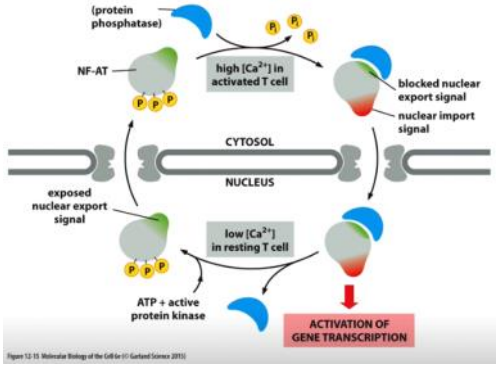
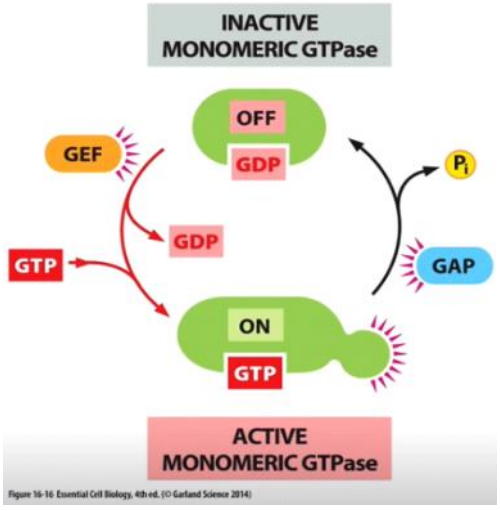
EXAM 2 MATERIAL

Monday, September 28, 2020 10:39 PM

Nuclear Transport

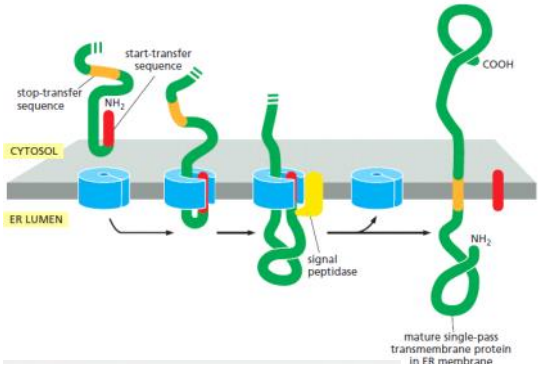
- General components needed for transport are:
  - Signal
  - Recognition of signal
  - Transporter
- Nuclear transport occurs at the nuclear pore, highly regulated complexes which control what can go in and what can leave the nucleus.
  - Nuclear pores are composed of **nucleoporins (nups)**, which have cytosolic fibrils that recognize and draw in importin proteins. In addition, a disordered region of channel nucleoporins also contributes to selectivity of the nuclear pores, such that only importins are allowed through with their cargo protein.
  - There are an incredibly large amount of nup proteins found in a nuclear pore, likely conferring an evolutionary advantage in multiple phenylalanine-glycine (FG) repeats, which allow for cross-linkage in membranes, allowing for more selectivity in nuclear pores.
  - The shuttling of proteins between the cytosol and the nucleus can control activation of gene transcription, which is essential for reacting to changes in the environment
    - One example of gene transcription is the regulation of calcium levels in the cell, as shown in the schematic to the right.
    - Another example would be feedback regulation of cholesterol biosynthesis. Typically, when cholesterol levels are high or normal in the cell, a protein which contains a release factor (which triggers cholesterol production when transcripted) would be found in the ER. However, when cholesterol levels start to get too low, the protein containing the release factor is transported to the golgi apparatus, which then is released and transported into the nucleus of the cell, where it triggers transcription and cholesterol biosynthesis.
- A **nuclear localization signal (NLS)** is a series of amino acids which, when read by special transporter proteins known as importers, allows for a cargo protein with an NLS to be transported into the nucleus. Similarly, a cargo protein with a **nuclear export signal (NES)** can be exported out of the nucleus.
  - Cargo proteins may also form complexes with nuclear import adaptor proteins which have a NLS, which allow for transport even if the cargo protein itself does not necessarily have an NLS.
- **Small GTPases** are molecular switches that control many different cell processes. Typically when a small GTPase is bound to GTP, is considered "powered on" whereas if it is bound to GDP, it is "unpowered." Many cycles are involved in the exchange of these small GTPases in the nuclear pore. One example is the cycle of Ran-GTP / Ran-GDP:
  - Ran-GTP is considered "activated" and is able to carry out its function, whereas Ran-GDP is "inactive" and unable to carry out its function until it is activated again.
    - **Guanine Exchange Factors (GEFs)** switch on the active Ran-GDP by removing GDP so that GTP may be substituted in its place.
    - **GTPase activating proteins (GAPs)** switch off the active Ran-GTP by hydrolyzing the GTP
  - Ran-GTP splits the importin from its cargo protein in the nucleus and carries the importin out of the nucleus while the cargo protein stays within the nucleus, whereas Ran-GDP is the by product of Ran-GAP hydrolyzing the Ran-GTP. When the GTPase switches off, the importin is released from the complex, and is once again able to bring in cargo proteins.





Endoplasmic Reticulum Translocation

- The ER is structurally and functionally diverse. There are two main types of ER:
  - Rough ER
    - Contains a sheet like structure coated in ribosomes
    - Site of membrane and secreted protein synthesis
    - Large percentage of total membrane - cells that secrete a lot of proteins, for example, will have a lot of rough ER as opposed to smooth ER.
  - Smooth ER
    - More tubular like structure
    - Site of lipid synthesis and calcium storage
    - Lack of concentrated ribosomes in comparison to rough ER.
- How does a protein get into the ER?
  - Bernhard Dobberstein and Gunter Blobel conducted an experiment in the 1970s to figure out how a protein entered the ER.
  - The researchers needed three main parts to figure out how a protein entered the ER of a cell:
    - Translation extract
      - Used to synthesize proteins
      - Contained cytosolic extracts and S-35 methionine, a special radioactively labeled amino acid which helps researchers to track where the amino acid is.
      - Also contains all the necessary components of translation, including amino acids, tRNAs, energy, so that the translation actually occurs.
    - ER Microsomes
      - Microsomes are samples of ER were isolated from ER rich cells (typically pancreatic cells) and were centrifugated to separate smooth ER from rough ER.
      - Microsomes allow for N linked glycosylation, attachment of sugars to the luminal side of the membrane.
    - mRNA for secreted protein
      - In the 1970s, recombinant DNA technology did not exist. Researchers therefore needed to isolate mRNA from an existing cell. This was typically done from antibody secreting tumors
  - The results of the reconstitution of protein translation from isolated fractions were run under a gel, one lane with the ER components and one without. The researchers then detected where the radioactive products were, and saw one band in the ER negative lane, but two bands in the ER positive.
    - Protease was then added to the ER - and ER + lanes. The sample without ER had been digested by the protease into many different fragments, whereas the sample with ER remained largely the same, but had only one band as opposed to the two original bands, suggesting that the ER+ sample had largely been protected from digestion.
    - The researchers concluded upon further study of the synthesized protein that there was a conserved sequence within all proteins synthesized that acted as a "passport" to enter the ER.
  - The **signal recognition particle (SRP)** is essential for travel of proteins into the ER. Ribosomes that are creating secretory proteins bind strongly to SRPs, whereas ribosomes creating cytosolic proteins have a weaker affinity for binding to the SRP.
    - The selectivity of the SRP allows for specific proteins to be targeted and moved into the ER, whereas other cytosolic proteins are formed in the cytoplasm without SRP interference.
- The mechanism of protein transport into the ER
  - SRP binds to the signal sequence of a newly emerging secretory ribosomal protein, pausing translation and directing the ribosome toward the ER.
  - SRP binds to receptor protein embedded in ER membrane (Sec61), activating a neighboring translocation channel, or a translocon
  - Polypeptide continues to be "threaded" through the translocon, with the signal peptide being cleaved off by signal pepsidase, leaving the protein synthesized in the ER of the cell.
  - A plug binds to the end of the translocon, closing off transport for newly synthesized proteins (putative)



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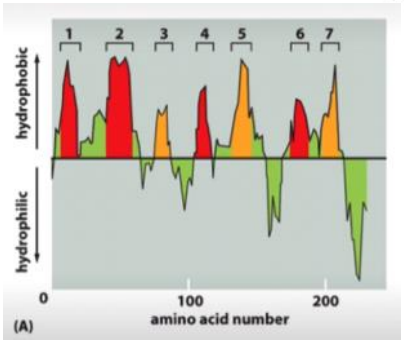
- Co-Translational Translocation vs Post-Translational Translocation
    - **Co-translational translocation** - the first peptide produced from the mRNA's end terminus is the signal sequence, which attaches to SRP and enters the ER membrane while the protein is translated.
    - **Post-translational translocation** involves the formation of the whole polypeptide first then the transport of that polypeptide to the ER membrane.
- Transmembrane proteins (single pass proteins) have *hydrophobic* stop transfer sequences which prevent the whole protein from being brought across the ER membrane (as shown to the right)
    - Similarly, multi-pass transmembrane proteins must also have stop transfer sequences, but in multi-pass transmembrane proteins, it is often the case that the start transfer sequence is embedded in the middle of the protein rather than at the very beginning. Multi pass transmembrane proteins may have many start and stop transfer sequences all within the polypeptide chain.
        - In the context of the hydrophobicity plot (Kyte Doolittle plot), the first two peaks must be start sequences, every odd numbered peak afterwards must be a stop sequence, and every even numbered peak afterwards must be a start sequence. (Shown to the right)
    - The integration of membrane proteins with internal signal sequences will depend on the charges of flanking sequences - typically the cytosolic side is more positively charged, whereas the ER lumen is more negatively charged.

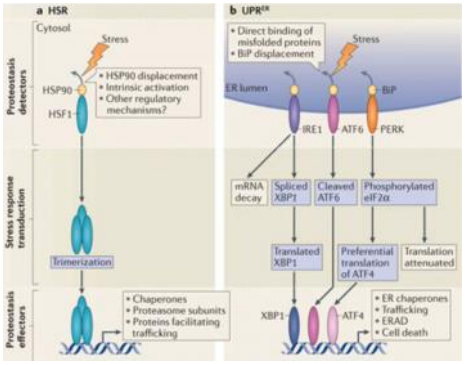


Protein Folding

- A newly synthesized protein follows a set of possible pathways to be folded correctly. If the protein is not folded correctly by entropy or chaperonin proteins, it may be digested by a proteasome. If not digested, the misfolded proteins may result in protein aggregates which cause stress and damage to the cell.
- Chaperone Proteins
    - Assist in the correct folding of proteins, with each type being very specific to their protein.
    - Two general classes of chaperone proteins:
        - Molecular Chaperones (monomeric)
        - Chaperonin (multimeric) - shaped like a barrel
- Protein Degradation
    - **Retrotranslocation** - the transport of a misfolded protein from the ER lumen back into the cytosol for its degradation.
    - Ubiquitin ligases target proteins to the proteosome for degradation - with the attachment of ubiquitin to a protein and ATP to power that attachment, misfolded proteins are broken down to their amino acid constituents.
        - When a misfolded protein undergoes retrotranslocation, the protein is immediately ubiquitinated upon exit of the ER lumen, as aggregation of misfolded proteins in the cytosol can potentially lead to greater cell damage - therefore, the E3 ubiquitin ligase complex is immediate to the protein translocator complex.

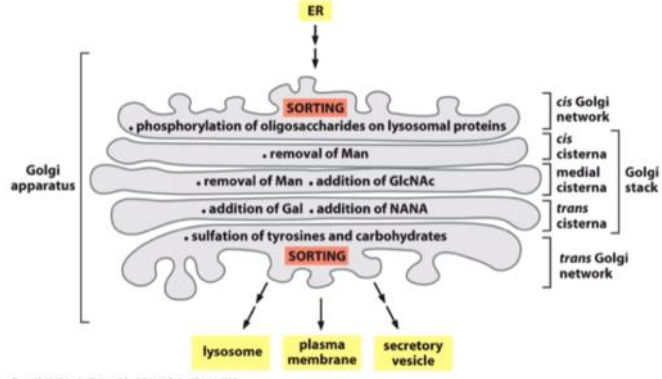
Unfolded Protein Response

- The accumulation of misfolded proteins have different effects in different parts of the cell. In the cytosol, the **Heat Shock Response** results from the accumulation of misfolded proteins. In the ER, the **Unfolded Protein Response** occurs, each with their own pathway.
- Specific mechanisms cells use to manage accumulation of misfolded proteins, in order of increasing invasiveness:
    - Expand chaperone expression (main impact of heat shock response in cytosol)
    - Upregulated proteins of Ubiquitin Proteasome Pathway
    - Degradation of transcripts
    - Decrease in translation
    - Expansion of ER membrane
    - Reduction of ER to Golgi trafficking
    - ER-associated degradation
    - Apoptosis



Membrane Trafficking

- The Golgi Apparatus
    - The Golgi is subdivided into three main parts, the cis-Golgi network, which faces the ER, the trans-Golgi network, which is opposite to the ER, and the cisternae, which is intermediate to the two.
    - The Golgi is the site of many types of post translational modifications, so specificity is determined by different enzymes that reside in distinct stacks of the golgi (as seen in the schematic to the right)
    - pH is a factor which allows for differential receptor affinity in the golgi complex, allowing for greater specificity within the golgi stacks, as to which enzymes are active on which stack. Generally, the further from the ER, the more acidic the environment of the secretory pathway.
- Vesicles can bud off of the ER and fuse with the golgi complex. From there, the proteins within the vesicles can fuse with the membrane of the cell or with other membranous structures within the cell.
- A general schematic of the transportation of secretory and endocytic pathways, where red represents the secretory pathway, green represents the endocytic pathway, and blue represents the retrieval pathway.



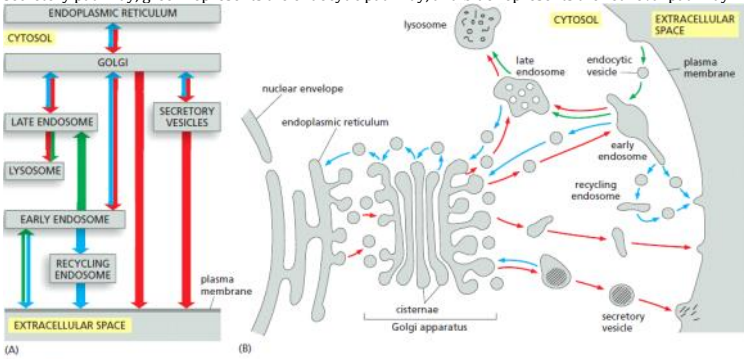
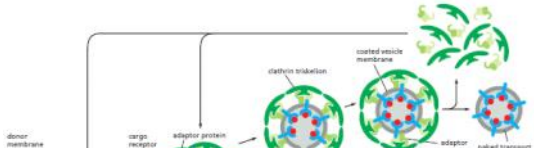


Figure 13–3 **A "road-map" of the secretory and endocytic pathways.** (A) In this schematic roadmap, which was introduced in Chapter 12, the endocytic and secretory pathways are illustrated with *green* and *red arrows*, respectively. In addition, *blue arrows* denote retrieval pathways for the backflow of selected components. (B) The compartments of the eukaryotic cell involved in vesicle transport. The lumen of each membrane-enclosed compartment is topologically equivalent to the outside of the cell. All compartments shown communicate with one another and the outside of the cell by means of transport vesicles. In the secretory pathway *(red arrows)*, protein molecules are transported from the ER to the plasma membrane or (via endosomes) to lysosomes. In the endocytic pathway *(green arrows)*, molecules are ingested in endocytic vesicles derived from the plasma membrane and delivered to early endosomes and then (via late endosomes) to lysosomes. Many endocytosed molecules are retrieved from early endosomes and returned (some via recycling endosomes) to the cell surface for reuse; similarly, some molecules are retrieved from the early and late endosomes and returned to the Golgi apparatus, and some are retrieved from the Golgi apparatus and returned to the ER. All of these retrieval pathways are shown with *blue arrows*, as in part (A).

- Vesicle budding is accomplished by **COATS**, or **coat proteins** that surround the vesicle which is displaced.
    - Coat proteins are responsible for selecting cargo proteins and physically deforming the membrane to



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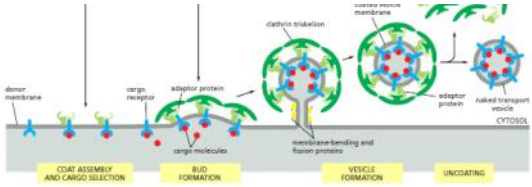
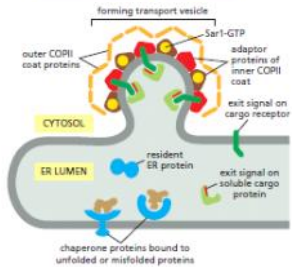
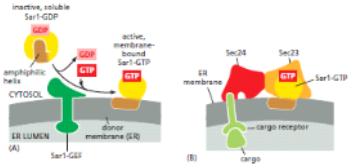


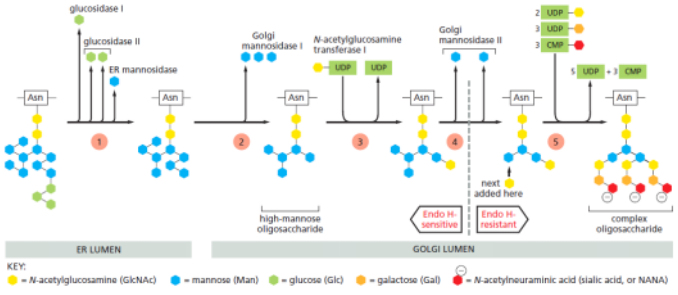




- Vesicle budding is accomplished by **COATS**, or **coat proteins** that surround the vesicle which is displaced.
  - Coat proteins are responsible for selecting cargo proteins and physically deforming the membrane to allow for the vesicle to leave its donor. After the vesicle leaves the donor, the COAT proteins must also disassociate with the vesicle (stop binding to the vesicle).
  - There are three distinct vesicle coat proteins: clathrin, COPI, and COPII, all of which form on specific membrane compartments.
    - **COPII** is found off of the ER membrane and coats vesicles destined for the golgi
    - **COPI** found off the golgi cisternae and the cis-golgi end (side closer to ER)
    - Clathrin COATS can be found on the trans-golgi (side further from ER) and everything that follows from vesicle secretion from the golgi.
- The Coatomer Proteins
  - Clathrin
    - Composed of a triskelion shaped complex of heavy and light chains
    - Mediates transport between the trans-Golgi network and the extracellular space and vice versa
    - Multiple clathrin complexes cover a vesicle in a lattice structure to select for proteins and transport the vesicle. Much like other coat proteins, clathrin must be removed from the vesicle (usually by chaperone proteins and auxilin) in order for the vesicle to be functional.
    - Relies on adapter protein complexes (AP complexes) to mediate the binding of the cargo receptor to the clathrin itself.
      - Different AP complexes assemble on different membrane compartments, increasing specificity for specific proteins in certain regions of the membrane.
  - COPI
    - Typically formed on Golgi compartments that are trafficked backwards, and may traffic vesicles back to the ER.
    - Relies on ARF1 small GTPase to assemble
  - COPII
    - Mediates transport between the ER and the cis-golgi
    - Relies on Sar1 small GTPase to assemble
- Mechanism of Action of Sar1-GTP and COPII

1. Sar1 GTPase is activated at the ER membrane with the binding of a GTP to the SAR1 by SAR1-GEF found in the membrane of the ER, allowing for a conformational change which exposes a amphiphilic lipid tail for the Sar1 to imbed itself into the ER membrane.
2. COPII inner coat proteins assemble between SAR1-GTP. These inner coat proteins are also known as adapter proteins (shown as Sec24 and Sec23 in the schematic to the right). These adapter proteins also contain GAP factors that are essential for shedding the COAT complex from the vesicle after it has bud from the ER membrane.
3. Cargo proteins bind to receptor protein in ER membrane
4. Receptor proteins recruited into inner COPII coat complex
5. COPII outer coat proteins assemble, causing conformational bending of plasma membrane.
6. If enough cargo is recruited, then the vesicle will pinch off, forming a COPII coated vesicle.
7. The coat proteins must disassemble from the vesicle using GAP (GTPase Activating Protein) factors that are found within the adapter proteins themselves.
   - Note that since the GAP factors are in the adapter proteins themselves, if the process of recruiting cargo proteins does not happen fast enough, the budding vesicle may fail to form entirely and the process must start anew.
   - Arf1 has a similar mechanism, but the main difference is that Arf1-GEF is localized in the cytoplasm.

- Targeting of vesicles to their acceptor membrane
  - Involves a new family of small GTPases known as Rabs, which confer specificity for targeting of transport vesicle.
  - Rab proteins will associate with the membrane of a transported vesicle mainly through the presence of **Rab GEF, which is localized to the donor membrane**, and will remain on the membrane even after the COAT proteins have dissociated from the vesicle.
  - Often times Rab proteins may recruit motor proteins to allow the protein to "walk" over a cytoskeletal track, directing the protein to the target membrane.
  - Rab proteins have a strong affinity for a rab effector protein, or a tethering protein, which is found in specific segments of the target membrane. **Rab GAPs are found at the target membrane.**
  - **Rab-GDI** is involved in the recycling of used Rab proteins from the target membrane to the donor membrane by escorting an inactive Rab protein (after Rab-GAP has interacted with it), resulting in a cycle of activation by Rab-GEF, deactivation by Rab-GAP, and shuttling back by Rab-GDI.
  - **v-SNARE (vesicular)** and **t-SNARE (target)** proteins provide more specificity and are essential for the fusion process between the donor and target membranes.
    - v-SNARE and t-SNARE proteins form a tight complex to each other that excludes water, allowing for the lipid membranes of both the vesicle and the target proteins to fuse together
    - When the v and t SNARE proteins complete membrane fusion, they must be dissociated with accessory proteins and energy in the form of ATP.
- Retrograde Transport
  - Retrograde transport is needed to traffic proteins from the golgi back to the ER. Often normal resident ER proteins may be accidentally brought into a vesicle and transported with cargo proteins to the golgi, and a mechanism is required to traffic the golgi back into the ER.
  - The ER requires many resident proteins for normal function, including BiP or calnexin. Misfolded proteins are retrotranslocated.
- Resident Proteins of the ER
  - Soluble resident proteins have a retention signal sequence, known as **KDEL**, named after the constitutive amino acids that make up the sequence. If a soluble resident ER protein is accidentally transported into the golgi, the KDEL sequence binds to an empty KDEL receptor embedded in the membrane of the golgi. COPI protein coats then bind to the KDEL receptor with the help of small GTPase Arf-1 for retrograde transport.
  - **EndoH**, an enzyme isolated from bacterial cells, is used to experimentally determine if a protein is trafficked past the cis-Golgi.
    - EndoH works by binding to exposed mannose chains on N-linked sugars and cleaving the oligosaccharide. If there are no exposed mannose chains, the EndoH will not be able to bind.
    - Between the cis-Golgi lumen and the Golgi cisternae, mannose will be blocked off from interacting with EndoH, as there are no exposed chains for the EndoH to react with the sugar chains. Therefore, proteins in the secretory pathway found before the cisternae are EndoH sensitive (meaning that they will be cleaved) and proteins found after the cis-Golgi lumen are endoH resistant (unable to be cleaved).



The Lysosome

- Lysosomes contain digestive enzymes that can break down intracellular and extracellular components.
- The lysosome is born from the endosome and golgi vesicles.
  - When a part of the plasma membrane is endocytosed into the cytosol, an endocytic vesicle is formed, resulting in an endosome. This early endosome will gradually become more acidic through the help of vacuolar ATPase pumps within its membrane adjusting proton concentration within the organelle.
  - The digestive enzymes, also known as **acid hydrolases**, of the lysosome originate from the rough ER and are secreted into the golgi as is commonplace in the secretory pathway.

    - When the enzymes leave the golgi, they are tagged with **mannose 6 phosphate (M6P)** so that the enzymes end up in the correct endosome. M6P also causes a conformational change in the digestive enzymes that deactivate them.
    - When the M6P tagged vesicle recognizes and fuses with a late endosome, the acidic environment in the interior of the endosome removes the M6P from the enzymes and activates the digestive enzymes, allowing for a late endosome to mature fully into a lysosome.
- There are three main cellular processes that the lysosome is involved in:
    - Autophagy - recycling or digestion of intracellular components.
    - Endocytosis - degrading of objects originating from outside of the cell.
    - Phagocytosis - the intake of large particles to the cell to be digested.