Objectives

1.White book: Read Chap 3 & p 77-98 & 108

2.Black book: Read Chap 3 & p75-96 & 106

Objectives:

1. List metric measurement units for microorganisms and convert to other metric units (m, mm, um, nm).
2. Identify parts & functions of the compound light microscope.
3. Define/calculate total magnification & resolution.
4. Compare, contrast, and identify uses (diseases/organisms) for brightfield, darkfield, fluorescent, electron-transmission, and electron-scanning microscopy.
5. Differentiate, compare, and explain the appearance and uses of each of the following: acidic & basic dyes, simple, differential & special stains, capsule, endospore, acid-fast and flagella stains.

Objectives, cont'd

6. List specific chemicals that are used for each type of stain in the objective above, primary stain, mordant, decolorizer, counterstain.
7. Gram stain: list the steps, purpose, and the appearance of GP & GN cells after each step.
8. Identify the 3 basic shapes of bacteria and secondary arrangements.
9. Describe the structure & function of the glycocalyx, flagella (including arrangement), axial filaments, fimbriae, pili. Identify flagellar arrangements.
10. Compare & contrast the cell walls of GP bacteria, GN bacteria, archaea, mycoplasmas, and mycobacteria. (Including composition, antibiotic & chemical resistance, presence of toxins, staining reactions, effect of penicillin, lysozyme, etc.)

Objectives, Cont'd

11. Identify the functions of the cell/plasma membrane, chromatophores/thylakoids, nucleoid, ribosomes, endospores (including location), inclusions.
12. Transport: passive (simple diffusion, osmosis, facilitated diffusion), active transport, hypertonic, hypotonic, isotonic, osmotic lysis, plasmolysis
13. Discuss several pieces of evidence that support the endosymbiotic theory of eukaryotic evolution.
14. Describe the overall structure and defining characteristics of prokaryotes, as compared to eukaryotes.
15. On given slides identify shape, gram reaction, arrangement, type of stain.

Measurement Units & Terms

1. **Units**
   - **A. Micrometer (µm) = ____**
   - **B. Nanometer (nm) = ___________**
     - **i. Example: Convert 21.5 nm to m**
       - _______________________
2. **Total Magnification**
3. **Resolution: Distance apart needed to see ___________________ (Ability to see ___________)**

Resolution & Refractive Index

A. Resolving power = ______________________________

____

N.A. depends on:

i. ____________________ of material between lens & slide.

ii. The _____________of most divergent light ray

B. To improve resolution:

i. .

ii. .

C. Improve conditions but NOT resolution:

i. .

ii. .

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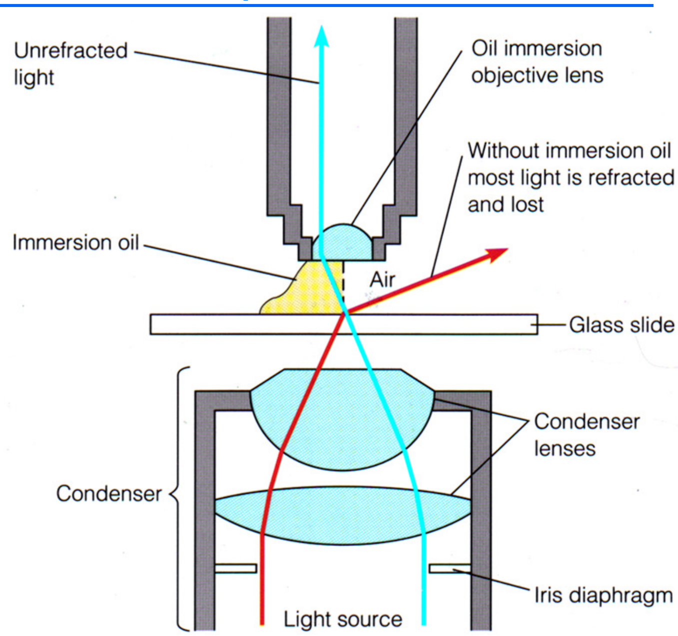
Fig 3.3 Refraction w/ & w/o Oil, p.59

Using oil does improve resolution, as it increases the numerical aperture, which will cause a better (smaller) resolving power number



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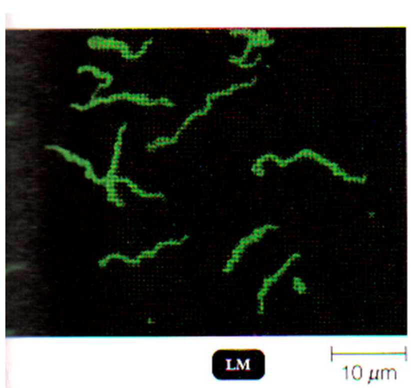
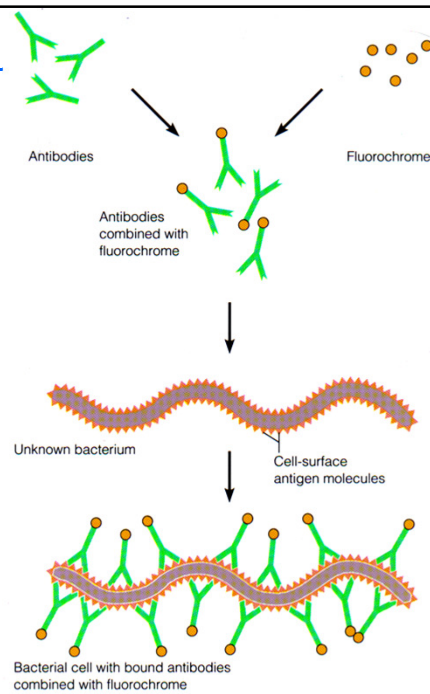
Types of Scopes-3 subtypes of Light microscopes

Scope	Enhanced by	Advantages	Uses
Light, Brightfield: Background ______ Visible light Res: Mag:	______________ ______________ ________ & light	Inexpensive Easy to use	Live specimens (unstained) Stained specimens Bacteria, protozoa
Light, Darkfield: Background ____ & microbes ____ Same	N/A	Easier to see ______________ ________ microbes	Live microbes: __________
Light, Fluorescent: Background ____ & __________ ______________ microbes Same	Fluorescent- __________dyes: Fluorescent dye on ____________ to microbe _________, microbe fluoresces	__________directly from specimen, w/o culture Detection of ______ microbes compared to other light microscopy	When immediate diagnosis needed When cultures aren't avail, or take long __________ __________

Fig 3.6 Immunofluorescent Staining Technique



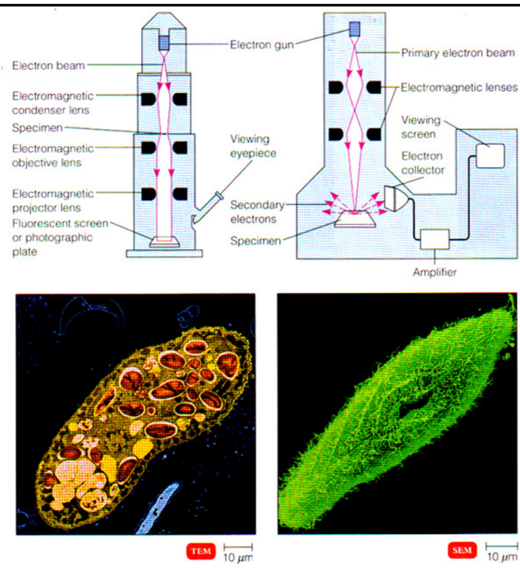
Demo-Fluorescent marker drawings



Scopes-Electron

Scope	Enhanced by	Advantages	Uses
Electron, Scanning Res; ____________ Mag; ____________		3-D Book from U of I	Surfaces structures - eukaryote to virus
Electron, Transmission Res ___________ Mag ____________	Stain w/+ salt of heavy metal	________ res & mag **DISADVANTAGE**: Need ________ slice as e- can't _________ All e- scopes- _____________ due to killing, & fixing under vacuum	Virus particles, bacterial flagella, ______ cell structures, protein molecules
Scanned-Probe Res 1/100 of atom		Res No special prep	Map atomic & molecular shapes & processes, ie. DNA, fibrin (clot) formation

Fig 3.8 Transmission vs. Scanning



(a) Transmission. (Top) In a transmission electron microscope, electrons pass through the specimen and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. (Bottom) This colorized transmission electron micrograph (TEM) shows a thin slice of a *Paramecium*. In this type of microscopy, the internal structures present in the slice can be seen.

(b) Scanning. (Top) In a scanning electron microscope, primary electrons sweep across the specimen and knock electrons from its surface. These secondary electrons are picked up by a collector, amplified, and transmitted onto a viewing screen or photographic plate. (Bottom) In this colorized scanning electron micrograph (SEM), the surface structures of a *Paramecium* can be seen. Note the three-dimensional appearance of this cell, in contrast

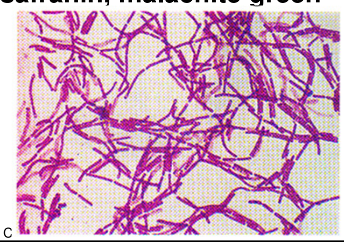
Stains-Slide Prep & Basic Stains

Slide Prep:

1. Smear
2. Fix – _______________ to slide (won't ________ off)
    A. .
    B. .
    C. .
    D. HOPEFULLY-preserves w/ _____________________

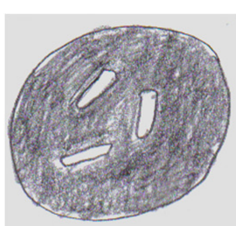
Staining

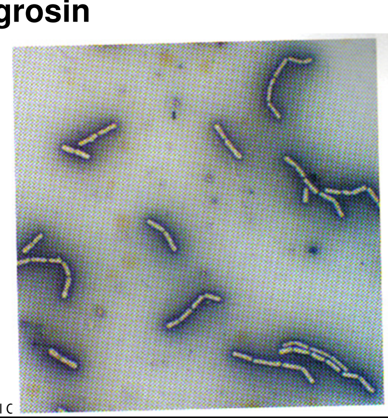
1. Basic dye/_______________ stain: Colored (___) ion of a salt
    A. Attracted to (_____) bacterial cell; stains _____________
    B. Crystal violet, methylene blue, safranin, malachite green



Acidic Dye / Negative Stain

2. Acidic dye / _________________ stain: Colored (___) ion
    A. _________________& stains __________________
    B. For cell ______________________, to detect ___________
    C. Advantage: ________________(no ______________ & stain ______________________________so accurate size & shape)
    D. Examples: Acid fuchsin, nigrosin

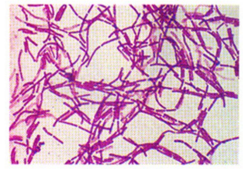




Mordant, Simple Stain, Differential Stain

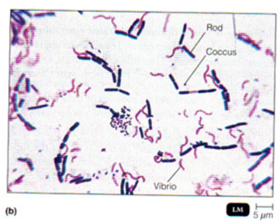
3. **Mordant**: Substance used to cause more ________ staining

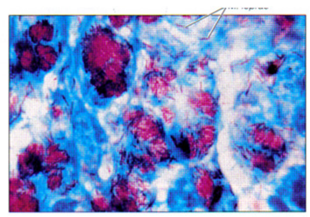
NOTE: This is not the stain that gives color, only helps the stain be more intense color



4. **Simple stain**: ______________ basic dye
   A. All microbes - ____________________
   B. Only for ____

5. **Differential Stain**: Use of ______________________ to ___________________________ groups of bacteria
   A. Examples: gram stain, acid fast stain





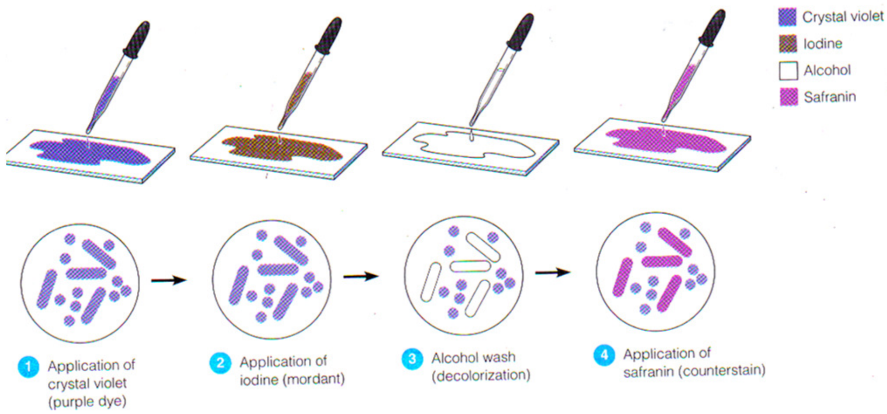
Gram Stain

6. **Gram Stain**: _______________________________ - due to ______________________ differences
   A. GP = gram positive, _____________, retain __________________ stain
      i. Us. ___________________ to penicillin
   B. GN = gram negative, red, ________________________________ stain & accepts ____________________________
      i. ________________________________ to penicillin
   C. Staining problems
      i. Need ____________________ cultures
      ii. Some bacteria stain ___________________________
      iii. ___________________________ timing is ___________________________
      iv. Potential ____________________ -structures/distortions that appear due to prep or staining procedures NOTE: this is potential problem w/all stains

Most common stain in medical microbiology

Know procedure-steps, purpose of each step/stain, appearance of cells after each step, how cell wall causes differential staining (Chap 4)

Gram Stain Diagram



- Shapes above?
- GN or GP?
- Combine?
- GNR/GNB & GPC

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Stains: Acid Fast & Capsule

7. **Acid Fast Stain**
   - A. **Acid-fast positive = ____________ (due to __________ in cell ____)**
   - B. **Acid-fast neg = _____________**
   - C. **ID __________________________ species, ____________________**

8. **Capsule Stain (w/ _________________stain)**
   - A. **Capsule = _____________________covering on outside of bacteria**
   - B. **Variation w/2 stains:**
     - i. .
     - ii. .
     - iii. **________________________of capsule left between the stains**
   - C. **Problems: capsule may ____________________________**

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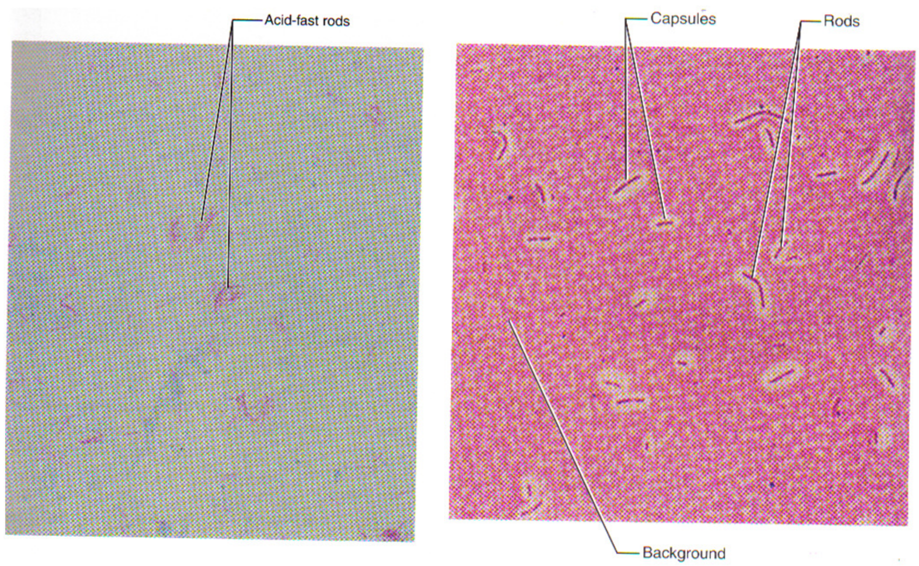
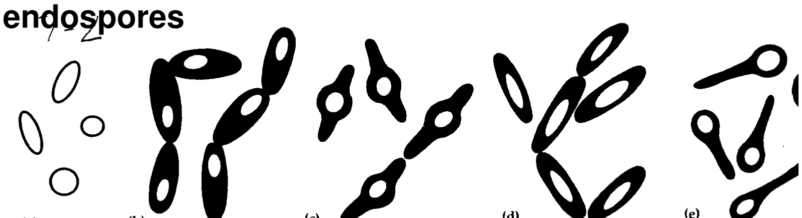
Pictures-Acid Fast & Capsule Stains

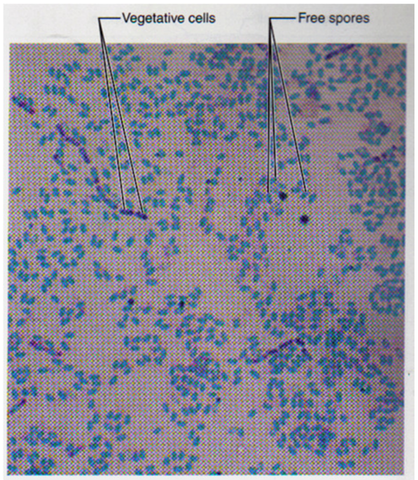


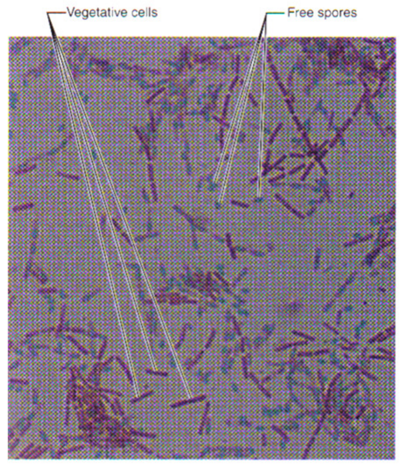
Stains: Endospore

9. **Endospore Stain**
   A. **Endospore**____
   ____
      i. **Position used to ID species**_______________________
      ____
   A. **Uses _____________ to force dye into ________________ endospores**



Stains; Endospore Pictures





1. Discuss vegetative vs. endospores. Free vs. still in cell.
2. Which of the 2 pictures above has been subjected to adverse conditions longer? Explain.

Stains; Flagella

10. Flagella Stain

A. Flagella = ________________

____

B. ________________________________

used to ID bacteria

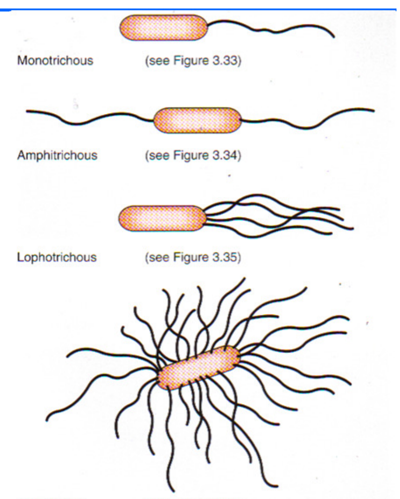


FIGURE 3.32 Flagella arrangements in bacteria. In *monotrichous* flagellation, a single flagellum is located at one end of the cell. In *amphitrichous* flagellation, a single flagellum is located at both ends of the cell. In *lophotrichous* flagellation, many flagella are grouped at one end of the cell. *Peritrichous* flagella are located all around the cell.

Chapter 4: Prokaryotic Cells

Prokaryote

1. .

2. .

3. .

4. .

5. Bacteria – cell wall ______________________________

6. Archaea – ______________________________________

Fig 4.5a Prokaryotic Cell

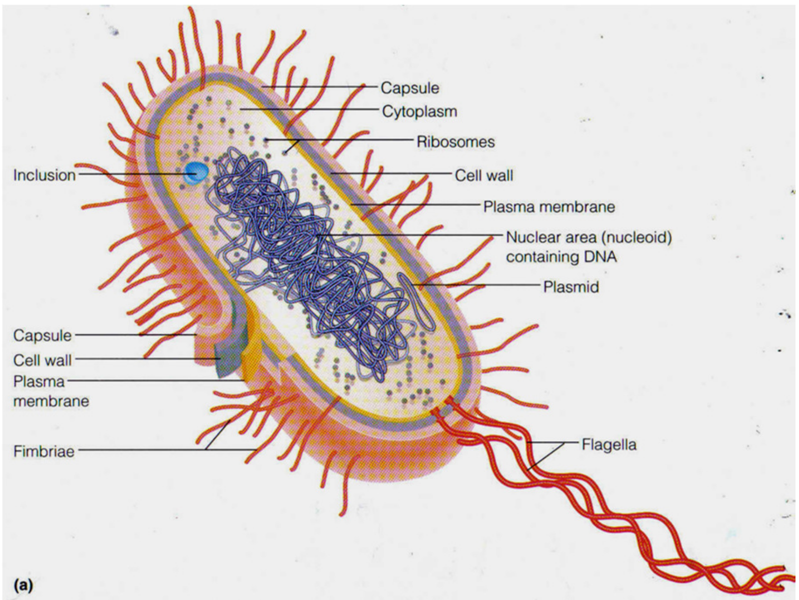
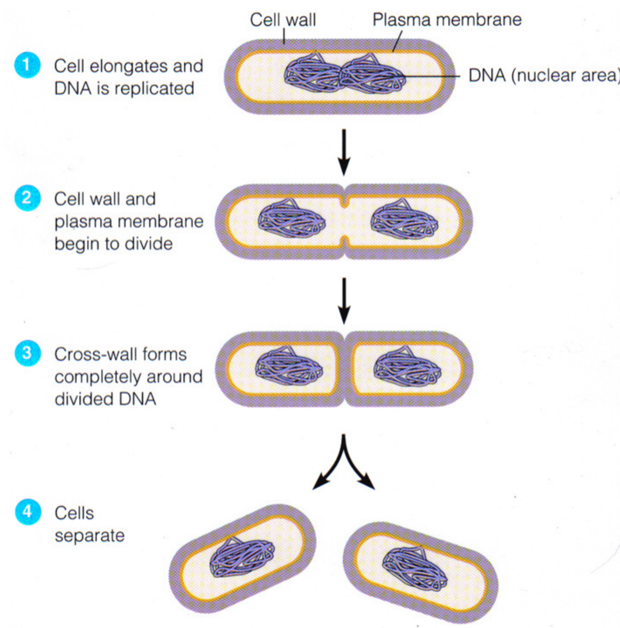


Fig 6.11a Binary Fission



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Arrangement

Size of bacteria 0.2 – 8 um vs. resolution of light microscope?

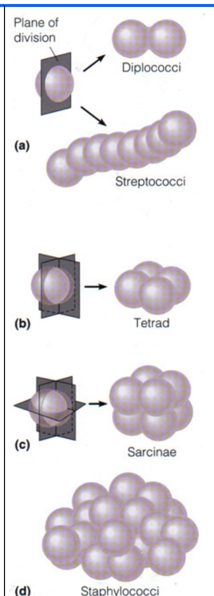
<u>Arrangement Review:</u>

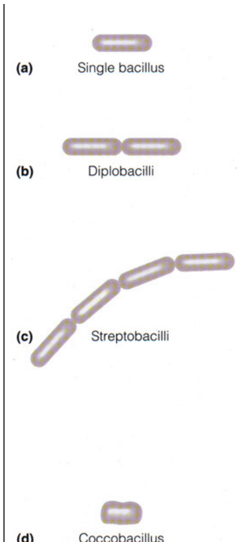
Shapes/Morphology?

Arrangements?

Other morphology terms:

1. ______________
2. ______________
_





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Cell Wall - Bacteria

Bacterial Cell Wall

1. .

2. Clinical importance

    A. .

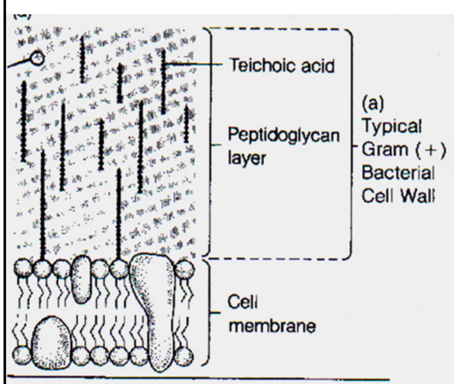
    B. .

3. .

4. Penicillin interferes ___________________________________

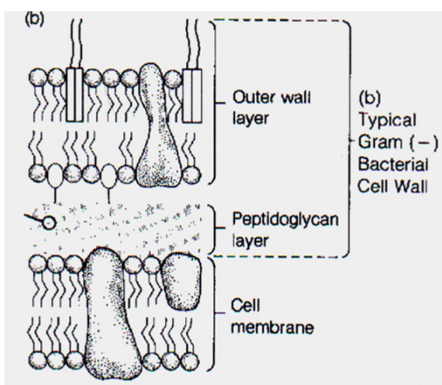
Diagram –Cell Wall Diagrams

Outside Cell



Inside Cell

Outside Cell



Inside Cell

Table – GP vs. GN Cell Wall Characteristics

GP Wall	GN Wall
1. .	1. .
2. Contains _______________	2. None
3. None	3. OUTER Wall Membrane A. Evades _______________ _______________ B. Contains _______________ C. .
4. None	4. Periplasm- _______________ _______________ (where peptidoglycan is) A. Contains _______________ _______________

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Gram Stain & the Cell Wall

Cell Wall & gram stain

1. Iodine = ____
2. Alcohol
   A. GP:
   B. GN:
   C. GP falsely stain GN when cell wall damaged due to ________
3. GPR/GPB only:
   A. ________________________: Bacillus & Clostridium
   B. ________________________: Mycobacterium (TB)

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Chemicals & the Cell Wall

Chemical Effects on Cell Wall

1. **Lysozyme:**
   - A. **Most effective on**
2. **Penicillin**

Atypical Cell Walls

Atypical Cell Walls

1. **Mycoplasma species:** ______________________________
   - A. **High amount ________________ in plasma membrane, ___________ from lysis**
2. **Mycobacteria- High _____________in wall**
   - A. .
   - B. .
3. **Archea; ______________________________________**

Structures External to Cell Wall

External Structures

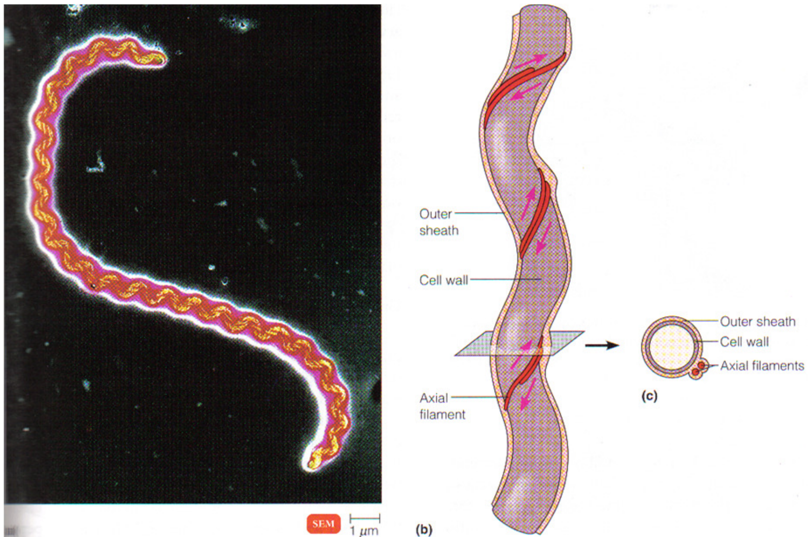
1. **Glycocalyx/Capsule:**
   - A. **EPS (Extracellular polysaccharide) & polypeptide polymer**
   - B. **.**
   - C. **Negative Stain, but uses 2 dyes**
     - i. **Basic stains _____________**
     - ii. **Acidic stains _____________**
     - iii. **_________________________**

External Filamentous Structures

2. Table:

Flagella	**Axial Filaments**	**Fimbrae**	**Pili**
Monotrichous - **Amphitrichous-** **Lophotrichous-** **Peritrichous-**	**Spiralled around cell within _____________** **(AKA endoflagella)**		

Diagram-Axial Filament



URE 4.9 **Axial filaments.** **(a)** A photomicrograph of the spirochete *Leptospira*, ing an axial filament. **(b)** A diagram of axial filaments wrapping around part of a chete. **(c)** A cross-sectional diagram of the spirochete, showing the position of filaments.

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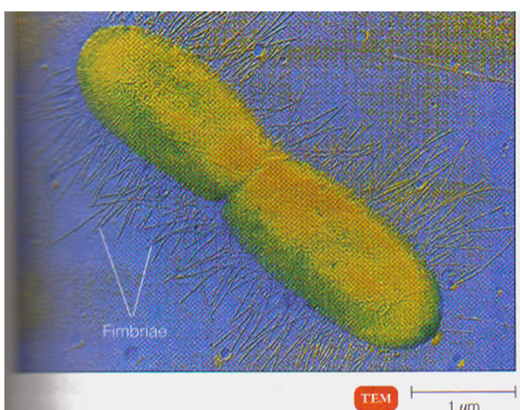
Photo-Fimbriae



RE 4.10 **Fimbriae.** The fimbriae seem to bristle from this cell, which is beginning to divide.

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Fig 8.26 Bacterial Conjugation

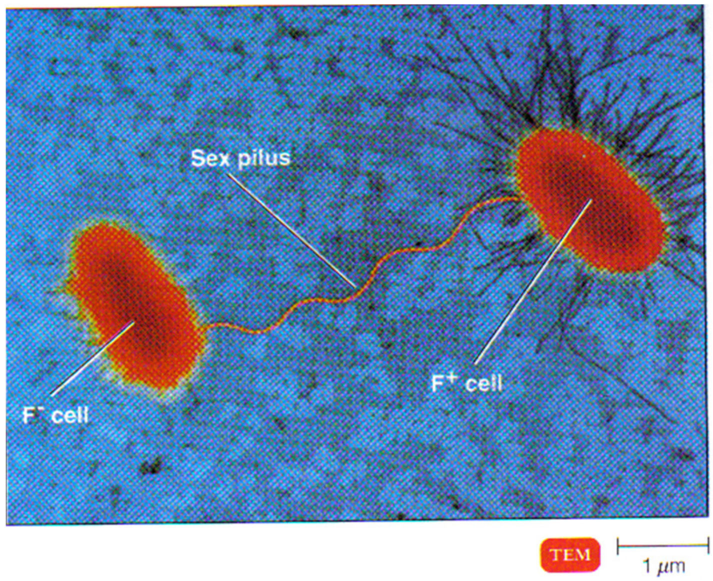
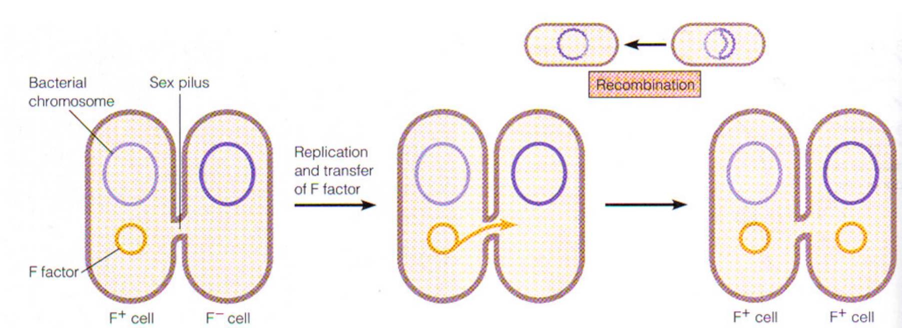


Fig 8.27 Conjugation in E. coli



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

External Filamentous Structures, Cont'd

3. NO ________________

4. Taxis: ____

   A. Chemotaxis

   B. Phototaxis

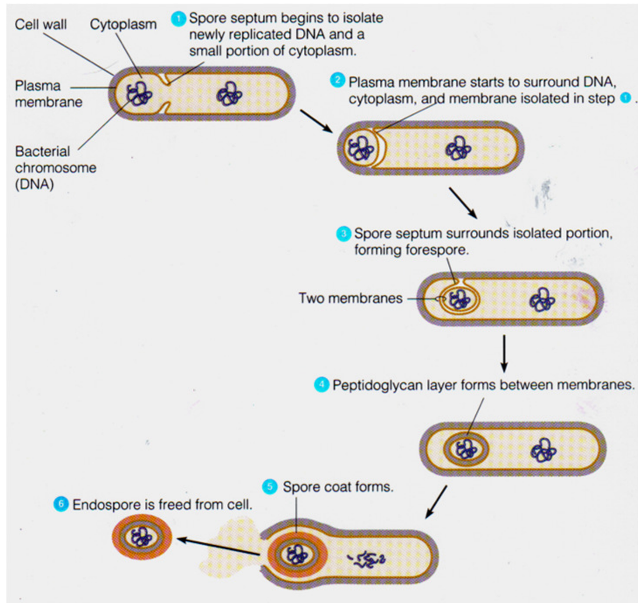
Discuss serovars

Endospores

Structure Internal to Cell Wall

1. Endospores: ______________________________structures to ________________ adverse conditions
   - A. .
   - B. Sporulation / Sporogenesis
   - C. Germination – return to ___________________state
   - D. .
   - E. Location: ____
   - F. Survive ____ ____
   - G. Stains:
     - i. Gram- _____________
     - ii. Endospore Stain:
       - Primary: basic stain ______________________________
       - Rinse: removes stain from _________________________
       - Counterstain: basic stain colors _____________________

Fig 4.20a Endospore Formation



(a) Sporulation, the process of endospore formation

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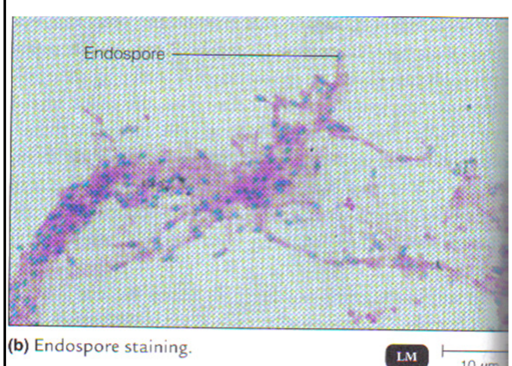
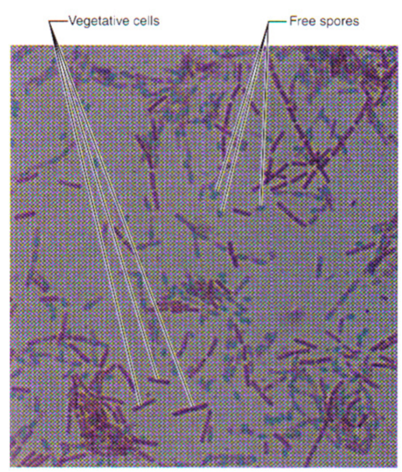
Endospore Stain Pictures



(b) Endospore staining.



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Plasma/Cytoplasmic Membrane

2. **Plasma Membrane**
   - A. .
   - B. .
   - C. Special: ______________________________

Alcohols, disinfectants, some antibiotics effective here

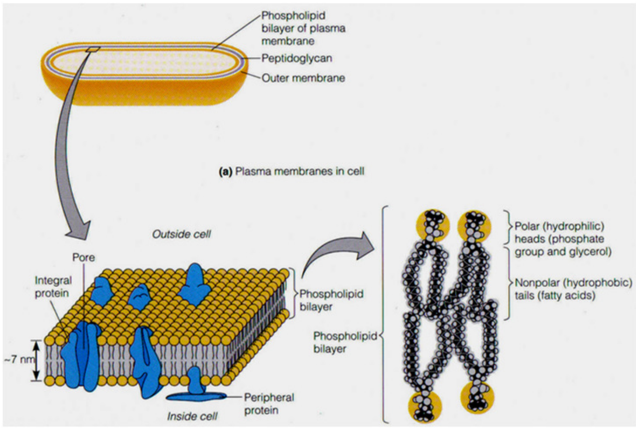

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Fig 2.11 Phospholipids

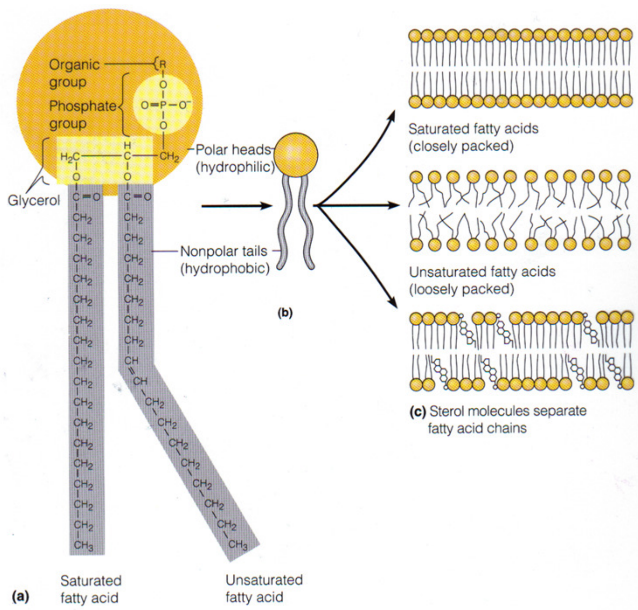

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Fig 4.16 Diffusion

D. **Diffusion:** ____

   i. **Simple diffusion**

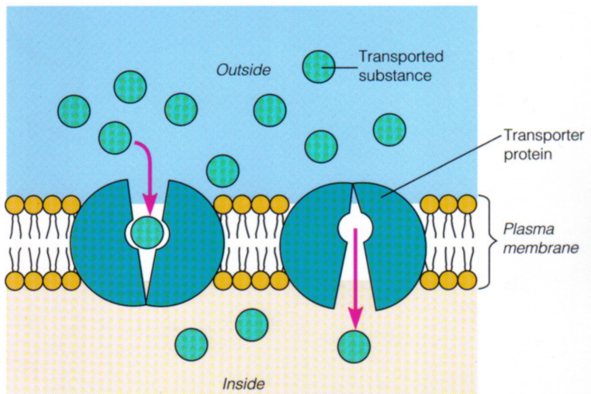
   ii. **Facilitated diffusion**

   iii. **Osmosis**

E. **Active Transport:** ____________________________________

Diagram on the right:

Which type of transport does it represent?



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Osmosis-Animal vs. Plant

Special terms reflect % solute, and therefore affect net direction of osmosis.

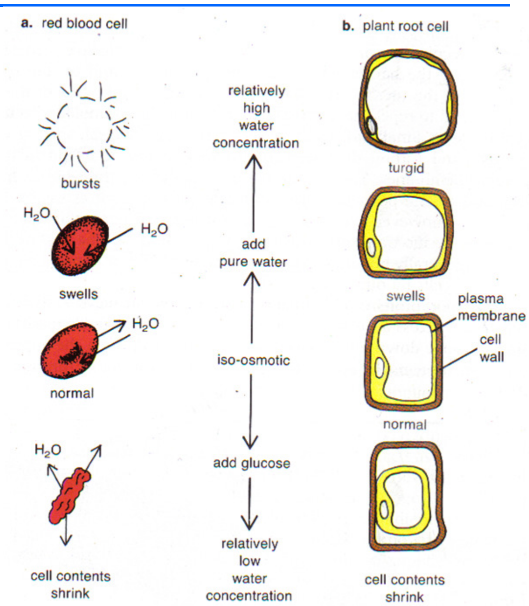
What do the following prefixes mean?

Iso?

Hypo?

Hyper?

Suffix is "tonic" = tension



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Osmosis & Solution Types

F. Osmotic Environments

- **i. Isotonic/isoosmotic solution: ___________________**
  - » .
  - » **Water movement __________________________**
  - » .
- **ii. Hypotonic solution: ____________________________**
  - » **Net H_2O moves _________________**
  - » .
- **iii. Hypertonic solution: ___________________________**
  - » **Net H_2O movement _____________________**
  - » .

Diagram from Bio-Osmosis & Plant vs. Animal

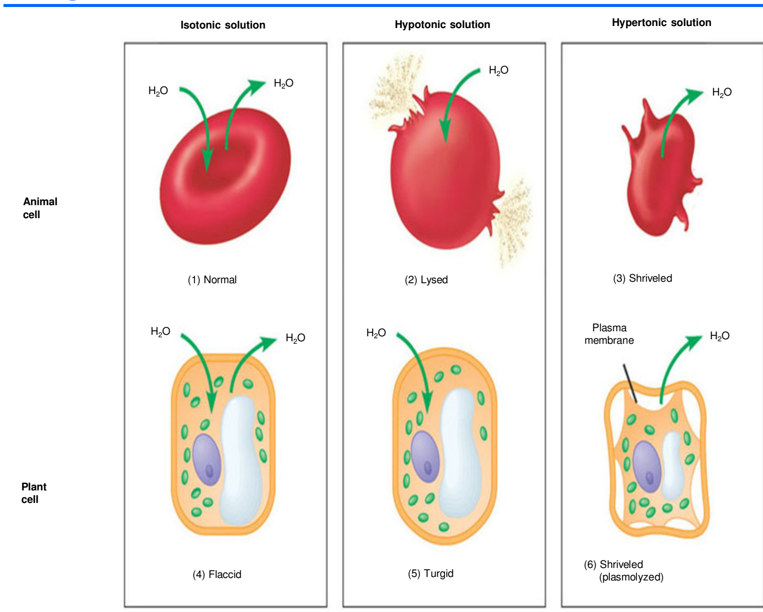
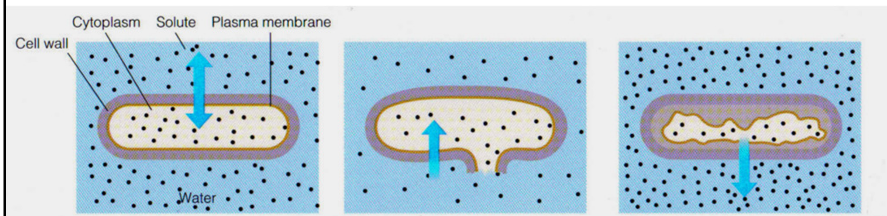


Fig 4.17 cde Osmosis in Varying Osmotic Environments



(c) Isotonic (isosmotic) solution—no net movement of water

(d) Hypotonic (hypoosmotic) solution—water moves into the cell and may cause the cell to burst if the wall is weak or damaged (osmotic lysis)

(e) Hypertonic (hyperosmotic) solution—water moves out of the cell, causing its plasma membrane to shrink (plasmolysis)

Internal Cell Structures continued

3. **Chromatophores/thylakoids:** ________________________________**structures**

4. **Nucleoid/nuclear area: No nuclear membrane**

   A. **Contains** ____

5. **Plasmids:** ____

   A. .

   B. **Conjugation: transfer through** __________________________

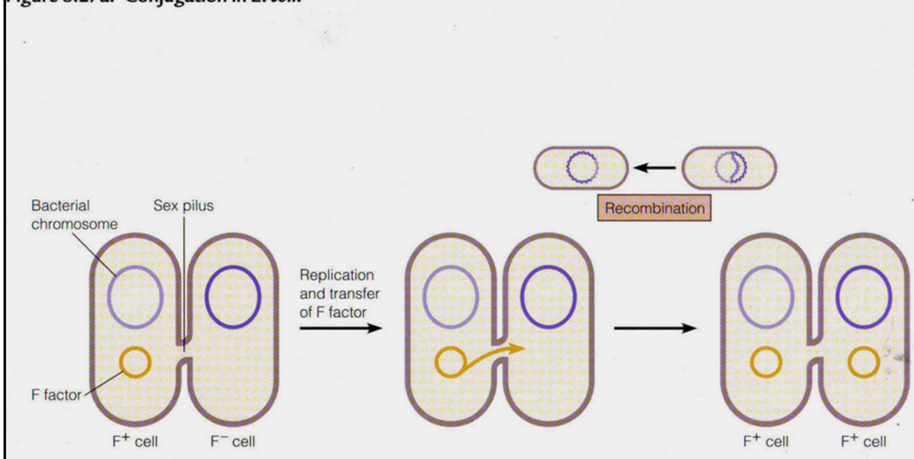
      i. **GN-**_________________________

      ii. **GP-**_________________________

   C. **Biotech:** ____

Fig 8.27a Conjugation-Plasmid Transfer

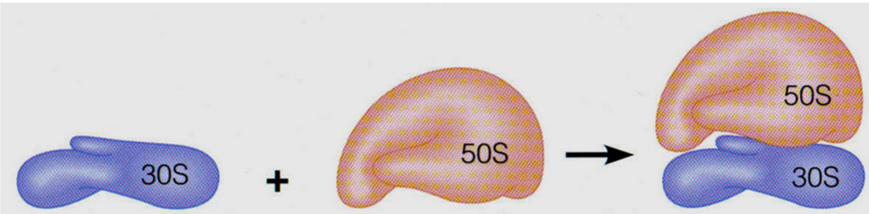
Figure 8.27a: Conjugation in *E. coli*.



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

Internal Structures cont'd-Ribosomes

6. **Ribosomes: ________________________________**
   - **A. 2 subunits: protein & RNA**
     - **i. Prokaryotic size: ________________________**
     - **ii. Euk: __________________________________**
   - **B. ____________ attach to subunit of ____________ size**



(a) Small subunit (b) Large subunit (c) Complete 70S ribosome

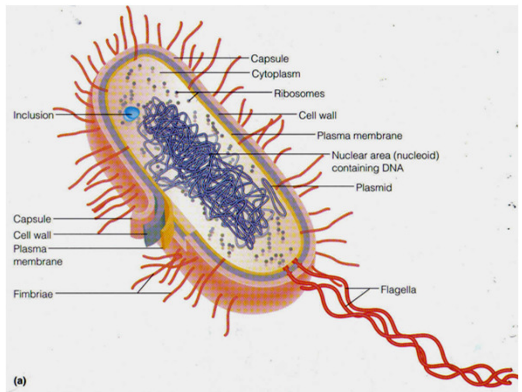
Internal Structures cont'd-Inclusions

7. **Inclusions:** ______________________________

   A. .

   B. .

Review Structures:



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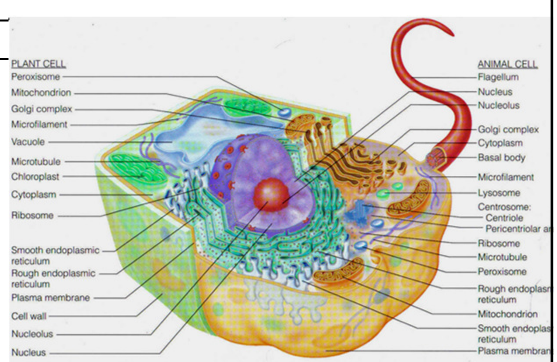
Eukaryotes

<u>Eukaryotes</u>

1. .

2. .

3. **If cell wall**
   A. **Algae:** ____________
   B. **Fungi:** ____________



(a) Highly schematic diagram of a composite eukaryotic cell, half plant and half animal

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Endosymbiotic Theory

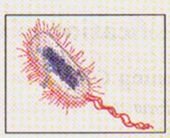
Endosymbiotic Theory

1. Eukaryotes evolved from ______________________________________ living inside ______________________________
2. Evidence
   - A. .
   - B. .
   - C. .
     - i. .
     - ii. .
     - iii. .

Table 10.2 Prokaryotic Cells vs. Eukaryotic Organelles

Table 10.2 *Prokaryotic Cells and Eukaryotic Organelles Compared*

	Prokaryotic Cell	Eukaryotic Cell	Eukaryotic Organelles (Mitochondria and Chloroplasts)
DNA	Circular	Linear	Circular
Histones	No	Yes	No
Ribosomes	70S	80S	70S
Growth	Binary fission	Mitosis	Binary fission



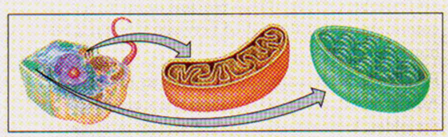


Fig 10.2 Endosymbiotic Theory

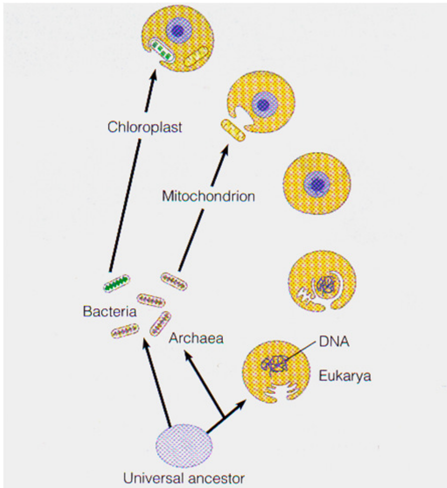
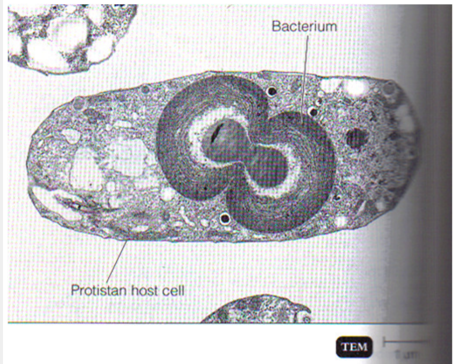


FIGURE 10.2 A model of the origin of eukaryotes. Invagination of the plasma membrane may have formed the nuclear envelope and endoplasmic reticulum. Similarities, including rRNA sequences, indicate that endosymbiotic prokaryotes gave rise to mitochondria and chloroplasts.



GURE 10.3 ***Cyanophora paradoxa.*** This organism, in which e eukaryotic host and the bacterium require each other for su val, provides a modern example of how eukaryotic cells migh ave evolved.

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