How Cells Are Studied

I. Electron Microscopy – not in color
II. Fluorescence Microscopy – excitation and absorption color
III. Fluorochromes – tag a protein with a fluorescent protein
IV. Immunofluoresence Microscopy -Antigen Staining
V. Quantum Dots – nanoparticle of semiconductor material, can be coupled to molecules – don't get photobleached, and can have any excitation color
VI. CLSM –sharper image microscope
VII. Scanning Electron Microscopy – Gives topography
VIII. Revealing Gene Expression Patterns – Promoter GFP –fusion, or fusion to actual protein
IX. FRET – Fluorescence Resonance Energy Transfer- protein-protein interaction, transfer of energy from fluorophore on protein x to fluorophore on protein y if the two proteins interact
X. FRAP – Fluorescence Recovery After Photobleaching – Bleach out signal and wait for it to diffuse back in, measures diffusion, protein trafficking
XI. FLIP – keep photobleaching at one particular part of cell and see how long it takes for all protein to lose fluorescence
XII. BiFC – tag protein a with N-terminal of YFP, tag protein B with C-terminal of YFP if they interact → fluorescence
XIII. Atomic Force Microscopy – Gillete Stadium pin; measures topography

LRR (Leucine Rich Repeat) Receptor Kinases and Plant Signaling –

I. Plants don't have: Ras, JAK, STAT, Notch, Wnt, Hedgehog, cAMP
II. Plants use Receptor Kinases, but not tyrosine kinases like animals: Serine-threonine kinases, and Leucine-rich repeat receptor kinases (most abundant)
    A. Example: Brassinosteroids – family of >50 compounds contribute to growth, division, differentiation, defense
        1. BRI1 encodes the main Br receptor, *bri1* mutants are BR-insensitive
        2. BRI1 has:
            a. leucine rich repeat – extracellular interacts with BR
            b. transmembrane region
            c. autophosphorylating kinase region
        3. Process:
            a. BKI1 prevents autophosphorylation
            b. Binding of Brassinosteroid (BL) causes BKI1 to dissociate and BAK1 to bind and enhance autophosphorylation
            c. Autophosphorylation of BRI1 leads to phosphorylation of BSK
            d. BSK phosphorylates BSU1 which is a phosphatase and dephosphorylates BIN2 – deactivating it
            e. Active BIN2 phosphorylates BZR1/2 keeping them in the cytoplasm

f. When dephosphorylated BZR1/2 move to the nucleus where they activate and repress BR-regulated genes
    1. BZR2/BES are transcription factors activated by BR that promote transcription; BZR1 repress transcription
g. BZR2/BES are transcription factors activated by BR that promote transcription; BZR1 represses transcription

ER to Golgi

I. Mechanisms of Vesicular Transport
II. Cis and Trans Golgi – location relative to ER
III. Transport Vesicles
    A. Form from specialized coated membranes where cargo accumulates, and bud off as coated vesicles
    B. Function of the Coate:
        1. Selects molecules for transport
        2. Creates curved shape of vesicle allowing budding off
    C. Types of vesicles – clathrin coated (Golgi to Plasma Membrane), COPI-coated (Golgi to Golgi), COPII- coated (ER to Golgi)
IV. Plant Cells filled with Golgi Stacks
V. Plant vs. Animals- no vesicular tubular cluster in plants, ER and Golgi closer together, Golgi is more mobile
VI. KDEL receptors –prevent movement of proteins to the Golgi
    A. KDEL + KDEL-sequence protein are sent to golgi where KDEL- sequence proteins are bound by KDEL in Golgi and returned to the ER
VII. Functions of the Golgi
    A. Protein Sorting and Trafficking
    B. Protein Glycosylation (N-linked Oligosaccharides)
        1. Complex Oligosaccharides
        2. High-mannose oligosaccharides
        3. What is the purpose of glycosylation
            a. helps solubilize folding intermediates, prevents aggregation
            b. marks progression through the system "Glyco-code"
            c. Protects against proteases
            d. Signaling: can change receptor specificity
    C. Synthesis of Cell Wall Polysaccharides (in Plants) – Pectin, Hemicellulose
        1. Sugars processed as they're moved from Cis to Trans; in the stacks they removal and addition steps occur, in the Cis and trans edges of golgi sorting steps occur
        2. Action happens on lumenal side of the membrane
    D. Two Models of Golgi organization:
        1. Vesicular transport – each protein travels across the golgi by leaving and returning in vesicles

2. Cisternal Maturation – each protein travels directly through the golgi

E. Examples of Plant Secretion Products
   1. Extracellular destination:
      a. N-linked glycoproteins: enzymes, defense, lignin
      b. O-linked glycoproteins: cell wall proteins
      c. Non-glycosylated secreted proteins
   2. Cell Membrane:
      a. Transmembrane e.g. cellulose synthase, ion pumps
      b. Extracellular side only
   3. Pure polysaccharides – wall matrix, hemicellulose, pectin

F. Cellulose is a pure polysaccharide made at the cell membrane by cellulose synthetase not in the Golgi

VIII. Sugars added in the Golgi lumen to proteins become extracellular

IX. Mitosis: Animals – Golgi Fragments; Plants – Golgi Separated into daughter cells

Vacuoles, Endocytosis, Exocytosis

I. Lysosomes and Plant Vacuoles

A. Plant Vacuoles can have different purposes – storage, digestion, turgor pressure regulation

B. Three Pathways to lysosome
   1. Phagocytosis,-exterior to lysosome
   2. Endocytosis –> late endosome -> lysosome
   3. Autophagy → interior organelle to lysosome

C. Animals Sorting of lytic Enzymes into lysosomes
   1. Addition of phosphate to Mannose-6 on Proteins from ER tagged to go to lysosome
   2. M6P receptor on membrane of trans golgi recognizes M6P, Clathrin coat M6P receptor and forms vesicle
   3. Vesicle transported to Lysosome; phosphate removed, vesicle recycled
   4. Specificity of phosphorylation lies in amino acid sequence signal patch, which recruits GlcNAc phosphotransferase

D. Plant vacuolar sorting signals are amino acid sequences
   1. NPIR sequence in N-terminal sequences
   2. No Specific sequence in C-terminal

II. Endocytosis

A. In animals – Clathrin coats form at the plasma membrane

B. Receptor-mediated endocytosis - Receptor-adaptor-clathrin form complex at membrane,
   1. Receptor comes in forming early endosome, which can be sent to other parts of the cell or to the plasma membrane
   2. The remaining endosome will become a late endosome and get degraded by lysosome