BIOL 200 (Section 921)
Lecture # 9-10
June, 29-30, 2006

UNIT 7: ENDOMEMBRANES

Readings:

ECB (2nd ed.) Chapter 15 (Whole chapter): Introduction, pp. 496-501; Protein Sorting pp. 502-504; Protein targeting to the Endoplasmic Reticulum, pp. 505-512; Vesicular Transport, pp. 512 – 516; Secretory Pathway, pp. 516 – 523; Endocytotic Pathway, pp. 523 -529. Questions: 15-3, 15-4, 15-6 to 15-9, 15-12, 15-13, 15-15, 15-16, 15-18, 15-19, 15-21).

ECB (1st ed.) Chapter 14 (Whole chapter): Introduction, pp. 447-452; Protein Sorting pp. 452-455; Protein targeting to the Endoplasmic Reticulum, pp. 458-462; Vesicular Transport, pp. 462 – 466; Secretory Pathway, pp. 467 – 472; Endocytotic Pathway, pp. 472 - 477. Questions: 14-3, 14-4, 14-6 to 14-9, 14-12, 14-13, 14-15, 14-16, 14-18, 14-19, 14-21).

I. Protein Targeting - Nucleus, Mitochondria, Chloroplasts and ER.

Learning Objectives

- To explain the nature of signals and sorting
- To explain the function of coat proteins and the signals carrried by vesicular conponents.
- To understand the role and importance of the signal recognition particle and the SRP Receptor in the targeting of proteins to the endoplasmic reticulum.
- To explain how single and multiple pass membrane proteins are inserted into the membrane through use of signal sequences, start transfer sequences and stop transfer sequences.

Main Points:

Sorting signals (signal sequence of 15-60 amino acids long) are necessary to direct a protein to a particular organelle. see Table 15-3

1. ER signal sequence-generally at N terminal, contains block of hydrophobic amino acids.
2. Nuclear localization signal-one or two short sequences with positively charged aa
3. Mitochondria/chloroplast-signal sequence at N terminal
4. KDEL-retention in the ER lumen

Proteins are transported into organelles by three mechanisms (Fig:15-5)

1. **Nuclear import**:-soluble, folded nuclear proteins made in cytosol, move into nucleus through nuclear pores. Nuclear import receptors in cytoplasm bind nuclear localization signal, bind nuclear pore complex, protein+bound receptor imported. Receptors recycle to cytoplasm (fig. 15-9).

2. **Protein translocators**- are protein pores in the membrane that facilitate proteins (unfolded) to snake through membranes (Fig. 15-.5).

-ER translocator-ribosome bound to ER during translation, protein crosses membrane in unfolded conformation.

-mitochondria and chloroplasts-outer and inner membranes come together in "contact points" where proteins translocated in unfolded conformation through translocator.(Fig. 15-10). Inside chaperones help proteins fold, signal sequence cleaved.

3. **Transport vesicles**-membrane bilayer vesicles pinch off donor compartment, fuse with recipient compartment and carry both soluble proteins in lumen and membrane proteins associated with bilayer.

II. Golgi, Vesicle Transport, Endocytosis, Exocytosis.

Learning Objectives

- Become familiar with the structure and overall function of the Golgi apparatus
- Understand how vesicles are formed and targeted.
- To be able to trace a molecule through each of these pathways describing all of the structures and process through which they pass.

Review Questions

- What do the terms cis and trans Golgi referto?
- What are COP coated vesicles and what is their role? How is their role different that that of clathrin coated vesicles
- What are snares and how do they regulate membrane fusion?

Main Points:

Golgi Structure: named after Camillo Golgi who discovered the Golgi apparatus in 1898.

- Golgi apparatus refers to all the stacks, total complement, of Golgi in a cell. Each Golgi stack consists of flattned membrane-bound sacks – each sack is called a cisterna,plural cisternae.
- Each cisterna has a unique complement of resident proteins that function within it.
- Each Golgi stack has a cis (forming) face (cis Golgi network-sorts and repackages escaped ER residents to return them to the ER) and trans (leaving) face (sorts lysosomal proteins from secreted and plasma membrane proteins).
- Facing the Golgi lumen are many membrane-bound enzymes that make and modify polysaccharides of products that are working their way through the stack

Golgi functions: Glycosylation, packaging and sorting of glycoproteins.

• In RER glycosylation started by covalent attachment of the oligosaccharide tree (14 sugars)
• Protein glycosylation continues in the Golgi and involves trimming of some sugars from the oligosaccharide tree and addition of other individual sugars

From ER, vesicular transport moves cargo and membranes along the secretory pathway (Fig.15-17)

- proteins destined for secretion move:
- to the cis (entry face) cisterna of the Golgi,
- between the stacks of the Golgi,
- from the trans (exit face) cisterna of the Golgi to PM

Mechanism of vesicle budding: protein coats drive vesicle formation.

- Form a protein shell around membrane and pull it into a sphere, then vesicle bud is pinched off by enzyme like dynamin (see Fig. 15-19)
- Coat must be shed before vesicle can fuse with target
- different donor compartments have different coats, examples given in Table 15-4.

COPII-coated vesicles (short for Coat Protein II)ER to cis-Golgi, "coatomer", soluble protein complex that forms coat is recruited from the cytoplasm.

COPI-coated vesicles-Golgi to ER recycling- (and intraGolgi traffic?-not known for sure)

Clathrin coated vesicles-PM to endosomes and Trans Golgi to lysosome

Transport vesicles fuse with target membranes:

1. docking, vesicles come into close approach. (Fig. 15-20)

-SNARE (matching sets of proteins found on either vesicle, V-SNARE, or target, T-SNARE).proteins believed to be important in docking and helping assemble protein complex that causes fusion.

2. fusion, membrane bilayers come together and lumen contents released into target while membrane proteins and lipids mix in fluid bilayer.