BARBARA IMPERIALI: I always like to just remind you that the sixth -- it's kind of an assignment, but the numbers-- we're going to do this news brief project, where it's a teamwork project if you choose. If you take a look at the piece that you have in your hands now, it asks you for a little bit of information on that, who you're going to be working with, if you choose to work with someone. Or you can work on your own. That's fine.

And we're looking to get a news brief that's of significance to research going on in the life sciences. And I've given you-- there are a couple of links in the sidebar of the website, so good places where you can find interesting material. What I'm super interested in for you, as a group where many of you are in the engineering fields, is to find something really cool at the interface between the life sciences and engineering, where engineering has a huge impact on the life sciences.

You have alternatives. You can download the coordinates of a protein and print it on a 3D printer and give us a summary of what the protein is, what it does, and submit your 3D print. I'll give it back to you afterwards, once we've had a look at it. But actually submit the 3D print.

And then the other opportunity is-- I think you'll remember back to when we were talking about molecular biology of the cell. I did kind of a clunky demo at the front of the class, nothing like Professor Martin's demos at all. This was me with the ethernet cables showing you what topoisomerase did. But in my demo, I didn't show you how topo also cuts a strand of DNA, holds it while the supercoiling unwinds, and then stitches it together. So I thought some of the engineers might be able to come up was something that was really better than that for me to use, for us to use, next year in class. So I'm really laying down the challenge there.

So I always like things in the news. I thought this was kind of interesting that the first vertebrates evolved in shallow waters. I thought those were really cool first vertebrates. I'd love to get one of them in a fish tank and keep it. But anyway, that's that.

It's truly amazing what you can see in the science reports, news briefs. I look at them whenever they come in. I get the posts every two or three days. And I'm kind of pleased to see that there's a lot of things that are in those news briefs that I feel that we're enabling you to read with some appreciation because of what we're covering in the class.

So what we're doing now is we're really taking a leap forward here into cells and organisms,

with respect to understanding how structure and function of individual macromolecules, proteins, nucleic acids, sugars, determine life, determine the dynamics of life that are necessary for an organism to really go through a life cycle, divide, have cells divide, go forward, have cells move. So what we're going to be talking about in the next lectures, which is section 6, is cellular trafficking and signaling. And so for the first lecture, which is 19 that we're on now-- so we're past the midway mark-- I'm going to be talking about trafficking. And that is how, within a cell, things get to where they need to be, or they get exported from a cell.

Because all of the actions of a cell-- I really like thinking about the cell as a circuit board, where there's a receiver that gets information. And then the complex circuitry determines what outcome you get at the end of the day. So many of the proteins that we've talked about need to be in specific places for the cell to function.

We have to have DNA polymerase in the nucleus. It's not going to be useful in the cytoplasm. We have to have a transcription factor that helps transcription go to the nucleus at the right time for transcription to occur. But we don't want it there all the time, because otherwise you'd have the light switch on the entire time. That wouldn't be useful. So we need to regulate where certain macromolecules are. We need to have the receivers on the surface of the cell to receive signals from outside.

This is not just pertinent for multicellular organisms. It's pertinent for unicellular organisms, for them to sense their environment, know what's going on around them. Is the salt concentration changing? Is it getting very hot? Is it getting cold? Is there enough oxygen? Even unicellular organisms need to receive signals and respond to them.

Multicellular organisms are way more complicated. Because you need to establish organs and different parts of a multicellular organism that have specialized function. So trafficking really is about what happens after you've made a replicated DNA in the nucleus, transcribed it, made a mature messenger that goes out to the cytoplasm in most cases. We'll talk about the exceptions to that case.

And then in the cytoplasm, when proteins are expressed, all the different things that happen that guarantee that the protein gets to a proper destination for function. And some of those are quite complicated. Because remember, if I'm going to park a receiver in the cellular membrane with the signals being captured from outside, I've got to get from the cytoplasm out there in a reliable way.

In lectures 20 and 21, I'll talk to you about cellular signaling with a focus on mammalian cells and the sorts of signaling processes that may go awry in cells, for example, proliferating cells. And then Professor Martin will really focus in on neuronal cells, optogenetics in lecture 22. So this bundle really allows you to call in the things that you've learned until now and apply them into much more intriguing and complex situations.

So here's a wonderful, sort of silly drawing of a triangular cell. There's always a joke in cell biologists, when they're trying to talk to mathematicians and mathematicians want to simplify everything. And so everything gets-- imagine a cell, and there's this box shows up on a screen. Well, we all know that cells aren't triangular or box-shaped. But nevertheless, I thought this one was particularly cool.

And so trafficking, the process of trafficking, is really all about, where is the information encoded into the protein that ensures that the protein is where it needs to be for the dynamics that we observe in living system? We've talked a lot about static things. We make the protein. Here's the protein. The protein folds.

We've talked a lot about things that are kind of fixed in time and space. But what we want to do is understand what makes a cell programmed to undergo a new function. For example, something as simple as cell division, we have to orchestrate a huge variety of activities in order for the cell division process to start to occur. Something as really simple a cell mobility, think about, how do cells move? They're not moving all the time, but sometimes they will move towards a signal. What triggers that kind of interactions?

So in looking at the cell, these are some of the older images, where certain organelles, for example, are stained so that you can see them. So peroxisomes are where degradation happens. The golgi and the ER are a part of what's known as the endomembrane system. You'll see a lot about this towards the later part of the class, where we talk about how things get outside the cell through the endomembrane system.

There's the surface plasma membrane. The cytoplasm is this sort of not really aqueous-- it's an open space. But it really isn't open. It's highly congested with all kinds of molecules, all kinds of structural proteins and so on. So don't think of the cytoplasm as a solution, but think of it as a much more gel-like structure with a lot of things happening in it.

The nucleus itself is also surrounded by a membrane, as is the endomembrane system. So

this would be the nuclear envelope. Within the nucleus, you have a structure called the nucleolus, where aspects of the nucleic acids necessary for protein biosynthesis are made. Then there are structural proteins like microtubules and actin.

But now, in this day and age, we don't have to deal with these vanilla images. We can actually use the methods that you've learned about in the last section, recombinant biology, to create new versions of proteins that have along with their sequence a marker that gives them a fluorescence-colored marker. So we are, later on in the semester, going to spend three lectures on fluorescence and cellular imaging, where you'll learn more about these fabulous proteins beyond just saying we've got a green one and a red one. We're going to give you all the background on the protein engineering that enabled those to become tools for biology.

But for now, I'm just going to show you how much more interesting the images of the subcellular structures are when you've labeled, for example, a particular protein that goes exclusively to the nucleolus with a blue fluorescent protein, or to the mitochondria. Remember, Professor Martin told you we always think of these as-- and I'm not going to do the push-up. I'm just going to say it, powerhouse of the cell. I'm not doing-- [LAUGHS] I'm not great with push-ups, to be honest.

But you see these sort of more tangled, extended structures. Vimentin is more of a structural protein. Here are the golgi, the endoplasmic reticulum, and the nucleus.

So the colored fluorophore proteins, or the fluorescent fluorophore proteins, actually allow us, in real time, to observe dynamics. Once a protein is made, where does it go? If we add a trigger to the cell to cause an interaction, can we observe that protein, for example, migrating to the plasma membrane. Can we watch proteins being made through the ER? A variety of different things that allow us in modern biology to really look at dynamics, not just static information.

And so what I'm going to talk to you about is the ways in which proteins are coded very early on in their genesis, in their biogenesis, in order to go to certain locales within the cell. So let me just give you a bit of a road map here with a protein. And where things may start-- so we have some options. Do we want to send the protein outside the cell or keep it inside the cell?

Obviously, two big default differences, if you're going to go to a particular venue inside the cell. Are we going to just stay in the cytosol? That's a sort of simple-- actually, that is the default position. Because you want to remember that most proteins are made on ribosomes in the

cytosol of the cell.

But the statistics are that about 50% of proteins end up somewhere else than the cytoplasm. They may end up in an organelle, back in the nucleus on the surface, or secreted. So there's a lot-- so it's a good, solid 50% that don't end up staying in the cytosol, where they were originally made.

Their alternative is to go to organelles. And if you're going to an organelle, remember, the ribosome is not membrane. It doesn't have a membrane perimeter. But many of the organelles do have membrane perimeters.

So we're talking here about the mitochondria. That is far too long of a word. The nucleus-- so I'm going to abbreviate things like peroxisomes, or various membrane-bordered organelles, where we're going to have to figure out, if something is made in the cytoplasm, how does it get into those organelles?

Now we've spoken a little bit about the fact that some proteins are made in the mitochondria. I'm going to get back to that in a moment. But all the proteins in the mitochondria are not made in the mitochondria. Some of them are shipped in.

Remember the thing the endosymbiont theory, where we said that mitochondria may have originated from bacteria and been engulfed into cells. Those bacteria obviously were originally self-sufficient. But a lot of the proteins that were expressed in the mitochondria were dispensed with, and mitochondria now use proteins that are encoded by the nuclear DNA rather than the mitochondrial. But to this day, some proteins remain encoded within the mitochondria.

So these are opportunities for where that may be. And I'm going to talk very specifically about signals that can get proteins into the mitochondria and into the nucleus. And it turns out that the barriers around those organelles are pretty different. I'll come back to that in a second when we get on the next slide.

With respect to going outside the cell, there are two options. One option is for the protein to remain in the plasma membrane but with part of its structure outside the cell. So the other option is for the protein actually to be spit out of the cell as a soluble entity that can travel around an organism, for example, in the bloodstream and go to a remote site. And that becomes very important in signaling. So we would call those proteins secreted and soluble.

So these would be membrane-bound. These would end up being soluble proteins. Let's take a look at the structure of the cell and look at where these various components are.

So if you see these dots, those are free ribosomes in the cytoplasm. They would start to express different proteins. A lot of proteins are expressed in the ribosome. But in some cases, proteins become expressed on ribosomes that are associated with the endoplasmic reticulum. And therefore, you start a process whereby proteins end up being shipped to the outside of the cell.

So where you see the speckles here, the free ribosome, and then the ribosomes bound to the rough endoplasmic reticulum, here, your destinies are on the right-hand side of that picture. And here, the destiny of these proteins ends up on the left-hand side of this sort of family tree that I'm showing you. There's obviously one more place where proteins are made, and that's in the mitochondria.

And if you remember the first question on your exam, it described the DNA that's in the mitochondria. Going back to the endosymbiont theory, that's a circular piece of DNA. And it sets it apart. And the ribosomes in the mitochondria look more like bacterial ribosomes than you eukaryotic ribosomes. So remember, all along, we're going to try in the second half of the course to bring back knowledge we've taught you, but sort of, in a sense, endlessly remind you to keep the big picture in mind. Because we've already spoken to you about it.

So this now is a nice pictorial vision of what I've just described to you. And I'm going to first of all talk about proteins that are made in the cytoplasm and may be shipped to various organelles, and how that's accomplished. And then in the second part of the class, I'll talk about how proteins are shipped to cell surface, or through expulsion from the cell.

So the key mechanisms whereby proteins are trafficked to new locations are first of all using targeting sequences that are part of the protein sequence. And this is a very common way in which proteins are trafficked. They are part of the sequence. They may be at the amino or the carboxy terminus. But they are woven into the structure of your protein.

So your protein comes along with a barcode saying where it's going to necessarily end up. And for the nucleus mitochondria and peroxisomes, for example, people have done extensive work with bioinformatics to basically look up protein sequences and find common themes of particular sequences that may be common to where a set of proteins may end up. Sometimes

those sequences may not be easy to see just at first glance. But now there are websites that you can very, very readily put your protein sequence into the web site, and it will say, it's got a nuclear localization sequence, or a mitochondrial-targeting sequence. So we can either do this by eye or we can use informatics analysis.

Informatics analysis is very valuable because sometimes information may be a bit more encrypted. And it may be a real struggle to slog through a lot of sequences. So you can really find out about the targeting sequences through bioinformatics. Because nowadays, the genomes of dozens and thousands of organisms are available readily online. And you can literally parse out information from the genomic information that gives you the proteomic information.

So that's one way, so with sequences that are targeted. In some cases, those targeting sequences remain part of the protein. But in other cases, in order to ensure that the protein stays put, the targeting sequences are removed. So that's another important point. You may keep the targeting sequence, or you may lose it through the action of another enzyme that cuts off the targeting sequence when destination has been reached.

Now, there's a second way that we can program where a protein may go. And these are rather useful transformations that make things even more dynamic. So let me walk you through a concept.

If you think of a protein that's made on the ribosome, it's got a targeting sequence. In order to get that protein to destination, you've got to make a new batch of protein that's going to go to its destination. It's going to end up in the mitochondria. You've got to make the protein de novo.

Sometimes when we need to have the action of a cell we can't wait that long. We can do things quickly and expect the cell to suddenly change what it's doing. Because we're sitting around waiting for the ribosome to make new copies of the protein. So the second way in which proteins are targeted to new destinations is through what's known as post-translational modifications.

This is so unfair, Adam. I saw you using the middle boards, but it looked so much easier.

So the second way to target a protein to a destination is using post-translational modification. What does this mean? What it means is that the protein is made. It's ready. It's waiting. But we

haven't engaged its final destiny. We haven't triggered it to go where it needs to be. But we're waiting for an enzyme to just carry out a seemingly minor modification of that protein. And then the protein will go to its destiny.

And I've shown you here examples of three types of modifications. One we will talk about today, because it's very simple to understand, lipidation. And then the other two, we'll talk about next time, phosphorylation and ubiquitination.

And these are all what are known as PTMs, Post-Translational Modifications. And they are changes that occur to an amino acid side chain within an already made protein to alter its destiny. And I'd like to talk about lipidation first, because I get to remind you about cellular membranes. So remember, we've talked about these semipermeable barriers that are around organelles and around cells.

And let's say that this is a membrane-- I've got to put my-- that exists between the cytoplasm and the outside of a cell. And let's say I have a protein lurking around in the cytoplasm, but I need it at the membrane. I need it to get involved in a signaling process. And I need it now to be there.

If I have a soluble protein, it's not associated with the membrane. But I can use another enzyme to attach a hydrophobic, greasy tail to that protein. So what it really wants to do is to get to the hydrophobic membrane. Lipidation is such a modification.

It's just the modification with a long-chain, often C16, C18, fatty acid that then renders the protein lipophilic and makes it want to move, and insert this lipophilic tail into the membrane, and part the protein of the plasma membrane. So the information is still, though, encoded within the protein.

How could that happen? How could I have made that information be in the protein? What might be the strategy there? It's still encoded, but it's secret. It's cryptic. Any ideas?

So I'm not going to just glom this group onto a protein. I'm going to put it somewhere specific. And so oftentimes, lipidation reactions occur site-specifically at particular sites within a sequence, and an enzyme recognizes that site and transfers the lipidic molecule to it.

So lipidation actually may occur, for example, of the amino terminus of a protein. But if there are certain features within that protein, you may then attach the lipidic group. So once again, using bioinformatics, you can look at the target protein of interest and predict that it's the

target of a post-translational modification reaction.

So once again, the information is programmed into the sequence, but it's quite cryptic. It could be within the middle of the sequence. There could only maybe be a couple of clues. But the clues are there nonetheless that can be parsed out using computer learning and screening of sequences to say that is a target for lipidation, or phosphorylation or such.

Is that clear to people? Does that make sense? The information is encoded, but you can't see that it's there. But the advantage of the post-translational modifications is that they occur on demand, as opposed to making a new protein de novo, and then having it go to a particular cellular location.

Later on, when we talk about phosphorylation, you will see that phosphorylation is the bread and butter of cellular signaling. It's the light switch in every room in the cell that turns on and off in order to make functions happen within the cell. And that's a really major, dynamic post-translational modification that has significant meaning.

So the reason on this little image-- I just wanted to show you the membrane and just remind you that the membrane is a supramolecular structure that's assembled with a hydrophobic core and polar head groups on both faces, as I've sort of indicated in this cartoon. So let's start with sequences that might take us to the nucleus.

Now, the nuclear membrane is rather a strange entity. Because the nuclear membrane isn't a simple membrane like the plasma membrane. It's actually a double-layered membrane. So if you look at a nuclear membrane-- and I'm just going to do a job of showing a portion of the nuclear membrane.

Within the nuclear membrane, there are pores, quite launch openings. And the membrane is actually a double membrane, where all of these lipid bilayers. So it's not a single membrane. It's a double membrane with large openings.

And you might say, well, that's no use. There's just these great big, gaping holes in the nucleus. Anything can come and go if it wants. But the nuclear pores are kind of a special structure. Because they have a protein that's kind of disordered, that creates a tangled network. That means that that pore isn't totally open, but there's some stuff that something's got to get through to get from one side to the other.

And my colleague Thomas Schwartz in biology works on the macromolecular structure of nuclear pores to understand these structures. Because these are also made through the auspices of having a lot of proteins that help create this structure. Otherwise, that membrane wouldn't stay in its proper format.

So in order for a protein to get into the nucleus, if it needs to, or leave the nucleus, it has to have some kind of mechanism to get through this structure that's plugging the nuclear pore. So this would be the inside of the nucleus. And this would be the cytoplasm.

So as shown on this slide, the nucleus, there's a particular protein sequence that's appended to a protein. That's known as the Nuclear Localization Sequence, or NLS. And what an NLS sequence is, it's a short sequence of amino acids that enables a protein to get to its proper destination.

And these sequences are quite well recognized. They may end up being highly basic sequences. So an example of an NLS would be Lys-- it's not very exciting, but it just goes on, Lys, Lys, Lys, arginine, lysine. And it may be bounded by hydrophobic residues or other types. So that would be a typical NLS sequence that's in a protein.

And I want to remind you that lysine and arginine all have side chains that at physiological pH are positively charged. So the nuclear localization sequence is something that's easily recognized because of this sort of short sequence that may be at the N- or C-terminus. I think there's either possibility. But it's a very clear sequence. You could look at your protein sequence and say, there's an NLS on that sequence.

And it's the NLS sequence alone that's responsible for getting the proteins in and out of the nuclear pore. Let's mostly focus on getting into the nucleus. Basically, you have a protein structure that has an NLS sequence at one terminus. And that NLS sequence binds to another protein.

Creatively, you had a little bit of chance to give proteins names in the exam. It's called importin. So it's an import protein that binds to the NLS, and as a consequence of that, will carry cargo. It will escort cargo into the nucleus of the cell. And it sends it through this meshwork of proteins.

That's a very loose mesh work of proteins. And they're not ordered proteins. They're highly disordered proteins. So they make more of a filter than a plug. But they are definitely

something that doesn't allow any old protein to go through that nuclear pore.

NLS tags are very easy to recognize, once again, through bioinformatics analysis. And what's really cool is that you can reprogram a protein to be where you want by manipulating the NLS. So this is rather a nice set of experiments.

Let's say we have a protein that we're going to micro-inject into the cytoplasm of the cell. And we want to program it to either go into the nucleus or stay outside the nucleus. That can be done readily by attaching a nuclear localization sequence to a protein along with a fluorophore dye or fluorescent protein that will allow you to observe that experiment. If you micro-inject into the cytoplasm, that protein that's got an NLS will get run into the nucleus through association of the NLS with importin. But if you chop that NLS, the protein the stuck, remains out in the cytoplasm.

Let's say you want to study a new protein. I just want to show you that these NLS sequence are totally independent of the cargo they carry. You can just stick an NLS on your favorite protein who you want to interrogate.

Let's take pyruvate kinase. It doesn't have anything to do with specific transport to the nucleus. But nevertheless, if you put-- if it doesn't have an NLS, it's fluorescently labeled, it stays outside in the cytoplasm. But if you put an analysis on it, you concentrate into that region of the cell.

So these experiments show you that what we know about these targeting sequences can be manipulated and used to enable you to move things around in the cell. So that's one particular type of mechanism. The next mechanism I want to describe to you is the mechanism that's used for mitochondrial transports. And it's a little bit different in its strategy.

So to get into the mitochondria, there is, again, a recognition sequence, in this case, a mitochondrial localization sequence that has particular characteristics. In this case, the mitochondrial localization sequence, let's say it's at the N-terminus of your protein. And it would be something that might be a mix of charges. Some Arg, Glu, Arg, Glu. So that's a typical MLS sequence.

And in this case, the charge at physiological pH is different from the nuclear localization sequence, because it's an alternating positive and negative charge. So this is pretty different from this. It doesn't say bioinformatics to figure that one out. So you can then pick out

mitochondrial localization sequences.

And so in this case, remember, mitochondria make some of their own proteins on their circular DNA. But they've abandoned expressing all the proteins that are needed in the mitochondria. And some proteins are transported into the mitochondria using these types of sequences. But the approach, the strategy, is different from getting into the nucleus.

In this case, the MLS sequence associates with a protein channel that is in a closed state. So here's a membrane. Here's the makings of a channel. But it's in a closed state.

But once the protein with the NLS sequence binds to that, that channel opens. It's triggered by the binding of that sequence to a portion of the protein that's outside that membrane. And that then allows the protein to be unfolded and transported into the mitochondria, where that sequence may be removed. And then protein refolds in the mitochondria.

So it's a very different strategy for that and the nuclear localization sequence. So you'll find, for many different organelles in the cell, there might be very specific localization sequences that you could look up and learn about. But one thing I want to mention to you is that these localization details are very important. And many diseases in cells are a consequence of proteins not being localized to the right place.

If you're not in the right place at the right time, then things will start to go wrong with the signaling or the processes of the cell. So diseases are frequently associated with mislocalization. So now what we're going to do is basically say, we've taken care of understanding things made in the cell. They either stay in the cytosol or they'll go to organelles based on particular types of strategies that are largely dependent on short tagging sequences, but in other cases, may be dependent on post translational modification.

All right. So here is a cartoon. But actually, I want to do something slightly different if it doesn't take too long.

Now, when we first talked about translation on the ribosome, what you see there in green and yellow is the ribosome. The dark band is a messenger RNA. The dark blue are transfer RNAs that are being helped with elongation factors to get to the ribosome. But what I want to point out here is the emerging sequence of polypeptide coming out through a tunnel on the ribosome.

Now, if a protein is going to be destined outside the cell, it is expressed with what's known as a

signal sequence. It's about a 20-amino acid residue sequence that is recognized by the signal recognition particle. And then translation slows down and clamps the ribosome on the endoplasmic reticulum membrane so that the new peptide starts being threaded into the endoplasmic reticulum through what's known as the translocon. So you're now not sending the protein out to the cytoplasm, but you're rather sending the protein into the endoplasmic reticulum. And you're also sending it down this branch of the protein biosynthesis pathway.

You see this piece of protein emerging. This hatched portion is the cytoplasm. The gray portion is the endoplasmic reticulum. So there is a complex machinery at play that enables proteins to be made in the cytoplasm but now targeted to a completely new location. And these are the proteins that are going to be destined to either stay in the plasma membrane or be secreted from the cell.

And this view here gives you a little bit more than the cartoon. So ribosome-- a signal peptide is made that is a green peptide sequence that's about 20 amino acids long. That is actually called a signal peptide. It's signaling for synthesis through the endomembrane network.

That causes the ribosomes to dock down on the cytosol ER membrane and keep on being synthesized so that proteins are made into that endomembrane system. And you can think of this cavernous endomembrane system as your tunnels out of a cell for either display on the surface of the cell or for secretion entirely in vesicles. So let's take a look at how that occurs.

When you make a protein in that way, see the dark dots, the rough ER? These are ribosomes that are attached to the membrane. Proteins are made into the membrane. And then the endomembrane system is not really just a tunnel or a labyrinth. But actually, each of those layers spits off vesicles that fuse with next layers to gradually make their way outside of the cells.

So here you see there are vesicles. You're always keeping proteins associated with membrane as you go through the endomembrane system. And here is a vesicle that's got protein in it. It may either release it to the outside of the cell, or the protein may be associated with the membrane of the vesicle and stay parked in the plasma membrane.

And so I just want to give you one final slide where I talk about the biogenesis of membrane proteins. Now, this is pretty complicated stuff. Because you have to remember what's inside and out. So I spent more time than I should have on this cartoon to show you which end of the

protein ends up outside the cell and which inside the cell, and how you make multi-membrane-spanning proteins.

So let's take a look at this in detail now, looking-- here's the ribosome. Here's the protein emerging. If there's signal sequence there, that ribosome docks down on the membrane and starts translating the protein, amino terminus first, into the endoplasmic reticulum. We'll all OK with that.

As synthesis continues, we may reach the stop codon on the messenger RNA. And what may happen is that the protein may remain associated with membrane. The amino terminus will be in the ER. And the C-terminus will remain on the other side. There are a number of different configurations.

But if we want to start to transport this protein to the surface of the cell, that will then stay associated with membrane but not in the form of the flat membrane that it was delivered into. But that membrane may pinch off into a spherical vesicle. But you still have the C-terminus outside and the N-terminus inside. That will then work its way through the endomembrane system, and ultimately, fuse with the cytosol. This is the really fun part.

And then, once it's fused with the cytosol, it has the option to be displayed on the outside of the cell. Why? You have a protein. The N-terminus is on the outside. The C-terminus is on the inside.

So that shows you the biogenesis of the cell surface protein that's stuck in the membrane through its membrane-associated domain. If you're not going to stay with the membrane, you can actually also simply release this into the vesicle for release of a soluble protein. I will not go through this. But there are miraculous steps that end up in the biogenesis of multi-transmembrane proteins.

Because each of those transmembrane domains gets made in the translocon and gets shuttled sideways. And you start piling up transmembrane domains that span the membrane. And in the next class, we're going to see how useful these proteins are in cellular signaling. So those are very important proteins to think about.

One last thing-- so let's think about this. For either configuration, either post-translational modification or using targeting sequences, when do we define where the protein's going to end up? Where's the information first defined? Anyone want to answer me and explain why?

Yes?

AUDIENCE: Would it be B, the mRNA sequence, because that would have a significant portion of the splicing?

BARBARA IMPERIALI: It's a good try. But you want to remember, yes, splicing is important. But when was the sequence actually in the entire pre-mRNA? When would that have been defined? Yeah? Sorry. Carmen?

AUDIENCE: Is it in the genomic DNA sequence?

BARBARA IMPERIALI: Yes. Because you never have information in the RNA that wasn't in the DNA. So the DNA has got the information there. Yeah, it may need a bit of splicing to put things in the right place. But the information is there in the DNA.

So you want to remember, for all of this targeting information, it's in the genomic information most commonly. It's the genomic information that has the patterns of sequences for post-translational modification. It's the genomic information that has things like NLSes and MLSes. They're already there.

But they are often encrypted. And there was a very nice point there, though. If you want to send to make a single chunk of a genome that encodes either a protein that's going to be exported through the secretory pathway or stay in the cytosol, you might splice in or out a signal sequence. So that's a really good way, using the same original DNA sequence, to actually get to proteins that fulfill different final destinies within the cell. So next time, we're going to talk about signaling. It's going to be a blast.