JOANNE STUBBE: So what we were doing last time is we were still focused the first two lectures were trying to understand the biosynthetic pathway for cholesterol bio-- it's good, thanks-- for cholesterol biosynthesis. And we almost got to where we wanted to go, but we didn't quite get there. So what we've been focusing on is a new way of forming carbon-carbon bonds using C5 units, isopentenyl pyrophosphate and dimethylallyl pyrophosphate. And to do that, we had an initiation process where these molecules were generated from acetyl CoA.

And then the last lecture we were focused on how we did the elongation process where we took a bunch of these IPP units, strung them together to make farnesyl pyrophosphate, which is C15, and I showed you that C15 could be linear or cyclized. And we went through the general rules of what you're going to see with all turpine chemistry, which is quite diverse, given that there are estimated to be 70,000 natural products in the terpenome.

So we had gotten to production of farnesyl pryophosphate and now the next step-- remember, cholesterol, if you look at its structure-- this is a precursor to its structure-- is a C30. And so the next step is quite an interesting enzymatic reaction which we're not going to talk about in any detail, but those of you who are interested can go look it up. But how do you take two C15s and form a C30 so you lose your pyrophosphates? And you can see when you generate this, now you have a linear c30, which, of course, is a complete hydrocarbon and is insoluble. So this now sort of defines that you need to be in the membrane to be able to do any further chemistry. So those of you who are interested in mechanisms of how things work, that's really sort of a fascinating system it's really pretty well worked out at this stage.

But today what I mean to do is focus on the next step is, how do we get from C30, which is this linear squalene hydrocarbon, into lanosterol, which is then the precursor to steroids but also the precursor to cholesterol, which is what we're focusing on in this particular module. So what we're going to be looking at is how we went from two FPPs-- we're still doing chain elongation-- to a C30.

And then the question is, how do you get from C30, which is linear, to a linear epoxide. And I'm not going to draw the whole structure out, but we're still linear. And then the next step is the step I want to talk about. So this is when lanosterol synthase. So that's where we're going in the next few minutes to get to our final product.

So if you look at this reaction, remember, we're going to do a cyclization. And what do you need to do to do cyclizations? What was the general rule that I gave you last time? Does anybody remember? If you want to cyclize something, we talked about it. We looked at a number of examples. What did we do in those examples? Anybody remember?

So here's a second example. I gave you two rules. If you go back and you look at your notes, we protonated the olefin and that triggered off the cyclization. And here, perhaps you could have protonated the olefin to trigger off the cyclization, but in the end, cholesterol has a hydroxyl group in the C3 position.

So the next step in the pathway, which also will involve, ultimately, protonation and ring cyclization, so those are the two rules I gave you during the last lecture, to get to this epoxide, we have to do some chemistry. Does anybody know what cofactors you would use to do this reaction? Anybody got any ideas from introductory biochemistry? You have a vitamin bottle. What vitamin would be involved in doing this transformation or could be involved with doing this kind of a transformation?

It's an oxidation. Requires oxygen gas. So what are the possibilities?

AUDIENCE: NAD.

JOANNE STUBBE: So NAD. Does NAD-- this is a good teaching point. Does NAD react with oxygen? Who suggested NAD? Why doesn't it make you react with oxygen? That's one of the things you learn in any introductory course. NAD does not react with oxygen. Why? What is the chemistry of NAD/NADH?

Whoa. Maybe I should be teaching 5.07. So NAD/NADH, we just went through this with conversion of acetyl CoA moiety of mevalonic acid to the alcohol. It involves hydride transfer. And if you tried to do this chemistry instead of two electrons at a time, one electron at a time, and you looked at the reduction potentials, it would be way uphill, thermodynamically. So NAD/NADH never does one electron in chemistry. So that's not going to be a possibility. Yeah?

AUDIENCE: You could use something that's like iron?

JOANNE STUBBE: So that would be one thing. And we're going to see iron-- heme irons play a key role in all of this process. This turns out to be a flavoprotein. That's the other redox active cofactor. So this is a flavin monooxygenase. You don't need to remember this. We understand the details. I'm not going to talk about the detailed mechanism, but flavin cofactors are extremely well understood. The chemistry of them is extremely well understood now.

So we've gotten to our oxidosqualene and now we've finally gotten to this really cool step. So how do we go from this step-- so this is this molecule here. And what I'm emphasizing again is we're going from a linear step into the cyclic product.

So remember, triggering off cyclization, there were two rules-- protonation, protonation of an olefin. In this case, you have some kind of protonation of the epoxide. Epoxides are not very good leaving groups. You need to protonate it. And that is then going to trigger off this cascade of reactions to allow you to generate a molecule with four rings. And this all occurs with a single enzymatic step.

And so the way you can visualize this happening-- and again, you don't need to copy this down. It's all-- if you look at your handouts ahead of time, there's some things that are written down that would take you 10 minutes to copy and then you probably get it written down incorrectly because you're looking like this is. The hard things that are hard to write down are all given to you in your handouts. You can write it down if you want, that's fine.

So what we want to do is we want a ring open, so we need to protonate the epoxide, and that generates what? A carbocation. And then now what happens? We generate another carbocation. And now what happens? We generate another carbocation. And now what happens? We generate another carbocation and we end up with a carbocation at this position.

So I'm going to draw the structure of this. So we have ring opened, and let me also emphasize that the key to this process occurring to give us lanosterol is the conformation of the linear molecule. So what do we see here? What does this look like? In terms of cyclohexanes, what does this look like? If you have cyclohexyl rings, what kinds of conformations do they have?

AUDIENCE: Chair?

JOANNE STUBBE: Chair and chair and boat. So the key here is that you have a chair conformation here. You have a chair conformation here, but here you have a boat conformation. And one of the

general rules I told you last time about terpene chemistry in general was, what do the enzymes do in the active site to transform something that's linear into something that's cyclic? They need to fold the molecule into the right conformation. And that can, in part, be done, the fact is the active site is very hydrophobic. We talked about that. And you can also have aromatics that could potentially-- I'm not drawing out all these intermediates, but could potentially facilitate not only the conformation but stabilization somewhat of the intermediates that you observe along the reaction pathway.

So here's another example of the importance of shape to defining the chemistry that's actually going to happen. And in contrast to the enzymes we talked about last time, which were type I. You probably don't remember that. But this is, again, a different super family involved that you observe, and it's observed quite frequently. So these are type II. So if you look up the structures, and in the article you had to read by Christiansen, the second type of structure. There are two general types of structure. This is the second type of structure involved in making interesting terpene molecules.

So what I'm doing now is showing you how we've cyclized this to leave us with a carbocation. And remember, if you have just a stick as opposed to a stick with a hydrogen, that's a methyl group. So here at the ring juncture, we have a hydrogen. We have a trans ring juncture.

And again, if we have a stick with nothing on it, it's a methyl group. And we're into a chair conformation again, and then we need to attach the last ring so we have three six-membered rings and a five-membered ring. And in the end, what have we generated? We've generated a carbocation.

So I've written this as a single step. Nobody has seen the intermediates. You could write it is multiple steps. I mean, the fact is it would be-- it's pretty hard to trap any of these carbocations, and people have spent a lot of time trapping them.

So what you see, I think, is quite amazing, but we aren't finished yet because we have a carbocation and we need to get rid of that. And what you need to do and this is-- you will have one of these problems on the problem set that will be due next week. You'll be given something simple, not as complicated as cholesterol. But what you need to think about is where do all these methyl groups end up in. What's the stereochemistry of the reaction?

So then this geometry becomes critical if you're thinking-- you need to think about the stereo electronic control of hydride and methyl anion equivalent migrations. So what you have in this

particular reaction is you're going to have-- and I like this example because, again, I gave you a set of rules that you can see that are associated, typically, with carbocation reactions in general, and this one does all of them. So one of the rules was that you have hydrogen migrate with a pair of electrons, so that's a hydride.

Again, you have a second hydrogen migrate with a pair of electrons. So I'm not drawing out all the intermediates. Now what we have is a methyl group migrate with its electrons. We now have a second methyl group migrate with its electrons. And in the end, we're left with a cation here, and the last step in many of these reactions is loss of a proton. So here we would have loss of a proton.

And if you look at the chemistry and you look at the final product, which I'm not going to draw out, you end up with this molecule. So this is a flat rendition of what I've actually drawn on the board. So this is an example of all of the chemistry I talked about as being general in all of these 70,000 terpenes. You'll find most of them don't do all of the chemistry. This one does all the different kind of chemistries associated with carbocation type chemistries that hopefully some of you have learned about in introductory organic chemistry classes.

So again, to me, this is the most amazing reaction I think I've ever seen. I told you already that I heard about this in 1969 when they'd just figured out that this could happen enzymatically. And this became the basis, for those of you, if there are any synthetic people here, people doing cascade reactions. Kim Jameson's lab does this, but back in those days, they were using this approach, trying to define the folding to do all these steps, just like nature had figured out how to do this. And if you look at the number of asymmetric centers, you end up with seven asymmetric centers and no other products that people could detect. So this is quite an amazing feat.

So this is the model that I just drew on the board. And so we still aren't quite there yet because if you look at this structure and you look at the final structure of cholesterol, you have a methyl group here, here, and you're going to have a methyl group here. So we have 1, 2, 3 methyl groups. And if you look at the final product, cholesterol, they're all gone. So you need 19 more steps to get to cholesterol. This is not a simple biosynthetic pathway.

So to get from cholesterol-- so this is lanosterol. So we've gotten to the precursor to steroids and cholesterol. And when we start talking about regulation, you'll see that lanosterol is, again, a central player because it can partition between different kinds of natural products that we

aren't going to be talking about, other kinds of natural products we aren't going to be talking about. But to get to cholesterol, which I'm abbreviating from now on as Ch, it's 19 steps.

So let's go over here. My goal is not to teach you about the chemistry of all this. I'm not sure how easily you can see it. Hopefully, you have the handouts with you, but we have this methyl group, this methyl group, and this methyl group that need to be removed over here. So that methyl group is gone and these two methyl groups are gone. So how do we do that? And so all of this reaction-- so we have loss of three methyl groups.

And all of these reactions are catalyzed by one kind of enzyme, which is a cytochrome P450 monooxygenase. So we're going to see that all the reactions are catalyzed by a cytochrome P450 monooxygenase, not a flavin monooxygenase. And if you look at the chemistry, flavins are not anywhere near as strong are oxidants as heme-dependent oxidation. So if you have something really hard to oxidize, you're never going to use a flavin. You're going to use a heme.

And what do we know about all these enzymes? I'm not going to talk about this in detail, but you have an iron 3 heme. And for those of you who don't remember what heme is, we're going to be talking about this in more detail in the section on reactive oxygen species. It's a protoporphyrin IX. That's what you see in hemoglobin. It's the exact same co-factor you see in hemoglobin, but what's distinct about this is that instead of having a histadine ligand, it has a thiolate ligand. And that's key to why P450s can catalyze these inactivated hydroxylations-- can catalyze hydroxylations of unactivated bonds where this hemoglobin reversibly binds oxygen.

So these P450s use this heme system in an oxygen system. And what did they do? And so what I do is refer you over here to-- let's look simply at 7 through 10 and we're removing this methyl group. So we're removing this little methyl group in the A ring. The first ring is the A ring. Sorry. So stereo specific, and so I'm not drawing the rest of the structure. And our goal is if we go through 9 through 10 and then 11 through 13, we want to get rid of both of these methyl groups.

And it's thought that one enzyme, but they don't know, can catalyze multiple oxidations. And why don't they know? Where do you think all was chemistry happens? You have cholesterol. What do we know about the structure of cholesterol? It's a grease ball. So where do you think the chemistry happens?

AUDIENCE: In the membranes.

JOANNE STUBBE: In the membranes, yeah. And so that's been-- P450s, you go to meetings, thousands of people still go to P450 meetings on the major targets of all kinds of therapeutics, and they're almost all membrane-associated, which has been problematic in terms of isolation and characterization. And here, despite a lot of effort, people really still don't know the sequence of events or have isolated and purified the enzymes. They're all in the ER, which is what we're going to come back to, and there are a membrane-associated.

So what happens in these reactions is you take a methyl group and then you oxidize it with one P450. So we somehow use oxygen-iron chemistry to do a hydroxylation reaction. Have you seen that before, in the first part of the semester? Anybody remember seeing it? Maybe you didn't see it. I missed a couple of lectures. Do you remember seeing hydroxylation reactions anywhere? Liz, do you talk-- was that in any of the natural products?

AUDIENCE: Sometimes [INAUDIBLE] P450s [INAUDIBLE].

JOANNE STUBBE: But what you'll see-- I think this would be, like, a decorating module that you saw in the non-ribosomal peptide synthetases. But here these things, as in the non-ribosomal peptide synthetases, are absolutely specific. And so you have one hydroxylation, you have a second hydroxylation, you have a third hydroxylation, which is chemically distinct.

And then the question is, how do you get rid of this altogether? Because our goal is to remove the methyl. That's what our goal is. So we've gone hydroxymethyl, the aldehyde, the acid. So now you have an acid next to the alcohol. How do you get rid of that? Has anybody-- what kind of chemistry could you do to allow you to lose the CO2?

AUDIENCE: [INAUDIBLE].

JOANNE STUBBE: You need to speak louder. Don't be-- I mean, just tell me what you think.

AUDIENCE: Decarboxylation.

JOANNE STUBBE: The what? Decarboxylation. But can you decarboxylate-- so you're right. We want to decarboxylate.

AUDIENCE: [INAUDIBLE].

JOANNE STUBBE: What do you have to do to decarboxylate?

AUDIENCE: You reduce the alcohol [INAUDIBLE].

JOANNE STUBBE: Reduce the alcohol?

AUDIENCE: [INAUDIBLE].

JOANNE STUBBE: What? What are you going to do? These are the kinds of-- you'll see these reactions happen over and over again in biochemical pathways.

AUDIENCE: Oxidize [INAUDIBLE].

JOANNE STUBBE: Right. You want to oxidize it. So what happens, if you look at this pathway over here, in this step, you use-- it should be NADP, so you use NADP. And what does that do? I'm not going to write this out, but it oxidizes this to a ketone. And now what do you have? You have a beta-ketoacid. And beta-ketoacids rapidly undergo decarboxylation reactions. So this is a strategy that nature uses over and over again in many biosynthetic pathways.

And the thing that's interesting, if you look at that pathway in detail-- and again, you're not responsible for that-- but then it does the same thing on the next methyl. So in the end, you end up with a carbon with two hydrogens here. But it's not straightforward, but this kind sequence of events you actually see a lot in metabolic pathways.

So I don't want to really say much more about this. In 19 steps, you need to remove three methyl groups. All the enzymes are ER bound, making it difficult to study the individual enzymatic reactions. And we would like to know the order, but we don't know it at this stage. What we know is what we see at the end.

So finally, I wanted to get here at the end of lecture 2. We've gotten here a little later. We've started with acetyl CoA. We've made the major building blocks for all terpenes, IPP and dimethyl APP. And we've gotten to form this very complicated molecule. Everything starts with acetyl CoA and you can-- this was classic work by Konrad Bloch, who won the Nobel Prize for this work, who then by doing label chasing, which you learned about, hopefully, in introductory chemistry, helped them to figure out this complex biosynthetic pathway, which isn't so easy because things are membrane bound and very lipophilic.

So we've gotten to cholesterol. So this module is on cholesterol and we've been able to biosynthesize it through an amazing sequence of reactions that have been studied over the

decades. But we can also get cholesterol-- we want to ask the question, first of all, why are we interested in cholesterol? I think you've already seen hints of that with the statins inhibiting HMG-CoA reductase. We have issues when cholesterol levels are too high or too low. We need to control the levels of cholesterol.

And the second way we can get cholesterol besides making it is we get it from our diet. So if we get it from the diet, the molecule we'll see is not very soluble. How is it distributed into the tissues? And then if you've distributed a lot of cholesterol from your diet, you certainly don't want to keep making cholesterol.

So the question is, how do you control those two events? What are the general mechanisms of regulation of the levels of cholesterol? And we're going to at the end look at some of the classic experiments that Brown and Goldstein did to understand how cholesterol, which from the diet can get into the bloodstream, can get transferred into cells. And so that's where we're going. And again, the reading is a reading I've already given you before.

So why do we care about this? We have a 30-step synthesis. We're getting it from the diet. We have key issues in homeostasis, which is what our focus is going to be. So why do we care about cholesterol? We care about cholesterol because it's associated with human health and coronary artery disease. Probably many of people who have had heart attacks.

And so elevated cholesterol levels have been known for some time to be associated with plaques, artherosclerotic plaques, which can lead to heart attacks and strokes. So what happens is the cholesterol deposits, you try to remove the cholesterol, you generate a lot of scar tissue, which then inhibits blood flow. And then you're in trouble if you can't figure out how to unblock the blood flow. So that's the main motivator and we'll see another main motivator is related to young children dying of heart attacks, which is what got Brown and Goldstein into the area of cholesterol homeostasis.

So there have been three Nobel Prizes given for work on cholesterol over the years. This is a classic paper. Some of the classic papers are actually quite interesting to read, and often the original papers get things wrong. So it was mostly right, but not completely right. But anyhow, I think if you put it into the context, 1928, how would you do experiments like that? We had no IR. We had no MR. We had no mass spec.

What did we have? We had ways of degrading things. People don't do that anymore. If you go back and you look at the discoveries before 1970 or something, these feats of pulling out the

structures with the right stereochemistry is really, I think, quite astonishing.

And I think what's most amazing to me is this old literature is actually much more reproducible than anything in the current literature. The current literature, we're spewing out papers, a lot of which will never get reproduced so we won't know if it's reproducible. But if you go back and you do anything, in the old days, you had to learn German because a lot of the original papers, all of the chemical papers, were in German. They did seminal experiments back in those days. And most of the time it was correct.

So anyhow, these guys figured out the structure almost. And then Konrad Bloch figured out, along with Fritz Lynen, figured out how you make cholesterol by labeling experiments. Now, many of you-- how many did label chasing in an introductory biochemistry course? Any of you have problem sets with label chasing? So it's quite distinct.

I taught with John Essigmann. All those problems were label chasing and I used to say, oh, no. Who wants to do label chasing? But the fact is now if you read any of the current papers in the literature, everybody is label chasing. And now we have much better ways of actually chasing labels using mass spec methods. So you can hardly pick up a journal nowadays without thinking about label chasing. So these guys who were way ahead of their time, but it was much harder in those days.

And then here are Brown and Goldstein. They won the Nobel Prize for the discovery of low-density lipoprotein and may still win another Nobel Prize for the regulatory mechanisms that we'll talk about at the end of lecture 5.

So the first thing I want to talk about in lecture 3 is focused on-- let's see. What do I want to do? Is focused on the properties of cholesterol. So we want to look at the properties of cholesterol. Then we're going to ask the question, how does cholesterol get from the diet to the bloodstream? And then we're going to ask the question, how does cholesterol get from the bloodstream into the tissues where it's essential for membrane controlling membrane fluidity?

So what do we know about cholesterol itself? If you look at the structure, what do we have? We have a grease ball and a little hydrophilic head. And so this cholesterol moiety, if you look at the structure up there, is really pretty rigid. And it, in fact, rigidifies. So this is rigid-- and in fact rigidifies membranes. And so you can see this if you go back and look at this. Hard to see these little things, but those are cholesterols stuck within the phospholipid bilayers.

And this is key since this is something that I think a lot of people are spending a lot more time on and we're getting much better at this now. People have stayed away from membranes because it's so-- and membrane proteins because it's so hard to work with and they stick to everything. How do you control all of this? And Brown and Goldstein really did some of the classic experiments that taught us how to deal with these type of really hydrophobic molecules.

And so cholesterol is pretty important. 10% of the membranes actually have-- of the lipids in the membranes are from cholesterol. So if you look at this, you would think it wouldn't be very soluble. And in fact, the solubility of this-- solubility is about five micromolar. So it isn't very soluble, but in fact, as an adult, we have 35 to 50 grams-- we each have 35 to 50 grams of cholesterol. And we know that per day 1 gram is derived from synthesis in the liver, so the predominant organ where cholesterol is made, like we just were describing, is the liver.

But we also have-- and I don't know how good these numbers are. I got them out of some book. So I'm not an expert in this, but anyhow, these are all rough numbers and you'll see these in other nutrient uptake systems. You want to have some vague idea of the contributions to the two distinct processes. We get from the diet, say, 200 to 300 milligrams from the diet.

So then if you think about this, cholesterol we're going to see is transported in the blood, and we'll see how that happens. Whoops. Transported in blood. And we know something about the amounts. And if you do a calculation, that says that you would have five millimolar cholesterol. So that's impossible. The number is squishy, but it's impossible. So you'd have this insoluble mess.

So the question is, how do you deal with it? And so that's what we need to think about. So how does cholesterol move-- I think [INAUDIBLE]. So how does cholesterol go from the blood to tissues, given the solubility problems? So here is again the structure of cholesterol. Again, it's pretty rigid and it inserts itself into membranes.

Where do you get cholesterol from? You all know you get cholesterol from beef and chicken and eggs. I guess there aren't very many-- do any of you eat at McDonald's? Or is that a passe thing? I love McDonald's anyhow. That was my favorite when I was in Wisconsin. There was only one restaurant near where the biochemistry department was and I went there every day, and my favorite thing was like two of those things slathered in cheese with French fries.

Anyhow, fortunately, I have very low cholesterol. But anyhow, you get that our diet is a major

source of cholesterol and what you eat can, in fact, be problematic and part of it really sort of depends on how lucky you are genetically, right? That's sort of the key thing.

So what do we do? We have this insoluble molecule and the question is, how do how are we going to get this insoluble molecule into the tissues where it's needed to control the fluidity of the membranes? That's the issue. So the second thing I'm just going to introduce you to, and this is taken from Voet and Voet. So many of you may have read that if you had 705 or something, but in 507 we don't cover this reaction. So I'm going to spend a few minutes going over it.

So what has to happen is cholesterol is found in lipoprotein particles. And we know a lot about the composition of these lipoprotein particles, which-- this is taken from Voet Voet. And what you can see is-- and I think, again, the relative amounts isn't all that important. But you can see you have-- and we're going to be focused on low-density lipoprotein, which is the major deliverer of cholesterol to the tissues.

And why is that true? So if we look at free cholesterol, we see we see 7% to 10%. I'm going to tell you about the structure of the lipoproteins in a minute. But most of the cholesterol is actually esterified with fatty acids, and you can see that the cholesterol esters are 35% to 40%. So if you look at the total amount of cholesterol in the LDL particles compared to all the other particles, it's much higher.

So what do you see and what do you have to worry about if you're getting this from the diet? So what would you expect to see? You would expect to see proteins. You would see phospholipids. So this is a phospholipid. You would expect to see triacylglycerol. Everybody know what triacylglycerol is? We expect to see fatty acids. We expect to see cholesterol.

And if you look over here, what people have done, have isolated different particles. How do they isolate the particles? The particles are isolated based on density differences. And if you look at all of these different compositions, they vary between very low-density lipoprotein, intermediate-density lipoprotein, high-density lipoprotein. They have different amounts of these different species. And in fact, most of them have very hydrophobic stuff in the outside and more hydrophilic stuff-- on the inside and more hydrophilic filled stuff on the exterior, which is more dense. And then that tells you something about how these things sediment by a subterfugation method and a density gradient.

So these lipoprotein particles-- and we're going to see this kind of method in next week's

recitations-- are separated by the centrifugation due to density differences. So let's just briefly look at LDL. That's what we're going to be dealing with today. And it's important because LDL is what we're going to try to take into the cell, and the composition of the LDL is key to thinking about how to studying that process in the classic Brown and Goldstein experiments.

So if we look at the cartoon of LDL that you see up there, what you see is a particle. They're sort of circular. The LDL particles, which is what we're going to be focusing on, low-density lipoprotein particles, have only a single protein and this protein is called the ApoB. It's a huge protein and it covers about 50% of the surface of the particle itself. Again, these things change in size. We'll see when we actually look at the transport process.

What do we know? It's on the exterior. On the exterior what you see all over the exterior, these things that are phospholipids. So the phosphate is on the outside, the fatty acids are on the inside. What else do you see on the exterior? You see a lot of cholesterol molecules, which I'm indicating like this. And then it turns out that the predominant-- and that maybe covers, I don't know, 20 Angstroms, but the particles are 200 Angstroms, 220 Angstroms.

So what's in the center? And what's in the center, so this is the interior. You basically have triacylglycerols and then you have cholesterol. And remember, cholesterol has one lone 3 prime hydroxyl group, and this is a esterified with a fatty acid. And so this is also in the interior. So that's the composition of the LDL particles. And the question is, again, where did they come from starting with stuff we get from the diet?

So that's what we're going to be focused on and that's what we're going to try-- the cholesterol is stuck on the surface and in the interior. Yeah?

AUDIENCE: I don't understand. Aren't there different splice variants ApoB and which one is the one that's involved now?

JOANNE STUBBE: There could be. There could be We're not talking about this in detail at all. I don't know how many splice variances there are. And I don't really know that much about all of these different proteins. You'd have to go read about them in detail. So I'm giving you sort of a cartoon general overview of what you need to think about.

There are splice variance of almost any protein. And in humans, you have in the PCSK that we talked about, there were nine isozymes. So isozymes and eukaryotic systems are something you don't have to worry about a lot for this lecture and for what I want to say. You don't have

to worry about that. And if you want to read about it, go for it.

So we have LDL particles and they are distinct from all these other particles which have different densities, different proteins. And there are two cartoons I want to use. This cartoon was taken from Voet and Voet. I think it was the third issue. The one from the fourth issue, which I'll show you in a minute, I think, is much better. So I'm going to change the handout.

But I just want to very briefly walk you through this. This is a really complicated process and, from my reading, is really not completely understood. But you have diet. And what do you have in your diet? Triacylglycerols, phospholipids, proteins. They get taken-- in the diet, they get into the intestine and somehow in that process they need to get packaged into one of these lipoprotein particles. And the lipoprotein particle that really is the predominant one that comes out of the intestine are these things called chylomicrons. And you can see they have a lot of proteins. They have a lot of triacylglycerol. They have a lot of phospholipids. Anyhow, the composition varies and the sizes also vary.

So these chylomicrons come from the diet. So how do these lipoproteins deliver LDL to the extrahepatic tissues? That's what we're really after. And so these chylomicrons-- let me just show you the next slide for a second. I think this is probably a better one, anyhow.

So these chylomicrons, somehow they have to package all this stuff into these lipoproteins. You know how that happens? I don't really know very much about it. Maybe somebody does. I don't know that much about it. So anyhow, it gets packaged into these little particles and then it goes into the intestinal lymph, which then goes into the bloodstream and then it needs to start circulating. So everything comes from the diet comes from these chylomicron particles.

So what happens when you go adjacent to adipose tissue or muscles? So what you need, if you're going to be involved with fat metabolism or you need energy to run down the street, you need fatty acids. So where do the fatty acids come from? They come from the triacylglycerol.

So what you have are lipases and all of these chylomicrons in the lipases. Then when you get near the tissue-- let's see. Here we get near the tissue, the muscle or the adipose tissue, a li-- does everybody know what a lipase is or do you want me to write that reaction on the board? Does everybody know what a lipase is? No? OK.

So a lipase-- so here is your triacylglycerol with different fatty acids. So this is a TAG. This is glycerol. It's stereo-- this is a chiral center. And so what happens then is lipase is simply an

esterase that hydrolyzes the bond. So I'm not going to draw the reaction out, but a lipase catalyzes, release-- this is a fatty acid. And it actually cuts off two of them, and so most of the time you have monoacylated fatty acids. But again, from what we're talking about, this is not really important because really what we want to do is get to the low-density lipoproteins.

So what you see when you start doing this is that if you drop off triacylglycerols or monoacylglycerols here, and you drop off something else to the other tissues along the way, the sizes of your particles change size. So they call these things, then, the remnants from your starting material. And it turns out there is a receptor that takes up chylomicron remnants into the liver. So the central player in all of this is the liver. So you got to take stuff-- we get it from the diet, but we got to get it into the liver and that's where everything is controlled. And that's predominantly where cholesterol is actually biosynthesized.

So if you start-- you bring in-- what are you bringing in? You're bringing in cholesterol because you've dropped off triacylglycerols and lipids and phospholipids to the tissues. So what's predominantly left? What's predominantly left is cholesterol. So from these chylomicrons you drop off fatty acids and monoacylglycerols to adipocytes or muscle, and then what you have left is cholesterol. You have a lot of stuff left, but cholesterol is a major thing, which is then going to go to the liver.

And so then once this gets into the liver, the liver has all this machinery to repackage things and they can make very low-density lipoproteins again. So you can go back and look at this. It's very complicated. They then in the bloodstream can drop off stuff along the way to tissues as well. And then they change into intermediate-density lipoproteins, which then can change into low-density lipoproteins.

And it's these low-density lipoproteins that are going to then deliver cholesterol, that has more cholesterol than any of these other particles, either two extrahepatic tissues or back into the liver. So you have a complicated set of transport systems that we're not going to spend any time on, but it's all related to the fact that cholesterol is basically not a happy camper in water. And so we've got to figure out how to move cholesterol around.

So that just summarizes-- what I didn't show you over here, you all have heard about high-density cholesterol, low-density cholesterol. And high-density cholesterol is distinct from all these other lipoprotein particles. And it sort of scavenges excess cholesterol from these extrahepatic cells and returns it to the liver. But unlike the look the receptor-mediated

endocytosis we're going to talk about with the LDL receptor, this receptor is completely distinct. I'm not going to talk about it, but the mechanism is distinct from these other receptors that people have also studied in some detail.

So the other thing that I wanted to briefly say is that in addition to cholesterol what you see-- and I'm not going to spend much time on this either, but I think it's worth mentioning-- is when you get cholesterol back into the liver, what can you do with that excess cholesterol? If you have too much of it, how do you control the levels? That's the key thing we're going to be trying to focus on. What have we learned, at least to some level, in control of cholesterol levels?

But it turns out in the liver-- so the key organ in all of this is the liver-- cholesterol can be metabolized to form molecules that have four rings just like cholesterol that are called bile acids. And these are multiple steps. I'm not going to draw out the steps, but if you look at a bile acid-- and I have cartoons of bile acids over here. So here's cholesterol and if you look at this-- it's hard to see it, but if you look at it, it really sort of it looks a lot like cholesterol. The only differences are you add additional hydroxyl groups.

So in cholesterol we have a 3-alpha hydroxyl group. In the bile acid you have two additional hydroxyl groups put on again by cythochrome P450s. So you have a hydroxyl group at C7. You have a hydroxyl group at 12 alpha, simply means the stereochemistry. So the stereo chemistry of the hydroxyl group is on the same face. So that's what I mean here. So what you have then is hydrophobic and hydrophilic.

And in addition, if you look at the very end, it turns out you have molecules glycine or taurine, which are on the handout, which has a negative charge. And again, it's on the same face. So we have a bunch of hydroxyls, something charge, and they act as emulsifying agents, and that's all you really need to know. So these become emulsifying and they really play sort of key role in also helping to take things back into the cell. And this is a really complicated process.

And in fact, I think it was 15 years ago, something, people used to try to remove bile acids as a mechanism of controlling cholesterol levels. And what you did was actually-- boy, I'm way over again. What you actually did was eat-- have any of you ever worked with Dowex in an exchange? You used to eat the resin you have in the lab called Dowex because it would bind the negatively charged materials. And so, really, it was very hard for people to stomach this, but that was before we had really sort of wonder drugs-- Dowex, eating Dowex in these little

grainy resins. You should go look in the lab if you're doing a UROP. That's how we used to treat high levels of cholesterol. So anyhow, bile acids also play a key role. We're not talking about this in detail.

So the next time we're going to come back and we now sort of see what the properties of cholesterol are, that they're in lipoproteins. And we want to focus on the key experiments that showed how LDL is taken into cells.