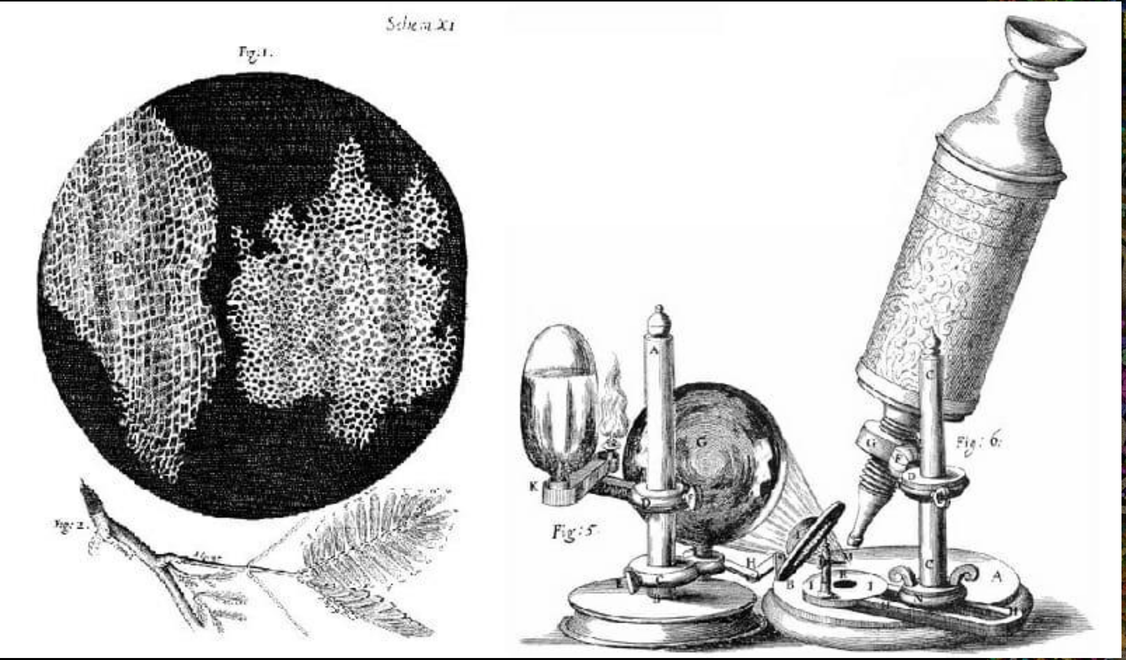

Ch 1&2: Introduction to Cells

Dr. Matthew Ellis

Learning Objectives for Today's Lecture (CH 1 & 2)

Upon completing this module, **you should be able to**:

Chapter 1:

1) Understand the cell theory.
2) Explain the major differences in microscopy techniques (light, fluorescent, confocal, electron).
3) Identify main eukaryotic organelle structures and functions.

Chapter 2:

1) Differentiate between ionic and covalent bonds.
2) Explain the types of bonds that occur between molecules in biological systems.
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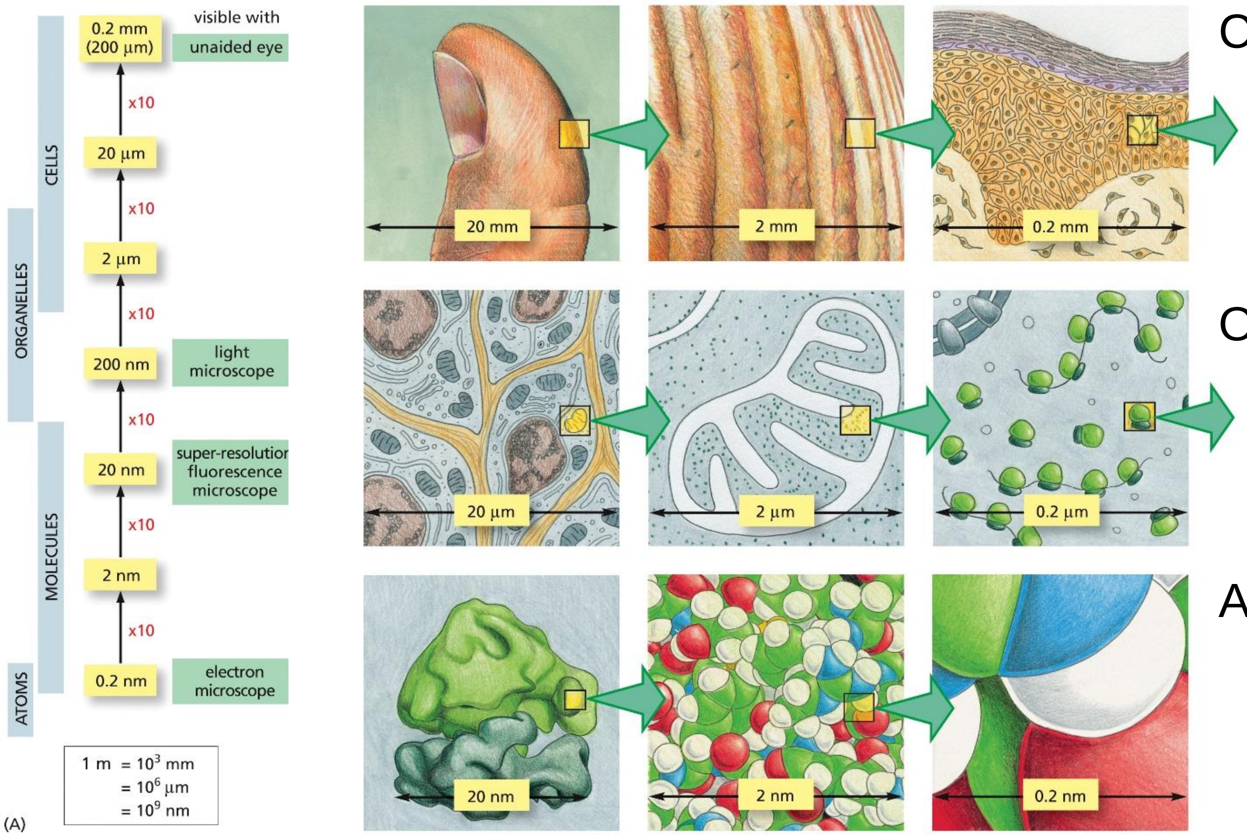
Advances in Microscopy Allowed Detailed Studies of Cells

- Early cell biology was *descriptive*, focusing on observation with little emphasis on explanation
  - Microscopes had limited ***resolution***, or ***resolving power*** (ability to see fine detail)
- By the 1830s, compound microscopes were used, improving both magnification and resolution
  - Structures only 1 micrometer (μm) in size could be seen clearly
    - 1 inch ≈ 2.5 cm; 1 cm = 10,000 μm

Scale of Resolution by Microscopy



Cell

Organelle

Atom

The Cell Theory Applies to All Organisms

- In 1839 Theodore Schwann (physician/physiologist) postulated the **cell theory:**

1. All organisms consist of one or more cells
2. The cell is the basic organizational "simplest unit" of life
3. All cells arise only from preexisting cells; cells reproduce.

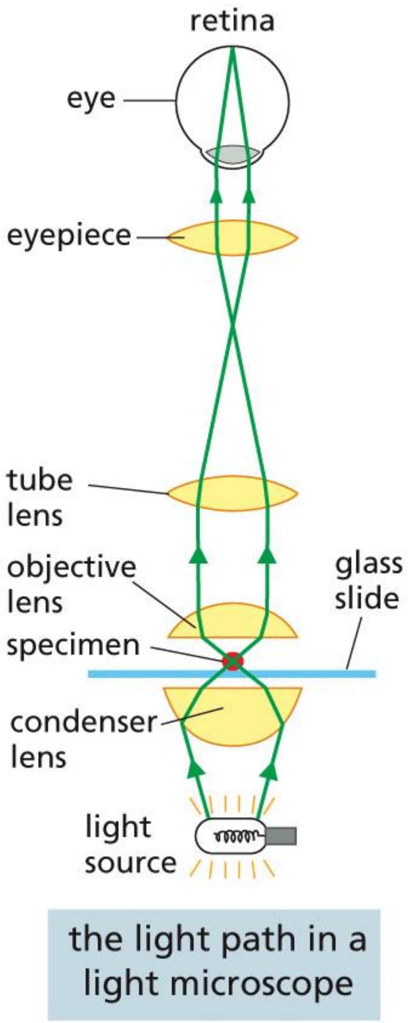
**Other characteristics of cells: (4) although different shapes and sizes all cells have a basic composition, (5) hereditary information carried in DNA is replicated in same way, (6) energy flow occurs within cells (7) composed of same chemicals and participate in same reactions*

Light Microscopes

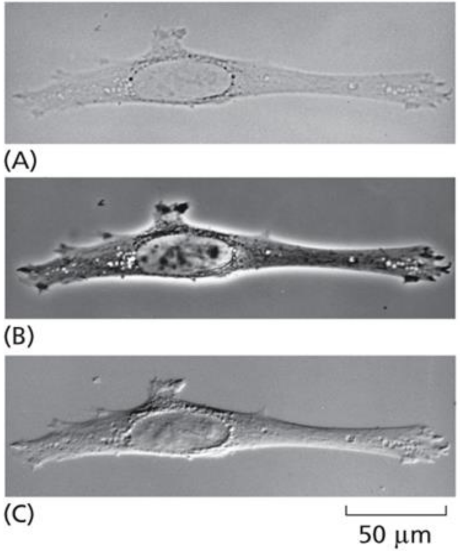
- Light microscopy allows us to magnify cells up to 1000 times and resolve details as small as 0.2 µm (200 nm), a limitation of the wavelike nature of light

- Operation of a light microscope:

  1. A bright light is focused on the specimen by lenses in the condenser
  2. Specimen must be prepared to allow light to pass through it
  3. Optical components (objective, tube, eyepiece) must be arranged to focus an image of the specimen in the eye



Imaging a Living Cell with Light Microscopy

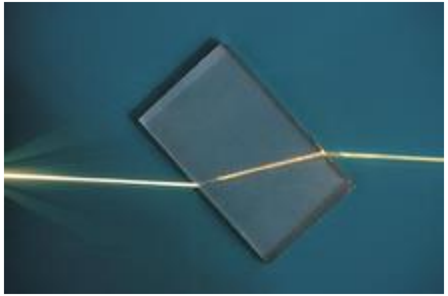


Bright-field optics

Phase contrast optics

Interference contrast optics

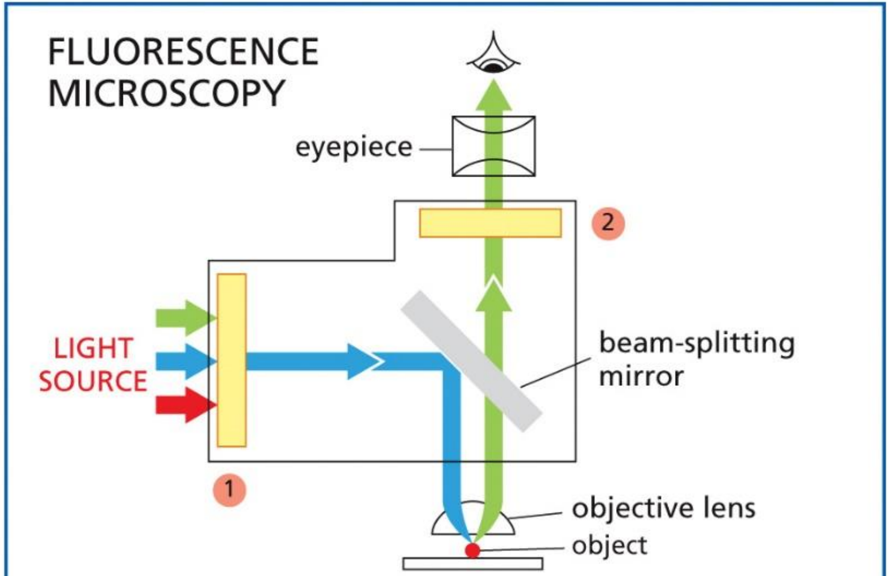
Refractive index



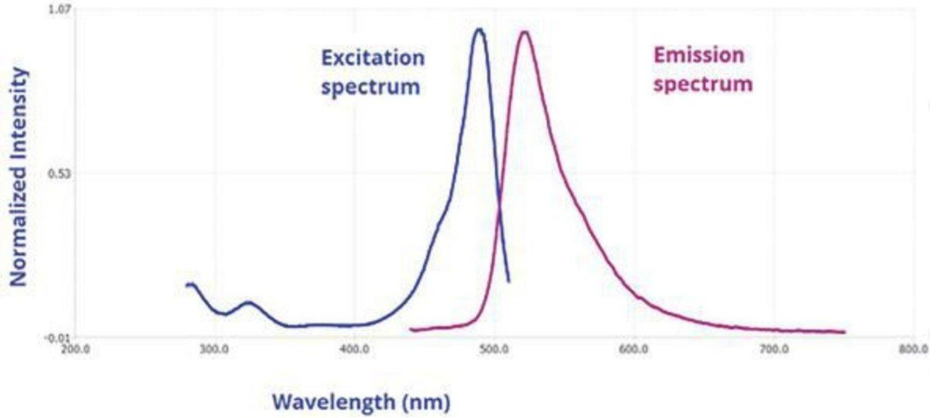
Light passes through glass slower than it does through air

B and **C** exploit differences in the ***refractive indices*** (measure of how light passes through a medium, such as liquid vs. air) of different components of the cell

All three images are obtained by the same microscope by changing the optical components



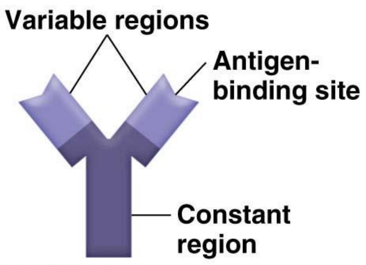
Fluorescent dyes used for staining cells are detected with the aid of a *fluorescence microscope*. This is similar to an ordinary light microscope, except that the illuminating light is passed through two sets of filters (*yellow*). The first (1) filters the light before it reaches the specimen, passing only those wavelengths that excite the particular fluorescent dye. The second (2) blocks out this light and passes only those wavelengths emitted when the dye fluoresces. Dyed objects show up in bright color on a dark background.



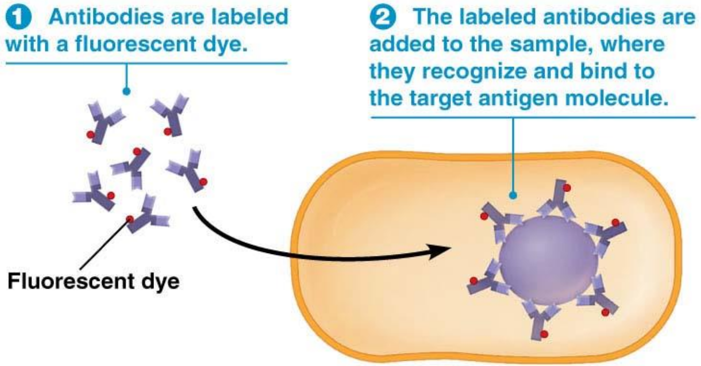
Shooting light into a molecule excites the atoms, pushing them to a higher energy state. Relaxation of these molecules to baseline through vibration releases heat, reducing the energy and increasing the wavelength.

Immunofluorescence (IF) uses antibodies to bind to specific target molecules to be visualized under a fluorescent microscope

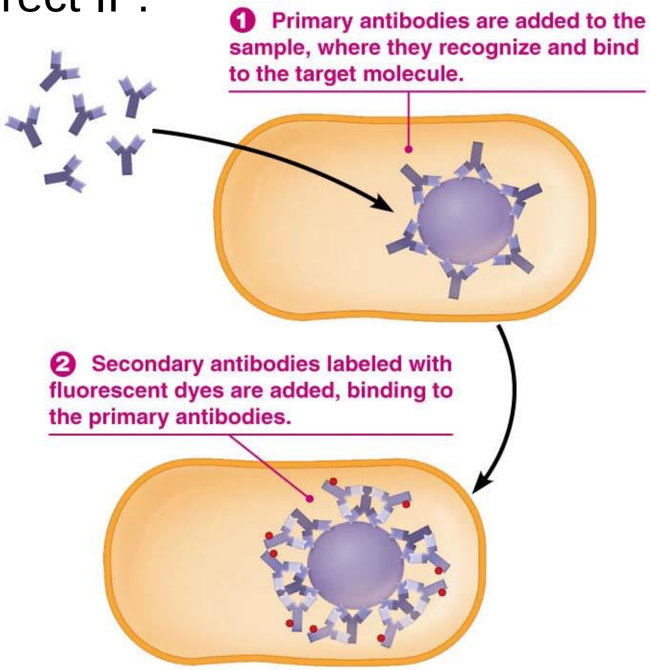
Antibody Structure:



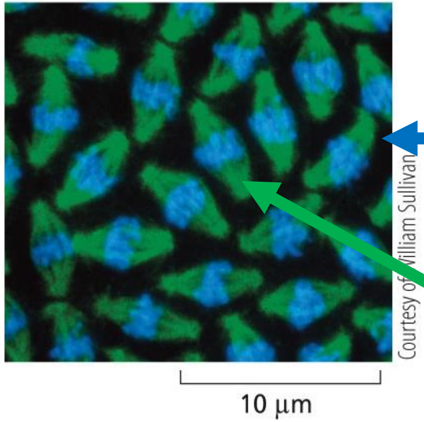
Direct IF:



Indirect IF:

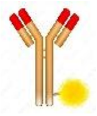


Imaging with a Fluorescent Microscope

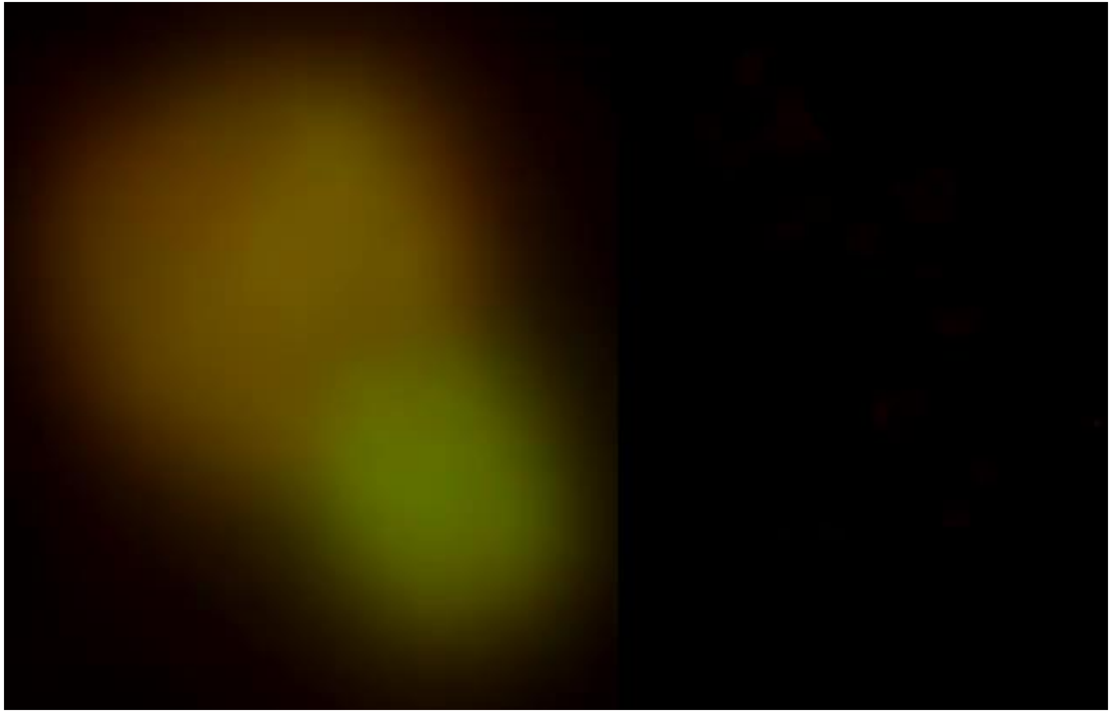


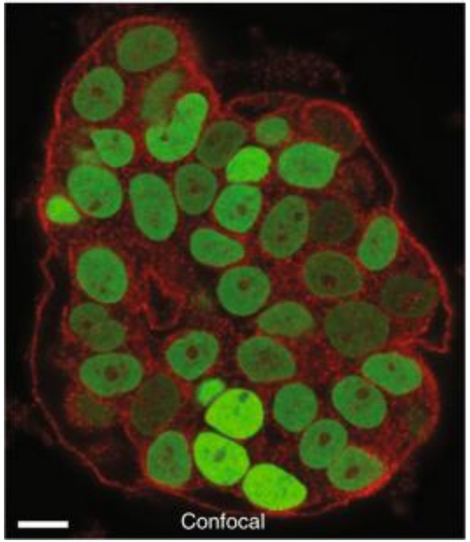
DNA (fluorescent dye)

Microtubules (fluorescent labeled antibody)



Confocal Fluorescence Microscopy uses a specialized microscope to image emitted fluorescence at a specific focal plane. Layering a series of these images together constructs a 3D image useful to understanding cell biology (as cells are three dimensional structures!)





https://microscopysolutions.ca/2012/05/31/any_way_you_slice_it/

https://www.nature.com/articles/s41596-020-0313-9

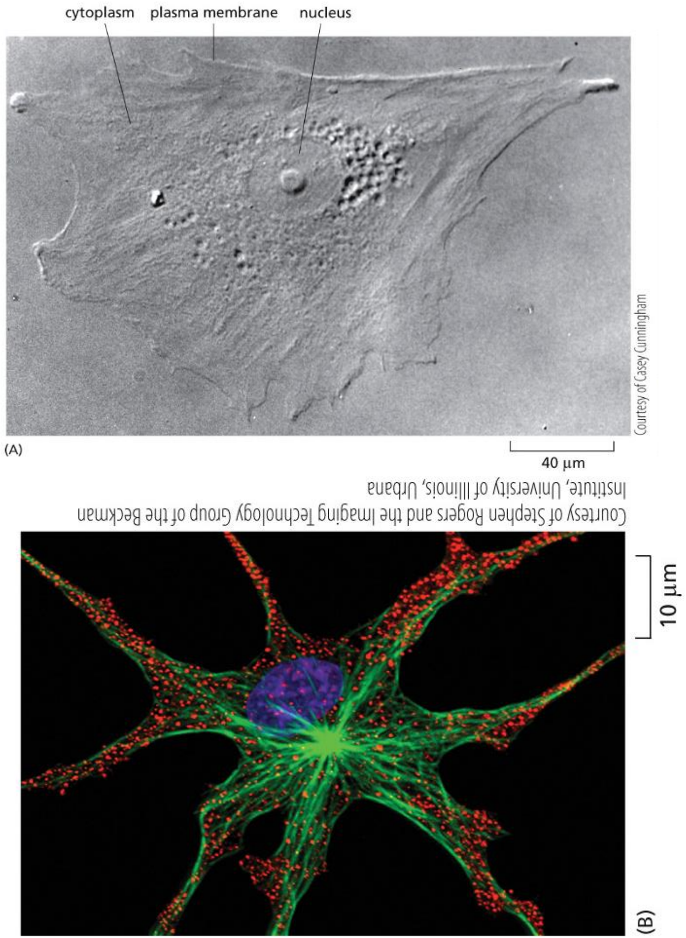
Electron Microscopy

- The **electron microscope** uses a beam of electrons rather than light, and was a breakthrough for cell biology
- Electrons have a **much shorter wavelength** than visible light allowing for **higher resolution images**
    - The limit of resolution (ability to distinguish items from one another) of electron microscopes is about 100 times better than light microscopes
    - The magnification (ability to make small objects appear larger) is much higher: up to 100,000x vs only 2000x for light microscopes

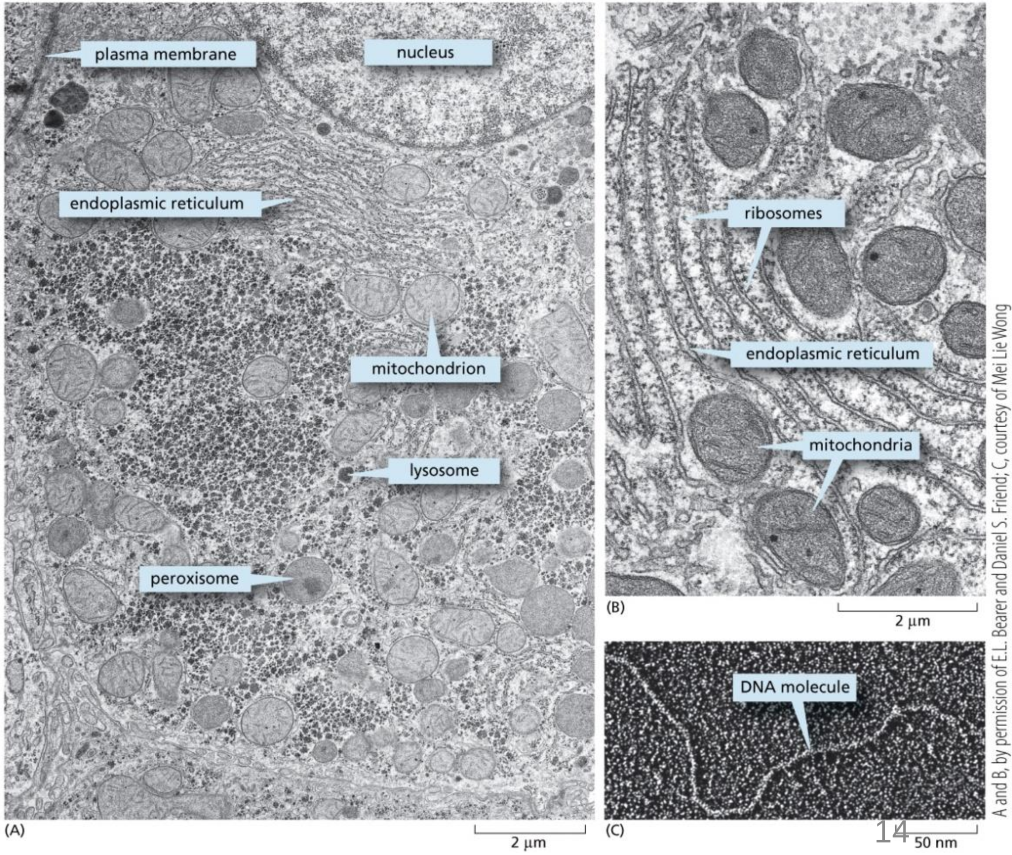
The Fine Structure of a Cell Is Revealed by Electron Microscopy

Light

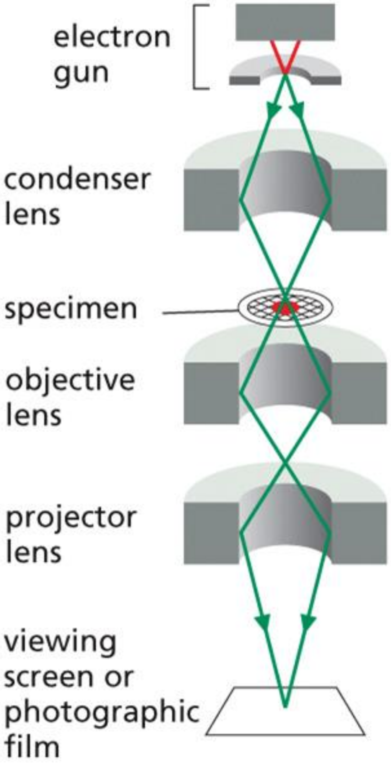
Confocal



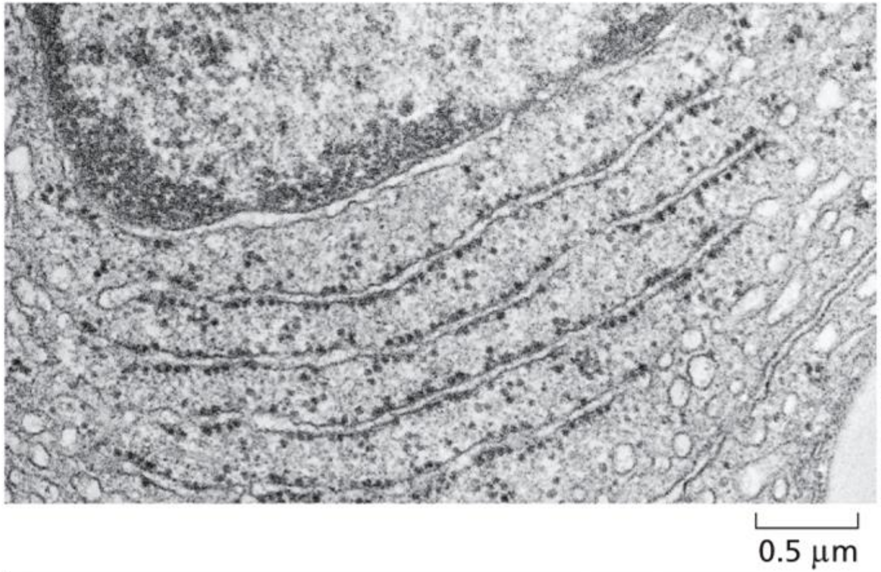
Electron



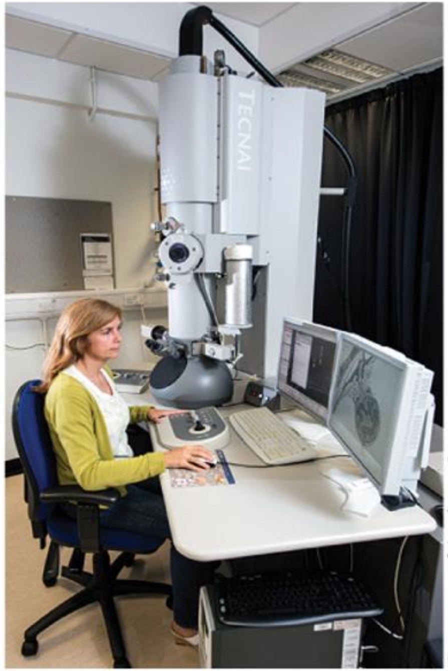
TRANSMISSION ELECTRON MICROSCOPY



The transmission electron microscope (TEM) is in principle similar to a light microscope, but it uses a beam of electrons, whose wavelength is very short, instead of a beam of light, and magnetic coils to focus the beam instead of glass lenses. Because of the very small wavelength of electrons, the specimen must be very thin. Contrast is usually introduced by staining the specimen with electron-dense heavy metals. The specimen is then placed in a vacuum in the microscope. The TEM has a useful magnification of up to a million-fold and can resolve details as small as about 1 nm in biological specimens.

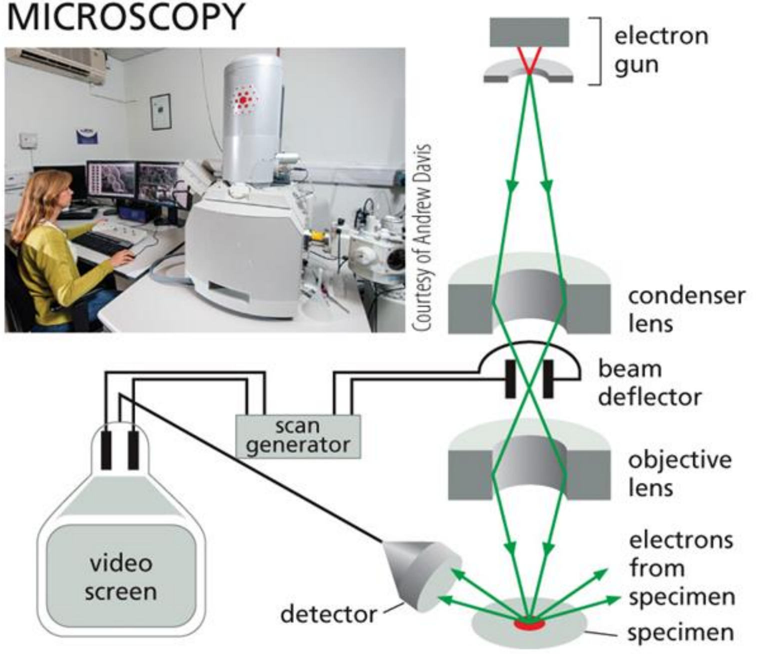


Courtesy of Daniel S. Friend

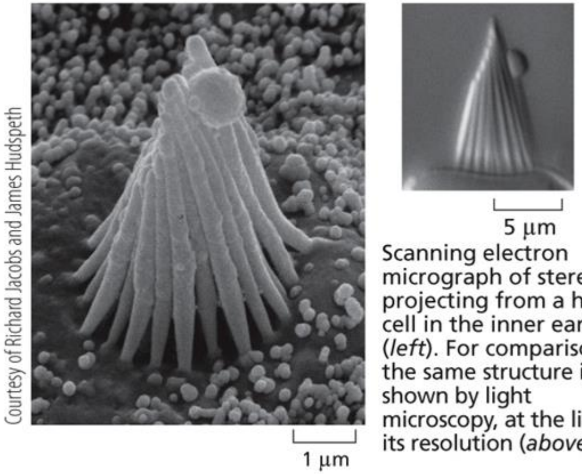


Courtesy of Andrew Davis

SCANNING ELECTRON MICROSCOPY



In the scanning electron microscope (SEM), the specimen, which has been coated with a very thin film of a heavy metal, is scanned by a beam of electrons brought to a focus on the specimen by magnetic coils that act as lenses. The quantity of electrons scattered or emitted as the beam bombards each successive point on the surface of the specimen is measured by the detector, and is used to control the intensity of successive points in an image built up on a video screen. The microscope creates striking images of three-dimensional objects with great depth of focus and can resolve details down to somewhere between 3 nm and 20 nm, depending on the instrument.



Scanning electron micrograph of stereocilia projecting from a hair cell in the inner ear (*left*). For comparison, the same structure is shown by light microscopy, at the limit of its resolution (*above*).

While TEM uses transmitted electrons (electrons that are passing through the sample) to create an image, SEM creates a surface-level image by detecting reflected or knocked-off electrons.

In general, if looking at a large area and only need surface details, SEM is ideal. If you need internal details of small samples at near-atomic resolution, TEM will be necessary.

SQUARECAP Q#1-3

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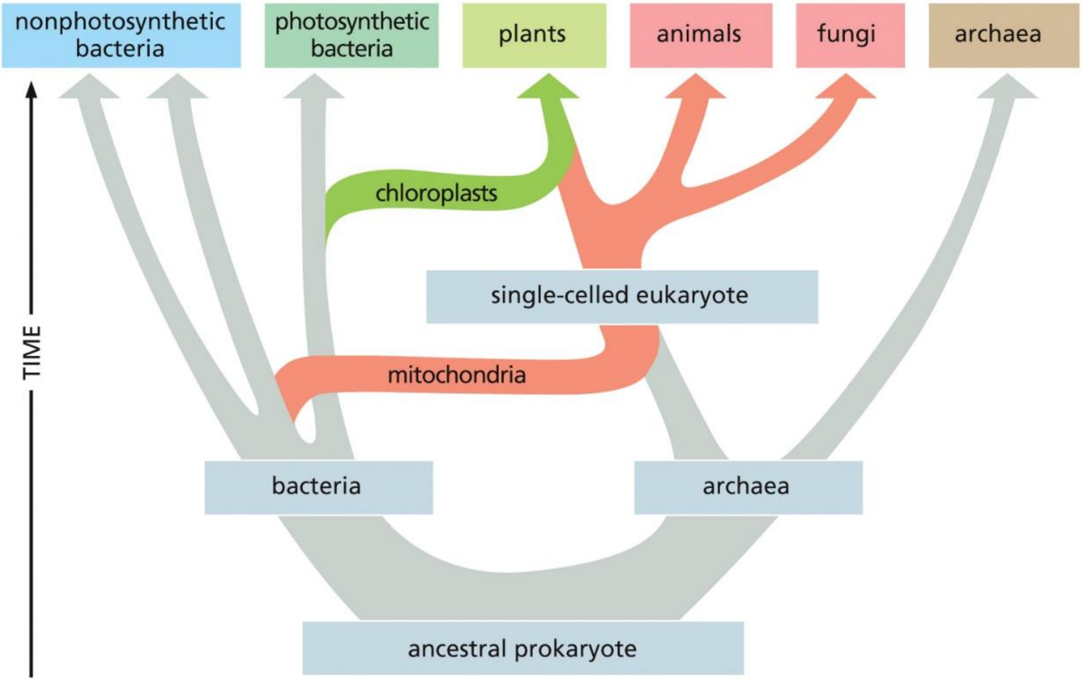
All Organisms Are: Bacteria, Archaea, or Eukaryotes

- Biologists recognize two types of cells:
  - **Prokaryotes:** small and simple, but most diverse and numerous (bacteria and archaea)
  - **Eukaryotes:** large and complex (plants, animals, fungi, and protists)
- The **main distinction** between the two cell types is the membrane-bounded nucleus and organelles within eukaryotes

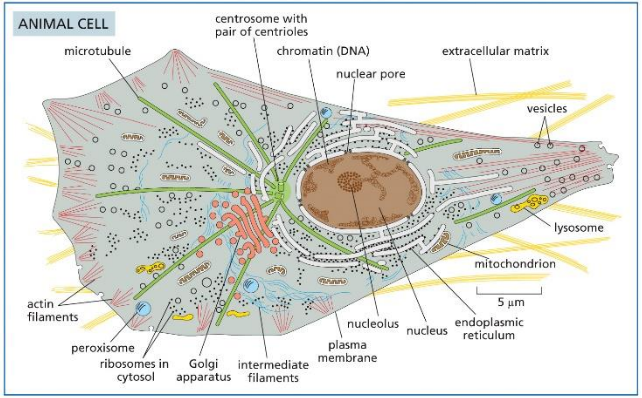


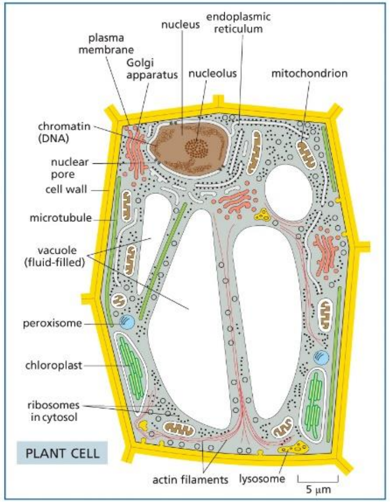
The Eukaryotic Cell in Overview: Structure and Function

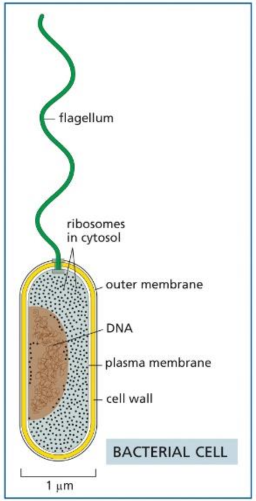
- A typical eukaryotic cell has a ***plasma membrane***, a ***nucleus***, ***membrane-bounded organelles***, and the ***cytosol*** interlaced by a ***cytoskeleton***

- In addition, plant and fungal cells have a rigid ***cell wall*** surrounded by an extracellular matrix

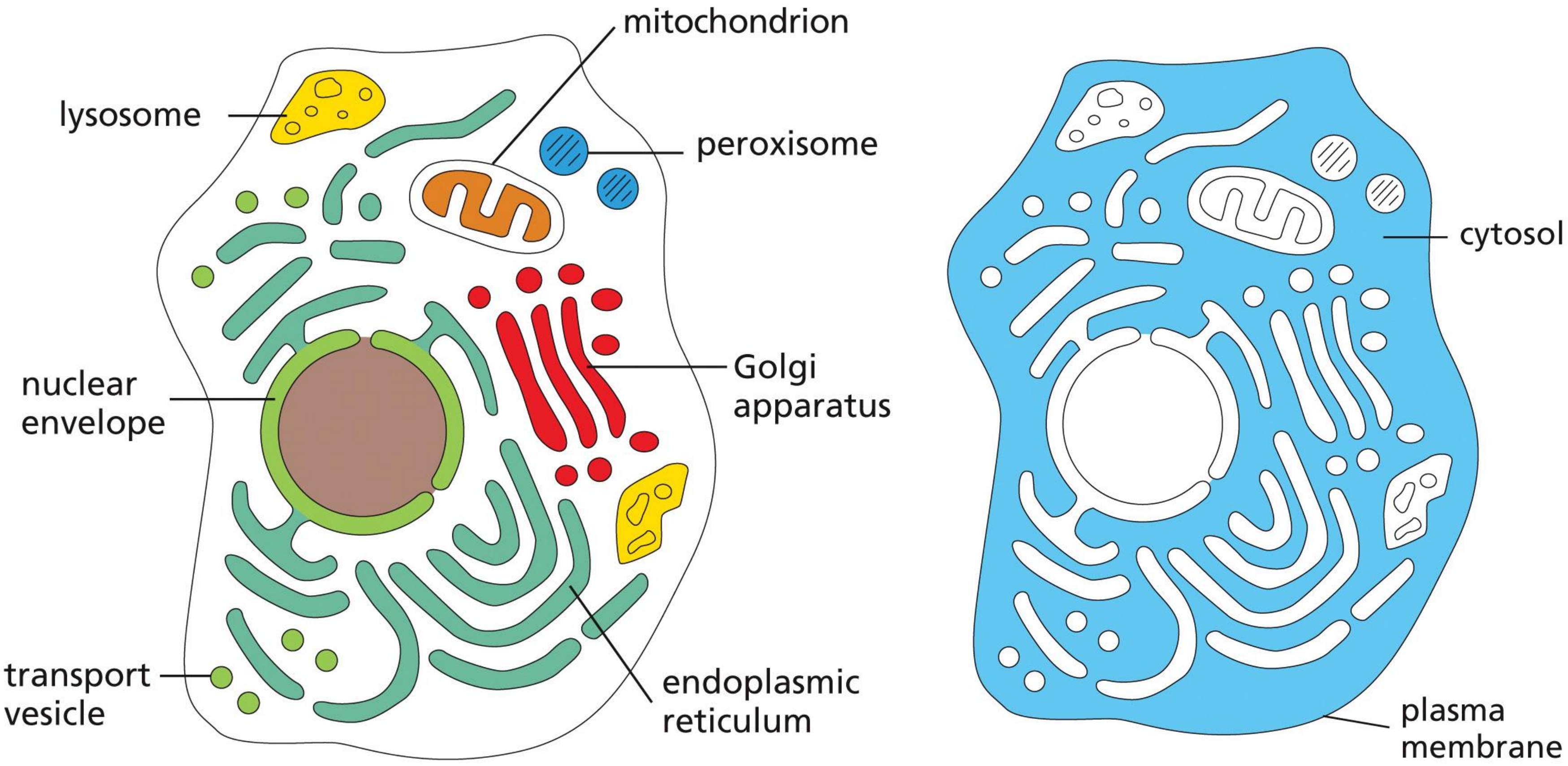
- Prokaryotes, in contrast, are relatively simple cells lacking these more orderly structures





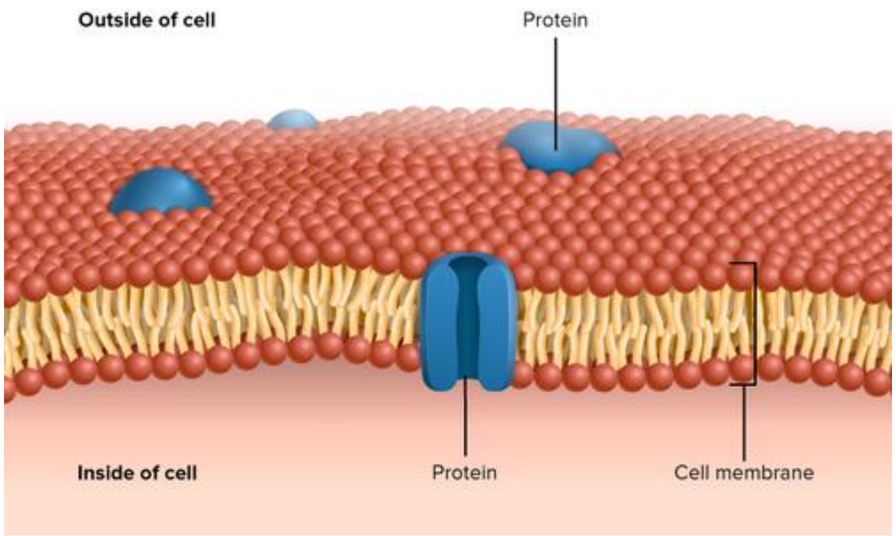


Eukaryotic Cell Components

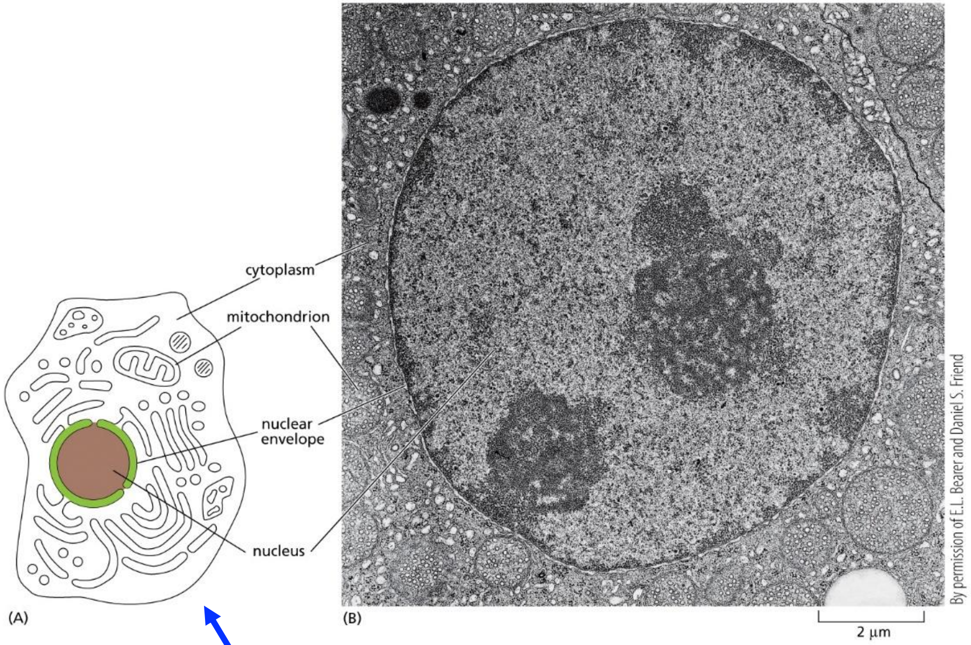


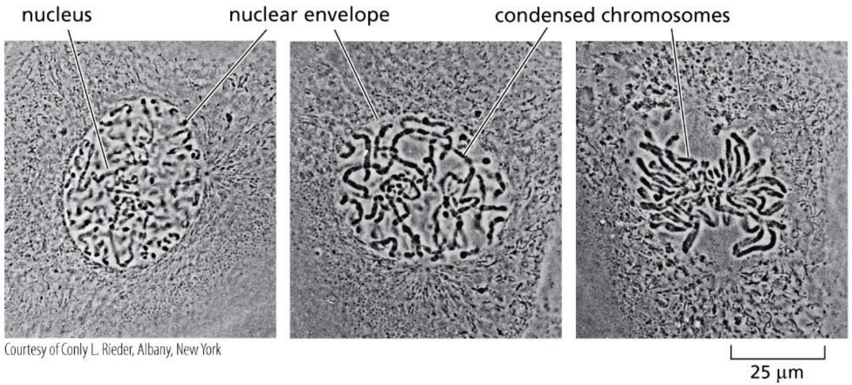
The Plasma Membrane Defines Cell Boundaries and Retains Contents

- The **plasma membrane** surrounds every cell and consists of a *phospholipid bilayer* and *membrane proteins*
- The membrane is formed through interactions between *amphipathic* molecules and water
  - Hydrophilic “heads” face outwardly toward the aqueous environment while hydrophobic “tails” point inwards, forming a semi-permeable membrane



The Nucleus stores all genetic material (DNA): the ‘brain’ of the cell

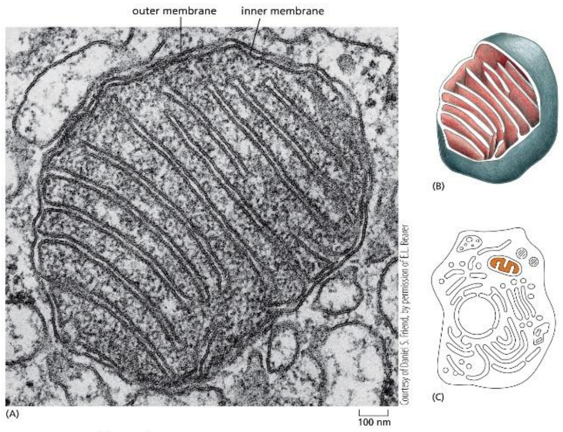


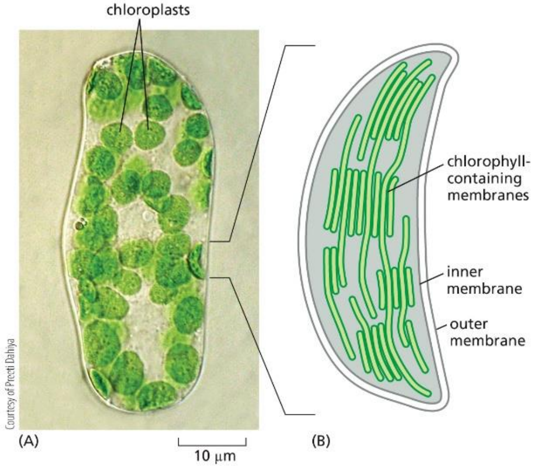


Use this blueprint of the cell to keep track of the various organelles!

Mitochondria and Chloroplasts Provide Energy for the Cell

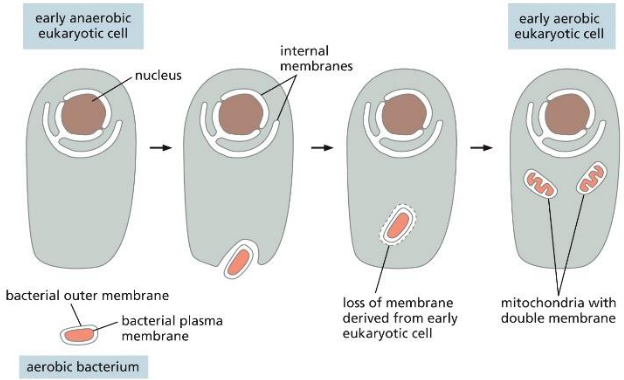
- The *mitochondrion* and the *chloroplast* are organelles involved in energy production for cells
  - The mitochondrion consists of inner folds (*cristae*) and assists with oxidation of sugars to produce energy in the form of the molecule adenosine triphosphate (**ATP**) in animal cells
  - The chloroplast harvests solar energy and converts it to chemical energy in the form of ATP in plant cells



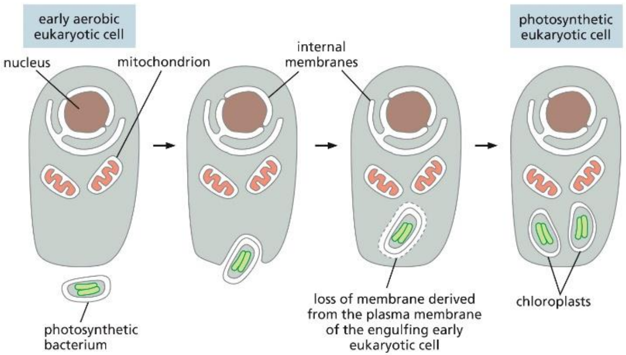


The Endosymbiont Theory Proposes that Mitochondria and Chloroplasts were derived from Bacteria

- **Semiautonomous organelles** with many similarities to bacteria:
  - Both resemble bacteria in size and shape and are surrounded by double membranes, the inner of which has bacterial-type lipids
  - Have circular DNA molecules with the ability to self-replicate



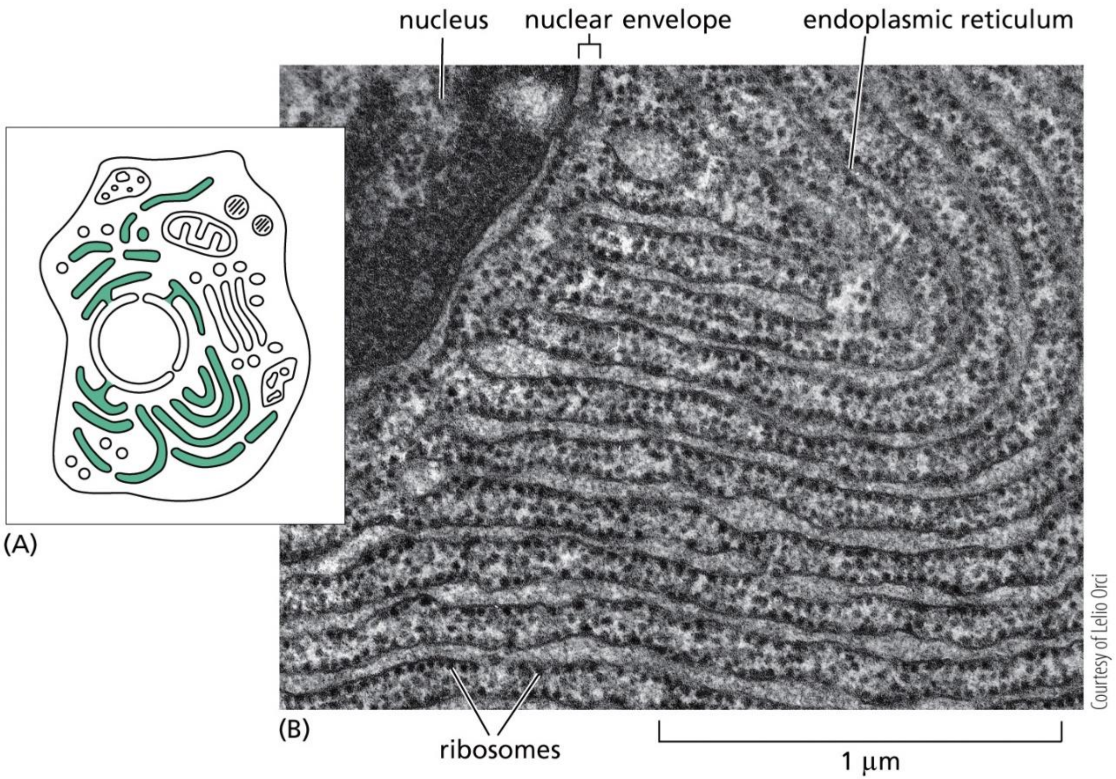
Mitochondria evolved from engulfed bacteria



Chloroplasts evolved from engulfed photosynthetic bacteria

The Endoplasmic Reticulum (ER) Smooth and Rough

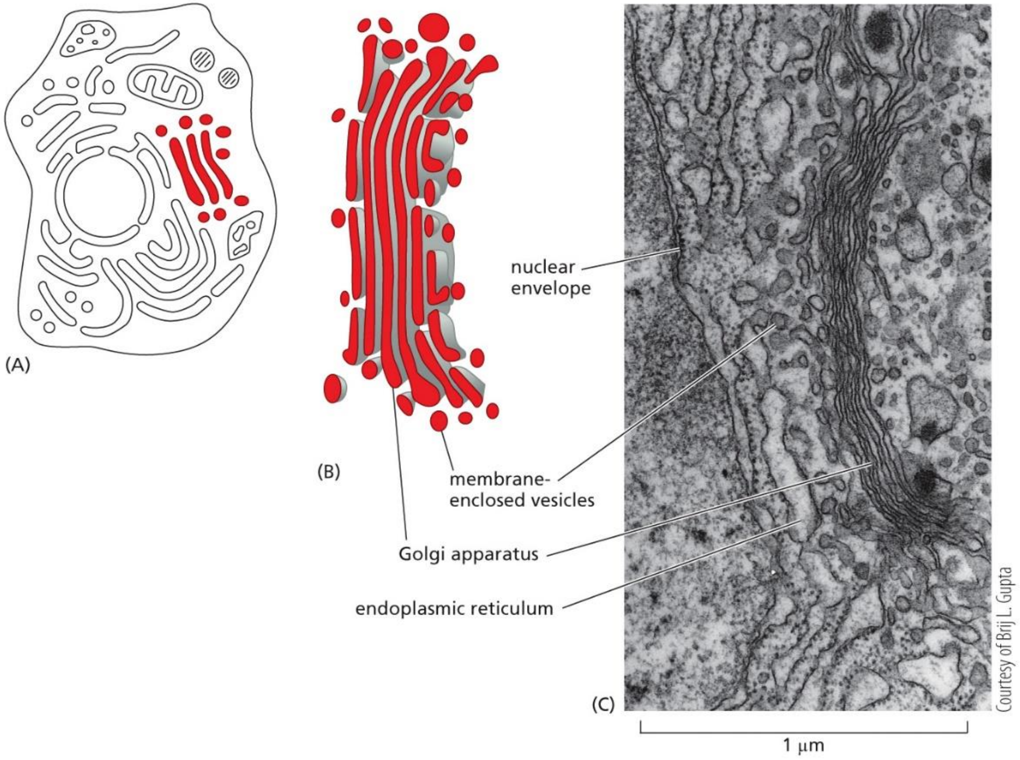
- Site of *lipid (smooth)* and *protein (rough)* synthesis and transport
- Major component of the *endomembrane system*: system of membranes within cell that work together to modify, fold, and transport lipids and proteins



The Golgi Apparatus is a “Processing Station”

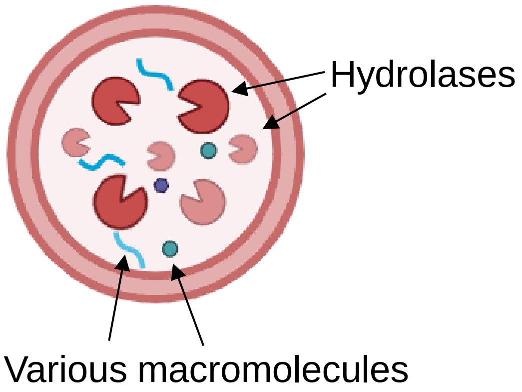
- The **Golgi apparatus,** another component of the endomembrane system closely related to the ER in proximity and function, consists of a stack of flattened vesicles (*cisternae)*

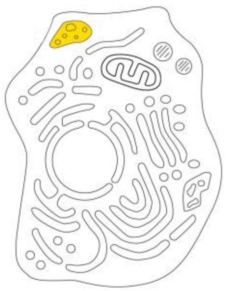
- Main function is to process and package proteins from ER that are destined for secretion out of cell or to other compartments within the cell



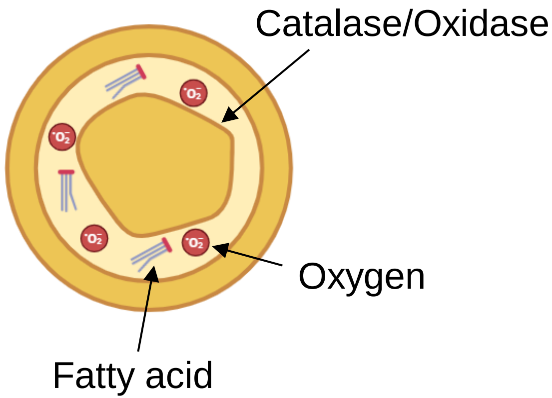
Lysosome and Peroxisome: The “Stomachs” of the Cell

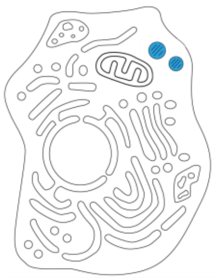
- **Lysosome:** Fuses with multiple vesicles to degrade **all macromolecules** inside (intracellular digestion)
  - Enzymes are **acid hydrolases** (proteases, DNAses/RNAses, glycosylases, lipases) that break down nucleic acids, proteins, and sugars
  - Hydrolases are only active at pH of 5 which is maintained inside lysosome





- **Peroxisome:** Essential in **fatty acid breakdown** (e.g. β-oxidation; essential for energy/heat production) using oxygen
  - Other functions include **detoxification of alcohol** and **hydrogen peroxide**
  - Enzymes are **catalases** and **oxidases**

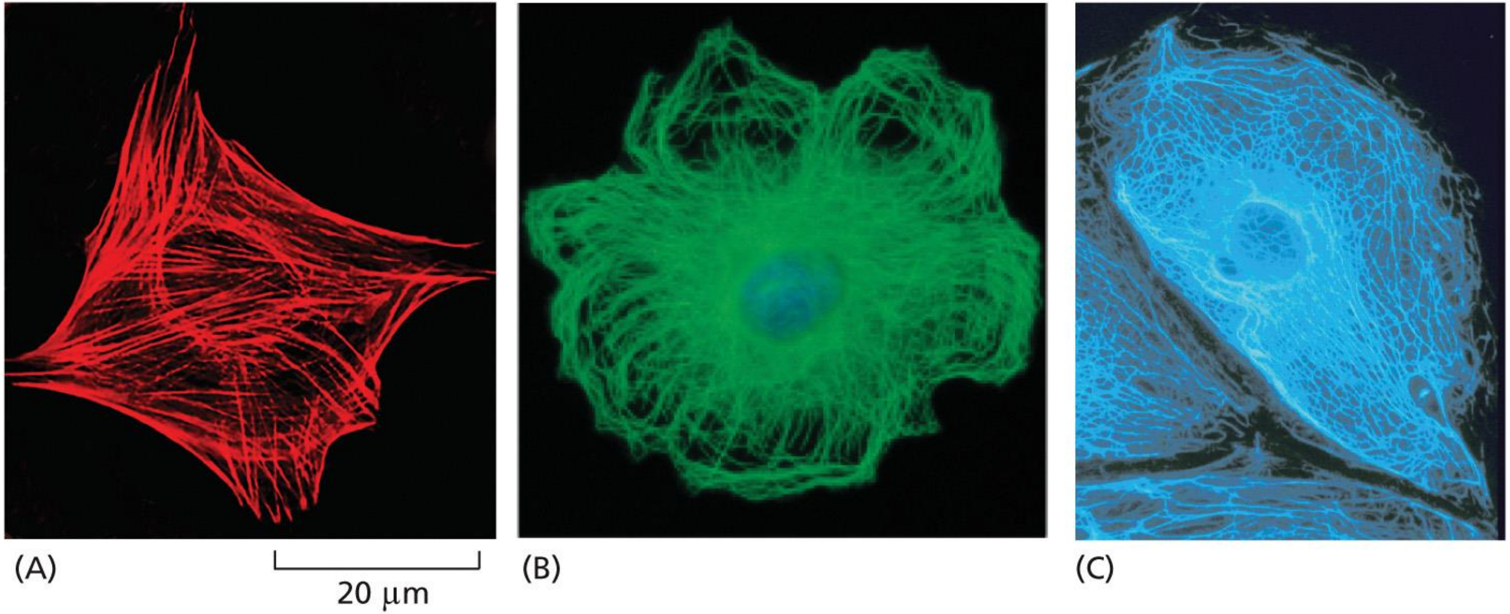




The Cytoskeletal System forms a network throughout the cell important for cellular structure, rigidity & movement (inside & out of cell) and come in three main classes:

Actin Filaments **Microtubules** **Intermediate Filaments**

A, Molecular Expressions at Florida State University; B, courtesy of Nancy Kedersha; C, courtesy of Clive Lloyd



Why should you care about organelles?

- Organelle dysfunction is linked to many diseases as well as the aging process
    - "Telomere shortening" in the nucleus leads to DNA mutations and programmed cell death as a natural effect of cell division
    - Oxidative damage to mitochondria decreases energy production in our cells leading them to stop dividing and functioning properly
- Understanding more about how organelles function and how to course correct when things go wrong is essential to prevent premature aging as well as many diseases

https://www.huffpost.com/entry/not-so-fast-3-ways-to-slo_b_12661378

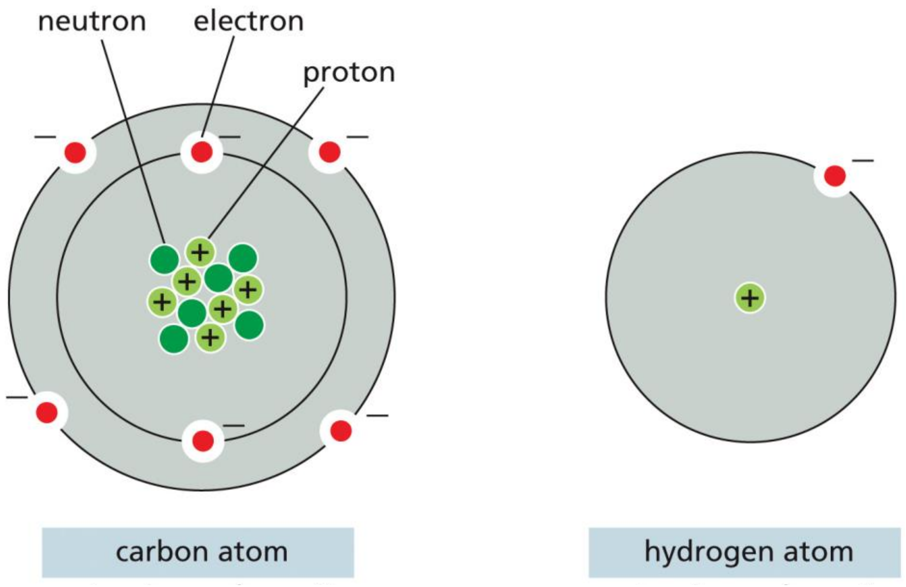
SQUARECAP Q#4-5

Learning Objectives for Today’s Lecture (CH 1 & 2)

Upon completing this module, **you should be able to**:

1) Understand the cell theory.
2) Explain the major differences in microscopy techniques (light, fluorescent, confocal, electron).
3) Identify main eukaryotic organelle structures and functions.
4) Differentiate between ionic and covalent bonds.
5) Explain the types of bonds that occur between molecules in biological systems.
6) List the main types of macromolecules and describe their subunits and functions.

Molecules are formed by groups of atoms held together by chemical bonds



- Atoms consist of a dense nucleus surrounded by an electron cloud, with the **outermost electrons** determining how atoms interact

Exchanging Electrons: Types of Chemical Bonds

- In order to fill outer shell and be stable, atoms with incomplete outermost shells tend to exchange electrons
- Electron exchange can be achieved by either *sharing* electrons between atoms through **covalent bonds** or by *transferring* electrons from one atom to another through **ionic bonds**

This table shows partially completed outermost electron shells for various elements in red, as dictated by the periodic table

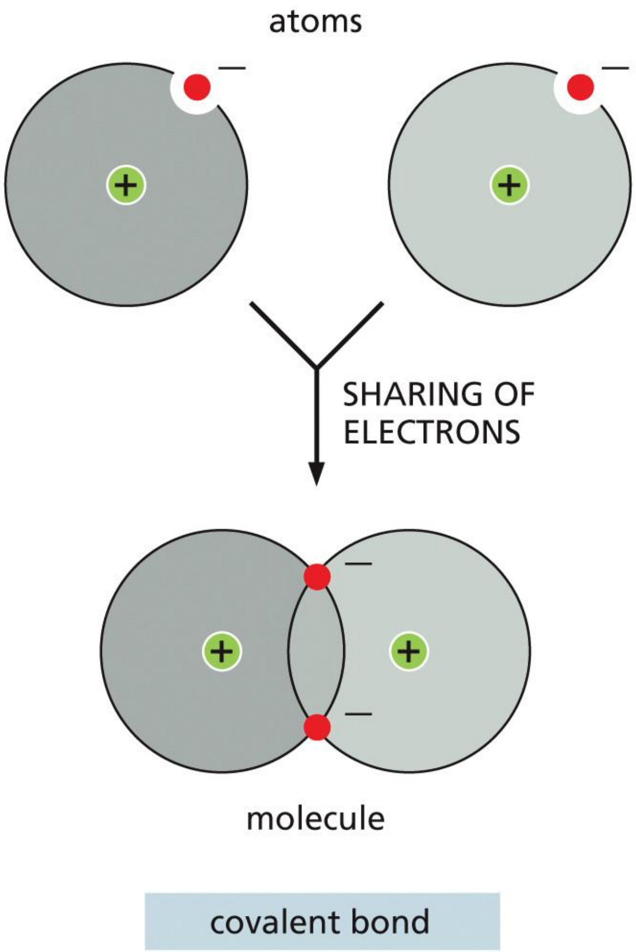
element		electron shell			
		I	II	III	IV
1	Hydrogen (H)	●			
2	Helium (He)	●●			
6	Carbon (C)	●●	●●●●		
7	Nitrogen (N)	●●	●●●●●		
8	Oxygen (O)	●●	●●●●●●		
10	Neon (Ne)	●●	●●●●●●●●		
11	Sodium (Na)	●●	●●●●●●●●	●	
12	Magnesium (Mg)	●●	●●●●●●●●	●●	
15	Phosphorus (P)	●●	●●●●●●●●	●●●●●	
16	Sulfur (S)	●●	●●●●●●●●	●●●●●●	
17	Chlorine (Cl)	●●	●●●●●●●●	●●●●●●●	
18	Argon (Ar)	●●	●●●●●●●●	●●●●●●●●	
19	Potassium (K)	●●	●●●●●●●●	●●●●●●●●	●
20	Calcium (Ca)	●●	●●●●●●●●	●●●●●●●●	●●

Common in cells

**Electron shells want to be filled and dictate chemical reactivity!*

Covalent Bonds

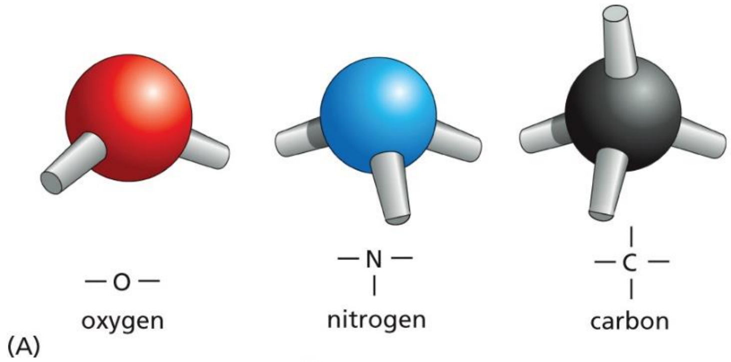
- Covalent bonds form by the <u>*sharing of electrons*</u>
  - <u>Co</u>- together; <u>valency</u>- # of electrons to offer
- Covalent bonds are strong enough to survive the conditions inside cells (10-100x stronger than ionic bonds)
- Electrons in covalent bonds are often shared unequally leading to **polarity** or partial positive and negative charge distribution across the bond

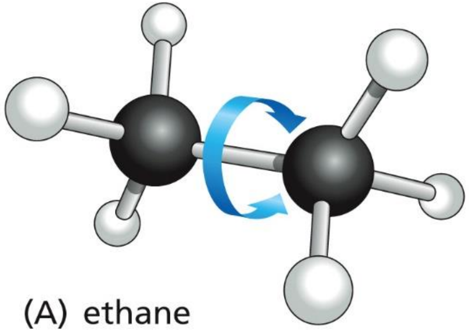


Covalent Bond Geometry

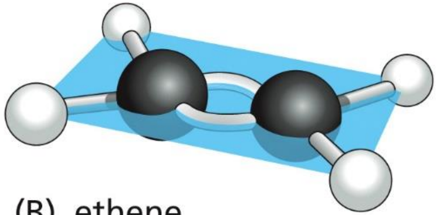
- The number of electrons needed to fill the outermost shell dictates the number of bonds that can be made with other atoms:
  - Oxygen: 2; Nitrogen: 3; Carbon: 4

- Molecules formed from these interactions have precise 3D structures defined by bond angles for each linkage with single covalent bonds allowing rotation between atoms and double bonds restricting rotation, bringing all atoms into the same plane





(A) ethane



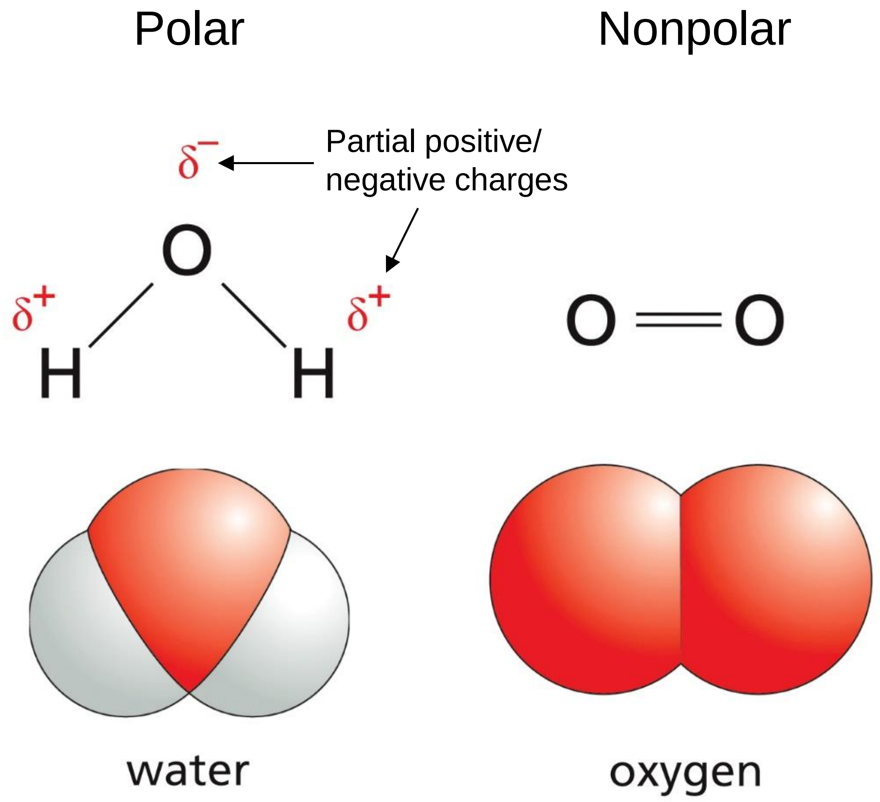
(B) ethene

Polar covalent bonds share electrons unequally

- Polar covalent bonds often form *between atoms of different elements*

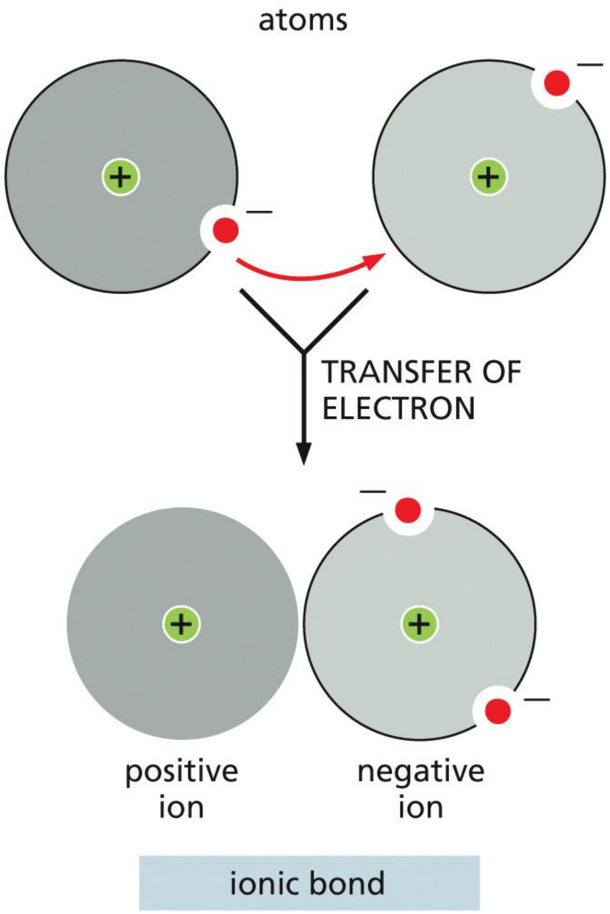
- **Electronegativity** (tendency to attract electrons) dictates how strongly an atom attracts electrons

  - For example, with water, O is more electronegative than H, and therefore pulls the electrons from H closer to itself, leading to a partial negative charge due to the additional electron proximity

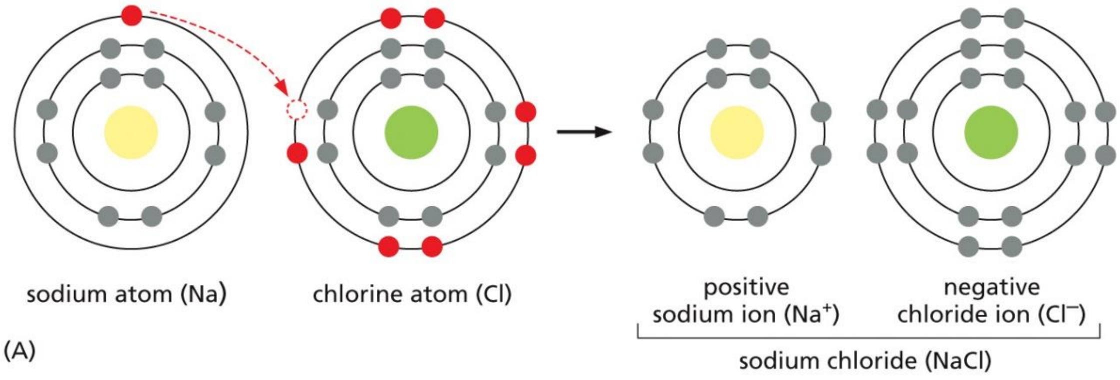


Ionic Bonds

- Ionic bonds form by the gain and loss of electrons due to *extreme differences in electronegativity*
  - These bonds occur when a completely filled outer shell can be attained most easily by donating or accepting electrons



Ionic Bond Example



Weak noncovalent bonds help bring molecules together in cells

Types of short-range attractive forces:

1. *Electrostatic interactions* (charged or polar based)

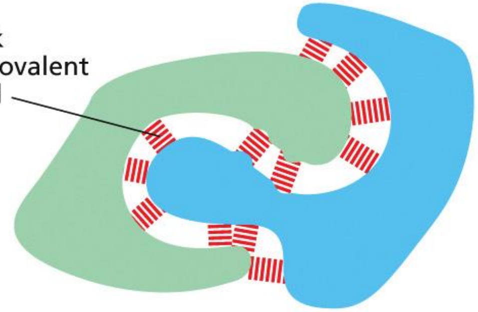
2. *Van der Waals forces* (transient proximity of atoms; non-specific)

3. ***Hydrogen bonds*** (weak, temperature sensitive interactions between δ^{+}on H and δ^{-} on an electronegative element of a separate molecule)

4. *Hydrophobic force* (repulsion of hydrophobic groups from water in aqueous solutions, such as in the formation of the cell plasma membrane)

WEAK NONCOVALENT CHEMICAL BONDS

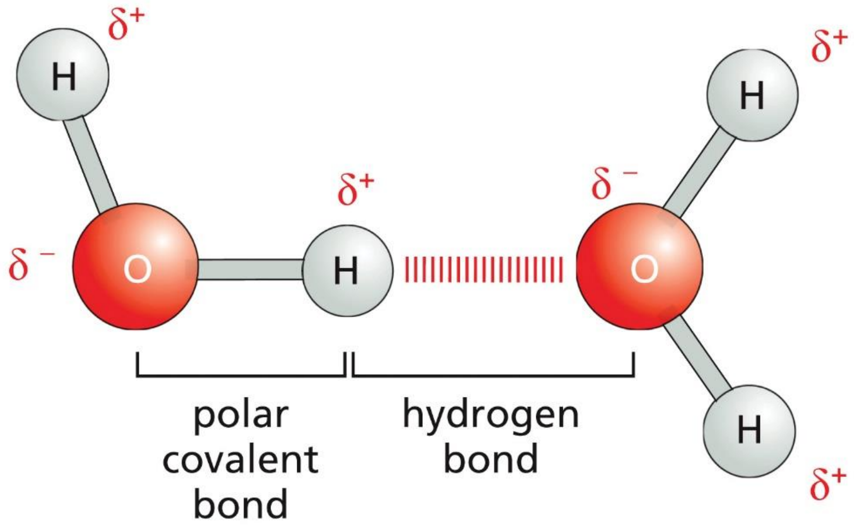
Organic molecules can interact with other molecules through three types of short-range attractive forces known as *noncovalent bonds*: van der Waals attractions, electrostatic attractions, and hydrogen bonds. The repulsion of hydrophobic groups from water is also important for these interactions and for the folding of biological macromolecules.



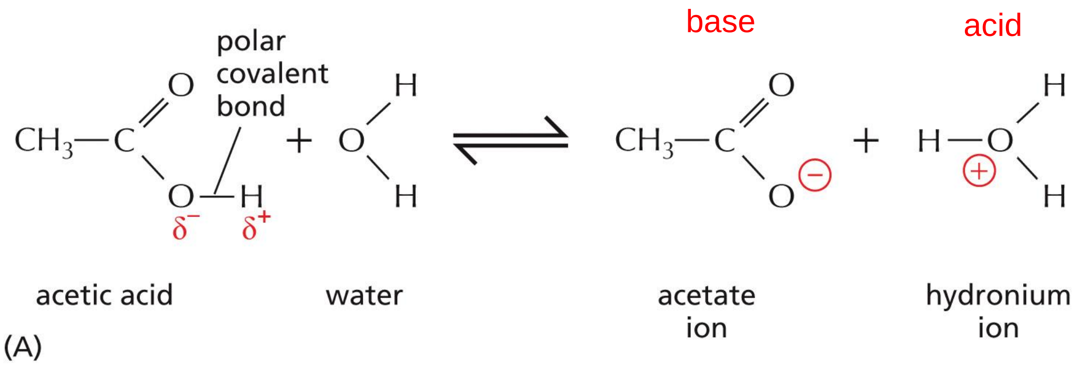
Weak noncovalent bonds have less than 1/20 the strength of a strong covalent bond. They are strong enough to provide tight binding only when many of them are formed simultaneously.

Hydrogen Bonds

- Most intracellular reactions occur in an aqueous, or water-filled, environment (70% of cell weight is water!)
- The two covalent H-O bonds in a water molecule are highly polar, creating partial positive and negative charges (δ)
- The positive charge on the H of a water molecule attracts to the negative charge of the O on a second water molecule to create a (weak) hydrogen bond



Acids and bases are formed by certain polar molecules in water



- Protons move continuously from one molecule to another in aqueous solutions
- **Acids:** substances that donate **H^+ or protons**
- **Bases:** substances that accept **H^+ or protons**

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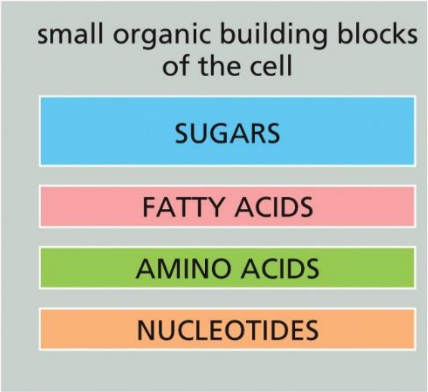
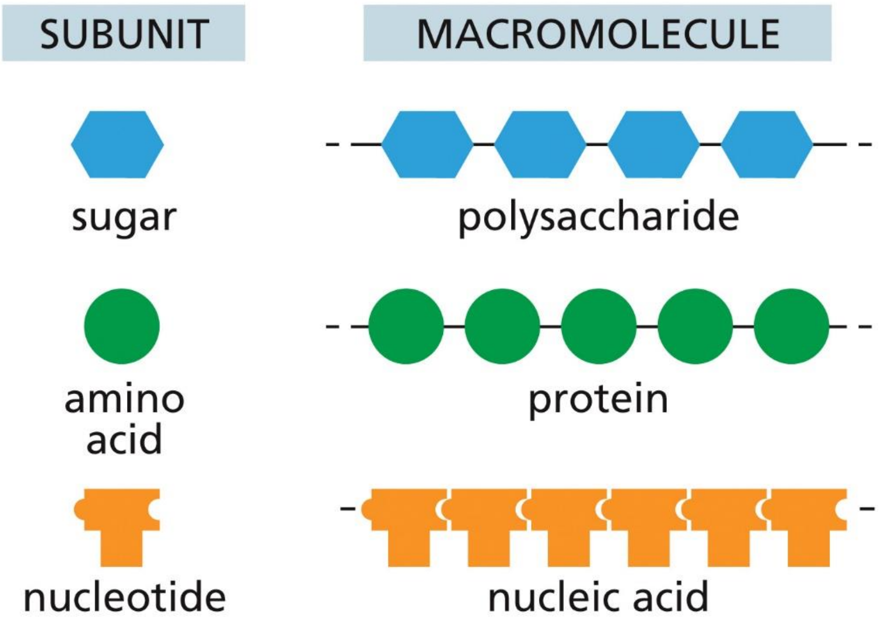
4) Differentiate between ionic and covalent bonds.
5) Explain the types of bonds that occur between molecules in biological systems.
6) List the main types of macromolecules and describe their subunits and functions.

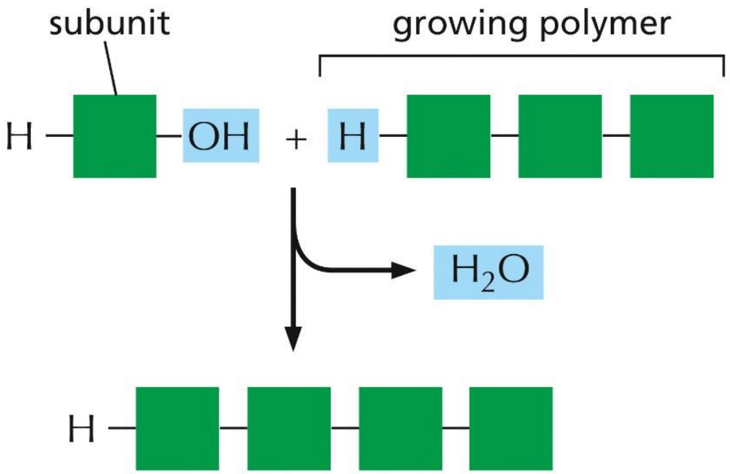
Macromolecules are built from four major families of small organic molecules



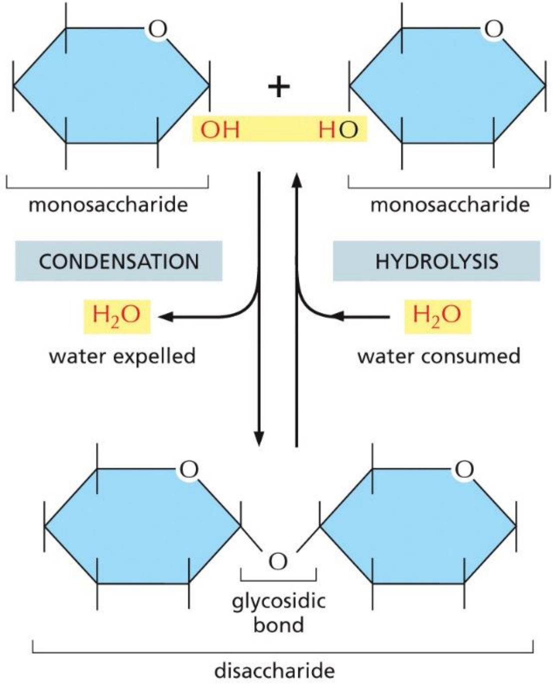
- Macromolecules also serve as energy sources in metabolic pathways and are found freely in the cytosol

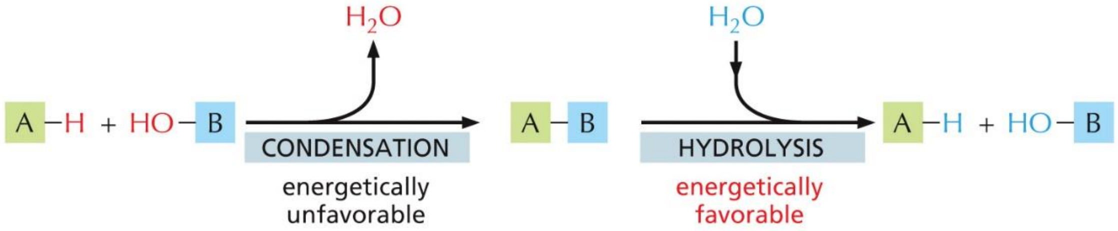
Macromolecules are polymers of monomers linked together by covalent bonds





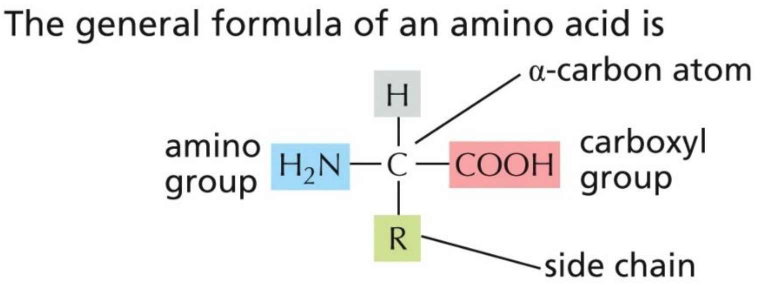
Polysaccharides: Two or more sugars (monosaccharides) can be linked by covalent *glycosidic bonds* to form disaccharide or polysaccharides

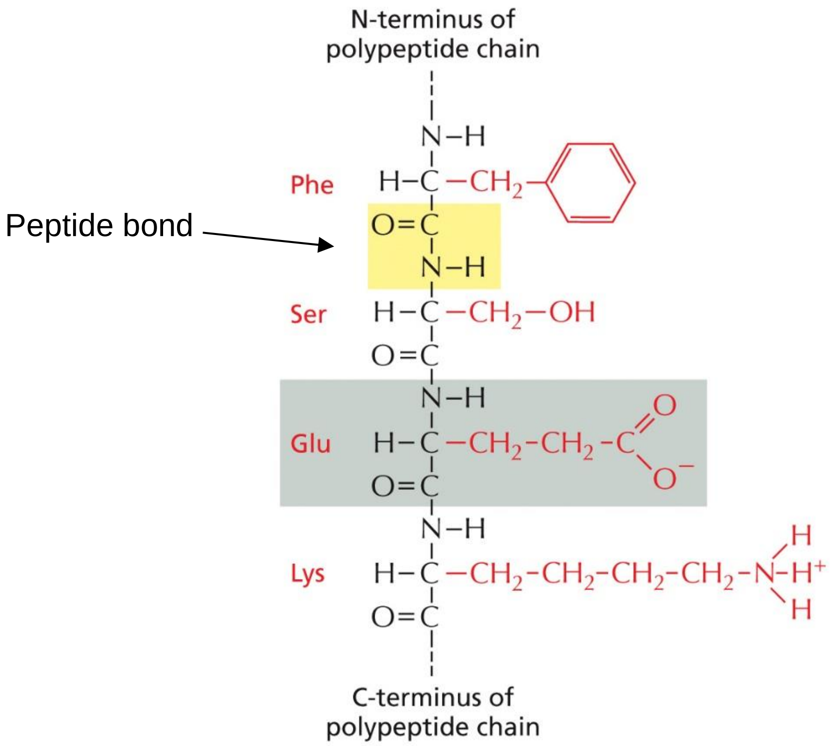




We will discuss reaction energetics in the next lecture!

Proteins: Amino acids subunits of proteins are linked together by *peptide bonds*

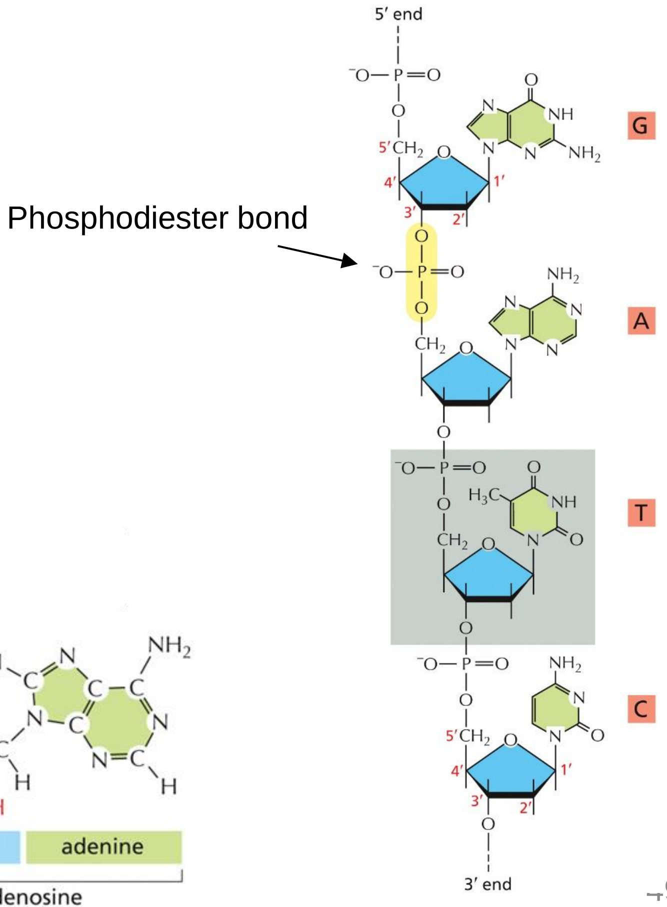


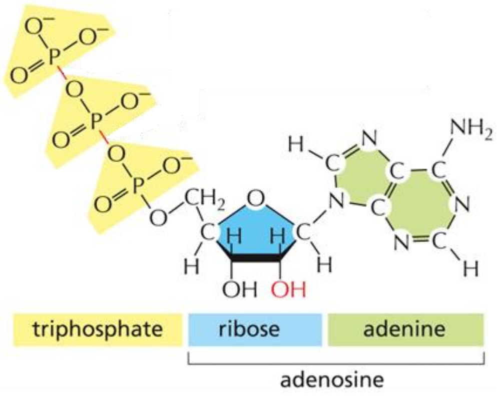


- Twenty unique amino acid side chains
- A polypeptide chain always has an amino group at one end (N-terminus) and a carboxyl group at the other (C-terminus)

Nucleic Acids: Nucleotide subunits combine through covalent *phosphodiester bonds* to form RNA and DNA

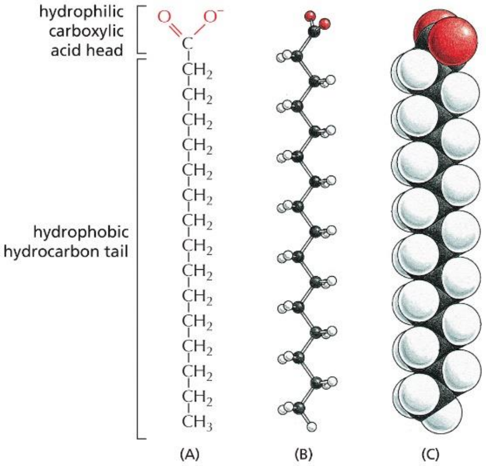
- DNA and RNA are built from **nucleotides**
  - Consist of nitrogen containing ring compound (*nitrogenous base*) linked to a five-carbon sugar (*ribose*) with one or more phosphate groups attached to it
  - Sugar can be ribose (**R**ibo**N**ucleic **A**cid) or deoxyribose (**D**eoxyribo**N**ucleic **A**cid)
  - Each nucleotide is named after the base it contains (A,T/U,C,G)



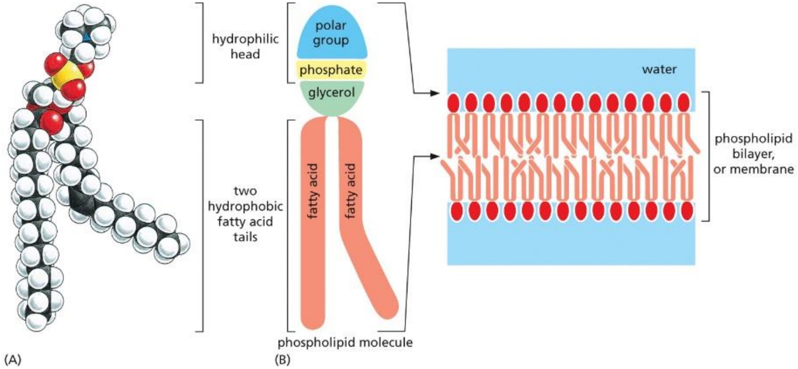


Fatty Acids have both hydrophilic and hydrophobic regions (amphipathic)

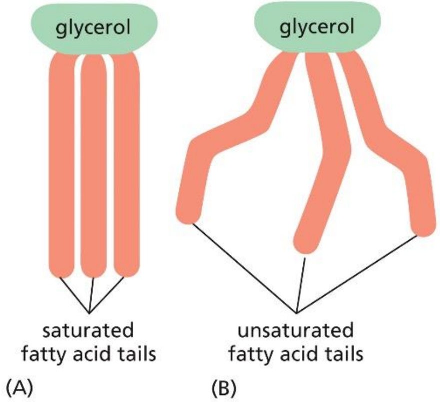
Palmitic acid

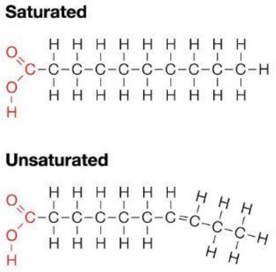


phosphatidylcholine



Triacyglycerols

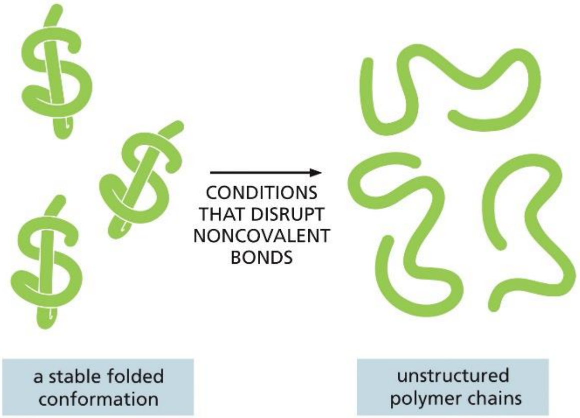


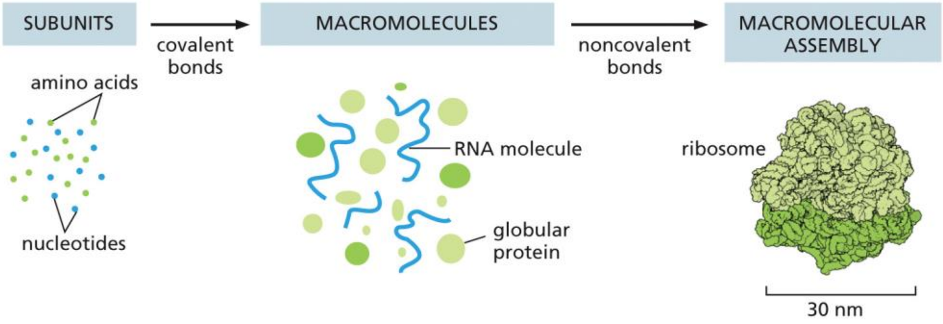


- Hydrophilic heads: water attracting (polar)
- Hydrophobic tails: water resistant (non-polar)
- Double bonds create kinks in hydrocarbon tail and prevent from packing tightly (unsaturated, healthy fat)

Noncovalent bonds specify the shape of a macromolecule and define interactions between macromolecules

- Electrostatic interactions, van der waals, hydrogen bonds, etc. ensure 3-D conformation and stabilize assembly of large complexes
- Shape determines chemistry and activity of macromolecules and their interactions with other molecules (such as enzyme and substrate interactions)





SQUARECAP Q#6-7

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Feedback/Reflection

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