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. .

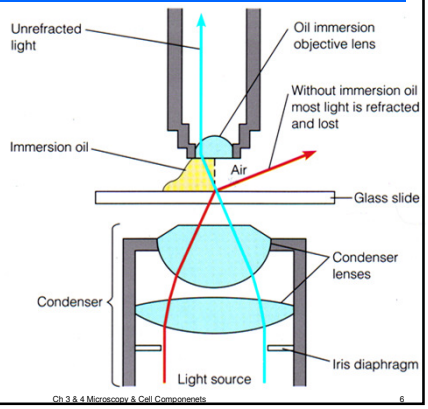
C. Improve conditions but NOT resolution:

i. .

ii. .

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

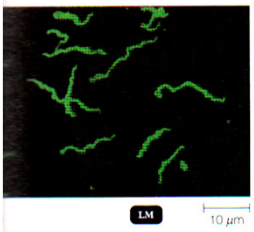
Using oil does improve resolution, as it increases the numerical aperture, which will cause a better (smaller) resolving power number

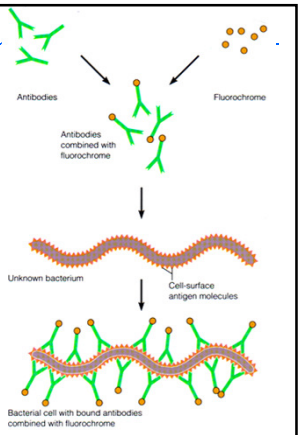


Types of Scopes-3 subtypes of Light microscopes

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

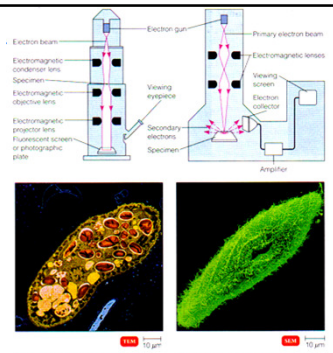


Fig 3.8 Transmission vs. Scanning

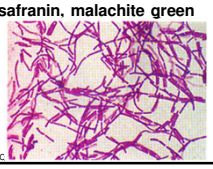
Stains-Slide Prep & Basic Stains

Slide Prep:

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

Staining

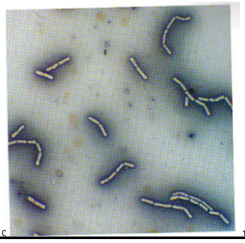
1. **Basic dye/_____stain:** Colored (___) ion of a salt
   - A. Attracted to (_____) bacterial cell; stains _____
   - B. Crystal violet, methylene blue, safranin, malachite green



2/4/2013 Ch 3 & 4 Microscopy & C 11

Acidic Dye / Negative Stain

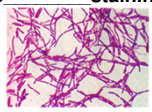
2. **Acidic dye /_____stain:** Colored (___) ion
   - A. _____& stains _____
   - B. For cell _____, to detect _____
   - C. Advantage: _____(no _____ & stain _____so accurate size & shape)
   - D. Examples: Acid fuchsin, nigrosin



2/4/2013 Ch 3 & 4 Microscopy & Cell C 12

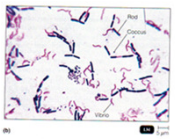
Mordant, Simple Stain, Differential Stain

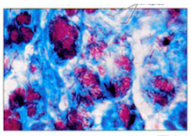
3. **Mordant**: Substance used to cause more _____staining
   NOTE: This is not the stain that gives color, only helps the stain be more intense color



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

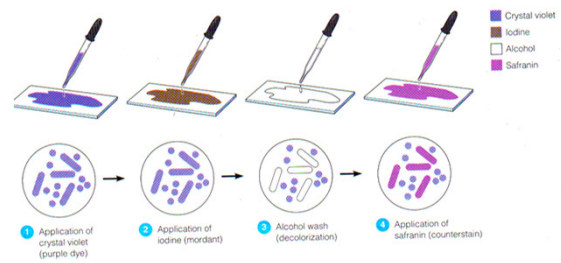
   A. GP = gram positive, _____, retain _____stain
      i. Us. _____to penicillin

   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

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

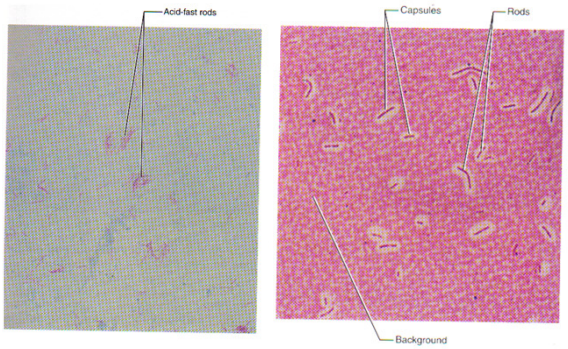
   B. Variation w/2 stains:
      i. .

      ii. .

      iii. _____of capsule left between the stains

   C. Problems: capsule may _____

Pictures-Acid Fast & Capsule Stains



Stains: Endospore

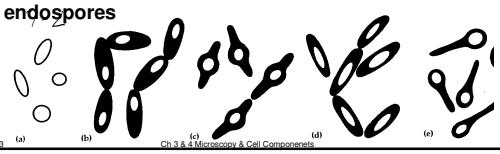
9. Endospore Stain
   A. Endospore_____

   _____

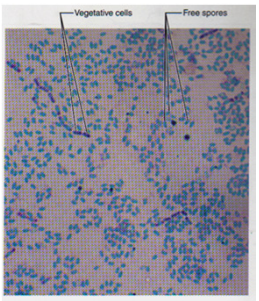
      i. Position used to ID species_____

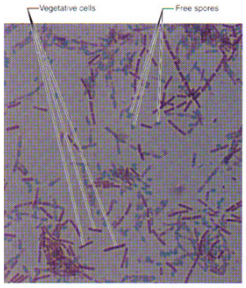
      _____

   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

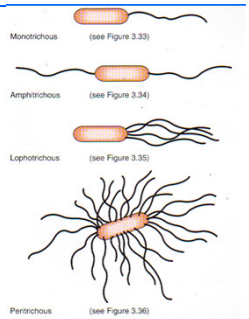


FIGURE 3.32 Flagella arrangements in bacteria. In *monotrichous* flagellation, a single flagellum is located at one end of the cell. In *amphitrichous* flagellation, a single flagellum is located at both ends of the cell. In *lophotrichous* flagellation, many flagella are grouped at one end of the cell.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

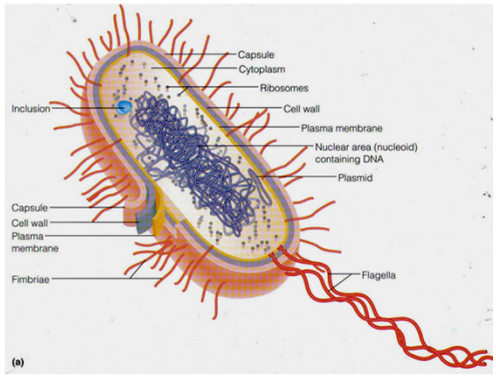
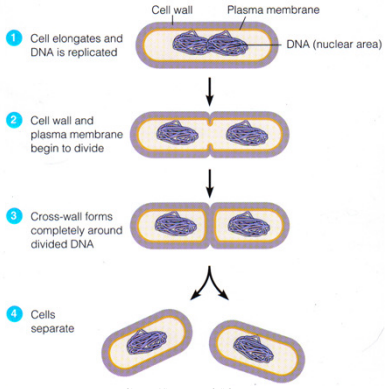


Fig 6.11a Binary Fission



Arrangement

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

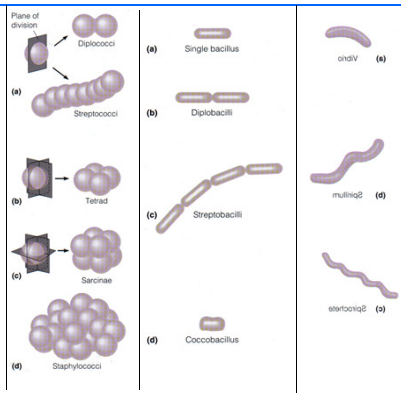
Arrangement Review:

Shapes/Morphology?

Arrangements?

Other morphology terms:

1. _____
2. _____



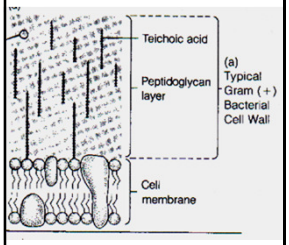
Cell Wall - Bacteria

Bacterial Cell Wall

1. .
2. **Clinical importance**
   - A. .
   - B. .
3. .
4. **Penicillin interferes** _____

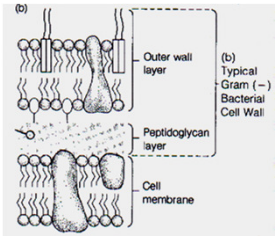
Diagram –Cell Wall Diagrams

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 _____ _____

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)

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. .

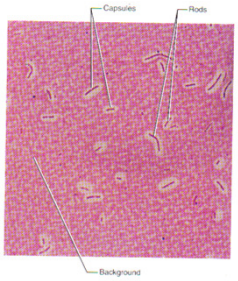
   B. .

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

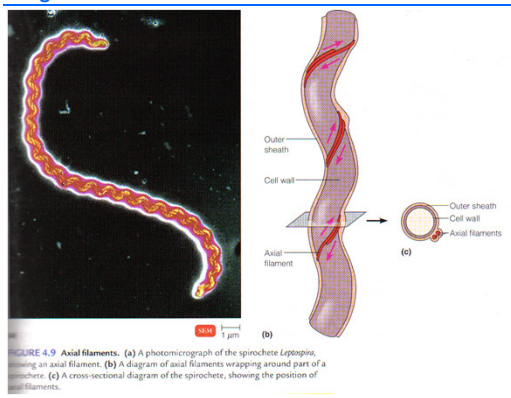
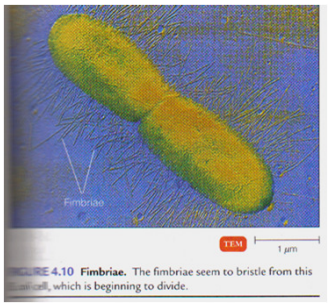


FIGURE 4.9 **Axial filaments.** **(a)** A photomicrograph of the spirochete *Leptospira*, showing an axial filament. **(b)** A diagram of axial filaments wrapping around part of a spirochete. **(c)** A cross-sectional diagram of the spirochete, showing the position of axial filaments.

Photo-Fimbriae



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

Fig 8.26 Bacterial Conjugation

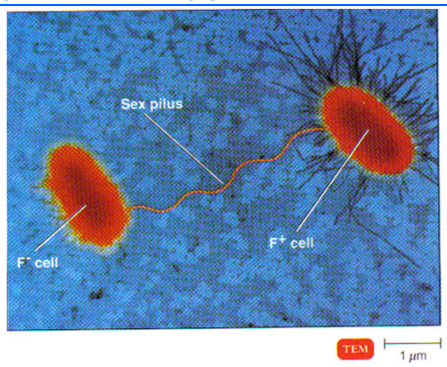
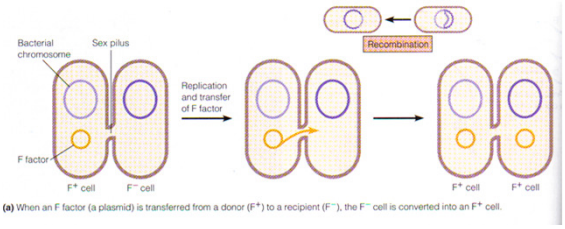


Fig 8.27 Conjugation in E. coli



(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

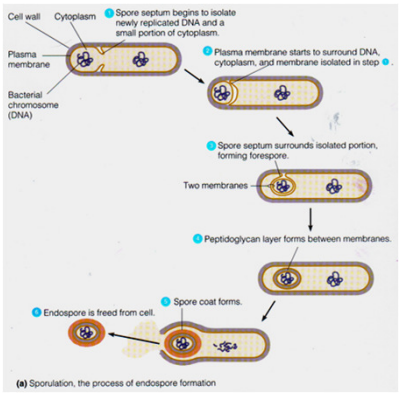
Discuss serovars

Endospores

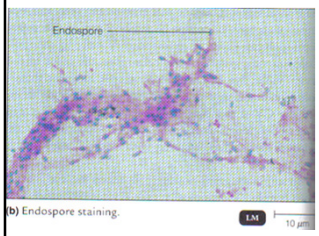
Structure Internal to Cell Wall

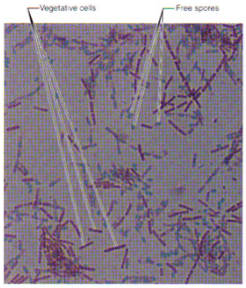
1. Endospores: _____structures to _____ adverse conditions
   - A. .
   - B. Sporulation / Sporogenesis
   - C. Germination – return to _____state
   - D. .
   - E. Location: _____
   - F. Survive _____
   - G. Stains:
     - i. Gram- _____
     - ii. Endospore Stain:
       - Primary: basic stain _____
       - Rinse: removes stain from _____
       - Counterstain: basic stain colors _____

Fig 4.20a Endospore Formation



Endospore Stain Pictures





Plasma/Cytoplasmic Membrane

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

Alcohols, disinfectants, some antibiotics effective here

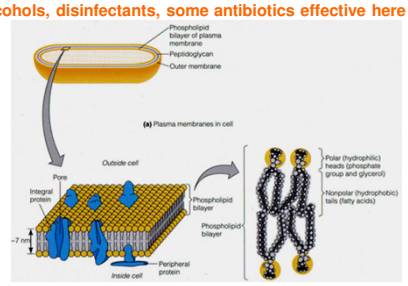


Fig 2.11 Phospholipids

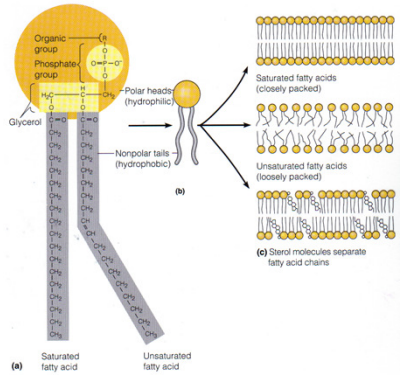


Fig 4.16 Diffusion

D. Diffusion: _____

i. Simple diffusion

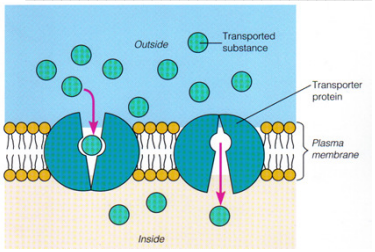
ii. Facilitated diffusion

iii. Osmosis

E. Active Transport: _____

Diagram on the right:

Which type of transport does it represent?



Osmosis-Animal vs. Plant

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

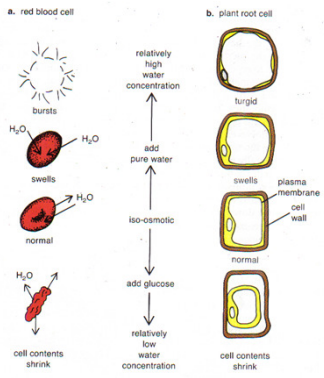
What do the following prefixes mean?

Iso?

Hypo?

Hyper?

Suffix is "tonic" = tension



Osmosis & Solution Types

F. Osmotic Environments

i. Isotonic/isoosmotic <u>solution</u>: _____

» .

» Water movement _____

» .

ii. Hypotonic <u>solution</u>: _____

» Net H_2O moves _____

» .

iii. Hypertonic <u>solution</u>: _____

» Net H_2O movement _____

» .

Diagram from Bio-Osmosis & Plant vs. Animal

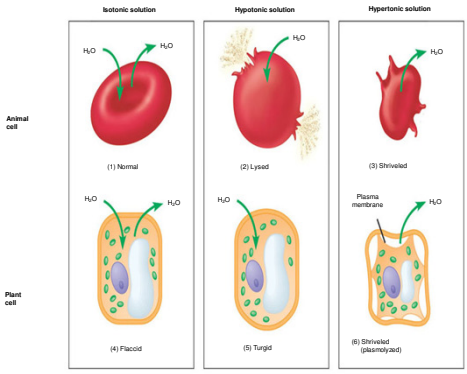
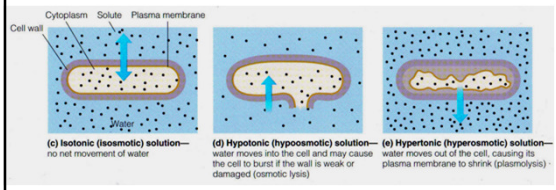


Fig 4.17 cde Osmosis in Varying Osmotic Environments



Internal Cell Structures continued

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

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

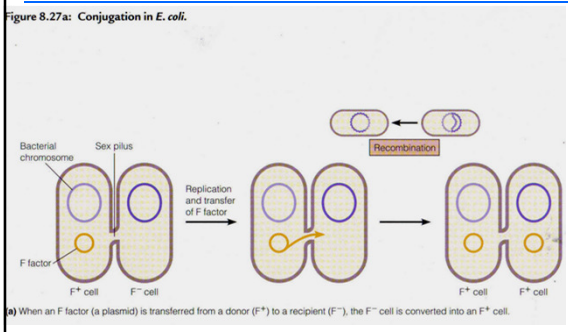
   A. **Contains** _____

5. **Plasmids:** _____
   A. .

   B. **Conjugation: transfer through** _____
      i. **GN-**_____
      ii. **GP-**_____

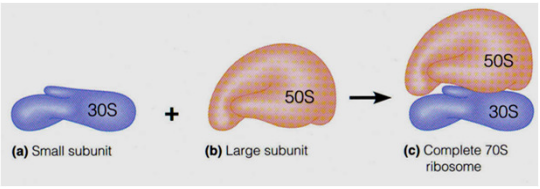
   C. **Biotech:** _____

Fig 8.27a Conjugation-Plasmid Transfer



Internal Structures cont'd-Ribosomes

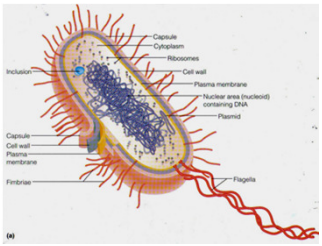
6. **Ribosomes:** _____
   - **A. 2 subunits: protein & RNA**
     - **i. Prokaryotic size:** _____
     - **ii. Euk:** _____
   - **B. _____ attach to subunit of _____ size**



Internal Structures cont'd-Inclusions

7. **Inclusions:** _____
   - **A. .**
   - **B. .**

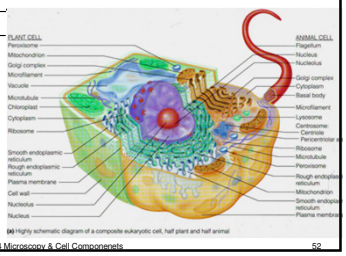
Review Structures:



Eukaryotes

Eukaryotes

1. .
2. .
3. If cell wall
   - A. Algae: _____
   - B. Fungi: _____



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

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

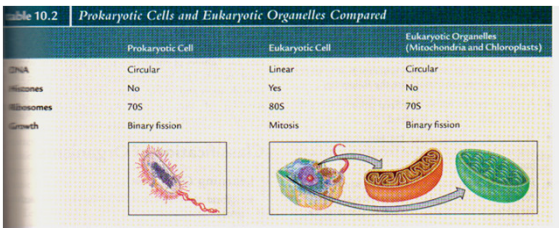


Table 10.2 Prokaryotic Cells and Eukaryotic Organelles Compared

	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

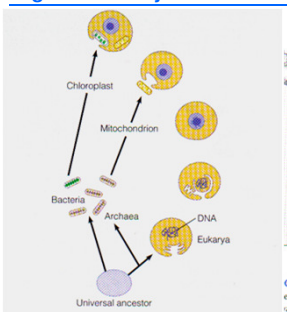
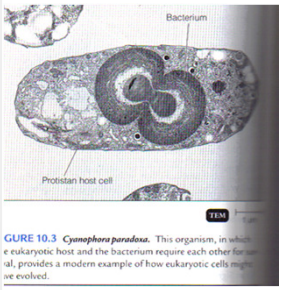


FIGURE 10.2 A model of the origin of eukaryotes. Invagination of the plasma membrane may have formed the nuclear envelope and endoplasmic reticulum. Similarities, including rRNA sequences, indicate that endosymbiotic prokaryotes gave rise to mitochondria and chloroplasts.



GURE 10.3 *Cyanophora paradoxa.* This organism, in which e eukaryotic host and the bacterium require each other for sur al, provides a modern example of how eukaryotic cells might ve evolved.