BIL 255 – CMB

Mircroscopy and Methods, Protocols & Instrumentation for observing cells in Cell & Molecular Biology
Methodologies, Techniques, & Procedures…

for observing Cell Structure in CMB

web links: use class web pages to hyperlink:


Cell Biology Dictionaries
A Table of Glossaries
Glossary of Techniques
National Human Genome Glossary
General Procedures & Protocols - Cell Bio
General Procedures & Protocols - Molecular Biology
Mallery's CMB Resources

mcb(5/e) pages 184-193 & 165-173
Early Methodology in CMB - 1910 to 2010

Equipment advances of last 50 years are the epitome of modern scientific age

MICROSCOPY is the technical field using microscopes to view cellular objects: development of microscopy revolutionized biology & remains essential tool of CMB

2 major classes of microscopy: Light (optical) microscopy and electron microscopy

**Light Microscopy**: - produces magnified images of small objects with compound lens
  objective lens - next to object (100x) and ocular lens (10x) = 1,000x magnification
  (technically complicated)  

*types of light microscopy*  (technically complicated & mcb fig 9.10*)

1876 **Abbe** optimizes microscope designs (lens & condensers)
1886 **Zeiss** - lens RESOLUTION near limits of light  
(0.2 um* = 200 nm)

**Specimen preparation:**
1900's - killing, fixing, embedding & sectioning: **microtome** (1 to 10 um thin tissue sections)
  selective staining: **stains** attach to specific molecules (picture*)
Tracing with molecular precursors & light microscopy:

**autoradiography** - 1924 Lacassagne - produces an autoradiograph, which is a light microscope image on photographic film or emulsion produced by the pattern of radioactive decay emissions (e.g., beta particles or gamma rays) from a distribution of a radioactive substance.

**fluorescence microscopy** - 1941 Coons - form of light microscopy where component of interest in a specimen has been specifically labeled with a fluorescent molecule as GPF (Green Fluorescent Protein or fluorescein).

**immunofluorescence microscopy** - fluorescently tagged antibodies bind specifically to a corresponding antigen as a probe for identifying a particular molecule in cells, tissues, or biological fluids: ex. rat intestine

**confocal fluorescence microscopy** - 1957 Minsky - confocal microscopes uses pinpoint illumination of a fluorophore in one focal plane to eliminate out-of-focus fluorescence. Since only fluorescence in a narrow focal plane is detected the image resolution is greatly enhanced providing a sharper image

1980 Alexrod - TIRF* (total internal reflection fluorescence) eliminates background light (pics)
1998 Live Cell Imaging confocal microscopy by PerkinElmer, Inc. (image of scope)
2007 Live Cell Video Microscopy (5.5 min - view at home)
Electron Microscopy

**TEM** 1931 *Ruska* - 1st transmission EM  
**TEM-photo** & mcb6eFig1.2b* & mcb9.5a*  
TEM passes e's through a specimen onto a viewing screen  
(resolution theoretical = 0.005nm, but effective resolution is = 0.1 to 0.2 nm*)  
1952 *Palade / Porter* - 1st TEM pics & EM stains – image due to differential scattering of e’s in specimen (stains as heavy metals - osmium tetroxide for membranes) stain = dark.  
specimens must be thin = 50 nm thick; cut via **microtome**  
1957 *Robertson* - unit membrane hypothesis (all membranes look alike in EM)  
2000 computer image averaging allows 3D modeling - **models of ribosome & Ca pump***

**fFEM** (cryoelectron microscopy) - an aqueous specimen is frozen in liquid N₂ (-196°C)  
1964 *Steere & Muhlethaler* - develops freeze fracture EM - **prep*** & **picks** (scroll down)  
2004 cryoelectron tomography – specimen rotated in electron beam & individual images are computationally fit into 3D reconstruction (tomogram) - **nuclear pores***

**SEM** (scanning electron microscopy) - **neuron*** & **virtual SEM***  
1965 *Charles Oatley* - 1st scanning EM (Stereoscan) uses metal shadowing to **coat** sample & bombardment with e's releases 2ⁿᵈary e’s when focused onto detector reveals 3D surface details

**Tagging** - 1981 antibody tagging with gold particle in electron microscopy - **fig 9.21***  
1974 Nobel Prize to G. Palade, C. deDuve, A. Claude - for their "inner workings of cells“  
Interpret EM's* & Microscopy provides for size relationship analyses*

Mallery Microscopy & Methods in CMB 5
RESULTS of MICROSCOPY...
Investigations of Cells - some major EUKARYOTIC ORGANELLES
a tour through a Virtual Cell*

The light microscope, so called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology. Live cells lack sufficient contrast and internal cell structures are colorless and transparent. Contrast is increased by staining with selective dyes, which involves killing and fixing the sample, which can introduce artifacts.

The electron microscopy uses a focused electron beam on fixed sectioned of cells, which are static (mcb9.5a*) to describe organelles, mostly by presence or absence of membranes...

The section on CELL ORGANELLES below* is a general review of freshman biology cell structure. Please REVIEW this material on your own, and we will question you on the material during testing.
the Results of Microscopy : Investigations of Cells....

some major EUKARYOTIC ORGANELLES

microscopy has used fixed sectioned cells which are static (mcb5.22a) divide organelles by presence or absence of membranes

Links to reviews of major cell organelles of animal & plant cells:

mcb5.19*(ans) & mcb5.19*(ans) –

Quick Review of Major Eukaryotic Cell Organelles
Double Membrane Bound Organelles:

1. **nucleus**...
   - synthesizes DNA, rRNA, tRNA, primary transcript (mRNA preccursor)
   - largest double membrane bound –
     - outer membrane contiguous with ER

   **peri-nuclear space** (2-5nm) is contiguous with lumen of ER
   - contains **pores** of protein complexes (*mcb 8.20a*)
     - regulates nucleoplasm-cytoplasm exchange
       - via NLS of 7 aa sequence @ C-terminus (pro-lys-lys-lys-arg-lys-val)

**nucleolus** - regions of rDNA that makes rRNA

**nucleoplasm** - 'cytoplasm' of the nucleus

**heterochromatin** - condensed (**dark EM color**) = inactive DNA *mcb6.33a*
**euchromatin** - non-condensed (**light EM color**) = active DNA

**lamins** - fibrous proteins adjacent to inner nuclear membrane
   - form frame for nuclear shape
2. **mitochondria**... conducts ATP production of cell via oxidative metabolism of glucose & fatty acids
   outer membrane (50:50 lipid/protein)
   contains porin (mcb10.18*) transports most ligands < 10K
   inner membrane (20:80 lipid/protein)
   strictly regulates most transport into mitoplasm
   **cristae** - infoldings of inner membrane (mcb9.8 & 12.6*)

3. **chloroplast**... largest green plant cell organelle (0.5-2.0 µm by 10 µm)
   double membranes
   with extensive inner membrane-limited sacks called **thylakoids** (mcb9.9*)
   absorbs light energy via **chlorophyllous** pigments
   converts light energy into ATP & NADPH (chemiosmosis)
   reduces CO₂ into CH₂O
Similarities of Mitochondria & chloroplasts...

1. make ATP/NAD(P)H via same mechanism
   - **chemiosmosis**: oxidative creation of H+ gradient coupled to ATP synthase

2. show **mobility** throughout cell

3. divide by **fission** independent of cell's division

4. autonomously **replicate their own DNA**
   - [mito: 16,569 nucleotide pairs: about 37 genes]
   - [chlp: 10fg or 120 genes - highly supercoiled & repetitive-up to 6 copies]

5. both contain **70s** - bacterial size **ribosomes**

6. **synthesize** their own **proteins** on own protein synthesizing machinery
4. **endoplasmic reticulum**... network of closed-flattened membrane sacks called **cisternae**
   found in all nucleated cells; involved in protein/lipid biosynthesis
   2 types: **SER** (smooth) - lacks ribosomes mcb9.5*
   - makes FA & lipids (esp. in hepatocytes)
   - detoxifies hydrophobic chemical including carcinogens & pesticides
   **RER** (rough) - membranes bound w ribosomes mcb9.4*
   - makes plasma membrane proteins & exportable proteins of ECM
   - abundant in cells making - antibody protein (plasma cells)
   - pancreas (digestive enzymes & hormones)

5. **Golgi Complex**... series of flattened membrane sacks (cisternae)
   that take up ER transport vesicles and process contents via glycosylation
   (adding carbohydrate residues)
   three divisions:
   - **cis** - where ER vesicles enter mcb9.5b*

   **medial** - where modifications (glycosylations) occur

   **trans** - vesicle packages & budded off here for secretion mcb9.6*


Single Membrane Bound Organelles:

6. **endosomes**... membrane bound vesicles of extracellular milieu internalized by ENDOCYTOSIS
   a. **endocytosis** - cathrin protein "coated" membrane pits, pinch of endosome vesicles
   b. **phagocytosis** - whole cells engulfed & passed to lysosomes for digestion
   c. **autophagy** - worn-out organelles fuse with lysosome

7. **lysosomes**... several hundred single membrane bound vesicles
   (exclusive to animals - plants use vacuoles)
   have acid pH environment to help denature proteins
   (H+ATPases* & Cl transporters --> HCl)
   contains **hydrolytic enzymes**
   (nucleases, proteases, phosphatases, glycosylases)
   cytosolic & nuclear proteins are not digested within lysosomes,
   but rather **proteasome***
   **Tay-Sachs** (tt): defective lysosomal enzyme degrades ganglosides,
   glycolipids buildup in neurons ≈ dementia, blindness, and death
8. **plant vacuole**... membrane limited interior space (up to 80% cell volume) containing membrane transporters that accumulate ions, nutrients, & wastes. 

   lumen holds digestive enzymes (acid pH optima).

**tonoplast membrane** permeable to water influx, helps establish turgor pressure (5-20 ATM)

9. **peroxisomes**... spherical (0.2-1.0 µm) organelle containing **oxidases** (catalase) that use O₂ to oxidize (removes e-'s) from molecules as H₂O₂ (& other toxins). degrade FA's to acetyl groups - used to make cholesterols (esp. impt in liver/kidney cells).

   X-linked adrenoleukodystrophy (ADL): no FA digestion occurs, leads to several neuro-linked defects & death.

   plants contain **glyoxysomes** which oxidize lipids (very similar to peroxisomes).