[SQUEAKING]

[RUSTLING]

[CLICKING]

MATTHEW VANDER HEIDEN: Hello, everybody. Last time, I introduced the idea of the TCA cycle, tricarboxylic acid cycle. Also known as the citric acid cycle, because citric acid is a tricarboxylic acid, as you'll see later today. Also known as the Krebs cycle, named after Hans Krebs, who discovered it in the early part of the last century. The TCA cycle is the series of reactions that occurs in the mitochondrial matrix and it allows the complete oxidation of two carbon units, derived from many things, including pyruvate, derived from glucose and glycolysis and enables the complete oxidation of that carbon to CO2.

Now, it's a cycle because those two carbon units from pyruvate or other sources enter the cycle, combine with 4-carbon oxaloacetate, and combine to make 6-carbon citrate. Hence the citric acid cycle, or TCA cycle, that is then oxidized back to 4 carbon units, forming a cycle that allows cells to release lots of energy. Complete oxygen enables, ultimately, complete oxidation of glucose to CO2. We've talked many times how this releases energy. And it also generates lots of intermediates for the cell that can be used to make other stuff.

Last time, I alluded to the fact that citrate can be used, say, to make fatty acids. Today, what I want to do is I want to dive into the details of how this TCA cycle occurs and the consequences of the way it works, and how that affects other aspects of metabolism. You'll see that it actually affects the ability of different organisms, whether or not they can make things from intermediates in this cycle, because of how the cycle works.

Now, to start. Of course, if we're going to start from pyruvate. Pyruvate, of course, has three carbons. And so if we're going to generate a two-carbon acetate group, we have to lose CO2.

And as I described a couple lectures ago, acetate is also the thing that we can derive from metabolism of ethanol, showing us two things-- metabolized glucose to pyruvate or ethanol itself-- can both be turned into acetate. But really, the donor is this molecule, acetyl-CoA. Which is basically also a carboxylic acid, but rather than being a carboxylic acid, instead makes this a thioester bond, which activates this carbon as a leaving group, such that it can combine with oxaloacetate, which you'll remember from gluconeogenesis, releasing that S-CoA molecule to generate six-carbon citric.

So, this is citric. You can see that we've made a bond from this carbon on the acetate, losing this S-CoA group to this carbon oxaloacetate to make this six-carbon citrate molecule, which is a one, two, three-- three-carboxylic acid, or tricarboxylic acid. Hence the name citric acid cycle, tricarboxylic acid cycle.

Now, these six carbons can then-- or this six-carbon citrate molecule can then be oxidized, generating two CO2 molecules that are released, and ultimately reforming oxaloacetate that can pick up another two-carbon acetyl-CoA to generate another citrate, and around and around the cycle goes, allowing in the end the net entry of two carbons, effectively from acetate, and release of two carbons as CO2. Now, as I alluded to, this can come from pyruvate. It can come from acetate itself, vinegar. It can come from alcohol. It turns out that when you break down fat, you also break it into two carbon units. And so this cycle becomes very useful for cells, because it allows the oxidation of many different molecules to completely turn that carbon into CO2.

Now, if we're going to do this from glucose, however, you'll remember that pyruvate has three carbons. And so if we're going to turn pyruvate into acetate, or acetyl-CoA, we have to lose a carbon of CO2. We have to lose this carbon as CO2.

Now, we saw this before, that we can do this via-- this is exactly how we generated ethanol when we did fermentation of pyruvate to ethanol, but that molecule didn't generate acetate, It generated acetaldehyde. The difference, of course, being whether or not this carbon gets oxidized in the case of acetate to the acid, or in acetaldehyde in ethanol metabolism, where it remains an aldehyde.

Now obviously, you can make ethanol and then turn that ethanol into acetate. And that's a pathway that I guess certainly would work. However, that's not the way it happens in most organisms. Most organisms actually directly produce acetate, or more correctly, acetyl-CoA from pyruvate. So let's take a look at that reaction.

So here once again is pyruvate. And if we turn that pyruvate into this two-carbon acetyl-CoA, let's look here what has to happen. Well, the first thing is that we have to decarboxylate this molecule. And so that generates a CO2. We have to lose that one carbon.

Also, as I pointed out, if we do this as if we did it in ethanol metabolism, we'd be left with an aldehyde. But this is an acid, and so this carbon also has to be oxidized. We know how to do that. We can donate those electrons to something like NAD, so the NAD is reduced to NADH. And we had to add this S-CoA molecule, which I'll come to in a minute. And so each of these steps ends up making a fairly complicated reaction.

Obviously, several co-factors are going to be involved. You should be able to guess that just by looking at it. Obviously, I already drew up there NAD.

If we're going to decarboxylate, remember this is a alpha carboxylic, an alpha ketoacid-- the ketone group is alpha to the carboxylic acid. And so if we're doing alpha decarboxylation, as you might guess, we need a co-factor. That co-factor, as I told you before, is the co-factor factor thiamine pyrophosphate. And finally, there's this S-CoA group. We need to describe what that is.

However, before delving into those, and there's actually a couple other factors that are needed as well, I want to mention one other issue about this reaction, in that this reaction happens in the mitochondria, because that's also where the TCA cycle happens. So, recall that if we divide the cell into two compartments like an eukaryotic cell, here we have the cytosol and the mitochondria in a eukaryotic cell. As we've already talked about, having different compartments helps facilitate different metabolic reactions, because you can have different conditions in the two compartments.

And so as we said, glycolysis occurs in the cytosol. Glucose to pyruvate. And last time I mentioned, the TCA cycle occurs in the mitochondria. And so that means if we're going to fully oxidize the pyruvate carbon to CO2 using the TCA cycle in the mitochondria, that pyruvate has to get from the cytosol to the mitochondria, or at least carbons from the pyruvate have to get there.

It turns out, you'll see in a minute, acetyl-CoA is a very large group. And so pyruvate itself is transported into the mitochondria. And that's where the reaction occurs to turn it into acetyl-CoA that can then enter the TCA cycle and oxidize. However, what this means is that a transporter is actually needed to get this across the mitochondrial membranes and into the matrix of the mitochondria where the TCA cycle happens.

And I like to mention this because it turns out the pyruvate carrier-- that is, the transporter-- the way that it actually gets that pyruvate from the cytosol into the mitochondria actually was an unknown thing about metabolism until 2012, so not that long ago. Sometimes one can be left with the feeling, listening to these metabolism classes or reading a textbook, that everything about metabolism has been known forever, but it's actually not true. Here's a very key, central part of the pathway that was actually just discovered, at least at the time of this lecture, less than 10 years ago. It also illustrates that not all metabolism is completely understood.

Now, let's get back to this reaction, how you turn pyruvate into acetyl-CoA. And I want to go through now and point out that several co-factors are needed. And so I already mentioned one of them, S-CoA, which is shorthand for coenzyme A. So, I need to tell you what that is.

You hopefully are already familiar with TPP plus, thiamine pyrophosphate. We talked about that when we talked about how you do alpha decarboxylation to generate ethanol from pyruvate, also used here for the alpha decarboxylation reaction. Redox reaction happens, and so we needed NAD plus to get converted into NADH.

We talked a lot about how that serves as an electron carrier, but it turns out that there's two additional electron carriers that are involved in this reaction. One of them is called FAD and the other one is called lipoic acid. Now you might say, why do we need all these electron carriers? Well, these are just different molecules that can carry two electrons, similar to NADH. And effectively, what these can do by having multiple electron carriers-- one can build chains of oxidation and reduction reactions.

And it turns out these chains of oxidation reduction reactions really become central to energy transfers in biology, because building these chains allows more easy stepwise release of energy as one moves across these oxidation reduction reactions. Which remember, as I alluded to earlier, really are at the core of bioenergetics and a lot of what allows energy release from these pathways. What I mean by this will be more explicit as we go through what some of these co-factors look like.

So, I'm not going to draw TPP plus or NAD again, but let's define what some of these other cofactors look like. So, let's start with coenzyme A. So, coenzyme A, it turns out, is useful. It's actually involved in lots of acylation reactions.

What's an acylation reaction? Well, that's basically if you're making a carbon-carbon bond by adding a molecule of greater than one carbon, so two carbons or greater, to something else. That's an acylation reaction, as we did with adding the two-carbon acetate to oxaloacetate to make citrate. And you'll actually see coenzyme A will come up in this in many, many lectures throughout the rest of the course.

Why this becomes useful is because it activates this basically carbon to the left of, in this case, the ketone. Because, by having that thioester there, ends up activating that carbon and allows it to carry out these acylation reactions. Coenzyme A itself is derived from a vitamin called pantothenic acid. And as a vitamin, this is something that you have to get from the diet.

Now, when we abbreviate coenzyme A, which is often abbreviated S-CoA, you get the sense that it's this little tiny molecule that's just stuck on sulfur-- stuck on to the end to make this thioester bond. But it turns out coenzyme A is actually a giant molecule, one of the reasons why you actually synthesize acetyl-CoA in the mitochondria, because it's hard to transport this giant molecule. And so this is what pantothenic acid looks like.

So, if I put an acid group here and a hydroxyl group there, that would be pantothenic acid, the thing that's in your cereal, on the side of your cereal box. Then there's these additional pieces. This is the active end of the molecule, that's that sulfur from the S-CoA, and then this side of the molecule is esterified to two phosphates, which are esterified to-- I'm not going to draw it out, but this would have an adenine base and a phosphate there. So basically, this is ADP, with a phosphate added to the 3 prime position of the ADP molecule, added to pantothenic acid, added to this short chain with the sulfur on the end. And this whole molecule together is coenzyme A.

And so when we say acetyl-CoA, it's this giant molecule, S-thioester to the carbonyl, the acid on acetate, to CH3. And so that would be, basically, acetyl-CoA. And so one convenient thing about this is it's much easier than drawing that big molecule, but it is a little bit misleading in terms of its size. So, that's coenzyme A.

Next one I want to talk about is FAD which stands for flavin adenine dinucleotide. So, flavin adenine dinucleotide is an electron carrier, just like NAD plus. And it's derived from the vitamin riboflavin, also referred to as vitamin B2. Another thing from the side of your cereal box, and basically looks like this.

So, like NAD, it's a dinucleotide. And so here's ADP, just like we do for CoA, or just like one end of NAD. One difference is that the other nucleotide down here actually isn't technically a sugar. It's ribitol instead of ribose.

What does that mean? It doesn't have an aldehyde. Instead, it is just a five-carbon chain where all of the carbons are alcohols. And so since there's no aldehyde, it doesn't form a ring. And the base on this end, as a nicotinamide, is this flavin group, which looks like this.

And so this is FAD, which is in the oxidized form. Turns out, in the oxidized form, FAD is yellow, hence riboflavin-- "flavin", yellow. And the reason it's yellow-- you see there's a conjugated double bond here across this part. It turns out this is the active part of the molecule, and it works as follows.

And so if you have a hydride ion, remember the way we can transfer two electrons. Can transfer the two electrons that way, and that allows it to generate. And I'll just draw the middle, here. Active part of the molecule.

So, that would be these two nitrogens. We've added two electrons to it. And so this is abbreviated FADH2, or the reduced form of FAD. And it is colorless, because now you no longer have that conjugated double bond system, loses its color. And so you can follow whether FAD is oxidized or reduced by just looking at color change. So, that's FAD. It's an electron carrier, carries electrons by a hydride transfer, very similarly to what we described for NAD, but obviously a different molecule.

And the last molecule, the last co-factor that we need for this reaction, is lipoic acid. Which, unlike most things in metabolism, doesn't have an abbreviation and also functions as an electron carrier. And so lipoic acid looks like this.

So, that is lipoic acid. This is in the oxidized form. And so where it's oxidized is here at this sulfur-sulfur bond. So, you can think of this as the same as a disulfide bond that you learned about from Professor Yaffe in proteins. And so this is the oxidized form of the disulfide bond. If I take this hydride, transfer two electrons across that bond, then it goes to-- and I'll just draw here the active end.

Then we go here to the reduced form of lipoic acid, and there's no abbreviation for oxidized or reduced lipoic acid. It's just lipoic acid, oxidized lipoic acid, reduced. And how they're oxidized and reduced is basically very similar to the disulfide bonds that you saw in proteins being oxidized, disulfide bond being oxidized, or it can be reduced to not be a disulfide bond.

Now, it turns out that these cofactors, our TPP, FAD, and lipoic acid, are all stably associated with different subunits of a multi-protein complex that assembles to catalyze that reaction to convert pyruvate to acetyl-CoA. The enzyme or enzyme complex that carries this out is referred to as PDH, sometimes abbreviated PDC, which stands for the pyruvate dehydrogenase complex. So, pyruvate dehydrogenase, PDH, pyruvate dehydrogenase complex, PDC.

This complex is basically three different polypeptides that come together to form a complex and catalyze that reaction. Now, where this complex sits-- so, this is the mitochondria. This is the matrix. That's where this pyruvate dehydrogenase reaction occurs. That's where the TCA cycle occurs. And this PDH complex is basically sitting here at the membrane on the matrix side of the membrane.

Now, the three different polypeptides that come together to form this complex are creatively named E1, E2, and E3, four. Enzyme one, two, and three. And each of these, as I alluded to, is associated with a different co-factor. And so E1 is associated with thymine pyrophosphate. E2 is associated with lipoic acid. And E3 is associated with FAD. Now, let's go through the mechanism for how this pyruvate dehydrogenase reaction works.

So remember, this is the active part of TBP plus. It is bound in the active site of E1. And it reacts with pyruvate to catalyze alpha decarboxylation, just like we described for conversion of pyruvate to alcohol. So, you're going to see exactly the same mechanism that we drew before.

So, that decarboxylates the alpha-keto acid, just like we saw to generate acetaldehyde. The difference is rather than resolve this such that this carbon has the same oxidation state and make acetaldehyde, instead the next step of this reaction is going to be oxidized by reducing lipoic acid. So, the active part of lipoic acid that is in the active site of the E2 subunit.

So this will now regenerate E1, but what we're left with, then, is this. Now, this intermediate bound to E2. Here's where coenzyme A can come in, which will then generate acetyl-CoA. But now we are left with E2 in the reduced state, rather than being in the oxidized state. So E2 has to be re-oxidized in order for this complex to carry out the next catalytic cycle, and the way that works is as follows.

So you have FAD bound on E3. And so you have a hydride ion from the oxidation of E3 that can be transferred to FAD. That will generate FAD from the oxidized to the reduced form. So re-oxidize lipoic acid on E2, reduce FAD to FADH2, and then that FADH2 can be re-oxidized back to FAD via transferring those electrons to NAD plus to generate NADH.

So in this case, FADH2 re-oxidized the FAD, NAD plus reduced to NADH. And so, effectively, what this happens is that enzyme E1 and E2 cooperate to call what's referred to as oxidative alpha decarboxylation of pyruvate, while adding -CoA, so that's where you get acetyl-CoA, with reduction of lipoic acid in E2, and then E3 re-oxidizes the lipoic acid in E2 while generating NADH.

This NADH, once it's generated, of course those electrons have to go somewhere, so they, like in glycolysis-- it also needs to regenerate to NAD at some point. This is ultimately the electrons that end up on oxygen, and it's really this series of electron transfers, with oxygen being a good electron acceptor, that ultimately allows controlled energy release during carbon oxidation. And cells, you'll see, can use that to make ATP or do other kinds of work.

So, the net reaction and/or another way to draw the pyruvate dehydrogenase reaction would be as follows, and that's taking pyruvate to acetyl-CoA. And so we're going to take coenzyme A and release CO2. This is done by TPP plus, as part of E1. That involves converting the lipoic acid in E2 from the oxidized to the reduced state. That lipoic acid then has to be re-oxidized. If something's oxidized, something else has to be reduced.

That's FAD on E3, which also then cycles between the oxidized and reduce state. And ultimately, those electrons ended up being transferred to NAD plus to generate NADH. And so the PDH complex is basically a chain of electron transfer reactions. And it's the first example of a chain of electron transfer reactions. We're going to see that there is the electron transport chain in the mitochondria, effectively does the same thing. And by coupling oxidation and reduction reactions across chains of molecules like this, effectively as a preview, allows the stepwise energy release of these oxidation reactions to occur.

So remember, if we burn glucose, completely oxidize it in one step, where those electrons are directly transferred to oxygen in combustion. Lots of energy released, but all in one step. By doing these stepwise electron transfers, we can then basically break up that energy release in a way that can be captured by cells to do work.

Here, the way energy is captured, it's not so obvious in this electron transport reaction. But effectively, you're using oxidation of the ketone on pyruvate, with decarboxylation to the acid to generate, rather than just the acid, a thioester bond. And that's that thioester bond-- as well as NADH, that's energy as well-- but it's really that thioester bond that then can be recaptured later to drive synthesis of citrate in the TCA cycle.

Now, next what I want to do is I want to discuss the TCA cycle reactions. Now that you see how you can get acetyl-CoA, at least from pyruvate, I want to discuss how you can now use that acetyl-CoA and oxidize it back to combine it with oxaloacetate, and oxidize it back to make citrate, and then oxidize it back to oxaloacetate to run the TCA cycle. OK, great.

So, the first reaction of the TCA cycle. Here's acetyl-CoA. It will combine with oxaloacetate. This reaction is catalyzed by an enzyme called citrate synthase, and generates the six-carbon tricarboxylic acid citrate. So, how does this reaction work?

Here's drawing acetyl-CoA in a slightly different way. If I redraw this as the enol, this will generate citrate via, effectively, that mechanism.

Next, citrate is converted by adding water across this carbon-carbon, or by removing water across this carbon-carbon bond, to generate this intermediate called cis-aconitate. So this intermediate is called cis-aconitate. so all I did was dehydrate across that bond so there's a double bond there. And then if I re-add water across that bond, I generate this molecule called isocitrate. So effectively, to convert citrate to isocitrate, I'm moving the hydroxyl group from that carbon to this carbon. To do that, I basically dehydrate, make a double bond, remove water, re-add water across that bond in the opposite direction to generate this molecule, isocitrate.

This reaction is carried out by an enzyme called aconitase, and converts citrate to isocitrate. I think it's a little easier to see this reaction if I draw it a slightly different way. So, this here is just drawing citrate by just slightly rotating the molecule to look like that. And so I'm basically removing water here. And then I'm now just adding water back in the opposite orientation to generate isocitrate.

Now, you'll notice when I drew this-- if you look at citrate, this is actually a symmetrical molecule, so the top half and the bottom half of citrate are identical. And so what's interesting about nature is that it treats these carbons-- the green carbons that came from acetyl-CoA-- different from the side of the molecule that comes from oxaloacetate. And effectively, nature always moves the hydroxyl group to this carbon that came from oxaloacetate, and never moves it to this carbon that came from acetyl-CoA.

This is an example where enzymes-- nature treats a symmetrical molecule like citrate in an asymmetrical way. And this has consequences for how carbon is actually traced through the entire TCA cycle. Because even though you might think things could get scrambled at citrate, they never do. Meaning an isocitrate-- it's always these green carbons that came from acetyl-CoA. You never get those green carbons on the other side of isocitrate. And so when we go through the TCA cycle, I'll keep these carbons green until the point where you can no longer distinguish which carbon came from came from which reaction.

The next reaction is we're going to oxidize this carbon of isocitrate. So if we're going to oxidize that carbon, those electrons have to go somewhere. And so if we oxidize the carbon, we can use NAD plus as an electron acceptor, reduce it to NADH. That generates this intermediate.

So hopefully this is clear to everybody at this point, but just in case. So, this carbon here and isocitrate. If I oxidize that alcohol to the ketone, now I generate a hydride ion. Those two electrons and the hydrogen can go to NAD plus and reduce it to NADH.

That generates this intermediate. It's called oxalosuccinate. Which then, if you notice, the oxalosuccinate is now a beta keto acid. So alpha, beta. The acid group is beta to the ketone, so it's a beta keto acid. Remember, beta decarboxylation is favorable, and so I can lose that CO2. And what I'm left with is this molecule, which is called alpha keto glutarate.

So this whole reaction here, the oxidation of the alcohol to the ketone, followed by beta decarboxylation of oxalosuccinate to alpha ketoglutarate, is carried out by an enzyme called isocitrate dehydrogenase. And of course, just to remind you, here's that beta keto acid in oxalosuccinate. And so that can decarboxylate, leading this enol. Which can, of course, rearrange back to the ketone that we see, an alpha ketoglutarate.

Now, if you look at alpha ketoglutarate, you'll notice that-- and I will redraw over here. So, this here is just me redrawing alpha ketoglutarate, which is often abbreviated alpha kG. Just drew it as a straight line.

Now, if you'll notice, alpha keto glutarate is a alpha ketoacid. And so here the acid group is alpha to the ketone. So, an alpha keto acid. It's effectively just like pyruvate, but has this additional pieces on it. And it turns out the next step in the TCA cycle is the exact reaction that we saw with pyruvate dehydrogenase. It's alpha decarboxylation, oxidative alpha keto acid decarboxylation, as follows.

So, we carry out. This is a molecule called succinyl-CoA. And so, you'll see what happened there is decarboxylated here, this carbon, while oxidizing this ketone to the acid and adding -CoA. That's a redox reaction. So NAD, NADH, it turns out this is exactly the same mechanism-- so I don't need to draw it again-- that I just showed you for pyruvate dehydrogenase. So it needs all the same cofactors-- TPP plus, lipoic acid, FAD. And in fact, it even shares some of the same enzyme complexes, subunits, as pyruvate dehydrogenase.

And so this is a reaction that's catalyzed by alpha ketoglutarate dehydrogenase. Like pyruvate dehydrogenase, this is a complex, and so it has a unique E1, which makes sense. Remember, E1 of pyruvate dehydrogenase was actually the subunit that bound the pyruvate. E1 of alpha ketoglutarate dehydrogenase is unique. It binds alpha ketoglutarate instead of pyruvate. However, they share the same E2 and E3s, and so the E2s and E3s would carry out exactly the same reaction and play the same part in the mechanism of how you do this alpha oxidative alpha decarboxylation to turn alpha ketoglutarate into succinyl-CoA.

Now, remember in pyruvate dehydrogenase, once we got that acetyl-CoA, we then use this CoA group to drive condensation with citrate. Well, in this case, what happens is you don't want to combine condensation here. Instead, what is going to happen is you want to use this CoA group to now generate ATP. And so this is going to couple release of the CoA to generate an ATP equivalent. It's actually GTP that's generated by the TCA cycle. And so, this is going to generate this molecule, succinate. And the enzyme that does this is called succinic bio-kinase.

So, let's go through over here how this enzyme works. So here's succinyl-CoA, basically using favorable loss of the CoA breaking the thioester bond to generate this acid anhydride. We saw an acid anhydride before in glycolysis. Remember, we made 1-3-bisphosphoglycerate, so that's a good phosphate donor. And then that can be used to generate succinate, and transferring that phosphate to GDP to make GTP, just like 1-3-bisphosphoglycerate, was able to transfer the phosphate from the acid anhydride to ADP to make ATP in glycolysis.

So, the next step is to oxidize this carbon-carbon bond in succinate. So if we're going to oxidize a carbon-carbon bond, those electrons have to go somewhere. So there's our carbon-carbon bond. And so if we generate hydride ion just like we did in other oxidation reactions, but this hydride ion is transferred not to NAD, but instead to FAD, a different electron carrier, to generate if FADH2.

Now, I should point out succinate, like citrate, is a symmetrical molecule. But at this point, nature doesn't tell the difference. Once it generates succinate, this molecule now gets scrambled. And so everything downstream of succinate, you no longer know which carbons came from acetyl-CoA. This generates this molecule called fumarate, and this reaction is carried out by an enzyme called succinate dehydrogenase, often abbreviated SDH for succinate dehydrogenase.

Now, the next reaction is, we're going to add water across this double bond of fumarate. And that generates this intermediate, malate. This reaction is carried out by an enzyme called fumarate hydratase. And it's simply adding a water molecule across that double bond.

Once we have malate, if you look what's the difference between malate and oxaloacetate, the difference is that in oxaloacetate, this carbon is a ketone. Whereas in malate, it's an alcohol. And so if we want to turn this carbon from the alcohol into the ketone, that is, of course, a oxidation reaction, and so those electrons have to go somewhere. Don't need to draw the mechanism again. It's basically just the hydride transfer to oxidize this to an alcohol, to the ketone.

That oxidation couples to a reduction of NAD plus to NADH. This is carried out by an enzyme called malate dehydrogenase. And doing this completes the TCA cycle, regenerating oxaloacetate, that can then recombine with another acetyl-CoA to go through another round of the cycle.

You'll notice that going through this cycle, there's two CO2s lost. One of them is lost here at the isocitrate to alpha ketoglutarate reaction. So, this decarboxylation from oxaloacetate to alpha ketoglutarate. That beta decarboxylation, that's the first CO2. The other one is lost here at the alpha ketoglutarate. The alpha ketoglutarate dehydrogenase step, where you have this alpha decarboxylation to take alpha ketoglutarate to succinyl-CoA oxidative alpha decarboxylation.

Now, what's cool about this, as you noticed, we just discussed all the reactions of the TCA cycle. And I showed you, reminded you, of some chemistry that you've already seen, but unless you count the chemistry we showed you earlier for how the PDH and alpha ketoglutarate dehydrogenase reactions work with E1, E2 and E3, with lipoic acid, FAD. That was obviously new for today. But other than that, everything else was chemistry that you've already seen.

And this really points out the point that I made earlier, that metabolism is really variations on relatively few reactions. We've just repurposed some of the same tricks, if you will, that we're used in glycolysis, and allowed it to now do an entire different pathway, the TCA cycle. It also points out how Hans Krebs was-- well, is still-- remarkable, able to figure out from chemistry alone, because there's actually quite a bit of logic to the way metabolism works.

Now, I want to say this again. Note there were two carbons that entered acetyl-CoA, and two carbons that were lost to CO2. But if you look, the green carbons remain in the same places until they get to succinate. and so the two carbons that enter are not lost on the first turn of the cycle. It's actually two carbons that came from oxaloacetate that are converted to CO2 as that acetyl-CoA goes through the cycle. And so to oxidize the exact carbons from acetyl-CoA to CO2 requires more than one turn of the cycle.

You'll also notice that the cycle is oxidation. And so oxidation reactions, of course, release energy. We've talked about that. And so it's favorable.

And the products, if you will, are three NADH molecules. You can say plus one more NADH if we're going all the way from glucose or from pyruvate. Glucose derived pyruvate because the pyruvate dehydrogenase reaction also generates an NADH to make that a acetyl-CoA. One FADH2, as well as one GTP molecule. And so lots of oxidation going on here. We completely oxidized two carbons to CO2, so that's energy release. But you notice you only get one GTP from the molecule.

Now, this GTP, of course-- that reaction, the succinic thiokinase reaction, like the reactions we saw on glycolysis, is such that it can generate GTP at a high DGP-GDP ratio, or ATP-ADP ratio. Remember, those are equivalent. Those energy charge are similar, and so that makes sense.

But most of the energy released is actually reducing NAD and FAD to NADH and FADH2. And of course, these need to transfer their electrons somewhere else, and that's the role of oxygen. Oxygen, remember, is a very good electron donor, and so it's the ultimate transfer of those electrons from these molecules to oxygen that also provide energy that the cell can use to do work, but it does so, in a way, by charging up different ratios in the cell.

So the NAD-NADH or the FADH2-FAD ratios. And just like we talked about, the ratio or the energy of ATP is in the interconversion between ATP and ADP. It's the ratio that drives the free energy change. The same thing exists for an NADH and NAD, FADH2 and FAD. And so charging up these ratios while passing through the TCA cycle, and the ultimate downstream transfer of those electrons to oxygen, really is where most of the energy is captured as carbon is oxidized through the TCA cycle.

And exactly how that works and how it can be related to ATP will be something that'll be more explicit in the coming lectures. Now, I want to point out apart from the oxidation, there's actually lots of intermediates made here. And it turns out, a bunch of these intermediates are useful for cells to make stuff.

So we talked about gluconeogenesis. Gluconeogenesis needs electron balance. We get NADH from the TCA cycle, and so you can think of gluconeogenesis as an alternative to fermentation to dispose of electrons. Well, you can use the NADH from the TCA cycle to run gluconeogenesis as well.

But beyond the cofactors, the carbon itself. So citrate, I've alluded to now a few times, is important as a precursor to make fat. We'll discuss that in later lectures, too. But other intermediates in this pathway are useful for various amino acids and nucleic acids. And so there's lots of things that can come from the TCA cycle that cells can find useful to do, not just catabolism, but also anabolic processes.

Now, the way the TCA cycle works, though, is that there's actually an issue if you want to use the intermediates from the TCA cycle to make stuff. And so what is that issue? Well, the TCA cycle functions at a site as a cycle. And so, if we're going to take things in and out of it, that has consequences for how the cycle runs.

Now there's a couple words for this that I want to just introduce to you. The first one is cataplerosis and the second one is anaplerosis. And so cataplerosis is the act of removing stuff from a metabolic cycle. So, we're going to remove citrate from the cycle to make fat. That's cataplerosis.

And anaplerosis is adding stuff back to a metabolic cycle so it can continue to function. Viewing this is really evident if you think of the TCA cycle as a chicken and egg problem. So, the very first time acetyl-CoA was generated, how do you start the TCA cycle in the first place? You can't add it to the TCA cycle unless you have oxaloacetate to combine with the acetyl-CoA, which can then generate another oxaloacetate.

So where does the first oxaloacetate come from? Well, we already talked about one reaction. We talked about it in the context of gluconeogenesis. That can solve this problem.

And so we have pyruvate. And so we talked earlier today how we can do oxidative decarboxylation of pyruvate to give acetyl-CoA. But we talked to in the gluconeogenesis lecture how we can add a CO2 pyruvate to generate oxaloacetate. So if I do those two reactions, now I have all the carbon I need to generate a citrate and start off the TCA cycle.

Now obviously, if I do cataplerosis and I remove that citrate I made to make fat, well, now I need two pyruvate again to generate the next citrate if I'm using this pathway, because every time I bring an acetyl-CoA into the cycle, I need an oxaloacetate to combine it with. And so if I remove something, I have to add something back. And so pyruvate to oxaloacetate, the pyruvate carboxylase reaction is an example of an anaplerotic reaction.

Now what this means, though, is that in order to do an anaplerosis, you have to be able to generate four-carbon oxaloacetate, or a four-carbon molecule. Now, pyruvate carboxylase, pyruvate to oxaloacetate, allows you to do that. We can take a three-carbon molecule and generate four-carbon oxaloacetate.

However, if we start from a two-carbon molecule like acetate or acetyl-CoA that enters the cycle, it's actually not so simple to take that two-carbon molecule and turn it into four-carbon oxaloacetate. And in fact, humans lack any enzymes that allow them to take a two-carbon unit, to take acetate or acetyl-CoA, and turn it into anything that's longer than net-- turn it into anything that's longer than two carbons.

And this has important implications for human physiology, because what it says is that we can't make glucose from anything that starts with something less than three carbons long. So if you drink alcohol and you metabolize that alcohol to acetate, it turns out you take fat and you break down fat also to acetyl-CoA, to acetate two carbons long. There is no way to turn those molecules into glucose, because you cannot generate the oxaloacetate to do the anaplerosis that's necessary to get it there.

What this means is that our body can only store calories that come from two carbon units, fat or alcohol, as fat. We can never turn them back into glucose or make glycogen. And this is very relevant for those of you that go to medical school, because it's relevant to our physiology.

And that is, when our bodies exhaust all of our stores of glucose, what happens? Our liver can no longer do gluconeogenesis. And so what happens? Now it has to switch over to doing something else. It has to work with two carbon units. And ultimately, this is ketone metabolism, which we'll talk about in a few lectures.

It also said that the body-- that this is also the basis of a very old adage that's out there that some of you may have heard-- that you need to have some other fuel if you're going to burn fat. The basis for that is that fat is turned into two carbon units, acetyl-CoA units.

And so if you're going to take those acetyl-CoA units and ultimately burn them away, turn them into CO2, you need a source of oxaloacetate, or your TCA cycle won't work. And you don't need a lot of something, but it is true. You can't start just with acetyl-CoA as a human and turn it into CO2, so you need some-- at least a little bit of oxaloacetate to get your TCA cycle started.

Now, that's a problem that we as humans and other mammals face, but it turns out there's lots of microbes out there that grow just fine on acetate or on alcohol, even if it's only carbon source. And so those organisms must have some way to build stuff from two carbon units. That is a way to use two carbon units and do an anaplerosis. And it turns out the way they do this is via something called the glyoxylate cycle.

And so the glyoxylate cycle is an alternative version of the TCA cycle that effectively uses two enzymes that we lack as mammals. And so I'll quickly tell you about it here. So, this is isocitrate from the TCA cycle. And some microbes have an enzyme called isocitrate lyase.

And what isocitrate lyase does is basically splits citrate in half, such that the top portion of the molecule is another TCA cycle intermediate, succinate. And the bottom portion of a molecule is this two carbon aldehyde called glyoxylate. Glyoxylate can react with acetyl-CoA by another enzyme that we lack as humans called malate synthase. And I don't have time to show the mechanism again, but malate synthase basically adds the two carbons from acetyl-CoA to the aldehyde, the carbonyl, the aldehyde carbonyl of glyoxylate in a reaction that is, for all intents and purposes, exactly what happens in citrate synthase. That will generate this molecule, which is malate, also in the TCA cycle.

And so having these two extra reactions, isocitrate lyase and malate synthase, gives microbes the ability to have acetyl-CoA be anaplerotic. And so how does that work? Well, that's because if we start with one oxaloacetate, four carbons, and acetyl-CoA, two carbons, can run the TCA cycle and make citrate, six carbons. Turn that citrate into isocitrate. Use isocitrate lyase to generate glyoxylate, two carbons. Plus succinate.

That succinate can run through succinate dehydrogenase to generate malate, which you can go through malate dehydrogenase to generate oxaloacetate. This glyoxylate can start with a second acetyl-CoA. Two carbons come together, generate malate. That generates a second malate molecule, which can then exit the cycle as malate or oxaloacetate or whatever you want.

And so basically, it allows two acetyl-CoAs to net generate an oxaloacetate, and so net generates a way to do an anaplerosis from two carbon units by having this malate synthase reaction and this isocitrate lyase reaction and run this alternative version of the TCA cycle, called the glyoxylate cycle. And it's a nice way how life-- again, no new chemistry here, just variations on what we've already shown-- repurposed the similar chemistries that it's already using as a way to live off of carbon sources that contain only two carbons, like ethanol or acetate.

So in closing today, the last thing I want to talk about, very briefly, is how the TCA cycle is regulated. And we don't need to spend a ton of time on this, because it really follows principles that make sense, particularly when we think about things that we've already talk about.

And so, regulation of the TCA cycles is of course important, and it's really a critical hub, both for anabolic and catabolic pathways. So, you needed to get energy to fully oxidize carbon, but it's also a useful place to get stuff. And before I talk about regulation, I just want to point out that many of the enzymes in the TCA cycle, even though we talk about it in the mitochondria and we're about to talk about regulation in terms of catabolism-- that is oxidation, ways to release energy-- many of these enzymes are also in other locations in the cell, because there's functions for them in making stuff that is very different than what goes on in the TCA cycle.

And the regulation that I'll tell you about, and that comes up on MCAT exams and stuff like that, usually talks about this pathway as a catabolic pathway, as a way to make CO_2, to make ATP. However, recognize that there's also variations on this pathway that can use in anabolic things, making stuff. And that regulation is something that really is not necessarily what we're going to talk about here and is a little bit less well-understood.

But at least the regulation, in terms of catabolism, if we put it in context of glucose metabolism-- so here's glycolysis, turning glucose into pyruvate. And then that pyruvate can operate through the pyruvate dehydrogenase reaction to generate acetyl-CoA. That acetyl-CoA can combine with oxaloacetate to generate citrate.

That citrate, of course, can be used to generate fat, as we've talked about. That can go to isocitrate. Isocitrate to alpha ketoglutarate. That's catalyzed by isocitrate dehydrogenase, which I will abbreviate IDH. Alpha ketoglutarate to succinate. More correctly, to succinyl-CoA by alpha ketoglutarate dehydrogenase, Alpha KGDH. And then this back to oxaloacetate.

And so the main enzymes that are typically discussed as being regulated are alpha ketoglutarate dehydrogenase, isocitrate dehydrogenase, and pyruvate dehydrogenase. I also drew glycolysis. And I'll just write over here, gluconeogenesis up here, because we can now see more fully how this plays in with citrate acting as a positive regulator with gluconeogenesis and an inhibitor of glycolysis. Again, we're full in citrate, stop running glycolysis, start running gluconeogenesis.

Now, the other regulation. If you have a lot of acetyl-CoA, stop making it from pyruvate. acetyl-CoA is a negative regulator of PDH.

This is carbon oxidation. Releases a lot of energy, generates a lot of ATP, generates a lot of NADH. If you have lots of those things, no reason to keep sending carbon into acetyl-CoA to go into the TCA cycle.

Ultimately, we have to send those electrons somewhere. So if there's nowhere to put them, low oxygen also inhibits pyruvate dehydrogenase. And of course, if you need more energy, if ADP is high, that activates pyruvate dehydrogenase. So at least in terms of glucose, complete glucose oxidation, a lot of regulation happens at pyruvate dehydrogenase.

And a lot of it really makes sense. High levels of ATP, high levels of NADH, also inhibit isocitrate dehydrogenase and alpha ketoglutarate dehydrogenase. Makes sense. Succinol-CoA high, inhibit alpha ketoglutarate dehydrogenase. High levels of ADP, need to release more energy, activate alpha ketoglutarate dehydrogenase.

Again, these are the feedbacks that people talk about on board exams. Good to know, but you can almost guess what they would be from first principles. Because remember, this is a pathway that releases a lot of energy. High energy high ATP, high NADH-- don't run the cycle, don't enter carbon in the cycle.

Low energy, high ADP, put carbon in the cycle, run the cycle faster. Makes sense. Also makes sense of the reciprocal regulation of glycolysis and gluconeogenesis.

Great. Next time, we will talk more about how we can oxidize fatty acids, fat, by accessing acetyl-CoA and entering it into the cycle. Thanks.