

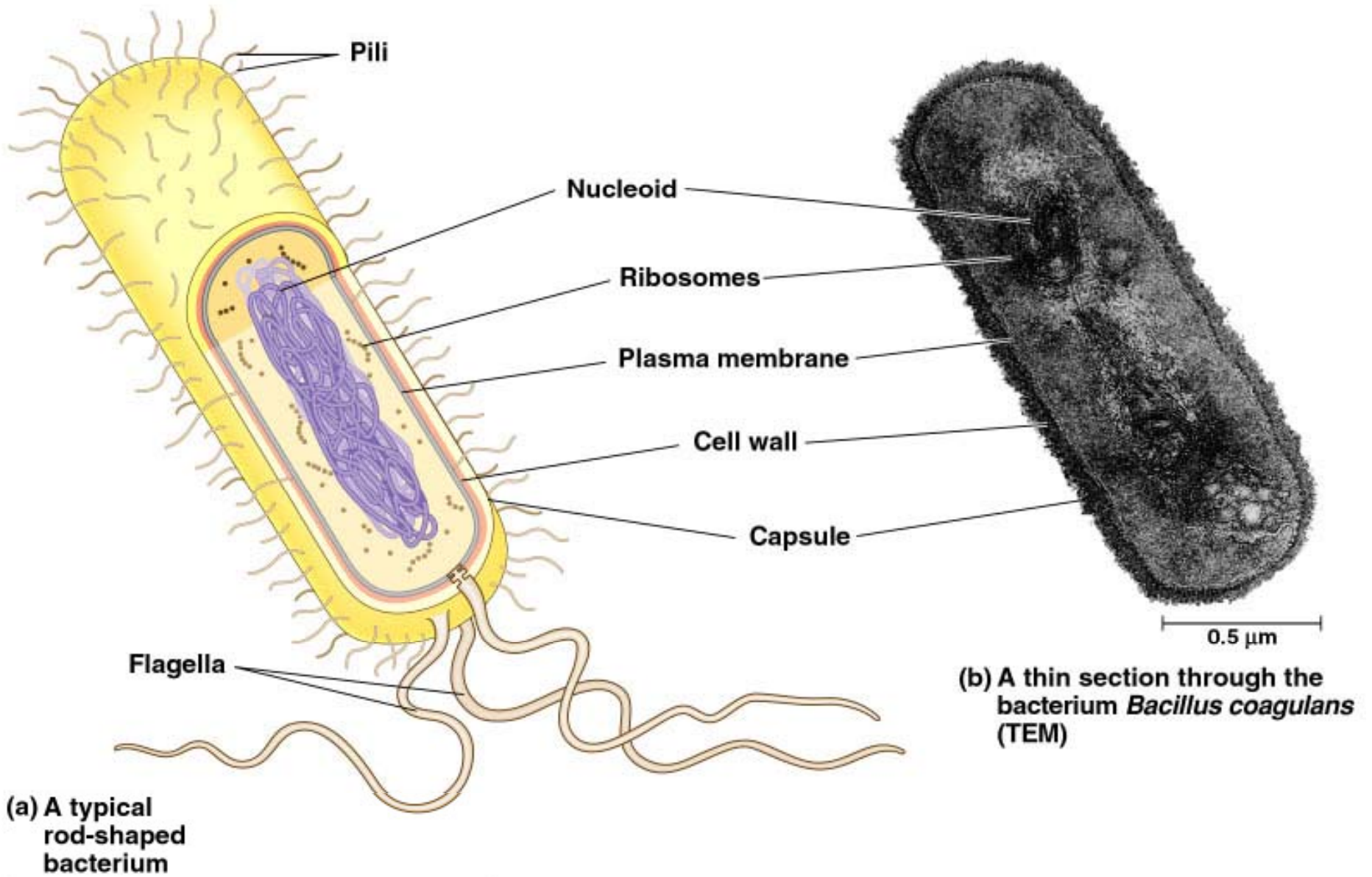
Comparing Bacteria, Archaea and Eucarya

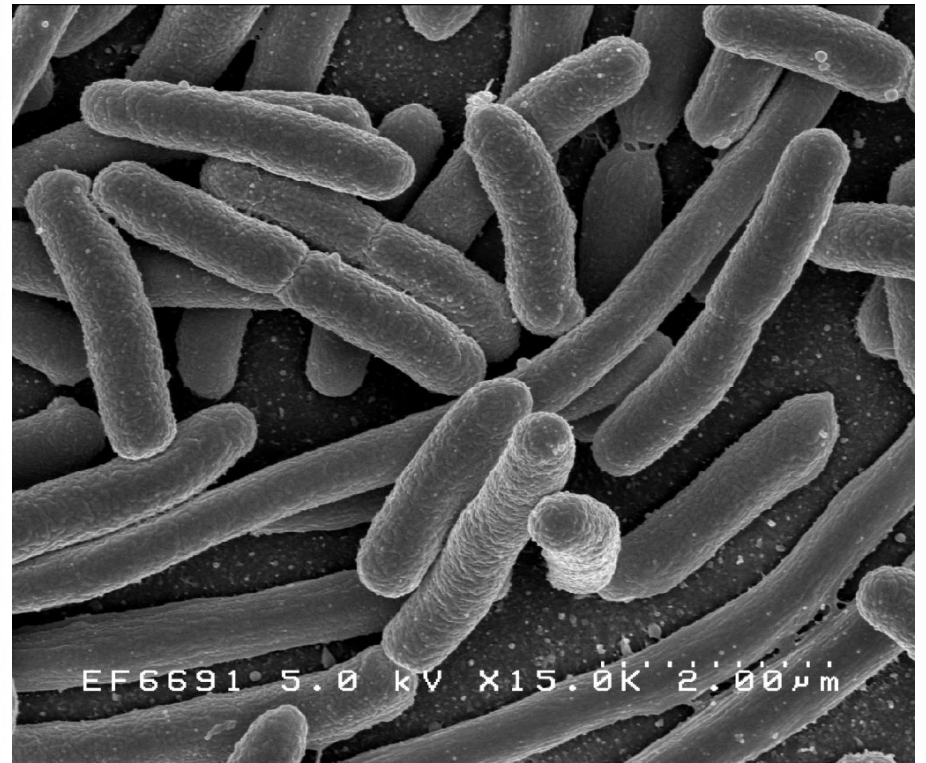
Basic unit of living organisms is the cell; the smallest unit capable of life.

“Features” found in all cells:

- **Ribosomes**
- **Cell Membrane**
- **Genetic Material**
- **Cytoplasm**
- **ATP Energy**
- **External Stimuli**
- **Regulate Flow**
- **Reproduce**

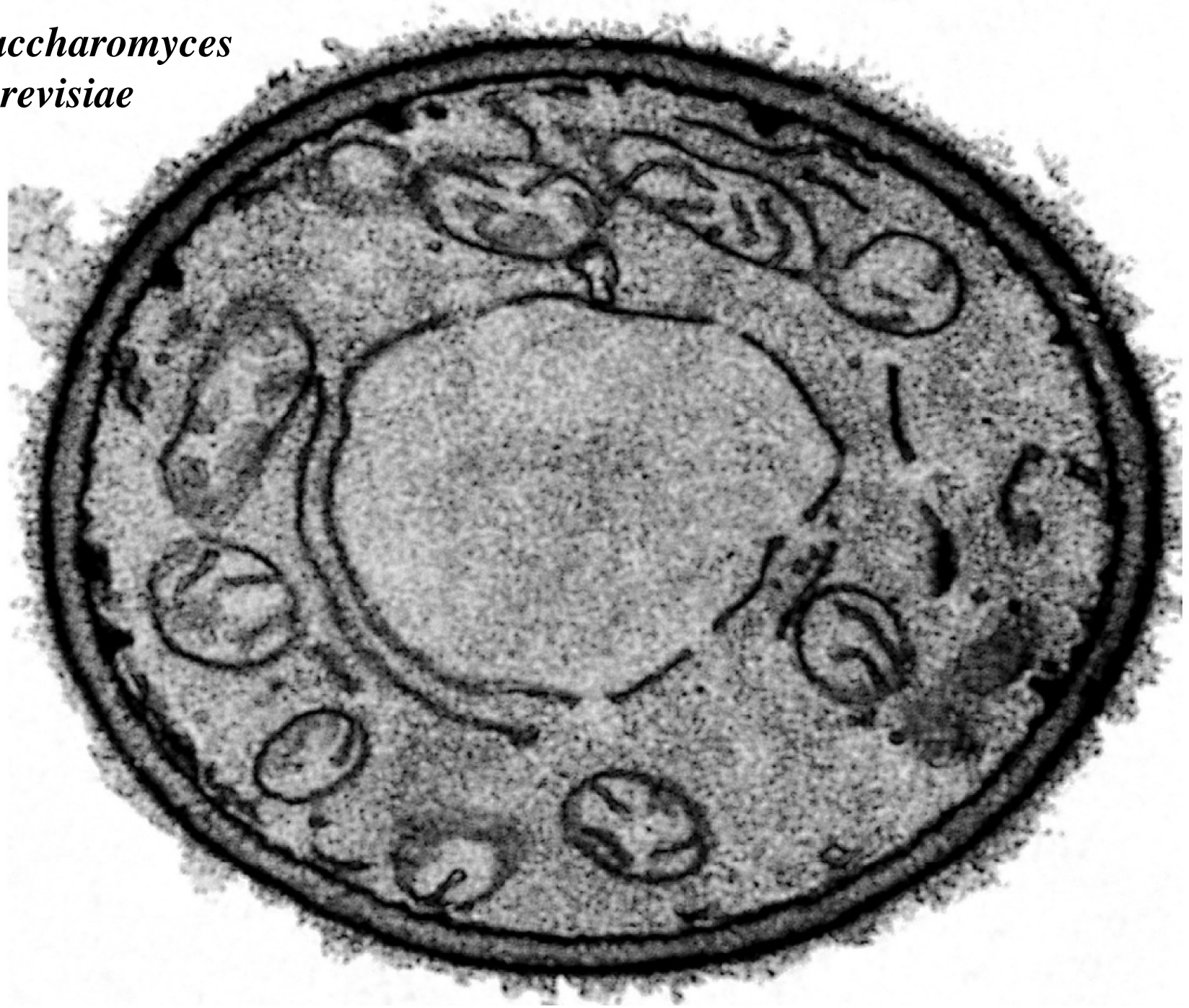
A Bacterial Cell



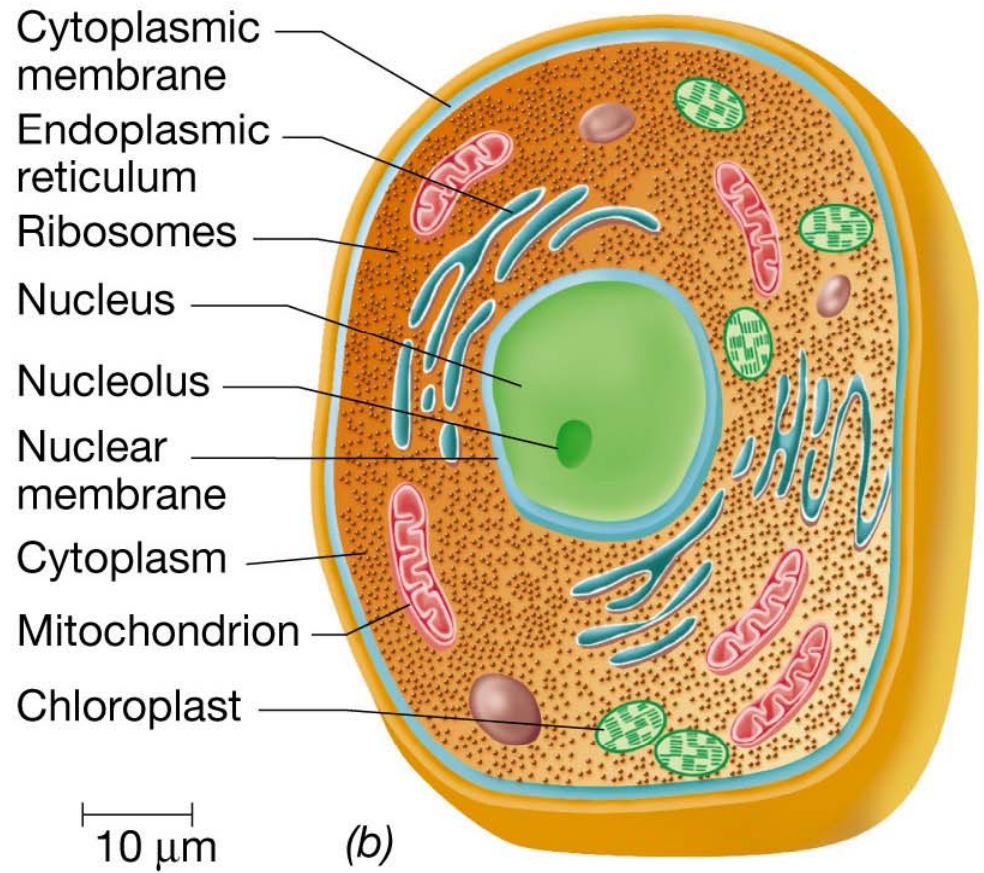
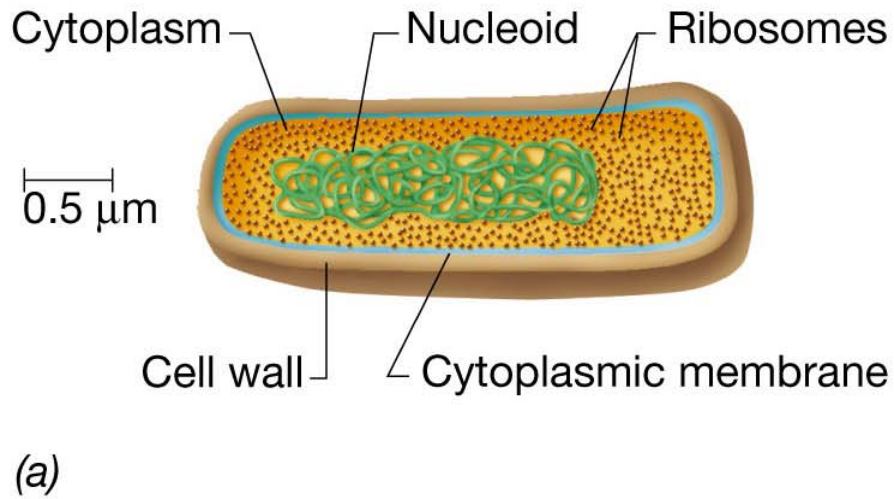


*Escherichia
coli*

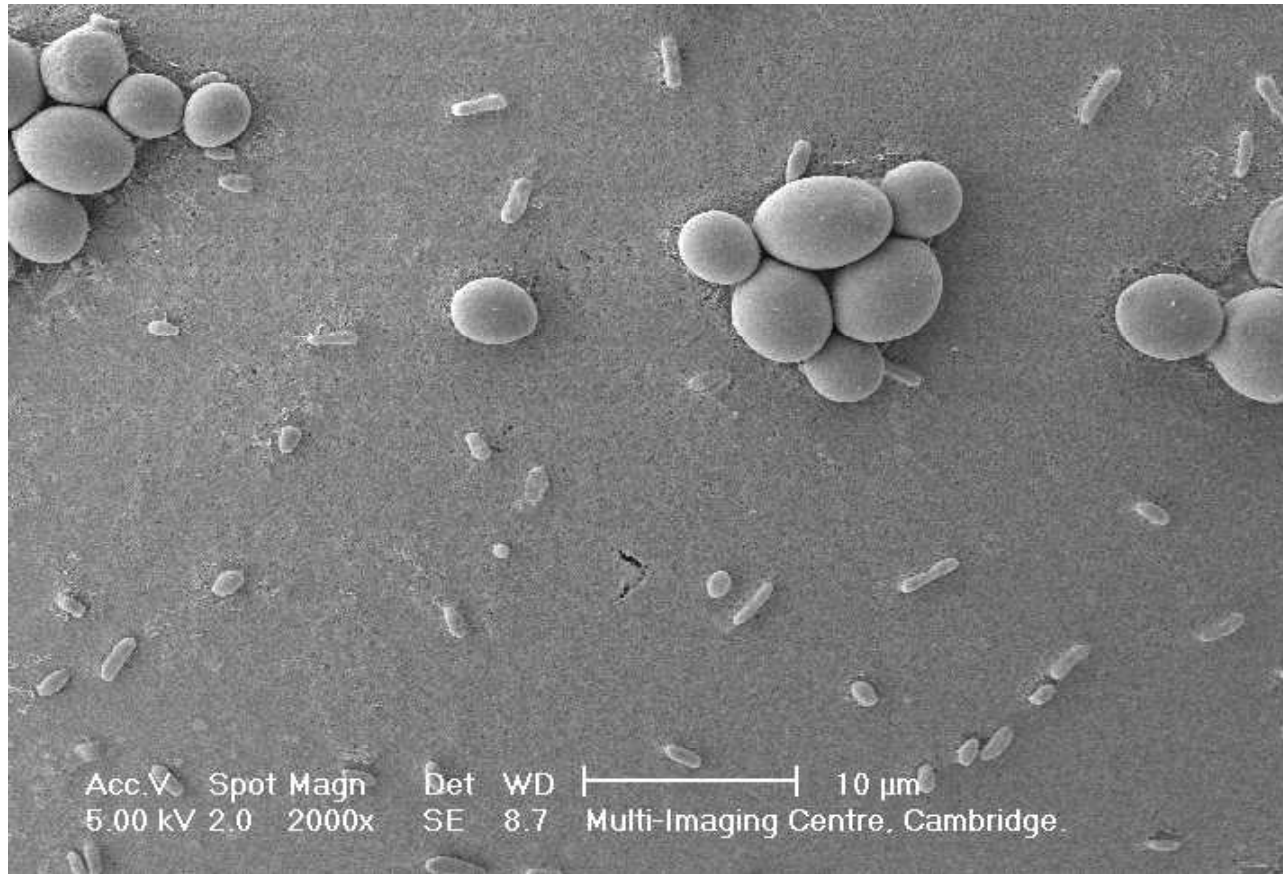
Saccharomyces cerevisiae



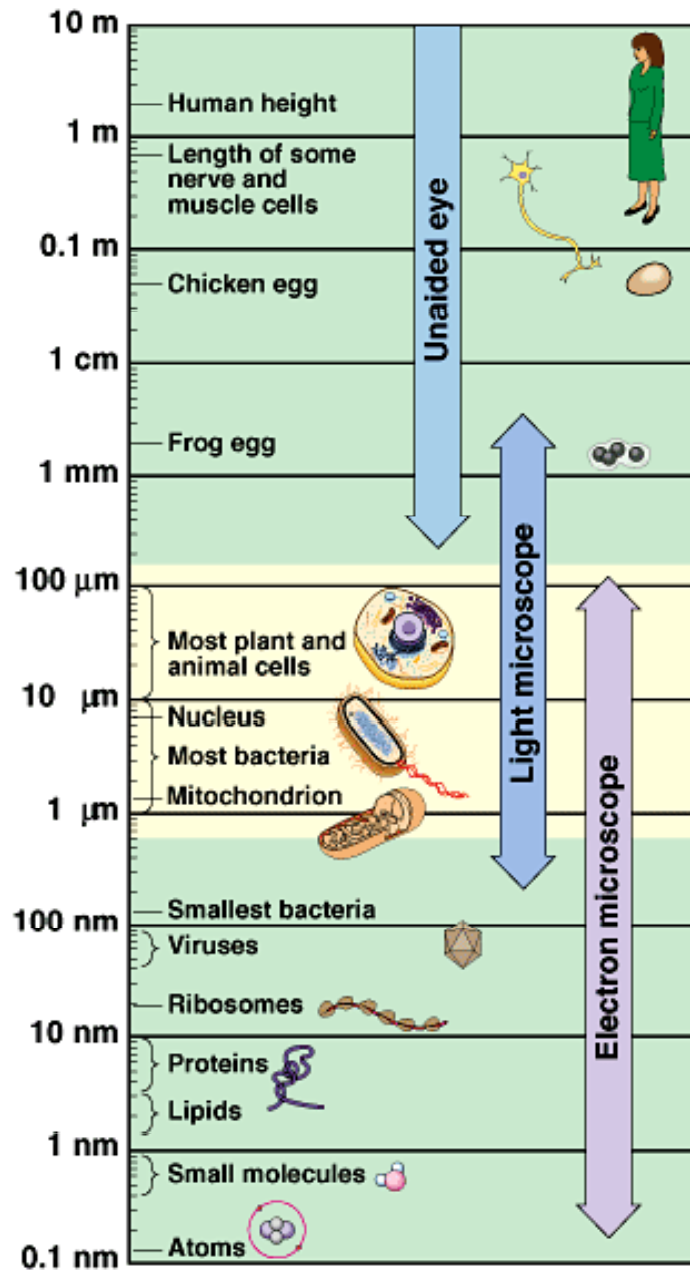
Elements of cellular structure



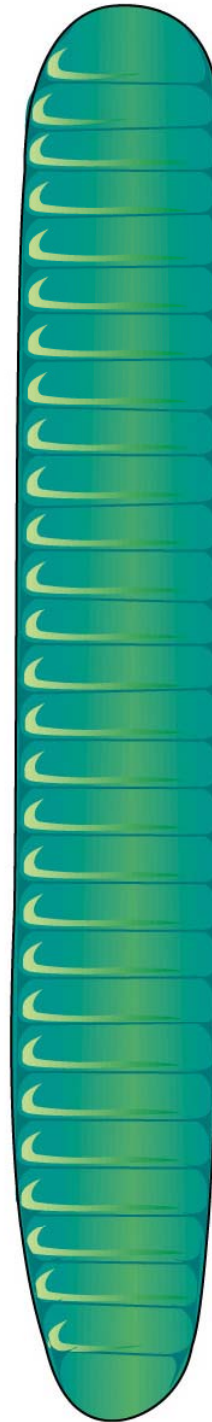
E. coli and S. cerevisiae



The size range of cells



Oscillatoria (a cyanobacterium)
8 × 50 μm



Bacillus megaterium
1.5 × 4 μm



Escherichia coli
1 × 3 μm



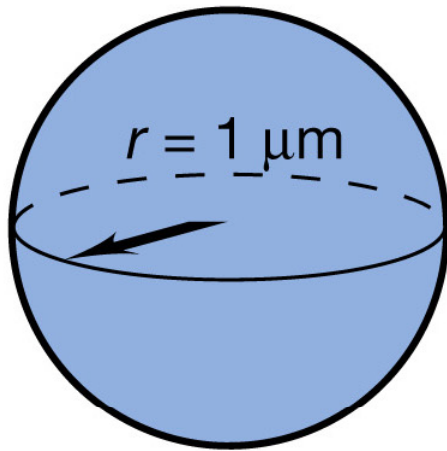
Streptococcus pneumoniae
0.8 μm diameter



Haemophilus influenzae
0.25 × 1.2 μm



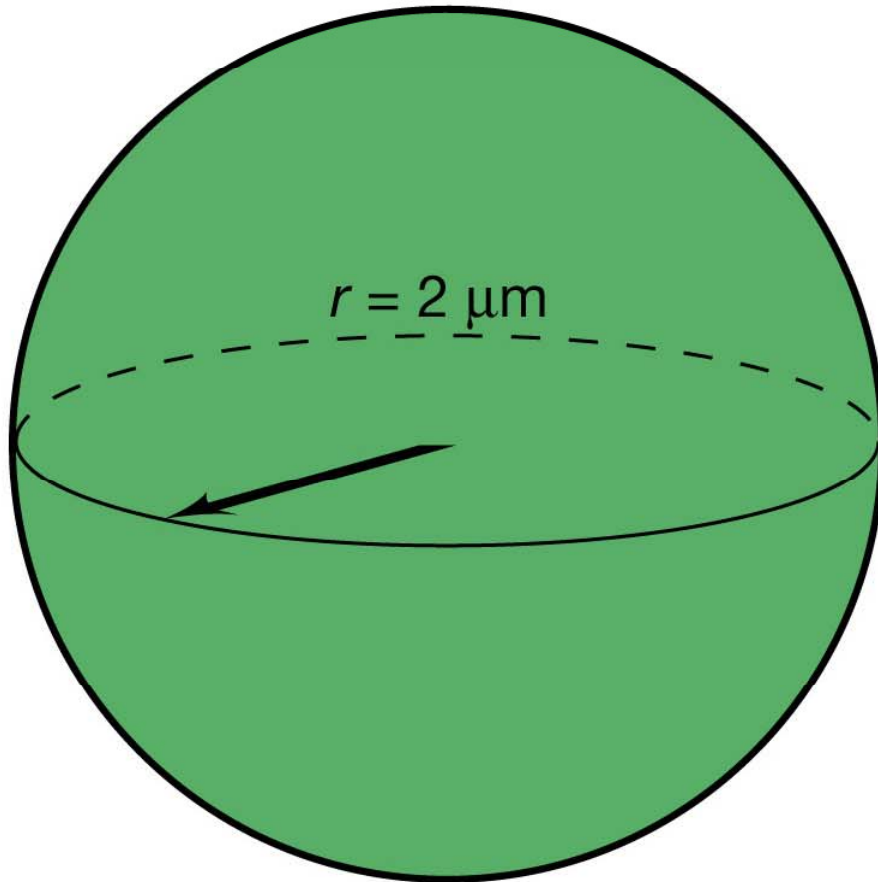
Size relationship among Bacteria



$$\text{Surface area } (4\pi r^2) = 12.6 \mu\text{m}^2$$

$$\text{Volume } \left(\frac{4}{3}\pi r^3\right) = 4.2 \mu\text{m}^3$$

$$\frac{\text{Surface}}{\text{Volume}} = 3$$

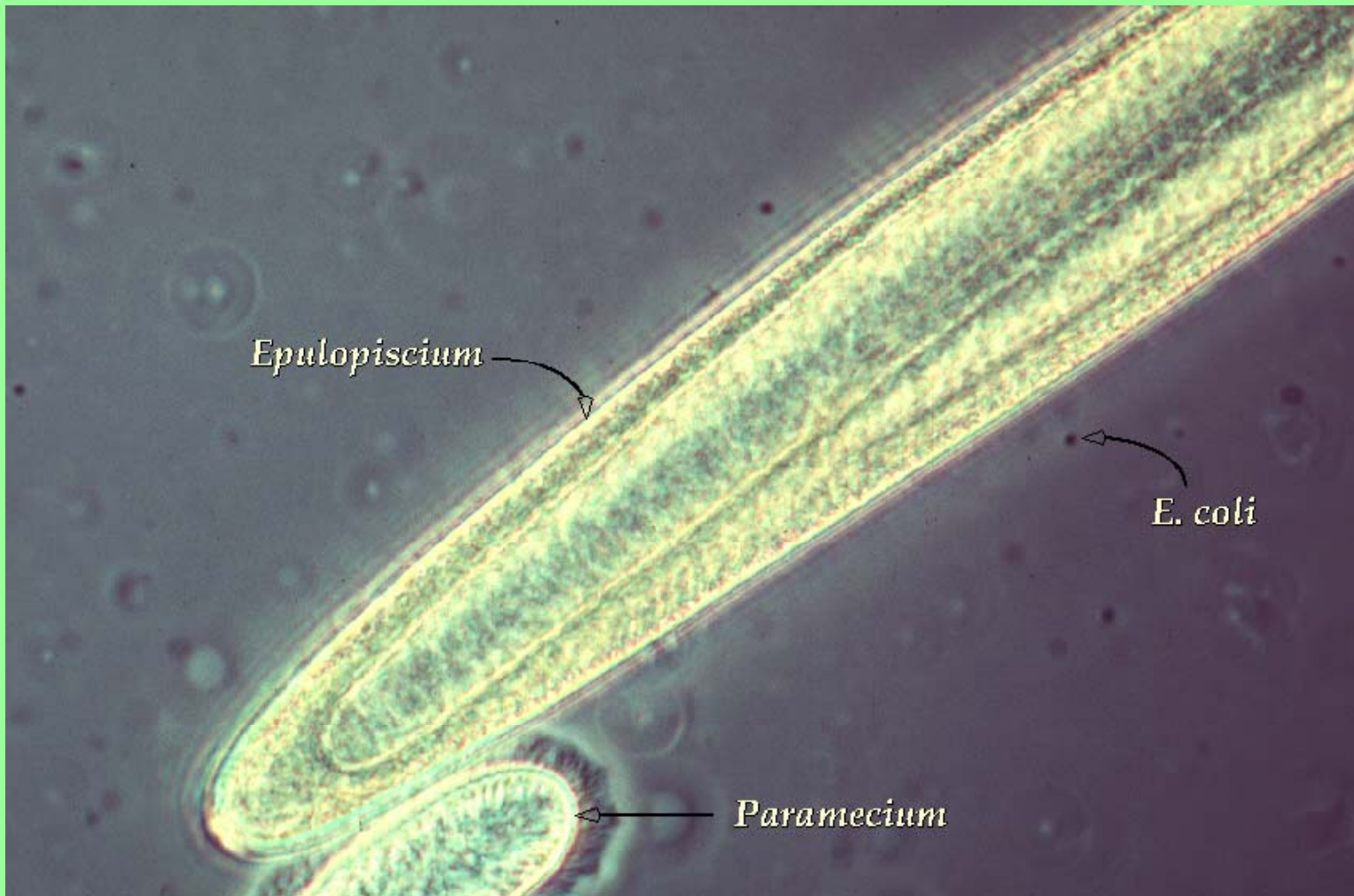


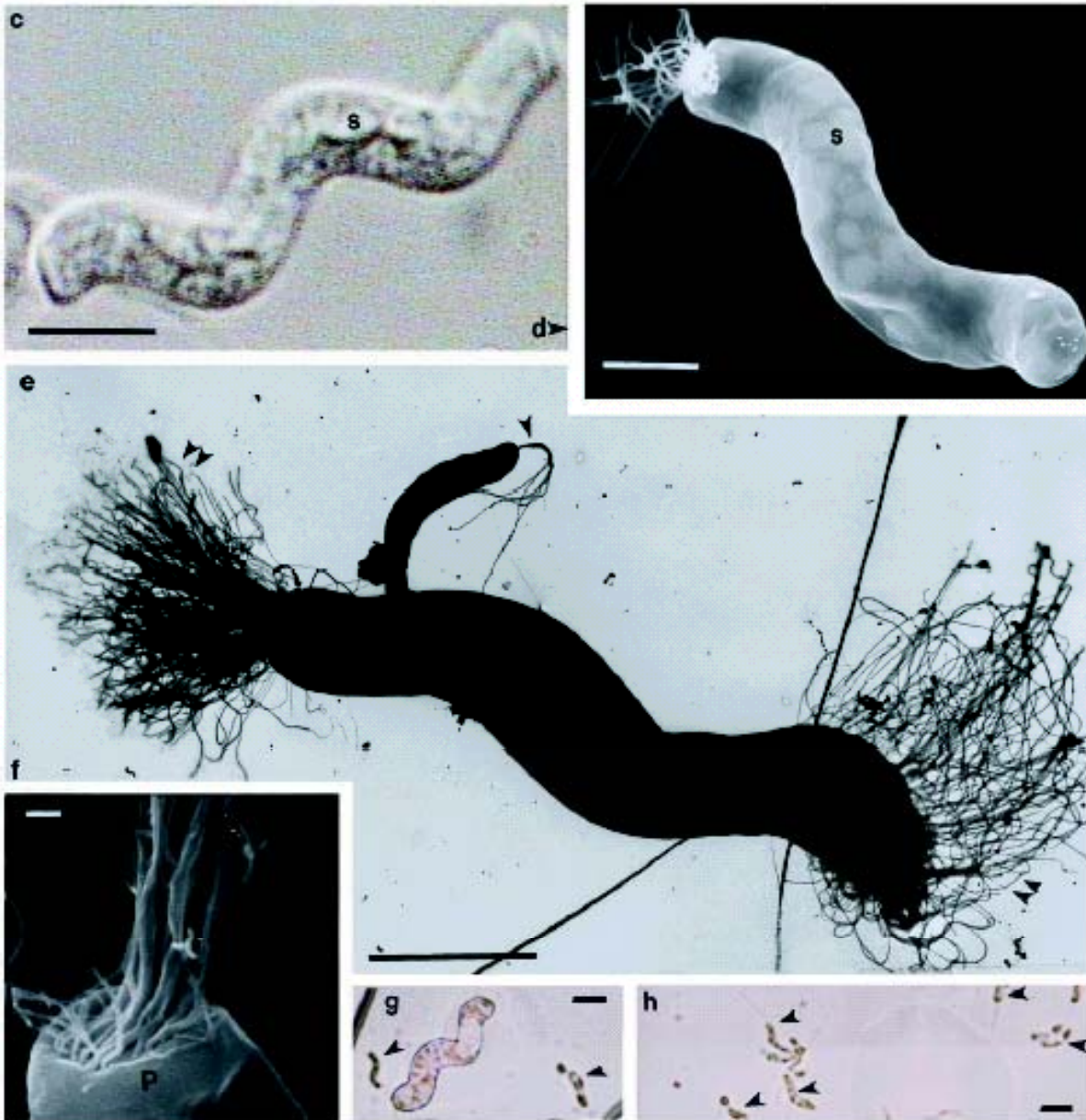
$$\text{Surface area} = 50.3 \mu\text{m}^2$$

$$\text{Volume} = 33.5 \mu\text{m}^3$$

$$\frac{\text{Surface}}{\text{Volume}} = 1.5$$

A Million times bigger than E. coli!





Titanospirillum velox

Up to 40 μm long

FIG. 1. (a) Mat surface at the Ebro Delta field site (3) showing lack of standing water. (Bar = 10 cm.) (b) Two spirilla cells (S, sulfur globule) shown by differential interference contrast (Nomarski). (Bar = 5 μm .) (c) Phase contrast microscopy of live spirillum cells. (Bar = 5 μm .) (d) Bipolar lophotrichous large spirillum in which only one pole has retained flagella. Sulfur globules are visible through the cell wall (scanning electron micrograph). (Bar = 5 μm .) (e) Negative-stain transmission electron micrograph of an entire bipolar lophotrichous large spirillum showing flagella "braids" (double arrowheads) compared with standard-sized spirilla (single arrowhead). (Bar = 5 μm .) (f) This scanning-electron micrograph of a cell terminus shows one vaulted end with residual flagella. The indentation coated by the polar organelle (P; see Fig. 2) is implied. (Bar = 0.5 μm .) (g) This Gram-stain brightfield preparation compares the two size classes, huge and standard, of Gram-negative spirilla. (Bar = 5 μm .) (h) Standard-sized spirillum Gram stain. The lighter spots are probably sulfur globules. (Bar = 5 μm .)

Thiomargarita namibiensis

Up to 500 μm wide

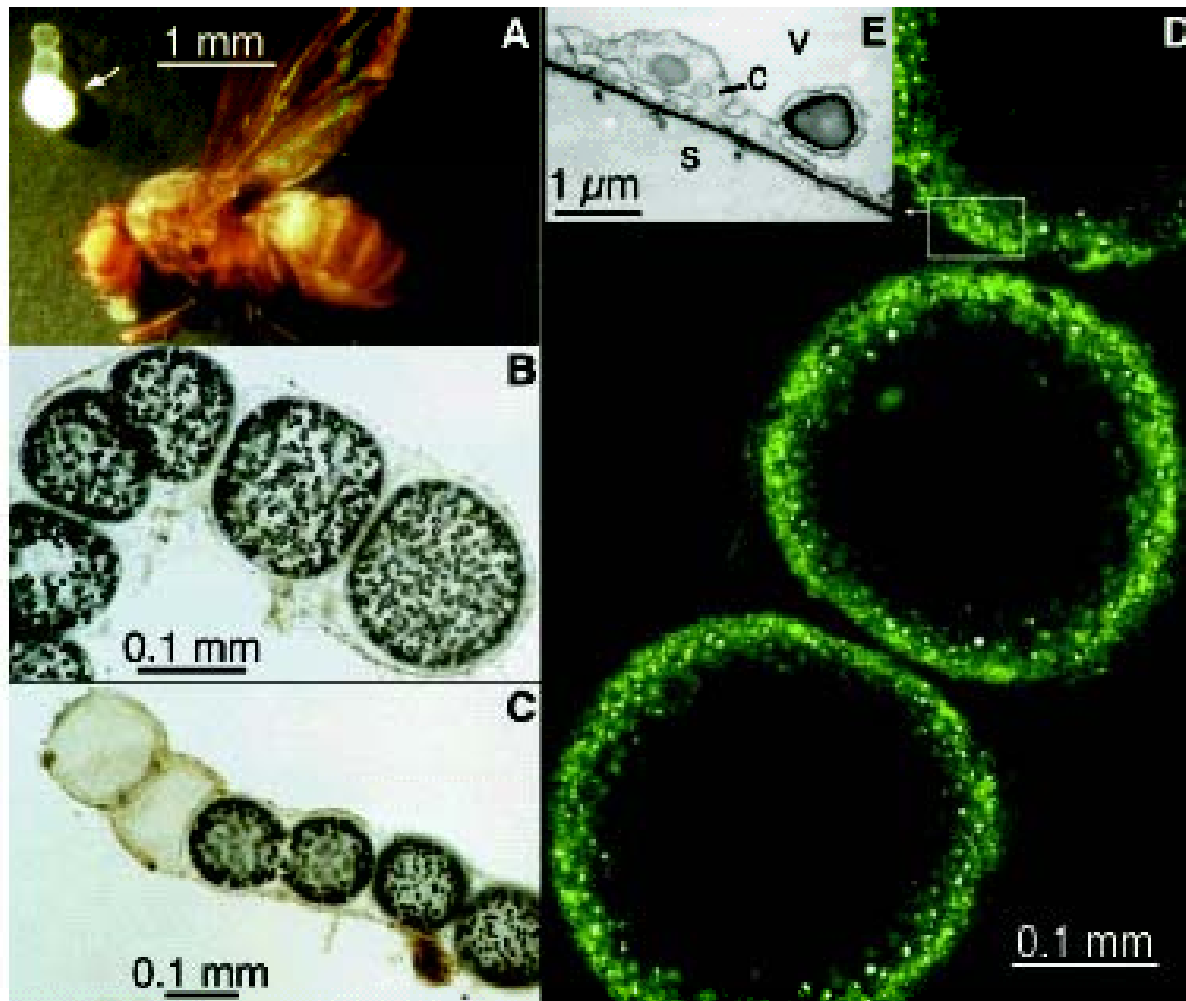
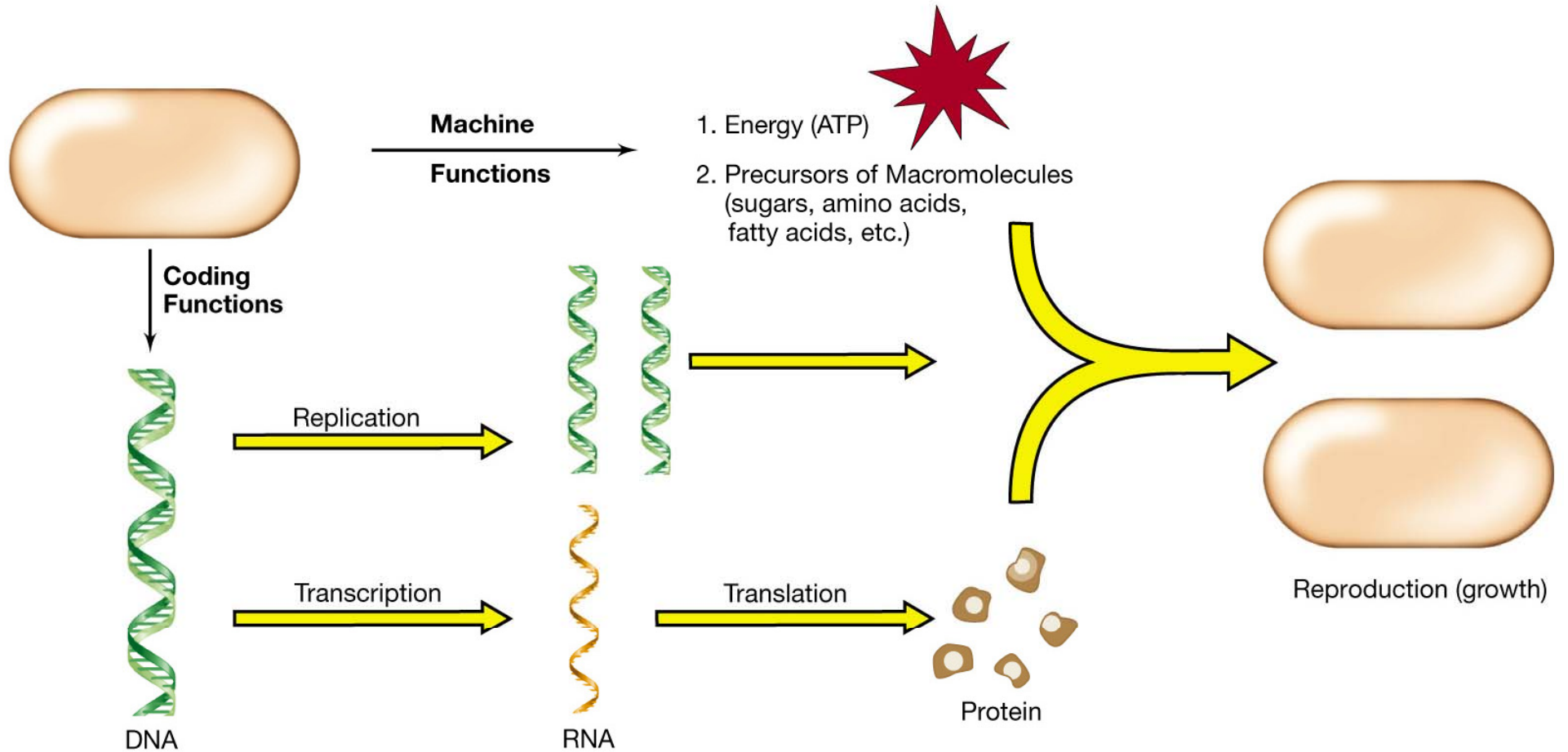


Fig. 1. *Thiomargarita namibiensis*. (A) The white arrow points to a single cell of *Thiomargarita*, 0.5 mm wide, which shines white because of internal sulfur inclusions. Above there is an empty part of the sheath, where the two neighboring cells have died. The cell was photographed next to a fruit fly (*Drosophila virilis*) 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).

The machine/coding functions of the cell



Central Dogma

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

Table 3.1 Molecular composition of a bacterial cell, *Escherichia coli*, during balanced exponential growth.^a

Component	Percentage of total weight ^b	Approximate number of molecules/cell	Number of different kinds
Water	70%	20,000,000,000	1
Proteins	16%	2,400,000	2,000 ^c
RNA: rRNA, tRNA, and other small regulatory RNA (sRNA), mRNA	6%	250,000	200
	0.7%	4,000	2,000 ^c
Lipids: phospholipids (membrane)	3%	25,000,000	50
lipopolysaccharide (outer membrane)	1%	1,400,000	1
DNA	1%	2 ^d	1
Metabolites and biosynthetic precursors	1.3%	50,000,000	1,000
Peptidoglycan (murein sacculus)	0.8%	1	1
Inorganic ions	0.1%	250,000,000	20
Polyamines (mainly putrescine and spermine)	0.1%	6,700,000	2

Mostly rRNA

^aValues shown are for a hypothetical “average” cell cultured with aeration in glucose medium with minimal salts at 37°C.

^bThe total weight of the cell (including water) is about 10⁻¹² gram (g), or 1 picogram (pg).

^cThe number of kinds of mRNA and of proteins is difficult to estimate because some genes are transcribed at extremely low levels and because RNA and proteins include kinds that are rapidly degraded.

^dIn rapidly growing cells, cell fission typically lags approximately one generation behind DNA replication; hence, two identical DNA copies per cell.

Source: Modified from Neidhardt, F., and H. E. Umbarger. 1996. Chemical composition of *Escherichia coli*, p. 14. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd ed. ASM Press, Washington, DC.

Take Home Message:

Proteins are #1 by weight

Lipids are #1 by number

Peptidoglycan is 1 jumbo molecule

RNA is mostly ribosomes

DNA is also a huge polymer

Macromolecules in a typical bacterial cell:

Not just “soup” – highly ordered cytoplasm

Contents (e.g., proteins) will vary, depending on conditions.

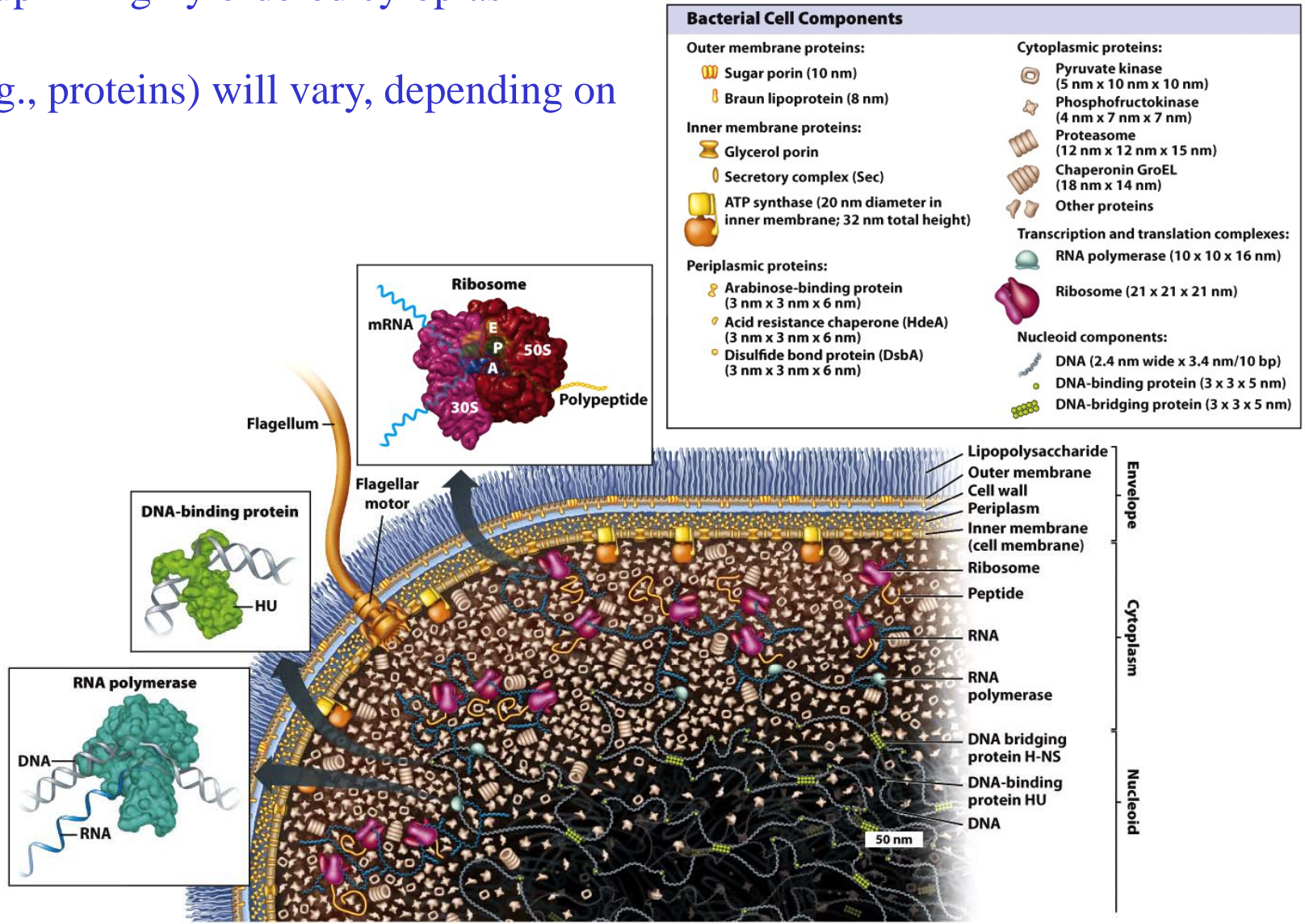
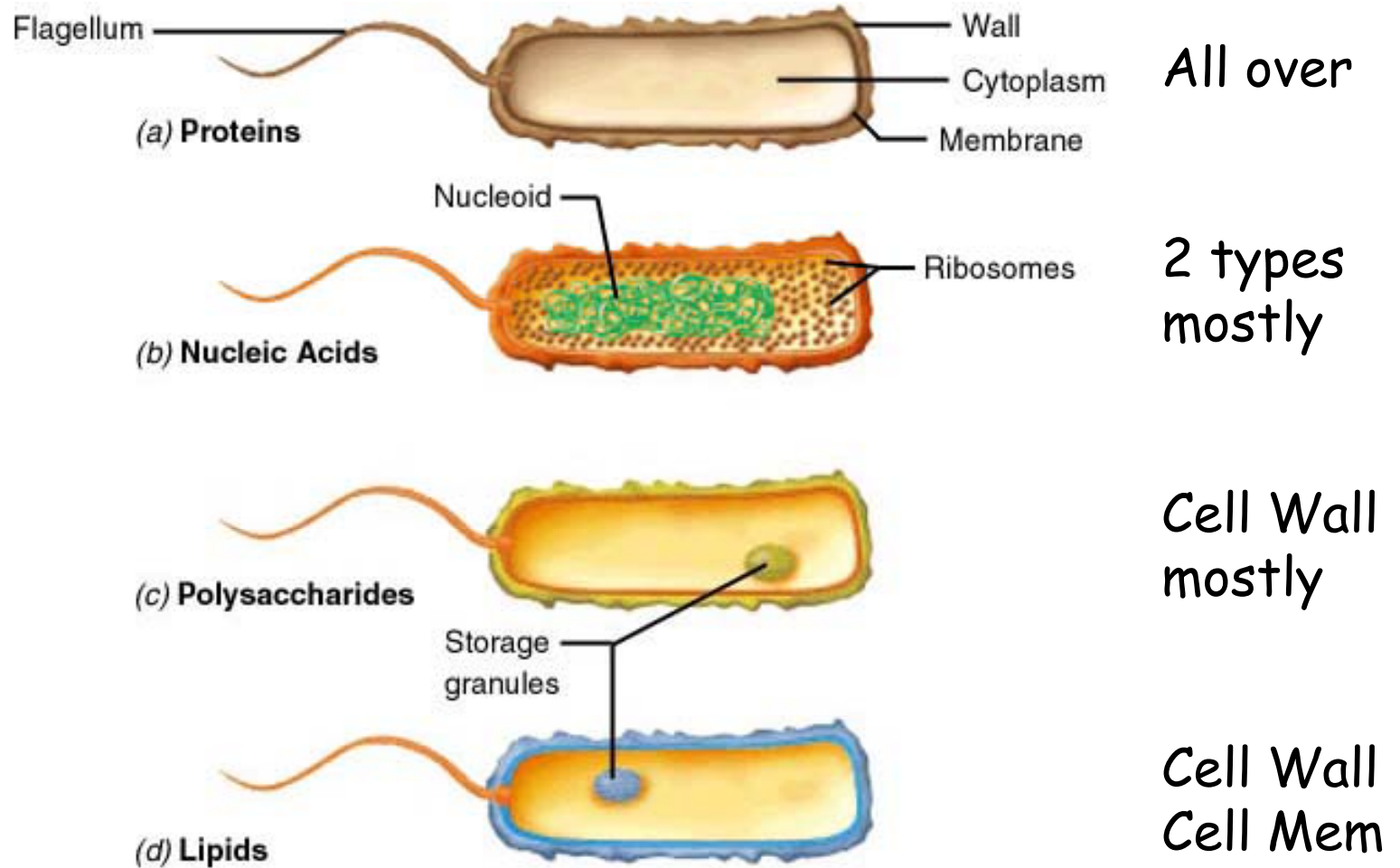


Figure 3.1 part 2 Microbiology: An Evolving Science
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Locations of macromolecules in the cell



Comparing Bacteria, Archaea and Eucarya

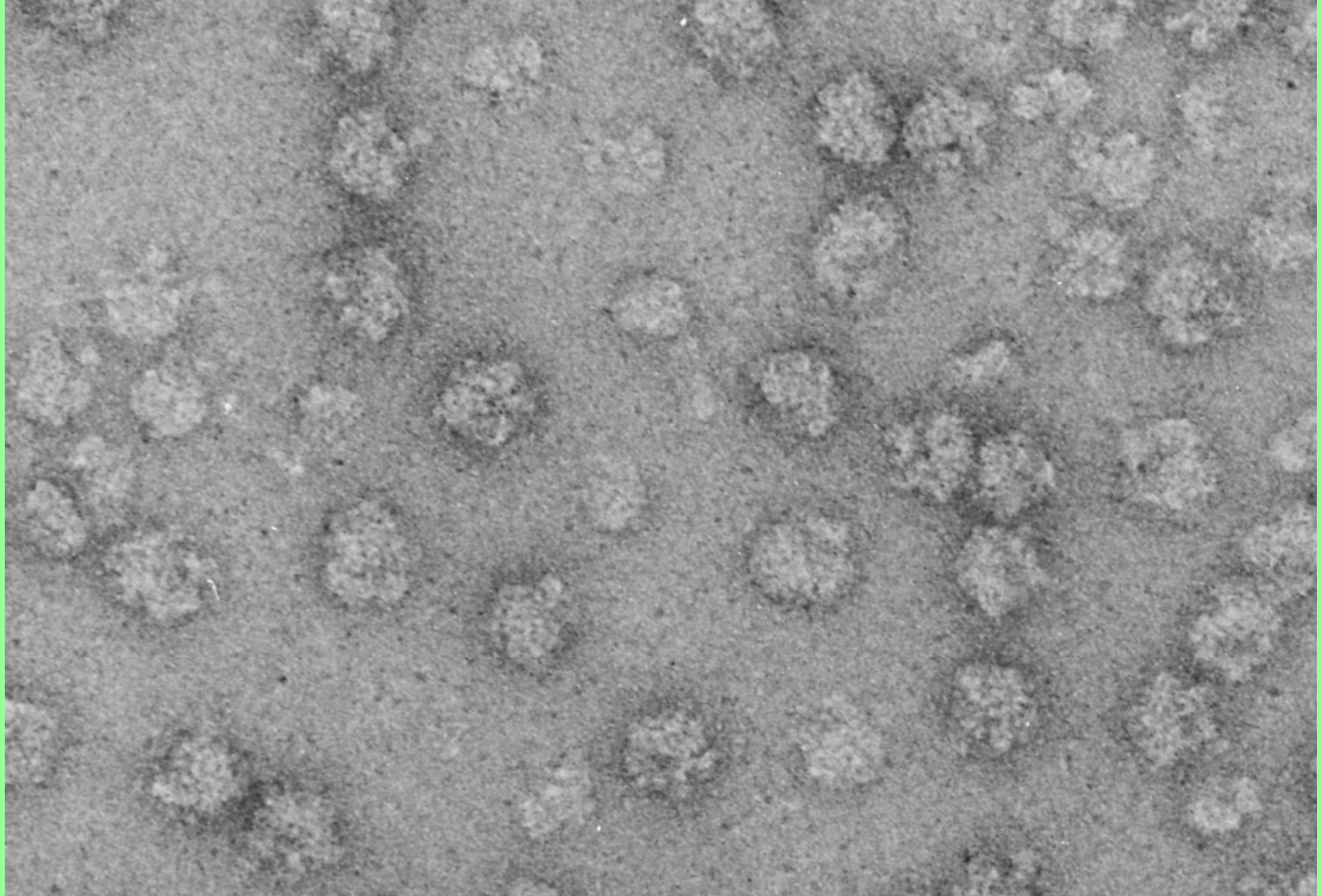
**Classification of microbial cellular features:
Invariant (or common to all)**

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

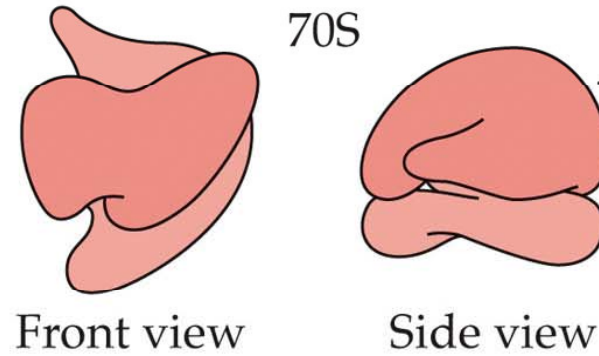
**Cell Membranes: The barrier between order
and chaos.**

Nucleoid Region: Curator of the Information.

Ribosome structure



(B) Prokaryotic ribosome
(*Escherichia coli*)



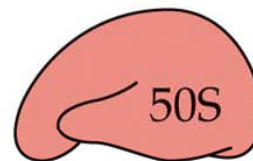
70S ribosome has two subunits.

Ribosome

30S subunit consists of 16S rRNA and 21 proteins.

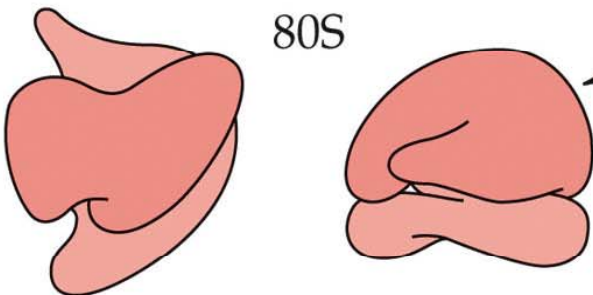


50S subunit consists of 23S rRNA, 5S rRNA, and 31 proteins.



Subunits

(C) Eukaryotic ribosome
(Rat)



80S

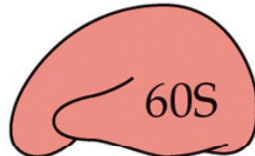
80S ribosome has two subunits.

Ribosome

40S subunit consists of 18S rRNA and 33 proteins.



40S




60S

60S subunit consists of 28S rRNA, 5.8S rRNA, and 49 proteins.

Subunits

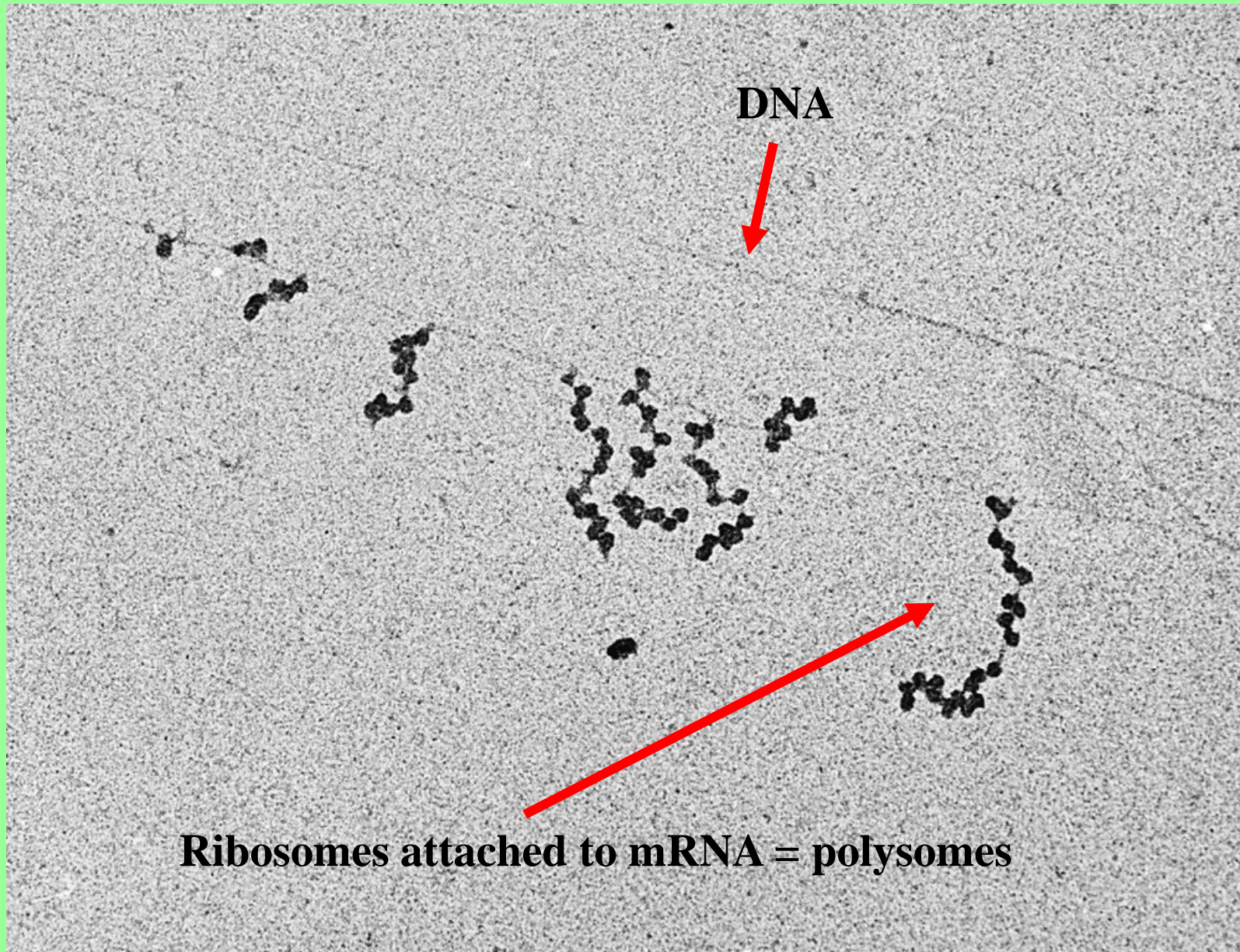
Table 7.6 Ribosome structure^a

Property	Prokaryote	Eukaryote
Overall size	70S	80S Most Complex
Small subunit	30S	40S
Number of proteins	~21	~30
RNA size (number of bases)	16S (1500)	18S (2300)
Large subunit	50S	60S
Number of proteins	~34	~50
RNA size (number of bases)	23S (2900)	28S (4200)
	5S (120)	5.8S (160)
		5S (120)

^a Ribosomes of mitochondria and chloroplasts of eukaryotes are similar to prokaryotic ribosomes ( Section 14.4).

S= Svedberg; a sedimentation coefficient that is NOT ADDITIVE!!!

Protein synthesis



Comparing Bacteria, Archaea and Eucarya

**Classification of microbial cellular features:
Invariant (or common to all)**

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

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

Nucleoid Region: Curator of the Information.

The Cytoplasmic Membrane

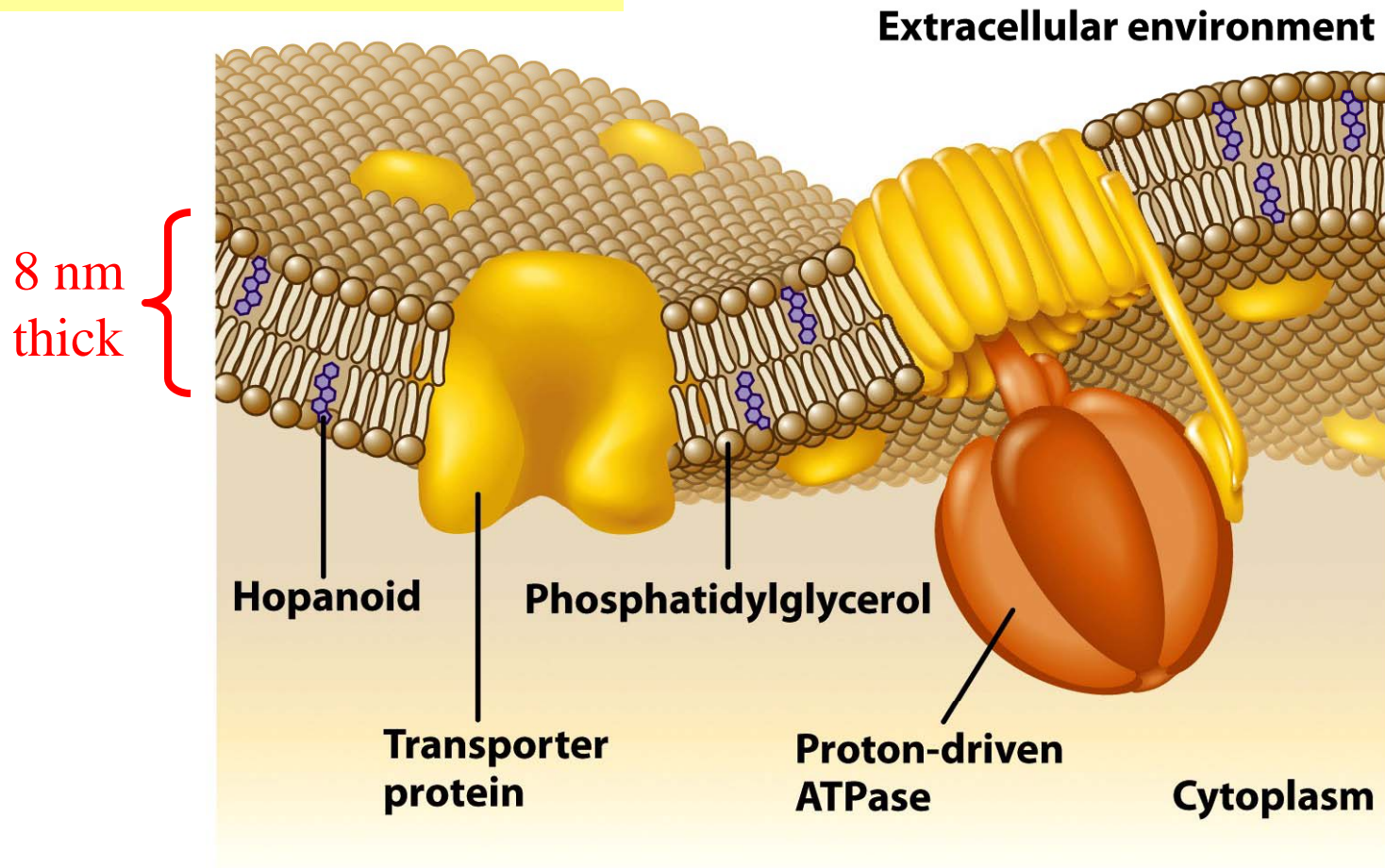


Figure 3.7 Microbiology: An Evolving Science
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Membrane has similar viscosity as oil: **Fluid Mosaic Model**

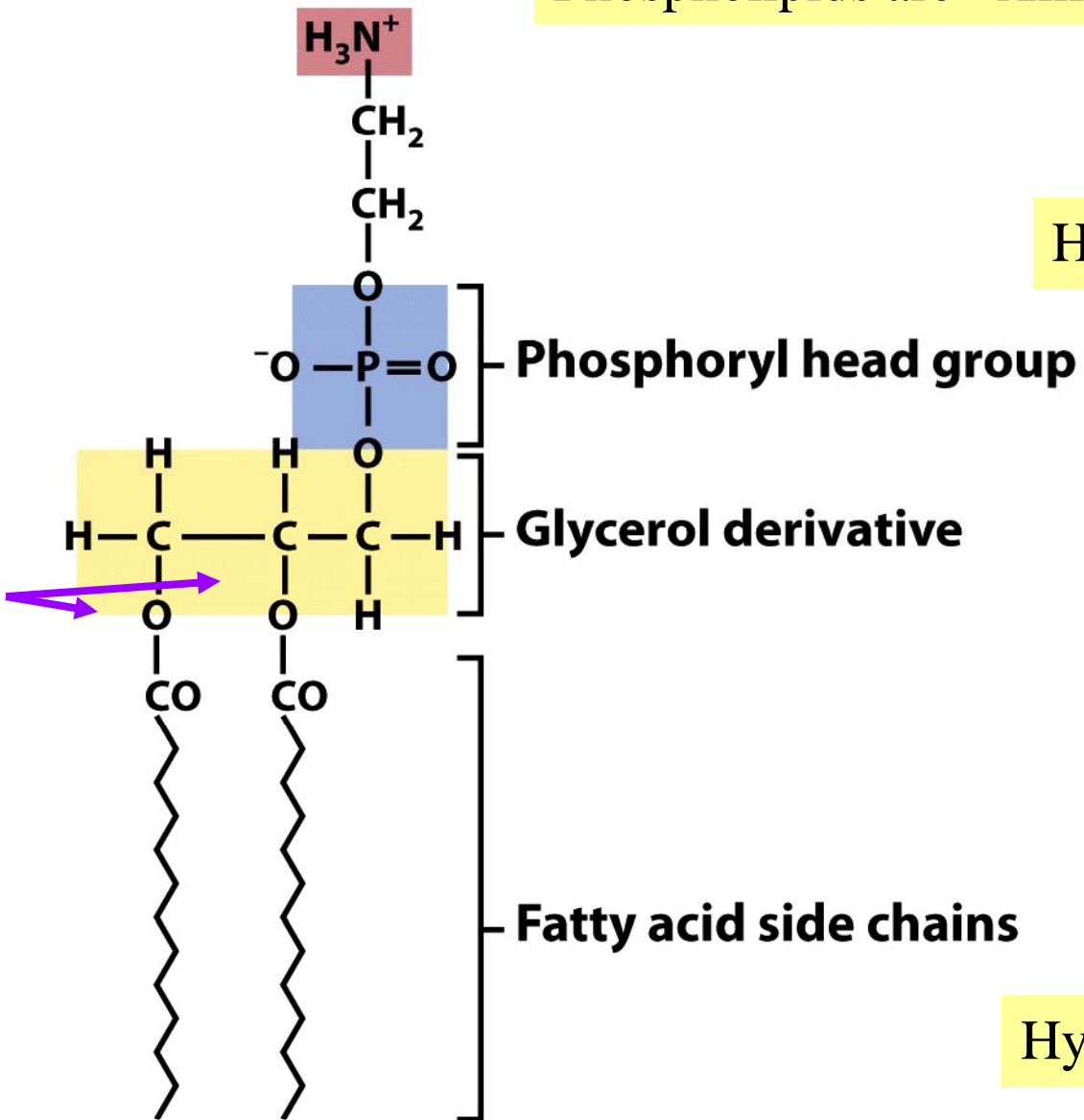
Stabilized by H bonds, hydrophobic interactions, and by Mg^{++} and Ca^{++} binding to phosphate heads

Phospholipids are “Amphipathic”

Hydrophilic

Hydrophobic

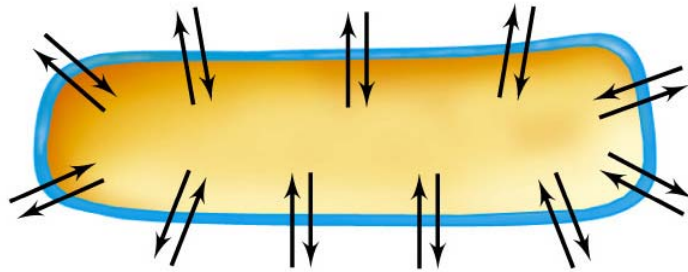
Ester linkages



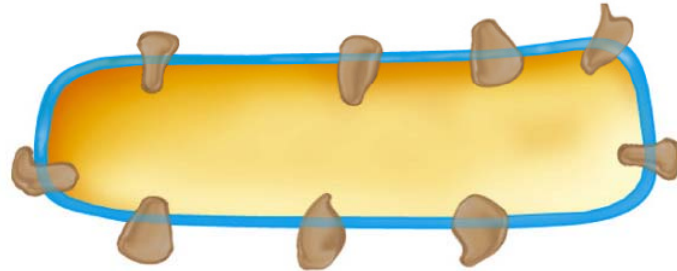
Phosphatidylethanolamine

Figure 3.8 Microbiology: An Evolving Science
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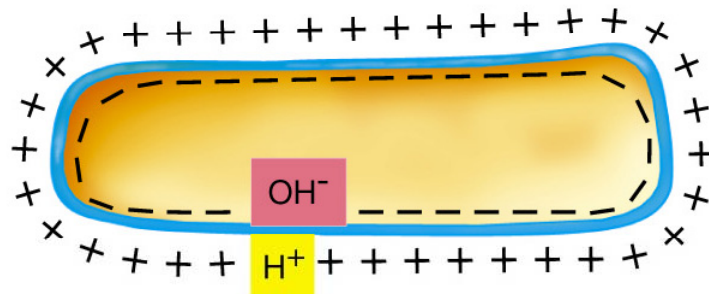
Functions of the cytoplasmic membrane



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

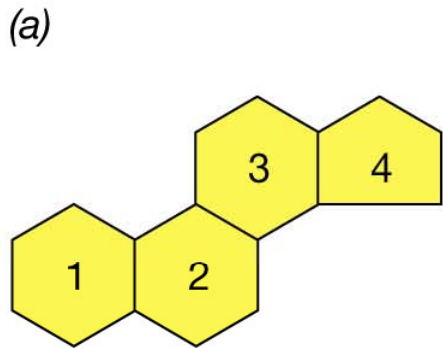
Energy Conservation — Site of generation and use of the proton motive force

Table 4.2**Comparative permeability of membranes to various molecules**

Substance	Rate of permeability^a	
Water	100	← Free diffusion of water (passive transport) assisted by aquaporins
Glycerol	0.1	
Tryptophan	0.001	} Active or passive transport (depends on conditions), but proteins aid movement
Glucose	0.001	
Chloride ion (Cl ⁻)	0.000001	
Potassium ion (K ⁺)	0.0000001	
Sodium ion (Na ⁺)	0.00000001	

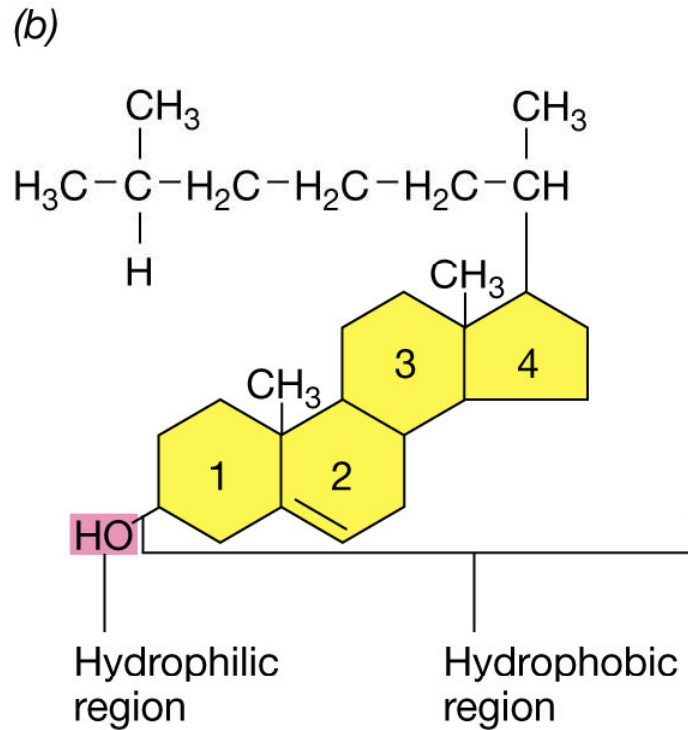
^a Relative scale—permeability with respect to permeability of water given as 100. Permeability of the membrane to water may be affected by aquaporins (see text).

Sterol

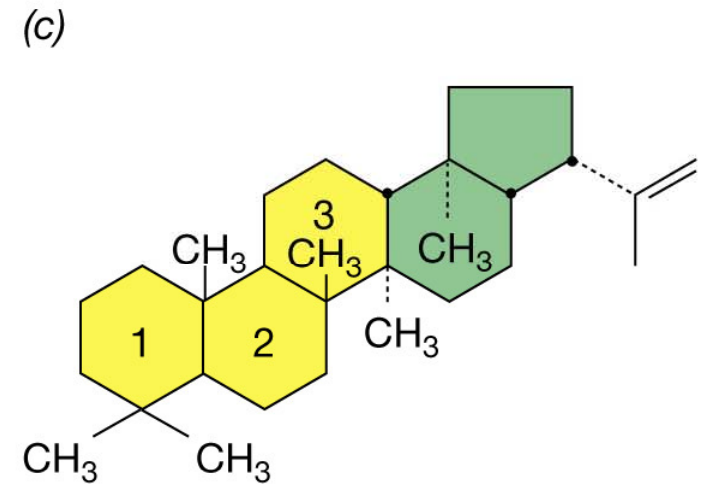


Few Bacteria

Cholesterol



Hopanoid (e.g., Diploptene)

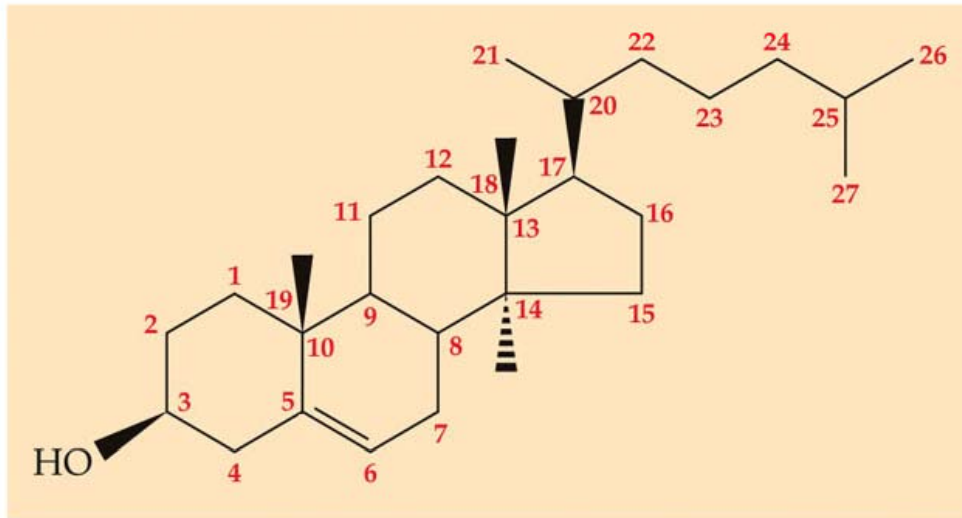


Many Bacteria

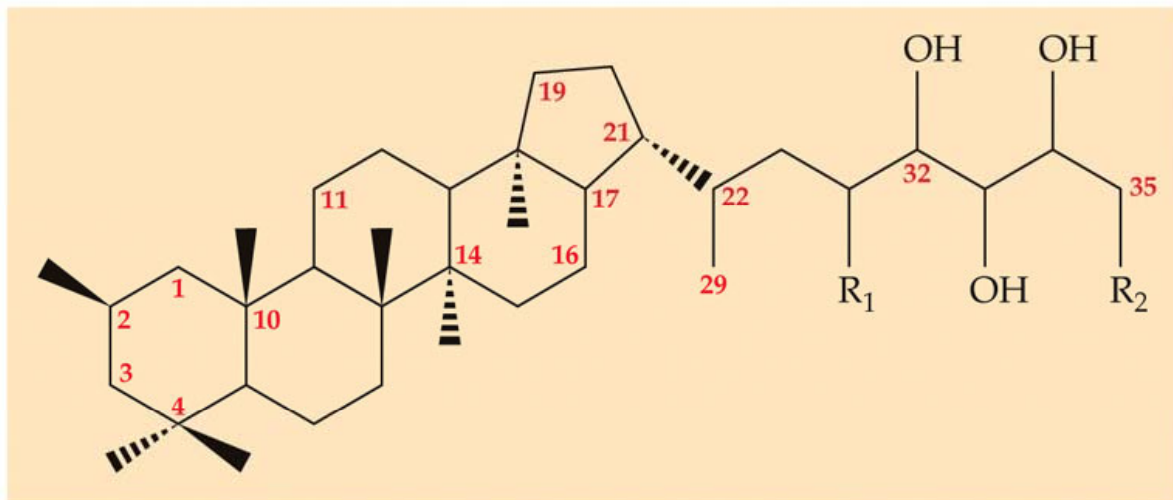
O₂ -

All rigid planar molecules

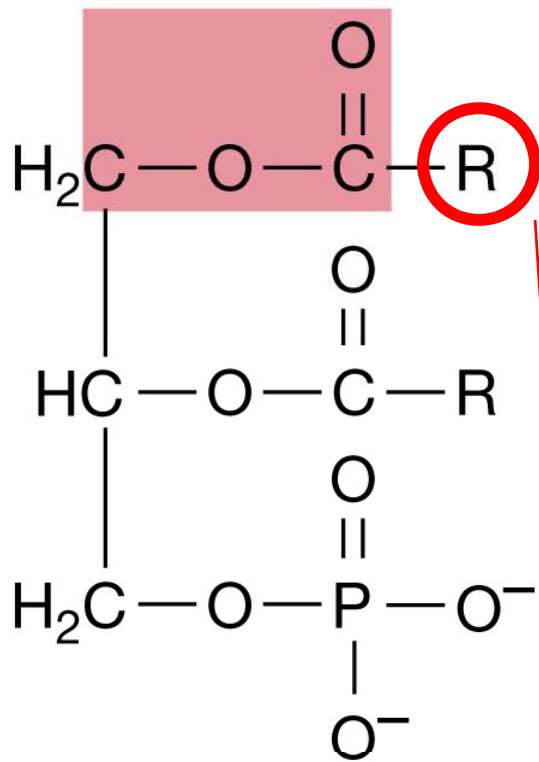
(A) Cholesterol



(B) A hopanoid from a cyanobacterium



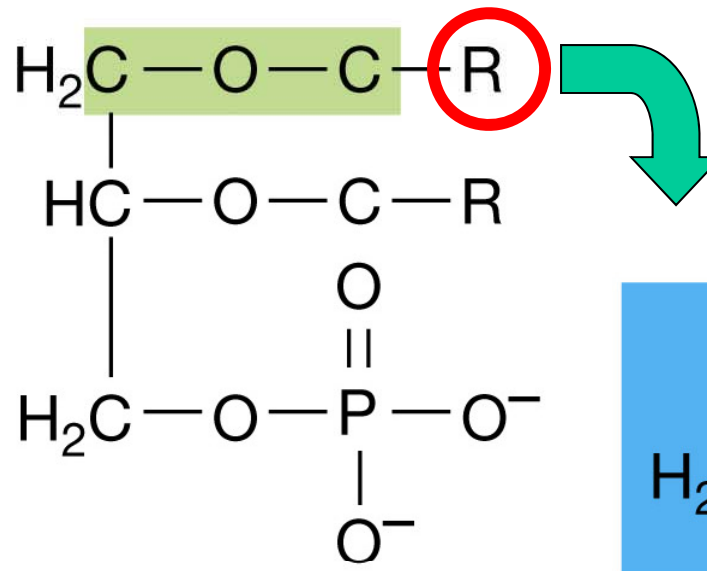
Ester Linkage



(a)

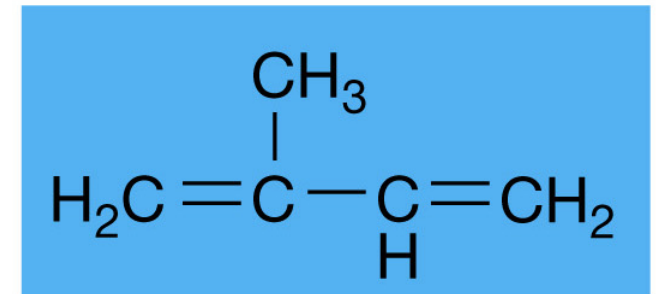
Fatty Acid

Ether Linkage



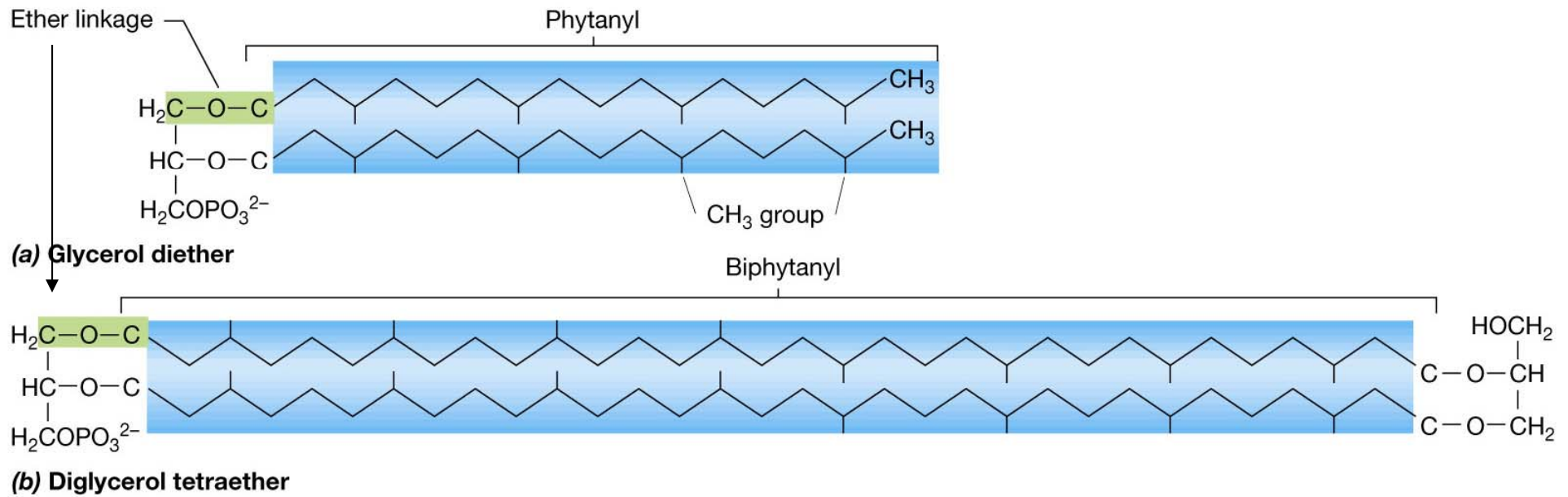
(b)

Isoprene Unit



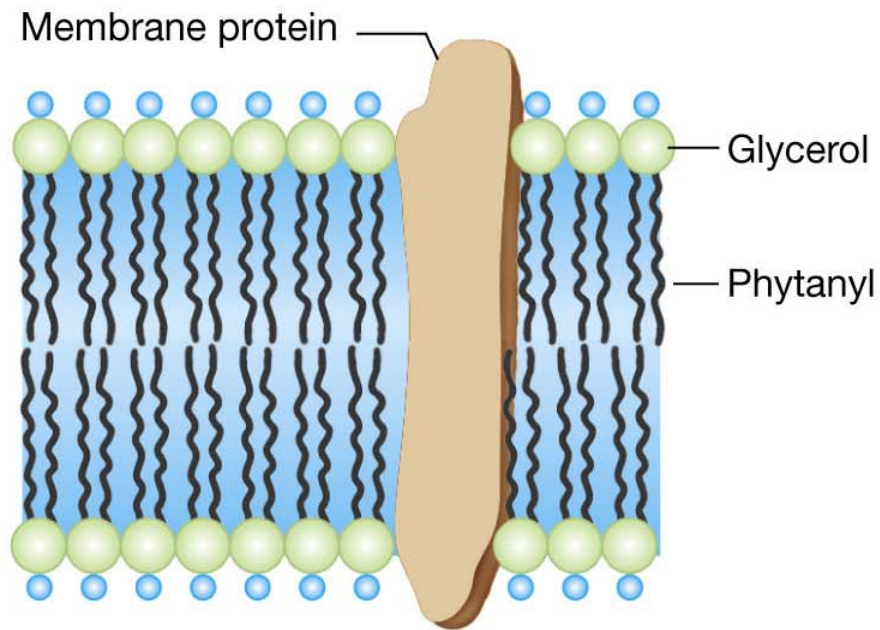
(c)

Major lipids of *Archaea* and the structure of archaeal membranes

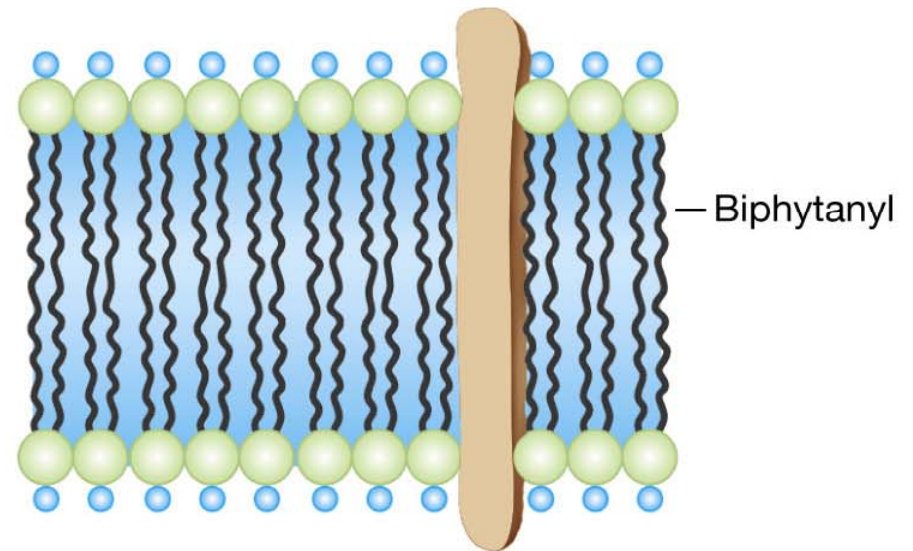


Lipid monolayer,
not a bilayer:
mostly found in
extremophiles

Major lipids of *Archaea* and the structure of archaeal membranes



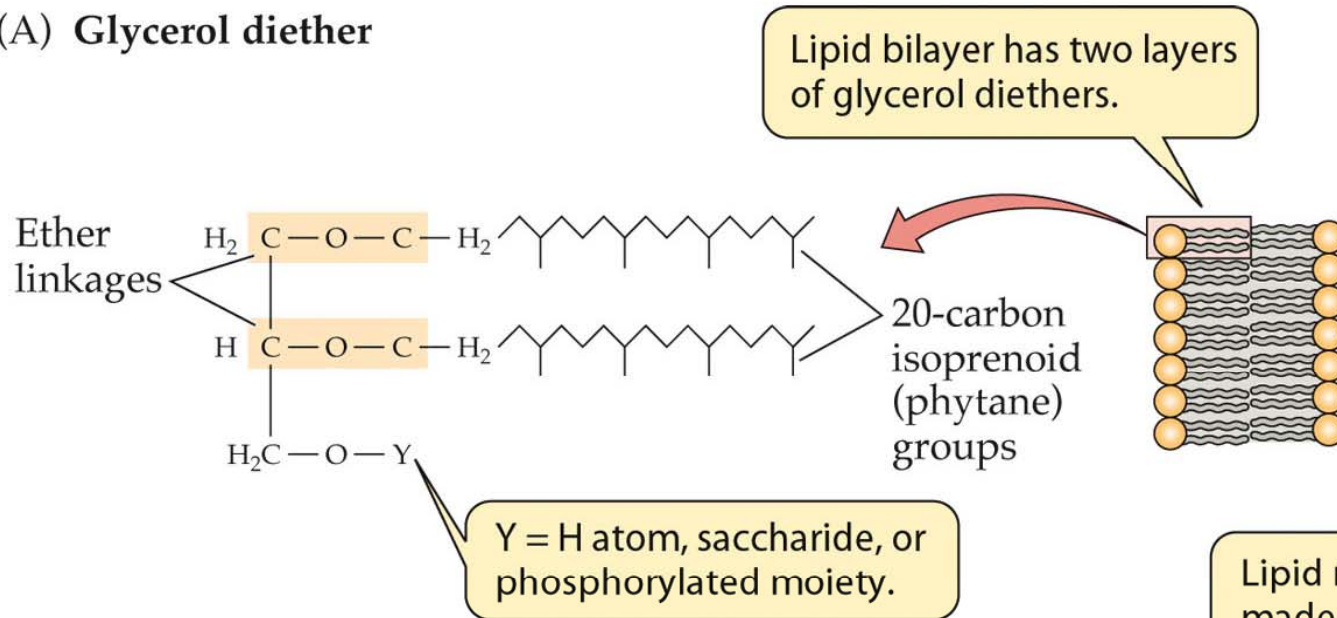
(c) Lipid bilayer



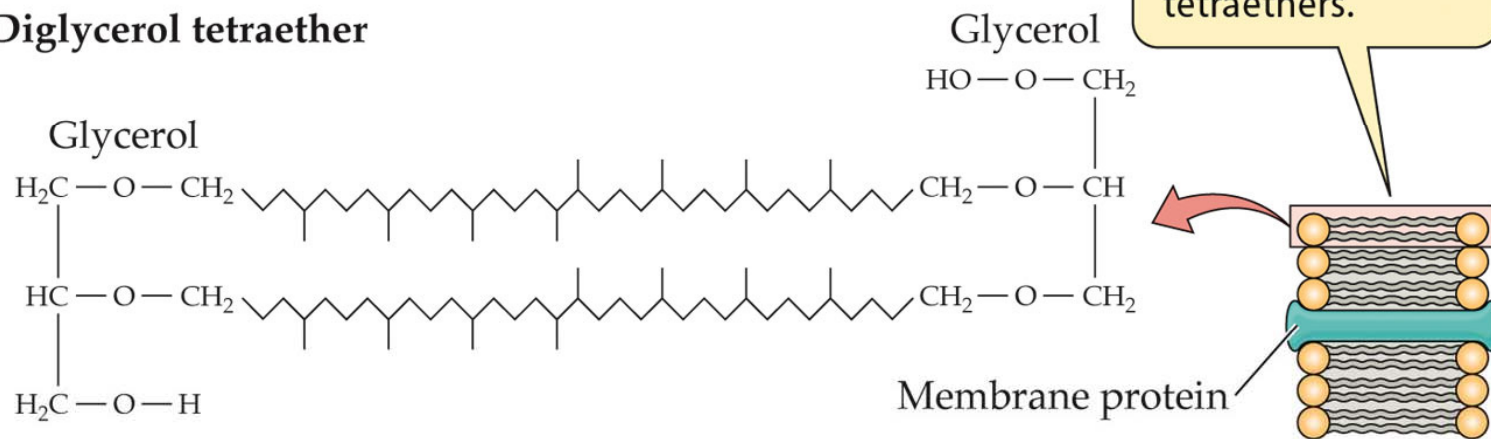
(d) Lipid monolayer

Archaeal cell membrane structure

(A) Glycerol diether



(B) Diglycerol tetraether



Passive Diffusion:

- small, uncharged molecules (O_2 , CO_2 , H_2O)
- weak acids and bases in protonated form (more hydrophobic)

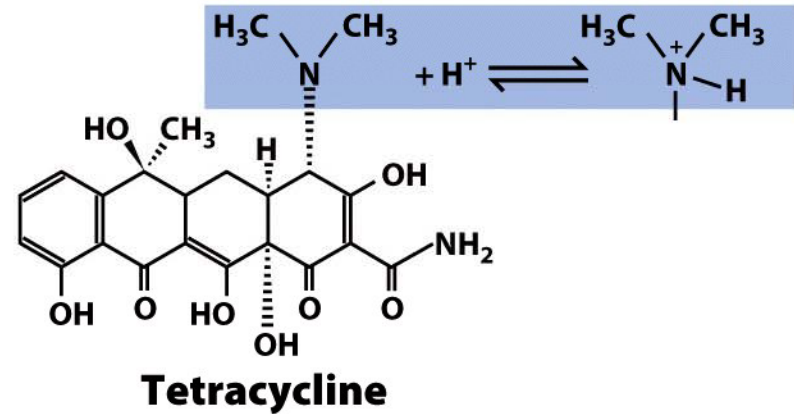
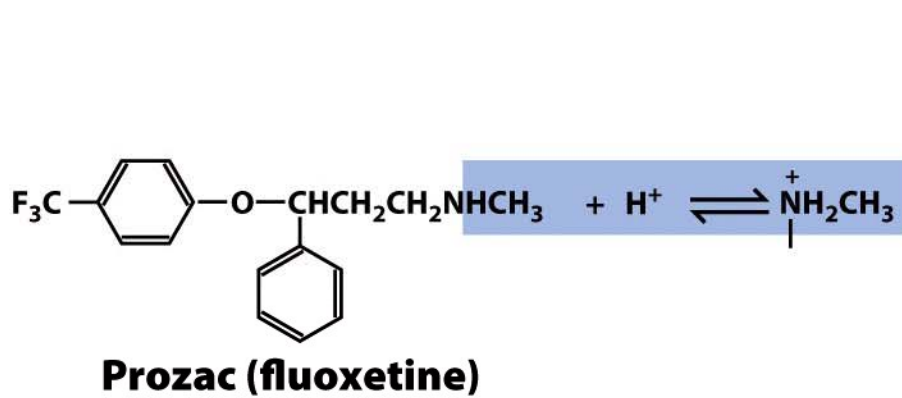
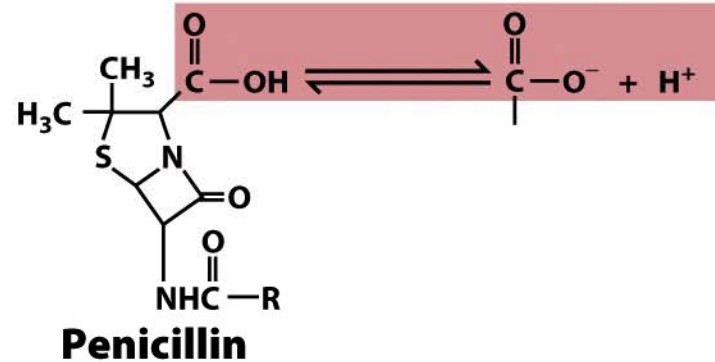
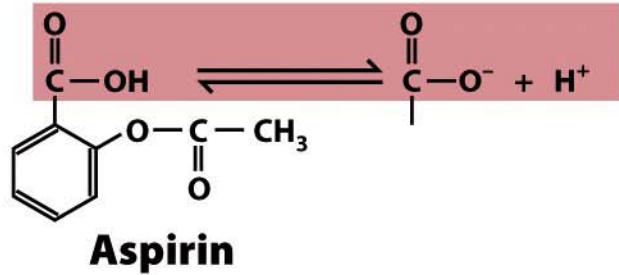


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Facilitated Diffusion (a type of passive transport):

Powered by solute's own concentration gradient

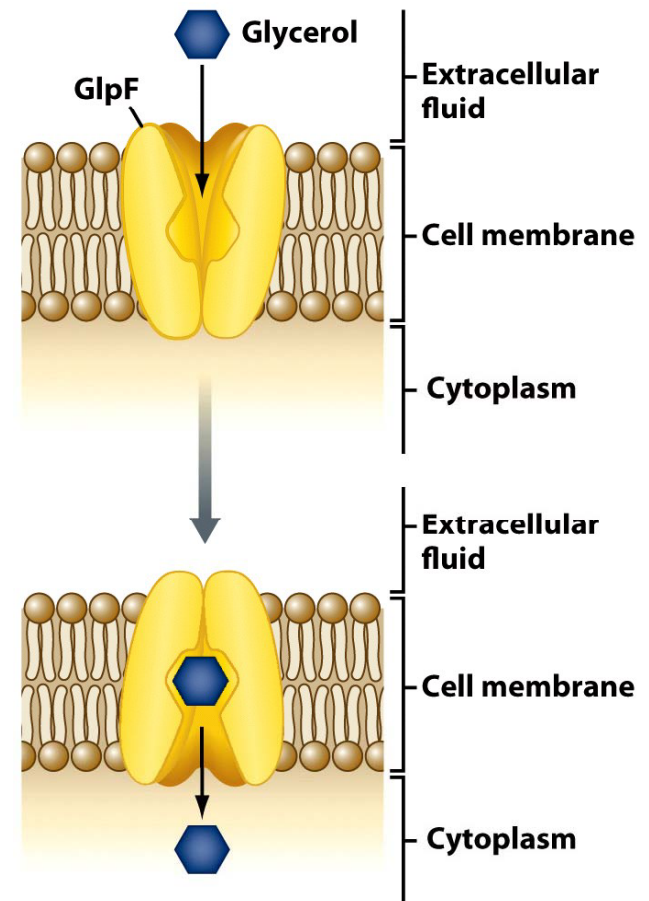
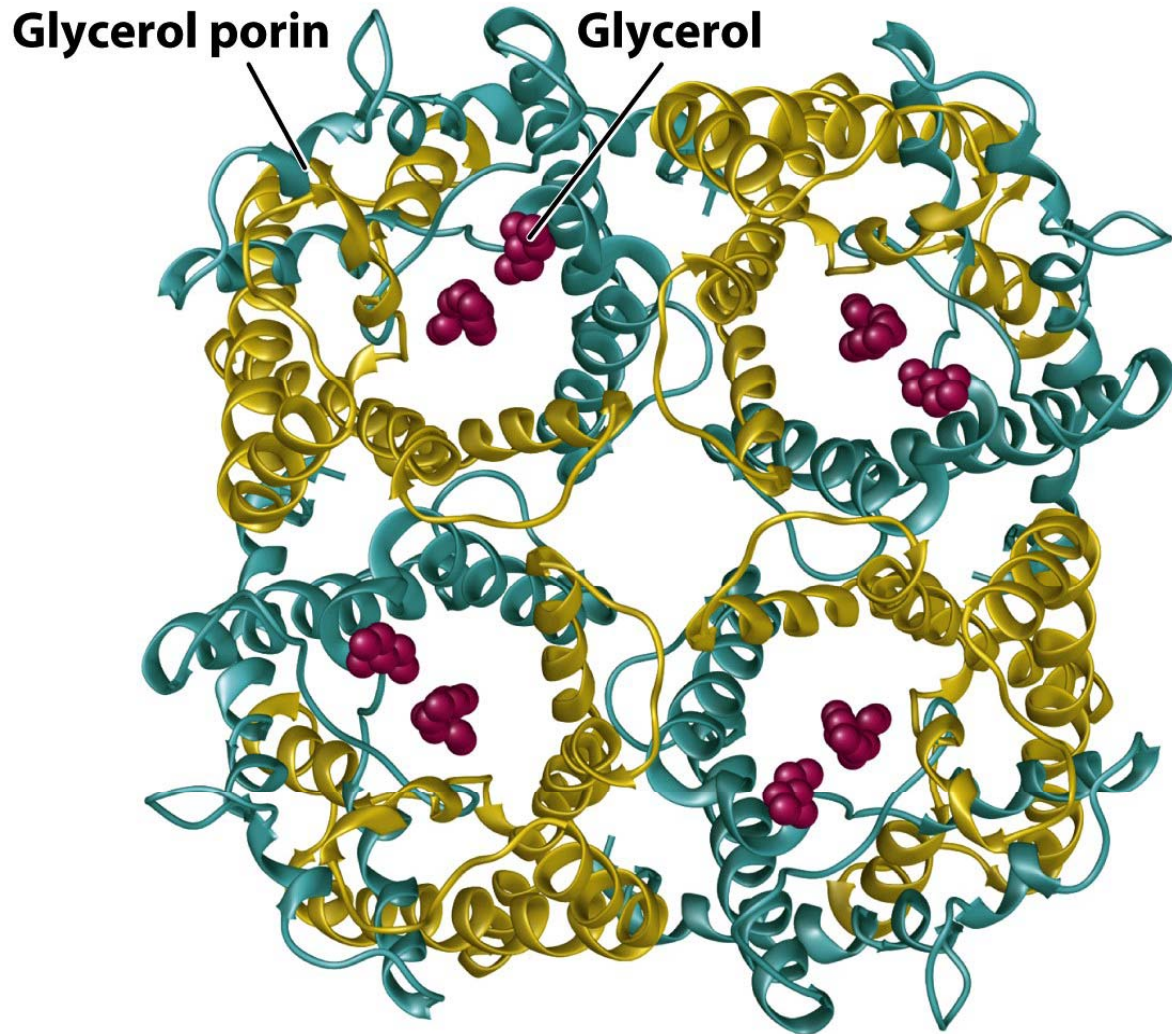


Figure 4.5b Microbiology: An Evolving Science
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Figure 4.5a Microbiology: An Evolving Science
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Aquaporins, glycerol transporters

But how do you get glucose into the cell?

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

Problem: Microbes 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?

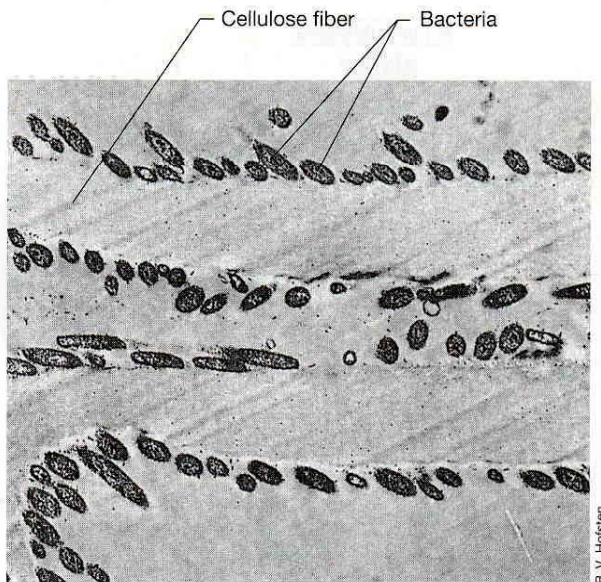
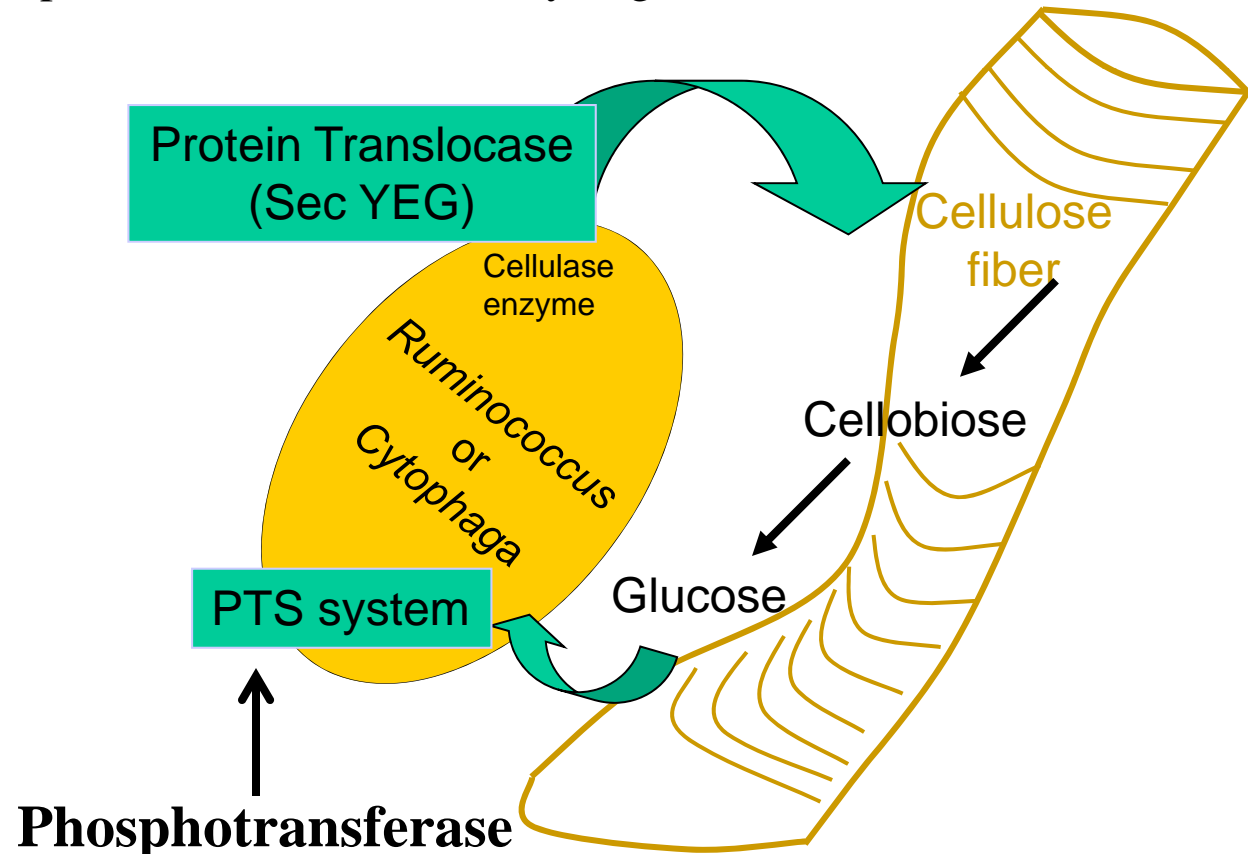


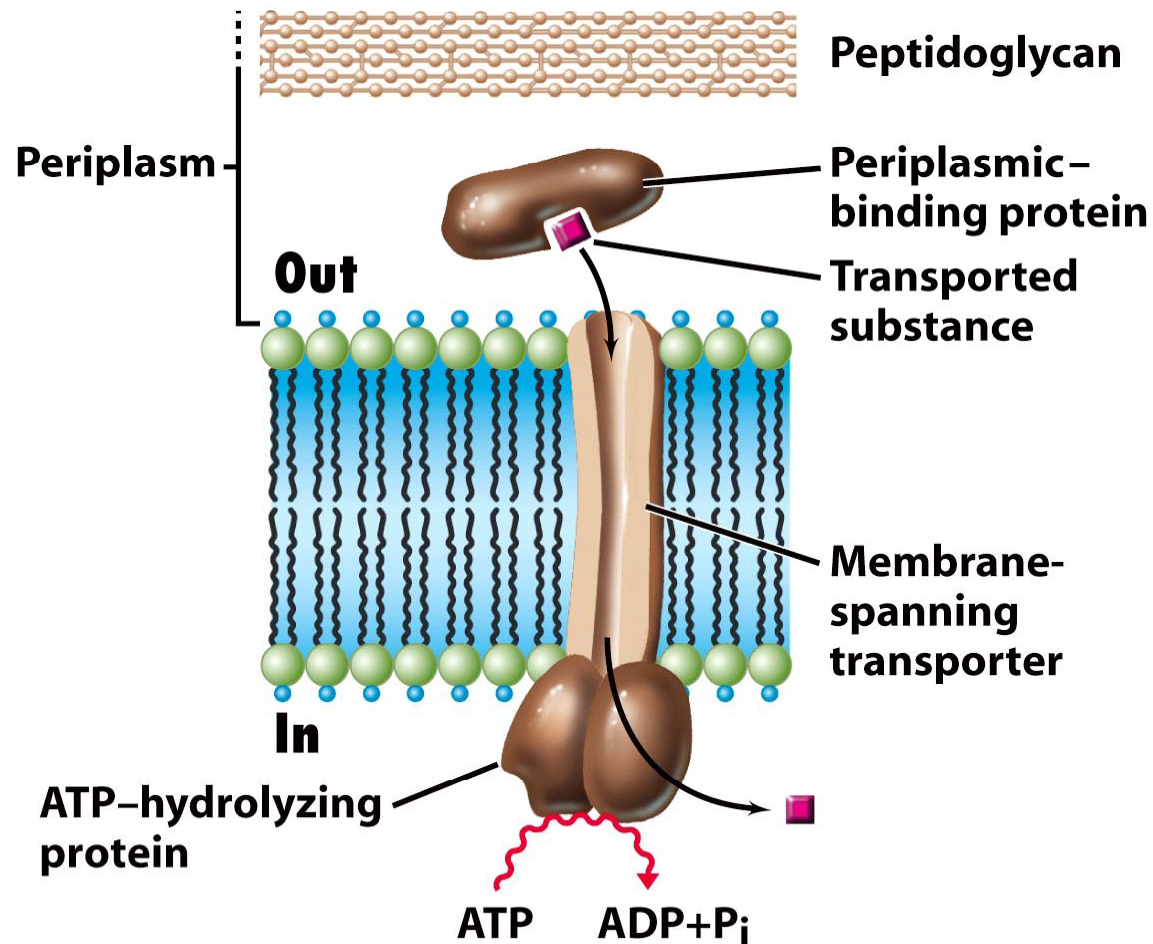
Figure 17.61 Transmission electron micrograph showing attachment of cellulose-digesting bacteria, *Sporocytophaga myxococcoides*, to cellulose fibers. Cells are about 0.5 μm in diameter.



Primary Transport (a type of Active Transport):

- ATP hydrolysis provides energy for transfer
- ABC transporters = ATP-Binding Cassette

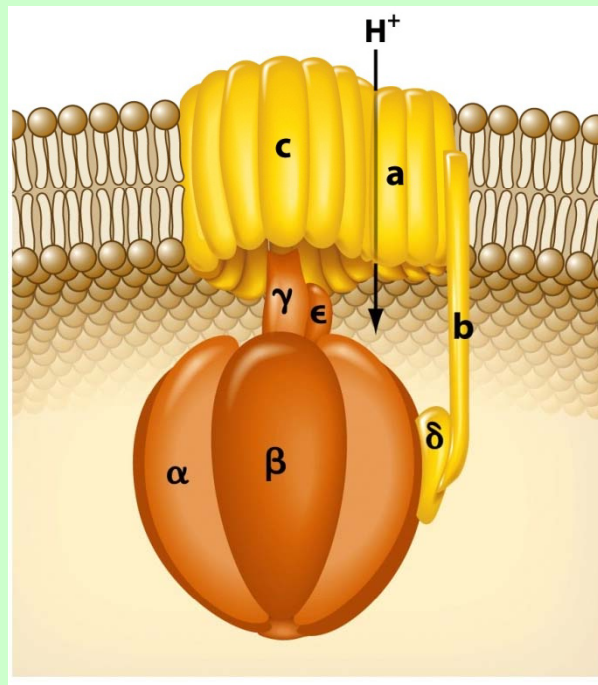
1. Periplasmic binding proteins “find” low-concentration solutes ($\geq 10^{-6}$ M)
2. Binding protein docks on membrane-spanning domain
3. Conformational change and ATP hydrolysis drive transport



Proton pumps (e.g. cytochrome oxidase) push protons out of cell; the electron transport chain is **anchored in membrane**.

Energy conservation: proton-motive force (PMF) is generated from protons.

- Osmotic force tries to push protons back into cell
- Electrical force tries to push protons back into cell



PMF is used to create ATP via the enzyme ATP synthase

Comparing Bacteria, Archaea and Eucarya

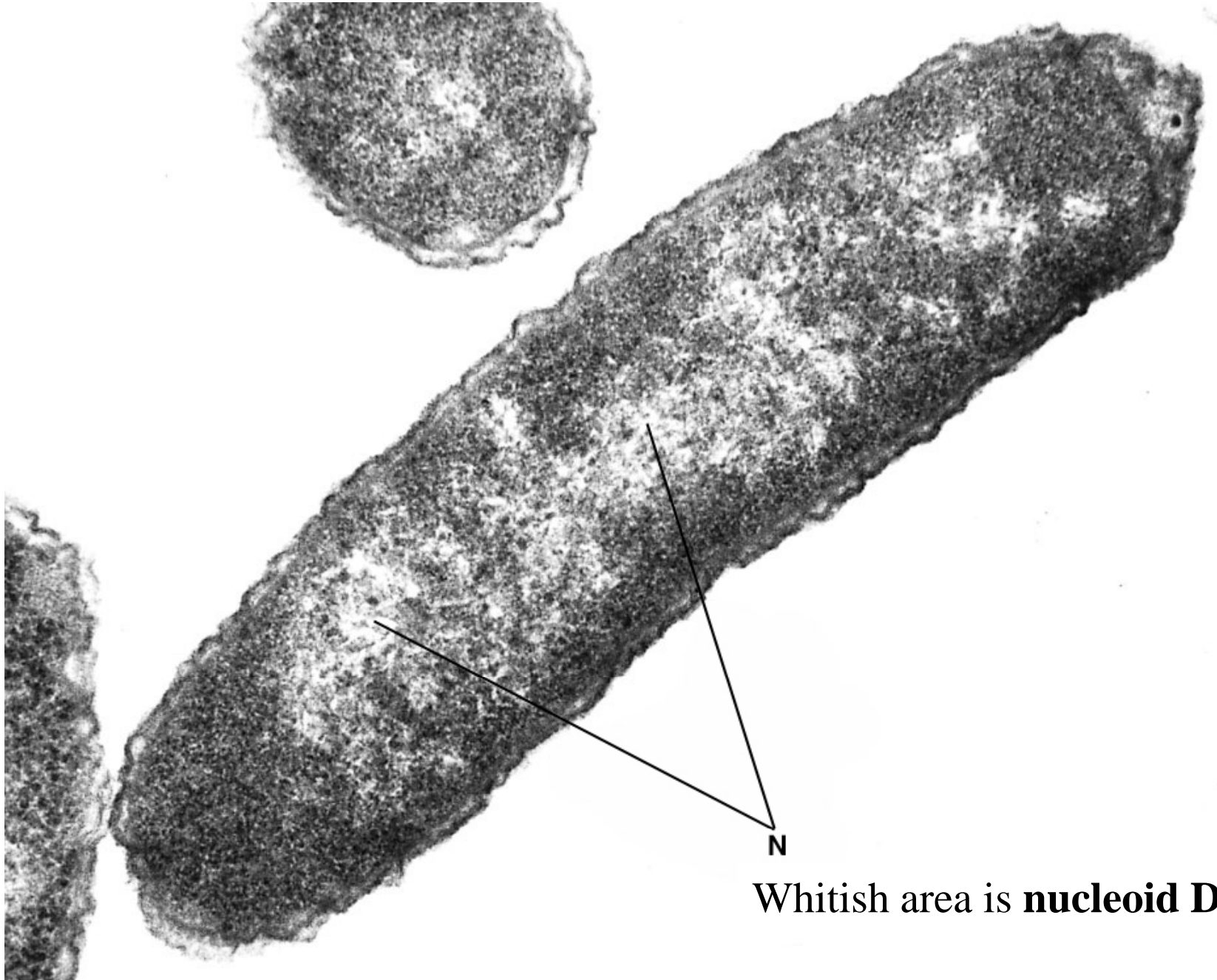
**Classification of microbial cellular features:
Invariant (or common to all)**

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

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

→ Nucleoid Region: Curator of the Information.

Appearance of nucleoid via TEM



Whitish area is **nucleoid DNA**

DNA strands released from cell



Bacterial & Archaeal DNA

Statistics:

Chromosomes: ~1; bears essential genes

Plasmids: 0 to hundreds; helpful genes

Actual Length: ~ 1 mm



Enigma:

How to fit 1 mm long chromosome into a 1 μm wide cell?

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

Supercoiling: tight twisting

Organization: wrapped around histone-like proteins (in Bacteria) or histones (in Archaea)



(a) Relaxed, covalently closed circular DNA

Break one strand
Seal



(b) Relaxed, nicked circular DNA

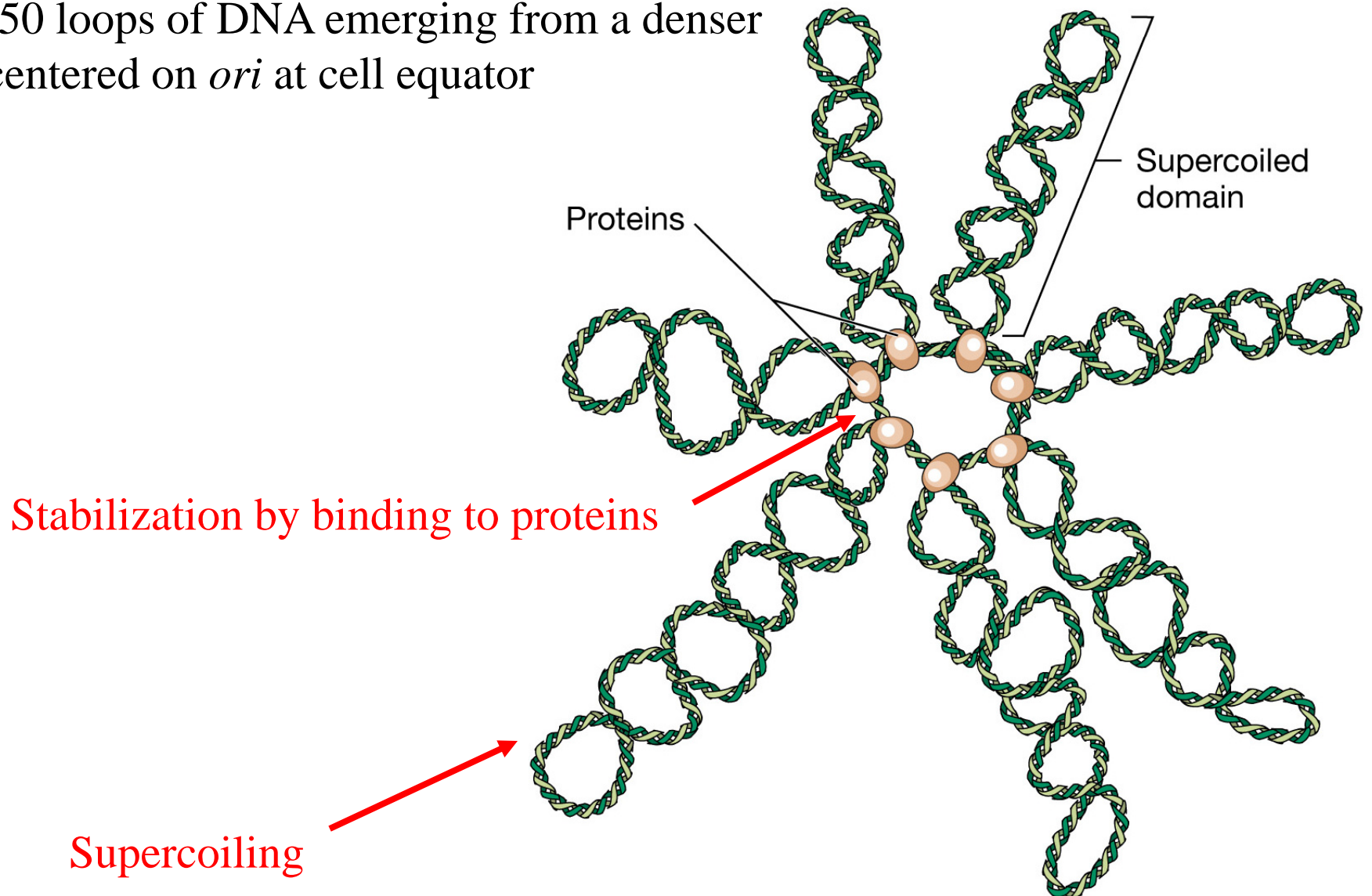
Break one strand
Rotate one end of broken strand around helix and seal



(c) Supercoiled circular DNA

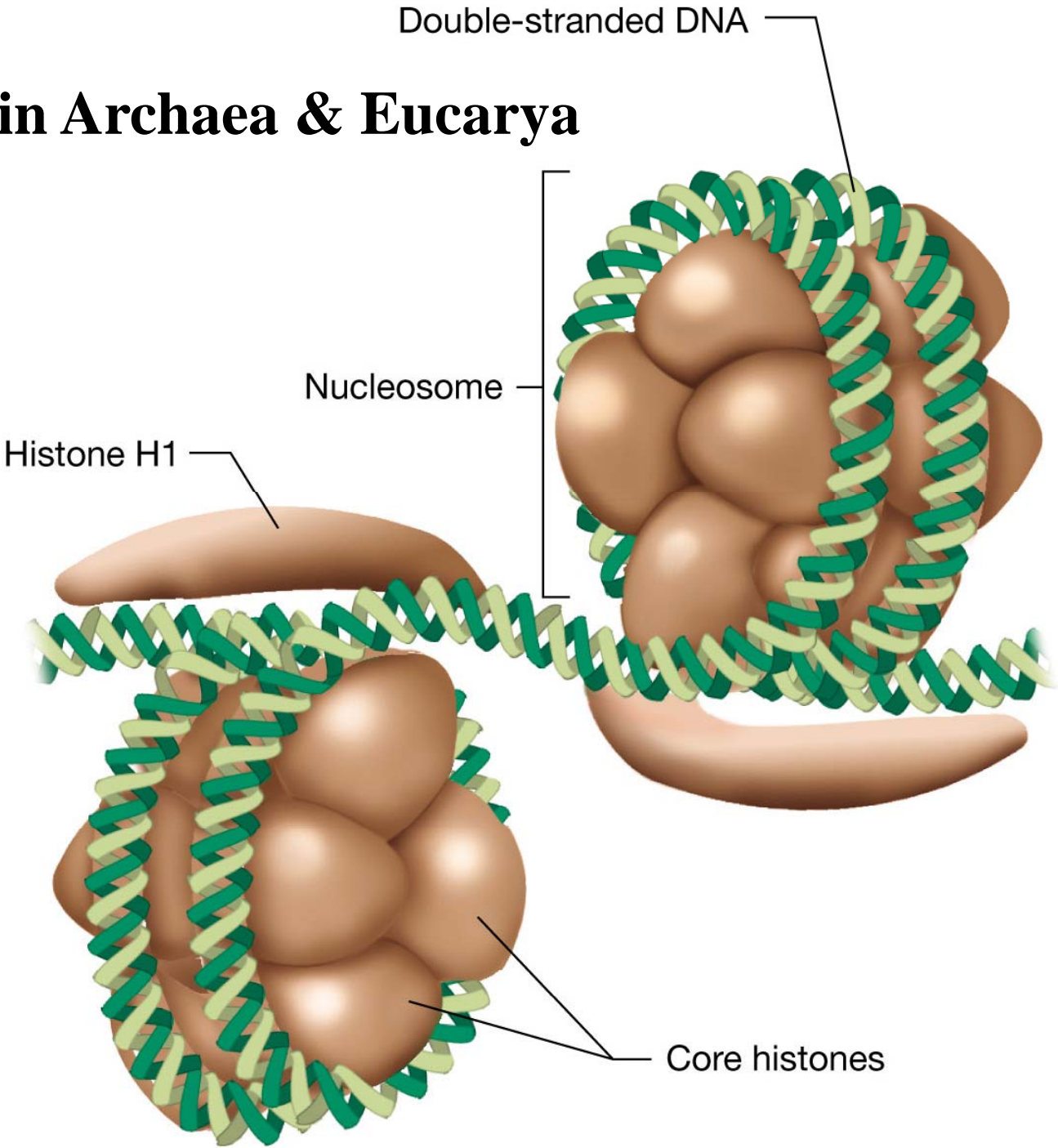
Condensation:

30 to 50 loops of DNA emerging from a denser core centered on *ori* at cell equator

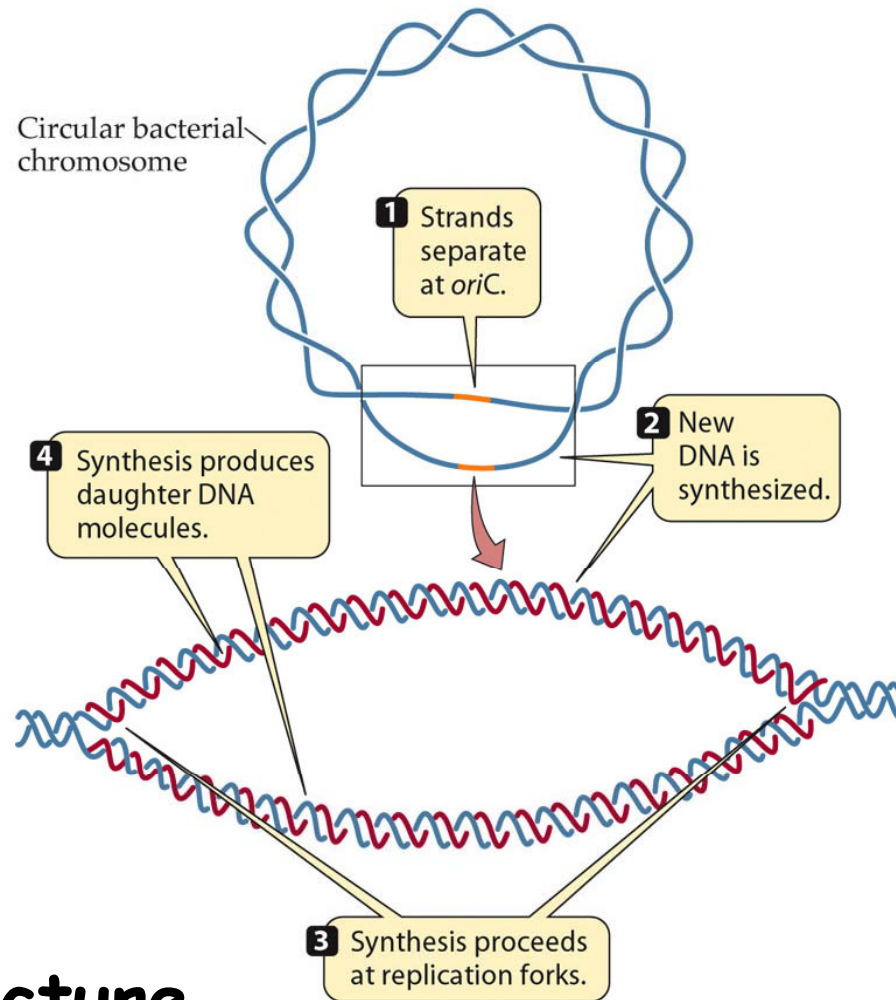


(d) Chromosomal DNA with supercoiled domains

Nucleosomes in Archaea & Eucarya



Overview of DNA replication

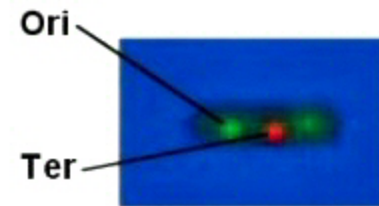


Theta Structure

Overview of DNA replication

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

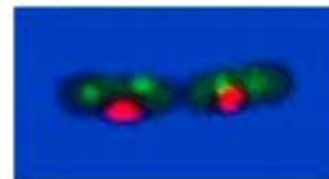
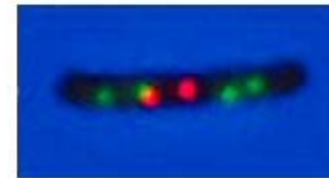
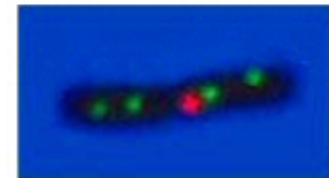
Lau et al (2003) Mol. Micro. 49:731



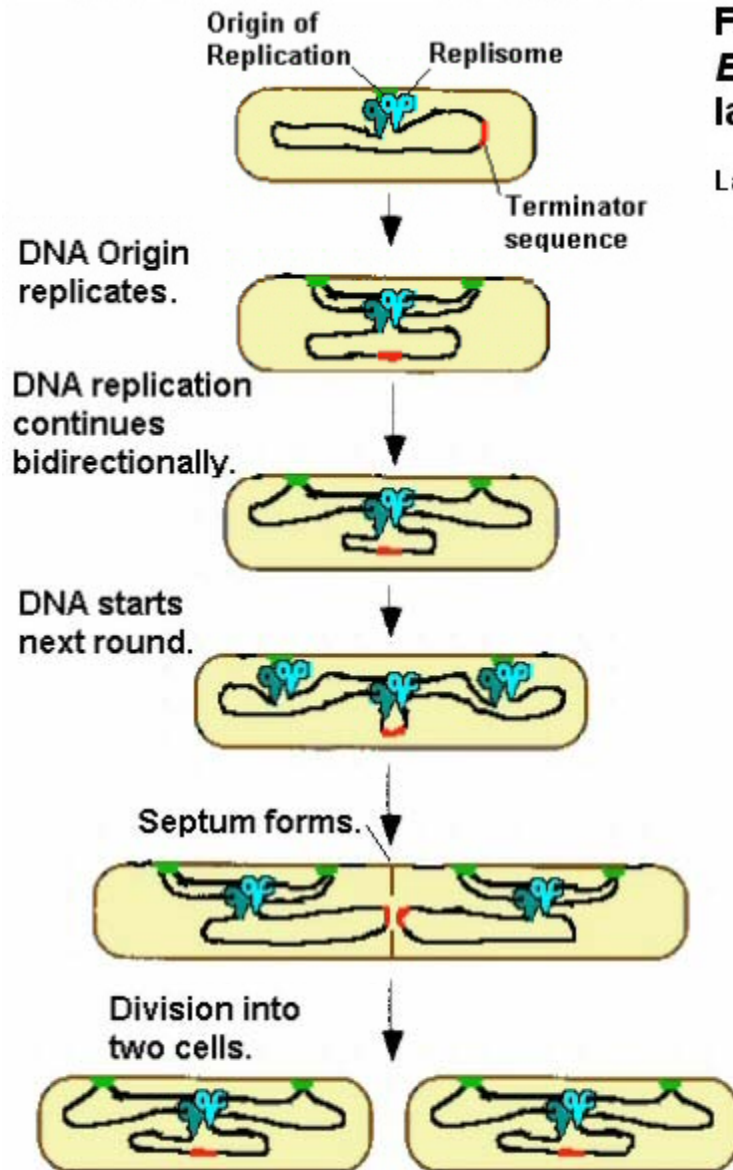
40 minutes to replicate *E. coli*
chromosome.

20 minutes for cell division.

How???

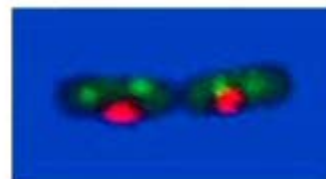
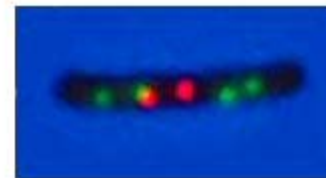
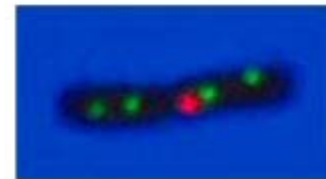
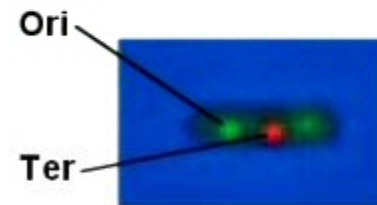


Overview of DNA replication



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

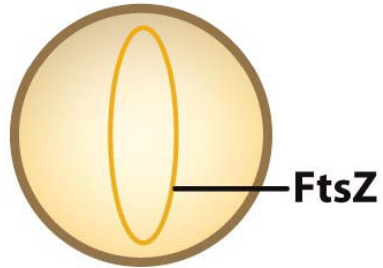
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Bacteria have cytoskeletons!

A.

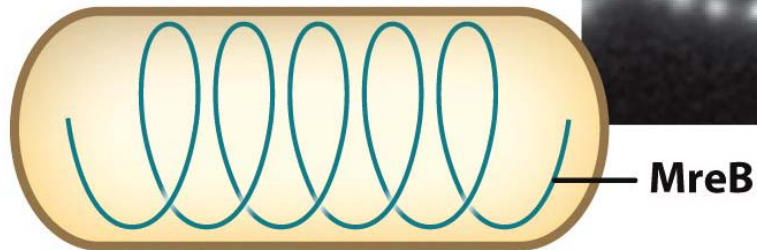
Staphylococcus aureus



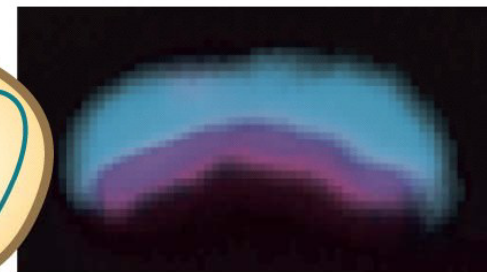
B.



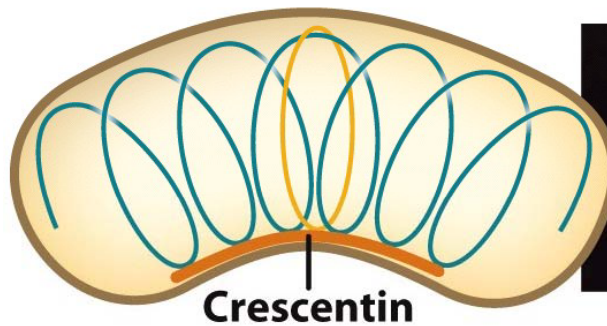
Escherichia coli



C.



Caulobacter crescentus



Bacteria divide by binary fission. FtsZ marks the spot.

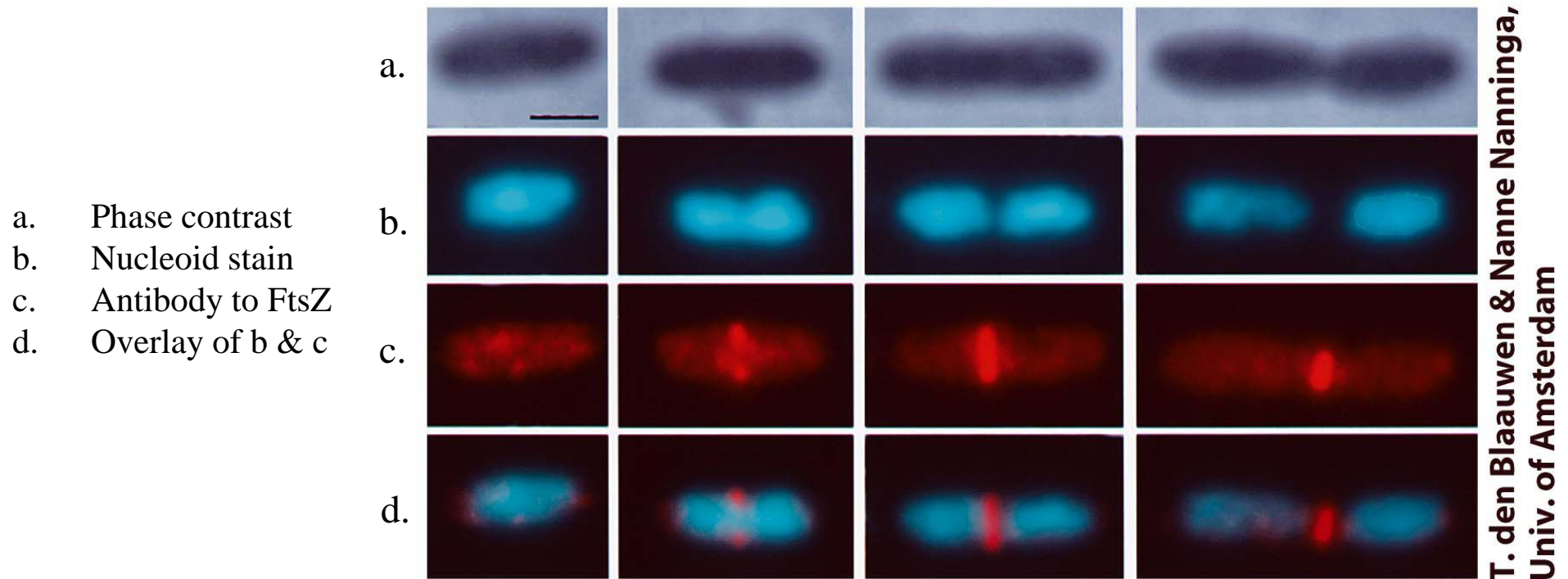


Figure 6-2b Brock Biology of Microorganisms 11/e
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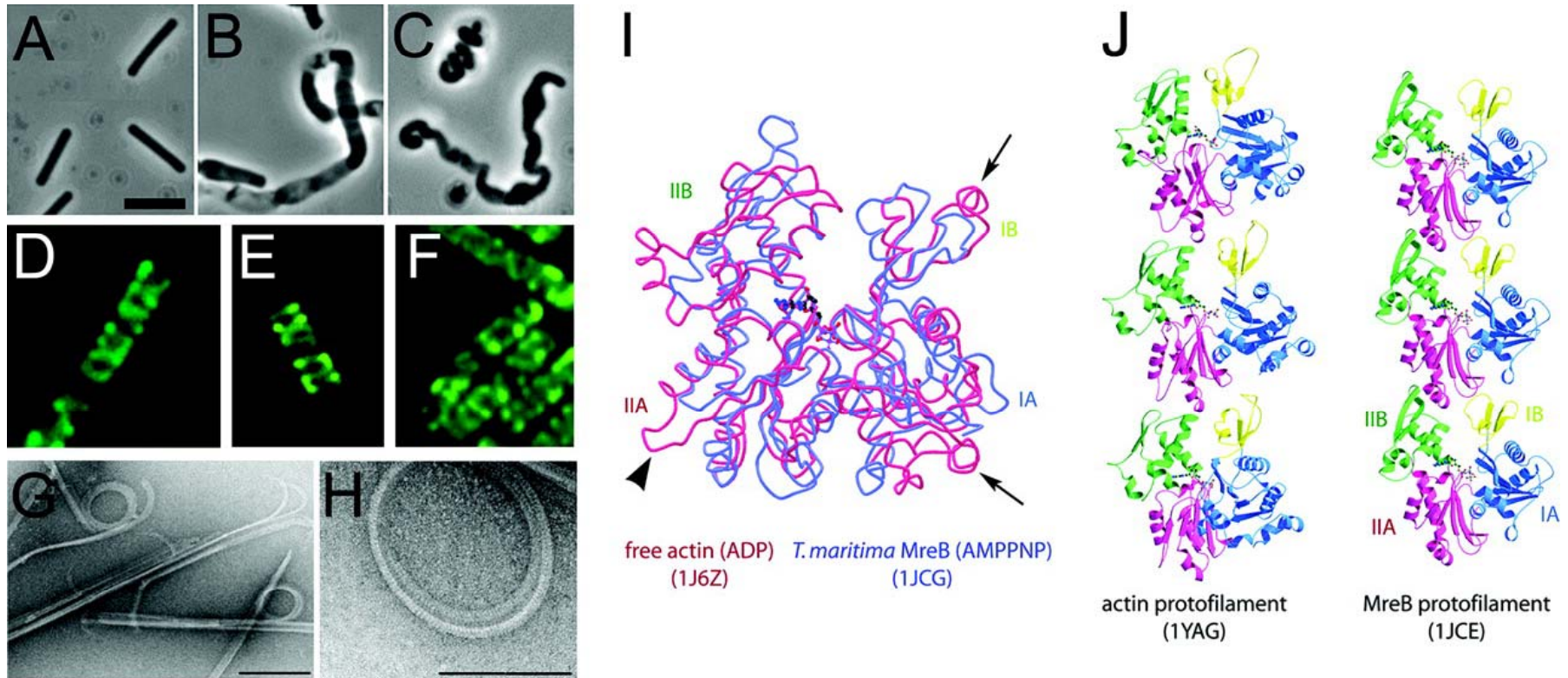
FtsZ is a structural analog (ancestor?) of eukaryotic tubulin

→ forms Z-ring at future site of cytokinesis

→ only 17% amino acid identity to tubulin but similar 3D structures and assembly properties

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.



*Gemmata
obscuriglobus*

Membrane encompassed
nucleoid