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JOANNE STUBBE: So we've been talking about iron metabolism in general in the first lecture. And in the second lecture we started to focus on iron metabolism in humans, and the third set of lectures is going to be iron metabolism and bacteria with a focus on hemes. And the two things you want to talk about in the lecture today are, how does iron get taken up into cells in humans, with a focus on receptor mediated endocytosis, and then we're going to start talking about hopefully iron regulation-- how you sense iron, ion regulation at the translational level. By sort of a unique mechanism, at least at the time of its discovery.

So in the last lecture, we introduced you to some key features about iron chemistry in general that we're going to use throughout this lecture and next lecture. So you need to go back and review your notes if you don't remember that. Or hopefully you've had it somewhere before, and it's a review from you from freshman chemistry or inorganic chemistry. And so iron metabolism-- what do we know?

We know the average human being has 3 to 4 grams of iron. We talked about this at the end of the last class, of how is the iron distributed. We all went through that most of our iron is in our red blood cells in the form of hemoglobin. But it's also-- so in the form of hemoglobin, it also can be stored in proteins called ferritins, which we're not going to spend much time on, but I will introduce you to today.

And then many of you may know that red blood cells die every 120 days. And we'll see that the iron is really continually recycled, and we'll talk a little bit about the mechanism of how that's regulated. So instead of excreting it, what happens is you recycle. The iron unit's recycled by macrophages in the spleen. And so the other place you see a fair amount of iron is in the macrophages. And the third place you see a fair amount of iron is in the tissues, because myoglobin, again, has to deliver oxygen to the respiratory chain.

So what I want to do now, and I'm going to go back and forth between the PowerPoint and notes. And so some things I'm going to write down some things not. Hopefully you have these

cartoons in front of you so you can write down some of the things that I will say here, and say it again and say it again.

So this is sort of the big picture that I took from some review. And most of these big pictures have some issues with them. But I think it still gives you the big picture. So here's a duodenum, where we can take up iron from the diet. And we'll talk about this in more detail, but a key player in allowing the iron from the diet to go into our system is going to be FPN-- that's going to be ferroportin, I'm going to describe this again. But you're going to see FPN over and over again. It allows iron to be transferred in the plus 2 state, and that's going to be important.

And so what we see, that if you look at iron from the diet, there's not that much. [AUDIO OUT] somebody's guess as to how much there is. A few milligrams. And the question is, where does it go in the bloodstream? And it goes to a protein that we're going to talk about that's a carrier for iron in the plus 3 state. So we're going to see plus 2, plus 3 into conversions over and over again. And sort of what the strategy that has evolved to be able to deal with these different oxidation states is.

We'll see that this little protein, TF, is transferrin, and we're going to look at transferrin for a-- very briefly, but it binds iron 3 and bicarbonate, and then delivers this to tissues, and also delivers it to marrow. And marrow, which is-- accounts for approximately, by mass, 4% of the body weight, makes all of our red and white blood cells. So that's going to be important. And so the marrow makes the erythrocyte, the heme for the erythrocytes makes the erythrocytes, and the erythrocytes are the red blood cells that have all the hemoglobin. So out of the 4 grams, you have 2 and 1/2 grams of hemoglobin.

And then these red blood cells die every 120 days, and instead of just discarding everything, they're recycled. And they're recycled by the macrophages in the spleen. And somehow you want to take the iron from these red blood cells and reuse it. And so there's a series of reactions that happen. Ultimately you get iron 2, and the iron 2-- here's again our iron 2 transporter, ferroportin, is going to take the iron that's recovered and put it back into transferrin, where, again, it can be distributed, depending on the sensing of iron.

Now, the major player in the sensing and storage of iron is the liver. So the liver, we're going to see there's a protein there not indicated on the slide called ferritin, and ferritin binds 4,500 molecules of iron. And this is also-- the liver is the organ that generates, biosynthesizes the

key regulator of iron homeostasis, which is a peptide hormone that we're not going to spend a lot of time on, but I'm going to show you what it does. So that's called hepcidin.

And what we'll see is hepcidin in some way controls the levels of ferroportin. So we also see that we lose some iron daily, but the iron losses are small. So we have a lot of iron units, but the iron is continually recycled, and the question is, how does that happen? So I just want to look at one place where, in the duodenum, where we're going to take up iron.

So what I'm going to do is-- this is a cartoon of what I just showed you in more detail. But I'm going to focus on iron absorption from the diet. And I want to make a couple points about this, which are general. And so what we'll see is we have enterocytes, so this is an enterocyte. And you have an apical brush border membrane. And then you have a second membrane which is going to get us into the bloodstream. So this is called a basolateral membrane.

So we get iron from our diets mostly in the plus 3 state. But to do anything with iron, probably because of the ligand exchange issues we talked about last time, the rate constants for exchange are much slower with iron 3 than iron 2. So from the diet, we have iron 3. And iron 3 needs to be reduced to iron 2. And that can be done-- we'll see this is going to happen over and over again. And this can be done by a ferric reductase.

And what we will see is in this membrane, we're going to have an iron 2 transporter. So in addition to the ferroportin I just briefly introduced you to, and will introduce you to again, we have an iron 2 transporter, that's called DMT 1. Again, the acronyms are horrible. But it's a divalent predominantly iron 2 metal transporter.

And we're going to see, when we think about regulation of iron homeostasis, this is going to be a key player. Because it takes iron from the diet into our cells. And in this membrane of the enterocyte, what we will see is that we have-- and this is what you saw in the previous slide-- you have ferroportin-- so I'm only going to write this down once. But this is going to take the iron 2 and then transfer it into, ultimately, the carrier in the bloodstream, which is going to be transferrin.

So here we have iron 2, but for it to get picked up by transferrin, it gets oxidized to iron 3. So what you're going to see over and over again is going back and forth between iron 2 and iron 3. And so this gets oxidized to iron 3. And these proteins-- there's a copper iron oxidase. And if you look at the handouts, you'll see that this is also called-- again, I don't expect you remember the names. What I think is key here is that you need to transfer this to the plus 3

oxidation state.

So now what happens in the plus 3 oxidation state-- so let's go over to the next board here-- we have a protein called transferrin, and we'll look at this a little bit. And transferrin is going to bind iron in the plus 3 state, but it also requires bicarbonate. So in the blood, is that unusual that you would require bicarbonate? Or why might you require bicarbonate? What do you know about blood cells and hemoglobin?

So we have iron 3 that's regenerated enzymatically, through some kind of oxidation reduction equipment. And we're going to see this, again, over and over again. And they each have different names, so that's confusing as well. But you're cycling between 2 and 3. And then transferrin, we have a structure of this picks up the iron in the plus 3 state, and also picks up bicarbonate. So where do you think that bicarbonate comes from in blood cells?

AUDIENCE: CO_2.

JOANNE STUBBE: Yeah, so it comes from CO_2. Why? Because a major function of red blood cells is to transfer CO_2 from the tissues back to the lungs. So CO_2 is not there, at pH 7, it gets rapidly hydrated to form bicarbonate and protons. And so this is unusual. I think this is one of the few systems where you have-- we'll see bicarbonate as a ligand.

So in addition to these enterocytes, which again are involved in iron uptake, we also have macrophages in the spleen. And so this, again, is due to the diet. And this is due to basically recycling-- iron recycling. And so what you have is macrophages in the spleen, and you have in the macrophages dead red blood cells, which I'll abbreviate RBC. And so the idea is we want to get the iron out of the red blood cells somehow to reuse it. So that's the goal.

And so somehow in a complicated process, we get iron 2 out. And then iron 2-- here we have our friend ferroportin, that I just showed you in the previous slide, is going to take and put into the extracellular mirror in the plasma the iron 2. So what happens to the iron 2? We just saw over here, the iron 2 gets oxidized to iron 3. The same thing is going to happen over here.

So we have iron 2 that needs to get oxidized to iron 3. And again, let's just call it a copper iron oxidase. I'm not going to go through the details. And then what happens to the iron 3? So the iron 3 then gets picked up by the transferrin. And then depending on what the needs are the cell, the transferrin can deliver. If you have a lot of iron, it could deliver it back to the liver. We'll see that's the storage place for the iron. So the iron 3 transferrin needs to get taken up, just

like we saw with cholesterol.

Or if we need iron in some other tissues, we'll see that there are receptors for iron 3 transferrin that can, again, take iron into the cells to meet the needs of the cell for iron requirement. Now, the one thing I wanted to tell you in the first slide, which I had forgot, was that in addition to all of these requirements for iron, and the predominant form being hemoglobin and myoglobin, what we see is that iron is found in only 4% of the metabolic enzymes. So iron is found in many proteins that catalyze all kinds of reactions, like we talked about last time. But that's a small percentage of the total amount of iron.

So this sort of diagram is pointing out a few things that sort of is indicative of iron mediated metabolism in many cases. And so what I briefly want to do is sort of summarize the functions of these different proteins. So this is phenomenological. And if you're going to have-- if you were given an exam on this, I'll give you the names of all of these things. Because I think the names are actually confusing.

So number one, we have DMT1. And again, when ion is trans-- it's a transporter of iron 2. And so that's an important thing to remember. But even though it's transferred into the cell and it moves around inside the cell as iron 2, likely because, again, the ligand exchange, though iron starts here and it needs to move here, and it needs to move here, and this is the way nature-- because of the exchangeability of the ligands-- has decided to move iron, and also oftentimes copper 1 around, instead of in the oxidized state that this transporter deals with iron 2.

The second key thing is ferroportin, and this goes-- brings, again, iron 2 to the extracellular milieu. And so this is bringing it inside the cell. This is bringing it extracellularly the outside the cell. And this leads to the next thing that we see over and over again-- while iron 2 is brought outside the cell, it then gets oxidized to do anything with it.

So then we have general ways of iron 2 being oxidized to iron 3. And this could be a copper iron oxidase. But again, there are multiple-- there are multiple names for these [INAUDIBLE]-- we'll see in a few minutes, steep is one. I mean, they have five different iron oxidases. And iron 3 is going to be the key for transferring this to ferritin, which is the way that iron is transferred, just like the LDL particles are the way cholesterol is transferred around the cell. Ferritin-- transferrin is the way the iron is transferred around the cell.

So so this iron 3-- so iron 3 is picked up by transferrin. And again, this is iron 3. I'll show you-- we have structures of all these proteins. This is, again, iron 3 bicarbonate. And then the

question is, how does this transferrin get into cells? So this is the major carrier. Iron. And it's carried in the plus 3 oxidation state.

Maybe, and we'll see that the KD for binding-- what do you think the KD for binding to a transferrin might be? Do you think it's weak? Do you think it's strong? And what would you, if you were designing something that was carrying around iron to all the tissues, what would you design? Something weak or strong? Say it was weak, what would happen?

Yeah, it comes unbound. And then if it gets reduced, into the realm where you have iron 2 and then you have reactive oxygen species. And so nature has developed, I would say, you've seen with siderophores you can get things 10 to the minus 35 for dissociation constants to 10 to the minus 50. The KD for this is 10 to the minus 23 molar for iron binding to transferrin.

And so the next thing that happens is that the iron binding to transference goes to the transferrin receptor. And so transferrin then binds to the transferrin receptor, just like the LDL particle binds to the LDL receptor. So this is the transferrin receptor. And so what you're going to see is that, in contrast with iron transported across-- in the case of the enterocyte, or in the case of ferroportin, where it's iron 2, this is all transferred in the iron 3 state.

So this-- again, this is important to see the differences in the oxidation states that are used to control uptake into the cell. And this occurs by-- we'll briefly look at this, but it's very similar to what you saw with the LDL receptor. The receptor mediated endocytosis. So we're going to look at a cartoon of this.

So there's one other player that I want to introduce you to. And this player becomes really critical because we don't have ways-- we don't produce a lot of excess iron and then export it. All the iron is recycled. So what controls that iron recycling? So the key regulator is a peptide hormone which I introduced you to in the previous slide, called hepcidin.

And we know quite a bit, actually, about the structure of this peptide hormone. And I'll tell you what its proposed function is. We're not going to spend a lot of time discussing this. But it is made in the liver. So it's bio synthesized in the liver. And it's basically-- its function is, it's a major site of regulation, and it controls iron from the diet, and iron cycling through extracellular factors, like the transferrin-- like transferrin.

So how does it do this? So here we have a little peptide hormone. It's made in the liver. And how can a control iron recycling? And so the one guy that we see now is ferroportin,

ferroportin. And so its major function-- it has a lot of functions, and it's complicated, and people are still studying this. But one of the major functions is to control the amount of ferroportin. So if you look at the way it's described, the hepcidin combined extracellularly to the ferroportin. So I'll draw a little cartoon of that.

And then targets it for degradation by the proteosome inside the cell. So that's the key feature of hepcidin that you need to remember. So we're going to see, if you look at-- if you look at a lot of the cartoons I've given you, you have your ferroportin, watch transfers iron from the inside extracellularly. I forgot my colored chalk today. I was on drugs or something. But people were bothering me up until five minutes. I didn't have time to think before this lecture. So I'm sorry I'm a little discombobbled here.

But this is hepcidin-- hep-cid-in. And so it binds to the extracellular side. And what does that does when it binds? It causes-- somehow things change, and it causes it to be degraded inside the cell by the proteosome. So. This interaction, extracellular, causes ferroportin to be degraded inside the cell by our friend the proteosome.

So does everybody sort of understand what the model is? So this is the key regulator. And you've seen ferroportin-- we only looked at two cell types. We looked at the enterocyte, and we looked at the macrophages in the spleen, both of which have ferroportins, but ferroportins that are in a number of additional cell types. And when we look at regulation, one of the key regulators of everything is going to be that we need to control are the levels of ferroportin. Because that allows all the iron to somehow be recycled. It's a key player controlled by hepcidin that allows the iron to be recycled to the different tissues.

So we have a number of proteins that I'm going to very briefly introduce you to, in addition to these guys. And so we're getting into more acronyms cities. But the additional proteins that we need to think about-- so involved in iron homeostasis. Our number one, the ferritin, which in the introductory slide-- and let me just show you.

So what I'm going to do, these are the list of proteins that I'm going to go through one by one and tell you a little bit. This is sort of an amazing protein. It has 24 protein subunits. It has two kinds of protein subunits. You don't need to remember this. But what is this function? It's a key-- and this is found in all organisms-- it's involved in iron storage. And why is this important? It's important because it keeps iron soluble so that it's not precipitating sort of as rust. There are, in yeast, if you look at some of yeast homeostasis, when things start going

awry you can you can look at it in an electron microscope, you see iron all over the inside of the mitochondria, just these big black blobs where the iron has precipitated and mineralized.

So we need to keep iron soluble, and we need to keep iron non-toxic. So what do I mean by non-toxic? In the last lecture, I told you that iron 2 can easily be oxidized to iron 3 by oxygen. We're going to talk about that in module 7 a little bit. And that can result in all kinds of damage inside the cell if it's not controlled. So this protein is sort of amazing. You can bind 4,500 irons, most of them are in the iron 3 state. But when you start out, it binds iron 2.

So iron 2, again, inside the cell is what gets transferred around in general. So iron 2 binds, and then each ferritin has an oxidase activity that I'm not going to go into in detail that can oxidize it to iron 3, which puts it into this mineral structure that you see in these 4,500 atoms of iron. OK, you don't see it there, all you see is the protein there.

So this gets oxidized to iron 3, and this is how it's stored in mineral form. So now the question is, say you needed iron. So we have a lot of iron, we want to keep it sequestered so we don't have to worry about reactive-- it doing chemistry that's aberrant. We want to keep it soluble. So we have iron stored in the plus 3 state in some kind of mineral form. How would you, if you wanted to use iron, now what would you do?

Do you think you can get it out of the iron 3 mineral? No. What do you have to do to it to make the ligands more labile? All you need to reduce it. So to use it, you now-- and people are still arguing about what the reductants are-- so you need to reduce iron 2 plus 2 so you can use it. So that's ferritin.

Does anybody have any questions about ferritin? It's got a complex structure, we have lots of structures of it. You can have-- every ferritin is sort of different, it has different ways of dealing with these issues of how you mineralize, and how you remove it. But this is a major storage protein in all organisms of ferritins. It's sort of an amazing structure.

So what we were talking about before is that we get iron 3 transferrin. What does iron 3 transferrin look like? So we take iron from the diet, or we're recycling iron from red blood cells. We need to get it to the plus 3 state, where it gets picked up by transferrin. That's what we need to do.

And so if you look at this-- So we've picked up iron 3 in transferrin. And the structures of transferrin are known. So now we need to look at transferrin-- whoops. And if you look at the

structure, it is composed-- the protein is composed of two domains, each of which can bind iron 3 bicarbonate. So it has two little lobes over here. You can see this lobe and this lobe, the N terminal and the C terminal lobe.

And they each bind-- if you look at this carefully, there is the iron, there is the bicarbonate. It has two tyrosines, a histidine, and an aspartate as ligands. And it's in an octahedral environment. So again, why bicarbonate? And people thought for a long time the bicarbonate was related potentially to how do you deliver this iron 3 out of the transferrin into something that's useful, namely the enzymes that are going to use it to catalyze transformations. And what is the bi-- is there a role for bicarbonate in that process?

So what's unusual about the transferrin, again, I get-- the KD is tight. What's most unusual is it's got bicarbonate, it's got two tyrosines, and it's got a histidine, and it's got an aspartate, and it's an octahedral environment. And how do you think-- what do you think the proteination state of the tyrosines are? Everybody know what tyrosine is? Do you think it's proteinated? Non-proteinated?

This brings up another sort of general principle we talked about last time. If you have water attached to a metal, what can it do to the pKa of the water? It decreases it so that you lose the proton under physiological conditions. What's the pKa of tyronsine? It's on the order of 10, 10 1/2. And in fact, this is bound-- it's the phenylate. So both of these are in the phenylate form. So both of these are phenylate.

And again, if you want to think more about this, both Liz and Lippert have taught a course, are teaching a course now, in bio inorganic chemistry, where you really sort of talk about the details of these kinds of interactions, which are key to the way everything functions.

So we have transferrin, and the unusual part is the binding of bicarbonate, and then, again, let me just re-emphasize it's in the plus 3 state, and you have fairly tight binding. And what we're going to see is, it's going to bind just like the LDL particles bind to the LDL receptors, it's going to bind to the transferrin receptor. So we now have a transferrin receptor. So this is the receptor.

And we know we have structures, actually, of the receptors. It's a 90 kilodalton dimer. So and its transmembrane. So you have-- so this is the transferrin receptor. I'm going to show you a cartoon of this in a minute. 90 killodalton dimer, and so this is extracellular. This is intracellular. And this is the membrane. So let me just show you that cartoon over here. So extracellular,

intracellular. And if you remember back to the LDL receptor, how did we trigger receptor mediated endocytosis?

We had a zip code. Here we also have a zip code. And the zip code is YTRF. So there's also, on the intracellular side, a zip code for triggering transferrin uptake. So those are the players that we need to think about. So the transferrin, in the transferrin receptor, have parallels with LDL. LDL receptor-- of course every one of these things is different. But this was one of the other systems that had been characterized quite extensively, the first one being the LDL receptor.

And so the model is shown here. This model hasn't really been-- this model's not completely correct. I'll tell you where things need to be changed a little bit. But really people haven't studied this model in a long time, even though there's a lot we don't understand. So here's the surface. Here's transferrrin, these little things here. Here's the transferrin receptor purple. So the transferrin binds to the transferrin receptor.

To get uptake into the cell, you need to have clustering. So that's not shown here, because this cartoon was drawn before we realized that you had a cluster-- the transferrin receptors. When you transfer, when you cluster, and you bind transferrin, again, just like we saw with the LDL receptor, in some way, you have machinery that attracts the clathrin, and then it's going to pinch off the clathrin coated vesicle. And they skip here the clathrin coated vesicle. So that should be in between-- this is clathrin.

And then what happens, just like in the LDL receptor, you remove the clathrin from the external part of your little vesicle. So that's what's indicated here. So what do we have? We have the transferrin receptor, and transferrin, and this is-- the internal pH of this system is about 5-5. So if you think about this, how would you-- how would you remove the iron from the transferrin? Why might bicarbonate be there?

So I just told you that bicarbonate in iron are bound to the transferrin. Can you think of a mechanism by which that could happen? X inside the cell, at lower pH? We don't know the answer to this. It's still open to debate. So-- but what happens to the bicarbonate at low pH? Think about hemoglobin. Think about 5.07 and hemoglobin. We spend so much time talking about bicarbonate as a key player inside red blood cells. What happens to bicarbonate in the presence of acid?

Yeah, so it forms carbonic acid. What happened to the carbonic acid? To CO_2 in the water.

Yeah. So this is something we banged into you over and over again in 5.07. There's an equilibrium that happens over and over again inside cells. So maybe that's a way to deliver the iron. I don't know. So we somehow lose iron. But the iron is in the plus 3 state. To get it into the cytosol, which is where we're going to use it, to deliver it to all of the proteins, what do we need to do? Hopefully you now remember this. We need to reduce it.

So steep is a reductase, a ferric reductase, that converts this into iron 2. Where did we see this guy before? DMT1. We've see that before as a key player in uptake into enterocytes. So you see these same players over and over again. You see this shift from iron 2 to iron 3 over and over again, actually, in yeast, where I know a lot about iron metabolism as well as in human systems. Now-- so we've got iron 2 out of the transferrin, transferrin receptor.

And then the iron 2 goes into the cytosol. And then we've got to figure out how to use it in a way so that we don't have oxidative stress and deliver it to the proteins to biosynthesize all our co-factors. So then the question is, remember in the LDL receptor, it got recycled. So what happens here is distinct from what happens in the LDL receptor. In that now the transferrin and the transferrin receptor are both recycled.

So that's distinct from what we briefly talked about in the case of cholesterol. So we have two ways of taking iron into the cell one-- is through these di-- iron 2 transporters, the DMT molecules, and the second way is through iron transfer-- iron transferrin which circulates in the blood and delivers it to all the tissues. So these are the major mechanisms of iron delivery, and recycling within the cell controlled by hepcidin, this peptide hormone.

So what I want to do now is look at how this iron is sensed. How do we control everything? And iron sensing-- So iron sensing, there are going to be two players. And so we're going to look at iron sensing. And I'm going to introduce you to the two players, and then I'm going to show you the general logic of how you control all these proteins we've talked about-- ferritin, DMT1, transferrin receptor-- all of these things are going to be controlled by the mechanism we're going to talk about now, which is regulation at the translational level.

So this is iron sensing by translational control. So who are the two players? They're written up there. But we have iron responsive element, and we're going to see that's a little piece of RNA. So-- and I'll show you what it looks like. So this is RNA, a little piece of RNA, stem loop piece of RNA, that has defined characteristics. I'm going to show you what it is.

And then we have iron responsive protein 1, or iron responsive binding protein 1. They're called both of these things, I don't remember what was in the articles you had to read. They're sort of used interchangeably. And there are two of these, so there's a 1 and there's a 2. And they're structurally homologous to each other, and I'll tell you a little bit about each one of these. So we also have a one and a two.

So those are the two guys. These are proteins. So these are proteins, that's why the name binding protein. So it turns out that iron responsive binding proteins are homologous to aconitase-- where you seen aconitase before? Yeah, so in the TCA cycle. It catalyzes the conversion of citrate to isocitrate. So-- and where is the TCA-- TCA cycle located? In the mitochondria.

So this is a TCA cycle enzyme found in the mitochondria. But what we'll see is, we're working on RNA, we're going to regulate somehow. We're going to use interaction between this protein and a piece of RNA to control the translational process, where is that located in the cytosol? So these proteins are located in the cytosol.

So if you think about what happens with aconitase, let me just write that down for you. So we have citrate. And I asked the question, do you think it's interesting that citrate is involved in this overall process that I'm going to be describing? What do we know about citrate, besides the fact that it's an intermediate in the TCA cycle?

So this is citrate. It undergoes a dehydration reaction. So we're going to lose water to form aconitate, cis-aconitate-- and then it becomes rehydrated. So that's the reaction you learned about a long time ago in the Krebs cycle or the TCA cycle. Why is it interesting that citrate is involved? I don't know why it's really involved. But do you think it's interesting? What is citrate, if you look at the structure of it? Yeah.

AUDIENCE: Combined iron.

JOANNE STUBBE: Yeah, combined iron. And in fact, there are iron siderophores that use citrate. I don't think this is an accident. And thinking about, again, how nature uses primary metabolites over and over again in ways other than what you see in primary metabolism. So what's unusual about this protein is the following. And this is the key to the way the sensing is going to work for the iron responsive binding proteins.

So if you look at-- if you go back and you look-- if you go back and you look at the Krebs cycle,

or you go back and you think about this, this is something that probably confused you all. You have an iron 3, a 4 iron 4 sulfur cluster. Remember I talked a little bit about this, trying to show you that this was going to be highlighted later on? and what we have in this 4 iron 4 sulfur cluster-- you have a cysteine attached to three of the irons. We have one iron that's unique, OK that doesn't have the cysteine that you see in normal 4 iron 4 sulfur clusters.

So this is the unique iron. So if you look at that over here-- so here's the cartoon of this. So here you have cysteine, cysteine, cysteine in the 4 iron 4 sulfur cluster. Here's citrate. And that iron-- so most of you probably learned in respiration, iron sulfur clusters are involved in electron transfer. They do one electron chemistry. They undergo oxidation reduction, which we briefly discussed in the last lecture.

But what's it doing here? What it's doing here is binding the citrate. So here's citrate. This is the hydroxyl that we're going to eliminate to lose water to form cis-aconitate. So this is the first example. But this was discovered by Helmut Beinert at Wisconsin many years ago, where the iron sulfur classes were doing something other than redox chemistry.

This is just the tip of the iceberg. Remember, I talked to you about radical SAM proteins, 100,000 proteins doing interesting chemistry. This is the first example of this. And these really are seminal experiments to figure out how this all worked. So the unusual thing is that most iron sulfur clusters look like this, and they all have 16 on each of the iron, and they do redox chemistry, but now we're finding that a lot of iron sulfur clusters have unique iron they can end up doing interesting chemistry as well, namely binding S-adenosyl methionine.

So if you go back and you think about what happens, this is helping dehydration. So you're going to dehydrate. But now you have to reorganize the thing. This is one where they talk about the Ferris-- spinning around the Ferris wheel if you look at an introductory TCA cycle thing, how this reorganizes. I don't think this is a very good picture. But it needs to reorganize because you're going to rehydrate another carbon, using the same iron.

So if you sit here and you stare at this, what you see is this carboxylate. Now, here was the initial carboxylate bound, this one wasn't bound. Now, this one ends up being bound. And now you're adding water back across this double bond. So the purpose of this system is simply to catalyze the dehydration reaction. So what the heck are we doing with an iron responsive binding protein being a cytosolic aconitase equivalent?

And so what I'm going to come back and tell you one Friday is, this is going to be the key

switch for iron sensing. Whether the iron is in the apostate, with no metal, or whether it moves to the 4 iron 4 sulfur cluster state. And we'll talk a little bit then about how those two states, and the presence of RNA, can control which of all these proteins I've thrown at you today actually get translated. OK.