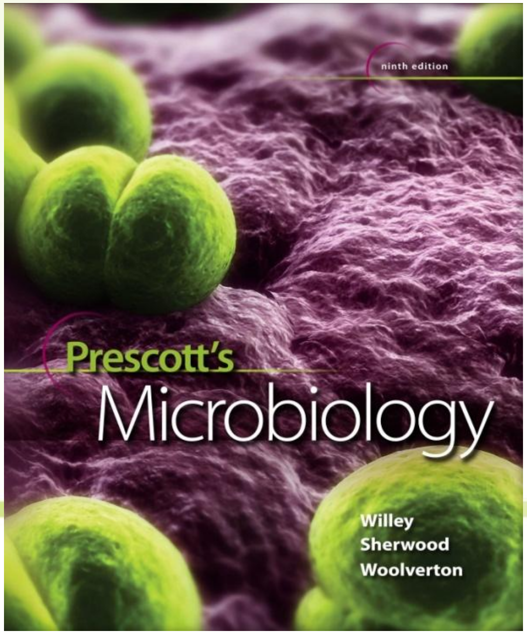
3

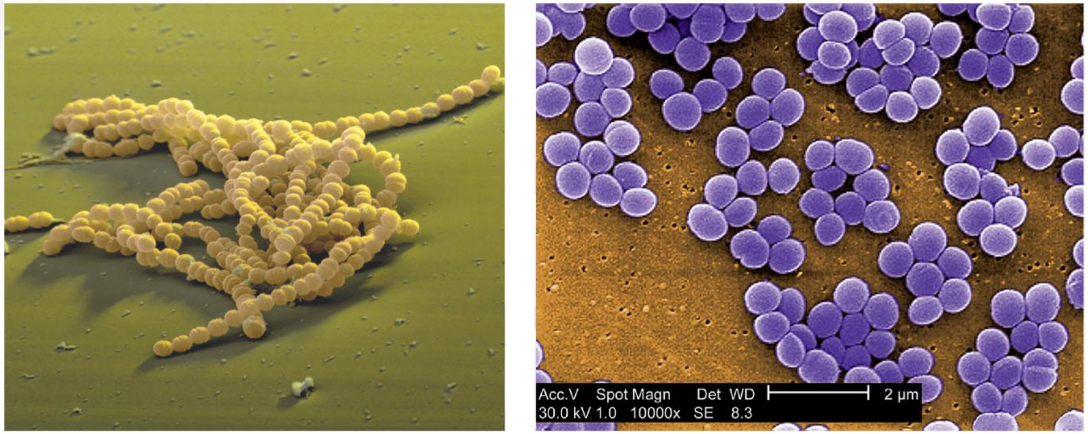


Bacterial Cell Structure

Size, Shape, and Arrangement

- Shape
  - cocci and rods most common
  - various others
- Arrangement
  - determined by plane of division
  - determined by separation or not
- Size - varies

Shape and Arrangement-1

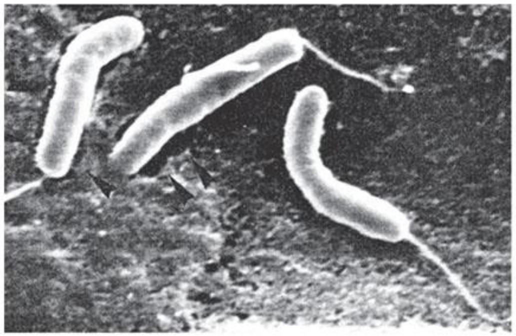


(a) *S. agalactiae*—cocci in chains **(b)** *S. aureus*—cocci in clusters

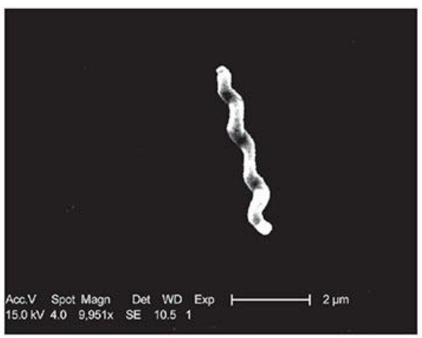
a: © Photo Researchers, Inc.; b: CDC/Janice Haney Carr

- Cocci (s., coccus) – spheres
  - diplococci (s., diplococcus) – pairs
  - streptococci – chains
  - staphylococci – grape-like clusters
  - tetrads – 4 cocci in a square
  - sarcinae – cubic configuration of 8 cocci

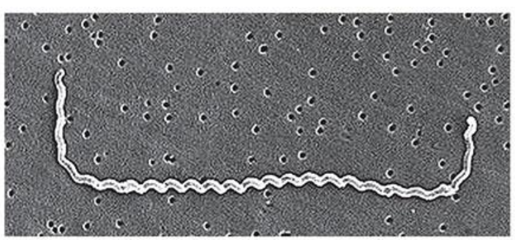
Shape and Arrangement-2



(a) *V. cholerae*—comma-shaped vibrios

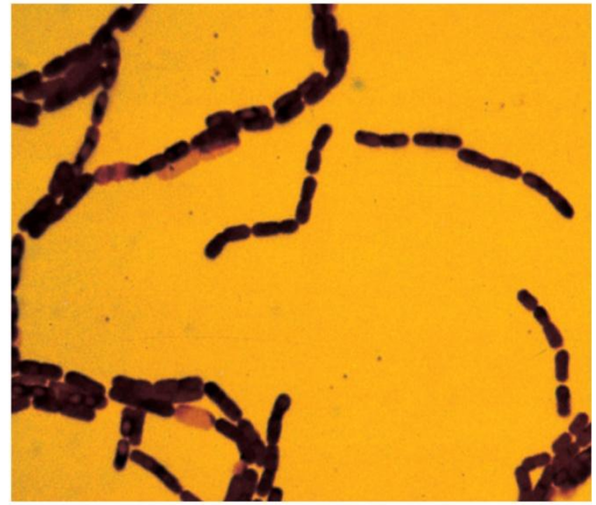


(b) *C. jejuni*—Spiral-shaped spirillum



(c) *Leptospira interrogans*—a spirochete

a: CDC; b: CDC/Janice Haney Carr;
c: CDC/NCID/HIP/Janice Carr



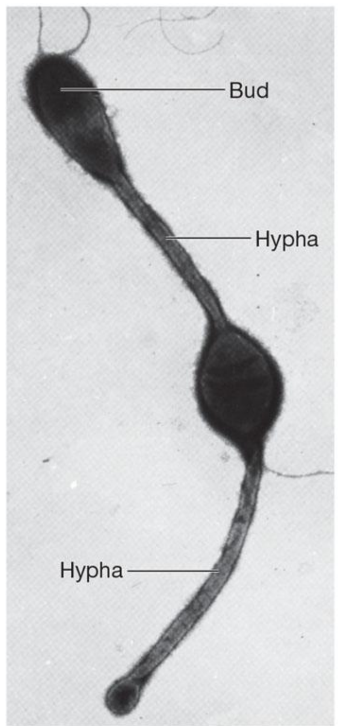
(c) *B. megaterium*—rods in chains

© George Wilder/Visuals Unlimited

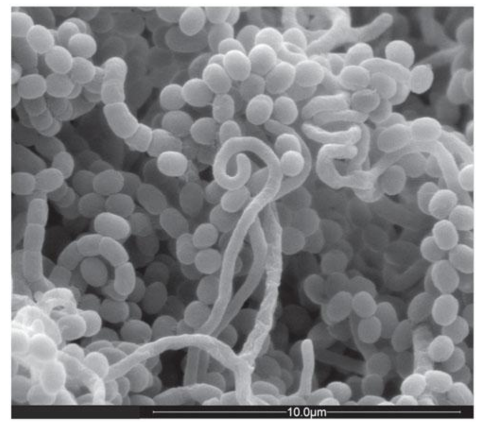
- bacilli (s., bacillus) – rods
  - coccobacilli – very short rods
- vibrios – resemble rods, comma shaped
- spirilla (s., spirillum) – rigid helices
- spirochetes – flexible helices

Shape and Arrangement-3

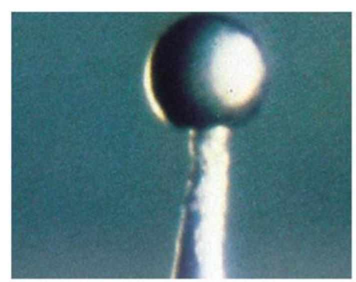
- mycelium – network of long, multinucleate filaments
- pleomorphic – organisms that are variable in shape



(d) *Hyphomicrobium*

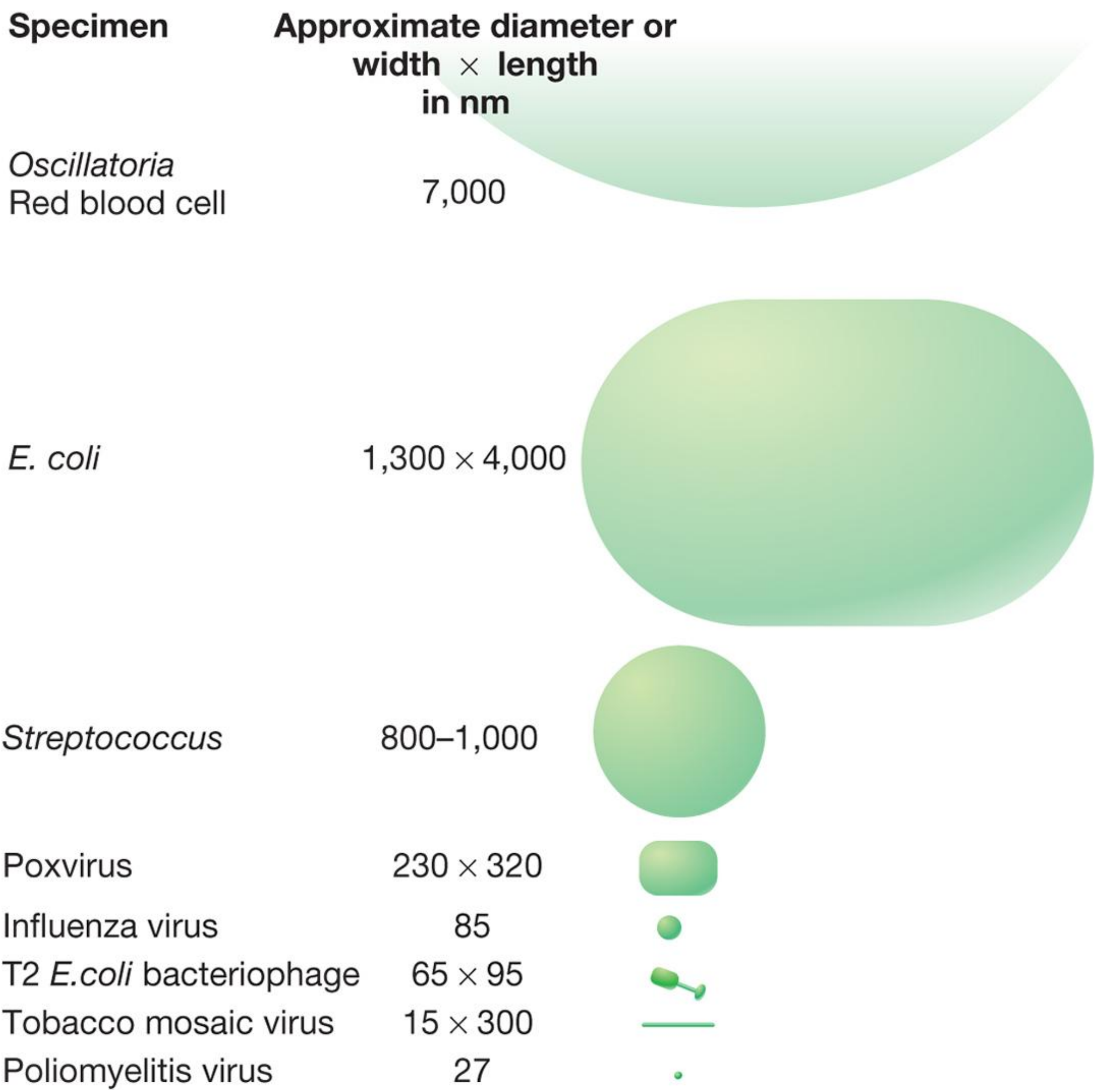


(e) *Streptomyces*—a filamentous bacterium



(f) *M. stipitatus* fruiting body

d: Reprinted from The Shorter *Bergey's Manual of Determinative Bacteriology*, 8e, John G. Holt, Editor, 1977 © Bergey's Manual Trust. Published by Williams & Wilkins Baltimore, MD; e: Dr. Amy Gehring; f: © M. Dworkin-Hi. Reichenbach/Phototake

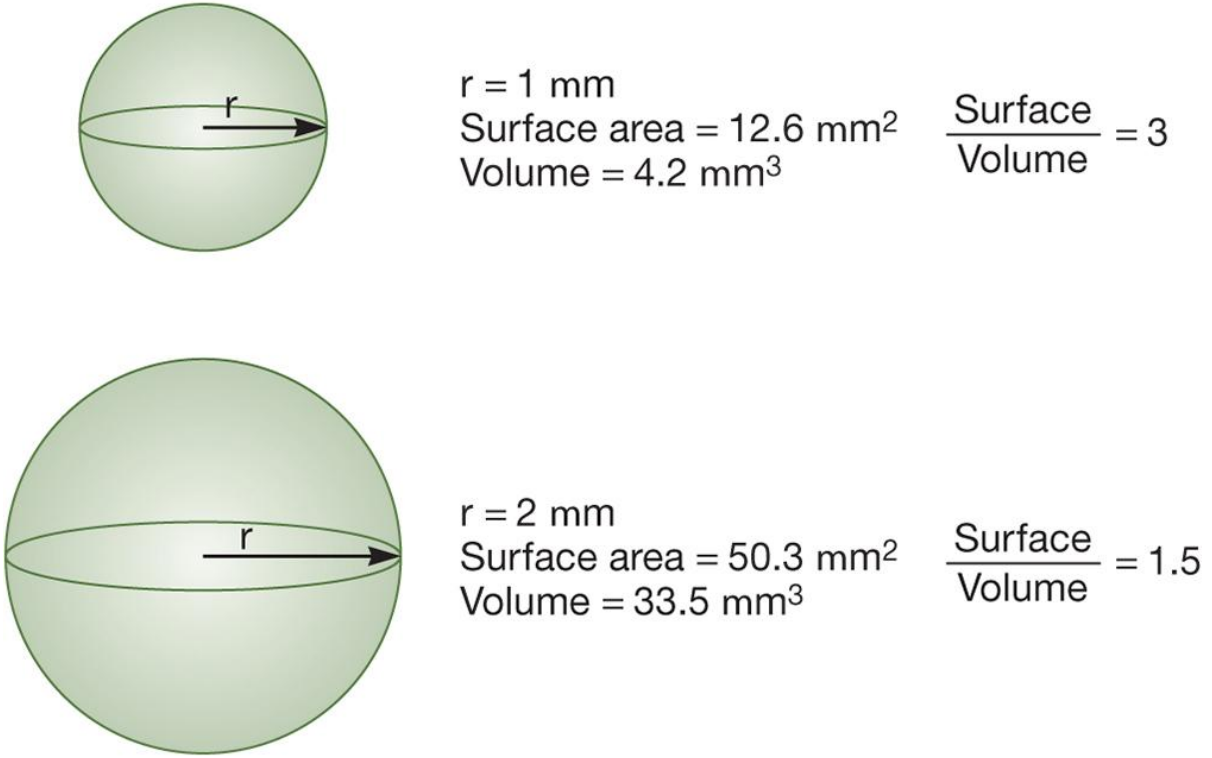


Size

- smallest – 0.3 µm (*Mycoplasma*)
- average rod – 1.1 - 1.5 x 2 – 6 µm (*E. coli*)
- very large – 600 x 80 µm *Epulopiscium fishelsoni*

Size – Shape Relationship

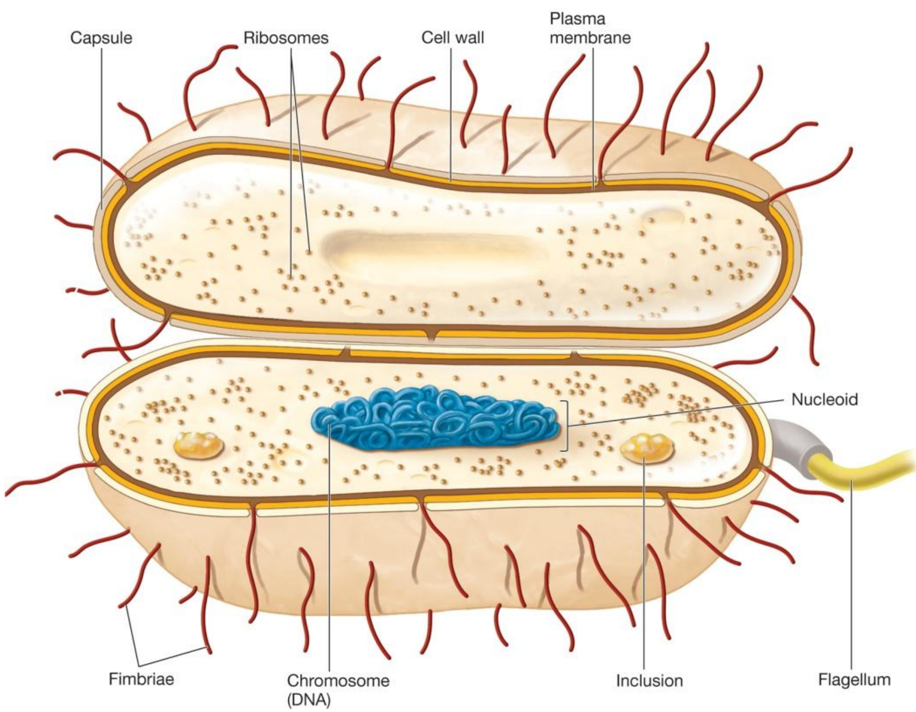
- important for nutrient uptake
- surface to volume ratio (S/V)
- small size may be protective mechanism from predation



Bacterial Cell Organization
Common Features

- – Cell envelope – 3 layers
- – Cytoplasm
- – External structures

Table 3.1	Common Bacterial Structures and Their Functions
Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	An inclusion that provides buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusions	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	In typical Gram-negative bacteria, contains hydrolytic enzymes and binding proteins for nutrient processing and uptake; in typical Gram-positive bacteria, may be smaller or absent
Cell wall	Protection from osmotic stress, helps maintain cell shape
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial conjugation and transformation, twitching and gliding motility
Flagella	Swimming and swarming motility
Endospore	Survival under harsh environmental conditions



Bacterial Cell Envelope

- Plasma membrane
- Cell wall
- Layers outside the cell wall

Bacterial Plasma Membrane

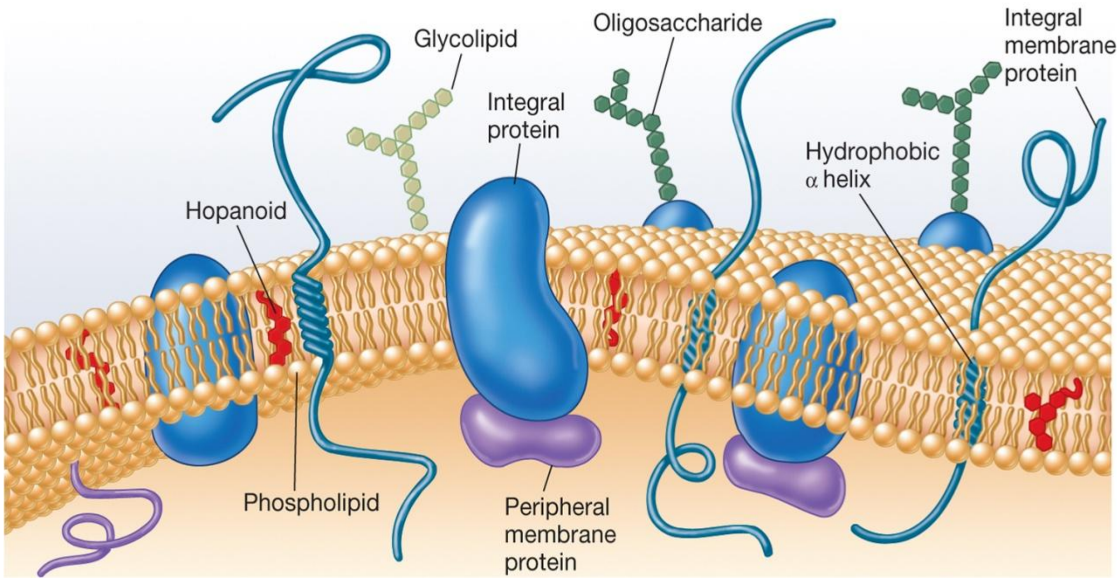
- Absolute requirement for all living organisms
- Some bacteria also have internal membrane systems

Plasma Membrane Functions

- Encompasses the cytoplasm
- Selectively permeable barrier
- Interacts with external environment
  - receptors for detection of and response to chemicals in surroundings
  - transport systems
  - metabolic processes

Fluid Mosaic Model of Membrane Structure

- lipid bilayers with floating proteins
  - amphipathic lipids
    - polar ends (hydrophilic – interact with water)
    - non-polar tails (hydrophobic – insoluble in water)
  - membrane proteins

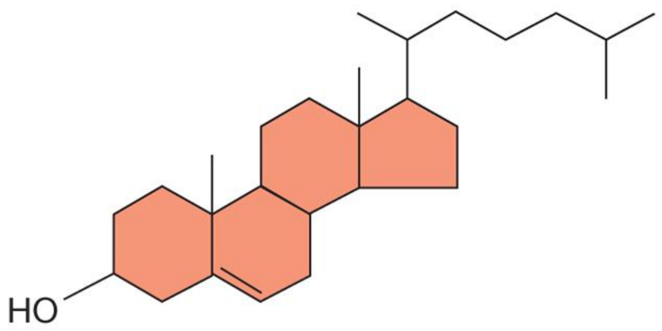


Membrane Proteins

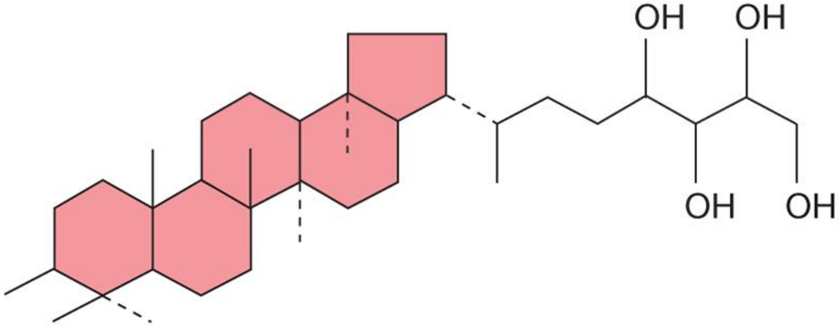
- Peripheral
  - loosely connected to membrane
  - easily removed
- Integral
  - amphipathic – embedded within membrane
  - carry out important functions
  - may exist as microdomains

Bacterial Lipids

- Saturation levels of membrane lipids reflect environmental conditions such as temperature
- Bacterial membranes lack sterols but do contain sterol-like molecules, hopanoids
  - stabilize membrane
  - found in petroleum



(a) Cholesterol (a steroid) is found in the membranes of eukaryotes.



(b) Bacteriohopanetetrol (a hopanoid) is found in many bacterial membranes.

Bacterial Cell Wall

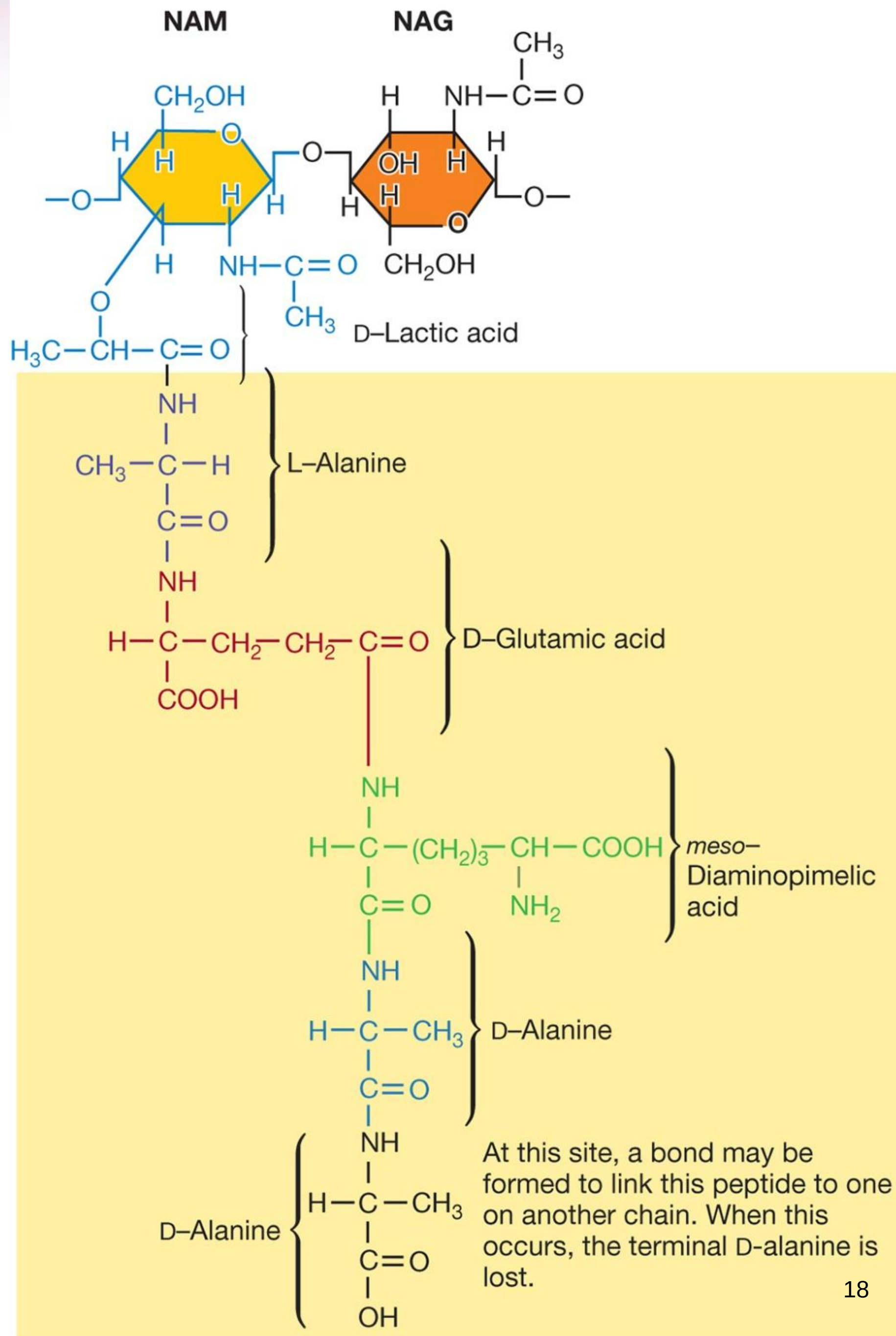
- Peptidoglycan (murein)
  - rigid structure that lies just outside the cell plasma membrane
  - two types based on Gram stain
    - Gram-positive: stain purple; thick peptidoglycan
    - Gram-negative: stain pink or red; thin peptidoglycan and outer membrane

Cell Wall Functions

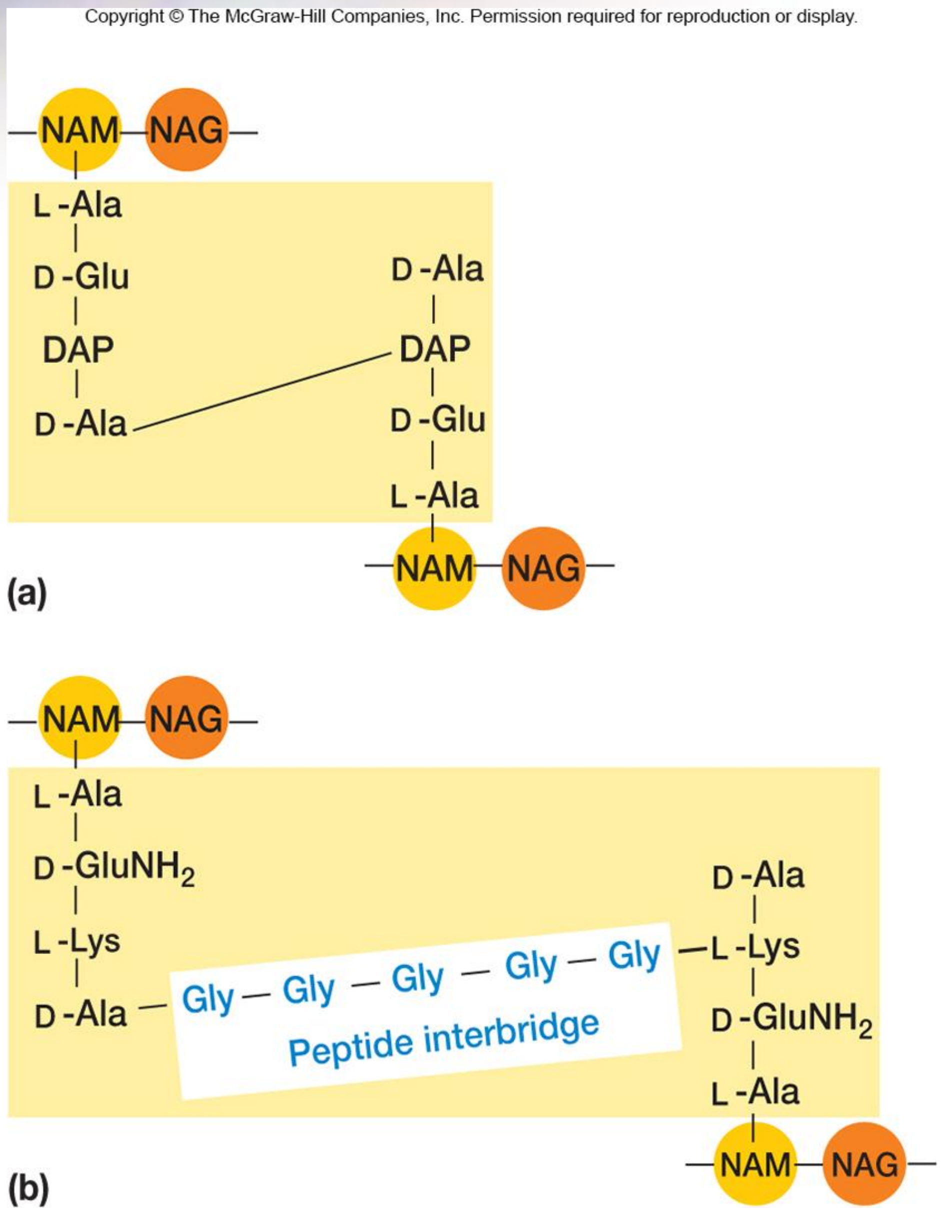
- Maintains shape of the bacterium
  - almost all bacteria have one
- Helps protect cell from osmotic lysis
- Helps protect from toxic materials
- May contribute to pathogenicity

Peptidoglycan Structure

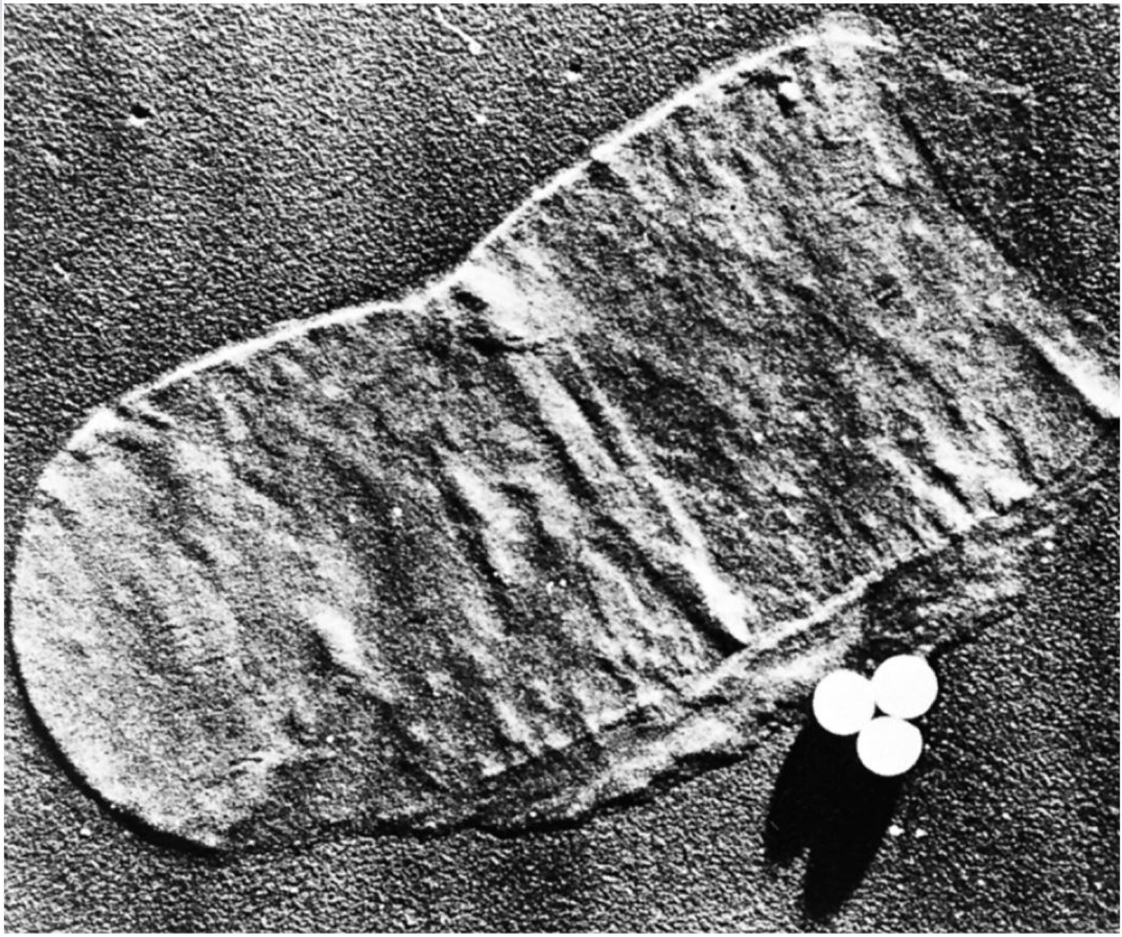
- Meshlike polymer of identical subunits forming long strands
  - two alternating sugars
    - *N*-acetylglucosamine (NAG)
    - *N*- acetylmuramic acid
  - alternating D- and L- amino acids



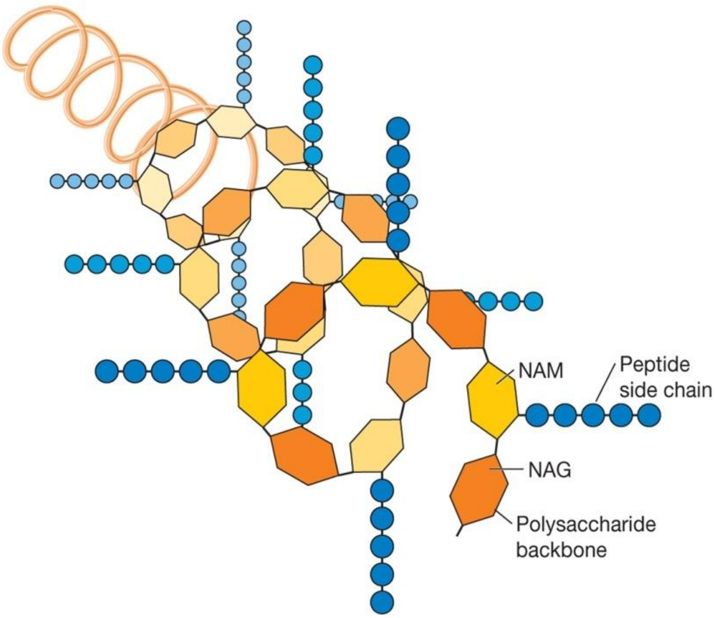
Strands Are Crosslinked



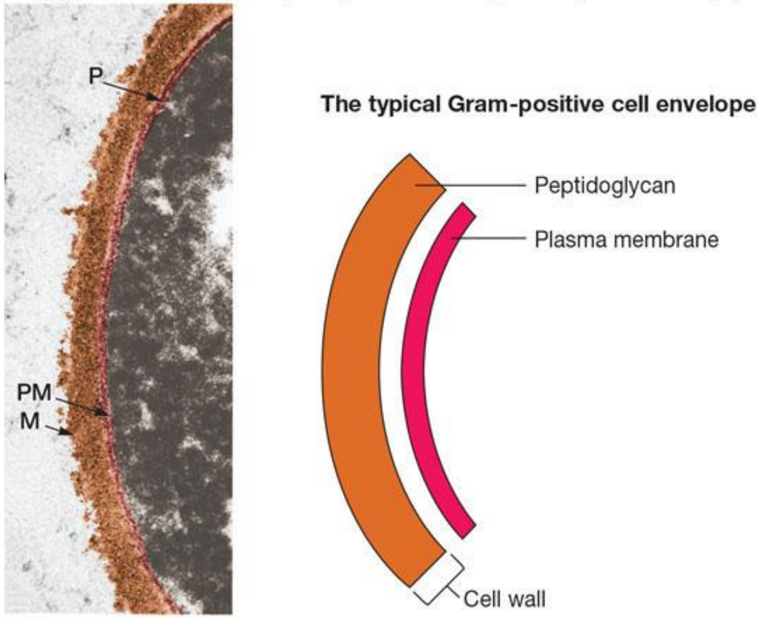
- Peptidoglycan strands have a helical shape
- Peptidoglycan chains are crosslinked by peptides for strength
  - interbridges may form
  - peptidoglycan sacs – interconnected networks
  - various structures occur



Courtesy of M.R. J. Salton, NYU Medical Center

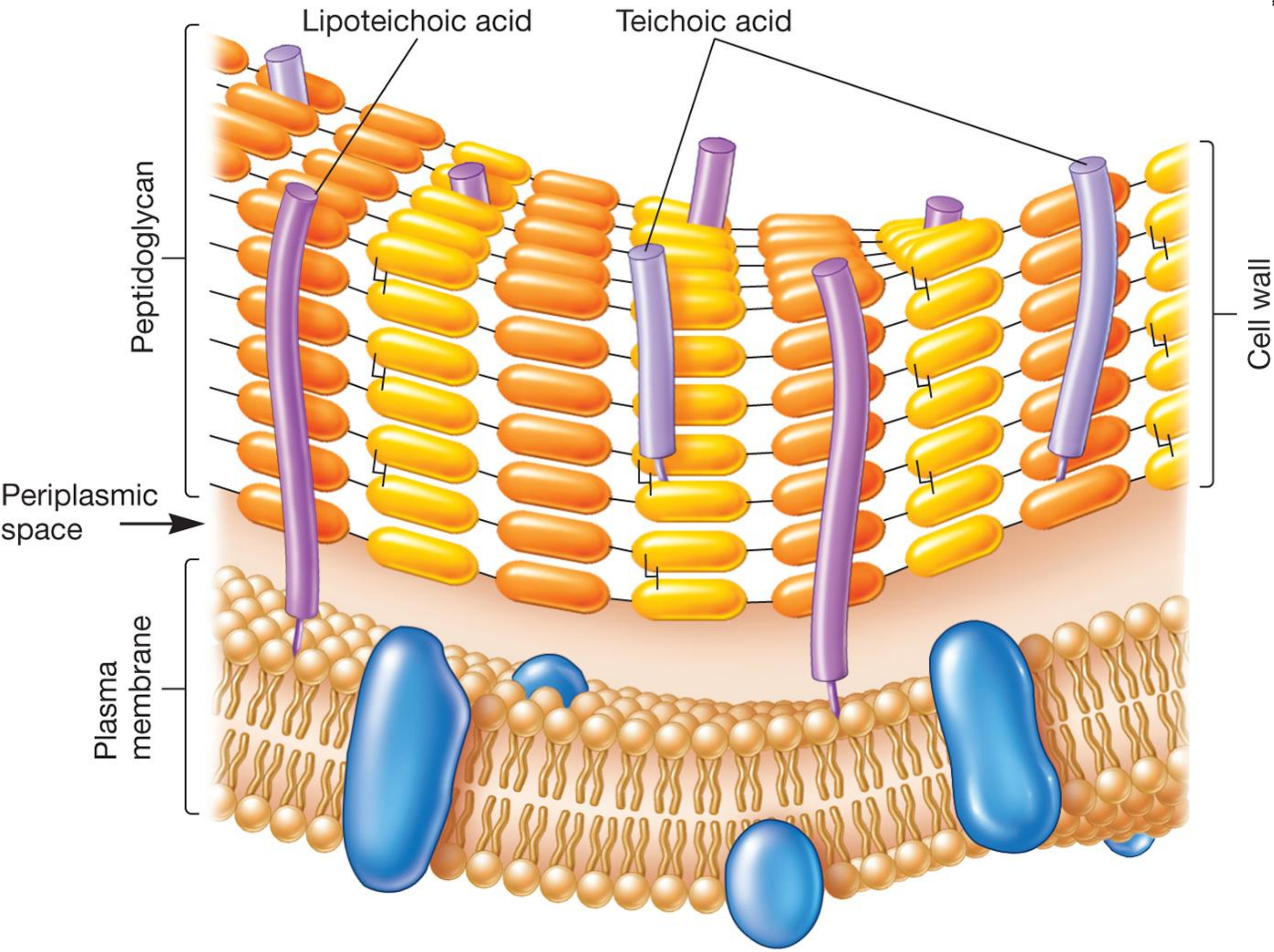


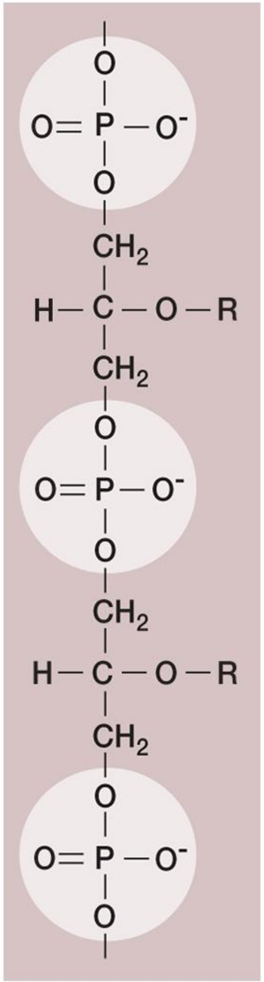
Gram-Positive Cell Walls



© T.J. Beveridge/Biological Photo Service

- Composed primarily of peptidoglycan
- May also contain teichoic acids (negatively charged)
  – help maintain cell envelope
  – protect from environmental substances
  – may bind to host cells
- some gram-positive bacteria have layer of proteins on surface of peptidoglycan


Lipoteichoic acid
Teichoic acid
Peptidoglycan
Cell wall
Periplasmic space
Plasma membrane

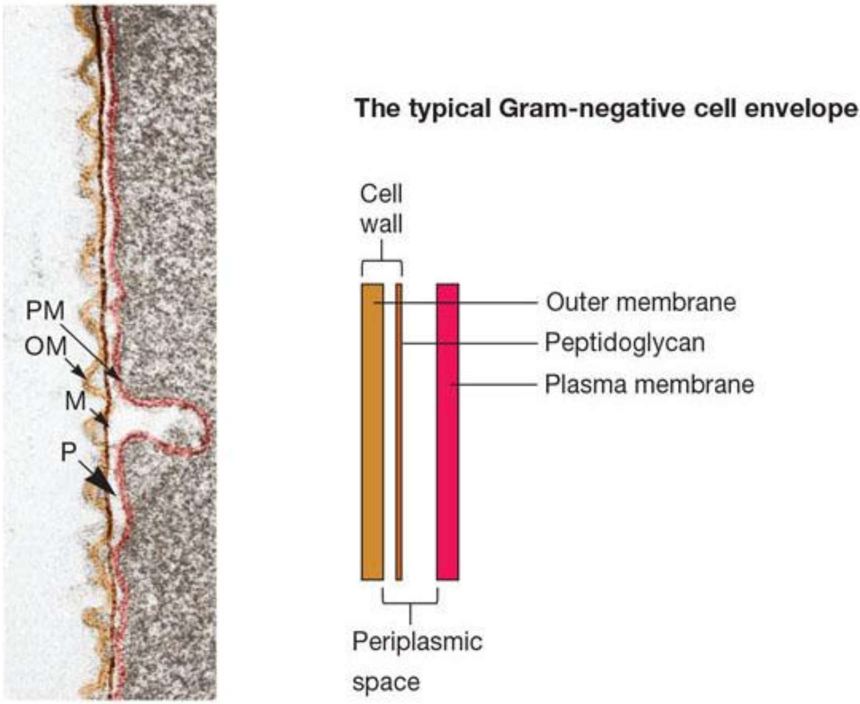

O
O= P – O⁻
O
CH2
H – C – O – R
CH2
O
O= P – O⁻
O
CH2
H – C – O – R
CH2
O
O= P – O⁻
O

Periplasmic Space of Gram + Bacteria

- Lies between plasma membrane and cell wall and is smaller than that of Gram-negative bacteria
- Periplasm has relatively few proteins
- Enzymes secreted by Gram-positive bacteria are called exoenzymes
  - aid in degradation of large nutrients

Gram-Negative Cell Walls

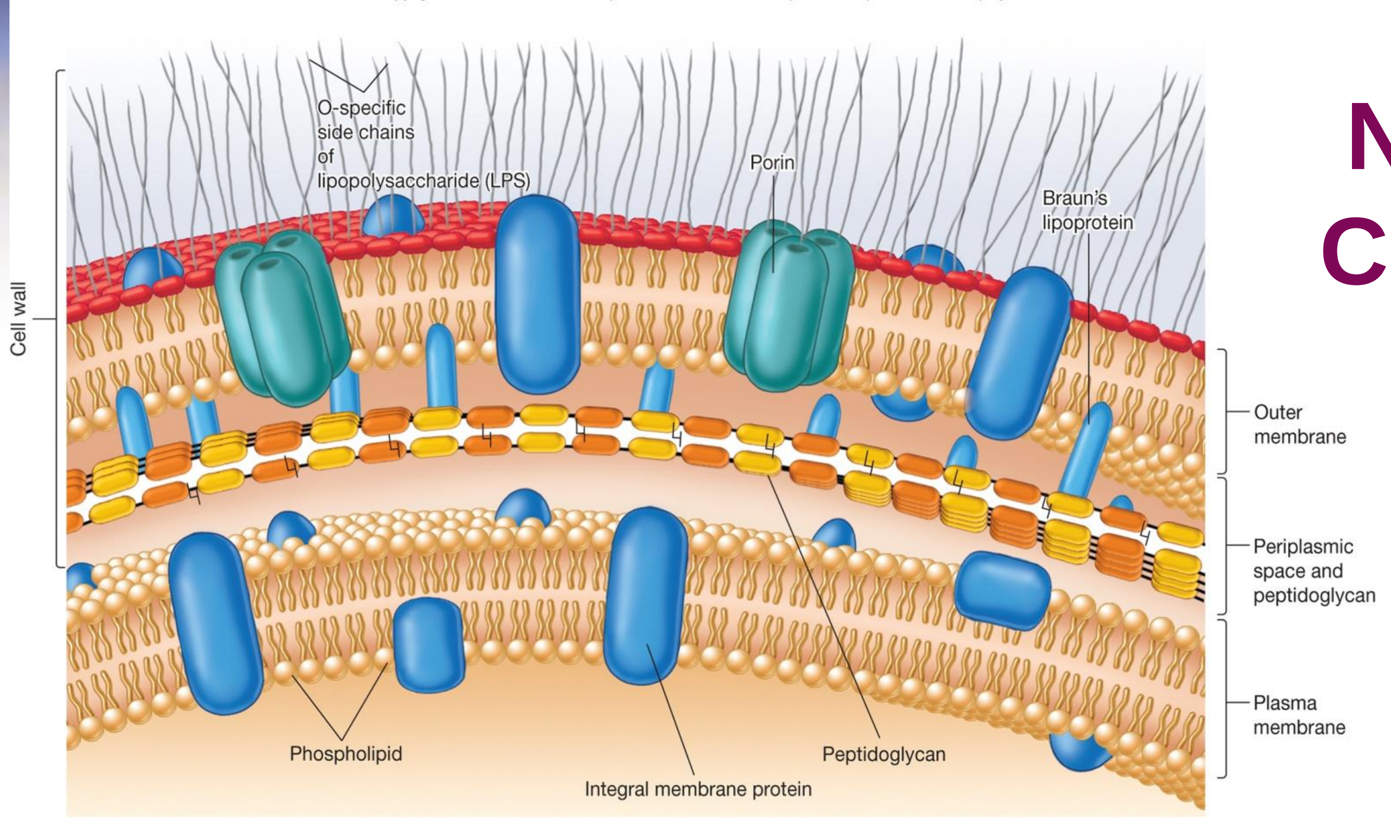
- More complex than Gram-positive
- Consist of a thin layer of peptidoglycan surrounded by an outer membrane
- Outer membrane composed of lipids, lipoproteins, and lipopolysaccharide (LPS)
- No teichoic acids



Gram-Negative Cell Walls

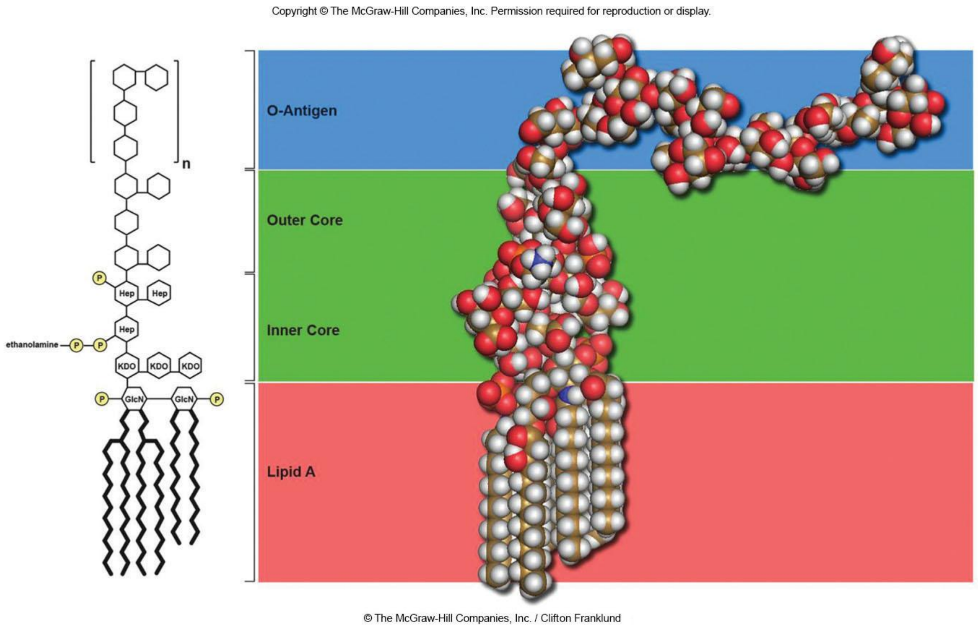
- Peptidoglycan is ~5-10% of cell wall weight
- Periplasmic space differs from that in Gram-positive cells
  - may constitute 20–40% of cell volume
  - many enzymes present in periplasm
    - hydrolytic enzymes, transport proteins and other proteins

Gram-Negative Cell Walls



- outer membrane lies outside the thin peptidoglycan layer
- Braun's lipoproteins connect outer membrane to peptidoglycan
- other adhesion sites reported

Lipopolysaccharide (LPS)



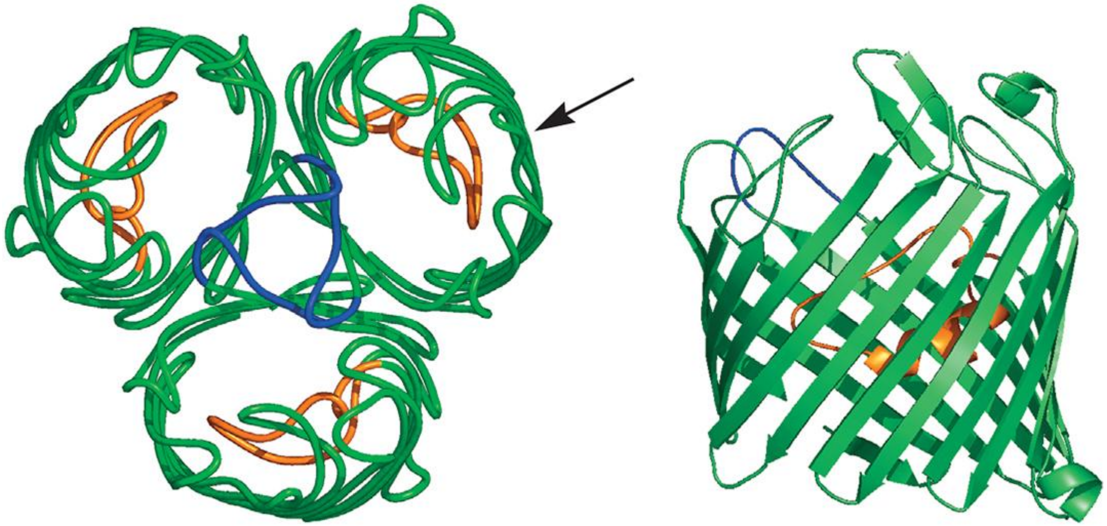
- Consists of three parts
  - lipid A
  - core polysaccharide
  - O side chain (O antigen)
- Lipid A embedded in outer membrane
- Core polysaccharide, O side chain extend out from the cell

Importance of LPS

- contributes to negative charge on cell surface
- helps stabilize outer membrane structure
- may contribute to attachment to surfaces and biofilm formation
- creates a permeability barrier
- protection from host defenses (O antigen)
- can act as an endotoxin (lipid A)

Gram-Negative Outer Membrane Permeability

- More permeable than plasma membrane due to presence of porin proteins and transporter proteins
  - porin proteins form channels to let small molecules (600–700 daltons) pass



(a) Porin trimer **(b)** OmpF side view

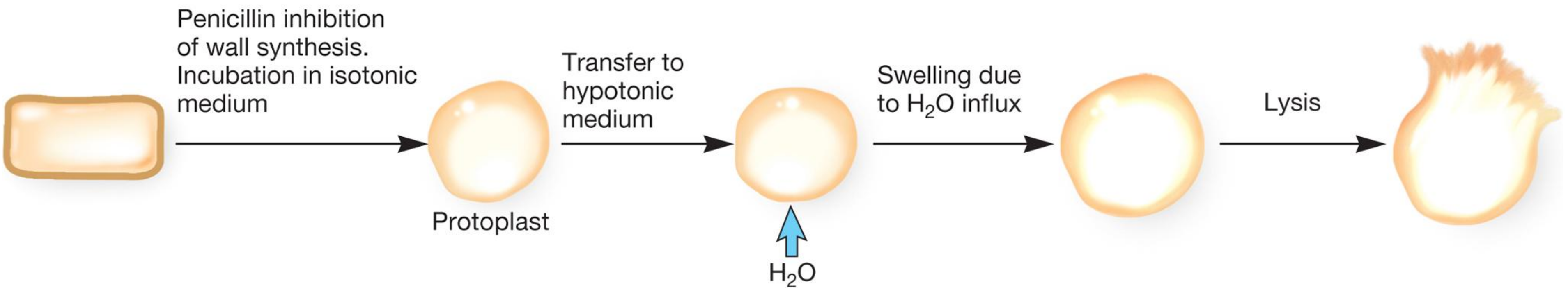
Mechanism of Gram Stain Reaction

- Gram stain reaction due to nature of cell wall
- shrinkage of the pores of peptidoglycan layer of Gram-positive cells
  - constriction prevents loss of crystal violet during decolorization step
- thinner peptidoglycan layer and larger pores of Gram-negative bacteria does not prevent loss of crystal violet

Osmotic Protection

- Hypotonic environments
  - solute concentration outside the cell is less than inside the cell
  - water moves into cell and cell swells
  - cell wall protects from lysis
- Hypertonic environments
  - solute concentration outside the cell is greater than inside
  - water leaves the cell
  - plasmolysis occurs

Evidence of Protective Nature of the Cell Wall



- lysozyme breaks the bond between N-acetyl glucosamine and N-acetylmuramic acid
- penicillin inhibits peptidoglycan synthesis
- if cells are treated with either of the above they will lyse if they are in a hypotonic solution

Cells that Lose a Cell Wall May Survive in Isotonic Environments

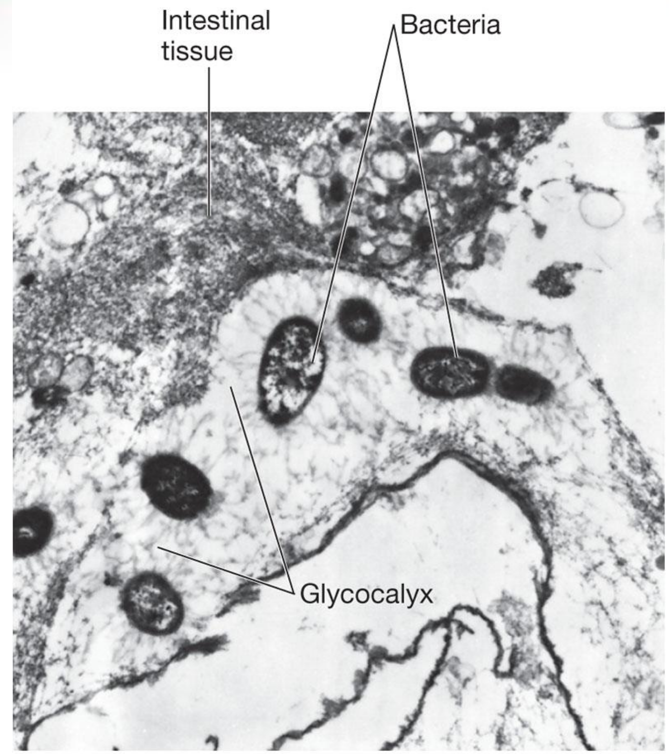
- Protoplasts
- Spheroplasts
- *Mycoplasma*
  - does not produce a cell wall
  - plasma membrane more resistant to osmotic pressure

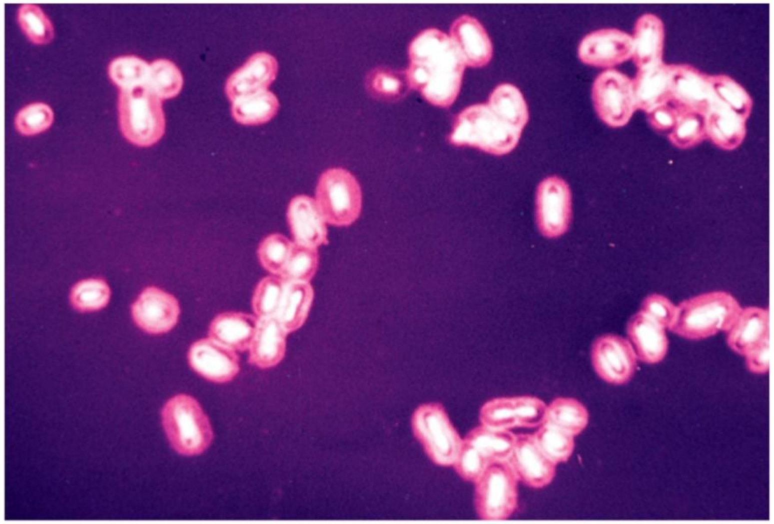
Components Outside of the Cell Wall

- Outermost layer in the cell envelope
- Glycocalyx
  - capsules and slime layers
  - S layers
- Aid in attachment to solid surfaces
  - e.g., biofilms in plants and animals

Capsules

- Usually composed of polysaccharides
- Well organized and not easily removed from cell
- Visible in light microscope
- Protective advantages
  - resistant to phagocytosis
  - protect from desiccation
  - exclude viruses and detergents





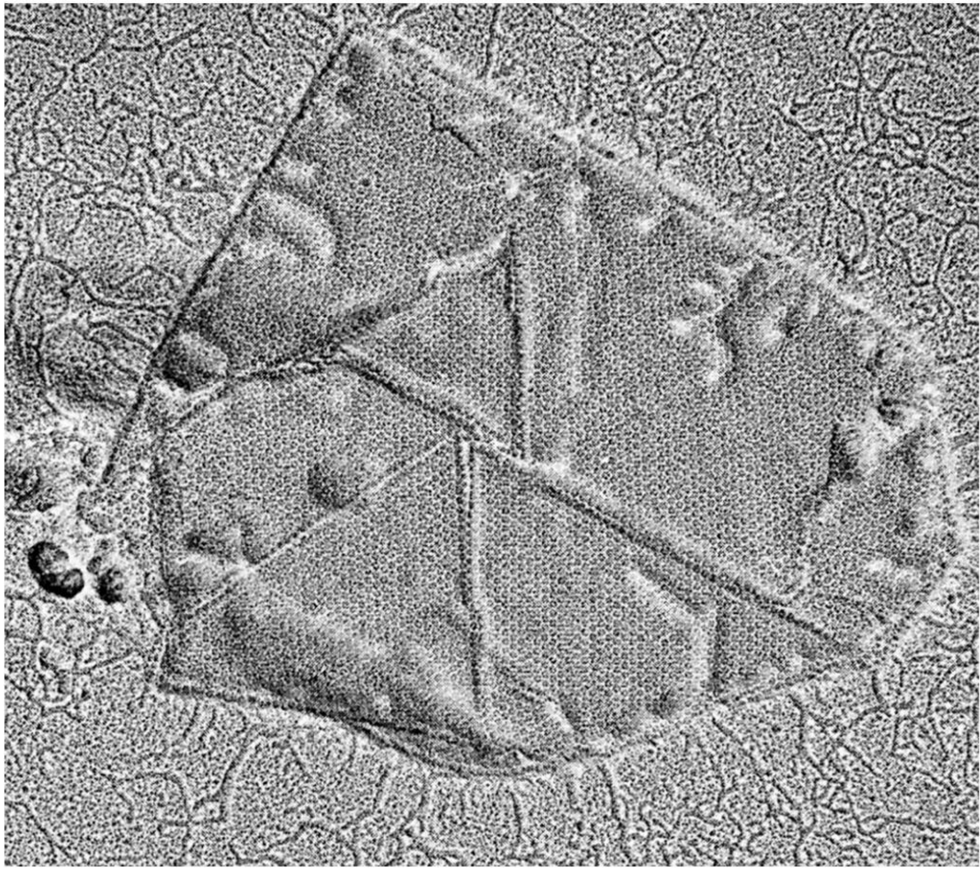
K. pneumoniae

Slime Layers

- similar to capsules except diffuse, unorganized and easily removed
- slime may aid in motility

S Layers

- Regularly structured layers of protein or glycoprotein that self-assemble
  - in Gram-negative bacteria the S layer adheres to outer membrane
  - in Gram-positive bacteria it is associated with the peptidoglycan surface



Dr. Robert G.E. Murray

S Layer Functions

- Protect from ion and pH fluctuations, osmotic stress, enzymes, and predation
- Maintains shape and rigidity
- Promotes adhesion to surfaces
- Protects from host defenses
- Potential use in nanotechnology
  – S layer spontaneously associates

Bacterial Cytoplasmic Structures

- Cytoskeleton
- Intracytoplasmic membranes
- Inclusions
- Ribosomes
- Nucleoid and plasmids

The Cytoskeleton

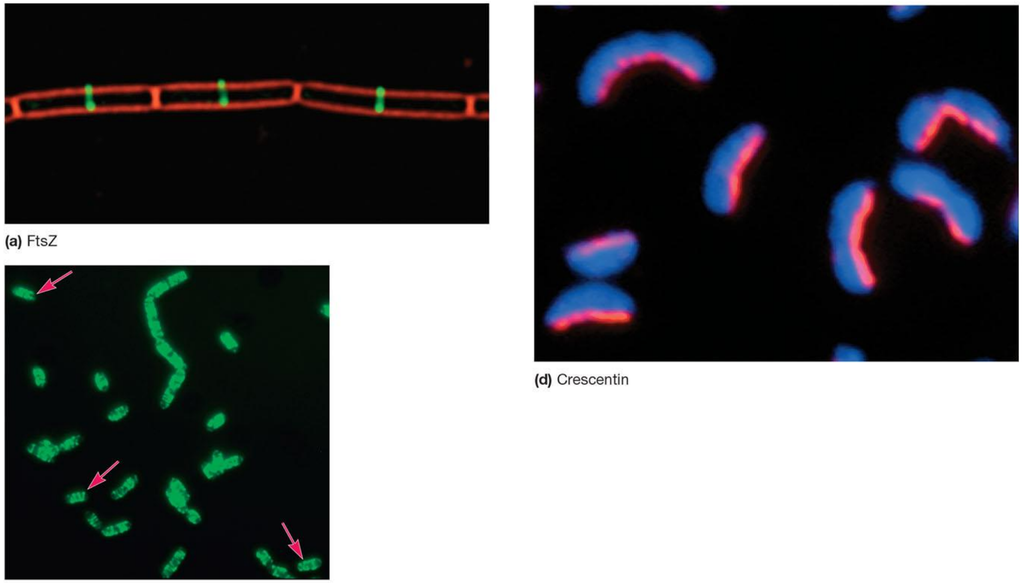
- Homologs of all 3 eukaryotic cytoskeletal elements have been identified in bacteria
- Functions are similar as in eukaryotes

Table 3.2 Bacterial Cytoskeletal Proteins

Type	Function	Comments
Tubulin Homologues		
FtsZ	Cell division	Widely observed in bacteria and archaea
BtubA/BtubB	Unknown	Observed only in *Prosthecobacter* spp.; thought to be encoded by eukaryotic tubulin genes obtained by horizontal gene transfer
TubZ	Possibly plasmid segregation	Encoded by large plasmids observed in members of the genus *Bacillus*
Actin Homologues		
MamK	Positioning magnetosomes	Observed in magnetotactic species
MreB/Mbl	Helps determine cell shape, may be involved in chromosome segregation, localizes proteins	Most rod-shaped bacteria
ParM	Plasmid segregation	Plasmid encoded
Intermediate Filament Homologues		
CreS (crescentin)	Induces curvature in curved rods	*Caulobacter crescentus*
Unique Bacterial Cytoskeletal Proteins		
MinD	Prevents polymerization of FtsZ at cell poles	Many rod-shaped bacteria
ParA	Segregates chromosomes and plasmids	Observed in many species, including *Vibrio cholerae, C. crescentus,* and *Thermus thermophilus*

Best Studied Examples

- FtsZ – many bacteria
  - – forms ring during septum formation in cell division
- MreB – many rods
  - – maintains shape by positioning peptidoglycan synthesis machinery
- CreS – rare, maintains curve shape



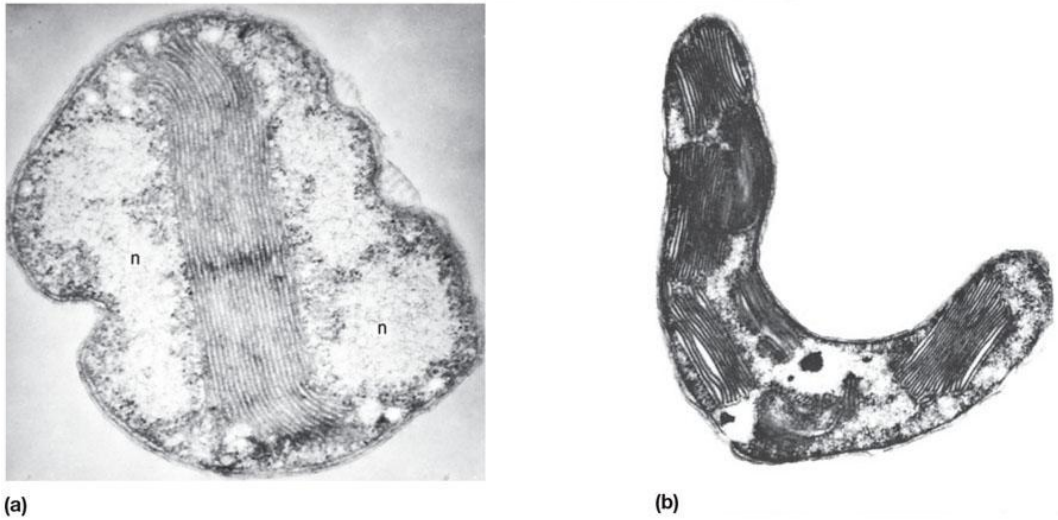
(a) FtsZ

(b) Mbl

(d) Crescentin

a: Dr. Joseph Pogliano; b: Image courtesy of Rut Carballido-Lo'pez and Jeff Errington; d: Dr. Christine Jacobs-Wagner

Intracytoplasmic Membranes



a: R.G.E. Murray & S.W. Watson, Journal of Bacteriology 89 (6): 1597, fig Nitrocystis Oceanus, 1965. American Society for Microbiology; b: Reprinted from *The Shorter Bergey's Manual of Determinative Bacteriology*, 8e, John G. Holt, Editor, 1977 © Bergey's Manual Trust. Published by Williams & Wilkins Baltimore, MD

- Plasma membrane infoldings
  - observed in many photosynthetic bacteria
  - observed in many bacteria with high respiratory activity
- Anammoxosome in *Planctomycetes*
  - organelle – site of anaerobic ammonia oxidation

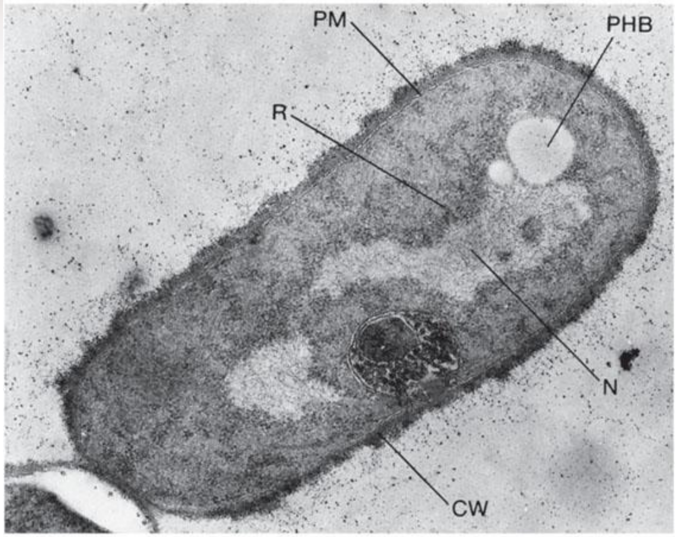
Inclusions

- Granules of organic or inorganic material that are stockpiled by the cell for future use
- Some are enclosed by a single-layered membrane
  - membranes vary in composition
  - some made of proteins; others contain lipids
  - may be referred to as *microcompartments*

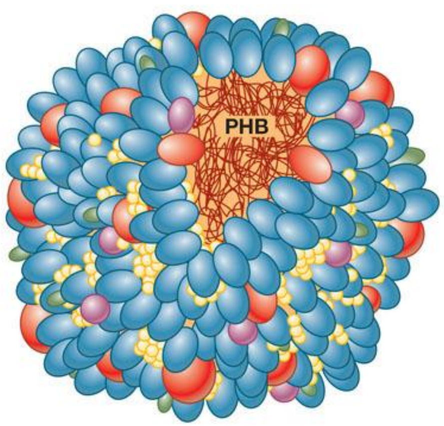
Storage Inclusions

- Storage of nutrients, metabolic end products, energy, building blocks
- Glycogen storage
- Carbon storage
  - poly-β-hydroxybutyrate (PHB)
- Phosphate - Polyphosphate (Volutin)
- Amino acids - cyanophycin granules

Storage Inclusions



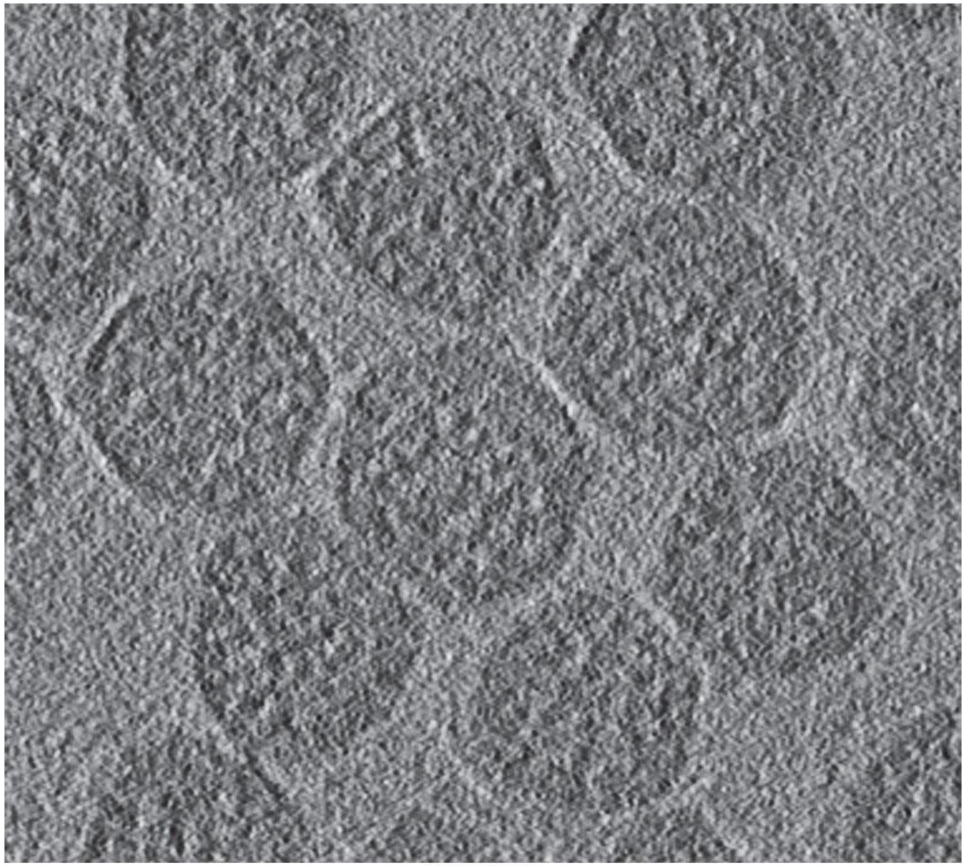
(a)



(b)

Microcompartments

- Not bound by membranes but compartmentalized for a specific function
- Carboxysomes - CO_2 fixing bacteria
  - contain the enzyme ribulose-1,5,-bisphosphate carboxylase (Rubisco), enzyme used for CO_2 fixation

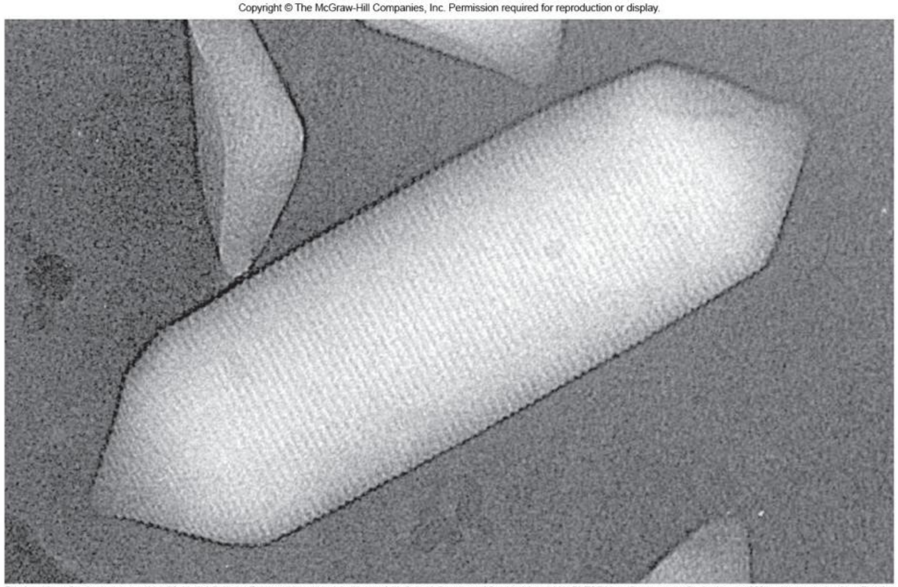


Michael Schmid

Other Inclusions

- Gas vacuoles
  - found in aquatic, photosynthetic bacteria and archaea
  - provide buoyancy in gas vesicles

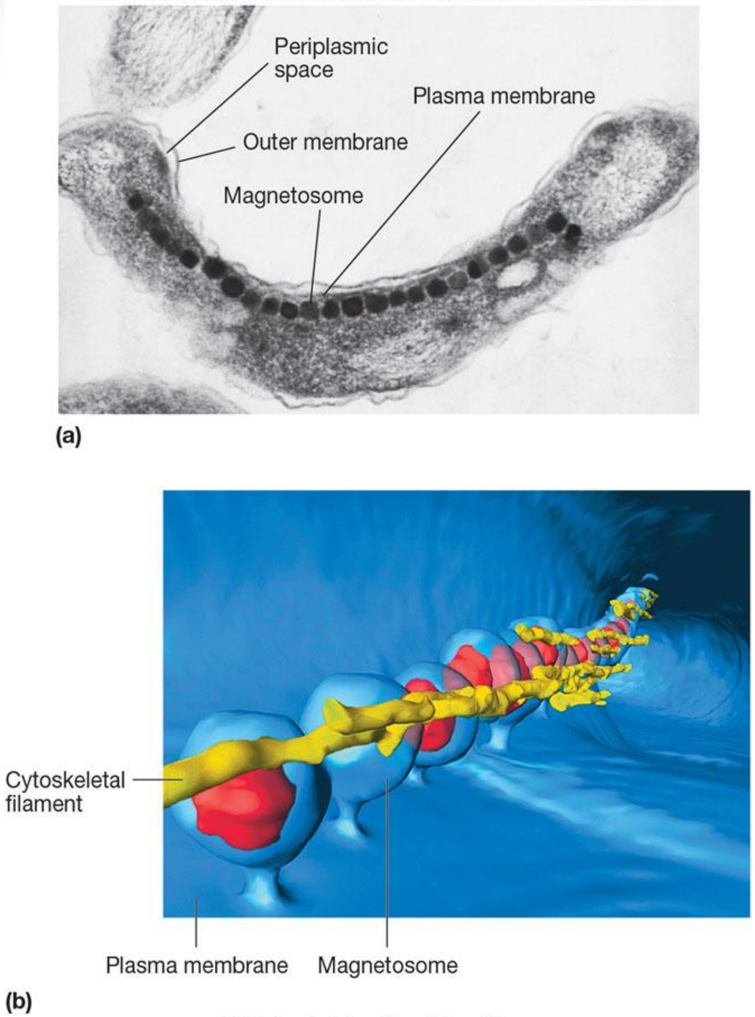
Courtesy of Daniel Branton, Harvard University



Reprinted from *Biophysical Journal* Vol. 86, Marina Belenky, Rebecca Meyers and Judith Herzfeld, Subunit Structure of Gas Vesicles: A MALDI-TOF Mass Spectrometry Study, January 2004, with permission from Elsevier

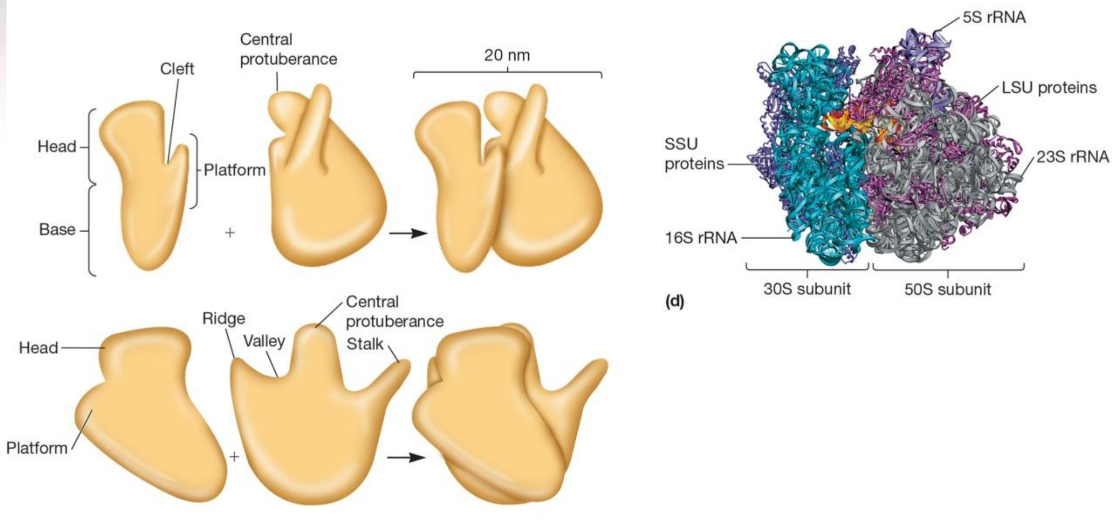
Other Inclusions

- Magnetosomes
  - found in aquatic bacteria
  - magnetite particles for orientation in Earth's magnetic field
  - cytoskeletal protein MamK
    - helps form magnetosome chain



a: Y. Gorby; b: Zhuo Li and Grant Jensen

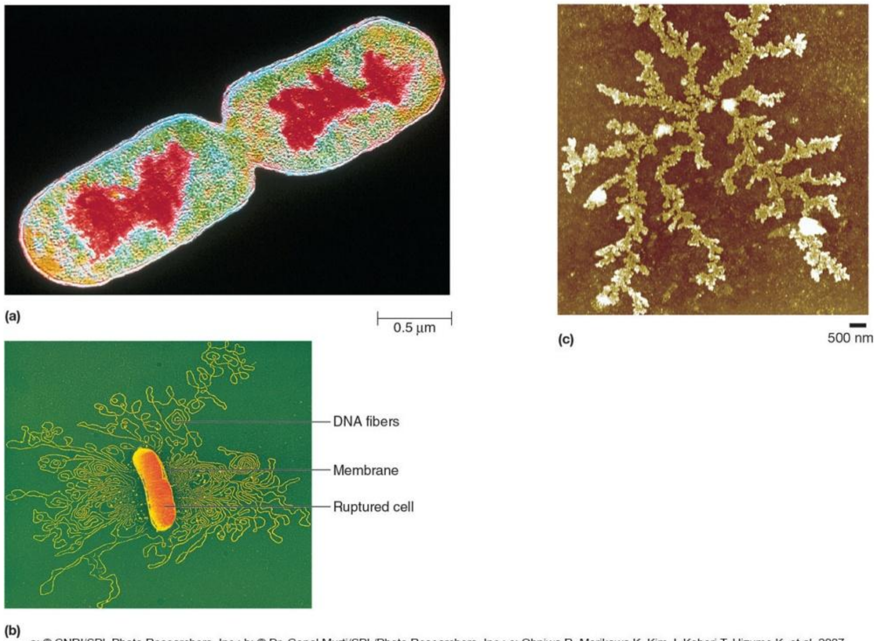
Ribosomes



(a) 30S subunit (b) 50S subunit (c) 70S ribosome

- Complex protein/RNA structures
  - sites of protein synthesis
  - bacterial and archaea ribosome = 70S
  - eukaryotic (80S) S = Svedburg unit
- Bacterial ribosomal RNA
  - 16S small subunit
  - 23S and 5S in large subunit

The Nucleoid



a: © CNRI/SPL Photo Researchers, Inc.; b: © Dr. Gopal Murti/SPL/Photo Researchers, Inc.; c: Ohniwa R. Morikawa K, Kim J, Kobori T, Hizume K, et al. 2007. *Microsec. Microana.* 13:3–12. Reprinted with the permission of Cambridge University Press

- Usually not membrane bound (few exceptions)
- Location of chromosome and associated proteins
- Usually 1 closed circular, double-stranded DNA molecule
- Supercoiling and nucleoid proteins (different from histones) aid in folding

Plasmids

- Extrachromosomal DNA
  - found in bacteria, archaea, some fungi
  - usually small, closed circular DNA molecules
- Exist and replicate independently of chromosome
  - episomes – may integrate into chromosome
  - inherited during cell division
- Contain few genes that are non-essential
  - confer selective advantage to host (e.g., drug resistance)
- Classification based on mode of existence, spread, and function

Table 3.3 Major Types of Bacterial Plasmids

Type	Function	Example	Size (kbp)	Hosts	Phenotypic Features[1]
Conjugative Plasmids[2]	Transfer of DNA from one cell to another	F factor	95–100	*E. coli, Salmonella, Citrobacter*	Sex pilus, conjugation
R Plasmids	Carry antibiotic-resistance genes	RP4	54	*Pseudomonas* and many other Gram-negative bacteria	Sex pilus, conjugation, resistance to Amp, Km, Nm, Tet
Col Plasmids	Produce bacteriocins, substances that destroy closely related species	ColE1	9	*E. coli*	Colicin E1 production
Virulence Plasmids	Carry virulence genes	Ti	200	*Agrobacterium tumefaciens*	Tumor induction in plants
Metabolic Plasmids	Carry genes for enzymes	CAM	230	*Pseudomonas*	Camphor degradation

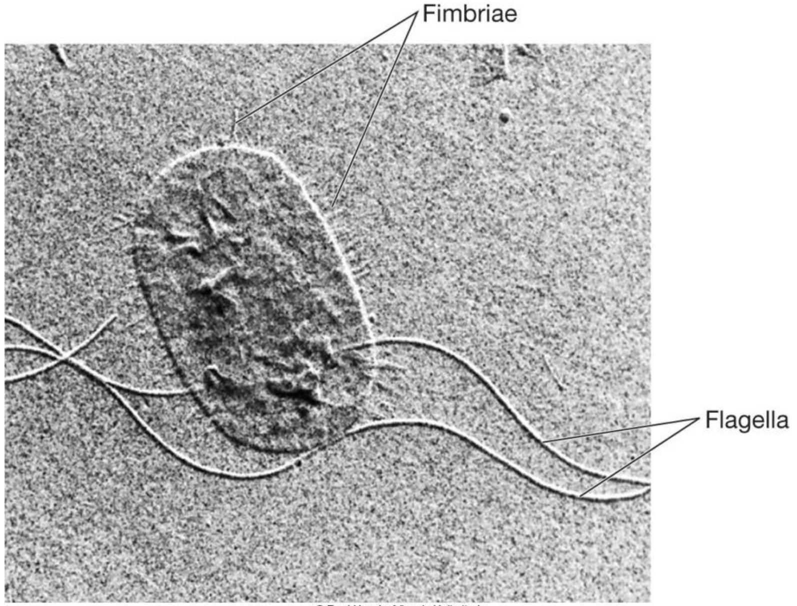
1 Abbreviations used for resistance to antibiotics: Amp, ampicillin; Gm, gentamycin; Km, kanamycin; Nm, neomycin; Tet, tetracycline.
2 Many R plasmids, metabolic plasmids, and others are also conjugative.

External Structures

- Extend beyond the cell envelope in bacteria
- Function in protection, attachment to surfaces, horizontal gene transfer, cell movement
  - pili and fimbriae
  - flagella

Pili and Fimbriae

- Fimbriae (s., fimbria); pili (s., pilus)
  - short, thin, hairlike, proteinaceous appendages (up to 1,000/cell)
  - can mediate attachment to surfaces, motility, DNA uptake
- Sex pili (s., pilus)
  - longer, thicker, and less numerous (1-10/cell)
  - genes for formation found on plasmids
  - required for conjugation



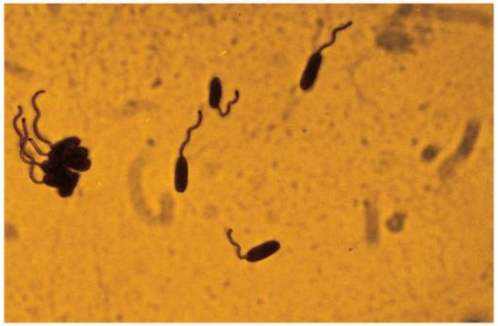
Flagella

- Threadlike, locomotor appendages extending outward from plasma membrane and cell wall
- Functions
  - motility and swarming behavior
  - attachment to surfaces
  - may be virulence factors

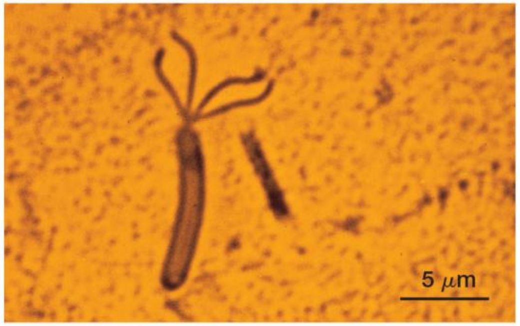
Bacterial Flagella

- Thin, rigid protein structures that cannot be observed with bright-field microscope unless specially stained
- Ultrastructure composed of three parts
- Pattern of flagellation varies

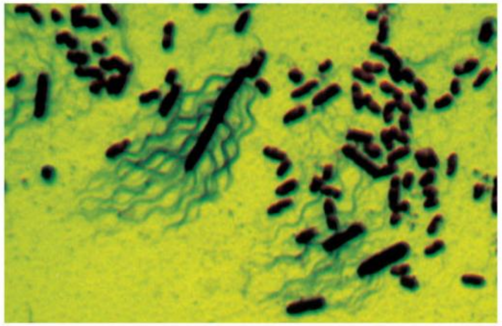
Patterns of Flagella Distribution



(a) *Pseudomonas*—monotrichous polar flagellation



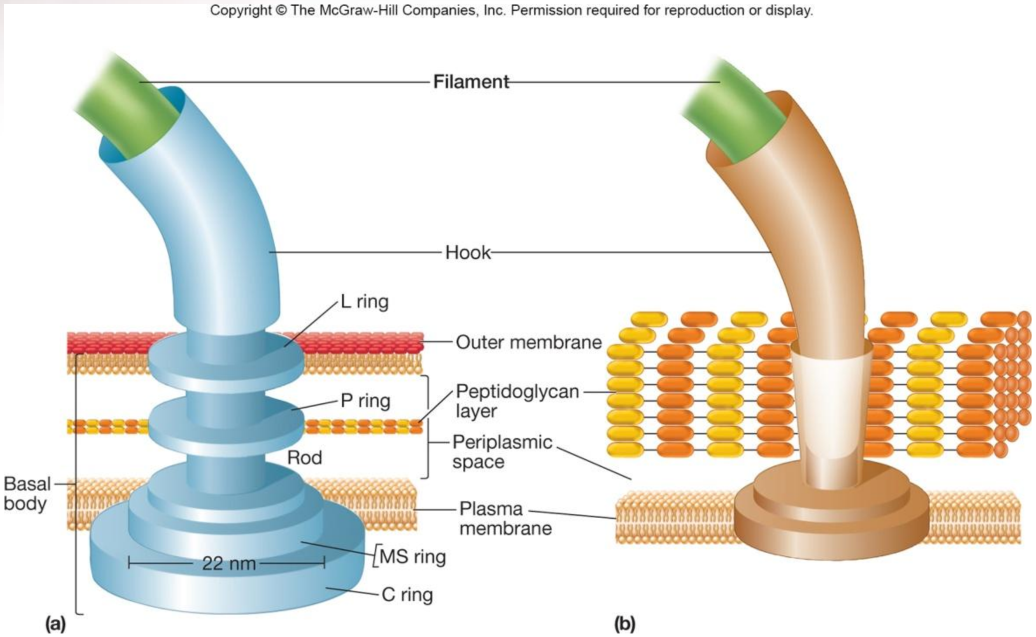
(b) *Spirillum*—lophotrichous flagellation



(c) *P. vulgaris*—peritrichous flagellation

- Monotrichous – one flagellum
- Polar flagellum – flagellum at end of cell
- Amphitrichous – one flagellum at each end of cell
- Lophotrichous – cluster of flagella at one or both ends
- Peritrichous – spread over entire surface of cell

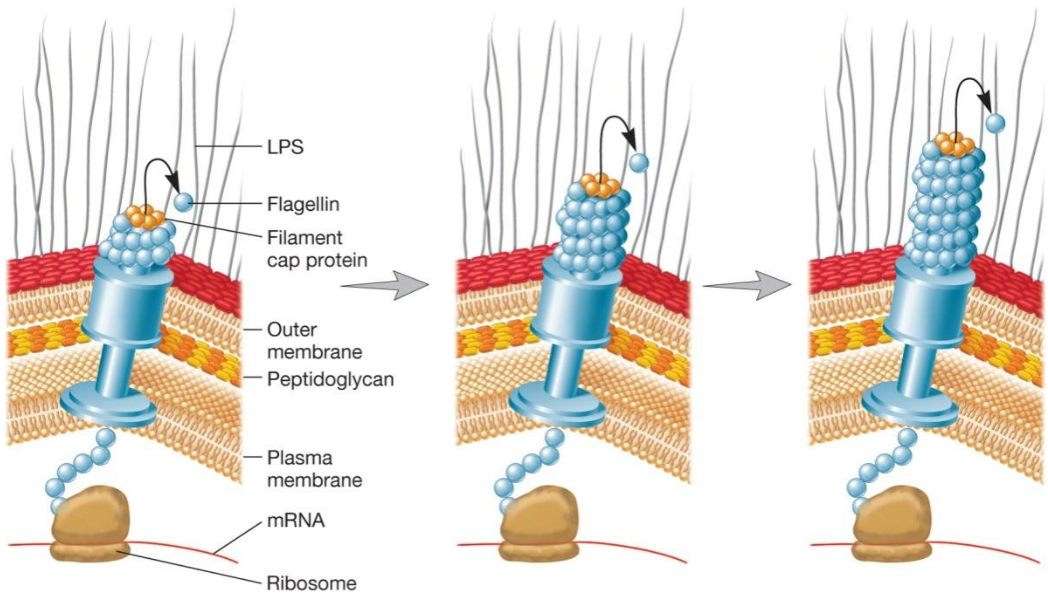
Three Parts of Flagella



- Filament
  - extends from cell surface to the tip
  - hollow, rigid cylinder of flagellin protein
- Hook
  - links filament to basal body
- Basal body
  - series of rings that drive flagellar motor

Flagellar Synthesis

- complex process involving many genes/gene products
- new flagellin molecules transported through the hollow filament using Type III-like secretion system
- filament subunits self-assemble with help of filament cap at tip, not base



Motility

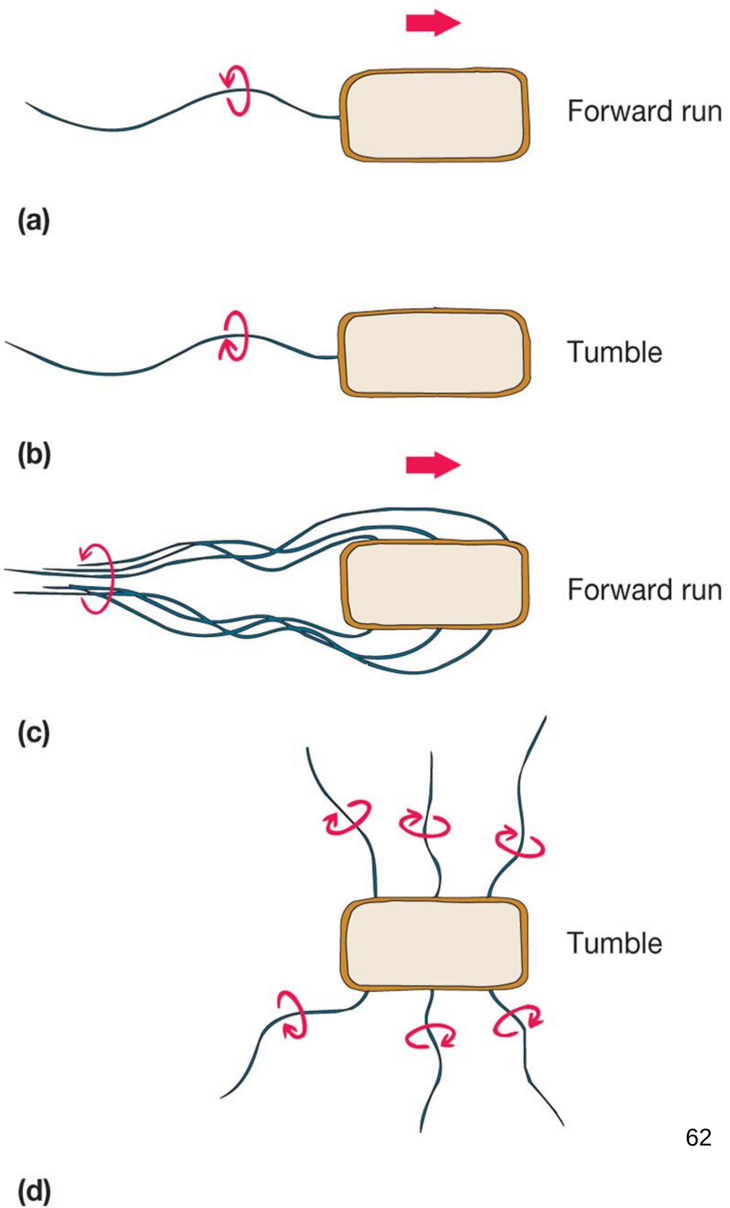
- Flagellar movement
- Spirochete motility
- Twitching motility
- Gliding motility

Motility

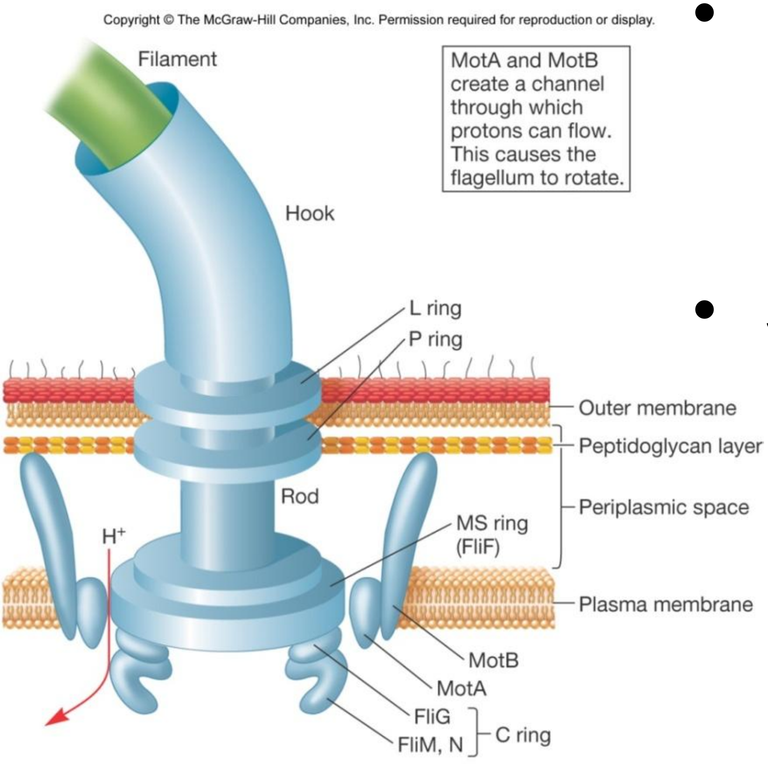
- *Bacteria* and *Archaea* have directed movement
- Chemotaxis
  - move toward chemical attractants such as nutrients, away from harmful substances
- Move in response to temperature, light, oxygen, osmotic pressure, and gravity

Bacterial Flagellar Movement

- Flagellum rotates like a propeller
  - very rapid rotation up to 1100 revolutions/sec
  - in general, counterclockwise (CCW) rotation causes forward motion (run)
  - in general, clockwise rotation (CW) disrupts run causing cell to stop and tumble



Mechanism of Flagellar Movement

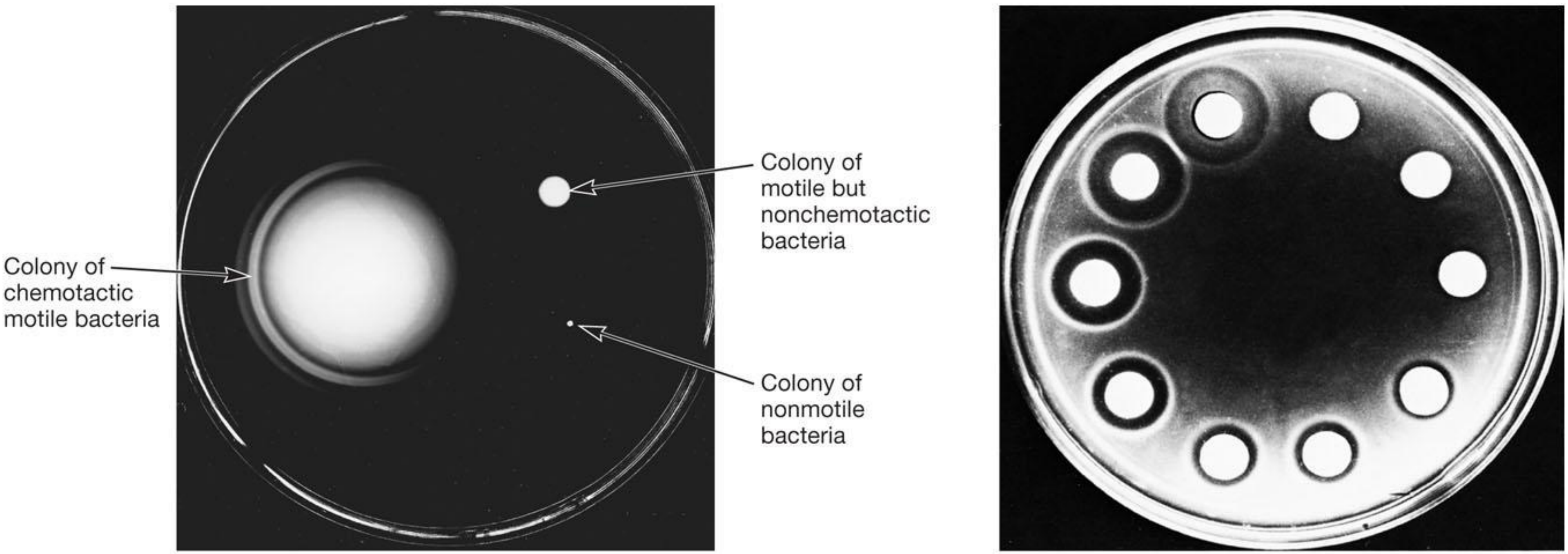


(a)

- Flagellum is 2 part motor producing torque
- Rotor
  - C (FliG protein) ring and MS ring turn and interact with stator
- Stator - Mot A and Mot B proteins
  - form channel through plasma membrane
  - protons move through Mot A and Mot B channels using energy of proton motive force
  - torque powers rotation of the basal body and filament

Chemotaxis

- Movement toward a chemical attractant or away from a chemical repellent
- Changing concentrations of chemical attractants and chemical repellents bind chemoreceptors of chemosensing system

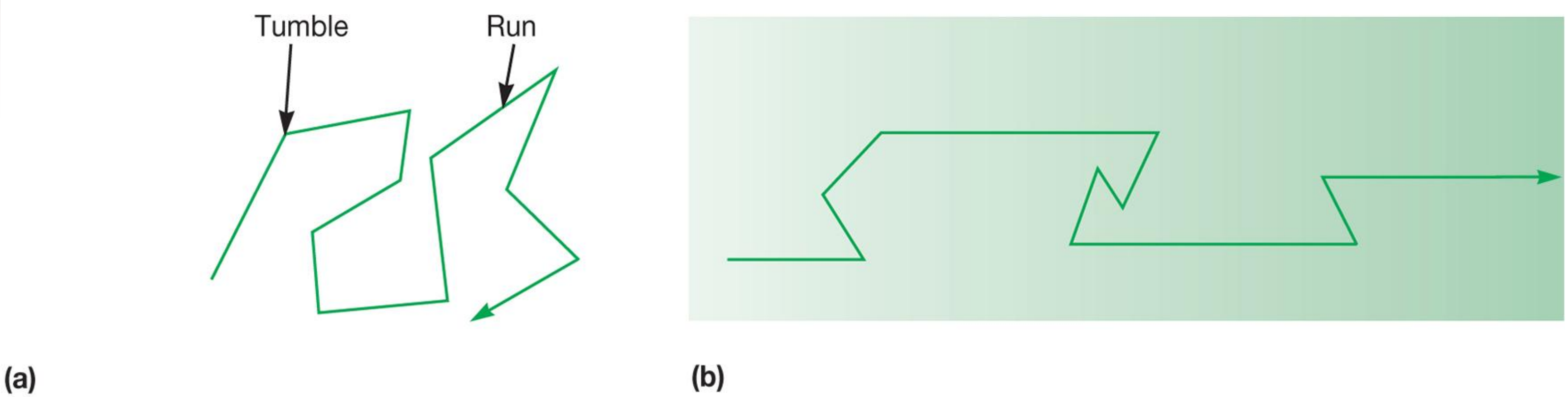


(a) Positive chemotaxis

(b) Negative chemotaxis

Courtesy of Dr. Julius Adler

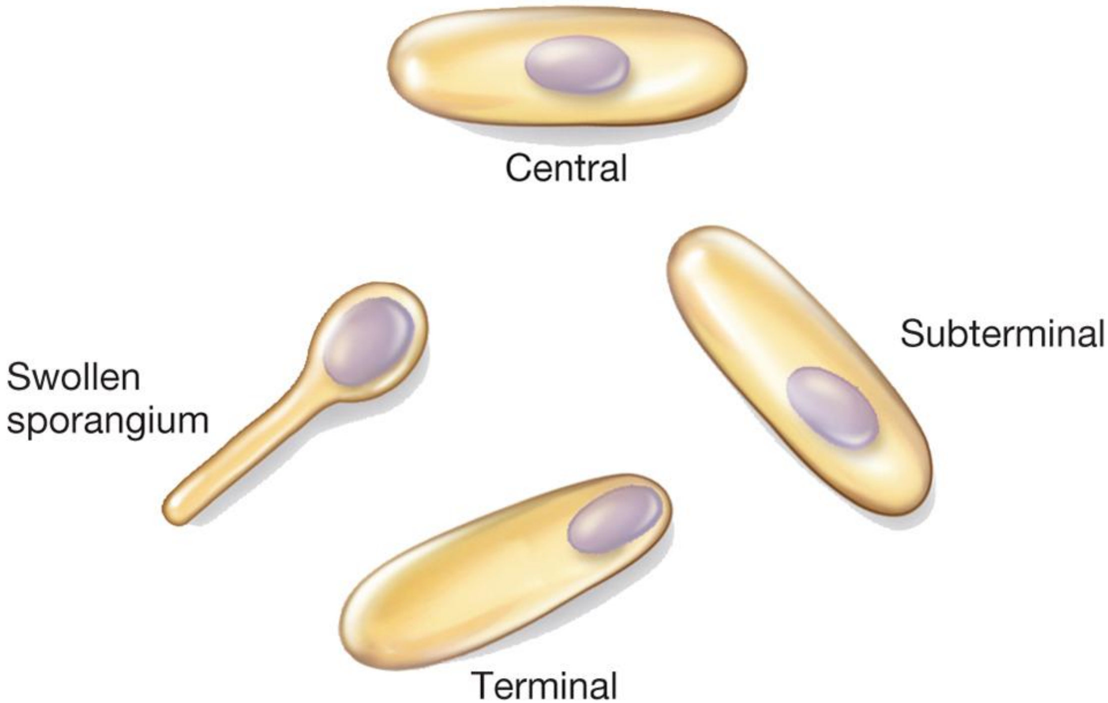
Chemotaxis



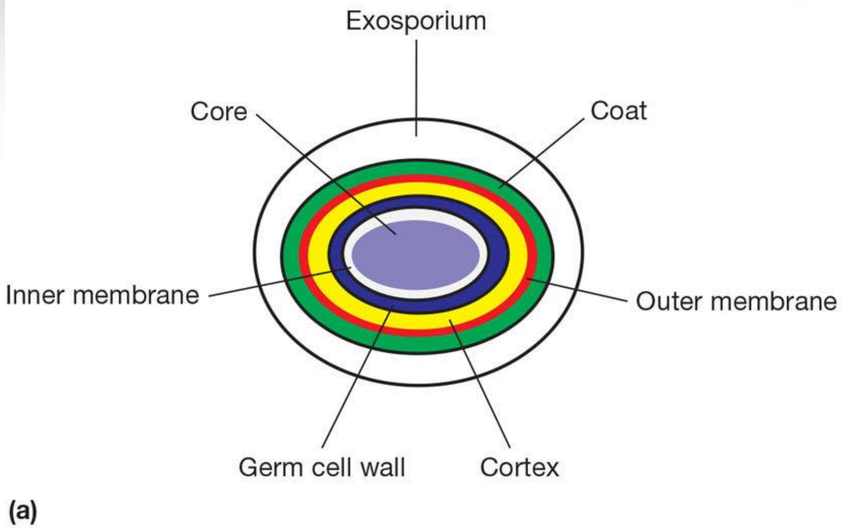
- In presence of attractant (b) tumbling frequency is intermittently reduced and runs in direction of attractant are longer
- Behavior of bacterium is altered by temporal concentration of chemical
- Chemotaxis away from repellent involves similar but opposite responses

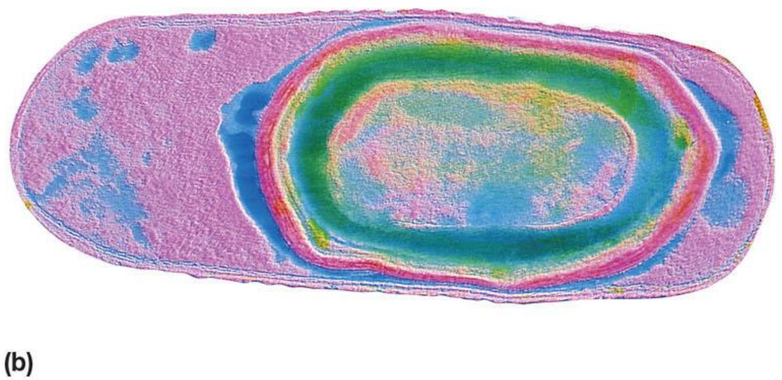
The Bacterial Endospore

- Complex, dormant structure formed by some bacteria
- Various locations within the cell
- Resistant to numerous environmental conditions
  - heat
  - radiation
  - chemicals
  - desiccation



Endospore Structure





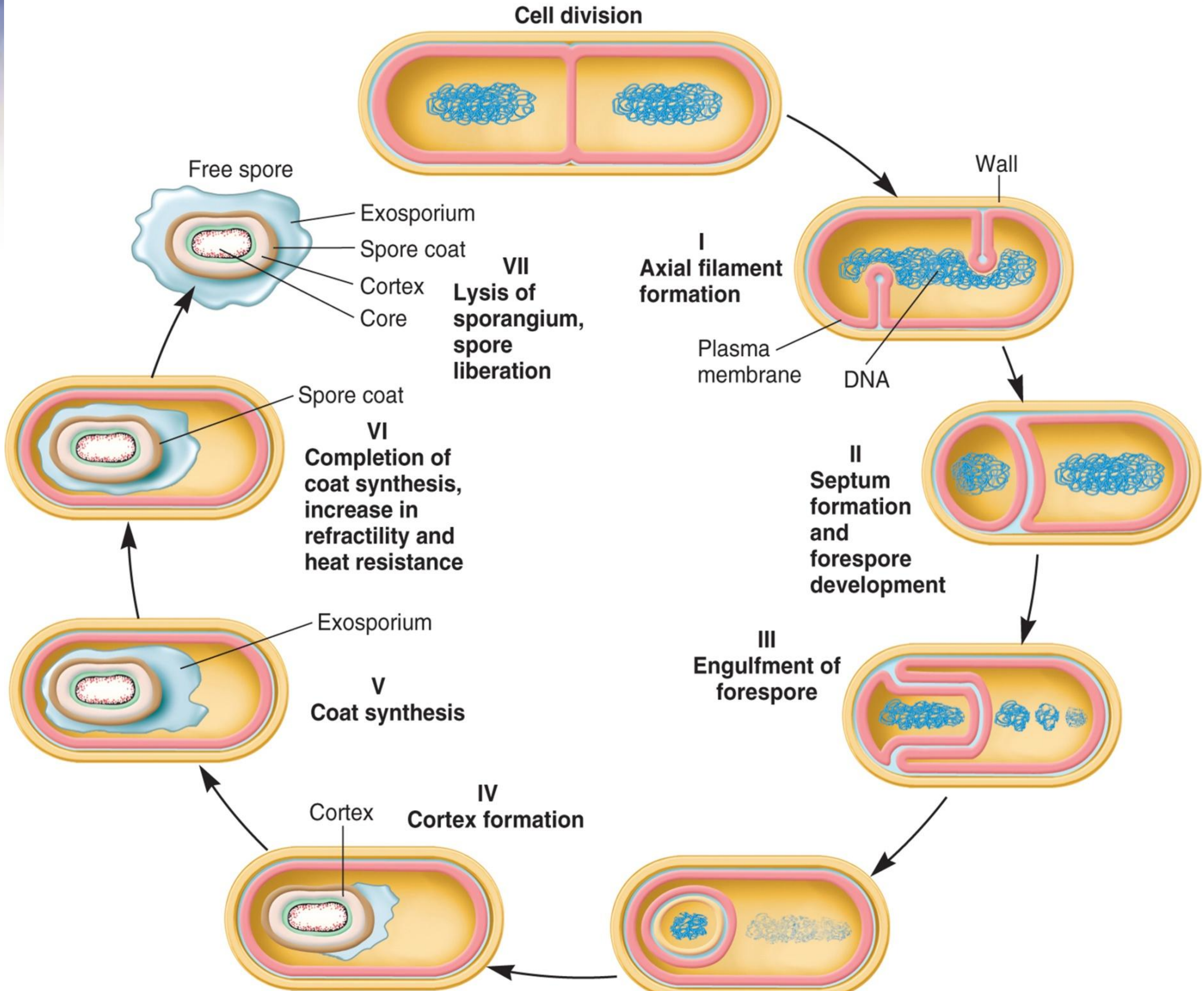
- Spore surrounded by thin covering called exosporium
- Thick layers of protein form the spore coat
- Cortex, beneath the coat, thick peptidoglycan
- Core has nucleoid and ribosomes

What Makes an Endospore so Resistant?

- Calcium (complexed with dipicolinic acid)
- Small, acid-soluble, DNA-binding proteins (SASPs)
- Dehydrated core
- Spore coat and exosporium protect

Sporulation

- Process of endospore formation
- Occurs in a hours (up to 10 hours)
- Normally commences when growth ceases because of lack of nutrients
- Complex multistage process


Cell division
Wall
I
Axial filament formation
Plasma membrane
DNA
II
Septum formation and forespore development
III
Engulfment of forespore
IV
Cortex formation
Cortex
V
Coat synthesis
Exosporium
VI
Completion of coat synthesis, increase in refractility and heat resistance
Spore coat
VII
Lysis of sporangium, spore liberation
Free spore
Exosporium
Spore coat
Cortex
Core

Formation of Vegetative Cell

- Activation
  - prepares spores for germination
  - often results from treatments like heating
- Germination
  - environmental nutrients are detected
  - spore swelling and rupture of absorption of spore coat
  - increased metabolic activity
- Outgrowth - emergence of vegetative cell