MOLECULAR CELL PHYSIOLOGY - Biomembranes & Subcellular Organization

I. The cell
- A. Cells are fundamental organizational units of all living things
  - 1. Three lineages based upon nucleotide sequences: bacteria, archaea, eukara
- B. Types of cells
  - 1. Procaryotic (bacteria and archaea)
  - 2. Eucaryotic (eukara)
    - a) Plant
    - b) Animal

II. Studying cells through different types of microscopy (magnification is important, but so is resolution)
- A. Light microscopy (resolution to 0.2 ? m)
  - 1. Cell (5-100 ? m or more), nucleus (5-15 ? m), chloroplast (5-10? m), mitochondrion (1-4 ? m), Golgi apparatus (0.5-2 ? m), ribosome (15-25 nm), plasma membrane (10 nm)
  - 2. Fresh samples can be used although they are often fixed, stained, and sectioned
- B. Fluorescence microscopy
  - 1. Sample absorbs light of one wavelength and emits another wavelength
  - 2. Immunofluorescent microscopy: fluorescent antibodies used to detect proteins
    - a) Confocal scanning and deconvolution pinpoint the plane of focus and refocus light emitted
- C. Phase-contrast microscopy
  - 1. Small differences in refractive index and thickness between parts can be viewed
    - a) Provides improved definition of subcellular structures
- D. Transmission electron microscopy or TEM (resolution to 0.10 nm)
  - 1. Electrons pass through sample and image is created on screen
  - 2. Sample must be really thin (about 50-100 nm)
  - 3. "Stain" with gold or osmium so that electrons scatter differently through cell parts
- E. Cryoelectron microscopy utilizes TEM and samples frozen in liquid nitrogen
  - 1. Computer averages images of particles to generate a 3-D model
- F. Scanning electron microscopy or SEM (resolution to 10 nm)
  - 1. Electrons scan the surface of sample and image is created on screen
  - 2. Sample is coated with a heavy metal such as platinum or gold

III. Purification of cells and cellular components (also see slide presentation)
- A. Cell purification by flow cytometry
  - 1. Based upon light scatter or fluorescence of different cells
    - a) Laser is used as light source and a detector / integrator enables aspirator / sorter (fluorescence-activated cell sorter) assembly to separate cells
- B. Disruption of cells to release cellular components
  - 1. Cells are suspended in a chilled, isotonic solution (standard is 0.25 M sucrose), along with buffers, stabilizers, and other chemicals to maintain proper environment
  - 2. If cell wall is present, enzymes (cellulase, pectinase, etc.) can be used to degrade it (protoplast is cell w/o cell wall)
  - 3. Suspended cells or tissue is homogenized to release cell contents
  - 4. Suspended cells may also be sonicated to rupture cell membrane
  - 5. Sample can be then be squeezed through cheese cloth to remove "clumps"
- C. Separation of organelles by centrifugation (provides crude separation)
  - 1. Differential-velocity centrifugation (600g to pellet nuclei;1,250g to pellet chloroplasts;15,000g to pellet mitochondria, peroxisomes, lysosomes; 100,000g to pellet plasma membrane, endoplasmic reticulum, large polysomes; 300,000g to pellet ribosomal subunits; any remaining protein is cytosol)
  - 2. Equilibrium density-gradient centrifugation uses layers of sucrose to separate organelles - typical is 40,000g or more for several hours
    - a) Sucrose density gradients vary - range between 1.00 and 1.25 g/mL

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2. Equilibrium density-gradient centrifugation (continued)
   b) Pellet contains nuclei; peroxisomes often end up near the bottom; chlorplasts and mitochondria in the middle; cytosol at the top
   c) Marker pigments reveal regions of the centrifuge tube (chlorophyll for chloroplasts) and marker enzymes (CCO for mitochondria, catalase for peroxisomes, acid phosphatase for lysosomes)

D. Some organelles can be purified because of unique protein coats such as clathrin, which is found in certain cytoplasmic vesicles

E. Purity of organelles can be checked by microscopy or markers

IV. Cell wall (note: animal cells do not have CW but do have a mixture of fibrous protein and polysaccharides called the extracellular matrix)

A. Provides mechanical strength, maintains shape, controls expansion, regulates transport, provides protection, aids signaling, stores food reserves

B. Primary wall forms outside (cellulose, hemicellulose, pectin, and protein)

C. Secondary wall forms to the inside of primary wall (cellulose, hemicellulose, and lignin)

V. Constituents of biomembranes (cell membrane found to the inside of cells walls or simply encompass cell if no wall is present).

A. Phosphoglycerides as a class of phospholipids
   1. Glycerol is parent compound with 2 fatty acyl side chains and a phosphate
   2. Phosphate can also be esterified to choline, serine, ethanolamine, inositol which allow strong interaction with water in the surrounding medium

B. Sphingomyelin is a another phospholipid that is found in plasma membranes

C. Cholesterol is a steroid found in the plasma membrane of animal cells

D. Glycoproteins and glycolipids help stabilize the conformation of membrane proteins

VI. Structure of biomembranes

A. Pure phospholipid bilayer membranes "spontaneously seal to form closed structures that separate two aqueous compartments"

B. All integral proteins and glycolipids bind asymmetrically to the lipid bilayer
   1. Each protein has a single, specific orientation with respect to the cytosolic and exoplasmic faces of a cellular membrane

C. Lipids and integral proteins can and do move laterally in biomembranes (within leaflets)
   1. Occasionally, membrane proteins can move between leaflets via flippases

D. The *fluid mosaic model*: proteins are free to diffuse in a "sea of lipid"
   1. Depending on the cell type, 30-90% of all integral proteins are freely mobile
   2. Information about integral protein structure can be revealed by freeze-fracturing followed by freeze etching (freeze in liquid nitrogen, sharp blow to sample, metal shadow with platinum and remove with acid to leave "shadow")

VII. Functions of biomembranes (mediated to a large extent by transport proteins)

A. Transport nutrients into and metabolic wastes out of the cell

B. Prevent unwanted materials from entering the cell

C. Prevent loss of needed metabolites

D. Maintain pH and osmotic pressure

VIII. Examples of biomembrane function

A. Continuum: nucleus / ER relationship allows a "flow of metabolites"

B. Isolation - mitochondrion cannot fuse with nucleus

C. Passage of metabolites
   1. Diffusion - from high concentration to low concentration
   2. Osmosis - movement of water through a differentially permeable membrane
      a) From high concentration to low concentration
      b) Selectivity - active transport

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- 3. Carrier systems
  - a) Chloroplast
    - 1) H^+ pumped into thylakoid lumen
    - 2) H^+ then transported to outer stroma and coupled to make ATP
  - b) Mitochondrion
    - 1) H^+ pumped out to intermembrane space
    - 2) H^+ then transported to inner matrix and coupled to make ATP

IX. Organelles in cytoplasm (cytoplasm includes organelles; cytosol surrounds organelles)

- **A. Nucleus**
  - 1. Genetic information – DNA
  - 2. Cell regulation - "the brains"
  - 3. Outer membrane is continuous with the rough ER and, sometimes the space between inner and outer nuclear membranes is continuous with ER lumen
  - 4. Most of the rRNA is synthesized in nucleolus
- **B. Chloroplasts**
  - 1. Photosynthetic organisms
  - 2. Photosynthesis
    - a) Thylakoids - light capture
    - b) Stroma - conversion to sugar
  - 3. Contain some DNA (proteins synthesized by ribosomes within organelle)
- **C. Mitochondria – can occupy up to 25% of cytoplasmic volume**
  - 1. Energy from sugars
  - 2. Outer membrane = 50% protein + 50% lipid
  - 3. Inner membrane = 80% protein + 20% lipid
  - 4. Contain some DNA (proteins synthesized by ribosomes within organelle)
- **D. Cytomembrane system**
  - 1. Packaging & transport
    - a) Endoplasmic reticulum
      - 1) Smooth ER: Synthesis of fatty acids and phospholipids
      - 2) Rough ER: Ribosomes of which produce some membrane and organelle proteins as well as those for excretion
    - b) Golgi apparatus (dictyosomes)
      - 1) Vesicles from ER fuse with cis Golgi sacs and migrate to medial then trans – eventually migrate in a second vesicle to the plasma membrane, etc.
    - c) Ribosomes
- **E. Microbodies**
  - 1. Peroxisomes – fatty acid oxidation in eukaryotic cells as well as photorespiration in plants
  - 2. Glyoxisomes - conversion of fats into sugars
- **F. Lysosomes**
  - 1. Found in animal cells
  - 2. Degradation of cellular components (acid hydrolases)
    - a) Endocytosis - extracellular materials taken by invagination of plasma membrane to form small vesicles
    - b) Phagocytosis – large particles are enveloped by plasma membrane and internalized
- **G. Vacuoles (like lysosomes, tend to have acidic pH) - storage, waste, water regulation**
- **H. Cytoskeleton**
  - 1. Microfilaments - contraction and movement of cells in animals
  - 2. Microtubules – assist in cell division and movement of organelles
- **I. Ergastic materials (crystals, starch, accumulated waste products)**