[SQUEAKING]

[RUSTLING]

[CLICKING]

MATTHEW VANDER HEIDEN: So today I want to continue our discussion of amino acid metabolism. And before we do that, I just want to come back for a few minutes and revisit the urea cycle and the Krebs bicycle that we discussed at the very end of the last lecture. I only want to do that because it-- a lot going on with this cycle. It can appear on the surface to be confusing, the molecules are hard to draw. And so I just wanted to draw it neatly here for you one last time and go through it again.

And so remember, if we start from the amino acid arginine, arginine can be converted into the non-proteinogenic amino acid ornithine, and in the process, release urea. That ornithine can then be in the mitochondria converted to citrulline, another non-proteinogenic amino acid. By picking up a carbon and a nitrogen from this molecule, carbamoyl phosphate, which is generated from CO2/bicarbonate, as well as ammonia and to phosphorylations with ATP to get carbamoyl carbon phosphate, that's added to ornithine to get citrulline.

That citrulline and then combine with the amino acid aspertate to give this molecule, argininosuccinate. So arginine plus a succinate on it. And then loss of fumarate leaves you with arginine. That fumarate a can, of course, go through the TCA cycle to regenerate malate, oxaloacetate, ultimately pick up nitrogen on oxaloacetate is that alpha-keto acid, is transaminated to aspertate, of course getting that nitrogen from glutamate to alpha-ketoglutarate.

And glutamate, of course, can get its nitrogen either by transamination with another amino acid to an alpha-keto acid, converting alpha-ketoglutarate to glutamate, or glutamate can be generated by this redox reaction where it picks up on ammonia via the enzyme glutamate dehydrogenase.

So, you'll notice in this cycle that ultimately the arginine, the urea group-- what is released as urea from arginine, comes from the carbamoyl phosphate. So that's one of these nitrogens that was picked up in the synthesis of carbamoyl phosphate. And the other one comes from aspertate, which, of course, can come from ammonia via glutamate dehydrogenase and transamination, or from transamination from nitrogen picked up from any other amino acid to ultimately end up on aspertate.

You'll notice that there's redox balance across this whole cycle. And so there's a NAD that's required to reconvert the malate to oxaloacetate to run this half of the reaction. But of course, if we pick up an ammonia, we also have a redox reaction involved in the glutamate dehydrogenase reaction.

You'll see that ATP is also-- quite a bit of ATP is needed. You need 2 ATP to generate each carbamoyl phosphate, as well as 2 ATP equivalents to generate argininosuccinate, because remember, if you go back to your notes from last time, you'll see that ATP to AMP plus pyrophosphate was part of the citrulline and aspertate generating argininosuccinate reaction. And the last thing is that CO2 is required to run the urea cycle. That's where the carbon in urea comes from. And that CO2 is, of course, picked up in the generation of carbamoyl phosphate.

Now, I think sometimes we can fall into the misconception when we talk about these that all cells generate urea. That's not true. So remember, different organisms use different strategies to excrete nitrogen. So in us as humans, the liver and the kidney would run the recycle as I have drawn it here as a way to ultimately produce urea that gets excreted in the blood-- I mean, sorry, in the urine, whereas most tissues in our body would simply ship off excess nitrogen from the catabolism of amino acids to the liver and the kidney by generating either glutamate or glutamine in the reactions that we talked about last time, and then those organs would deal with-- use the urea cycle to produce urea and get rid of excess nitrogen.

But of course, there's other animals that will use other strategies secreted as ammonia directly or produce the purine uric acid, which, of course, we'll talk about next time, how one can interconnect all this amino acid nitrogen metabolism with nucleic acid nitrogen metabolism. I just want to stress that this glutamate dehydrogenase reaction is a really key reaction that is necessary to get nitrogen in and out of the system. And once you get nitrogen in and out of the system, you can then use these transamination reactions to move nitrogens around between amino acids and alpha-keto acids using pyridoxal phosphate as we discussed last time.

This is shown up above also as the bicycle reaction. And again, just stresses that you sort of are running the urea cycle on the right, this other TCA-- modified TCA cycle, if you will, on the left, and ultimately illustrates how you pick up those nitrogens and CO2 to generate urea.

But, of course, if we're going to talk about how you would make amino acids, which is really the topic of today, how you break down and synthesize the carbon skeletons of different amino acids, well, obviously here's arginine metabolism. You can imagine that you could use the same reactions to produce or break down arginine, and that is what happens. But I also want to talk about the remaining amino acids. In the remaining amino acids, it's really about how do we deal with the carbon skeleton, the alpha-keto acids. And of course, these are linked via this pyridoxal phosphate-driven transamination reaction.

Now, I don't have time to in this course to go through how each amino acid carbon skeleton is produced and broken down. We'll discuss some major themes. However, if I don't cover your favorite amino acid, because of course, all people should have a favorite amino acid, you can look up the pathways to make it or break it down, and I promise that if you paid attention through the whole class, you have the skills to understand that particular pathway even if we don't study it in class.

So I want to remind you that in general for amino acid catabolism-- and this is in general. Of course, there are exceptions. Arginine would behave as I just described for the urea cycle. But for most amino acids, you start with transamination. So what does that give you? That gives the nitrogen to alpha-ketoglutarate to generate glutamate. And so that glutamate can then be shipped off to generate ammonia if you're a fish, generate urea if you're a human, et cetera.

And what does that leave you with? That leaves you with a alpha-keto acid related to the amino acid that can then be oxidized to CO2 using various pathways-- burn it and get energy, just like we've described for carbohydrates and fatty acids, or, of course, in some cases produce glucose or some other molecule that can then be used to support gluconeogenesis in the liver, keep glucose high in our blood. And so that's generally how most amino acids are stabilized.

It's very clear how this works. If we start with some of the more obvious ones-- so we talked last time about how alanine, the amino acid, is related-- so transamination, get the alpha-keto acid. That alpha-keto acid is pyruvate, aspertate. Amino acid undergoes transamination. The alpha-keto acid is oxaloacetate. Glutamate undergoes transamination. alpha-keto acid is alpha-ketoglutarate. It's very clear how all of these can end up in the TCA cycle or gluconeogenesis for The cell to either oxidize for energy or to produce something like glucose that can be used elsewhere.

Now of course, there's other amino acids out there that we've already talked about. So remember asparagine, very similar to aspertate. Glutamine similar to glutamate. So those were the ammonia group on the side chain. We can simply remove those. Should be clear how to then get those into the TCA cycle or gluconeogenesis.

A little bit of metabolic trivia. Humans, we actually don't have an enzyme to break down asparagine. Other organisms do. And then the other one that we alluded to last time was proline, which is cyclized and oxidized glutamate. And so I'll just illustrate here quickly how proline can be metabolized just so you can see this.

So here's the amino acid proline. And so proline, to show you, if we do oxidation of this carbon-nitrogen bond, so that utilizes these an electron acceptor. That electron acceptor happens to be FAD being reduced to FADH2. That generates this molecule. We can then add water across that double bond.

Now we can open up the ring like this. So just opened up the ring. Now I'm going to oxidize this aldehyde to the acid. So if I do that, something else has to be reduced. That something else can be NAD+ or NADP+ depending on the enzyme. There's enzymes that use either NAD or NADP to make NADPH or NADH respectively. And then what I'm left with is this amino acid glutamate.

And so that's how I turn proline into glutamate. Two additional oxidation steps generating FADH2 and NAD or NADPH. And so that's how you would derive energy from the catabolism of proline, by feeding it into glutamate, which can then, of course, be transaminated to alpha-ketoglutarate, and the rest of the carbon enter the TCA cycle.

OK. So that covers 1, 2, 3, 4, 5, 6 amino acids. 7 if you count arginine that we talked about with the urea cycle. That leaves 13 additional amino acids. Many of them are first transaminated to the alpha-keto acid, and then ultimately use-- the carbon skeleton can be used to generate another intermediate that's either involved in the TCA cycle or glycolysis.

And shown here on the slide is basically just a figure from a textbook that contains all of the various-- all of the 20 amino acids from protein and what they generate either in the TCA cycle or up here. So you can see here [INAUDIBLE] oxaloacetate, fumarate succinyl-CoA, alpha-ketoglutarate, acetyl-CoA pyruvate, acetyl acetate. Which, of course, as we saw last time, can be used to generate 2 acetyl-CoA or be used to generate the ketone body beta-hydroxy butyrate.

Now, obviously if you can generate any of these things, we already know the pathways about how you can oxidize them, or, in some cases, how you can turn them into glucose. But now I want to return to this idea that we've come to a bunch of different times that really comes down to this fact of what we talked about with the TCA cycle, how the TCA cycle can't net do anaplerosis from two carbon units. That is, you can't turn acetyl-CoA or acetoacetate, because that's just two acetylcholine molecules, into anything that can generate glucose. that We don't have the glyoxylate cycle. Organisms that have the glyoxylate cycle can do this, but we as humans cannot do that.

Now, many of our amino acids can be used to produce things like fumarate, succinyl-CoA, oxaloacetate. All of that stuff can easily be turned into glucose. And so if we starve ourselves or go on a low-carbohydrate diet, well, what happens is, our liver will break down amino acids and use those amino acids to make glucose, but we can only make glucose from amino acids if those amino acids are broken down into something that's not acetyl-CoA or acetoacetate because we don't have a glyoxylate cycle.

Just remember I mentioned a couple lectures ago when we talked about the ketogenic diet, a true ketogenic diet isn't just low carbohydrates, it's also low protein because your liver will turn that protein back into glucose. And so if you really want to be ketotic, you have to limit both glucose and-- both sugars and proteins, because fat, of course, will only be broken into two carbon units, and you can't turn those into glucose, so you instead turn them into ketones.

Now, not all amino acid skeletons, though, can be used to make something that can generate glucose. Some can only make stuff-- acetyl-CoA, acetoacetate-- that can be used to generate ketones. And so this defines another way to classify amino acids, is so-called glucogenic amino acids or ketogenic amino acids. And this is a direct consequence of what those amino acid carbon skeletons are broken into.

And so a glucogenic amino acid is really any amino acid where the product of breakdown is greater than 2 carbons that is not acetyl-CoA. Whereas ketogenic amino acid is an amino acid where the product of breakdown is acetyl-CoA. And I should mention, or acetoacetate, because acetoacetate, as we saw, is 2 acetyl-CoAs.

So acetyl-CoA, acetoacetate can be turned into the ketone body beta-hydroxybutyrate, an alternative fuel for the brain. If you break down an amino acid and all you get is acetyl-CoA or acetoacetate, you can't turn that carbon skeleton back into glucose; therefore, it's a ketogenic amino acid. All other amino acids that generate oxaloacetate, succinyl-CoA, whatever, something greater than 2 carbons, not acetyl-CoA, it can then be turned into glucose, therefore, it's a glucogenic amino acids.

Now if we come back over here to our slide and you look at the various amino acids, most of the amino acids are turned into something that can be turned into glucose. And so most amino acids are so-called glucogenic. However, there's a few amino acids that are ketogenic, and there's some amino acids that are both glucogenic and ketogenic.

And so if you look here through the slide, what you'll find is that tyrosine and isoleucine-- so there's isoleucine, here's tyrosine. So they're turned into ketones, but they can also be-- here's tyrosine, here's isoleucine-- can also be turned into something that can be used to make glucose, and so they're referred to as both glucogenic and ketogenic.

Now, as I said before, we don't have time to go through how to break down all of the amino acids, but I will take you through how you break down the three branched chain amino acids. And I will do that because, first of all, it will illustrate that you already know a lot about metabolism. You'll see that how these are broken down is just variations on pathways that we've already discussed. And so it really drives home the fact that the complexity of metabolism is really just nature repurposing relatively few reactions over and over and over again in a way that ultimately builds complexity and diversity, but in the end, is actually quite consistent across the whole thing.

And you'll also see examples of how amino acid skeleton breakdown can end up either as glucogenic or ketogenic, and that's because of the various branched chain amino acids. So there's, of course, leucine, valine, and isoleucine. So those are the three branched chain amino acids. You'll see the leucine is ketogenic, valine is glucogenic, and isoleucine, as I just said, is both ketogenic and glucogenic.

So three very amino acids. In genetic terms, very conservative substitutions within a protein. Very related structures. However, the way they're broken down, in the case of leucine, generates only things that are ketogenic; valine generates things that are glucogenic; and isoleucine generates both. And so we'll go through each of these and see how that happens.

So let's start with leucine. So leucine is a-- I said above is a ketogenic amino acid. And so leucine, like many amino acids, starts break down by transamination. So this transamination will, of course, take the nitrogen from leucine, an alpha-keto acid, alpha-ketoglutarate, generate glutamate, as well as the alpha-keto acid of leucine, which, just to remind you leucine looks like, looks like this.

So this is the side chain over here of leucine. Here's the alpha-keto acid related to leucine. If I take this alpha-keto group, turn it into an amino group, that's leucine. So this is the alpha-keto acid related to leucine. So the next step of leucine breakdown involves oxidative decarboxylation of this alpha-keto acid.

So if you remember, we've seen this reaction before. We did it in pyruvate to generate acetaldehyde, an ethanol metabolism. We did it in-- well, we did alpha decarboxylation there, and then we did oxidative alpha decarboxylation when we turn pyruvate into acetyl-CoA. And we turned alpha-ketoglutarate into succinyl-CoA.

Well, that exact reaction, which we saw with pyruvate decarboxylation, as well as what we saw with alpha-ketoglutarate dehydrogenase generating acetyl-CoA and succinyl-CoA is what happens here for the alpha-keto acids produced from breakdown of leucine and other branched chain amino acids.

And so if you look back in your notes, we went through the mechanism of this in detail. Remember, this involved edition of CoA, loss of CO2, it's an oxidation reaction. So remember, the electrons needed to go somewhere, and so they ended up going to NADH. So that's oxidized. NAD+ is reduced to NADH.

And remember, this involved that mini-electron transport chain with FAD, lipoic acid, and TPP+. And in the end, that releases the CO2 and you end up with this branched chain acyl-CoA. So same reaction is PDH, alpha-ketoglutarate, dehydrogenase. Just like those two reactions, remember, that had an E1, an E2, and an E3 subunit. So this actually uses the exact same E2 and E3 subunit as those reactions. The E1 subunit is, of course, different because it's unique to the branched chain amino acids, whereas in pyruvate dehydrogenase, to be unique to pyruvate and alpha-ketoglutarate dehydrogenase, you need the alpha-ketoglutarate.

In this case, this enzyme is called BCKDH for Branched Chain Keto Acid Dehydrogenase, and allows the oxidative decarboxylation to generate this branched chain acyl-CoA. Now this branched chain acyl-CoA looks a lot like a fatty acid, and in fact, it's metabolized very much like we would do fatty acid oxidation.

So what's the first step in beta oxidation of fatty acids? Well, we would introduce a double bond here. And so that's exactly what happens next. So this would be a membrane-bound FAD containing enzyme, part of the electron transport chain. Going to oxidize that carbon-carbon bond. There's that generate. All right.

Now, if we are doing fatty acid oxidation, we would, of course, add water across the bond. We'll do that in a minute. Before that happens, the next step, though, is that to facilitate ultimately break down of this, we're going to end up adding our carboxyl group to the end of the molecule. So if we're going to add CO2 to the end of the molecule, how would we do that? Well, we've seen that reaction many times before as well.

So if we're going to add a CO2 group, that's going to come from biotin. So the next enzyme would have biotin in the active site that can pick up a CO2. How does it pick up the CO2? Well, it picks up the CO2 via bicarbonate plus ATP, and that's going to go to-- that's going to phosphorylate the bicarbonate.

And then when we transfer that phosphorylated bicarbonate onto biotin, we'll release the PI, then the CO2 from biotin can be added to carboxylate. That molecule, just like we've saw before with other carboxylation reactions, and that will generate this intermediate. This intermediate here. OK. So no new chemistry. You've seen all this many times before.

Now we're going to continue with-- what we would do is if we were breaking this down-- oxidizing it as a fatty acid. And so now we're going to add water across that double bond. OK. Now we added water across that double bond. And then now we're just going to do this reaction, which will generate from this side of the molecule an acetyl-CoA.

And if you look on the other side of the molecule, what we are left with is acetoacetate. Acetoacetate can be broken down into two acetyl-CoAs. If I break it in half, add CoA to each one of them by reactions I showed you before as well, or, of course, we can reduce this ketone to the alcohol and make the ketone body beta-hydroxy butyrate. You can look that up from the end of the last lecture.

But in the end, this is how one can break down the leucine skeleton. You get a acetyl-CoA or acetoacetate. Can't generate glucose from any of those molecules as humans because we don't have a glyoxylate cycle. Therefore, leucine is a ketogenic amino acid. All right.

Valine. Let's look at valine. So valine is glucogenic despite the fact that it's very similar to leucine and broken down in a way that's very similar to leucine. So, how do we break it down? So first step, transamination. Nitrogen from valine to alpha-ketoglutarate to generate glutamate, and as well as the branched chain alpha-keto acid that's related to valine. So that's the alpha-keto acid related to valine. One carbon shorter than side chain, one carbon shorter than leucine.

Just like we did with leucine, we're going to metabolize the alpha-keto acid with the branched chain keto acid dehydrogenase. Same reaction. Oxidative decarboxylation here. That releases CO2, adds CoA. Reaction involves also NAD+ to NADH. Requires lipoic acid, TPP+, FAD, just like we described before. I'm not going to draw out all the pieces of it again, but we're going to end up with the branched chain acyl-CoA.

Metabolize this branched chain acyl-CoA as if it's a fatty acid. And so first step, oxidize this carbon-carbon bond. That electrons go to FAD, make FADH2. This product, add water across that double bond. Water across that double bond.

Next, if we oxidize this alcohol twice-- so oxidize it once, we'll get the aldehyde. Oxidize it again, we can get the carboxylic acid. And so oxidizing twice means NAD+ times 2 and NADH times 2. And now that becomes the acid. OK. So same molecule, but now this alcohol becomes an acid.

I drew it that way on purpose. If you look back in your notes, this is methyl methylmalonyl-CoA, which we saw before in our discussion of odd chain fatty acid metabolism. And you'll remember that there was this B12-dependent reaction where I can basically swap the positions of this proton and this thioester group here. This is not aero-pushing. This B12-dependent reaction that I described before where I can rearrange methylmalonyl-CoA to make this molecule, which is succinyl-CoA.

Succinyl-CoA oxidized in the TCA cycle, generate ATP, but also can use it as a substrate anaplerosis for the TCA cycle, so it can be used to generate glucose. And so that's why valine is a glucogenic amino acid. All right. Last branched chain amino acid is isoleucine. So isoleucine is both ketogenic and glucogenic. So let's see how that happens.

So if we start with isoleucine, first we undergo transformation. alpha-ketoglutarate to glutamate, and you get the branched chain alpha-keto acid. So that's isoleucine as an alpha-keto acid rather than an amino acid. Next step, do the alpha decarboxylation with branched keto acid dehydrogenase. That removes that CO2, oxidizes that carbon to the acid, and we end up with this branched chain acyl-CoA. Same first steps as we saw before.

Metabolize that as if it's a fatty acid. So oxidize that carbon-carbon bond. Add water across the double bond. OK. Get this intermediate. Now oxidize this alcohol to the ketone. Electrons go to an NAD+, which is reduced to NADH.

Now we can use CoA to take off this end of the molecule here, which is acetyl-CoA, hence ketogenic. The remainder of the molecule is this three-carbon acyl-CoA. We saw this from odd chain fatty acid metabolism. This is propionyl-CoA.

Remember, to deal with that, we can add CO2. So that, of course, requires biotin, and ATP. So remember, we had CO2 to this molecule. That gives us methyl-- not going to draw it all out for the sake of time, but that gives us methylmalonyl-CoA. Methylmalonyl-CoA shown up there, so it's adding CO2 to this molecule. And then we can do the vitamin B12 rearrangement to get succinyl-CoA. And so that can be turned into glucose. And so it generates an acetyl-CoA and a succinyl-CoA, isoleucine is both glucogenic and ketogenic.

And so a little tedious to go through how you break down all three of those branched chain amino acids, but it really illustrates that it's just variations on the same chemistry that we've seen before with fatty acid metabolism, TCA cycle reactions, that ultimately generate intermediates that can be fed into the TCA cycle, oxidized further to CO2, generate ATP, generate NADH, electron transport chain, oxidative phosphorylation, supply the cell with energy to do various kinds of work.

Or can be turned into a ketone if you're a human and you start with acetyl-- and what you get is acetyl-CoA or acetoacetate. Or even into glucose if you start with something like succinyl-CoA that can be turned back into glucose by the liver, defining the difference between the glucogenic and the ketogenic amino acids.

All right. Now going through branched chain amino acid metabolism also allows transition into a discussion of another topic which are the so-called inborn errors of metabolism. So what's an inborn error of metabolism? Well, sometimes individuals are born that have deficiencies in enzymes that are necessary to carry out some function of metabolism. Some aspect of the biochemistry we've been learning about.

Now you can imagine that some of these genetic deficiencies, if it's somewhere smack in the middle of central carbon metabolism and you can't generate energy, it's not going to be compatible with life. But it turns out that some of them are quite compatible with life, like deficiency in branched chain keto acid dehydrogenase.

So BCKDH deficiency is something that children are sometimes born with, and this deficiency results in a disorder called maple syrup urine disease. So it's called maple syrup urine disease because the unit smells like maple syrup, but effectively what this is is this is a disorder where there's a deficiency in the activity of branched chain keto acid dehydrogenase.

That means the cells can't break down branched chain amino acids. They end up generating some otherwise side toxic product. Some of it ends up in the urine, smells like maple syrup. But it also can result in damage to the CNS and problems with thinking, mental retardation, et cetera. And so it can be quite devastating to have loss of this enzyme, which prevents you from breaking down branched chain amino acids, a so-called inborn error of metabolism.

Now this is a relatively rare disease. All of these are. But if you go to medical school, these are questions because they lend themselves to questions that often show up on various board-type exams. And so it's good to know that deficiencies of some of these enzymes can result in diseases. So maple syrup urine disease caused by an inability to break down branched chain amino acids because you're deficient in the enzyme branched chain amino acid-- or branched keto acid dehydrogenase, which catalyzes the stuff I showed you up there in the breakdown of all three branched chain amino acids.

By no means is this the only one. Often these are associated with amino acid breakdown. And so two of the other more common-- all of these are very rare, but relatively more common ones is a disease called alkaptonuria. So what's alkaptonuria? Well, that's missing an enzyme-- so enzyme deficiency in tryptophan metabolism.

And another one is fennel phenylketonuria, which is an enzyme deficiency in phenylalanine metabolism. Branched chain amino acids are very common, but tryptophan, phenylalanine, two of the more rare amino acids, it turns out these are two of the amino acid breakdown deficiencies that have less severe phenotypes, and so they're somewhat-- more people walking around with those conditions.

If any of you have had children or someday when you have children, what will happen is that you will notice that when babies are born, one of the first things that's done with infants is they have a blood test sent out. They do a little heel stick, collect some blood, and send it to the state lab where they test for various inborn errors of metabolism.

What they're looking for is things like phenylketonuria, maple syrup urine disease, et cetera. Why do they want to find these things? Well, it's because if you have too much of the product, too much phenylalanine, too much tryptophan, too much branched chain amino acid, and you can't break them down, it's not the lack of the ability to break them down that's necessarily the problem. The problem is is that the inability to break them down leads to overwhelming a system building up of toxic intermediates that damage the brain.

And so by doing these newborn screening panels, it's greatly enhanced the management of these things, because often you can control the symptoms by dietary intervention. Simply limiting the phenylalanine or the branched chain amino acid or the tryptophan intake in the diet is a treatment for these diseases, and so therefore, if you know about them early, limiting exposure to those things limits the exposure to the toxic breakdown products that can't be metabolized because of the deficiency, and therefore, limits the effects on the brain.

If you drink diet soda and you look at the side of your diet soda can, what's written on the side of it is phenylketonurics, contains phenylalanine. Why is that written there? Well, because if you happen to be a phenylketonuric, you shouldn't drink diet soda. Why? Because the artificial sweetener in there contains phenylalanine, and so this would give you a big dose of phenylalanine, and if you can't break down phenylalanine, that's a problem. So it's a warning to the people in the population who happen to have phenylketonuria not to drink diet soda.

All right. Now, I want to say a few other things about amino acid metabolism, only because these things will come up for you as you go through other aspects of biology. And that is that amino acids are very versatile starting points to build all kinds of hormones and neurotransmitters and bioactive molecules.

So I know some people-- some of the MIT students in the class are course 9. That's the brain and cognitive science. Classes you take, you'll learn a lot about different neurotransmitters. Well, one of the canonical neurotransmitters is an amino acid itself. It's the amino acid glutamate. So obviously that's important for the brain to work.

But what are the other neurotransmitters? Well, there's things like dopamine, epinephrine, norepinephrine. These are all bioactive molecules, have to come from somewhere. Well, it turns out all of these ultimately come from the metabolism of tyrosine, which, of course, can also come from the metabolism of phenylalanine. And so don't have time to go into it, but you can look up the pathways to generate these various neurotransmitters from tyrosine if you're interested.

What's the other big neurotransmitter? Well, it's acetylcholine. What's acetylcholine? Well, let's break it down. Well, it's an acetyl group, so that comes from acetyl-CoA. And choline-- and what's choline? Well, choline comes from, ultimately as I showed you-- alluded to before, ethanolamine, and ethanolamine comes from serine. We'll come back to this later.

And so serine metabolism, as well acetyl-CoA, is how you get acetylcholine. It's also choline the phospholipids and ethanolamine for the phospholipids. If you're not into the brain but into other aspects pathophysiology-- so thyroid hormone, very common thing that many people need replacement of, very important to manage physiology in our body. Thyroid hormone also comes from thyroxine.

Melanin, the pigment that makes our skin different shades, melanin, the skin pigment, it is basically produced from dopamine-- or actually, a dopamine metabolite. So dopamine precursor. Which comes from tyrosine as well. And so if you're interested in any of these particular molecules, of course, you can look up in the book and you'll see how they're made, and I promise, you have the ability to understand it, it's repurposing some reactions that we've already seen. There's some unique ones in there like melanin synthesis is actually parts of it are non-enzymatic, even.

But this-- I like to mention this because obviously we don't have time to study and discuss the entire complexity of the metabolic map, but really, you understand the basics, the central parts of it, and it's the same reactions repurposed out in different directions that allows this complexity and this diversity of metabolism, making all of these different biomolecules that are necessary for the cells to do things, and, of course, you can go look these up if you're interested in any of them specifically. And regardless, you should have some appreciation of how these things all interconnect with the other pathways that we've talked about.

All right. All right. So now I want to turn to amino acid synthesis and say a few words about how amino acids are built. So of course, we've covered some of these already, and as a very general statement, many of them are made by transamination. So first you build the alpha-keto acid, and then you transaminate that alpha-keto acid from glutamate to generate the amino acid.

Now of course, the pathways that you use to make amino acids are not the same as the pathways that you use to break down amino acids. So again, if you paid attention for the entire course, it should be clear for both thermodynamic energetic reasons that you can't have the same pathways to build and break stuff down because all pathways delta G has to be less than 0.

So we saw this with glucose and fatty acids. We can repurpose-- some steps can be the same, but the ones that allow the energetic driving have to be different because you have to couple one direction to energy consumption and the other one could be spontaneous if it releases energy, and you have to have pathways that can work in either direction.

But, in general, if you can build an alpha-keto acid like oxaloacetic acid or alpha-ketoglutarate or pyruvate, well, obviously we can transaminate all of these things to get the amino acid. So alpha-ketoglutarate to glutamate can also be from glutamate dehydrogenase. That's how you can get new nitrogen into the system.

And that way we can generate aspartame glutamate and alanine, and aspertate into asparagine, asparagine by adding the ammonia to it. That would be a very similar reaction to glutamate into glutamine. That's glutamate synthase, asparagine synthase. Same mechanism describe glutamate synthase last time to make glutamine. And of course, proline, as I showed you earlier, is cyclized, and in this case, oxidized-- or sorry, cyclized then reduced glutamate.

And then-- so that's six of the amino acids. We covered arginine earlier, that seven. And so 13 other amino acids. Here's another figure from a book just showing the starting material to make all of the various amino acids. Obviously we know how to make oxaloacetate, phosphoenolpyruvate, erythrose 4-phosphate, pyruvate, ribose 5-phosphate, 3-phosphoglycerate, and alpha-ketoglutarate. And so generation of those by the pathways we've already talked about then allows those molecules to be shunted into pathways to generate all of the various amino acids.

Now I want to reiterate, humans, we can't make all 20 amino acids. Animals can't do that. Many organisms, of course, can. We lost the ability to make nine of our amino acids, and in fact, if you count the two conditional ones, really 11 of amino acids, even though we can turn phenylalanine into tyrosine, we still need a source of phenylalanine to do so.

And so each of these nine or 11, depending on how you count it, essential amino acids must be eaten in the diet. So there are obviously pathways to do them, we just don't have the enzymes in humans to do it. And then the other ones we do retain. You can obviously look up the pathway that's used by whatever organism to make your favorite amino acid. Today what I want to discuss is how we will make-- today I want to discuss how we will make serine and glycine, because it turns out, serine and s metabolism play a key role in other pathways, including nucleotide synthesis which we will cover in the next lecture.

Serine glycine metabolism also is a way to make one carbon units that are more reduced than CO2. And these will be critical for one-carbon transfer reactions that you'll encounter throughout other aspects of biology. So, serine and glycine, very important amino acids. And so just to be clear about this, so it turns out serine-glycine metabolism, important for nucleotides among carbon units, we'll see that a lot.

But serine and glycine also used to make phospholipid head groups. Nucleotides, one-carbon units we're going to talk about. I want you to appreciate, you also need serine to make phospholipid head groups. So what do I mean by that? Well, of course, there's phosphatidylserine. Obviously that's serine is the head group, and so serine-- the amino acid is required for that. But also used to generate ethanolamine.

So what is ethanolamine? Well again, just to remind you of this, so here is the amino acid serine. So, to generate ethanolamine, it's decarbonization of serine to do that. So loss of this CO2. This is a reaction I don't have time to go into, you can look it up. It is another repurposing of pyridoxal phosphate. And that generates this molecule, which is ethanolamine. That ends up head group on phosphatidylethanolamine.

And if I want to turn it into choline, either for acetylcholine as a neurotransmitter or for phosphatidylcholine as a head group, it's basically adding 3 methyl groups onto this nitrogen of ethanolamine. And so those three methyl groups, that's a carbon that's more reduced than CO2. That's a so-called one carbon unit. It turns out, as you'll see, those also come from serine.

Last thing is serine is necessary to make head groups for a class of lipids called sphingolipids. There's a-- sphingosine is another amino alcohol that is generated from serine and ends up being the head group for sphingolipids. Especially if you're interested in the immune system of the brain, these end up being important molecules.

And so you can look up how they're related to serine, but just appreciate that for now that they-- another important downstream thing that's generated from serine metabolism. Also illustrating how amino acids can be very important to generate lots of other biomolecules. OK. So, how do we make serine?

Well, if we go over here, you'll see that serine is downstream of 3-phosphoglycerate from glycolysis. So what's 3-phosphoglycerate? OK, here's 3-phosphoglycerate from glycolysis, or the product of rubisco if you like. So photosynthesis. So there's 3-phosphoglycerate.

So if I oxidize this alcohol to ketone, electrons have to go somewhere. NAD+ reduced to NADH, I get this intermediate. It's phosphohydroxypyruvate So pyruvate with a phospho on it, hydroxypyruvate. This molecule can undergo a transamination reaction. So pick up nitrogen from glutamate, make the alpha-keto acid. Take this alpha-keto acid and generate phosphoserine. The amino acid that's phosphorylated, phosphoserine. And then all I need to do is dephosphorylate it, and I end up with the amino acid serine. Cool.

All right. To make glycine from serine-- so remember, glycine is one basically the amino acid with no side chain on it. So I need to remove this carbon and the alcohol. So I'll just redraw serine this way. So that's redrawing serine. Need to lose that group to get glycine. This is helped along by pyridoxal phosphate. So a reminder, this is pyridoxal phosphate.

OK. So this is exactly what we drew, is the first steps in transamination. Except if instead of basically going like this, I do this to break this carbon-carbon bond, what do I get? Well, I release a one-carbon unit. In this case, it's the one-carbon unit as formaldehyde. The one-carbon aldehyde, formaldehyde. And generate this intermediate.

And it's just exactly what we showed before for resolving the transamination. And I am left with glycine, as well as I regenerated pyridoxal phosphate for the next catalytic cycle of the enzyme. And so illustrates the flexibility of pyridoxal phosphate. In this case, helping convert serine into glycine and a one-carbon unit shown here as formaldehyde.

Now the enzyme that carries out this reaction is an enzyme called SHMT, which stands for serine hydroxymethyltransferase. Serine hydroxymethyltransferase. And it actually doesn't release the formaldehyde directly. Instead, it transfers that formaldehyde to an important one-carbon donor for many one-carbon reactions that is called folate species or folic acid.

So remember, if we transferred CO2 in a carboxylation reaction, we use a cofactor biotin. Biotin's useful to transfer CO2. But if we're going to do something like transfer the methyl groups shown up there for choline synthesis, that's transferring carbon that's more reduced than CO2, and you don't use biotin for that. Instead, you use a cofactor called folate that's derived from the vitamin folic acid.

So folic acid, very important vitamin. It's the primary thing that's included in prenatal vitamins. Why is that? Because deficiency in folic acid was shown a while back to cause neural tube defects-- so developmental defects where the neural tube does not close. This leads to things like spina bifida.

One of the real successes of public health intervention was the recognition of this and fortifying various cereals with folic acid. And doing so has greatly reduced the incidence of these birth defects involving folate deficiency. Folate deficiency's also a common cause of anemia. And so folic acid is a vitamin that lots of people take for that reason. And in fact, folic acid, also very important in the history of cancer treatment. And if you want to learn more about that, take 745, which is offered in the fall.

All right. So, folic acid is a complex molecule that is converted in cells to a molecule called THF, which stands for tetrahydrofolate. So I'll draw out folic acid for you and tetrahydrofolate. OK.

So, this here is folic acid. The folic acid molecule is tetrahydrofolate. It has a peptide bond here to a glutamate molecule, and it's actually a polyglutamate tail that is there to trap it in cells. It's this central part of the molecule here that actually carries the one-carbon unit.

And so the way that the nitrogens are numbered, this is nitrogen-5. This is nitrogen-10. That will be important in a minute. But it's really this sort of central part here of the molecule that I will draw over and over again as the one-carbon unit-carrying species. To show how this works, it's easier if I start off by showing how it can pick up a more oxidized one-carbon unit. So the one-carbon carboxylic acid is this, that's formate.

And so if I phosphorylate formic acid, this formic acid can then be picked up by the-- so here's that carrying part of the one-carbon-- the box part of tetrahydrofolate. And so this can pick up formic acid like this.

OK. So that gives us this molecule, which is called N10-formyltetrahydrofolate, THF. I can create a ring out of this molecule. This. OK. And if I remove water from this ring, we draw it like this. OK. This is called N5,N10-methenyl THF.

And if I reduce that molecule, it's NADPH. So hydride ion. OK. Then I end up with this molecule, which is N5,N10-methylene THF. And if I further reduce this molecule, then I end up with this molecule, which is N5-methyl THF.

Appears incredibly complex what's going on here, but I went through this, because what I want you to realize is that each of these species are simply tetrahydrofolate carrying a one-carbon unit in a different oxidation state. So this N10 formyl THF is carrying formic acid, the one-carbon acid. I can reduce it to N5,N10-methylene THF. That's carrying the one-carbon aldehyde, formaldehyde. Or I could reduce it further. This is carrying the one-carbon methyl group as N5-methyl THF.

And it turns out that this allows a lot of flexibility in carrying out one-carbon reactions. Here is a same thing written from a textbook. The direction-- the order is shown a little bit differently, the molecules are rotated. And it includes a couple other species that are added in for histodine metabolism.

But if you look at this, you'll see it's exactly what I just drawn where you get these key species. Where N5-methyl THF is effectively carrying a methyl group. So a one-carbon methyl donor. N5,N10-methylene THF is carrying the formaldehyde. And N10-formyl THF is carrying the formate group to donate as a one-carbon unit.

This will be the donor, ultimately, for things like choline synthesis. Formaldehyde-- and N5,N10-methylene and formyl THF will be important in nucleic acid synthesis and we'll see that next time. And so now if we go back to our SHMT reaction, I can show you how these things really work.

And so here's a THF molecule. And so here is the intermediate in serine-glycine conversion found to pyridoxal phosphate, et cetera. And that will release glycine and eventually PLP. So glycine and PLP can be generated from that. And what ends up here on the folate is-- which is N5,N10-methylene THF.

And if I take this and I oxidize it, I can take the aldehyde to the acid. So oxidation. NAD+ is reduced to NADH. Now I get an N10-formyl THF. And if instead I reduce it, so electrons from NADPH go to NADP+. So NADPH gets oxidized, electrons go there. And then I get an N5-methyl THF. This gives me a donor of methyl groups. This gives me a donor of formyl groups.

All right. Very last thing I want to talk about today is we'll talk a lot about how we use the more oxidized species next time to generate nucleotides, but I want to say a few words about how these methyl groups are used to be involved in methylation reactions, things like choline synthesis shown up there, but also not just choline synthesis. Also can be used for reactions involved in, say, epigenetics.

And so for those of you who will never think about biochemistry again after taking this class but we'll take-- think about genetics, you'll certainly know the gene expression is controlled by methylation of DNA and histones and things like that. Those methyl groups have to come from somewhere, and they come ultimately from these methylation reactions, the serine there.

Now I don't have time to go through all of one-carbon metabolism. This is very complex. But it turns out the one-carbon donor for most methylation reactions isn't N5-methyl THF, even though that's where the methyl group comes from. It's actually this molecule.

So this here is the amino acid methionine. And if I put it here onto an adenine, what I end up with is this molecule called S-adenosylmethionine or SAM. This methyl group on SAM is very labile. And so it is the universal methyl donor to be used in methylation reactions, choline synthesis, epigenetic methylation of DNA or histones, whatever.

And if you transfer that methyl group off, you're left with this non-proteinogenic amino acid that's called homocysteine. And that homocysteine is here onto an adenine to make S-adenosylhomocysteine, which is abbreviated as SAH.

SAH picks up-- needs to regenerate SAM. Methyl group has to come from somewhere. This comes from N5-methyl THF. Of course, generates THF. And then THF can pick up another methyl group, ultimately from serine-to-glycine conversion and reduction with NADH to make N5-methyl THF.

I show here on the slide, here's just a picture of one-carbon metabolism that I took from a review article. And so here's SAM metabolism. Lots and lots of complicated reactions that are linked to cystine metabolism and polyamine metabolism. Obviously don't have time to get into this, but I want you to appreciate that this happens, where SAM and SAH come from, how they're involved in one-carbon metabolism, because this will come up again in your future endeavors if you do something with biology.

All right. Final topic next time is nucleic acid metabolism, and then we will have covered all of the basics of metabolism. Thanks.