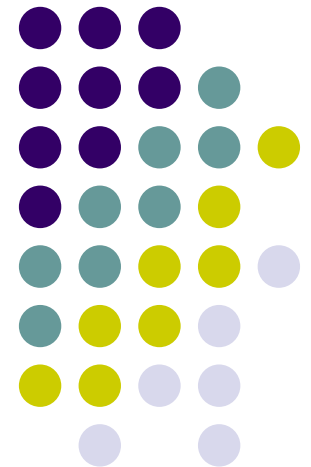


# Prokaryotes

## Cell Structure and Function



# Characteristics of Living Things

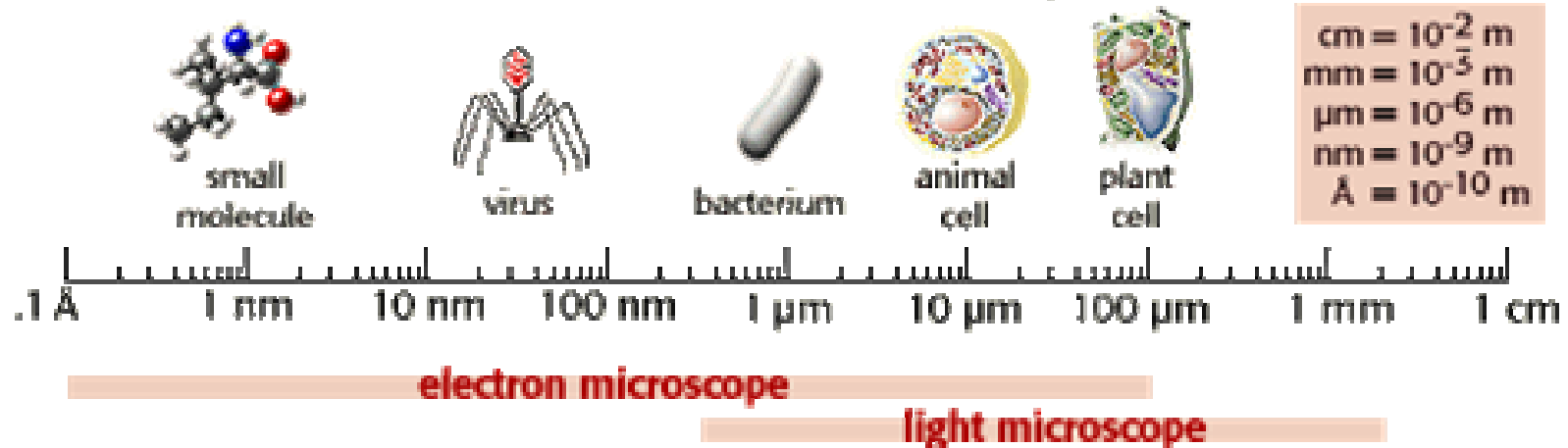


- Metabolism
  - Nutrient source
  - Chemical processes
- Growth
- Responsiveness
- Reproduction
  - Asexual
  - Sexual

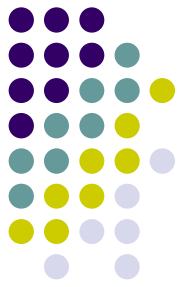
# Size Comparison



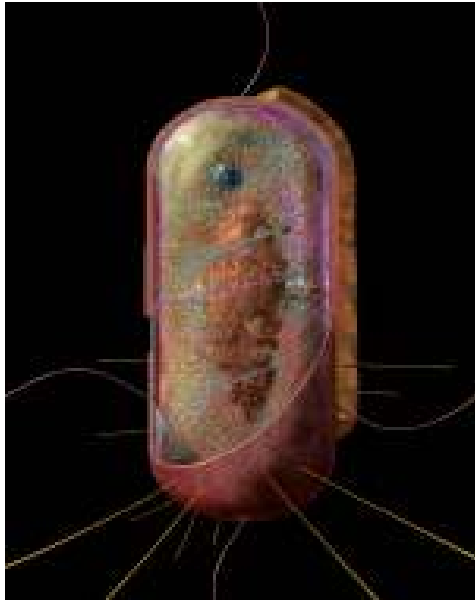
Relative sizes of cells and their components



# Comparison

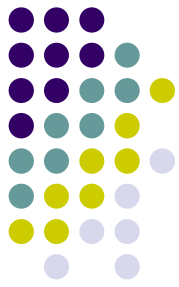


- Eukaryotes
- Prokaryotes

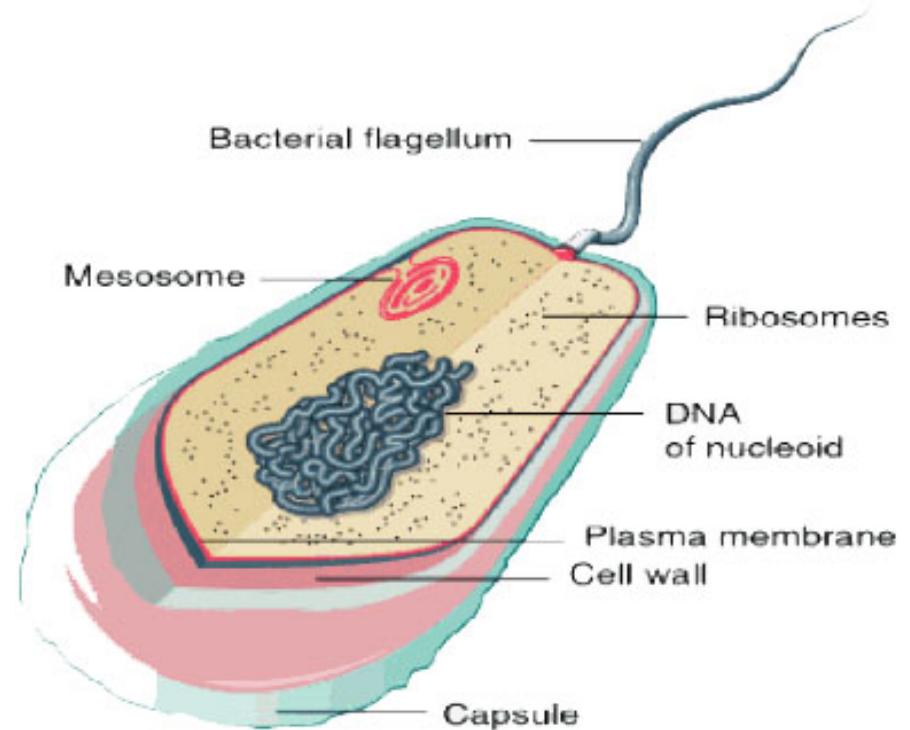


- DNA
- Nucleus
- Other Organelles
- Cell wall
- Plasma Membrane
- Other characteristics
  - Size
  - Shape
  - Location

# Prokaryotic Structure & Function Topics



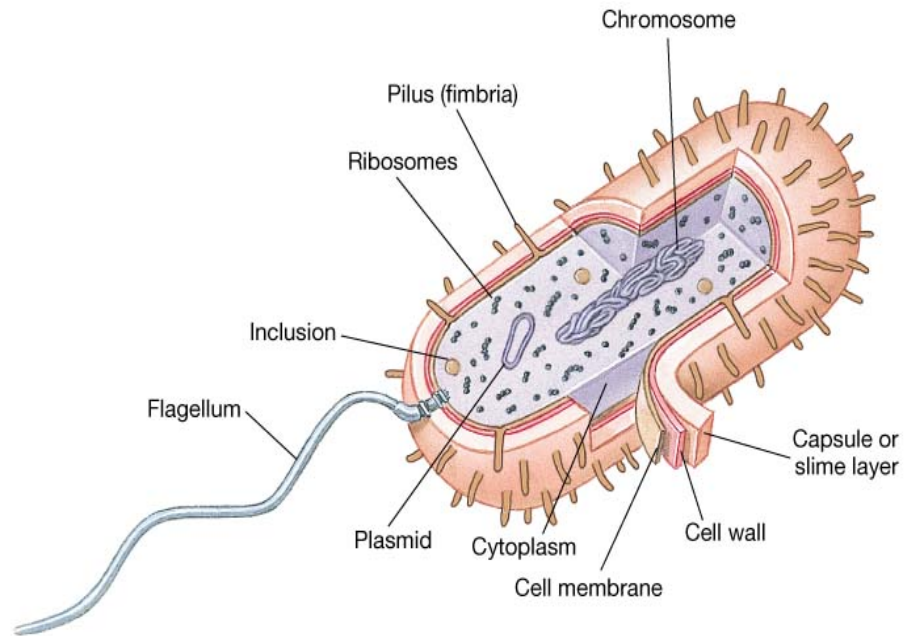
- Cell Wall Appendages
- Cell Wall
- Plasma Membrane
- Cytoplasm
- DNA region
- Organelles
- Reproduction
- Antimicrobial actions



# Cell Wall Appendages

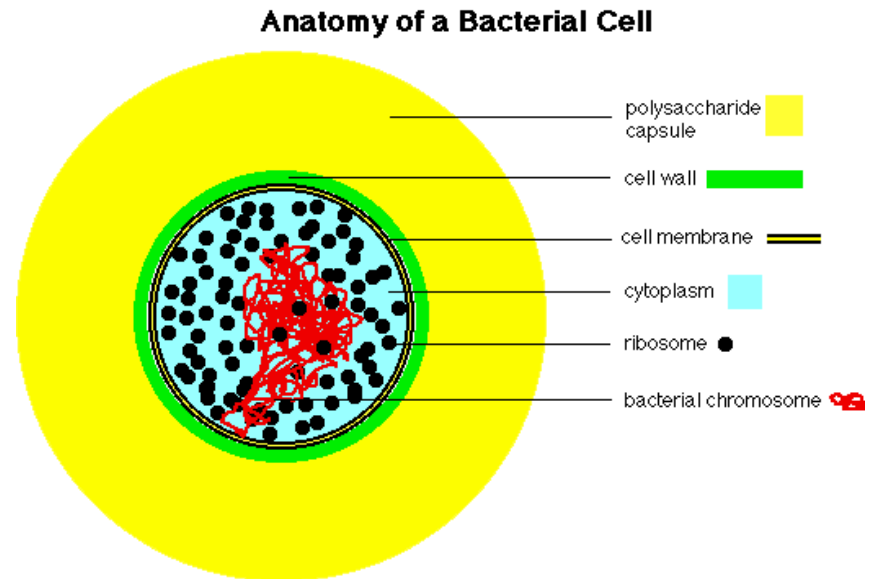


- Glycocalyx
  - Capsule
  - Slime Layer
- S-layer
- Flagella
- Fimbria / Pili

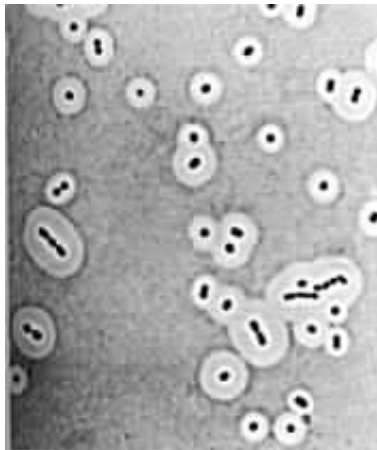
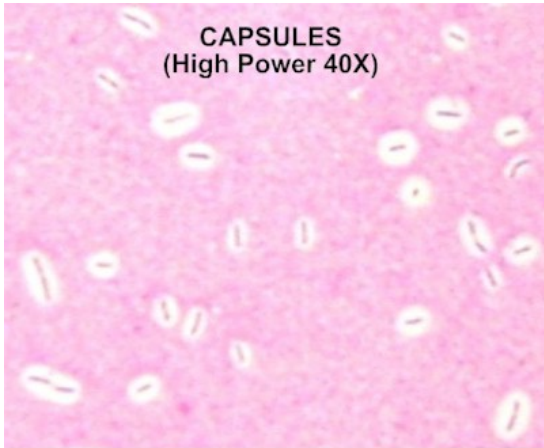
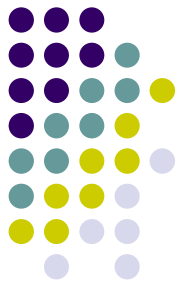


# Glycocalyx

- Secreted by all bacteria in some form
  - Produced inside
  - Extruded to outside
- Aid in survivability
- Aid in pathogenicity
- Negatively charged
- Composition
  - Polysaccharides
  - Polypeptides
  - Both



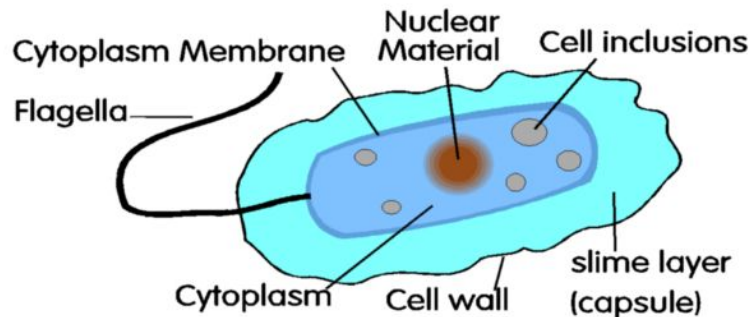
# Glycocalyx: Capsule



- Organized repeating units
- Thick
- Firmly Attached
- Function
  - Protect from desiccation and other environmental hazards
  - Evade host defenses via phagocytosis



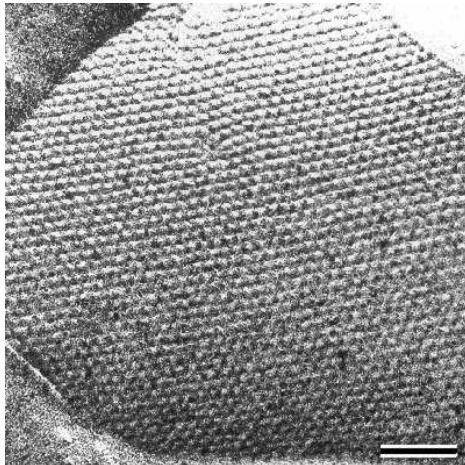
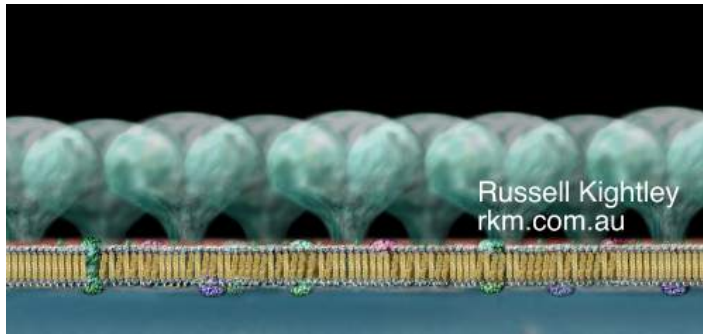
# Glycocalyx: Slime Layer



Cell structure

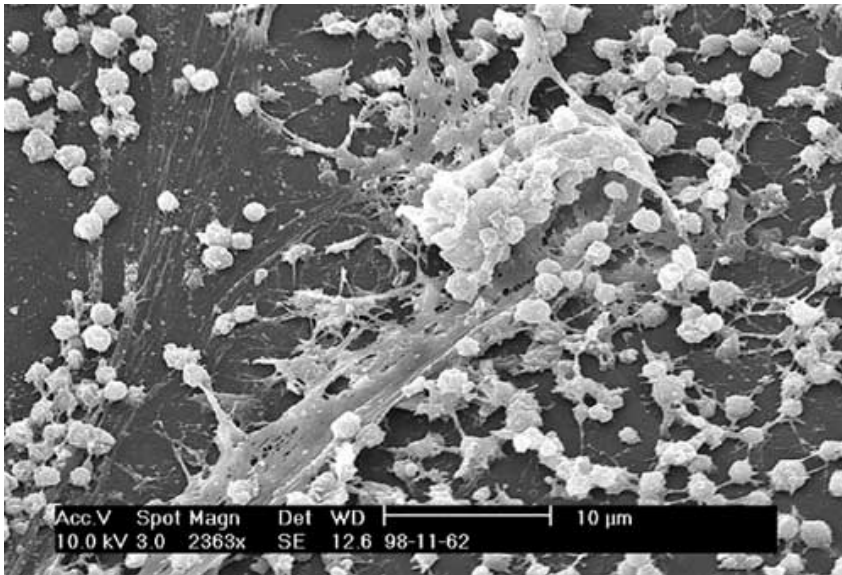
- Unorganized
- Thin
- Loosely attached
- Viscous
- Water soluble
- Function
  - Adherence
  - Protection
  - Trap nutrients

# S - Layer



- Glycoprotein
  - Viscous
  - Thin
  - Organized in crystal lattice
  - Anchored to plasma membrane
  - Some G + and G –
    - Eubacteria
    - Archeae
- Function
  - Strengthen and reinforce phospholipid bilayer
  - Adherence
    - Colonize
    - Resist flushing
  - Protection
    - Environment
    - Immune

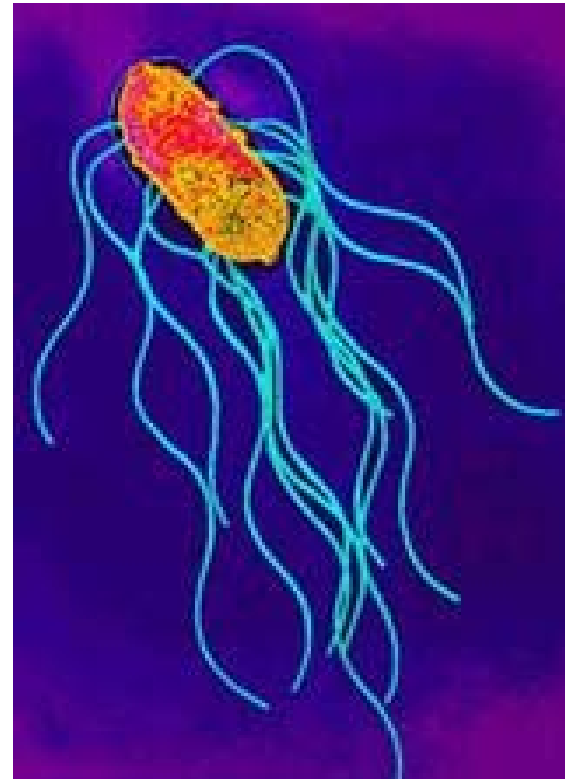
# Biofims

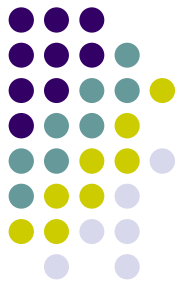


- Microbial community attached to surface
- Resistant
  - AB
  - Immune
- Types
  - Environmental
  - Infectious
    - Dental Plaque
    - Endocarditis
    - Kidney Stones
    - CF

# Flagella

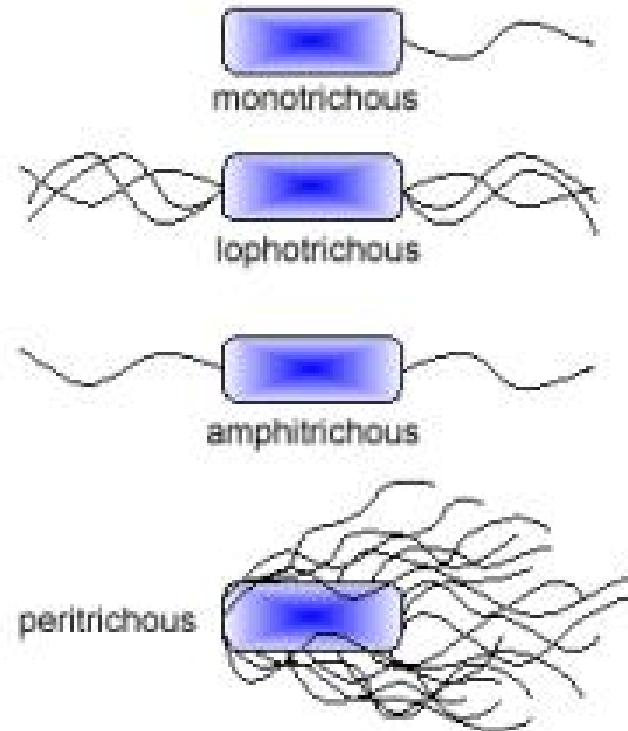
- Appearance
  - 10 -20 micrometers
  - thin
- Arrangement
- Parts
- Function





# Flagellar Arrangement

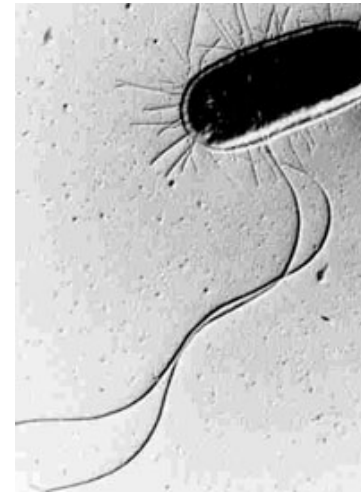
- Monotrichous
- Amphitrichous
- Lophotrichous
  - Tuft
  - One or both poles
- Peritrichous
- Variations
  - Axial Filaments
  - Atrichous



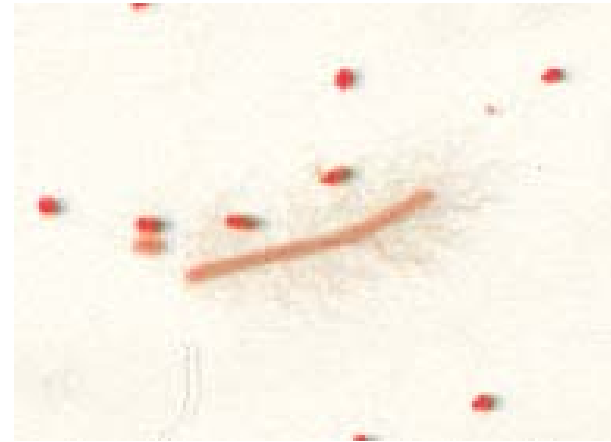
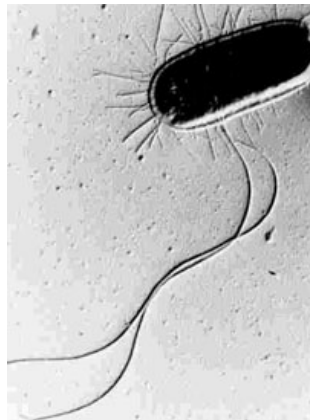
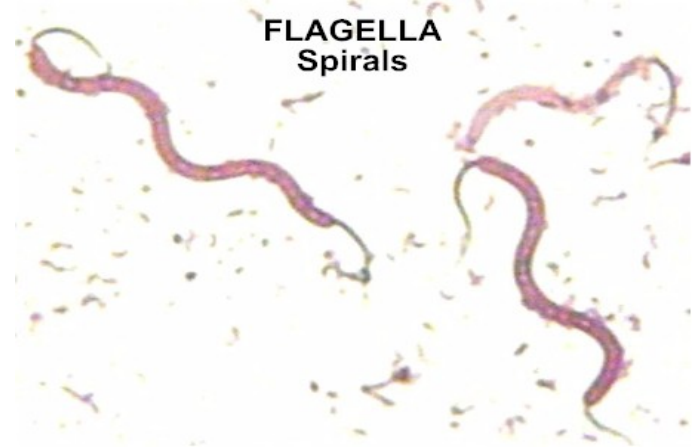
# Flagella Examples



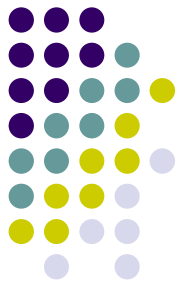
- Monotrichous:
  - *Pseudomonas aeruginosa*
- Amphitrichous:
  - *Spirillum volutans*
- Lophotrichous:
  - *E. coli*
- Peritrichous:
  - *Proteus vulgaris*



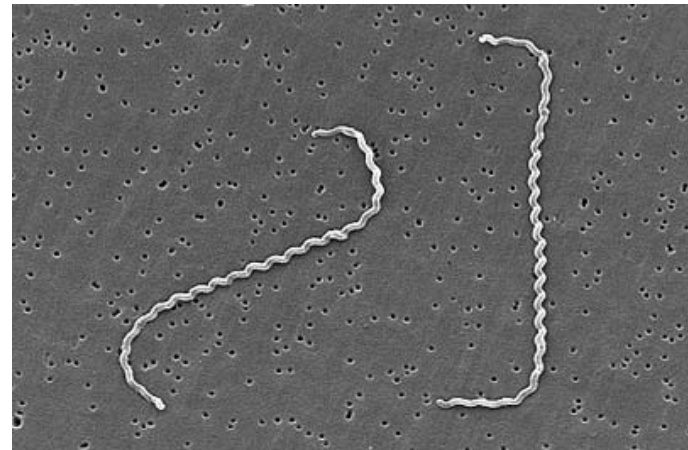
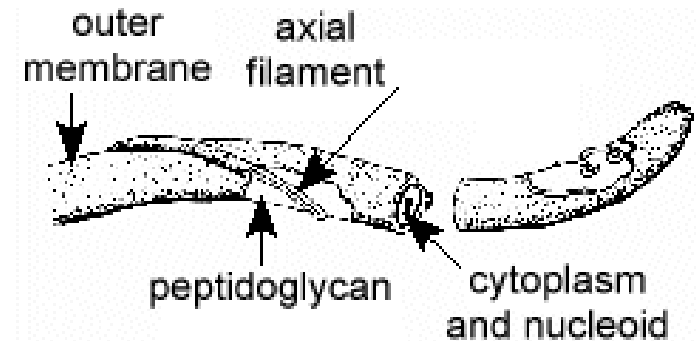
# Flagella



# Endoflagellar Arrangement

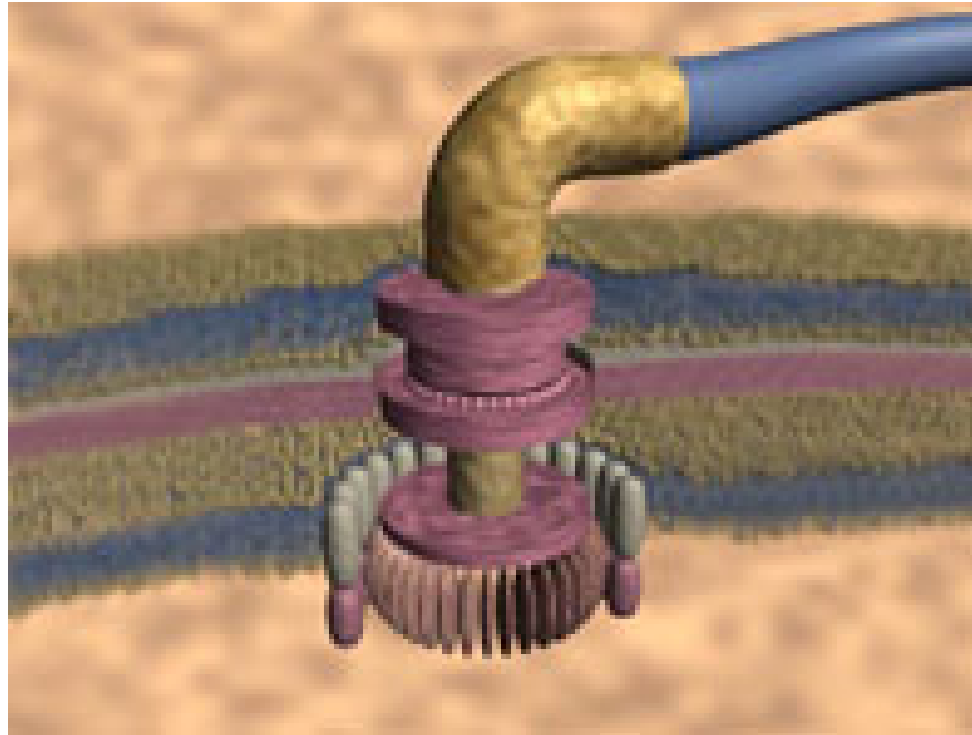


- Amphitrichous
- Spiral around outer membrane
- Axial Filament rotation
- Corkscrew motility
- Spirochetes

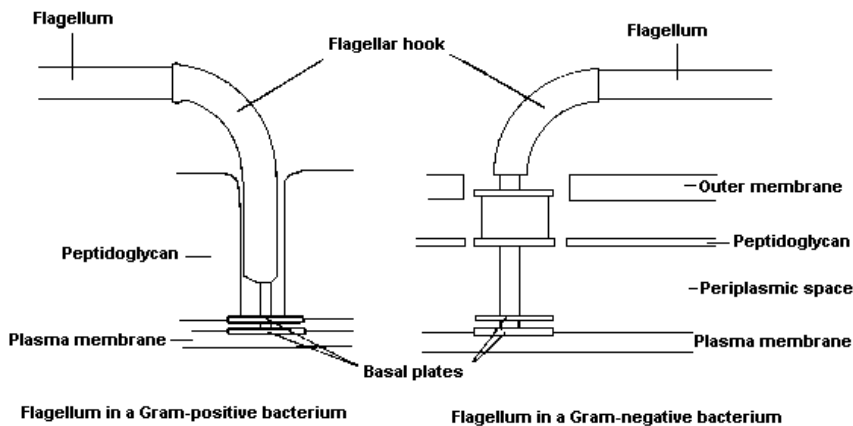




# Flagella Anatomy: Exoflagella



# Flagellar Parts: Exoflagella

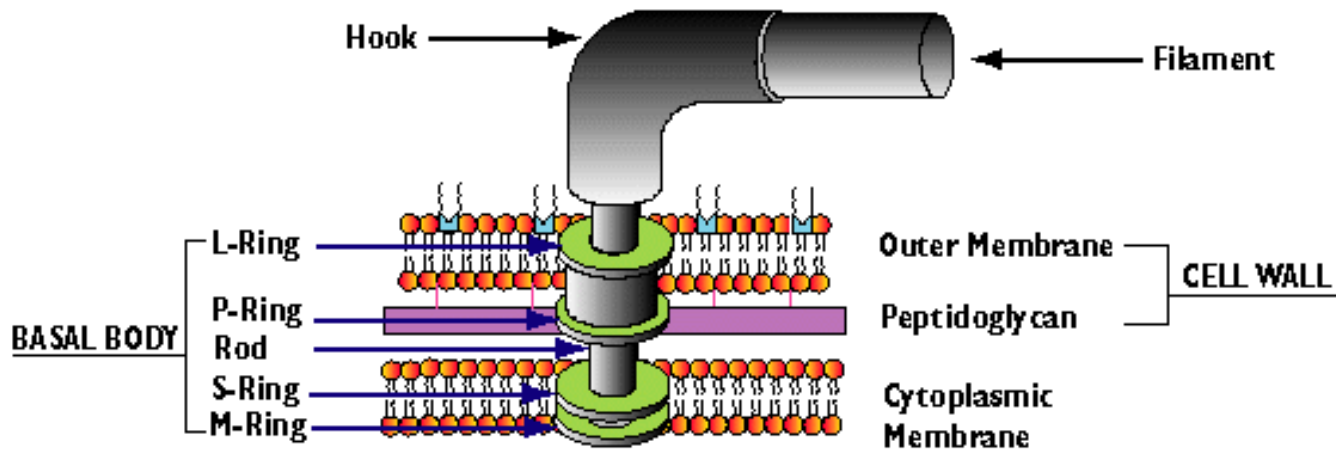


- Filament
  - Flagellin chains in helix
  - Hollow core for repair
  - H protein antigen
- Hook
  - Protein coupling
  - L or curved shape
  - Function: rotation
- Basal Body
  - Function: anchor
  - Central Protein Rod
  - Ringed protein structures
    - 4 rings for Gram Negative
    - 2 rings for Gram positive

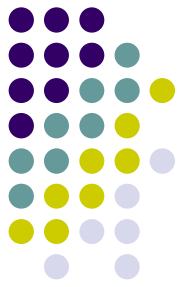
# Gram Negative Flagella



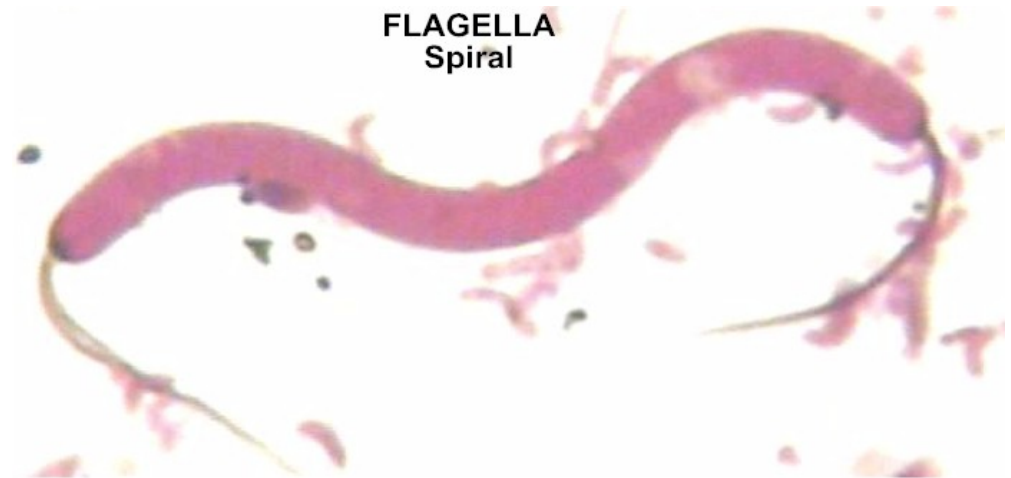
## FLAGELLA STRUCTURE IN GRAM NEGATIVE ORGANISM

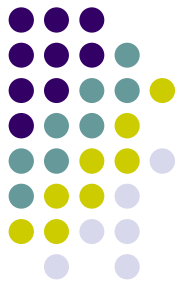


# Flagellar Stain



- Special Stain
- Determine
  - Presence
  - Number
  - Location
- Stains used
  - Carbofuchsin
  - Pararosaniline
  - With mordants

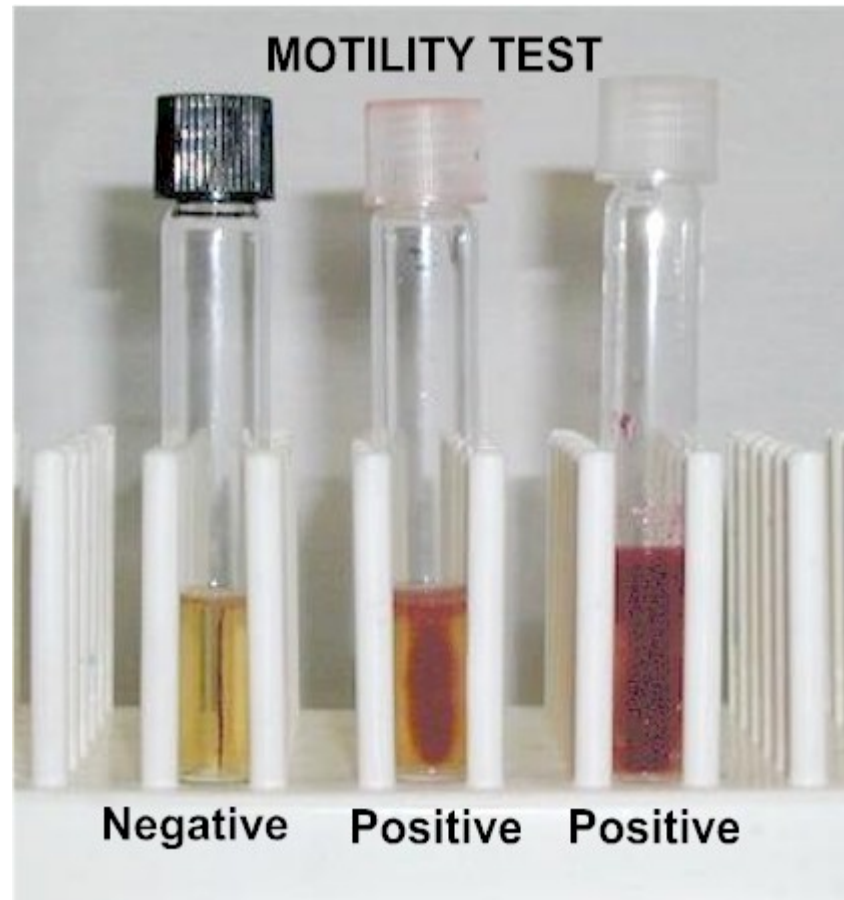




# Flagellar Motility

- Rotation
- ATP proton pump
- Flagellar Motor
  - MotA
  - MotB
  - Driven by proton Gradient [outside to inside cell]
- Moves toward food source
  - Clockwise= random movement [tumble]
  - Counterclockwise=forward movement [run]

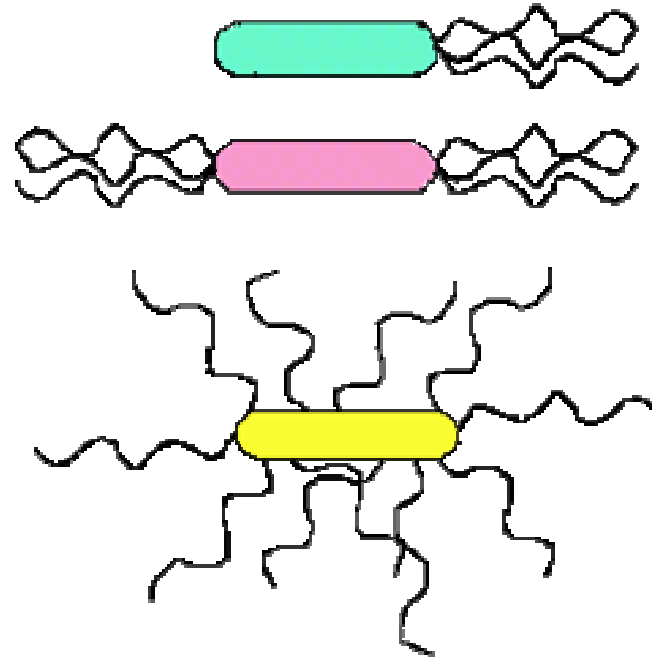
# Motility Tests

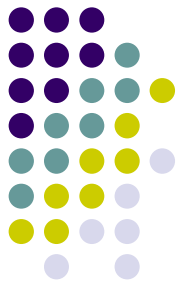


# Flagella Summary



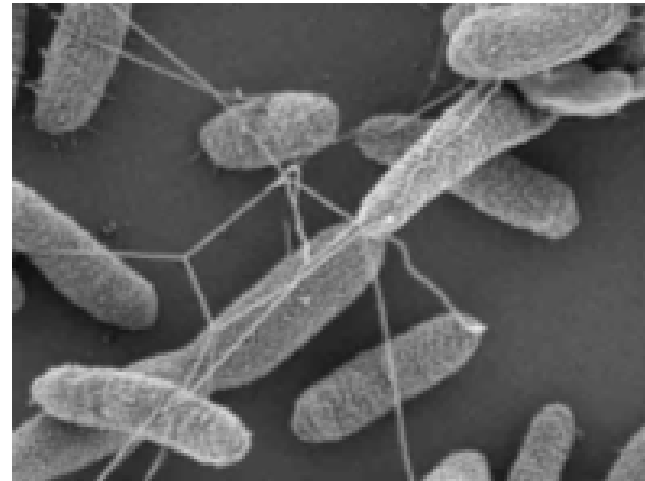
- Function: Motility
  - Phototaxis
  - Chemotaxis
  - Positive taxis
  - Negative taxis
- Proteins allow for classification into groups called serovars
- Types
  - Endoflagella [spirochetes]
  - Exoflagella Arrangement
    - Monotrichous
    - Amphitrichous
    - Lophotrichous
    - Peritrichous
- Anatomy
  - Filament
  - Hook
  - Basal body





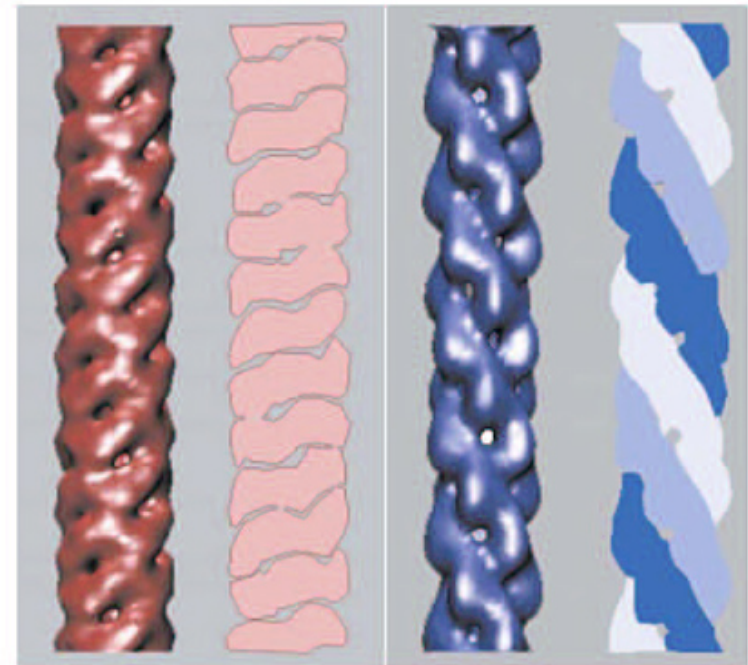
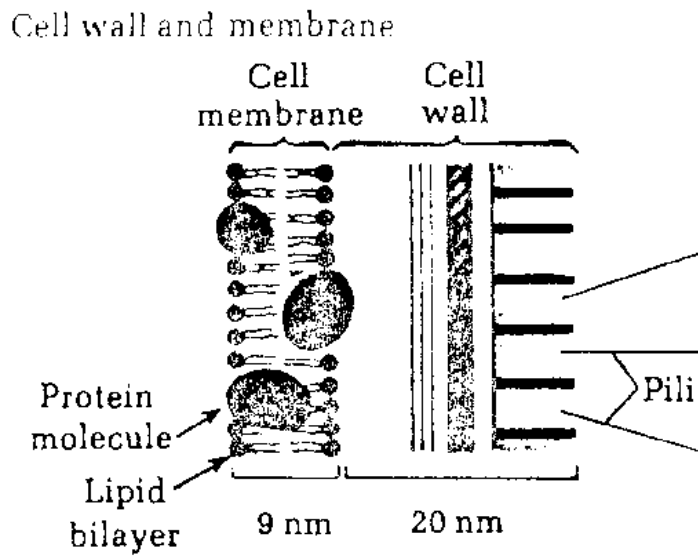
# Pili

- Pilin protein
- Thin hollow tubes
- Number
  - Average 1-2
  - Some have up to 10-100
- Glycoprotein or glycolipid tip
- Length varies
  - Shorter: adherence
  - Longer: conjugation





# Pili Structure

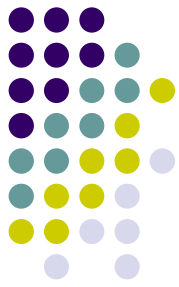
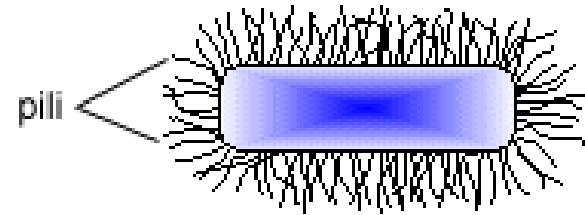


P-pili  
*Escherichia coli*

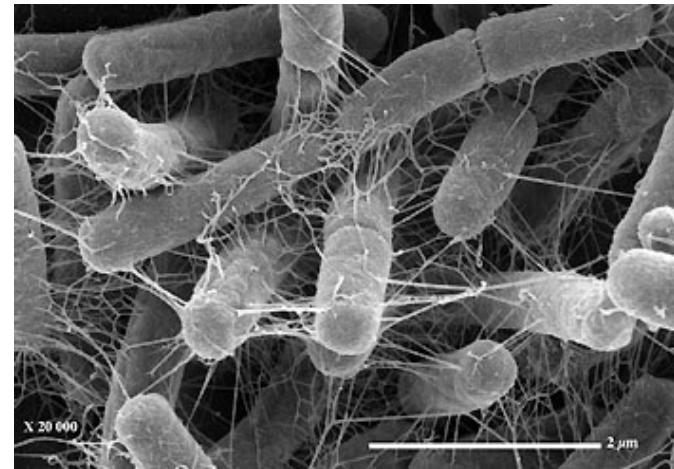
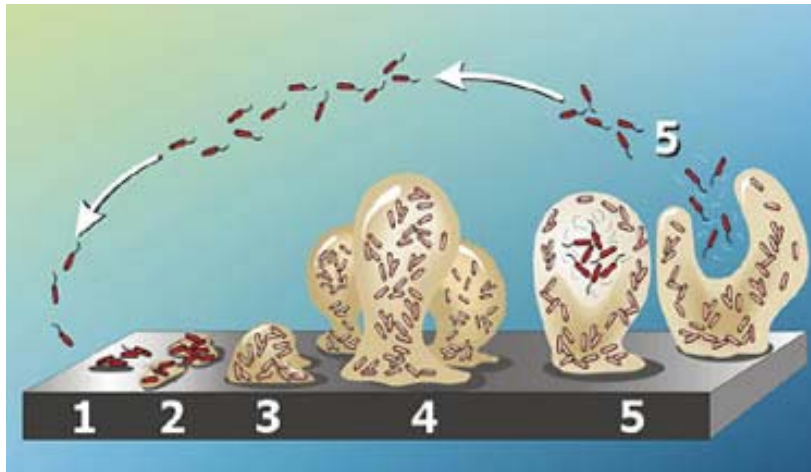
Hib pili  
*Haemophilus influenzae*

# Fimbria

- Gram Negative bacteria
- Shorter than flagella  
aka “Short pili”
- Sticky
- Function
  - Adherence
  - Resist flushing
  - Biofilm

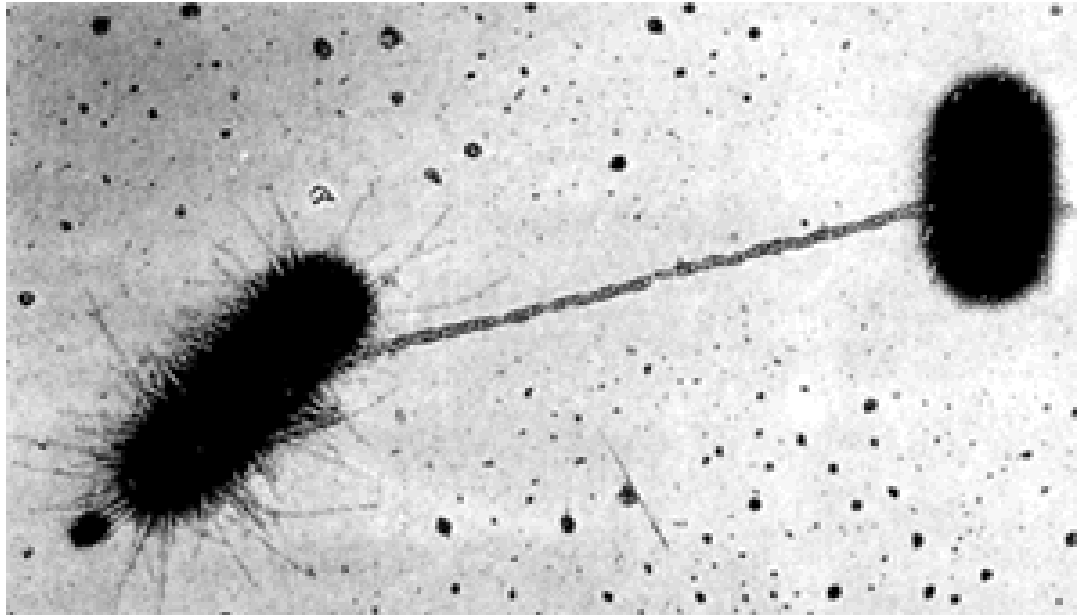
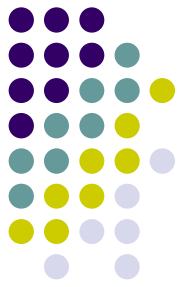


# Biofilms



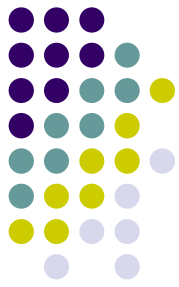
- Attachment to surface of host tissue or other microbes
- Cell to cell attachment
- Production of extracellular polysaccharides [eps]
- Planktonic colonization

# Conjugation (Sex or F) Pili

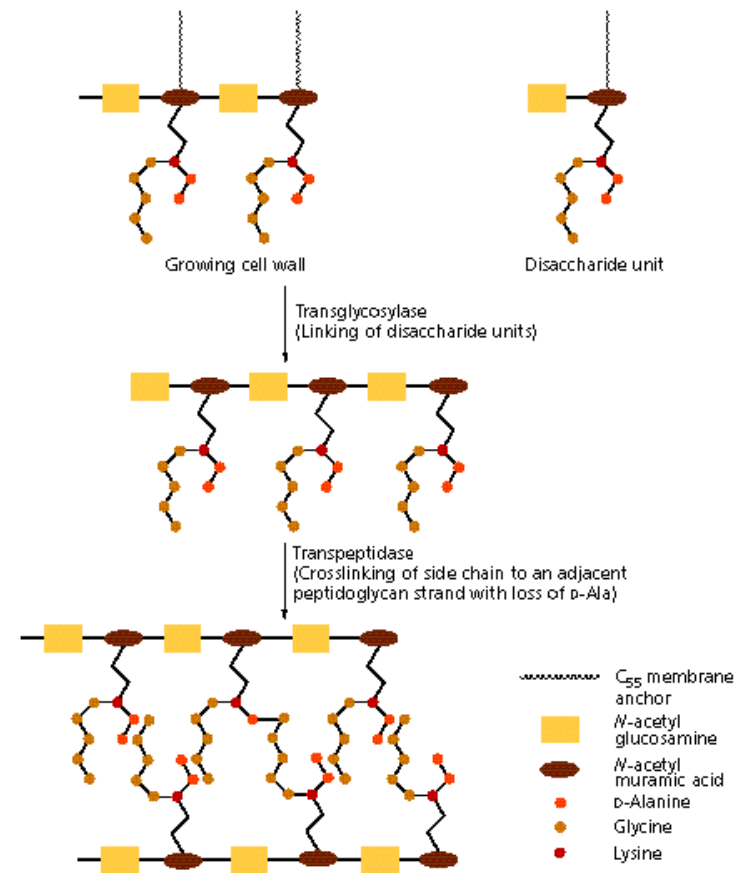


Transfer DNA

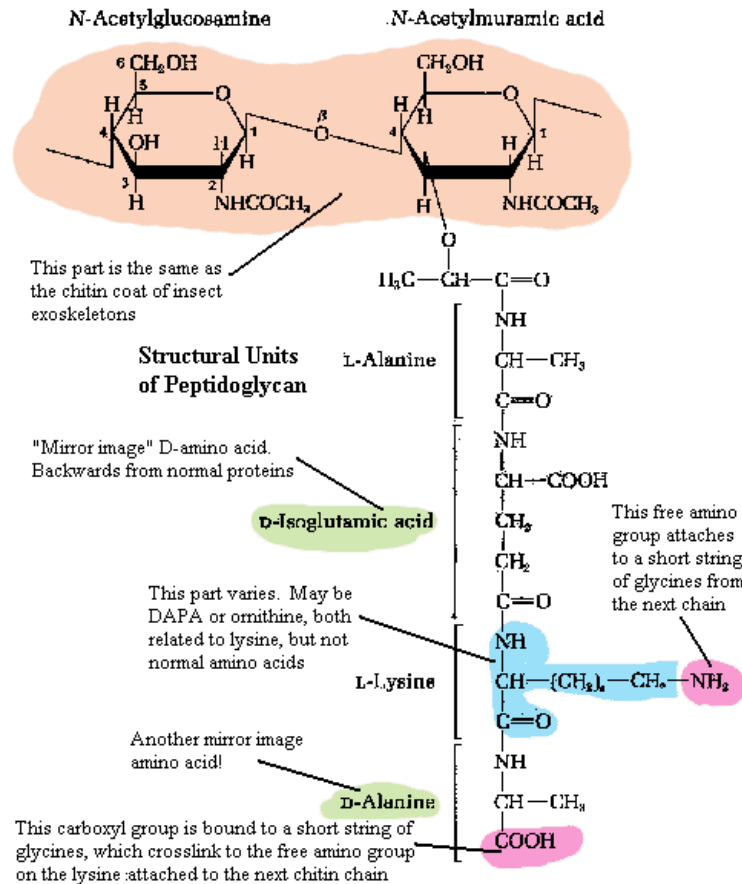
# Cell Wall Structure



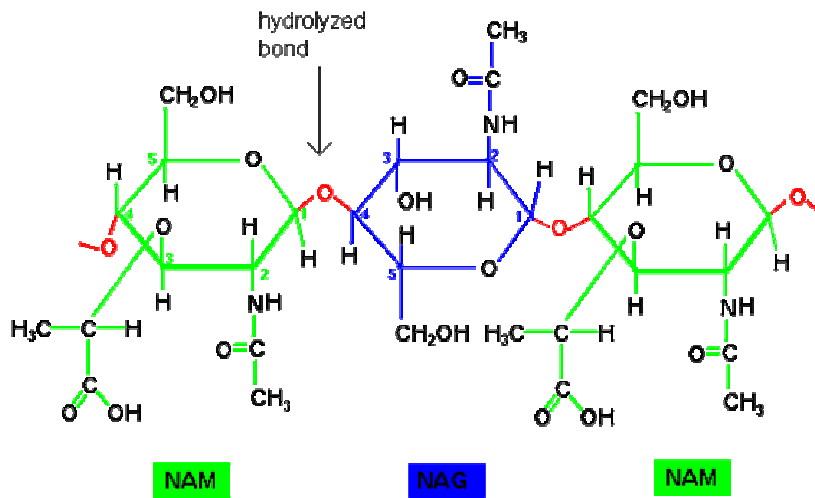
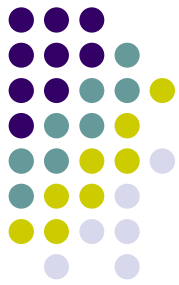
- Peptidoglycan
  - Complex polysaccharide
  - Alternating Amino Sugars
    - Linkage
      - Transglycolation
      - Beta 1-4 Linkage
    - Structure
      - NAM (muramic acid)
      - NAG (glucosamine)
  - Crossbridge
    - Linkage: Transpeptidases
    - Structure
      - Tetrapeptide
        - From NAM
        - Bonded together
        - Short chains



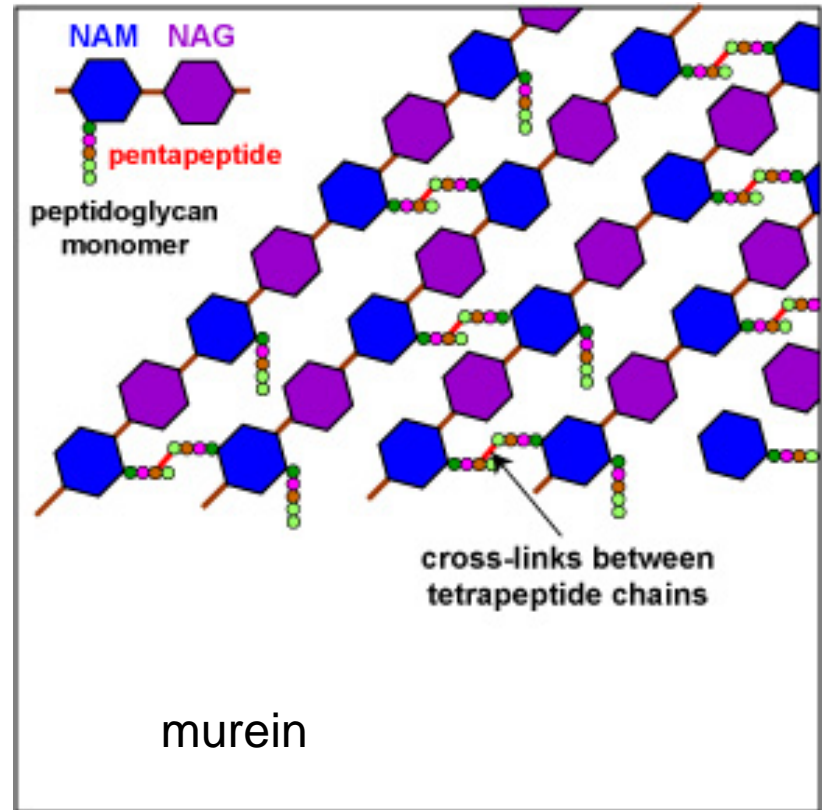
# Peptidoglycan Structural Unit



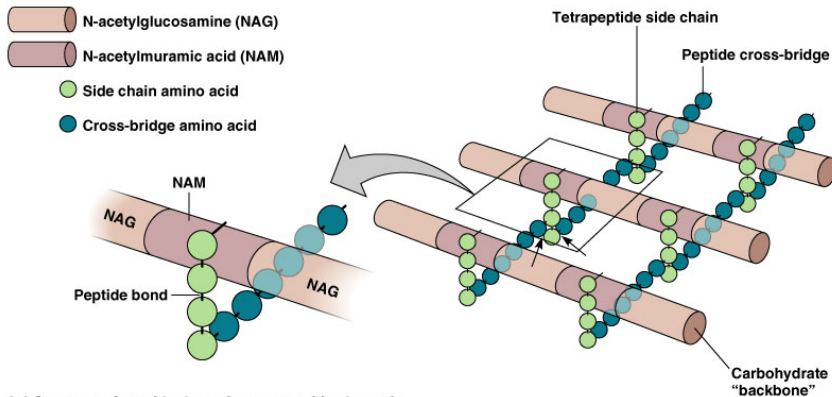
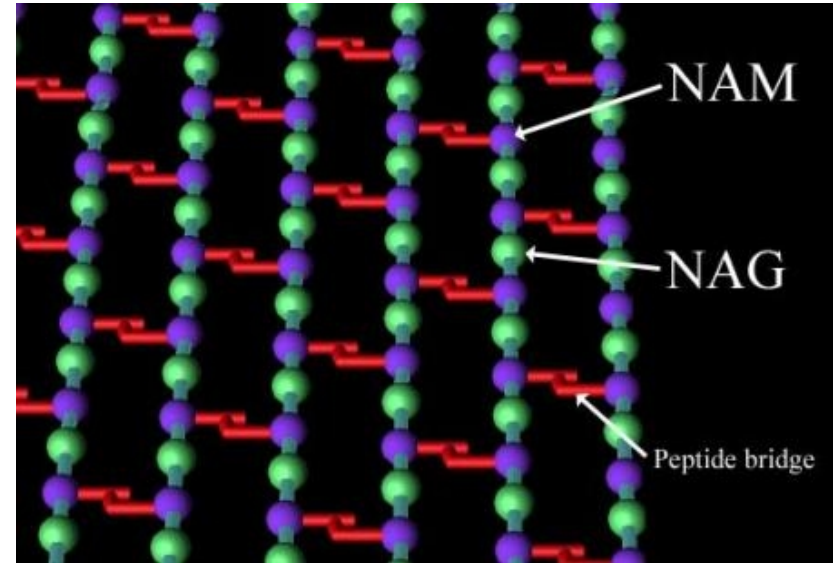
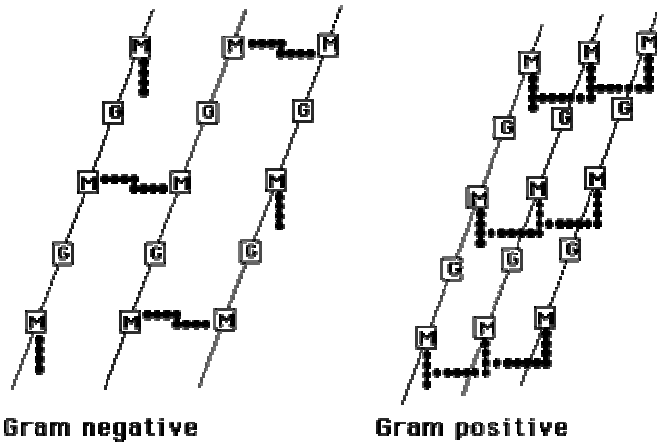
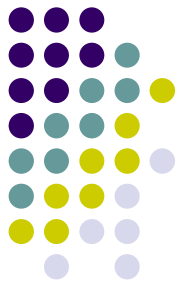
# Peptidoglycan Linkage



$\beta$  (1-4) glycosidic bonds in red



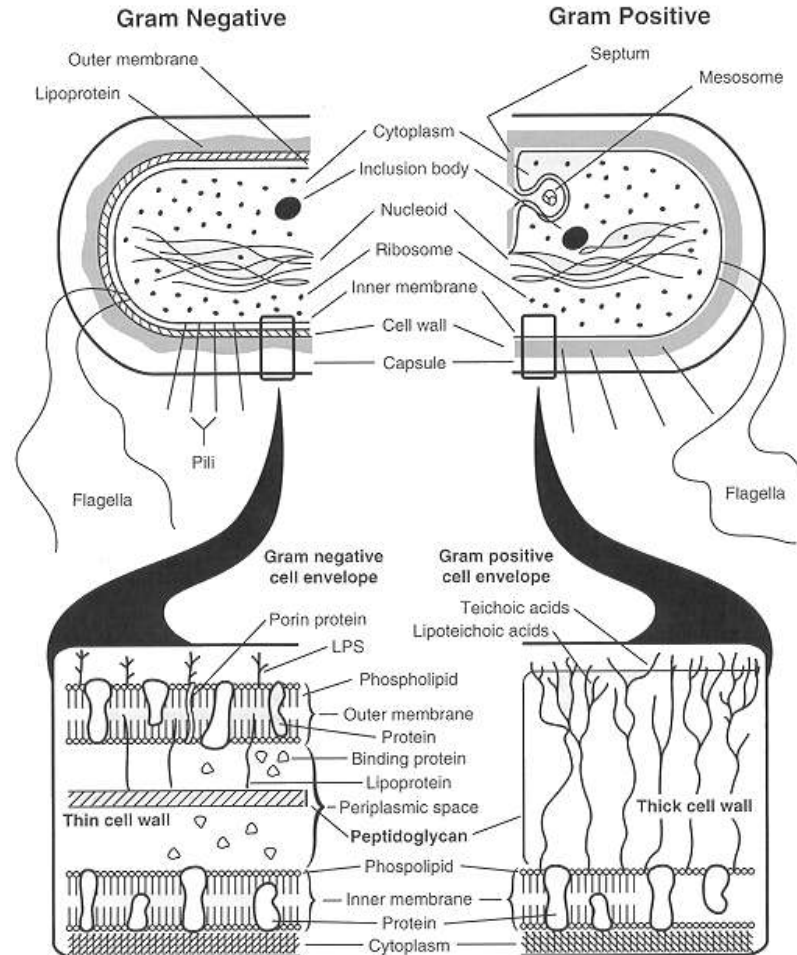
# Peptidoglycan Structure Compared



**(a) Structure of peptidoglycan in gram-positive bacteria**



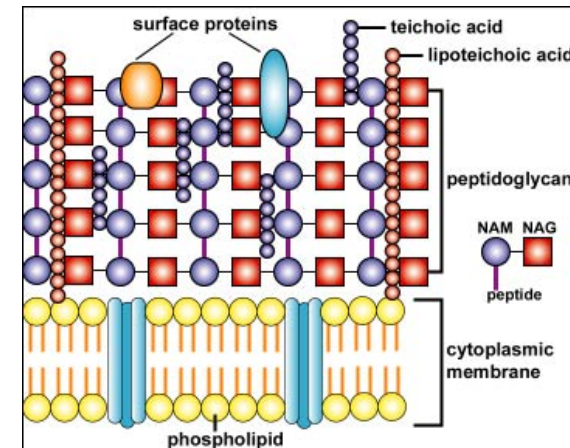
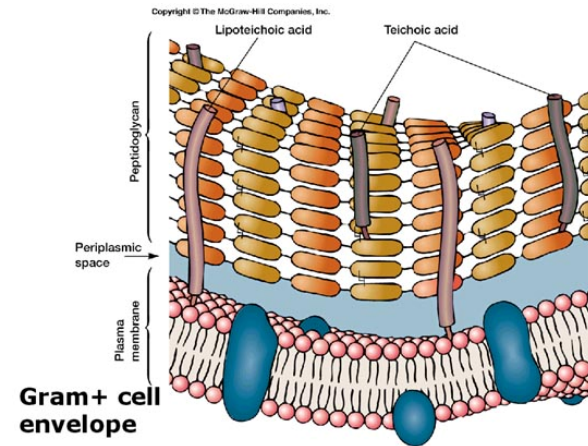
# Cell Walls



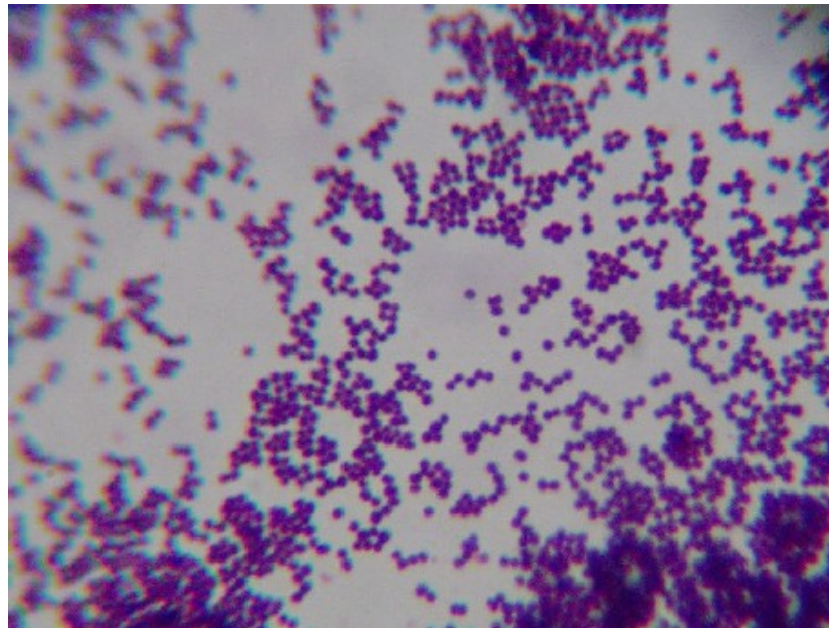
# Gram Positive Cell Wall



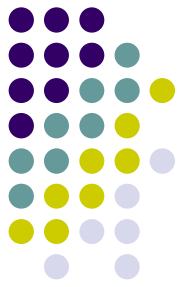
- Composition
  - Thick peptidoglycan 60-90% of cell wall
  - Teichoic acids
  - Lipoteichoic acids
  - Proteins
    - Surface
    - Tetrapeptides
- Function
  - Shape
  - Prevent osmotic lysis [peptidoglycan]
  - Teichoic Acids
    - Polymers of glycerol or ribitol
    - Reinforce cell wall
    - Ion flow
    - Protection
    - Antigen Specificity
  - Proteins
    - Enzymes
    - Adhesins
    - Invasins
- Examples
  - *Staphylococcus*
  - *Streptococcus*
  - *Clostridium*



# Gram Positive Bacteria

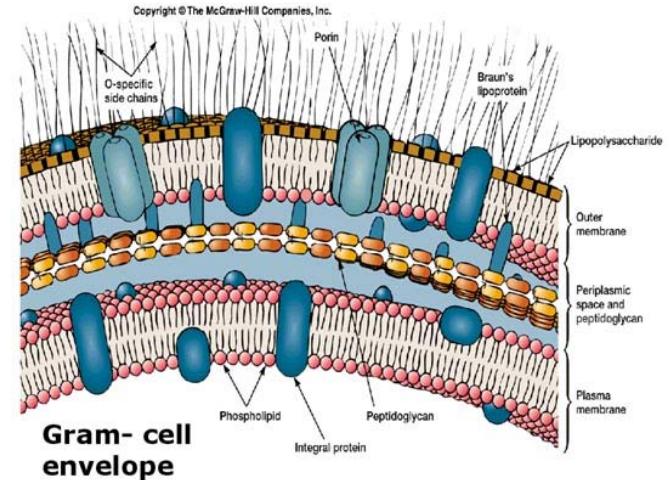


# Gram Negative Cell Wall



- Composition

- Inner Cell Wall
  - Peptidoglycan
    - 1-2 layers make up 20%
    - Intermediate cross linkage
    - NO teichoic acid
- Outer Cell Membrane
  - Lipid bilayer
    - Phospholipids
    - Lipopolysaccharides [LPS]
      - Lipid A
      - O Antigen
      - Core Polysaccharide connects A-O
      - LPS = Endotoxin
  - Lipoproteins
  - Proteins
    - Vary based on species
    - Porins

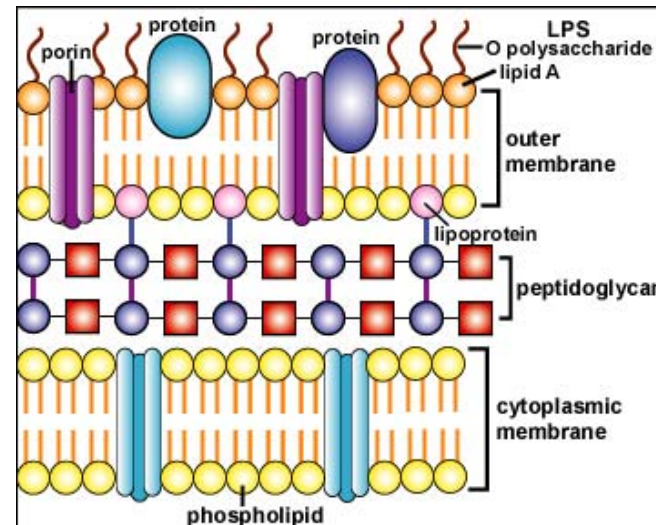


- Function

- Prevent osmotic lysis
- Semipermeable
- Reinforce membrane [LPS]
- Proteins
  - Adhesins
  - Enzymes
  - Invasins
  - Resist phagocytosis
  - Passage of nutrients

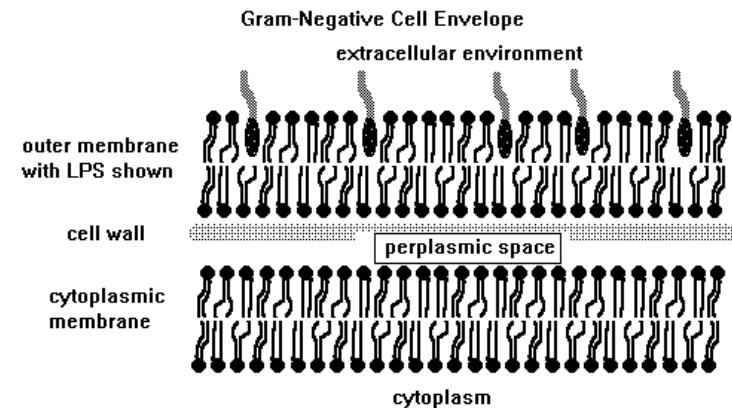
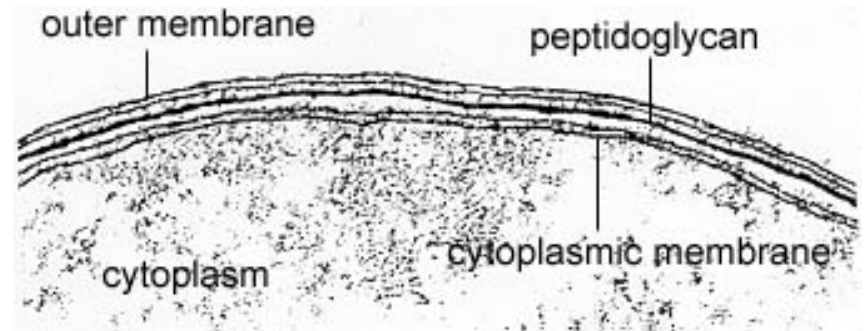
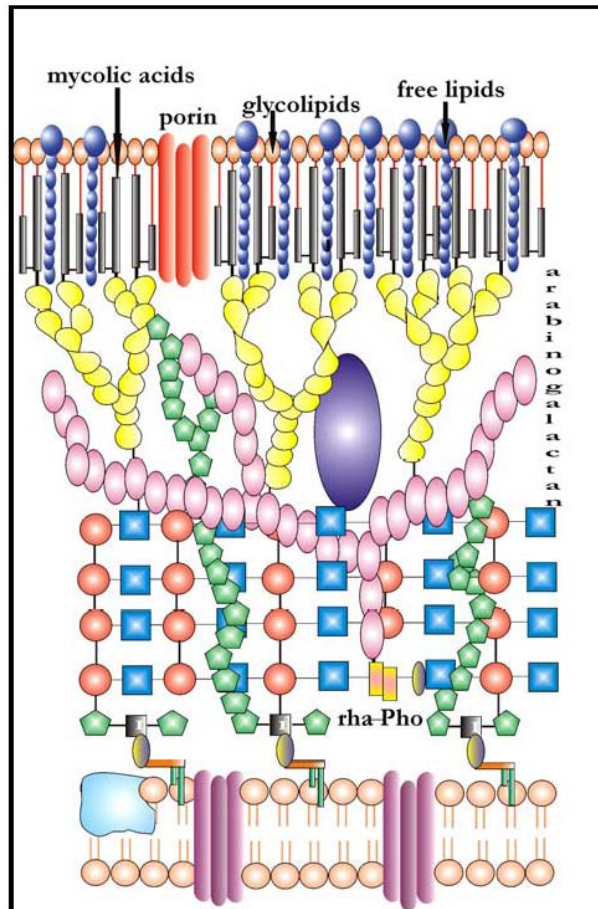
- Examples

- *Neisseria*
- *E coli*
- *Pseudomonas*
- *Proteus*

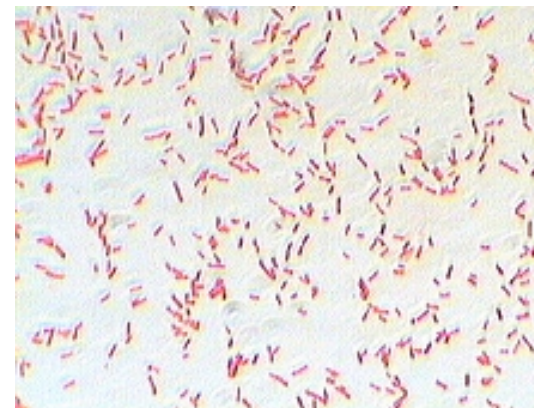
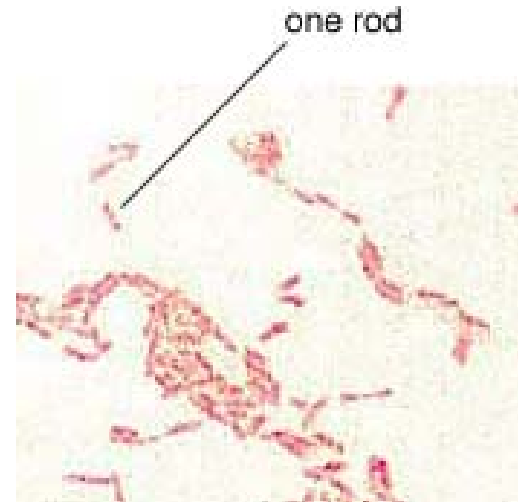
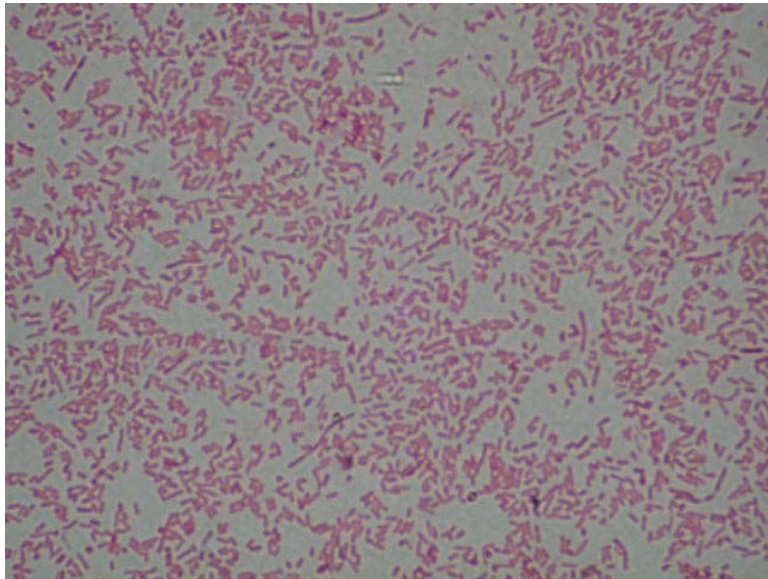




# Gram Negative Cell Wall



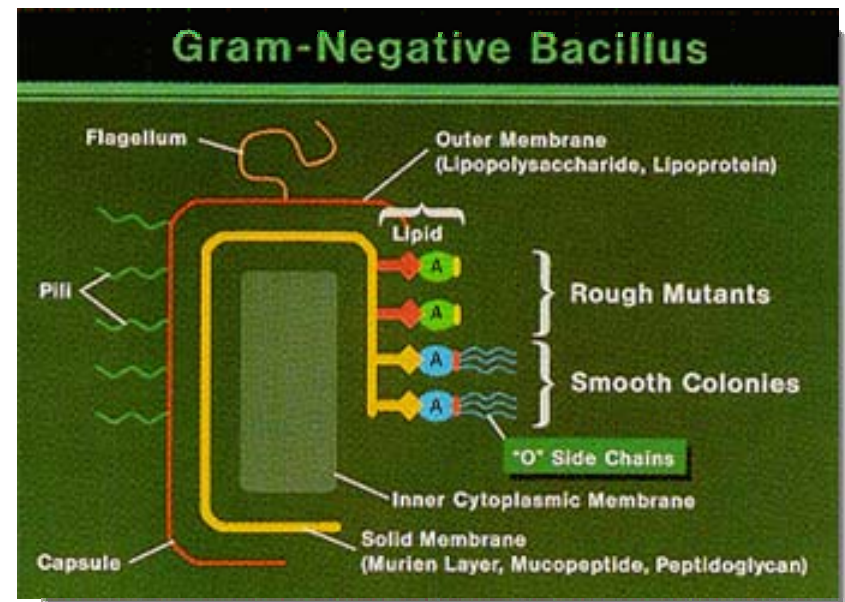
# Gram Negative Rods



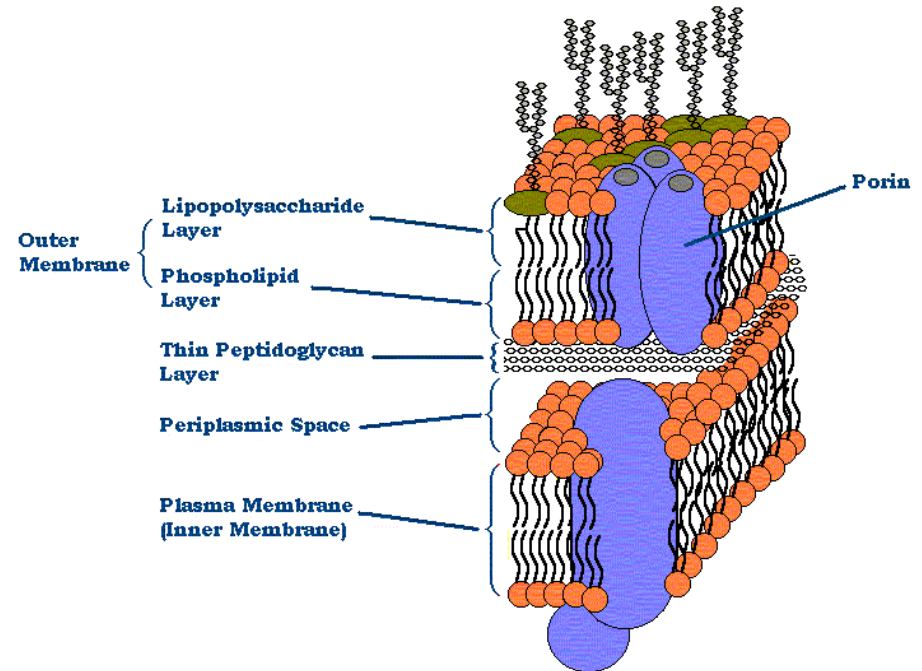
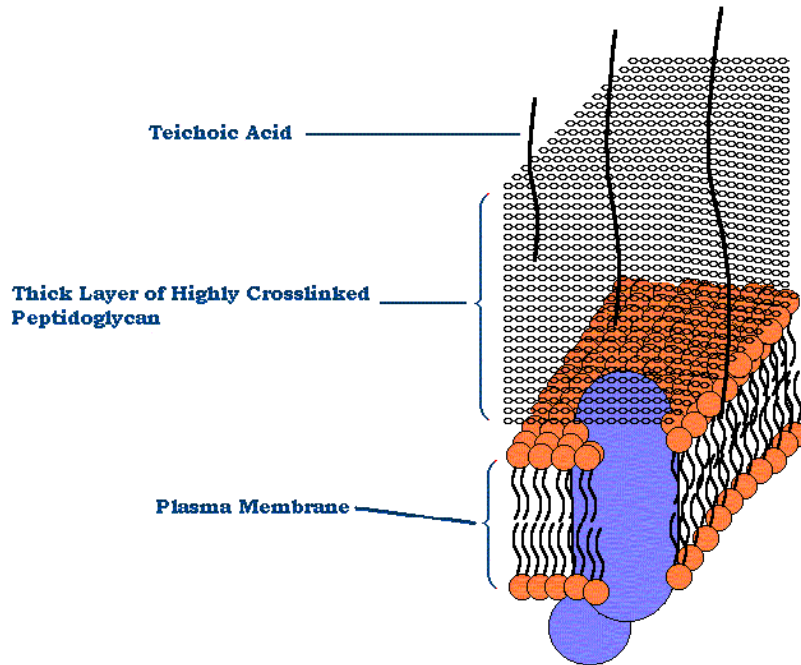
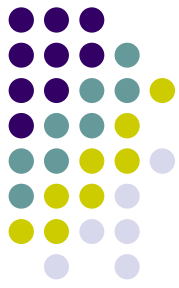
# LPS



- O Antigen
  - Hexoses
  - Antigenic specificity
  - Smoothness to organism
- Core Polysaccharide
  - NAG
  - Phosphorylated sugars [7,8 C]
- Lipid A
  - Lipopolysaccharide
  - Glucosamine moieties
  - Toxic component
- Endotoxin
  - Hydrophobic
  - Resistant: heat, acid
  - Released when cell wall disrupted



# Cell Wall Comparison

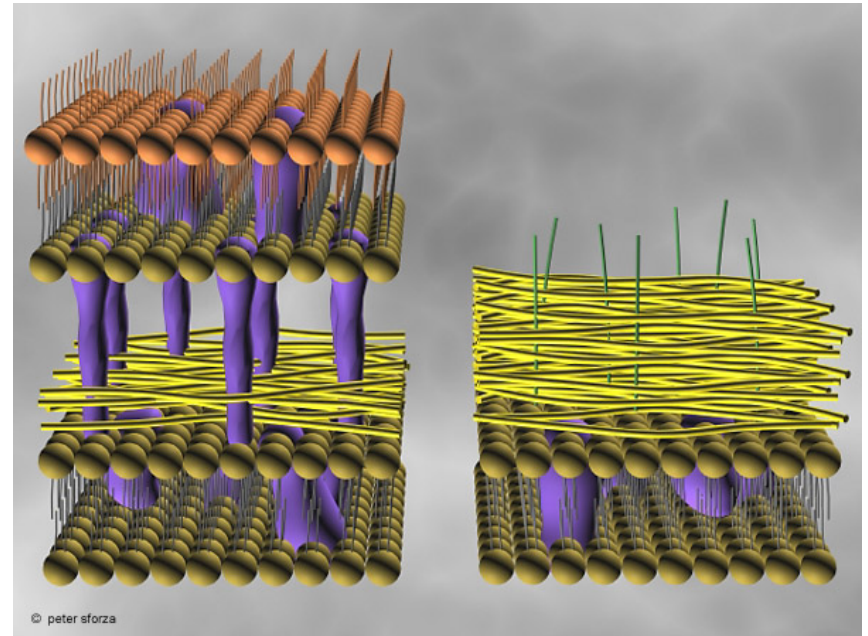
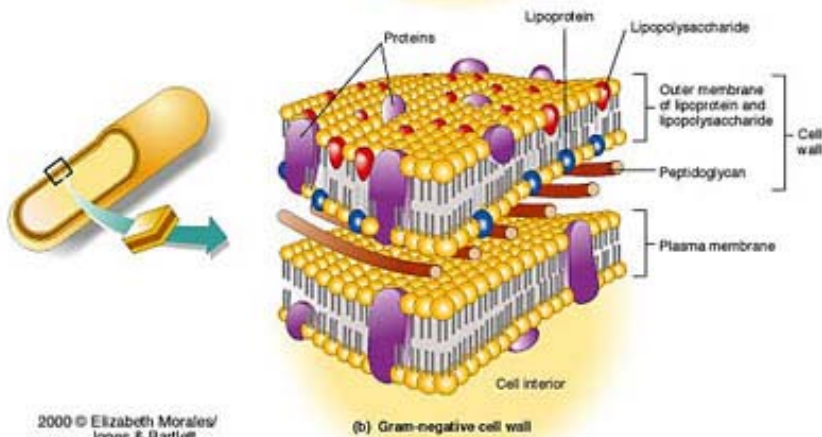
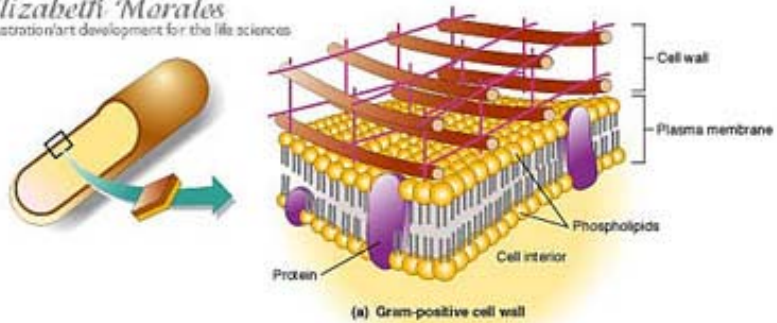




# Cell Wall Comparison #2



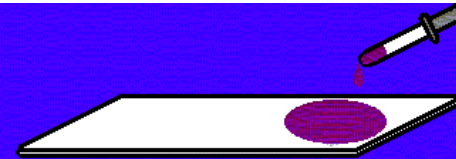
Elizabeth Morales  
Illustration/art development for the life sciences



# Gram Stain



1. Prepare a heat-fixed bacterial smear.



2. Cover the smear with crystal violet for 60 seconds, then rinse with water.



3. Cover the smear with Gram's iodine for 30 seconds, then rinse with water.



4. Briefly decolorize the smear with acetone-alcohol, then rinse with water.

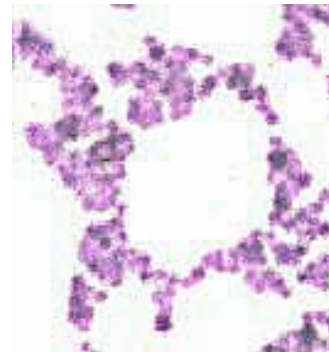
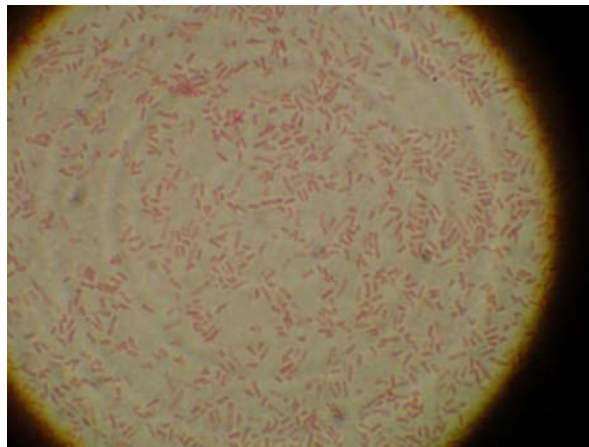
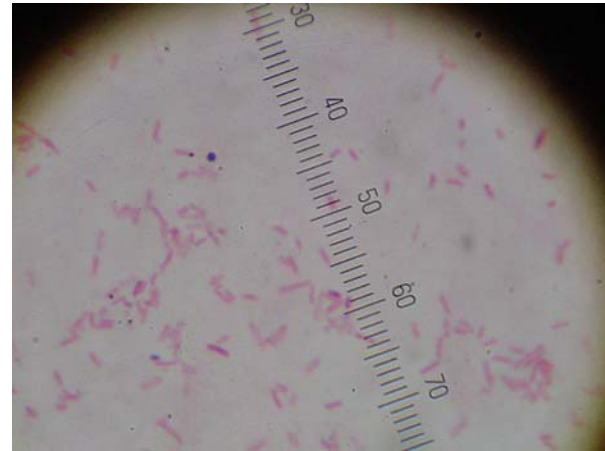
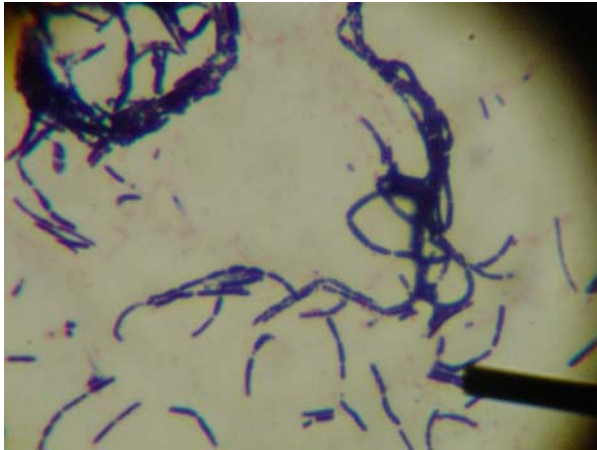
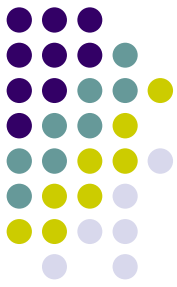


5. Cover the smear with saffranin for 60 seconds, then rinse with water and dry.

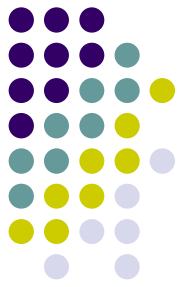


6. Observe the dried smear with the microscope, using oil-immersion.

# Gram Stain Results



# Acid Fast Cell Wall



- Composition

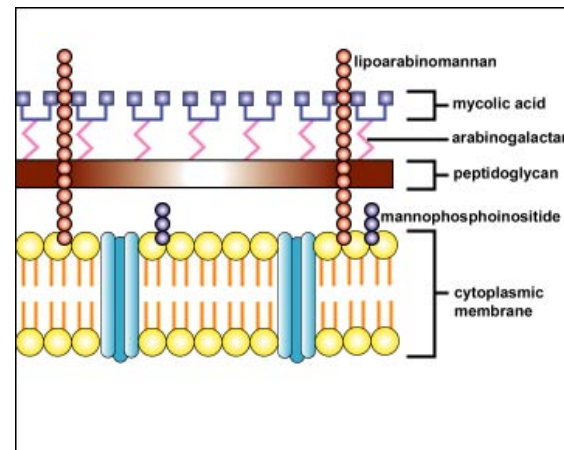
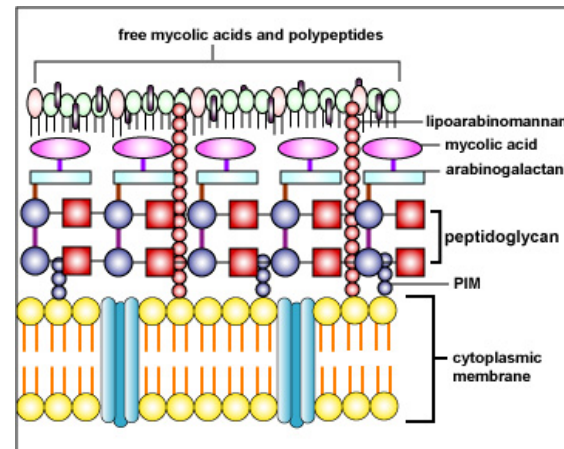
- Peptidoglycan
- Arabinogalactan linkage
- Glycolipid: mycolic acid
- Lipids
  - Free
  - Glycolipid
    - Lipoarabinomannan
    - Phosphatidylinositol mannosides
  - Peptidoglycolipid

- Functions

- Prevent osmotic lysis
- Impede entry of chemicals
  - Slower growing organism
  - Resistant to phagocytosis

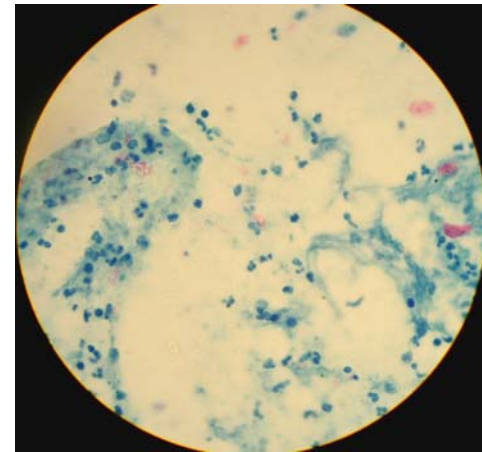
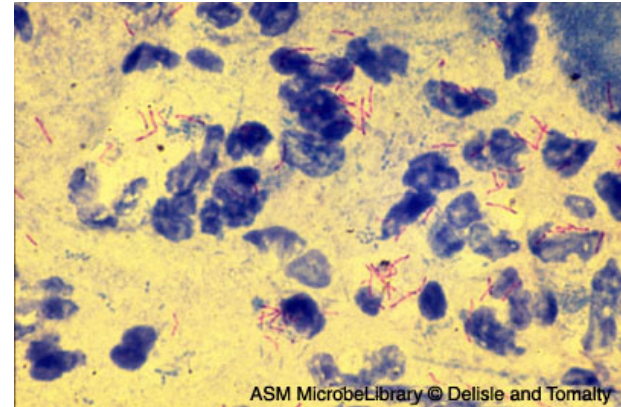
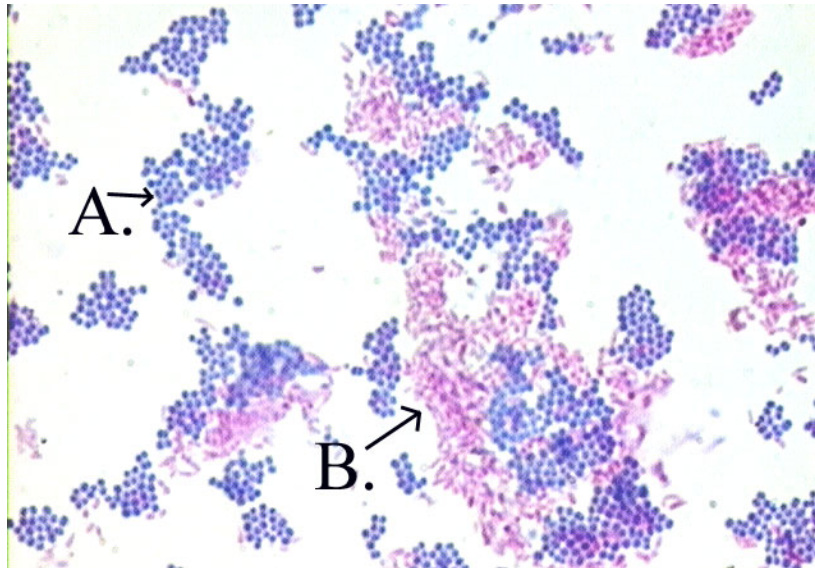
- Examples

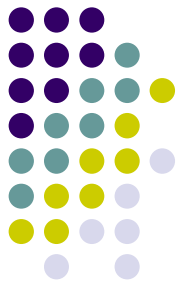
- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Nocardia*



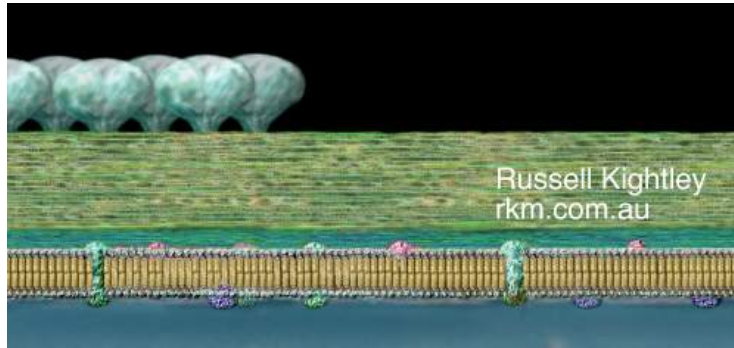


# Acid Fast Stain

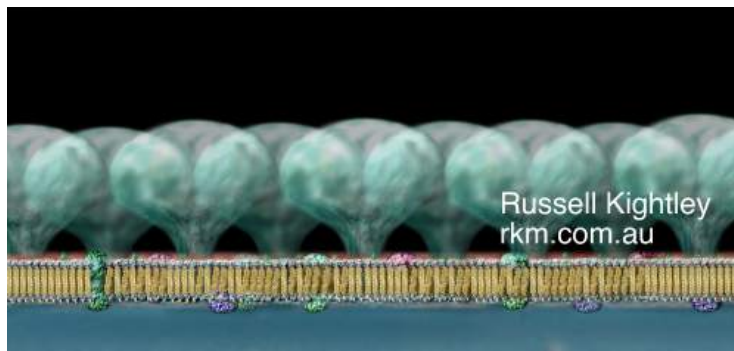




# Atypical Cell Walls



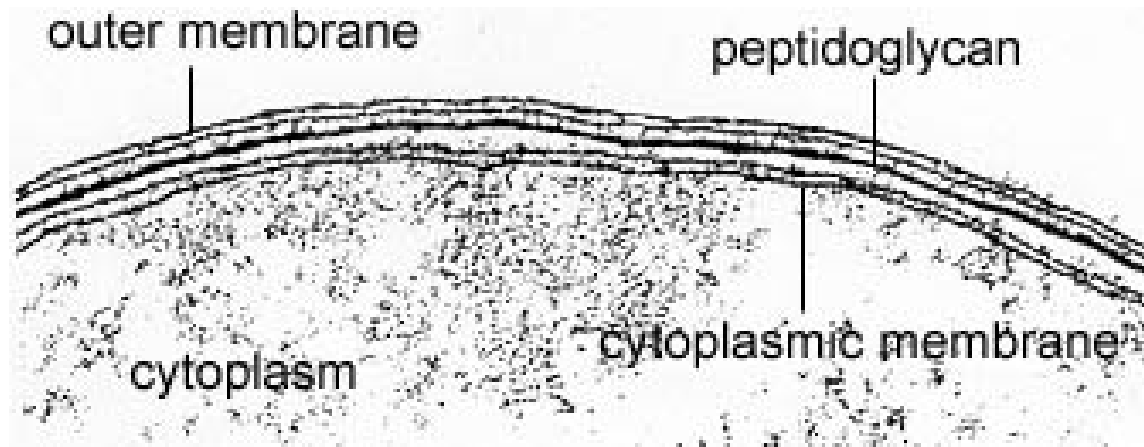
Gram positive cell wall



Gram negative cell wall

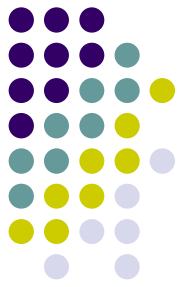
- Archaeobacteria
  - No peptidoglycan
  - Polysaccharides
  - S-layer [+/-]
  - Proteins
  - Gram +
    - Thick
    - Stain purple
  - Gram –
    - Protein layer
    - Stain pink

# Periplasm



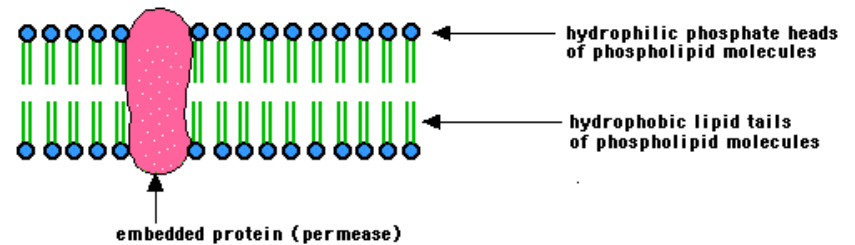
- Composition
  - Gelatinous
- Location
  - Between peptidoglycan and cytoplasmic membrane
- Function
  - Proteins
    - Enzymes for nutrient digestion [hydrolytic]
    - Facilitate transfer of nutrients
    - Storage for toxins to be released into environment

# Plasma Membrane



- Composition
  - Phospholipid
    - Polar
    - nonpolar
  - Proteins
    - Integral
    - Peripheral
    - +/- polysaccharides
  - No sterols, hapanoids
- Function
  - Encloses cytoplasm
  - Selectively permeable
  - ETC
  - Peptidoglycan synthesis
  - Aids in DNA replication
  - Flagella basal protein rings
  - Waste removal
  - Endospore formation

Fluid-mosaic model of the plasma membrane





# Plasma Membrane Transport

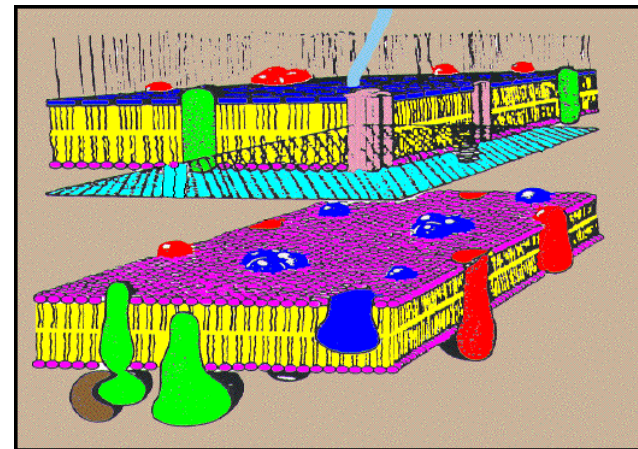
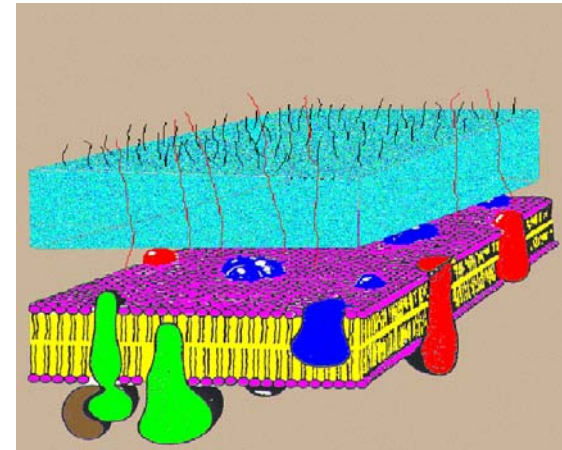


- Passive

- Diffusion
- Osmosis
- Facilitated Diffusion
  - Uniporter
  - Channel Proteins
    - Water
    - Ions

- Active

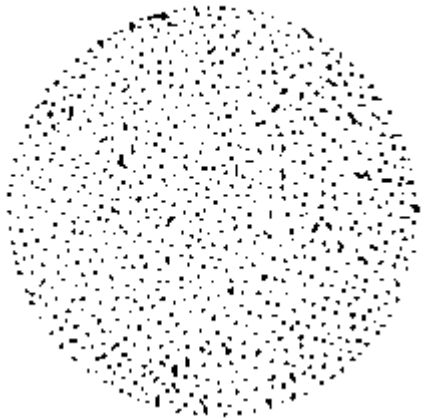
- Antiporter
- Symporter
- ATP binding cassette
  - Gram negative bacteria
  - Periplasm proteins
- Group Translocation
  - Chemical alteration
  - Membrane impermeable
  - Example: Sugars
    - Glucose
    - Mannose
    - Fructose



# Cytoplasm: Cytosol



Cytosol with Free Ribosomes



- Composition
  - 80% water
  - Proteins
  - CH<sub>2</sub>O
  - Lipids
  - Ions
- Function
  - Metabolism
  - Enzymes
    - Exoenzymes
    - Endoenzymes

# Organelles of Cytoplasm

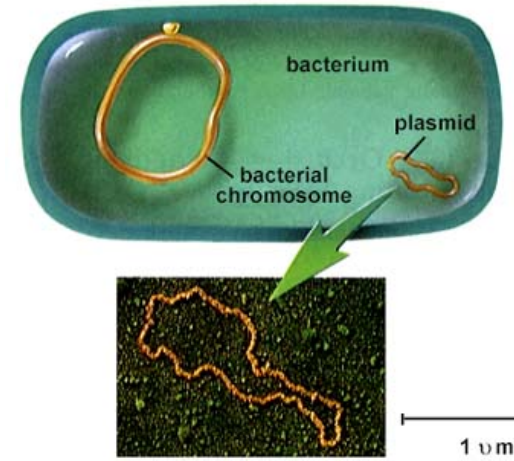
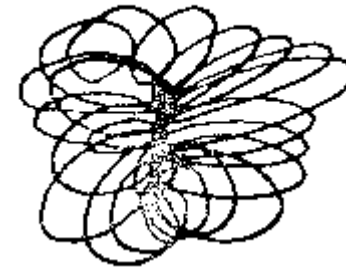


- Nucleoid region
- Ribosomes
- Plasmids
- Mesosomes

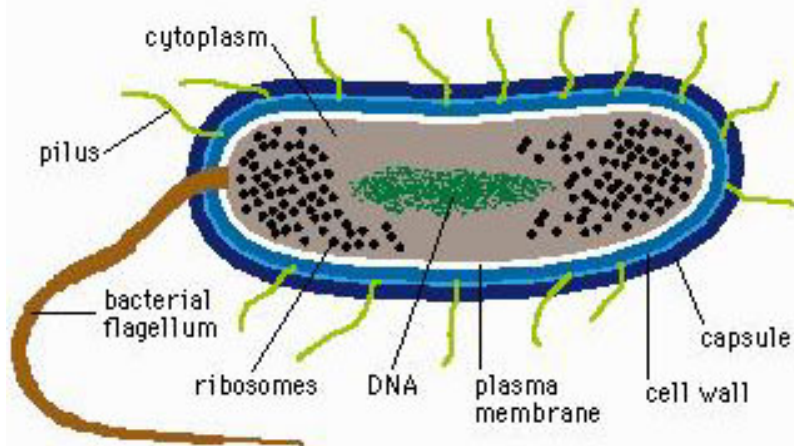
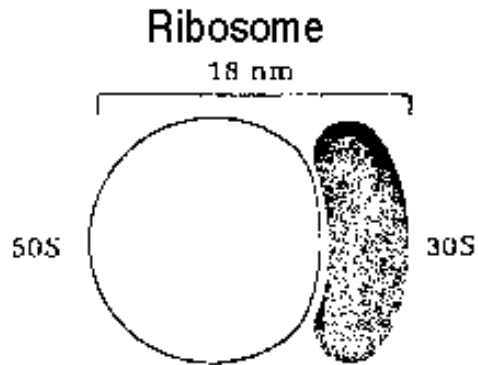
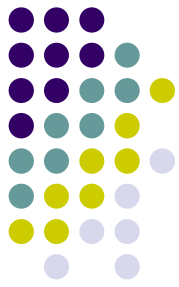
# Nucleoid

- Composition
  - Chromosome
    - Single haploid molecule
    - Double stranded DNA
    - Helical
    - Supercoiled around proteins via topoisomerases
- Function
  - Genome
  - Chemical reactions

Nuclear Zone



# Ribosomes: 70S



- Composition

- rRNA
- Protein
- Subunits
  - 50S
  - 30S

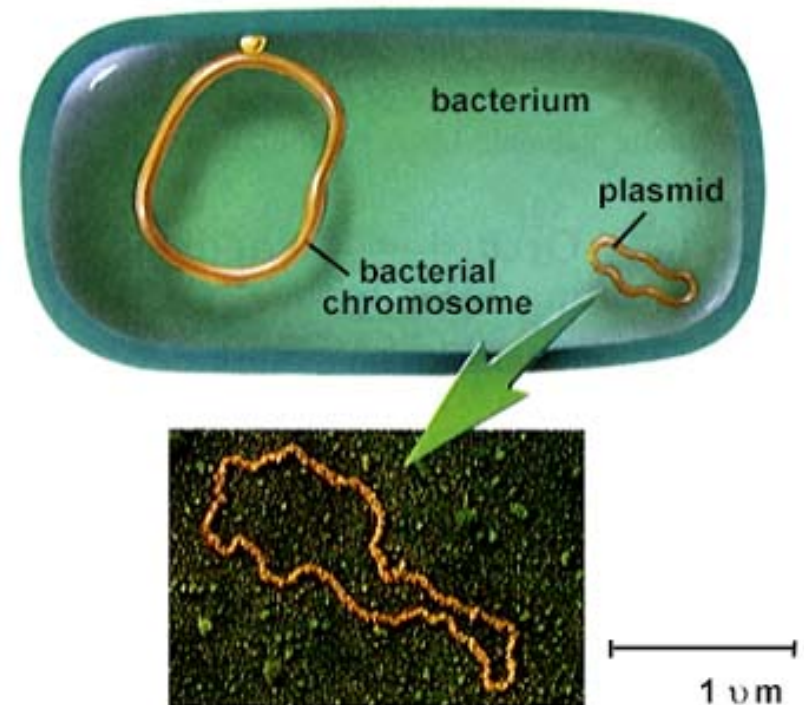
- Function

- Protein synthesis
- tRNA
- mRNA

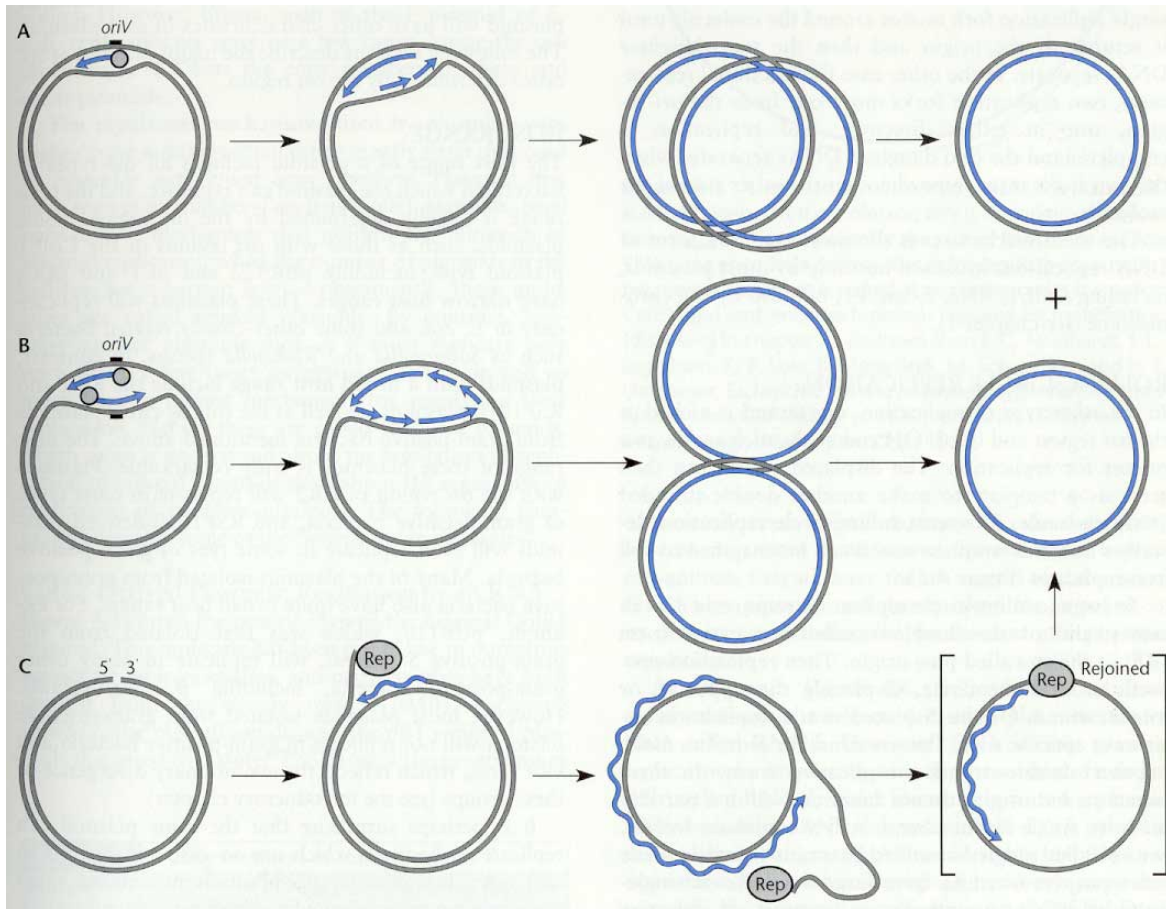
# Plasmids



- Composition
  - DNA
    - Small, helical
    - Double stranded
    - Independent replication
    - 5-100 genes
    - 1-700 copies in cell
- Function
  - Types
    - R plasmids: AB resistance
    - F plasmids: Fertility
  - Protein synthesis
    - Unique proteins
      - Exotoxins
      - Endotoxins

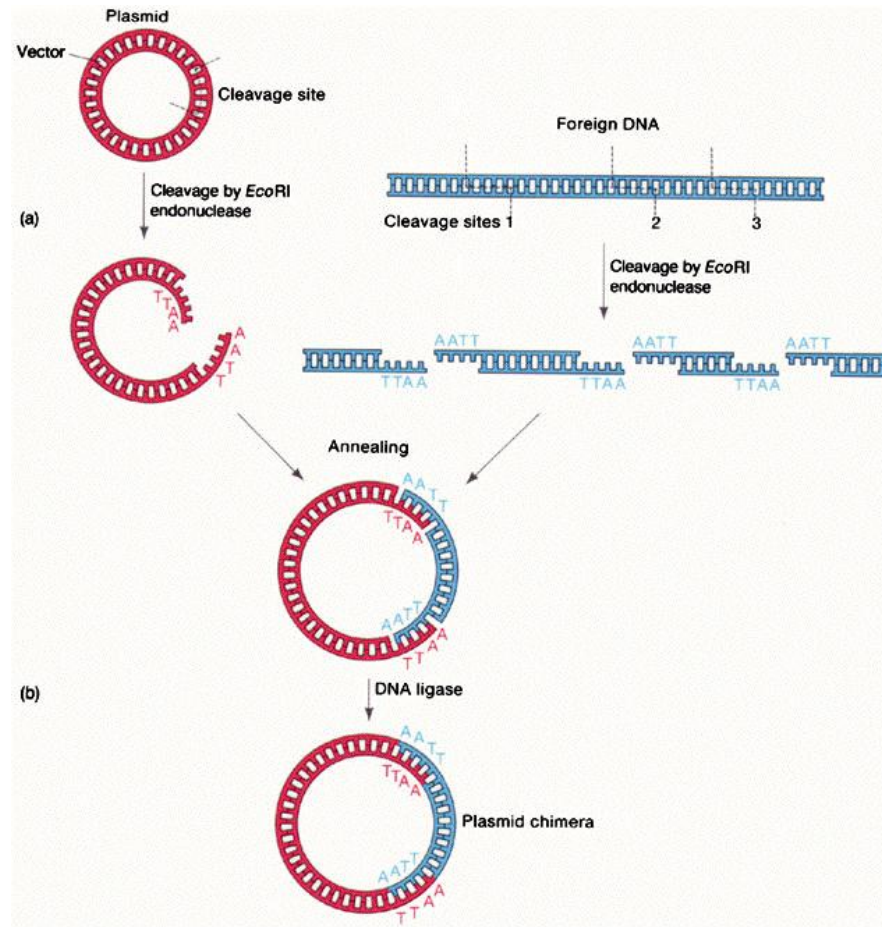


# Plasmid Replication



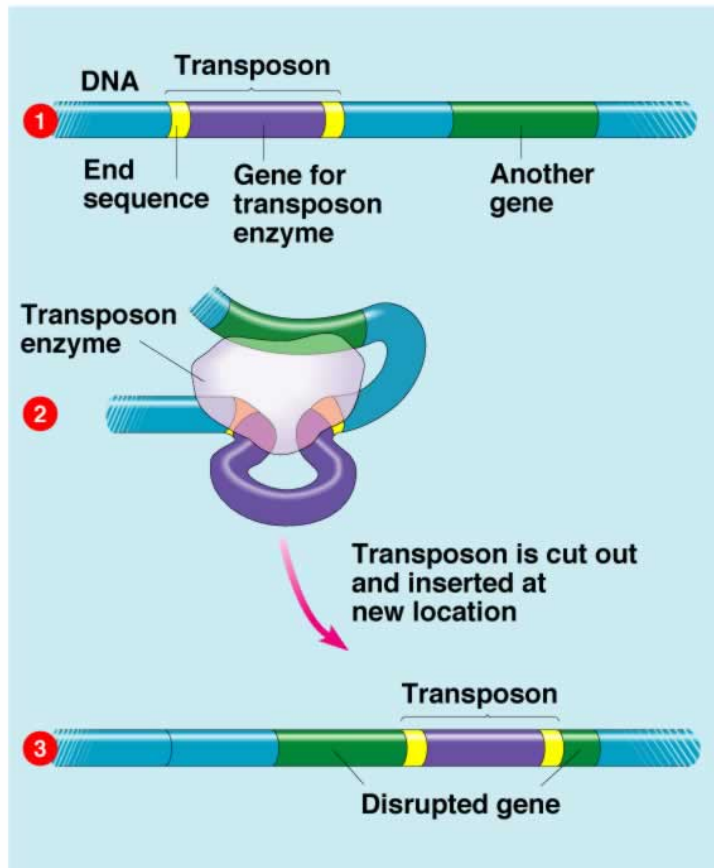


# Plasmid Transformation





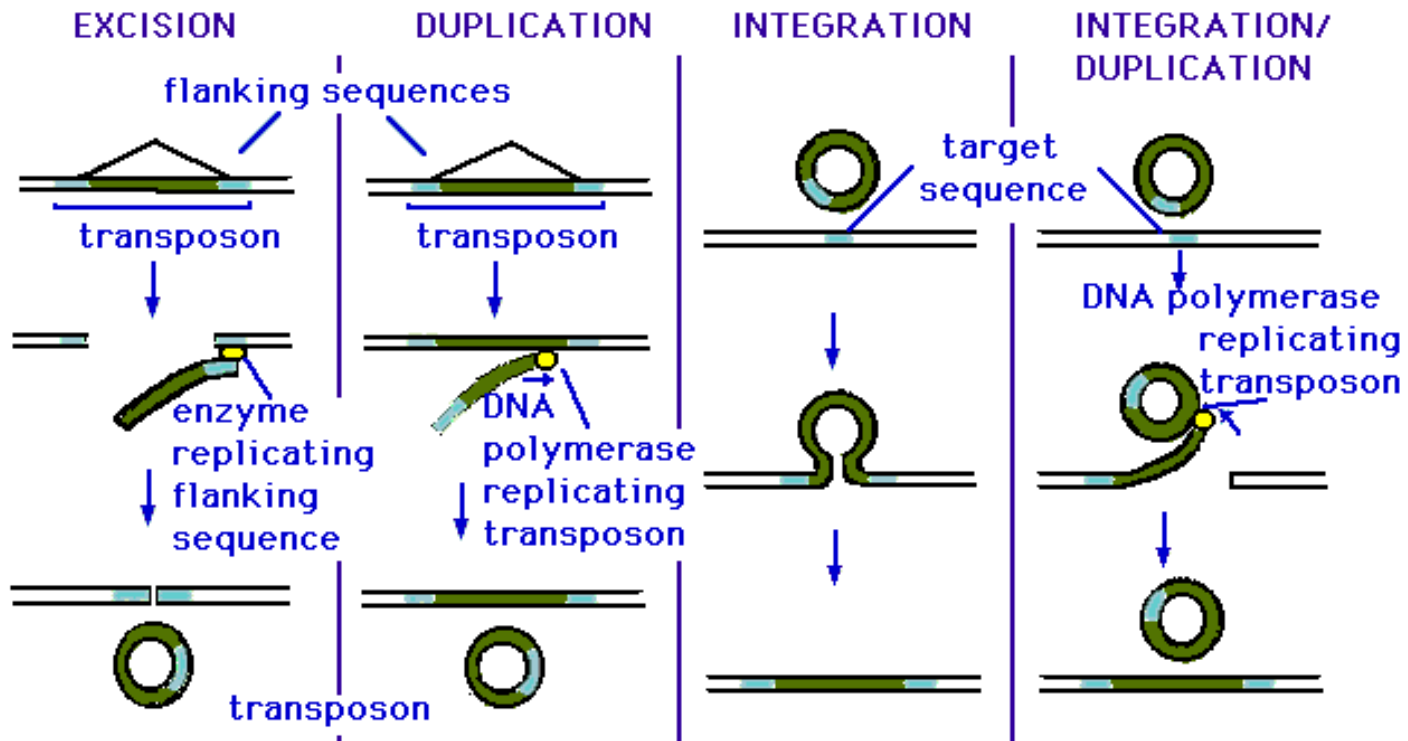
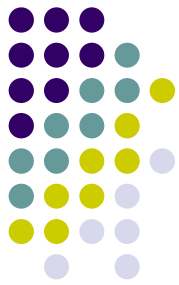
# Transposons

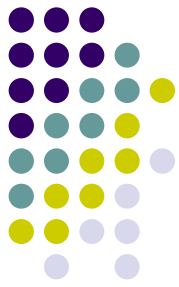


©Addison Wesley Longman, Inc.

- “Jumping Genes”
- Composition
  - DNA
  - Nucleiod
  - plasmid
- Function
  - Code for enzymes to transpose
  - Cut out
  - Re-insert
  - AB resistance

# Transposon cycle

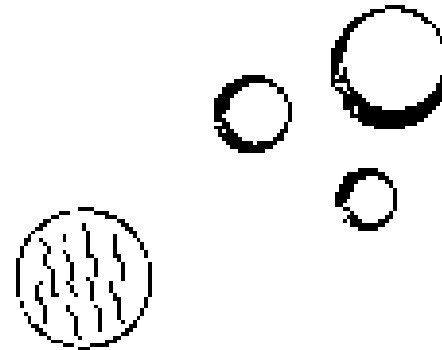


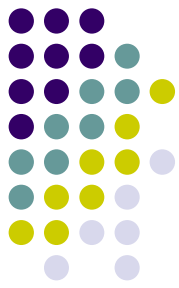


# Other Organelles

- Storage Granules
  - Sulfur
  - Glycogen
  - Volutin or Metachromic [Phosphate]
  - Nitrogen
- Gas vacuoles
- Photosynthesis
- Magnetosomes

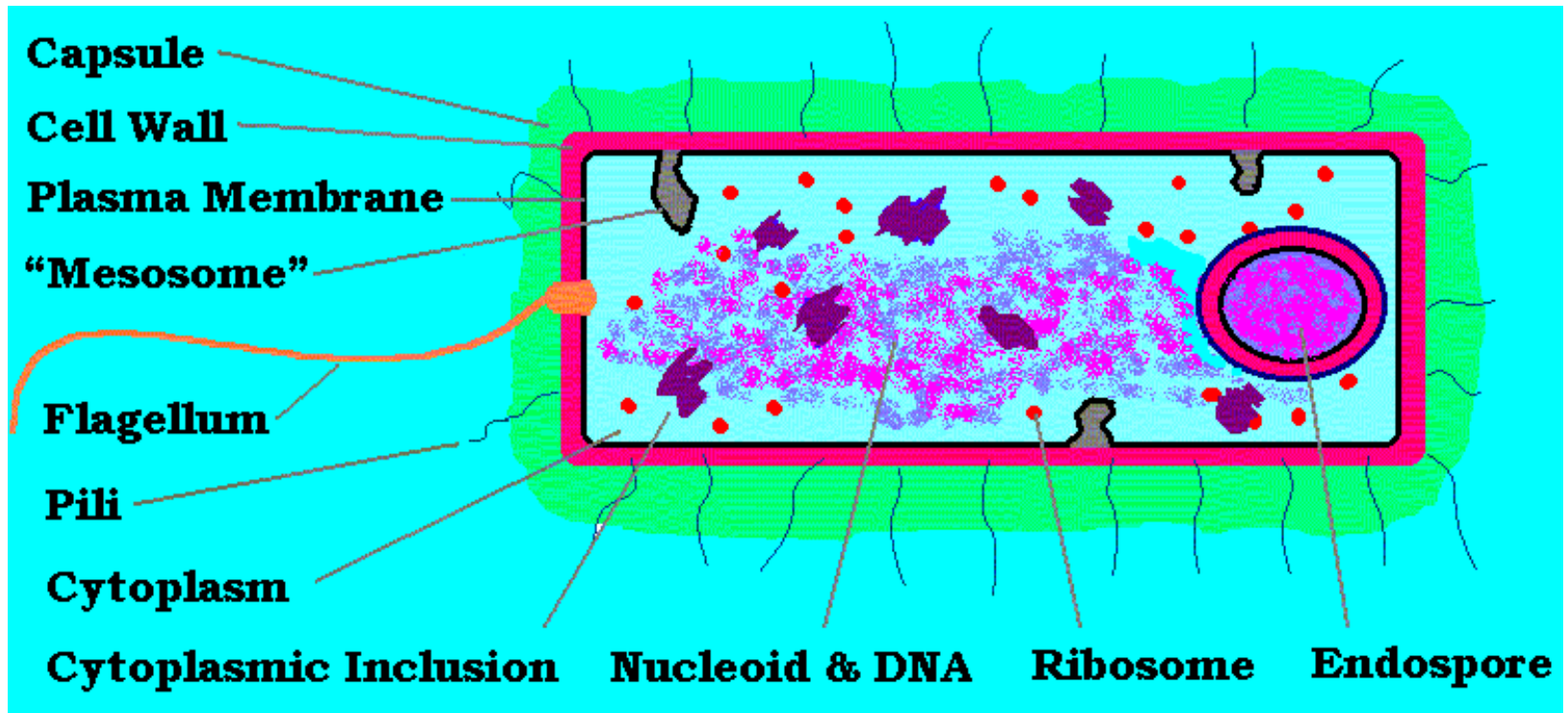
## Storage Granules





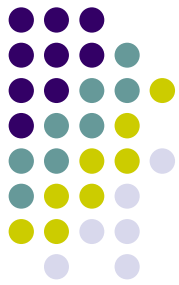
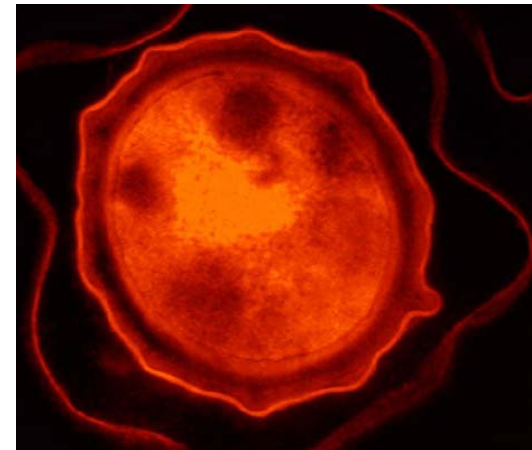
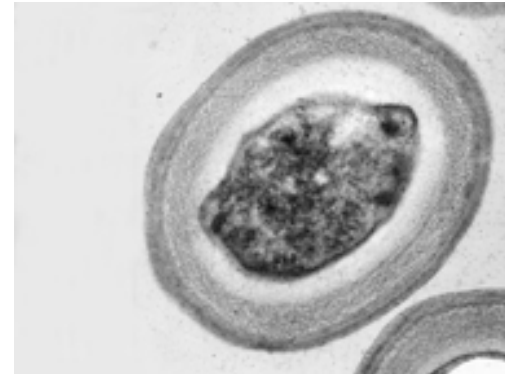
# Mesosomes

- Infoldings of cytoplasmic membrane
- Considered artifact of slide preparation



# Endospores

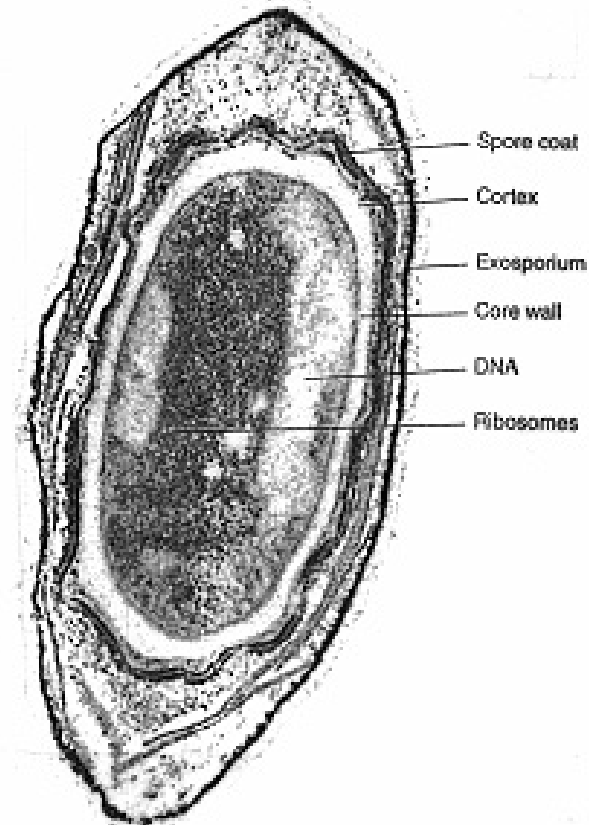
- Dormant alternate life forms
- Some Gram positive organisms
- Identified
  - Size: large, small
  - Shape: oval, rectangular, circular, club-shaped
  - Location : terminal, subterminal, central
- Resistant
  - High temperatures
  - Disinfectants
  - Radiation
  - drying
- Survival
- Germination
  - Favorable Conditions
  - Spore coat ruptures
  - Vegetative cell forms



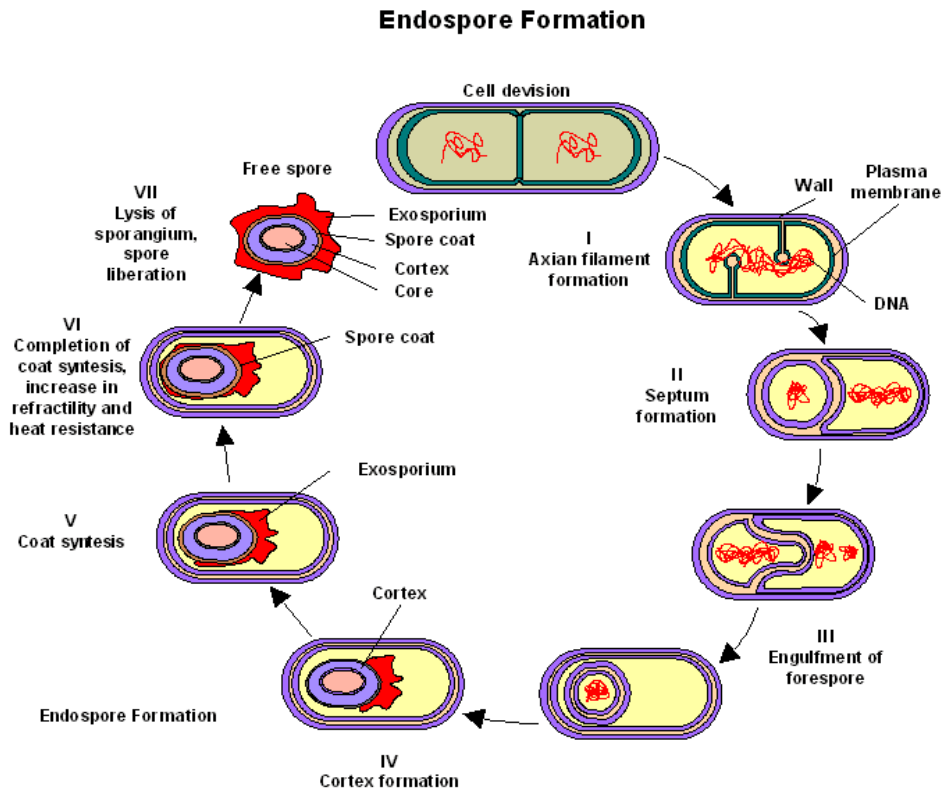
# Spore Structure



- Resistant coats
  - Cortex
  - Spore coat
  - +/- exosporium
- Nucleoid [DNA]
- Ribosomes
- Enzymes



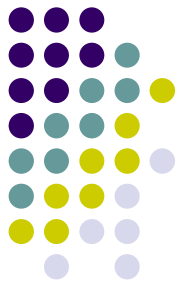
# Spore Formation



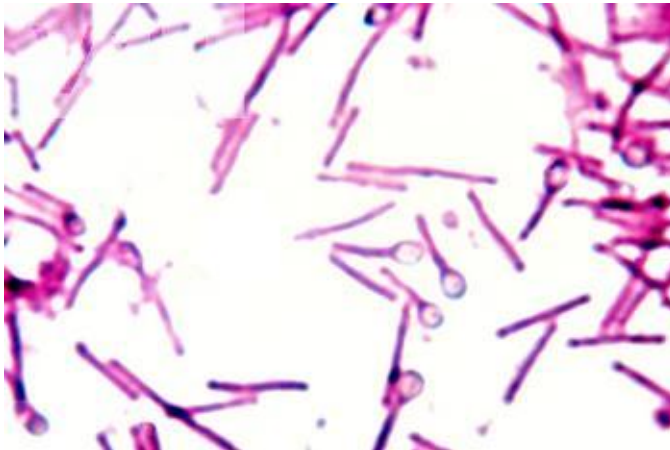
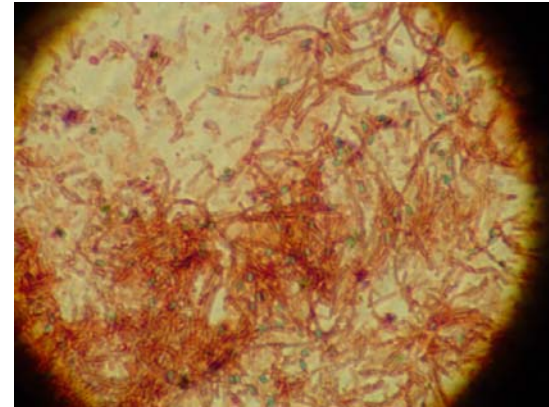
- Sporulation

- DNA replicates
- Membrane septums
  - One at end
  - One around DNA [forespore]
  - Both synthesize peptidoglycan to form Cortex [inside layer]
  - Calcium salts added
  - Spore Coat = keratin around cortex
  - Exosporium = outer lipid/protein

# Spores: Gram Stain



*Bacillus*

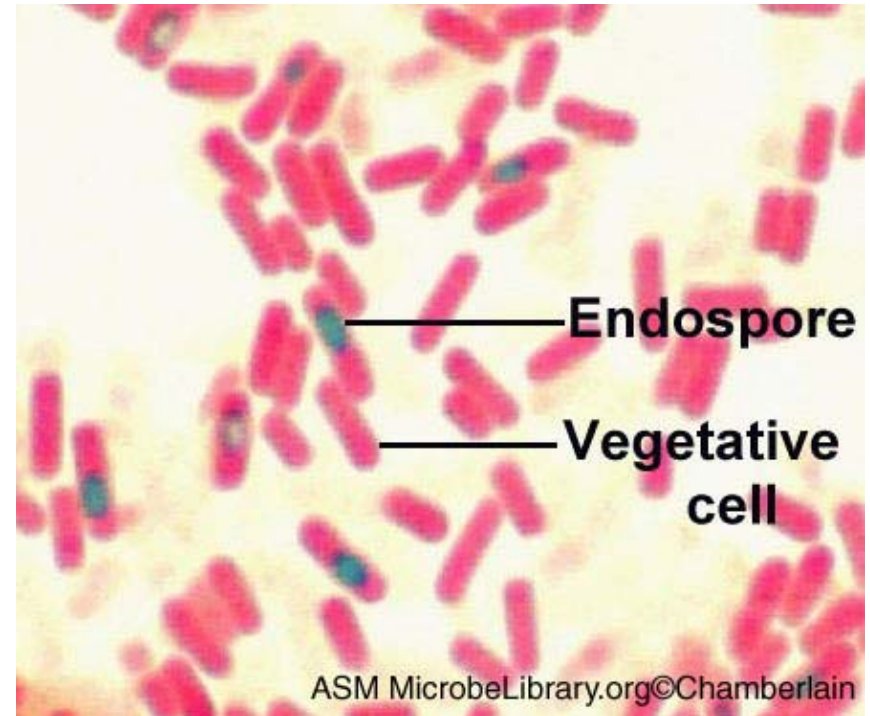
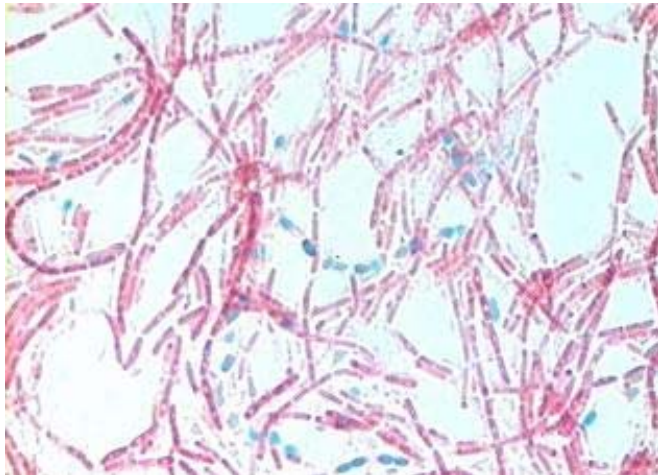
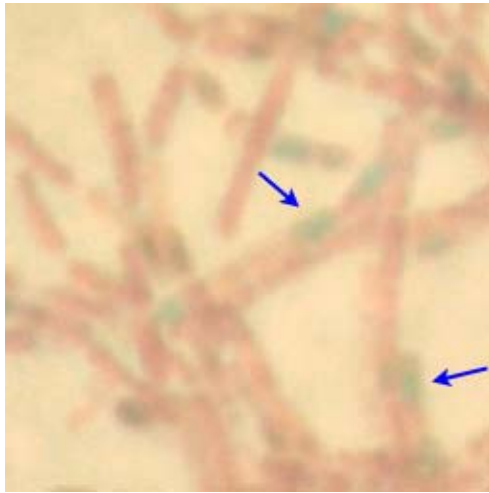


*Clostridium*





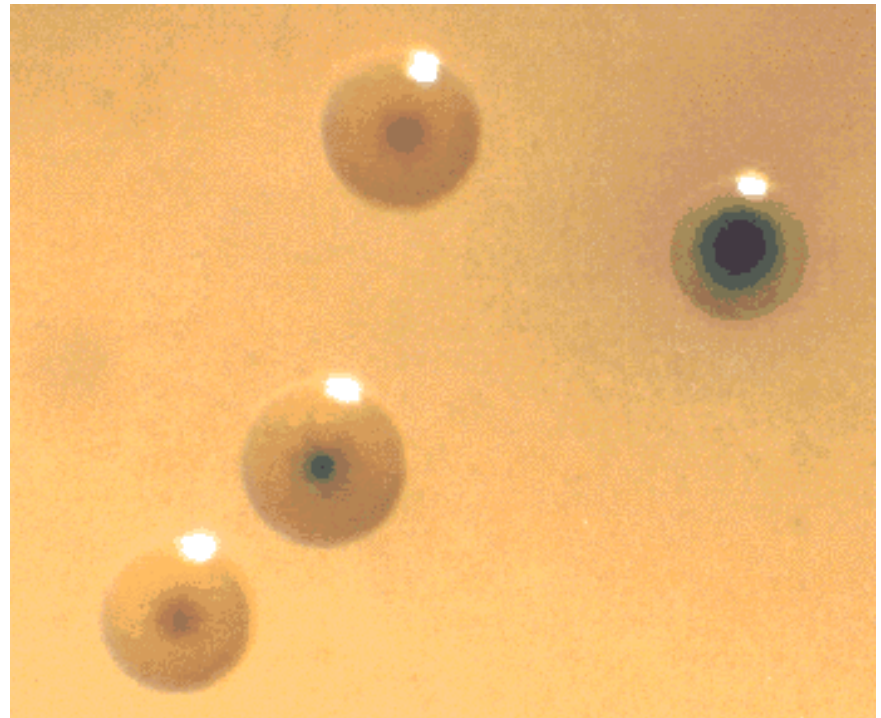
# Spores: Spore Stain



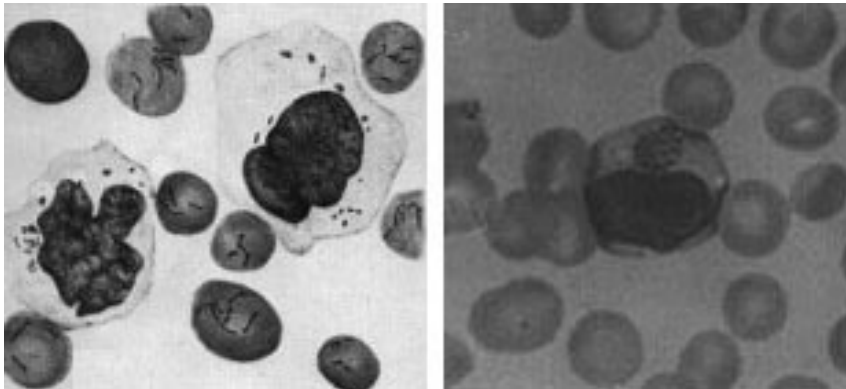
# Atypical Bacteria



- Mycoplasmas
  - Smallest free living cell
  - Lack cell wall  
[no peptidoglycan]
  - Sterols in cytoplasmic membrane
  - Mycolic acid [60%]
  - Examples
    - *Mycoplasma pneumonia*
    - *Mycoplasma hominis*

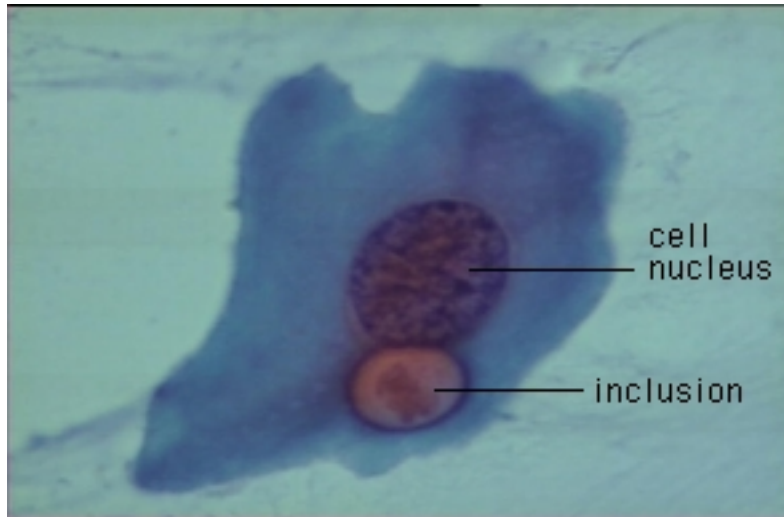
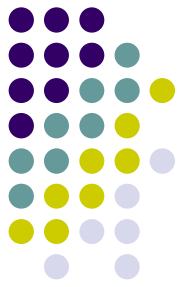


# Atypical Bacteria



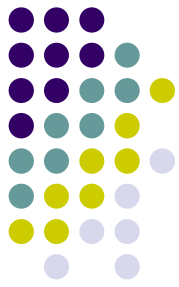
- Rickettsia
  - Pleomorphic
  - Obligate intracellular parasites
  - Arthropod vectors
  - Examples
    - *Rickettsia rickettsii*
      - tick
      - RMSF
    - *Rickettsia prowazekii*
      - Louse
      - Epidemic Typhus fever
    - *Rickettsia typhi*
      - Flea
      - Endemic Typhus fever

# Atypical Bacteria

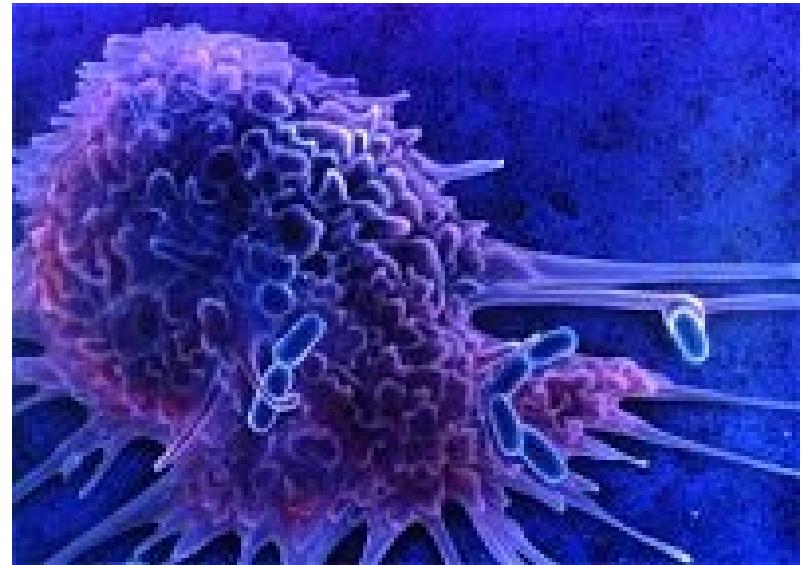


- Chlamydia
  - Coccoid
  - Lack peptidoglycan
  - Obligate intracellular parasites
  - Examples
    - Chlamydia trachomatis
      - STD -> PID
    - Chlamydia pneumoniae
      - Entire air way

# Immune System



- Cytokines
  - Interleukins
  - TNF
- Complement Activation
  - Inflammation
  - Phagocytosis



# Virulence Factors for Pathogenicity



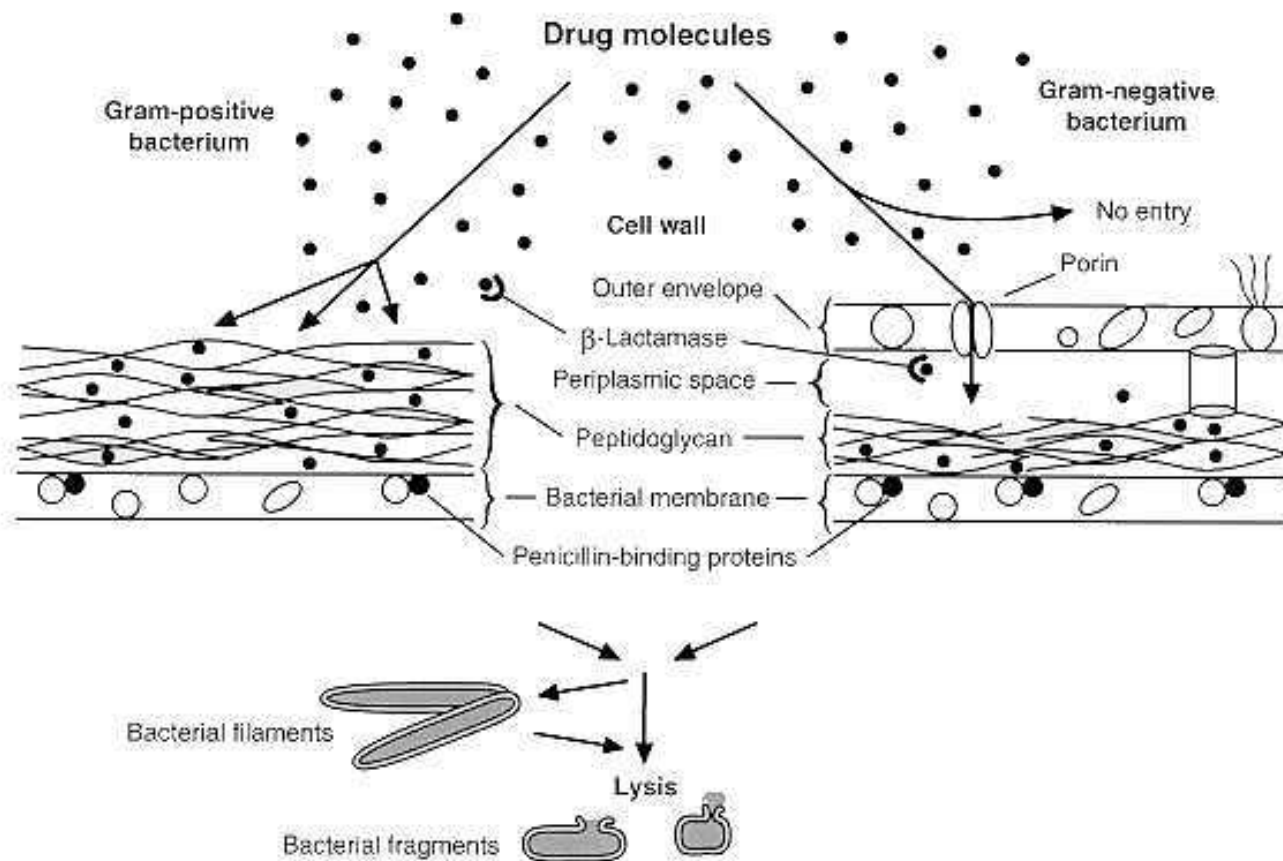
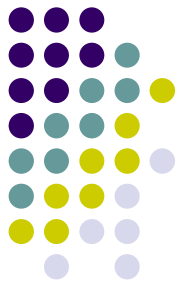
- Capsule [K antigen]
- Flagella [H antigen]
- Fimbria/Pili [adhesins]
- Outer membrane
  - LPS endotoxin
  - Proteins [porins]
- Cell Wall
  - Peptidoglycan
  - Proteins [A, M, T, R]
- Periplasmic Space [enzymes]
- Plasma membrane
- Endospores
- Plasmids
- Exotoxins
  - Enzyme action [hyaluronidase, DNase, collagenase]
  - Detergents [hemolysins, Staph a-toxin]
  - Alter cell metabolism [pertussis, cholera, diphtheria toxins]
  - Block Nerve function [tetanus, botulinum toxins]
  - Others: dermonecrotic, erythrogenic

# Physical and Chemical Actions on Bacterial Structures



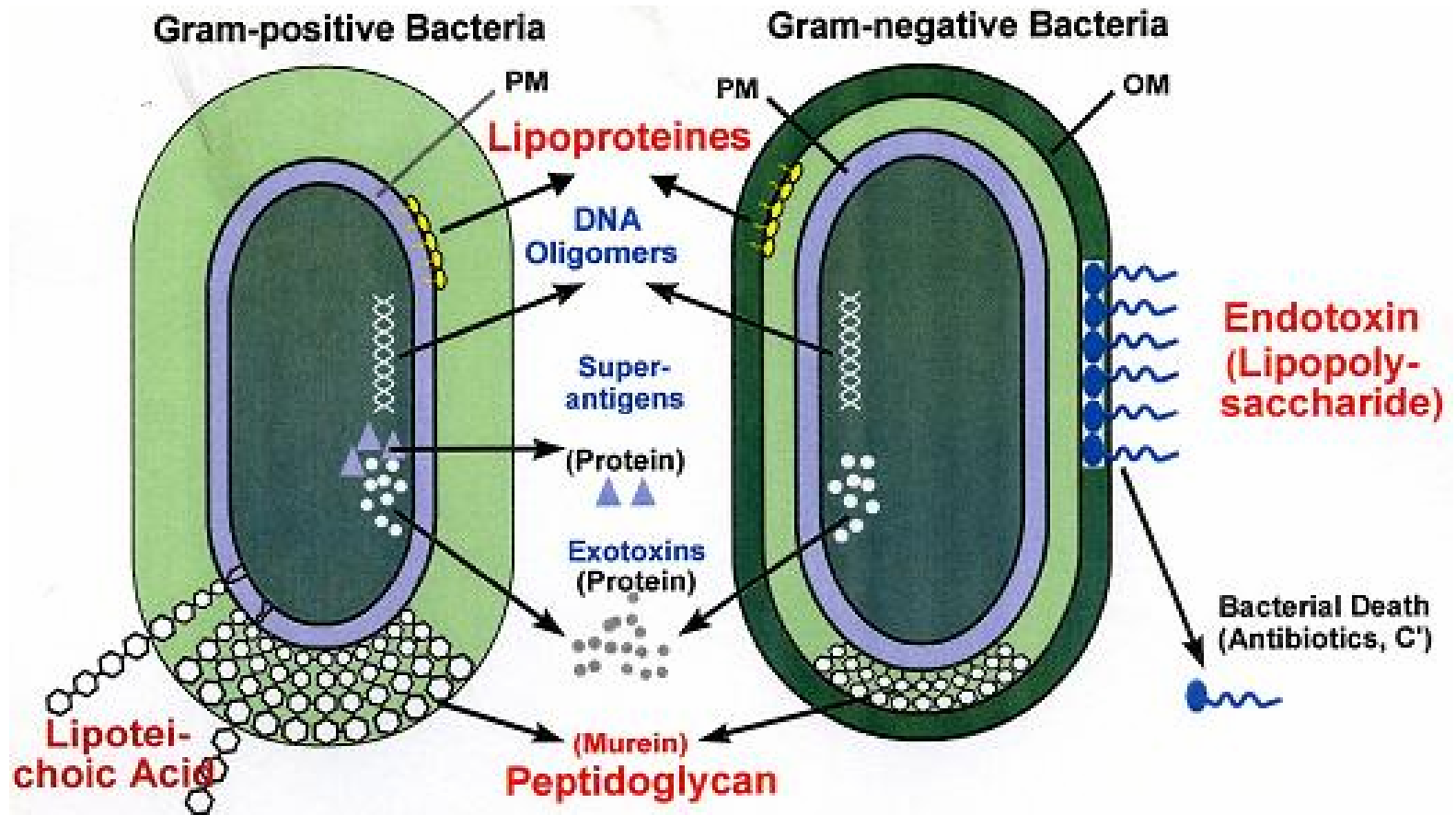
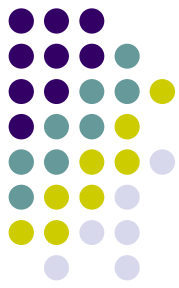
- Plasma Membrane
  - Polymyxins
  - Disinfectants: alcohol, chlorhexidine
- Ribosomes
  - 30S: -cyclines
    - bind reversibly to distort
    - tRNA/mRNA cannot align
  - 50S: macrolides [-mycin]
    - Bind reversibly
    - Inhibit elongation

# Chemical Actions on the Bacterial Cell Wall

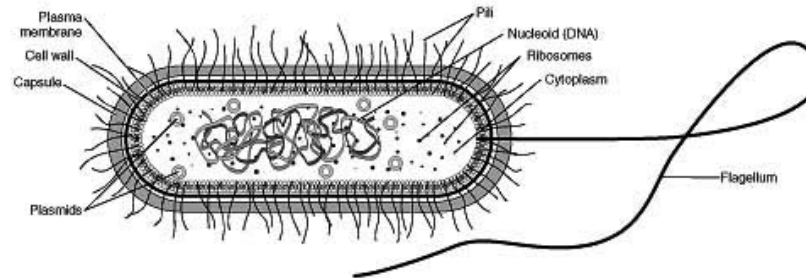




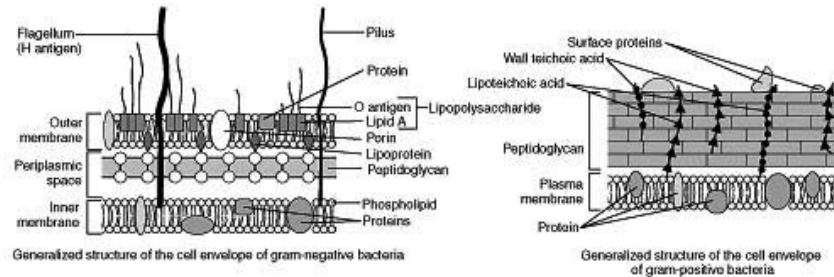
# Summary



# Questions?

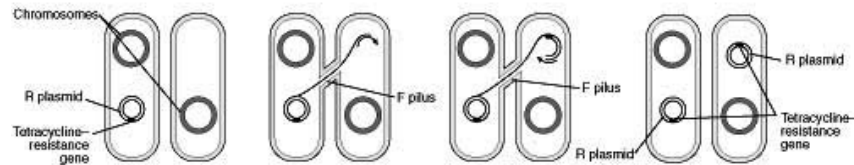


Generalized structure of a monotrichous bacterium (Not all structures occur in all cells)



Generalized structure of the cell envelope of gram-negative bacteria

Generalized structure of the cell envelope of gram-positive bacteria



Transfer of drug resistance, in this case to tetracycline, between bacteria by transfer of the R plasmid during conjugation