JOANNE STUBBE: --that Brown and Goldstein carried out, which in conjunction with many other experiments and experiments by other investigators have led to the model that you see here. And so we'll just briefly go through this model, which, again, was the basis for thinking about the function of PCSK9 that you learned about recitation last week, as well as providing the foundation for thinking about the recitation.

This week, we really care how you sense cholesterol levels in membranes, which is not an easy thing to do given that it's lipophilic and so are many other things. OK. So the LDL receptor-- that was their model, that there is a receptor-- is generated in the endoplasmic reticulum.

If you looked at the handout, you'll see that it has a single transmembrane-spanning region, which means it's inserted into a membrane. And the membrane where it functions, at least at the start of its life, is in the plasma membrane. So somehow, it has to get from the ER to the plasma membrane. And this happens by forming coated vesicles.

We'll see a little bit of that, but we're not going to talk about this methodology in any detail. But Schekman's lab won the Nobel Prize for this work, either last year or the year before, of how do you take proteins that are not very soluble and get them to the right membrane. And they do this through coated vesicles that, then, move through the Golgi stacks that we talked about at the very beginning. And then, eventually, they arrive at the plasma membrane and become inserted. So these little flags are the LDL receptor.

OK. So that's the first thing that has to happen. And I just know that this whole process is extremely complex. And patient mutants are observed in almost every step in this overall process. It's not limited to the one set of types of experiments, where something binds and doesn't bind to LDL receptor that we talked about last time.

So the next thing that has to happen-- again, and we haven't talked about the data for this at all, but not only do these receptors have to arrive at the surface, but they, in some way, need

to cluster. And it's only when they cluster that they form the right kind of a structure that, then, can be recognized by the LDL particles that we've talked about. And so they bind in some way. And that's the first step in the overall process.

And then, this receptor, bound to its cargo, its nutrients-- and, again, this is going to be a generic way of bringing any kinds of nutrients into cells. It's not limited to cholesterol-- undergoes what's now been called receptor-mediated endocytosis. And so when the LDL binds to the receptor, again, there's a complex sequence of events that leads to coding of the part that's going to bud off, by a protein called clathrin.

Again, this is a universal process. We know quite a bit about that. And it buds off. And it gives you a vesicle. And these little lines along the outside are the clathrin coat. I'll show you a picture. I'm not going to talk about it in any detail, but I'll show you a picture of it.

So the LDL binding, we talked about. We talked about binding in internalization. Those are the experiments we talked about last time in class that led, in part, to this working hypothesis. And so we have clathrin-coated pits. And it turns out that there's a zip code. And we'll see zip codes throughout-- we'll see zip codes again, in a few minutes, but we'll see zip codes which are simply short sequences of amino acids that signal to some protein that they're going to bind.

So how do you target clathrin to form these coated pits? How do you form a pit, anyhow, in a circle? And how does it bud off? And where do you get the curvature from? Many people study these processes. All of these are interesting machines that we're not going to cover in class.

So you form this coated pit, and then it's removed. So once it's formed, and you've got a little vesicle, it's removed. And then it can go on and do another step. And another step that it does is that it fuses with another organelle called an endosome, which is acidic pH. How it does that, how it's recognized, why does it go to the endosome and not directly to the lysosome-- all of these things, questions, that should be raised in your mind if you're thinking about the details of how this thing works, none of which we're going to discuss.

But it gets into the endosome, and then what you want to do is separate the receptor from its cargo, the LDL. And we know quite a bit about that. If you read-- I'm not going to talk about that either, but if you read the end of the PowerPoint presentation, there's a model for actually how this can happen.

And you can separate the receptor from the cargo. And the receptors bud off, and they are recycled in little vesicles to the surface, where they can be reused. The LDL particles can also, then-- and what's left here can then fuse with the lysosome. And that's, again-- we've talked about this-- it's a bag of proteases and a bag of esterases, hydrolysis, lipids. That's what we have in the LDL particle-- hydrolysis.

We talked about ApoB being degraded with iodinated tyrosine, last time. That's where this happens and gives you amino acids and gives you cholesterol. OK. And then, again, depending on what's going on in the environment of the cell, the cholesterol would then be shuttled, somehow, to the appropriate membranes.

OK. So you can see the complexity of all of this. If the cholesterol is present, and we don't need anymore in the membranes, then it can become esterified with long-chain fatty acids. Those become really insoluble, and they form these little globules inside the cell. And then the process can repeat itself. And the question we're going to focus on in lectures 4 and 5, really, are how do you control all of this.

OK. So this is the model. And so I think what's interesting about it is people have studied this in a lot of detail. It was the first example of receptor-mediated endocytosis. So we know something about the lifetime of the receptor. We know it can make round trip from surface inside, back to the surface in 10 minutes.

We also know it doesn't even have to be loaded to make that round trip. It could be one of the ones that isn't the clustering of the receptors, which is required for clathrin-coated vesicles to form. And so you can tell how many trips it makes in its lifetime. And so the question, then, what controls all of this?

But before we go on and do that, I just want to briefly talk about, again, mutations that have been found in the LDL receptor processing. And they're really, basically, at every step in the pathway. So the initial ones we found, that we talked about, we'll come to in a minute. But we had some patients with no LDL receptor express at all. So somehow, it never makes it to the surface. OK?

There are other examples-- and these have all been studied by many people over the decades-- that it takes a long time to go through this processing. And it gets stuck somewhere in the processing. That may or may not be surprising, in that you have transmembrane insoluble regions. And if the processing goes a little astray or some mutation changes, then

you might be in trouble.

So we talked about this last time. We talked about that they had just looked at 22 patients. Some of the patients had no binding of LDL to the surface of the fibroblast that they were using as a model, at all. Some have defective binding. So if they compared it to a normal, they had a range of dissociation constants.

And we'll talk quite a bit about dissociation constants, not this week but next week, in recitation. It's not so easy to measure dissociation constants when things bind tightly. And thinking about how to measure them correctly, I think, is really important. And I would say, probably, I could pull out 10 papers out of current journals, really good journals, where people haven't measured dissociation constant correctly, when you have tight binding. So this is something that we put in because I think it's important that people need to know how to think about this problem.

So anyhow, let's assume that Brown and Goldstein did these experiments correctly, which I'm sure they did. And they got a range of binding. And we also saw that the patient we looked at, JD, had normal binding. That indicates he was the same as normal patients, but something else was problematic. And that something else wasn't that it failed to form coated pits, but that it failed to bring this into the cell. So it failed to internalize the LDL. That was JD's defect.

We also, in recitation last week-- hopefully, you've had time, now, to go back and look at the paper a little bit. But LDL, in the model we were just looking at, gets recycled. It goes in and gets back to the surface. But what happens if, on occasion, instead of budding off into vesicles and returning to the surface, it, with the LDL cargo, goes to the lysosome and gets degraded?

Well, that was the working hypothesis for what PCKS did. It targeted to the wrong place and degraded it. And the phenotypes of those patients were interesting, and that's why it was pursued. So there are many, many defects. And despite the fact that we have these statins, people are still spending a large amount of time thinking about this because of the prevalence of coronary disease.

So I'm not going to talk about this, but I'm just going to show you two slides. And you can go back and think about this yourself. But this is the LDL receptor. We know quite a bit about it now. And one of the questions you can ask yourself, which is an interesting question we're not going to describe-- but you have LDL particles that are different sizes.

How do you recognize all these different sizes? And how does the clustering do that? And so that's done up here. And there's calcium binding. We know quite a bit about that, but I don't think we really understand the details. You have a single transmembrane helix in the plasma membrane. And this is the part-- this part up here-- that actually binds the LDL particle.

And the last thing I just want to briefly say, because we're going to see this again but without going through any details, remember that eventually we form what are called clathrin-coated pits. That's a picture of what the clathrin-coated pits look like. And the key thing-- and I just wanted to mention this briefly because we're going to see this again, over and over-- is the LDL receptor, itself, has a little zip code. And that's enough-- it's at the tail. That's enough for it to attract this green protein called to AP-2, which is key to starting clathrin binding, and formation of the curvature, and eventually being able to bud off these vesicles surrounded by clathrin.

And when you do that, you start budding. And then, somehow, it turns out there's a little machine, a GTPase-- we've seen GTPases all over the place-- that's involved-- this is the name of it-- that allows you to bud off. And you use ATP energy to do all of this. We've seen this over and over again.

And so the point I wanted to make here is we've seen this with these seminal experiments, by Brown and Goldstein. But in fact, we now know that this is sort of a generic mechanism for taking nutrients into the cell. So it's not limited to LDL receptor and LDL. And in fact, we're going to see, we're going to talk about, in module 7, Epidermal Growth Factor Receptor. And we're going to talk, in module 6, the receptor that takes iron into the cell, both of which do this kind of signaling.

So this is a generic mechanism to do that. All of these things are interesting. We know quite a bit about it. And if you want to study that, you could have spent another weeks worth of lectures studying this. So the idea, then, is that we have nutrient sensing. And this is a general way to try to get nutrients into the cell, that is, you have a receptor, and it's undergoing receptor-mediated endocytosis.

So that's the end of lecture 3. I think I'm one lecture behind, but that's not too bad. So what I'm going to do now is-- let's make sure I get this right-- I'm going to start on lecture 4. And now we're sort of into the question of how do we sense cholesterol. OK.

So what I've done in the original handout, I had lecture 4 and 5 in the single PowerPoint.

They're still in a single PowerPoint, but I've just split them into two. So I'll tell you how I've split them. So lecture 4 is going to be focused on sensing and transcriptional regulation. And lecture 5 will be focused on sensing and post-transcriptional regulation by a protein-mediated degradation.

So I'm going to split that in two parts. And so today's lecture will be mostly focused on transcriptional regulation. And the key issue is how do we sense cholesterol-- what is the mechanisms by which we sense cholesterol. And the outline for the lecture is that the transcriptional regulation involves a sterol-responsive element. So this is sterol-responsive element.

This is a DNA sequence of about 10 base pairs. And it also involves a transcriptional factor, so TF. This is a transcriptional factor-- transcription factor. And this is called SRE-BP. So this is Sterol-Responsive Element Binding Protein. So BP is Binding Protein.

OK. So the first thing I'm going to talk about, then, is the discovery of SRE-BP. So that'll be the first section. And then what we're going to do is we want to know what are the players that allow us to understand how this transcription factor works. What we'll see that's sort of amazing-- it was amazing at the time, but now it's been found in a number of systems-- is where would you expect a transcription factor to be located?

AUDIENCE: In the nucleus.

JOANNE STUBBE: In the nucleus. OK. And what they found from their studies that it's located in the ER membrane. So this was a major discovery. So this protein is located in the ER membrane. They didn't know it at the time. But now, you're faced with the issue, transcription factors do work in the nucleus. So somehow, we have to get it from the ER membrane into the nucleus.

And so to do that, what we need are players for SRE-BP to go from the ER to the nucleus. And we're going to see that these players are called SCAP, and they're called INSIG. And we'll come back, and we're going to talk about those in some detail.

And then the last thing we'll focus on is-- we'll see it throughout. I'm going to give you-- what I usually do when we're talking about some complex mechanism, I give you the model upfront so you sort of see where you're going. Hopefully, you've all had time now-- we've been in this module for a long time-- to read the review articles. But we want a model for transcriptional regulation. So that's where we're going.

And so what I want to do, before we get into the model, is come back where we started to try to keep you grounded on what we're doing. And what we're doing here is our cartoon of the cell that I showed you in the very beginning. We know that metabolism of hydrocarbons, fatty acids, and cholesterol all focus on a central player. And the central player is acetyl CoA.

Acetyl CoA can be obtained from fatty acids in the diet. We've talked about the distribution of fatty acids using lipoproteins, including LDL. And we get to acetyl CoA-- this all happens in the mitochondria. But acetyl CoA cannot get across membranes. And that's true. There are a number of things that can't get across membranes. And so carriers in the mitochondrial membrane are key to metabolism.

And I think once you look at it and think about metabolism overall, it's not so confusing. But you might not have chosen those. If you were the designer, you might not have chosen these to be the carriers to move in between organelles. So I think this happens quite frequently, so you need to pay attention to it.

And so what happens in this case is acetyl CoA combines with oxaloacetic acid to form citrate. Citrate is an intermediate in the Krebs cycle. The TCA cycle is part of all of central metabolism. We're going to see citrate again. It plays a central role in iron homeostasis as well.

And citrate-- there is a transporter that gets this into the cytoplasm. So here's the cytoplasm. There's an enzyme citrate, lyase that uses ATP to generate acetyl CoA. OK. So acetyl CoA is a central player. And really, what we're thinking about now, in general-- I'm going back through this-- is what do we expect sterol-responsive element-binding protein to regulate. And I'm going to show you it doesn't just regulate cholesterol homeostasis. There's a big picture [AUDIO OUT] all of this.

So you can make-- you talked about this as a prelude to the polyketide synthases, the natural products Liz introduced you to. Anyhow, you can make fatty acids. Fatty acids can do a number of things. If you have a ton of them, then you can react them with glycerol to form triacylglycerol. And they're insoluble messes. If you look at the structures, they form little globules.

So we have all these little insoluble globules inside the cell. And people are actually quite interested in studying these things. Now, we don't know that much about whether they are proteins or metabolic enzymes that could be sitting on the surface of these globules. A lot of

people are trying to figure that out.

But also, fatty acids are required in the presence of glycerol 3-phosphate, which comes from the glycolysis pathway, the other pathway that everybody learns about in an introductory course, to form phospholipids, which are the key component of all of your membranes. Alternatively, acetyl CoA, depending on the regulation of all of this-- that's the key-- gets converted to hydroxymethylglutaryl-CoA and mevalonic acid. Mevalonic acid-- that reduction between these two is a target of statins-- then ends up making cholesterol. And where does cholesterol have to go?

So cholesterol is made, and a lot of it's happening in the membranes. A lot of it is associated with the ER, but only a small amount of the total cholesterol is in the ER membrane. Somehow, it's got to be transferred to all these other membranes. So that's a problem we haven't talked about. That's a big problem. Most of the cholesterol is in the plasma membrane. If you have excessive of cholesterol, you can esterify it, and, again, form little droplets of fats, which have fatty acids and cholesterol.

So that's the big picture. And so this is the picture of the regulatory network. So I'll say this is a PowerPoint for the regulatory network. And it's governed by-- it turns out there are three SRE-BPs. They have a slightly-- and they're structurally homologous to each other, and they work in ways that they interact with other protein factors and control this whole homeostatic process between fatty acids and cholesterol biosynthesis.

So I think there are two things that you need to think about. So we want to control basically its lipid metabolism. And I should say at the outset, we're focusing on SRE-BP, but some of you, in maybe a more advanced biology course, know that there are other transcription factors involved in regulating cholesterol homeostasis. This is a major one, and that's all we're going to talk about in this class.

But what else do you need to make molecules, if you're going to make fatty acids, if you were going to make cholesterol? What you need is NADPH. So that's the other thing that you need to think about when you're looking at the regulatory network. So we need to control-- how do we make lipids? Where did they come from? They come from acetyl CoA.

And the second thing we need to think about is a source of energy to actually form the molecules. We're after the long-chain fatty acids. Go back and look at that-- or cholesterol, if you go back and look at the pathway we talked about in the first couple lectures.

So NADPH is at the center. And I forgot to point out before and probably many of you have heard of but never really thought about malic enzyme in the cytosol. You can go back and think about that, but that's a major source of NADPH. What is another source of NADPH in the cytosol. Anybody know? Where do you get most of your NADPH from? It's key to biosynthesis of any kind of anabolic pathways. Does anybody know?

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: No. OK. Did you ever hear of the pentose phosphate pathway? Well, hopefully, you've heard of it. Reproducing it might be challenging, but the pentose phosphate pathway is central to providing us with NADPH. It's central for controlling reactive oxygen species, which is going to be module 7. It's central for providing NADPH for nucleotide metabolism.

So the pentose phosphate pathway and malic enzyme are the key sources of NADPH. And if you're becoming biochemists, I think, now, all of these pathways, these central pathways that we talked about in 5.07, should just-- you don't need to know all the details, but you need to know how things go in and out. And it's central to thinking about anything.

And if you ever do any genetic studies, you can never figure out anything unless you know how all these things are connected. So knowing these central pathways and how things go in and out and connect is really critical in thinking about many, many kinds of reactions you might be doing in the lab. Because you might see something over here, but it might be way over here that you had the effects. And knowing these connections, I think, is why I spent another-- whatever-- five minutes describing the regulation.

OK. So if we look at this, what we see here-- and this is an old slide, so this might have changed. But all of the enzymes in italics are all regulated by SRE-BP. So here's acetyl CoA. What do we see in this path, where we're making cholesterol? So many of the enzymes-- we're not going to talk about them-- that we talked about when we went through the pathway are all regulated by SRE-BP and is predominantly-- again, there's overlap of the regulation between the different forms of the sterol-responsive element-binding protein.

But you can see, we have HMG CoA reductase, which is the rate-limiting step. So that might be expected. But many of the other enzymes that are also controlled by this transcription factor. And the one that turns out, I think, to be quite interesting for most recent studies is-- remember, we briefly talked about how you get from a linear chain, and then we had to use a

monooxygenase to make the epoxide. That enzyme is a key regulatory enzyme, people now think. It wasn't thought to be so not all that long ago.

So anyhow, all of these enzymes that we've talked about are regulated in some way by SRE-BP. But it doesn't stop there. If you go over here, you sort of have a partitioning between acetyl CoA also going into lipids and forming phospholipids or triacylglycerols, depending on whether you store or whether you're dividing and need more membranes. So all of this, again, it's about regulation.

And if you look at this, you can see that many of the enzymes in this pathway, for formation of monoacylglycerol and triacylglycerols are also involved. OK. So that gives you the big picture that I want you to think about. So when you wonder where you're going, you should go back and take a look at the first few slides.

So what I want to do now is really focus on the first thing. The first factor was how did they identify. So this is identification of SRE-BP. And so probably most people wouldn't talk about this, but I think it's sort of amazing. So I'm going to just show you what had to be done. And this is not an easy set of experiments.

First of all, transcription factors, in general, aren't present in very large amounts. To get them out, they also stick to DNA. So that poses a problem. Unlike using his tags and all this stuff, none of that stuff works to isolate transcription factors. And this was all done before the-- a long time ago.

And so this was this is quite a feat. And the key to this feat was that Brown and Goldstein recognized that in the front of the gene for HMGR-- Hydroxymethylglutaryl-CoA reductase-- in the LDL receptor, they found a 10-- I'm not going to write out the sequence-- base-pair sequence that was the same.

So that suggested to them that there's a little piece of nucleic acid with 10 base pairs that might be recognized by a protein, which could be the transcription factor. So this was the key, this 10 base-pair sequence. And I'll just say, see PowerPoint.

And this is the SRE, before the genes, again. And this has now been found in front of many genes. I just showed you that many, many genes are regulated, in some way, by these proteins. But this was an observation they made a long time ago. OK.

So where would you expect-- we just went through this. Where would you expect SRE-BP, the

transcription factor, to be located? You'd expect it to be in the nucleus. OK. That's a reasonable expectation. And so what step might you do, in the very beginning, to try to help you purify this protein? And let me just tell you at the outset that the protein had to be purified 38,000-fold.

OK. Now, you guys, none of you have ever experienced, really, protein purification, starting with kilograms of anything. I have done that and spent three months purifying a microgram of protein. And I would argue that some people still need to do that, because when you do recombinant expression, lots of times, you miss a lot of stuff. So somewhere along the way, somebody needs to really know what the endogenous protein is like, and not the recombinant protein.

So we're going to have to do a 38,000-fold purification. And I would say that's not uncommon. I've done 20 liter by 20 liter gradients that take three weeks to get through the gradients and looking for your proteins. So if your protein is not stable, even if you're in the cold room, what happens? Or if there are proteases, it gets degraded. So I'm just saying, transcription factors are not easy to deal with. And this was sort of an amazing feat.

Anyhow, they started with-- over here-- 100 liters of tissue culture cells. So most of you have probably seen tissue culture plates. And that's what you work with. They started with 100 liter, and that's why they're using HeLa cells, because you can grow them on this scale. You can probably grow a lot of things on this scale, now. We have much better ways than-- this was a long time ago.

So their approach was-- so the first thing-- I got sidetracked again. But the first thing is that if it's in the nucleus, what would you do to try to enrich in the transcription factor? What would be the first thing you might do after you've isolated the cells?

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: I can't hear you.

AUDIENCE: Maybe, something involving nuclear-binding proteins that transport things into [INAUDIBLE]-- that have transported things into the--

JOANNE STUBBE: OK. So I still can't hear you. You're going to have to speak louder. I'm going deaf. And I will get a hearing aid, but I don't have one now. So you have to speak loud, and you have to

articulate. Yeah?

AUDIENCE: Wait, so just the absolute first step?

JOANNE STUBBE: Yeah.

AUDIENCE: How we're just lysing cells and pelleting them?

AUDIENCE: Yeah.

JOANNE STUBBE: But is there a certain way you would pellet them?

AUDIENCE: You would have to do a sucrose gradient.

JOANNE STUBBE: You would do some kind of gradient to try to separate the-- well, you have to pellet the cells first. But then, what you want to do is separate the nucleus from all the organelles. The issue is-- we already told you this-- most of the protein is not found in the nucleus. And that was part of this. They didn't know that at all, but that's what they did.

They did some kind of a gradient to separate nuclei from the rest of it, because they were trying to enrich, which was a totally reasonable thing for them to have done. OK. So I'm not going to write that down, but that's the first thing they did.

The second thing they did is they made an affinity column all out of the SRE. So this is a nucleotide affinity column. And they ended up using that a couple of times. And they ended up using a couple of other kinds of columns and eventually got protein out after a lot of effort. After a lot of effort, they got protein out.

And the size of the protein-- so they went through this column. And they went through additional columns. I'm not going to go through the-- and they ended up with proteins that were actually smaller than the SRE-BP, but they still bound to the affinity column. So they ended up with proteins-- I don't remember.

And again, the details of this really aren't so important. But they ended up with smaller proteins. Somewhere, I have the size written down. 59 to 68 kilodaltons. So either protein had been degraded, or we will see the protein has been processed, or was being processed during all this workup. And there are many things that could have happened to this process.

But what this allowed them to do-- and this was the key to allowing them to do this better-- was

they could generate antibodies. So they took this protein that they isolated, and they generated antibodies. And we're going to be talking about antibodies this week. But we're going to be, also, talking about use of antibodies with fluorescent probes, the last recitation, as well.

So what did this allow them to do? The antibodies, then, allowed them to go back into the cells and look for expression of SRE-BP. And instead of finding it in the nucleus, what they found was that most of it was localized in the ER membrane.

So these antibodies revealed that SRE-BP is predominantly in ER membrane. And again, this question of antibodies-- which Liz brought up-- and the question of specificity, and, moreover, the question of sensitivity is really key. Because now, when you're looking at eukaryotic cells, we know things move around. They move around all over the place, and they move around dependent on the environment.

So you could easily miss location. This might be the predominant one under the conditions you looked, but it could be somewhere else. And I think they didn't realize so much about that back in these days, but we now know that a lot. So anyhow, that was a surprise. And then, that provided the basis for them going back and thinking much more about this system.

And so what I'm going to show you is the model that's resulted. And if some of you have started working on problem set 7 that's due this week-- the problem deals with some of the experiments-- then I'm going to tell you what the answer is. And you're supposedly looking at the primary data from where this model came-- a small amount of the primary data from where this model came.

OK. So this is the model. And I'll write this down in minute. But the model is at low sterol concentrations. So at low sterol concentrations, what do we want to do? We want to-- this transcription factor-- I should write this down somewhere. But the transcription factor activates transcription. It could repress transcription, but it activates.

So if you have low sterols, what do you want to do? You want to turn on the transcription factor. So it needs to somehow move from this location in the membrane to the nucleus. So that's where this model is coming from. And we'll walk through it step by step.

So what you'll see-- these are cartoons for the factors we're going to be looking at. So this SRE-BP has two transmembrane regions. We'll come back to that. This little ball here, which

turns out to be at the N terminus, is a helix-loop-helix, which is a DNA-binding motif. We'll come back to this in a minute. I'm just giving you an overview, and then we'll come back.

There's a second protein. And this is the key sensor that we're going to see of cholesterol levels, called SCAP. And it also resides in the ER membrane. And it has a little domain on it that recognizes and interacts with part of SRE-BP.

And so this is located in the lumen. And these guys, especially this guy, is located in the cytosol. And we don't want it inside the lumen, we want it on the outside so it can go into the nucleus eventually.

So what happens is somehow, when you have low sterols-- and we're going to look at the model for how this happens-- both of these proteins, SCAP and SRE-BP, are transferred by coated vesicles-- we'll come back to this in a minute-- into the Golgi. So they go together into the Golgi.

And I would say that, right now, a lot of people are asking the question, once you do the processing to get SRE-BP into the nucleus, what happens to SCAP. And there are lots of papers, now, that are focusing on the fact the SCAP can recycle from the Golgi back to the ER. So it's never as simple. This thing's continually going on that not that much is wasted.

So this can actually recycle. And I'm not going to talk about that. And then, in the Golgi apparatus, there are two proteins, called S1P and S2P. And they're both proteases. We'll come back to this in a second.

So what's unusual is that we want to get this guy into the nucleus. And one of the proteases cuts here. So then we get this piece. And then the second protease cuts here, and then we get a little soluble piece that can move into the nucleus. Now, this is also revolutionary, in that nobody had ever known there were proteases that are actually found in membranes. Now, we know there are lots of proteases found in membranes.

And any of you work in Matt's lab? What is the factor that is regulated just like SRE-BP? Do you know? OK. So go look up the AFT4. Anyhow, so to me, what is common is once we found this, we've now discovered this in many other systems. So this system is a paradigm for many things that people have discovered since the original discovery.

But of course, the thing that's amazing-- first of all, this was amazing. The fact that this thing is in the membrane and gets to the nucleus is amazing. And at low cholesterol, what you want to

do is activate transcription. And you saw all the genes that could be activated in the previous slide. And it's complicated. There are many factors involved.

And so the key question, then, is how do you sense this movement from one place to the other and what do we know about that. So what I'm going to do is look a little bit at the model. So the model will start with-- and the players. So this is part 2-- the players.

And the players are-- so if you look at the ER membrane, what we have is two domains. And whenever you see a line through the membrane, that means a single trans helix membrane spanning region. We see that a lot. So I'm not going to write that out. But this is really sort of a single transmembrane helix.

And the key thing is at the N terminus, you have the helix-loop-helix. And this binds to DNA. So this is a DNA-binding motif. And so this is the protein SRE-BP. And so the second protein-- and this is the protein you're focused on for your problem set-- has a SCAP. 2, 3, 4, 5, 6, 7, 8. So it has eight transmembrane helices. And they've studied all of this using some of the methods that you're going to be looking at in your problem set.

And to me, there's a couple of things that we're going to be talking about in detail, but your problem sets are focused on-- all right. So I haven't really shown you where the loops are, but there are a couple of loops, loop one and loop six, which is what the problem set is focused on. And how do you know these are interesting and important. And we'll come back to this in a little bit.

So now, at low sterols-- so we want to turn on the machinery to make more cholesterol-- so that low sterols. And one of the key questions is what is the structure of the sterol. Can more than one do that? We'll see different sterols turn on different domains. And we'll see that there's a domain within SCAP-- so this protein here is called SCAP. And we'll see that SCAP has a sterol-sensor domain, as does another protein called INSIG, as does HMG-CoA reductase.

So somehow, you have these transmembrane regions that can bind some kind of sterol, that then changes the conformations, that is going to allow all of this chemistry to happen. So here, for example, we're not going to talk about this now. We're going to talk about that in the last lecture. But here's SCAP with its sterol-sensing domain.

So what happens, then, is this has to move. And as I said before, this can return. This moves

to the Golgi. So this is the Golgi. And the Golgi are complicated. And so I haven't defined where within the Golgi this is. And these are transferred by COPII vesicles. OK.

And so what you then have, again, is your 1, 2, 3, 4, 5, 6, 7, 8. And you have your sterol-responsive element-binding protein. And now what you see-- and so nothing happens in terms of processing, until you get into the Golgi. And then, there's one protein, S1P, which is a protease.

And I'm not going to go into the details of it, but if you look over here, what's unusual about this protease? If I gave you this cartoon, what would you say about that protease? Is it unusual compared to, say, trypsin or chymotrypsin. Can you see it? You can pull out your handouts. What are the catalytic groups?

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: Huh? Where have you seen those before?

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: Yeah, so they're aspartic acid, histidine, and serine. You see these over and over and over again. There are 150 serine-type proteases. OK. But what's unusual about this? Huge-- huge. OK. And then, the other thing that's unusual about it is that you have a transmembrane region. So it's completely different from serine proteases, so there's got to be some little domain that's actually doing all of this.

So I just want to note that it's huge. But you could still pick up D, H, S and know that that's the protease domain. And you could study that. You could mutate serine to alanine or something.

And then you have S2 domains. So we've gotten here. And this protease ends up clipping. so within the membrane-- so somehow, these things got to come together. And the active side of this protease needs to clip SRE-BP.

So it does that. And when does that, what you end up with-- I'm not drawing the whole thing out, but what you end up with, then, is your helix-loop-helix. So this part is still embedded in the membrane. And then you have your second protease. I don't know. I probably have the wrong numbers.

So this is S2P. And if you look at S2P, what's unusual about it and what people picked up on is

that it has another little sequence motif. And this is what you see over and over again, in enzymology. Once you sort of know something in detail, you know, even though there's no homology between the proteins at all, you can pick up little motifs, just like you can pick out little motifs that are zip codes that move things around inside the cell.

This little motif is the key player that tells you that this is probably a zinc-dependent metalloprotease. So this turns out to be a zinc metalloprotease. And this, then, does cleavage. But now, we actually-- it's pretty close to the membrane. OK. It does cleavage.

And now what you've done is you've released this thing. It pulls itself out of the membrane. And what you can do, then-- I'll just put this in here for a second. But what you can do now is we now move to the nucleus. And we have our pieces of DNA. And we have our SRE. And now we have this helix-loop-helix that activates transcription.

OK. So this is really sort of what I just told you in the other cartoon. And I just want to repoint out again that we now believe that these SCAP proteins can recycle back into the ER and be used again. And so controlling the levels of all these things-- we're going to see at the very end-- is also related to protein-mediated degradation that we're just now beginning to appreciate.

OK. So here's the model. This now sets the stage for you to solve problem set 7 that's due. Because the key question you want to ask yourself is how do we know about the structure of SCAP. And so problem set-- sorry, I'm over again. But problem set 7 is focused on how do you know that this little loop here, this little loop here, and this little zip code plays a key role in this whole process of moving from the ER into the Golgi. OK.

And we'll come back and talk about this briefly. We're not going to talk in detail about the experiments. And then we're going to move on and look at the post-transcriptional regulation of cholesterol homeostasis.