Week 7

Bulk Transport

7.1 Exocytosis and Endocytosis

11/8:

- Two parts of lecture today:
  - How a protein that is formed in the ER reaches the plasma membrane.
  - How proteins in the plasma membrane get to other parts of the cell (endocytotic pathway/other plasma membrane locations).
- Krishnan has really enjoyed teaching this class :)
- ER to Golgi transport.
  - The golgi is the pathway to the plasma membrane.
  - We don't know too much about the Golgi.
  - Glycobiology.
  - Will become one of the most important organelles in the future because it's very important and we don't know that much about it.
  - The Golgi is very hard to model (it's a stack of pancakes, and these are hard to distinguish).
  - Proteins that go to the plasma membrane and lysosomes and get secreted all have to pass through the golgi.
- COPI and COPII coated vesicles.
  - How a protein starts its journey.
  - If there's a protein in the ER lumen, there's a massive sugar transferred from dolichol to a particular asparagine.
  - Every protein that get's secreted gets so labeled.
  - Don't worry about what happens to that sugar rn, but it's like an assembly line (different steps of the packing process, all delocalized).
  - If you have a huge amount of transport proteins, you're going to have errors (things getting sent out that shouldn't), so you need a way to bring them back.
  - When the ER buds, we have to concentrate the proteins inside it.
  - The vesicle is covered in a protein called COPII (the COPII complex) that signals it as outbound from the ER.
  - ER-Golgi intermediate compartment does exist.
  - Proteins that shouldn't have left get sent back (in vesicles coated with COPI).

  - Anything in the ER (most proteins that need to be secreted) need to fold.
    - There are chaperones in the ER (such as BIP, calnexin) that help other proteins fold.
    - Many big proteins (90% of them) don't fold properly, so proteins need ways to make sure that they're only excreting the right proteins.
    - The ER is host to many unfolded proteins. Chaperones recognize anything that is misfolded and mark them for degradation.
  - How do proteins get into the vesicle?
    - Proteins have an exit signal that interacts with a cargo receptor.
    - Cargo receptors cluster on the surface.
  - Now the patch buds out.
  - These receptors usually have a lumenal domain and a cytosolic domain.
  - Adapter proteins recognize a bound form and cause proteins to cluster. COPII then assembles on the outside of the vesicle.

- The basics of vesicular fusion.
  - A lot of fission and fusion today.
    - Big in neuroscience and neurobiology, but also occur in cell biology.
    - These occur because of t-SNAREs and v-SNAREs (allow vesicles to fuse).
  - Vesicle fusion: **Homotypic fusion** and **heterotypic fusion**.
    - You also have **N-ethylmaleimide sensitive factor**.

- **Homotypic fusion**: The fusion of two like membranes.
  - E.g., two lysosomes fusing to form a bigger lysosome.

- **Heterotypic fusion**: The fusion of two different kinds of membranes.
  - E.g., a COPI-coated vesicle and the ER.

- **Vesicular SNARE**: Occurs on every organelle. *Also known as* **v-SNARE**.

- **Target SNARE**: The specifying SNARE. *Also known as* **t-SNARE**.

- **N-ethylmaleimide sensitive factor**: *Also known as* **NSF**.
  - Pries apart t-SNAREs and v-SNARES, allowing fusion.
  - The two helical domains will then move apart.

- KDEL is an ER-retrieval sequence.
  - How do we send proteins that accidentally localized to vesicles back?
  - Original way out:
  - Bulk-phase endocytosis: Letting the vesicle fill up naturally and the sending it back; not specific.
  - Alternatively, you can attract your proteins to the future vesicle (receptor-mediated endocytosis).
  - The purpose of the receptor is to concentrate your substance.
  - How does cargo that's exited this way get sent back?
  - KDEL receptors bind the sequence KDEL in a protein. A bit in the golgi, but most of it is in the ER. But its function is to grab ER retrieval sequences on the N- or C-terminus. pH dependence.
  - How will an ER retrieval sequence differ from a localization sequence?
    - The fundamental difference is one is regulated, and the other is spontaneous.
  - What we still don't know: How does a vesicle get switched from a COPII coat to a COPI coat?

- Spatial position of the Golgi in the animal and plant cell.
  - 5-6 stacks in human cells.
  - 100-200 stacks in plant cells (plant cells have to secrete a huge amount of material to maintain a cell wall).
  - More stacks (cisterni) lead to a more advanced golgi.
  - Cisterni are well below the wavelength of light, so we need electron microscopy.
- Molecular compartmentalization of the Golgi apparatus.
  - Our protein gets dolichol-marked.
  - Different reactions in different cisterna.
  - Different enzymes in each cisterna.
  - Each location in the Golgi acts on the protein differently before its eventually secreted due to the difference in enzymes stored in each region.
  - Cis-Golgi: beginning of the Golgi (closer to ER).
  - Stack.
  - Then trans-Golgi (end; opposite side from the ER).
  - Krishnan goes over an experiment showing what's localized in what compartment (different tagging proteins tag different enzymes, revealing localization in an electron micrograph).
- Oligosaccharides processing in the Golgi.
  - N-acetylglucosamine (GLcNAc).
  - Mannose (Man).
  - Galactose (Gal).
  - Sialic acid (NANA).
  - These all get attached. How far along a cargo has gone is decided by the sugar ordering.
  - Initial trimming of mannose and glucose. Trimming takes off a lot of these sugars, and then we replace with specific sugars (specifically those 4 above).
  - When a protein/lipid that's gone through the entire process reaches the plasma membrane, it will be able to show 2-types of sugar: Complex oligosaccharides (with a high concentration of negatively charged sialic acid at the end) and high-mannose oligosaccharides that do not get sialic acid added because the sugars arranged on the protein are inaccessible.
- What is the purpose of glycosylation?
  - Most important slide of the first part of the lecture!
  - In the ER lumen, we have two initial enzymes (glucosidase I and II) that chop off glucoses.
  - Manosidase in the ER takes away mannoses.
  - Leaves behind a sugar that's good to go to the Golgi.
  - Golgi mannosidase takes off 3 mannose residues at a time (the accessible ones).
  - In the medium golgi: We start adding GlcNAc, then add galactose, then silylation.
  - GlcNAc is added by N-acetylglucosamine transferase I.
  - Something attached to gludine, which is a good leaving group (highly anionic).
  - All these glycosylated molecules are present inside the comparment.
  - How does localization happen?
    - We have membrane proteins that will take in particular molecules and will work with them in one particular compartment.

  - Another mannosidase event (Golgi mannosidase II).
  - Now proteins are Endo-H resistant.
  - Addition of 2 more GlcNAc molecules, then galactose, then silylation. These give us our complex oligosaccharide.
  - The rules of the molecule Endo-H (of bacterial origin) tells you how far a sugar has gone in its journey. Helped us figure out many enzymes and transporters involved in the pathway.
  - You can work out the molecular weight of a protein.
  - High gets sent one place, low gets sent another place. This helped us investigate stuff.

- Two models of Golgi-protein transport.
  - We still don't know this (it's being researched, largely at UChicago).
  - What is the model of protein transport in the Golgi?
  - Does a *cis*-Golgi gradually mature (Golgi cisterni "grow up", gaining/losing proteins along the way), or do we have proteins transferred between fixed cisterni. The other one has vesicles budding out to either go forward to the next cisterni or back to the previous cisterna.
  - **Cisternal maturation model** vs. **vesicle transport model**.
  - If you want to know which is currently winning, write to UChicago's Ben Glick :)

- What keeps the golgi together (why are all of our pancakes stuck together)?
  - There are hydrophobic tentacles called golgins that wind together and prevent cytosolic fluid from getting between the pancakes.
  - At the time of cell-division or during apoptosis, we need to disentegrate the golgi because we can't have ?? hanging around.
  - This is induced by a kinase which phosphorylates the golgins, causing fragmentation.
  - Once the cell membrane comes back, the golgi reform and you get new ones.
  - When a cell divides, its organelles must divide, too, and we only have one golgi.
  - We build a new cisterni atop the old one.

- Onwards and outwards: Exocytosis and secretion.
  - There is a basal level of secretion that happens all the time, and there is regulated secretion where you have to release a massive amount of something all at the same time.
    - Regulated example: Insulin.
  - What needs to be secreted **basally** (all the time)? Mucus!

- Protein sorting at the TGN.
  - Once something comes to the trans-golgi network, where can it go? To the lysosome, outside the cell, and constitutive (e.g., placing things in the cellular membrane).

- Secretory vesicle maturation.
  - Very important!
  - How do we send out a huge amount of glucose, or melanin?
  - We concentrate molecules/proteins into dense core secreted granules (100-200 nm), extremely high concentration.
  - Longer peptide has a secretion clock. At each point, you have a pausing condition.
  - You take advantage of processing to pack tight.

  - Recall the tight junction from the first lecture (intestinal cell). Different parts of a membrane have different properties. Non-leaking proteins. You need a way to get proteins to exactly one part of the plasma membrane (how this works still isn't understood very well).

- Sorting plasma membrane proteins in polarized cells.
  - Two models: Direct and indirect sorting.
  - One model is different vesicles go to different locations. The second is you get random input into the plasma membrane, and you then have sorting at the endocytotic level with the help of an endosome that takes ones in the wrong place to the right place.
  - One recycling endosome goes to the cell surface, the other one elsewhere.

- Golgi to lysosome transport.
  - Lysosome proteins are all set up for proteolysis (chopping things up) and glycosidases. Take old cellular machinery, chop it up into its component parts, and let it get recycled.
  - A cell *must* recycle stuff, or it will need to make so much more amino acids.
  - Lysosomes are *highly* acidic and contain degratory proteins.
  - Why don't lysosomes digest themselves?
  - pH 5 and the proteins still work. Usually this pH would denature the protein, but instead lysosome proteins are built for this.
  - Hydrolases chop stuff up; they've evolved to withstand the highly acidic environment.
  - Mannose phosphate receptor in the Golgi: A bus to the lysosome and back (takes hydrolases from the trans-golgi to the pre-lysosomal compartments). Not a lysosome resident protein. Why doesn't it get denatured? Glycosylation of the receptor protects it from the hydrolase and the lysosome; can't purely operate at pH 5 but has to be stable at multiple.
  - Glycosylation tree branches around a specific protein protect it from the actions of the lysosome.

- Transport of newly synthesized lysosomal hydrolases to endosomes.
  - The mannose phosphate receptor is our bus.
  - We have a standard lysosomal hydrolase.
  - Carries a mannose as it enters the Golgi.
  - Addition of phospho-GlcNAc allows us to put a phosphate onto manose, and then it goes away. It's like a cofactor. Then it gets cut off.
  - Protonation allows the protein to fall off and enter the lysosome.
  - Then a retromer coat allows our receptor to be retrieved.

- Recognition of a lysosomal hydrolase.
  - Start with a lysosomal hydrolase carrying a glycosylation on the N-terminal .
  - GlcNAc phosphotransferase transfers a phosphate onto the lysosomal
  - UDP-GLcNAc transfers a phosphate and a GlcNAc to the lysosomal hydrolase, kicking out UMP.
  - Then the enzyme releases the lysosomal hydrolase. We then remove the GlcNAc, leaving mannose 6-phosphate behind (M6P).

- Ways to enter the lysosome.
  - Phagocytosis: Take in a bacteria to test it; use the lysosome to break it down into pieces that can be used by the rest of the cell (e.g., for defense).
  - Endocytosis: Take things in from the outside and digest them.

  - Autophagy: We automatically create a vesicle around something inside and move it to the lysosome.

- Endocytosis.
  - We take stuff in from the outside to an early endosome (vesicle within a cell; an intracellular sorting organelle).
  - Microtubule mediated transport to wherever we need, e.g., the lysosome, the trans-Golgi network, etc.

- Different mechanisms of endocytosis.
  - Macropinocytosis: An appendage sticks out and closes in.
  - Clathrin-coated vesicle: ...
  - Noncoated vesicle: ...
  - Caveolae: ...
  - Phagocytosis: ...

- Clathrin-mediated endocytosis.
  - Clathrin shapes rounding.
  - Makes things go to specific plases.
  - Receptor-mediated endocytosis.

- Receptor-mediated endocytosis.
  - Receptors on the plasma membrane draw external stuff into the cell.

- Recycling endosomes.
  - We don't want to destroy things we'll use again.
  - Membrane proteins that aren't needed go to an endosome, and when they're needed, they bud off and go back to the membrane.

- Degrading proteins: Autophagy.
  - Nucleation and extension: Bits of phospholipid engulf cytosol and organelles.
  - Transport to the lysosome.
  - Digestion: There, acid hydrolases break down the material.

- Transcytosis.
  - Moving something through a cell and to the other side. Think intestinal cells.
  - Endocytosis on the one side. Transport to the early entosome. Multiple pathways from there.
  - We can have an empty transport vesicle go back to the plasma membrane to replenish the phospholipidds there.
  - A full one can bud off to go for degradation in the endolysosome.
  - A full one can bud off and go to a recycling endosome for transcytosis to the far cell wall.

- Exocytosis.
  - Needed for cytokinesis, phagocytosis, plasma membrane repair, and cellularization.

7.2 Office Hours (Krishnan)

- Exam format:
  - There will be about 10 questions on the paper, so about 12 mins/question.
  - 15/65 points on protein transport and localization.
  - Some questions on sequencing.
  - There will be one hydropathy index plot question.
  - Review strategies: Go through the four lectures that have been taught.
  - The guest lecture will not be covered at all on the midterm.
- What is oxidative stress?
  - Not Krishnan's question; won't be testable.
- What primer do you create if you haven't sequenced yet?
  - If you want to sequence my CFPR gene, for example, we have the whole human genome at this point in time, so you can assume that mine will be similar. Thus, we design based on past precedent.
  - If you want to do whole-genome sequencing, you sonicate your DNA and don't know what starts and ends where. In this case, use an adapter to specifically target certain parts.
- What are these adapters?
  - There are many ways to attach an adapter. You can use a poly A polymerase which adds tons of adenines to the end and then adapt to that.
  - You can also use ligase to attach the $5'$ end of your adapter to the $3'$ end of your DNA duplex. Similar if you have a $3'$ adapter.
- What does it mean that apyrase is an eraser?
  - An apyrase converts ADP to AMP. It is basically a reset mechanism that allows you to add the next nucleotide.
  - Recall that in pyrosequencing, you have a bead with clonal DNA copies all over it. Dumping in a certain dNTP...
- How do the variable lengths in PCR come in? How do they eventually become the same length?
  - See the videos on Canvas.
- In Maxam-Gilbert sequencing, is the strand cleaved before or after the modification.
  - Chemically, each modified nucleotide is significantly altered during the reaction, and the phosphate backbone breaks on both sides of it. You will see this in PSet 3, Q2a.
  - Thus, it is best if we describe cleavage as happening "before" the modification, since everything after the last base before the modification will either be cleaved away or entirely destroyed.