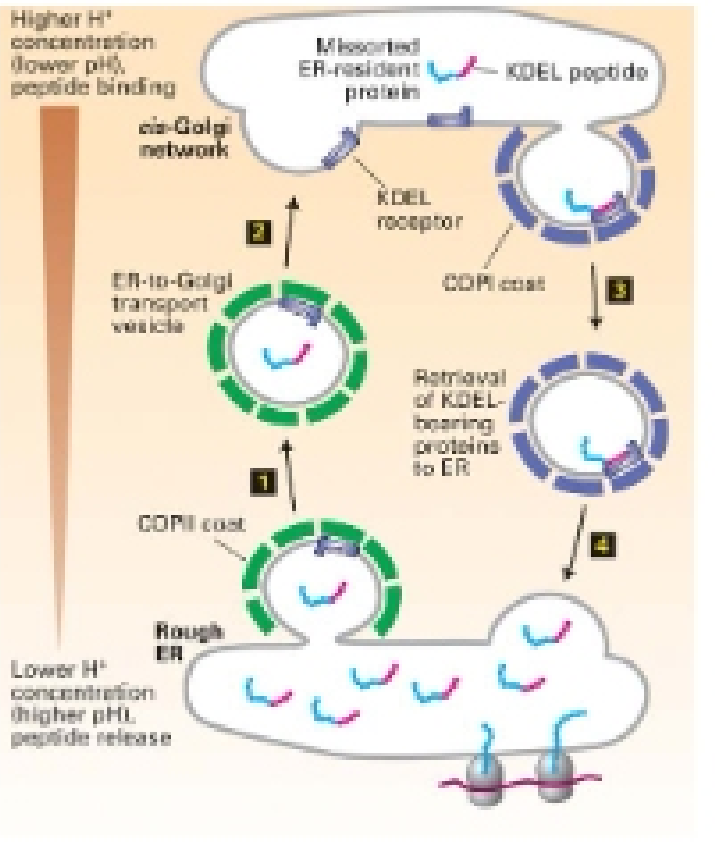
CBIO 3400 – Week 6 Study Questions

1. What is the KDEL sequence? How does it affect the sub-cellular localization of proteins? Describe the complete mechanism regarding the KDEL sequence and protein trafficking.

KDEL is a sorting signal sequence attached to the KDEL receptor protein in the membrane of the Golgi; this KDEL receptor "grabs" soluble ER-resident proteins from the Golgi. The low (acidic) pH of the Golgi allows the KDEL receptor to attach to the ER-resident proteins within the Golgi. When secretory vesicles break off of the Golgi, the KDEL receptors go with the vesicles, still holding on to the ER-resident protein it grabbed in the Golgi. When the secretory vesicles meet and fuse with the ER membrane, the high (basic) pH of the ER causes the KDEL receptor protein to release the missorted ER-resident protein into the lumen of the ER. In general, the KDEL sequence affects the sub-cellular localization of proteins by bringing them back to the ER from the Golgi.

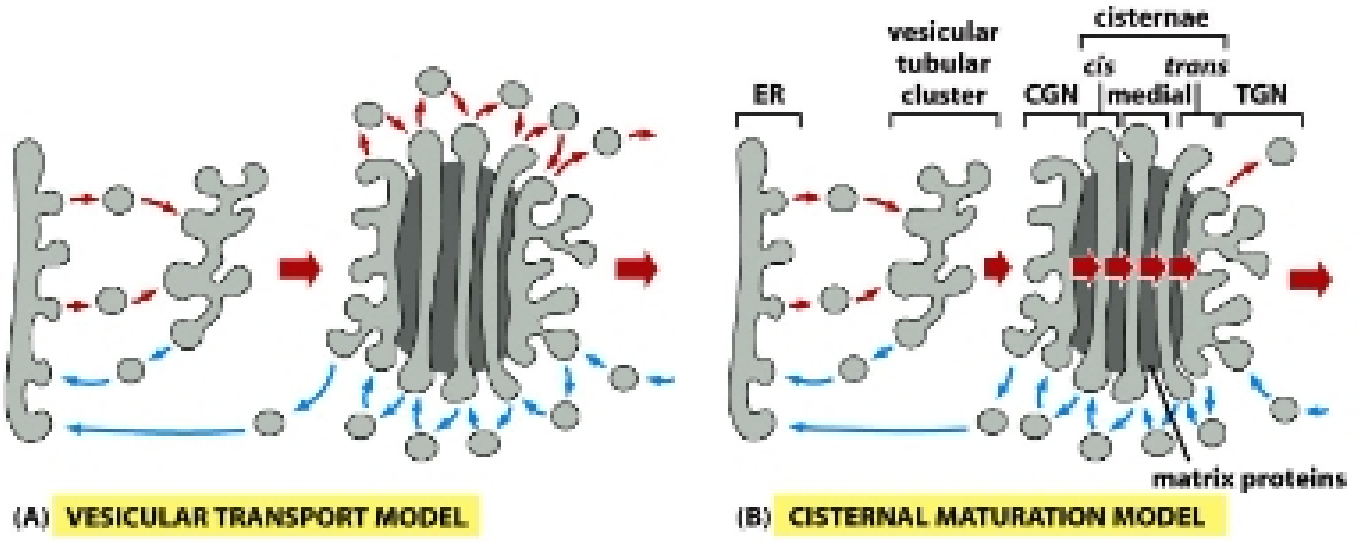
The complete mechanism regarding the KDEL sequence and protein trafficking is as follows:

1. A vesicle containing an ER-resident protein (with KDEL peptide attached) begins to form from the membrane of the rough ER. The membrane of the soon-to-be vesicle contains a KDEL receptor and is surrounded by a COPII coat. The ER-resident protein is NOT associated with the KDEL receptor at this time.
2. The vesicle buds off of the rough ER membrane and heads toward the Golgi.
3. The vesicle fuses with the Golgi, releasing the missorted ER-resident protein (with KDEL peptide attached) into the Golgi lumen and the KDEL receptor into the Golgi membrane.
4. The missorted ER-resident protein (with KDEL peptide attached) binds to the KDEL receptor, and a vesicle begins to form from the membrane of the Golgi. The membrane of the soon-to-be vesicle contains the KDEL receptor with its attached missorted ER-resident protein and is surrounded by a COPI coat. (To reiterate, the ER-resident protein IS associated with the KDEL receptor at this time.)
5. The vesicle buds off of the Golgi and heads toward the rough ER.
6. The vesicle fuses with the rough ER, releasing the ER-resident protein (with KDEL peptide attached) back into the ER lumen and the KDEL receptor back into the rough ER membrane.



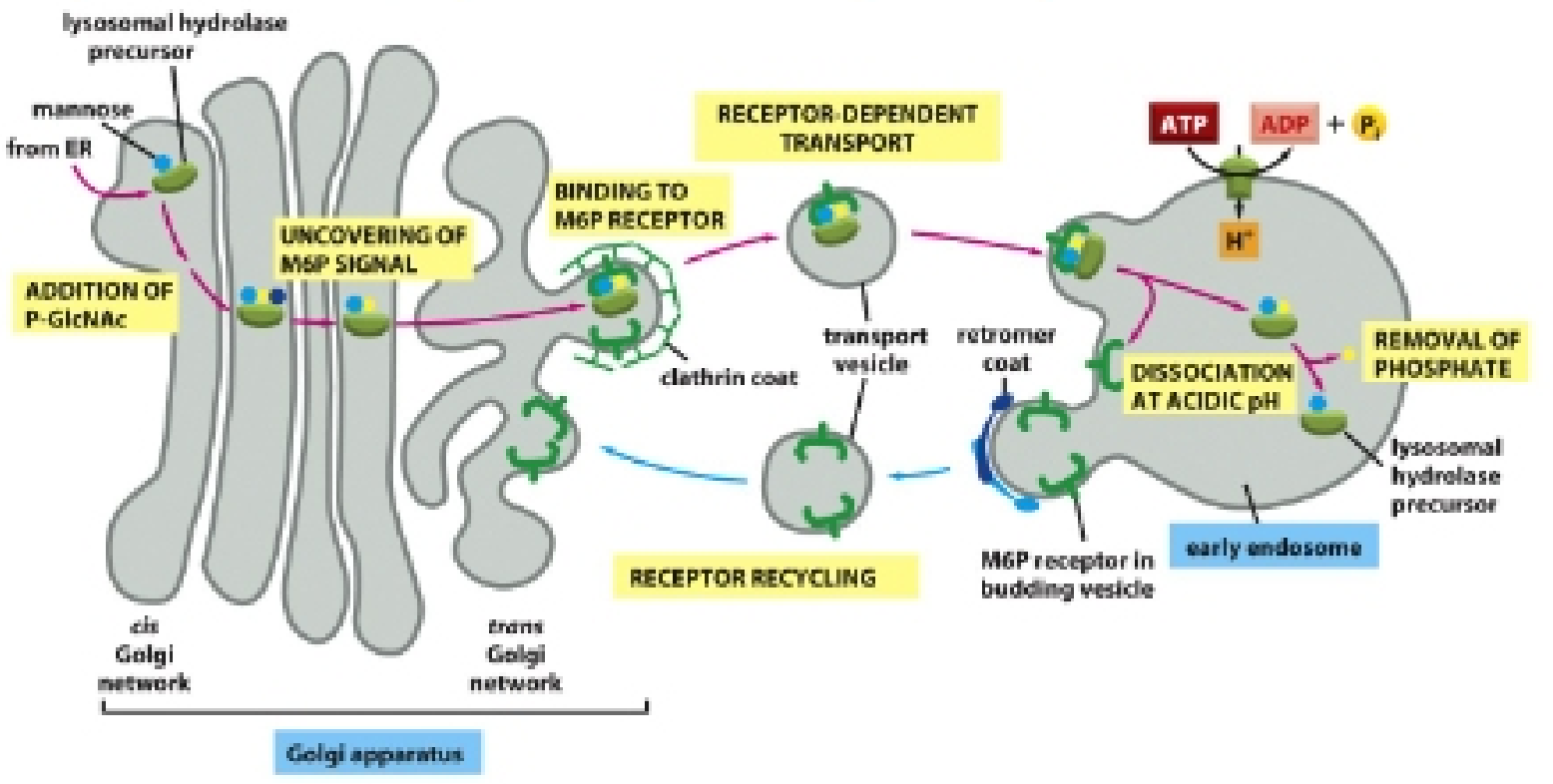
2. How do proteins migrate through the Golgi? Compare and contrast two existing models.

There are 2 existing models regarding how proteins migrate through the Golgi: (1) the vesicular transport model and (2) the cisternal maturation model. In the vesicular transport model, proteins are brought from the ER to the Golgi in vesicles that fuse to the CGN; these vesicles then bud off and fuse again with each cisternae in the Golgi until they finally bud off of the TGN and head toward either the plasma membrane or the endosome. In the cisternal maturation model, proteins are also brought from the ER to the Golgi in vesicles that fuse to the CGN; however, the proteins are then transported directly through the membranes of the cisternae (NO USE OF VESICLES) until they reach the TGN. Upon arriving at the TGN, the proteins bud off in vesicles and head toward either the plasma membrane or the endosome. Large Golgi products pass through the cisternae in this manner (via cisternal maturation/progression).



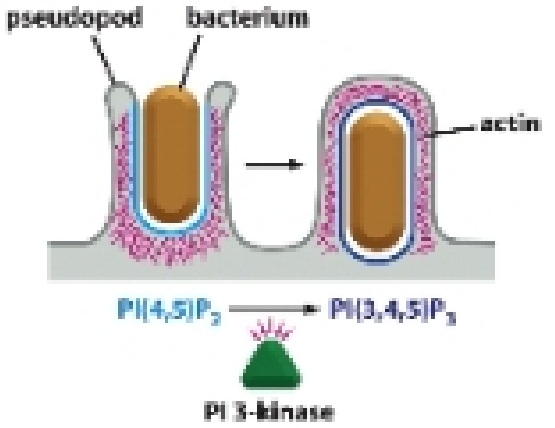
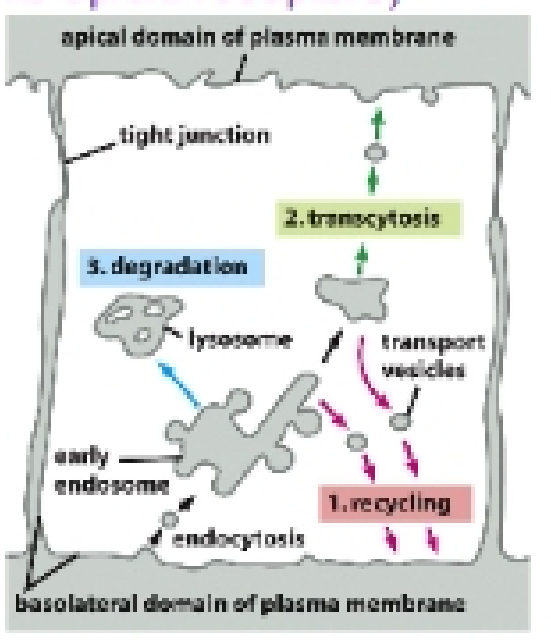
3. Describe how lysosomal hydrolases are transported from the Golgi to the lysosome.

Lysosomal hydrolases moves from the ER → vesicles → *cis*-Golgi → *trans*-Golgi → vesicles → lysosomes. Lysosomal proteins have a complex "signal patch." Mannose-6-PO_4 (M6P) acts as the sorting signal. M6P receptors bind M6P-modified proteins and coat adaptors with a clathrin coat. GlcNAc phosphotransferase adds GlcNAc-PO_4 to mannose in the CGN. GlcNAc is removed in the TGN, leaving only mannose-PO_4 behind. M6P receptors can then bind to lysosomal proteins in the TGN, where the pH is 6.7, but must release their cargo in the late



endosome, where the pH is 6.0; hence, M6P receptor binding is pH-dependent. The removal of the PO_4^- from the cargo and the low pH in the lysosome make transport unidirectional. After the PO_4^- is removed from M6P, it becomes mannse-6, which cannot bind the M6P receptor; M6P receptors are thus recycled from the early endosome back to the TGN using a retromer coat.

4. Compare and contrast three different kinds of endocytosis.

PHAGOCYTOSIS	PINO-CYTOSIS	RECEPTOR-MEDIATED ENDOCYTOSIS
• solids, phagosomes • pseudopodia growth around a particle is driven by actin polymerization  • a triggered process [antibodies are best known trigger]: particle binds to cell membrane via receptors → signal transmitted to cell • Rho-GEF activation: Rho GTPase → actin polymerization → PI-3K	• fluids, small vesicles • involved budding off and clathrin-coated vesicles • occurs with all eukaryotic cells • recycling of plasma membrane • clathrin-coated pits → clathrin-coated vesicles → early endosomes • specific example = caveolae • caveolae require other proteins to pinch off vesicle → use dynamin instead of clathrin coat to pinch off vesicle → transcytosis → may fuse to endosome (or not)	• macromolecules bind to specific transmembrane receptors • receptors cluster in clathrin-coated pits • some always associate with clathrin pits, while some only associate with ligand/cargo • 1,000 receptors/pit → mixed receptor population • endocytosis and fusion to early endosome • 3 different fates: (1) recycling (e.g., LDL receptor, transferrin receptor), (2) transcytosis (e.g., Fc receptor binds to antibodies), and (3) degradation (e.g., signaling/hormone receptors like opioid receptors)  • receptors retrieved from the endosome and sent back to the Golgi are recycled • transcytosis is when receptors move to a different part of the plasma membrane • degrading receptors lowers [receptor] after cell stimulation