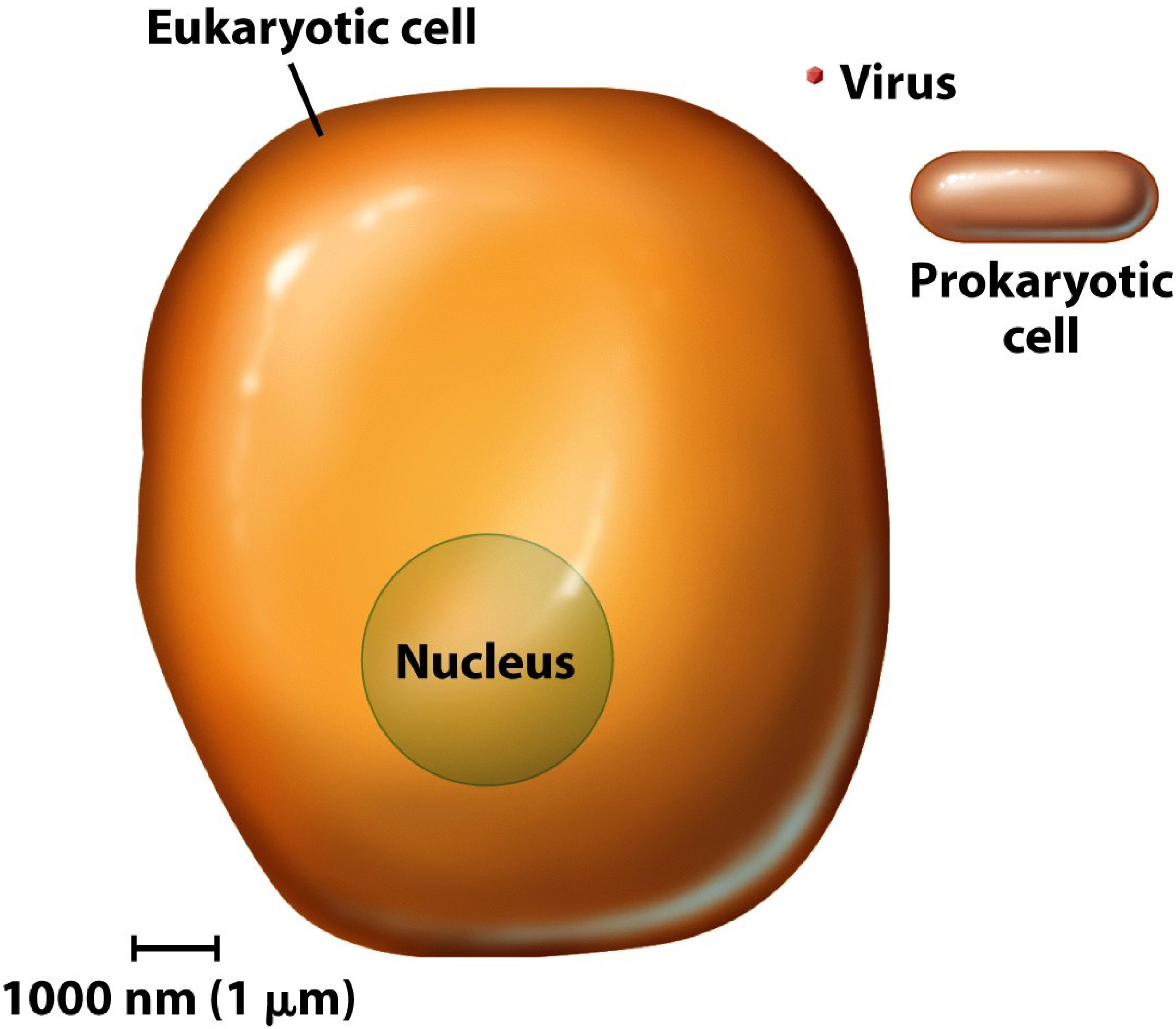
Prokaryotic Cell Features


Eukaryotic cell
Virus
Prokaryotic cell
Nucleus
1000 nm (1 µm)

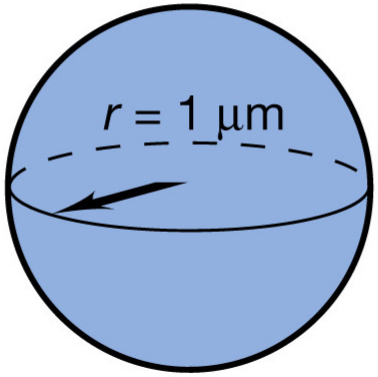
Size

The small size of prokaryotic cells affects their physiology, growth rate, and ecology.

Due to their small cell size, most prokaryotes have the highest surface area–to–volume ratio of any cells.

This characteristic aids in nutrient and waste exchange with the environment.

As a cell increases in size, its surface area-to-volume ratio decreases

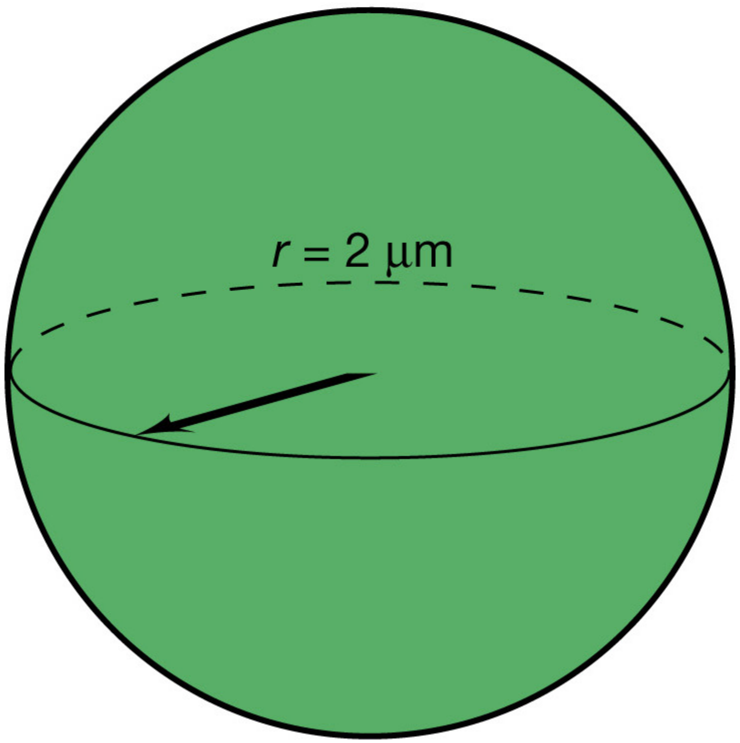


Surface area ($4\pi r^2$) = 12.6 μm^2

Volume ($\frac{4}{3}\pi r^3$) = 4.2 μm^3

$$\frac{\textbf{Surface}}{\textbf{Volume}} = \mathbf{3}$$

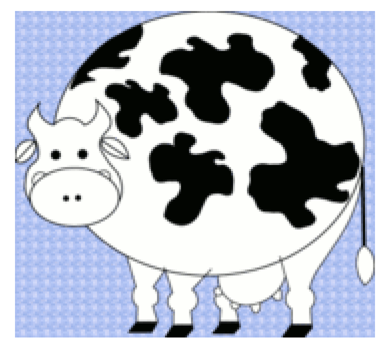
For a sphere,
S / V = 3/r



Surface area = 50.3 μm^2

Volume = 33.5 μm^3

$$\frac{\textbf{Surface}}{\textbf{Volume}} = \mathbf{1.5}$$



Prokaryotes maintain a high surface area to volume ratio by their smallness.

1. **TRANSPORT RATE: Efficient transport of raw materials in and wastes out**

2. **GROWTH RATE: Nutrient exchange limits growth rates.**

   Many prokaryotes = high metabolic activity (fast growth, reproductive rate).

3. **EVOLUTIONARY RATE: Given the same amount of nutrients, small cells can have more individuals in a population than larger cells. Thus, more cell division, and more mutation, more evolutionary change.**

Eukaryotes larger cell size (in general)

Different answers for the transport problem

1. lots of internal membrane channels, invaginations and surface area
2. cytoplasmic streaming
3. organelles (compartmentalize cell functions)
4. bulk uptake - endocytosis (phagocytosis/pinocytosis)

Table 4.1 **Cell size and volume of prokaryotic cells, from the largest to the smallest**

Organism	Characteristics	Size[a] (μm)	Cell volume (μm^3)
Thiomargarita namibiensis	Spherical sulfur chemolithotroph	750	200,000,000
Epulopiscium fishelsoni	Chemoorganotrophic bacterium	80 × 600	3,000,000
Beggiatoa sp.	Filamentous sulfur chemolithotroph	50 × 160	1,000,000
Achromatium oxaliferum	Ellipsoid sulfur bacterium	35 × 95	80,000
Lyngbya majuscula	Filamentous cyanobacterium	8 × 80	40,000
Prochloron sp.	Prochlorophyte	30	14,000
Thiovulum majus	Spherical sulfur chemolithotroph	18	3,000
Staphylothermus marinus	Hyperthermophile	15	1,800
Titanospirillum velox	Rod-shaped sulfur chemolithotroph	5 × 30	600
Magnetobacterium bavaricum	Magnetotactic bacterium	2 × 10	30
Escherichia coli	Chemoorganotrophic bacterium	1 × 2	2
Mycoplasma pneumoniae	Pathogenic bacterium	0.2	0.005

[a]Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter.

Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Ann. Rev. Microbiol. 55*: 105–137.

Table 4-1 Brock Biology of Microorganisms 11/e
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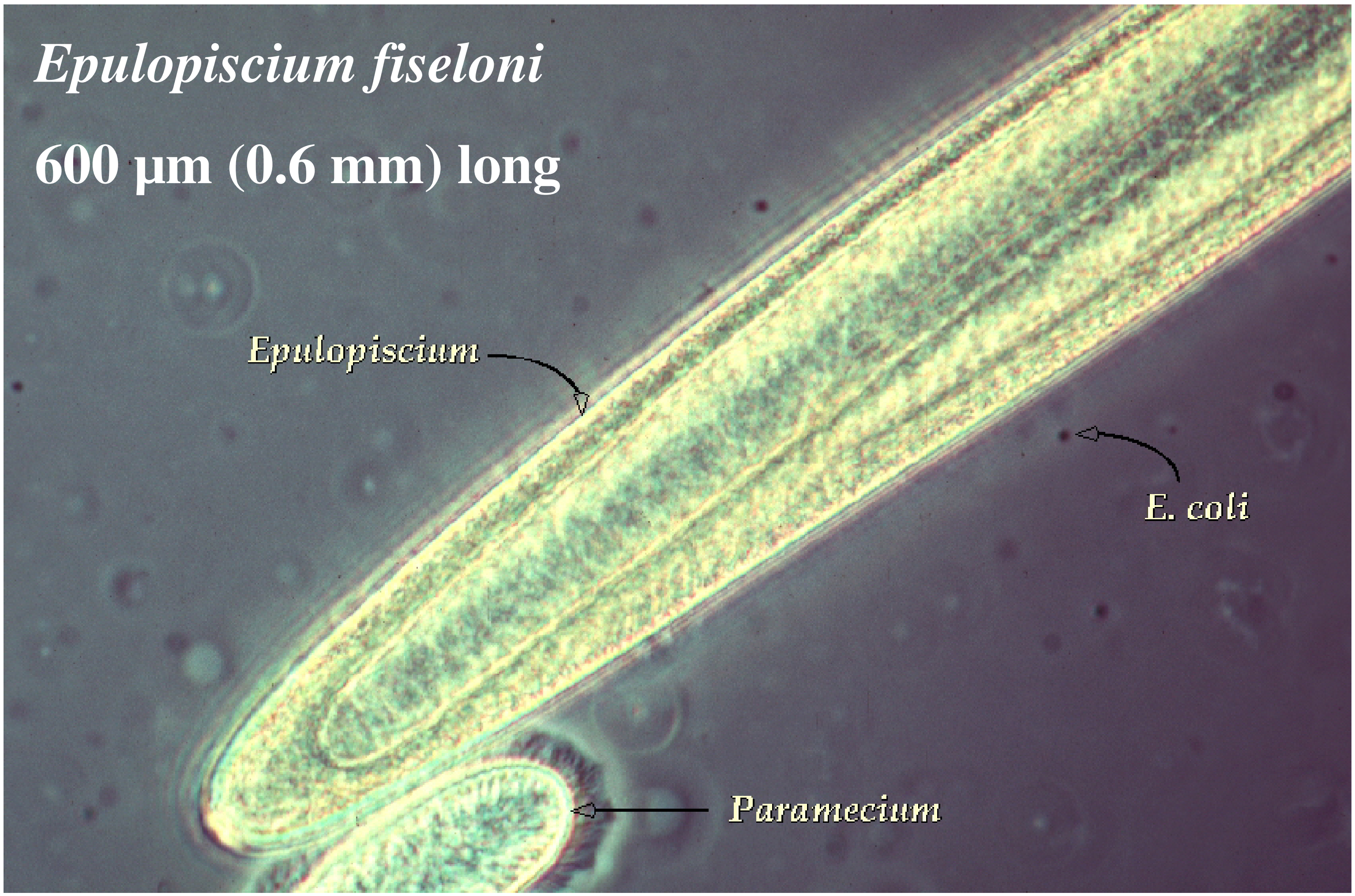
Introducing a prokaryote that is one million times bigger than *E. coli*! Sturgeonfish symbiont.



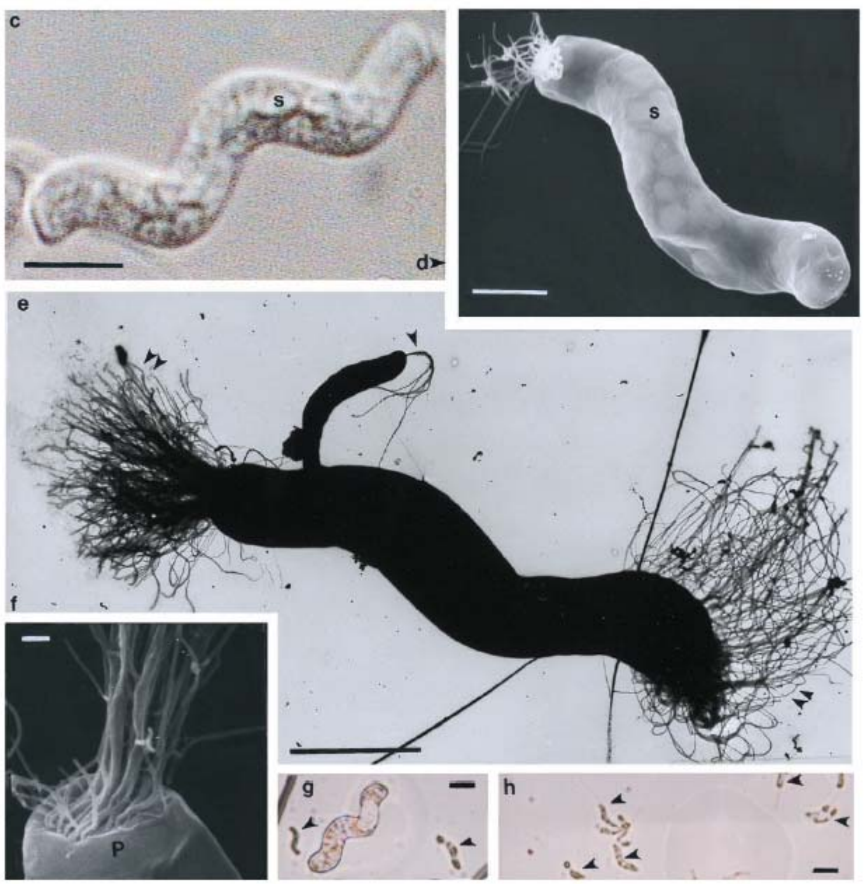
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 11584–11588, September 1999
Microbiology

Titanospirillum velox: A huge, speedy, sulfur-storing spirillum from Ebro Delta microbial mats

(Adrianus Pijper/bacterial motility/polar organelle/sulfur globules)

RICARDO GUERRERO*†, AARON HASELTON‡, MÓNICA SOLÉ§, ANDREW WIER¶, AND LYNN MARGULIS||

*Department of Microbiology, University of Barcelona, E-08028 Barcelona, Spain; Departments of ‡Entomology, ¶Microbiology, and ||Geosciences, University of Massachusetts, Amherst, MA 01003; and §Center for Molecular Biology, Autonomous University of Madrid, E-28049 Madrid, Spain



20 - 40 μm long

Individual flagella twist together to form "thick" flagella

FIG. 3. Huge spirilla rendered from both life and micrographs.

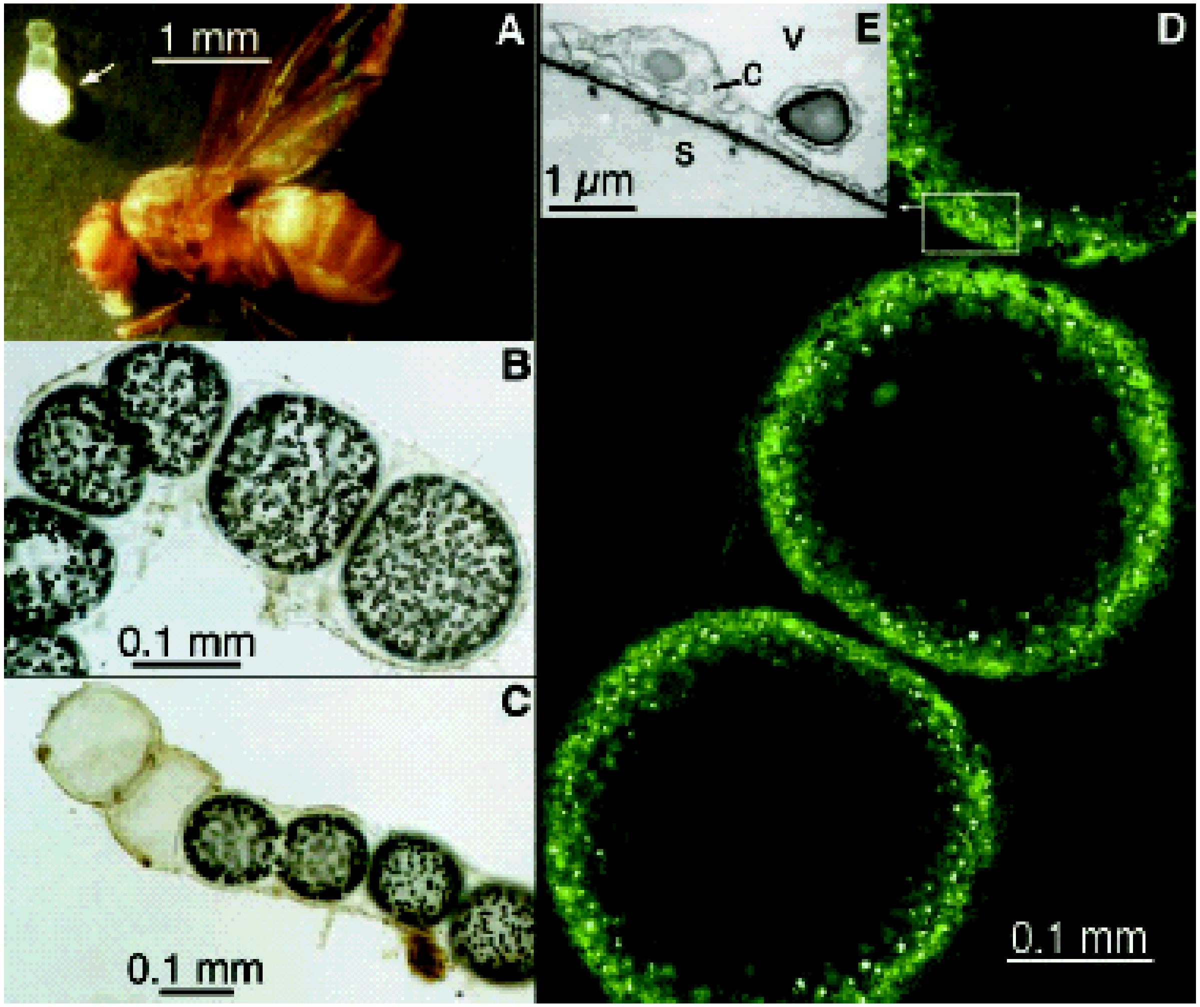


Fig. 1. *Thiomargarita namibiensis*. (A) The white arrow points to a single cell of *Thiomargarita*, 0.5 mm wide, which shines white because of internal sulfur inclusions. Above there is an empty part of the sheath, where the two neighboring cells have died. The cell was photographed next to a fruit fly (*Drosophila viriles*) of 3 mm length to give a sense of its size. (B) A typical chain of *Thiomargarita* as it appears under light microscopy. (C) At the left end of the chain there are two empty mucus sheaths, while in the middle a *Thiomargarita* cell is dividing. (D) Confocal laser scanning micrograph showing cytoplasm stained green with fluorescein isothiocyanate and the scattered light of sulfur globules (white). Most of the cells appear hollow because of the large central vacuole. (E) Transmission electron micrograph of the cell wall [enlarged area in (D)] showing the thin layer of cytoplasm (C), the vacuole (V), and the sheath (S).

Largest known bacterium:

Thiomargarita namibiensis

Some nearly 1 mm wide

Large central vacuole (dark) contains nitrate sequestered to use in the oxidation of reduced sulfur compounds.

String of pearls: reflective sulfur granules

How do we see microbes?

1. **Light Microscopy**

   Up to 1,500 X magnification (yours in lab: 1000 X)

   Limit of resolving power is ~0.2 μM, or about 1/3 the width of an average bacterial cell

   Light gathering ability decreases as magnification increases

   Immersion oil helps gather light rays that would otherwise be lost from specimen

How do we see microbes?

2. Electron Microscopy (EM):

Up to 1,000,000X magnification

(1,000 X more than light microscope)

"Light" => electron beam

"Lenses" => electromagnets to focus beam

Limit of resolving power is ~0.2 nm (molecules)

Can see internal structures and external appendages

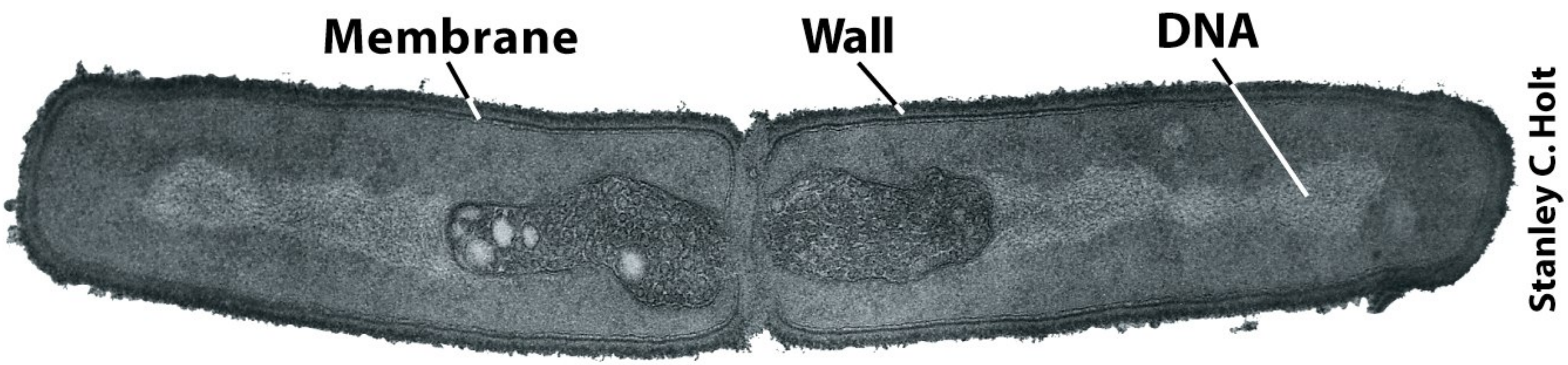


Figure 4-10a Brock Biology of Microorganisms 11/e
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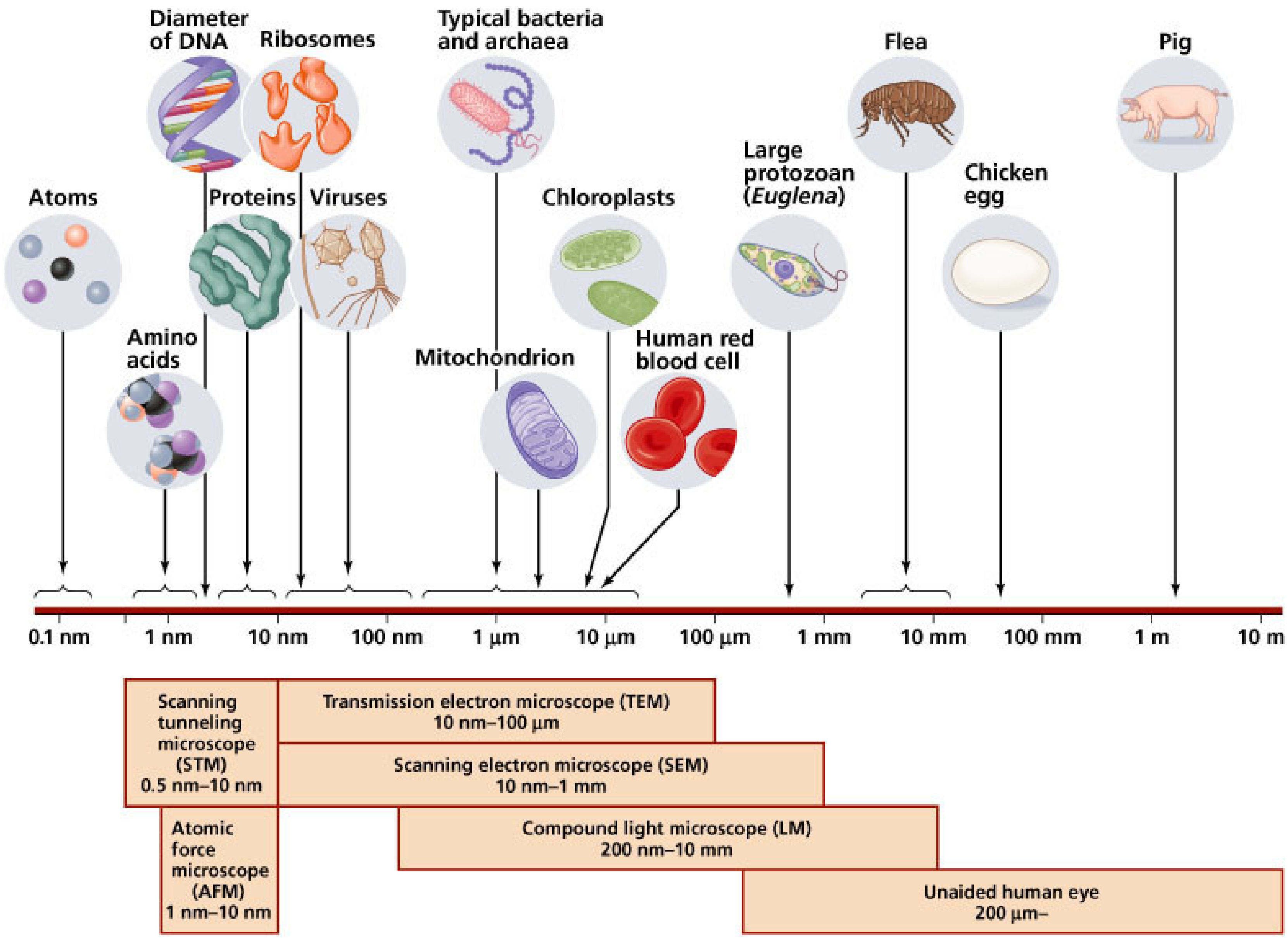
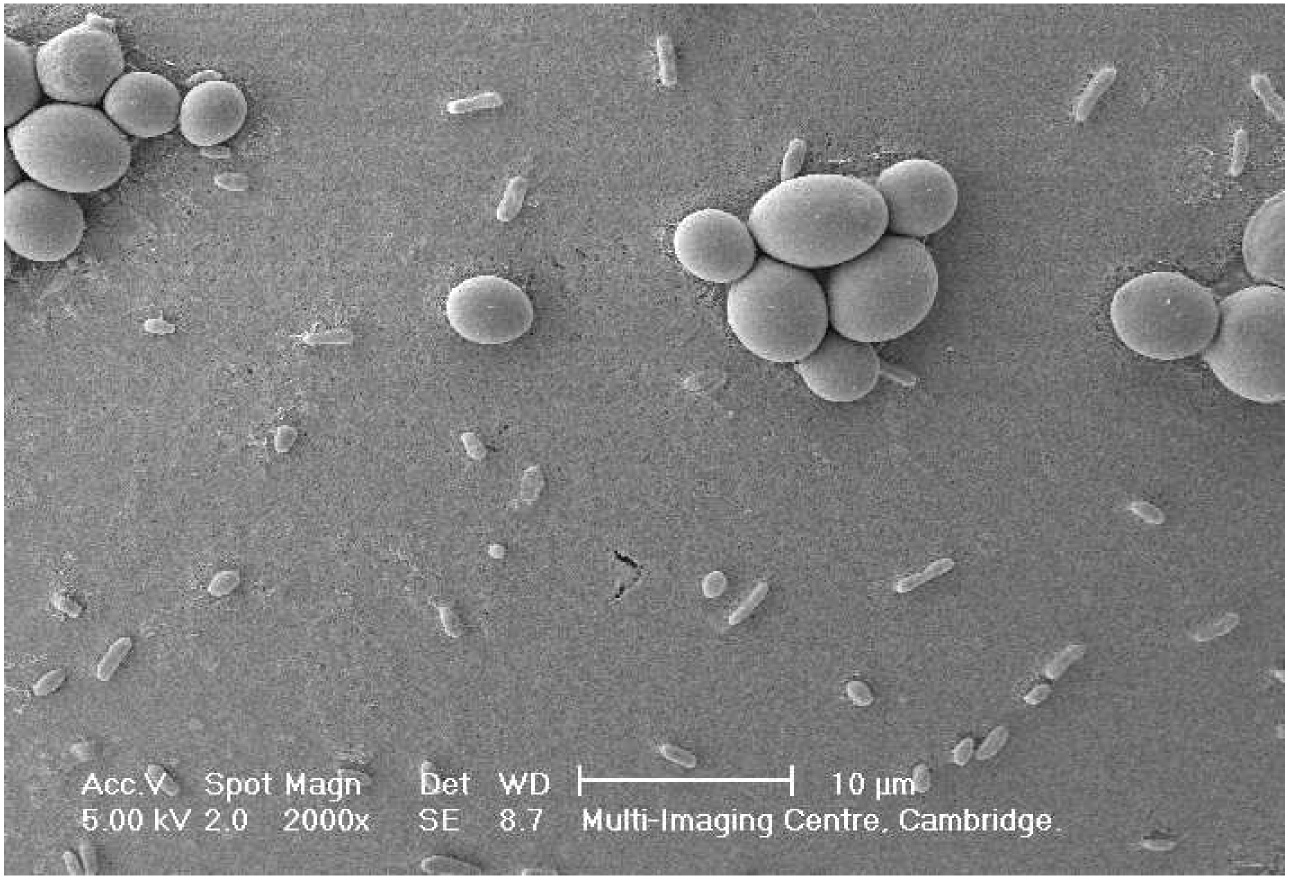

Diameter of DNA
Ribosomes
Typical bacteria and archaea
Flea
Pig
Large protozoan (*Euglena*)
Chicken egg
Atoms
Proteins
Viruses
Chloroplasts
Amino acids
Mitochondrion
Human red blood cell
0.1 nm
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
10 mm
100 mm
1 m
10 m
Scanning tunneling microscope (STM) 0.5 nm–10 nm
Transmission electron microscope (TEM) 10 nm–100 µm
Scanning electron microscope (SEM) 10 nm–1 mm
Atomic force microscope (AFM) 1 nm–10 nm
Compound light microscope (LM) 200 nm–10 mm
Unaided human eye 200 µm–

Figure 4.3

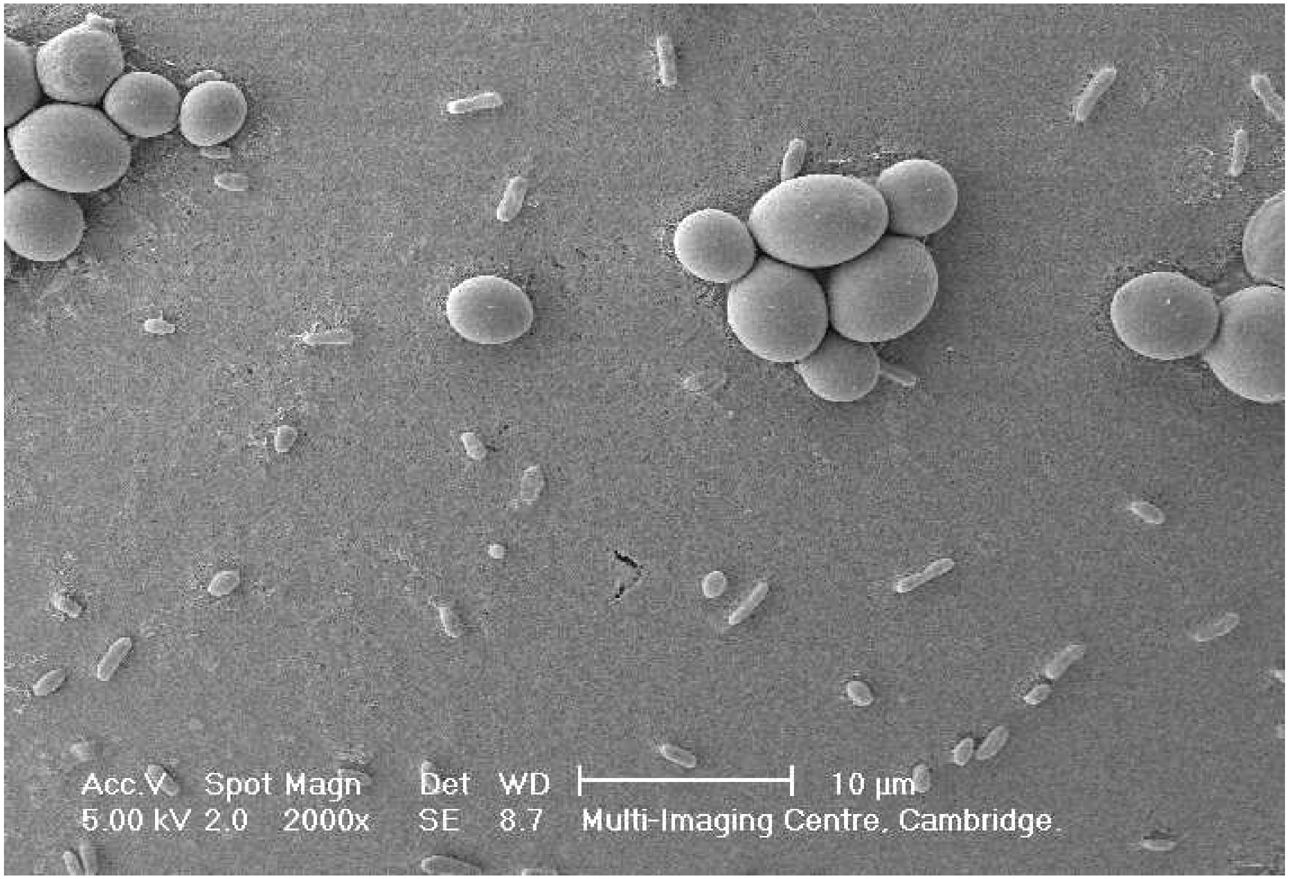
Comparing Prokaryotic and Eukaryotic Cells

Comparing Prokaryotic and Eukaryotic Cells

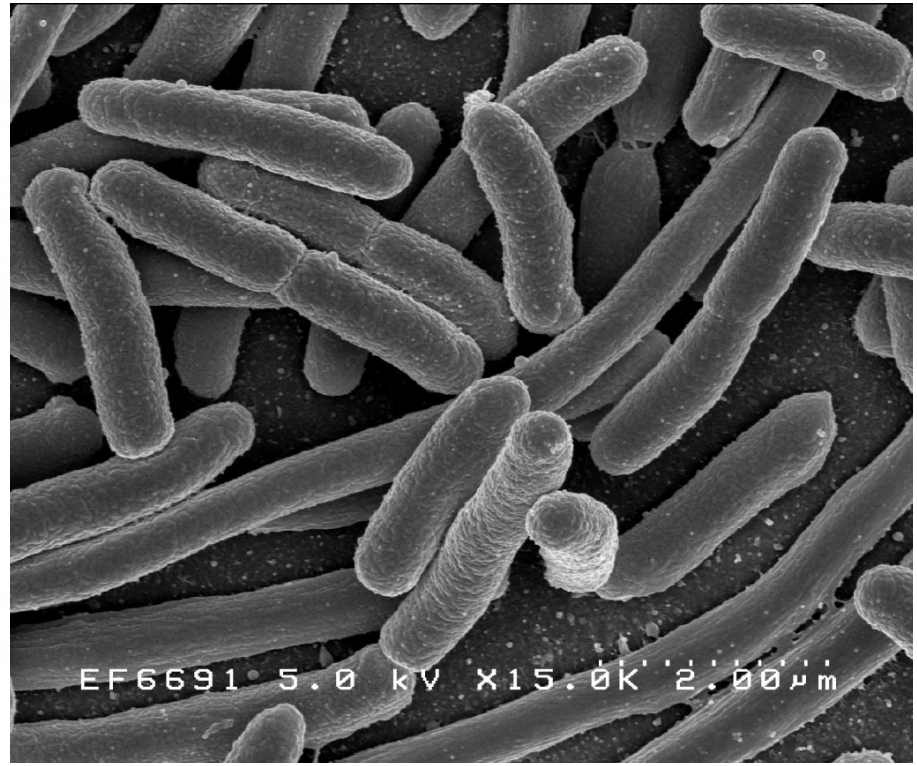


Compare the sizes of the bacterium *Escherichia coli* and the single-celled fungus (yeast) *Saccharomyces cerevisiae*

Comparing Prokaryotic and Eukaryotic Cells



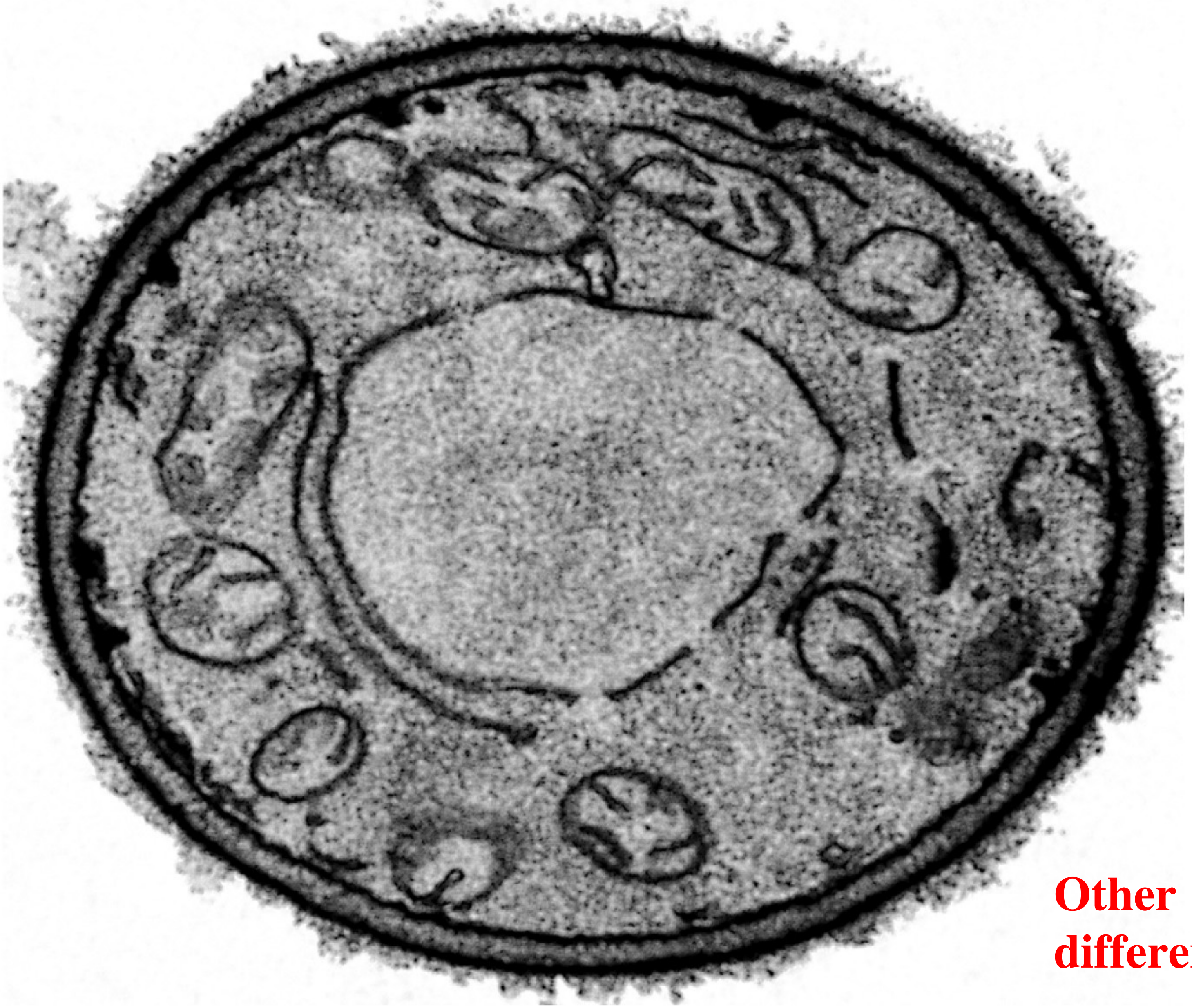
Compare the sizes of the bacterium *Escherichia coli* and the single-celled fungus (yeast) *Saccharomyces cerevisiae*



Compare:

cells of the bacterium *Escherichia coli*; the cells measure 1 x 3 µm…

…to cells of the eukaryotic fungus *Saccharomyces cerevisiae* (cells measure 8 μm in diameter).



Other differences?

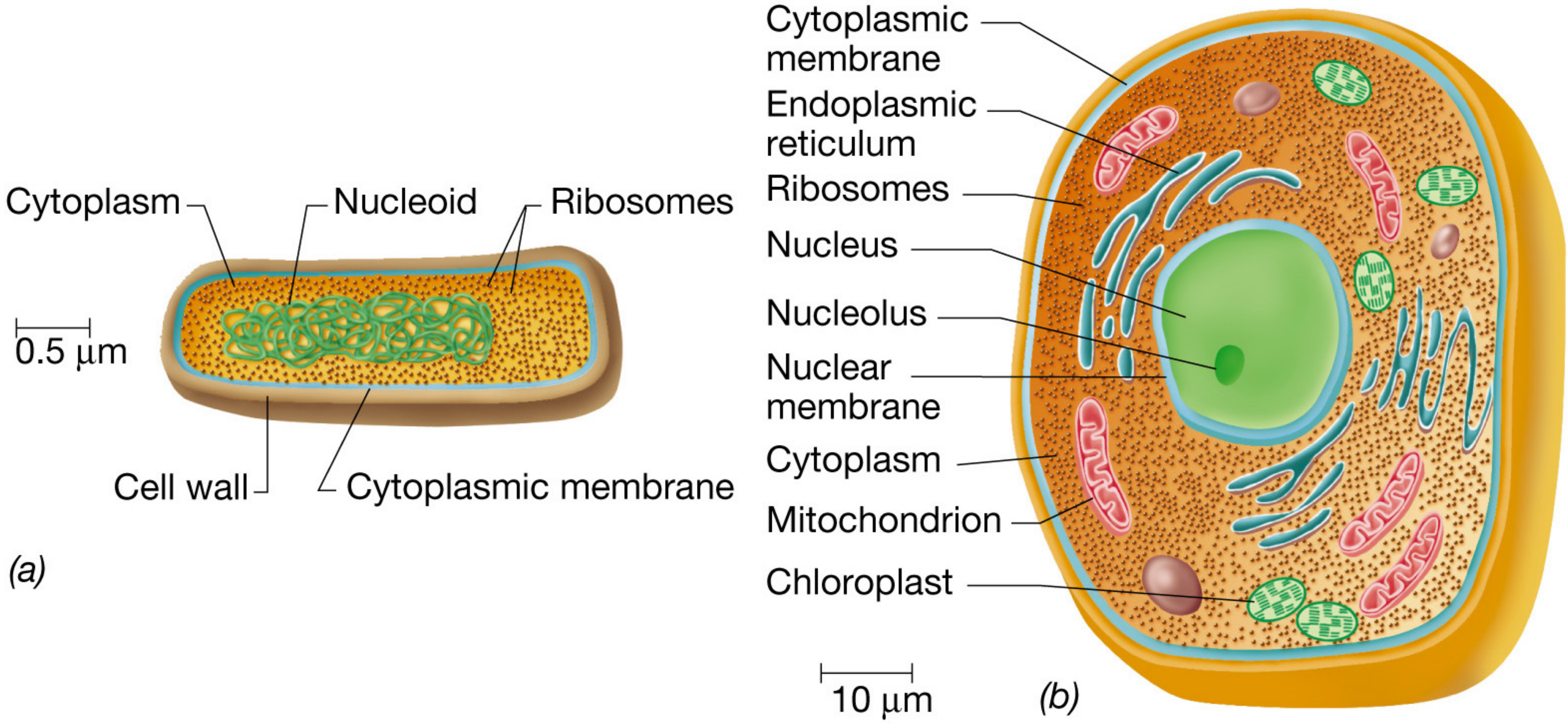
Comparing Prokaryotic and Eukaryotic Cells

Basic unit of living organisms is the cell; the smallest unit capable of life.

What's common to all cells?

STRUCTURES	CHARACTERISTICS
Ribosomes	ATP is energy "currency"
Cell Membrane	Respond to external stimuli
Genetic Material	Regulate influx/efflux
Cytoplasm	Reproduce

Elements of cellular structure



Prokaryotic Cell Features

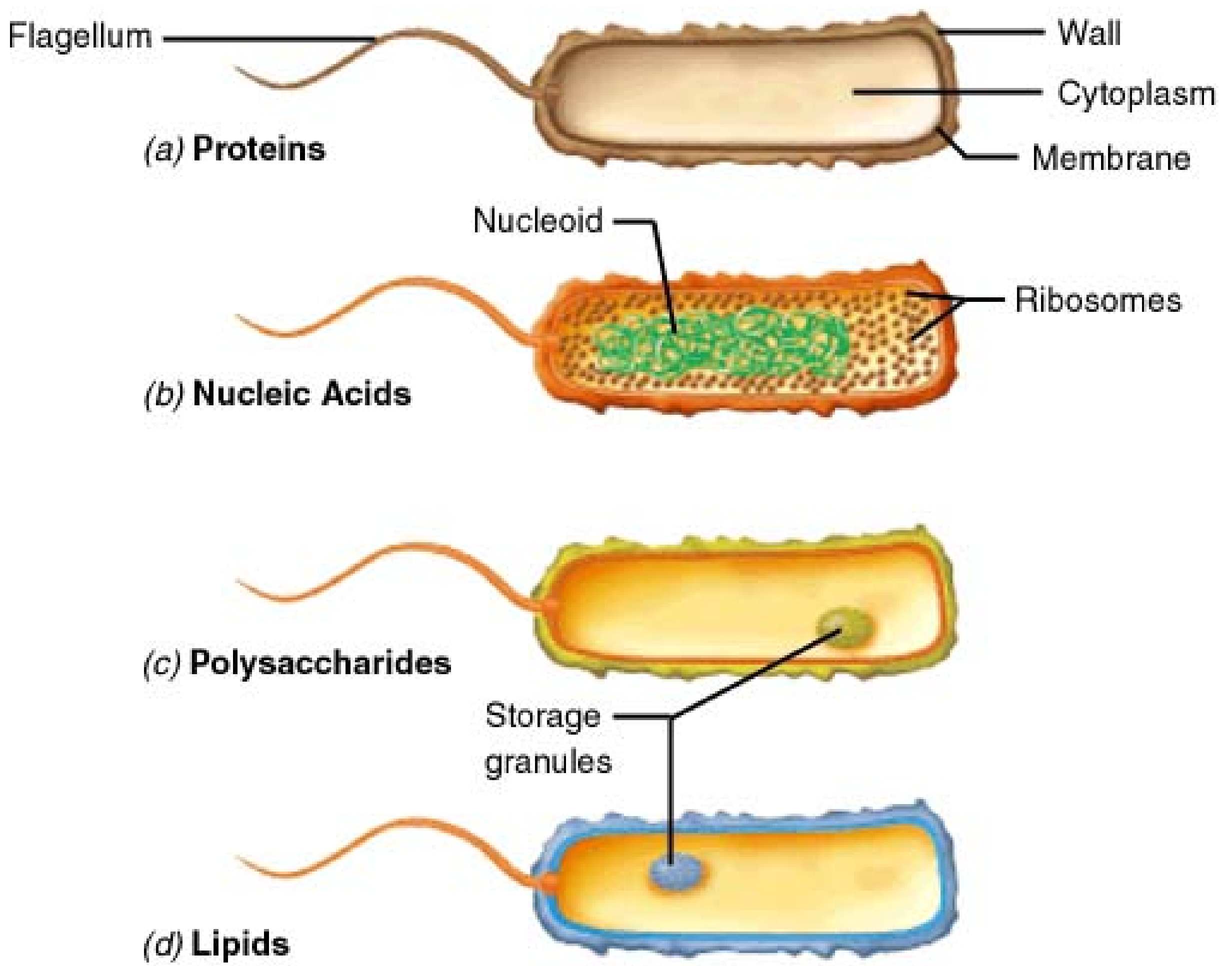
Invariant (or common to all)

Nucleoid Region: Curator of the Information.

**Ribosomes: Sites for protein synthesis –
aka the grand translators.**

**Cell Membranes: The barrier between order
and chaos.**

Macromolecules in the cell reflect main structural elements, and are localized for their functions



All over

Nucleoid (DNA), ribosomes (RNA)

Cell wall, mostly

Cell membrane (phospholipids), cell wall (Lipid A)

Chemical features of a "typical" bacterial cell
(*E. coli*)

70-85% Water

TABLE 2.2 Chemical composition of a prokaryotic cell[a]

Molecule	Percent of dry weight[b]	Molecules per cell	Different kinds
Total macromolecules	96	24,610,000	~2500
Protein	55	2,350,000	~1850
Polysaccharide	5	4,300	2[c]
Lipid	9.1	22,000,000	4[d]
Lipopolysaccharide	3.4	1,430,000	1
DNA	3.1	2.1	1
RNA	20.5	255,500	~660
Total monomers	3.0		~350
Amino acids and precursors	0.5		~100
Sugars and precursors	2		~50
Nucleotides and precursors	0.5		~200
Inorganic ions	1		18
Total	100%		

a Data from Neidhardt, F. C., et al. (eds.), 1996. *Escherichia coli* and *Salmonella typhimurium—Cellular and Molecular Biology*, 2nd edition. American Society for Microbiology, Washington, DC.

b Dry weight of an actively growing cell of *E. coli* ≅ 2.8×10^{-13} g; total weight (70% water) = 9.5×10^{-13} g.

c Assuming peptidoglycan and glycogen to be the major polysaccharides present. Glycogen = glucose polymer

d There are several classes of phospholipids, each of which exists in many kinds because of variability in fatty acid composition between species and because of different growth conditions.

Take Home Message:

Proteins are #1 by weight

Lipids are #1 by number

Prokaryotic Cell Features

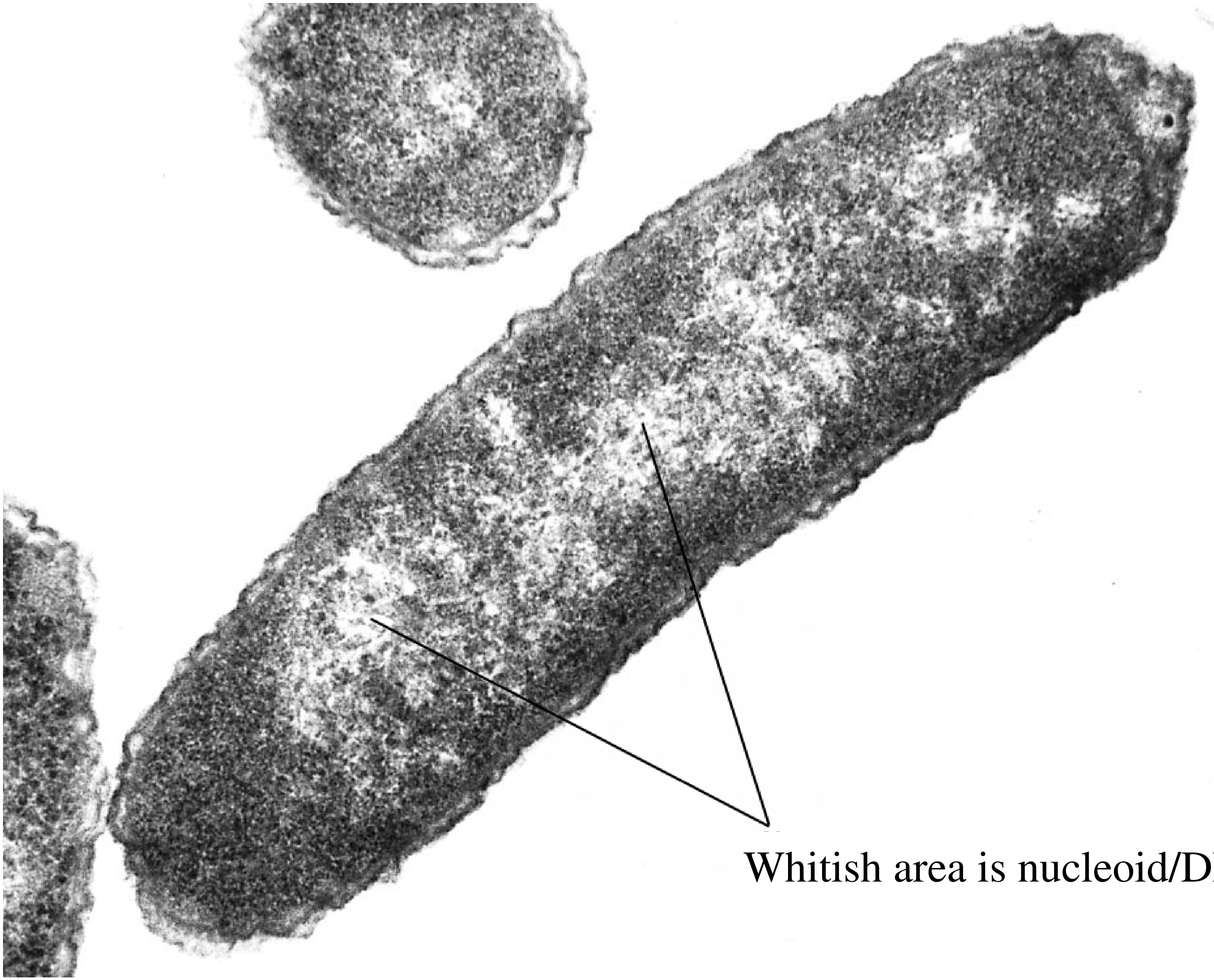
Invariant (or common to all)

Nucleoid Region: Curator of the Information.

**Ribosomes: Sites for protein synthesis –
aka the grand translators.**

**Cell Membranes: The barrier between order
and chaos.**

Appearance of DNA by TEM



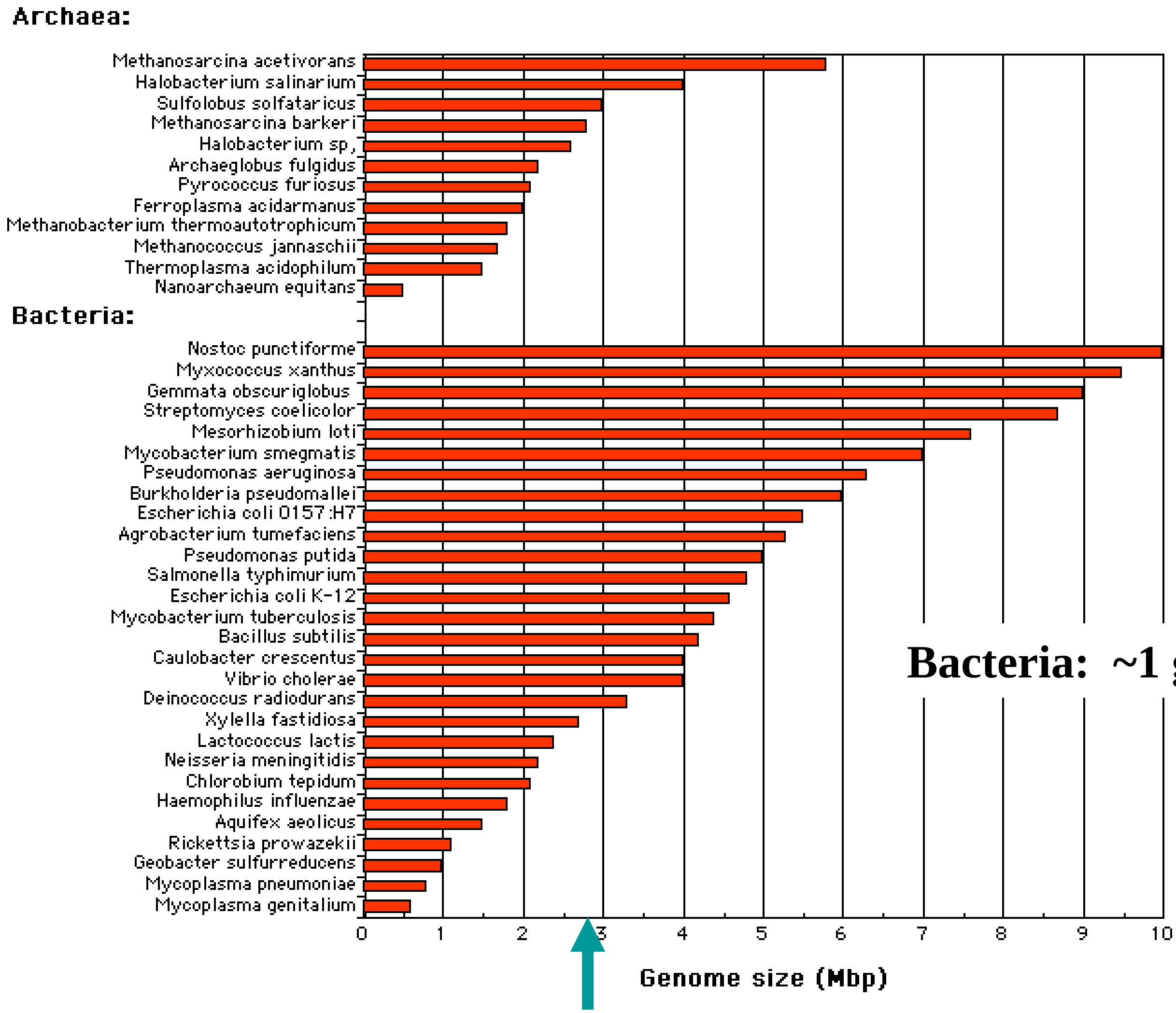
Prokaryotic DNA

Statistics:

Chromosomes: 1; bears essential genes
Plasmids: 0 to hundreds; helpful genes
Circumference: ~ 1 mm

Enigma:

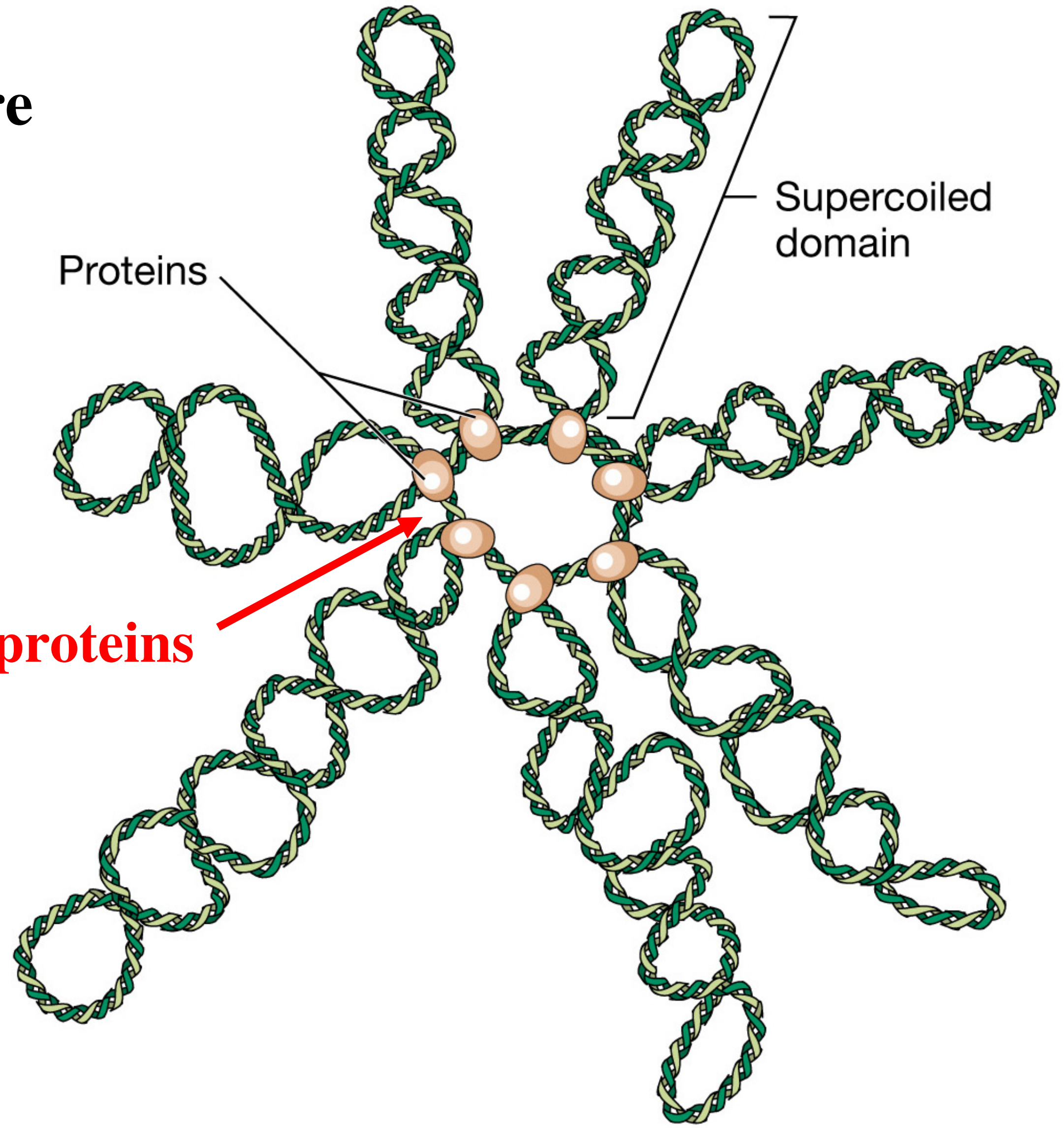
How to fit 1 mm long chromosome into a 1 uM wide cell?
Condensation: 30 to 50 loops of DNA emerging from a denser core
Supercoiling: tight twisting
Organization: wrapped around histone-like proteins



Bacteria: ~1 gene/1000 bp

Smallest eukaryotic genome: *Microsporidia*, 2.9 Mbp
Human Genome: 3,200 Mbp, 30,000 genes – less compact

Condensation:
30 to 50 loops of DNA emerging from a denser core



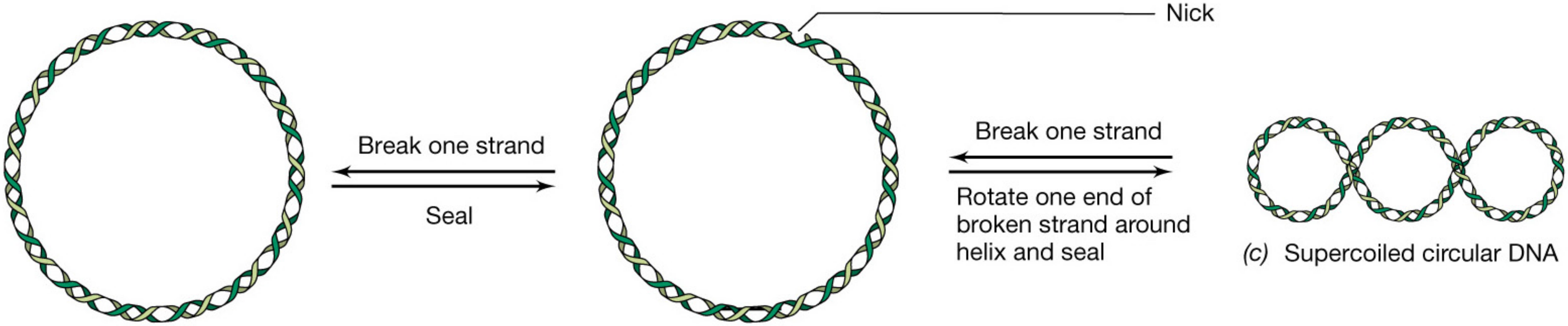
(d) Chromosomal DNA with supercoiled domains

Stabilization by binding to proteins

Supercoiling

Chapter 7.3

Supercoiling: tight twisting of DNA



(a) Relaxed, covalently closed circular DNA

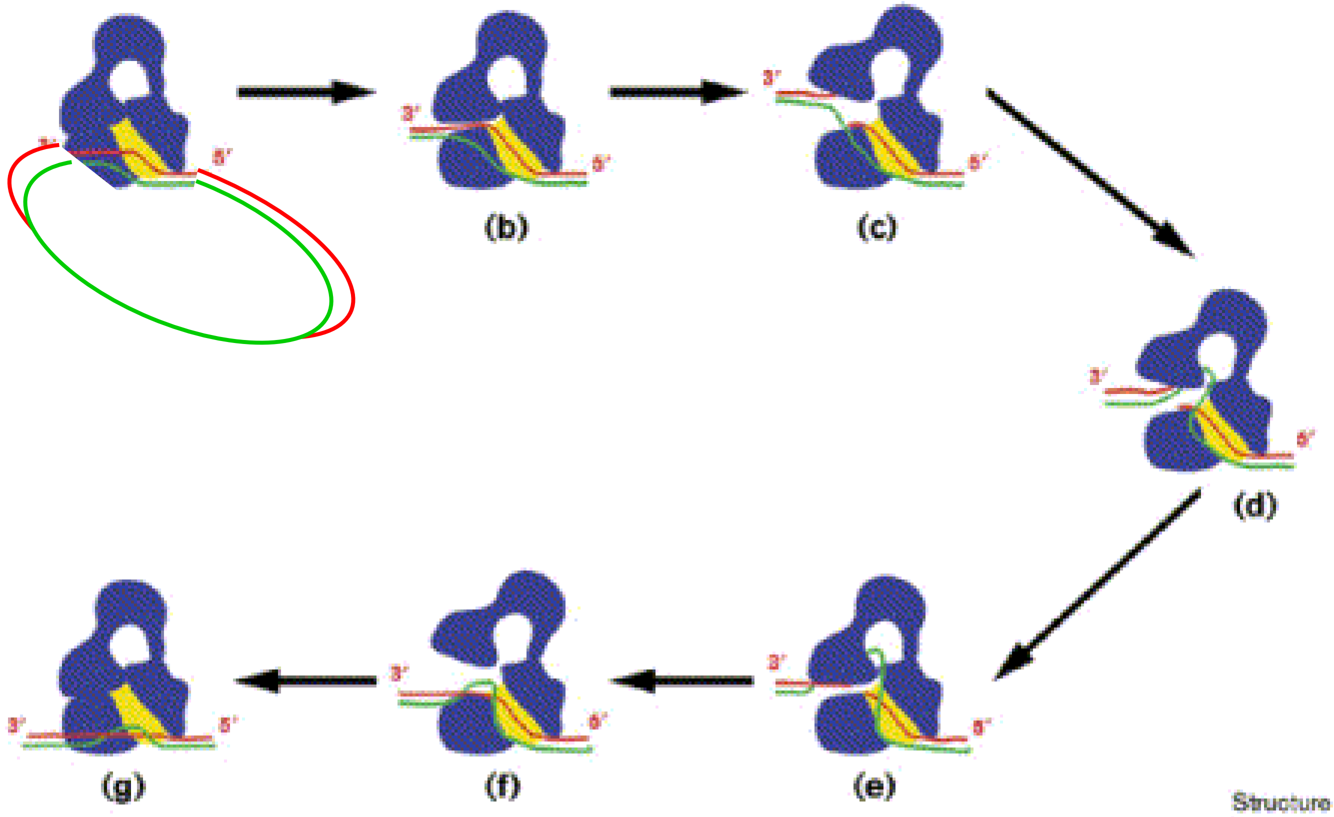
(b) Relaxed, nicked circular DNA

(c) Supercoiled circular DNA

Coiling and uncoiling: Topoisomerase I - IV (target of some antibiotics)

Positive supercoiling in some hyperthermophiles

Chapter 7.3



Proposed mechanism of action for type IA DNA topoisomerase of *E. coli*.

Yellow patch = putative binding groove for ssDNA

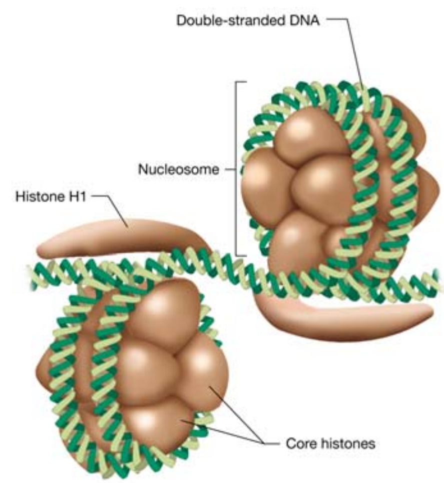
Mondragón and DiGate, 1999. Structure 7(11): 1373.

Bacteria and archea have histone-like proteins

Together, HU and IHF bind 10-20% of DNA in cell (depending on growth phase) and participate in chromosome supercoiling, DNA bending, and transcriptional regulation

HU: similar to eukaryotic histone H2B; binds dsDNA without sequence specificity

hupA*, *hupB* = genes encoding HU heterodimer in *E. coli
***hupAB* mutants are:**
- **sensitive to cold and heat shock**
- **sensitive to UV irradiation**
- **unable to start dividing quickly**
- **filamentous in shape, with long cells**

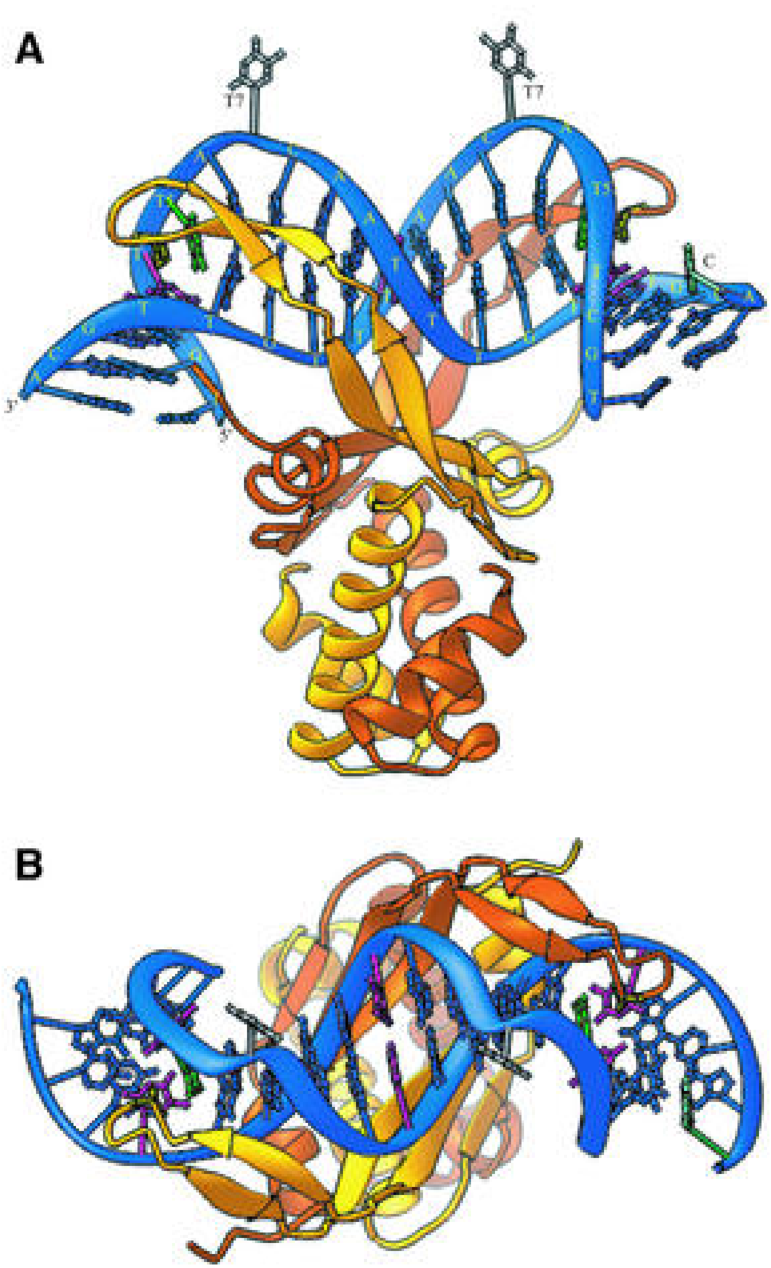


IHF: binds dsDNA at specific site

Other histone-like proteins (each binds ≤ 1% of DNA):

HN-S: binds curved, dsDNA without sequence specificity

Fis: binds dsDNA at specific site



IHF-DNA cocrystal structure

HU-DNA cocrystal structures

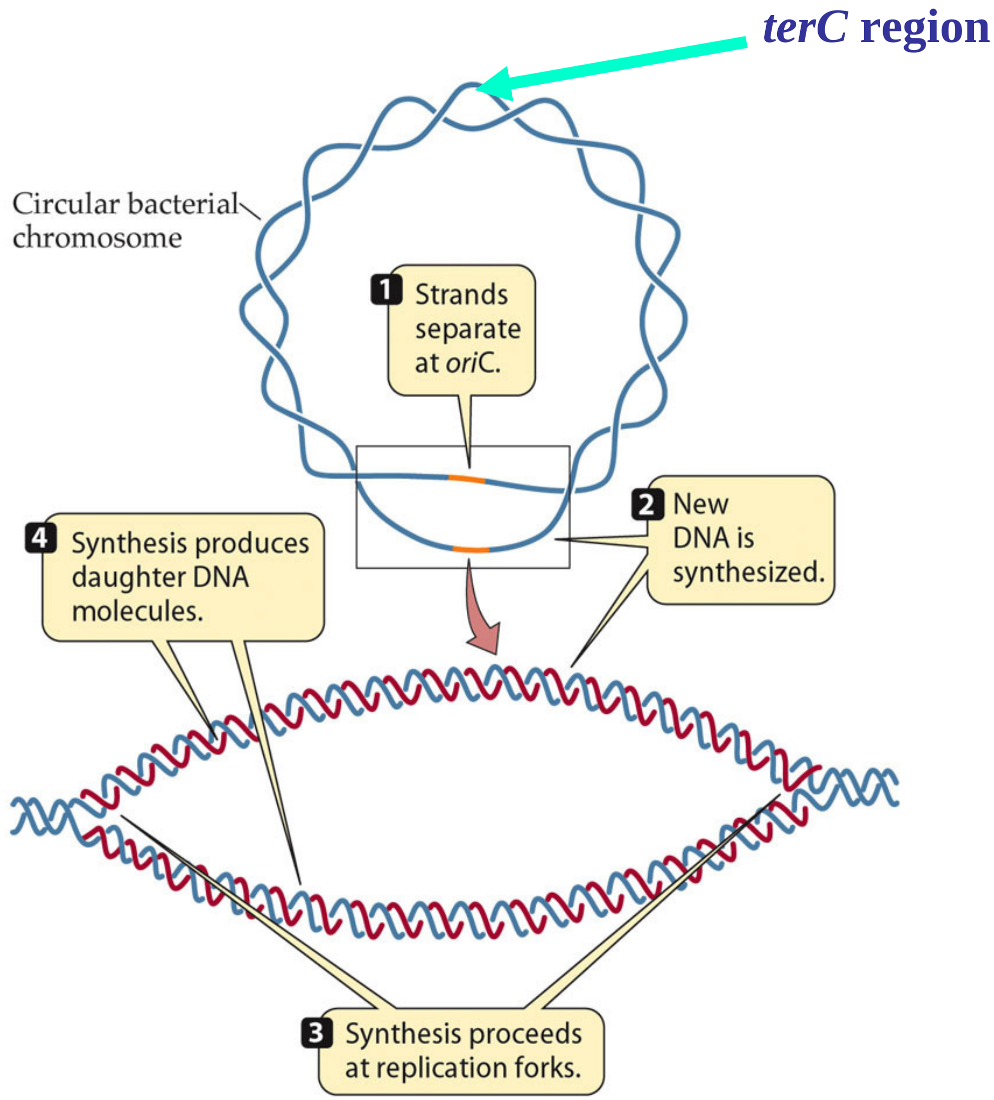
Swinger et al., 2003. EMBO 22(14): 3749–3760

Overview of DNA replication

Replisome at *oriC*: 25-40 proteins

-initiation
-elongation
-termination at *terC*
-50 Kbp/min
-1 in 10^{10} nucleotide errors

(i.e. 1 in 2000 progeny will have a point mutation)

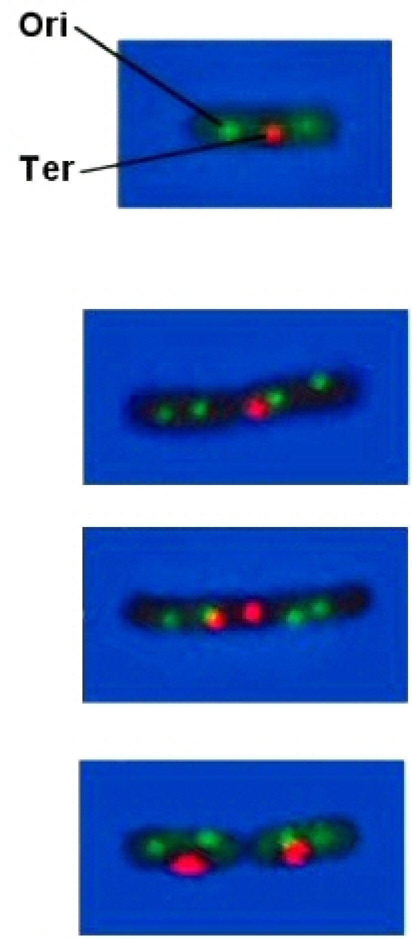


Chapter 7.5 - 7.6

Overview of DNA replication

Fluorescence microscopy:
E. coli cells with fluorophores labeling Ori and Ter

Lau et al (2003) Mol. Micro. 49:731



40 minutes to replicate *E. coli* chromosome.

20 minutes for cell division.

How???

Chapter 7.5 - 7.6

Chemical features of a "typical" bacterial cell (*E. coli*)

TABLE 2.2 Chemical composition of a prokaryotic cell[a]

70-85% Water

Molecule	Percent of dry weight[b]	Molecules per cell	Different kinds
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d There are several classes of phospholipids, each of which exists in many kinds because of variability in fatty acid composition between species and because of different growth conditions.

Prokaryotic Cell Features

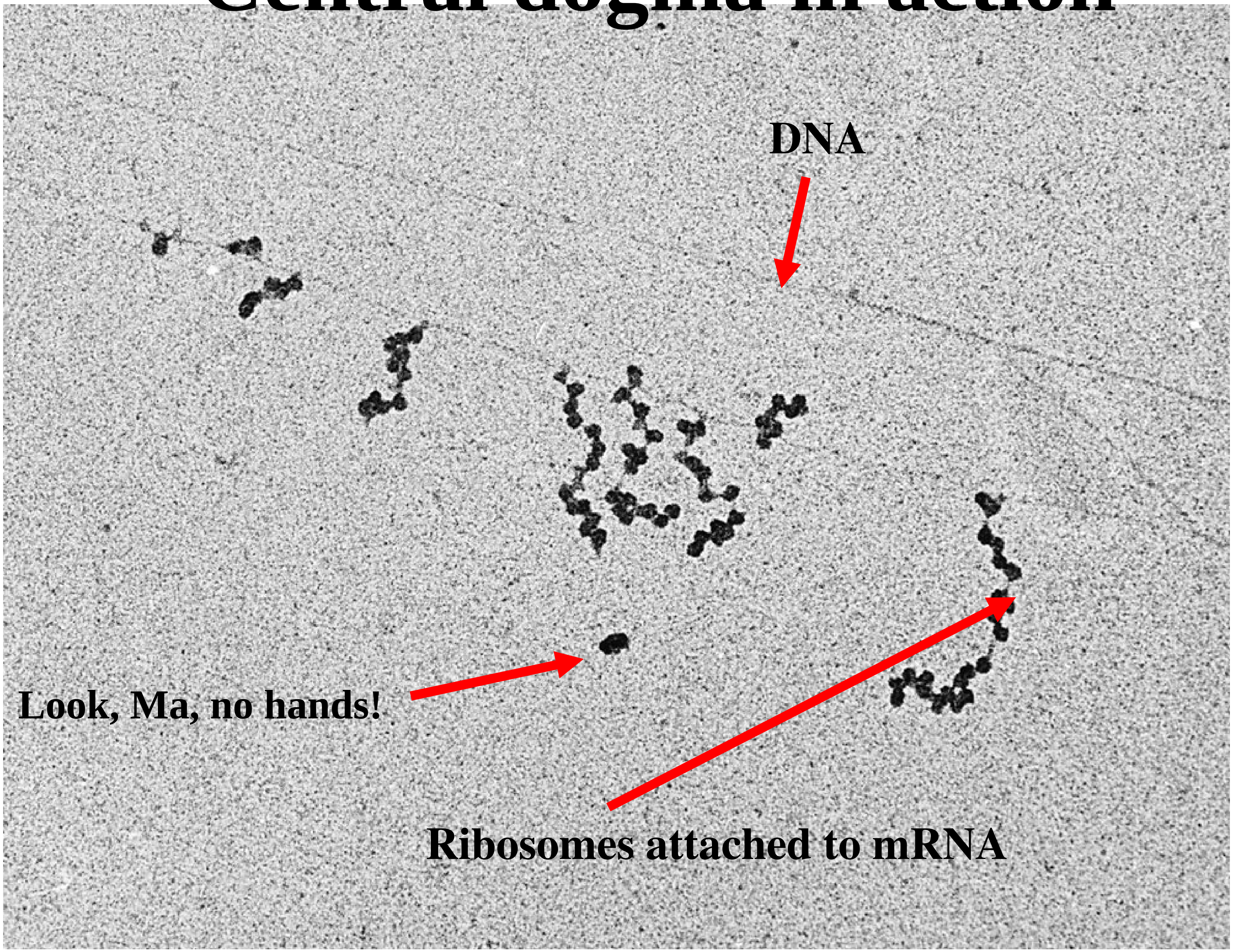
Invariant (or common to all)

Nucleoid Region: Curator of the Information.

***Ribosomes: Sites for protein synthesis –
aka the grand translators.***

Cell Membranes: The barrier between order and chaos.

Central dogma in action



Prokaryotic Cell Features

Invariant (or common to all)

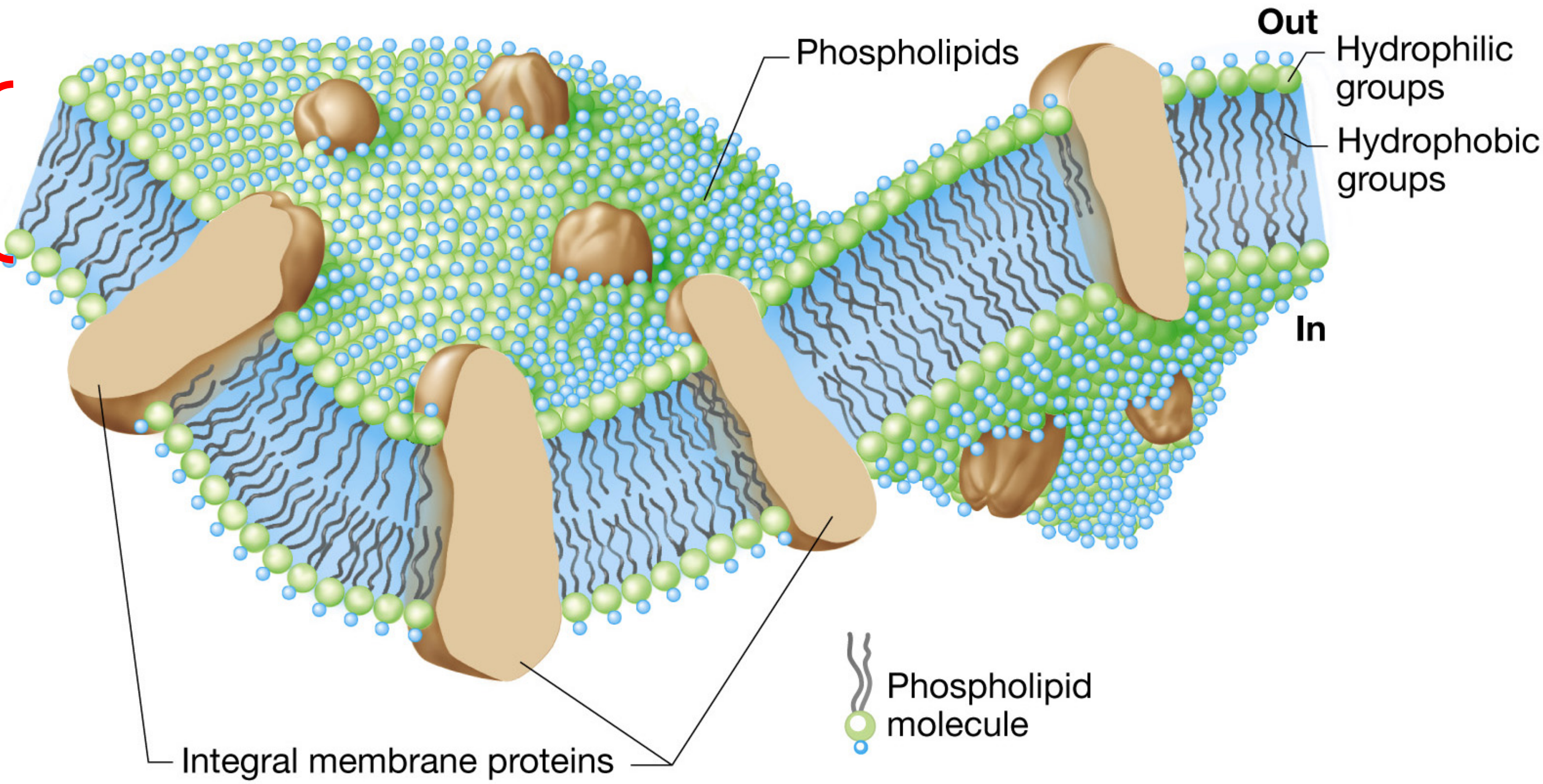
Nucleoid Region: Curator of the Information.

Ribosomes: Sites for protein synthesis – aka the grand translators.

Cell Membranes: The barrier between order and chaos.

The cytoplasmic membrane

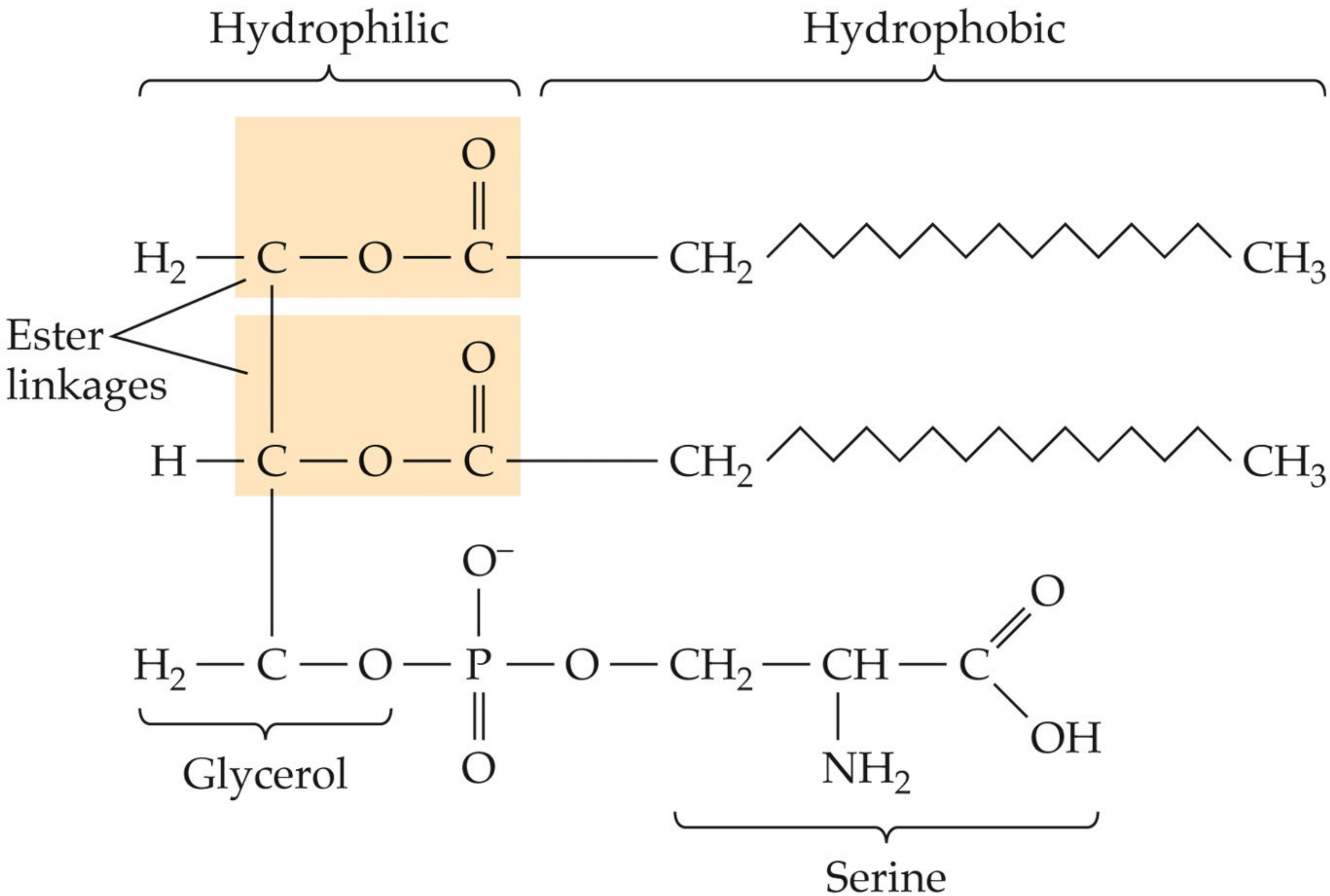
8 nm thick



Membrane has similar viscosity to oil: Fluid Mosaic Model

Stabilized by H bonds, hydrophobic interactions

Stabilized by Mg^{++} and Ca^{++} binding phosphate heads

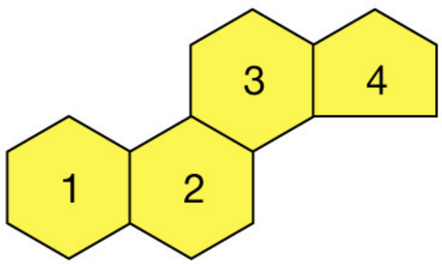


Phospholipids are amphipathic

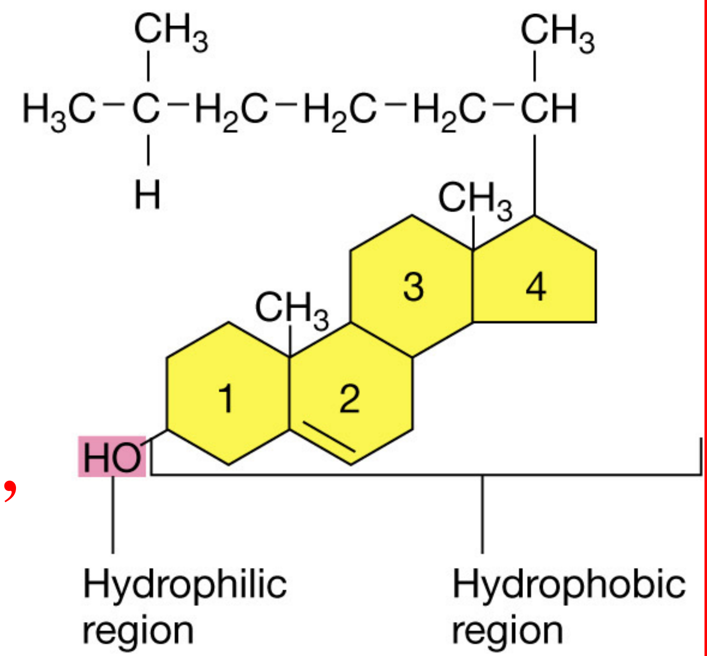
General Sterol Core:
Four rings

Example:
Cholesterol

(a)



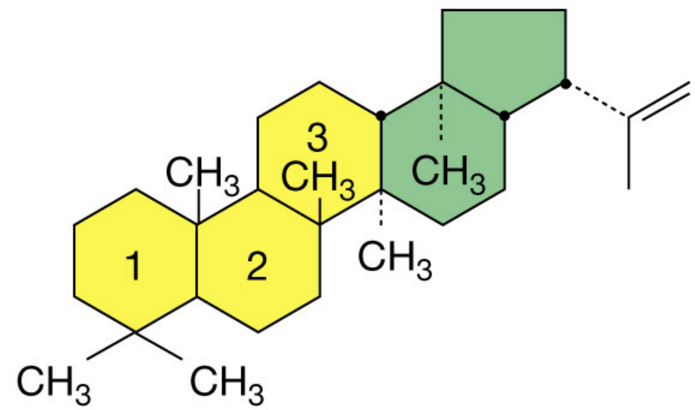
(b)



All Eukaryotes,
a few Bacteria

Similar molecules:
Hopanoids
(e.g., Diploptene)

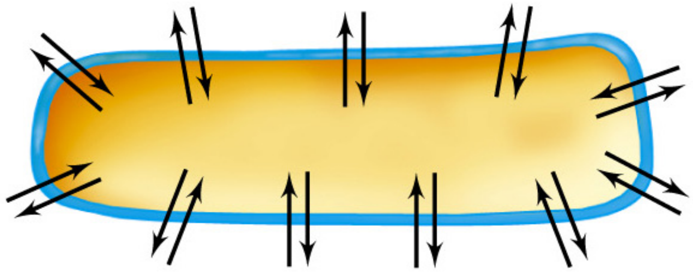
(c)



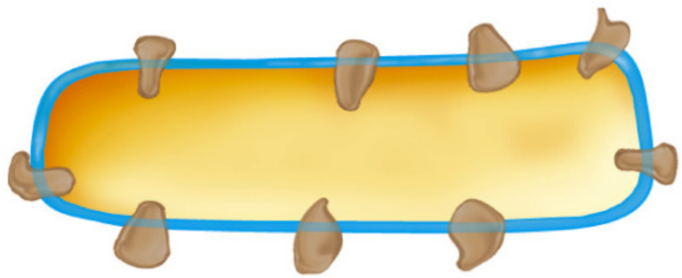
Many Bacteria

All sterols are rigid planar molecules
Fatty acids are flexible

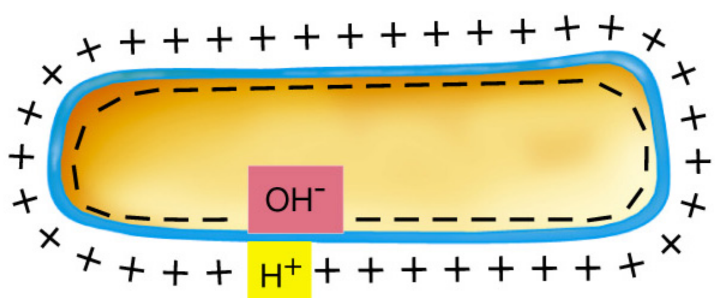
Functions of the cytoplasmic membrane



Permeability Barrier — Prevents leakage and functions as a gateway for transport of nutrients into and out of the cell



Protein Anchor — Site of many proteins involved in transport, bioenergetics, and chemotaxis



Charge separation: Potential energy Analogous to a battery

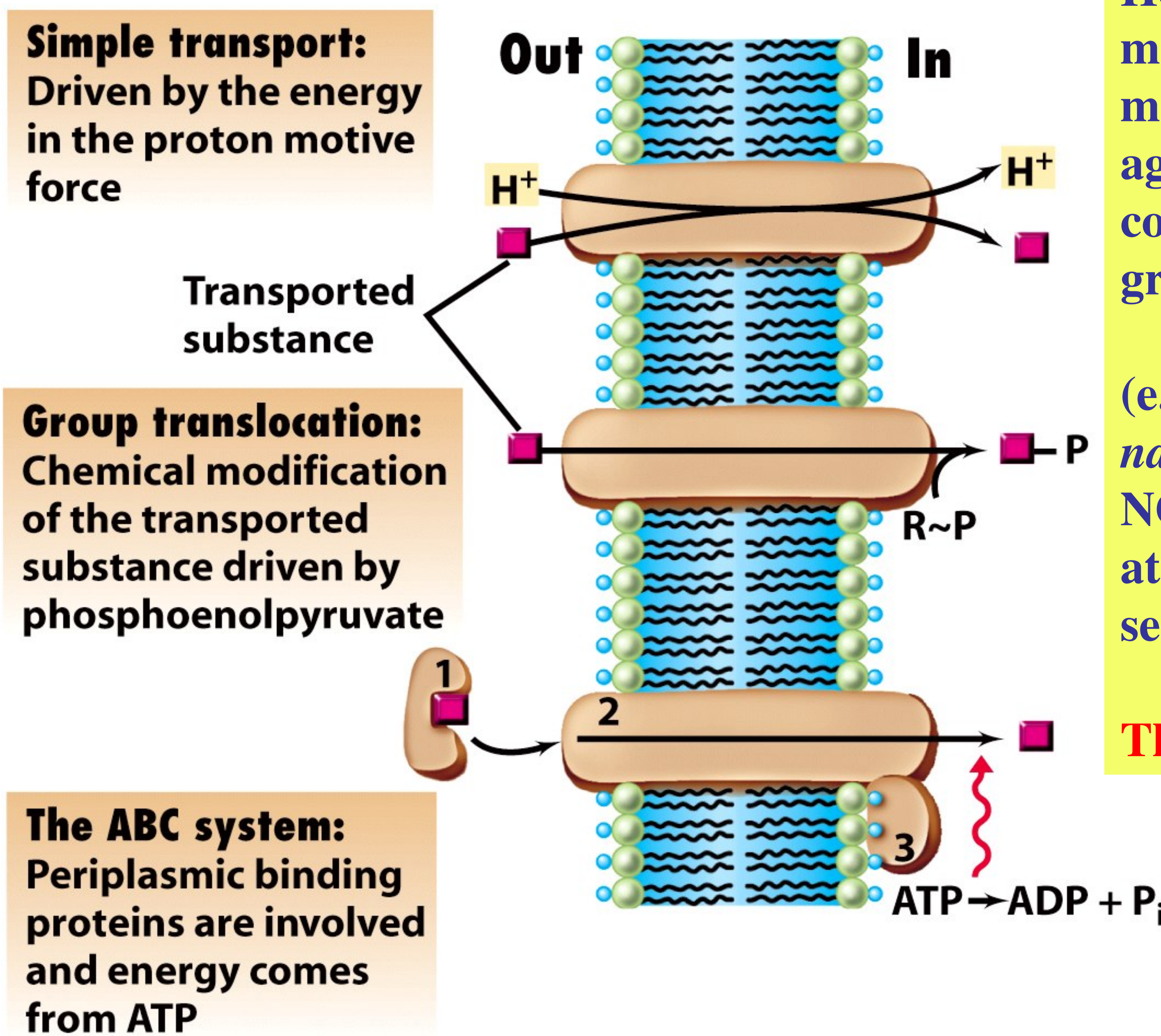
Energy Conservation — Site of generation and use of the proton motive force

Table 4.2 Comparative permeability of membranes to various molecules

Substance	Rate of permeability [a]
Water	100
Glycerol	0.1
Tryptophan	0.001
Glucose	0.001
Chloride ion (Cl^-)	0.000001
Potassium ion (K^+)	0.0000001
Sodium ion (Na^+)	0.00000001

Free diffusion of water
But assisted by aquaporins

[a] Relative scale—permeability with respect to permeability of water given as 100. Permeability of the membrane to water may be affected by aquaporins (see text).



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How do these molecules cross membrane… often against a concentration gradient?

(e.g. *Thiomargarita namibiensis*, internal NO_3 concentrations at 10,000X that of seawater!)

The gatekeepers!

Simple transport: Driven by the energy of the proton motive force

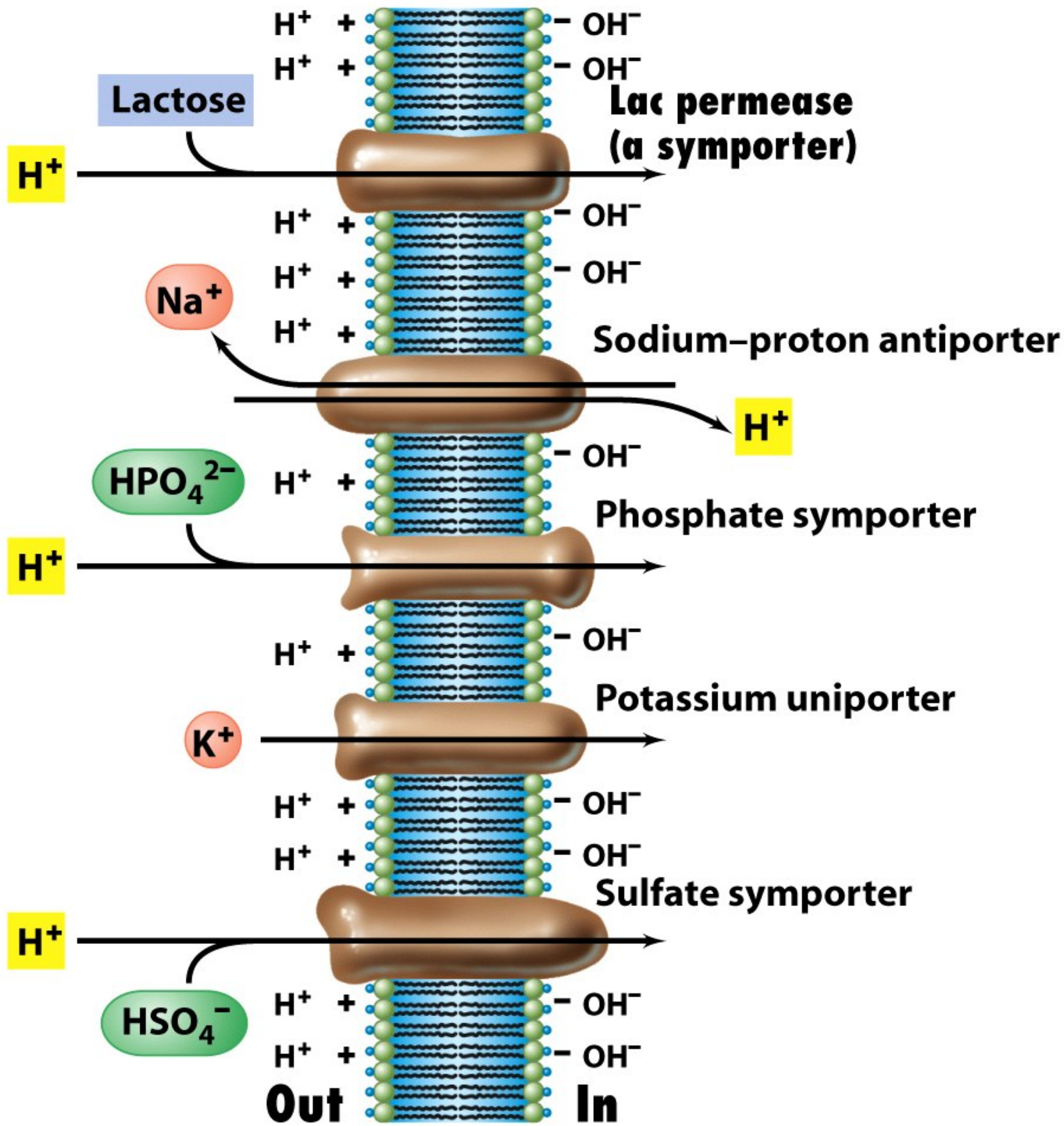

Figure 4-24 Brock Biology of Microorganisms 11/e
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When protein binds substrate, changes conformation which "squeezes" the molecule to the other side of the membrane.

Group translocation: chemical modification of the transported substance

Example: the glucose phosphotransferase system

1. **PEP provides energy for transport**
2. **Phosphorylation alters glucose so it does not accumulate as glucose inside, increasing concentration gradient**
3. **Glucose-6-phosphate is first step in glycolysis anyway**

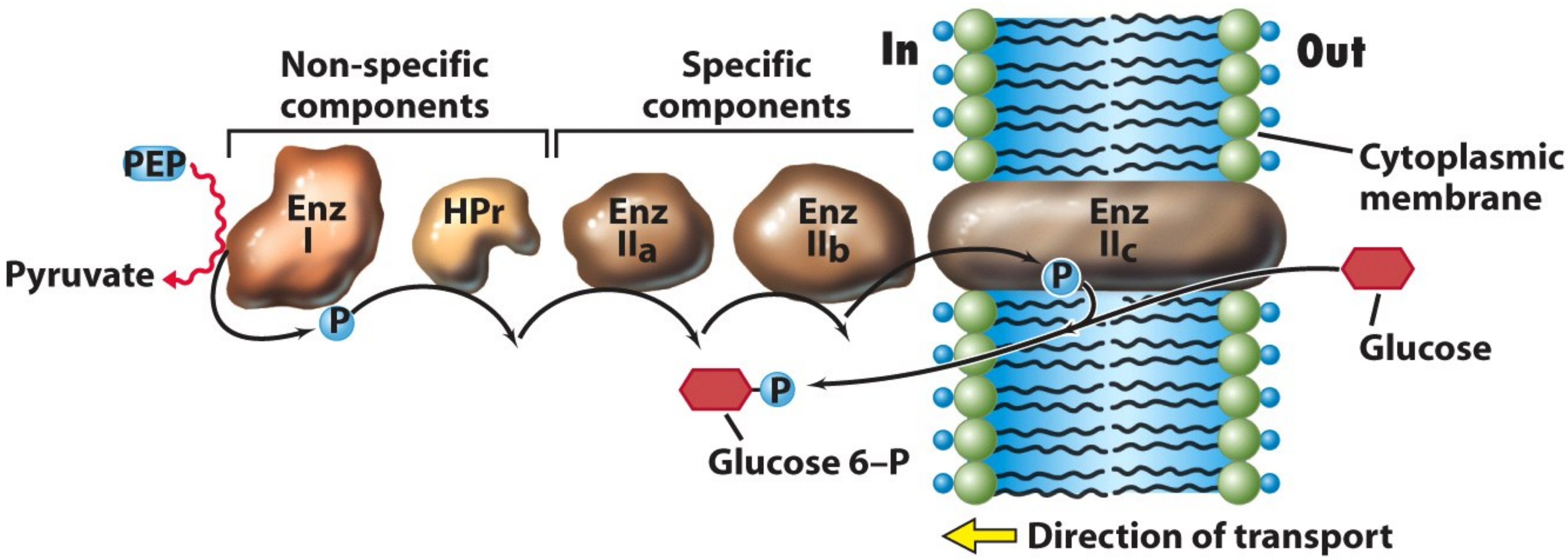

Figure 4-25 Brock Biology of Microorganisms 11/e

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ABC transporter: ATP hydrolysis provides energy for transfer

1. Periplasmic binding proteins "find" low-concentration solutes (as low as 10^{-6} M)
2. Binding protein docks on membrane-spanning domain
3. Conformational change and ATP hydrolysis drive transport

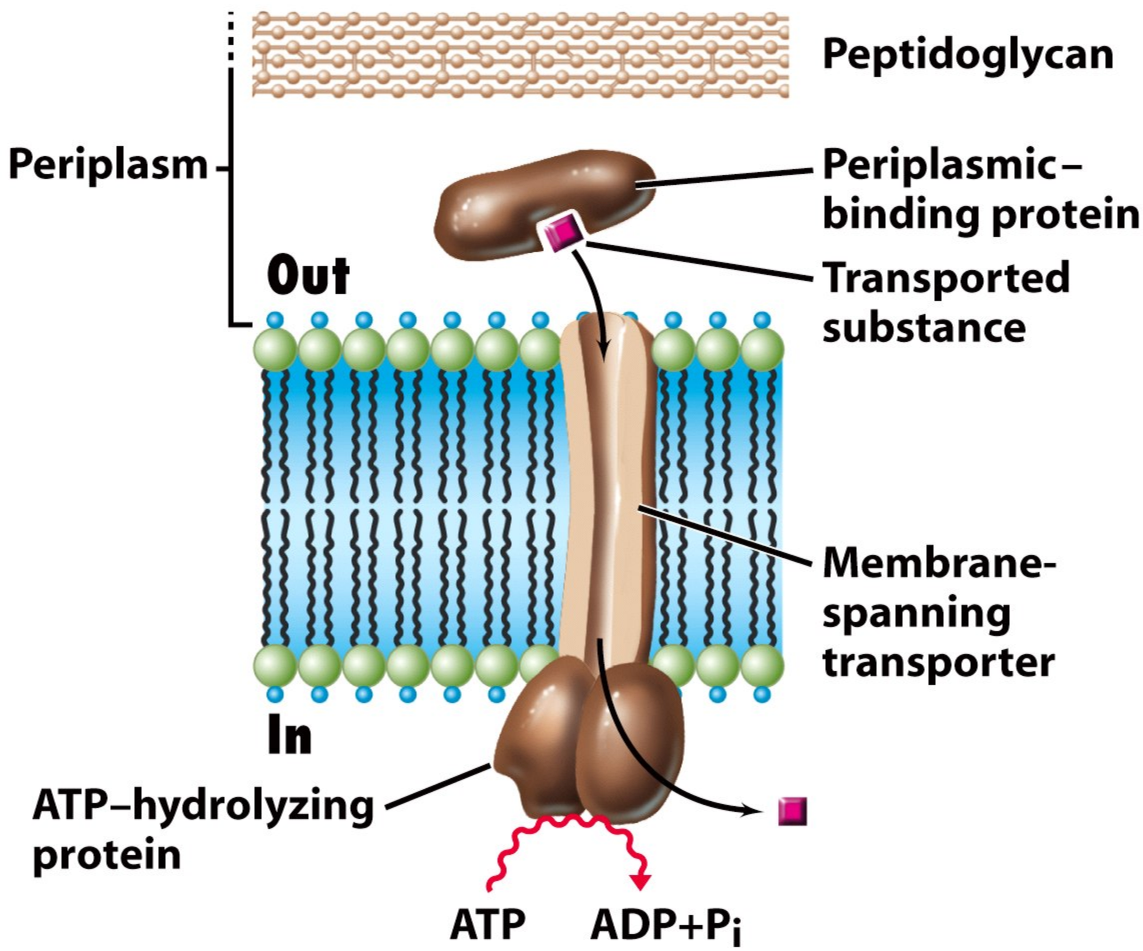

Figure 4-26 Brock Biology of Microorganisms 11/e
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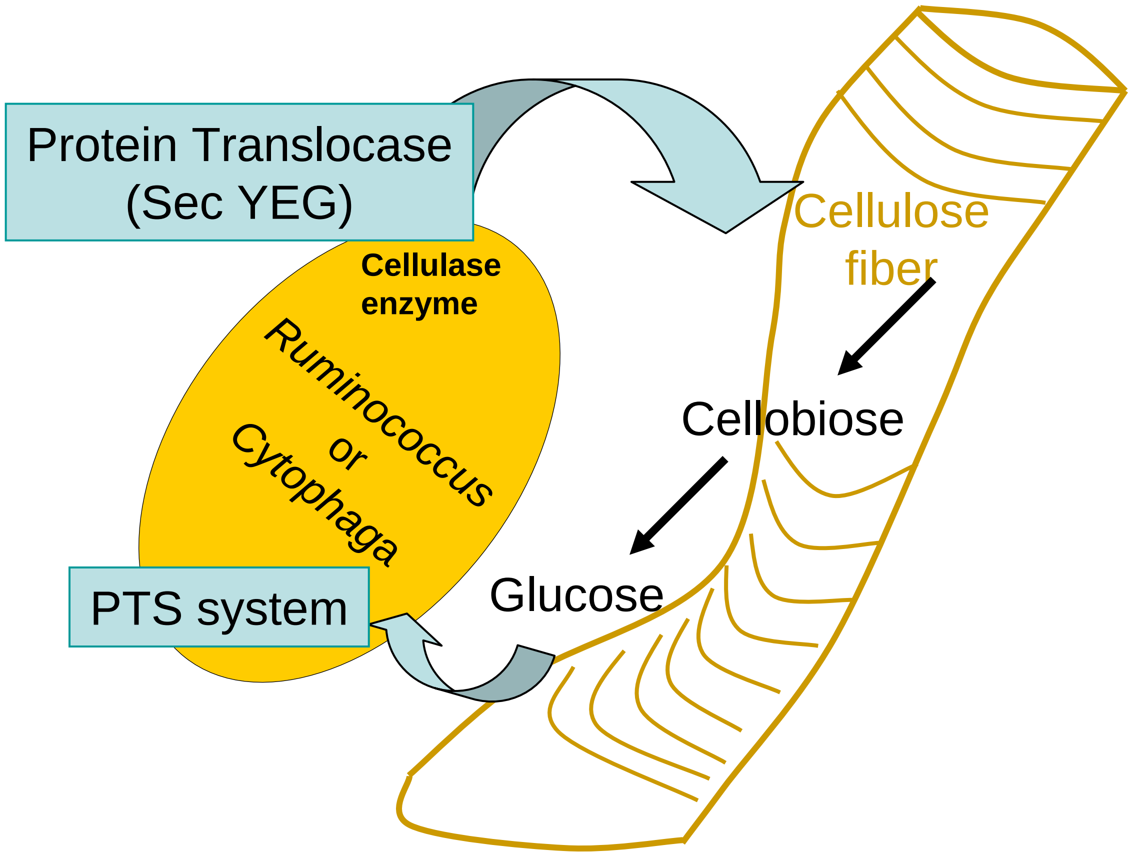
Small molecules transported in; what about transporting proteins out?

- Important because prokaryotic digestion is extracelluar
- Many protein secretion systems (at least six) – complex molecular machines
- Secrete proteases, lipases, nucleases, etc.

The overall strategy for feeding in bacteria and archaea:

Suppose you wished to use bacteria in a landfill to break down paper. Paper consists of a polymer, cellulose. It is a readily metabolizable macromolecule - it is a good *carbon and energy source* for bacteria

<u>Problem</u>: Prokaryotes do not ingest – no phagocytosis, no pinocytosis – everything must be solubilized before transport into the cell. How do you get the cellulose into the cell?



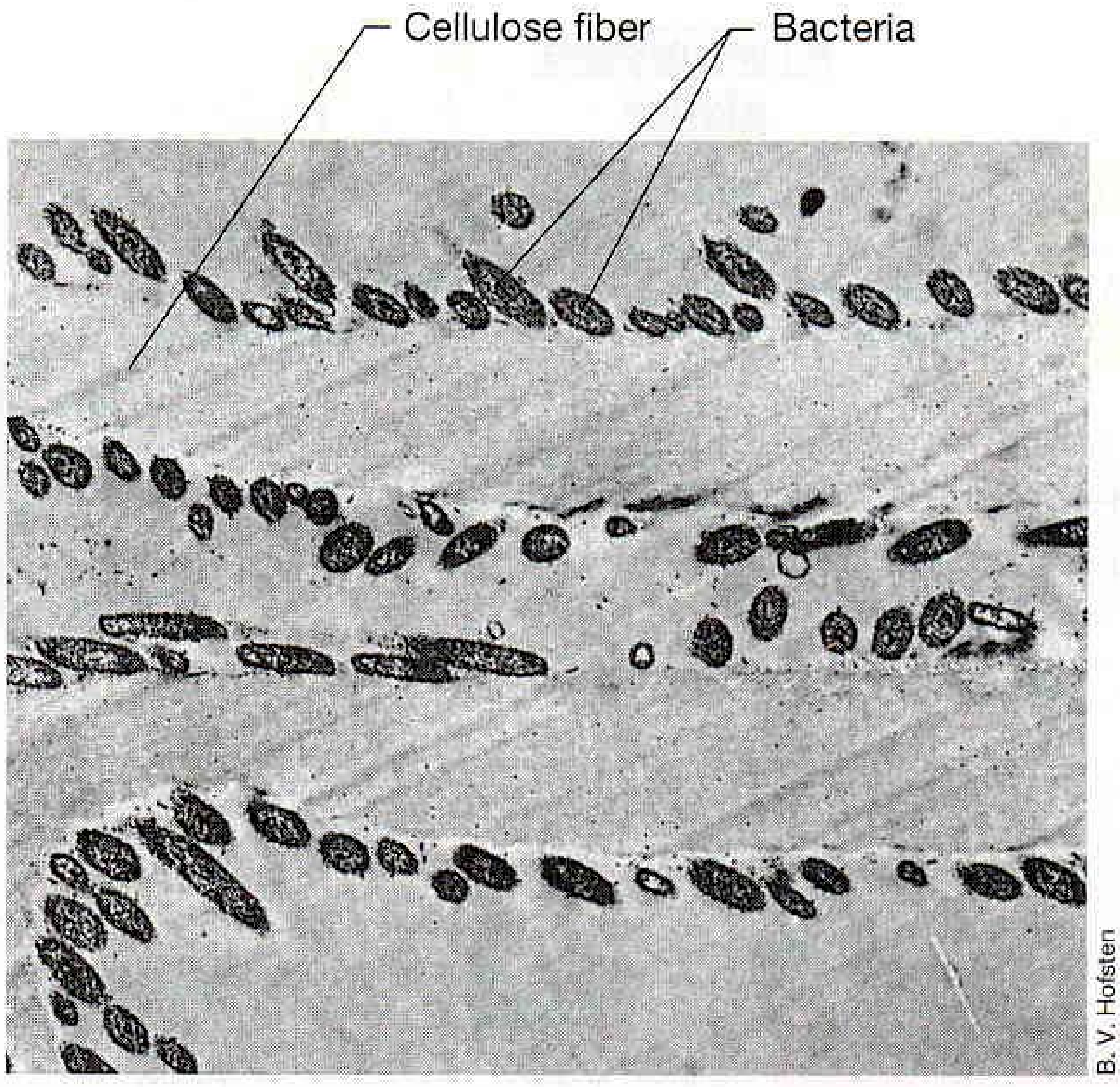
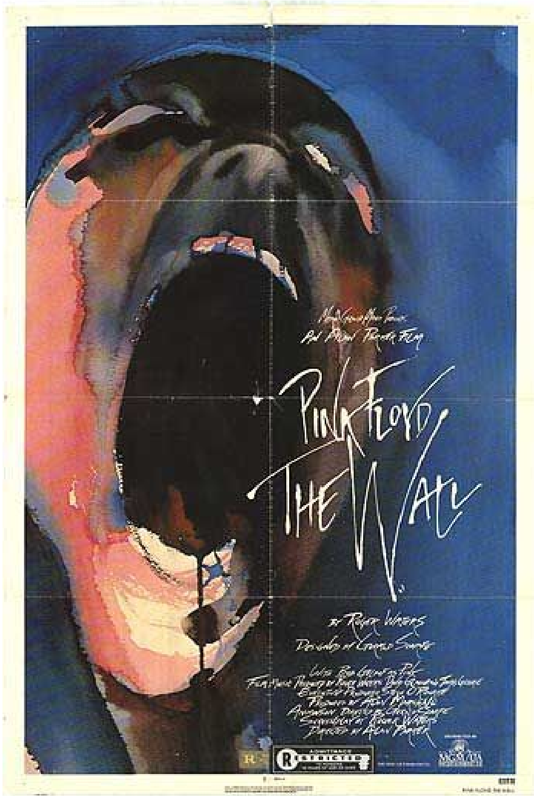


Figure 17.61 Transmission electron micrograph showing attachment of cellulose-digesting bacteria, *Sporocytophaga myxococcoides*, to cellulose fibers. Cells are about 0.5 μm in diameter.



Prokaryotic Cell Features

Cell Wall

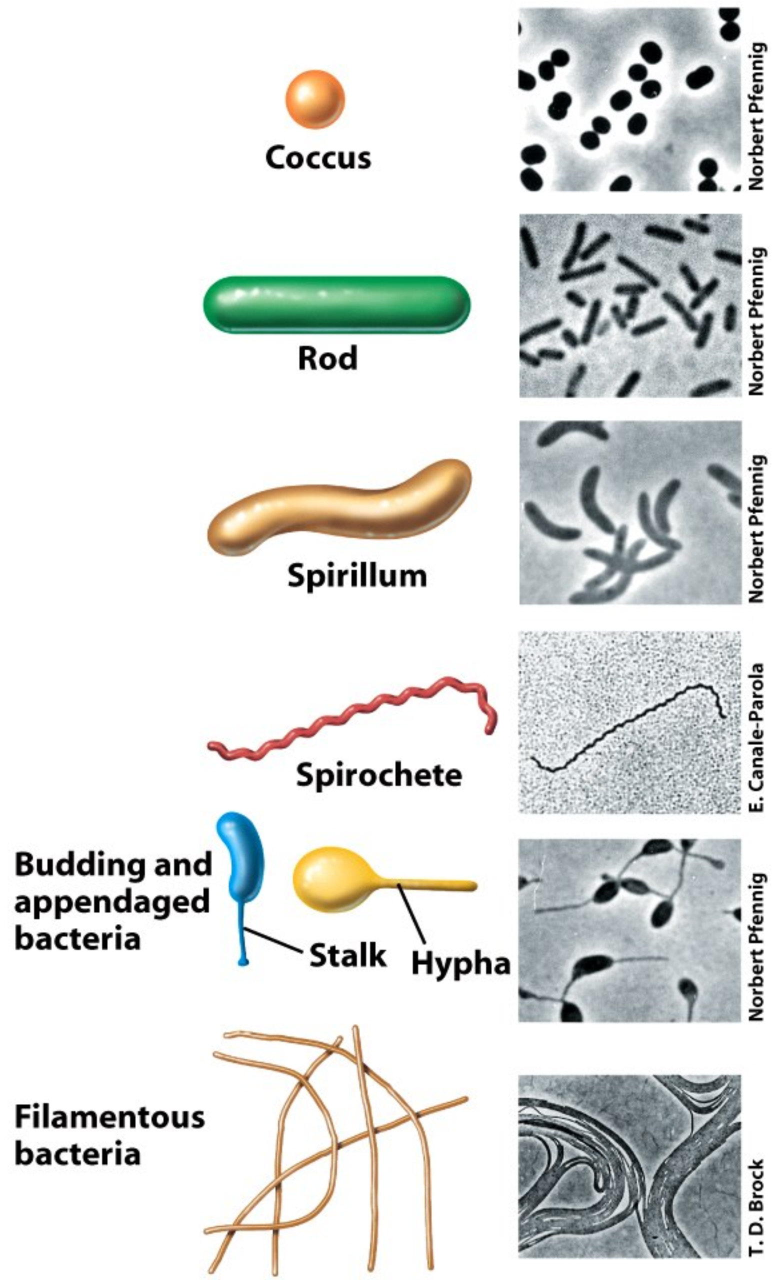
Classification of prokaryotic cellular features: Variant (or NOT common to all)

-Cell wall (chemistry varies; some don’t have one)

-Endospores (heavy-duty life support strategy)
-Capsules/Slime Layer (exterior to cell wall)
-Bacterial Flagella (appendages for movement)
-Pili (conduit for genetic exchange)
-Inclusion Bodies (granules for storage)
-Gas vesicles (vertical movement in liquid medium)

Prokaryotic cells can have a wide variety of **morphologies**, which are often helpful in identification.

Cell walls are integral in maintaining these unique shapes.



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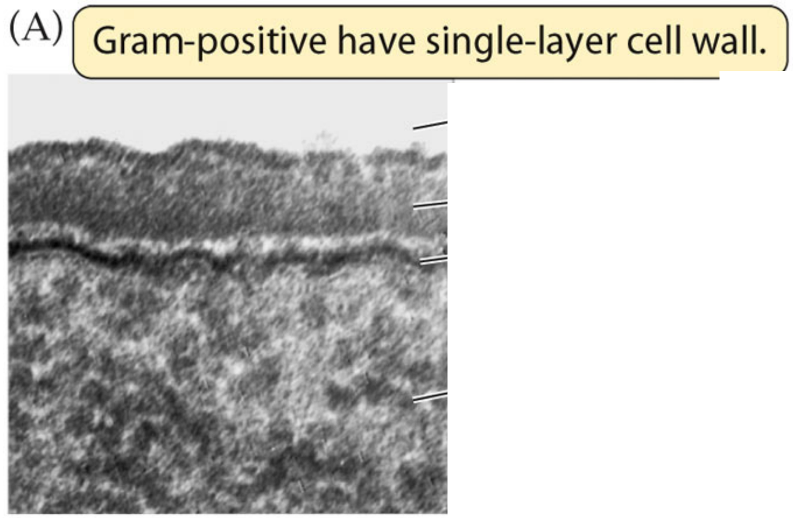
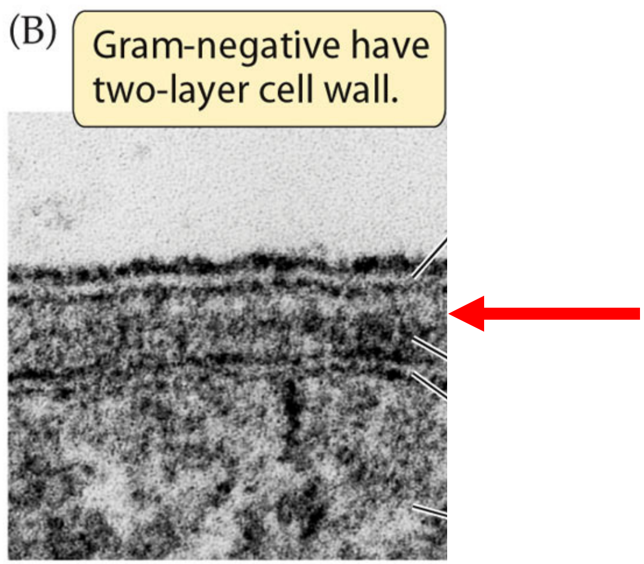
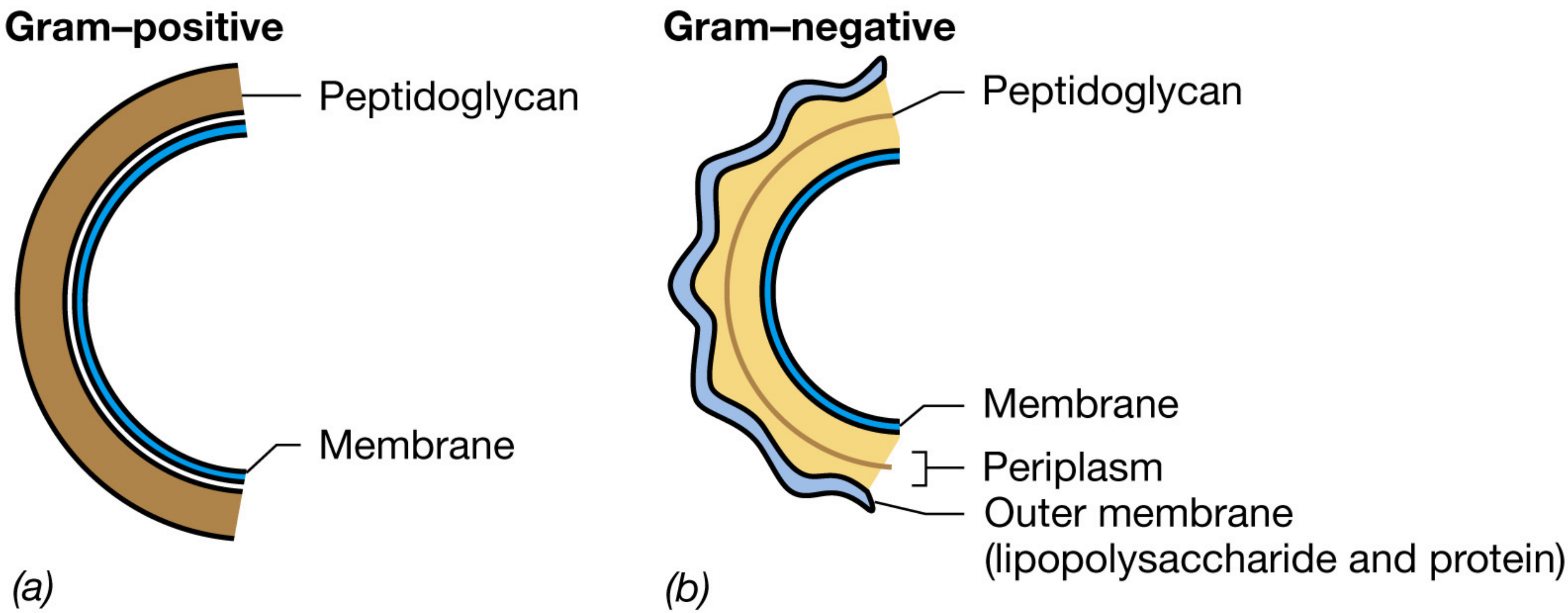


What's different between the cell envelopes of gram positive and gram negative bacteria?



What's this gray area between the two phospholipid bilayers in the gram negative bacterium?

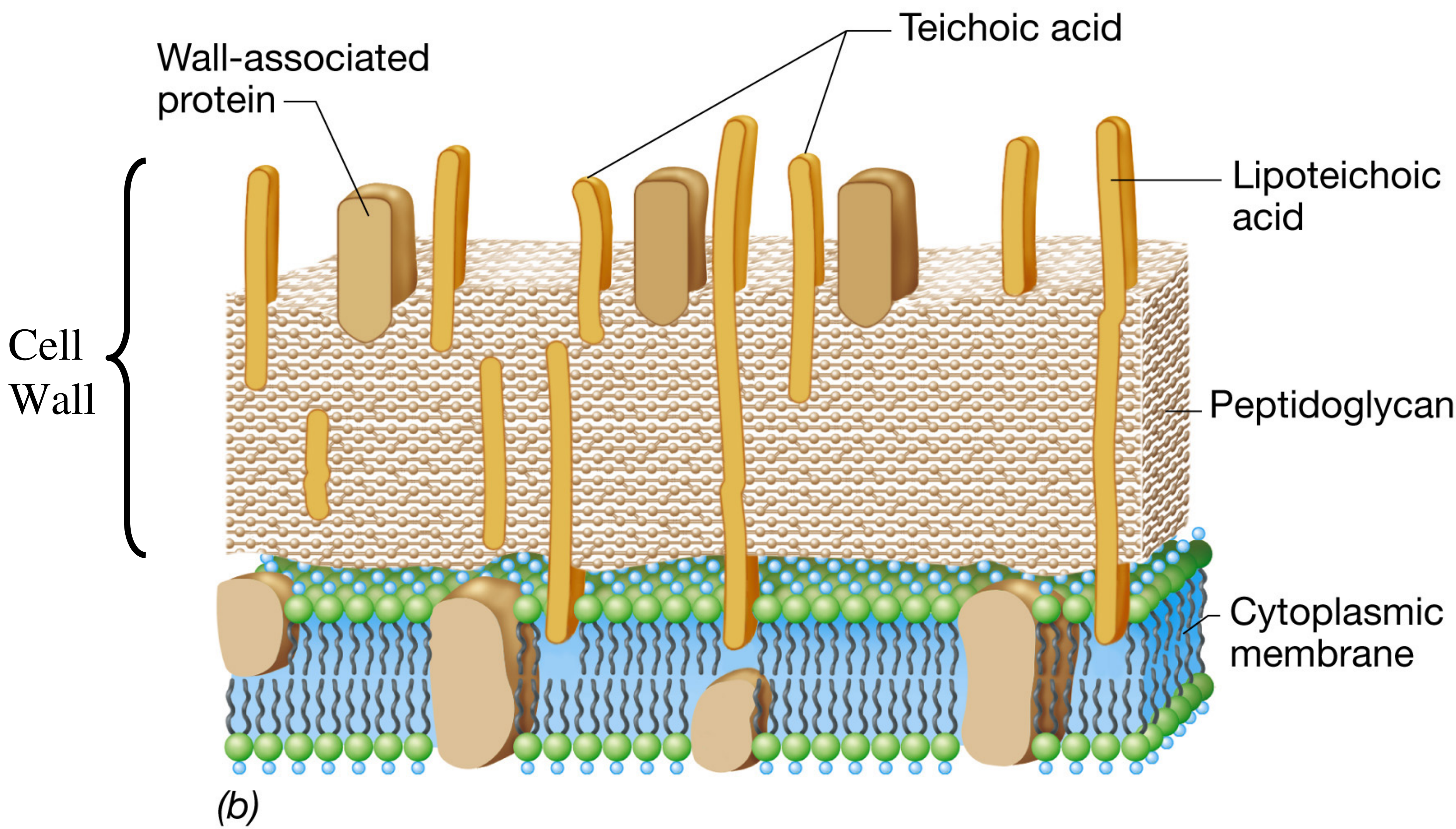
Cell walls of *Bacteria*



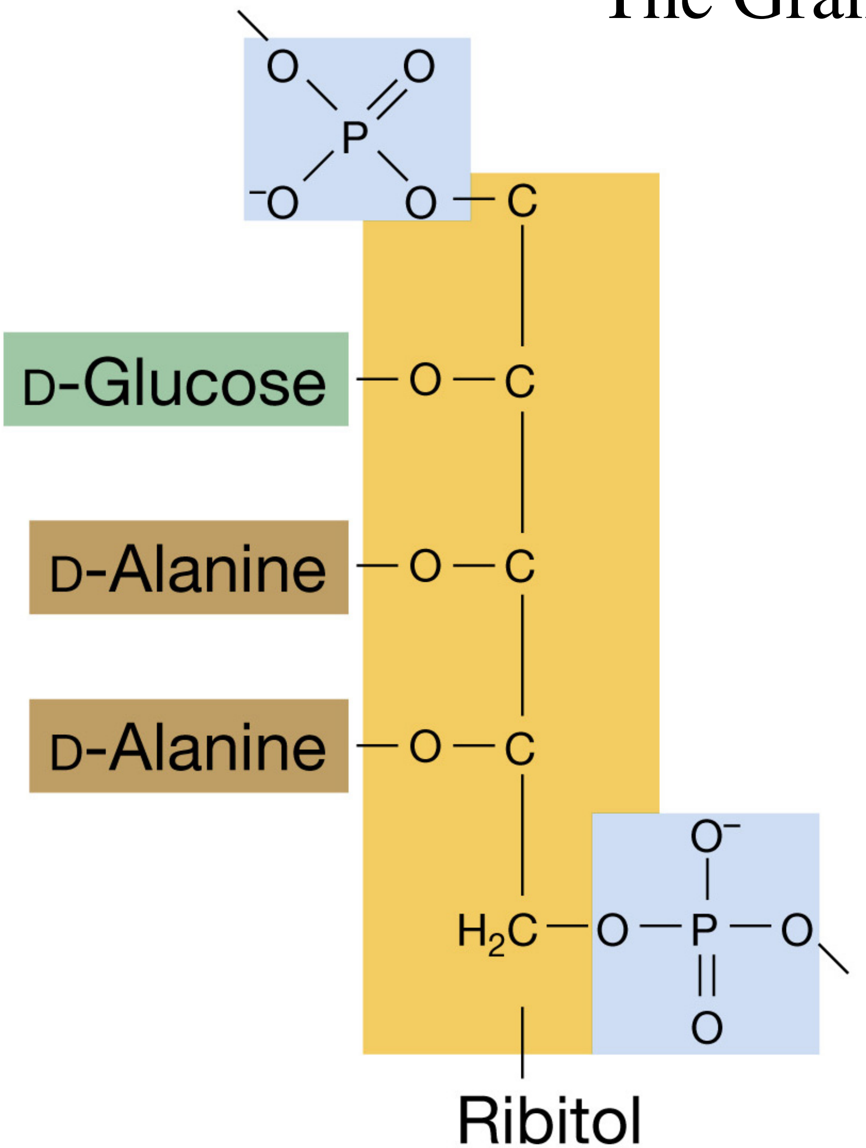
More layers of PG
One membrane

Few layers of PG
Two membranes

The Gram+ cell wall



The Gram$^+$ cell wall



(a)

Teichoic acid polymers are formed from repeating units of this structure.

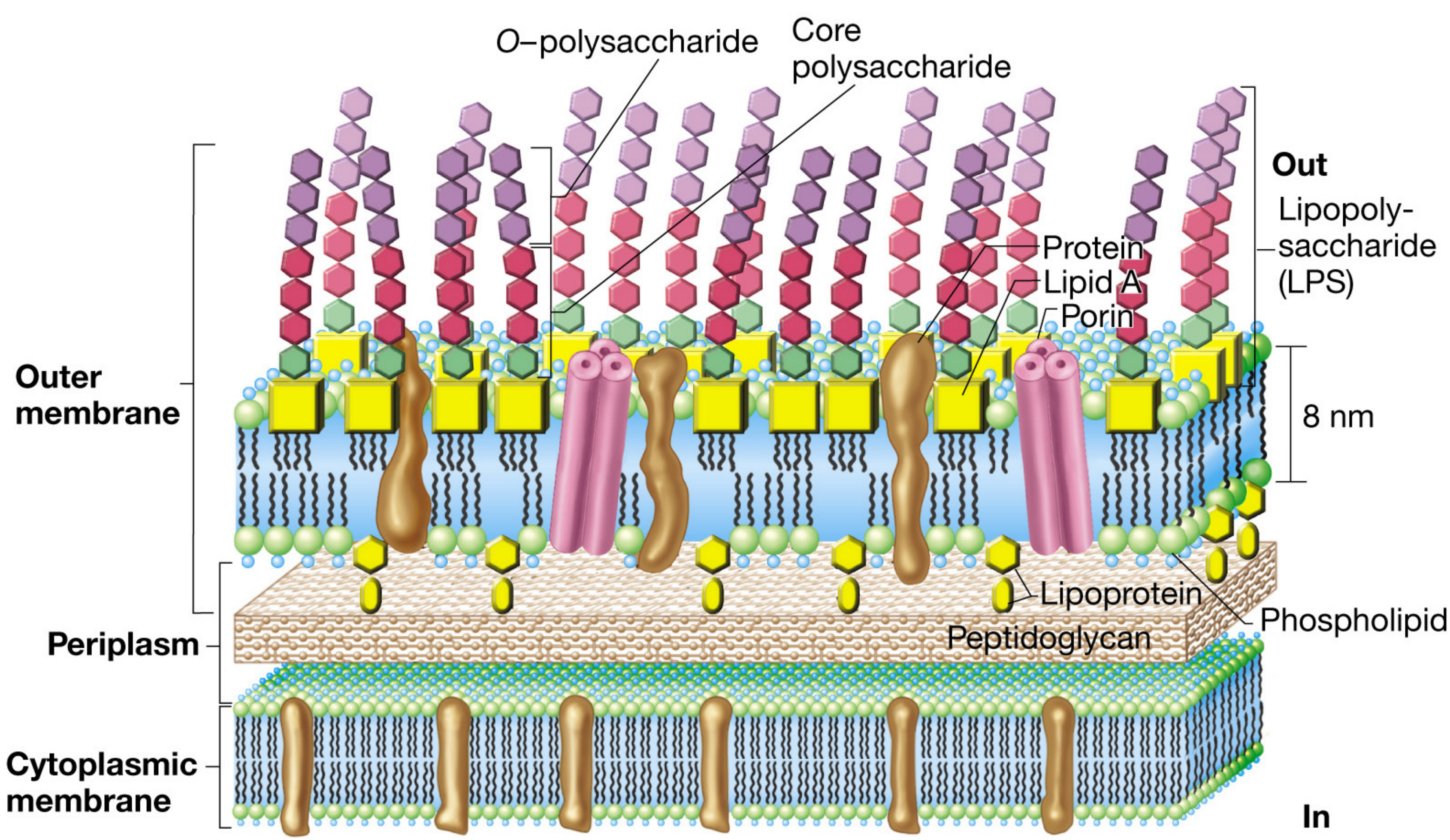
-negatively charged, contribute to negative charge of cell surface

-found in wall, membrane, capsule

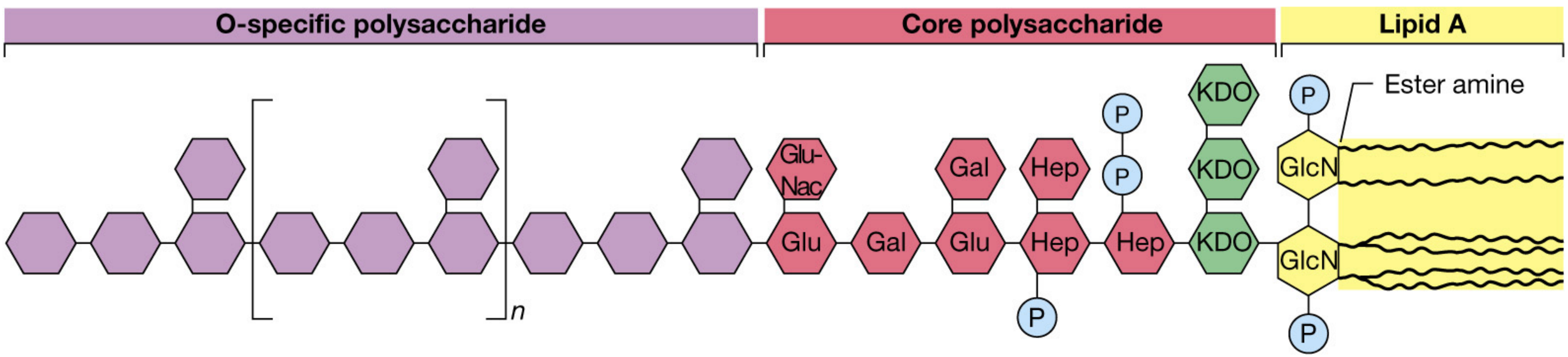
-may be covalently attached to membrane lipids

The Gram^{-} cell wall

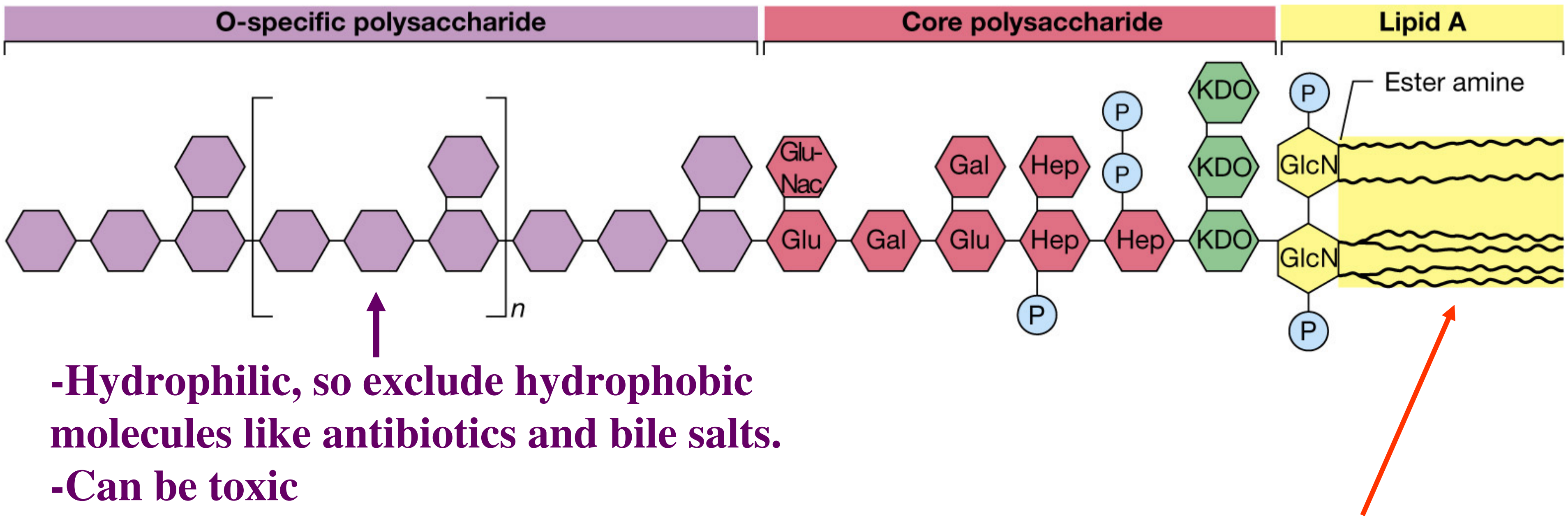
Cell Wall



Structure of the lipopolysaccharide of Gram-negative *Bacteria*



Lipopolysaccharide (LPS) of Gram - *Bacteria*
(precise chemical structure varies by species*)*



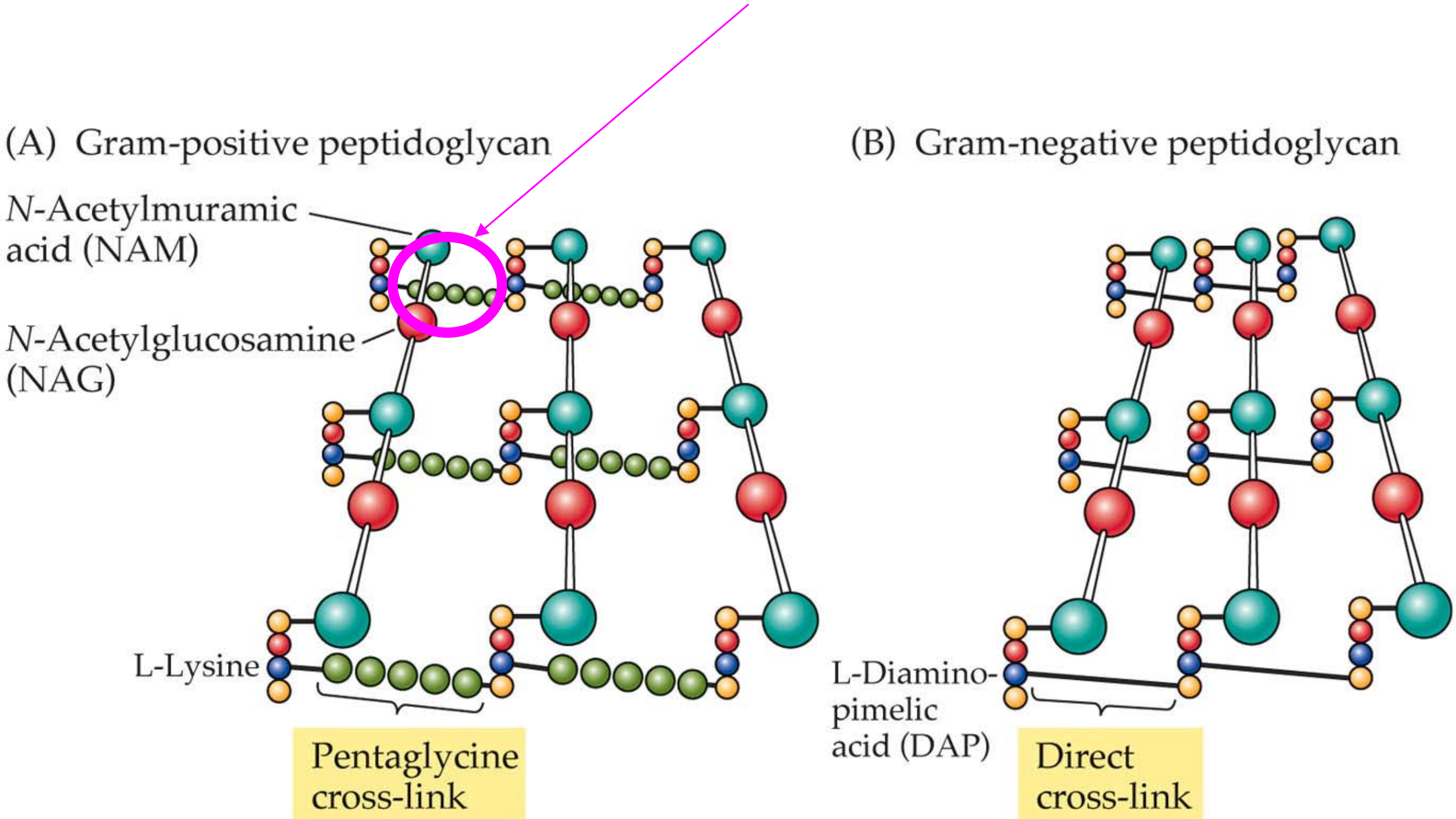
-Hydrophilic, so exclude hydrophobic molecules like antibiotics and bile salts.
-Can be toxic
-Serve as an "epitope" or "antigen" – the body raises antibodies to these, but VERY strain-specific.

-Embedded in lipid layer.

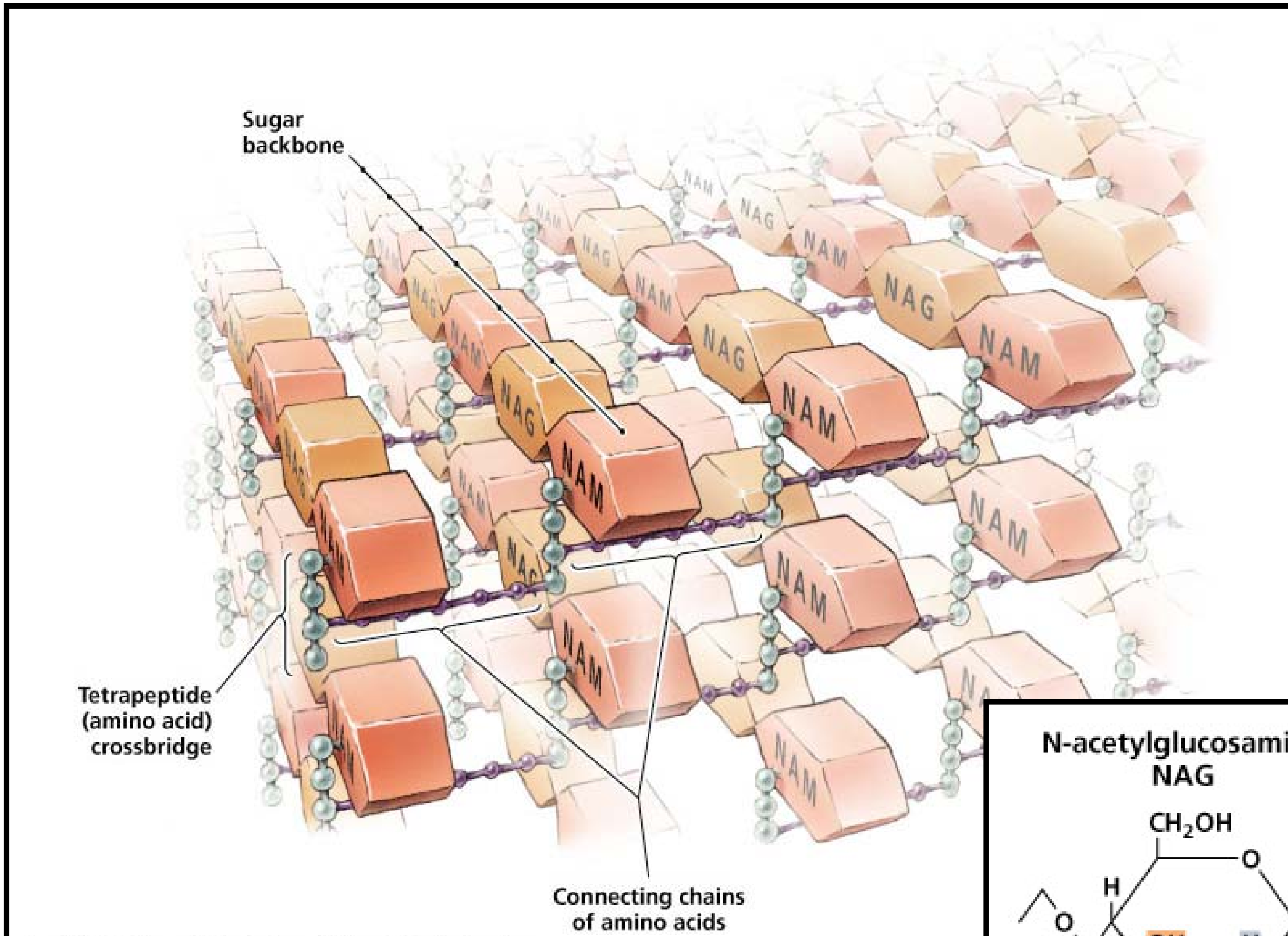
-"Endotoxin", causes fever and shock in mammals if released from membrane (when bacteria lyse)

Free (whole) LPS also triggers host defense by binding a receptor in macrophages

Comparing peptidoglycan of gram-positive and gram-negative bacteria



Peptidoglycan



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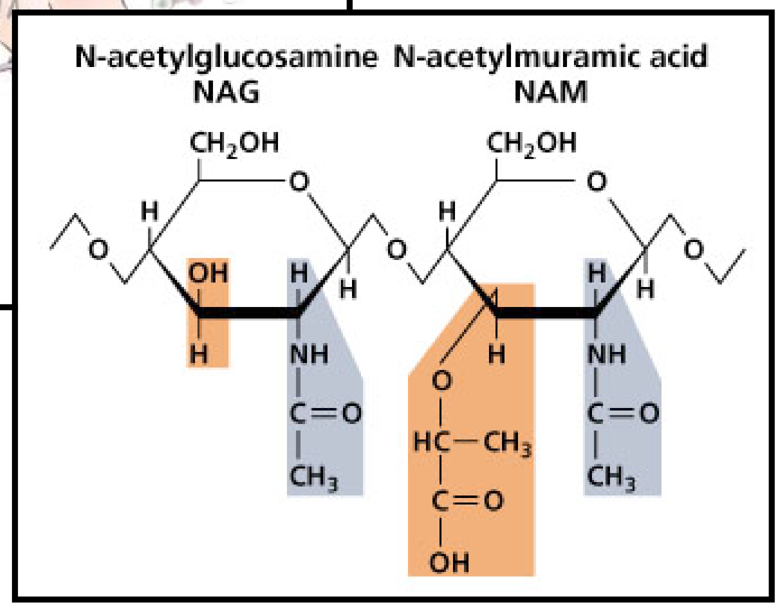
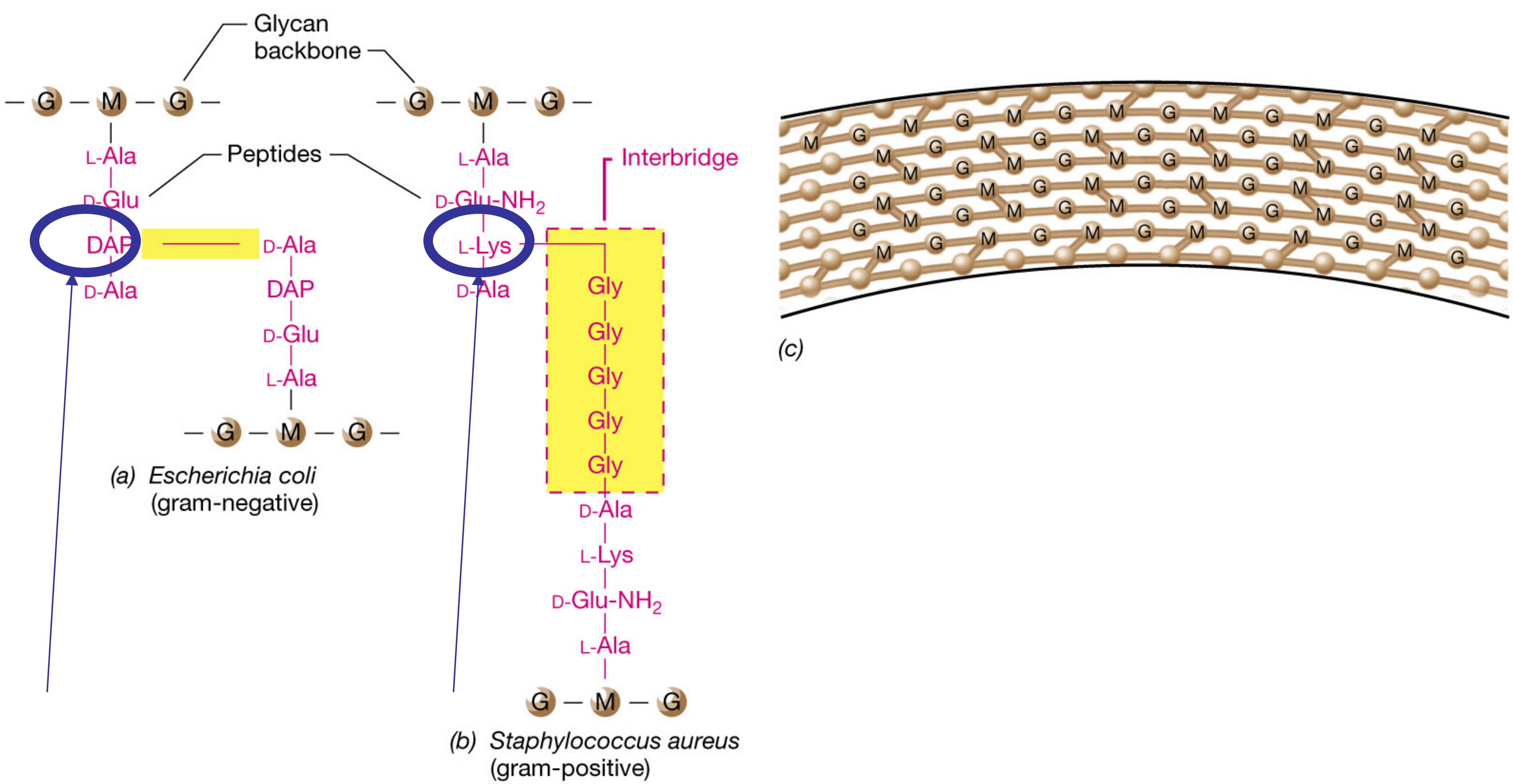


Constant: glycan (NAM and NAG), β-1,4 glycosidic linkages horizontally

Variable: type of peptide bridge, connects sheets vertically

Peptidoglycan: peptide bridge varies between G- and G+ cells.

DAP or lysine to alanine or glycine…

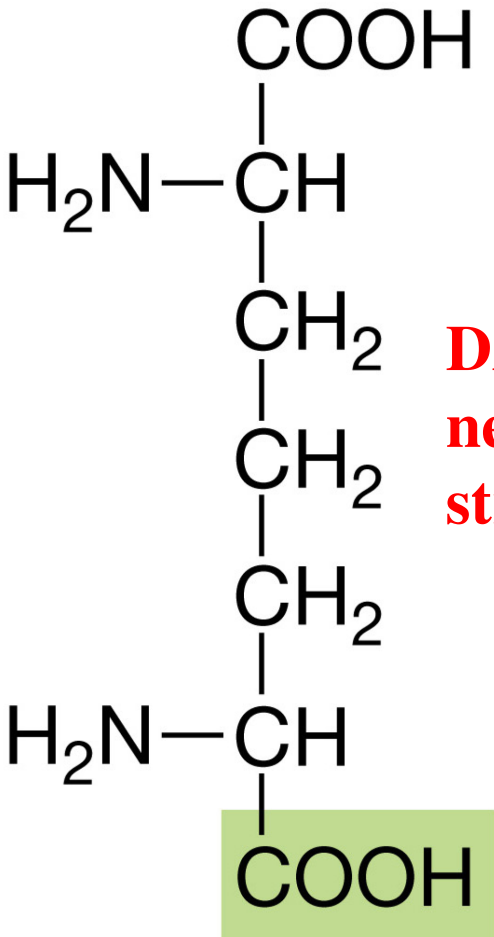


(a) *Escherichia coli* (gram-negative)

(b) *Staphylococcus aureus* (gram-positive)

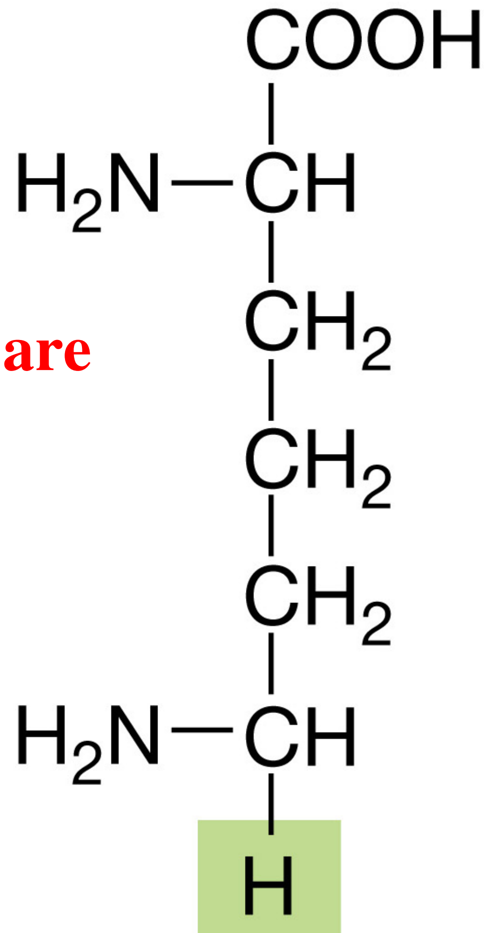
(c)

Comparing peptidoglycan of G$^+$ and G$^-$ bacteria



(a)

DAP and lysine are nearly the same structure.

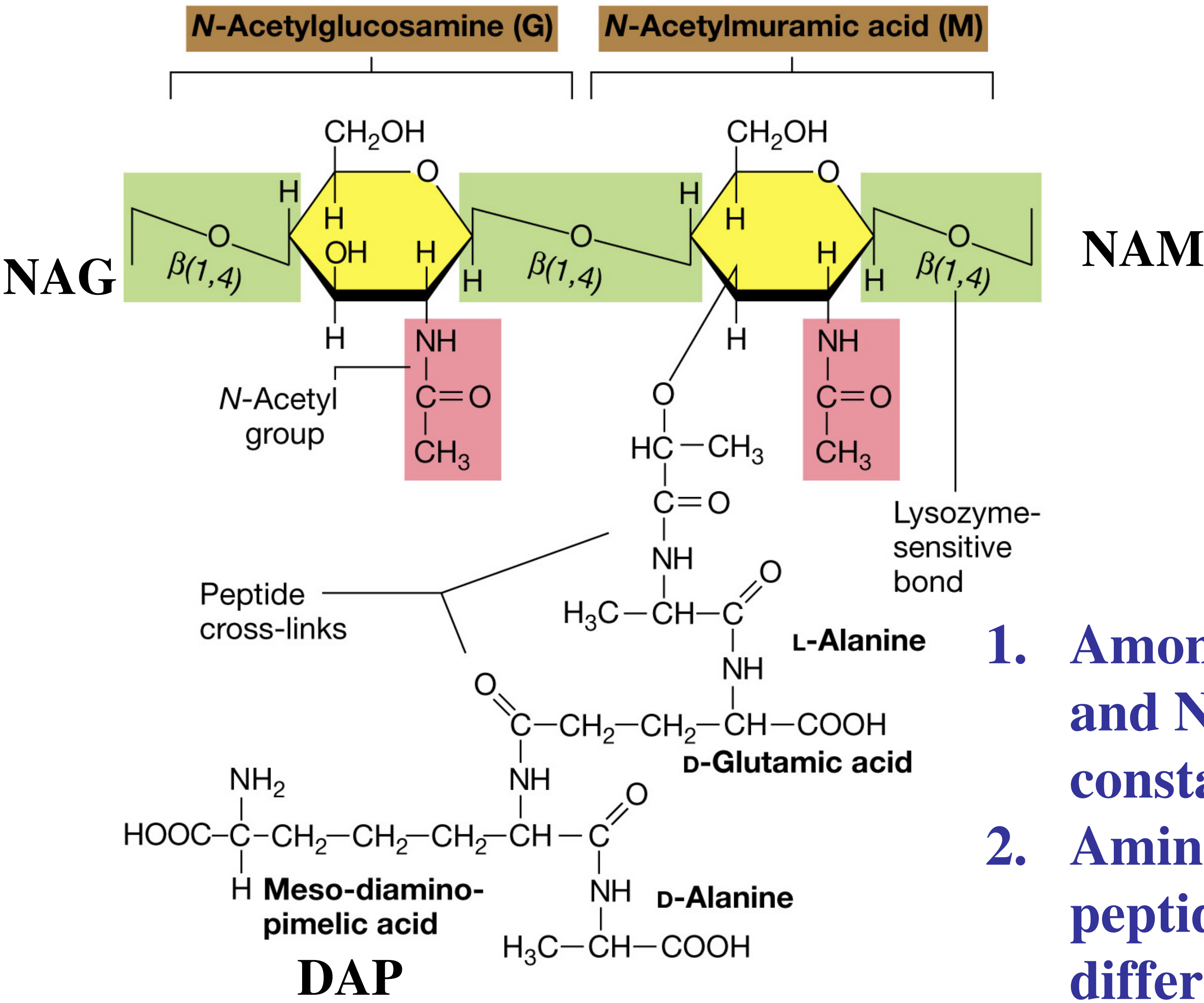


(b)

DAP or Diaminopimelic acid

Lysine

Structure of one of the repeating units of peptidoglycan

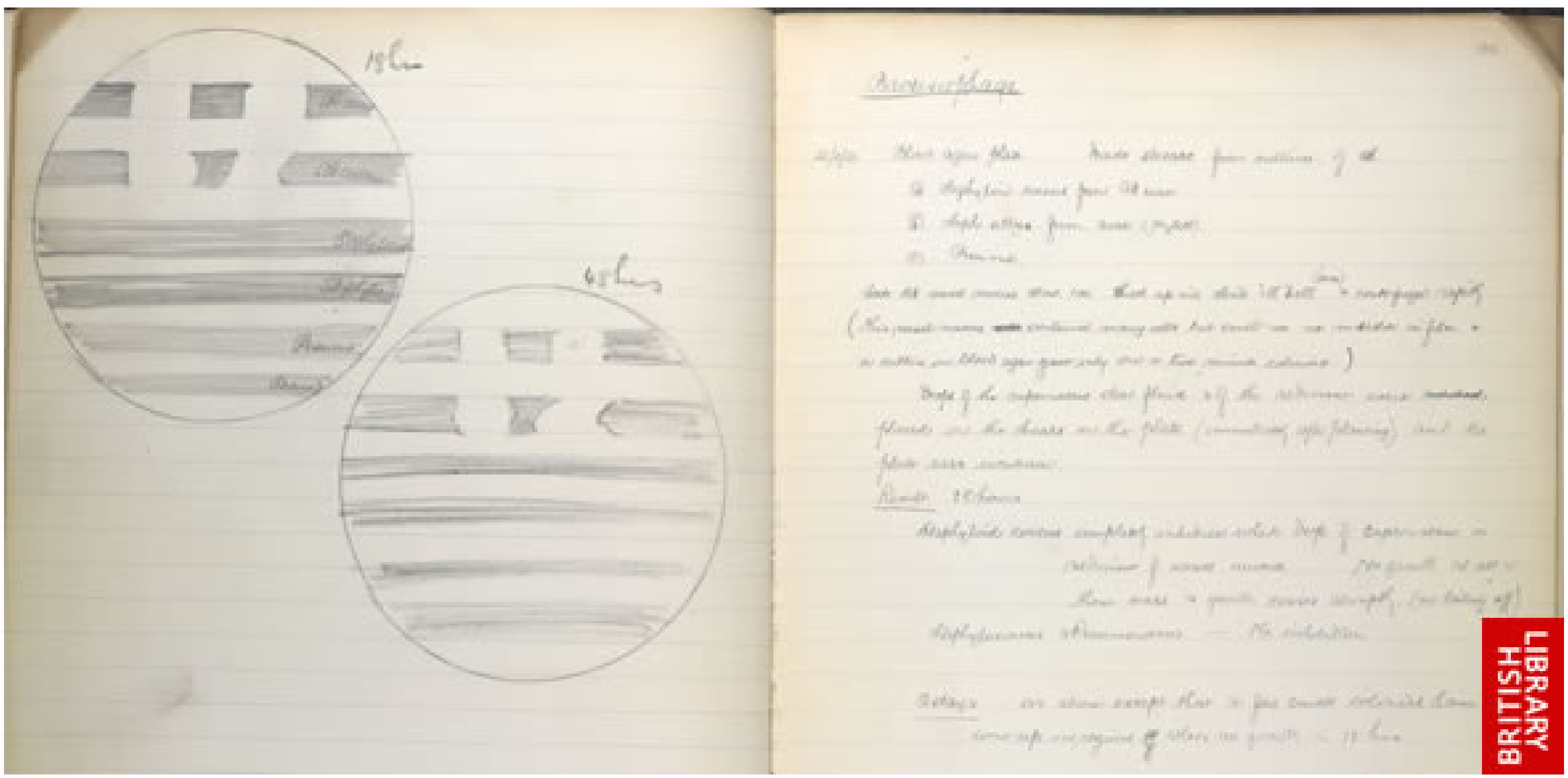


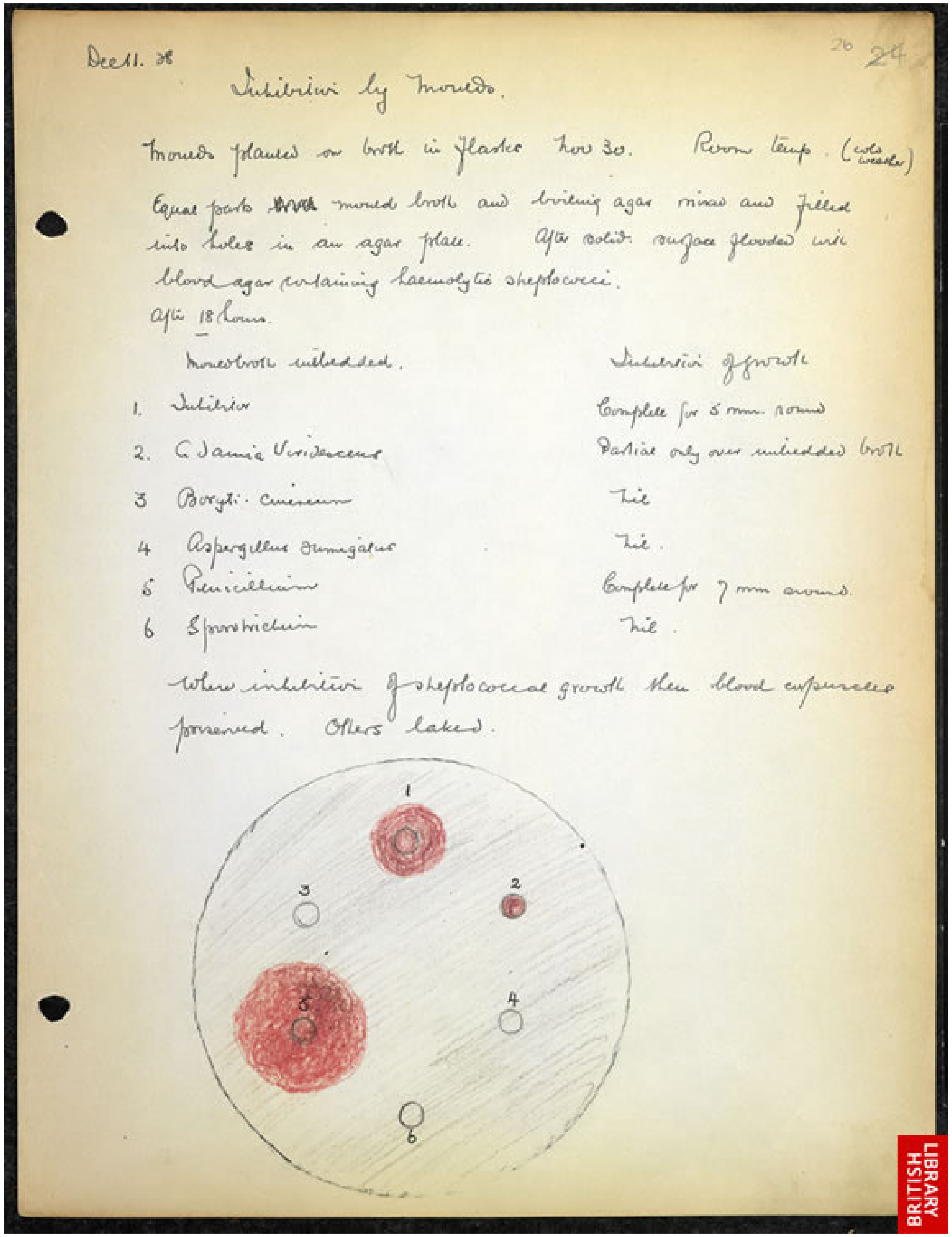
1. **Among Bacteria, NAG and NAM are constant.**
2. **Amino acids of the peptide portion can differ.**
3. **Why D-amino acids??**

Alexander Fleming served as a doctor in WWI, where he observed that gas gangrene and tetanus were caused by bacteria.

They grew deep in wounds (anaerobic) and couldn't be reached by antiseptics applied to the surface. He became curious about "chemotherapy" for microbial infections. After the war, as a researcher, he continued this interest.

After a bad cold, he curiously spread his nasal secretions on a petri plate… and discovered "lysozyme". This is an animal protein found in secretion that serves as a major defense against Bacteria.

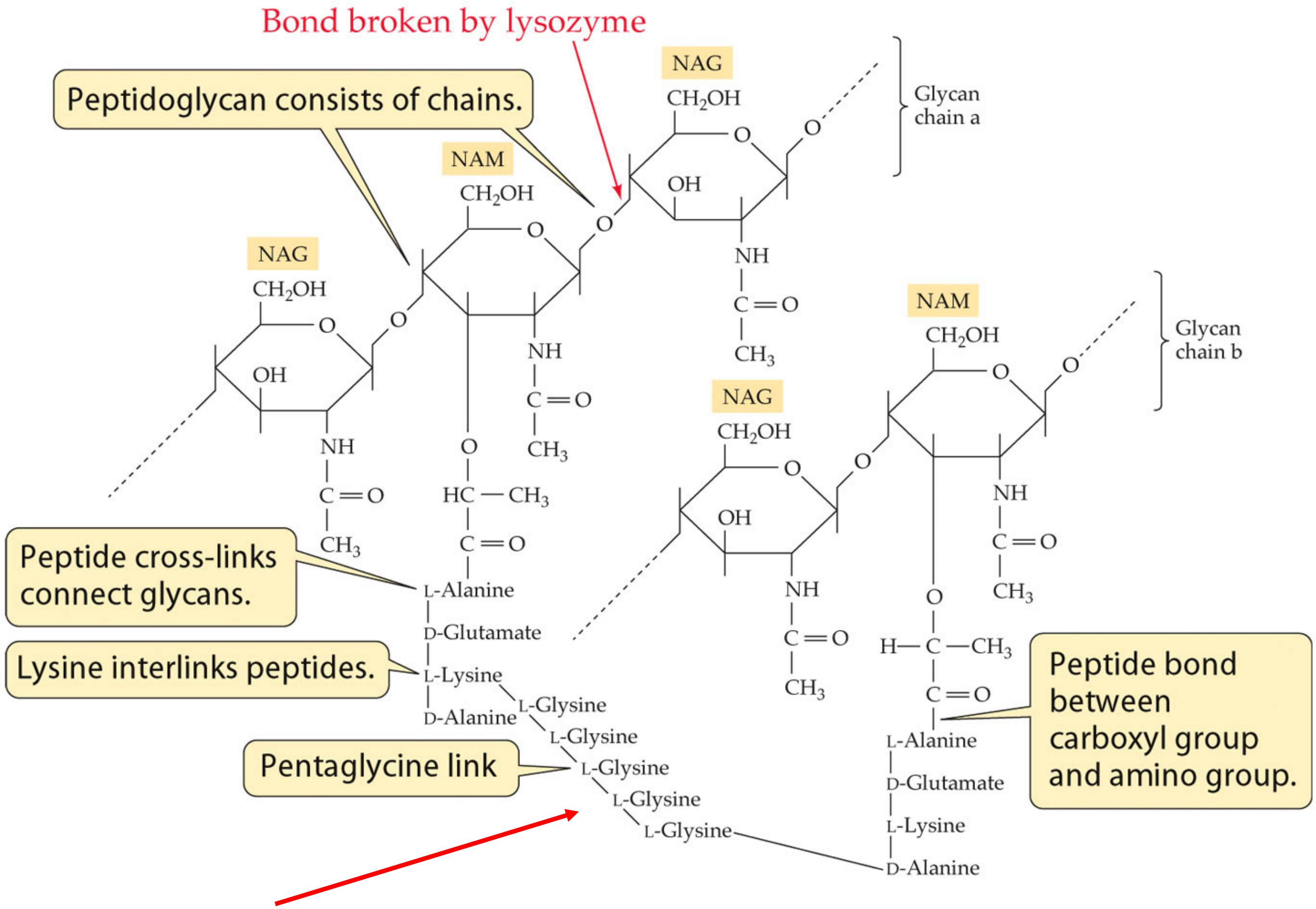




Seven years later, returning from vacation, he noted inhibition of Gram positive bacteria (*Micrococcus luteus*) on petri plates in the sink that had become contaminated with fungi.

He investigated further and realized that this was due to a water-soluble substance secreted by the fungus *Penicillium* (although not by other fungi) and which was antibacterial.

Peptidoglycan of a gram-positive bacterium



Pentaglycine crosslinking of tetrapeptides is prevented by penicillin

Was Fleming really smart, or did he (because chance favored his prepared mind) stumble upon what fungi and mammals found out long ago: that peptidoglycan is the "Achille's Heel" of Bacteria?

Why **is it the Achille's Heel??**

Chemical features of a "typical" bacterial cell (*E. coli*)

70-85% Water

TABLE 2.2 Chemical composition of a prokaryotic cell[a]

Molecule	Percent of dry weight[b]	Molecules per cell	Different kinds
Total macromolecules	96	24,610,000	~2500
Protein	55	2,350,000	~1850
Polysaccharide	5	4,300	2[c]
Lipid	9.1	22,000,000	4[d]
Lipopolysaccharide	3.4	1,430,000	1
DNA	3.1	2.1	1
RNA	20.5	255,500	~660
Total monomers	3.0		~350
Amino acids and precursors	0.5		~100
Sugars and precursors	2		~50
Nucleotides and precursors	0.5		~200
Inorganic ions	1		18
Total	100%		

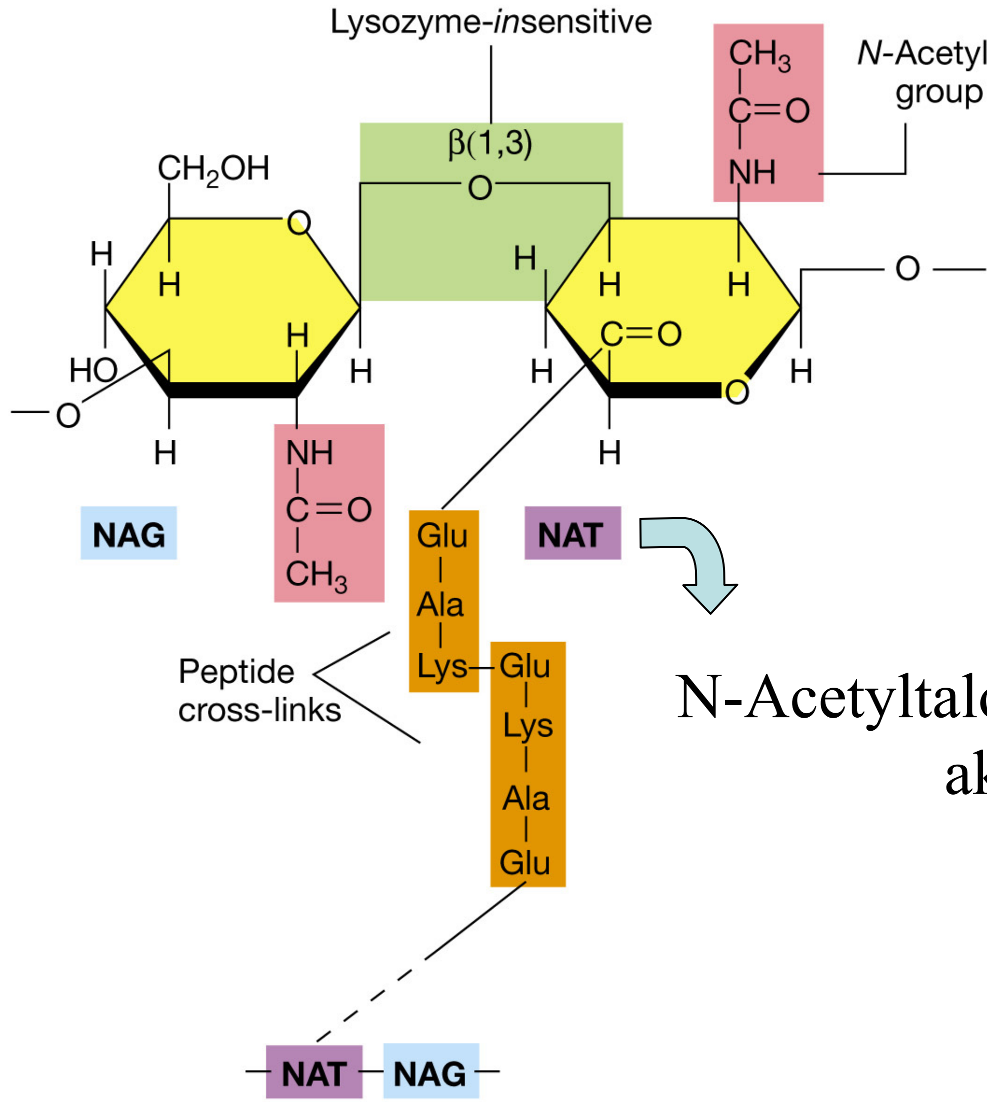
a Data from Neidhardt, F. C., et al. (eds.), 1996. *Escherichia coli* and *Salmonella typhimurium—Cellular and Molecular Biology*, 2nd edition. American Society for Microbiology, Washington, DC.

b Dry weight of an actively growing cell of *E. coli* $\cong 2.8 \times 10^{-13}$ g; total weight (70% water) $= 9.5 \times 10^{-13}$ g.

c Assuming peptidoglycan and glycogen to be the major polysaccharides present. Glycogen = glucose polymer

d There are several classes of phospholipids, each of which exists in many kinds because of variability in fatty acid composition between species and because of different growth conditions.

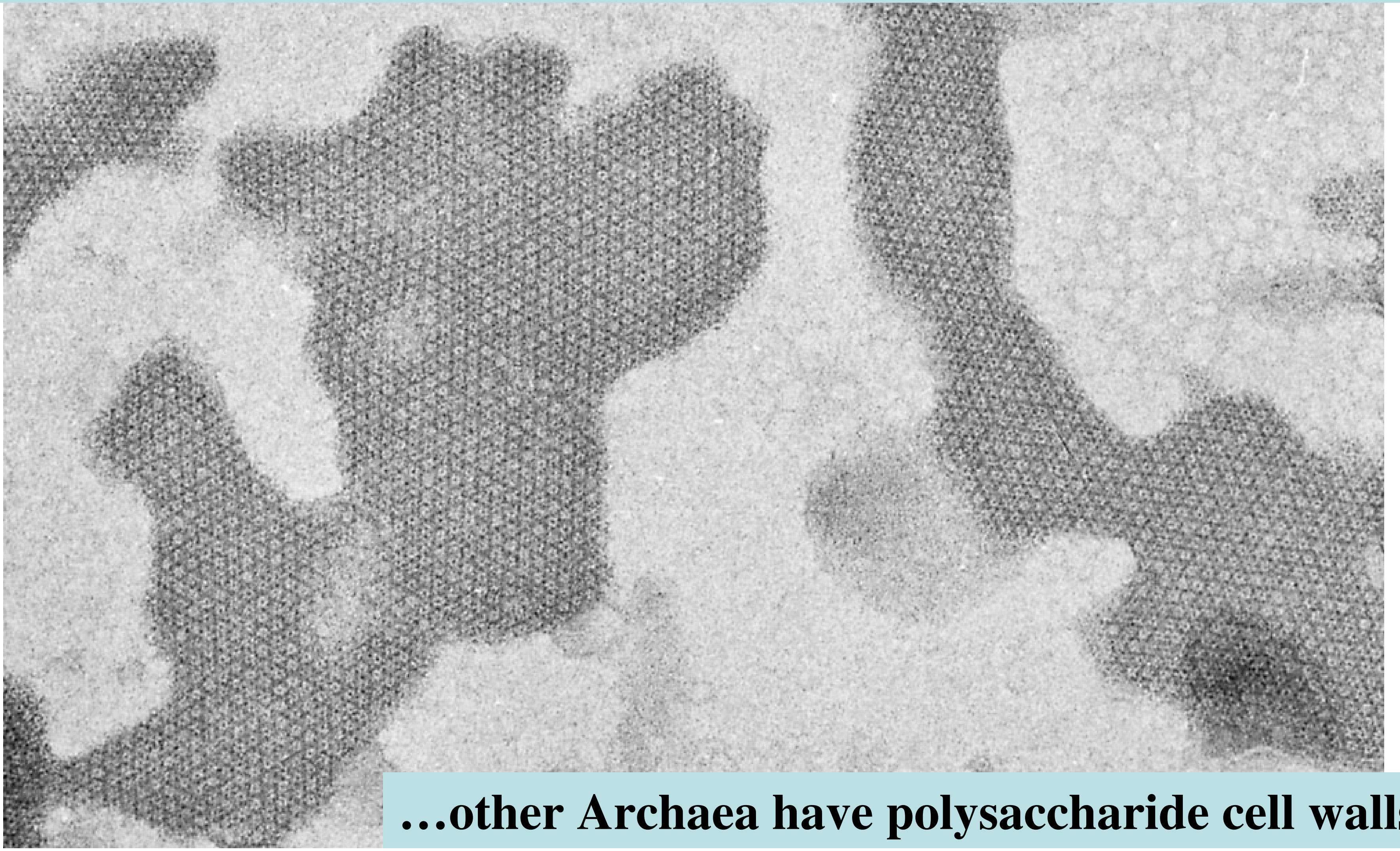
Cell walls of *Archaea:* some (not all) have pseudopeptidoglycan



N-Acetyltalosaminuronic acid
aka NAT

Cell walls of *Archaea:*

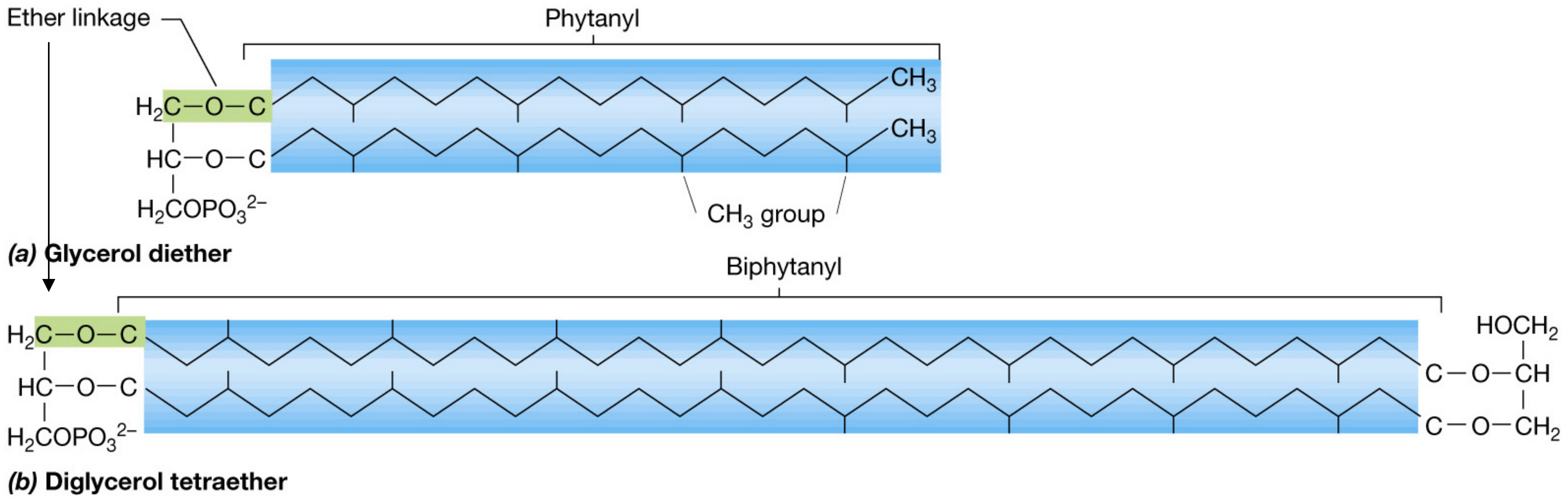
Some *Archaea* & *Bacteria* have a protein jacket outside the membrane called the "paracrystalline surface layer", or S-layer. The S-layer sometimes serves as cell wall for *Archaea.*



…other Archaea have polysaccharide cell walls

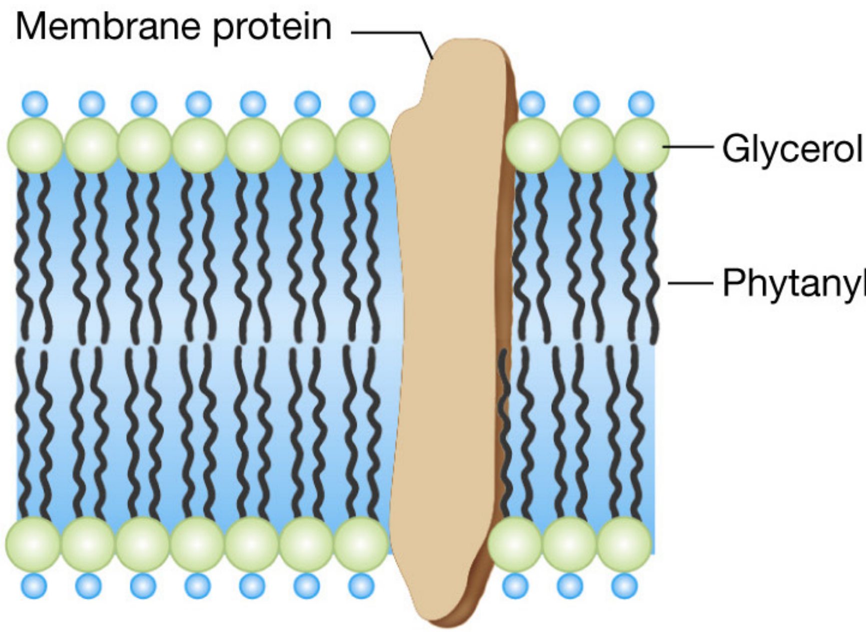
S-layers function as a **selective sieve**, allowing the passage of low-molecular-weight substances while excluding large molecules and structures.

Membranes of *Archaea:* structure of major lipids found in archaeal membranes

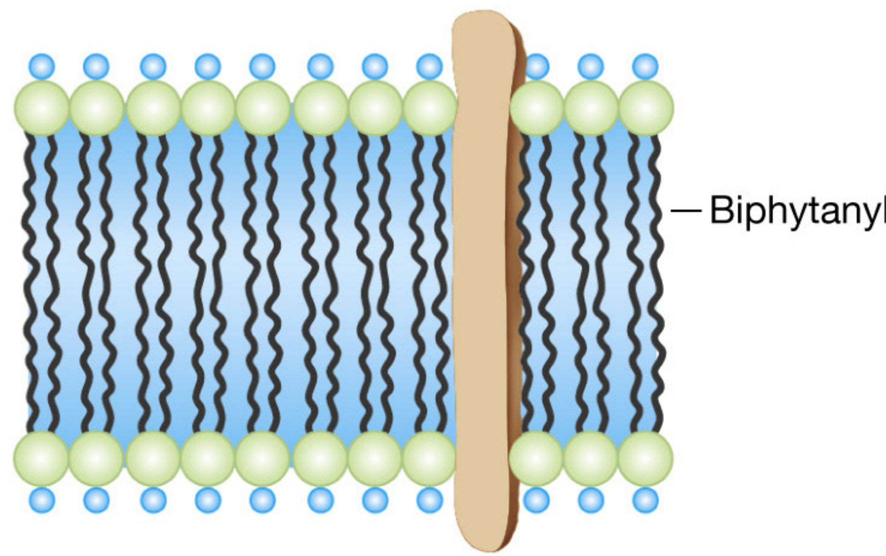


Lipid monolayer,
not a bilayer:
mostly found in
hyperthermophiles

Membranes of *Archaea:*
structure of major lipids found in archaeal membranes



***(c)* Lipid bilayer**

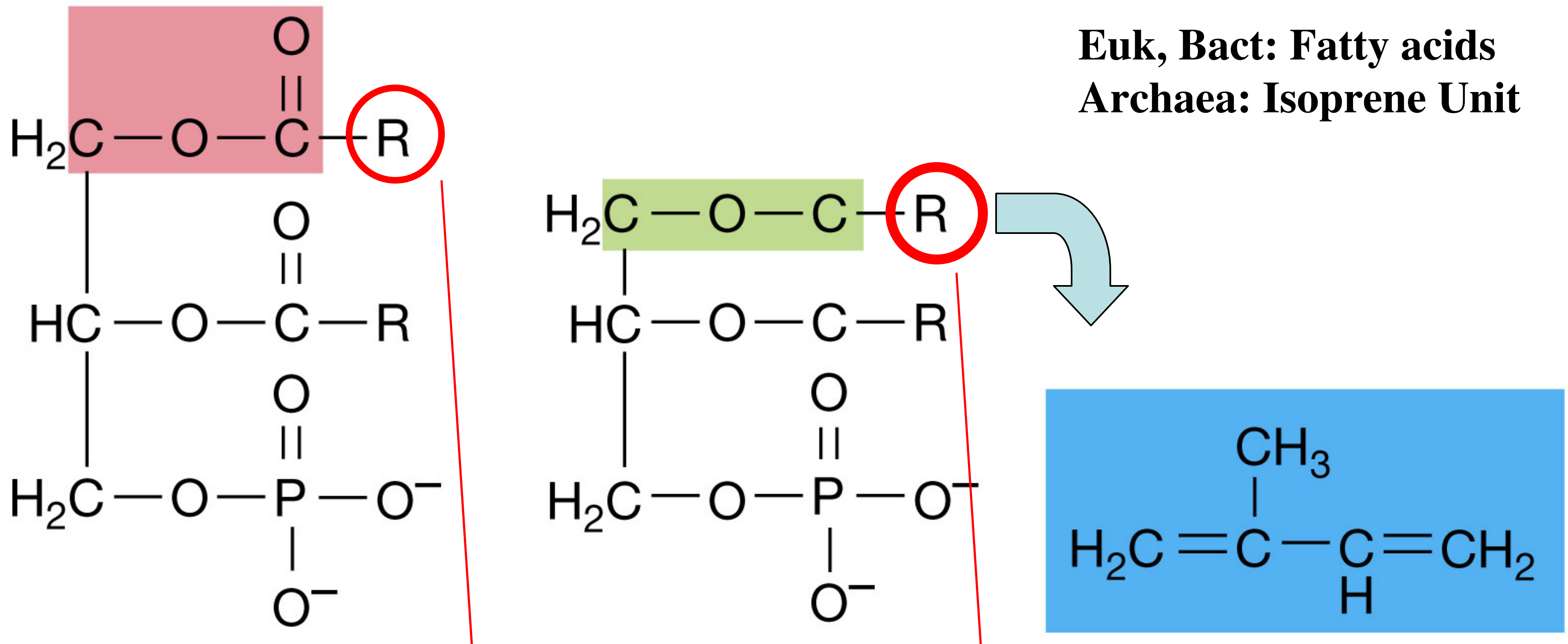


***(d)* Lipid monolayer**

Eukaryotes and Bacteria:
Ester Linkage

Archaea:
Ether Linkage

Euk, Bact: Fatty acids
Archaea: Isoprene Unit



(a) *(b)* *(c)*

Fatty Acid

Isoprene polymer

Classification of prokaryotic cellular features: Variant (or NOT common to all)

-Cell wall (chemistry varies; some don't have one)

-Endospores (heavy-duty life support strategy)

-Glycocalyx: capsules or slime layer (exterior to cell wall)
-Bacterial Flagellae and Injectisomes
-Fimbriae and Pili
-Inclusion Bodies (granules for storage)
-Gas vesicles (vertical movement in liquid medium)

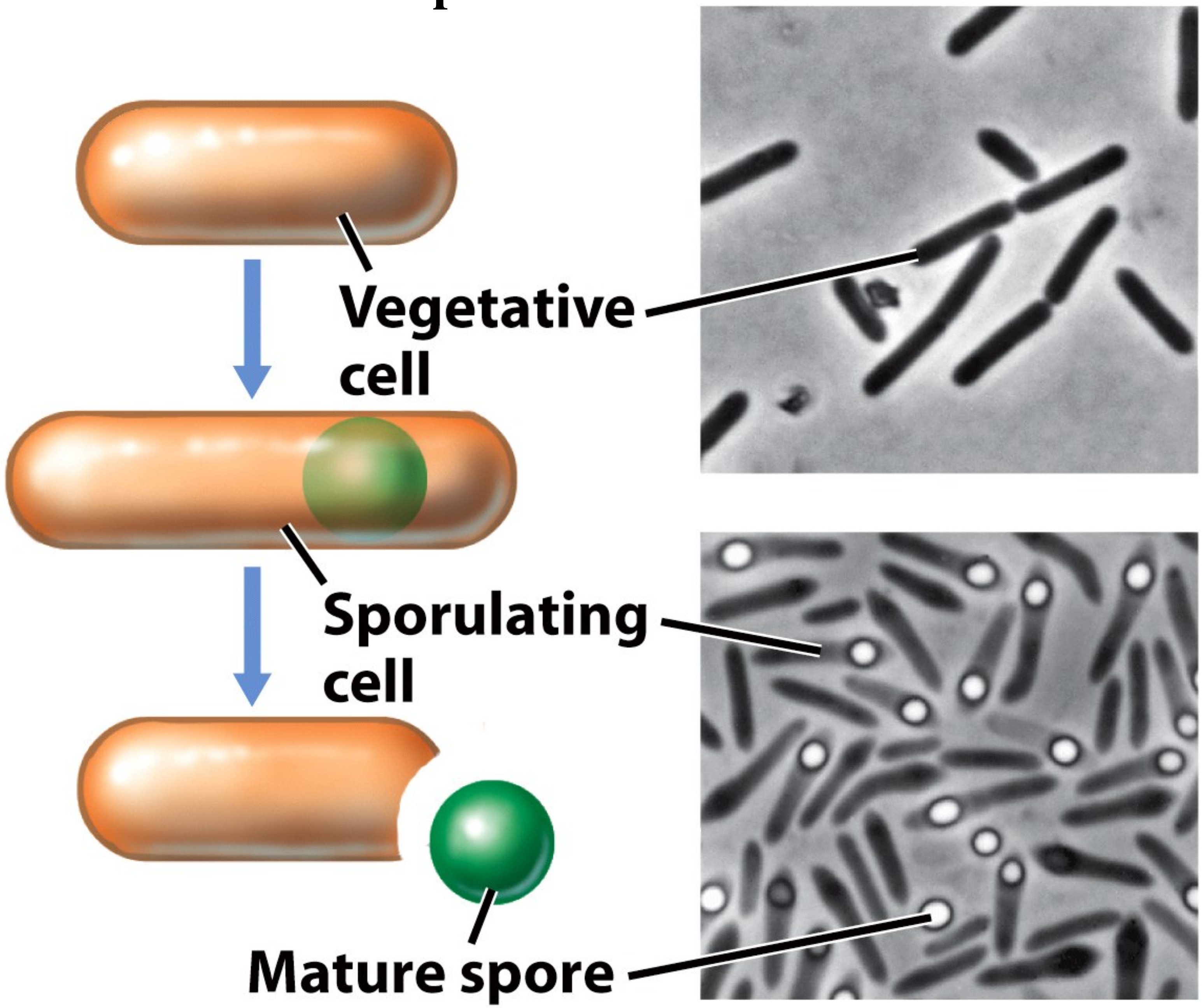
Spores remain viable in the environment after long periods of dormancy.

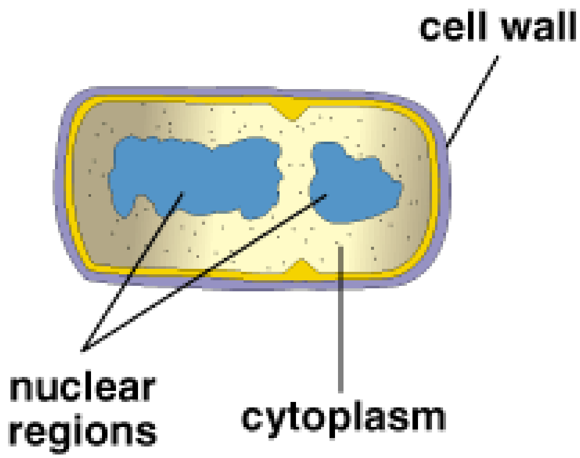
Extreme reports of endospore revival:

1. Bacillus sphaericus **found in the guts of bees preserved in 40 million year old Dominican amber**

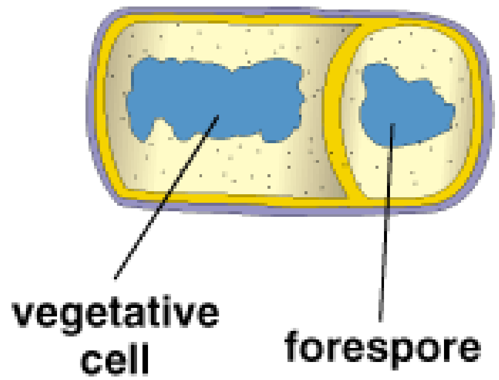
2. Virgibacillus **spp. found in salt crystals in the 250 million year old Salado Formation in New Mexico**

Formation of the endospore

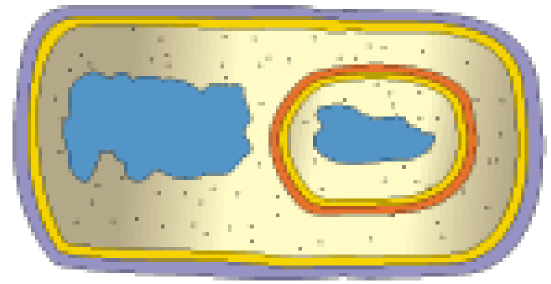




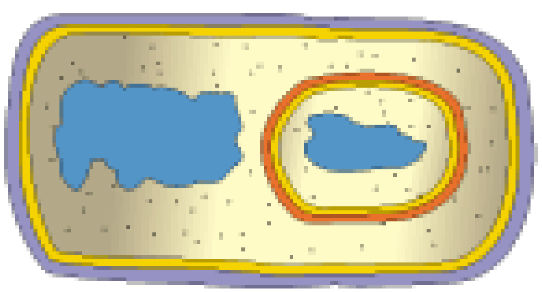
a. The bacterium undergoes unequal division.



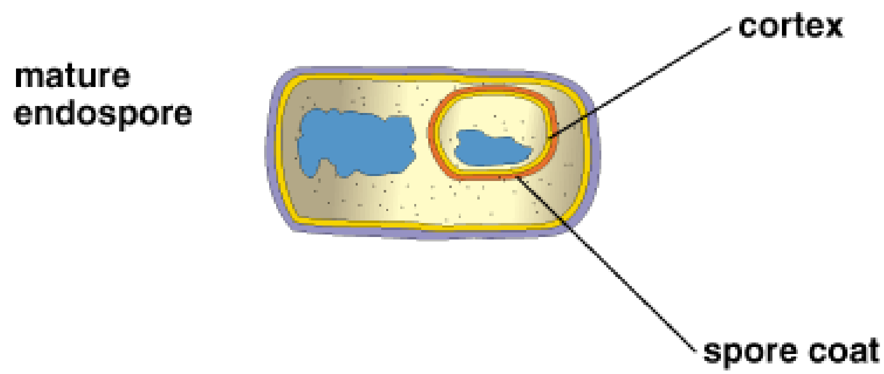
b. It forms a forespore.



Mother cell phagocytoses forespore, forming double membrane. Peptidoglycan layers form between membranes.



d. The forespore matures into an endospore by using materials from the surrounding vegetative portion to build a protective wall.



e. The wall is composed of an inner cortex and an outer spore coat. The endospore also dehydrates during maturation.

Endospores are a highly resistant differentiated bacterial cell produced by certain gram-positive *Bacteria*.

-mostly soil bacteria of phyum Firmiculites (evolved just once)

-most common in *Clostridium*, *Bacillus*

-agents of **survival**

-metabolically inert, highly dehydrated (10-15% water)

-most resistant biological structure known: heat up to 150^{o}C, dryness, UV, strong acids, disinfectants

-can survive 100's (thousands? millions? *) of years

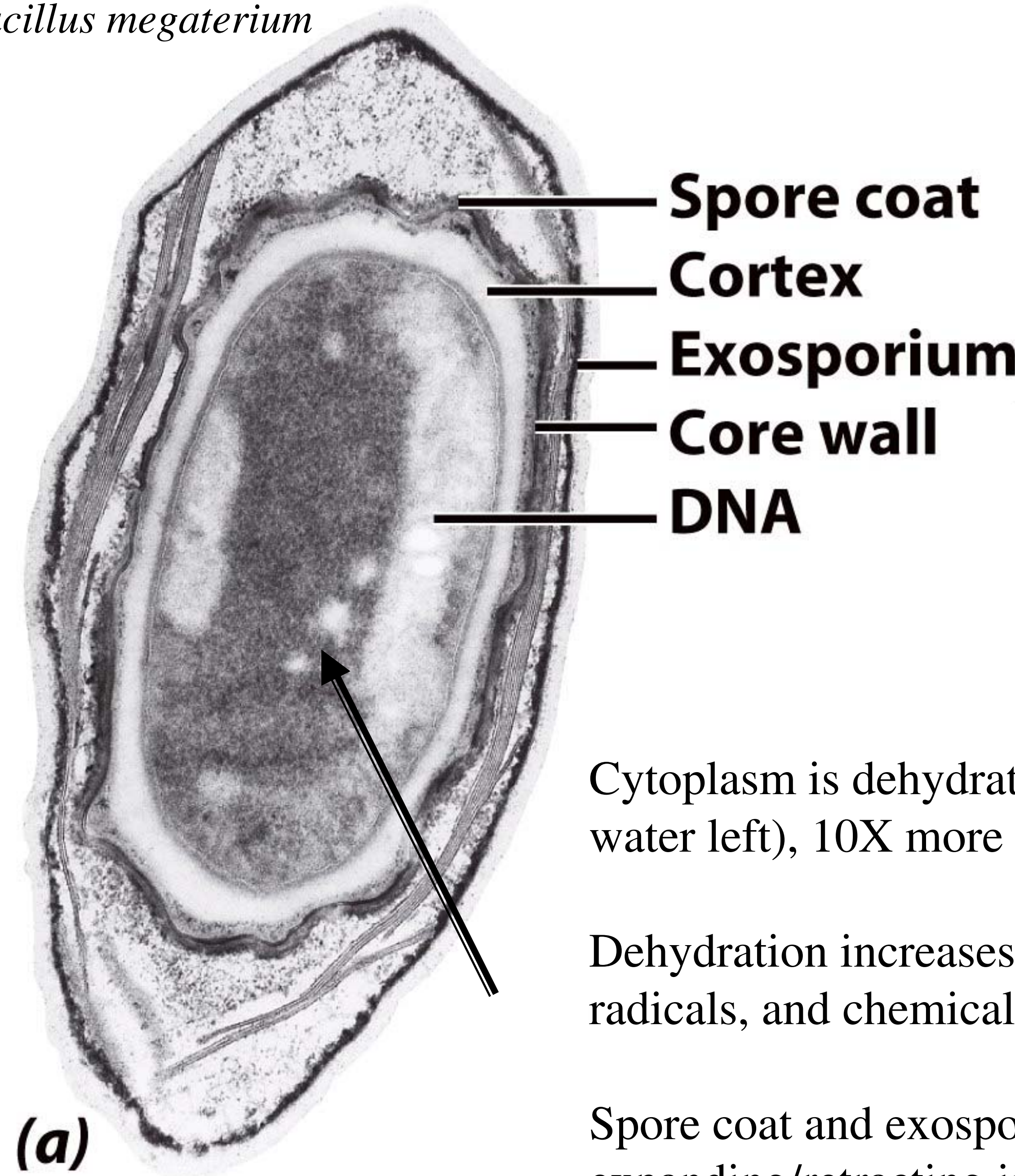
Exospores are formed by pinching off of tips of filamentous bacteria (and of fungi)

-*Streptomyces*, *Myxobacteria*

-agents of **dispersal**

* Text, p. 88

Bacillus megaterium



2. Spore-specific proteins

4. Thick, loose peptidoglycan

1. Thin protein coating

3. Thin peptidoglycan surrounding spore protoplast

Cytoplasm is dehydrated (only 10-25% of original water left), 10X more acidic than cell, and gel-like.

Dehydration increases resistance to heat, free radicals, and chemicals and inactivates enzymes.

Spore coat and exosporium are structurally flexible, expanding/retracting in response to humidity

Table 4.3 **Differences between endospores and vegetative cells**

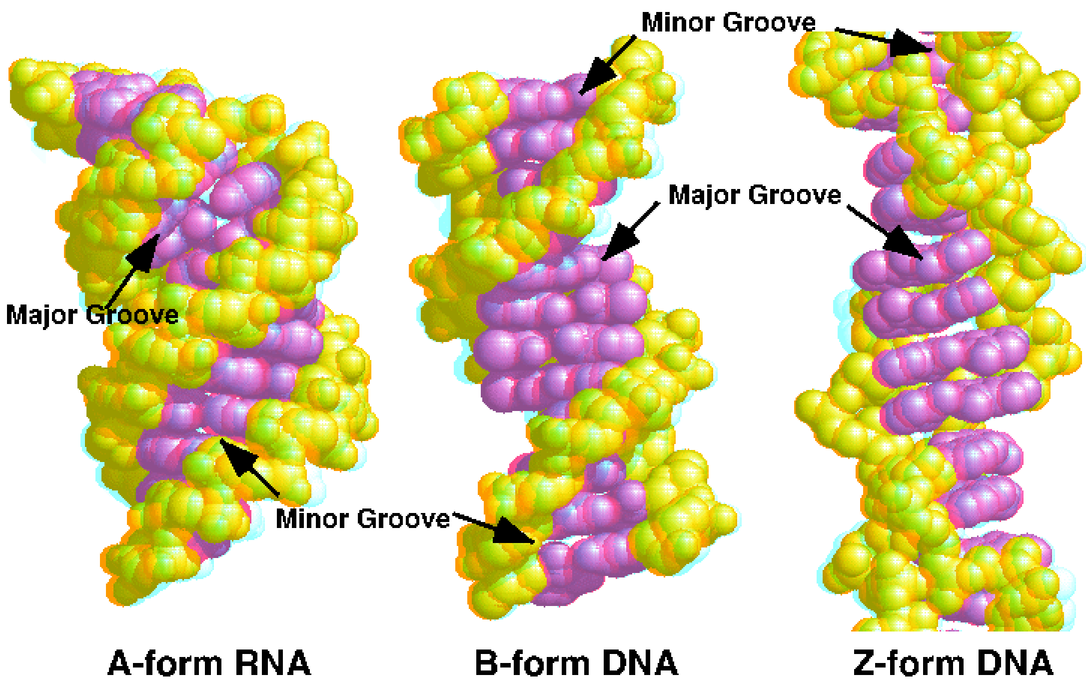
Characteristic	Vegetative cell	Endospore
Structure	Typical gram-positive cell; a few gram-negative cells	Thick spore cortex Spore coat Exosporium
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Metabolism (O_2 uptake)	High	Low or absent
Macromolecular synthesis	Present	Absent
mRNA	Present	Low or absent
Heat resistance	Low	High
Radiation resistance	Low	High
Resistance to chemicals (for example, H_2O_2) and acids	Low	High
Stainability by dyes	Stainable	Stainable only with special methods
Action of lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble proteins (product of *ssp* genes)	Absent	Present
Cytoplasmic pH	About pH 7	About pH 5.5–6.0 (in core)

Melanin

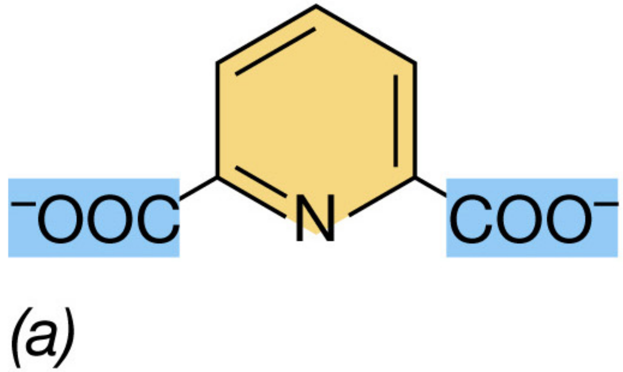
SOD,catalase

Spore coat protects PG

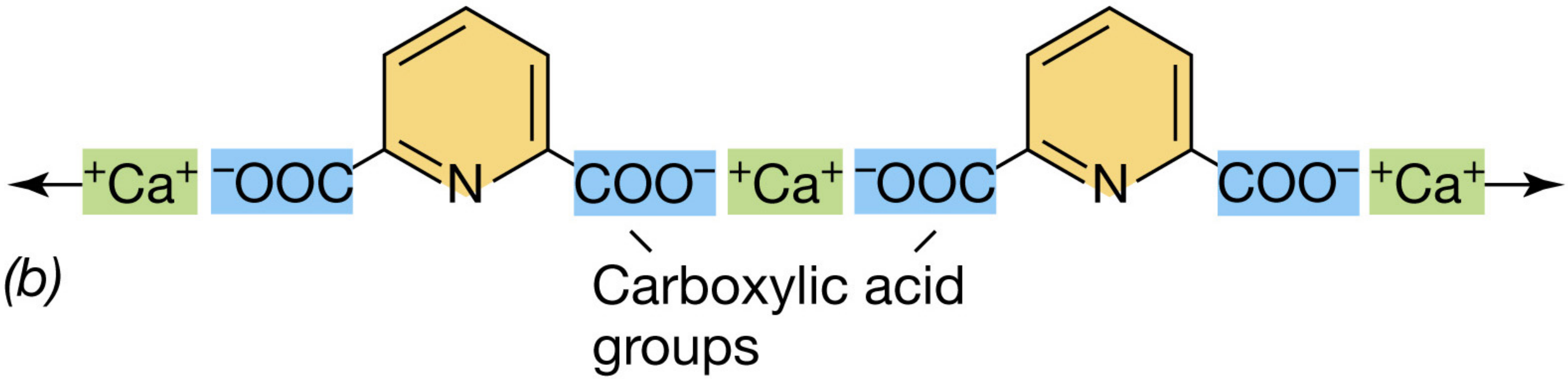
Bind and protect DNA; change it from normal B form to compact and resistant A form.



Minor Groove
Major Groove
Major Groove
Minor Groove
A-form RNA
B-form DNA
Z-form DNA



(a) Structure of Dipicolinic Acid



(b) Crosslinked with Ca^{++}

-10% of dry weight of endospore
-Found in all endospores regardless of species
-Reduces water availability
-Intercalates into DNA and stabilizes against heat denaturation

Classification of prokaryotic cellular features: Variant (or NOT common to all)

-Cell wall (chemistry varies; some don't have one)
-Endospores (heavy-duty life support strategy)

-Glycocalyx: capsules or slime layer (exterior to cell wall)

-Bacterial Flagellae and Injectisomes
-Fimbriae and Pili
-Inclusion Bodies (granules for storage)
-Gas vesicles (vertical movement in liquid medium)

Prokaryotes may contain cell surface layers composed of any of these:

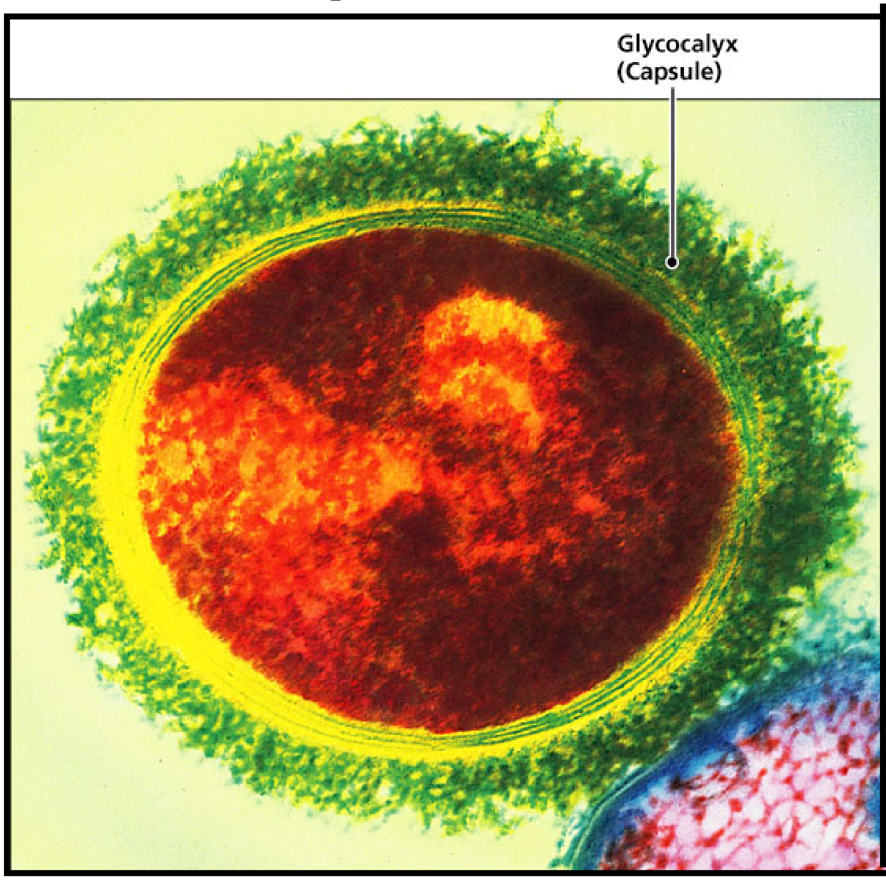
-a two-dimensional array of protein called an **S-layer**

-polysaccharide **capsules**

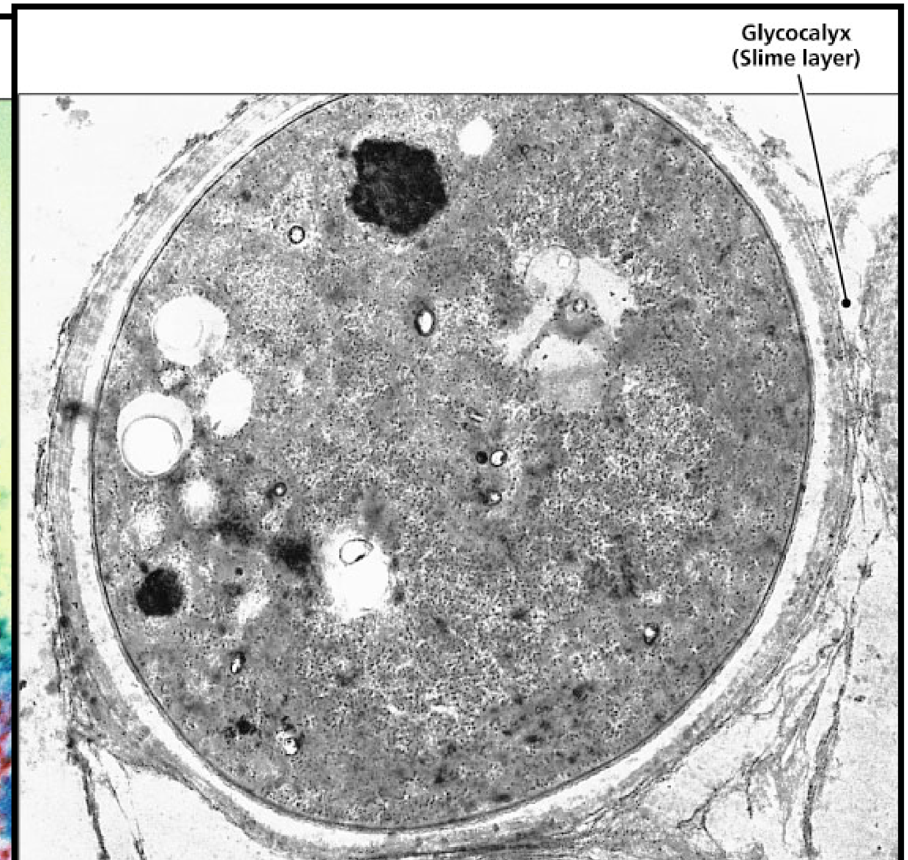
-a more diffuse polysaccharide matrix (**slime layer**).

Glycocalyx

S. pneumonia



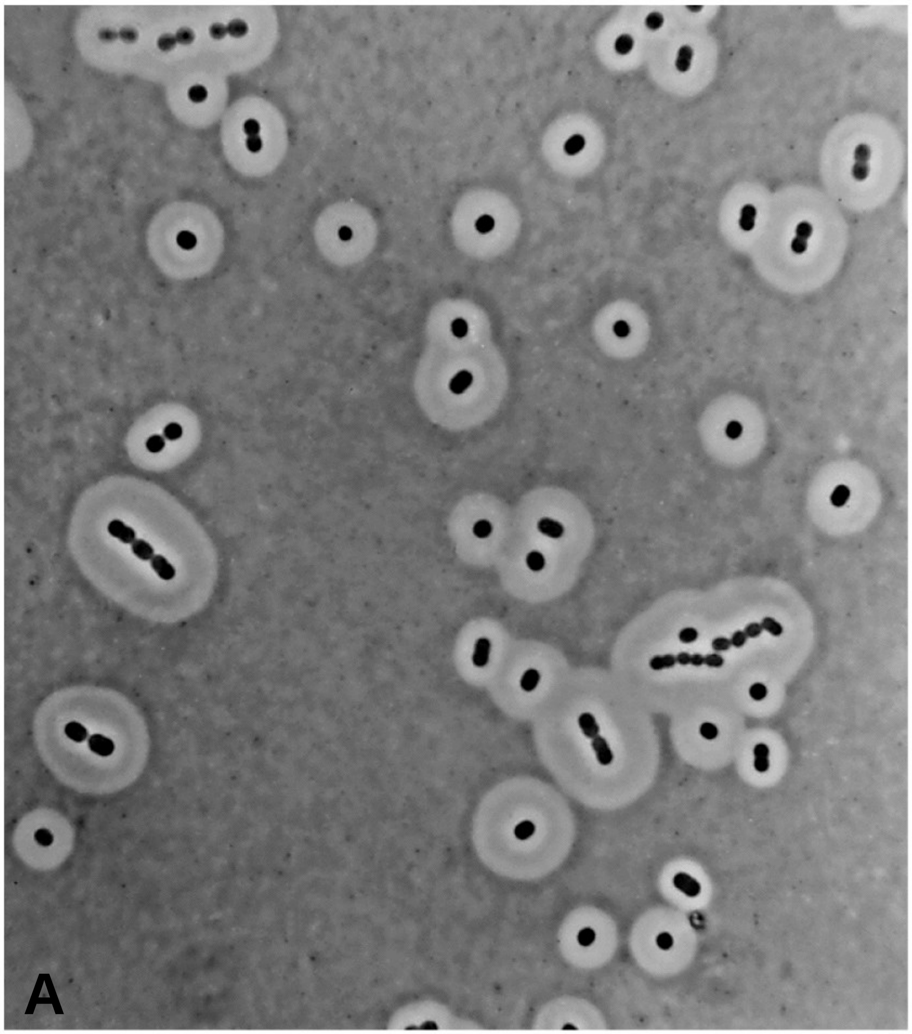
Bacteroides



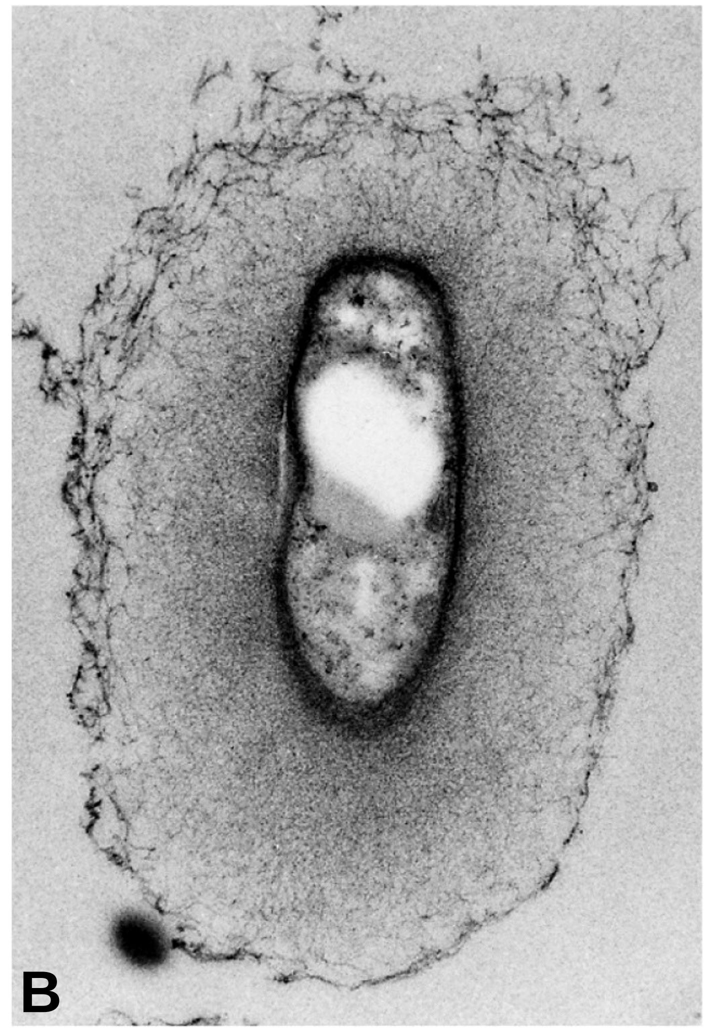
- Sugar & protein coating
- Tight - **capsule**
- Loose - **slime layer**
- Sticky
- Immune evasion

Bacterial Capsules:

(a) *Acinetobacter* sp.



(b) *Rhizobium trifolii*



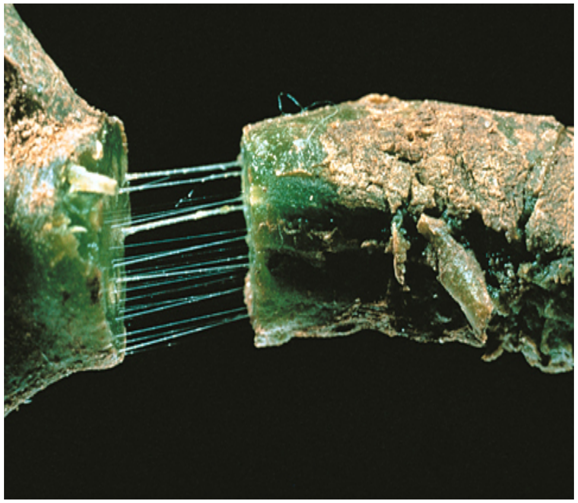
negative stain

Glycocalyx (slime) is a virulence factor in a Select Agent

Ralstonia solanacearum, causal agent of Bacterial Wilt of Tomato

Serious agricultural threat that quickly wipes out crops; for decades confined to "warm regions" of the world but becoming prevalent in temperature climates

Wilting, stunting, discolored vascular tissue, ooze from surfaced cut stems



Classification of prokaryotic cellular features: Variant (or NOT common to all)

-Cell wall (chemistry varies; some don’t have one)
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-Bacterial Flagellae and Injectisomes

-Fimbriae and Pili
-Inclusion Bodies (granules for storage)
-Gas vesicles (vertical movement in liquid medium)

Flagellar Motility

• Motility in most microorganisms is due to flagella.

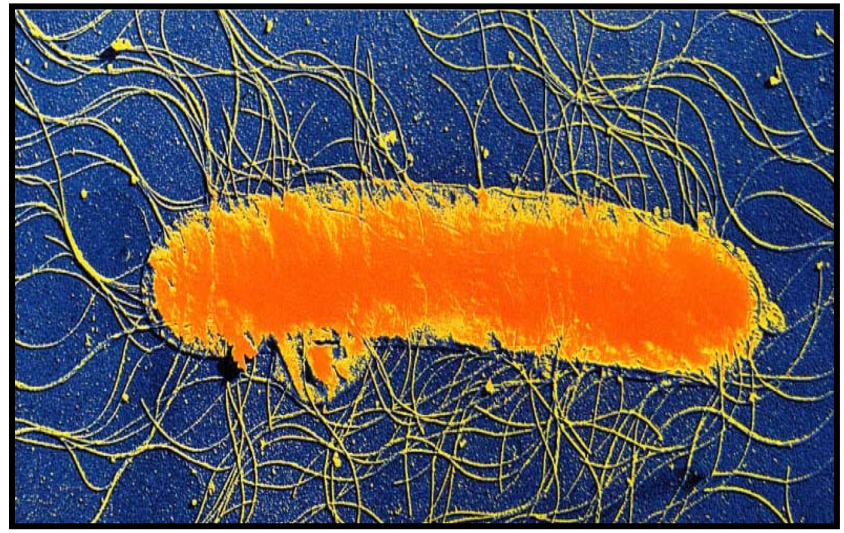
• In prokaryotes the flagellum is a complex structure made of several proteins, most of which are anchored in the cell wall and cytoplasmic membrane.

•The flagellum filament:

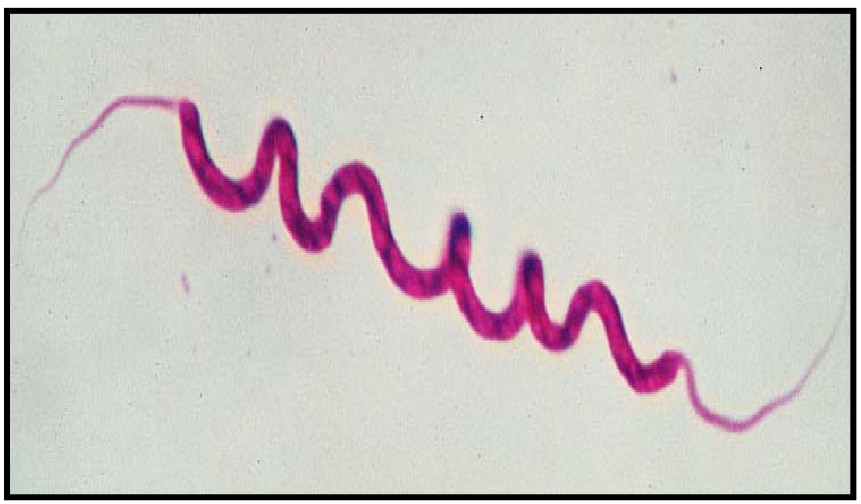
- -is made of a single kind of protein (flagellin)
- -rotates at the expense of the proton motive force (which drives the flagellar motor).

Flagella

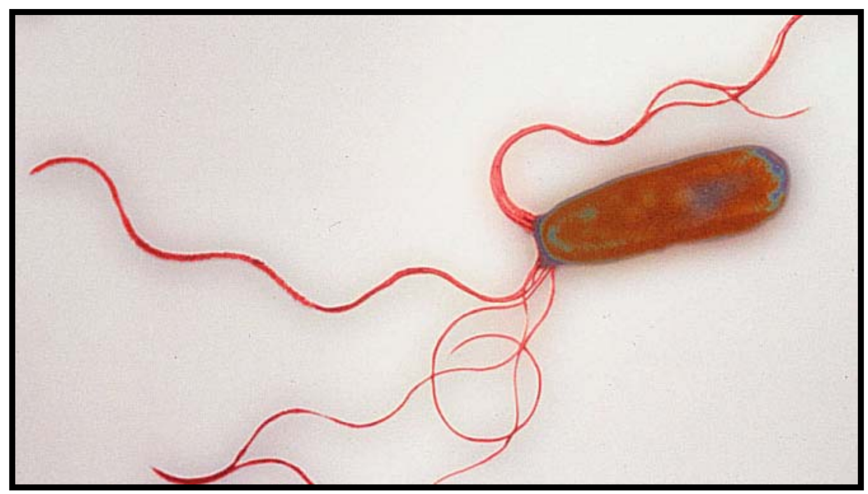
•Monotrichous



•Peritrichous

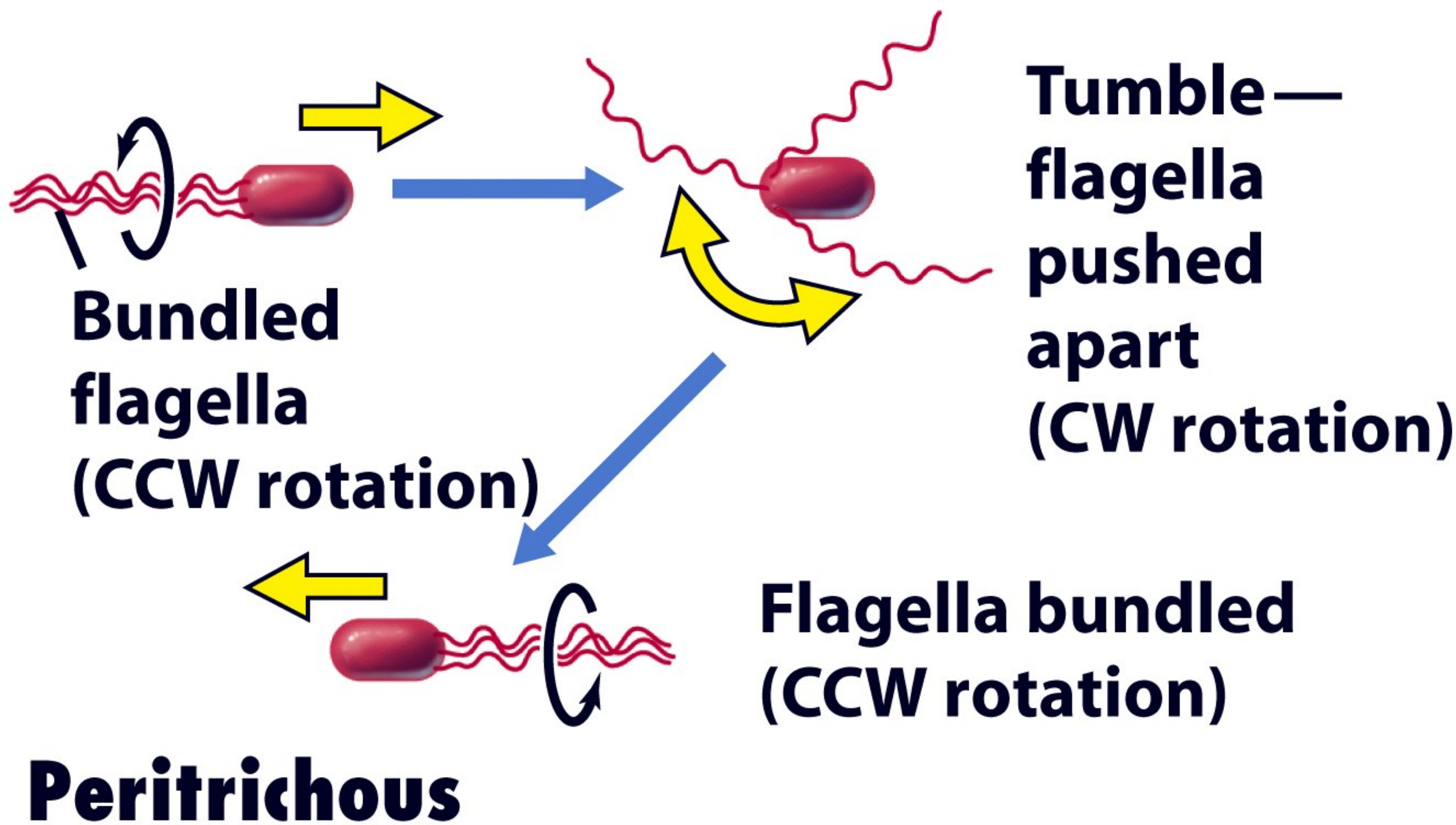


•Amphitrichous



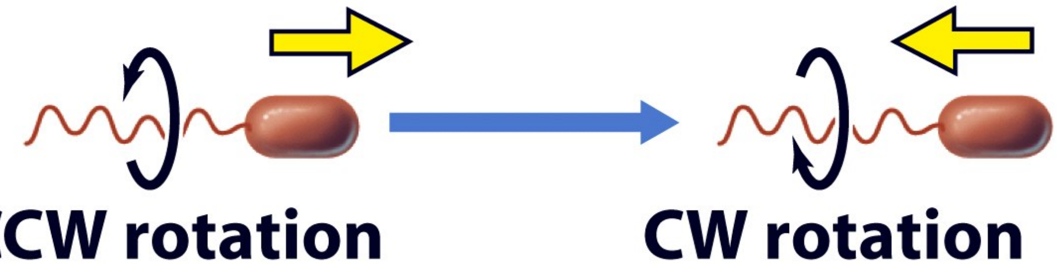
•Lophotrichous

Flagellar Motility

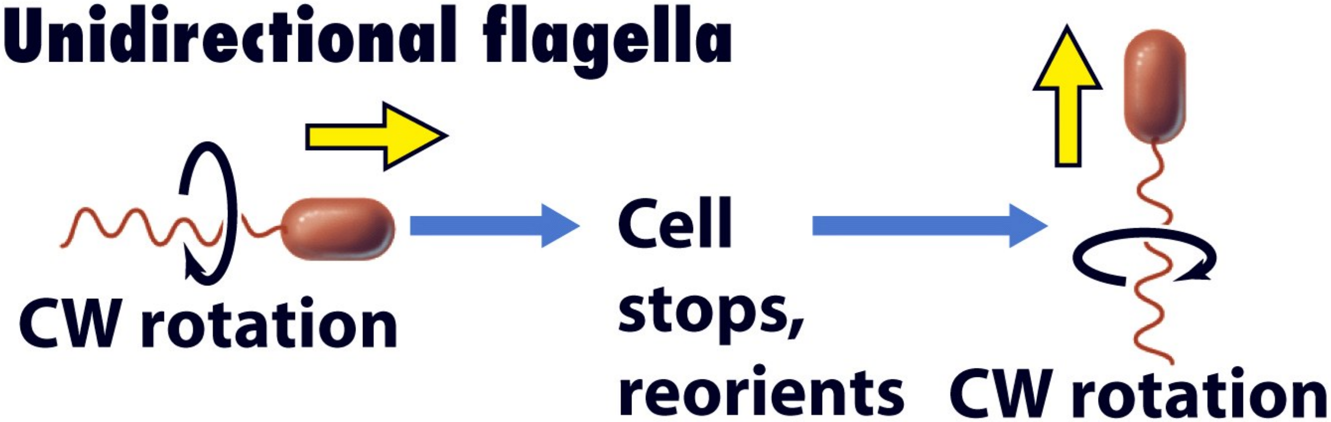


Flagellar Motility

- **Reversible flagella**



- **Unidirectional flagella**



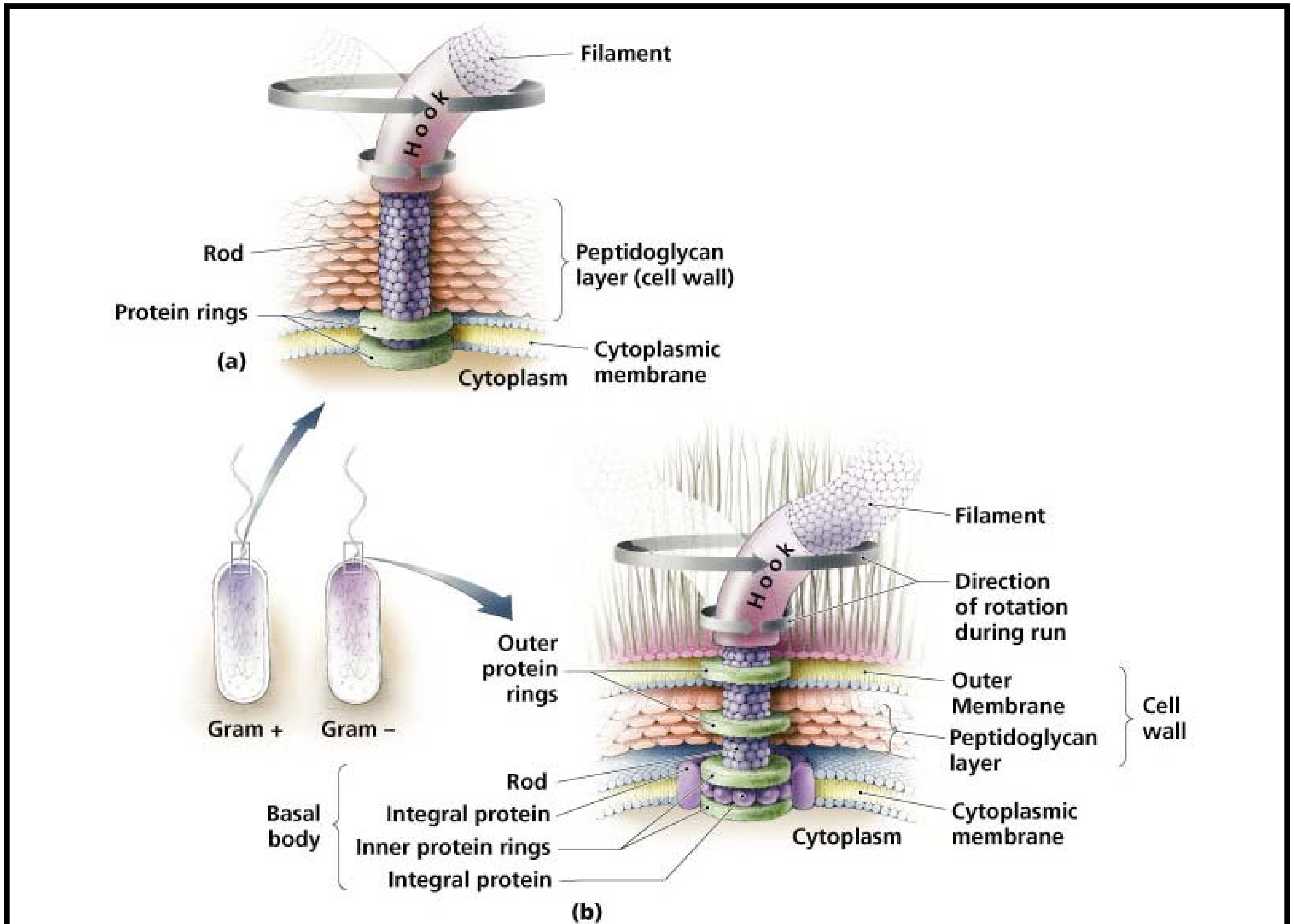
Polar (both)

Flagellar Structure

Complex structure

Rotates to swim

Immunogenic (flagellin recognized by mammalian immune system)



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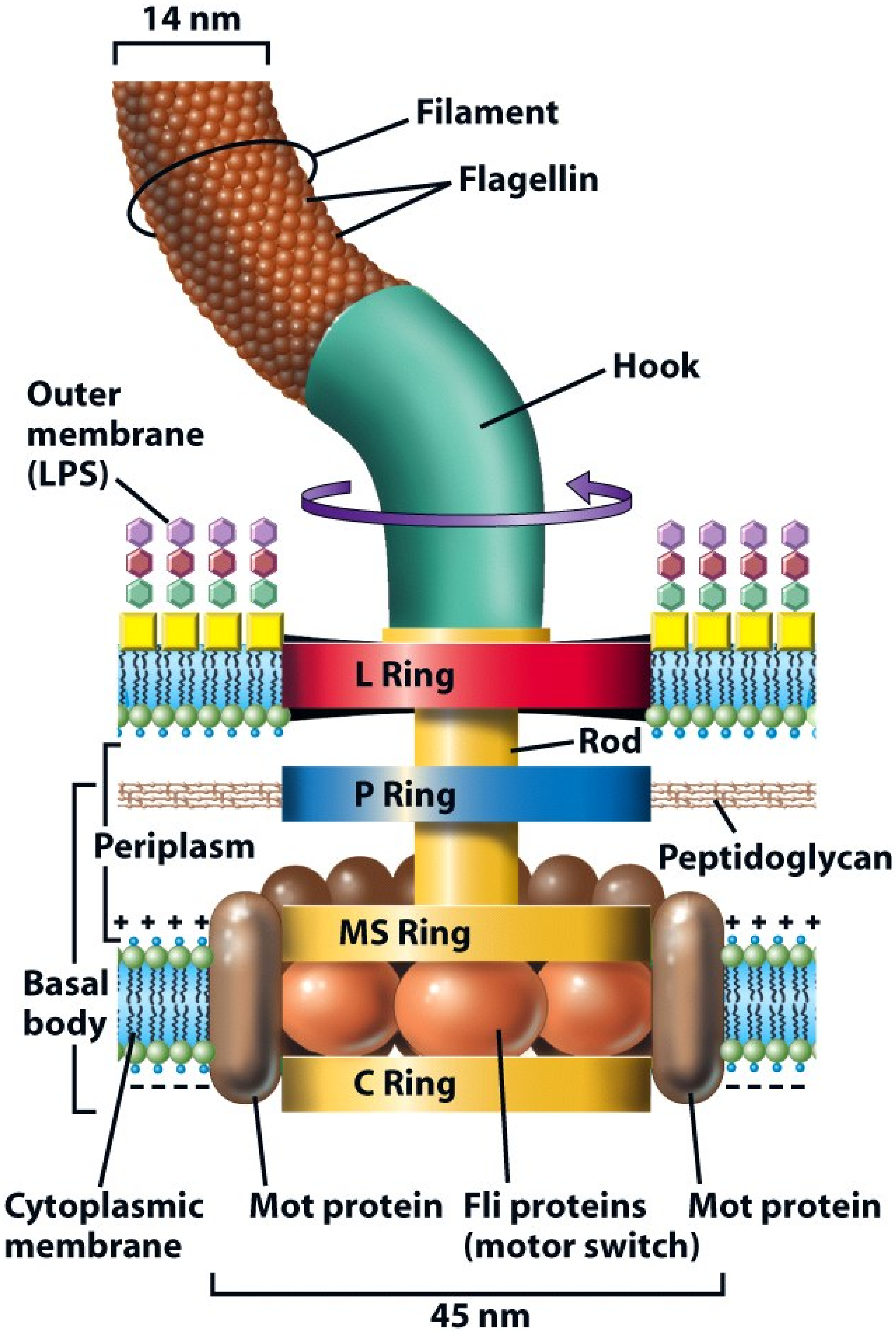


Flagellar Structure

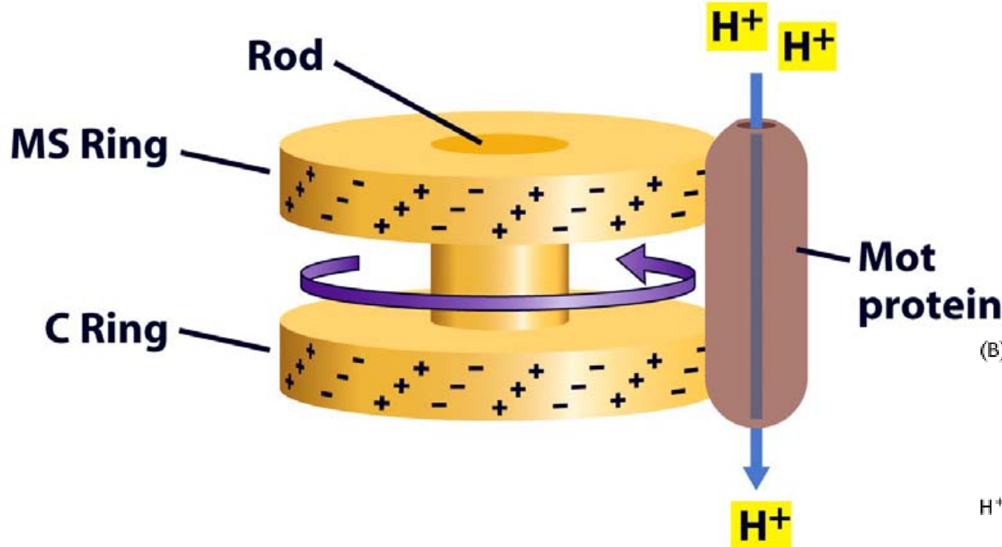
Rotor = C & MS rings; central rod. Turns. MS ring (membrane/supramembrane ring) is made of same protein (FliF) as central rod.

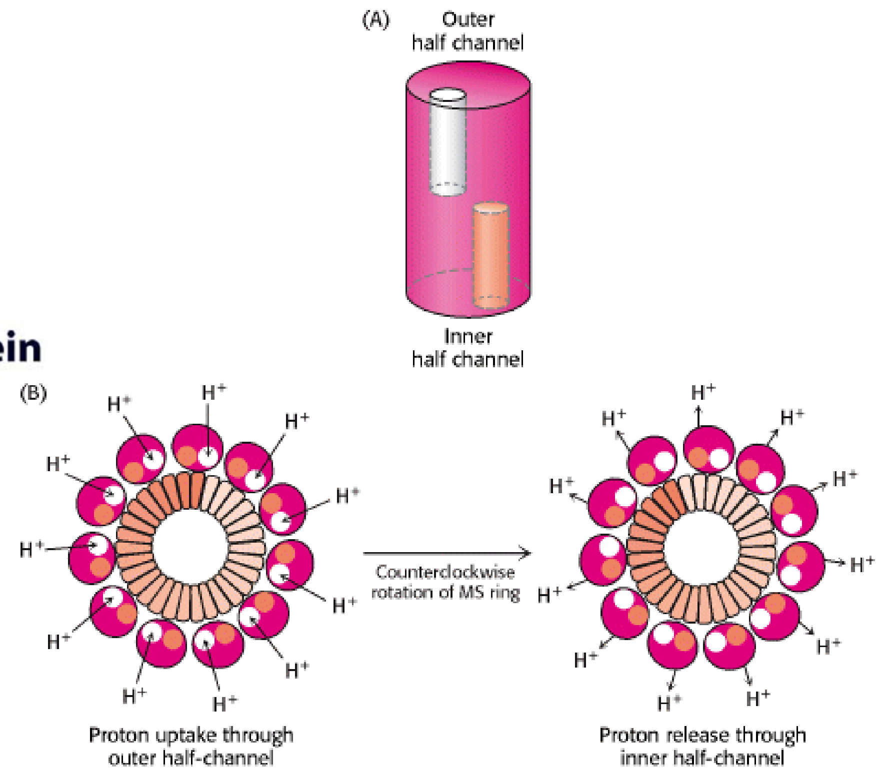
Switch complex: FliM & FliN (comprise C ring), and FliG (precise location unknown) . Dictates direction (CW/CCW).

Stator: MotA and Mot B. Generate torque.

Bushing surrounding central rod: L&P rings (LPS, peptidoglycan rings).

Bacteria: 60 cell lengths/second
Cheetah: 25 body lengths/second





Models for Flagellar Rotation

(A) Mot proteins (MotA/MotB complex) form two half-channels.

(B) Protons are taken up into the outer half-channel and transferred to the MS ring OR protons, during transfer, cause changes in electrostatic interactions between MotAB and MS ring.

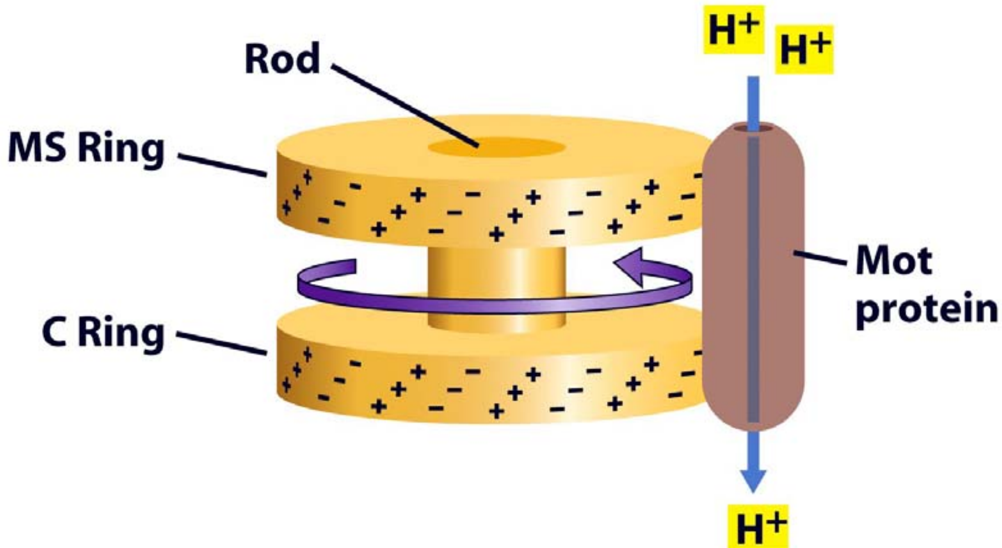
The MS ring rotates in a CCW direction, and the protons are released into the inner half-channel. The flagellum is linked to the MS ring and so the flagellum rotates as well.

(C) Electrostatic interactions between MotA and FliG facilitate rudder-like switching of direction.

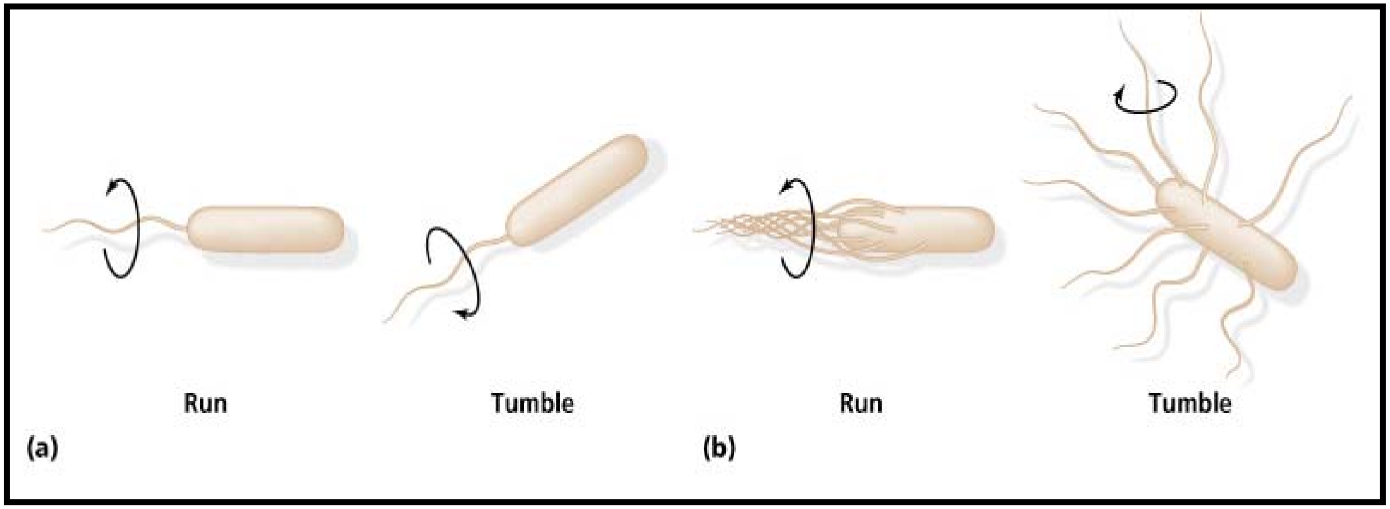
Estimate: 1000 protons/1 turn

In alkalophiles and marine *Vibrio* species, the fuel for flagellar rotation is a Na^+ gradient rather than H^+.

Why?

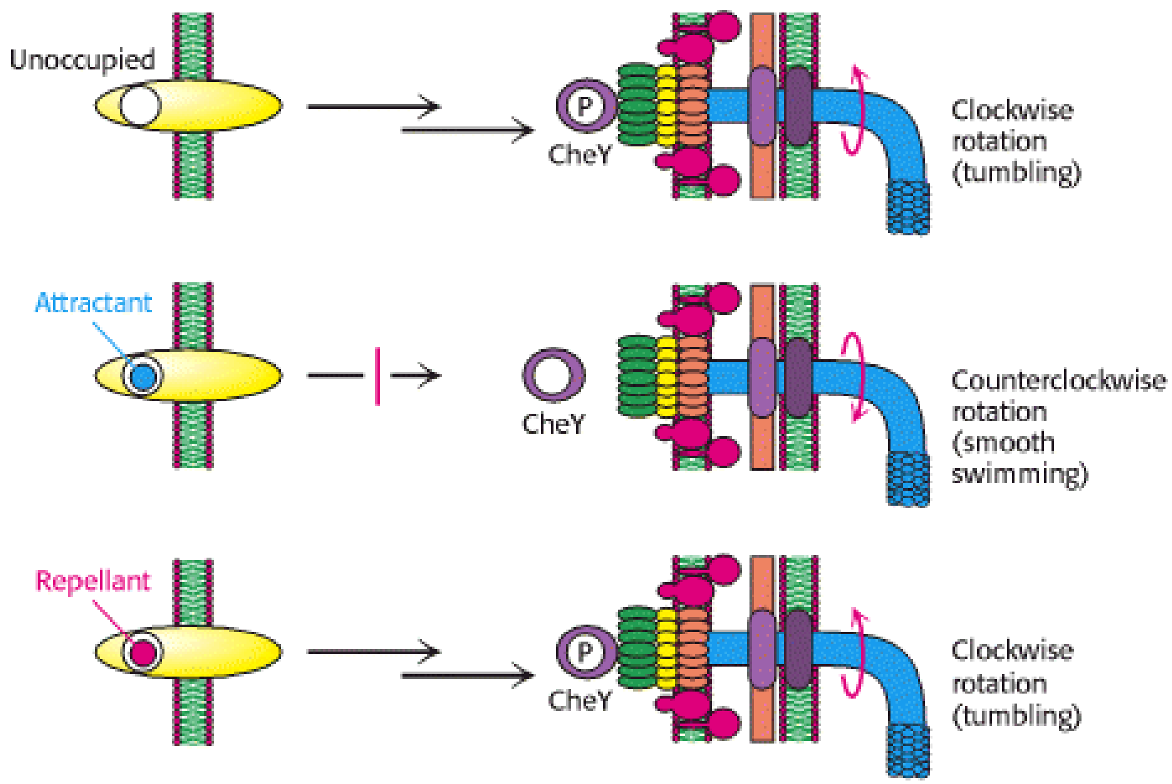


Flagellar Motility: Chemotaxis



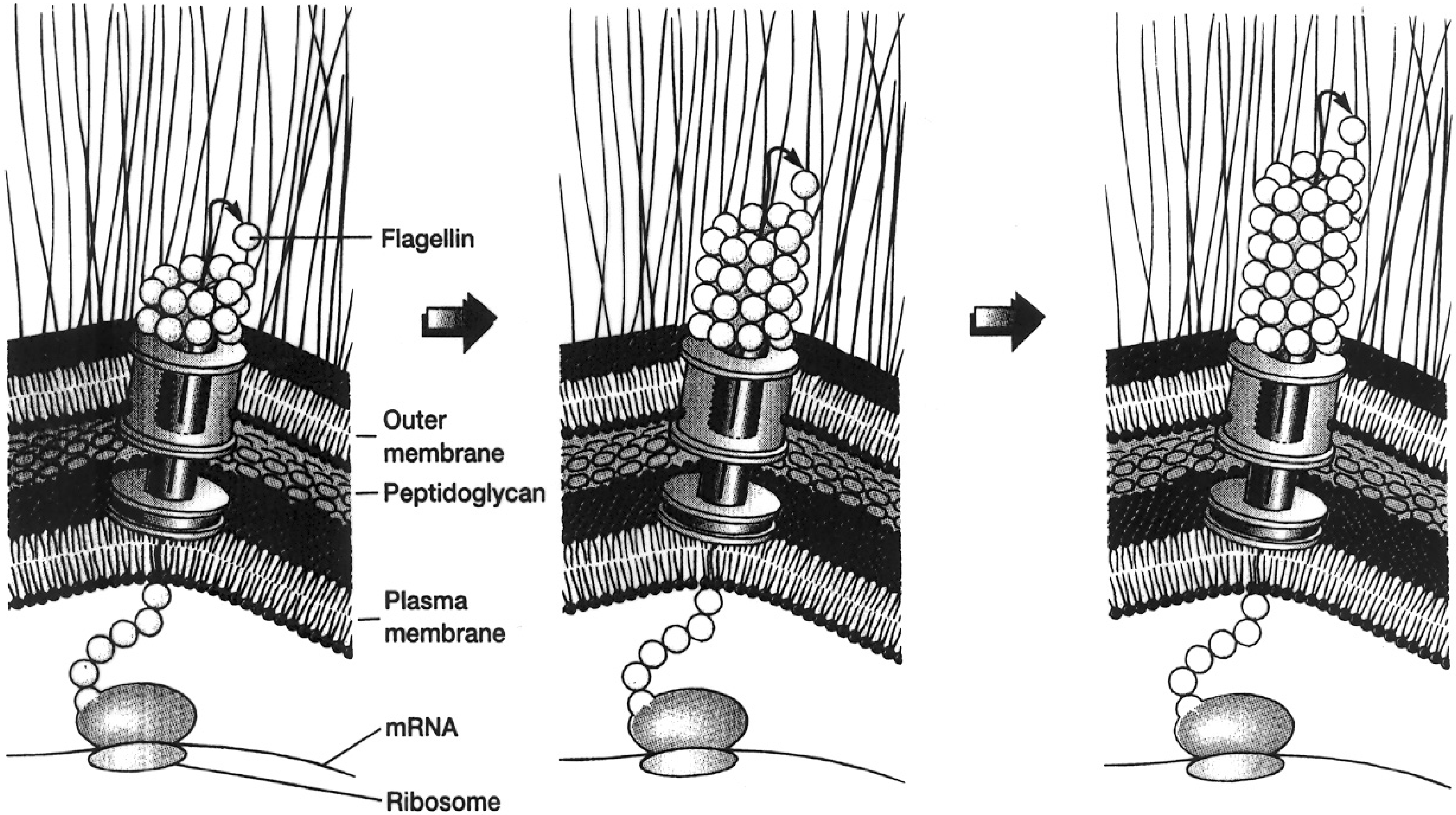
- CCW rotation moves forward
- CW rotation tumbles
- In isotropic environment, runs of 1 second interspersed with tumbles
- In a spatial gradient of attractant, length of runs increase if they carry cells up the gradient
- No change (or slight decrease) in length of runs down the gradient

Flagellar Motility: Chemotaxis signaling pathway



Chemotaxis Signaling Pathway. Receptors in the plasma membrane initiate a signaling pathway leading to the phosphorylation of the CheY protein. Phosphorylated CheY binds to the flagellar motor and favors CW rotation. When an attractant binds to the receptor, this pathway is blocked, and CCW flagellar rotation and, hence, smooth swimming results. When a repellant binds, the pathway is stimulated, leading to an increased concentration of phosphoylated CheY and, hence, more frequent CW rotation and tumbling.

Flagellar Assembly



Growth of Flagellar Filaments. Flagellin subunits travel through the flagellar core and attach to the growing tip.

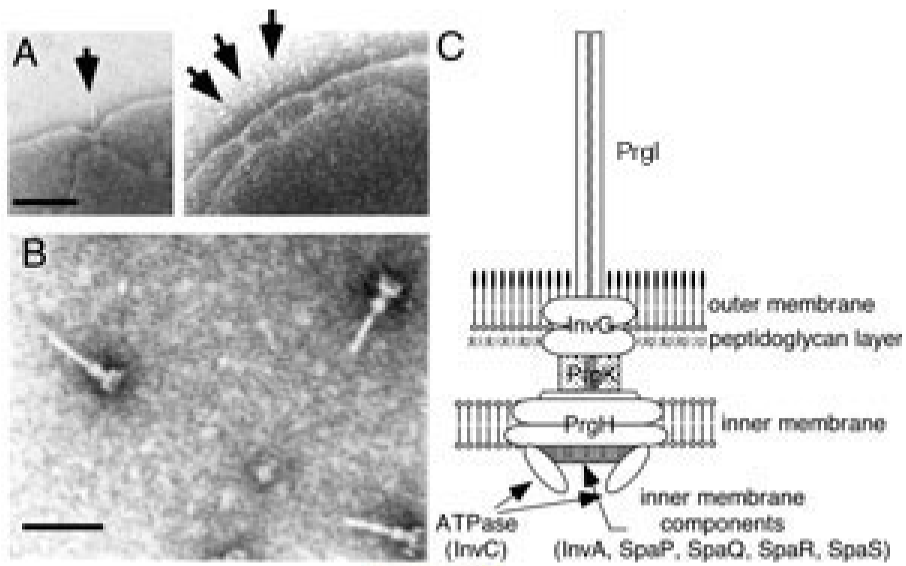
The flagellum contains a built-in secretion apparatus that serves for the export of the hook and filament components. In some instances this secretion apparatus also secretes nonflagellar proteins.

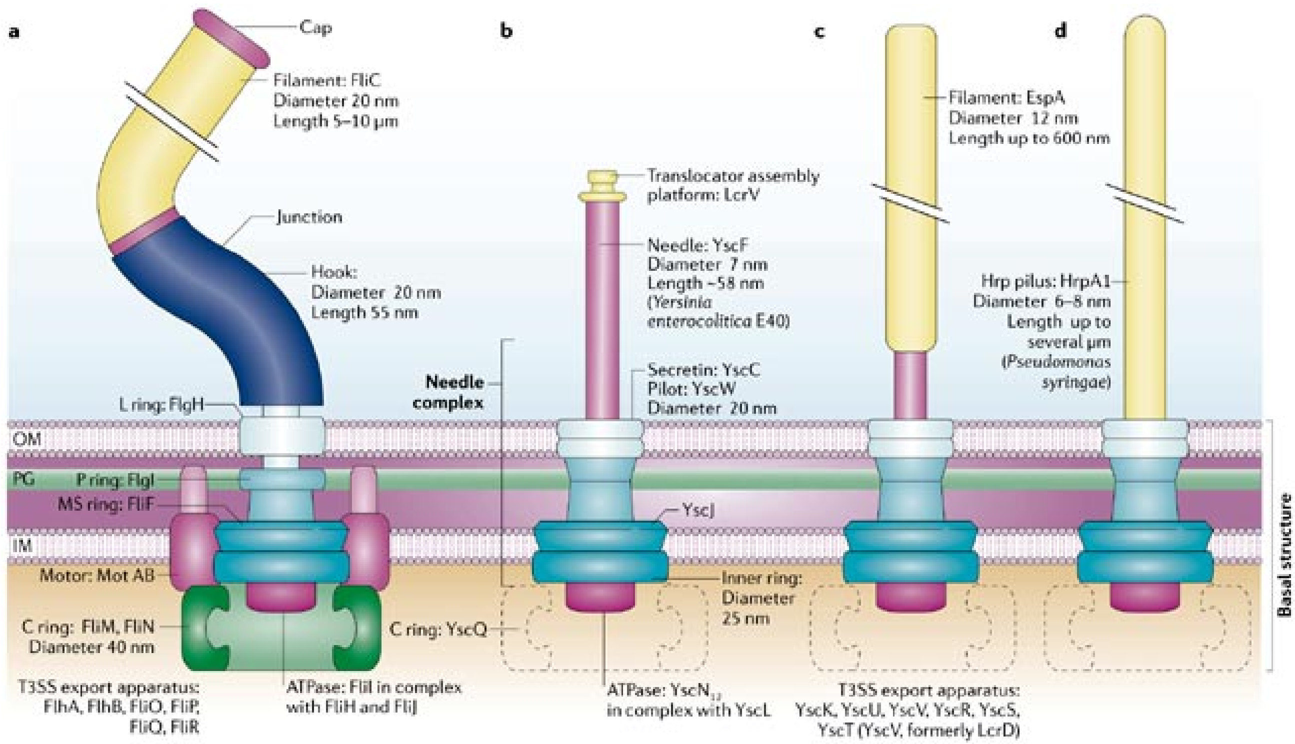
Flagellae are related to injectisomes

The name ‘Type III secretion’ (T3S) refers to a secretion pathway that is common to the flagellae of eubacteria and the injectisomes of some Gram-negative bacteria.

The injectisome can be structurally viewed as a flagellum topped by a needle instead of a hook and a filament.

T3S inject effector proteins via a needle complex directly into host cytoplasm!!





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Schematic representation of:

a. the flagellum

b. injectisome from *Yersinia pestis*, causal agent of Black Plague

c. injectisome from enteropathogenic *E. coli*

d. injectisome from plant pathogens

Cornelis *Nature Reviews Microbiology* **4**, 811–825 (November 2006) | doi:10.1038/nrmicro1526

Flagellae are related to injectisomes

The needle of the injectisomes:

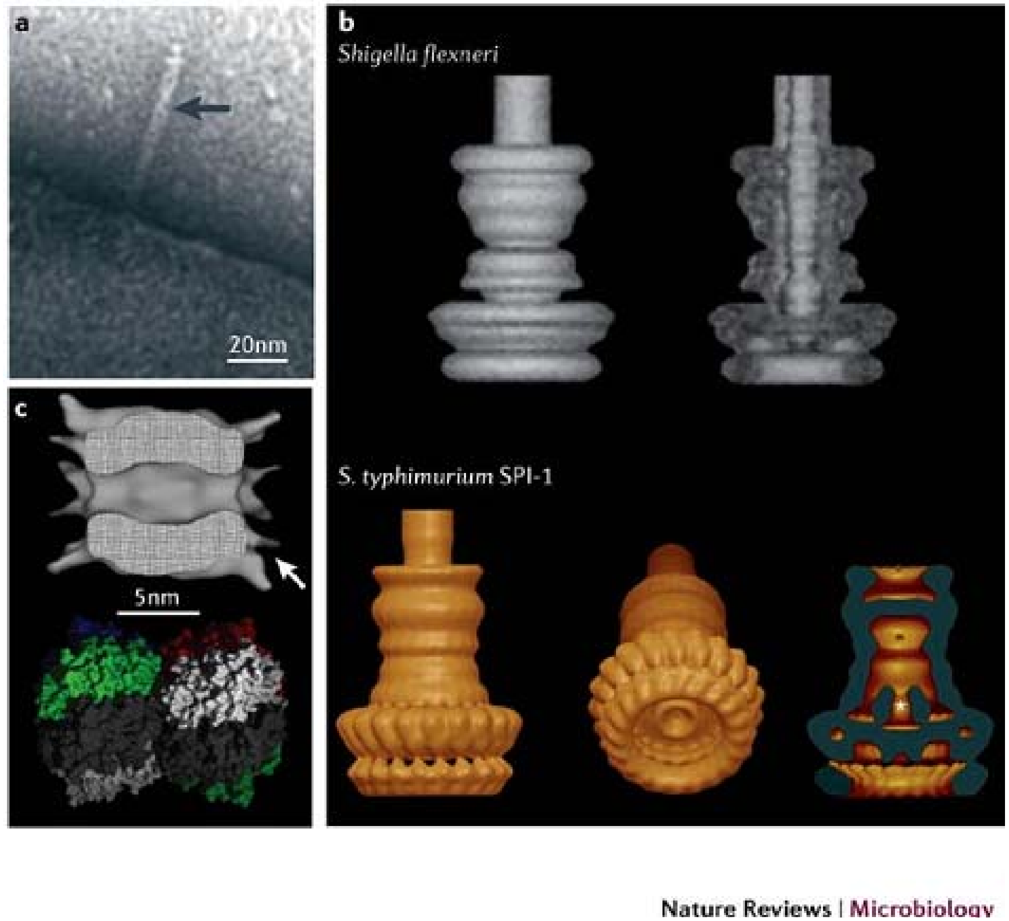
- straight hollow tube
- ~10 nm OD/ 2.5 nm ID
- needle 45-80 nm long

- made by the polymerization of (varied from species to species, but related) single major subunits

The hook/filament of flagellae:

- curved hollow tube
- ~20 nm OD
- hook ~ 55 nm long
- filament ~ 5,000 – 10,000 nm long

- made by the polymerization of a single major subunit (FliC/flagellin)



a Transmission electron micrograph of *Yersinia enterocolitica* E40. One needle (arrow) protrudes from the cell surface.

b 3D structure of the needle complex encoded by *Shigella flexneri* (top) and by *Salmonella enterica* serovar *Typhimurium* (bottom), reconstructed by averaging cryo-electron micrograph images.

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Injectisome family	Species	Description	Taxon
Chlamydiales	*Chlamydia trachomatis*	Obligate intracellular human pathogen (trachoma, genital infections)	Chlamydiaceae
	Chlamydia pneumoniae	Obligate intracellular human pathogen (acute respiratory disease)	Chlamydiaceae
Hrp1	*Pseudomonas syringae*	Plant pathogen	γ-proteobacteria
	Erwinia amylovora	Plant pathogen	γ-proteobacteria
	Pantoea agglomerans (formerly Enterobacter agglomerans)	Environmental and human commensal, rarely pathogenic.	γ-proteobacteria
	Vibrio parahaemolyticus	Human pathogen (seafood-borne gastroenteritis)	γ-proteobacteria
Hrp2	*Burkholderia pseudomallei*	Human pathogen (meloidosis)	β-proteobacteria
	Ralstonia solanacearum	Plant pathogen	β-proteobacteria
	Xanthomonas campestris	Plant pathogen	γ-proteobacteria
SPI-1	*Salmonella enterica*	Human pathogen (gastroenteritis)	γ-proteobacteria
	Shigella flexneri	Human pathogen (dysenteria)	γ-proteobacteria
	Burkholderia pseudomallei	Human pathogen (meloidosis)	β-proteobacteria
	Chromobacterium violaceum	Emerging human pathogen (evoking meloidosis)	β-proteobacteria
	Yersinia enterocolitica	Human pathogen (gastroenteritis, mesenteric adenitis)	γ-proteobacteria
	Sodalis glossinidius	Tse-tse fly symbiont	γ-proteobacteria
SPI-2	*Escherichia coli* EPEC	Human pathogen (gastroenteritis)	γ-proteobacteria
	Escherichia coli EHEC	Human pathogen (uremia, hemolysis)	γ-proteobacteria
	Salmonella enterica	Human pathogen (gastroenteritis)	γ-proteobacteria
	Citrobacter rodentium	Mouse pathogen, model for EPEC	γ-proteobacteria
	Chromobacterium violaceum	Emerging human pathogen (evoking meloidosis)	β-proteobacteria
	Yersinia pestis	Rodent and human pathogen (plague)	γ-proteobacteria
	Yersinia pseudotuberculosis	Rodent and human pathogen	γ-proteobacteria
	Edwardsiella tarda	Human pathogen (gastroenteritis)	γ-proteobacteria
Rhizobium	*Mesorhizobium loti*	Plant symbiont (Nitrogen fixation)	α-proteobacteria
	Rhizobium sp	Plant symbiont (Nitrogen fixation)	α-proteobacteria
Ysc	*Yersinia pestis*	Rodent and human pathogen (plague)	γ-proteobacteria
	Yersinia pseudotuberculosis	Rodent and human pathogen	γ-proteobacteria
	Yersinia enterocolitica	Human pathogen (gastroenteritis, mesenteric adenitis)	γ-proteobacteria
	Pseudomonas aeruginosa	Animal, insect and human (cystic fibrosis, burned, immunocompromized patients) pathogen	γ-proteobacteria
	Aeromonas salmonicida	Fish pathogen	γ-proteobacteria
	Photorhabdus luminescens	mutualistic with entomophagous nematodes	γ-proteobacteria
	Vibrio parahaemolyticus	Human pathogen (seafood-borne gastroenteritis)	γ-proteobacteria
	Bordetella pertussis	Human pathogen (whooping cough)	β-proteobacteria
	Desulfovibrio vulgaris	Sulphate reducing environmental bacteria	δ-proteobacteria

EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*.

Plant and mammalian pathogens share this mechanism of injecting host cells with bacterial proteins:

Salmonella, *Shigella*, *Yersinia*, etc.

Cornelis *Nature Reviews Microbiology* **4**, 811–825 (November 2006) | doi:10.1038/nrmicro1526

Injectisomes allow bacteria to deliver effector proteins not only across the two bacterial membranes but also across the eukaryotic cell membrane.

Effector proteins reprogram the target cells to the benefit of the bacterium (or on rare occasions, to the benefit of both organisms).

How does the cell know which of its proteins are effector proteins? There's no evidence for a signal sequence in the mRNA or in the primary amino acid sequence. Rather, experimental evidence supports patterns in the 3D protein structure that are recognized by chaperones.

Along with effectors, "translocators" are also secreted – a set of proteins (generally three, as in *Salmonella, Shigella,* and *Yersinia*).

In *Pseudomonas aeruginosa*, PopB, PopD, and PcrV are the translocator proteins.

Question: **Do these form pores in the mammalian cell membrane?**

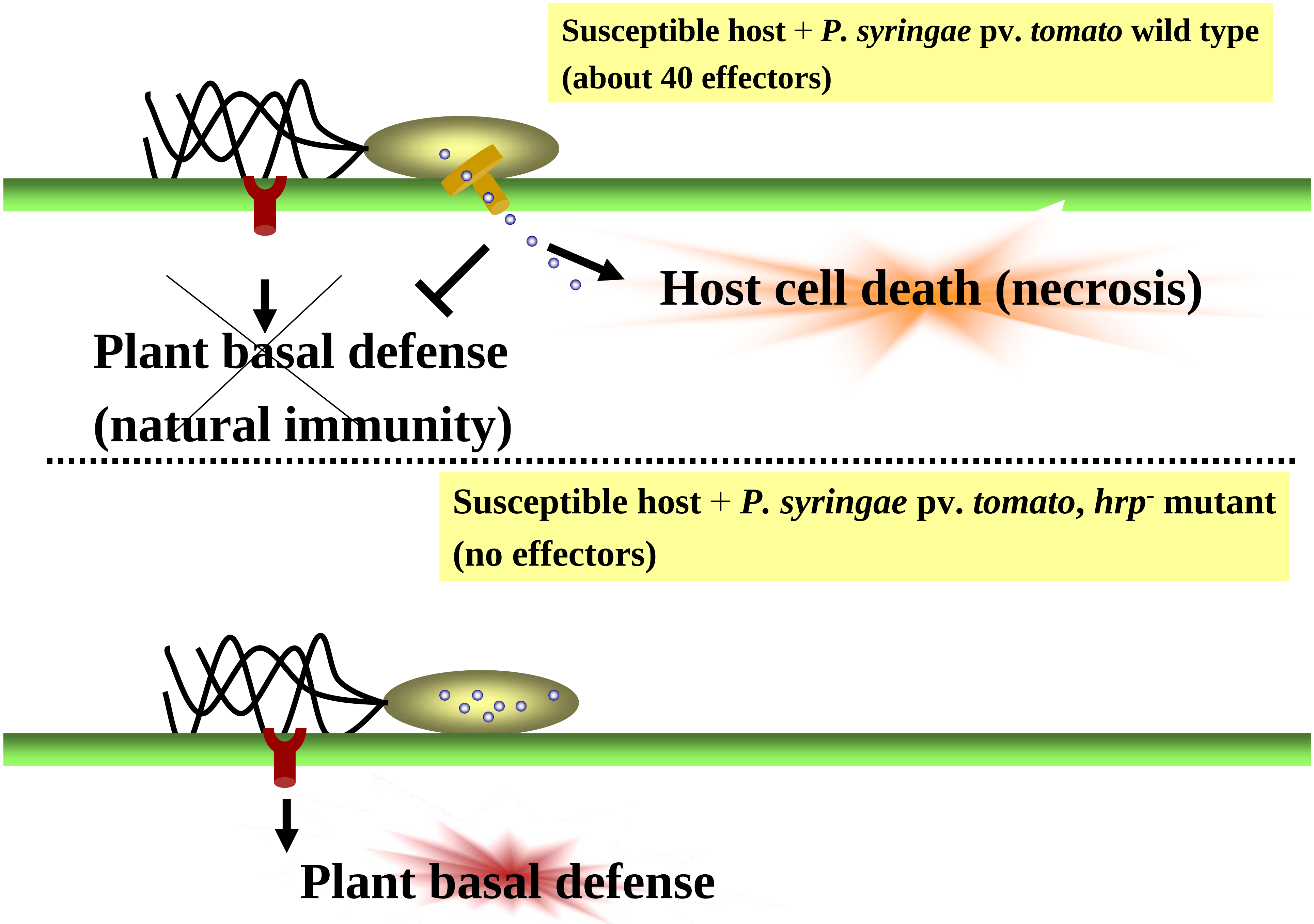
Experiment: **treat erythrocytes with *P. aeruginosa*. Extract proteins from membrane and look for bacterial proteins.**

Result: **only PopB and PopD present; PcrV absent.**

BUT: **When the *pcrV* gene is mutated, *P. aeruginosa* cannot form pores. PcrV is absolutely required for pore formation.**

Conclusion: **PopB and PopD are probably hydrophobic pore-formers and PcrV is a chaperone.**

Journet, Hughes, and Cornelis. 2005. Molecular Membrane Biology 22: 41-50



Susceptible host + P. syringae pv. tomato wild type
(about 40 effectors)
Host cell death (necrosis)
Plant basal defense
(natural immunity)
Susceptible host + P. syringae pv. tomato, hrp- mutant
(no effectors)
Plant basal defense

Flagellae and Injectisomes: Take Home Messages

- Motility in most microorganisms is due to flagella.

- In prokaryotes the flagellum is a complex structure made of several proteins, most of which are anchored in the cell wall and cytoplasmic membrane.

- The flagellum filament, which is made of a single kind of protein, rotates at the expense of the proton motive force, which drives the flagellar motor.

- Flagellae and injectisomes are evolutionarily related.

- Injectisomes are usually associated with pathogenic microbes. They are used for injection of effector proteins into host cells. Effector proteins alter host metabolism.

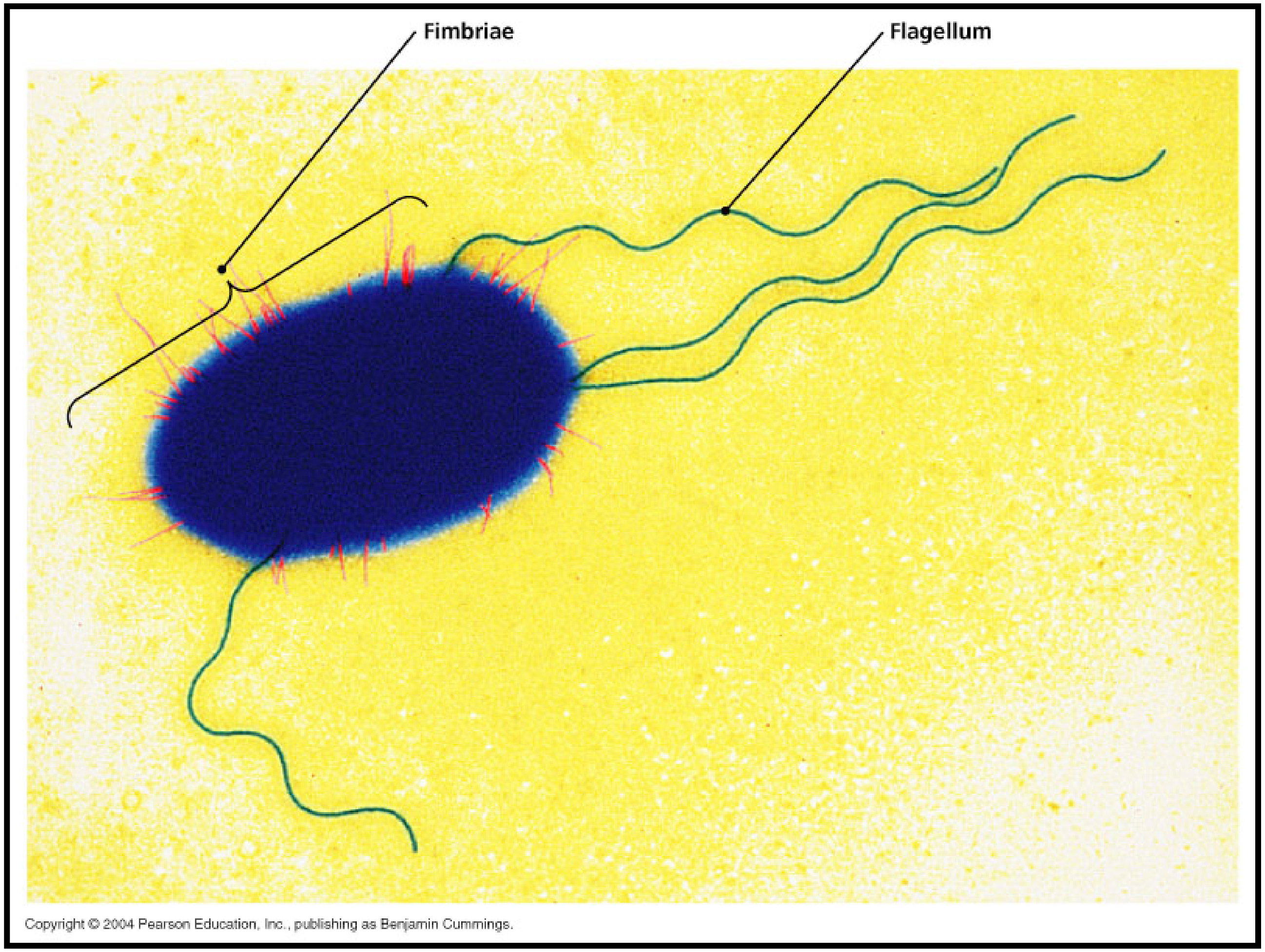
Classification of prokaryotic cellular features: Variant (or NOT common to all)

-Cell wall (chemistry varies; some don’t have one)
-Endospores (heavy-duty life support strategy)
-Glycocalyx: capsules or slime layer (exterior to cell wall)
-Bacterial Flagellae and Injectisomes

-Fimbriae and Pili

-Inclusion Bodies (granules for storage)
-Gas vesicles (vertical movement in liquid medium)

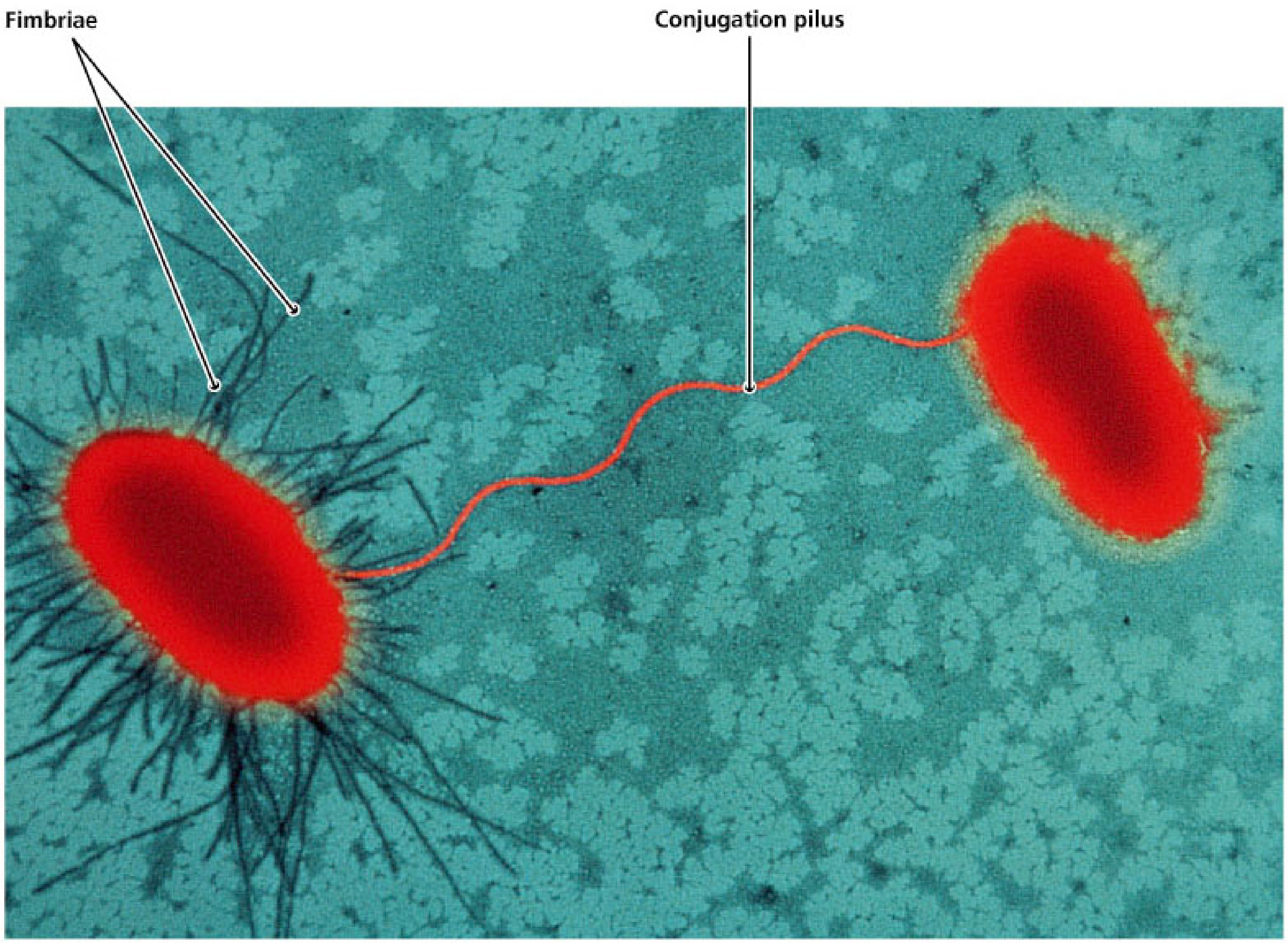
Fimbriae



E. coli

- **Uropathogenic *E. coli* use fimbriae to adhere to urinary tract**
- **Hold 'tighter' in high flow, release and swim in low flow**
- **Critical pathogenicity factor**

Conjugation



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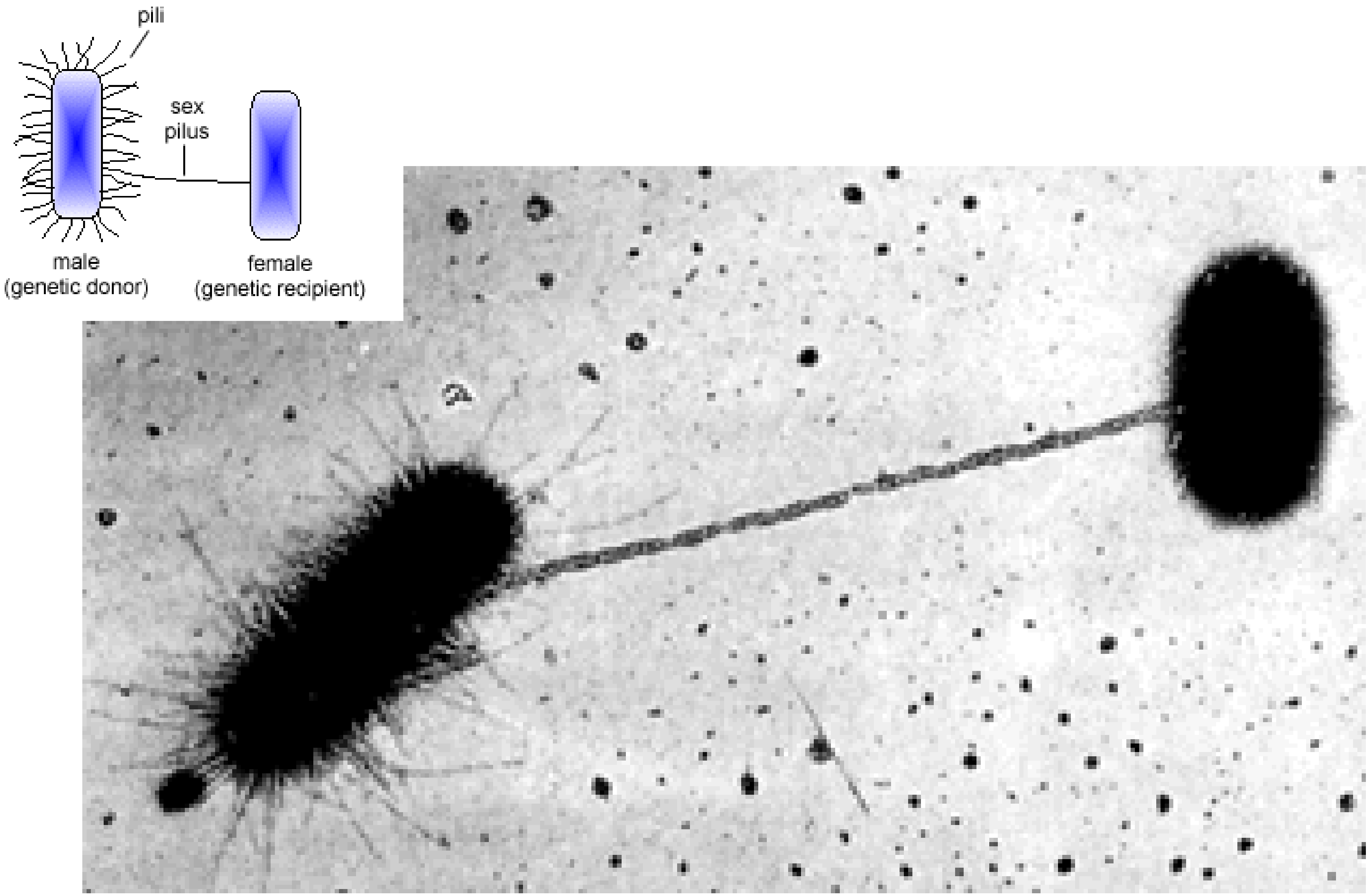
- •Conjugal transfer occurs from 'male' to 'female'
- •Spreads plasmids and antibiotic resistance
- •*Agrobacterium*: DNA to eukaryotic cells… others can too!



“Sex” Pili used in bacterial conjugation of *E. coli* cells

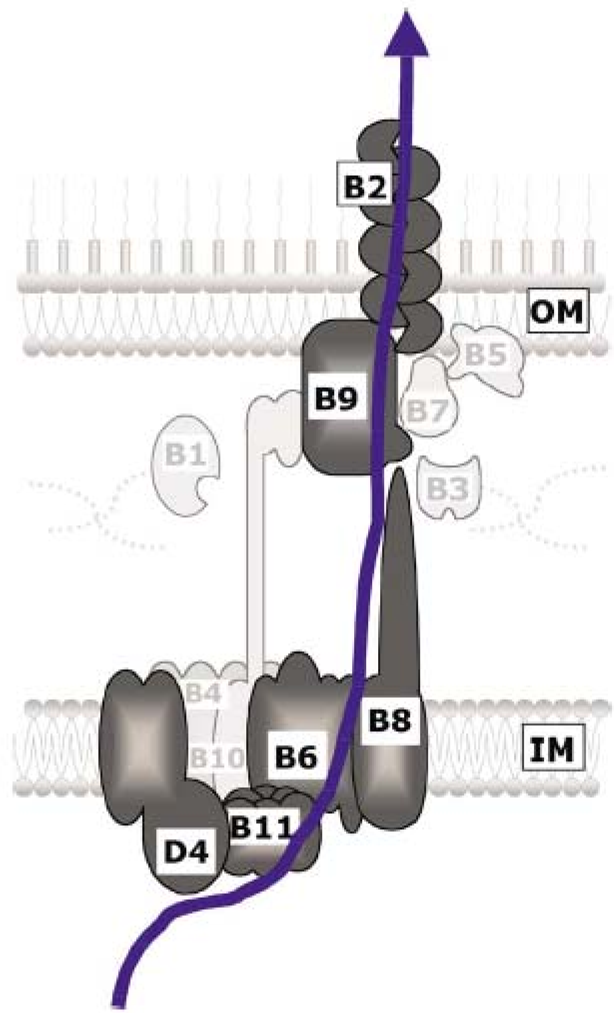
The pilus responsible for the genetic engineering revolution:

- ***Agrobacterium tumefaciens:*** **ubiquitous gram-negative, soil-dwelling bacterium**
- **Plant pathogen, causes crown gall disease**
- **Very broad host range (dicots)**
- **Uses a pilus to inject tumor-inducing (Ti) plasmid into plant cells**

(i) Formaldehyde treatment of intact cells to crosslink pilus subunits to the T-DNA substrate as it exits the cell

(ii) Detergent-solubilization and immunoprecipitation of individual pilus subunits

(iii) PCR amplification for detection of the T-DNA in the immunoprecipitates



Christie and Casales, 2005. Molecular Membrane Biology 22: 51-61.

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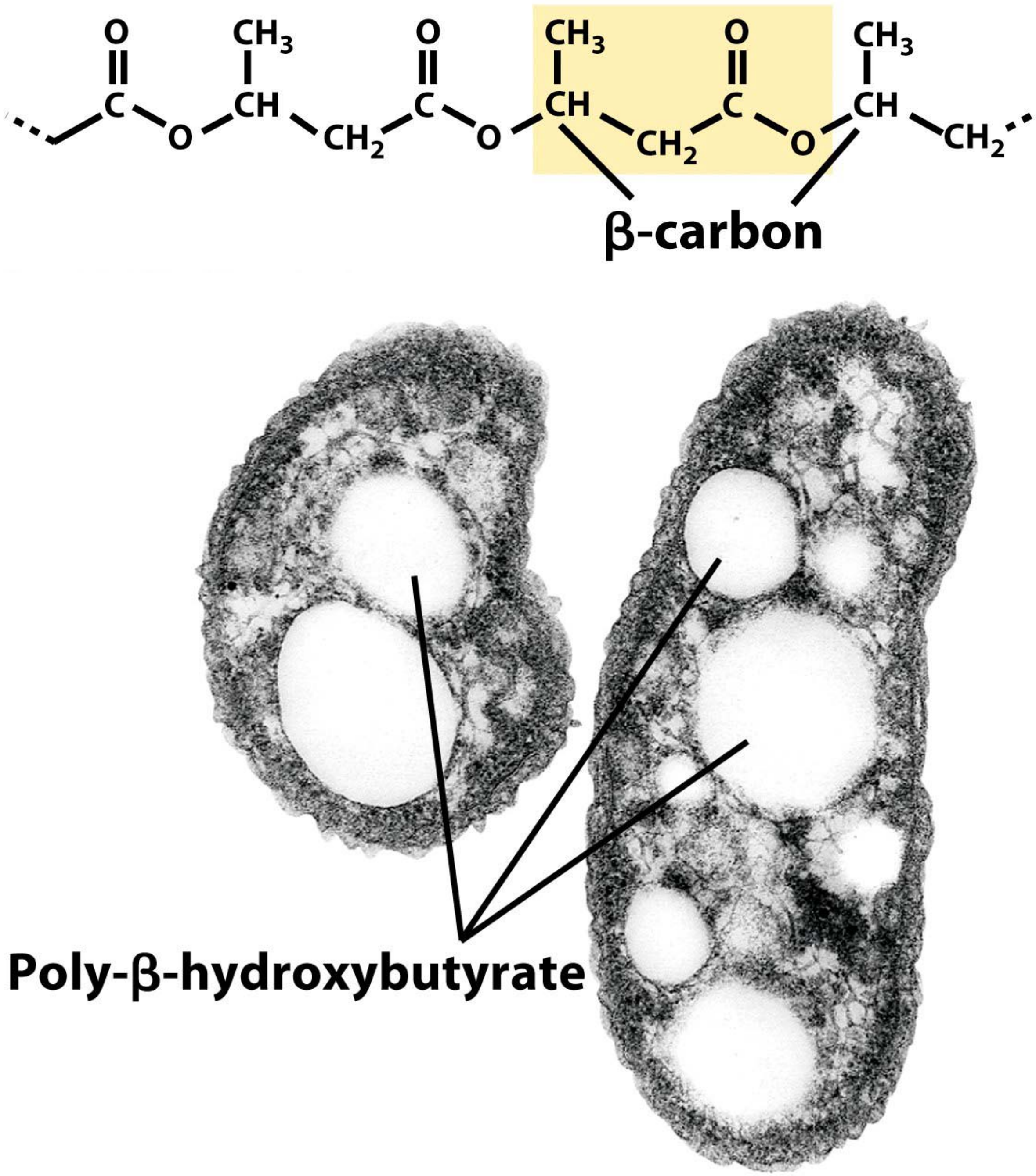
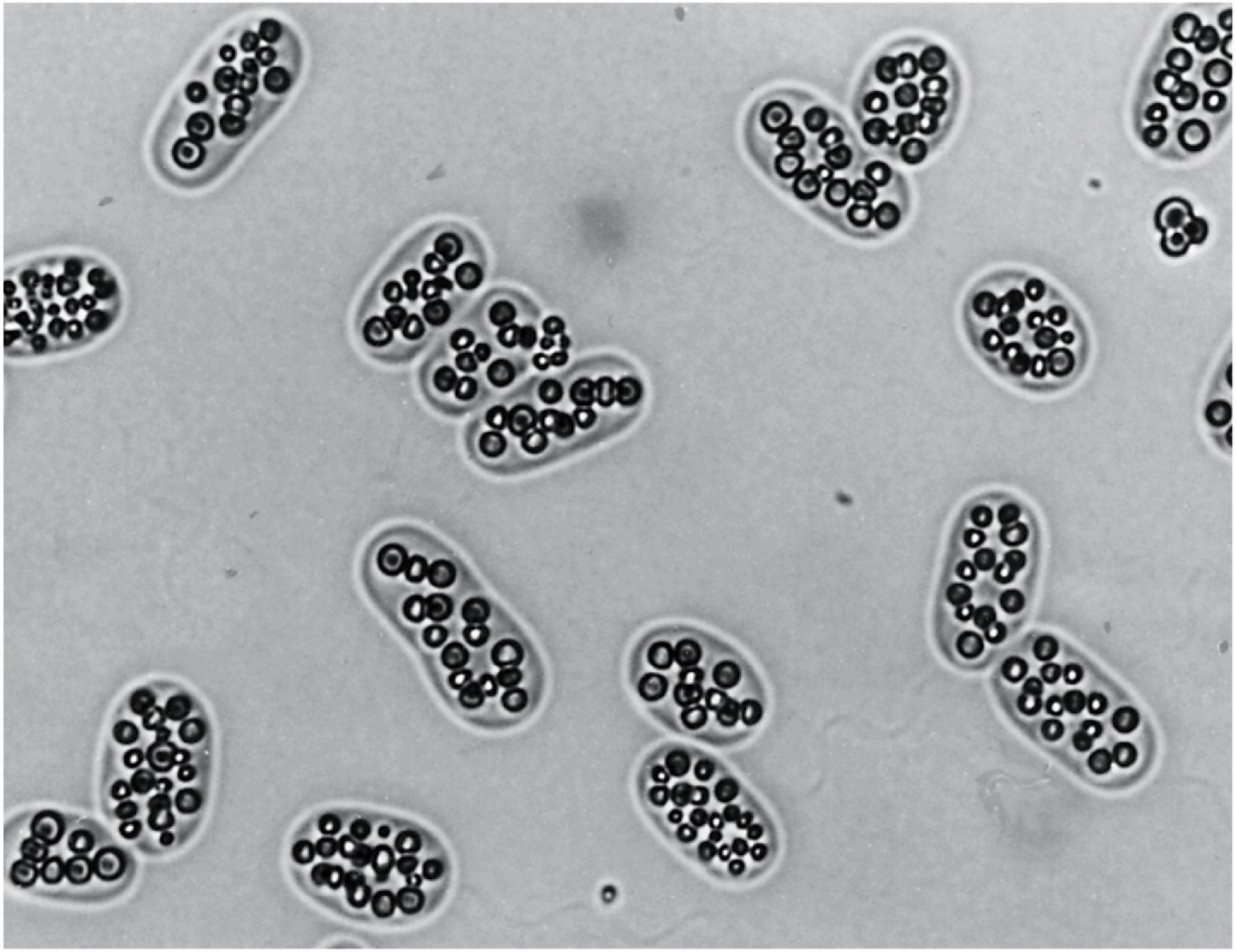
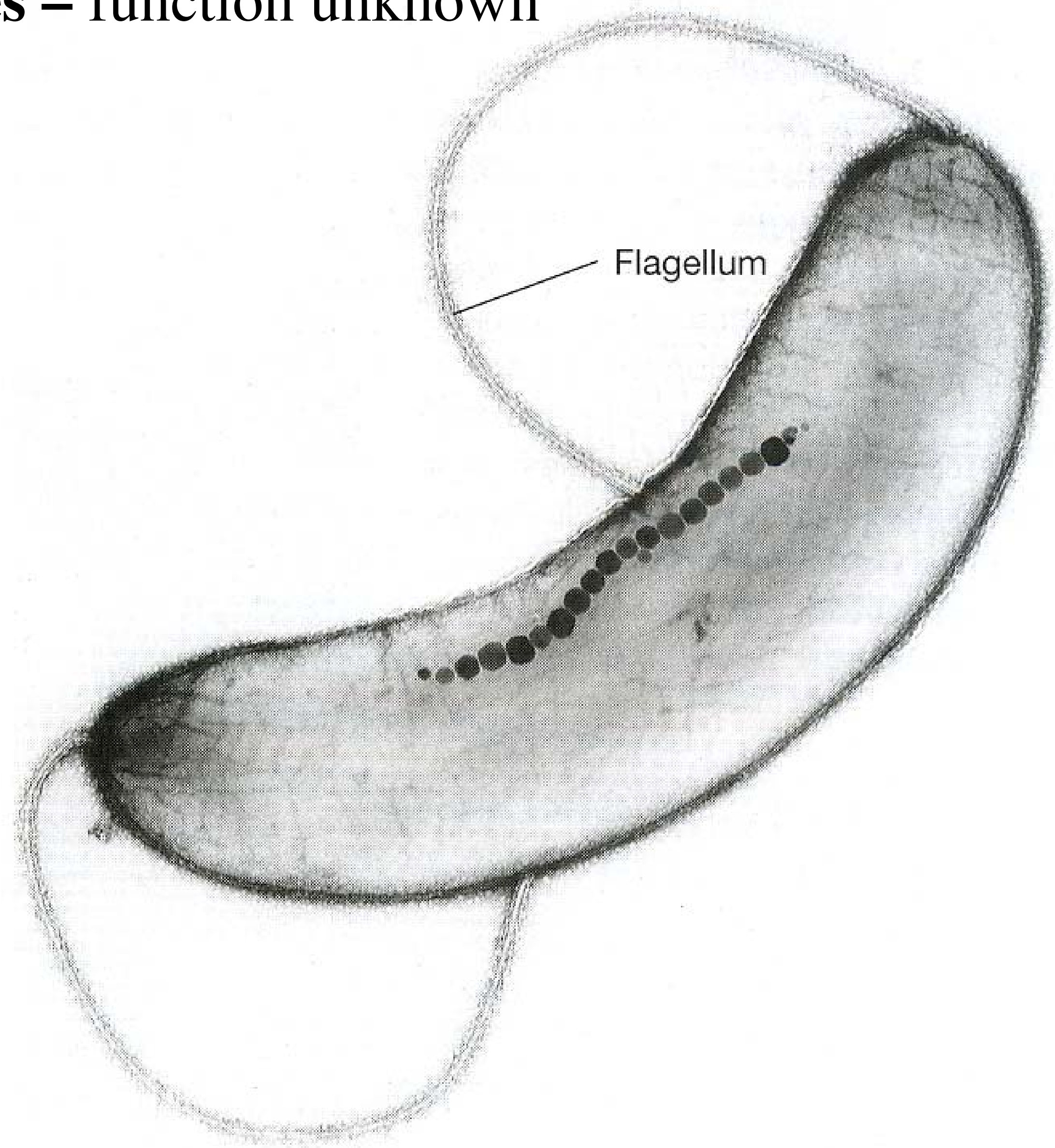


Storage of PHB (or other carbon polymers like glycogen; energy reserve like Gu or battery)



Sulfur globules inside the purple sulfur
bacterium *Isochromatium buderi:* oxidation of H_2S

Magnetotactic bacteria with Fe_3O_4 (magnetite) particles called **magnetosomes –** function unknown



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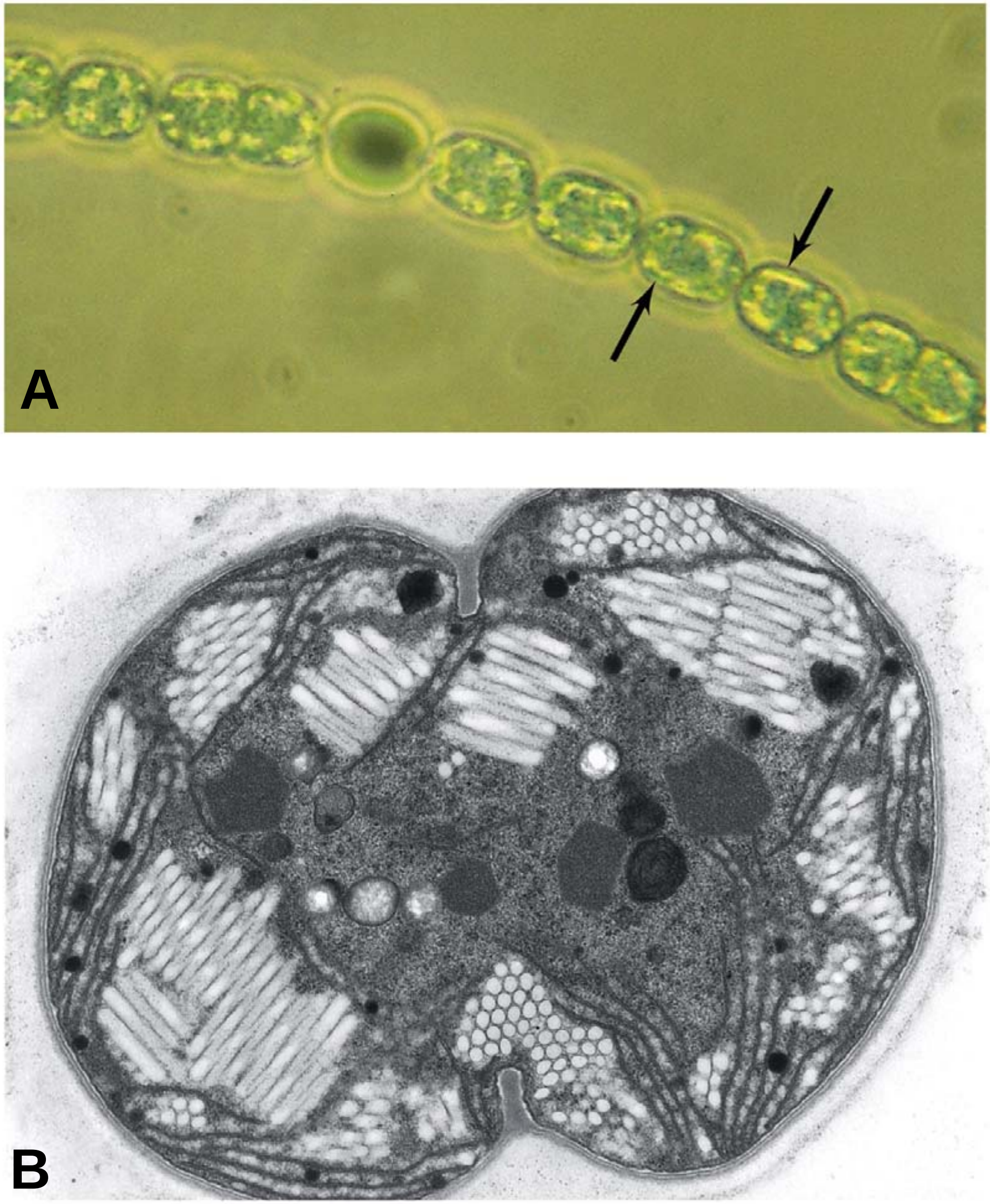
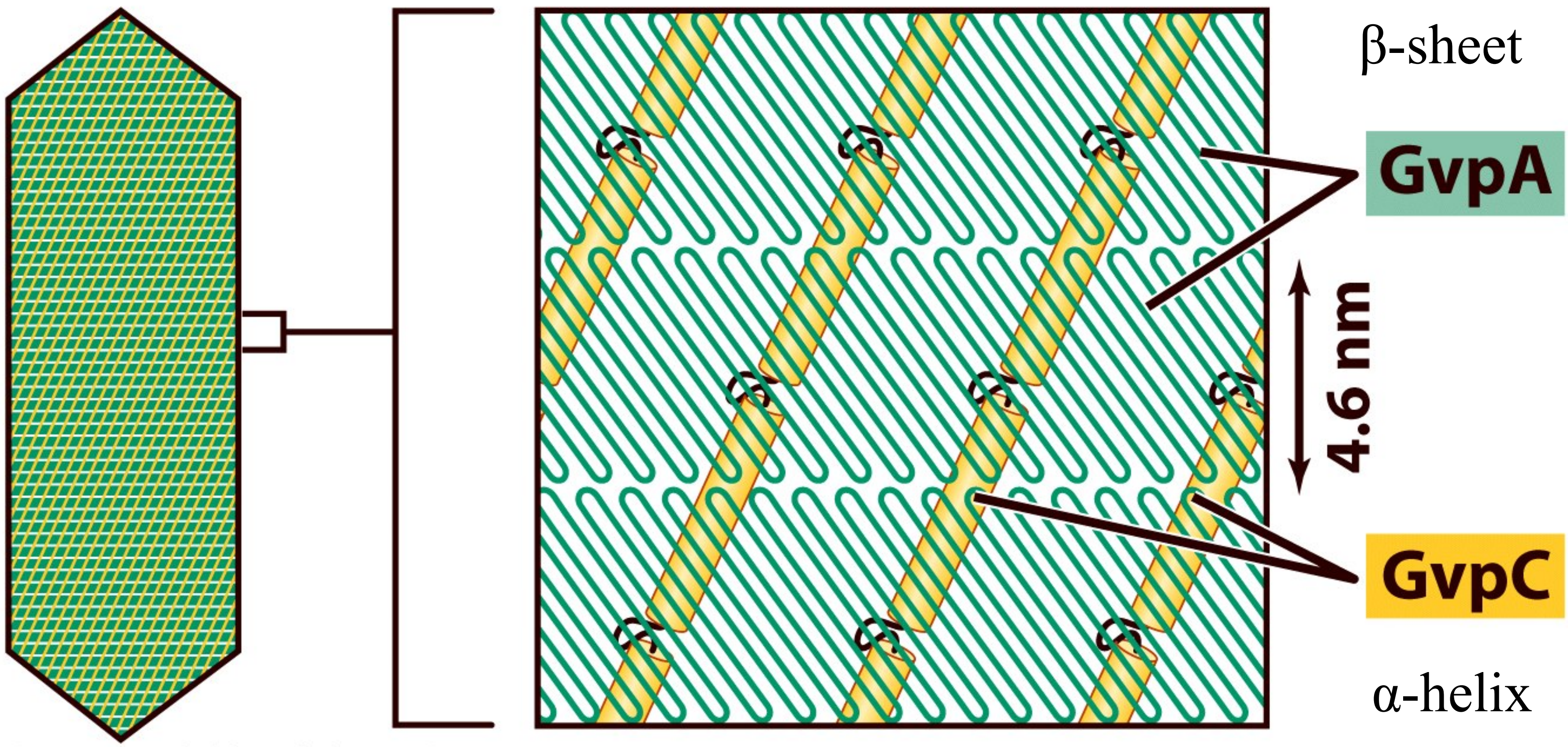
-**Gas vesicles (vertical movement in liquid medium)**



Gas Vesicles

(a) *Anabaena flos-aquae*
(b) *Microcystis* sp.

Model of how the two proteins that make up the gas vesicle, GvpA and GvpC, interact to form a watertight but gas-permeable structure (GoreTex).



Classification of prokaryotic cellular features: Variant (or NOT common to all)

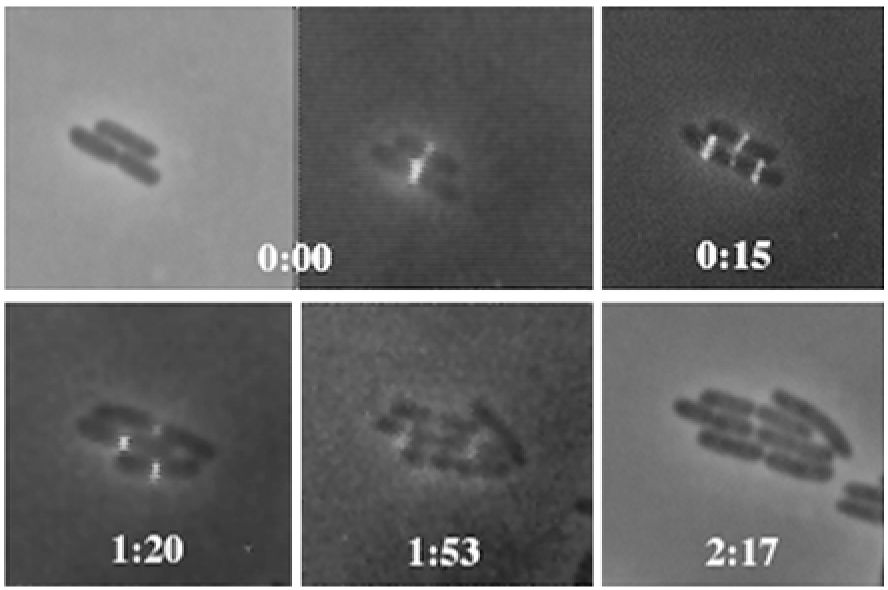
Odds and Ends:

Eukaryotic cytoskeleton homologs

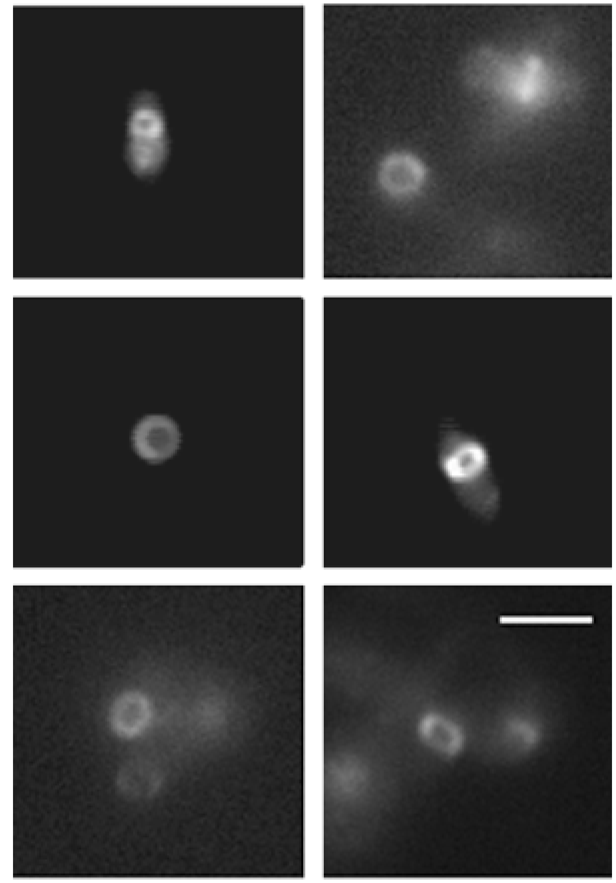
Biofilms

FtsZ is a structural homolog (ancestor?) of eukaryotic tubulin

-forms Z-ring at future site of cytokinesis
-only 17% amino acid identity to tubulin
but similar 3D structures and assembly properties



Growth and division of *E. coli* microcolonies expressing FtsZ-GFP and FtsZ

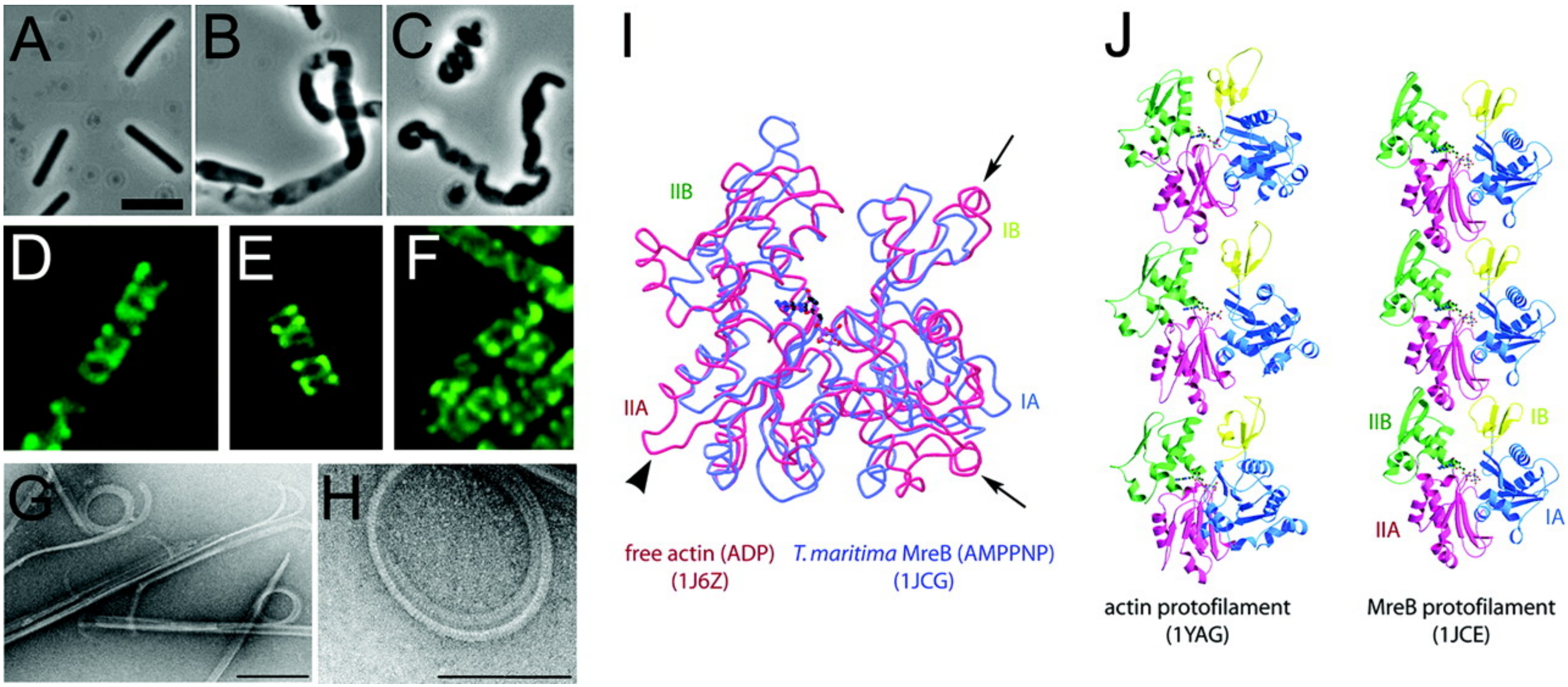


Fluorescing FtsZ ring in immobilized, live cells perpendicular to the plane of the coverslip.
Bar = 2 µm.

Sun and Margolin. 1998. J. Bacteriol., 180(8): 2050-2056.

MreB is a homolog (ancestor?) of actin

-cell shape determinant
-present in rod- and spiral-shaped cells but absent from cocci
-only 15% amino acid identity but similar 3D structure



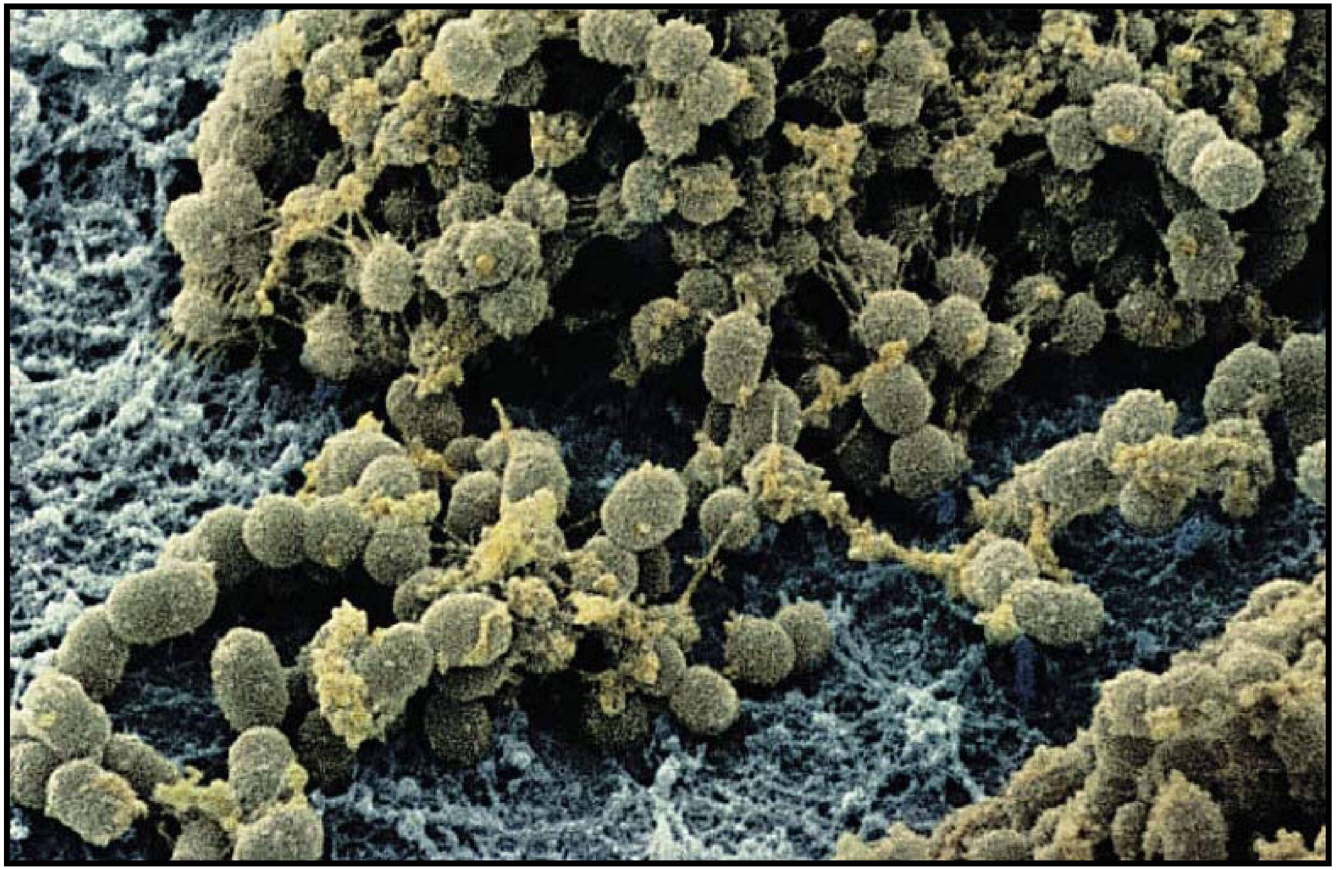
A-C: WT and mreB mutants of *B. subtilis* (note cell shapes)

D-F: Helical filaments formed by MreB-like proteins in *B. subtilis*

G&H: MreB filaments

I &J: Actin and MreB structures overlaid. Only 15% amino acid identity but similar 3D structure.

Biofilms



- **Capsule and fimbriae** promote adherence
- Bacterial communities
- Chemical communication alters lifestyle
- Protect against antimicrobics
- Colonization of medical equipment
- 2/3 of human infections
- Cystic fibrosis and dental plaque