the DNA. Removes supercoils two at a time.
- The competing action of these two enzymes governs the overall supercoiling of bacterial DNA.

Transfer of conjugative plasmids requires relaxases, proteins that cleave one plasmid strand sequence specifically. Mob relaxases nick at origin of transfer (oriT) to initiate the process of DNA mobilization.

DNA replication and transcription
Negative supercoiling

- Negative supercoiling makes it easier to denature base pairs.
- Negative supercoils favour:
- Local unwinding (underwound) of the DNA, allowing processes such as:

1. DNA replication;
2. Transcription;
3. Recombination.

DNA replication and transcription
DNA topoisomerases and helicases

- The supercoiled DNA must be uncoiled and relaxed in order for:

1. DNA polymerase to bind for DNA replication, and
2. RNA polymerase to bind for transcription of the DNA.

- During transcription, RNA polymerase makes a copy of a gene from the DNA to mRNA as needed.
- This process is similar in eukaryotes and prokaryotes.

DNA replication

DNA topoisomerases and helicases

- Antiparallel DNA strands have 2 different modes of replication:

1. Continuous replication (leading strand);
2. Discontinuous replication in fragments (lagging strand).

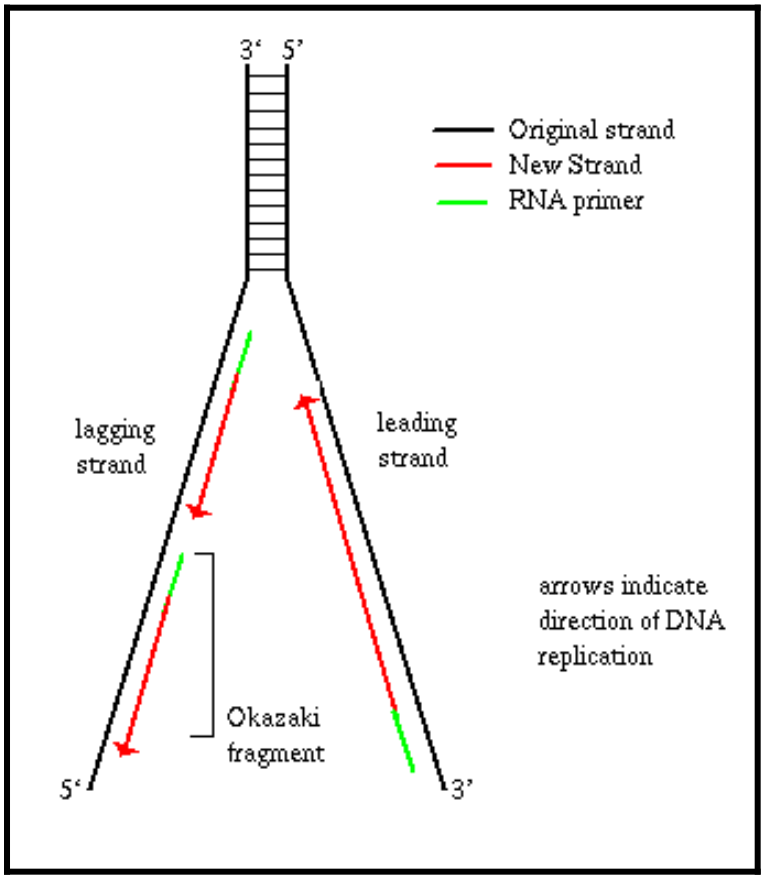
- Replication fork (unzipping area)

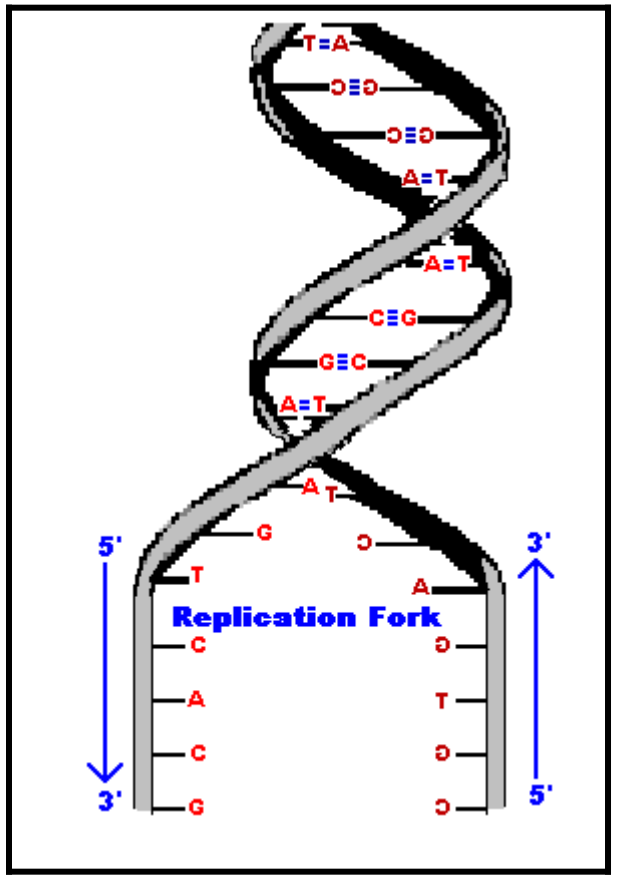
1. Leading strand (template 3' --> 5') replication continuously;
2. Lagging strand (template 5' --> 3') replication in fragments.

- DNA polymerase use a strand of DNA as a template upon which to build the new (polymerized) strand.

DNA replication

DNA topoisomerases and helicases





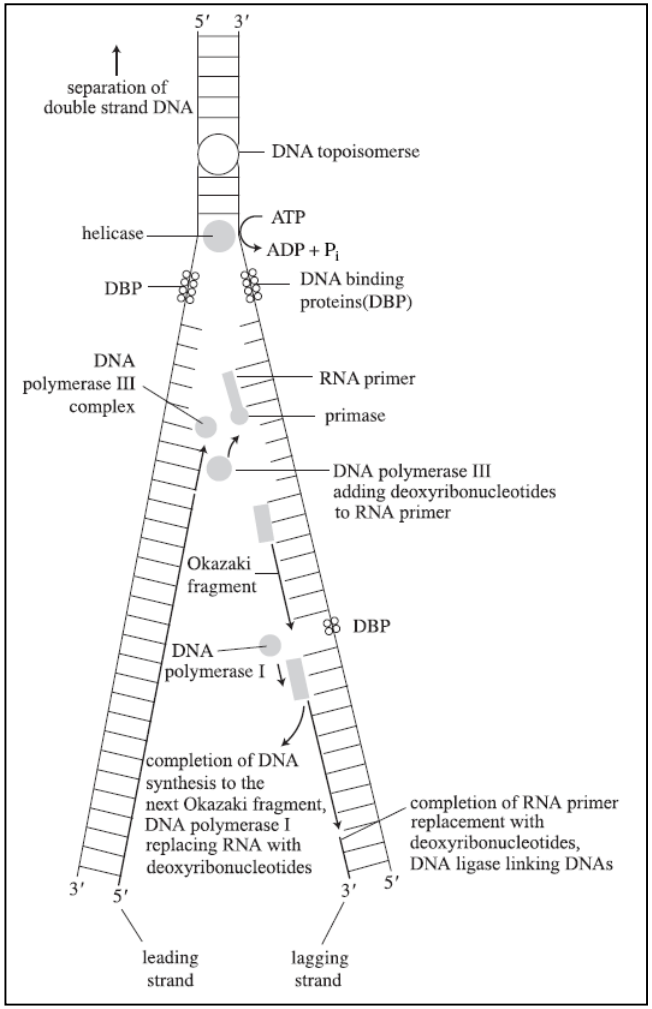
As the two DNA strands separate (unzip) and the bases are exposed, the enzyme DNA polymerase moves into position at the point where synthesis will begin.

DNA replication

DNA topoisomerases and helicases

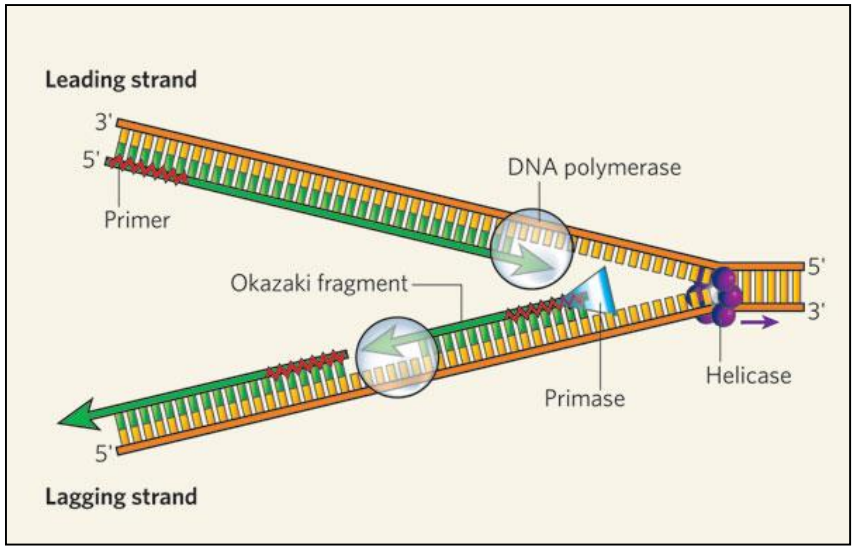
1. Topoisomerases relaxes the supercoiled DNA.
2. Helicases separates the double strand to single strand.

- If the supercoiling is not relieved, it will physically prevent the movement of helicase.
- DNA topoisomerase converts supercoiled DNA to a relaxed form before the helicase separates the double strand to single strands, thereby breaking the hydrogen bonds to start replication.



DNA is dynamic and has high energy
But not stiff or static as first thought
Replication

- An important property of DNA is that it can replicate, or make copies of itself.
- Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases.
- This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.

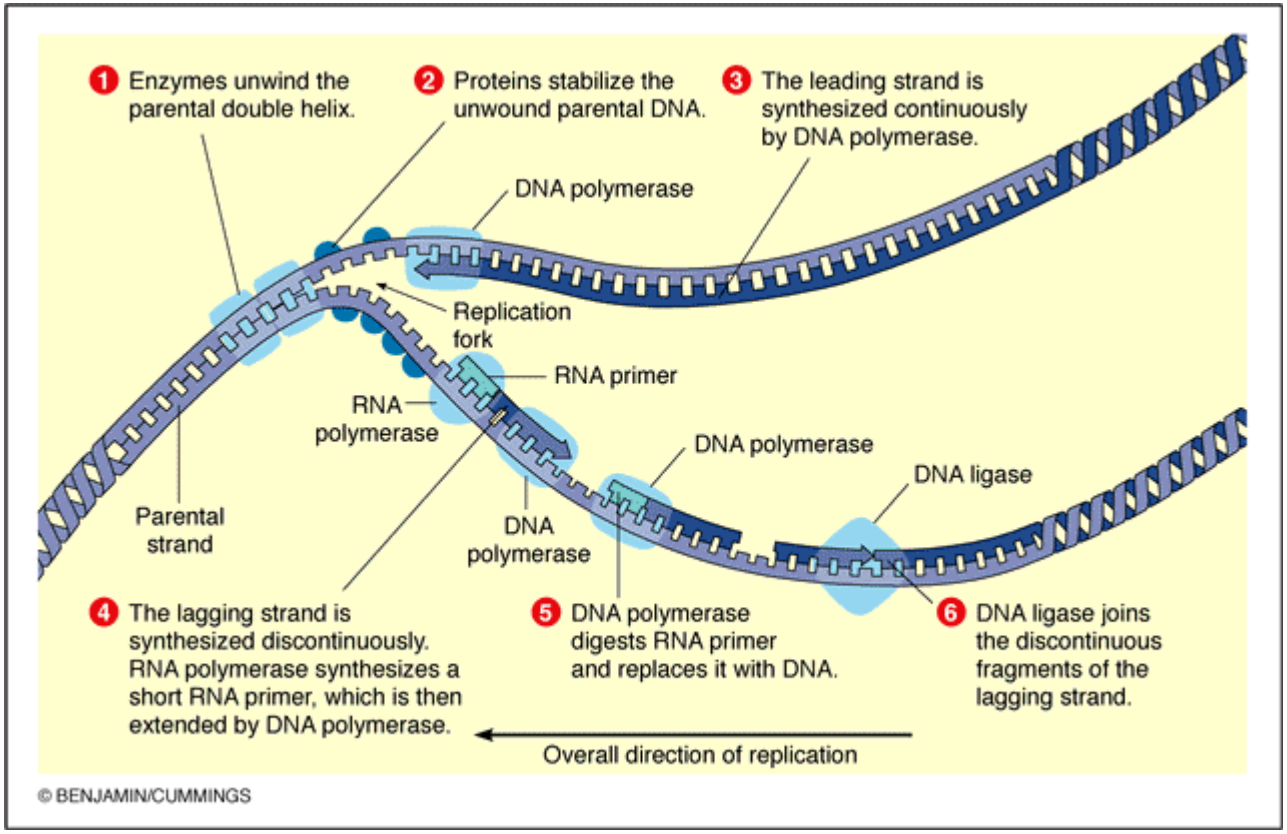


Okazaki fragments are short, newly synthesized DNA fragments that are formed on the lagging template strand during DNA replication. Primase adds RNA primers onto the lagging strand, which allows synthesis of Okazaki fragments from 5' to 3‘.

DNA replication

DNA topoisomerases and helicases

DNA and RNA polymerases



RNA polymerase synthesizes a short RNA primer (a short strand of RNA) that initiates DNA replication. DNA ligases are essential for the joining of Okazaki fragments during replication. Reiji Okazaki, the Japanese molecular biologist who discovered the role of Okazaki fragments.

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation(transcription+translation)

1. Each cell expresses, or turns on, only a fraction of its genes.
2. The rest of the genes are repressed, or turned off.

- Gene regulation (transcription + translation) is an important part of normal development.
- Genes are turned on and off in different patterns during development to make a brain cell act different from a liver cell or a muscle cell.

All these cells contain the same DNA, but their genes are expressed differently (turned "on" or "off"), which creates the different cell types.

Genetics Home Reference,2015;..

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- In eukaryotic cells such as human we start out as one cell (a single fertilized egg), all our cells have the same DNA.
- That first cell divided over and over again until a new baby was made. In the end, we all are made up of trillions of cells (37.2 trillions) with nearly identical genes.
- But ca. 300 different cell types are not same in function.
- The cells in our heart, for instance, work very differently from the cells in our eyes. The same is true for our skin and our liver cells.

Human cells have little colour and so resolution and contrast would be low without the use of stains. Methylene blue stains the cell and its organelles differentially so that the cell is more clearly seen and some organelles stand out more than others, e.g. the nucleus.

Wydeven,2011; jacusers.johnabbott.qc.ca,2014;..

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- These cells are different because they use the same set of genes differently.
- So even though each of our cells has the same 20,000 or so genes, each cell can select:

1. which ones it wants to "turn on", and
2. which ones it wants to keep "turned off".

- Genes tell each cell in our body what to do and when to do it.
- For example, genes have the instructions for making liver cells, carrying oxygen in our blood, or helping us break down sugar.
- How to they do all of this? By making proteins.

Transcription factors(TxF's)

How do transcription factors work?

Genes turned on and off

- Transcription factors are proteins that bind to DNA near the start of transcription of a gene.
- General transcription factors (GTFs), also known as basal transcriptional factors, are a class of protein transcription factors that bind to specific sites (promoter) on DNA to activate transcription of genetic information from DNA to messenger RNA.

In bacteria, transcription initiation requires:
1. an RNA polymerase, and
2. a single general transcription factor(sigma factor).

Wikipedia,2016

Transcription factors(TxF's)

How do transcription factors work?

Genes turned on and off

- 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.

- In prokaryotes: activator and repressor proteins act on operators (DNA sequences just downstream of the promoter).
- In eukaryotes: activator proteins act on enhancer DNA sequences; repressor proteins act on silencer DNA sequences.

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.

Approximately 2000 human transcription factors easily accounts for the unique regulation of each gene in the human genome during development. Therefore, approximately 10% of genes in the genome code for transcription factors.

Transcription factors (TxF's)

How do transcription factors work?

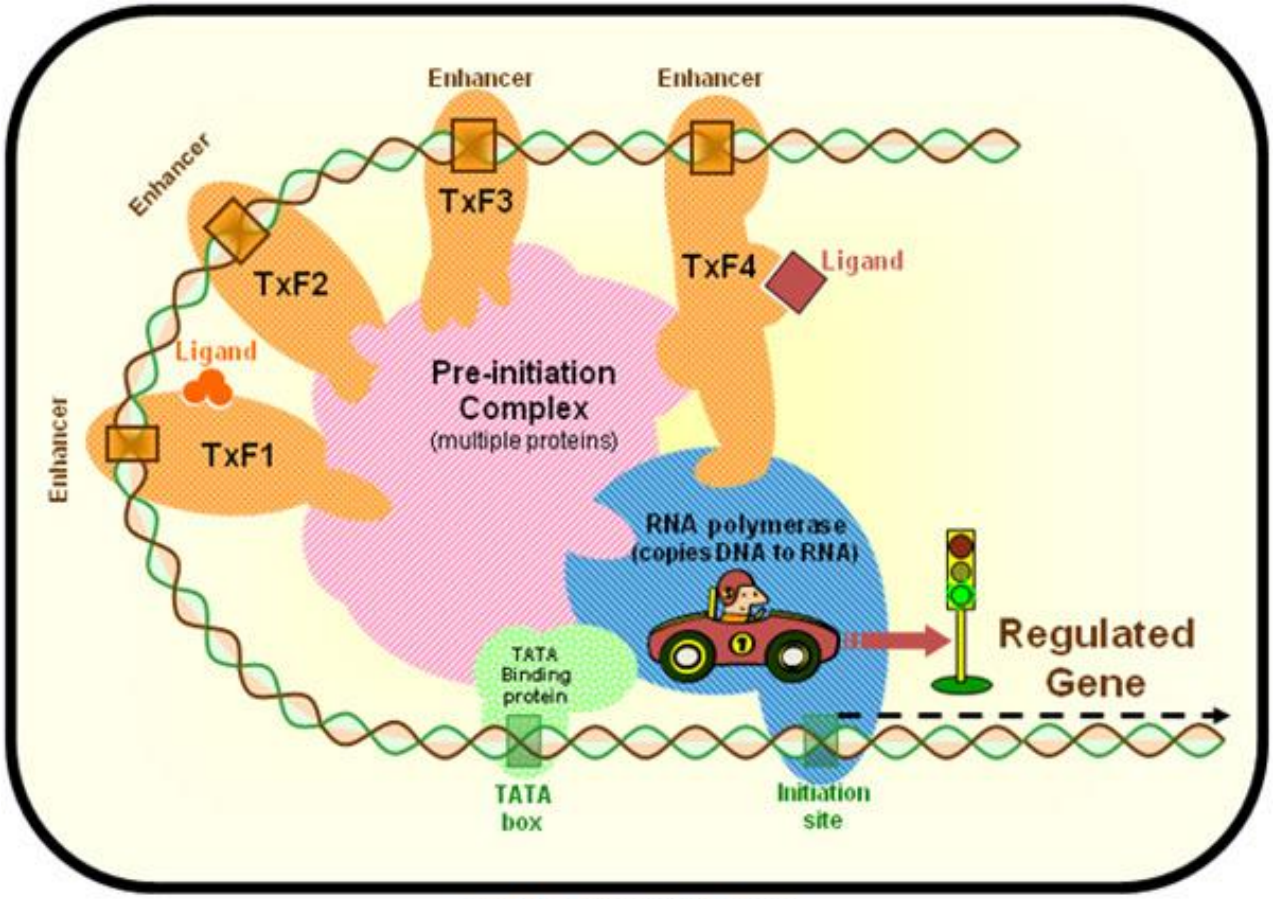


Figure 2 - Transcription Factors

Monsanto Co.,2010

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- But not every protein can do every job.
- Proteins are specialized to do certain things like carry oxygen in the blood or recognize bacteria in our bodies.
- And because of this, some proteins are only needed during certain times or in certain areas of the body.
- For example, proteins that build bone don't need to be in our hair!
- So how do the proteins know which places in the body to be?
- The answer is that genes are able to be turned on and off in different places and at different times.

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- Just like a chef reads a recipe to make a dish, cells read a gene to make a protein.
- Each gene makes a specific protein that does a specific job.

1. Cells need to make thousands of different proteins in the right amounts,
2. at the right time, and
3. in the right place in order to work properly.

- **Promoter (P)** - aids in RNA polymerase binding;
- **Operator (O)** - "on/off" switch - binding site for the repressor protein.

Note: constitutive or housekeeping genes are constitutively expressed (they are always turned ON).

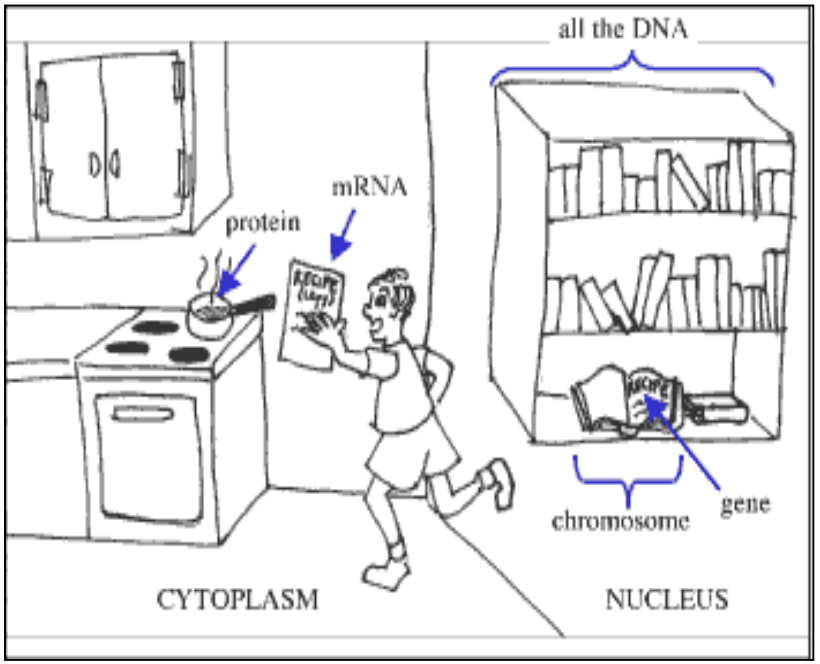
DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- Gene regulation can occur at any point during gene expression, but most commonly occurs at the level of transcription (when the information in a gene's DNA is transferred to mRNA).
- Signals from the environment or from other cells activate proteins called transcription factors.
- These proteins bind to regulatory regions of a gene and increase or decrease the level of transcription.
- By controlling the level of transcription, this process can determine the amount of protein product that is made by a gene at any given time.

Genetics Home Reference,2015;..

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation

- It might help to think about our set of genes as a cookbook and each of our cell types as a different cook.
- So the breakfast cook focuses only on breakfast recipes and the lunch chef only on lunch ones.



Wydeven,2011

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation (transcription+translation)

1. Most genes contain the information needed to make functional molecules called proteins.
2. A few genes produce other molecules that help the cell assemble proteins. e.g. rRNA and tRNA (in synthesis of ribosomes and proteins).

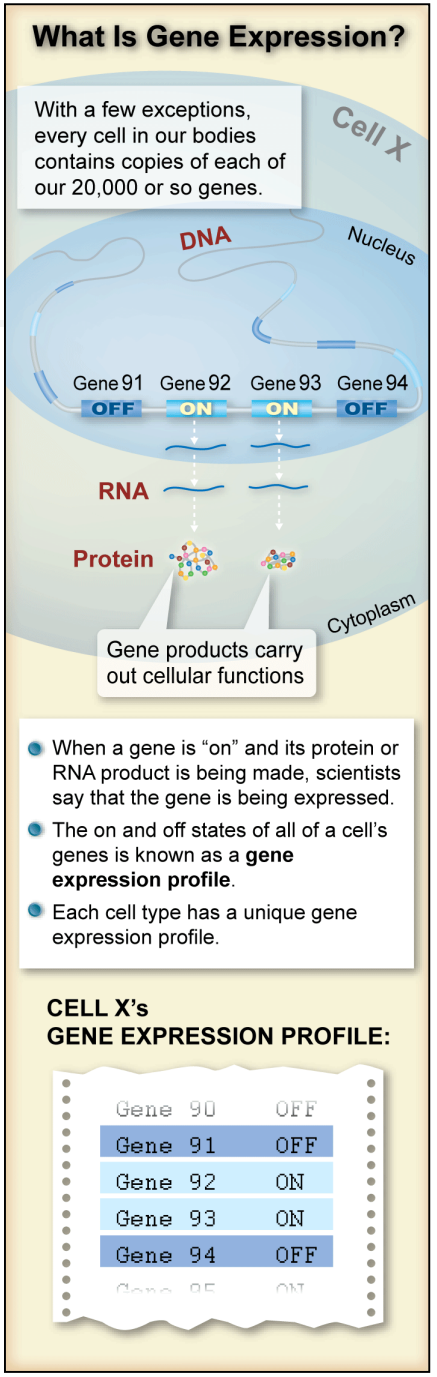
- The journey from gene to protein is complex and tightly controlled within each cell.
- It consists of two major steps:

1. Transcription, and
2. translation.

- Together, transcription and translation are known as gene expression.

Genetics Home Reference,2015;..

DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation (transcription+translation)



DNA is dynamic and has high energy
But not stiff or static as first thought
Gene regulation (transcription+translation)

- So when everything is working OK, each cell type only reads the genes it needs. That way it only makes the proteins it needs too.
- For example, early on our cells need to divide and divide so we can grow from one to trillions (10^{12}) of cells.
- All of our cells have the growth genes churning our proteins to make the cells keep dividing.
- But when we are done growing, we don't want our cells to keep dividing. These genes stop making proteins and cells stop dividing so much.
- If the cells didn't stop growing, this growth would cause cancer.

Wydeven,2011;..

DNA replication and transcription

Gene regulation

What is the epigenome

- Epigenetics is the study of mechanisms that switch genes on or off.
- It refers to external modifications to DNA that turn genes "on" or "off."
- "epi-" means above in Greek. Epigenetics literally means "above" or "on top of" genetics.
- Epigenetic changes alter the physical structure of DNA.
- These modifications do not change the DNA sequence, but instead, they affect how cells "read" genes.
- Chemical modifications to the DNA or proteins, can alter gene expression.

Genetics Home Reference; Live Science;.

DNA replication and transcription

Gene regulation

Epigenetics

- Not all of the genes are turned on at the same time in every cell, and so they must be regulated.
- Reading and controlling the genome to express genes can come from many different sources such as:

1. DNA methylation- a process by which methyl groups are added to DNA. Methylation modifies the function of the DNA, typically acting to suppress gene transcription.
2. Histone modifications (in eukaryotes), and
3. Transcription factors.

DNA replication and transcription

Epigenetic changes

DNA methylation in bacteria

- Little is known concerning how widespread epigenetic control is in the bacterial world and the roles that epigenetic regulatory systems play in bacterial biology, including pathogenesis.
- Epigenetic mechanisms evolved as a form of cellular defense, targeting incoming viral and other foreign DNA sequences for degradation.
- Bacteria also use epigenetic mechanisms to control phase variation.

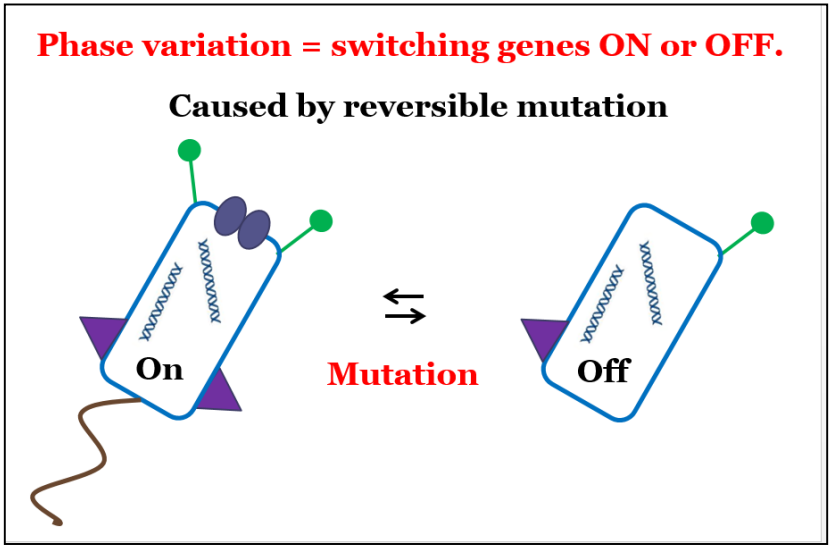
Casadesu´s and Low,2006

DNA replication and transcription

Epigenetic changes

DNA methylation in bacteria

- Phase variation:
- Phase variation is the switching on and off of genes in response to mutation.
- These mutations are reversible, meaning genes can be switched on and off interchangeably.

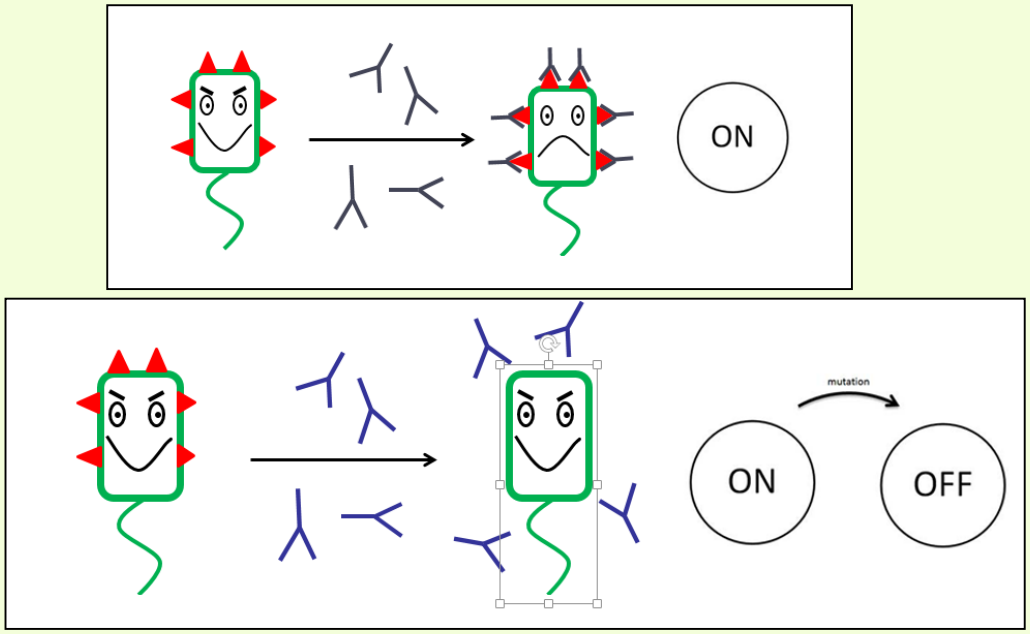


DNA replication and transcription

Epigenetic changes

How can phase variation benefit bacteria?

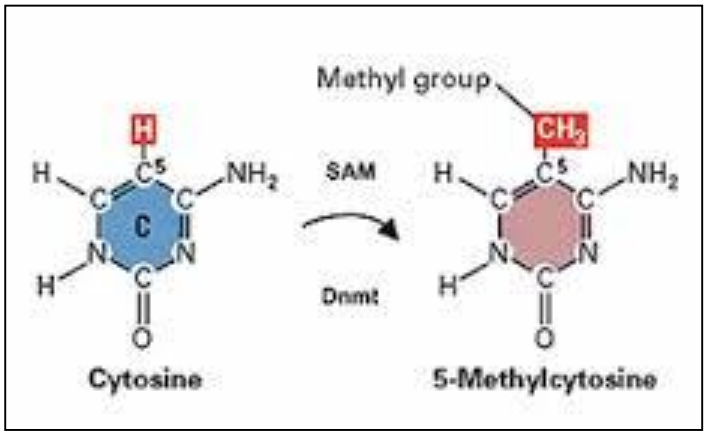
- Phase variation:
- Switching some genes off can help the bacteria to escape the immune system.

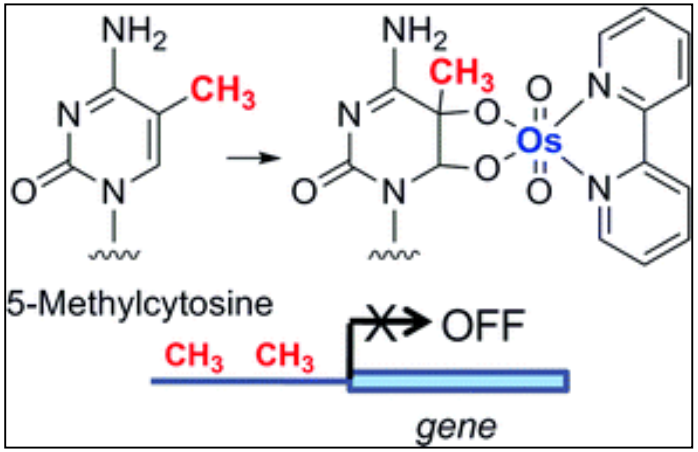


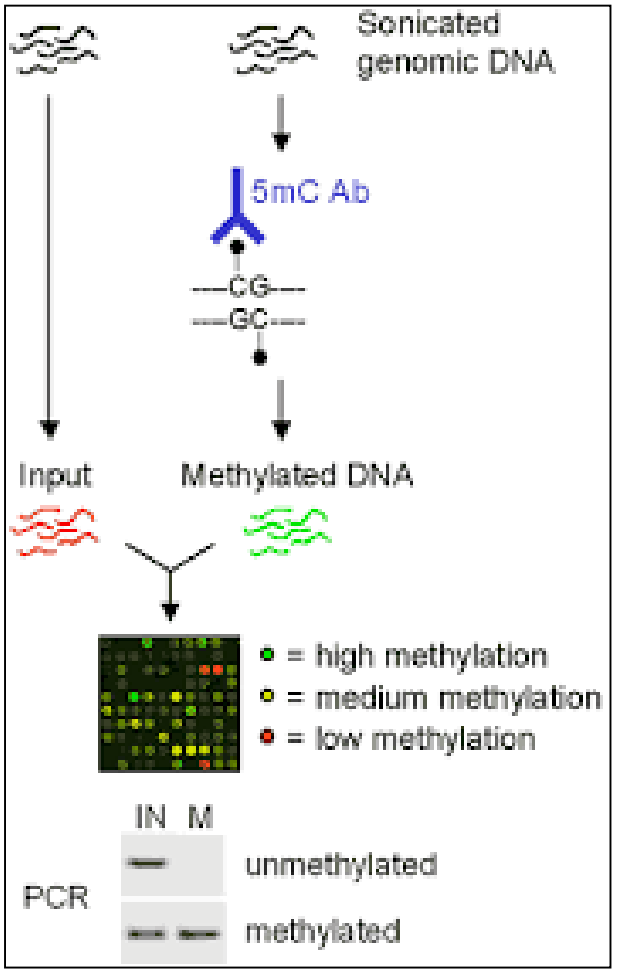
DNA replication and transcription

Gene regulation

DNA methylation in bacteria







DNA replication and transcription

Epigenetic changes

DNA methylation in bacteria

- Epigenetic changes regulate the activity (expression) of all the genes within the genome.
- A common type of epigenomic modification is called methylation.
- DNA methylation is the addition of a methyl group, or a "chemical cap," to part of the DNA molecule, which prevents certain genes from being expressed.
- Methylation involves attaching small molecules called methyl groups, each consisting of one carbon atom and three hydrogen atoms, to segments of DNA.

DNA replication and transcription

Epigenetic changes

DNA methylation in bacteria

- When methyl groups are added to a particular gene, that gene is turned off or silenced, and no protein is produced from that gene.
- Methylation modifies the function of the DNA, typically acting to suppress gene transcription.
- In the bacterial kingdom, the most prevalent base modifications are in the form of DNA methylations, specifically to adenine and cytosine.
- The methylation of native DNA acts as a sort of primitive immune system, allowing the bacteria to protect themselves from infection by bacteriophage.

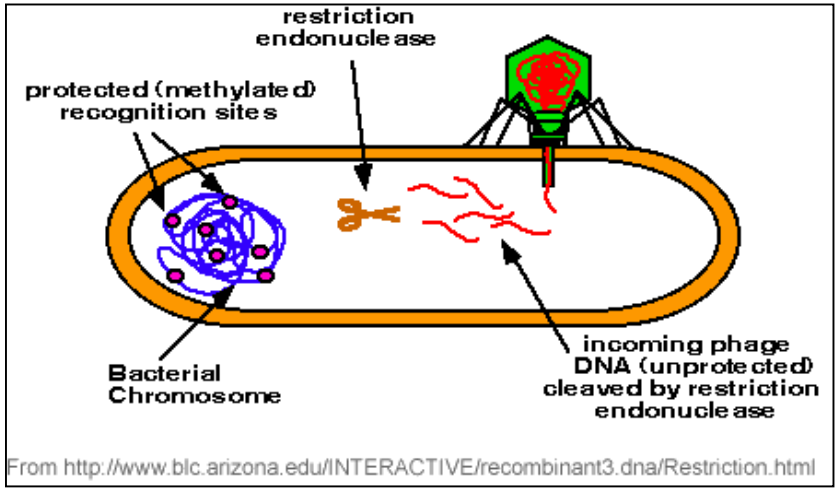
Genetics Home Reference;..

DNA replication and transcription

Epigenetic changes

DNA methylation in bacteria

- When foreign phage DNA enters a bacterium, it is recognized as foreign and these cellular enzymes cut it up, restricting the ability of the phage to infect the bacterium.
- The phage genome is cut at specific sites by the restriction enzyme. e.g. EcoR1.



Genetics Home Reference,...

DNA replication and transcription

Epigenetic changes

Demerits

- Because errors in the epigenetic process, such as modifying the wrong gene or failing to add a compound to a gene, can lead to abnormal gene activity or inactivity, they can cause genetic disorders. Conditions including cancers, metabolic disorders, and degenerative disorders have all been found to be related to epigenetic errors.
- Note: Environmental influences, such as a person's diet and exposure to pollutants, can also impact the epigenome (negative epigenetic modifications).

Genetics Home Reference;..

DNA expression

DNA replication, transcription and Translation

1. Replication: Copying DNA before cell division using DNA polymerase.
2. Transcription: Making an RNA copy (mRNA) of DNA using RNA polymerase.
   Note: Transcription involves copying in the same language (e.g., court transcription).
3. Translation: Making a protein from the mRNA.

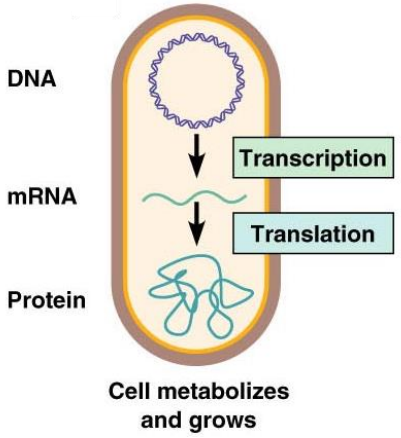
- Note: The nucleic acid language is being translated into the protein language.

DNA ---(replication)---> DNA

DNA ---(transcription)---> RNA ---(translation)---> Protein

Reverse transcription(RT-PCR- convert RNA to cDNA)

RNA ---------> DNA



Bjorkman,2011;..

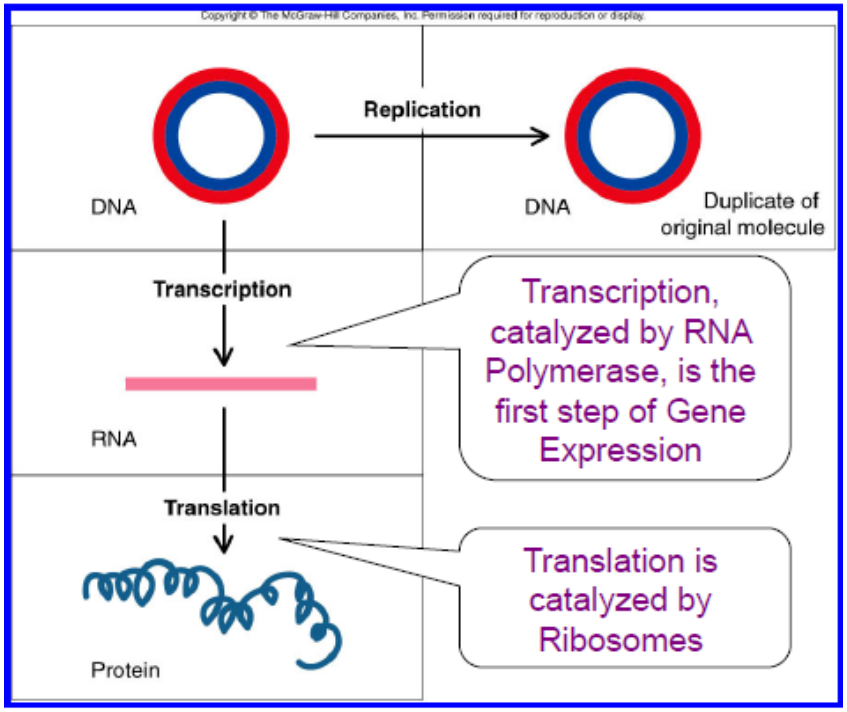
DNA replication, transcription and Translation

Central Dogma

DNA polymerase and RNA polymerase functions

1. **Synthesis of DNA.** Replication is under the control of the enzyme DNA polymerase.
2. **Synthesis of RNA.** Transcription is under the control of the enzyme RNA polymerase.

- Transcription is the name given to the process where the information in a gene in a DNA strand is transferred to an RNA molecule.



Prokaryotic DNA replication occurs inside the cytoplasm, whereas, in eukaryotes it occurs inside the nucleus.

DNA replication, transcription and Translation

Central Dogma

- The production of protein takes place in two steps.
- In the first step, called transcription, the permanent DNA message (1) is copied into a temporary messenger RNA (mRNA), by RNA polymerase (2).
- This mRNA message can be read by a complex cellular "machine" called a ribosome (3).
- In this second step, called translation, the ribosome assembles amino acids in an order specified by the mRNA to create a specific protein (4).

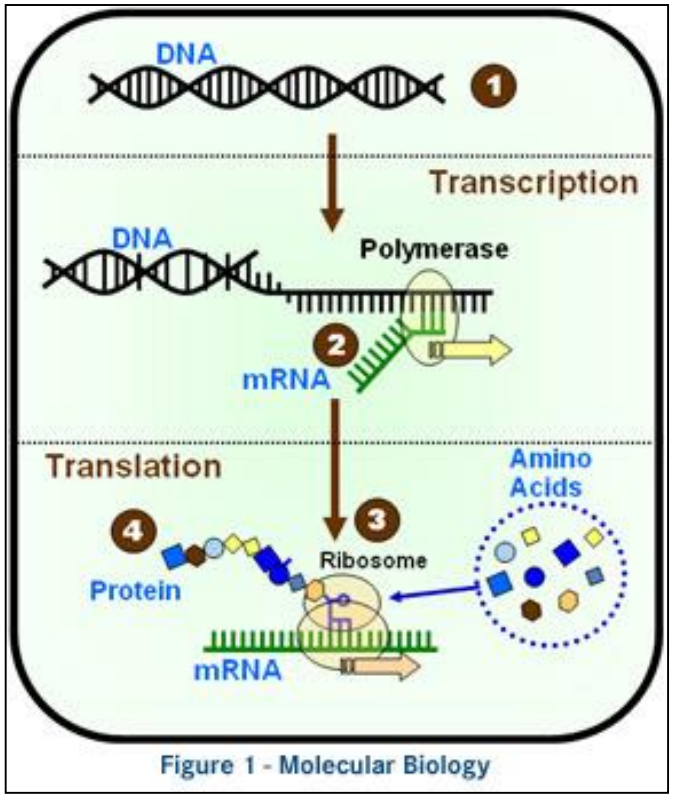
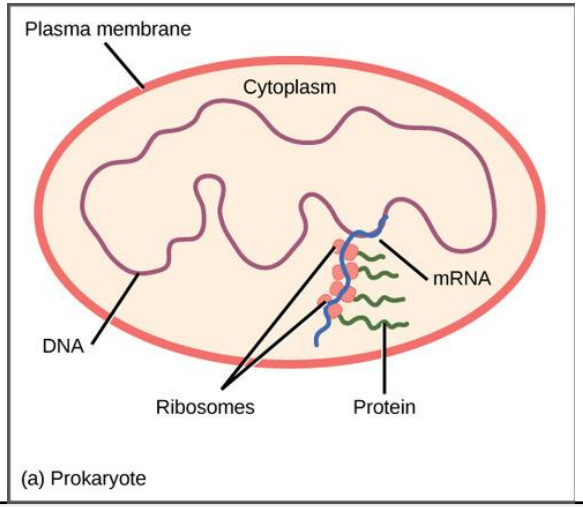


Figure 1 - Molecular Biology

Monsanto Co.,2010

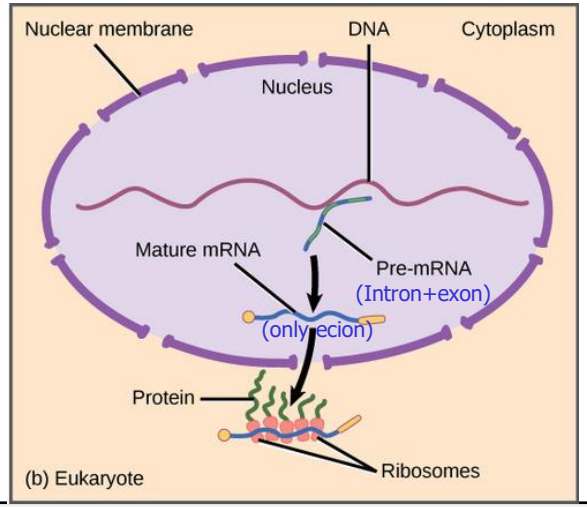
DNA replication, transcription and Translation

Prokaryotic vs. Eukaryotic transcription



In a prokaryotic cell, transcription and translation are coupled; that is, translation begins while the mRNA is still being synthesized. This is because there is no nucleus to separate the processes of transcription and translation. When bacterial genes are transcribed, their transcripts can immediately be translated.

Pearson Education;..

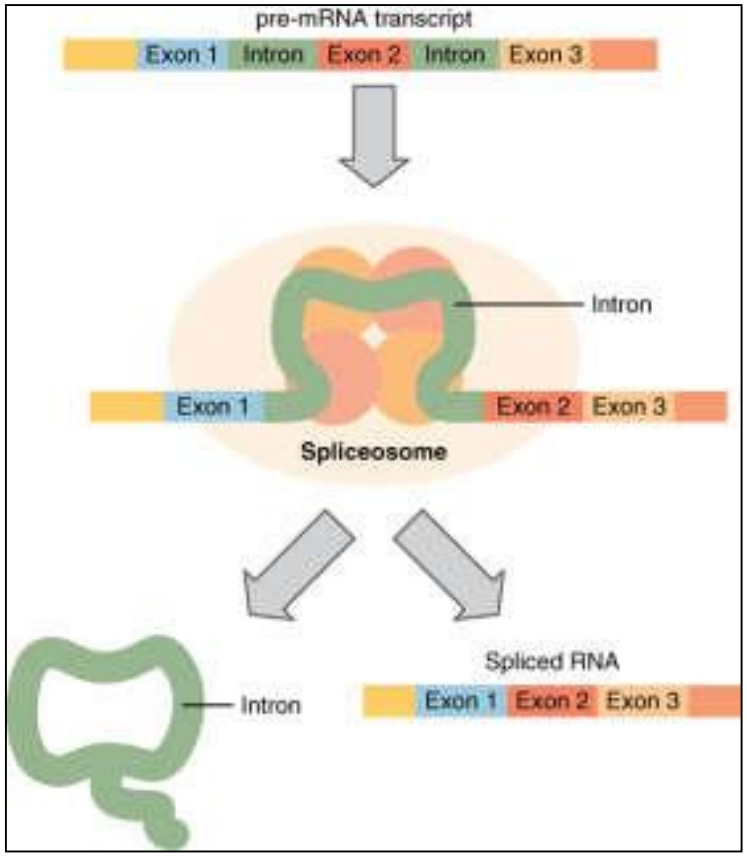


In a eukaryotic cell, transcription occurs in the nucleus, and translation occurs in the cytoplasm. This is because transcription occurs in the nucleus to produce a pre-mRNA molecule.
The pre-mRNA (immediate product of the transcription of one strand) is typically processed to produce the mature mRNA (only exons). The mRNA transcript is subsequently exported through nuclear pores (pores in the nuclear envelope) to the cytoplasm for translation.

DNA replication, transcription and Translation

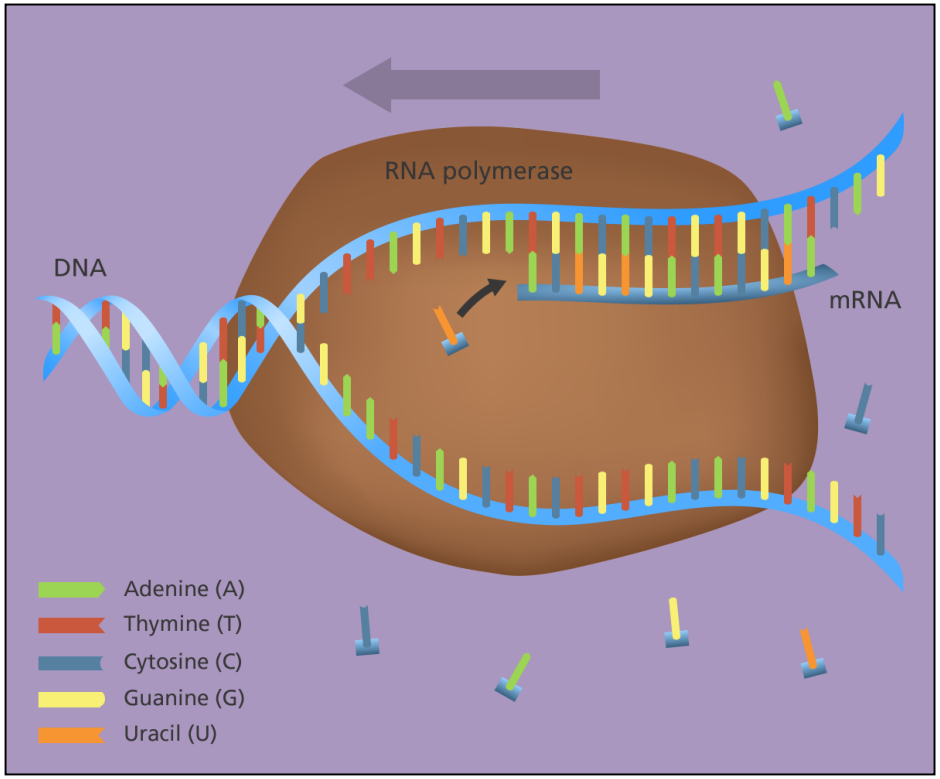
Pre-mRNA vs. mature mRNA

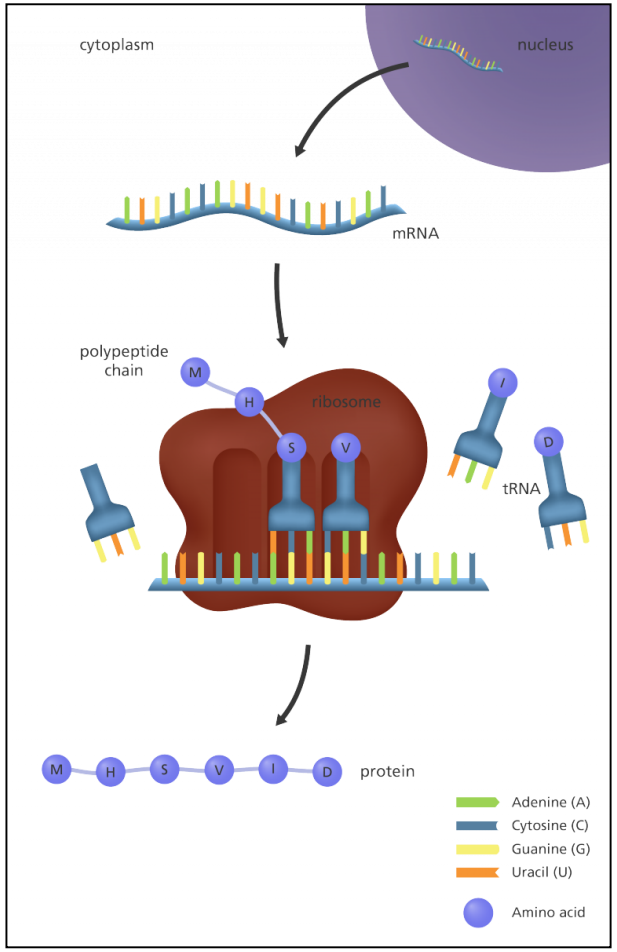
- The first (primary) transcript from a protein coding gene is often called a pre-mRNA and contains both introns and exons.
- Pre-mRNA requires splicing (removal) of introns and put together the rest of the DNA sequences (exons) then the now mature RNA can exit the nucleus and travel in the ribosomes where it will be translated.



Protein synthesis

Transcription and Translation





Facts yourgenome_org.htm

Chromosome replication

Sites of DNA replication

Prokaryotes vs. Eukaryotes DNA replication

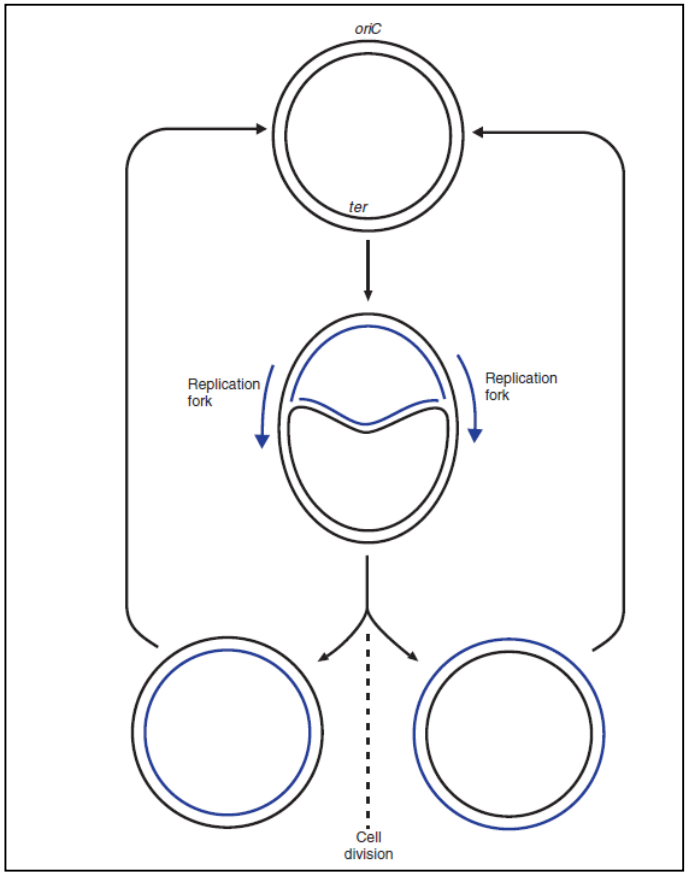
1. Most bacterial chromosomes are circular with one replication origin.
2. Eukaryotic chromosomes each contain one linear DNA molecule and multiple origins of replication.

- Replication of a bacterial chromosome normally starts at a fixed point (the origin of replication, *oriC*) and proceeds in both directions to a termination point (*ter*) that is approximately opposite to the origin, producing two double-stranded molecules.

Dale and Park,2004

Chromosome replication

- Bidirectional replication starts at *oriC* and continues to the termination site *ter*, producing two double-stranded molecules.



Dale and Park,2010

Synthesis of DNA
DNA replication

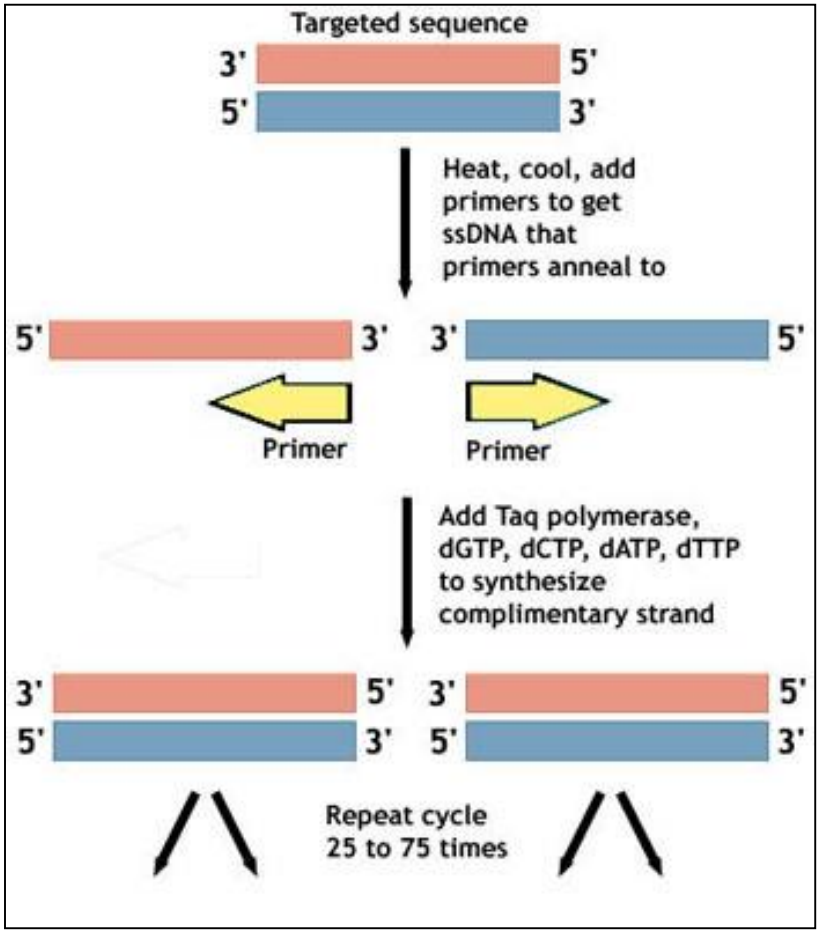
- *Taq* polymerase (isolated from the bacterium *Thermus aquaticus*) often abbreviated to *Taq* Pol or simply *Taq*.
- This is the enzyme that is in charge of replicating DNA. This is the polymerase part of the name polymerase chain reaction (PCR).
- Use of the thermostable *Taq* polymerase enables running the PCR at high temperature (~60°C and above), which facilitates high specificity of the primers and reduces the production of unspecific products, such as primer dimer.

Synthesis of DNA

DNA replication

New pieces of ssDNA are made

- Taq polymerase catalyzes the generation of new pieces of ssDNA that are complimentary to the portions marked by the primers.
- The job of Taq polymerase is to move along the strand of DNA and use it as a template for assembling a new strand that is complimentary to the template.



Transcription

RNA polymerase in different cells

Sigma factor+core RNA polymerase= a holoenzyme

- RNA polymerases are essential to life and are found in all organisms and many viruses.
- Most RNA polymerases are multimeric enzymes and are composed of a variable number of subunits.
- In bacteria:
- The core RNA polymerase complex is:

1. sufficient for transcription elongation and termination, but
2. is unable to initiate transcription.

- Transcription initiation requires a sixth, dissociable subunit called a sigma factor.

EMBL-EBI 2013;..

Transcription

RNA polymerase in different cells

Prokaryotic vs. Eukaryotic transcription

- RNA polymerase (RNAP) is the enzyme responsible for transcription.

1. Bacterial RNA polymerase- single and relatively simple, containing 5 different proteins. All transcription is performed by a single type of RNA polymerase.
2. Archeal RNA polymerase- Archaea have a single type of RNAP, responsible for the synthesis of all RNA. RNA polymerase from Archaea contains 8 proteins.
3. The eukaryotic RNA polymerase- achieved by three different types of RNA polymerase (RNA pol I-III).

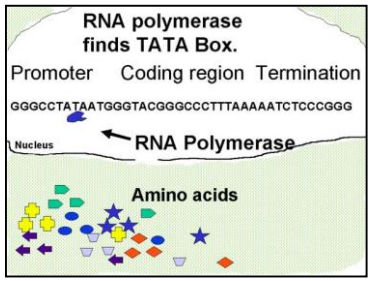
- RNA polymerase II responsible for most mRNA transcription contains 12 proteins and is similar to the RNA polymerase in the archaea.

Ebright,2000;The Microbial World,2013;..

Transcription

RNA polymerase in different cells

Prokaryotic vs. Eukaryotic transcription



	Bacteria	Archaea	Eukarya
RNA polymerase	Single. 5 proteins. A sixth, dissociable subunit called as sigma factor Rifampicin sensitive	Single.8-10 proteins Rifampicin resistant	Three.12 proteins (RNA polymerase II) Rifampicin resistant
Transcription start site	Variable often contains a -35 and -10 region(bases)	TATA box (The core DNA sequence 5'-TATAAA-3')	TATA box (The core DNA sequence 5'-TATAAA-3')
Starting amino acid	formylmethionine	methionine	methionine

- Bacterial RNA polymerase is simple (contains 5 proteins) and a sixth, dissociable subunit called as sigma factor.
- The eukaryotic RNA polymerase (termed RNA polymerase II) contains 12 proteins.
- Archaeal RNA polymerase contains 8-10 proteins.

The Microbial World,2013

Prokaryotic vs. Eukaryotic RNA Polymerase

The core of the bacterial RNA polymerase (RNAP) consists of five subunits

Two alpha, a beta and a beta are conserved from bacteria to mammals

- In eukaryotic cells, there are three different RNA polymerases (RNA Pol).
- Prokaryotic cell has one.
- Each RNA Pol is responsible for a different class of transcription:

1. PolI transcribes rRNA (ribosomal RNA),
2. PolII mRNA (messenger RNA), and
3. PolIII tRNA (transfer RNA) and other small RNAs.

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.

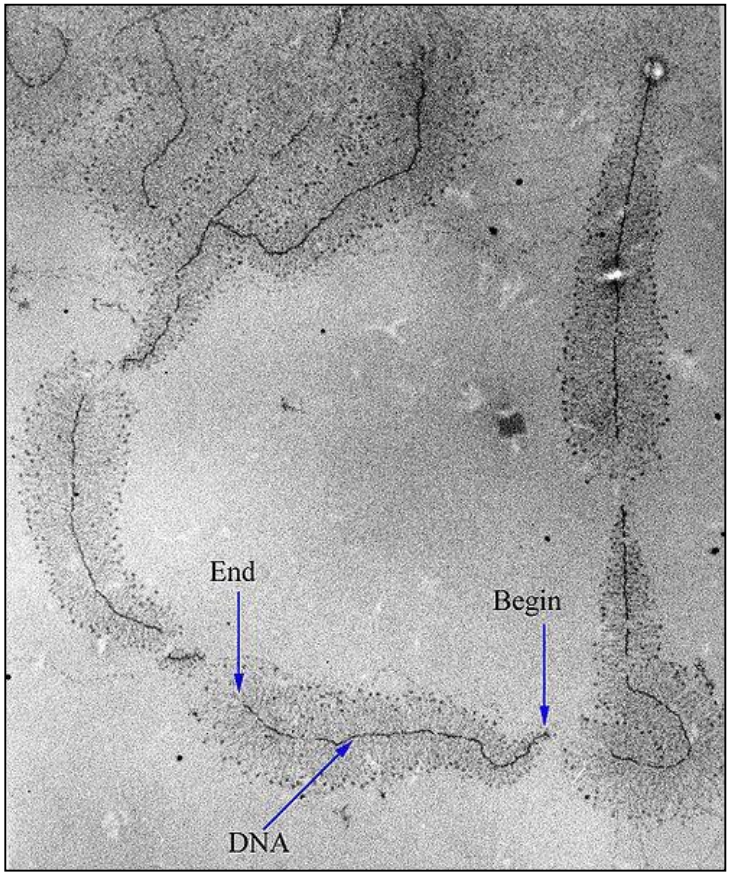
Source: Data adapted from Ebright R.H. 2000 *J. Mol. Biol.* **304:** 687–698, Fig. 1, p. 688. © 2000 Academic Press.

The eukaryotic RNAPs recognize different promoters. RNA pol II can bind to a DNA sequence within the promoter of many genes, known as the TATA box, to initiate transcription.

Eukaryotic DNA transcription

An electron-micrograph of DNA strands decorated by hundreds of RNAP molecules too small to be resolved

- Each RNAP is transcribing an RNA strand, which can be seen branching off from the DNA.
- "Begin" indicates the 3' end of the DNA, where RNAP initiates transcription;
- "End" indicates the 5' end, where the longer RNA molecules are completely transcribed.



Wikipedia

RNA Polymerase
Bacteria vs. Archaea

- Bacteria and Archaea belong to prokaryotic group.
- Bacterial and archaeal RNA polymerases versatile enzymes for they transcribe all types of genes, i.e.
- tRNA,
- rRNA, and
- mRNA genes.
- In terms of promoter elements each of them differs in contents.

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.

Prokaryotic RNA Polymerase

Only a single RNA polymerase

- Bacteria have only a single RNA polymerases.
- This one RNA polymerase (with the help of sigma factor) synthesizes all classes of RNA:

1. mRNA,
2. rRNA,
3. tRNA.

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.

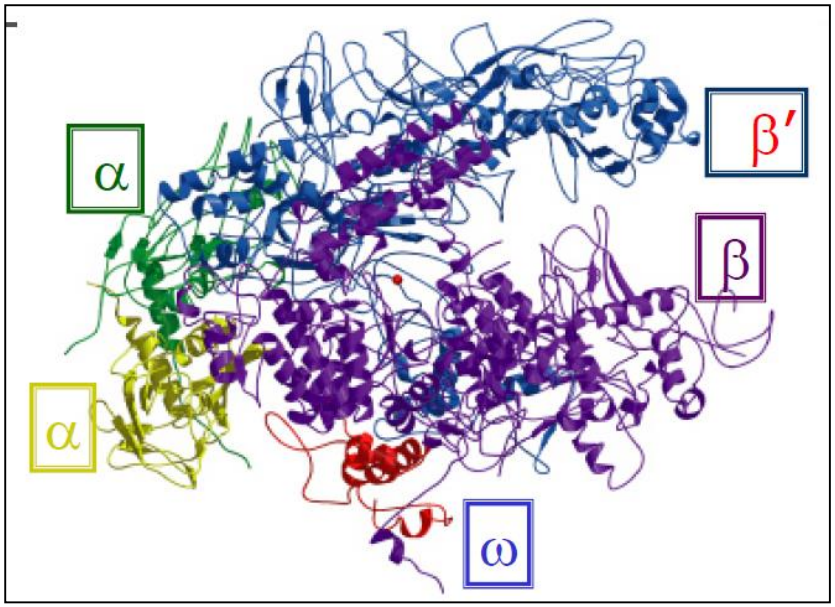
Source: Data adapted from Ebright R.H. 2000 *J. Mol. Biol.* **304:** 687–698, Fig. 1, p. 688. © 2000 Academic Press.

Note: the sigma factors are a class of proteins which are needed for recognizing the core promoter region for transcriptional initiation.

Prokaryotic RNA polymerase

The enzyme without the sigma subunit is called the 'core' enzyme and with sigma subunit is called the holoenzyme

- **The core enzyme has five subunits ($\beta\beta'\alpha_2\omega$):**
- Two alpha, beta, beta', sigma and omega subunits.
- Only the first three subunits (alpha, beta, beta') are required for polymerase activity and RNA synthesis.
- A sigma factor (σ factor) is a protein needed only for initiation of RNA synthesis.
- It is a bacterial transcription initiation factor (equivalent of transcription factors in eukaryotes).



RNA polymerase core enzyme is a multimeric protein α, β, β′, ω.
The β′ subunit is involved in DNA binding. The β subunit contains the polymerase active site.
The two α subunits acts as scaffold on which the other subunits assemble.
ω may facilitates assembly of RNAP and stabilizes assembled RNAP.

Prokaryotic RNA polymerase

alpha subunit

The two domains

- **The α subunit (RpoA):**
- The alpha subunit consists of:

1. a N-terminal domain (NTD), and
2. a C-terminal domain (CTD), connected by a short linker.

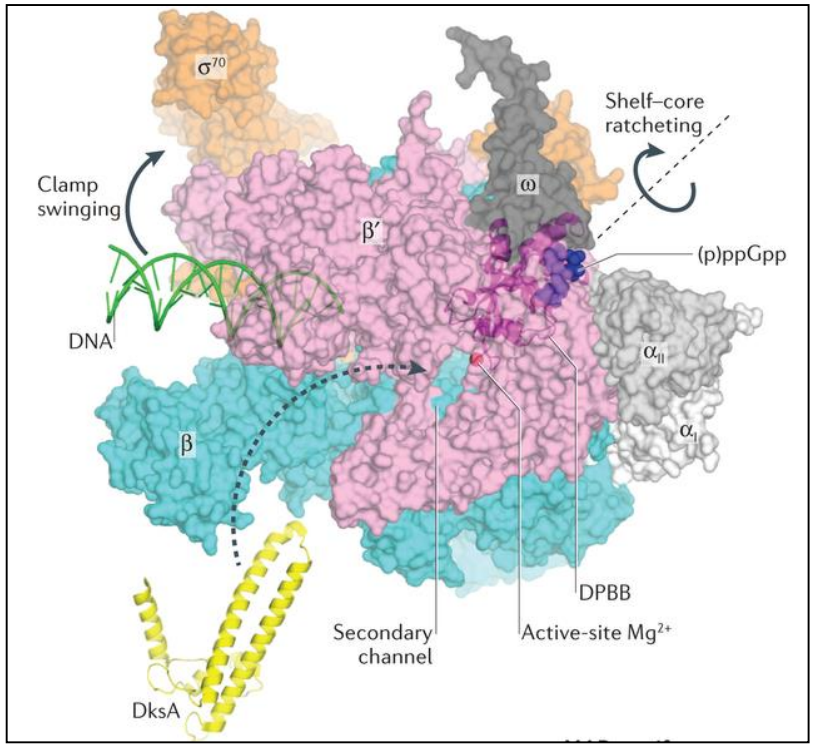
- The NTD is essential for RNA polymerase assembly;
- The α subunit C-terminal domain (CTD), recognizing promoter upstream elements.
- The CTD is necessary for transcription regulation.

Prokaryotic RNA polymerase

Sigma factors is partially or totally dependent on ppGpp

β′ subunit binds DNA

- The β′ subunit is involved in DNA binding.
- Its Mol. wt is 155-160 KD, coded for by rpoC gene.
- This is perhaps one of the largest proteins produced by the bacteria.
- The strong affinity for DNA stems from its COO- terminal, which has a zinc finger motif, this region provides strong DNA binding forces.
- Beta and beta′ together bind to both strands of DNA, but catalytic activity is located in β subunit.



Hauryliuk *et al.*,2015;…

Prokaryotic RNA polymerase

Number and rate of synthesis within a cell

1. Concentration of RNA polymerases (RNAPs) in the cell is approximately 7000 units per cell (1500 to 11,400), and
2. Sigma factors, is about 2300 and odd per cell.
3. At any given time more than 5000- 6000 enzymes are engaged in transcriptional activity.
4. The rate of synthesis under optimal conditions is about 25 to 75 nucleotides or more per second.

Transcription

RNA polymerase in different cells

Gene promoters

- Each gene has a promoter that allows it to be turned on or off in all the right places, at all the right times.
- Promoters, like genes, are made up of A's, G's, C's and T's all lined up in a certain order.
- Promoters are usually located very close to the gene they control.
- Each promoter is a set of instructions for what proteins should sit on it.
- The cell then looks at what proteins are there and decides whether or not to read the gene.

The Tech Museum of Innovation,2013

Transcription

RNA polymerase in different cells

Gene promoters

- Promoters are DNA sequences located in the 5' region adjacent to the transcriptional start site.
- RNA polymerase and transcription factors bind to the promoter to initiate production of an mRNA transcript.
- Transcription always proceeds from the 5' end of a strand of DNA to the 3' end.
- The old polymer is read in the 3' to 5' direction and the new, complementary fragments are generated in the 5' to 3' direction.
- Interactions of proteins at the promoter regulate gene activity by:

1. Activating, or
2. repressing transcription.

Transcription

RNA polymerase in different cells

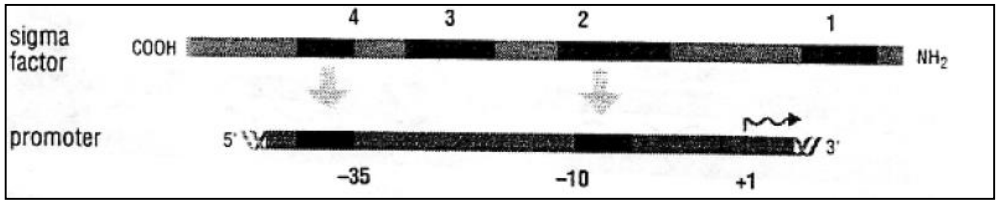
Gene promoters

- A promoter is the DNA sequence required for correct initiation of transcription.

1. Map ends of mRNA on DNA;
2. Mapping sites on DNA for protein binding.

- It affects the amount of product from a gene, but does not affect the structure of the product.

1. Most promoters are at the 5’ end of the gene.
2. Promoters are located near the transcription start sites of genes.



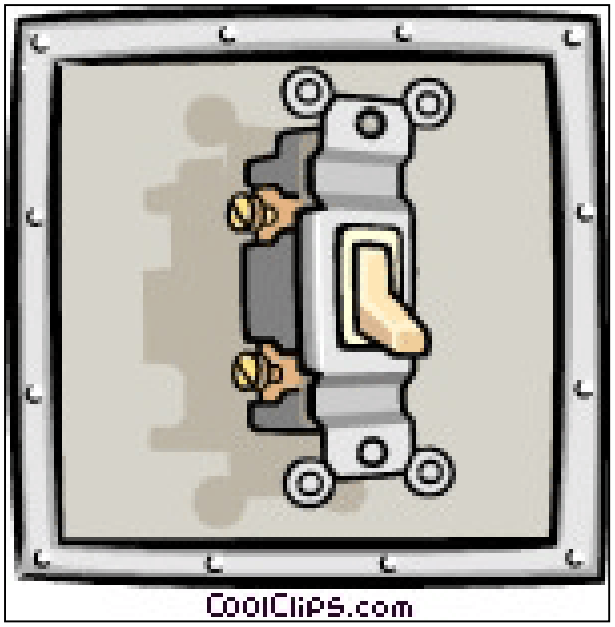
Transcription

Gene is the light bulb and its promoter is the light switch

- Let's think of turning on a gene, like turning on a light bulb in a room.
- Our gene is the light bulb and its promoter is the light switch.

1. Promoter (P) - aids in RNA polymerase binding;
2. Operator (O) - "on/off" switch - binding site for the repressor protein.
3. TFs are called activators when they turn genes on.
4. TFs are called repressors when they turn off a gene.

- Note: the light can't be turned on at all.



A gene's light switch is called a promoter.
TFs can either turn genes on or off.

Transcription

Gene is the light bulb and its promoter is the light switch

Constitutive vs regulated promoters

- Constitutive promoters: they are active in all circumstances in the cell. Their activities may be affected by the levels of RNA Polymerase or particular σ factors.
- Regulated promoters (inducible promoters): These are regulated, becoming active in the cell only in response to specific stimuli (biotic/abiotic stresses). E.g.

1. drought-inducible promoters
2. cold- and heat-shock-induced promoters.

There are virtually hundreds of inducible promoters that vary according to the organism source and cells or tissues where they regulate gene transcription. Many synthetic promoters have been generated and characterized.

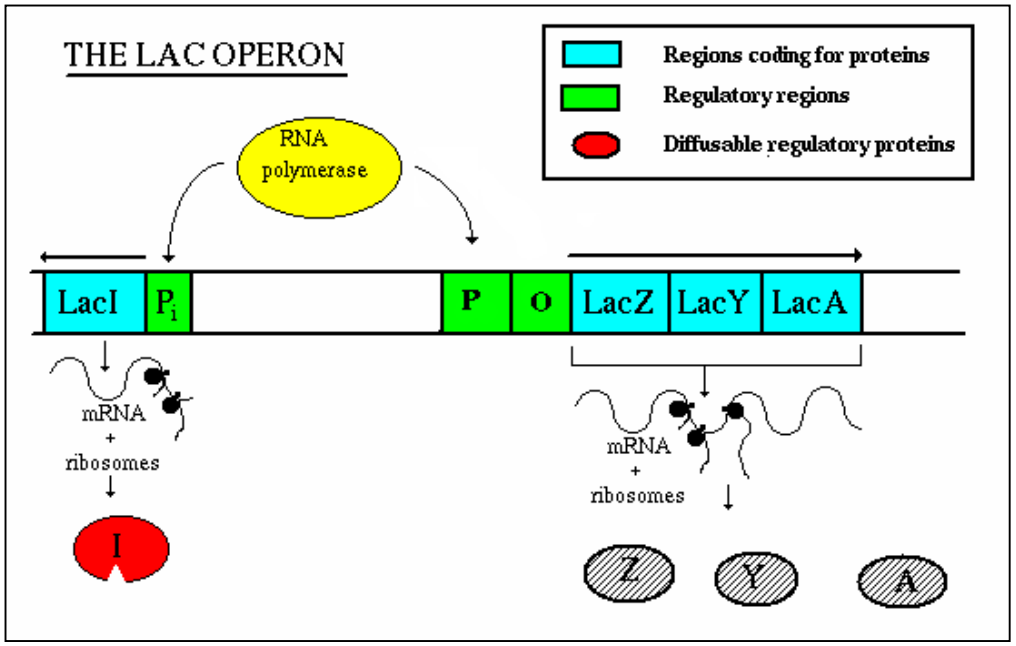
Transcription in bacteria

The *lac* operon

Gene is the light bulb and its promoter is the light switch

- **Function of the operon: to produce enzymes which break down lactose (milk sugar).**

1. Promoter (P) - aids in RNA polymerase binding;
2. Operator (O) - "on/off" switch - binding site for the repressor protein.

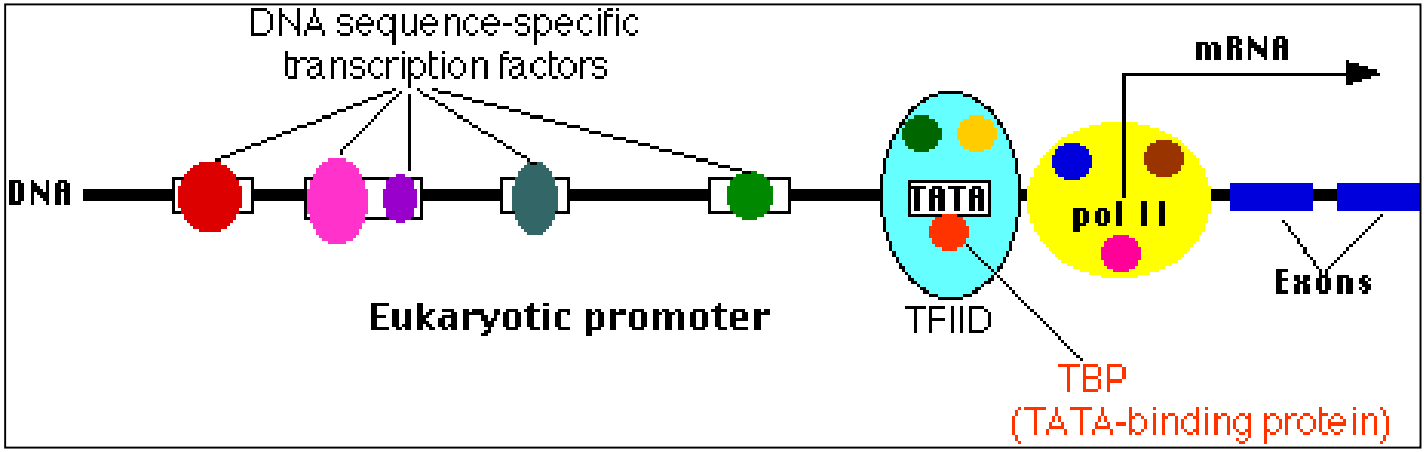


Transcription in eukaryotes

Basal promoter

Gene is the light bulb and its promoter is the light switch

- The basal promoter contains a sequence of 7 bases (TATAAAA) called the TATA box (this is very similar to the -10 box or Pribnow box found in prokaryotes).
- It can be bound by Transcription Factor IID (TFIID read T F 2 D) which is a complex of some 10 different proteins.



Transcription

RNA polymerase in different cells

Gene promoters in all organisms

- RNA polymerases from all organisms recognize a variety of start sequences or promoters.
- In bacteria, a promoter for mRNA transcription is recognized by the sigma protein and has two recognition zones about 10 and 35 bases before the transcription start site.
- In archaea and eukaryotes the transcription recognition sequence is a TATA sequence (termed the TATA box) and transcription is regulated by various protein transcription factors that bind to regions near the TATA box and then recruit RNA polymerase.

Transcription

RNA polymerase in different cells

Gene promoters in all organisms

- Transcription factor (TF) binding sites or motifs (TFBMs):
- Any protein that is needed for the initiation of transcription is defined as a transcription factor.
- Structural motifs such as helix-turn-helix proteins are common types of motifs that are:

1. found in different transcription factors, and
2. responsible for binding to DNA.

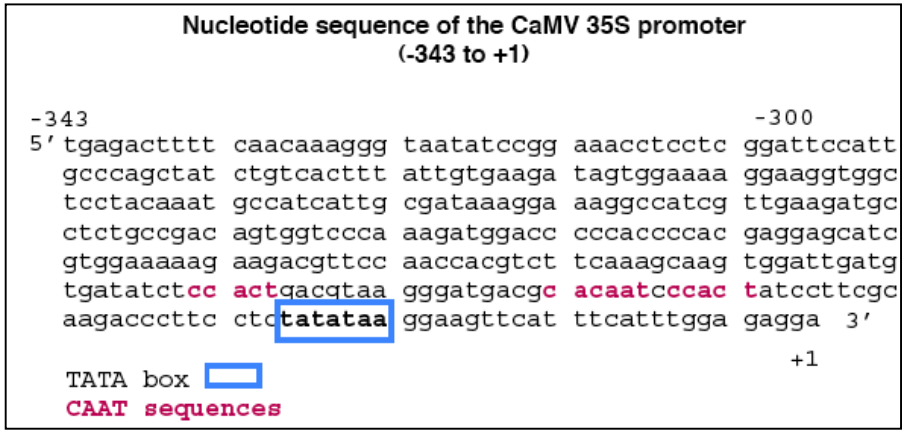
The sigma-70 factor domain-4 contains a helix-turn-helix (H-T-H) motif that mediates interaction with the -35 element in promoter DNA.

Transcription

Gene promoters in virus

Nucleotide sequence of the CaMV 35S promoter

- Promoter: Site where RNA polymerase binds and initiates transcription.
- Promoters can be about 100–1000 base pairs long.
- The 35S promoter is a very strong constitutive promoter, causing high levels of gene expression in dicot plants.
- However, it is less effective in monocots, especially in cereals.
- The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors.



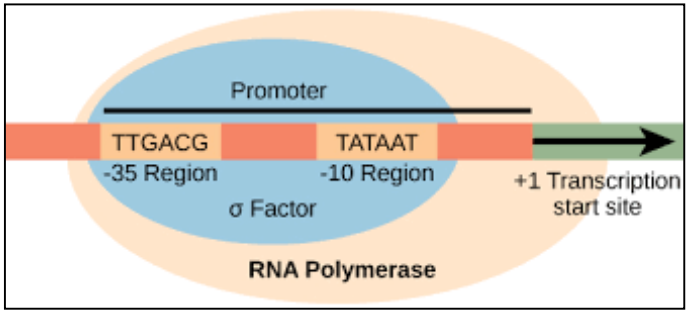
Transcription

Bacterial promoters

Gene promoters in bacteria

- In bacteria, the promoter contains two short sequence elements approximately -10 and -35 nucleotides(bases) upstream from the transcription start site.

1. The sequence at -10 (the -10 element) has the consensus sequence TATAAT.
2. The sequence at -35 (the -35 element) has the consensus sequence TTGACA.



Transcription

Bacterial promoters

Gene promoters in bacteria

- Motifs reside ~10 bases and ~35 bases.
- *E. coli* promoter consists of two motifs described as:

1. -10 box 5′-TATAAT-3′(or−10 promoter motifs/sequences), and
2. -35 box 5′-TTGACA-3′ (or−35 promoter motifs/sequences).
3. Both are six nucleotide motifs.

- These sequences are usually found in the 5' promoter region of a gene.

Sami and Nagatomi,2008;Case,2012;..

Motifs

Conserved regions of protein or DNA sequences

- In genetics, a sequence motif is:

1. a nucleotide, or
2. amino-acid sequence pattern.

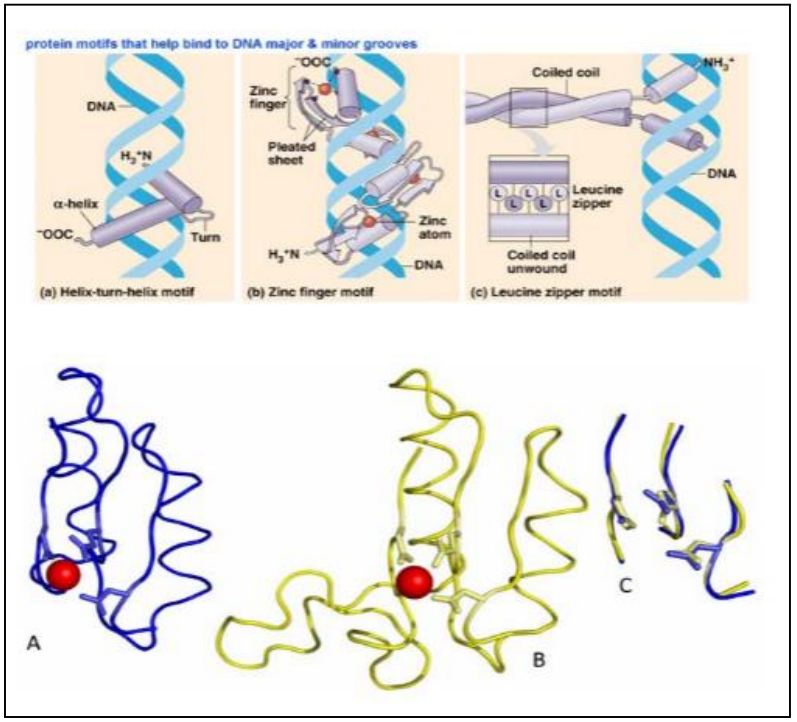
Motifs
DNA-binding motif

- The word motif can sometimes mean the same thing as domain - for example, someone might refer to a DNA-binding motif in a protein.
- However, a motif is typically smaller than a domain, can occur in DNA, RNA, and proteins.
- A structural motif in a protein is something like a helix-loop-helix or a beta-hairpin turn that can appear in multiple different kinds of protein domains, and doesn't necessarily have the same exact function in those different domains, but typically has a fairly conserved sequence that is very similar.

Protein motifs

An amino-acid sequence pattern

- Protein motifs such as helix-turn-helix,.. helps RNA polymerase binds to DNA for transcription.
- helix-turn-helix motif was found both in eukaryotes and in prokaryotes.



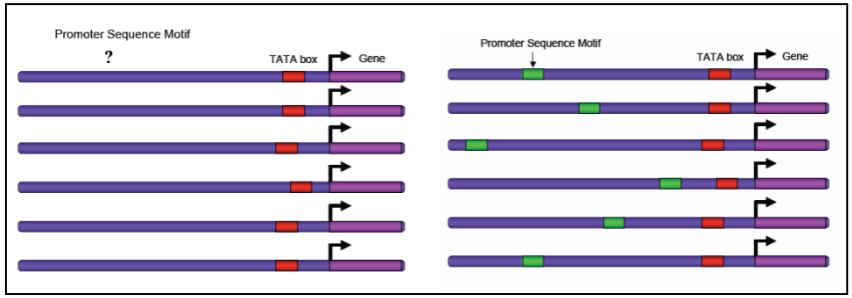
A motif can occur in DNA, RNA, and proteins, and has to do with the specific sequence. E.g. the zinc finger motif is found in protein domains that bind DNA, RNA, and other proteins.

Prat Thiru;..

DNA motifs

A nucleotide sequence pattern

- In eukaryotes: the TATA promoter sequence is an example of a highly conserved DNA sequence motif.
- In prokaryotes: Either
- -10 box 5′-TATAAT-3′, or
- -35 box 5′-TTGACA-3′ promoter motifs/sequences.
- These sequences are usually found in the 5' promoter region of a gene.



Prat Thiru;..

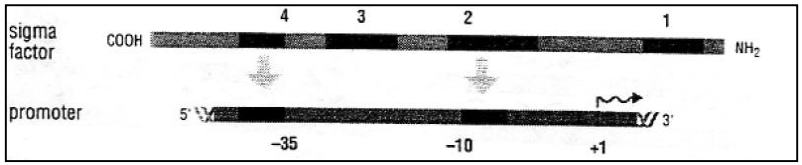
Transcription by RNA polymerase proceeds in a series of steps

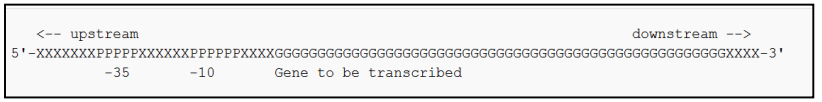
Bacterial promoters

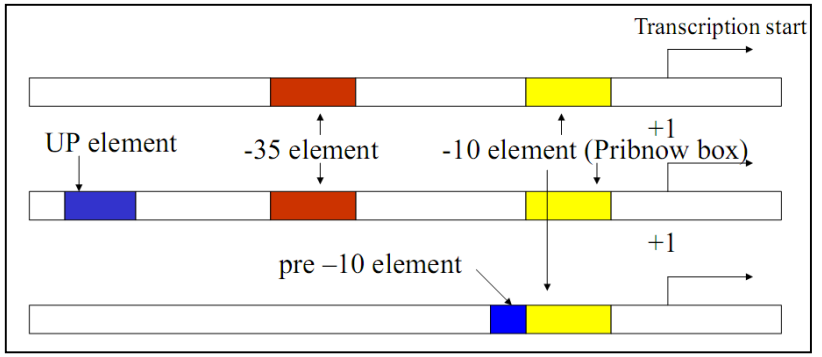
- Most bacterial promoters have –35 and –10 elements (regions).

1. Some have UP element (upstream promoter element).
2. Some lack –35 element, but have extended –10 region.

- Note also that molecular biologists use a numbering system which has no zero!
- The first nucleotide of the RNA transcript is numbered +1;
- Position +1 is the transcription start site.
- The nucleotide immediately upstream from that is numbered -1.



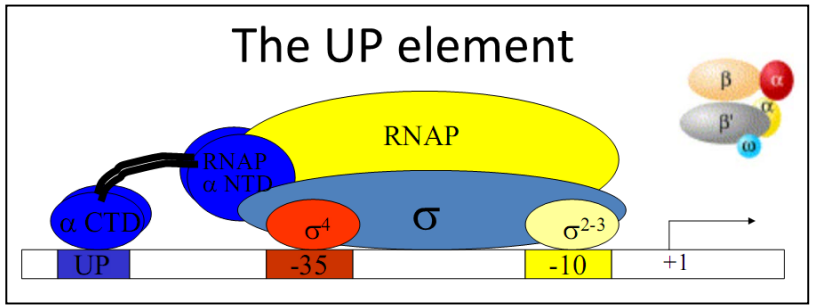




- Pribnow Box: A region of DNA to which RNA polymerase binds before initiating the transcription of DNA into RNA.
- The -35 region and the -10 ("Pribnow box") region comprise the basic prokaryotic promoter.
- The DNA is unwound and becomes single-stranded ("open") in the vicinity of the initiation site (defined as +1).
- Upstream" (toward 5′ end) of the initiation point.

Transcription by RNA polymerase proceeds in a series of steps
Bacterial promoters- UP element

- Some bacterial promoters have UP element (upstream promoter element).
- UP element is an AT rich motif present in some strong promoters (e.g. rRNA).
- UP element interacts directly with C-terminal domain(CTD) of RNA polymerase a subunits.



Transcription

Gene promoters

Binding position in promoter

- As promoters are typically immediately adjacent to the gene in question, positions in the promoter are designated relative to the transcription start site, where transcription of DNA begins for a particular gene. i.e.,
- Positions upstream are negative numbers counting back from -1.
- for example -100 is a position 100 base pairs upstream.
- Region protected from nucleases by binding of RNA polymerase is -50 to +20.

Wikipedia,2015;..

Transcription
Bacterial promoters

- In bacteria, the promoter contains two short sequence elements approximately -10 and -35 nucleotides upstream from the transcription start site.
- A combination of approaches shows that the -10 TATAAT and -35 TTGACA sequences are the essential DNA sequences in most *E. coli* promoters.

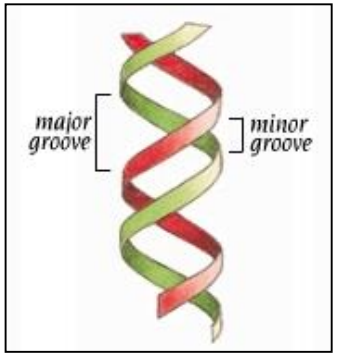
1. The sequence at -10 has the consensus sequences/motif TATAAT.
2. The sequence at -35 has the consensus sequences TTGACA/motif.

Wikipedia,2015;..

Transcription

Bacterial promoters

DNA binding motifs



1. The helix-turn-helix motif (HTH), used by most σ-factors, maintains its specificity and accuracy by binding in the major groove of DNA, where it can interact with the base pairs in the DNA double-helix.

- One such DNA-binding motif, the helix-turn-helix motif helps specifically recognize DNA promoters at both the -35 and -10 positions.
- The HTH motif is commonly used for transcriptional repression. HTH motifs are observed in the *lac* repressor.

2. As mentioned earlier the β' subunit of RNA polymerase has a zinc finger motif, provides strong DNA binding forces.

The sigma-70 factor domain-4 contains a helix-turn-helix (H-T-H) motif that mediates interaction with the -35 element in promoter DNA.

Transcription

Bacterial promoters

Zinc finger motif

- Both zinc ions are bound to β′ of RNA polymerase.
- The β′ residues coordinating zinc are conserved throughout eubacteria and chloroplasts.
- Four cysteine residues in the *Escherichia coli* RNAP largest subunit, β′ was locate that one of the two zinc ions tightly associated with the RNA polymerase enzyme.
- In the absence of zinc, or when zinc binding is prevented by mutation, the RNAP gets non-functional.

Marko *et al.*,1999

Transcription

Bacterial promoters

σ^{70}(RpoD) is the housekeeping sigma factor or also called as primary sigma factor, transcribes most genes in growing cells

- The bacterial core RNA polymerase complex, which consists of five subunits, is sufficient for transcription elongation and termination but is unable to initiate transcription.
- Transcription initiation from promoter elements requires a sixth, dissociable subunit called a sigma factor, which reversibly associates with the core RNA polymerase complex to form a holoenzyme.
- Sigma factors direct bacterial core RNA polymerase to specific promoter elements that are situated 10 and 35 base-pairs upstream of transcription-initiation points.

Holoenzyme =core enzyme + sigma factor

```
  <-- upstream                                                    downstream -->
5'-XXXXXXXPPPPPXXXXXXPPPPPPXXXXGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGXXXX-3'
          -35       -10       Gene to be transcribed
```

Transcription

Bacterial promoters

σ^{70}(RpoD) is the housekeeping sigma factor or also called as primary sigma factor, transcribes most genes in growing cells

- Every cell has a "housekeeping" sigma factor that keeps essential genes and pathways operating.
- In the case of *E. coli* and other gram-negative rod-shaped bacteria, the "housekeeping" sigma factor is σ^{70}.
- Promoter sequences are recognized only by RNA polymerase holoenzyme containing sigma-70.
- The sigma 70 subunit of RNA polymerase recognize and contacts both the -35 and the -10 boxes.

Holoenzyme =core enzyme + sigma factor

```
  <-- upstream                                                    downstream -->
5'-XXXXXXXPPPPPXXXXXXPPPPPPXXXXGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGXXXX-3'
          -35       -10       Gene to be transcribed
```

Wikipedia,2015

A sampling of *E. coli* regulatory systems:

1. Nutrient limitation:
   - ✓ Carbon, nitrogen and phosphorous.
2. Growth limitation:
   - ➢ Stringent response (ppGpp), stationary phase, oxygen.
3. Stress:
   - ➢ Osmoregulation, oxygen stress, heat shock, envelop stress and pH shock.

TABLE 13.1 A sampling of *E. coli* global regulatory systems

System	Response	Regulatory gene(s) (protein[s])	Category of mechanism	Some genes, operons, regulons, and stimulons
Nutrient limitation				
Carbon	Catabolite repression	*crp* (CAP, also called CRP)	DNA-binding activator or repressor	*lac, ara, gal, mal,* and numerous other C source operons
	Control of fermentative vs. oxidative metabolism	*cra* (*fruR*) (CRA)	DNA-binding activator or repressor	Enzymes of glycolysis, Krebs cycle
Nitrogen	Response to ammonia limitation	*rpoN* (NtrA)	Sigma factor (σ^{54})	*glnA* (GS) and operons for amino acid degradation
		ntrBC (NtrBC)	Two-component system	
Phosphorus	Starvation for inorganic orthophosphate (P_i)	*phoBR* (PhoBR)	Two-component system	>38 genes, including *phoA* (bacterial alkaline phosphatase) and *pst* operon (P_i uptake)
Growth limitation				
Stringent response	Response to lack of sufficient aminoacylated-tRNAs for protein synthesis	*relA* (RelA), *spoT* (SpoT)	(p)ppGpp metabolism	rRNA, tRNA, ribosomal proteins
Stationary phase	Switch to maintenance metabolism and stress protection	*rpoS* (RpoS)	Sigma factor (σ^{S})	Many genes with σ^{S} promoters; complex effects on many operons
Oxygen	Response to anaerobic environment	*fnr* (Fnr)	CAP family of DNA-binding proteins	>31 transcripts, including *narGHJI* (nitrate reductase)
	Response to presence of oxygen	*arcAB* (ArcAB)	Two-component system	>20 genes, including *cob* (cobalamin synthesis)
Stress				
Osmoregulation	Response to abrupt osmotic upshift	*kdpDE* (KdpD, KdpE)	Two-component system	*kdpFABC* (K^+ uptake system)
	Adjustment to osmotic environment	*envZ*/*ompR* (EnvZ/OmpR)	Two-component system	OmpC and OmpF outer membrane proteins
		micF	Antisense RNA	*ompF* (porin)
Oxygen stress	Protection against reactive oxygen species	*soxS* (SoxS)	AraC family of DNA-binding proteins	Regulon, including *sodA* (superoxide dismutase) and *micF* (antisense RNA regulator of *ompF*)
		oxyR (OxyR)	LysR family of DNA-binding proteins	Regulon, including *katG* (catalase)
Heat shock	Tolerance of abrupt temperature increase	*rpoH* (RpoH)	Sigma factor (σ^{32})	Stimulon, Hsps (heat shock proteins), including *dnaK, dnaJ,* and *grpE* (chaperones), and *lon, clpP, clpX,* and *hflB* (proteases)
Envelope stress	Misfolded Omp proteins	*rpoE* (RpoE)	Sigma factor (σ^{E})	>10 genes, including *rpoH* (σ^{32}) and *degP* (encoding a periplasmic protease)
	Misfolded pilus	*cpxAR* (CpxAR)	Two-component system	Overlap with RpoE regulon
pH shock	Tolerance of acidic environment	Many	Many	Complex stimulon

The 70 family of sigma factors

Major or primary sigma factor vs. alternative sigma factors

- Sigma (σ) factors control the promoter selectivity of bacterial RNA polymerase (RNAP).
- Most bacteria express a multiplicity of sigma factors.

1. Primary σ factors:

- All bacteria contain a primary σ factor that is responsible for transcription of housekeeping genes necessary for growth and survival.

2. Alternative σ factors:

- In addition, many bacteria encode multiple alternative σ factors.
- The level and activity of the alternative σ factors are highly regulated and can vary depending on environmental or developmental signals.

The 70 family of sigma factors

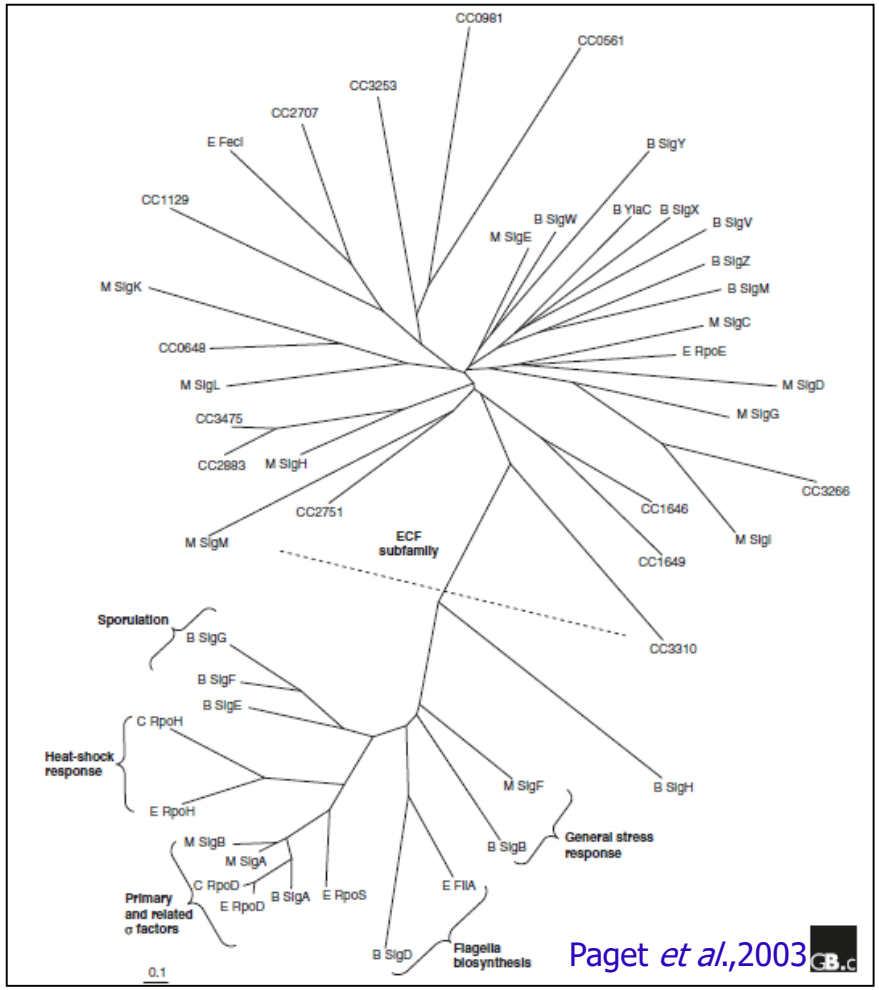
Major or primary sigma factor vs. alternative sigma factors

- **Alternative σ factors:**
- The alternative sigma factors are required for the transcription of specific subsets of genes.
- The synthesis of alternative σ factors allows the coordinated activation of discrete sets of genes through the recognition of distinct promoter sequences and thereby contributes to:
- stress responses,
- motility,
- endospore formation, and
- numerous other adaptive responses.

The 70 family of sigma factors

Major or primary sigma factor vs. alternative sigma factors

- Phylogenetic relationships between members of the 70 family of sigma factors from four diverse bacteria:
  1. *E. coli* (E);
  2. *Caulobacter cresentus* (CC or C);
  3. *B. subtilis* (B); and
  4. *Mycobacterium tuberculosis* (M).
- Note that the primary and related factors comprise groups 1 and 2, the group 3 factors are divided into functionally related groups (sporulation, flagella biosynthesis, general stress response and heat-shock response), and the divergent ECF(extracytoplasmic function) factors comprise group 4.



Paget *et al.*,2003

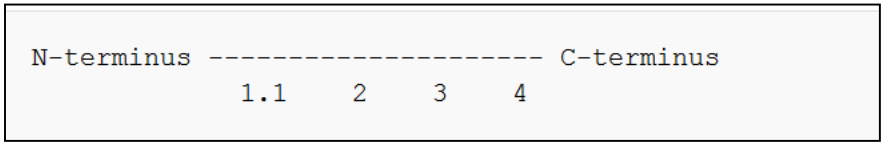
The 70 family of sigma factors

Regions and sub-regions

Function of each regions and sub-regions

- Sequence alignments of the sigma70 family members reveal four conserved regions.
- Sigma factors have four main regions that are generally conserved, although the highest conservation is found in regions 2 and 4, which are involved in:

1. binding to RNA polymerase,
2. recognizing promoters, and
3. separating DNA strands (so-called 'DNA melting').



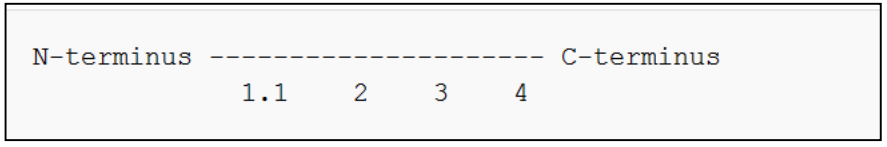
Wikipedia,2015;..

The 70 family of sigma factors

Regions and sub-regions

Function of each regions and sub-regions

- The four main regions are further subdivided into sub-regions: e.g. 2 includes 2.1, 2.2, etc.).
- Sub-region 1.1 is found only in "primary sigma factors" (RpoD σ^{24}, σ^{28} or RpoS or σ^{38} in *E.coli*). It is involved in ensuring the sigma factor will only bind the promoter when it is complexed with the RNA polymerase.
- Sub-region 2.4 recognizes and binds to the promoter -10 element (formerly called pribnow box).
- Sub-region 4.2 recognizes and binds to the promoter -35 element.



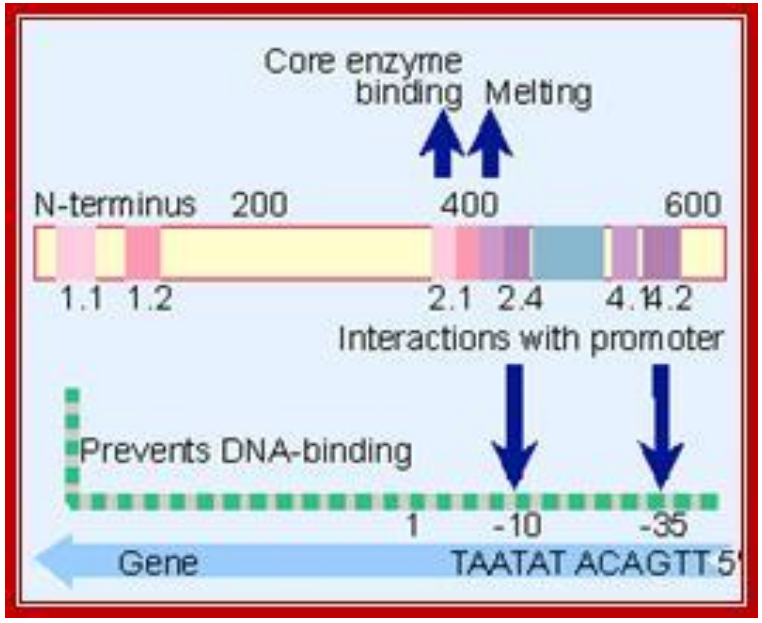
Region 1 ensuring the sigma factor will only bind the promoter.
Region 3 primarily is involved in binding the core RNA polymerase in the holoenzyme.

The 70 family of sigma factors

Regions and sub-regions

Function of each regions and sub-regions

- Sub-region 2.2, which may be involved in the binding of the sigma factor to the core RNA polymerase, and
- Sub-region 2.4 recognizes and binds to the promoter -10 element.
- sub-region 4.2, which seems to harbor a DNA-binding 'helix-turn-helix' motif involved in binding the conserved -35 region of promoters recognized by the major sigma factors.



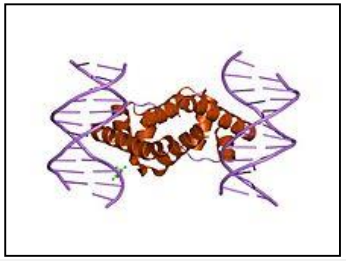
Region 4 is involved in binding to the -35 promoter element via a helix-turn-helix motif.

EMBL-EBI,2013;..

Transcription

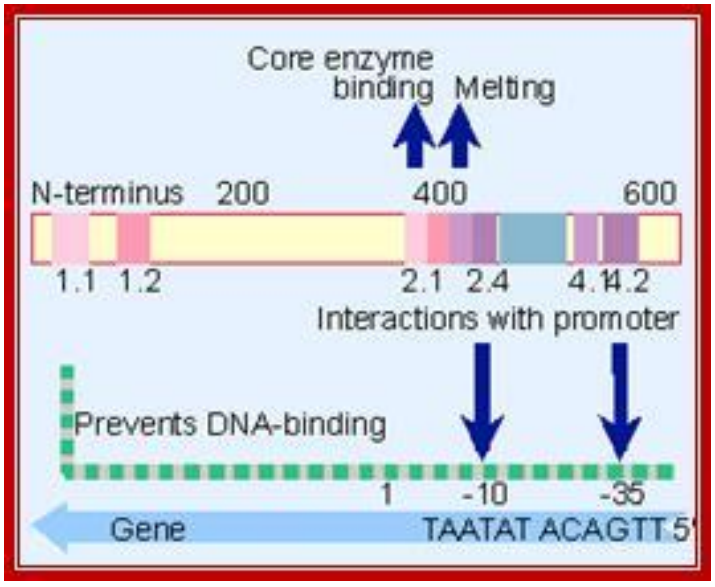
Bacterial promoters

35 and -10 sequences

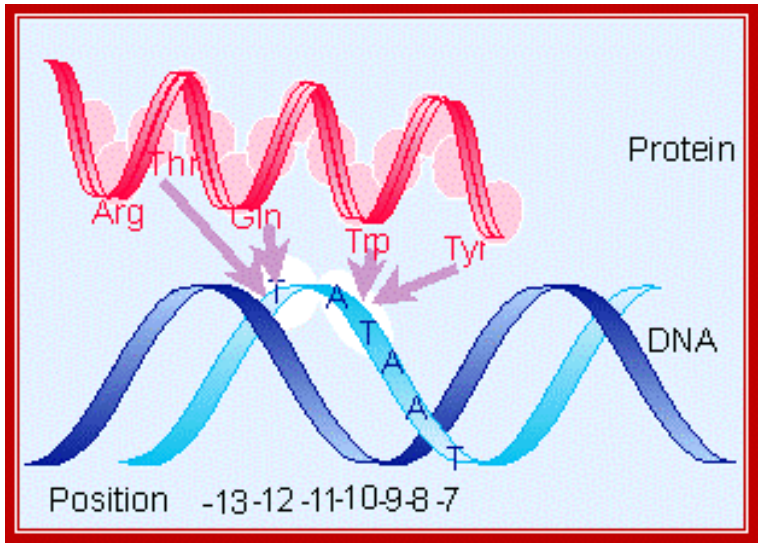


Crystal structure of *Escherichia coli* sigma70 region 4 bound to its -35 element DNA.

- The sigma subunit of RNA polymerase contacts both the -35 and the -10 boxes.
- Region 2.4 recognizes and binds to the promoter -10 element.
- Region 4.2 recognizes and binds to the promoter -35 element.



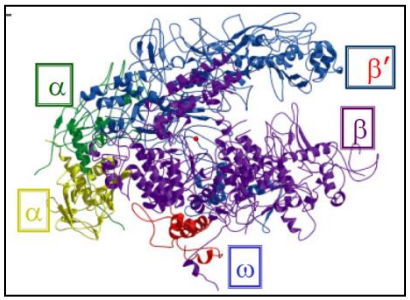
The sig-70 has four domains, called, from the N-terminal, as 1, 2, 3 and 4. The C- terminal 4th domain has helix turn helix motifs and recognizes -35 sequences and bind.



Sigma protein specific amino acids contact specific DNA sequences.

Transcription

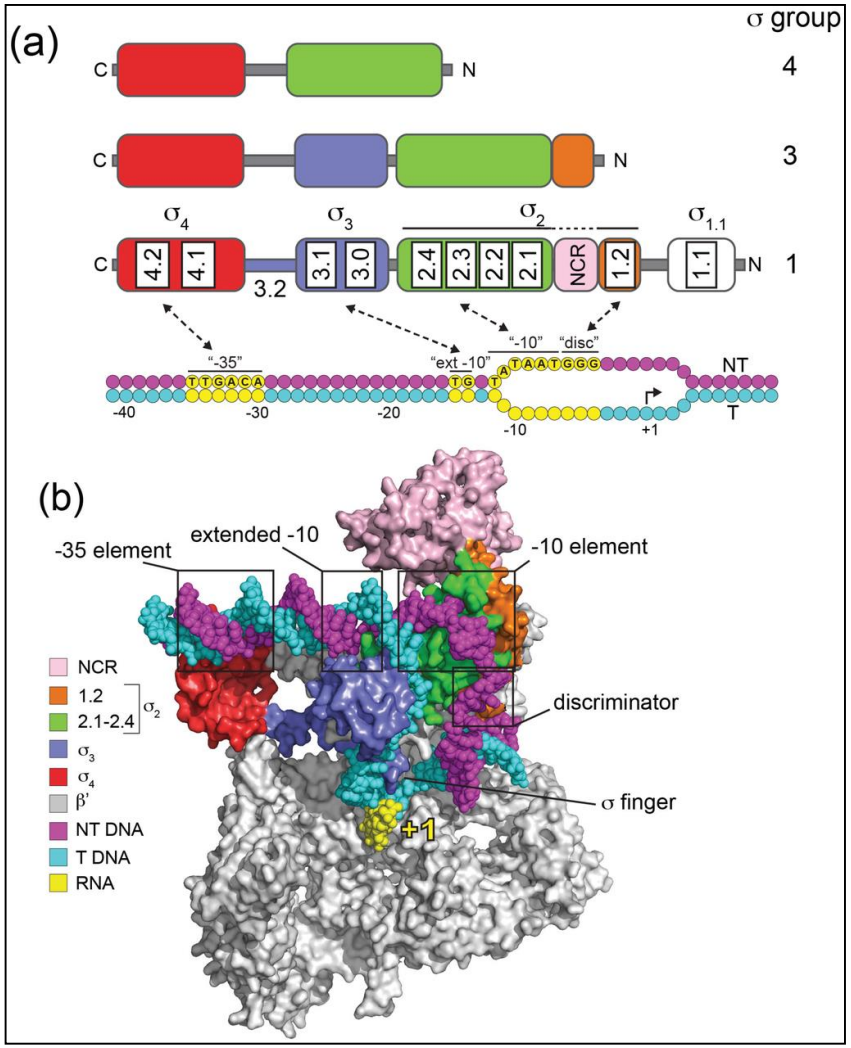
Domain organization, promoter recognition and structural organization of the σ^{70} family



- The sigma-70 factor domain-4 contains a helix-turn-helix (H-T-H) motif that mediates interaction with the -35 element in promoter DNA.
- The domain also mediates interaction with the RNA polymerase subunit RpoA.
- The rpoA gene, encoding the α- subunit of RNA polymerase.

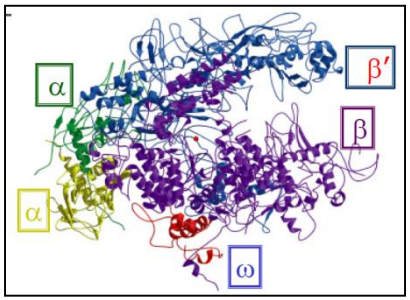
Region 1 ensuring the sigma factor will only bind the promoter.
Region 3 primarily is involved in binding the core RNA polymerase in the holoenzyme.

Wigneshweraraj and Hinton,2015;.

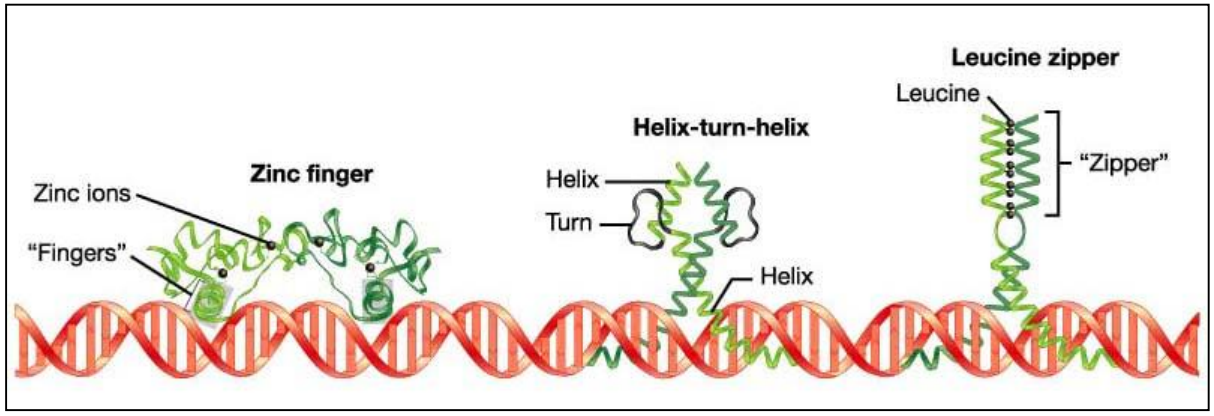


Transcription

Domain organization, promoter recognition and structural organization of the σ^{70} family



- The sigma-70 factor domain-4 contains a helix-turn-helix (H-T-H) motif that mediates interaction with the -35 element in promoter DNA.
- HTH(homeodomain) - three different planes of the helix are established and bind to the grooves of the DNA.



Control of Genetic Systems in Prokaryotes and Eukaryotes.htm

Transcription

RNA polymerase in different cells

Promoters and transcription factors

- The proteins that sit on the promoter are called transcription factors (TFs).
- TFs and promoters work together:

1. to turn a gene on (and make a protein), or
2. to turn a gene off (and stop making a protein), or even
3. to just make more or less protein.

The Tech Museum of Innovation,2013

Transcription

RNA polymerase in different cells

Promoters and transcription factors

1. Genes can either be on or off all of the time. Or
2. be turned on only under special situations.

- How a gene is controlled is determined by these TFs.
- But some genes, called housekeeping genes, are on all the time, in every cell.

1. Housekeeping genes and their promoter (or light switch) has TFs that keep it in the "on" position all the time.
2. Other genes are only needed during certain times, like fighting an infection or making more bone. The promoters for these genes have TFs that tell the cell to only turn them on during these times or in these places.

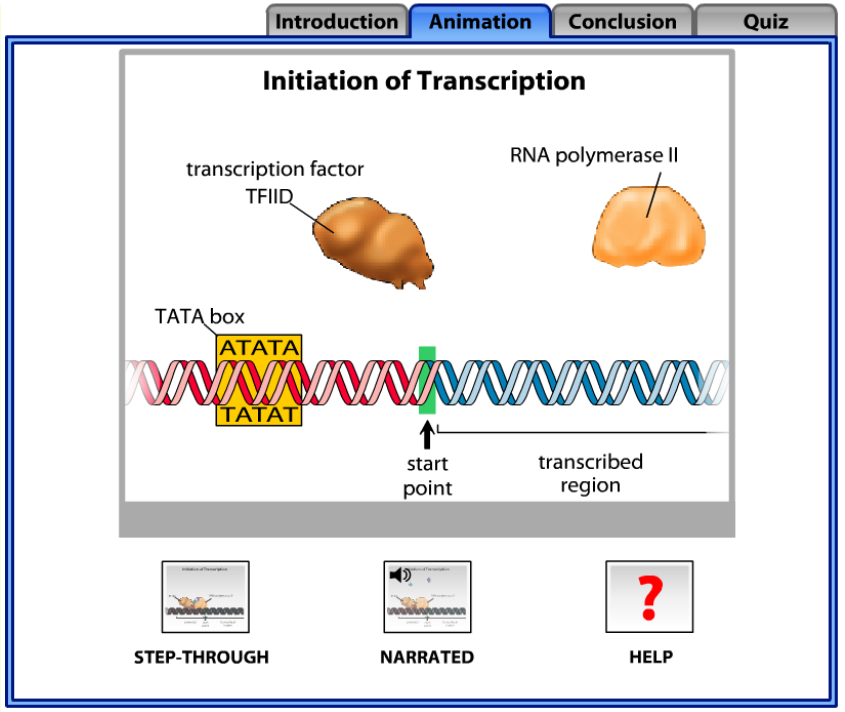
The Tech Museum of Innovation,2013

Eukaryotic DNA transcription

Transcription recognition sequence is a TATA sequence

RNA pol II together with one of transcription factors constitutes the basal (or minimal) transcriptional apparatus that is needed to transcribe any class II promoter

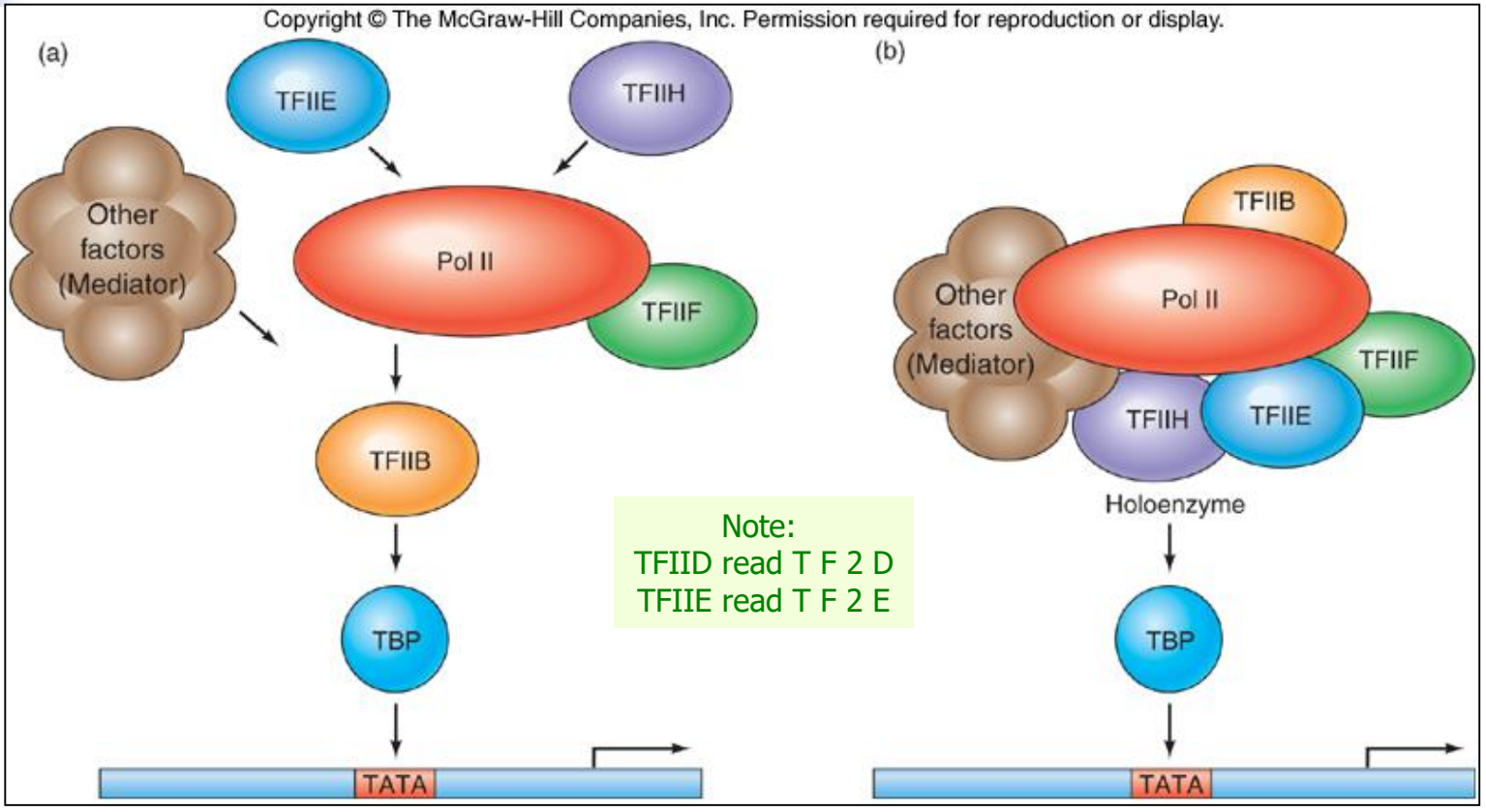
- RNA pol II enzyme cannot initiate transcription itself, but is absolutely dependent on auxiliary transcription factors (called TFIIX, where "X" is a letter that identifies the individual factor here is TFIID).
- Sigma factors are absent in eukaryotes.
- Instead, general transcription factors act as sigma factors in the eukaryotes (equivalent of sigma).



General TF and other proteins are bound to RNA pol II in a complex called RNA polymerase holoenzyme.

Eukaryotic DNA transcription

RNA pol II together with one of transcription factors constitutes the basal (or minimal) transcriptional apparatus that is needed to transcribe any class II promoter. Complex Components in Yeast

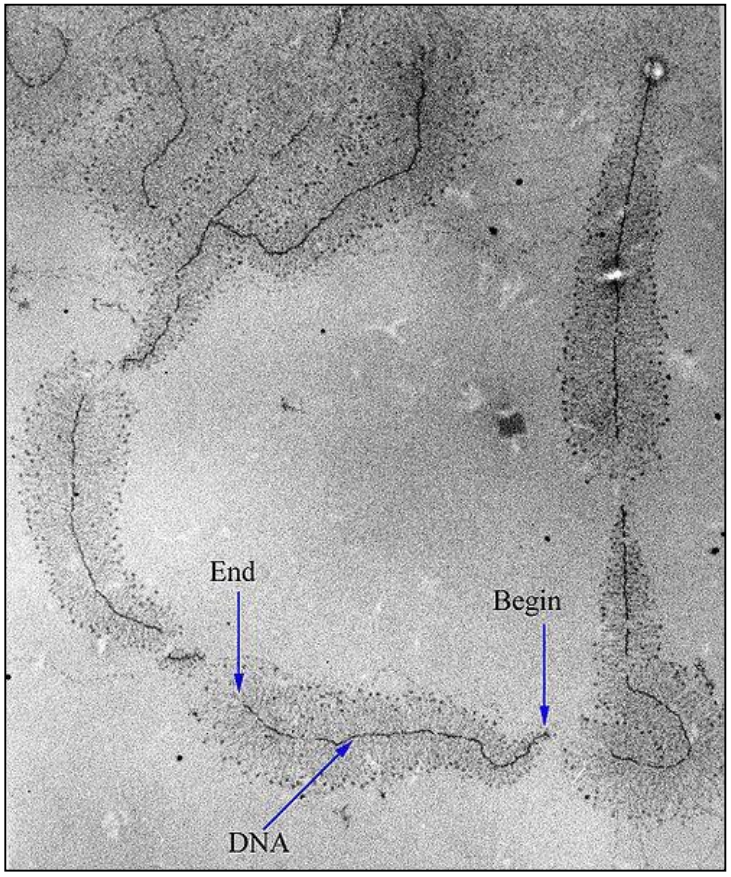


Eukaryotic transcription initiation factors assemble an initiation complex, which dissociates at the end of initiation. Prokaryotic transcription initiation factors do not assemble an initiation complex.

Eukaryotic DNA transcription

An electron-micrograph of DNA strands decorated by hundreds of RNAP molecules too small to be resolved

- Each RNAP is transcribing an RNA strand, which can be seen branching off from the DNA.
- "Begin" indicates the 3' end of the DNA, where RNAP initiates transcription;
- "End" indicates the 5' end, where the longer RNA molecules are completely transcribed.

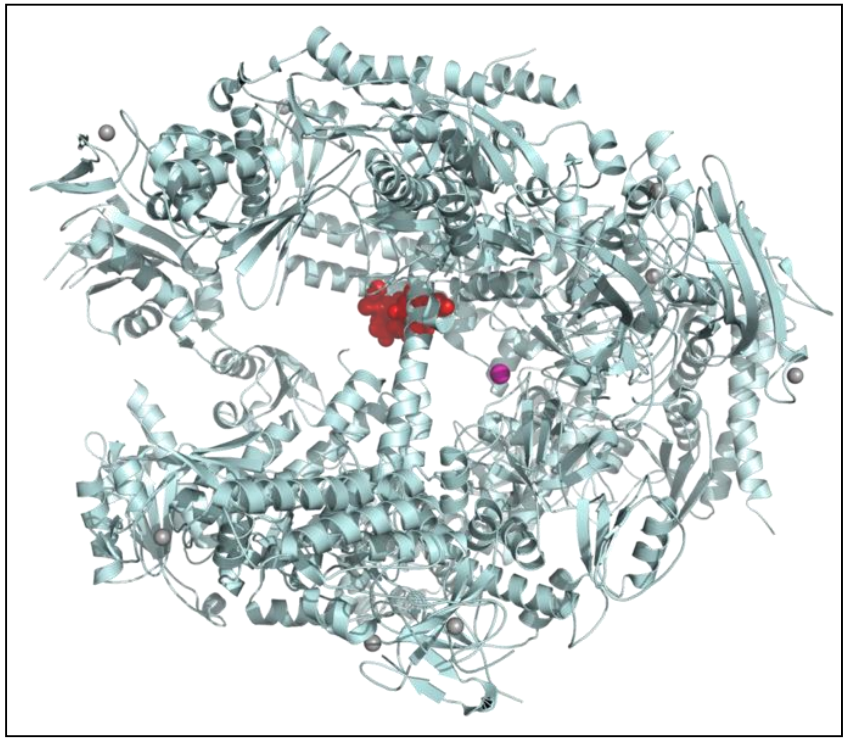


Wikipedia

Eukaryotic DNA transcription

An electron-micrograph of DNA strands decorated by hundreds of RNAP molecules too small to be resolved

- Structure of eukaryotic RNA polymerase II (light blue) in complex with α-amanitin (red), a strong poison found in death cap mushrooms that targets this vital enzyme.



Wikipedia

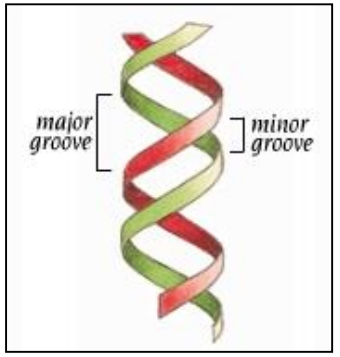
DNA-binding proteins
Required for expression of new proteins

- The expression of proteins responsible for moment-to-moment chemical and structural tasks is critical to the functioning of living cells.
- This requires that the cell regulate the expression of new proteins.
- DNA-binding proteins play an important role in this process.
- These proteins interact with DNA by means of various structural motifs, and can stimulate or repress transcription of messenger RNA, depending on the properties and location of the DNA sequence to which they bind.

DNA-binding proteins

DNA-binding domains

Structural DNA-binding motif



- Each structural motif contains features that are highly conserved among many organisms.
- Some proteins bind DNA in its major groove, some other in the minor groove, and some need to bind to both.
- Only a few structural motif are responsible for binding DNA in a large number of different DNA-binding proteins.
- Structural motifs are common types of motifs that are responsible for binding to DNA can be found in different transcription factors.

Atlas Genet Cytogenet Oncol Haematol, 2003

DNA-binding proteins

DNA-binding domains(DBDs)

DNA-binding motif

- **Sequence-specific DNA-binding motifs**
- Many DNA-binding domains must recognize specific DNA sequences, such as DBDs(DNA-binding domains) of transcription factors that activate specific genes.
- Sequence-specific DNA-binding proteins generally interact with the major groove of DNA.
- **Non-specific DNA-binding motifs**
- Nonspecific DNA binding generally involves simple attachment through either of the DNA grooves.
- E.g. structural proteins around DNA. These proteins organize the DNA into a compact structure.

DNA-binding proteins

DNA-binding domains

DNA-binding motif

Motif	Examples of proteins with this motif
1. Sequence-specific DNA-binding motifs	
Helix-turn-helix family	
Standard helix-turn-helix	*Escherichia coli* lactose repressor, tryptophan repressor
Homeodomain	*Drosophila* Antennapedia protein
Paired homeodomain	Vertebrate Pax transcription factors
POU domain	Vertebrate regulatory proteins PIT-1, OCT-1 and OCT-2
Winged helix-turn-helix	GABP regulatory protein of higher eukaryotes
High mobility group (HMG) domain	Mammalian sex determination protein SRY
Zinc-finger family	
Cys_2His_2 finger	Transcription factor TFIIIA of eukaryotes
Multi-cysteine zinc finger	Steroid receptor family of higher eukaryotes
Zinc binuclear cluster	Yeast GAL4 transcription factor
Basic domain	Yeast GCN4 transcription factor
Ribbon-helix-helix	Bacterial MetJ, Arc and Mnt repressors
TBP domain	Eukaryotic TATA-binding protein
β-Barrel dimer	Papillomavirus E2 protein
Rel homology domain (RHB)	Mammalian transcription factor NF-κB
2. Non-specific DNA-binding motifs	
Histone fold	Eukaryotic histones
HU/IHF motif(a histone-like motif)	Bacterial HU and IHF proteins
Polymerase cleft	DNA and RNA polymerases

DNA-binding proteins

DNA-binding domains

Structural DNA-binding motif

- DNA-binding motifs are regions of regulatory proteins which bind to DNA. e.g.

1. helix-turn-helix motif (originally identified in bacterial proteins, this motif has since been found in hundreds of DNA-binding proteins from both eukaryotes and prokaryotes);
2. Helix-loop-helix motif (eukaryotic transcription factors);
3. zinc finger motif (common DNA-binding motifs found in eukaryotic transcription factors, and have also been identified in prokaryotes);
4. leucine zipper motif (eukaryotic transcription factors).

DNA-binding proteins

DNA-binding domains

Structural DNA-binding motif

1. **Zinc finger motif:** It contains one or more zinc ions which are crucial for the structural stability.
2. **Helix-turn-helix motif(HTH):** It consists of two α helices and a short extended amino acid chain between them (composed of three elements). Not to be confused with the basic **helix-loop-helix domain(HLH)**. Helix-turn-helix motif was found both in eukaryotes and in prokaryotes.
3. **Leucine zipper motif:** It is formed by two α helices, which are held together by hydrophobic interactions between leucine residues. The leucine zipper generally appears as a dimer of a helices that form a coiled coil.
4. **Helix-loop-helix motif(HLH):**
   It is characterized by two α helices connected by a loop.

DNA-binding proteins

DNA-binding domains

Structural DNA-binding motifs

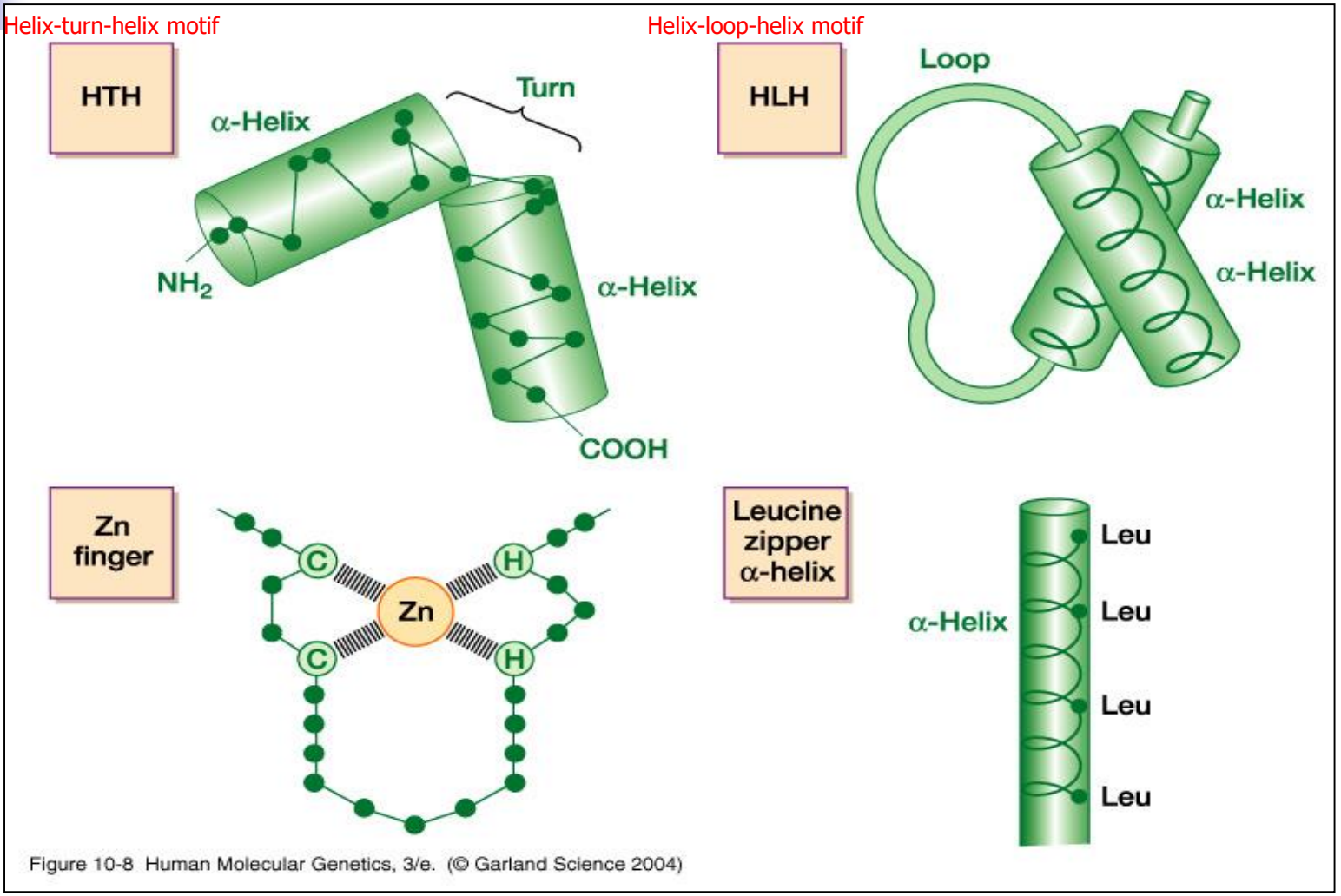


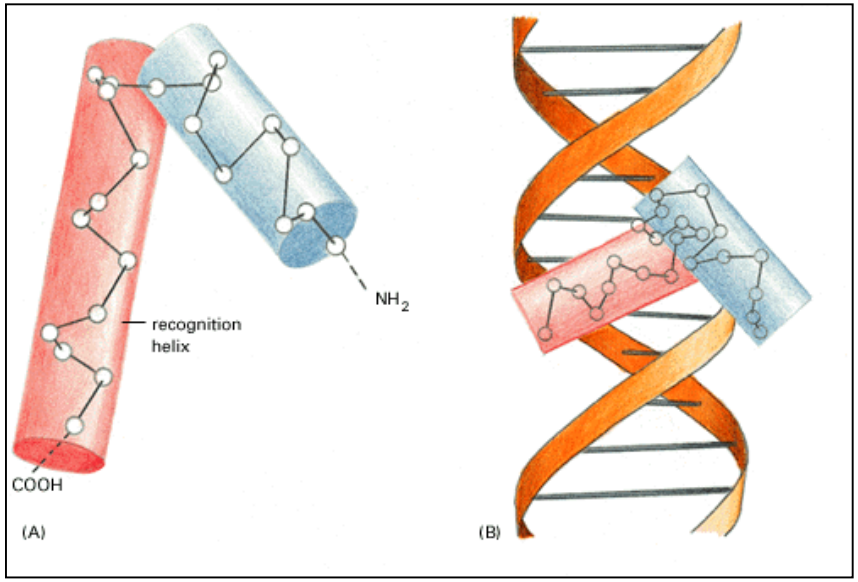
Figure 10-8 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Sequence-specific DNA-binding motifs
Helix-turn-helix motif
Interacts with the major groove of DNA

A. Each white circle denotes the central carbon of an amino acid.

B. The carboxyl-terminal alpha helix (red) is called the recognition helix because it participates in sequence-specific recognition of DNA.

- As shown in (B), this helix fits into the major groove of DNA, where it contacts the edges of the base pairs.



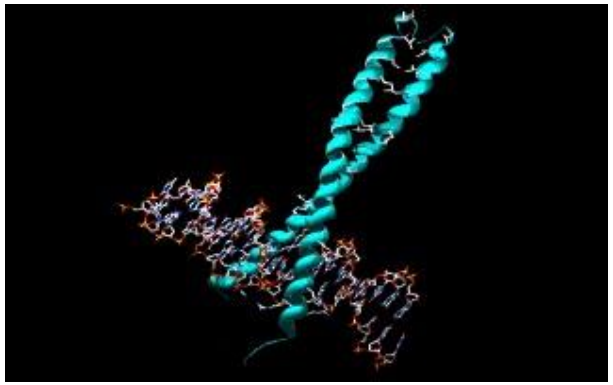
HTH consists of two α helices.

Alberts *et al.*,1994

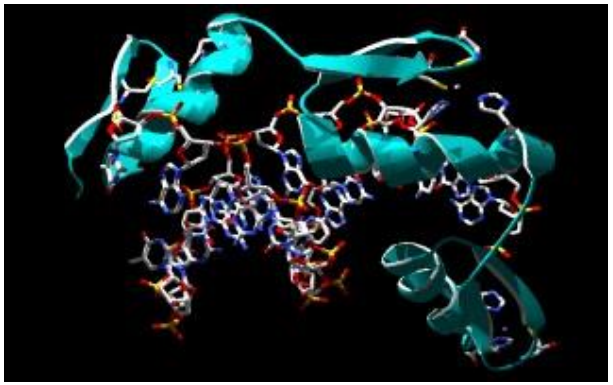
DNA-binding proteins

DNA-binding domains

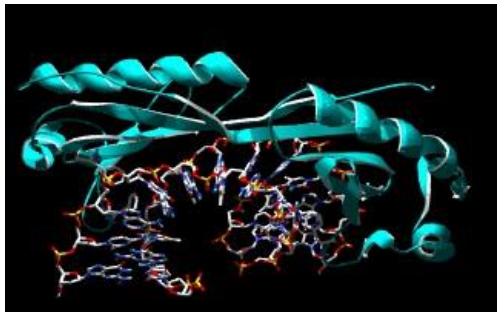
Structural DNA-binding motifs



Leucine zipper



Zif 286 protein (zinc finger)



TATA-binding Protein

It binds a highly conserved sequence (TATAAAAG) in the promoter of eukaryotes.

DNA-binding proteins

DNA-binding domains

DNA-binding motif

- Protein-protein and protein-nucleic acid interactions are essential functions of many proteins.
- DNA-binding proteins are proteins that are composed of DNA-binding domains.
- The term domains in biochemistry and molecular biology is usually used to refer to a part of a protein that has a conserved structure and function.
- The role of the DNA-binding domain is to bring the transcription-activation domain into the vicinity of the promoter.

1. A domain can contain several motifs.
2. Motifs are short sequences and domains are longer ones.

DNA-binding proteins

DNA-binding motifs

DNA-binding motifs

- Transcription factor (sometimes called a sequence-specific DNA-binding factor) is a protein that binds to specific DNA sequences.
- TFs acting as flags on DNA and regulating the transcription of genes.
- In general, transcription factors are dimeric, each with one helix containing basic amino acid residues that facilitate DNA binding.

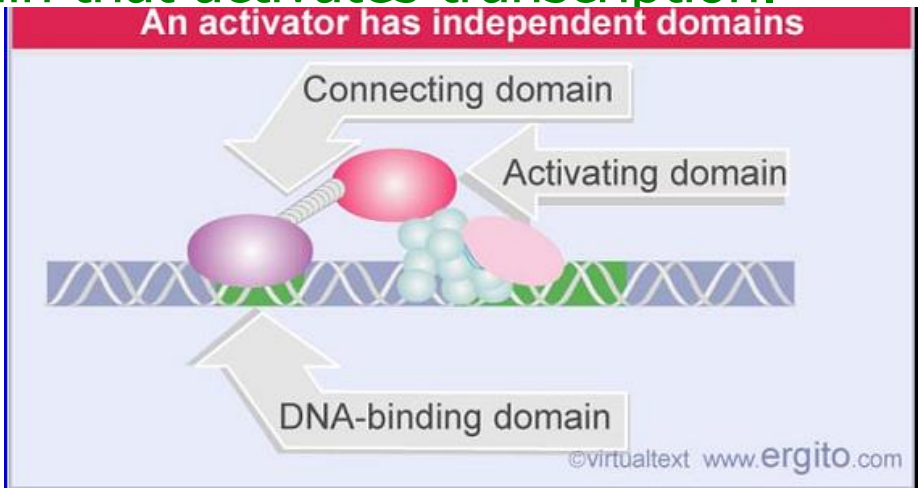
DNA-binding proteins

DNA-binding domain

DNA-binding motifs

- All transcription factors have two domains that are necessary for their function.

1. A DNA-binding domain (motifs that binds DNA such as helix-turn-helix, helix-loop-helix, leucine zipper, zinc finger,..);
2. A domain that activates transcription.



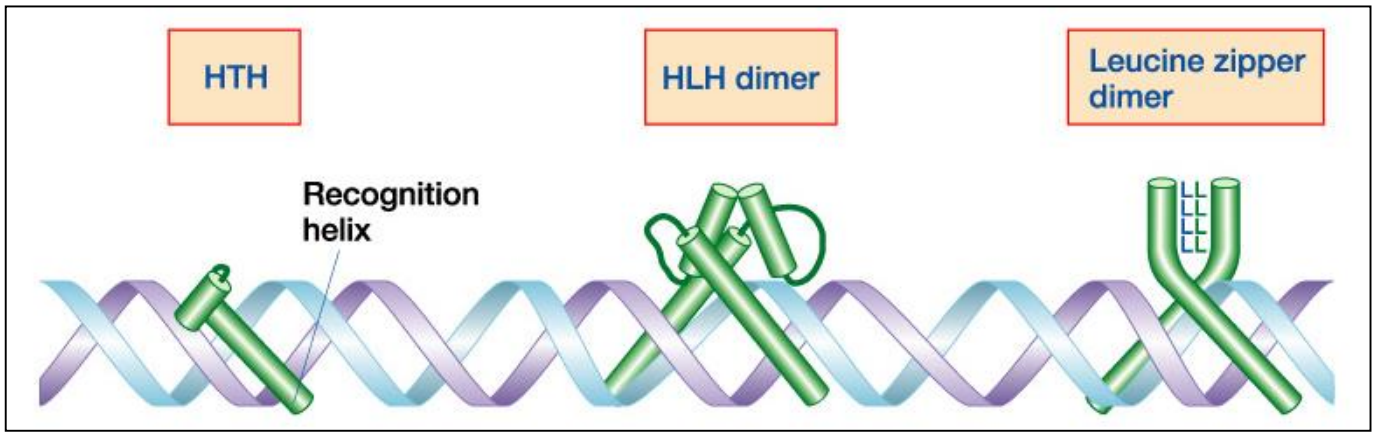
The role of the DNA-binding domain is to bring the transcription-activation domain into the vicinity of the promoter.

DNA-binding proteins

DNA-binding motifs

Structural DNA-binding motifs

- A DNA-binding domain (DBD) contains at least one motif that recognizes double- or single-stranded DNA. This specific binding region is called a promoter.
- The transactivation domain (TAD) is where other proteins (co-regulatory proteins) bind to the transcription factor(in bacteria sigma factor).



M. F. Kusie; Wiki,..

Prokaryotic DNA transcription
Phases of transcription

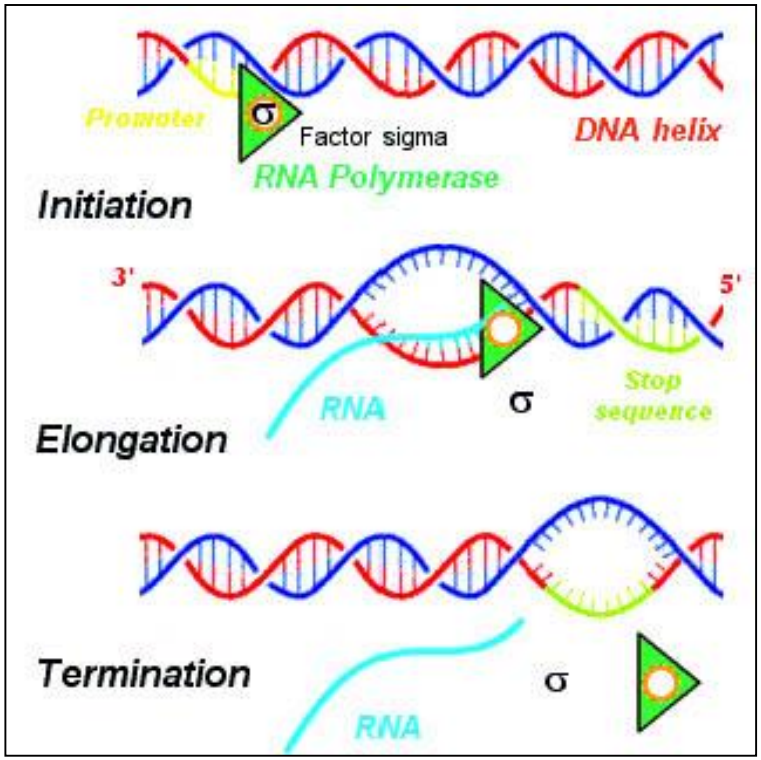
1. Initiation: Binding of RNA polymerase to promoter, unwinding of DNA, formation of primer.
2. Elongation: RNA polymerase catalyzes the processive elongation of RNA chain, while unwinding and rewinding DNA strand.
3. Termination: termination of transcription and disassemble of transcription complex.

Prokaryotic DNA transcription

mRNA transcription is recognized by the sigma protein

Transcription proceeds in one direction

- In bacteria, all transcription is performed by a single type of RNA polymerase.
- The sigma subunit dissociates from the RNA polymerase core enzyme shortly after transcription begins.
- Nucleotides are added onto the 3' end of the growing RNA chain.



However, a recent study has shown that σ^{70} can remain attached in complex with the core RNA polymerase, at least during early elongation. It was indicates that sigma plays roles during early elongation.

Prokaryotic DNA transcription

mRNA transcription is recognized by the sigma protein

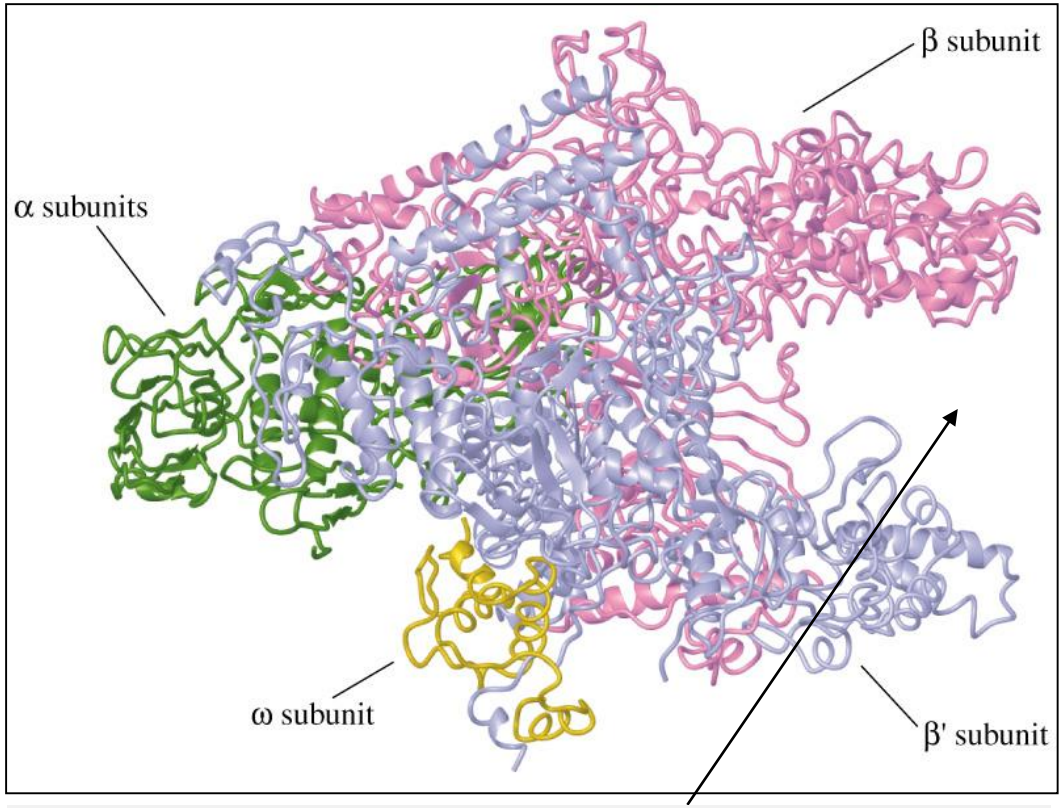
RNA polymerase copy DNA to RNA(mRNA)

- In initiation process, RNA polymerase holo-enzyme (RNA polymerase+sigma factor) binds to DNA and scans for promoter sequences (start sequences).
- The sigma factor is the subunit of the RNA polymerase complex that recognizes the specific promoter sequence of DNA that the RNA polymerase complex should bind to.
- Scanning occurs in only one dimension, 100 times faster than diffusion limit.
- During scanning enzyme is bound non-specifically to DNA.
- Can quickly scan 2000 base pairs.

Holoenzyme is the core enzyme saturated with sigma factor 70. A biochemically active compound formed by the combination of an enzyme (core enzyme) with a coenzyme or cofactor(sigma factor).

Prokaryotic RNA polymerase

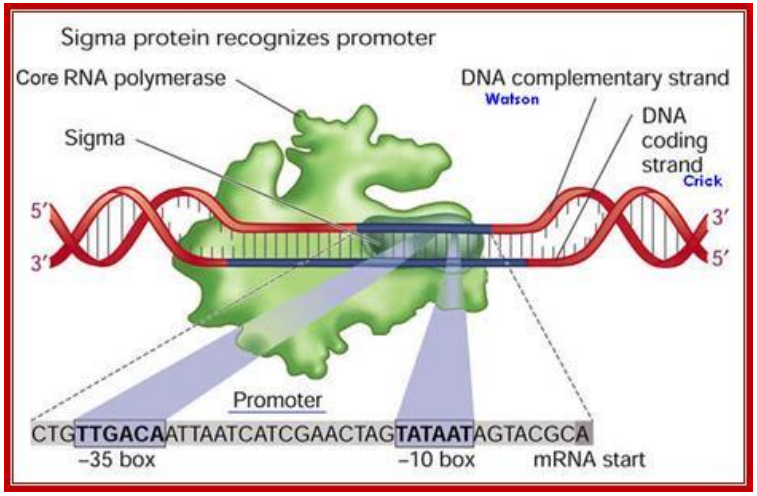
The enzyme without the sigma subunit is called the 'core' enzyme



Site of DNA binding and RNA polymerization.

Prokaryotic RNA Polymerase

- Unlike the eukaryotes, bacteria have sigma factors.
- A sigma factor (σ factor) is a protein needed only for initiation of RNA synthesis.
- The sigma factor is the subunit of the RNA polymerase complex that recognizes the specific promoter sequence of DNA that the RNA polymerase complex should bind to.

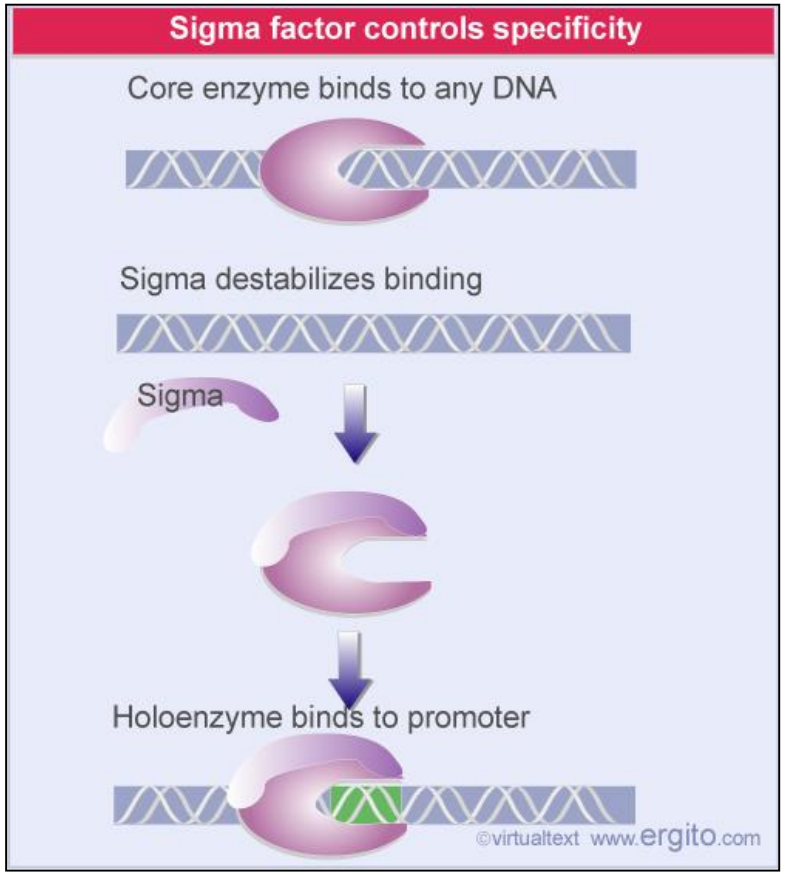


Prokaryotic DNA transcription

Initiation

Sigma factors

- Sigma factors are specialized bacterial proteins that bind to RNA polyerases for transcription initiation.
- Several distinct sigma factors have been identified.
- Sigma factors are discriminatory, as each binds a distinct set of promoter sequences.



Holoenzyme is the core enzyme saturated with sigma factor 70.

Transcription and Gene Expression

Transcription in bacteria

Sigma factors

A sigma factor (σ factor) is a prokaryotic initiation enzyme factor that must be part of RNA polymerase for attachment to promoter sites on DNA.
Shortly thereafter, RNA polymerization begins (the first nucleotide bears a triphosphate on it 5' OH) and the sigma subunit is released.

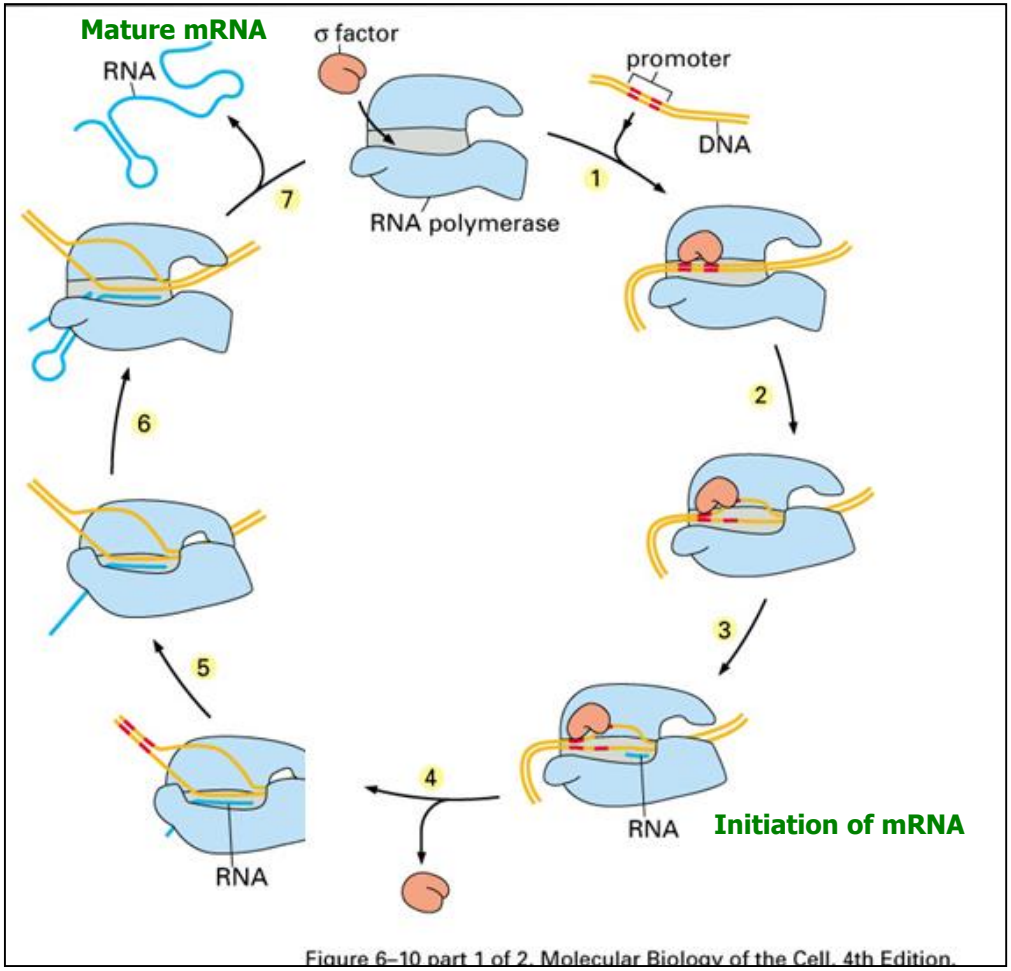
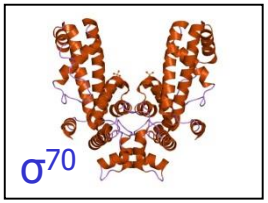


Figure 6–10 part 1 of 2, Molecular Biology of the Cell, 4th Edition.

Prokaryotic DNA transcription

Initiation

Sigma factors or sigma factor regulons



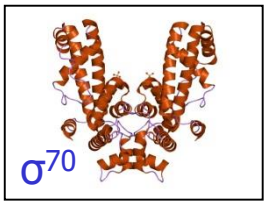
- Sigma factor (also known as the regulon) is a protein needed only for initiation of RNA synthesis.
- Sigma factors are a major regulator of prokaryotic gene expression.
- It is well known that bacteria use different sigma factors to control the initiation specificity at different promoters, including those promoters whose genes encode virulence factors.

Paget *et al.*,2003; Wilson *et al.*,2015

Prokaryotic DNA transcription

Initiation

Sigma factors



- Including both:

1. Major or abundant primary (housekeeping) sigmas, and
2. Minor alternative (secondary) sigmas from diverse organisms.

- Every molecule of RNA polymerase holoenzyme contains exactly one housekeeping" sigma factor (primary sigma factor), transcribes most genes in growing cells.
- Primary sigma factor is present in all growth conditions.

1. The number of sigma factors varies between bacterial species.

- Thus there are members of the σ^{70} family of sigma factors.

Paget *et al.*,2003;..

Sigma factors

Bacterial transcription initiation factor

Different numbers and specialized sigma factors

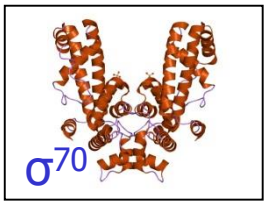
- Sigma factor (σ factor) is a protein needed only for initiation of RNA synthesis.
- It is a bacterial transcription initiation factor that enables specific binding of RNA polymerase to gene promoters.
- The number of sigma factors varies between bacterial species.
- Different sigma factors are utilized under different environmental conditions.
- These specialized sigma factors bind the promoters of genes appropriate to the environmental conditions, increasing the transcription of those genes.

Wikipedia

Prokaryotic DNA transcription

Initiation

Sigma factors



- Every molecule of RNA polymerase holoenzyme contains exactly one sigma factor subunit.
- Sigma factors allow sequence-specific binding of RNA polymerase to bacterial promoters.
- The number of sigma factors varies between bacterial species.
- *E. coli* has seven sigma factors.
- The 10 sigma factors thus far identified in *B. subtilis*.
- *Streptomyces avermitilis* and *Streptomyces coelicolor* with 13 and 14 σ^{70} genes, respectively.
- Sigma factors are distinguished by their characteristic molecular weights.
- For example, σ^{70} refers to the sigma factor with a molecular weight of 70KDa.

Prokaryotic DNA transcription
Major and minor sigma factors

Name	Function
σ^{70}(RpoD) (major)	Housekeeping sigma factor transcribes most genes in growing cells
σ^{19} (FecI)	the ferric citrate sigma factor, regulates the fec gene for iron transport
σ^{24}(RpoD)	the extracytoplasmic/extreme heat stress sigma factor
σ^{28}(RpoD)	the flagellar sigma factor
σ^{32} (RpoH)	RNA polymerase sigma factor RpoH. The heat shock sigma factor, it is turned on when the bacteria are exposed to heat
σ^{38} (RpoS)	the starvation/stationary phase sigma factor
σ^{54} (RpoN)	the nitrogen-limitation sigma factor

Prokaryotic DNA transcription

Anti-sigma factors

Anti-sigma factor 70 Rsd against sigma factor 70

- Anti-sigma factors bind to sigma factors and inhibit transcriptional activity.
- Anti-sigma factors are antagonists to the sigma factors, which regulate numerous cell processes including flagellar production, stress response, transport and cellular growth.
- For example, anti-sigma factor 70 Rsd protein in *E. coli* is present in the stationary phase and blocks the activity of sigma factor 70 which in essence initiate gene transcription.

Note: There are also anti-sigma factors that inhibit the function of sigma factors and anti-anti-sigma factors that restore sigma factor function.

Thermophilic organisms
Bacteria and archaea

- All thermophilic organisms are prokaryotes and archaea.
- These protect their DNAs from degradation by:

1. Salts like potassium and magnesium are found at higher levels in thermophilic archaea. These salts protect double-stranded DNA from phosphodiester bond degradation.
2. Polyamines (positively charged organic compounds that are derived from amino acids) also protect against degradation.
3. Positively supercoiled DNA (by making many reverse DNA gryase)appears to resist degradation more than negatively supercoiled DNA.
4. Gistone-like proteins are found in thermophilic organisms.

Porterfield,2015

Protein Folding

Heat-shock proteins (HSPs)

Quality control of protein folding

- Heat shock proteins (HSP) are a family of proteins that are produced by cells in response to exposure to stressful conditions.
- HSPs are found in virtually all living organisms, from bacteria to humans.
- Similar phenomena in prokaryotes and other eukaryotes.

1. In eukaryotes this regulation is performed by heat shock factor (HSF) and
2. In bacteria by sigma factor (σ^{32}).

Wikipedia,2015

Protein Folding

Heat-shock proteins (HSPs)

Quality control of protein folding

- The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress.
- They were first described in relation to heat shock but are now known to also be expressed during other stresses including:

1. exposure to cold,
2. UV light, and
3. during wound healing or tissue remodeling.

Ron *et al.*,2000;..

Protein Folding

Heat-shock proteins (HSPs) in bacteria

Quality control of protein folding

- The bacterial heat-shock response is not limited to changes in temperature and is a general stress response, but also induced by other environmental changes, such as:

1. the addition of ethanol,
2. heavy metals,
3. high osmolarity,
4. pollutants,
5. Starvation, or
6. interaction with eukaryotic hosts (for diseases).

Note: heat-shock proteins have been shown to play an important role in pathogenesis.

Protein Folding

Heat-shock proteins (HSPs)

Quality control of protein folding

- Heat-shock proteins are named according to their molecular weight.
- For example,

1. Hsp40 (heat shock protein kilodaltons in size). This is also known as chaperone DnaJ.
2. Hsp60 (heat shock protein 60 kilodalton in size)
3. Hsp70 (heat shock protein 70 kilodaltons in size)
4. Hsp90 (heat shock protein 90 kilodaltons in size).

Hsp70 family chaperone:

The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress. Prokaryotes express three Hsp70 proteins: DnaK, HscA (Hsc66), and HscC (Hsc62).

Protein Folding

Heat-shock proteins (HSPs)

Quality control of protein folding

- The heat-shock response controls the expression of more than 20 genes that code for:

1. chaperones,
2. proteases and
3. regulatory proteins.

Ron *et al.*,2000

Protein Folding

Heat-shock proteins (HSPs)

DnaK-DnaJ-GrpE chaperone system+σ^{32}

- The heat-shock response is the mechanism by which cells react to increases in temperature to prevent damage, and it involves the expression of the almost universally conserved heat-shock genes.
- Many heat-shock proteins (HSPs) are molecular chaperones or proteases and function by:

1. Facilitating refolding of damaged proteins, or
2. Eliminating proteins that cannot be repaired.

- σ^{32} (RpoH) - the heat shock sigma factor, it is turned on when the bacteria are exposed to heat. Due to the higher expression, the factor will bind with a high probability to the polymerase-core-enzyme.
- σ^{32} (RpoH) directly controlled by the DnaK-DnaJ-GrpE chaperone system.

Protein Folding

Heat-shock proteins (HSPs)

Quality control of protein folding

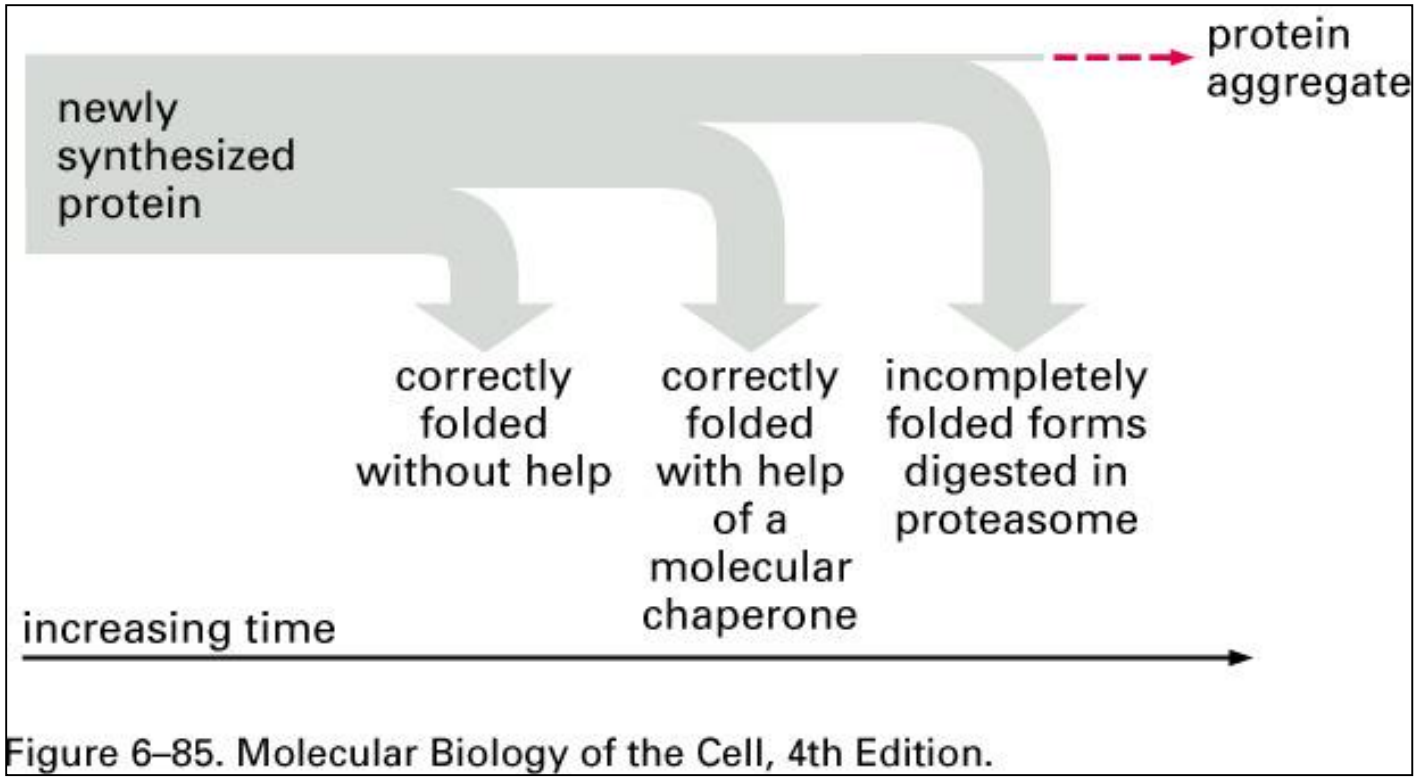


Figure 6–85. Molecular Biology of the Cell, 4th Edition.

Protein Folding

Heat-shock proteins (HSPs)

Protease action on unneeded/damaged proteins

- The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.

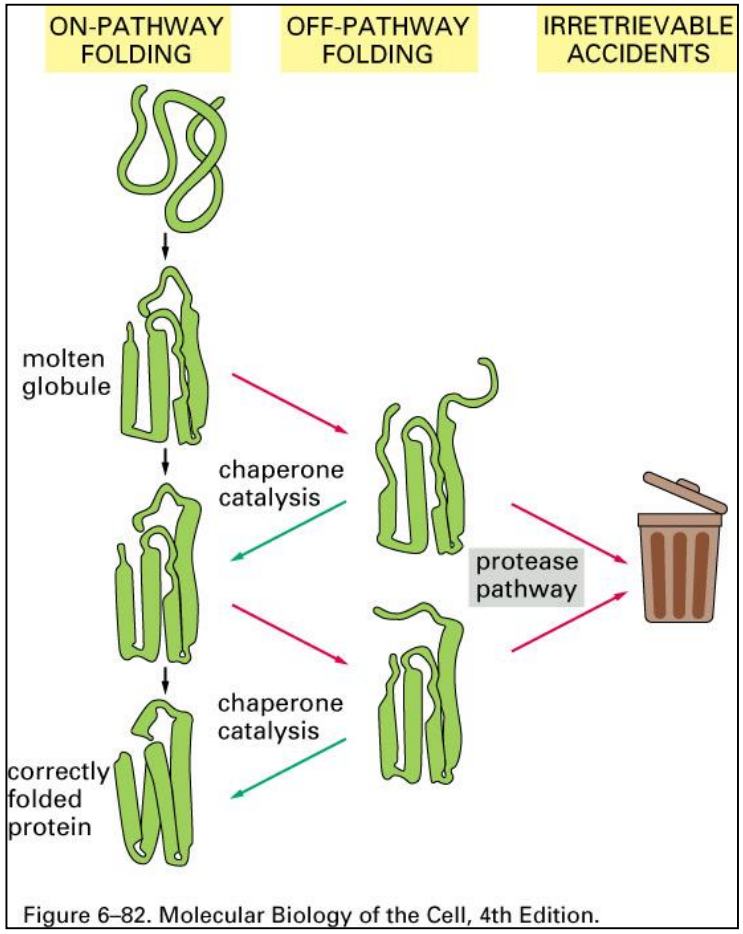


Figure 6–82. Molecular Biology of the Cell, 4th Edition.

Protein Folding

Heat-shock proteins (HSPs)

The DnaK chaperone system

- The chaperon Hsp70 is the product of the bacterial *dnaK* gene.
- The co-chaperones DnaJ and GrpE are products of *DnaJ* and *grpE* genes, respectively.
- The chaperone system formed by association of DnaK, DnaJ and GrpE chaperons.

The DnaK chaperone system mediates inactivation and degradation of σ^{32} probably through association with the heat shock promoter specific σ^{32} subunit of RNA polymerase.

Sigma 32 (sigma RpoH) factor
Heat shock control

- σ^{32} has greater affinity for RNA polymerase, core enzyme than σ^{70} at high temperatures.
- σ^{32} is also responsible for genetic responses to other environmental insults.
- σ^{32} regulation is a multivalent process consisting of transcriptional, translational and postranslational controls.

Protein Folding

Heat-shock proteins (HSPs)

DnaK-DnaJ-GrpE chaperone system+σ^{32}

- σ^{32} (RpoH) is turned on when the bacteria are exposed to heat.
- This heat-shock sigma factor (σ^{32}) is coded by the *rpoH* gene and binds to specific heat-shock promoters located upstream of heat-shock genes.

dnaK gene codes for ⟶ DnaK chaperone (HSP70) ⟶ DnaK-DnaJ-GrpE chaperone system regulates activity of the bacterial heat shock transcription factor σ^{32} (RpoH).

Protein Folding

Heat-shock proteins (HSPs)

The DnaK chaperone system

- In *Escherichia coli* the genes encoding HSPs form a regulon that is positively controlled by:

1. the *rpoH* gene product;
2. the heat shock promoter-specific σ^{32} subunit of RNA polymerase.

- The level and activity of σ^{32} are limiting for heat shock gene transcription.

Gamer *et al.*,1996

Protein Folding

Heat-shock proteins (HSPs)

DnaK-DnaJ-GrpE chaperone system+σ^{32}

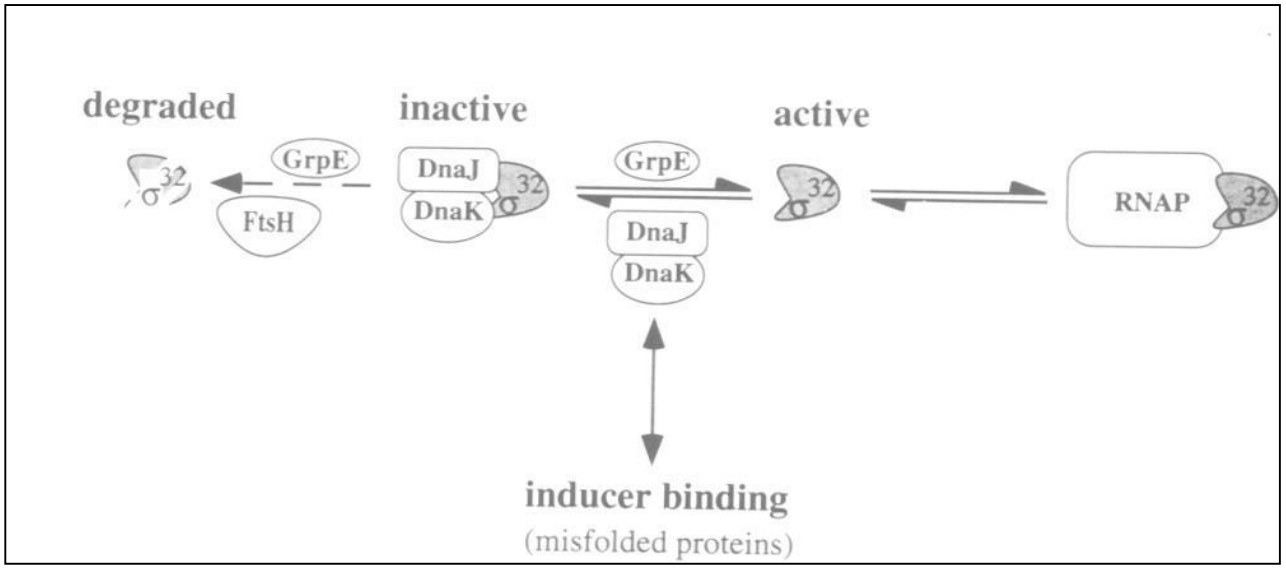
1. During heat shock (positive response), the intracellular concentration of RpoH increases, due to slightly increased transcription, increased synthesis and stabilization of the protein.
2. In the absence of heat shock, or after heat shock(negative response), activity is inhibited by transient association with DnaK-DnaJ-GrpE system, which reduces the amounts of free active RpoH, makes it unstable and mediates its degradation by the FtsH protease.

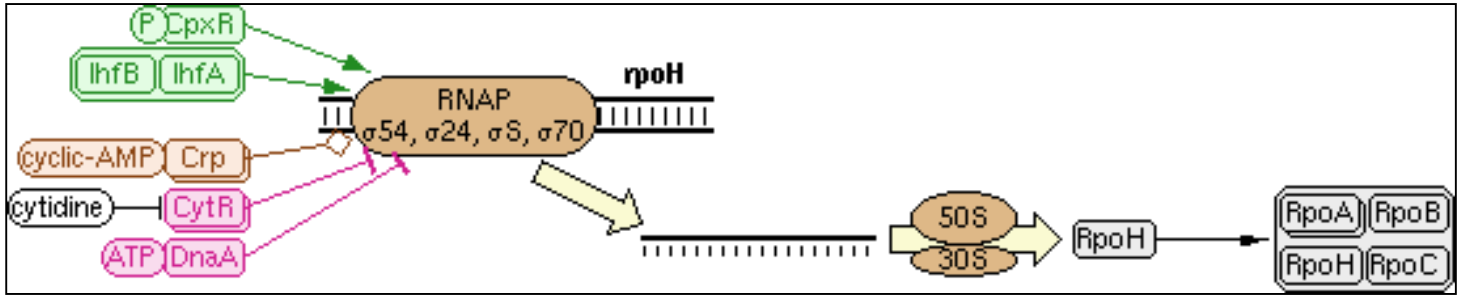
The DnaK chaperone system mediates inactivation and degradation of σ^{32} probably through association with the heat shock promoter specific σ^{32} subunit of RNA polymerase.

Protein Folding

Heat-shock proteins (HSPs)

The DnaK chaperone system





Gamer *et al.*,1996;Biocyc.org

Protein Folding

Heat-shock proteins (HSPs)

DnaK-DnaJ-GrpE chaperone system+σ^{32}

- Induction occurs mainly at the post-transcriptional level, via translational thermoregulation:

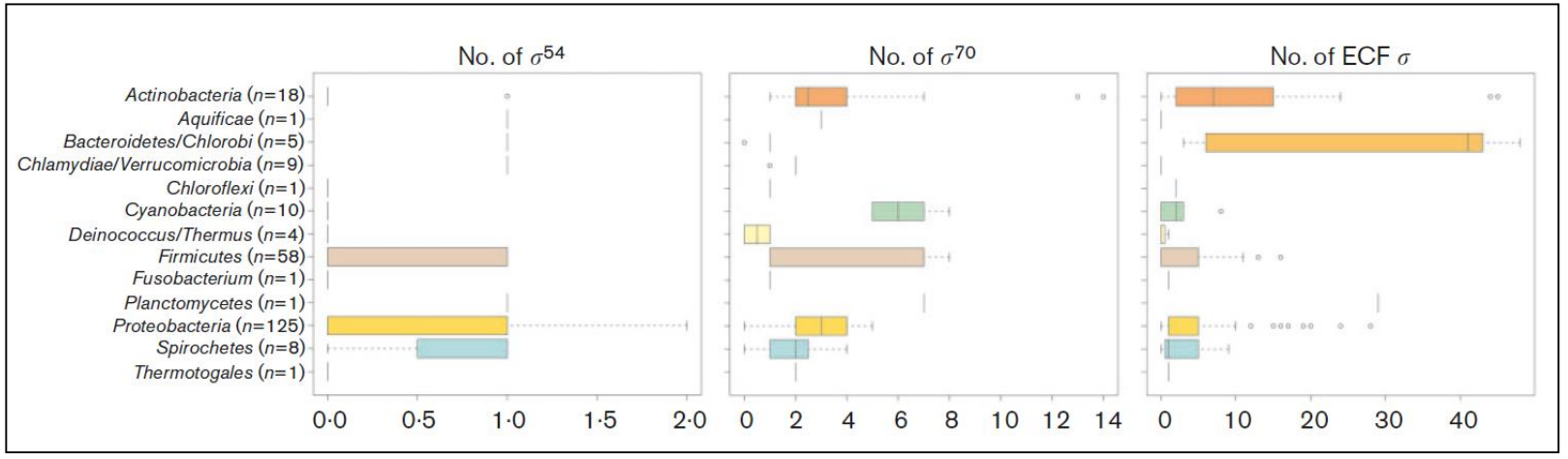
1. At low temperature, the structure of the rpoH mRNA blocks its translation,
2. while at high temperature, melting of the mRNA secondary structure facilitates ribosome binding and synthesis of the RpoH protein.

UniProt

Prokaryotic DNA transcription

Box and whisker plot of the number of sigma factor proteins in 13 different bacterial phyla

- *P. fluorescens* Pf5 is the largest genome and it also has by far the largest number of sigma factors – a total of 33 (1 σ^{54}, 4 σ^{70} and 28 ECF σ^{E}).
- The σ^{70} group is shown in the middle.
- The extracytoplasmic function (ECF) Group IV sigma factors are conserved across both Gram-positive and Gram negative species. σ^{E}, an ECF sigma factor, important for bacterial responses at the cell surface
- nitrogen-responsive σ factor.



Kill *et al.*,2015

Prokaryotic DNA transcription

Alternative sigma factors

Minor alternative (secondary) sigmas

- Bacteria and especially those capable of persisting in diverse environments, such as *Escherichia coli* provide particularly valuable models for exploring how single-celled organisms respond to environmental stresses.
- Bacteria have developed sets of specific response genes that are regulated by a subset of the σ^{70}-like sigma factors in order to respond to a changing environment. E.g.

1. When the cell is put under stress by high temperature.
2. Sporulation sigma factors in *Bacillus subtilis*.

- Because it is the sigma subunit that is responsible for promoter recognition, different sigma subunits may allow different promoters to be recognized.

Sofer,1999;..

Sigma factors
Alternative sigma factors

- Alternative sigma factors and promoter recognition sequences.
- *In *B. subtilis*.
- All others are in *E. coli*.

-35 region	Spacer	-10 region	Sigma factor	Regulated genes
TTGACA	N_{16-18}	TATAAT	σ^{70}	General housekeeping genes
CTTGAAA	N_{13-15}	CCCATNT	σ^{32}	Heat shock genes
CTGGNA	N_6	TTGCA	σ^{54}	Nitrogen assimilation genes
		CTACACT	σ^{38}	Stationary phase/General stress genes
GTTTAA	N_{12-14}	GGGTAT	σ^{B}	General stress genes*
TAAA	N_{15}	GCCGATAA	σ^{28}	Flagella genes

Dale and Park,2004

Prokaryotic DNA transcription

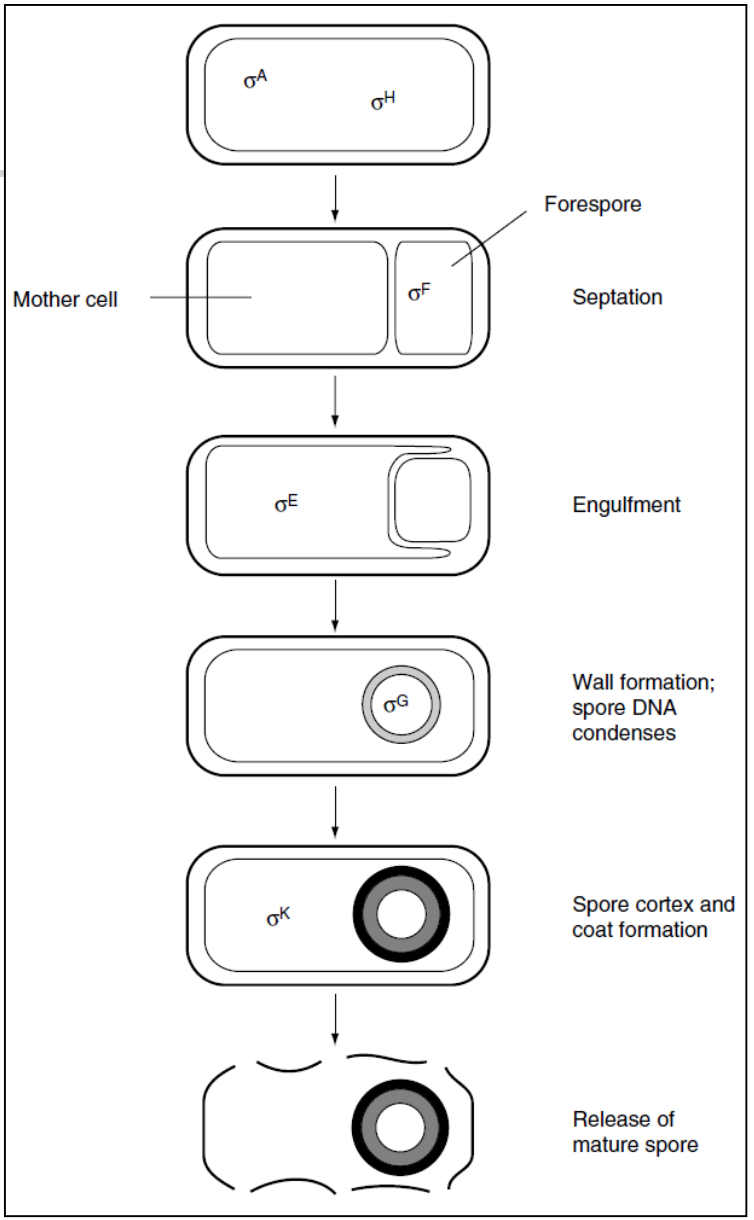
RNA polymerase holoenzymes containing other sigma factors recognize different core promoter sequences

Factor	Gene	Use	-35	Separation	-10
σ^{70}	*rpoD*	General	TTGACA	16 - 19 bp	TATAAT
σ^{32}	*rpoH*	Heat Shock	CCCTTGAA	13 - 15 bp	CCCGATNT
σ^{28}	*fliA*	Flagella	CTAAA	15 bp	GCCGATAA
σ^{54}	*rpoN*	Nitrogen starvation	CTGGNA	6 bp	TTGCA

Alternate sigma factors are used to express specific sets of genes.

Sigma factors
Sporulation in *Bacillus*

- The diagram shows the stages at which each sigma factor first becomes active.
- Sporulation is governed by a complex transcriptional regulatory programme that controls the expression of more than 100 genes and involves the sequential activation of six different σ-factor.



Dale and Park,2004

Prokaryotic DNA transcription

Alternative sigma factors

Minor alternative (secondary) sigmas

- σ^S(RpoS) and σ^B (SigB) have been identified as general stress responsive alternative sigma factors in Gram-negative and in Gram-positive bacteria, respectively.
- σ^B, a Group III sigma factor encoded by *sigB*, was initially identified and characterized in *B. subtilis*, but has also been identified in other Gram-positive bacteria.
- The *B. subtilis* σ^B-dependent general stress regulon is large: over 200 genes are expressed following bacterial exposure to heat, acid, ethanol, salt stress, entry into stationary phase, or starvation for glucose, oxygen, or phosphate.
- *sigB* mutants are sensitive to oxidative stress.

Boor,2006

Alternative sigma factors and their roles in bacterial virulence

The stationary phase sigma factor RpoS

- Heat-shock proteins are also essential for stationary phase.
- The alternative sigma factors RpoS (σ^{38}) has been shown to regulate the expression of genes in response to stationary phase, nutrient deprivation, and oxidative and osmotic stress.
- These are environments which are physiologically relevant to those encountered by many microbial pathogens during the natural course of infection.
- The RpoS sigma factor has been shown to be important for virulence in a number of plant and animal bacterial pathogens including *P. aeruginosa*.

Kazmierczak *et al.*,2008

Alternative sigma factors and their roles in bacterial virulence

The stationary phase sigma factor RpoS

- Among the pathogenic bacteria, *Pseudomonas aeruginosa* is perhaps the best understood in terms of the virulence factors regulated and the role the Quorum sensing plays in pathogenicity.
- Regulation of Quorum sensing by RpoS in *Pseudomonas aeruginosa*.
- RsmA, RpoS, QocR all negatively regulate the Rhl or Las Quorum sensing systems, thus preventing early activation of these systems.

Whiteley *et al.*,2000; Deep *et al.*,2000

Alternative sigma factors and their roles in bacterial virulence

PvdS of *Pseudomonas aeruginosa*

- Some bacterial pathogens are known to express virulence genes in low iron environments.
- In *P. aeruginosa*, the siderophore pyoverdine is a virulence factor.
- PvdS is required for virulence and appears to regulate only virulence-related genes.
- The genes involved in pyoverdine synthesis are located in three clusters on the *P. aeruginosa* chromosome and regulated by ECF sigma factor PvdS.

Extracytoplasmic function (ECF) sigma factors, σ^E

Kazmierczak *et al.*,2008; Brooks and Buchanan,2008

Alternative sigma factors involved in virulence

- As alternative sigma factors have been shown to regulate expression of both virulence and virulence-associated genes, these sigma factors can contribute both directly and indirectly to bacterial virulence.

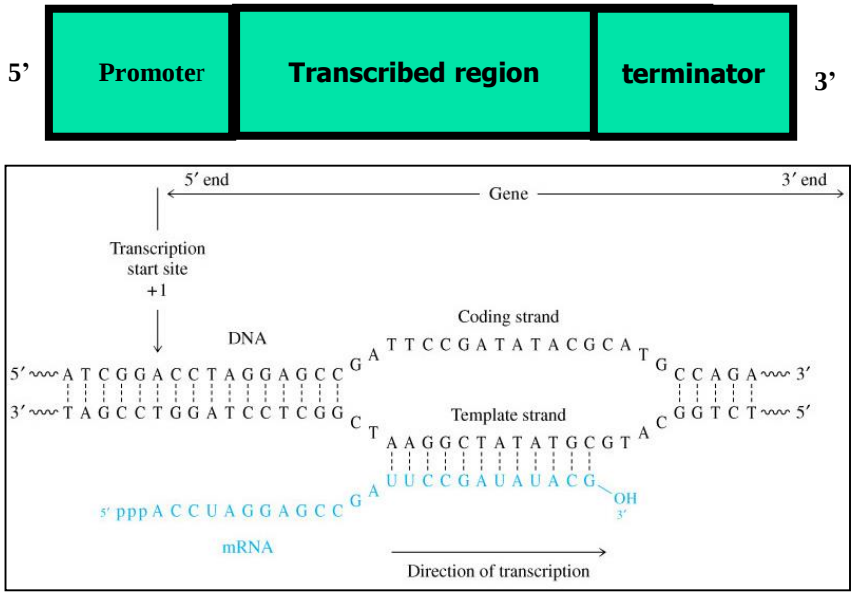
Family, class, and sigma factor[a]	Bacterial species[b]
σ^{70} family	
Stress response	
σ^{B}	*B. anthracis*, *L. monocytogenes*, *M. tuberculosis*, *S. aureus*, *S. epidermidis*
σ^{S}	*E. coli*, *P. aeruginosa*, *S. enterica* serovar Typhimurium, *S. enterica* serovar Typhi
σ^{F}	*M. tuberculosis*
ECF	
RpoE	*H. influenzae*, *S. enterica* serovar Typhimurium, *V. cholerae*
AlgU	*P. aeruginosa*
PvdS, FpvI	*P. aeruginosa*
σ^{C}	*M. tuberculosis*
σ^{D}	*M. tuberculosis*
σ^{E}	*M. tuberculosis*
σ^{H}	*M. tuberculosis*
HrpL	*Erwinia* spp., *P. syringae*
σ^{28}	
FliA	*C. jejuni*, *H. pylori*, *S. enterica* serovar Typhimurium, *V. cholerae*, *Y. enterocolitica*
σ^{54} family	
σ^{N}	*C. jejuni*, *H. pylori*, *L. monocytogenes*, *P. aeruginosa*, *P. syringae*, *V. cholerae*, *V. parahaemolyticus*

Kazmierczak *et al.*,2008

Transcription and Gene Expression

General gene structure

- Promoter: sequences recognized by RNA polymerase as start site for transcription.
- Transcribed region: template from which mRNA is synthesized.
- Terminator: sequences signaling the release of the RNA polymerase from the gene.

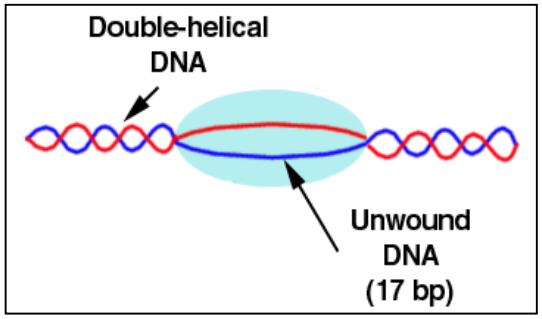


Prokaryotic DNA transcription

Initiation

Sigma factors

- Sigma (sometimes called sigma factor) is loosely bound to the enzyme, and, in fact, can be separated from it. The enzyme without the sigma subunit is called the 'core' enzyme.
- After binding at the promoter sequence, RNA polymerase unwinds the DNA in the vicinity of the enzyme and thereby separates the two strands.
- Shortly thereafter, RNA polymerization begins (the first nucleotide bears a triphosphate on it 5' OH) and the sigma subunit is released.



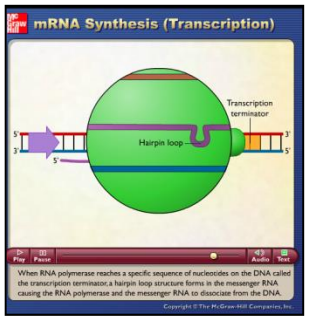
Sofer,1999

Prokaryotic DNA transcription
Elongation

- During the elongation phase, the enzyme moves along the DNA.
- A bubble representing the separation of the two strands of DNA in the vicinity of the enzyme accompanies this movement.
- This means that the DNA is being unwound ahead of the bubble and rewound behind it.
- Remember, RNA synthesis(transcription) is 5' to 3‘.
- Synthesis continues until the enzyme comes to a terminator sequence on the DNA, at which point RNA polymerase ceases RNA synthesis, releases the RNA, and falls off the DNA.

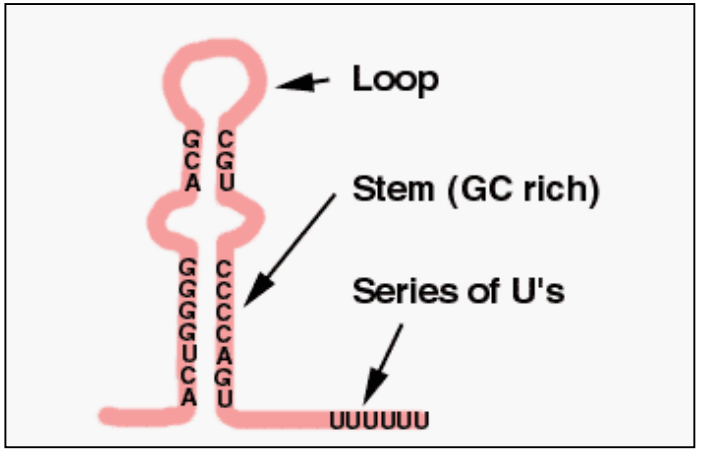
Sofer,1999

Prokaryotic DNA transcription
Termination



- Termination occurs in two ways in bacteria.
- When RNA polymerase encounters a characteristic two part sequence:

1. One part of the sequence causes the newly synthesized RNA to form a stem-loop structure.
2. The second part of the sequence produces a series of uridines (ribose and uracil) usually 6 or more.



The formation of the stem-loop structure in the RNA appears to cause the RNA polymerase to pause, and during this pause, the DNA/RNA hybrid dissociates and so does the enzyme, thereby stopping RNA synthesis.

Sofer,1999

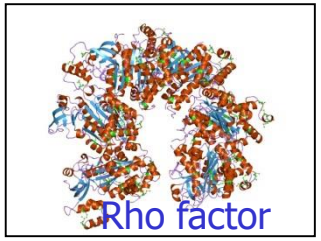
Cytoplasm of Prokaryotes

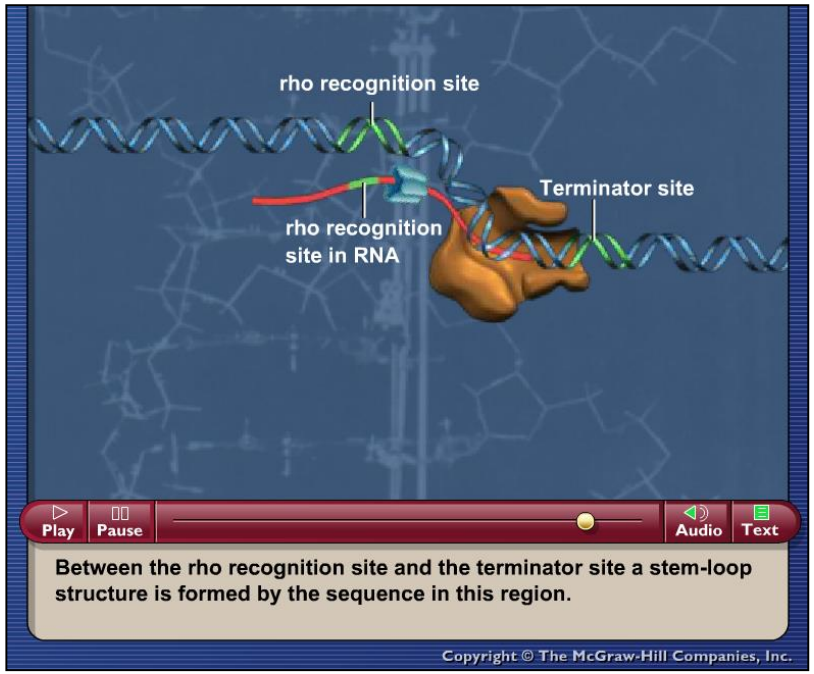
Cytoplasmic granules and inclusion bodies

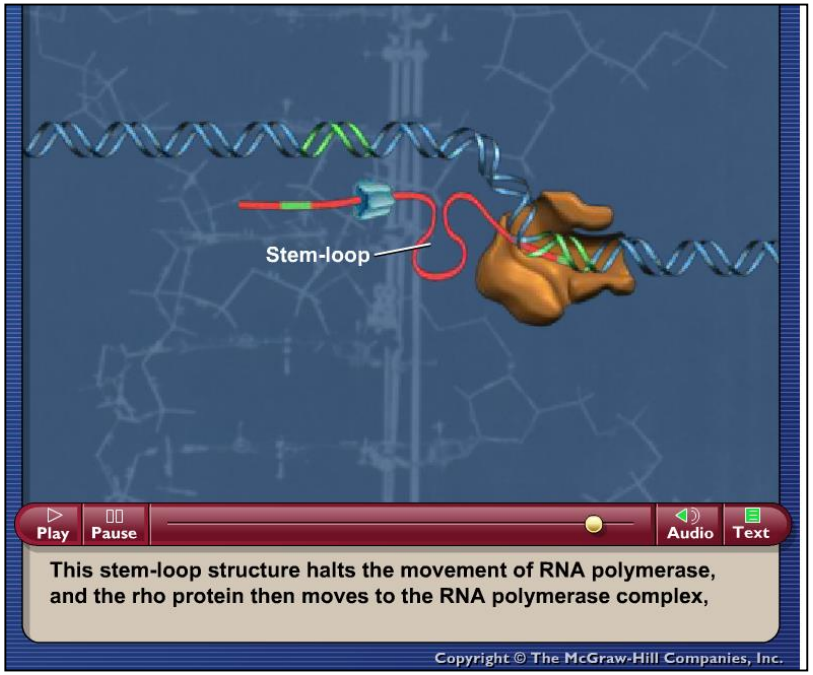
The bacterial genomes

Plasmids

Prokaryotic DNA transcription
Termination



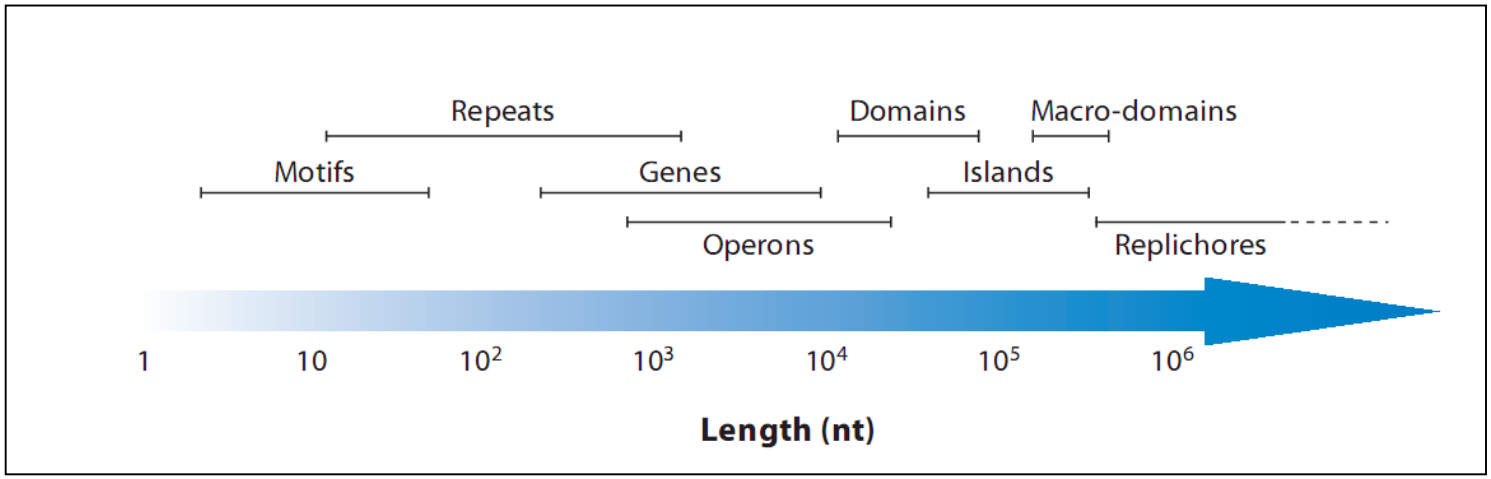




A ρ factor (Rho factor) is an additional protein, which causes RNA transcription in prokaryotes to be stopped. It is a ~274.6 kD hexamer of identical subunits. It terminates transcription in *Escherichia coli*.

The bacterial genomes
Chromosome vs plasmid sizes

- The scales of genome organization.
- These features shape chromosome organization at very different scales, from small motifs to very large chromosomal regions.



Rocha,2008

The bacterial genomes
Chromosome vs plasmid sizes

- Chromosome refers to the primary replicon.
- The chromosome is always the largest replicon in the genome and contains the majority of the core/essential genes.

1. The average and median bacterial genome (chromosome) sizes are ~ 3.87 Mb and ~ 3.65 Mb, respectively.

- The average and median plasmid sizes are ~ 78.9 kb and ~ 46.2 kb, respectively.

The relationships between bacterial plasmids and chromosomes are unclear (Zheng *et al.*,2015).

diCenzo and Finan,2017

The bacterial genomes

Chromosome vs plasmid sizes

The major features of genomes and all replicon classes

- Plasmids could represent up to 30% of the bacterial genomes (Fournes *et al.*, 2018).

Genome organization	Genome size (Mb)			Chromosomal GC content (%)			Chromosomal SCUO[a]		
	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum
Overall	3.64	0.16	13.1	49.04	14.55	74.91	0.28	0.13	0.7
Nonmultipartite	3.41	0.16	13.1	47.36	14.55	74.91	0.27	0.13	0.7
Multipartite	5.56	2.48	9.73	61.29	28.83	72.94	0.31	0.15	0.56

[a]SCUO (synonymous codon usage order) was calculated with CodonO (99) and is a measure of the extent of codon usage bias, with higher values indicating greater bias.

[a]SCUO (synonymous codon usage order) was calculated with CodonO and is a measure of the extent of codon usage bias, with higher values indicating greater bias.

diCenzo and Finan,2017

The bacterial genomes

Chromosome vs plasmid genomes

Longer sequences tended to have higher G+C content values

- Based on complete bacterial genome sequence data, we demonstrate a correlation between bacterial chromosome length and the G+C content of the genome, with longer genomes having higher G+C contents.

Bacterial genomes			
Organisms with highest bacterial genome G+C content			
1	*Anaeromyxobacter dehalogenans* 2CP-C (CP000251.1)	5013479	74.9
2	*Anaeromyxobacter* sp. K (NC_011145.1)	5061632	74.8
3	*Streptomyces rubrolavendulae* strain MJM4426 (CP017316.1)	6543262	74.8
4	*Corynebacterium sphenisci* DSM 44792 (NZ_CP009248.1)	2594799	74.7
5	*Cellulomonas fimi* ATCC 484 (NC_015514.1)	4266344	74.7
Organisms with lowest bacterial genome G+C content			
1	*Candidatus Zinderia insecticola* CARI (CP002161.1)	208564	13.5
2	*Candidatus Carsonella ruddii* CE isolate Thao2000 (CP003541.1)	162589	14.0
3	*Candidatus Carsonella ruddii* HC isolate Thao2000 (CP003543.1)	166163	14.2
4	*Candidatus Carsonella ruddii* CS isolate Thao2000 (CP003542.1)	162504	14.2
5	*Candidatus Carsonella ruddii* HT isolate Thao2000 (CP003544.1)	157543	14.6
Plasmid genomes			
Plasmids with highest G+C content			
1	*Streptomyces autolyticus* CGMCC0516 plasmid unnamed3 (NZ_CP019460.1)	30888	87.5
2	*Streptomyces autolyticus* CGMCC0516 plasmid unnamed8 (NZ_CP019465.1)	15591	83.3
3	*Streptomyces cattleya* NRRL 8057 plasmid pSCAT (FQ859184.1)	1809491	73.3
4	*Streptomyces cattleya* DSM 46488 plasmid pSCATT (CP003229.1)	1812548	73.3
5	*Streptomyces* sp. FR-008 plasmid pSSFR2 (CP009804.1)	24272	72.9
Plasmids with lowest G+C content			
1	*Candidatus Baumannia cicadellinicola* strain B-GSS plasmid (CP011788.1)	3465	20.3
2	*Blattabacterium* sp. (*Nauphoeta cinerea*) plasmid (NC_022551.1)	3674	20.6
3	*Borrelia burgdorferi* B31 plasmid lp21 (CP009673.1)	18777	20.6
4	*Streptobacillus moniliformis* DSM 12112 plasmid pSMON01 (CP001780.1)	10702	20.9
5	*Brachyspira intermedia* PWS/A plasmid pInt (CP002875.1)	3260	21.0

Almpanis *et al.*,2018

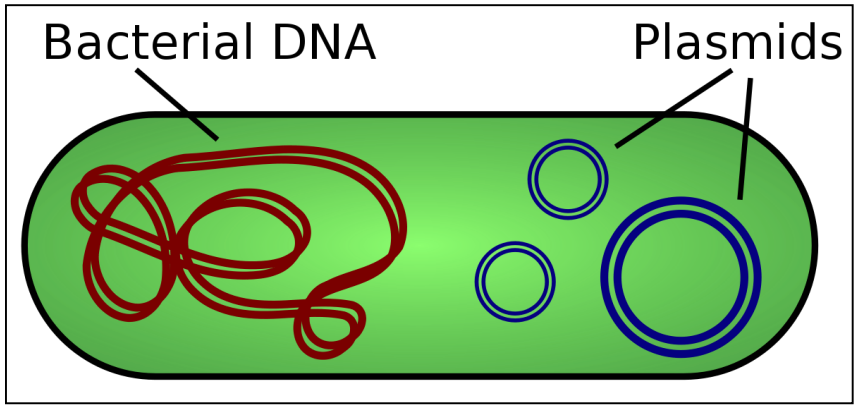
The bacterial plasmids

Small circular mini-chromosomes with their own origin of replication

- A plasmid is a small, often circular DNA molecule found in bacteria and other cells.
- Plasmids are:

1. separate from the bacterial chromosome, and
2. replicate independently of it.

- They generally carry only a small number of genes, notably some associated with antibiotic resistance.



Wikipedia,2020

The bacterial plasmids

Small circular mini-chromosomes with their own origin of replication

1. Plasmids are generally circular.
2. Some are linear plasmids.

- For transformation of foreign DNA:

1. *E. coli* prefer circular plasmid, while
2. *Bacillus subtilis* get higher transformation efficiency with linearized plasmid.

- Linear DNA is actively taken up by the cells and found in:

1. Most bacteria (Gram-ve and Gram+ve),
2. Number of eukaryotes, and
3. Even in the mitochondria of some plants contain independent pieces of DNA called plasmids.

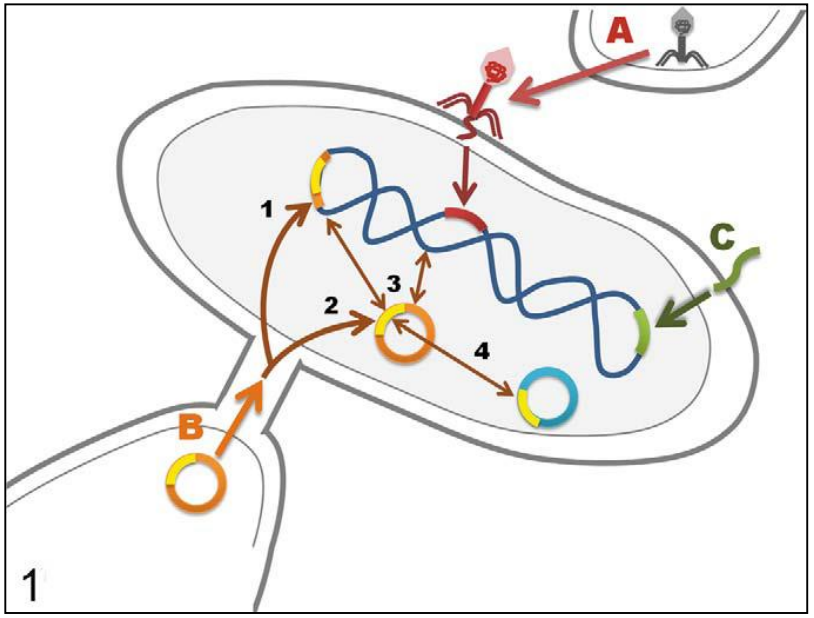
The bacterial plasmids

Mechanisms of DNA transfer between and within bacteria

A. Transduction: injection of DNA into a bacterium by a phage.

B. Conjugation: plasmid in a donor bacterium is transferred through a pilus into a recipient bacterium; plasmid may integrate into the chromosome (1) or remain in the cytoplasm (2); plasmid may be transferred between cytoplasmic and chromosomal locations (3); plasmid may exchange insertion sequences or transposons with other plasmids (4) or the chromosome.

C. Transformation: uptake of naked DNA from the environment.



Gyles and Boerlin,2014

The bacterial plasmids

Cryptic plasmids

- Plasmids are not essential to their bacterial host.

1. Plasmids can be easily gained or lost by a bacterium,
2. Plasmids can be transferred between bacteria as a form of horizontal gene transfer.

- Plasmids have been identified in a large number of bacterial genera.
- Some bacterial species harbor plasmids with no known functions (cryptic plasmids) which have been identified as small circular molecules present in the bacterial DNA.

Plasmids

Antibiotic resistance plasmids

- Genomic islands (GIs) contain many antibiotic resistant genes are referred to as antibiotic resistance islands.
- Genomic islands (GIs) encoding antibiotic resistance determinants.
- These genes are frequently carried as genomic islands on transmissible plasmids and provide ready sources of resistance determinants.
- These genes on plasmids encodes enzymes for resistant against antibiotics, heavy metals, radiation, etc.

Antibiotic resistance it may be needed when cells are exposed to that antibiotic but not needed when cells are growing in the absence of the antibiotic.

The bacterial plasmids
Origin

- Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes.
- When a bacterium divides, all of the plasmids contained within the cell are copied such that each daughter cell receives a copy of each plasmid.

Nature Education,2014

Plasmid stability

- One of the characteristic features of plasmids is their instability.
- Plasmid-borne features are often lost from a population at a higher frequency than would be expected for the normal processes of mutation.
- The extent of this instability varies enormously from one plasmid to another.
- There are three quite distinct phenomena associated with the concept of plasmid stability:

1. Plasmid integrity,
2. Partitioning at cell division, and
3. Differential growth rates.

Dale and Park,2004

Host range of bacterial plasmids

- The host range of a particular plasmid is usually limited to closely related genera.
- Some plasmids, however, are much more promiscuous and have a much broader host range.
- The broad-host-range (BHR) plasmids have been defined as those plasmids that can self-transfer themselves and can stably replicate and maintain in bacterial species from at least two subgroups within the Proteobacteria (e.g., between α- and β Proteobacteria).

Szpirer *et al.*,1999; Sen *et al.*,2011

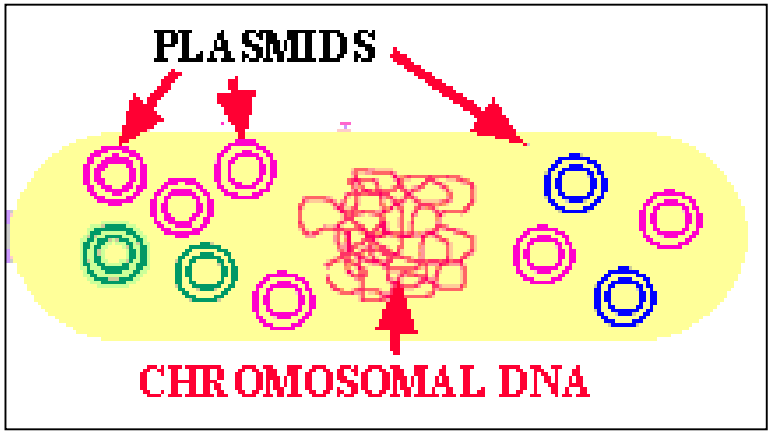
Numbers of plasmids

- A bacterial cell may contain:

1. no plasmids,
2. one plasmid, or
3. many copies of a plasmid.

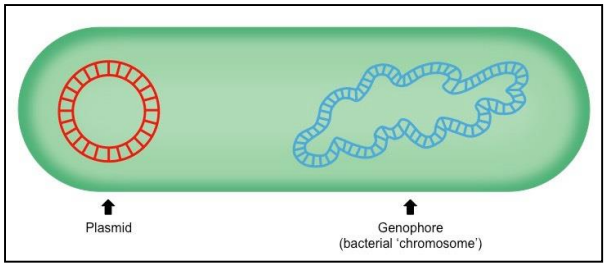
- The number of copies of the plasmid would depend of:

1. its size, and
2. the characteristics of its replication origin.



Some bacteria can survive without plasmids. e.g. a considerable number of intracellular bacteria such as *Brucella*, *Rickettsia*, and some endosymbionts. Some other may carry megaplasmids that acts more than little chromosomes. e.g. *R. solanacearum*.

Plasmid size



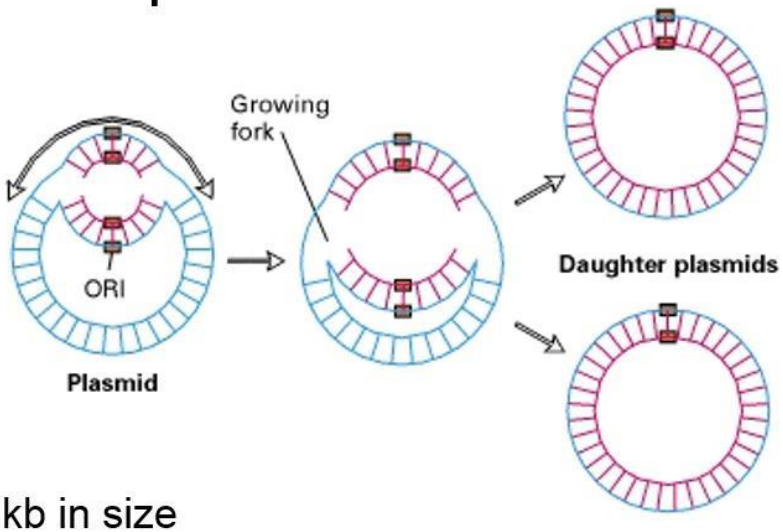
- In general, bacterial plasmids can be classified into two groups on the basis of the number of genes and functions they carry.
- Plasmids vary in size.
- Most are between 1,000 to 25,000 base pairs vs. 4,000,000 bp in the genome.
- A very large plasmid ranging in size from 100-1700 Kb.
- A kilobase (Kb) is 1000 bases of DNA, while a megabase (Mb) is 1,000,000 bases.
- Mbp = mega base pairs = 1,000,000 bp or one million base pairs or 1000 kb.

Their size can range from very small mini-plasmids of less than 1-kilobase pairs (Kbp) to very large megaplasmids of several megabase pairs (Mbp).

Plasmid replication

Plasmid origin of replication(ORI)

Plasmid Replication



- 1 – 100 kb in size
- Replicate independent of host DNA
- Plasmids are segregated to daughter cells during cell division

Reference: Molecular Cell Biology. 4th edition.
http://www.ncbi.nlm.nih.gov/books/NBK21498/#A1586

Numbers of plasmids
Xylella fastidiosa

- The sequence of the genome of *X. fastidiosa* pathogenic strain 9a5c revealed:

1. A 2.7-Mb circular chromosome, and
2. Two indigenous circular plasmids:

- one of 1.3 kb (pXF1.3) and
- a second one (pXF51) of 51 kb.

Simpson *et al.*,2000

Plasmids

Antibiotic resistance plasmids

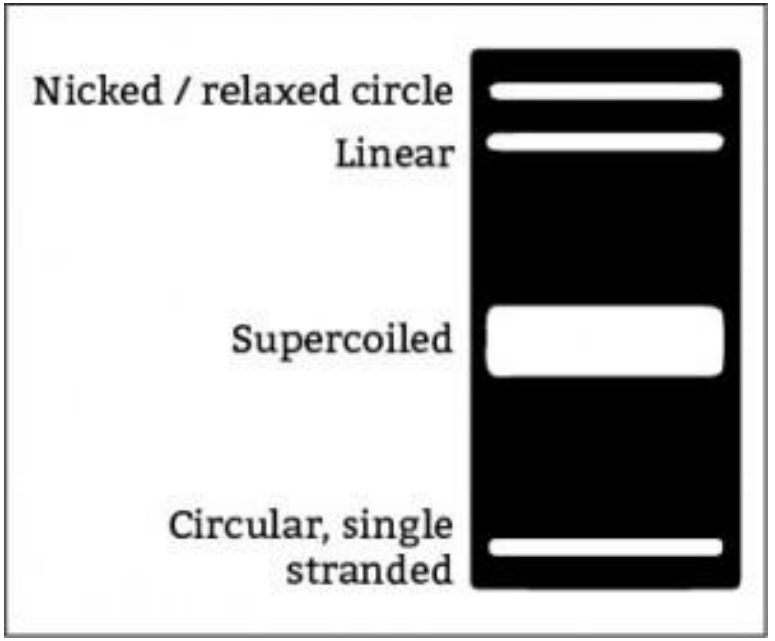
- Genomic islands (GIs) contain many antibiotic resistant genes are referred to as antibiotic resistance islands.
- Genomic islands (GIs) encoding antibiotic resistance determinants.
- These genes are frequently carried as genomic islands on transmissible plasmids and provide ready sources of resistance determinants.
- These genes on plasmids encodes enzymes for resistant against antibiotics, heavy metals, radiation, etc.

Antibiotic resistance it may be needed when cells are exposed to that antibiotic but not needed when cells are growing in the absence of the antibiotic.

Plasmid size

Demonstration of plasmids in cell extracts by agarose gel electrophoresis

- When plasmid DNA is isolated and run on an agarose gel, you may observe 2, 3 or even 4 or more bands.
- Hopefully the majority of your isolated DNA will be supercoiled DNA, but other forms can also crop up.
- Linear DNA generally migrates between the nicked circle and the supercoiled forms.
- Nicked/relaxed circular plasmid was relaxed by topoisomerases.



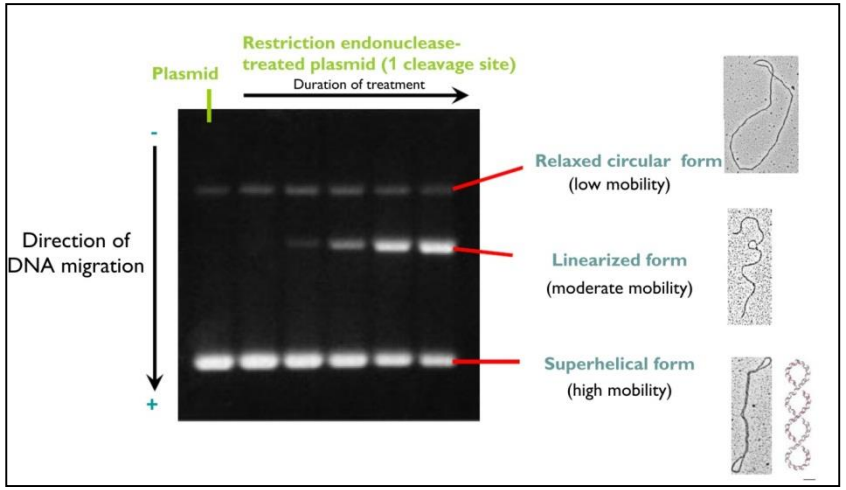
Tirabassi,2014

Plasmid size and shape

Mobility of DNA molecules

Gel electrophoretic image of plasmid DNA

- Besides their size, the electrophoretic mobility of DNA molecules is also significantly affected by their shape.
- Circular plasmid DNA has a compact structure, and its hydrodynamic size is much smaller—and its electrophoretic mobility is therefore greater—than that of linear DNA molecules of the same size, as the latter form a freely moving entropic chain.



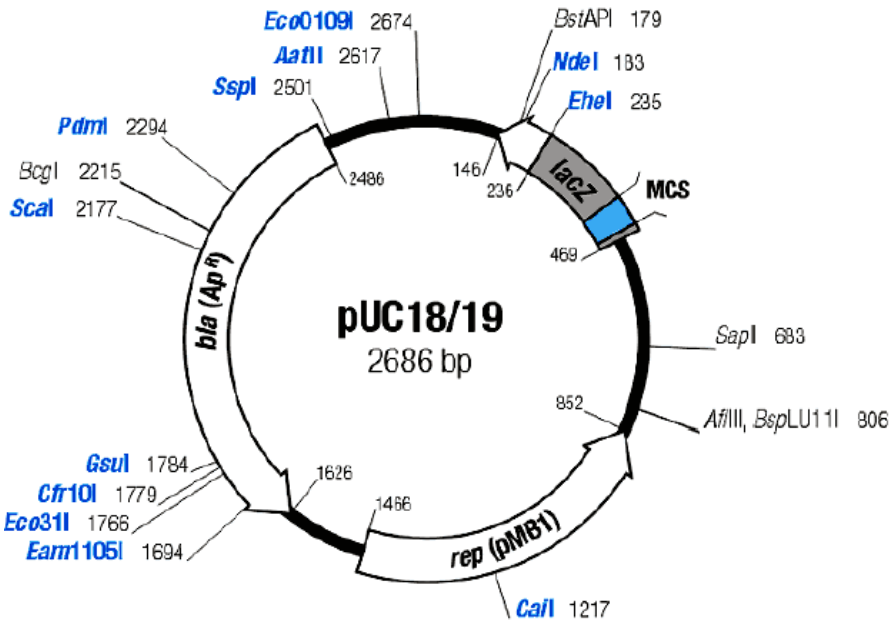
Hegyi *et al.*,2013

Types of plasmids

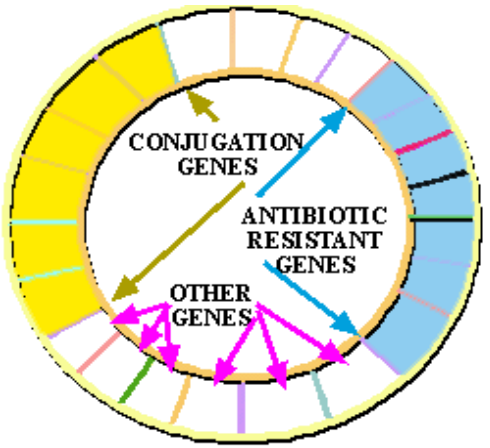
Based on their function plasmids can be classified into many types as:

- Resistant plasmids: They encode enzymes for resistant against antibiotics, heavy metals, radiation, etc.
- Conjugative plasmids: Some plasmids e.g. F^+ Plasmid are transmitted from one bacterium to another (even of another species) mostly through conjugation. These plasmids encode the conjugative sex pili necessary for their own transfer.
- Virulent plasmids: They confer invasive and infective ability of pathogen. e.g. by producing toxin or agents that damage host tissues.
- Bacterocinogenic plasmids: Plasmids those produce bacterocins those act against other bacteria e.g. subtilicins by *Bacillus subtilis*.

Vector/fertility plasmids



A plasmid vector, pUC 18/19



CIRCULAR PLASMID DNA

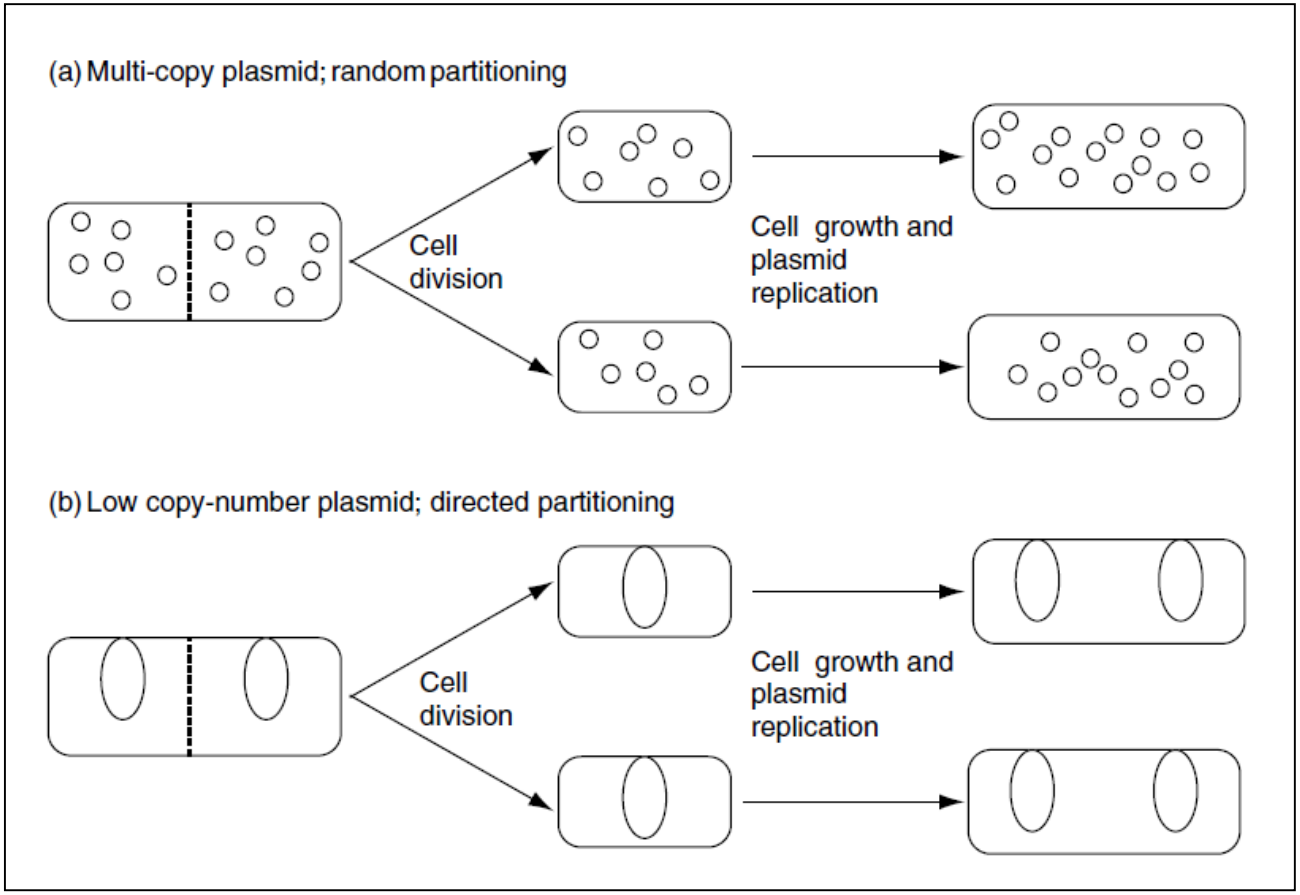
Fertility (F) plasmid

Common feature of all plasmids
Replicon

- One common feature of all plasmids is a specific sequence of nucleotides termed an origin of replication (ori).
- This sequence, together with other regulatory sequences, is referred to as a replicon.
- The replicon allows a plasmid to replicate within a bacterial cell independently of bacterial chromosome.
- If the plasmid makes many copies of itself per cell, it is termed a "relaxed" plasmid.
- If it maintains itself in fewer numbers within the cell it is termed a "stringent" plasmid.

Partitioning of plasmids at cell division

Relaxed and stringent plasmids



Dale and Park,2004

Functions of plasmids

- **I. ALL Plasmids:**
  1. Self-replication
- **II. By Some Plasmids:**
  1. Self-transfer
  2. Resistance to antimicrobial agents
     * Antibiotics
     * Chemotherapeutic agents (sulfa drugs)
     * Heavy metals
  3. Pigment production
  4. Toxin production (pathogenicity)
  5. Phage sensitivity (enhance resistance to bacteriophage)
  6. Antibiotic production
  7. Bacteriocin production against closely-related bacterial strains
  8. Induction of plant tumors
  9. Hydrogen sulfide production
  10. Catabolic functions (petroleum fraction degradation)

Symptoms associated with phytopathogenic bacterial groups

Plasmid-borne genes to the host-pathogen relationship

Symptom	Bacterium	Host plant	Plasmid traits
Blights, cankers, knots	*Ps. syringae* pathovars	Pathovar-specific ranges	*avr, vir*, coronatine, Sm^r, Tc^r, Cu^r, UV^r, hormones
Blights	*X. campestris* pathovars	Pepper, cotton and other hosts	*avr* genes, Cu^r, Sm^r, Tc^r
Galls	*Pantoea gypsophilae*	*Gypsophila* (flowering plants in the carnation family)	*hrp* and pathogenicity genes, hormones
Necrosis	*Er. amylovora*	Apple, pear and related ornamentals	Thiamin (Vitamin B1) and EPS biosynthesis
Tissue soft rot	*B. cepacia*	Onion	Endopolygalacturonase production
Tissue soft rot of storage organs	*P. carotovorum*	Wide range, including potato	Cryptic
Wilt	*R. solanacearum*	Potato, tomato, banana, aubergine(egg plant)	Virulence
Wilt	*Pa. stewartii*	Corn	Cryptic

Vivian *et al.*,2001

Phytopathogenic strains of *P. syringae* containing plasmids

Pathovar	Reference
P. syringae pv. *angulata*	Piwowarski and Shaw,1982
P. syringae pv. *atrpurpurea*	Sato *et al.*,1983
P. syringae pv. *coronafaciens*	Piwowarski and Shaw,1982
P. syringae pv. *glycinea*	Curiale and Mills,1983
P. syringae pv. *lachrymans*	Coplin,1989
P. syringae pv. *papulans*	Burr *et al.*,1988
P. syringae pv. *phaseolicola*	Quant and Mills,1984
P. syringae pv. *savastanoi*	Comai *et al.*,1982
P. syringae pv. *striafaciens*	Beck-Von Bodmann and Shaw, 987
P. syringae pv. *syringae*	Gonzales *et al.*, 1984
P. syringae pv. *tabaci*	Obukowicz and Shaw, 1983 & 1985
P. syringae pv. *tomato*	Denny, 1988; Bender and Cooksey,1986

OECD,1997

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

Types of RNA in bacterial cells
tRNA, rRNA, and mRNA

- Although there are multiple types of RNA molecules, the basic structure of all RNA is similar.

1. tRNA travels from nucleus (DNA) to cytoplasm in a cell. Some 30-40 different tRNAs have been identified in bacterial cells and as many as 50-100 in animal and plant cells.
2. tRNA and rRNA very abundant relative to mRNA.
3. mRNA is transcribed at higher rates than rRNA and tRNA.
4. Abundance is a reflection of the relative stability of the different forms of RNA.

Types of RNA in bacterial cells
mRNA

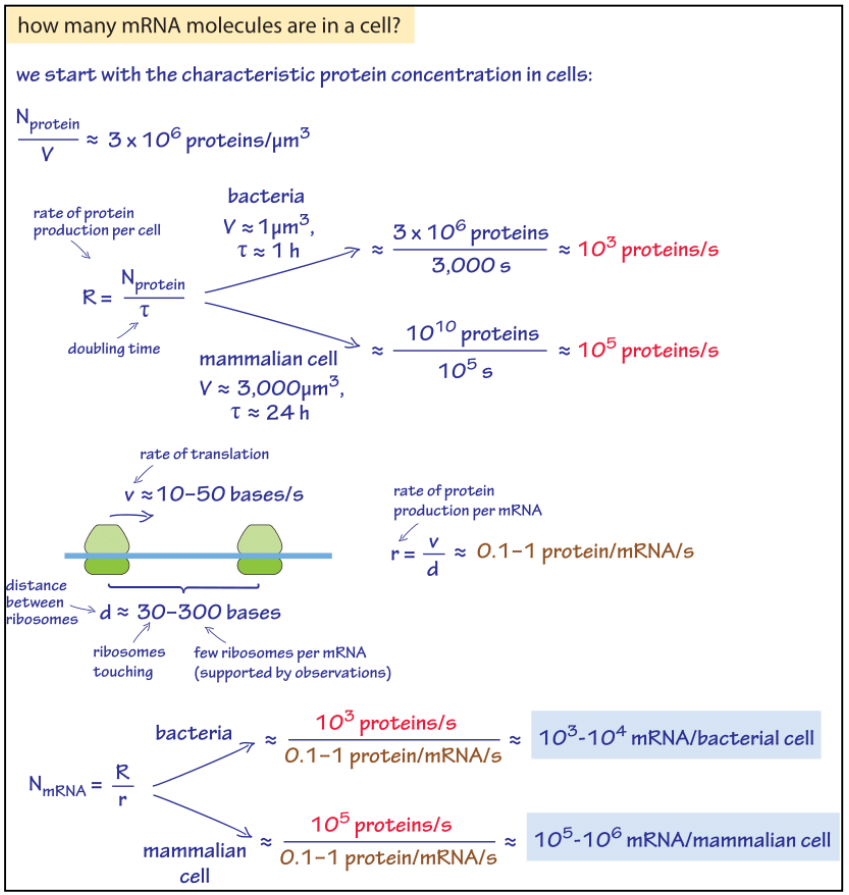
- The majority of RNA molecules are tRNAs and rRNAs.
- Ribosomal RNA (rRNA) associates with a set of proteins to form ribosomes.
- Ratio of rRNA to mRNA (~97:3).
- mRNA accounts for only 1-5% of the total cellular RNA although the actual amount depends on the cell type and physiological state.
- Approximately 360,000 mRNA molecules are present in a single mammalian cell.

Types of RNA in bacterial cells
mRNA

- Unlike eukaryotic mRNA each contains information coding for only one protein, in prokaryotic mRNAs, each may encode more than one protein.
- When a bacterial cell needs more or less of a pathway's enzymes, it simply transcribes more or less of that pathway's mRNA.

Types of RNA in bacterial cells
mRNA

- As shown in this back of the envelope calculation we can derive an estimate for rapidly dividing cells of 10^3-10^4 mRNA per bacterial cell and 10^5-10^6 mRNA per the 3000 μm^3 characteristic size of a mammalian cell.



Types of RNA in bacterial cells
mRNA

- mRNA life in prokaryotic cell is short (few seconds to two minutes) as mRNA is unstable.
- mRNA in eukaryotic cell has a life of few hours to few days; it is quite stable.
- In prokaryotes translation is a faster process, each mRNA adds about 20 amino acids per second.
- in eukaryotic cell, mRNA adds one amino acids per second, thus a slower process.

Types of RNA in bacterial cells

RNA content of E. coli Cells

tRNA, rRNA, and mRNA

Type	Steady State Levels	Synthetic Capacity	Stability
rRNA	83%	58%	High
tRNA	14%	10%	High
mRNA	3%	32%	Very Low

A stable condition that does not change over time or in which any one change is continually balanced by another. Expression level of each gene in these types are in steady (continuous) state.

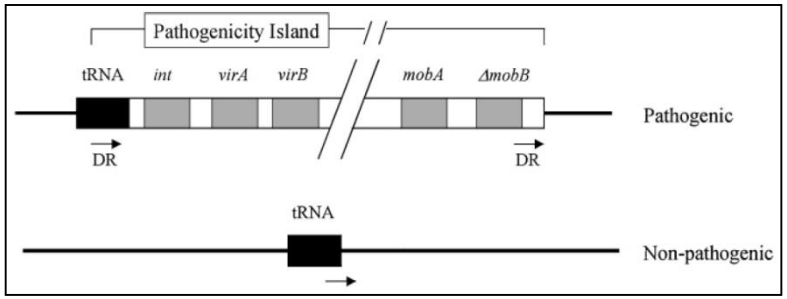
Types of RNA in bacterial cells

- **1. mRNA:** This is known as 'messenger' RNA because it carries the message, the base sequence from the nucleus to the ribosome.
- This message is represented by a series of letters, which represent the molecules that form an mRNA molecule.
- Bearing that in mind, the following could represent an mRNA sequence: ACUGGCCCCUAA.
- Ribosomes read the sequence three molecules at a time. So, to a ribosome, the sequence would look more like this: ACU-GGC-CCC-UAA.
- Each of these three-letter sequences codes for something specific, usually an amino acid.

Types of RNA in bacterial cells

DNA is just one type of nucleic acid(NA). Some other types are RNA, mRNA, and tRNA. All of these "NAs" work together to help cells replicate and build proteins

- **2. Transfer RNA (tRNA):** The main function of tRNA molecules is to bind mRNA at the ribosome for protein synthesis.
- The type of RNA that brings amino acids to the ribosomes.
- Transfer RNA is responsible for carrying amino acids to the messenger RNA.
- tRNA molecules attach to specific amino acids and match them to the correct codon during protein synthesis.
- But some tRNAs have functions unrelated to translation.
- E.g. Some tRNA genes are frequently associated with bacterial pathogenicity islands.



Types of RNA in bacterial cells

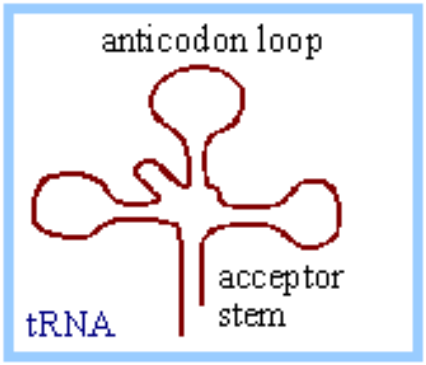
DNA is just one type of nucleic acid (NA). Some other types are RNA, mRNA, and tRNA. All of these "NAs" work together to help cells replicate and build proteins

- **3. Ribosomal RNA (rRNA):** Each subunit of the ribosome has an rRNA molecule which holds together the protein components of the ribosome structure.
- **4. Small RNA molecules with enzyme activity (sRNA):** Many other classes of functional RNAs participate as enzyme subunits, as splicing components, and as regulator of transcription. These are believed to be ancient remnants of the "RNA World" before DNA-based cell evolved.
- **5. Antisense RNA:** In bacteria, some RNA molecules are transcribed as the complement of genes whose expression can be turned off by antisense RNA hybridization to the messenger RNA.

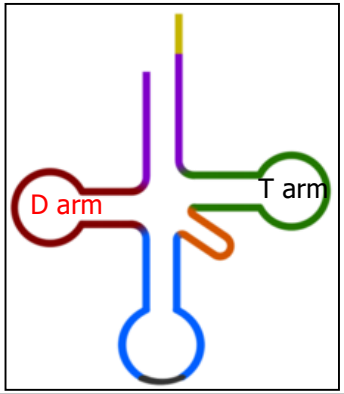
Transfer RNA

tRNA structure

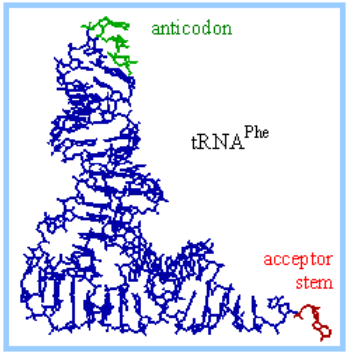
- Transfer RNA (abbreviated tRNA) is a small RNA (usually about 74-95 nucleotides) that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation.
- It has a 3′ terminal site for amino acid attachment.
- D arm and T arm conventionally used in describing bacterial and eukaryotic systems.



RNA molecules usually have secondary structure, consisting of stem and loop domains.



Acceptor stem in purple, Variable loop in orange, D arm in red, Anticodon arm in blue with Anticodon in black, T arm in green.



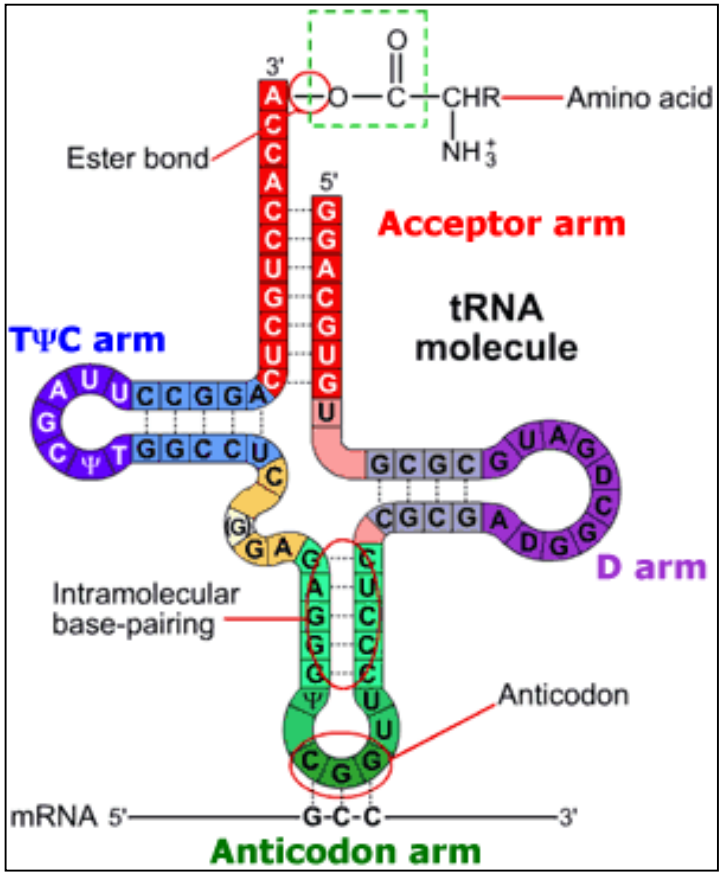
Structure of tRNAPhe, determined by crystallographic analysis.

Transfer RNA

tRNA structure

- The tRNA molecule is made up of four general regions:

1. the acceptor arm (red): amino acid attachment site;
2. the T-arm (purple): contains thymine;
3. the anticodon arm (green): the anticodon triplet base-pairs with the codon triplet of the messenger RNA, and
4. the D-arm (orange): contains the modified base dihydrouracil (an intermediate in the catabolism of uracil). The D-stem is also believed to contains some identity elements for recognition.



Ψ pronounced as psi= associated with thymidine and cytosine.

Ribosomal RNA

Conserved macromolecules

Structure and functions

- Ribosome (round shaped organelle) is a complex of ribosomal ribonucleic acid (rRNA) and protein.
- There must be 60-80 different proteins in every ribosome.

1. The rRNA (ribosomal RNA) of the ribosomes in eukaryotes are synthesized in the nucleolus, within the nucleus.
2. The protein component of ribosomes are synthesized by ribosomes, since it's a protein and all proteins are made by ribosomes.

- The rRNA passes through nuclear pores into the cytoplasm and bind to small ribosomal subunit, then recruits the large subunit of the ribosome.

Ribosomal RNA

Conserved macromolecules

Structure and functions

- Ribosomal RNA (rRNA) as component of ribosome are located in specific sections of the genome called nuclear organizers.

1. Ribosomal RNAs are synthesized in a specialized region of the cell nucleus (in eukaryotes) called the nucleolus.
2. Ribosomal proteins are synthesized in the cytoplasm and transported back to nucleus and together with the rRNA produced in nucleolus, they are assembled into complete ribosome.
3. The subunits (ribosomal RNAs+ ribosomal proteins) are then returned to the cytoplasm for final assembly.

Ribosomal RNA

Conserved macromolecules

Structure and functions

- Nearly two-thirds of the mass of a ribosome is rRNA.
- These rRNAs are never translated (into proteins).
- The rRNAs form extensive secondary structures and play an active role in recognizing conserved portions of mRNAs and tRNAs.
- These complex structures, which:

1. physically move along an mRNA molecule,
2. catalyze the assembly of amino acids into protein chains.

Ribosomal RNA

Conserved macromolecules

Structure and functions

- rRNA helps build the proteins.
- It decodes (translate data or a message from a code into the original language) mRNA into amino acids and provides peptide bonds for amino acids.

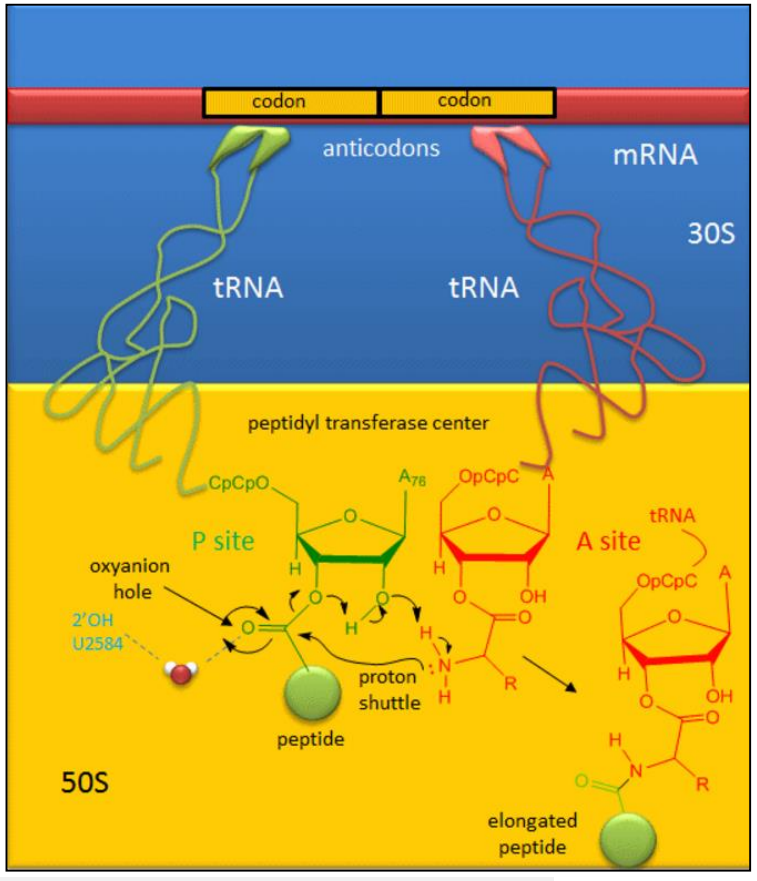
rRNAs are catalytic RNA molecules or ribozymes (ribonucleic acid enzyme). Ribosome itself is an unusual catalyst, composed of three RNAs.

Ribosomal RNAs
Functions

1. Ribosomal RNA (rRNA) is a type of non-coding ribonucleic acid (RNA) that is a primary and permanent component of ribosomes, the small, cellular particles that form the site of protein synthesis in all living cells.
2. As non-coding RNA, rRNA itself is not translated into a protein, but it does provide a mechanism for decoding messenger RNA (mRNA) into amino acids and interacting with the transfer RNAs (tRNAs) during translation by providing peptidyl transferase activity.

Ribosomal RNAs
Functions

1. Ribosomal RNA (rRNA) associates with a set of proteins to form ribosomes.
2. These complex structures, which physically move along an mRNA molecule, catalyze the assembly of amino acids into protein chains.
3. They also bind tRNAs and various accessory molecules necessary for protein synthesis.



Ribosomal RNA (rRNA) is a component of the ribosomes, the protein synthetic factories in the cell.

Ribosomal RNA

Conserved macromolecules

Structure and functions

- Prokaryotes (organisms that lack a nucleus) generally have fewer sets of rRNA genes and ribosomes per cell.
- For example, in the bacterium *Escherichia coli*, seven copies of the rRNA genes synthesize about 15,000 ribosomes per cell.
- In eukaryotes (organisms that possess a clearly defined nucleus), anywhere from 50 to 5,000 sets of rRNA genes and as many as 10 million ribosomes may be present in a single cell.

The *rrf*, rrs and *rrl* rRNA genes encode for the structural rRNA molecules required for ribosome assembly and function (5S, 16S and 23S rRNAs, respectively).

Conserved macromolecules

Ribosomal RNAs

Function of rRNAs

- Also it is now believed that the rRNA plays a major role.
- This includes:

1. The participation of rRNA in mRNA selection,
2. tRNA binding (in A, P and E sites),
3. Ribosomal subunit association,
4. Proofreading, factor binding,
5. Antibiotic interaction (e.g. antibiotics which inhibit protein synthesis react with the rRNA rather than ribosomal proteins),
6. Termination and the peptidyltransferase function.

The 3' end of the 16S ribosomal RNA (in a ribosome) binds to a sequence on the 5' end of mRNA. Many studies have confirmed that base pairing between the SD sequence in mRNA and the 3' end of 16S rRNA is of prime importance for initiation of translation by bacterial ribosomes.

Size of conserved macromolecules rRNAs

- The RNA in the ribosome comes in three basic sizes:
- Two large rRNA molecules (16S & 23S rRNAs)
- One small rRNA molecule (5S rRNA)

1. The 16S rRNA molecule, found in the smaller ribosomal subunit (SSU rDNA), has a chain length of 1542 nucleotides(nt),
2. whereas the 23S rRNA molecule, a component of the larger ribosomal subunit (LSU rDNA), contains 2904 nucleotides(nt).
3. Each larger subunit (LSU) contains, in addition, one very short rRNA molecule that sediments at 5S rRNA molecule and has 120 nucleotides.

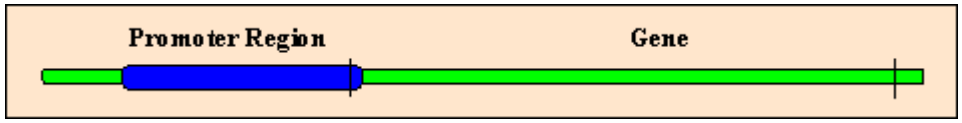
Swedberg units(S): Measure of the rate of sedimentation of a particle in a centrifuge, where the sedimentation rate is associated with the size of the particle.

Operon

Prokaryote genes are grouped in operons

Structure and functions

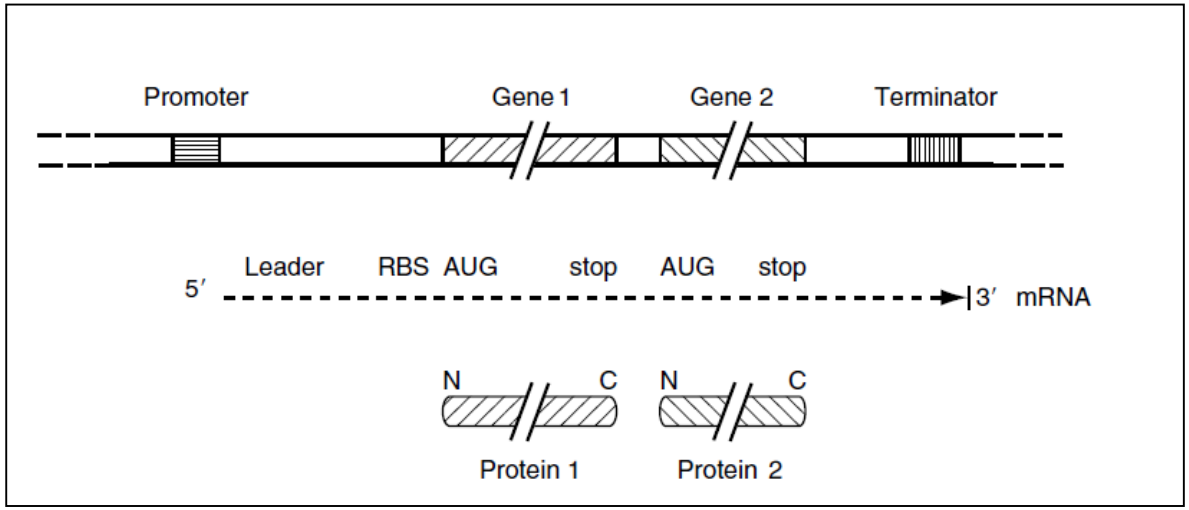
- Common in bacteria but rare in eukaryotes.
- In bacteria, genes with related functions are often (but not always) located together in a group known as a operon.
- Enzymes in many biosynthetic pathways of bacteria and viruses are encoded by operons.
- An operon has a single promoter and is transcribed into a single polycistronic mRNA molecule, which carries the information for several proteins.
- Promoter: Region of DNA where RNA polymerase attaches and initiates transcription.



Dale and Park,2004;..

A typical operon

- A typical operon is transcribed from a single promoter into a polycistronic mRNA from which several independent polypeptides can be translated.
- Promoter region: region of DNA usually upstream from the gene which regulates gene activity.

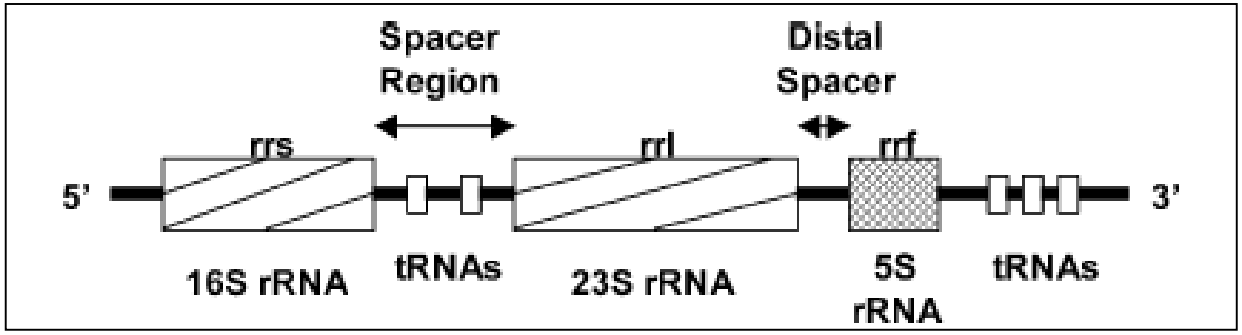


Dale and Park,2004;..

Ribosomal RNA operon(rrn)

Genomic structure of rRNA genes in bacteria and archaea

- The ribosomal RNA operon (rrn) has also frequently been used to design primers that allow highly sensitive detection, but due to its universal nature, the level of discrimination lies at the species or genus levels.
- In bacteria and archaea, ITS is located between the 16S and 23S rRNA genes.



Tavares *et al.*,2010;..

Ribosomal RNA operon(rrn)

Predicting the number and organization of operons

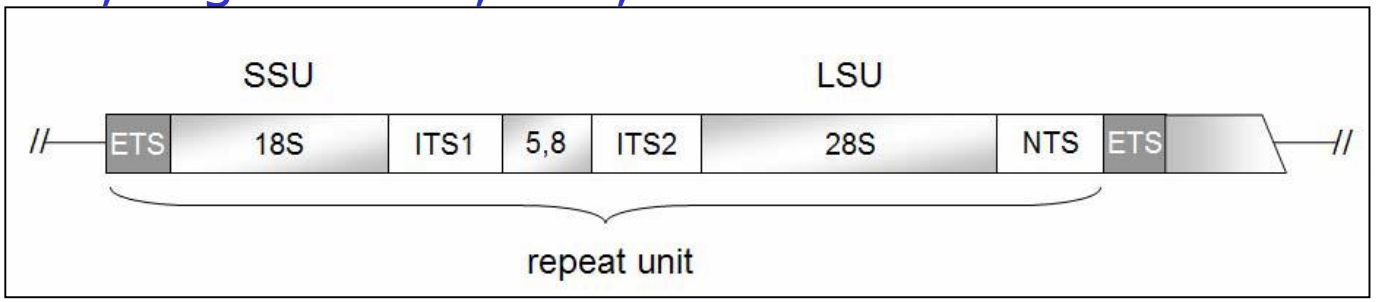
- As a result, predictions can be made based on an organism's genomic sequence.
- Number and organization of operons has been studied most critically in *E. coli*.
- 4288 annotated protein-coding genes are organized into 2584 operons.
- One prediction method uses the intergenic distance between reading frames as a primary predictor of the number of operons in the genome.
- Longer stretches exist where operons start and stop, often up to 40-50 bases.

Wikipedia,2016

Ribosomal RNA operon(rrn)

Genomic structure of rRNA genes in eukaryotes

- In eukaryotes, the coding region for rRNA consists of 18S, 5.8S and 28S rRNA genes, which are separated by internal transcribed spacers (ITS) and flanked by an external transcribed spacer (ETS) and a non-transcribed spacer (NTS).
- A section from one ETS to another represents a repeat unit.
- LSU, large subunit; SSU, small subunit.



Hillis and Dixon 1991

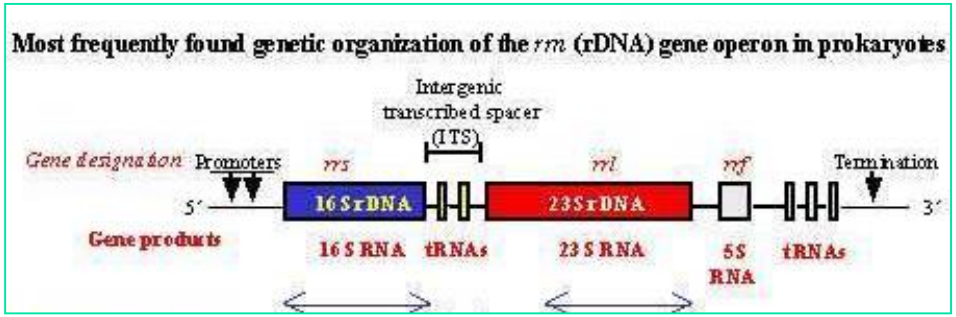
Ribosomal RNA operon(rrn)

Used to design primers

- The rrn locus consisted of:

1. a 16S rRNA gene (rrs), followed by an intergenic transcribed spacer (ITS) containing two genes of $tRNA^{Ile}$ and $tRNA^{Ala}$,
2. a 23S rRNA gene (rrl),
3. an ITS devoid of tRNA genes, and
4. a 5S rRNA gene (rrf).

- The internally transcribed spacer region (ITS) between the 16S and 23S rRNA genes appears to be more variable than 16S and 23S rRNA genes.



Ribosomal RNA operon (rrn)
Phytoplasma

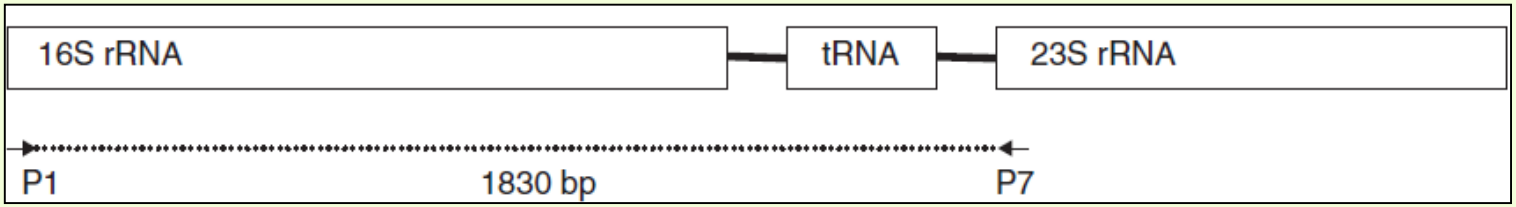
- Many phytoplasma strains have two rRNA operons:
- *rrn*A
- *rrn*B
- While *rrn*A and *rrn*B may be identical or nearly identical in some phytoplasma strains, apparent *rrn* interoperon sequence heterogeneity exists in other strains.

Wei *et al.*,2007

16S-23S rRNA operon

Phytoplasmas

- 16S-23S rRNA operon showing the position of some of the various universal primers that have been developed for PCR amplification of this region from phytoplasmas.
- e.g. the universal primer pair P1/P7 can be used to prime the amplification of a 1.8 kb product of 16S ribosomal RNA (rRNA) gene, the spacer region between the 16S and 23S rRNA gene, and the start of the 23S rRNA gene regions of the phytoplasma genome.



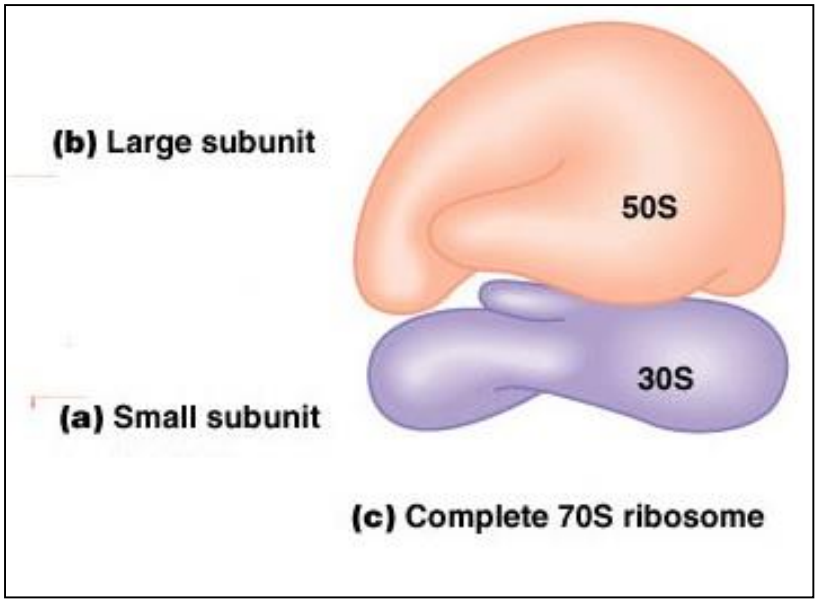
Weintraub and Jones,2010

Bacterial ribosome

The cellular protein factory

Sites or workplaces of protein synthesis

- A ribosome is a cell organelle. It functions as a micro-machine for making proteins.
- Ribosomes are composed of special proteins and nucleic acids.
- Prokaryotic bacteria contain 70S ribosomes, nearly 20,000 per cell.
- In *E. coli*, there are between 10,000 and 70,000! ribosomes present in each cell at any given time.



Bacterial ribosome

The cellular protein factory

Sites or workplaces of protein synthesis

- Rapidly growing cells usually have a large number of ribosomes.
- This accounts for about 25% of the total cell mass.
- They are large (2.5 MD molecular weight or larger) macromolecular complexes composed of:

1. RNA (two-thirds of ribosome's mass), and
2. protein (1/3).

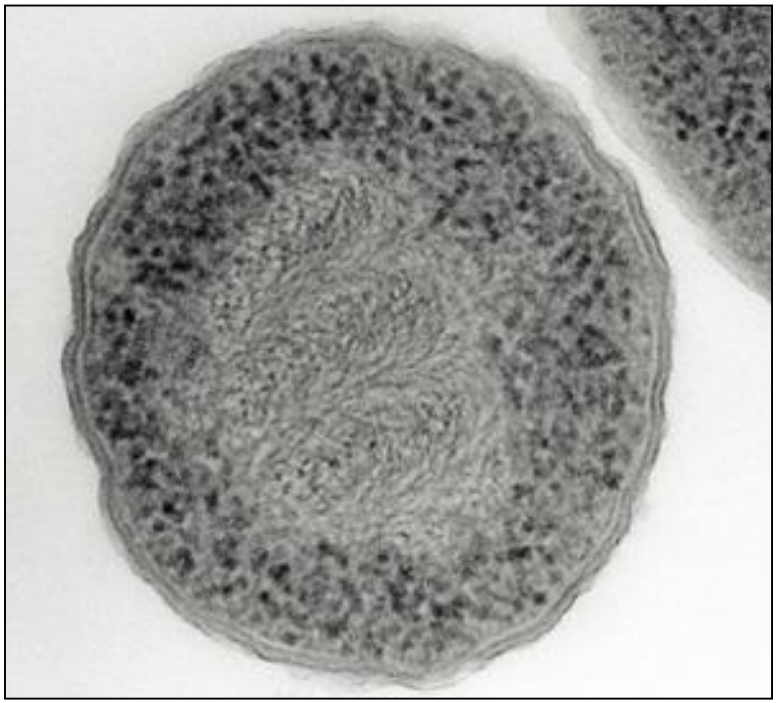
One Dalton is equivalent to the mass of one proton. One million Daltons in size (or one mega Dalton). One hydrogen weighs 1 dalton (Da). The average amino acid weighs 110 daltons.

British Society for Cell Biology;..

Bacterial ribosome

An electron micrograph of a prokaryote (*Escherichia coli*), showing DNA and ribosomes

- This *Escherichia coli* cell has been treated with chemicals and sectioned so its DNA and ribosomes are clearly visible.
- The DNA appears as swirls in the center of the cell, and the ribosomes appear as dark particles at the cell periphery.
- Courtesy of Dr. Abraham Minsky (2014).



Nature Education,2014

Bacterial ribosome

The cellular protein factory

Sites or workplaces of protein synthesis

- As the central piece of the translational machinery, ribosomes are constantly involved in the synthesis of proteins required during the entire life cycle of all organisms.
- Functions:

1. Translate encoded information from the cell nucleus provided by messenger ribonucleic acid (mRNA),
2. Link together amino acids selected and collected from the cytoplasm by transfer ribonucleic acid (tRNA), and
3. Export the polypeptide produced to the cytoplasm where it will form a functional protein.

The TRANSLATION of information and the Linking of AMINO ACIDS are at the heart of the protein production process.

Bacterial ribosome

Ribosomes (nucleoprotein particles)

Sites or workplaces of protein synthesis

- Ribosomes in prokaryotes use a slightly different process to produce proteins than do ribosomes in eukaryotes.
- Ribosomes are classified as eukaryotic or prokaryotic in type, on the basis of:

1. Sensitivity to various antibiotics;
2. Functional interchangeability of soluble factors and ribosomes from different sources; and
3. Structure and sedimentation characteristics, e.g.

- 70s (prokaryotic), or
- 80s (eukaryotic).

Eukaryotes have 80s type of ribosomes, 10-20 million per cell, are found in cytoplasm. Viruses use host ribosomes to make proteins for them.

Eukaryotic ribosome

The cellular protein factory

Sites or workplaces of protein synthesis

- Ribosomes are found 'free' in the cytoplasm or bound to the endoplasmic reticulum (ER) to form rough ER.
- In a mammalian cell there can be as many as 10 million ribosomes.
- Ribosomes have only a temporary existence.
- When they have synthesized a polypeptide the two sub-units separate and are either re-used or broken up.

Ribosomes can join up amino acids at a rate of 200 per minute. Small proteins can therefore be made fairly quickly but two to three hours are needed for larger proteins such as the massive 30,000 amino acid muscle protein titin.

Ribosome
Polysome
Eukaryotes and prokaryotes

- Can a single mRNA transcript act as the template for multiple ribosomes simultaneously?
- A polyribosome (polysome) is an mRNA that is simultaneously being translated by several ribosomes.
- Usually a ribosome can only work on one mRNA strand at a time. i.e. it can only create one protein during a single translation event.
- But they are multiple ribosomes can bind to any mRNA strand to form polyribosomes(polysomes).
- In eukaryotes polysomes are only found in association with membranes.
- In prokaryotes, polysomes are found free in the cytoplasm.

Ribosome

Polysome

Eukaryotes and prokaryotes

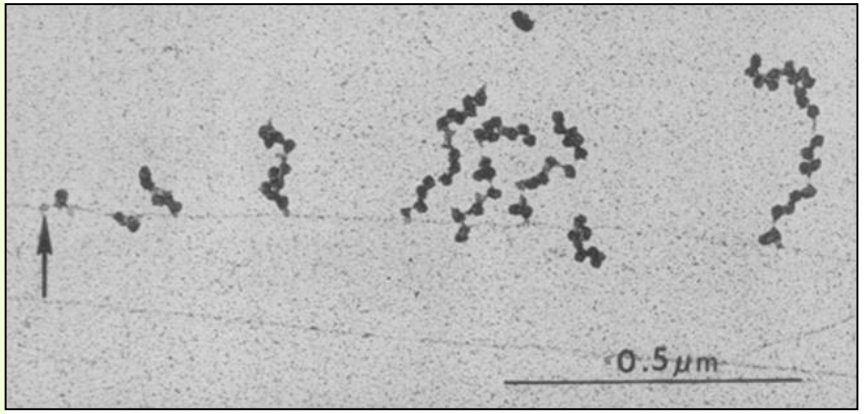
- Can a single cell contain many different monocistronic mRNAs all at once?
- Usually in bacterial cells, several ribosomes are working parallel on a single RNA, forming what is called a polyribosome or polysome.
- As mRNA synthesis proceeds, more ribosomes attach to the elongating strand to form a polysome.
- Whereas in eukaryotes mRNA contains the codon sequence for a single polypeptide, prokaryotic mRNAs may be polycistronic.
- Polycistronic mRNAs have multiple initiation codons and ribosomes may attach to any of these independently.

Ribosome
Polysome
Prokaryotes

- The ribosomes within the polysome follow each other, one after the other, down the entire length of the mRNA.
- In prokaryotes, the last ribosome is immediately followed by an enzyme whose job is to break down the mRNA strand.
- By the time translation is completed the mRNA no longer exists.

Ribosome
Polysome
Prokaryotes

- A portion of an *E. coli* chromosome being transcribed (left to right) and being simultaneously translated.
- The arrow points to the putative site where RNA polymerase is first bound to the DNA.
- The chains of dark bodies are polysomes, that is, several ribosomes on the same mRNA molecule.



Note that the size of the polysomes increases with distance from the upstream site where transcription began.

Elio,2013

Ribosome

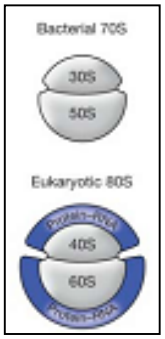
Polysome

Prokaryotes

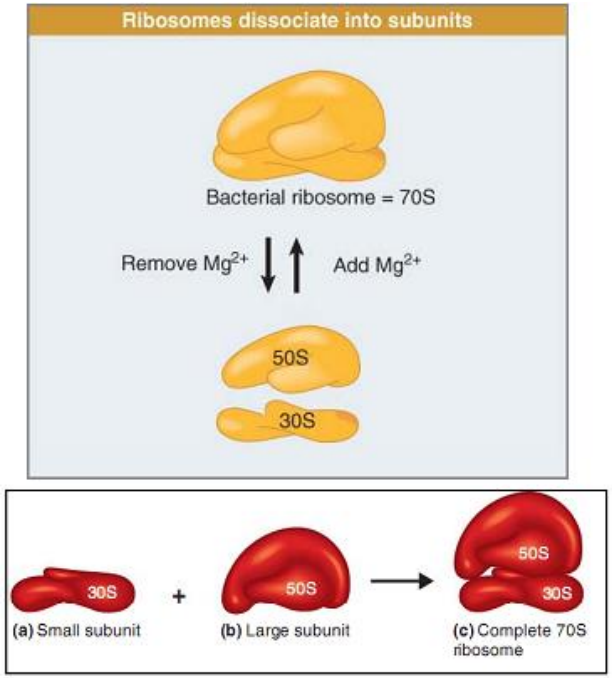
- As each mRNA strand begins, the first of a series of ribosomes binds to the mRNA and initiates translation.
- As the mRNA grows, additional ribosomes are added to the polysome, each one ultimately translating the entire protein.

Bacterial ribosomes

Sites of protein synthesis



- Each ribosome is actually made up of two subunits and they can be separated into individual smaller subunits by lowering Mg^{2+} concentration and they can be sedimented into individual components.
- The letter S refers to Svedberg units, which indicate the relative rate of sedimentation during ultra-high-speed centrifugation.
- Sedimentation rate is a function of the size, weight, and shape of a particle.



Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit. Ribosomes have only a temporary existence.

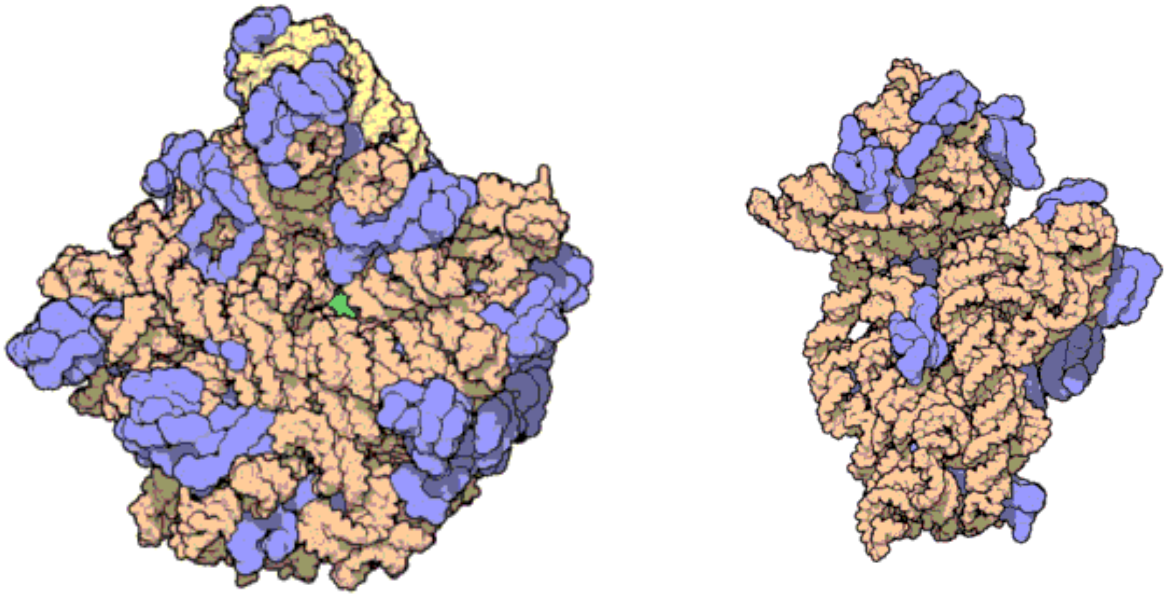
Bacterial ribosome

What is unique about the structure of bacterial ribosome?

- Electron microscopy was the main technique used to discover the structure of the bacterial ribosome.
- Ribosomes are freely suspended in the cytoplasm.
- Existence of ribosomes is temporary, after the synthesis of polypeptide the two sub-units separate and is reused or broken up.
- In bacterial cells, ribosomes are synthesized in the cytoplasm through the transcription of multiple ribosome gene operons.
- In eukaryotes, the process takes place both in the cell cytoplasm and in the nucleolus, which is a region within the cell nucleus.

Bacterial ribosomes

Consisting of two subunits

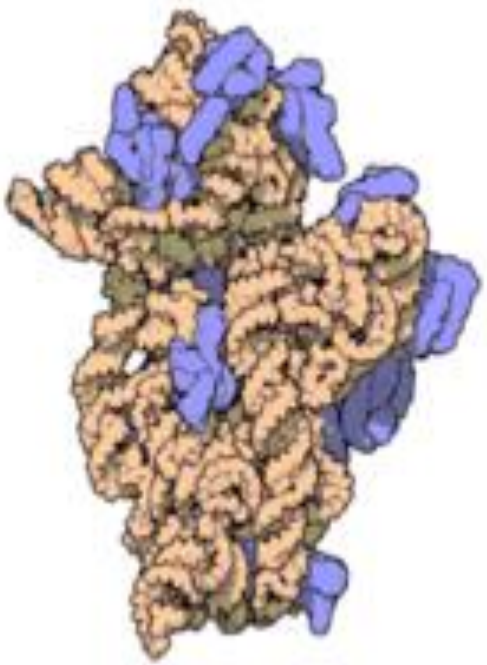


50S (left) and 30S (right).
Larger one, "50S" is located upper side of ribsomes. 50S has rather rigid structure, while 30S has fairly flexible one.

Erin Husson

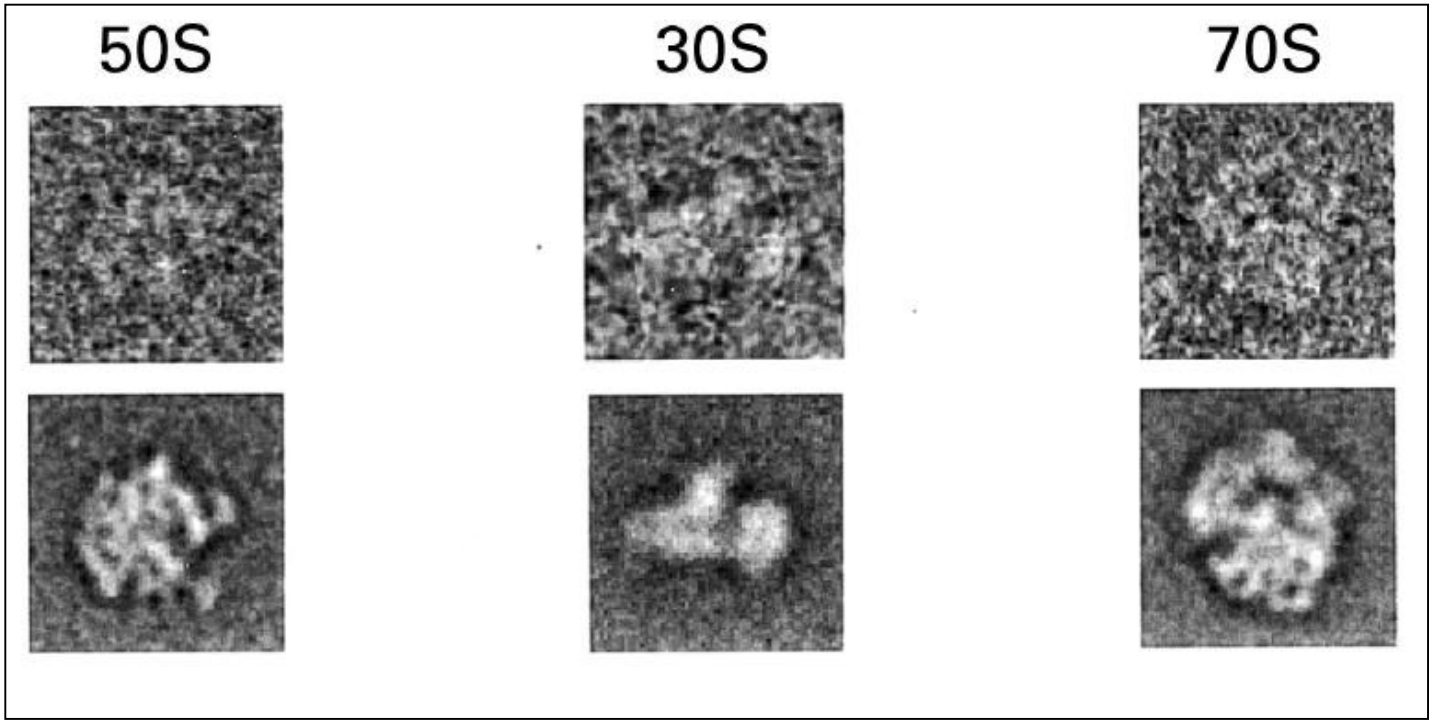
Atomic structure of the 30S Subunit

- 30S is a complex of ribosomal RNA and ribonucleoproteins which functions in mRNA translation.
- Proteins are shown in blue and the single RNA strand in orange.
- The 30S subunit is the site of inhibition for antibiotics such as tetracycline.



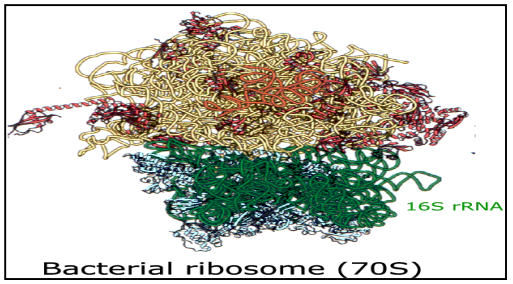
Bacterial ribosome

Ribosomal subunits



Complete structures of both ribosomal subunits have been determined using X-Ray crystallography.

Bacterial ribosome
RNA and protein components



- Each subunit is composed of:

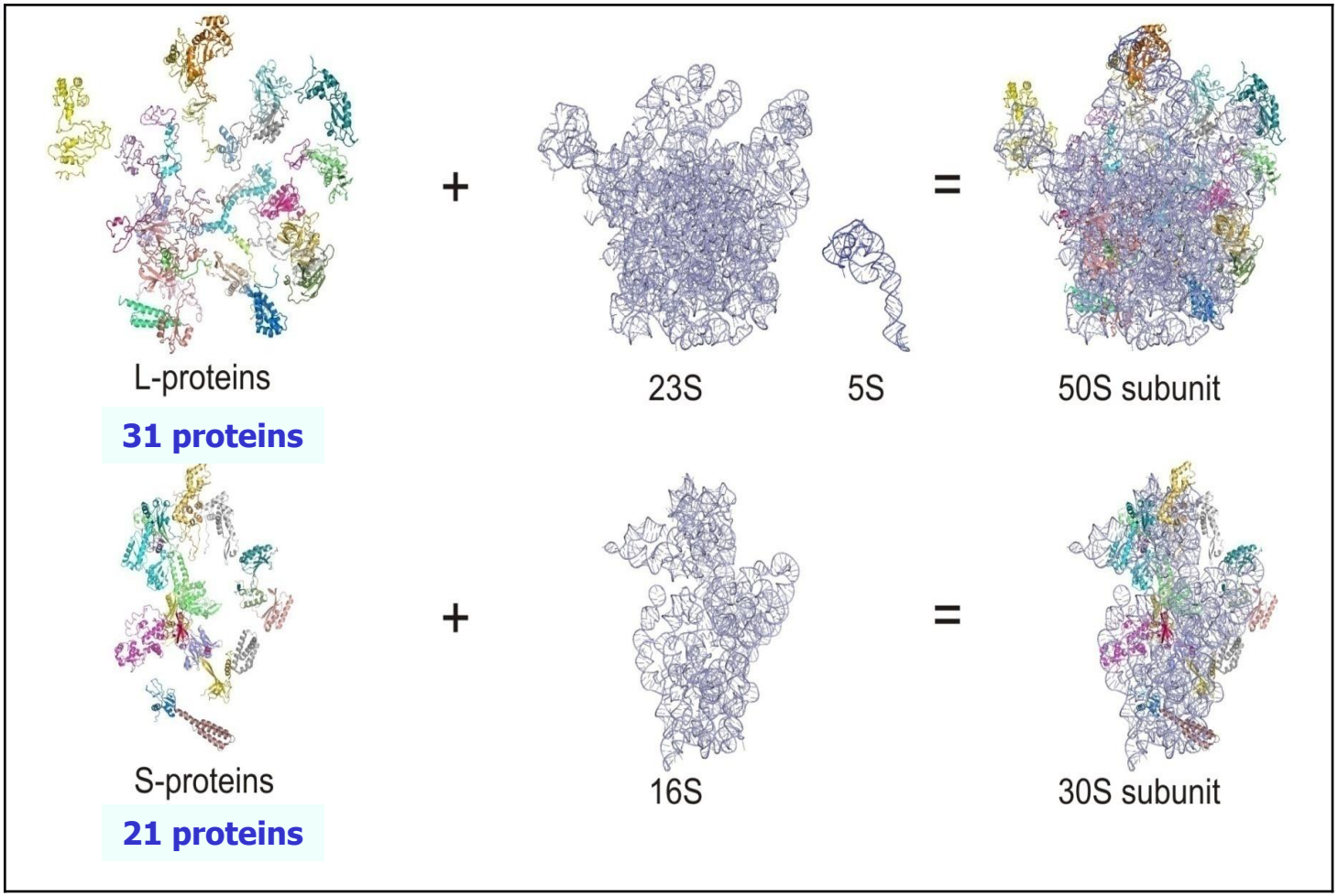
1. Ribosomal RNA (rRNA), and
2. Ribosomal proteins (r-proteins).

- The larger (50S) subunit has two RNA molecules (23S and 5S) plus 31 different polypeptides (large r-proteins or L-proteins).
- The smaller one (30S) contains a single RNA molecule (16S) and 21 polypeptides (small r-proteins or S-proteins).

	30S subunit	50S subunit
rRNA	16S	23S and 5S
Proteins	21	31

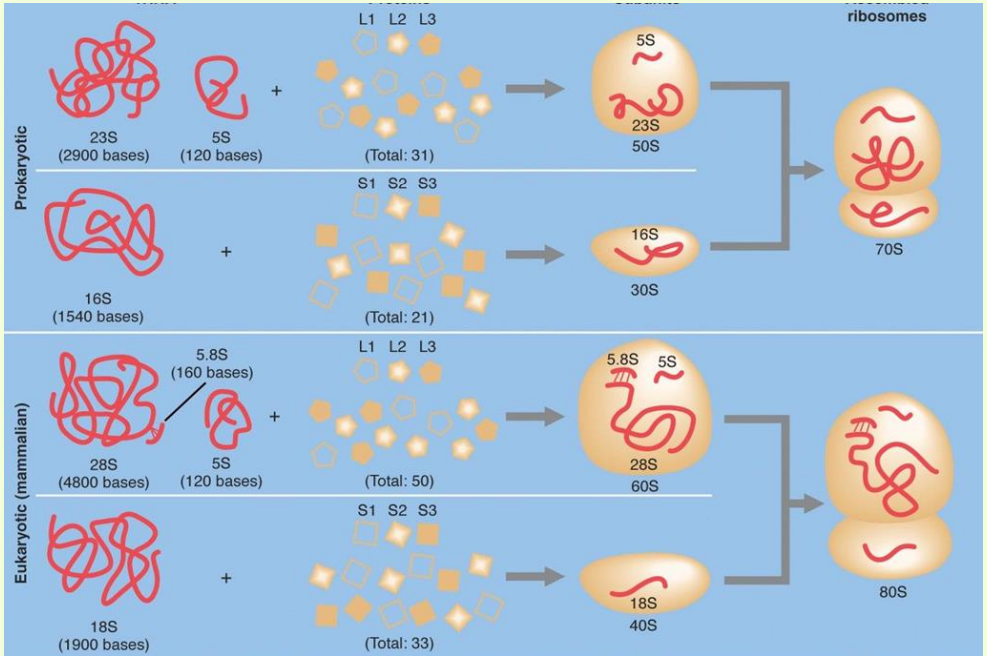
Ribosomal subunits

Part protein and Part RNA



Ribosomal subunits
Part protein and Part RNA

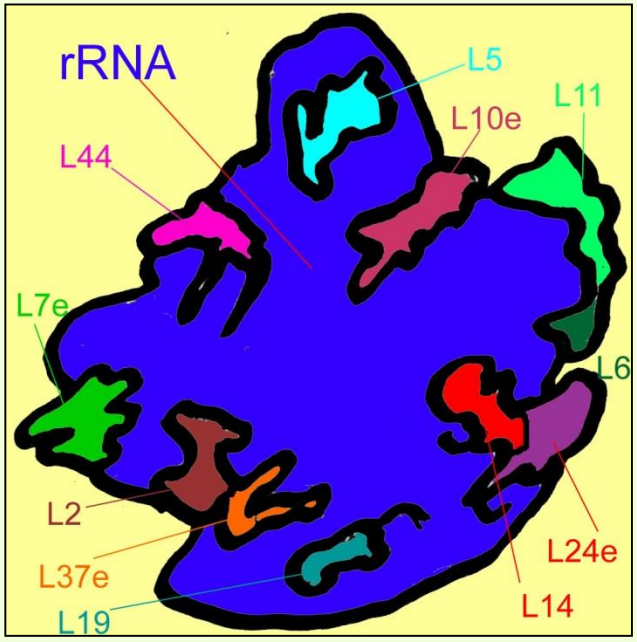
- The small one, 30S, is composed of 16S rRNA (1542 nucleotides) and 21 ribosomal proteins.
- The 50S (large) subunit containing 34 different proteins (L1-L34), 23S rRNA of 2904 nucleotides.

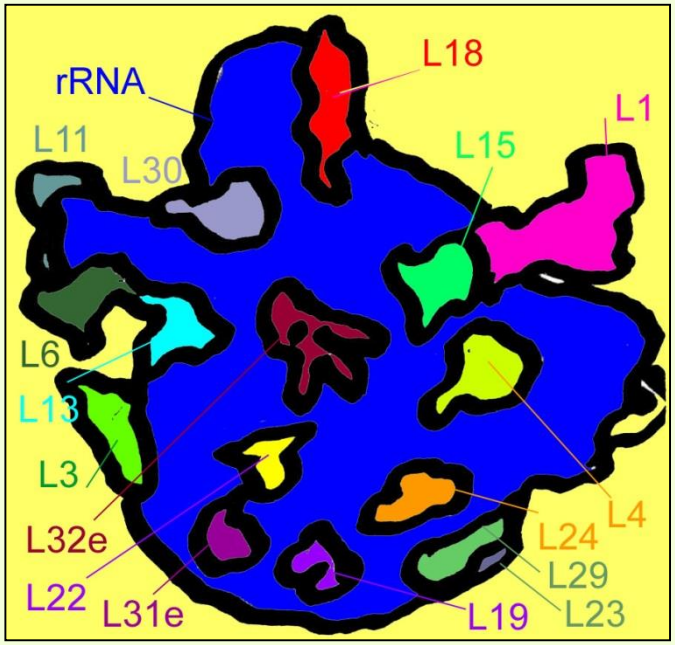


Powers,2010;..

Ribosomal subunits
Part protein and Part RNA

- Below are images (views of two opposite sides) of the large subunit of a prokaryotic ribosome with the ribosomal proteins labelled.





bio.sunyorange.edu/updated2

Ribosomal subunits

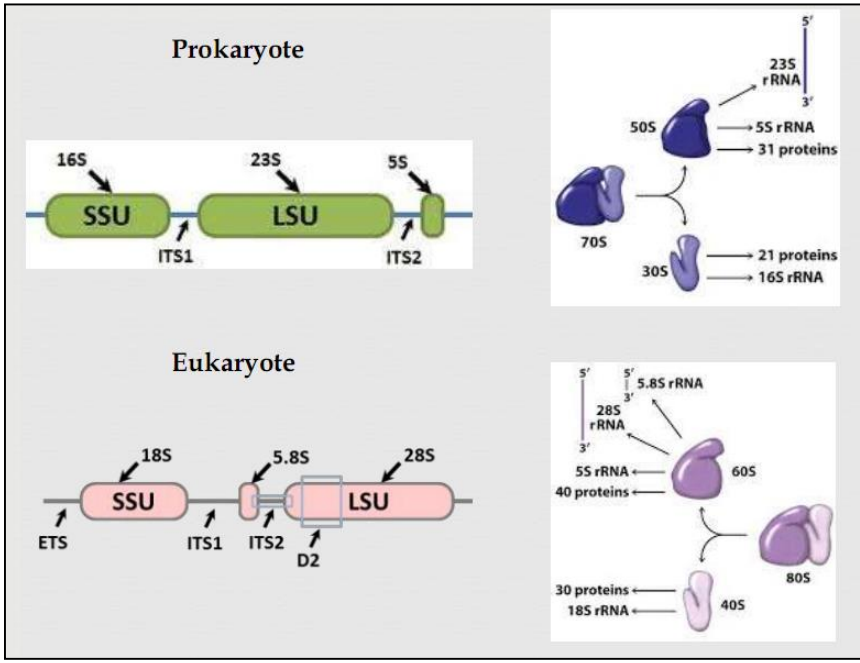
Part protein and Part RNA

- The small one, 30S, is composed of:

1. 16S rRNA (1542 nucleotides) and
2. 21 ribosomal proteins.

- The 50S (large) subunit containing:

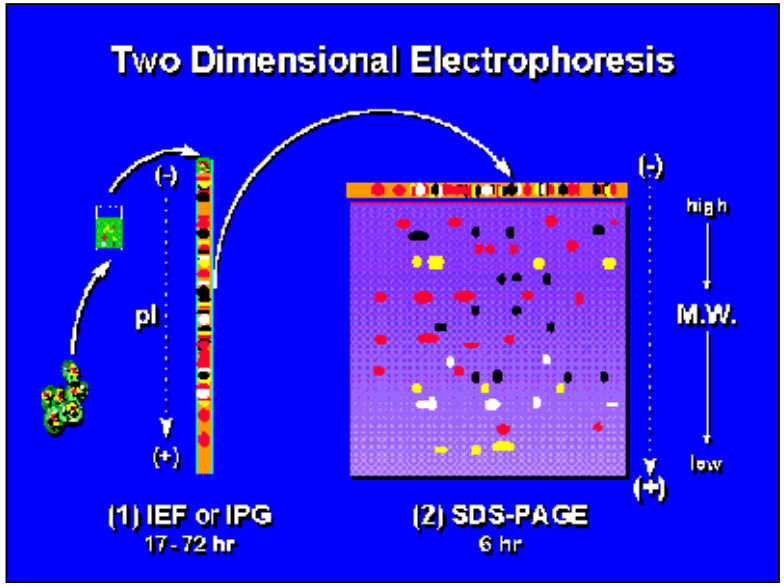
1. 34 different proteins (L1- L34), and
2. 23S rRNA of 2904.



Powers,2010;Sophie Arbefeville

Ribosomal subunits
Part protein and Part RNA

- Riboproteins, isolated from small and large subunits, have been numbered as small and large riboproteins.
- They are numbered as S1 to S21 and L1 to L31 respectively.
- The numbering is based on the mobility of each of the riboprotein subunits on a 2-D polyacrylamide gel.
- The S1 is the largest protein found at the left top most corner of the gel and S21 is the smallest, found at the right bottom most corner of the gel.



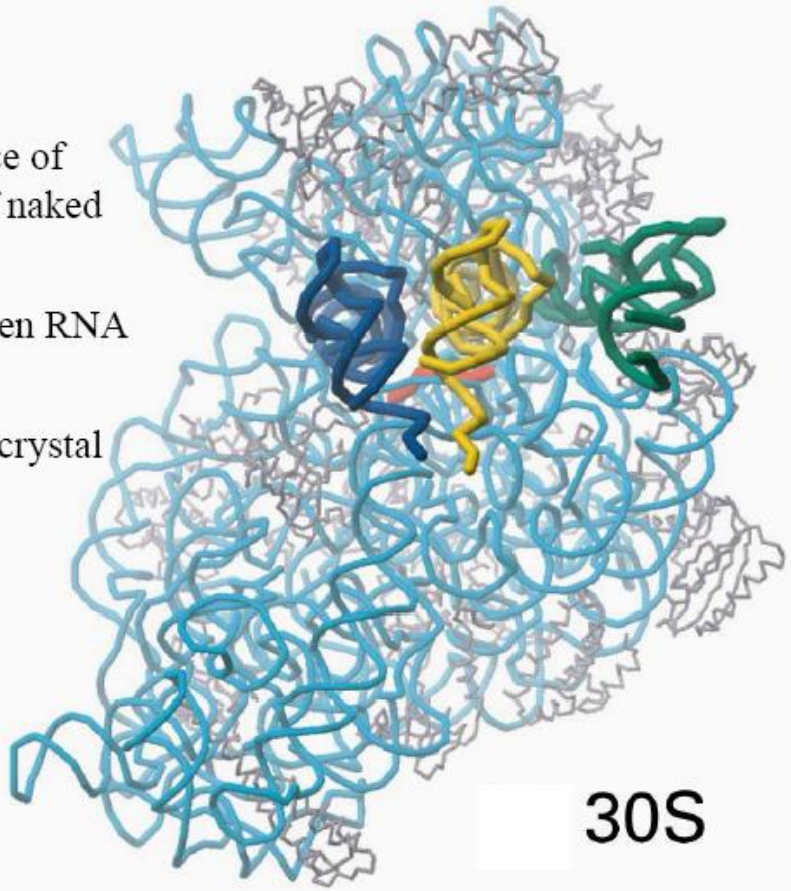
3D Crystal Structure of ribosomes
Small subunit (30S)

A. Small Subunit

-Surface that ends up on interface of subunit is a long double helix of naked rRNA (no proteins!)

-In general, proteins fit in between RNA helices

- Excellent agreement between crystal structure and biochemical data.



Ribosomal RNAs

Three key features of the variation in rRNAs

- rRNAs are not identical between species.
- For our purposes there are three key features of the variation in rRNA sequence between species.

1. The rRNA molecules in the ribosome fold over into complex three dimensional shapes. The specific shape that they take is highly conserved between species.
2. When a single species splits into two distinct evolutionary lineages, differences can accumulate in the sequence of the rRNAs between the two lineages.
3. Some regions of rRNAs evolve (i.e., diverge) slowly and others diverge rapidly. Some regions are basically the same across most or all taxa.

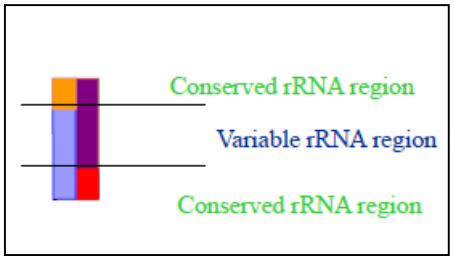
16S ribosomal DNA

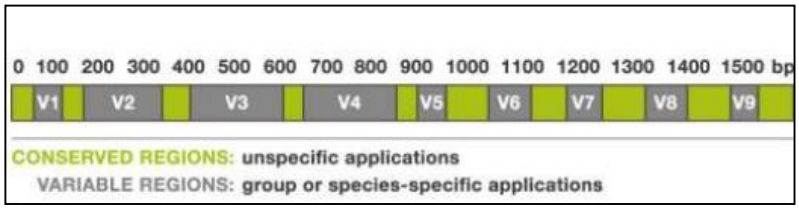
1.5 kb in length, consists of 8 highly conserved regions and 9 variable regions

- The 16S rRNA gene is approximately 1500 bases in length and contains regions that are:

1. Highly 'conserved' (i.e., have the same sequence in all bacteria and archaea), and
2. Highly 'variable' (i.e., have sequences that are unique at the genus or species level).

- Thus the conserved regions of the gene can be used to bind primers for PCR and sequencing, and the variable regions to determine the identity of the organism.

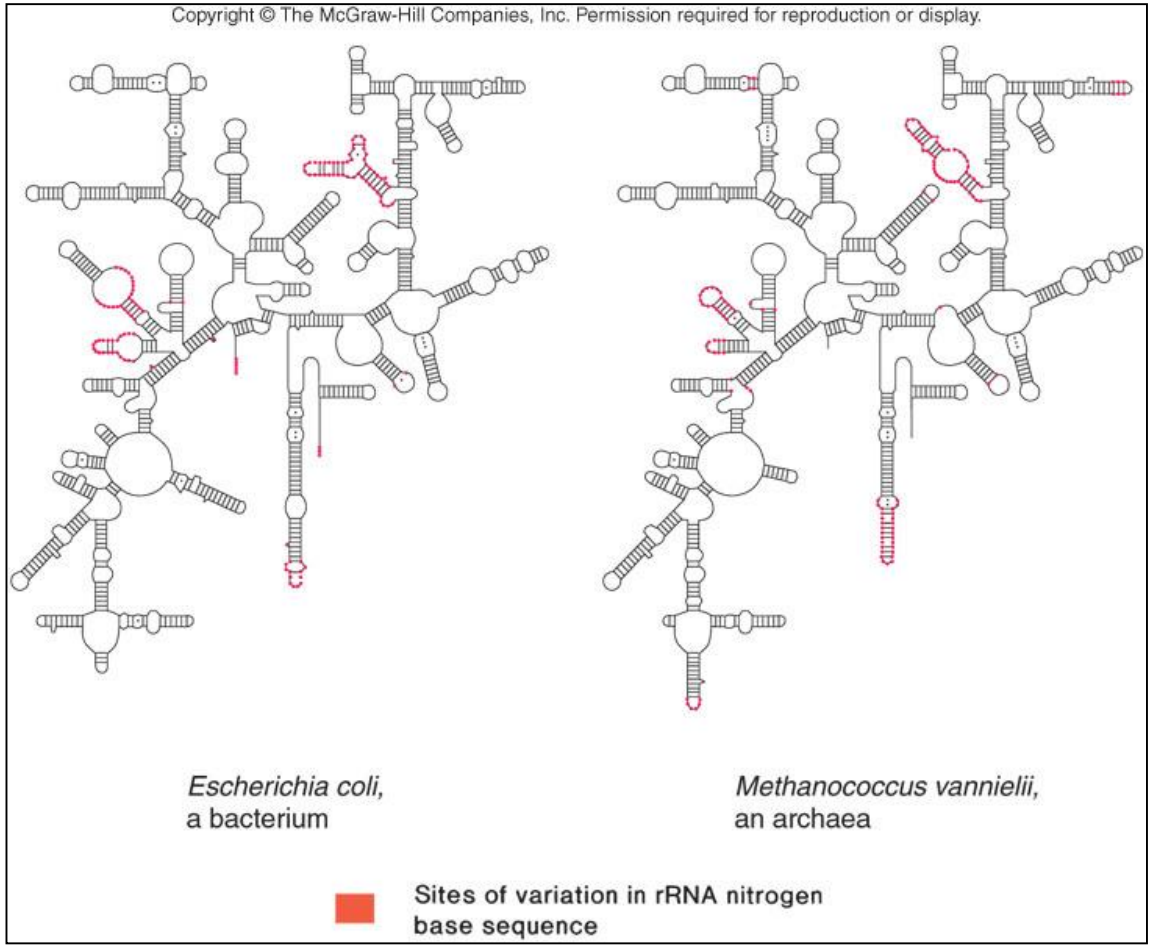




Moran,2010; Sophie Arbefeville

16S ribosomal DNA

1.5 kb in length, consists of 8 highly conserved regions and 9 variable regions



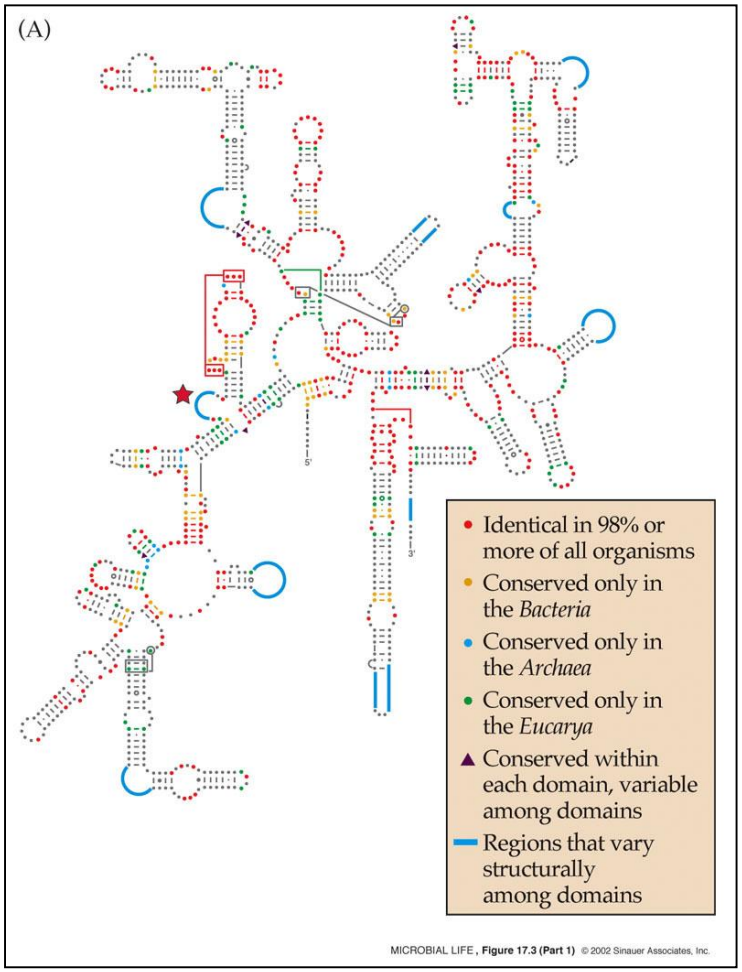
Powers,2010

16S rRNA

Fold into a complex, stable secondary structure, consisting of stems and loops

- The sequences of some of the loops are conservative across nearly all bacterial species because of the essential functions involved.
- Whereas the features of the structural parts (stems) are largely variant and specific to one or more bacteria.
- The variant regions, V1–V9, of the 16S rRNA genes (rDNAs) have been used for species identification.

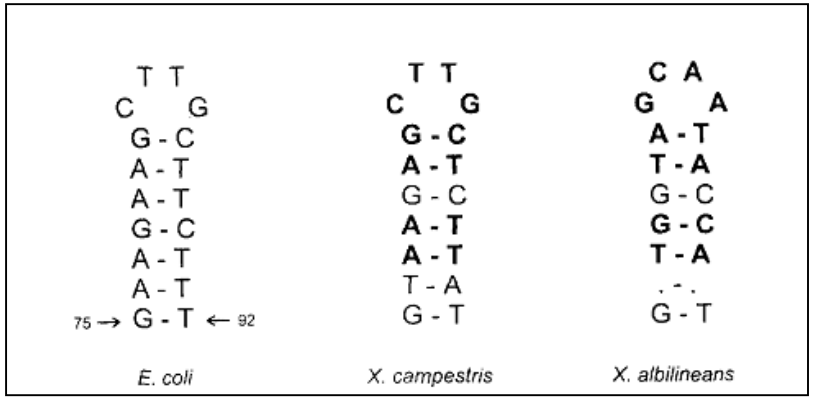
Karlm,2004; Wang and Qian,2009



16S rRNA

Fold into a complex, stable secondary structure, consisting of stems and loops

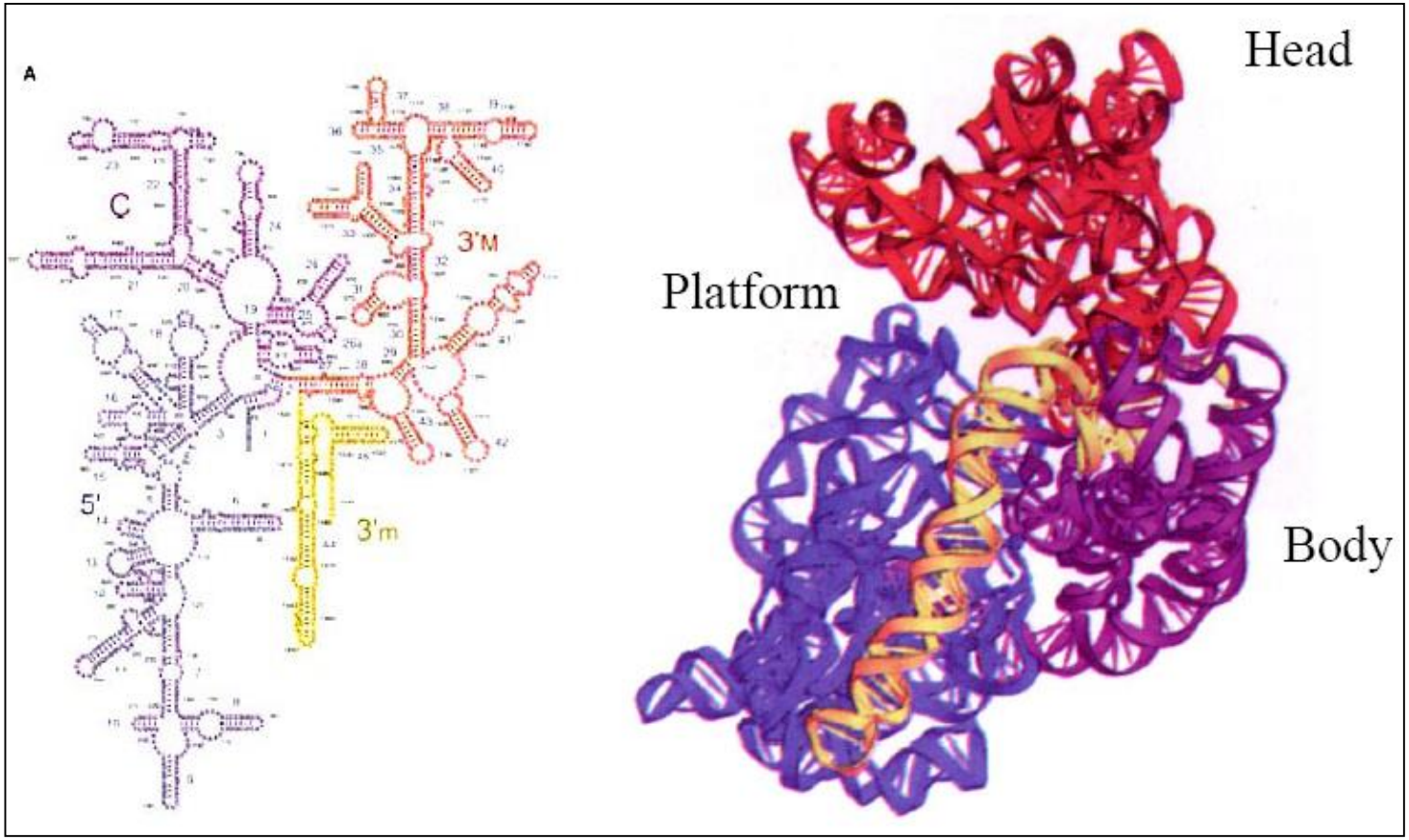
- Diagnostic area in helix 6 (V1 region) of the 16s rDNA which distinguishes the cluster around *X. albilineans* from the *X. campestris* core.
- Variable positions are shown in **boldface type**.
- Dots indicate gaps.
- *E. coli* 16s rRNA gene sequence numbering was used.



Hauben *et al.*,1997

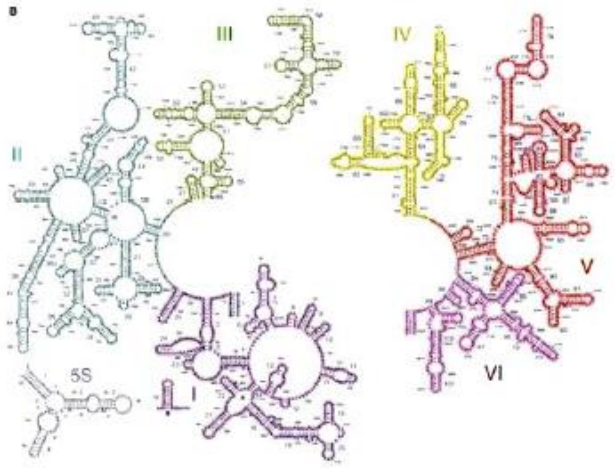
3D Folding of rRNA for small subunit

16S rRNA

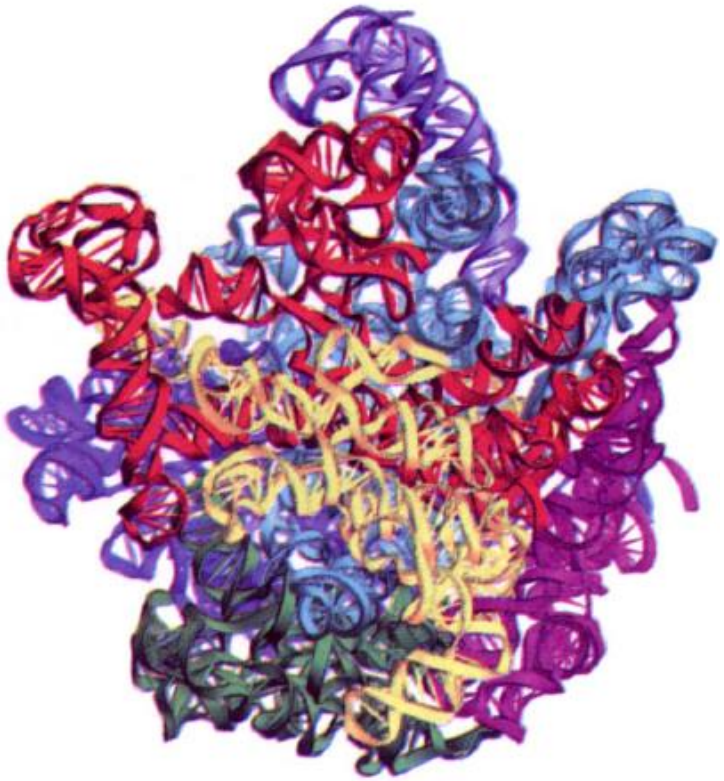


3D Folding of rRNA for Large Subunit

5S & 23S rRNAs



5S rRNA



23S rRNA

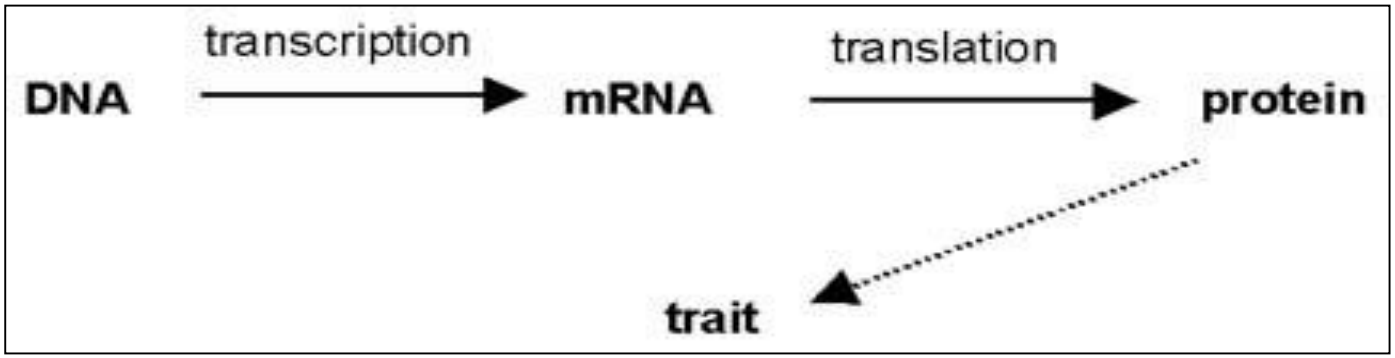
Cytoplasm of Prokaryotes

Gene expression for protein synthesis

The bacterial genomes(Protein synthesis)

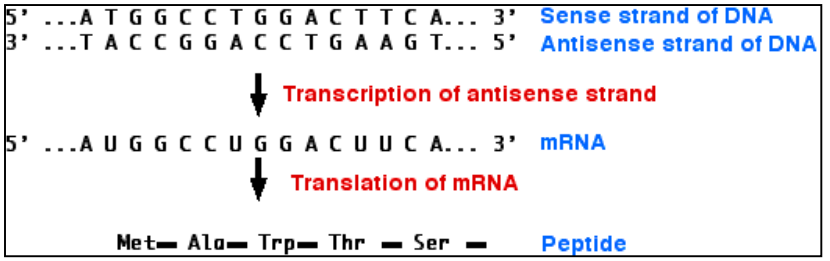
The central dogma of molecular biology
Protein synthesis

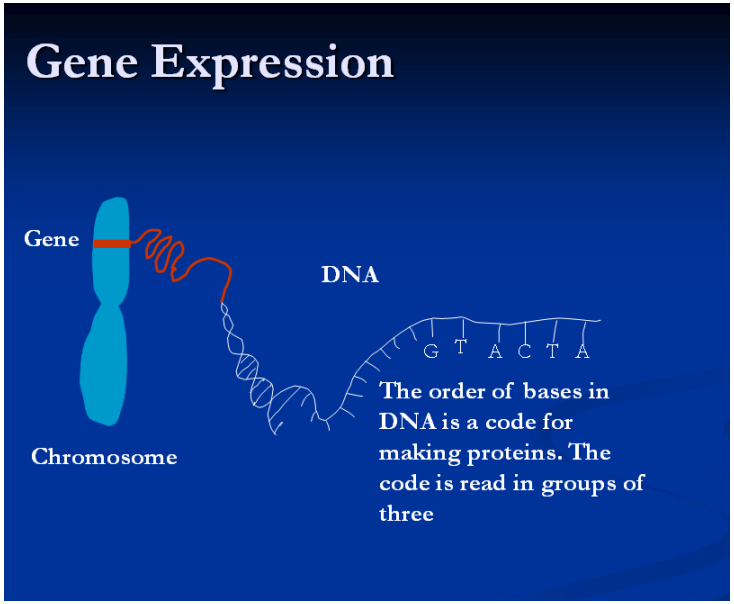
- The DNA contains the master plan for the creation of the proteins and other molecules and systems of the cell, but the carrying out of the plan involves transfer of the relevant information to mRNA in a process called transcription.



Gene expression for protein synthesis
Transcription and translation

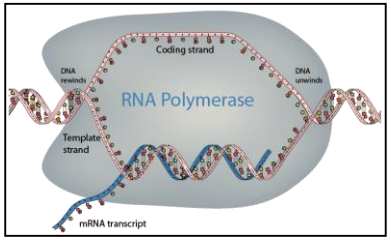
- The process of protein synthesis, including:
1. DNA to mRNA (transcription)
2. mRNA to protein (translation)
    - Initiation
    - Elongation
    - End of translation.

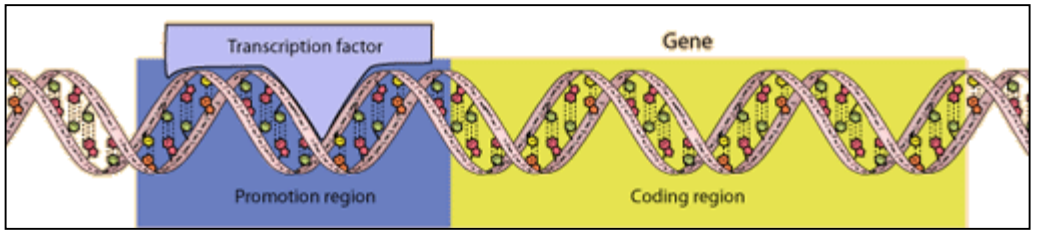




Gene expression for protein synthesis
Transcription
RNA polymerase are universally conserved in all organisms -- from bacteria to humans

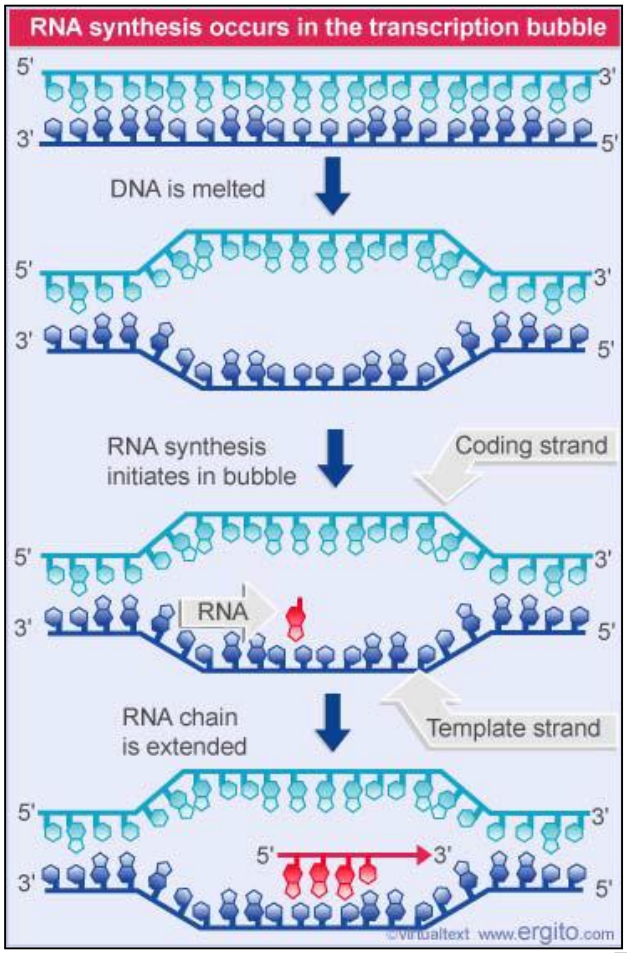
- Transcription is the process by which the information in DNA is copied into messenger RNA.
- During transcription, the enzyme RNA polymerase uses DNA as a template.
- Note that any place in a DNA molecule, either strand may be serving as the template. i.e. only a particular segment of DNA is copied into RNA by the enzyme RNA polymerase.
- As RNA polymerase moves along the DNA template, it unwinds the duplex at the front of the bubble (the unwinding point), and rewinds the DNA at the back (the rewinding point).





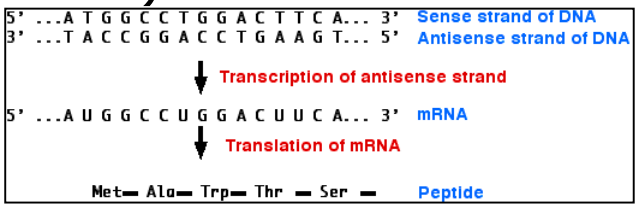
Gene expression for protein synthesis
Transcription
RNA polymerase

- It begins when the RNA polymerase binds with transcription promoting regions of DNA.
- RNA polymerase separates the two strands of DNA in a transient "bubble" and uses one strand as a template to direct synthesis of a complementary sequence of RNA.



Gene expression for protein synthesis
Transcription
RNA polymerase

- RNA polymerase attaches to the DNA at a specific area called the promoter region.
- Once the enzyme has attached to the DNA, it unwinds the double helix over a short length, and splits the two strands apart.
- The first thing that the enzyme has to do is to find the start of the gene on the coding strand of the DNA.
- This allows RNA polymerase to transcribe only a single strand of DNA into a single stranded RNA polymer called messenger RNA (mRNA).

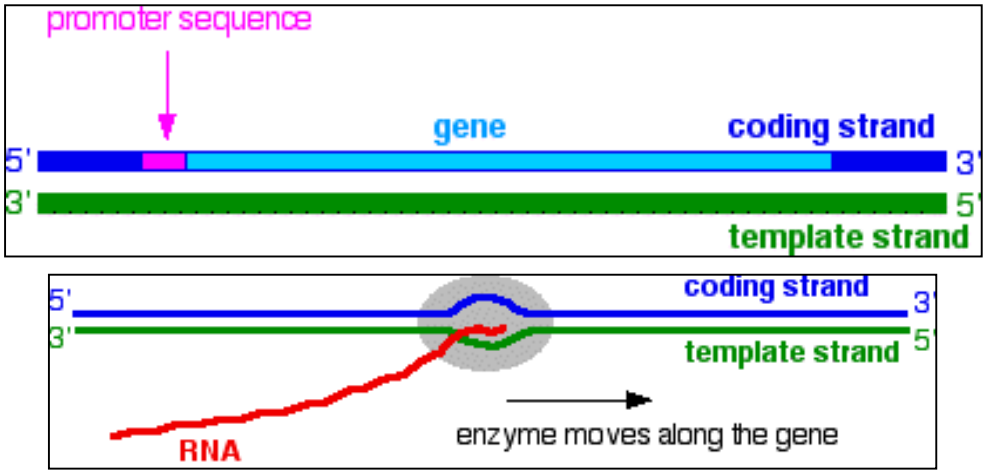


Gene expression for protein synthesis
Transcription

- Messenger RNA will be translated into a polypeptide.
- Messenger RNA comes in a wide range of sizes reflecting the size of the polypeptide it encodes.
- Most cells produce small amounts of thousands of different mRNA molecules, each to be translated into a peptide needed by the cell.
- Many mRNAs are common to most cells, encoding "housekeeping" proteins needed by all cells.
- Other mRNAs are specific for only certain types of cells. These encode proteins needed for the function of that particular cell.

Gene expression for protein synthesis
Transcription

- The RNA polymerase proceeds to "read" one strand moving in its 3′→5′ direction.
- Also the enzyme RNA polymerase knows where to stop after it reaches the end of the gene.
- When transcription is complete, the transcript is released from the polymerase and, shortly thereafter, the polymerase is released from the DNA.



Gene expression for protein synthesis
Genetic code
RNA codons or DNA codons

- The genetic code can be expressed as either RNA codons or DNA codons.
- A codon is a sequence of three DNA or RNA nucleotides that corresponds with a specific amino acid or stop signal during protein synthesis.
- RNA codons occur in mRNA and are the codons that are actually "read" during the synthesis of polypeptides (the process called translation).
- Full set of codons is called the genetic code.

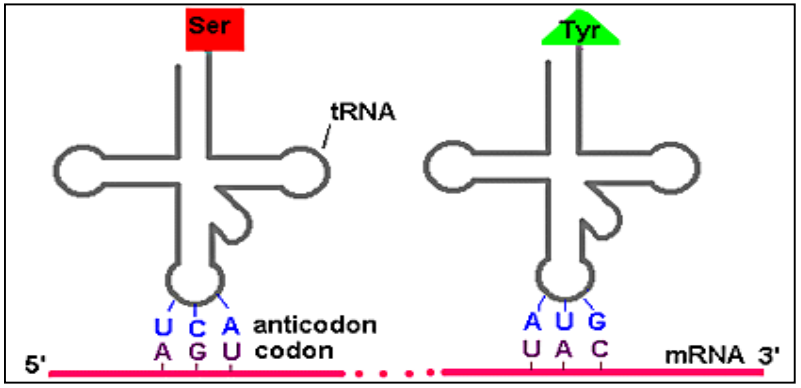
Nucleotide: Basic building blocks of DNA and RNA. Consists of Adenine, Guanine, Thymine, Cytosine, and Uracil.

Gene expression for protein synthesis
Genetic codes

- The genetic code is said to be universal because a codon represents the same amino acids in almost all organisms.
- There are more than one codon for the same amino acid.
- The magic number in the 'case of codons' is 3.
- Putting 3 nucleotides together will provide for 64 possible amino acids.
- Many of these 3 groups code for the same amino acid.
- Arginine and leucine are encoded by 6 triplets, isoleucine by 3, methionine and tryptophan by 1, and all other amino acids by 4 or 2 codons.

Gene expression for protein synthesis
Genetic codes
mRNA codons and tRNA anticodons

- The process of translation of genetic information into the assembling of a protein requires first mRNA, which is read 5' to 3' (exactly as DNA), and then transfer ribonucleic acid (tRNA), which is read 3' to 5'.
- tRNA is the taxi that translates the information on the ribosome into an amino acid chain or polypeptide.



Genetic code

DNA codon table (64 codons)

Standard genetic code

1st base	2nd base T		2nd base C		2nd base A		2nd base G		3rd base
T	TTT	(Phe/F) Phenylalanine	TCT	(Ser/S) Serine	TAT	(Tyr/Y) Tyrosine	TGT	(Cys/C) Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine	TCA		TAA	Stop (*Ochre*)	TGA	Stop (*Opal*)	A
	TTG		TCG		TAG	Stop (*Amber*)	TGG	(Trp/W) Tryptophan	G
C	CTT		CCT	(Pro/P) Proline	CAT	(His/H) Histidine	CGT	(Arg/R) Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine	ACT	(Thr/T) Threonine	AAT	(Asn/N) Asparagine	AGT	(Ser/S) Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	ATG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine	GCT	(Ala/A) Alanine	GAT	(Asp/D) Aspartic acid	GGT	(Gly/G) Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GTG		GCG		GAG		GGG		G

Wikepedia,2015

Genetic code

RNA codon table (64 codons)

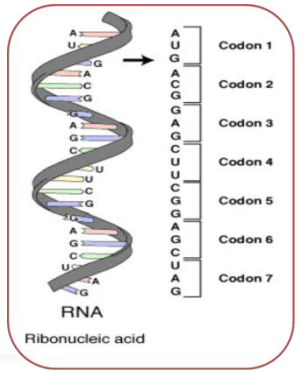
Standard genetic code

1st base	2nd base U		2nd base C		2nd base A		2nd base G		3rd base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

Wikepedia,2015

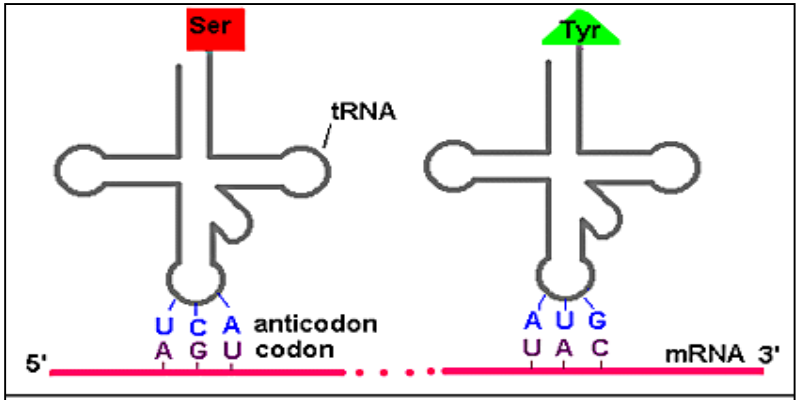
Genetic code

RNA codon table (64 codons)



- A codon is a set of 3 bases (nucleotides) which code for an amino acid.
- There are in total 64 different codons which produce 20 different amino acids. These include:

1. 61 of them code for 20 amino acids (AA);
2. 1 Start codon (ATG);
3. The last 3 codon (UAG,UGA,UAA) don not code for amino acids; they are termination or stop codons.



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Genetic code
Codons and anticodons

- A set of three nucleotide bases on an mRNA molecule is called a codon.
- A set of three nucleotide bases on a tRNA molecule is called an anticodon.
- Anticodon in tRNA complementary to a codon on mRNA.
- An anticodon in a tRNA molecule aligns with a particular codon in mRNA under the influence of the ribosome, so that the amino acid carried by the tRNA is added to a growing protein chain
- Even though there are only 20 amino acids that exist, there are actually 64 possible tRNA molecules:

$$4 \times 4 \times 4 = 64 \text{ possible combinations}$$

- The 20 amino acids can be linked in different combinations and in different numbers. For example:

alanine-valine-tryptophan.....serine

Genetic code

Gene Prediction

ORF in prokaryotes(bacteria and archeae) and eukaryotes

- Biologists often get a piece of DNA sequence and want to know what's in it.
- One of the most obvious questions to ask is, does it contain a gene?
- Because genomes of organisms (except prokaryotes) consist of many non-coding regions (introns),

1. it's not clear that a random piece of DNA will always have a gene.
2. And if there is a gene, where does it begin and end?

- A simple strategy for finding genes is to look for open reading frames.
- An open reading frame is the section of a sequence between a start codon and a stop codon.

Genetic code

ORF in prokaryotes(bacteria and archeae) and eukaryotes

Coding region of the gene (DNA/mRNA)

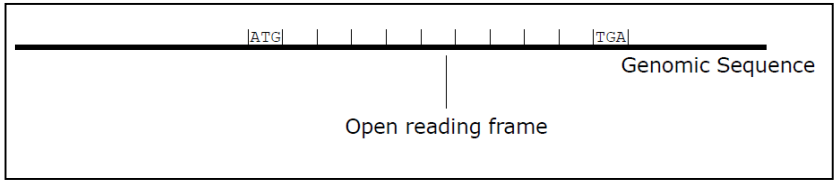
- An ORF is a sequence of DNA that starts with start codon "ATG" (not always) and ends with any of the three stop codon (or termination codon): TAA, TAG, TGA.
- Stop codon (or termination or nonsense codon) specifies the end of translation or transcription.
- Computer analysis of DNA sequence can predict the existence of genes based on ORFs.
- Long open reading frames may be a gene. However, genes are usually much longer than this (long ORF).

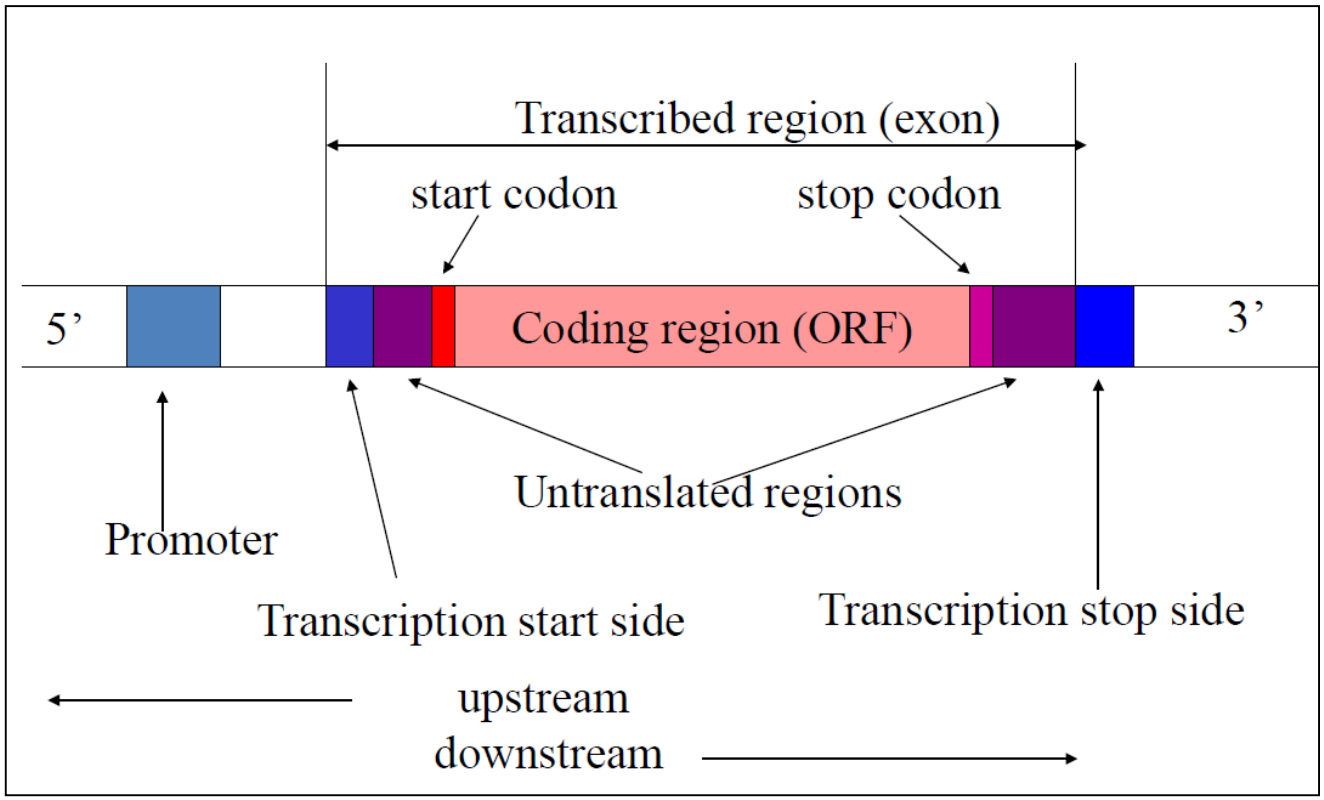
Stop codons (TAG, TGA and TAA in DNA, and UAG, UGA and UAA in mRNA).

Genetic code

ORF in prokaryotes(bacteria and archeae) and eukaryotes

Coding region of the gene (DNA/mRNA)





Genetic code

Search for an open reading frame in the mRNA

- The open reading frame can end with several different stop codons: UAA, UGA, or UAG.
- For example, the following mRNA contains a short open reading frame that begins at position 3 (starting from 0) and ends at position 12 (the first letter of the stop codon):

  AGAAUGGCCUGGUAAGGC open reading frame found in mRNA.

Genetic code

Search for an open reading frame in the DNA

Gene Prediction: Computational Challenge

- An ORF is a sequence of DNA that starts with start codon "ATG" (not always) and ends with any of the three stop codon (or termination codon): TAA, TAG, TGA.

atgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgc
ggctatgcaagctgggatccgatgactatgctaagctgggatccgatgacaatgcatgcggctatgctaatgaatggtct
tgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggctatgctaatgaatggtcttgggatttacctt
ggaatatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgcggctatg
caagctgggatccgatgactatgctaagctgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaat
gcatgcggctatgctaatgcatgcggctatgcaagctgggatcctgcggctatgctaatgaatggtcttgggatttaccttg
gaatgctaagctgggatccgatgacaatgcatgcggctatgctaatgaatggtcttgggatttaccttggaatatgcta
atgcatgcggctatgctaagctgggaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgct
aatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcatgcggctatgctaagct
catgcggctatgctaagctgggaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaat
gcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcatgcggctatgctaagctcgg
ctatgctaatgaatggtcttgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggctatgctaatga
atggtcttgggatttaccttggaatatgctaatgcatgcggctatgctaagctgggaatgcatgcggctatgctaagctgg
gatccgatgacaatgcatgcggctatgctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcgg
ctatgctaatgcatgcggctatgctaagctcatgcgg

Gene!

Is exon the same as open reading frame

- In protein-coding genes, the open reading frame (ORF) that codes for a specific portion of the complete protein are located in the exons.

1. However, an exon is any part of a gene but not all the gene. In eukaryotes, this will contain both introns and exons.
2. It is incorrect to say that all exons code for proteins, since there are many known noncoding exons.

Genetic code

Polycistronic mRNAs

ORF in prokaryotes(bacteria and archeae) and eukaryotes

- An open reading frame is the section of a sequence between a start codon and a stop codon.
- Most of the eukaryotic mRNAs are monocistronic.
  - Monocistronic mRNA contains the genetic information to translate only a single protein chain (polypeptide).
- In prokaryotes (bacteria and archeae), mRNA molecules are polycistronic.
  - Polycistronic mRNA carries several open reading frames (ORFs), each of which is translated into a polypeptide.

Cistron: a segment of DNA that is equivalent (synonym) to a gene and that specifies a single functional unit (as a protein or enzyme).

Genetic code

Polycistronic mRNAs

ORFs in prokaryotes(bacteria and archeae)

- Polycistronic mRNA carries several open reading frames (ORFs), each of which is translated into a polypeptide.
- Most of these mRNAs found in bacteria and archaea.
- These polypeptides usually have a related function (they often are the subunits composing a final complex protein) and their coding sequence is grouped and regulated together in a regulatory region, containing a promoter and an operator.

Promoter, operator and enhancer regions regulate the transcription of the gene into an mRNA. Negative control involves the binding of a repressor to the operator to prevent transcription.

Genetic code

Polycistronic mRNAs

ORFs in prokaryotes(bacteria and archeae)

- Usually in bacteria a single mRNA codes for multiple proteins.
- There are many ORFs that are lined one after the other; such mRNAs are called polycistronic mRNAs.
- A single polycistronic mRNA molecule, carries the information for synthesis of several proteins.
- In these cases there is another start codon a few distance ahead of a previous stop codon.

Polycistronic mRNA

Monocistronic mRNAs vs. polycistronic mRNAs

Prokaryotic mRNA encodes more than one protein

- A mRNA found in prokaryotes that encodes more than one protein.
- Each eukaryotic mRNA contains information coding for only one protein, hence monocistronic, whereas prokaryotic mRNAs may encode more than one protein and are said to be polycistronic.
- Most but not all eukaryotic mRNAs encode single gene product.

Genetic code

Polycistronic mRNAs

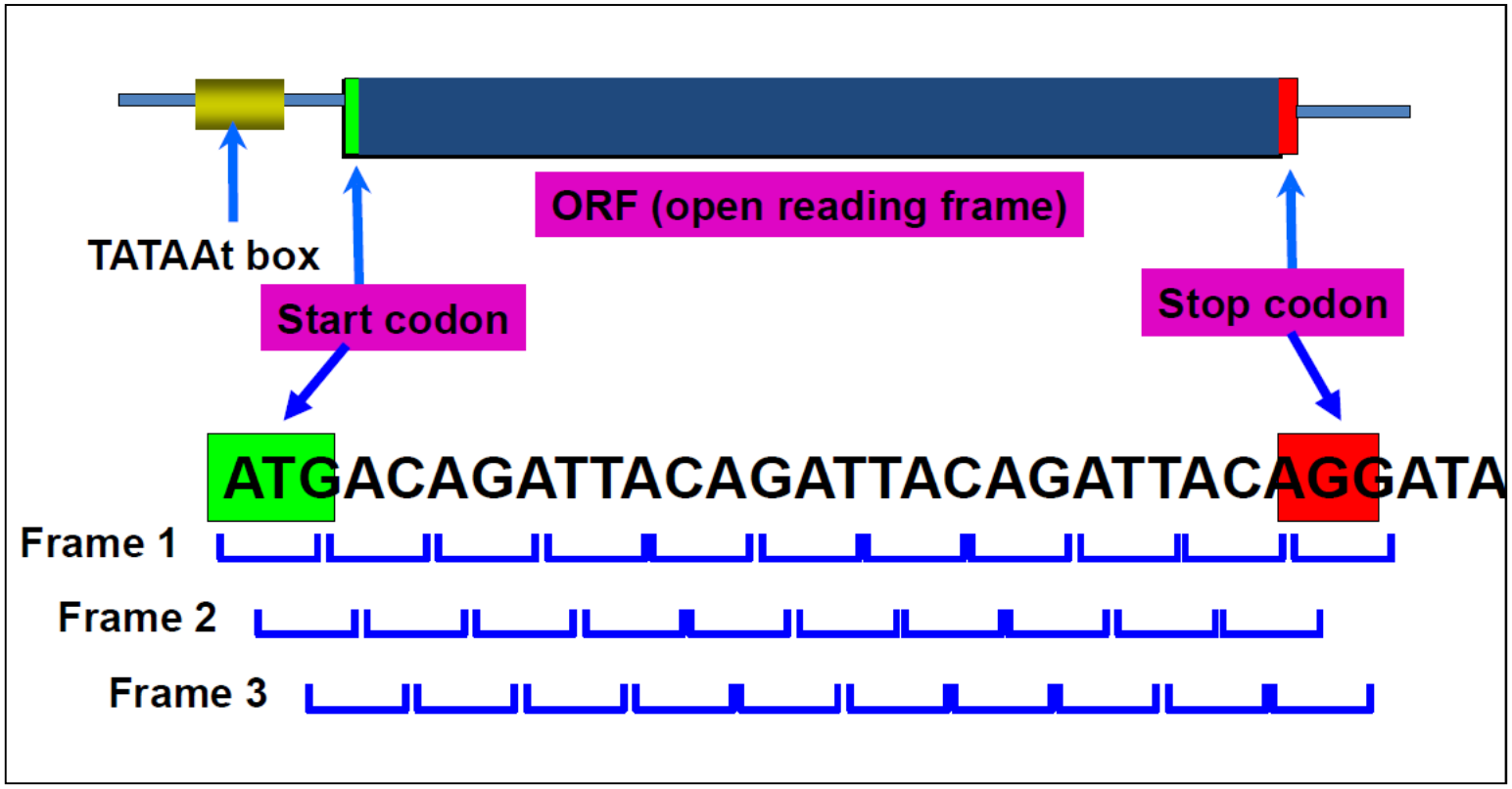
ORFs in prokaryotes (bacteria and archeae)

- Usually in bacteria a single mRNA codes for multiple proteins.
- There are many ORFs that are lined one after the other; such mRNAs are called polycistronic mRNAs.
- A single polycistronic mRNA molecule, carries the information for synthesis of several proteins.
- In these cases there is another start codon a few distance ahead of a previous stop codon.

Prokaryotic gene structure

Polycistronic mRNAs

ORFs in prokaryotes (bacteria and archeae)

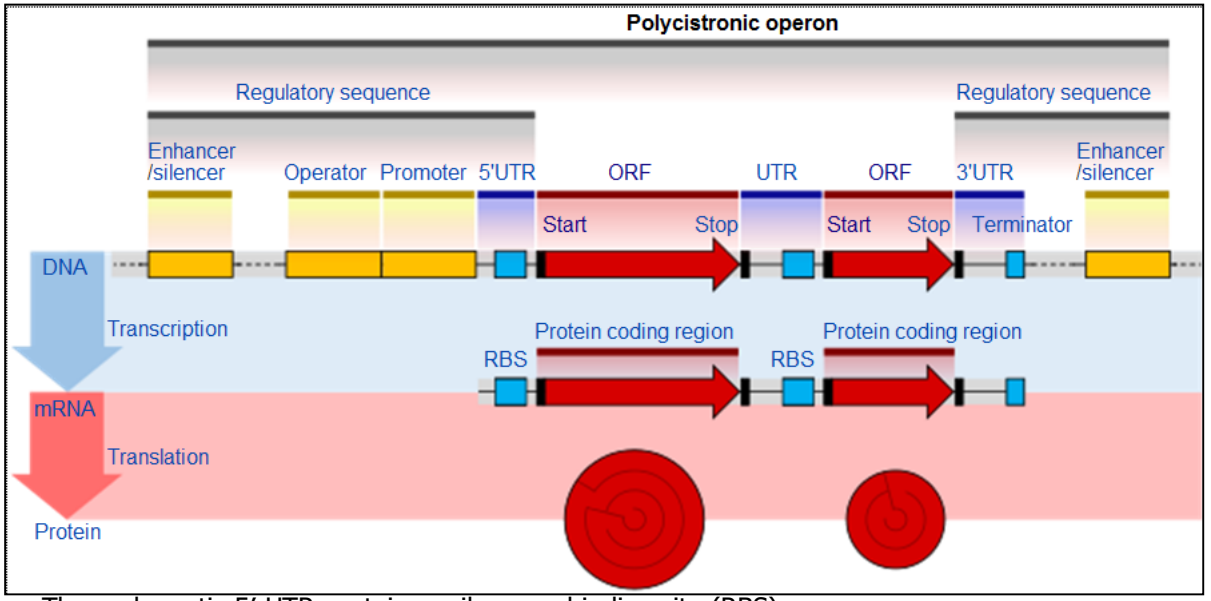


Genetic code

Polycistronic mRNAs

The structure of a prokaryotic operon of protein-coding genes

- Regulatory sequence controls when expression occurs for the multiple protein coding regions (red).
- Promoter, operator and enhancer regions (yellow) regulate the transcription of the gene into an mRNA.
- The mRNA untranslated regions, UTR (blue) regulate translation into the final protein products.



The prokaryotic 5′ UTR contains a ribosome binding site (RBS).

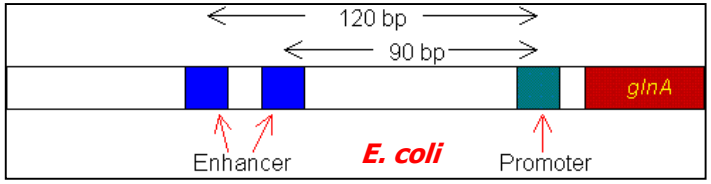
Enhancer

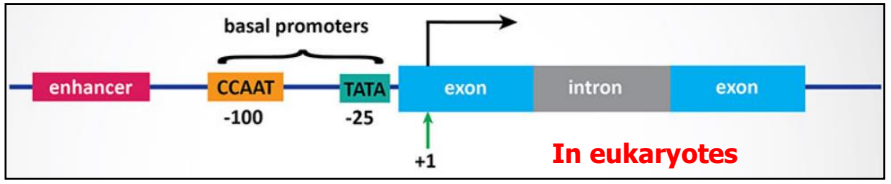
Binds transcription factors and enhance transcription

In prokaryotes and eukaryotes

- Enhancer is the DNA element that, upon binding with transcription factors (activators), can enhance transcription.
- It may be located either upstream or downstream of the transcriptional initiation site.
- However, most of them are located upstream.

1. In prokaryotes, enhancers are quite close to the promoter, but
2. eukaryotic enhancers could be far from the promoter.



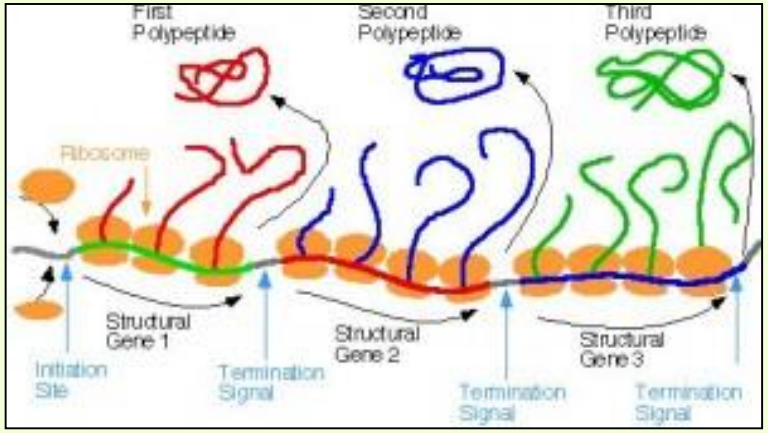


Genetic code

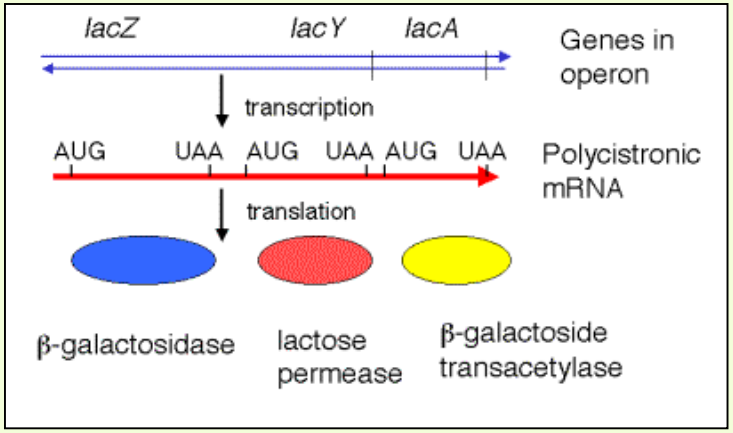
Polycistronic mRNAs

ORFs in prokaryotes(bacteria and archeae)

- These polypeptides usually have a related function (they often are the subunits composing a final complex protein).



- A polycistronic operon in *E. coli*.
- Three different enzymes (proteins) were synthsized.



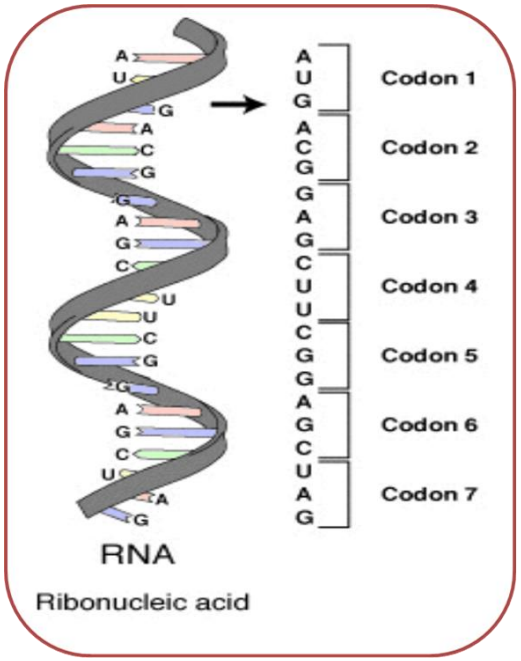
Genetic code

Full set of codons is called the genetic code

- 3. **61 of the 64 possible codons** (triplets) code particular amino acids.

AAA	Lys	CAA	Gln	GAA	Glu	UAA	STOP
AAG		CAG		GAG		UAG	
AAC	Asn	CAC	His	GAC	Asp	UAC	Tyr
AAU		CAU		GAU		UAU	
ACA	Thr	CCA	Pro	GCA	Ala	UCA	Ser
ACG		CCG		GCG		UCG	
ACC		CCC		GCC		UCC	
ACU		CCU		GCU		UCU	
AGA	Arg	CGA	Arg	GGA	Gly	UGA	STOP / Trp
AGG	STOP	CGG		GGG		UGG	Trp
AGC	Ser	CGC		GGC		UGC	Cys
AGU		CGU		GGU		UGU	
AUA	Ile / Met	CUA	Leu	GUA	Val	UUA	Leu
AUG	Met	CUG		GUG		UUG	
AUC	Ile	CUC		GUC		UUC	Phe
AUU		CUU		GUU		UUU	

wobble



Genetic code

Full set of codons is called the genetic code

- 3. **61 of the 64 possible codons** (triplets) code particular amino acids.
- Most amino acids are specified by more than one codon.
- This means that we don't need 61 different tRNA molecules for all 61 codons.
- The "wobble hypothesis," explains this phenomenon.
- According to wobble hypothesis:

1. The code often degenerated;
2. Inosine could enable one tRNA to recognize more than one codon.

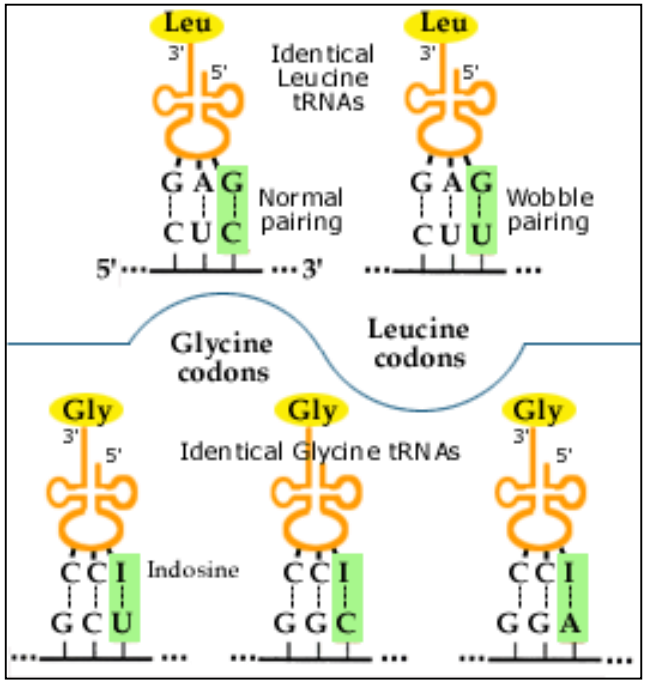
The table shows which codons code for which amino acids

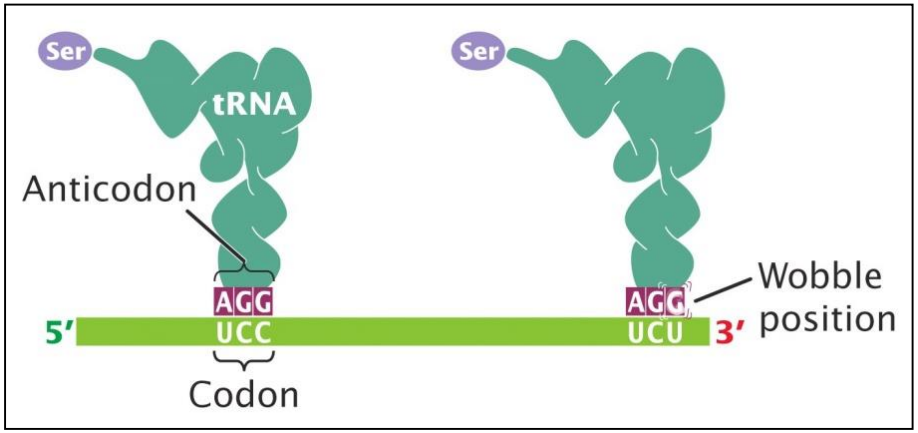
AMINO ACID	RNA CODON
ALANINE	GCC, GCA, GCG, GCU
ARGININE	AGA, AGG, CGU, CGA, CGC, CGG
ASPARAGINE	AAC, AAU
ASPARTIC ACID	GAC, GAU
CYSTEINE	UGC, UGU
GLUTAMIC ACID	GAA, GAG
GLUTAMINE	CAA, CAG
GLYCINE	GGA, GGC, GGG, GGU
HISTIDINE	CAC, CAU
ISOLEUCINE	AUA, AUC, AUU
LEUCINE	UUA, UUG, CUA, CUC, CUG, CUU
LYCINE	AAA, AAG
METHIONINE (INITIATION)	AUG
PHENYLALANINE	UUC, UUU
PROLINE	CCA, CCC, CCG, CCU
SERINE	UCA, UCC, UCG, UCU, AGC, AGU
THREONINE	ACA, ACC, ACG, ACU
TRYPTOPHAN	UGG
TYROSINE	UAC, UAU
VALINE	GUA, GUC, GUG, GUU
STOP	UAA, UAG, UGA

Genetic code
Wobble position/rules

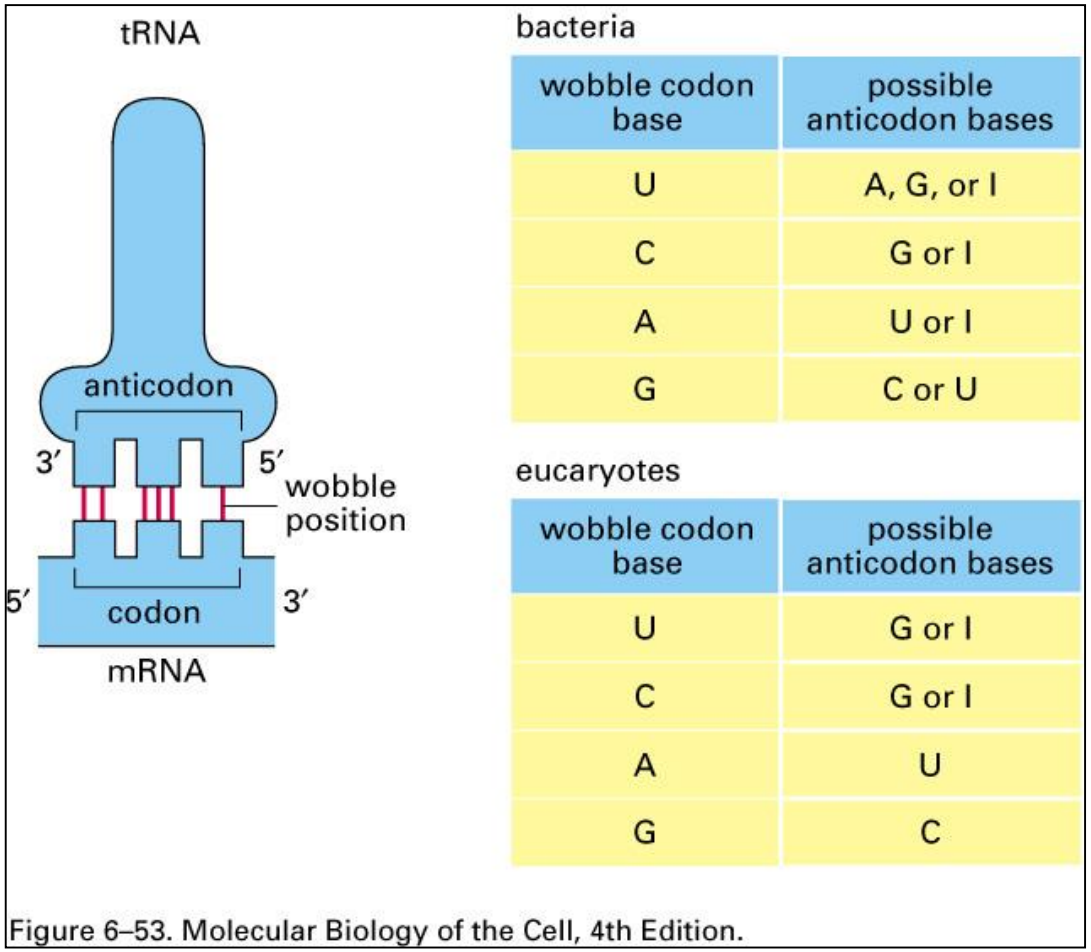
- Due to the degeneracy of the genetic code, the third base is less discriminatory for the amino acid than the other two bases.
- This third position in the codon is referred to as the wobble position.
- This flexibility at the "wobble" position allows some tRNAs to pair with two or three codons, thereby reducing the number of tRNAs required for translation.
- The following wobble rules mean that the 61 codons (for 20 amino acids) can be read by as few as 31 anticodons (or 31 tRNAs).
- In addition to the usual base pairs, one can have G-U pairs and I in the anticodon 1st position can pair with U, C or A.

Genetic code
Wobble position





Genetic code
Translation: Wobbing Effect



bacteria

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

eucaryotes

wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

Figure 6–53. Molecular Biology of the Cell, 4th Edition.

Protein synthesis

Translation

Initiation, elongation and termination

- Translation occurs in a structure called the ribosome, which is a factory for the synthesis of proteins.
- The ribosome has a small and a large subunit and is a complex molecule composed of several ribosomal RNA molecules and a number of proteins.
- Translation of an mRNA molecule by the ribosome occurs in three stages:

1. initiation,
2. elongation, and
3. termination.

Protein synthesis

Translation

Initiation, elongation and termination

- Translation is the process by which a protein is synthesized from the information contained in a molecule of messenger RNA (mRNA).
- During translation, an mRNA sequence is read using the genetic code, which is a set of rules that defines how an mRNA sequence is to be translated into the 20-letter code of amino acids, which are the building blocks of proteins.
- The genetic code is a set of three-letter combinations of nucleotides called codons, each of which corresponds with a specific amino acid or stop signal.

Protein synthesis

tRNA is involved in the translation of the nucleic acid message into the amino acids of proteins

- That start codon is usually AUG in both eukaryotes and prokaryotes, although eukaryotes use GUG on rare occasions.
  During initiation, the small ribosomal subunit binds to the start codon of mRNA sequence, AUG.
- Then a transfer RNA (tRNA) molecule carrying the amino acid methionine binds to what is called the start codon of the mRNA sequence.
- The start codon in all mRNA molecules has the sequence AUG and codes for methionine.
- Next, the large ribosomal subunit binds to form the complete initiation complex.

Protein synthesis

Initiation

The first phase of translation

- In bacteria, the mRNA binds by hybridization of a special sequence to the Shine-Dalgarno sequence of the 16s rRNA, part of the 30s subunit.
- The ribosome then finds the first AUG sequence (the start codon contain methionine) on the mRNA, where it binds the anti-codon of a Met-tRNA, at the P site.
- This led to formation of the first peptide bond during translation initiation and protein synthesis.

KAP Genetics and Development;..

Initiation

The first phase of translation

- The adaptor molecule for translation is tRNA.
- A charged tRNA has an amino acid at one end, and at the other end it has an anticodon for matching a codon in the mRNA; i.e. it "speaks the language" of nucleic acids at one end and the "language" of proteins at the other end.
- The machinery for synthesizing proteins under the direction of template mRNA is the ribosome.

Initiation

The first phase of translation

Comparison of the main characteristics of three domains

- On of the main differences is in the sequences of nucleotides of the cell's ribosomal and transfer RNAs.

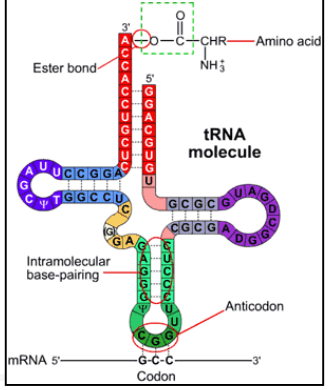
Characteristic	Archaea	Eucarya	Bacteria
Ribosomes	70S	80S	70S
Initiator tRNA	Methionine	Methionine	Formylmethionine
Introns in tRNA	Yes	Yes	No

fMet is present in proteins made by prokaryotes but not in those made by eukaryotes. fMet is a derivative of the amino acid methionine in which a formyl group has been added to the amino group. fMet is coded by the same codon as methionine, AUG.

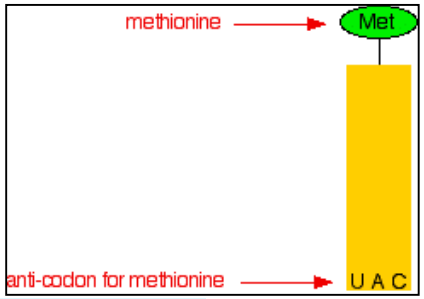
Initiation

The first phase of translation

$tRNA^{fMet}$ as initiator tRNA in bacteria



- 4 nucleotide bases: adenine, guanine, cytosine, and thymine (Uracil if in RNA) code for 20 amino acids.
- The messenger RNA code for methionine is AUG.
- If you look at the code in the anti-codon for methionine, it is UAC.
- That is exactly complementary to AUG.
- The U in the anti-codon will pair with the A in the messenger RNA; the A in the anti-codon pairs with the U in the mRNA; and the C in the anti-codon pairs with the G in the mRNA.



Initiation

The first phase of translation

$tRNA^{fMet}$ as initiator tRNA in bacteria

- In bacteria, the initiation codon is recognized by a specific tRNA molecule, $tRNA^{fMet}$.
- Methionine (abbreviated as Met or M), is coded by the initiation codon AUG which also indicates mRNA's coding region where translation into protein begins.
- After this tRNA molecule is charged with methionine, the amino acid is modified, to N-formylmethionine.

Initiation

The first phase of translation

$tRNA^{fMet}$ as initiator tRNA in bacteria

- Aminoacylated tRNA molecules normally bind to a site on the ribosome known as the A site (Acceptor), while their anticodon region pairs with the mRNA.
- Only after peptide bond formation is the tRNA able to move to a second site on the ribosome, the P (Peptide) site.
- The fMet-$tRNA^{fMet}$ (i.e. the $tRNA^{fMet}$ charged with formylmethionine) is unique in being able to enter the P site directly.

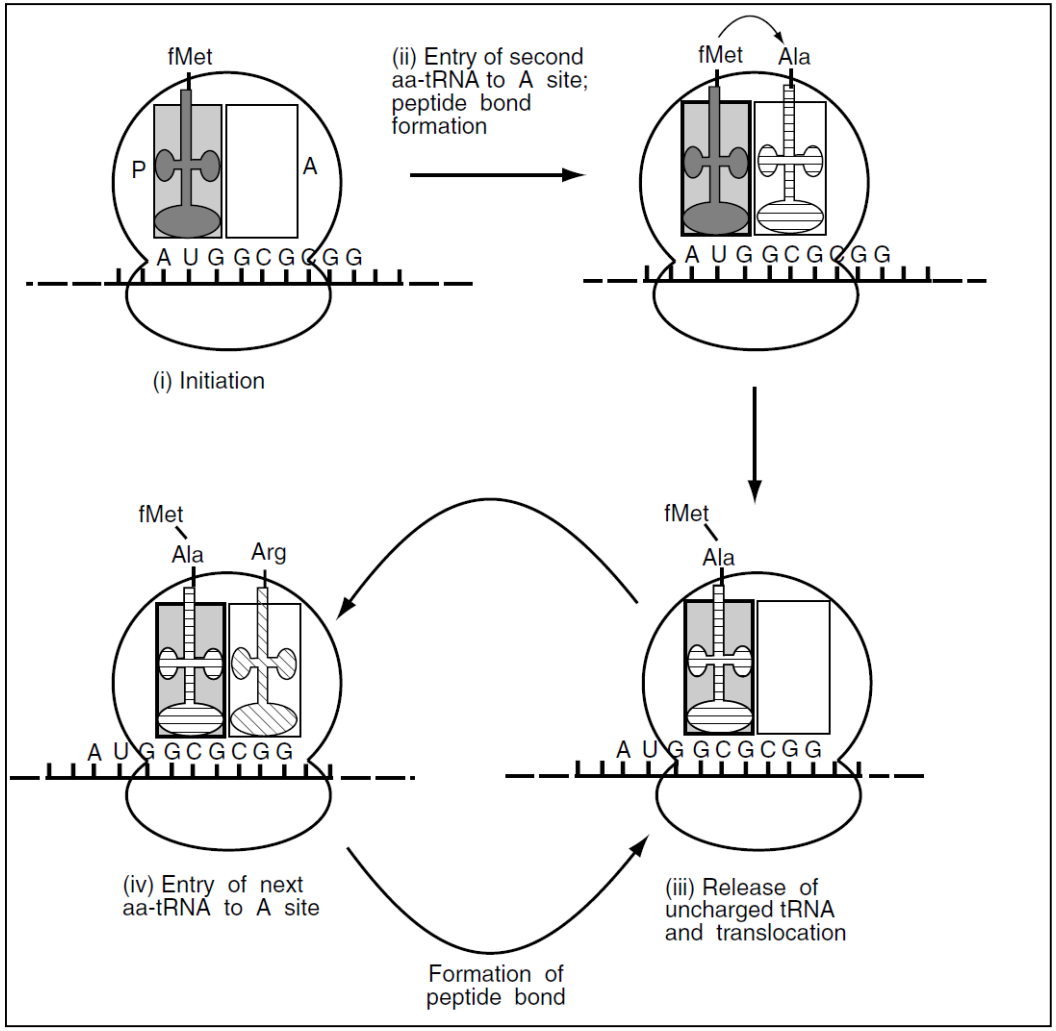
Initiation

The first phase of translation

$tRNA^{fMet}$ as initiator tRNA in bacteria

Methionine (abbreviated as Met or M), is coded by the initiation codon AUG.

In bacteria, the initiation codon is recognized by a specific tRNA molecule, N-formylmethionine ($tRNA^{fMet}$).

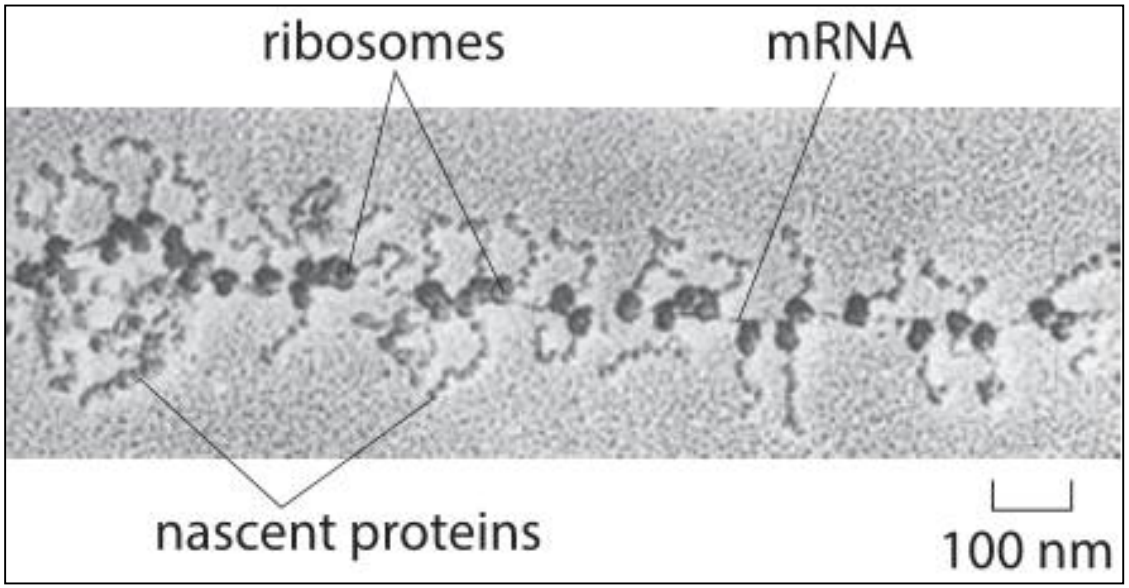


Dale and Park,2004

Protein synthesis

Translation

Initiation, elongation and termination



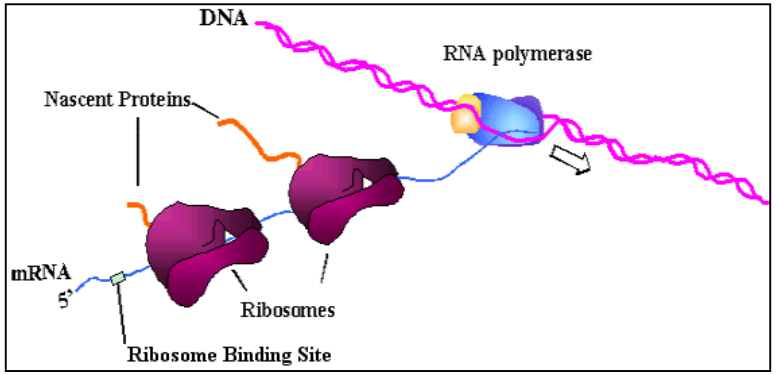
Ribosomes on mRNA as beads on a string.

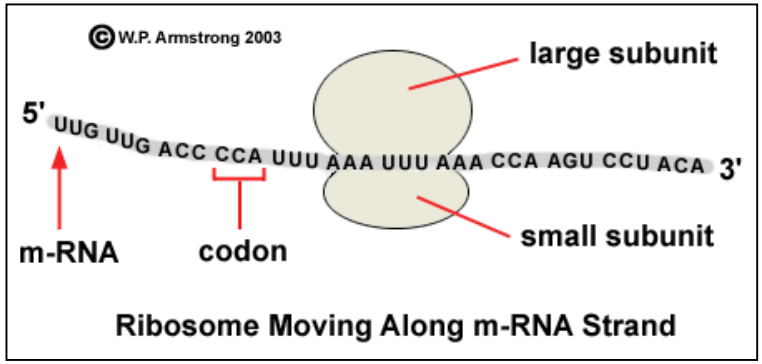
Protein synthesis

Translation

Initiation, elongation and termination

- As soon as mRNA starts getting transcribed, ribosomes attach to translate.
- In bacteria, nearly all translation occurs on growing mRNA still being transcribed.
- In eukaryotes, 15% of translation occurs on growing mRNA.
- The remaining 85% of translation occurs after the mRNA is processed and exits the nucleus, into the cytoplasm.

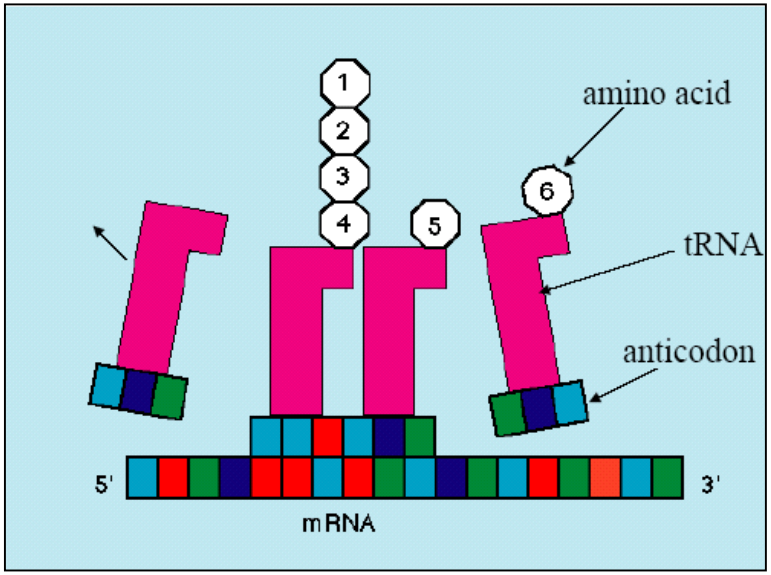


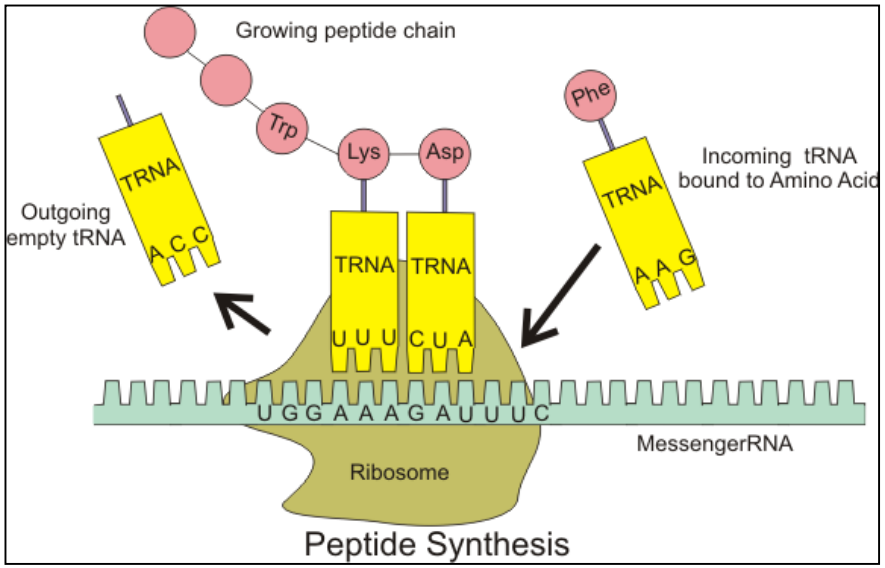


Protein synthesis

Translation

Initiation, elongation and termination



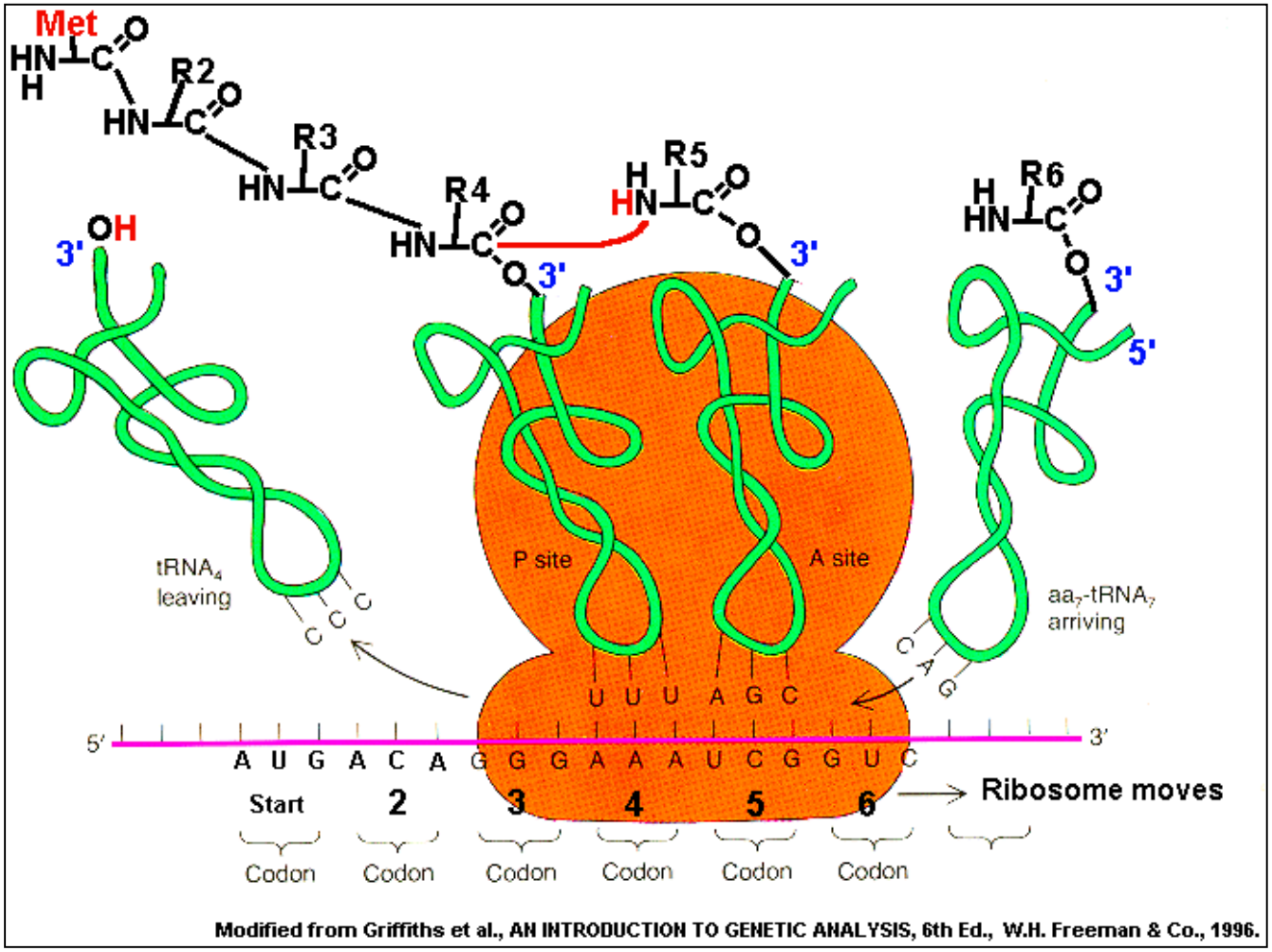


Anticodon in tRNA complementary to a codon on mRNA.

Protein synthesis

Translation

Initiation, elongation and termination

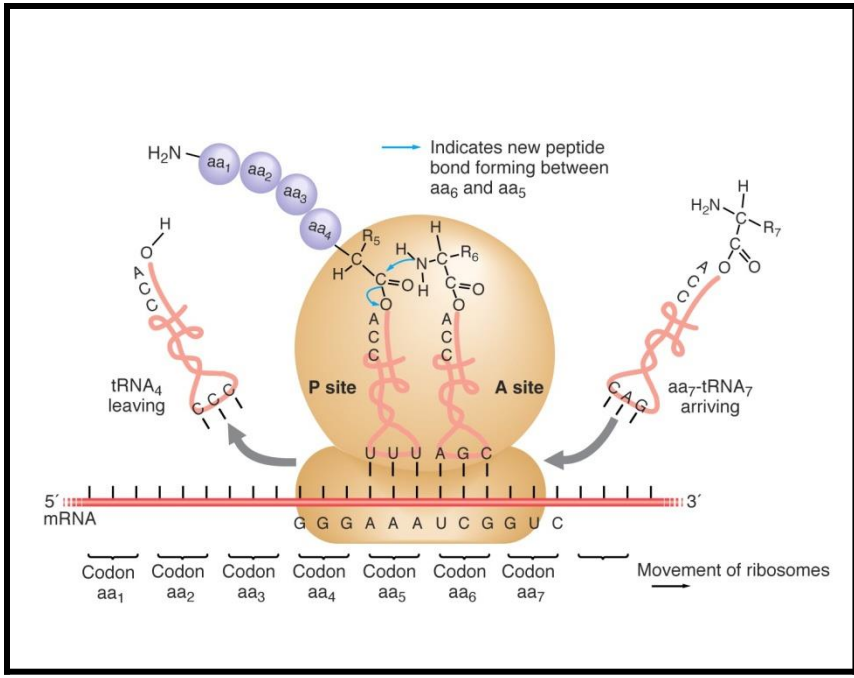


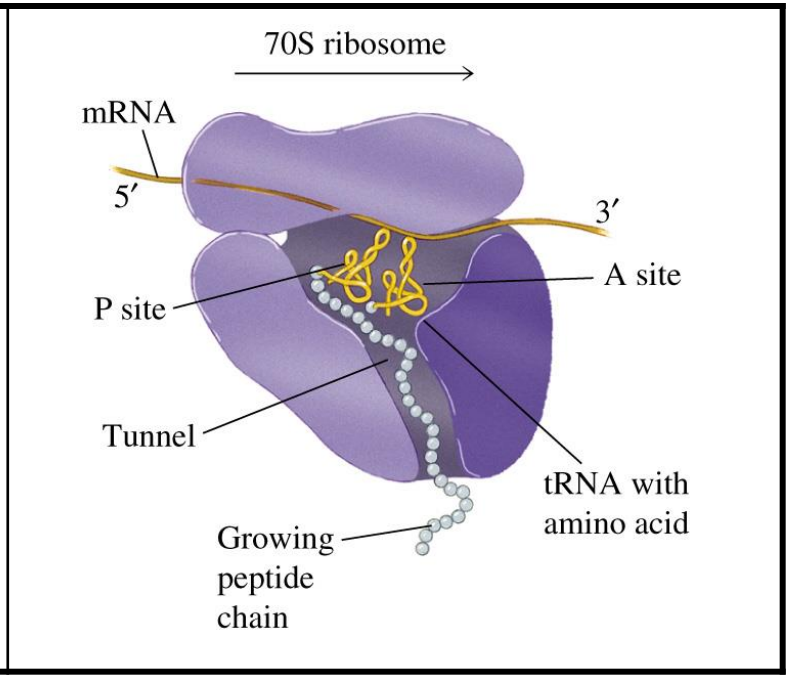
Modified from Griffiths et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th Ed., W.H. Freeman & Co., 1996.

Protein synthesis

Translation

Initiation, elongation and termination

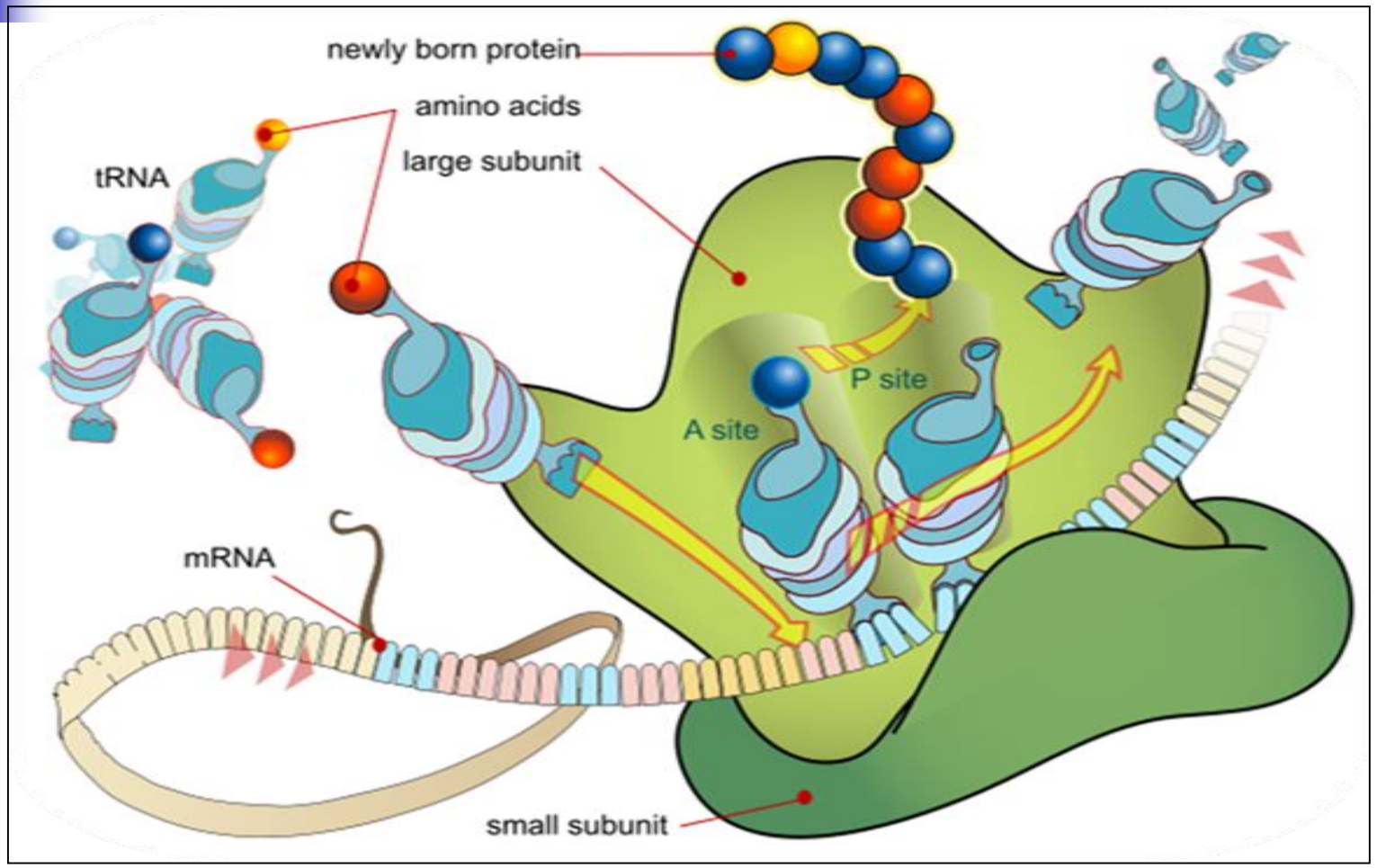




Protein synthesis

Translation

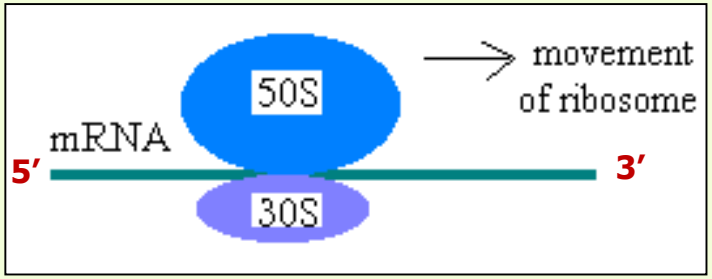
Initiation, elongation and termination



Ribosomal function

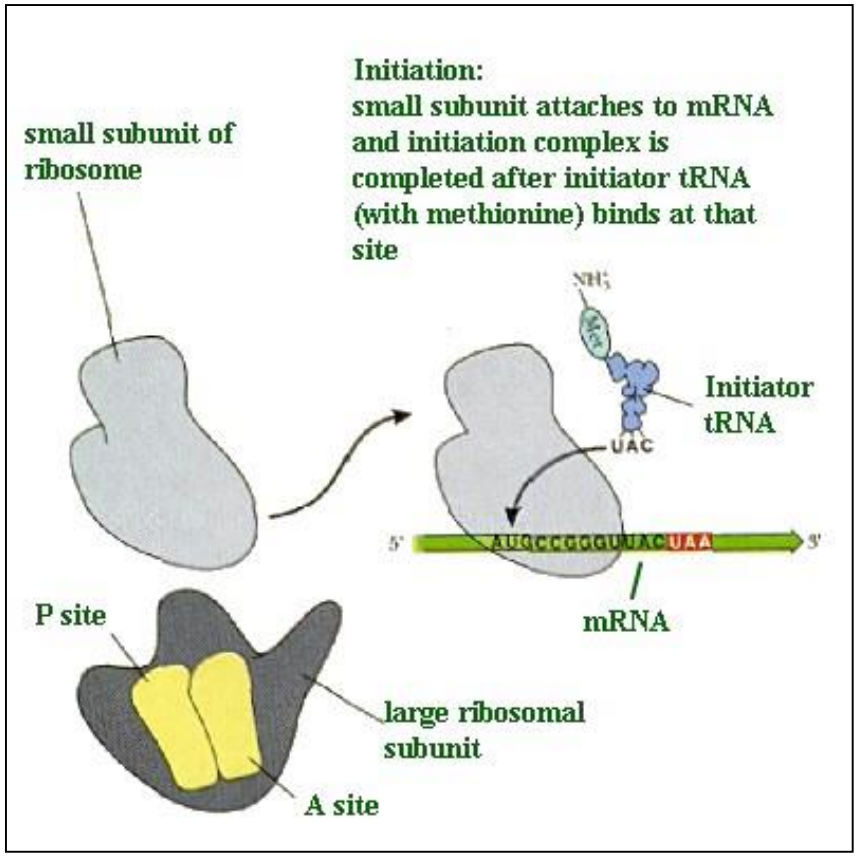
The small ribosomal subunit which reads the mRNA, and the large subunit which joins amino acids to form a polypeptide chain

- When a ribosome needs to be formed for translation, the subunits 30S and 50S attach to each other and form a 70S unit.
- A 30S subunit carrying initiation factors binds first to an initiation site on mRNA.
- Then 50S subunits to join the 30S-mRNA complex.
- Messenger RNA, a molecule of RNA is translated in the 5′-to-3′direction.



Ribosomal function

- Translation begins when mRNA attaches to the 30S.
- tRNA comes and binds to mRNA where nucleotide code matches.
- This triggers 50S binding to 30S.
- 50S is where all tRNAs will bind.

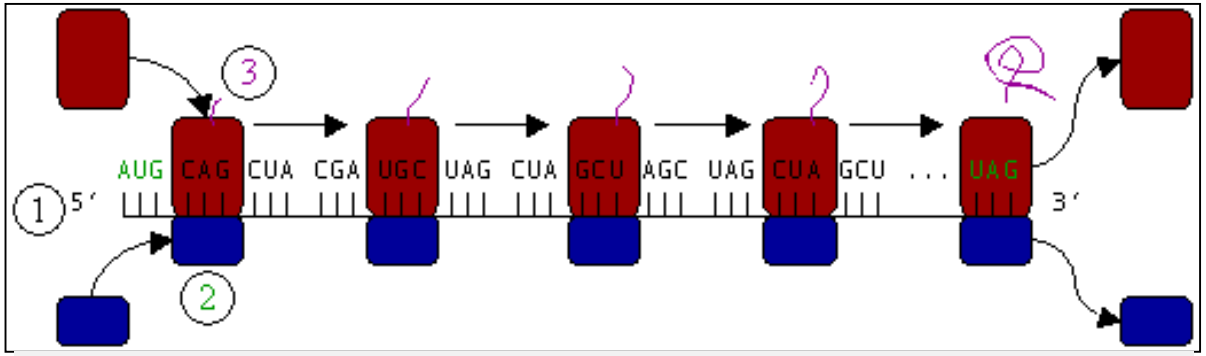


Erin Husson

Ribosomal function

Alternate start codons (non AUG) are very rare in eukaryotic genomes

- The ribosome begins at the start codon of RNA (AUG) and ends at the stop codon (UAG).
- **Alternative start codons:**
- *E. coli* uses 83% AUG, 14% GUG, 3%, UUG and one or two others (e.g., an AUU and possibly a CUG).

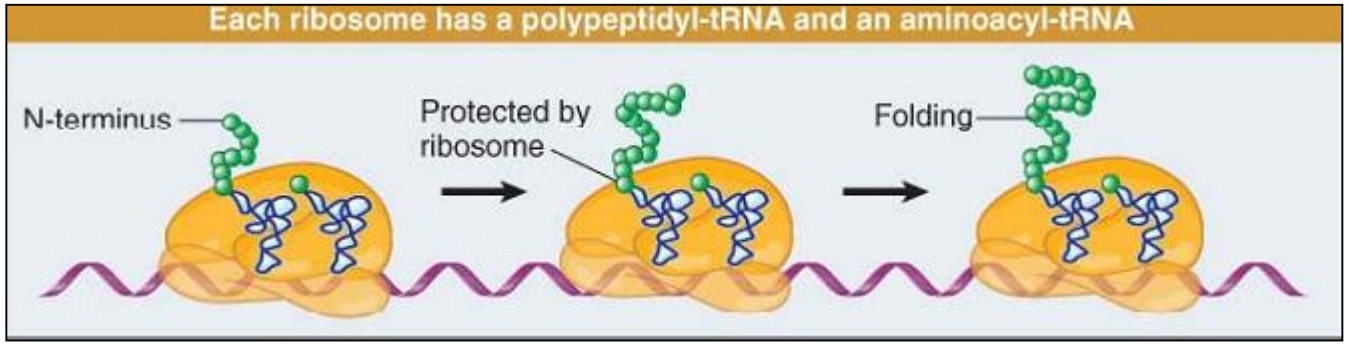


In the standard genetic code, there are three different stop codons:
in RNA: UAG, UAA, UGA
in DNA: TAG, TAA, TGA

Wikipedia,2015

Ribosomal function

- Many ribosomes bind to one mRNA.
- An mRNA is simultaneously translated by several ribosomes.
- Bacterial mRNA is unstable and has a half-life of only a few minutes.



Bacterial ribosomes

Three sites for protein synthesis. All binding sites were identified on the large ribosomal subunit

- The ribosome complex has three tRNA binding sites:

1. A (aminoacyl) site,
2. P (peptidyl) site, and
3. E (exit) site.

- The path of a tRNA through the ribosome is:

  A site --> P site --> E site

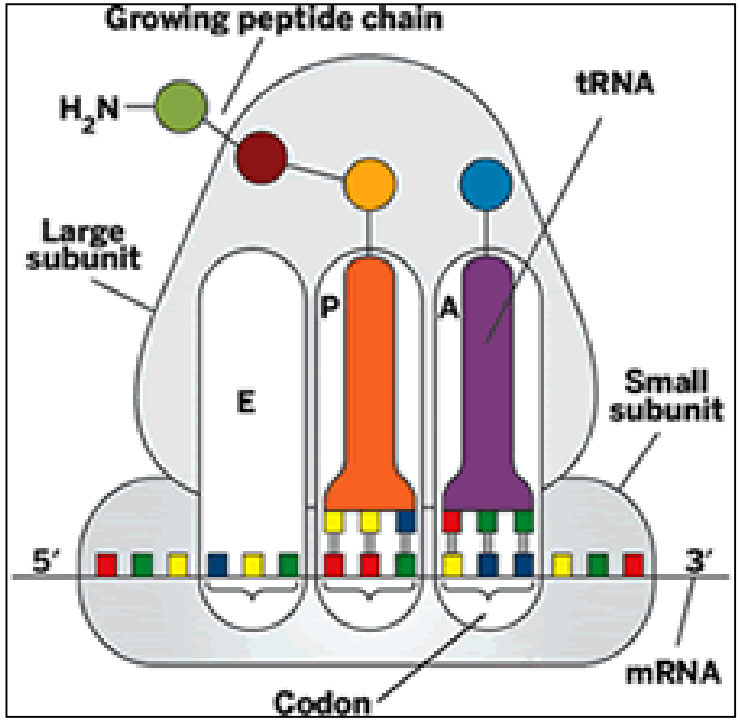
- Ribosome move down mRNA translating the protein with the tRNA moving from the P site to the E site.
- When the ribosome reaches the end of the mRNA molecule, a stop codon on the mRNA signals for termination of translation, and the protein chain is released for use by the cell.

Bacterial ribosomes

Three sites for protein synthesis. All binding sites were identified on the large ribosomal subunit

1. tRNAs containing amino acids enter the ribosome in a special pocket, or binding site, called the acceptor site (A site).
2. The peptidyl site (P site) carries the growing peptide chain.
3. The E site is the exit site for the tRNA that was previously in the P site.

- Once E,P,A sites are empty and tRNA is released the ribosome dissociates.



Borman,2007;..

Protein synthesis
Elongation stage

- During the elongation stage, the ribosome continues to translate each codon in turn.
- Each corresponding amino acid is added to the growing chain and linked via a bond called a peptide bond.
- Elongation continues until all of the codons are read.

During translation elongation, the mRNA template provides specificity. As the ribosome moves along the mRNA, each mRNA codon comes into register, and specific binding with the corresponding charged tRNA anticodon is ensured. If mRNA were not present in the elongation complex, the ribosome would bind tRNAs nonspecifically(UC Davis GeoWiki).

Protein synthesis
Termination stage

- Lastly, termination occurs when the ribosome reaches a stop codon.
- The stop codons are, UAA, UAG, UGA.
- This tells the cell to stop translation.
- Since there are no tRNA molecules that can recognize these codons, the ribosome recognizes that translation is complete.
- The new protein is then released, and the translation complex comes apart.

Gene expression

What is meant by gene expression?

Gene expression efficiency

- Gene expression refers to genes being 'turned on' and producing a product.
- The product could be an enzyme, a structural protein, or a control molecule.
- Studies of gene expression typically measure the production of mRNA.
- Most mechanisms that control gene expression do so by controlling transcription, the synthesis of mRNA.

Gene expression in prokaryotes
Gene expression efficiency

- Prokaryotes only transcribe genes that their end-proteins are needed at the time.
- They do this in order to save up energy and increase efficiency.
- The regulation of gene expression is depended mainly on their immediate environment, for example on the presence and absence of nutrients.
- Gene expression in prokaryotes occurs primarily at the level of transcription.

Gene expression in prokaryotes
Gene expression efficiency

- Genes can be expressed with different efficiencies.
- Gene A is transcribed and translated much more efficiently than gene B.
- This allows the amount of protein A in the cell to be much greater than that of protein B.

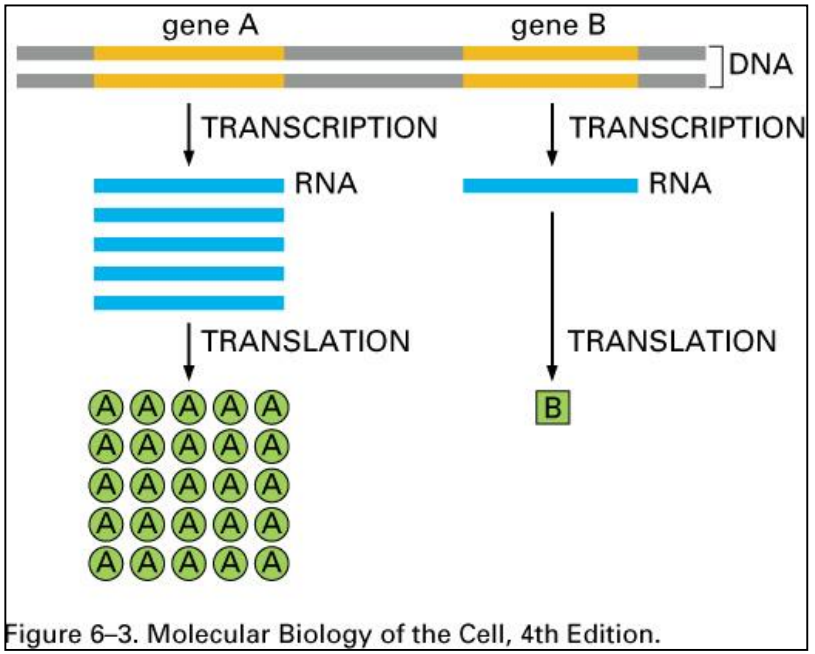


Figure 6–3. Molecular Biology of the Cell, 4th Edition.

Reproduction in bacteria

Cell division (Binary fission, budding and intracellular offspring production)

Reproduction in bacteria

Reasons for cell division

- **Cell division is the process by which cells produce new cells.**
- **Reasons for Cell Division:**

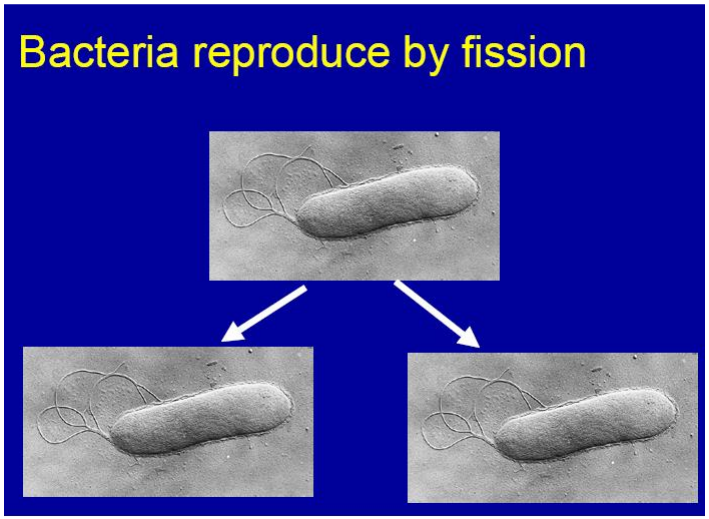
1. **Cell growth**
2. **Repair & replacement of damaged cell parts**
3. **Reproduction of the species.**

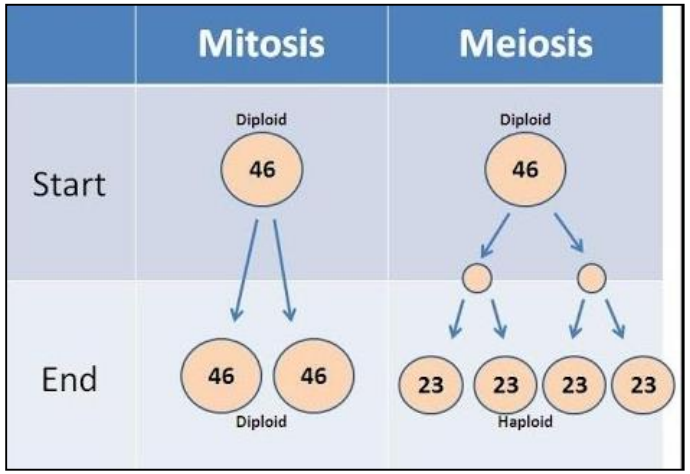
Wikipedia

Bacterial cell division

The cell cycle

- There are three major types of cell division, which are:
  1. Binary fission,
  2. Mitosis, and
  3. Meiosis
- Replication of DNA in procaryotes is simple (binary fission).
- Mitosis and meiosis take place in eukaryotic cells and are more advanced.





Cell division

Binary fission, budding and intracellular offspring production

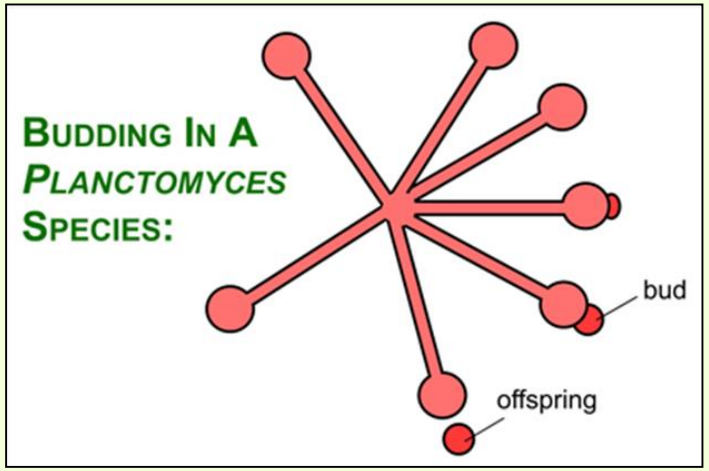
- Most bacteria rely on binary fission for propagation.
- Conceptually this is a simple process; a cell just needs to grow to twice its starting size and then split in two.
- But, to remain viable and competitive, a bacterium must divide at the right time, in the right place, and must provide each offspring with a complete copy of its essential genetic material.
- Some other bacterial lineages reproduce by budding. e.g. phytoplasmas
- Still others form internal offspring that develop within the cytoplasm of a larger mother cell. e.g. *Epulopiscium* spp.

Cornell university,2015;..

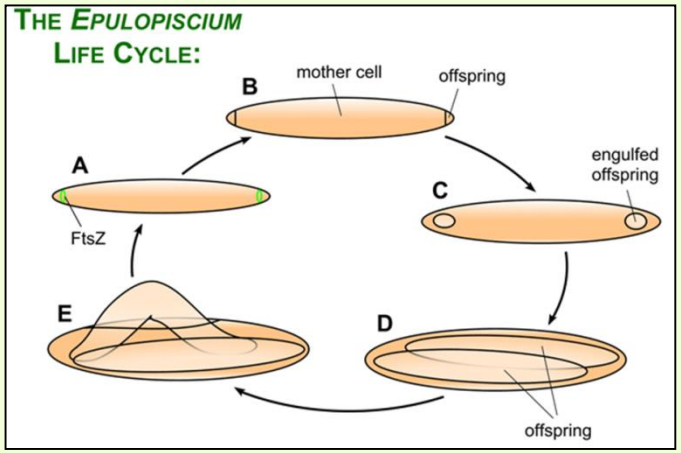
Cell division

Budding and intracellular offspring production

- Some members of the Planctomycetes, Cyanobacteria, Firmicutes (a.k.a. the Low G+C Gram-positive bacteria e.g. phytoplasma) and the prosthecate Proteobacteria.



- *Epulopiscium* spp., *Metabacterium polyspora* and the Segmented Filamentous Bacteria (SFB) form multiple intracellular offspring. For some of these bacteria, this process appears to be the only way to reproduce. Intracellular offspring development in these bacteria shares characteristics with endospore formation in *Bacillus subtilis*.

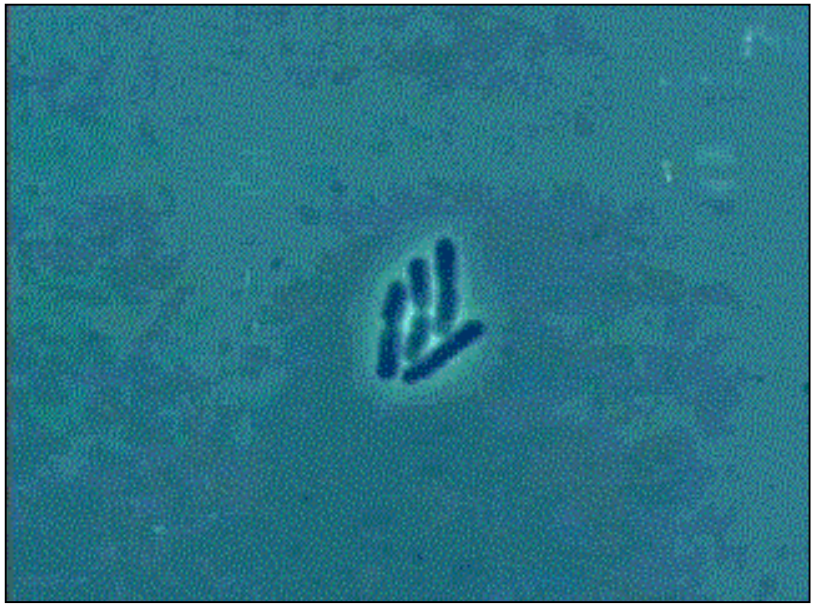


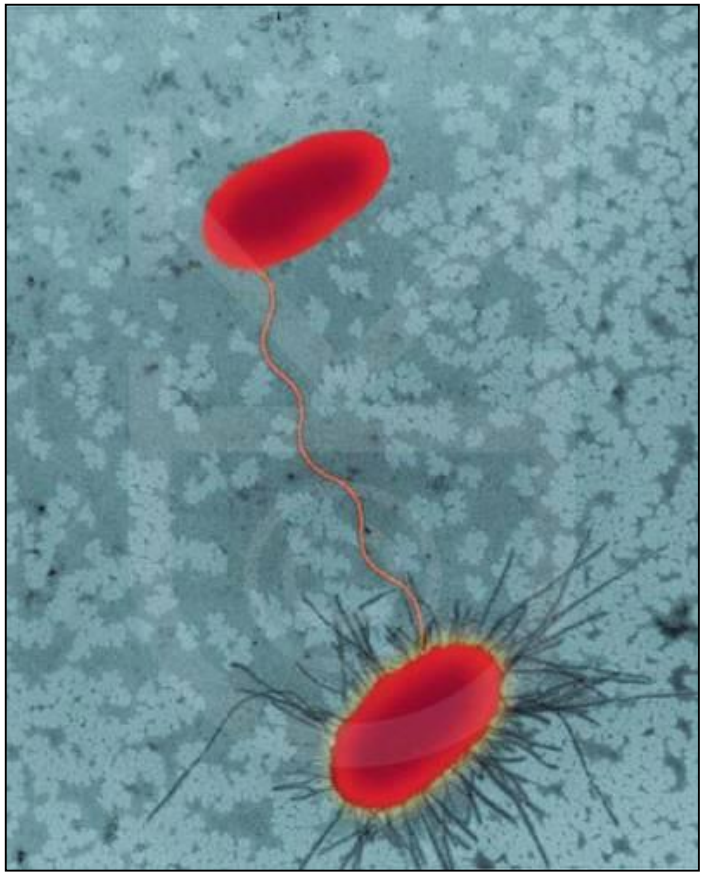
Cornell university,2015;..

Bacterial cell division

Binary fission

Bacteria can reproduce sexually by conjugation or asexually by binary fission

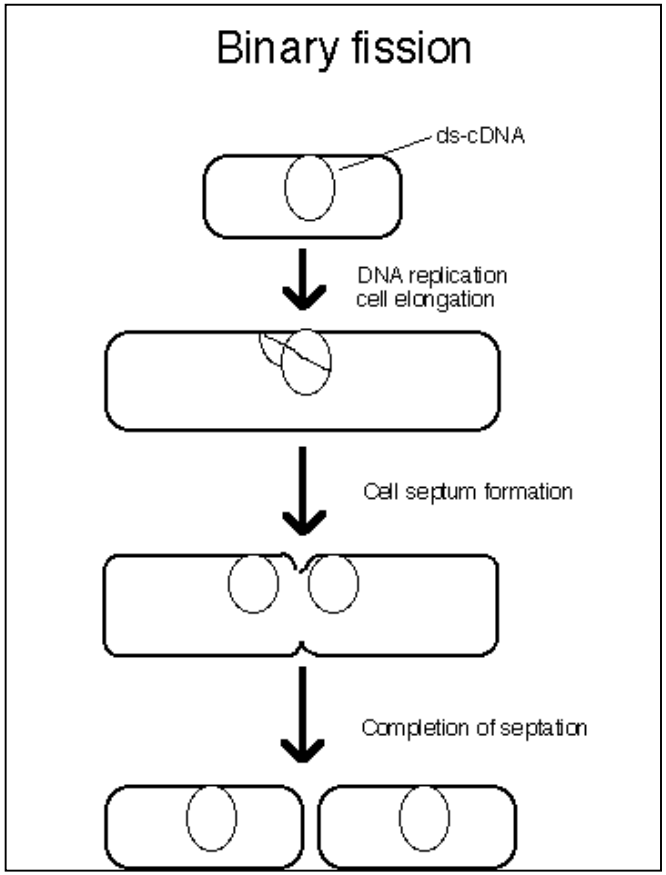


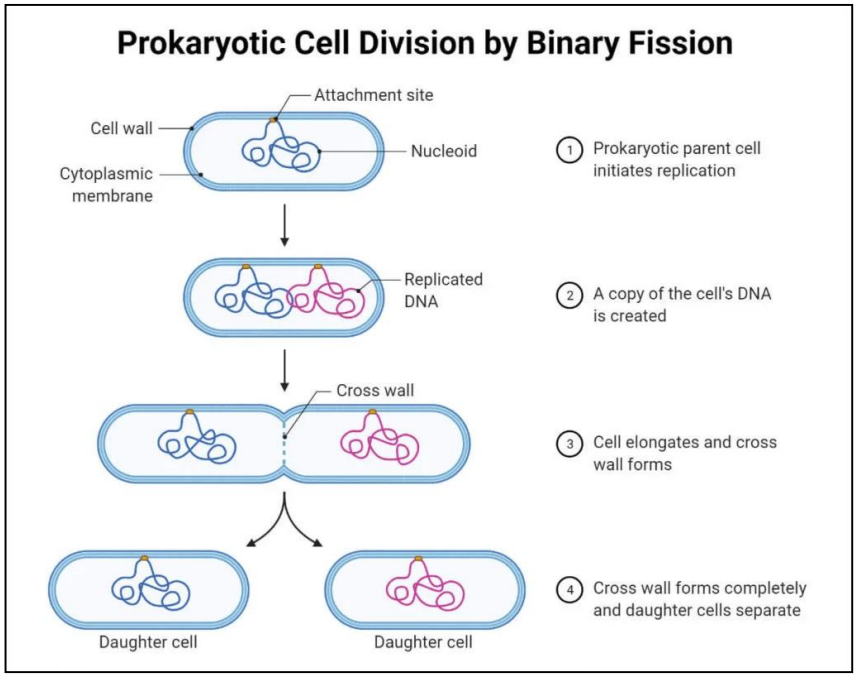


Passovoy

Bacterial Cell Division

Prokaryotic cell division by binary fission

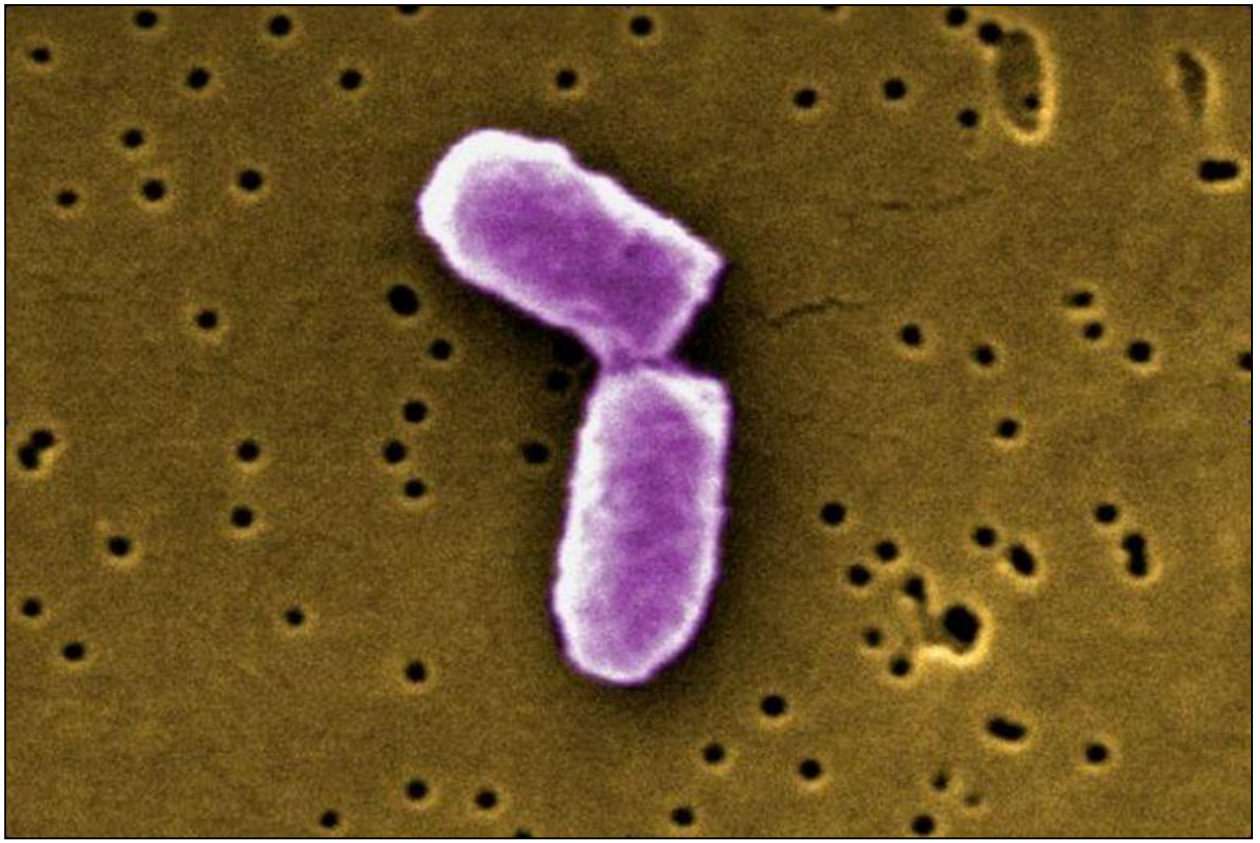




Passovoy; Microbe Notes,2024

E. coli bacteria undergoing binary fission

The cell wall is dividing resulting in the formation of two cells



Bailey,2017

Cytoskeletal elements in bacteria
Cytoskeletal proteins

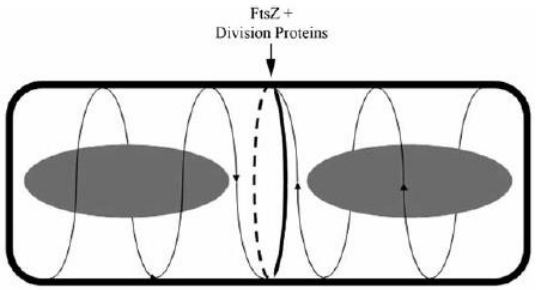
- We now know that bacteria have considerable intracellular organization, with several cytoskeletal elements, including:

1. Tubulin-like proteins (FtsZ),
2. Min proteins (found in rod-shaped bacteria, responsible for the inhibition of inappropriate assembly of the divisome near the poles of the cell),
3. Actin-like proteins (found e.g. in *Spiroplasma*) and some other proteins.

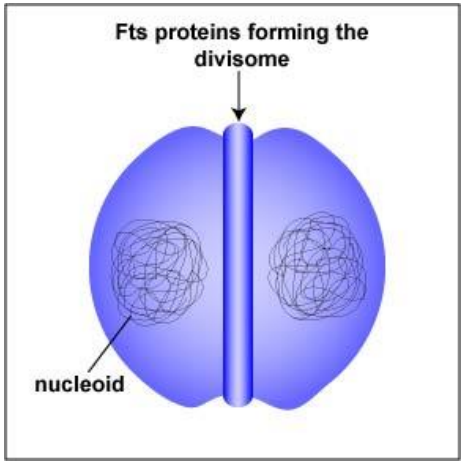
El-Sharoud,2008

Cell division
FtsZ ring formation

- In bacteria, the first known step in cell division is the localization of FtsZ into a ring structure (the Z ring) at the nascent division site.
- The Tubulin-like proteins called as Fts proteins form the cell division apparatus known as the divisome or septasome.
- Proteins at the divisome are thought to synthesize the peptidoglycan and new membrane material that both splits the bacterium into two daughter cells and subsequently enables each to grow to full size.



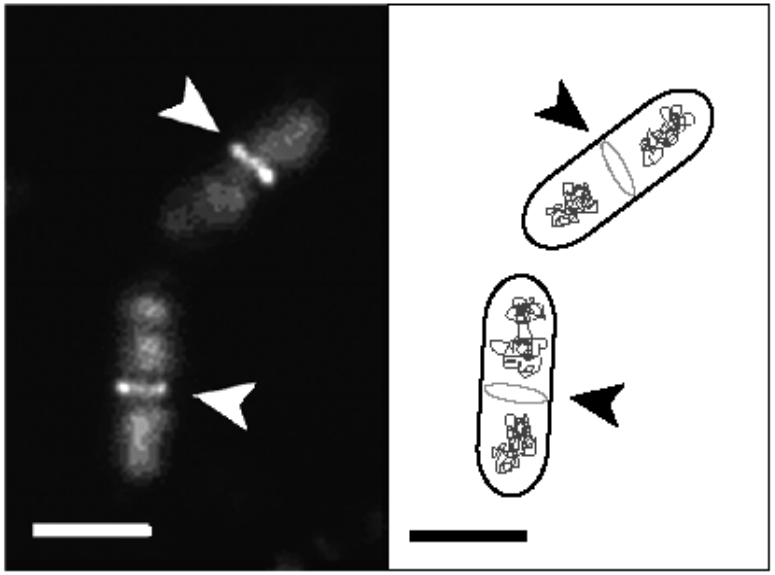
FtsZ, a tubulin-like protein in prokaryotes, naturally assembles into dynamic filaments (arrowed lines), El-Sharoud,2008.



Cell division

Immunofluorescently labelled FtsZ and DAPI-stained DNA

- Left, subcellular localization of FtsZ and in DNA *Bacillus subtilis* cells as visualized by an overlay of immunofluorescently labelled FtsZ and DAPI-stained DNA.
- FtsZ localisation is indicated by the arrows.
- Right schematic depicts:
- The cell membrane (oval outline),
- The position of the Z ring (shown by the gray ring structure denoted by the arrows), and
- DNA (represented by the twisted lines) close to the poles of the cell.
- Scale bar represents 1μm.



El-Sharoud,2008

General terms and abbreviations

- **Daltons:** One hydrogen weighs 1 dalton (Da). The average amino acid weighs 110 daltons. A one hundred residue protein weighs ~11,000 Da, or 11 kilodaltons (kD).
- **Downstream:** identifies sequences proceeding farther in the direction of expression; for example, the coding region is downstream of the initiation codon.
- **Epigenetics:** refers to external modifications to DNA that turn genes "on" or "off." Non-genetic influences on gene expression. These modifications do not change the DNA sequence.
- **Guanosine:** A purine nucleoside (guanine linked to ribose). It is a component of RNA and its nucleotides are important in metabolism.
- **Molecule:** A group of atoms bonded together, representing the smallest fundamental unit of a chemical compound that can take part in a chemical reaction.
- **Sessile bacteria:** Bacteria living within a biofilm.
- **Tanscription :** 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.

General terms and abbreviations

- **Upstream:** identifies sequences in the opposite direction from expression; for example, the bacterial promoter is upstream of the transcription unit, the initiation codon is upstream of the coding region.

Selected References

- Ammar *et al.*,2004. An attachment tip and pili-like structures in insect- and plant-pathogenic spiroplasmas of the class Mollicutes. Arch Microbiol 181: 97-105.
- Bové , J.M. and M. Garnier. 2003. Phloem-and xylem-restricted plant pathogenic bacteria. Plant Sceience 164:423-38.
- Cabeen, M. T. and Jacobs-Wagner. C. 2005. Bacterial cell shape. Nature Reviews, Microbiolgy, pp.601-610.
- Charles *et al.*, 2003. The Motility of Mollicutes.
- Cullimore, D.R.2000. A Simplified Guide to bacteria in water. Part 1.
- El-Sharoud, Walid. 2008. Bacterial Physiology- A Molecular Approach. 371 pp., Springer.
- Lecture 4. 2002. Procaryotic Cell Structure and Function. pp.1-10.
- Lecture 6. Structural Features of Prokaryotic Cells. Htm.
- Prescott,2006. Procaryotic cell structure and function, Chapter 3. pp.39-78.
- Ribosome. Wikipedia, the free encyclopedia. 2006.
- Lecture 2. Ribosome Structure and Control of Translation in Bacterial Systems. BCH 5413.
- Salton,M.R.J and K-S. Kim. Structure. Medmicro chapter 2. htm.
- Todar, K. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.
- Weintraub, P. G. and P. Jones (eds.)2010. Phytoplasmas: genomes, plant hosts, and vectors. CABI,331 pp.