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 - cathepin 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

2. **lysosomes**... several hundred single membrane bound vesicles 
   (exclusive to animals- plants use vacuoles) 
   have acid pH environment to help denature proteins 
   (H^+ATPases* & CI transporters $\rightarrow$ 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, 
   glysolipids buildup in neurons $\approx$ dementia, blindness, and death

3. **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)

4. **peroxisomes**... spherical (0.2-1.0 µm) organelle containing oxidases 
   (catalases) that use O2 to oxidize (removes e-')s from molecules as H2O2 
   (& other toxins). degrade FA's to acetyl groups - used to make 
   cholesterol (esp. impt in liver/kidney cells). 
   X-linked adrenoleukoprydystrophy (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 all nucleated cells; involved in protein/lipid biosynthesis 
   2 types: SER (smooth) - lacks ribosomes mcb5.22* 
   - makes FA & lipids (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*

Mallery Methodologies in CMB
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 (*mcb12.19*)
     - 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*) transmits most ligands < 10K
   - inner membrane (20:80 lipid/protein) strictly regulates most transport into mitoplast
   - cristae - infoldings of inner membrane (*mcb5.26*)

9. chloroplast... largest green plant cell organelle (0.5-2.0 µm by 10 µm)
   - double membranes with extensive inner membrane-limited sacks called thylakoids (*mcb5.27*)
     - absorbs light energy via chlorophylous 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

Cell Culture- growing isolated cells in defined media
   - single cells in controlled conditions... form colonies (clones) req: 37°C, pH, slats, essential amino acids, vitamins, glucose, serum (growth factors - insulin & transferrin [Fe])
   - 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