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Good morning. Good morning. Today I'm going to talk about, this is lecture 14, and I'm going to talk about protein localization.

Now, some of you may remember that earlier in the semester I was walking around with this sling. And so to help me from writing on the board, even though it is this arm, I have made a PowerPoint presentation of most of the things that I would have written on the board. And for your ease and comfort, this PowerPoint presentation will be posted online so you won't have to write down everything in my slides. So just sort of sit back. And I will write a few things on the board, so you can write those down.

OK. So now you guys have heard about central dogma from Professor Eric Lander and you've heard about gene regulation last Friday.

Here is something that you're familiar with, this image here depicting central dogma.

DNA replicates to DNA. This is replication. Replication.

DNA is translated, excuse me, transcribed to RNA.

Transcription. And RNA is translated to protein.

Central dogma. Where does this occur? Where does replication occur in a cell? Nucleus. Good.

Where does transcription occur in a cell? Nucleus.

I heard nucleus. That is correct. And where does translation occur? Cytoplasm. Ah, and yet we know that these processes require proteins to do them.

OK. You've just described where these processes occur in a eukaryotic cell. Let's say you're a bacterium.

In bacteria, where does replication, transcription and translation occur?

Where? Cytoplasm. OK. So now we've made bacteria look very simple. But they're not that simple. And so let's take a look here.

Here's a bacterial cell. I've drawn what could be E.

coli. I has an outer membrane, an inner membrane, and the space in between is the periplasm. Now, here is its circular chromosome. I've transcribed some gene to an RNA and a ribosomal pop on and make a protein which his in the cytoplasm.

Yet some proteins are localized to the inner membrane, others are localized to the periplasm, and some are

localized to the outer membrane, and others are actually exported completely outside the cell. Even more complicated is a eukaryotic protein, because not only does it have a plasma membrane where proteins are localized. It has a bunch of organelles. There's the nucleus and there's mitochondria and there's endoplasmic reticulum and Golgi apparatus.

And it, too, translates RNA by ribosomes in the cytoplasm.

So how do these proteins get back to the nucleus or go into the mitochondria or get into the organelles? So what we're going to do in the next few slides is we're going to follow the process, because they're so similar in bacteria and eukaryotic cells, of how proteins get to the membrane and how they get outside the cell.

And then I'll go back and talk about how proteins get into some of the organelles. So let me show you what some of the proteins are.

So an example of a cytoplasmic protein in bacteria is beta galactosidase. You've heard about it.

It breaks down lactose. It's in the cytoplasm. An example of a membrane protein is a lactose receptor.

The lacY permease that's on the surface of the cell brings lactose in. An example of a fully secreted protein is a toxin.

For instance, bacillus anthracis makes anthrax toxin.

It's completely exported from the cell. In a eukaryotic cell there's a bunch of cytoplasmic proteins. There are all of the glycolytic enzymes. And, for instance, biosynthetic amino acid enzymes like histidine synthesis enzymes, those are cytoplasmic. For a membrane protein there are receptors, like the receptor for insulin, a hormone, a peptide hormone, or growth factor receptors, every receptor that's membrane-bound. And a fully secreted protein.

Some cells like pancreatic cells secrete insulin.

Some cells like some of your immune cells secrete antibodies.

OK. So it was not clear how these cytoplasmically made proteins, proteins that were made in the cytoplasm got to this location.

And the person who worked on this was George Palade.

And this was in the fifties. And he studied pancreatic cells because they're master secretors. And he was able to perfect his microscopic technique. And you can see here this is a pancreatic cell. This is endoplasmic reticulum

studded with ribosomes. These are mitochondria.

This is the nucleus. Here is another picture that he took.

And here is the rough endoplasmic reticulum studded with ribosomes.

Here's Golgi apparatus. And then there are like little vesicles.

So he did this experiment where he decided he would pulse label proteins as they were being synthesized in a pancreas, directly in a pancreas. So what he did was he injected radioactive amino acids directly into the pancreas of hamsters.

I guess I could draw a little hamster here. And he directly injected radioactive isotopes.

And what he's doing is these radioactive amino acids will be incorporated into proteins as they're being translated, and he can follow the population of freshly translated proteins through the cell. So he injects hamsters with the radioactive amino acids.

And then at various time points he adds, he also injects glutaraldehyde.

So first the label, then glutaraldehyde. And what this does is it fixes the cells in its tracks. Whatever the cell is doing it just stops. And he removes the pancreas and he looks at the cells. This is fixes the cells.

So, Tom, I don't know what's going on here. Can we not use this, Tom? All right. It's just doing it on its own. It has some time thing?

Oh. All right. So what he found was at the early time points, now, what I did, I did this, OK? He didn't see yellow. What I did was I added yellow to his original slide to show you at the earliest time point he found the label associated with the endoplasmic reticulum. At the next time point he found the label associated with the Golgi apparatus. And then at even later time points he found the label in secretory vesicles.

So this is my representation of what he found.

So here's a cell, nucleus, mitochondria.

The early time points the label was in the ER followed by the Golgi.

I didn't do that. Yeah, take it out.

Followed by vesicles. It's not working. OK.

Sorry for that. I'm sorry. So he won a Nobel Prize for this work because it is the pathway that still holds true today.

Now, he won the Nobel Prize in '74. And at about that time, maybe '71, another scientist names Cesar Milstein was working on immunology.

And what he did was he fused a cancer cell with a cell that constantly secreted antibodies, so he ended up having an immortalized antibody producing cell. And he was doing some research and he did in vitro analysis. And he found that antibodies that were produced in vitro were longer than the ones that were actually coming out of the cell. So he proposed that there was an N-terminal end. He looked and saw that the N-terminus was different, and he proposed that it would possibly be cleaved upon export. OK? And so this is, he won a Nobel Prize, not for this work but for his work in immunology.

And this was also correct. And so this is from his lecture, Nobel lecture. It says in vitro synthesis of immunoglobulin light chains, that's what he was doing, to our delight we ran into the unexpected observation of the existence of a biosynthetic precursor of light chain. Further experiments led us to propose the extra N-terminal sequence was a signal for vectorial transport across the membrane during protein synthesis.

This was the first evidence that indicated the signal for secretion was an N-terminal segment rapidly cleaved upon protein synthesis.

OK. So now there was a student of Palade. He was a post-doctoral student. His name was Guther Blobel.

And he saw this experiment in 1971. And he thought how do we know it's not an artifact of in vitro science? Well, how do you know that the ribosomes are not just hopping on earlier in the message?

Maybe that's why it's a longer protein. And he didn't buy it.

So he wanted to further pursue this. And he did it in the Palade manner. So what he did was he took a test tube and he added message for exported protein, ribosomes and charged tRNAs. And when I say charged tRNAs I mean tRNAs that have amino acids attached to them. And so if you add those three things to a test tube you find a protein is made. So then he added microsomes. What are microsomes?

OK. We're going to pause this.

Now, I told you what Palade found out. He found out that proteins were first seen in the rough ER in the lumen. This is the lumen right here. This is where he first observed radioactivity.

Now, if you take endoplasmic reticulum and you shear it, it forms little tiny vesicles, little vesicles with ribosomes on the outside. And they're called microsomes for small things. Microsomes. And they're essentially little rough

endoplasmic reticulum vesicles. So when Blobel added to the same test tube that had the RNA, the ribosomes, the tRNA.

When he added microsomes he found that the protein was still in the supernatant. There was no protein found in the lumen of the microsomes.

So he figured, OK, it needs something.

Let me go extract something from the cytoplasm.

And he extracted lots of fractions. And he added these cytoplasmic fractions. And he found that one fraction actually was able to cause the peptide to enter the microsome. And if he added this fraction late in the reaction, the protein would never get into the lumen of the microsome. But if he added the fraction late, I mean, excuse me, early, if he added the fraction early they would get in. So let me just summarize. So message ribosomes, tRNA, you find protein in the supernatant. Message ribosomes, tRNAs, plus microsomes, protein in the supernatant, not in the microsomes.

You add a fraction that works sometimes, but if it's added late the protein is in the supernatant. But if you add that fraction early the protein is in the lumen of the microsomes. So he interpreted this result as that there was an amino acid sequence at the beginning of an exported protein. And that's recognized by a complex that was in the fraction. This complex is required to get the protein to the lumen of the ER. And to get to the lumen of the ER the protein has to be just beginning to be translated.

Now, since not all proteins have the same N-terminus, Blobel predicated, like Milstein, whatever the sequence was it would later be cleaved. And he won a Nobel Prize for this in 1999. And it wasn't just for this, because he went on and he actually figured out the entire pathway.

In the next few slides I'm going to show you what he discerned.

One thing I want to just point out, though, the experiment he did was heterologous. So the extract came from wheat germ, the microsomes came from dog, and yet it still worked. And it was right because this pathway is universal. And let me show you how universal. It's used in bacteria and it's used in eukaryotic cells.

So here's a bacterium, it's translating a message.

Here's the signal starting to be translated, it's an exported protein.

The same thing, in the cytoplasm a signal sequence is being newly made. And here's a close look of the signal sequence. It's about 20 amino acids long.

It has a couple of positive charges at its extreme N-terminus.

In the middle there's about seven to twelve hydrophobic amino acids variable. And this called a signal sequence.

OK, so let's take a look at what happens. OK. Now we're in the cytoplasm of a eukaryotic cell. Here is a signal sequence emerging from a ribosome here. What recognizes it is SRP.

That's what he named his complex for signal recognition particle.

So SRP binds to the signal sequence. And, if you recall, it takes it to the ER to be translated. Here's a picture of the ER.

And there's a docking protein or SRP receptor. So the SRP binds to the docking protein, it brings with it the signal sequence which is attached to the ribosome, which is attached to the message. Adjacent to the docking protein is a translocon which is a channel composed of proteins. The ribosome pops onto the translocon. The SRP floats away. And notice that the signal sequence is in the membrane, excuse me, starts to enter through the membrane and translation resumes.

The signal sequence is cleaved by a signal peptidase within the ER, it cleaves off the signal and translation continues.

And if it's a fully secreted protein it's fully internalized within the lumen of the ER and the ribosome pops up.

If it's a membrane protein the signal is cleaved again, translation resumes, and then it gets imbedded in the membrane.

And so I'm going to shut this off, otherwise it's going to keep going on its own here. So if it's a membrane protein what does it have? It has a transmembrane stretch.

You've seen this maybe before in problem sets. So it's a transmembrane stretch.

Or transmembrane domain. It's about 20, 22 amino acids long.

It can be 30 maybe. So we'll say 20 to 25 amino acids of hydrophobic residues.

It is a stop transfer sequence. Stop transfer for going across an ER membrane. It anchors it in the membrane.

It forms alpha helix.

If this part is the lumen of the ER right in here then this is the cytoplasm.

OK. So that's how membranes and proteins look of this kind. Now, as you can see here, it's in the membrane. It's imbedded in the membrane.

And I've drawn a different membrane protein over here because this protein is going to work its way to the far side of the endoplasmic reticulum. OK? As the fully secreted protein will also work its way. In the endoplasmic reticulum sugars get put on these proteins. So when they get to the far side they bleb off into little transport vesicles.

OK? So here's the cytoplasmic protein completely within the lumen of the vesicle. Here's the membrane protein imbedded in the membrane of the vesicle. And if you remember Palade sequence, the next stop is the Golgi. So the head over to the Golgi, they bind, they fuse, and what was imbedded in the membrane is still imbedded in the membrane. And the fully secreted protein is within the lumen of the Golgi. Here the sugars are modified. I put little bows on them. And they work there way over to the far side of the Golgi where they bleb off again into secretory vesicles.