JOANNE STUBBE: Where we were at at the end of the last lecture was trying to figure out what do we do with the fact that cholesterol-- its solubility is five micromolar. Yet if you look inside your blood, the levels would be 5 millimolar. And so the question is, how does it gets transported? And it gets transported in a complex fashion. We need to deal with that with any kind of very insoluble lipophilic materials. And I briefly introduced you to lipoproteins, which are mixtures of different kinds of lipids, triacylglycerols, phospholipids, cholesterol, cholesterol esters.

And the key question we learned in the first couple lectures that cholesterol could be biosynthesized. And what we started focusing on in the last lecture was that it can be taken up by the diet. That's what we're focusing on now. And then after we do a little more background, then how is it taken up and then how is this all regulated? How do you control biosynthesis versus cholesterol from the diet. What are the sort of major mechanisms?

So at the end of the last lecture I'd given you a second picture. And the PowerPoint-- the original PowerPoint didn't have this figure. This is taken out of a new Voet and Voet-- the newest Voet and Voet-- which I think better describes what's going on. But really sort of what you need to know is you form these particles, chylomicrons, if you look at the handout I gave you have lots of proteins, all kinds of lipids, cholesterol. And they get into the bloodstream and they pass off as they go through adipocytes or as they go through muscle. The surface of these cells have lipases, phospholipases that can clip off the fatty acids that you need for metabolism at most cells.

And what happens is the size of these particles just change. And so in the end, you remove the triacylglycerols and you remove phospholipids. And what you're left with is more of a cholesterol. And that-- and so what happens is the chylomicrons change size. They call them the remnants. And there are receptors on liver cells, which can take up these remnants, these lipoprotein remnants. And then they repackage them into other lipoproteins. And again, the differences in the lipoproteins we talked about very briefly, we have an outline.

Somebody measured these with a-- again, they're variable, but they're based on density. And so the liver repackages these things to a particle that's very low density, lipoprotein. And then again, they can dump off components into the tissues where you can use the lipids to do metabolism, changing the size, intermediate density, eventually low density lipoprotein which is what we're focused on now. And then today what we're focused on is how does the low density lipoprotein get taken up by the liver? And also, can it get taken up by other kinds of cells?

And if you have excess cholesterol produced in any of these extrahepatic cells, it can be taken up to form particles called high density lipoproteins. And they can come back. So they act as cholesterol scavengers, come back and deliver it back into the liver by a mechanism that is really different from what we're going to be talking about today. So that's the overview picture. And so what I want to do now is focus on the question, why do we care about cholesterol and what was the motivator for Brown and Goldstein's discovery of the low density lipoprotein receptor.

So this is the motivator. They were seeing when they were at medical school, a number of children that presented at an early age. These guys were six and eight. And the way they present, if they turn out to have both genes, both copies of the gene are messed up for low density lipoprotein receptor, that's called familial hypercholesterolemia. The way they present is they have these little xanthomases that are apparently yellow.

And what they are is they're full of cholesterol. OK, and so if you have someone that's heterozygous rather than homozygous-- these guys are homozygous-- you still see these but you see it at a much later time in their life. And so again, what it is, it's a function of the fact that you have too much cholesterol and this is the way-- one of the ways-- it manifests itself.

The second way it manifests itself is if you look at the concentration of low density lipoprotein and the plasma, which is given in milligrams for 100 mils, what you see is the concentrations of cholesterol are actually 5 to 10 times higher. So that's the manifestation. And children that manifest at this early age die of heart attacks by the time they're 30. And so this was the motivator. They were trying to figure out what is the basis or bases for this disease.

So that's what I said. This is a dominant effect. At the time, the gene or genes responsible for this were not known. It turns out from the data that I've gotten from some paper, one in 500 people are heterozygotes. That's quite prevalent, actually. But the ones that manifest

themselves in this really terrible way early on is something like one in a million. And so-- but even the heterozygotes, Brown and Goldstein study all of these people, also manifest in this way. They have elevated cholesterol levels. And so this was is a huge problem.

And so they decided they wanted to really devote their life to it. And I think they didn't know this in the beginning, but it's really associated with one gene. Most diseases are much more complicated than that. And so I think because of the, quote, "simplicity" unquote, you'll see it's not so simple, they were able to make progress. And these experiments were carried out really sort of in the-- started in the 1970s.

So I think Brown and Goldstein-- we talked about the cholesterol biosynthetic pathway. And we talked about what was rate limiting. So hopefully you all know that the rate limiting step is the reduction of hydroxymethylglutaryl CoA down. So the CoA is reduced all the way down to an alcohol and that product is mevalonic acid. And if you can't remember this, you should pull out the biosynthetic pathway.

And that was proposed to be by other people working in this field to be the rate limiting step in this overall process. And when you take an introductory course in biochemistry, you talk about regulation. I guess it depends on who's teaching it, how much you talk about regulation. But of course, one of the major mechanisms of regulation that's sort of easy to understand in some fashion, is that oftentimes the end product of a pathway can come back way at the beginning and inhibit the pathway.

So that's called feedback inhibition. We saw that cholesterol biosynthesis was 30 steps. And if you go back and you look at the pathway, you know, I think this is step four or five. I can't remember which one it is. And so the model was-- and there was some evidence that suggested that from what had been done in the literature-- that cholesterol was potentially acting as a feedback inhibitor. And that's what their original working hypothesis was.

So the hypothesis was-- this is how they started it out. And what we'll do is just look at a few experiments of how they were trying to test their hypothesis and then how they change their hypothesis to come up with a new model for cholesterol regulation. So you start out with acetyl CoA and you go through mevalonic acid. And then we get to cholesterol. And so the model was that-- this is HMG reductase-- that this was a feedback inhibitor. And that it inhibited by allosteric regulation.

And that's true of many pathways. And often, that's one out of many mechanisms that are

involved in regulation. So the first problem they faced-- and for those of you who want to read about this in more detail, the original experiments, I'm just going to present a few simple experiments and I'm going to present them in a simple way. OK, everything with human cells is more complicated than the way I'm presenting it.

But for those of you would like to read a little bit more about the actual experiments, there are two papers that I think are particularly compelling. And in previous years, I've actually used these papers in recitation. OK, so this is one of them. I'll put the other one up later on so that you can look at the detail, more about the experimental details. And I think in these particular experiments, what you're being introduced to, which most students don't experience, is the fact that you have-- all you do with these insoluble membrane-like proteins and how do you deal with membrane proteins.

Most of us-- I haven't had any experience with this at all. So this week's recitation, for example, sort of shows you what they had to go through to be able to answer these questions. And it's complicated. And I think reading the experimental details in the end, if you're going to do something like this, this provides a nice blueprint of how you try-- how you try to design experiments.

And you'll see some of the complexity from the few experiments I'm just going to briefly describe. OK, so what they needed was a model system. And of course, you can't do experiments on humans. So what they wanted to do was have some kind of tissue culture system. So they wanted a model system. And there was some evidence in the literature that human fibroblast skin cells were actually able to biosynthesize cholesterol.

So they wanted to ask the question, do these skin cells recapitulate what people had seen from the biological studies in humans? And so the first experiments I'll show you, does recapitulate that. It didn't have to. But then this became their model, human fibroblast cells became the model for which they're carrying out all of these experiments that we're going to very briefly look at. OK, so the experiments, I think, are simple, at least on the surface.

Although I think it wasn't so easy to figure out how to do these experiments. So what they wanted to do, they had patients-- whoops. I didn't want to do that. Anyhow, sorry I'm wasting time. OK, this patient is JD. And all of the experiments I'm going to show you is JD. But they had 25 other patients. And what you'll see is they all manifest themselves in different ways. And we're going to see that that, in the end, becomes important in sorting out really what was

going on.

OK, so the first set of experiments they did was the following. So they had some kind of normal control. And then so we have a normal-- so we have skin cells from a normal person. And this is the control. And then you have the FH patient, JD. And in the two papers I'm going to reference, they did a lot of experiments on JD's fibroblasts. And so they did some simple experiments.

And remember, the rate limiting step is proposed to be hydroxymethyl-- HMG CoA reductase. And so they wanted to first ask what happens if you treat the cells, so you have them growing. OK, and you let them grow for a certain period of days. And then what you do is you take the media, change it, and remove low density lipoproteins from the media. I don't know whether they removed them all. They said they removed 5%. I don't know what the percent that was there.

And so we're going to do that for both the experiment and the control. So this is the experiment. This is the normal person. This is the experiment, the FH patient. And if you look at the axis in measuring HMG CoA reductase activity. So what they're going to do is look at plus or minus LDL. So in this panel, they've removed the LDL, OK? And if we remove the LDL, you remove the cholesterol, what might you expect to happen to the normal HMG CoA reductase levels or activities?

If you remove the cholesterol from the plasma, what might you expect to happen to the activity? What would you want to do? Would you want to turn it on? Would you want to turn it off?

STUDENT: Turn on.

JOANNE STUBBE: Turn it on, right. So that's what they're going to be assaying. They remove it and if you look at the normal patient, the normal control, what's going to happen is the biosynthesis is turned on. So it'll look at this, then, you need to have-- and this goes back to the things we've talked about a little bit about in class-- and in fact, the original recitation that we had on radioactivity was completely focused on Brown and Goldstein's work. So we're going to see that they use a lot of radioactivity and all the assays I'm going to be describing today.

So what we're going to be doing is revisiting radioisotopes. They couldn't have done that without these radioisotopes. And this is converted to this. OK, what's the cofactor for this

reaction? So, I'm not going to draw up the rest of this. This is mevalonic acid. What's the cofactor required for this process? Any DPH. So you have any DPH. OK, so how would you assay this?

So we're doing this now in tissue culture systems. That's what-- we are doing this in fibroblast cells in tissue culture. So we don't have very much material. You might have a plateful of cells. How would you do the assay? So this is the first thing you have to figure out. And I would say, almost everything in this class, when you're studying the biology, first thing you have to do is figure out a robust assay. This case, I think it turned out to be quite easy.

But it's not necessarily easy in many cases. So this is something, as a chemist, you bring a lot to the table. Yeah?

STUDENT: You would measure the change in the absorption at 340.

JOANNE STUBBE: 340. So that's the way chemists would do that. Why can't you do that here?

STUDENT: You have to isolate the HMG CoA reductase or somehow be able to parse it from everything--

JOANNE STUBBE: Well, you might be able to do it in crude extracts if you had a lot of it. But it's tough. NADPH is used in hundreds of reactions. It's a great assay because the absorption change is removed from where most of the material absorbs, which is, you know, 280, 260, 280. It's not that sensitive. The extinction coefficient is 6,300 molar inverse centimeter inverse. And the bottom line is if you look at it, it's nowhere near sensitive enough. So if it's not sensitive enough, then what do you need to go to?

That's what-- the radioactivity. So what you're going to be doing here is-- so you could use either 14c-- hopefully you remember that's a beta emitter, which then gets converted into mevalonic acid. And then you need a way of separating starting material from products. And there are many ways that one could do that. But in the original paper, they use TLC. And that's how they monitored their reactions. And you need to have material that's of hot enough radioactivity so you can see these into conversion.

So that's the assay that they used. And so in the PowerPoint, I decided not to draw out. So if you PowerPoint, you look at the data, what do you see? What you see is that if you look at the experiment where they removed the low density lipoprotein from the media-- so they've taken it out. They've grown the cells they have HMG CoA reductase activity. What do you see immediately-- and the control and the patient's cells are growing exactly the same way. What

do you see immediately?

You see a huge difference in the amount of activity. So this is 2. This 150 or something. And so there could be a number of reasons for all of that. And so the question is, what is the basis for this increase in activity due to increased huge amount, the amount of HMG CoA reductase. Has the activity changed? Is there a mutation that changes the activity? There are lots of explanations.

And so what they then did, when they remove this, they started doing assays over 24 hours. And they crack open the cells and do this radioactive assay. And then they looked at the rate of formation of mevalonic acid. And so what do you see with the normal control? You see exactly what you might predict. So if the cholesterol levels become low, you might want to biosynthesize it.

But then what do you see with a homozygote, the JD patient? What you see is the levels start out high you have complete absence of regulation by changing the concentration of cholesterol. That's what you're seeing. So it seems like a simple experiment. It is a simple experiment. The basis for these observations is still open to debate. But the experiment turned out to be straightforward.

Then what they did is at 24 hours, they then started adding low density lipoprotein back into the media. So they start over here, they removed it. They add it back. Here's with non. Here's with two micrograms per mL. Here's with 20 micrograms per mL. And what do you see with a normal patient? With a normal control? What you see with the normal control is a loss of activity.

So that's exactly what you would expect that cholesterol-- you have a lot of cholesterol, you don't need to make it anymore. So this data, then, this simple data told you-- the control told you that minus LDL, you increased HMGR activity. And plus LDL, you decreased activity. And what about the patient? The FH JD patient?

So here what you see is that removing cholesterol from the plasma has no effect. What about adding it back? Has no effect. So somehow the patient is-- the patient's cells is oblivious to the presence or absence of cholesterol. So in this case, plus or minus LDL had no effect. So we say loss of cholesterol regulation, which could be due to feedback inhibition, it could be due to something else. We'll see it is due to something else.

And so this was consistent with what they predicted. And they furthermore learned that these fibroblast cells might be a good model for actually studying what's going on in the liver. I mean, you always have this issue. You have to figure out what you can study as a model system since we don't work on the humans. And so you always have to worry about how that extrapolates to humans.

So basically, you're looking at cholesterol in the media. You're looking at cholesterol not in the media. And these are the experiments we just described. And so one of the questions you can ask, then, is what happens now? Another thing that can happen is what if cholesterol can't get into the cell? So what they did is another experiment where they-- they did two things to look at the HMG CoA reductase activity in the normal control and in the FH patient.

And one of them was they repeated this experiment in the presence of ethanol, where they dissolved the cholesterol. And apparently that allows the cholesterol to get across the membrane. OK, so we're bypassing what we now know is going to be a receptor. So they did a second experiment and they used ethanol cholesterol. And it goes across membrane.

And then they looked at the HMG CoA reductase activity. And the activity of both the patient and the normal controls was the same. OK, so the activity, HMGR activity the same. They don't report the details of this experiment. But another way you could do this is you could pull out the protein or partially purify the protein in crude extracts and try to measure the activity using this assay. And if you have a good measure of the amount of protein, which is key, so you can measure specific activity, micromoles of product or nanomoles of product produced per minute per milligram of protein, you could actually see that the HMG CoA reductase activity was the same in the wild type in the normal and in the patient.

So you could also measure this using assay. And again, the result was that they were the same in both the normal and the patient. So then the elevated levels could be-- elevated levels, you saw in the very beginning of the HMG CoA reductase activity, could be due to the fact, they had a huge amount of protein, more so than you do with the fibroblasts. And so, there's no reason to think a prior if you looked at that previous slide, that the control, that normal control in the wild type-- I don't know what the scatter is in the data for HMG reductase activities, but that's something you need to think about.

But a 60-fold change is a huge change. So this data, the initial set of data said that, yeah, cholesterol may be acting as a feedback inhibitor. But here, we can get cholesterol into the cell

and the activities are the same. So they needed to come up with an alternative hypothesis. OK, so they then, using these two sets of data, came up with an alternative hypothesis. So they concluded that it's not cholesterol feed back regulated.

And so then they set out to do a second set of experiments based on a new hypothesis. And the new hypothesis is that there would be some protein that might be involved in taking up the LDL particle, which has a cholesterol into the cell. So the new hypothesis was there is an LDL receptor, so r is receptor. That's how I'm going to abbreviate it. That's key to taking up LDL. And so that's what's shown here.

And so then the question is, what sets of experiments do they do next. So this is a second set of experiments that was done in a paper that's also quite interesting. And so, for those of you who want to look at the details of this, this was published in 1976. And so this is where the data that I'm going to show you on this slide came from. Because I think they actually put it in one of the two review articles I gave you to read. But if you want to read the original data, the papers aren't that long.

And they go through the details of the rationale of how they design their experiments. OK, so what we want to do now is test the idea that to get cholesterol into the cell, there is an LDL receptor. And that that's going to play a key role in controlling cholesterol levels. That was the working hypothesis. OK, so how would you go about testing this experimentally?

So these are the results of the experiments. And the question is, how would you go about testing this experimentally if this were your hypothesis? And so if you think about it, you might like to know, does the LDL particle bind to the surface of the cell? Does it bind? OK, so that would be one thing you could do. And in fact, Brown and Goldstein were treating many, many patients. So they had fibroblasts for many patients, 20 to 25 patients. They all had different phenotypes.

And again, these were differences in the phenotypes actually helped them to try to dissect this process. And so could it bind? And so we can ask the question, how would we look at binding? I'm going to ask you that question. We're going to have a recitation on binding, I think, not this week, but next week. Then it gets into the cell. OK, so how do you know it gets into the cell?

And so that's another question. Inside, outside. And then the next question is, what is LDL? Hopefully you remember it's a lipoprotein that has a single protein on it, apoB. And then it's full with cholesterol, cholesterol esters, and phospholipids. What happens to that stuff once it's

inside the cell? OK, so those are the questions in this experiment that they set out to ask. OK, so what I want to do-- so binding, internalization, and then the fate of LDL inside the cell.

So that's what they were focused on. So what I want to do is show you the tools that they developed to try to answer these questions. OK, I'm going to show you a few things because this isn't such an easy set of experiments to carry out. And then what they observed on the normal cells and the patient cells. OK, so the tools that I want to talk about are the following.

OK, so we just talked about the fact that, to do the assay, we needed radioactivity. We needed to be sensitive enough. If you're going to be looking at binding on the surface, how do you do that? Do you think there are a lot of receptors? Are there a few receptors? So you might not know that. But in general, there aren't huge numbers of receptors. So measuring binding to the surface of the cell usually requires a very sensitive assay.

So the first thing they needed to do was they decided that they needed to make the LDL radial labels. And if you go back and you look through your notes in recitation three where we talked about radioactivity, we saw that we have beta, c14 beta, which is what they used up there. But they also used i125, which is a gamma, which is much more sensitive.

And so what they decided they needed to make was i125 labeled LDL. So if you haven't radio labeled, can you somehow see it sitting on the surface of the cell? So the question is, how can you do that? Well, we talked about the composition of the LDL particle. There is cholesterol. There's cholesterol esters, phospholipids, and one protein. And so what they're doing to put the iodine in is putting it into only the protein. OK, so what they use is a method called Bolton Hunter, which uses radial level iodide and a reagent.

I'm not going to go through-- you can look it up if you're interested-- I'm not going to go through the details. And what it does is it takes a protein-- this is still actually widely used. So this would be apoB. And it iodinates at the ortho position. So what you end up with, then, is iodinated apoB. So that's going to be your handle. You can make this a very high, specific activity.

OK, so that's one thing that they needed to do. OK, the second thing that they needed to do is if they're going to look for binding to the surface, how would you design that experiment? What might you need to do to figure out how you're going to look at binding only and not binding and uptake? What parameter could you change that would help you do that?

Temperature. So everything-- and you'll see this also in experiments this week-- the temperature is really critical. Why? Because hopefully you all know lipid bilayers are very fluid. And if you cool the temperature, you prevent uptake and other things. You have to test all this out. They did a huge number of controls. So the second thing that they wanted to do is they used temperature.

So four degrees, they're going to use to look at binding. Or if they're looking at a time course and they want to stop the reaction, and the reaction is normally done at 37 degrees-- so uptake experiments would be at 37 degrees. OK, so again, temperature is the key parameter. You could, if you wanted to a time course and stop the reaction, you could cool with down to four degrees. I mean, this was a hypothesis they had.

And so that's the second tool that they're going to use. And the third tool, which I think isn't necessarily so intuitive, is if you're looking at something binding on the surface, you have to always worry about non-specific binding. You'll talk about that in the recitation. On this that's always a problem. You're using really hot, iodine-labeled materials, so you could get neuron specific binding.

And so how do you-- so you need to wash it. So if the LDL particle bound loosely to the LDL receptor, that makes the problem extremely challenging because when you're trying to wash away the excess as you change the concentration of the LDL, you're going to start to lose-- you're going to have an equilibrium and you're going to start to lose binding.

It binds really tightly so they had to have some kind of a wash. So they figured out and optimized a wash. So you need to have a wash. So if you have a wash and then you're still looking at the receptor with the particle bound-- so that's the LDL-LDL receptor-- then the question is-- and it's tight binding-- how do you get that off? And remember, you're also going to have LDL that's been internalized.

So the creative approach they used was to use the molecule heparin. OK, so heparin-- I'm not going to draw out the structure-- but this is a third tool and this was key. And so they have heparin-sensitive and heparin-resistant. And what does this mean? Heparin turns out to-- it's a sugar. Many of you have probably heard about it. It plays a key role in blood coagulation.

But anyhow, from the point of view of today's lecture, you just need to know it's a sugar and it's got sulfates all over the outside of it. So it's negatively charged. So heparin is a sulfated sugar. So basically, you have something like this with SO3 minuses on the outside. And so what

happens is if-- what you want to do is release the LDL particle from the receptor. And apparently, treatment with heparin at certain levels-- I think they tried a lot of things-- was able to release the surface LDL.

So this is involved in release of surface bound. So then what you have left after you release this, is you could still have radio label that's been internalized. So that then becomes heparin-resistant. And so you can count that. And so then you have bound and internalized. Now, if you're studying this as a function of time, what can happen to-- once you internalize the LDL particle, what can happen to the iodinated LDL particle? What can happen?

So this is something else you need to think about in these assays. So now we have internalized LDL, i125 label. What can happen-- if you remember from recitation this past week, you remember what happened to the LDL? So you got protein. You got lipids. What's going to happen? You might not know the details.

That's what this whole-- that's what Brian and Goldstein uncovered, which we're going to talk about in the next few minutes. But LDL, you have a protein. What can happen to proteins? They can get degraded. So if you have the apoB, what can happen is inside the cells-- so this is inside-- you could have proteases that degrade this down to peptides. This happens in a lysosol where you still have iodinated tyrosine. Or it can be broken down all the way to just iodinated tyrosine.

So if you're breaking this down all the way here, the iodinated tyrosine could likely exit the cell. So you need to really think-- so what do you do to control for this aspect of the metabolism? What happens to the LDL inside the cell? And so to do this, how would you distinguish LDL itself from, say-- as a chemist, what could you do to distinguish LDL protein from LDL on small peptides or LDL as an amino acid?

So the key question was, what sort of bulk method do you use to try to distinguish between these two things. So then you can incorporate that into the analysis, which is what's on the slide here. So what happens if you treat proteins in general with acid? They what? They hydrolize? So peptide bonds are really strong. If you want to break a peptide bond, you have to heat it for 16 hours at 100 degrees. So that's not going to happen.

So that's not an option. But what else happens? What do you do when you put a protein into acid? What happens to the protein? It what? Yeah, it crashes out. So proteins in general, not all proteins, most proteins precipitate, but these kinds of things would be soluble.

So they've been able to take advantage-- so you have to, again, treat the cells in a certain way so that you can look at what's still retained in the LDL versus what's undergone degradation. OK, we're going to see that's key to the model we're going to come up with. OK, so those are the tools that they needed to develop. And so the question is, then, what did they observe? OK, so we're doing these same experiments. We're looking for binding on the outside, internalization, and breakdown. That's what we're looking for.

And so here is the patient and here is the control. So if we look at here, these guys are the binding. So Brown and Goldstein, in this particular paper, which is underneath here but in the cell paper, looked at 22 patients. And out of the 22 patients, most of them were binding deficient. They could see no binding at all. Some of them were binding modified. That is, they had lower levels of binding. And this one patient, JD, had normal binding.

So in this experiment, we're looking at here-- so in the PowerPoint, this is one of 22 patients they had normal binding. And the others-- and that's because we'll see that there are multiple ways you can have defects in your LDL receptor. We'll come back to that in a minute. But you can have deficient binding or you could have no binding. Or you could have normal binding. So those are all possible.

And the one that we've taken the data for here and that's described in the paper, is normal binding. And they did a lot of experiments I'm not describing to try to show you that this experiment, which suggests normal binding, is in fact normal binding. They looked at off rates. They looked at competition with HDL and LDL. And so if you look at that, if you look at the levels of binding, they really aren't very different between the experiment and the control.

And so now what happens, if you look at the normal, what happens is with time, the LDL on the surface goes away. And that's because it's becoming internalized. Whereas down here, what happens? You started out the same, but now you can see over-- this is hours down here, it really hasn't changed very much. It's not becoming internalized. And so then they wanted to use their method to look at internalized LDL. And so internalized LDL, using the heparin-resistant versus heparin-sensitive, that's the assay they used, what you see is as the surface binding at least early on decreases, the amount internalized increases.

But what happens over here to the patient? With the patient, you get nothing internalized. And the other question is, what happens to the LDL-- and it's labeled on the protein-- does that get

degraded? And so using a method with TCA, they used a couple of different methods, what they see is that you slowly degrade the protein into small pieces. And again, with the patient, it's not internalized so you can't get degradation.

So this type of experiment with this particular patient and also with the other patients that I talked about, one through 21, they drew a strong conclusion that there are two things that have to happen for cholesterol to get into the cell. Number one, it has to bind. And number two, there's got to be some mechanism for internalization. So the conclusions from this is we need binding, which is consistent with the LDL receptor. And then we need, in some way, internalization.

And of course, JD was the only one out of all of these patients where they can study internalization because in the other patients they didn't-- they had really poor binding or no binding at all. So they needed to have this spectrum of patients to be able to start to sort out what was going on in these experiments. So I think on the surface, the experiments look pretty-- you'll look at them, they look like they're really simple.

But technically, they're not so simple. And if you care about the technical details, which we'll see again in this week's recitation dealing with these membrane proteins and stickiness, becomes the key how creative you can be. And usually, we're not really plugged into that. And you usually don't do experiments like that unless you work in a lab that is focused on membrane-bound proteins.

So this resulted in the model. So this kind of experiment and many other experiments resulted in the model for receptor mediated endocytosis. And you've seen this before. You saw this in recitation last week because we saw interference with the PCSK9 with receptor mediated endocytosis. So we're back where we started last week. And the first slide I showed you was this slide.

And so what is the model? So there are many, many more experiments that have gone into coming up with this model. And the model is really still incomplete. I have a cartoon here, the whole process, every step along the pathway, how you go here and there and what the kinetics are, it's all complicated. But this is the working hypothesis. And so the first thing is you make the LDL receptor. It's a membrane protein, has a single transmembrane spanning region.

Is made in the ER. And because of this transmembrane spanning region, it's got to be

transported to the surface. And it's done so in little coded vesicles, which keeps things soluble. And it does this by passing through the Golgi stacks, which we talked about at the very beginning. Eventually, it gets to the surface. These little things here are the LDL receptors.

You can go home and sleep on this and look at it again because I'm over. And it just seems like I just started and it's already over. I'm sorry. OK, I must have spent too much time talking about something I wasn't supposed to talk about. But anyhow, hopefully you now all can go back and look at this and think about this, because we're going to be talking about this again in recitation this week.