

# BIL 255 – CMB

## **Methods, Protocols & Instrumentation in Cell & Molecular Biology**

# **Methodologies, Techniques, & Procedures used in Cell & Molecular Biology**

[Cell Biology Dictionaries](#)

[A Table of Glossaries](#)

[Glossary of Techniques](#)

[National Human Genome Glossary](#)

[General Procedures & Protocols - Cell Bio](#)

[General Procedures & Protocols - Molecular Biology](#)

**mcb(5/e) pages 184-193 & 165-173**

## Early Approaches in CMB - 1910 to 1960

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

Light Microscopy (0.2 um) History types\* mcb 5.42

1876 Abbe optimizes microscope designs (lens & condensers)

1886 Zeiss - lens resolution near limits of light

1900's - embedding & sectioning : microtome

(1 to 10 um thin tissue sections\*) selective staining :

stains attach to specific molecules (picture)

1924 Lacassagne - autoradiography\*, preparation,

tracking, images - definitions & procedures

1941 Coons - fluorescence microscopy\*

fluorescent tagged antibodies & Green Fluorescent Protein  
gene from jellyfish protein allows dynamic tagging

## Electron Microscopy (2.0nm)

mcb 5.50

- 1931 **Ruska** - 1st Transmission Electron Microscope (TEM)
- 1952 **Palade / Porter** - EM stains for ultrastructure **fig**  
microtome
- 1957 **Robertson** - unit membrane hypothesis
- 1964 **Muhlethaler** - develops freeze fracture EM -  
preparation & coating
- 1965 **Charles Oatley** - 1st Scanning EM (Stereoscan)
- 1974 Nobel Prize to G. Palade, C. deDuve, A. Claude  
- for their "inner workings of cells"

# Investigations of Cells.... the Results of Microscopy :

## major EUKARYOTIC ORGANELLES

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

### Single Membrane Bound Organelles:

1. **endosomes**... membrane bound vesicles of extra-cellular 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  
[mcb5.20a](#) & [endosomes & lysosomes](#)

2. **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 gangliosides,  
glycolipids buildup in neurons ≈ dementia, blindness, and death

3. **plant vacuole**... membrane limited interior space (up to 80% cell volume)  
containing membrane transporters that accumulate ions, nutrients, &  
wastes. [mcb5.24](#)\*  
lumen holds digestive enzymes (acid pH optima).  
**tonoplast membrane** permeable to water influx,  
helps establish turgor pressure (5-20 ATM)

4. **peroxisomes**... spherical (0.2-1.0  $\mu\text{m}$ ) organelle containing oxidases ([catalase](#)) that use  $\text{O}_2$  to oxidize (removes e-'s) from molecules as  $\text{H}_2\text{O}_2$  (& other toxins). degrade FA's to acetyl groups - used to make cholesterols (esp. impt in liver/kidney cells).

X-linked adrenoleukpdystrophy (ADL): no FA digestion occurs, leads to several neuro-linked defects and death. [mcb5.21\\*](#)

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

5. **endoplasmic reticulum**... network of closed-flattened membrane sacks called cisternae

found in al nucleated cells; involved in protein/lipid biosynthesis

2 types: **SER** (smooth) - lacks ribosomes [mcb5.22\\*](#)

- makes FA & lipds (esp. in hepatocytes)

- detoxifies hydrophobic chemical including carcinogens & pesticides

**RER** (rough) - membranes bound w ribosomes [mcb5.21\\*](#)

- makes plasma membrane proteins & exportable proteins of ECM

- abundant in cells making - antibody protein (plasma cells)

- pancreas (digestive enzymes & hormones)

6. **Golgi Complex**... series of flattened membrane sacks (cisternae) that take up ER transport vesicles and process contents via glycosylation (adding carbohydrate residues)

3 divisions:

**cis** - where ER vesicles enter [mcb5.22b](#)\*

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

**trans** - vesicle packages & budded off here for secretion [mcb5.23](#)\*

## Double Membrane Bound Organelles:

### 7. **nucleus**...

synthesizes DNA, rRNA, tRNA, primary transcript (mRNA precursor)

largest double membrane bound –

outer membrane contiguous with ER

**peri-nuclear space** (2-5nm) is contiguous with lumen of ER [mcb5.19\\*](#)

contains **pores** of protein complexes ([mcb 12.18\\*](#))

- 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 [mcb5.25\\*](#)

**euchromatin** - non-condensed (light EM color) = active DNA

**lamins** - fibrous proteins adjacent to inner nuclear membrane

- form frame for nuclear shape

8. **mitochondria**... conducts ATP production of cell via oxidative metabolism of glucose & fatty acids  
outer membrane (50:50 lipid/protein)  
contains porin ([mcb5.14\\*](#)) transports most ligands < 10K  
inner membrane (20:80 lipid/protein)  
strictly regulates most transport into mitoplasm  
**cristae** - infoldings of inner membrane ([mcb5.26\\*](#)) [pic1](#) & [pic2](#)
9. **chloroplast**... largest green plant cell organelle (0.5-2.0  $\mu\text{m}$  by 10  $\mu\text{m}$ )  
double membranes  
with extensive inner membrane-limited sacks called **thylakoids** ([mcb5.27\\*](#))  
absorbs light energy via **chlorophyllous** pigments  
converts light energy into ATP & NADPH (chemiosmosis)  
reduces  $\text{CO}_2$  into  $\text{CH}_2\text{O}$

## Similarities of Mitochondria & chloroplasts...

1. make ATP/NAD(P)H via same mechanism
  - **chemiosmosis**: oxidative creation of H<sup>+</sup> 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

**Cell Culture**- growing isolated cells in defined media  
single cells in controlled conditions... form colonies (clones)  
req: 37°C, pH, salts, essential amino acids, vitamins,  
glucose, serum (growth factors - insulin & transferrin [Fe])  
[mcb6.36\\*](#) & [mcb22.3\\*](#) [American Type Culture Collection](#)  
[Society for \*in vitro\* Biology & Plant Tissue Culture](#)

**Single Cell Analysis...**

[cytochemistry](#) ([JHC](#) & [Histochemical Society](#))

[cytometers](#) an instrument system for analyzing cells

[autoradiography](#) (see above)

[microspectrophotometry](#)... a technique for obtaining  
measurements of spectral absorption of stained  
microscopic material of cells.

[Cell sorters](#) - fluorescent tagged cell isolations