JOANNE STUBBE: So what I want to do today is continue where we left off to try to get further in into this module on regulation of iron right now in terms of humans. And we're talking about the fact that regulation occurs at the translational level. And so I'm going to introduce to you the model.

And I introduced you, last time, to two key players that we'll look at in a little more detail-- proteins and little pieces of RNA. And what happens is the proteins bind to the little pieces of RNA and prevent the translation of the messenger RNA into protein, or prevent degradation of the messenger RNA, allowing the translation to proceed. So that's really the take-home message. And I'll show you what the model is.

So the last time, we were talking about one of the protein players, IRP1 and IRP2. And I told you that IRP1 was a cytosolic aconitase and that you had seen the aconitase reaction, which I drew in the board last time, which is conversion of citrate into isocitrate. If you look at the model up there, citrate to isocitrate, you're simply losing a molecule of water. And then over here, you're generating isocitrate. And the chemistry is facilitated by the presence of a single unique iron in the form of iron 4 sulfur cluster, which was the first example of these kinds of clusters doing chemistry in addition to electron transfer reactions that you've been exposed to before.

OK. So the question then is, what is the signal? And so we're going to see that the signal is going to be related to-- let me just get myself organized here. So the signal-- the question is, what's recognized under low iron and high iron conditions? So that's what we'll be talking about, and how does this switch work to allow translation or not allow translation to occur.

And the other player that we need to be introduced to before we look at how this signal works is the iron responsive element. And this is a piece of RNA-- and I'll show you that on the next slide-- within the messenger RNA. OK. So you have a structure like this, and there are so many base pairs, in this little stem loop, that's part of the messenger RNA. And you have a three nucleotide sequence, and this is a bulge sequence.

And what we're going to see is that there are many of these structures. People have now done a much more extensive-- this was the model that came forth a long time ago when it was discovered. It was discovered a long time ago, but people have since done a lot of bioinformatics analysis to try to define really what do we know about this little sequence here. Is it three nucleotides? Is it more? People now think it's a little bit more, but it-- there's variability in that you need a bulge.

And so what's going to happen is our iron responsive protein is going to interact with this bulge, and that's going to be what's related to depending on the location of this bulge within the message. So we'll see this bulge can be at the 3 prime end or the 5 prime end. And this location and its interaction with this protein is going to regulate the translational process. So that's what I'm going to be presenting to you.

So what do I want to say? What I want to say then is for the iron response protein 1, it has, as we saw with the mitochondrial aconitase of 4 iron 4 sulfur clusters. So that could be a switch. We're doing iron sensing. What's going to cause us-- what is the sensor of iron that allows us to translate or not translate all of the proteins-- transfer and receptor, transfer and ferroportin, all of the things we were introduced to in the previous lecture.

So this protein has a 4 iron 4 sulfur cluster. And when it loads the cluster, as with mitochondrial aconitase, the protein is active. So it's found in the cytosol as opposed to the mitochondria. And it can convert citrate to isocitrate. OK.

So the question is, what is the switch that allows this IRP1 to interact with this little piece of RNA-- the stem loop piece of RNA? And so the switch is that you have to lose this cluster. And what you generate then is apoIRP1. And apoIRP1-- so somehow the cluster magically disappears. And when it disappears it can bind to the IRE.

So in the apo form-- that means no metal-- it binds to IRE. Whereas in 4 iron 4 sulfur loaded form, it does not bind. So what that would imply-- if you think about it sort of superficially-- if you have low iron and there's no iron sulfur cluster, the apo form is going to bind. OK. I'm going to show you the model in a minute. OK. So the switch really is related to-- in this case, the sensor is related to-- the fact that we have a 4 iron 4 sulfur cluster.

So we also have-- I told you before-- in addition to an IRP1, we have an IRP2. And IRP2 also looks, structurally, like a cytosolic aconitase, but it has no aconitase activity. OK. So we have

the second protein, IRP2. It's also a cytosolic aconitase lookalike, but it has no activity.

And why does it have no activity? You've seen, over here, the iron sulfur cluster is required to do the dehydration reaction. So it's required for activity in the mitochondrial enzyme. This has no iron sulfur cluster. So it has no iron sulfur cluster.

And what it has in addition, even though it looks like IRP2-- the structurally homologous-- it has a 73 amino acid insert. So this is a distinction between the two. OK. But now, this raises the question here-- at least superficially, you can understand that you might be able to sense iron, because you have a cluster, and you can go to no cluster. OK. And you can go back and forth.

And so, remember, in a couple of lectures ago I told you about biosynthetic pathways, and I showed you a picture of iron sulfur cluster assembly-- very complicated. At the end of the notes in this part of the lecture, you'll see what the model is. I'm not going to go through that. But how you assemble and disassemble, even though this model has been around for a long time, is only recently beginning to be understood. It's not trivial, because there are 10 steps to assemble a 4 iron 4 sulfur cluster. OK.

But here, we don't even have any iron. So how is the IRP2, which binds to the same IREs-- and again, in vivo we don't really know all of this. People are trying to sort that out as what the what the functions of the different proteins actually are. But how does it sense?

And so I just told you that the apo form of the IRP1 binds. That's also true of the IRP2. And in fact, it can only be apo, because it can't bind a cluster. So the active form, the binding form, the apoIRP2 binds the IRE. And then the question is, what is the switch?

And so what we'll see is that the switch relates to the fact that IRP2 gets degraded. So when IRP2 is degraded, it can't bind. And that's how you turn the thing off.

So then that takes you back a step further-- how do you target IRP2 for degradation? And this goes back to one of the reasons that I'm going to spend some time talking about degradation in mammalian systems. And so it turns out-- how does this relate then to iron sensing?

And what I'm going to show you is that you have an E3 ligase. I'll show you this in cartoon form. And I'll just say, see PowerPoint. We're not going to-- it's not really completely understood, so I'm not going to talk about it in detail. But what it has attached to it is an FXBL5 domain that looks like a protein we've seen before earlier that has an iron in it. So many of you probably don't remember hemerythrin, but that's the little enzyme in worms that reversibly

binds oxygen. So that was incredible. It's structurally homologous to that little protein.

So this is-- again, the details are not known. But it can bind iron and it can sense oxygen. So if you're at low iron, there's no iron bound to this little domain. And so there's a consequence. I'll show you what that is. But if it's high iron, it has a different consequence.

So the sensing is back a step. Its back a step into-- remember, I told you E3 ubiquitin ligases are multienzyme complexes. So this is part-- you'll see in a minute-- of the multienzyme complex. And so under certain sets of conditions when you have high iron, what happens is this is targeted for degradation. I'll show you what the model is for how this works.

So the models are the same. That is, apo in both cases bind. In one case, the iron sensor is directly related to the IRP itself, because it has an iron sulfur cluster. And in the second case, it's indirectly related to an iron cluster that's associated with the E3 ubiquitin ligase.

So another point I think I want to raise-- and this will get us into the next module, which I'm not going to spend very much time on-- the reason that we have iron module juxtaposed to the reactive oxygen species is they're really intimately linked. We've talked about how iron 2 can generate hydroxide radical or hydrogen peroxide. These are iron sulfur clusters are also oxygen-sensitive. This is oxygen-dependent.

So again, what you're seeing is not only do we have iron sensors, but we will see that iron sensing and oxygen sensing are linked. And I would say-- I was trying to make up your exam, and I was trying to put in a linkage so you would all of a sudden see this, and the more I read, the more confused I got. So the fact is, there are many, many papers published on this now, and the linkages-- the proteins involved-- do many things. And so sorting this out into a very simple model is really still tough. But what I what I believe right now is both iron and oxygen sensing are linked through this type of a model.

So let me now just show you a little bit about IRP1. We know a lot about IRP1, because we have structures. So this is the structure actually of the cytosolic aconitase, and this is with the 4 iron 4 sulfur cluster bound.

So what happens when you get to the apo form? What happens in the apo form, you now have a little piece of RNA bound. And this little piece of RNA always has a bulge with a cysteine in it. And it always has some kind of a loop. And we'll see in a second that the sequence of that loop can be variable. But here you can see that.

So these little balls here that are iron sulfur clusters are the cytidine bulge in this loop. So you see the thing changes confirmation and as binding to an IRE. So that is the switch. And then the question is, how does that work at level of controlling translation, which I'm going to show you in a second. OK.

So where do we see these iron-responsive elements in our messenger RNA? So messenger RNA-- go back and look at all of the players I introduced you to the last time. We have a transferrin receptor that's involved in uptake. We have DMT1. That's a dimetal transporter involved in iron uptake.

So intuitively, you should ask the question, if you are at low iron, do you want to take up more iron? So you want to turn on the transferrin receptor. You want to turn on the DMT1 protein. So I think most of it makes intuitive sense. The linkage to oxygen, I think, is less intuitive.

If you have a lot of iron, what do you want to do? You want to store the iron. So you in some way want to make more of the ferritin.

And then the other thing, this HIF 2 alpha is a transcription factor hypoxia-- inducible transcription factor-- that's linked to many, many things-- a huge number of people are working on this now-- one of which is this linkage to iron. But it senses anaerobiasis. And so you can see, it's also linked by one of these little elements. And the next slide just shows you a more recent one where people started doing a lot of bioinformatics on this. The previous slide was from a few years ago.

And again, the details, what you need to see is you, in all cases, have stem loops, a little bulge of a cytosine, and then you have some kind of a loop at the top of the stem loop. And if you look down here and you go through-- so these little stem loops are going to be either at the 3 prime or the 5 prime end of your messenger RNA. And so, for example, one of the things you see is aminolevulinic acid. Does anybody know what pathway that's involved in?

AUDIENCE: Heme synthesis.

JOANNE STUBBE: Yeah. So it's a rate-limiting step in heme synthesis. So there would be a place that would make sense. Remember, I told you all the iron is in heme and hemoglobin. OK. So almost all of these stem loops that you'll see, if you go back and you look through your notes, will make sense in terms of the big picture of how you want to control the levels of these proteins to deal

with high iron or to deal with low iron. OK. So that's iron 2.

And so here's the picture of IRP2. And this is the model for how IRP2 works. And so here's the case when you have high iron. And when you have iron, this part here, the Fbox, Skp1, and Cul are all part of the SCF E3 ubiquitin ligase. And I don't expect you to remember the names, but remember I told you the E3 ubiquitin ligase is the one that does what? It attaches ubiquitin on to the proteins, targeting it for degradation.

So this little part is the ubiquitin ligase. Here's your E2. Remember, you always need an E2 and an E3. And somehow the E2 is attaching this onto the IRP2, which is targeting it for degradation by the proteasome. OK. So this is exactly like the model we put forth a couple of lectures ago.

So again, this is the part that's most interesting. If you go back and you look at hemerythrin, which irreversibly binds oxygen with two irons, you have a diirons site. And earlier in your notes, I showed you what that site looks like. This site is intact because the protein is folded.

Under conditions of very low iron, what happens is this becomes unfolded. And then this part of the protein gets targeted for degradation by another E3 ligase-- not this one. And then you've lost your sensor. So the IRP2 remains stable.

So you might think this is complicated-- and maybe I didn't spend enough time going through this-- but you should go back, and you should look at the explanation again. So the two key switches are here and here. This one is the more complicated switch. Everybody thought everything was understood once they found this little iron binding protein that models hemerythrin, but nothing could be farther from the truth. We still really don't understand, overall, how this fits into the big picture. But it's not an accident that iron and oxygen are required to fold this into this little bundle that looks like hemerythrin.

So those are the switches. And so now, what I want to do is put forth a model. So let me see. So what is the model for how you want to turn these things off and on? OK.

So we have two things-- we have IREs-- Iron-Responsive Elements-- that can bind in front of the message to be transcribed or at the end. So what we're going to look at two sets of conditions-- one is under conditions we have low iron, and one is under conditions where you have high iron. How do you sense those conditions? So that's the question.

So again, we're sensing low versus high iron. So let's look at low iron first. So what we're going

to see is we're going to have a stem loop. So here's my messenger RNA, and here's the 3 prime end, and here's the 5 prime end. And here, you initiate translation.

And so if it binds-- if this protein IRP2 or IRP1, both in the apo form-- IRE-- and they do both bind. People are trying to sort all of this out. So this is IRP1 or IRP2. What happens to the translation? What happens to the translation is it's inhibited. So if you have this little stem loop in the front of your message, you inhibit translation.

And so what I'm showing you now-- and then I will give you some examples-- is the key to thinking about this. And most of it actually is intuitive once you remember what all the factors are that are involved in iron homeostasis that we've already gone over. So binding inhibits translation.

OK. So then we have the second case at low iron. And again, we have a 5 prime end, and then we have a 3 prime end, again, of our messenger RNA. And here is the initiation of translation. And in this case as well, you have the same sort of structures of stem loops. They are similar but distinct. And what can happen with the apo form of IRP1 or IRP2? Again, it can bind to these stem loops.

So you can have-- this chalk is not working-- your proteins, they're all bound-- they may or may not be all bound. I don't think we know that much. But what you see is the number of stem loops at the 3 prime end is variable. It depends on the message. So number of stem loops is variable.

So what does this binding do? What this binding does is it prevents the messenger RNA from being degraded. So it basically stabilizes the messenger RNA. So this model binding prevents messenger RNA degradation. So it stabilizes the messenger RNA. So that's the model.

OK. So now, let's just look at a couple of examples. And then what you can do later on is go back and think about this more of what's going on. So again, this is the model. And let me just make sure I go through the ones I want to do.

So we're still at low iron. And we'll do two at low iron. And then we'll look at the consequences of what happens at high iron, and does it make intuitive sense based on what we think the function of these proteins are that are going to be translated? So let's look at low iron. So we're at low iron. And let's look at ferritin.

OK. So what is ferritin? It's the iron storage protein. So under low iron, do we want to store iron? No. So if you have the choice of these two modes of regulation, what would you choose? Where would you put your iron-responsive element? At the 5 prime end the 3 prime end?

So we're at low iron. We don't want to store iron. So we don't want storage. So what would we do? If these are the two choices-- and these are the two choices from experimental data. There are many other variations on this you could have imagined, but this is the model that everybody agrees on at this stage.

So where would you put your stem loop? Yeah. You'd put it at the 5 prime end. And why would you put it at the 5 prime end? Because it prevents conversion of your message from ferritin into the protein. So you have less of the ferritin.

So what you see now is you have-- again, so this is a stem loop at the 5 prime end prevents translation and have lower concentration of ferritin. So that's exactly what you would expect. Some of the others are less intuitive, but we've seen ferroportin. Remember, ferroportin is the iron 2 transporter in many cells which allows the iron to come from the inside to, get picked up by transferrin, and redistributed to the tissue.

So it, in conjunction with the hepcidin peptide hormone we briefly talked about plays a really important role actually in controlling where the iron ends up going. And in fact, what you would like to be able to do-- say you had not very much iron, where would you want to put your iron? Would you want to put your iron in some metabolic pathway that's not so important, or would you want to put your iron in a metabolic pathway that's very important? You would want to put it into the pathway where you really need it to survive.

And so this is a subtle tuning on all of this. And so an example of how this can be tuned if you look at an iron-responsive element binding protein is succinate dehydrogenase. Any of you ever heard of succinate dehydrogenase? And where have you heard of it? You have heard of it, you just probably don't remember it.

[INTERPOSING VOICES]

JOANNE STUBBE: Yeah. So it's in the TCA cycle. So it converts succinate, which is a hydrocarbon, into an olefin, an alpha beta unsat-- into fumarate. So remember the TCA cycle, you can tune it down or you can tune it up. So if you really were desperate for iron, you would probably tune down the TCA cycle. So in fact, if you look, you'll see a stem loop in front of succinate dehydrogenase which

prevents its translation and tunes down the pathway. So there's a subtle example of how nature has-- at least is the way we rationalize the experimental observations of what nature has done.

Now ferroportin, which is the way I started on this, sets priorities. And it does this in conjunction with hepcidin, which we already talked about. Remember, hepcidin can target ferroportins for degradation. And this allows the iron to be distributed in defined ways within the cell. And in fact, what you want to do, in this case, is have the stem loop at the 5 prime end so that you don't export the iron inside the cell to the outside.

So that's what it does. And some of these, as I'm saying, are easier to rationalize than others. The ferritin one is really easy to rationalize. The ferroportin is easy to rationalize based on what I just told you. But what you see also is that in some of these systems-- I don't know how much you guys thought about RNA, but you know messenger RNA can be spliced.

In different cells it's spliced differently. You've also seen that cell types, in terms of iron homeostasis, the enterocyte, the macrophage system in the spleen, red blood cells are much more important, it might be, if you're in some other tissue, the splicing site is different, and you don't have a stem loop. So you can alter the regulation by alternate splicing systems.

So these are these two are at the 5 prime end. What about the transferrin receptor? So let me put this down here. What about the transferrin receptor? What would you expect at low iron-- we're still at low iron-- the regulation to be from the transferrin receptor? What do you want to do at low iron?

So this is another example, low iron. Let's look at the transferrin receptor. What does the transferrin receptor do?

Hopefully you know this. Yeah.

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: You need to speak louder, I can't hear anything you said. You just went like this. That didn't mean anything to me.

AUDIENCE: It helps to intake iron.

JOANNE STUBBE: Yeah. So it helps to intake iron. So if you have low iron, what do you want to do?

AUDIENCE: You want to increase--

JOANNE STUBBE: Yeah. So you want to increase that. So where would you put the stem loop?

AUDIENCE: 3 prime end.

JOANNE STUBBE: Yeah. So you put it at the 3 prime end, because that stabilizes the messenger RNA of the transferrin. So here, at low iron, you want to increase iron uptake. And that means that if you have the 3 prime end, you're going to stabilize the message.

So you can go through each one of the proteins that we discussed in the last lecture. And before you look at it, try to rationalize under different sets of conditions. This is low iron. What would you expect to happen at high iron?

Here, let's just look at this one so I don't have to draw this again. But what would happen to the transfer receptor at high iron? Do you want to take more iron into the cell? No, you don't.

So what you want to do is get rid of the transferrin receptor. So now what do you do? At high iron, if you're IRP1, you switch to pick up the iron sulfur cluster. It no longer binds. And so now what happens? So this is all bound. So it's stabilized and bound. In this case now, messenger RNA is degraded.

So the big players in iron homeostasis, I think, are easy to rationalize. If once you know-- this might not be so rational why you would stabilize messenger RNA or whatever, but this is the way nature designed this. Once you remember this-- and remember, the switches are just apo binding, and somehow they sense iron and they no longer bind, whatever the details are-- you should be able to understand in different kinds of cell types how you might regulate the iron at the translational level.

So I think that is all I wanted to say. This is just a summary of what we've done in the human part. And we're thinking about-- I gave you a big picture of what happens in humans. This is the summary of that big picture with all these factors that are regulated at the translational level by the iron-responsive binding proteins. And so you can go back and look at this cartoon. Whether you want to store it, whether you want to distribute it, whether you want to put some in the mitochondria-- all of that kind of stuff is regulated at the translational level.

So in this module, the second lecture, which was longer than I wanted it to be-- but that's life--

was focused on the big picture for human and how iron is transported. And uptake, which we talked about by divalent metal irons, transporters, and by transferrin in the plus 3 state, and this question of regulation at the translational level.

Now everything-- the hepcidin, we didn't touch on very much. Very complicated, but it plays a major role systemically. Whereas these others-- what we were just talking about is more specific for each cell type. And different cell types want to have regulation in different ways. So the bigger picture is the hepcidin. And it was discovered a while back, but I still would say we don't understand a lot about what's going on in terms of that hormonal regulation.

So now what I want to do, as advertised, is move into bacteria, and how do bacteria do the same thing. They have the same problem. We talked about metal homeostasis-- exact same problem in human and bacteria. But in the end, the bacteria want to survive and we want to survive, so we have the battle between the bacteria and us for iron.

So what I want to do is introduce you to the bacteria-- generically, how they take up metal to use for the same things that we use it for-- a little less complicated, maybe, than humans. And then what we're going to do is I'll introduce you to this war between bacteria and humans. And then we're going to focus on one bacteria that's a major issue nowadays-- Staphylococcus aureus-- because of resistance problems.

This is a problem that Liz's lab has worked on. And specifically, I'm going to give you one example of how Staphylococcus aureus gets iron out of our hemoglobin. That's going to be the example. And the system that you'll see, it's amazingly cool. But you'll see, there's still many things we don't really understand in a lot of detail.

So what I want to do now is introduce you-- how am I doing timewise? OK. So what I really would like to do is draw this out on the board, because it's complicated. And I know what happens if you use PowerPoint, you go through it at 100 miles an hour. But I'm going to be using more PowerPoint to get through something. So anyhow, this is an overview of where we're going if you forget.

So what I wanted to do, at least a little bit, we're going to be focusing on gram-positive and gram-negative systems. And I want to tell you what is the difference. You all know or have heard about gram-positive and gram-negative bacteria. They use different strategies. They use the same strategies, but they use distinct strategies because of their structures.

And so what I want to do is give you an overview, and then we'll focus specifically on Staph aureus. So in gram-positive, here we have our plasma membrane. So this is the plasma membrane. And this big guy here is PG-- the peptidoglycan.

And we'll see, in Staph aureus, the peptidoglycan is going to play a key role. So you need to understand the structure of the peptidoglycan. So I am going to spend a little bit of time describing to you the structure. It's also the major target of many antibacterial agents that are currently used. Why? Because it's unique to bacteria.

So you have only this plasma membrane. There's no outer membrane. That's going to be distinct from gram-negative. And so the question is, how do you get iron from the outside to the inside?

And so one of the ways you can take in iron is-- you've already seen this, and you've talked about it in detail in the first half of the course-- siderophores. And we've already talked about the fact that we have many, many different kinds of siderophores. And somehow these siderophores-- and we'll look at a few structures-- can get to the outside of a cell.

They pick up iron in the plus 3 state, and then they need to bring it back to the plasma membrane. And then somehow it needs to get transported across the plasma membrane. This is a transporter. Most of them are called ABC transporters and they require ATP. And when they get across, they take the siderophore with the iron into the cell.

So that looks simple enough. We'll see that the strategy of gram-negative bacteria is going to be distinct, because it has an outer membrane. So how would you get the iron out of the siderophore? And so I'm going to push this up, and I'll come back down again. So how do you get the iron out of the siderophore?

And of course, what that depends on is the reduction potential of the iron. So we will see with enterobactin, in which you already looked at, the reduction potential under neutral conditions is minus 750 millivolts. Minus means that it's really hard to reduce. It wants to be oxidized. It's outside the realm of what you can do inside the cell. So And we want to reduce iron 3 to iron 2. Why? Because we increase the exchangeability of our ligands. That's why that was introduced before.

So you could reduce this, potentially. And so I'll just put a question mark there. And so then what you have is a siderophore. So let me just write this down so you don't forget. So this is

the siderophore. And then you have your iron.

So what happens to the siderophore with no iron? It can now get recycled back to pick up more iron. So let me just put this here. This is recycled. And we're going to be focusing on here taking up iron from siderophores, but we'll see that you can take up iron from hemes.

And you have the same issue. You're going to use the same strategy. You'll bring it into the cell. You've got to get the iron out of the heme, and you have to recycle it.

So if you can't reduce it, what do you do? Does anybody remember what you do with enterobactin? Anybody remember the KD? We talked about this last time, but it bonds like a son of a gun. It's hard to reduce. You have ester linkages in enterobactin. If you go back and look at the structure, there are proteins that can hydrolize the ester linkages. So ring opens-- makes it bind less tightly, and so it can be released.

So in the case of enterobactin, you have an esterase. So let me just show you that, and then we'll come back again to the gram-negative. But if you look at the siderophores, there are 500 siderophores. Here is enterobactin. here are the esters. You can hydrolyze them to release. You can't reduce, because, again, the more negative, the more it wants to be oxidized.

And the range of reduction inside the cell is maybe minus 500. You can't get that much above that. But if you look down here at citrate-- remember, we were talking about citrate-- unusual in that citrate is part of this aconitase IRP1 and IRP2 system. But what's the reduction potentially are completely different. So if you had iron citrate, you could easily reduce it under physiological conditions. So the strategies you need to be able to release the iron to then use the iron for what you want to do is distinct depending on the siderophore.

So if we go back, now let's just look over here and I'll draw that in parallel. So what's the difference between gram-positive and gram-negative? So let's draw that out. And then what I'm going to show you, rapidly, is, again, the strategies with heme are subtly different, but very, very similar. We have different sets of proteins.

So with gram-negative we have an outer membrane. So this is gram-negative. And what we have in the outer membrane are proteins. It has a lot of proteins. And it has a big protein with a ball in it. And these proteins-- it has 27 beta strands, and these are beta barrels.

So there are many, many of these proteins. In fact if any of you heard Dan Cohn's talk this past semester, he's figured out how do these things get made down here and get inserted in

the outer membrane. It's an interesting problem. So these are beta barrel proteins, and they have 27 strands.

We then have a peptidoglycan. But the peptidoglycan is distinct. It's much smaller. It doesn't take up anywhere near as much space.

And then you have your plasma membrane. And then in the plasma membrane-- so this is a plasma membrane-- you still need to do the same thing. You need to get your siderophore into the cell.

So what do you have here? You still have transporters. And those transporters are going to allow your siderophore to go into the cell, just like we saw with the gram-positive. So over here then, we have a siderophore-- again, the same types of siderophores. So somehow it needs to get inside the cell.

And we have many of these beta barrels, and a lot of them are specific for a given siderophores. There are many, many of these things. We'll look at E. coli. There are 10 different ways to get iron from the environment into the cell. That tells you how important all of this is. And it turns out that you also have, inside the cell, a periplasmic binding protein that can pick up the iron when it gets transferred across here. So you have a periplasmic binding protein.

And one of the questions is, how does the siderophore get transferred? And to do that in gram-negative bacteria, you need a machine. And that machine is composed of three proteins. It's called the tan protein.

If you look over there in pink, you have tanB. It's exbB. And this should be not C, but exbD. So there are three proteins required. And they somehow can use the proton motor force from the inner plasma membrane to allow transport across the outer membrane.

So in all of these, one has tanB. So this is tanB. And tanB can recognize part of the beta barrel. So it interacts with the beta barrel protein. And this is exbD and exbB. And again, you generate a proton motor force which allows the siderophore to get into the cell.

It then gets transferred to a periplasmic binding protein. And then what does it have to do? So from here, it has to go through our transporter. So let me put this up here, just like we just did before.

So your siderophore-- so you can't see the bottom of my transporter-- comes through So this is the plasma membrane. And what do you have? You have the same problem. You have to get the iron out of the siderophore. And so the problem is exactly the same and gram-negative and gram-positive. So you somehow have to get it in. It's more complicated to get it in with gram-negative because of the different constructions of the peptidoglycans and the outer membrane.

The other thing I wanted to say about the other outer membrane is-- which I don't know if you guys know, but I think it's incredibly important and is a major issue in a human disease-- the fact that, in addition to this outer membrane in these beta barrels, the whole outer surface is covered with sort of amazing molecules called lipopolysaccharides. So the whole outer surface is covered with LPS-- I'm not going to write it out-- lipopolysaccharides. Which, actually, one of my best friends elucidated the whole pathway for how that works. It's a beautiful, beautiful set of biochemical studies to figure out how this thing is made.

It's got lipids. It's got all these sugars. It's got all this stuff hanging off of it. And this thing is really important in human health. If you read about infections, they're always talking about lipopolysaccharides.

So what I'm going to do next time, just by way of showing you to introduce you to this-- you can see here in the next cartoon we have the same problem when we want to take up hemes as opposed to siderophores. And we're going to focus on hemes. So this is a cartoon, very similar to the one you just saw. And there are a couple of proteins on the outside that you need to think about. How are you going to get the heme across the peptidoglycan or into the cells?

So the model is very similar. You should look at that. And then we'll see this is what your problem set is going to be on. This is for Staph aureus. And we'll see that if you get a heme, there's going to be bucket brigade that can transfer the heme through proteins covalently bound to the peptidoglycan into the cell. It's sort an amazing system, and that's what we're going to talk about for probably the first half of the next lecture. So you're going to have to read on that on your own to solve the. problem.