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MATTHEW VANDER HEIDEN: So today we're going to begin by going over the Calvin cycle, which I know I went quite quickly on just to introduce it at the end of the last lecture. And so we'll spend a little time there first. And then we'll move on to discuss the main topic of today, which is the pentose phosphate pathway. But before we get there, I just want to remind you about the Calvin cycle, which you'll recall happened in three phases.

So there was the fixation, reduction, and regeneration phases. And so remember, fixation was this special reaction that occurs only in photosynthetic organisms catalyzed by rubisco ribulose-1,5-bisphosphate carboxylase/oxygenase, where a ribulose-1,5-bisphosphate molecule combines with CO2, and then is split to produce 2,3-phosphoglycerates. This was the reaction that Calvin described as the dark reaction, that now bears the name of this as the Calvin cycle.

Once you get those 2,3-phosphoglycerates, you can then enter them into gluconeogenesis. This, of course, happens in the stroma of the chloroplast in eukaryotic photosynthetic organisms, and it's exactly the reactions for the reduction phase that we learned about for glycolysis or gluconeogenesis when we described it at the beginning of the course. And so that 3PG is turned into glyceraldehyde 3-phosphates and dihydroxyacetone phosphate.

Of course, that has to go backwards through the phosphoglycerate kinase as well as the glyceraldehyde 3-phosphate dehydrogenase step that we learned about when we discussed glycolysis and gluconeogenesis. And so therefore, our cost ATP, as well as need a source of electrons to go through glyceraldehyde 3-phosphate dehydrogenase, and that those come from NADPH when this is occurring in photosynthetic organisms and the chloroplast. Once you get these trioses, of course, you can continue with gluconeogenesis, combine them at the aldolase step to make fructose 1,6-bisphosphate.

That can be-- take the phosphate off by FPPase to give you fructose 6-phosphate. That can then be isomerized to glucose 6-phosphate, which, of course, could be further-- remove the phosphate and go to glucose, or be turned into glucose 1-phosphate or whatever else to build starch or another storage sugar for the plant-- combine glucose and a fructose, say, to make sucrose, table sugar. And so dose reduction steps we didn't spend a lot of time on, because it's really gluconeogenesis with the use of NADPH rather than NADH, as we described it when we describe gluconeogenesis back at the early part of the course.

Now, once you run this part of the cycle that, of course, gives you glucose. But of course, in order to net run this as a cycle, much like we saw for the TCA cycle, that if this is going to run as a cycle, you need a source of carbon acceptors for the next CO2. Anaplerosis, that we described in the TCA cycle-- remember, we needed a source of oxaloacetate that could net accept the next acytal-CoA to continue running the cycle. Same thing happens here. You need a source of ribulose-1,5-bisphosphate so rubisco has something to add CO2 to.

And so anaplerosis of this cycle involves regeneration of ribulose-1,5-bisphosphate. And that's the confusing set of reactions that I introduced last time that we'll spend much more time talking about today. And so, effectively, this comes from both 6 and 3 carbon units-- so glyceraldehyde 3-phosphate and fructose 6-phosphate with a series of 2- and 3-carbon swaps that ultimately generate these three pentoses, xylulose 5-phosphate, ribose 5-phosphate, and another xylulose 5-phosphate. Once you have those xylulose 5-phosphate, you can see differs from ribulose-1,5-bisphosphate by the stereochemistry at the three position. And so an epimerase reaction can fix that.

We talked about those earlier. And then, of course, you need to phosphorylation the 1 position on the sugar to give you ribulose-1,5-bisphosphate. That's how you deal with the 2 xylulose 5-phosphates. And ribose 5-phosphate, of course, you need to change it from the aldose to the ketose. So that's an isomerase reaction plus a phosphorylation to give you ribulose-1,5-bisphosphate.

Now, if you run this entire cycle, if you're going to net generate a hexose-- a glucose or some other hexose-- for the plant to store as a storage sugar-- of course, if it's coming from CO_2, you need to run the cycle six times to take six CO_2s and net generate one hexose that can be stored for later. And so I just want to start by showing you that this accounting can work. So if we start here at the top with six ribulose-1,5-bisphosphates and add 6 CO_2 molecules, you're going to net generate 12 3-phosphoglycerates. Those 12 3-phosphoglycerates can be turned into, of course, 12 glyceraldehyde 3-phosphate.

So that's going to cost 12 ATPs. And 12 is from the light reactions and photosynthesis. And so now you have 12 glyceraldehyde 3-phosphates. I'm going to divide them up in the following way.

If I take two of those and place them over here, then take the remaining 10, use five of those here and turn five more into dihydroxyacetone phosphate-- so that gives me 5 plus 5 plus 2 equals 12 total. Of these five glyceraldehyde 3-phosphates and five dihydroxyacetone phosphates, I can run them through the aldolase step to generate five fructose 1,6-bisphosphates, turn those five fructose 1,6-bisphosphates into five fructose 6-phosphates. And, of course, I need one of those to ultimately generate a glucose 6-phosphate or whatever hexose that I'm going to have as my net product.

If I do that, that leaves me four left over. And so I'm going to allocate them such that I have two here and two here. So the two I have here can be combined with the remaining two glyceraldehyde 3-phosphates to generate two xylulose 5-phosphates and two erythrose 4-phosphates, a 5-carbon sugar, and a 4-carbon sugar. This 5-carbon sugar-- this xylulose 5-phosphate-- I can then use a epimerase and ATP. So that's two ATPs running back through. And that's going to net give me two ribulose-1,5-bisphosphates, which I'll draw up there later.

Coming back down here, I have this two erythrose 4-phosphates and two fructose 6-phosphates. So this was a two carbon swap. So I took the two carbons for this step to generate the xylulose 5-phosphate and erythrose 4-phosphate. I took two carbons from fructose 6-phosphate added them to glyceraldehyde 3-phosphate. That's how I got the 5-carbon xylulose 5-phosphate. And that left me with a 4-carbon erythrose 4-phosphate. So all the carbons add up.

Now I take this 4-carbon erythrose 4-phosphate and the two remaining fructose 6-phosphates. And if I move three carbons from here to there, what I end up getting is a 7-carbon sedoheptulose 7-phosphate and a 3-carbon glyceraldehyde 3-phosphate. And so two in, two out-- now I'm going to move two carbons again-- to carbons from sedoheptulose 7-phosphate to the glyceraldehyde 3-phosphate.

And that's going to give me 2. 7 minus 2 is 5. 3 plus 2 is 5. So two 5-carbon sugars, two ribose 5-phosphates, and two xylulose 5-phosphates-- xylulose 5-phosphates through the epimerase, costing me two ATP. Ribose 5-phosphate through isomerase, costing me two ATP. And when I net generate is two ribulose 5-phosphates here, two ribulose 5-phosphates there, two ribulose 5-phosphates there. 2 plus 2 plus 2 equals 6. And so by running this cycle six times, six ribulose-1,5-bisphosphate in, six CO_2 in, six ribulose-1,5-bisphosphate out, and one hexose out. So all the accounting adds up.

I know there's a lot of moving pieces there. It's confusing. But we'll see as we go through today why it works this way. However, before we get there, I just want to stress what we've actually accomplished here.

So basically, we started with six CO_2 molecules. And we added to that 12 NADPHs. And so those 12 NADPHs came there through the 3-phosphoglycerate to generate the glyceraldehyde 3-phosphate-- so running through the GAPDH reaction using NADPH. And so we had to do that 12 times plus a total of 18 ATP molecules. And so 12 of them up there through the phosphoglycerate kinase cost me 12 ATPs, plus I needed an additional six ATPs to regenerate the ribulose-1,5-bisphosphates re-phosphorylation the six pentoses that I generated through the regeneration phase of the Calvin cycle.

And so that's 12 plus 2 plus 2 plus 2, or 12 plus 6 equals 18. And what I net generate is one glucose molecule from my six CO_2. And, of course, to do this, I needed a lot of energy input. That comes from the 12 NADPHs and the 18 ATPs, which, of course, are the products of the light reactions of photosynthesis.

And so you'll recall that if we go back to our chloroplast, the light reactions are happening here at the thylakoid membranes of the chloroplast. And that's where we're generating our ATP and our NADPH from the light reactions. And then here in the stroma of the chloroplast is where we're doing the dark reactions, and so perfectly positioned where the ATP and from the light reactions are there to fuel the dark reactions, the Calvin cycle, and net have this turn CO_2 and generate a pentose-- glucose, fructose, combine them to make a sucrose, or a bunch of glucose that we can build as alpha-1,4 polymers to make starch, depending on the organism and how it chooses to store its sugars.

So that is the Calvin cycle. But what I want to do now is look a bit more closely on why it works this way-- that is, why nature chose this confusing system with two and three carbon swaps. Wouldn't it just have been easier to just, I don't know, take CO_2s and build a glucose molecule together? Well of course, the reason it does this has to do with the chemistry of how the reactions happen.

And if we look more closely at the various steps and these swaps that take place in the regeneration phase, basically what we see is in every single situation-- so it's basically this reaction, this reaction, and this reaction, which are the swap part of the regeneration phase-- you see that in every case, we're moving two carbons. And so here two carbons were removed from fructose 6-phosphate to glyceraldehyde 3-phosphate. So 6 minus 2 equals 4. 3 plus 2 equals 5.

That's how we get erythrose 4-phosphate and xylulose 5-phosphate. Here we started with our fructose 6-phosphate and our erythrose 4-- phosphate, moved three carbons from here to there. So 6 minus 3 equals 3. 4 plus 3 equals 7. So that's a three carbon swap. And then here, again, is another two carbon swap-- so three carbon glyceraldehyde 3-phosphate, 7-carbon sedoheptulose 7-phosphate.

Move two carbons from here to there. 7 minus 2 is 5. 3 plus 2 is 5. And that's how we get those two pentoses. Now, if we look more closely at this, what you'll see in every case, the swap involves a ketose and an aldose.

So here we have fructose 6-phosphate. It's a ketose. Here we have glyceraldehyde 3-phosphate. It's an aldose. And what we end up with is the aldose getting longer by two carbons to become a ketose and the ketose getting shorter by two carbons to become an aldose.

Same thing happens here-- here we have a ketose and an aldose. We do the swap. We have the aldose getting longer by 3 carbons to become a ketose, and the ketose becoming shorter three carbons, in this case, to become an aldose. And lastly, same swap we showed before-- here we have a ketose and an aldose. The ketose gets shorter by two carbons to become an aldose. And the aldose gets longer by two carbons to become a ketose.

So I recognize that it's very hard to follow that when I'm saying aldose and ketose over and over and over again. But effectively in every single case, what we're doing is we're moving two carbons or three carbons and we're moving them from a ketose to an aldose. And so that means that our product, when we do that, is we end up getting a longer ketose.

So our former aldose plus 2 or plus 3 carbons becomes a longer ketose. And then we end up getting a shorter aldose from the former ketose minus two or minus three carbons. So if you go and look at that, you will see in every single case where we're doing those two and three carbon swaps, we're effectively following that rule.

So these reactions, which I've abbreviated up there, are catalyzed by enzymes called TK, which stands for transketolase or TA, which stands for transaldolase-- TK, transketolase, TA, transaldolase. I want to point out that these are the enzymes that catalyze the transketolase is going to catalyze the two carbon swaps. And the transaldolase is going to catalyze the three carbon swaps.

And so twice we used transketolase, once we used transaldolase. Transketolase moved the two carbons from the ketose to the aldose, giving you a longer ketose and a shorter aldose. And transaldolase also catalyzed, in this case, the 3-carbon swap, giving you a longer ketose and a shorter aldose following the exact same rules. Now, these enzymes is why it works this way.

And I want to point out that the enzyme names are absolutely not helpful for remembering what they do. Both enzymes use a ketose donor and an aldose acceptor. Both generate products where the former aldose becomes longer as a ketose and the former ketose becomes shorter as an aldose. So don't rely on the enzyme names to remember what they do.

You'll see partially why they're named what they are as we go through the mechanisms. But for now, just remember that transketolase, two carbons, transaldolase, three carbons-- different numbers of carbons moved, but always follow the exact same rules. I know this is confusing and so it's probably better to go through exactly what is going on using a couple examples.

So let's start off here with a ketose. So here is a ketose. This is, of course, fructose 6-phosphate. And so fructose 6-phosphate is a ketose.

If we use this step here as an example-- fructose 6-phosphate and glyceraldehyde 3-phosphate-- so here we start with our ketose. And if we also use with it a aldose-- so here is glyceraldehyde 3-phosphate, which is, of course, an aldose. So if we subject this to a transketolase reaction, which, remember, moving two carbons, we're going to take the two carbons from the ketose and move them to the aldose. So if we do that, what we end up with is-- if we take two carbons from here, we end up with a shorter aldose. And we end up with a longer ketose.

So transketolase reaction-- ketose donor of two carbons, give them to an aldose, acceptor of two carbons. That gives me an aldose that's shorter-- minus 2 carbons. So the bottom four carbons from fructose 6-phosphate now become a erythrose 4-phosphate, an aldose.

And here we have our aldose accepts the two carbons. And so now we generate a ketose that is longer by two carbons. And that gives me this longer ketose-- so in this case, xylulose 5-phosphate. So this is the exact transketolase reaction that is right there-- this first part, fructose 6-phosphate glyceraldehyde 3-phosphate give me an erythrose 4-phosphate and a xylulose 5-phosphate. So two carbons move from a ketose to an aldose, gives me a shorter aldose and a longer ketose.

Now, if we do exactly the same thing, but this time rather than do a transketolase reaction, do a transaldolase reaction-- so now I'm going to move three carbons. So same thing-- ketose donor, aldose acceptor. So now I'm going to end up with-- if I donate three carbons from that ketose, what I end up with is 3 carbons remaining.

And if I start with this 3-carbon as an acceptor, move three carbons over, now I end up with a longer ketose. So instead do a transaldolase reaction-- take three carbons from the ketose, transfer them to the aldose. That means the ketose now gets shorter by three carbons and becomes an aldose. And the former aldose gets longer by three carbons and becomes a ketose.

And so if I use these two things as substrate-- fructose 6-phosphate and those are glyceraldehyde 3-phosphate, what do I generate? I generate another glyceraldehyde 3-phosphate and fructose 6-phosphate, where all I do is scramble which carbons are aware. And so this, obviously, is not a reaction I showed for how you do anaplerosis of the Calvin cycle, but clearly can happen.

These transketolase and transaldolase enzymes are highly reversible. And so this is, of course, is happening-- scrambling carbons around, but there's no net generation or consumption of anything, because, of course, we're just moving carbons between a ketose and an aldose, generating the same ketose and the same aldose using these particular substrates. But if we look at how it worked where we showed it here-- fructose 6-phosphate erythrose 4-phosphate-- we end up moving those three carbons onto erythrose 4-phosphate. That gives us sedoheptulose 7-phosphate and leaves us with a shorter aldose glyceraldehyde 3-phosphate.

And so I color-coded the carbons here to show how transketolase and transaldolase work. And if you go back over here through the Calvin cycle, you will note that every single one of the reactions where I drew transketolase and transaldolase follows those exact rules. So why does it work this way? Well, it works this way because of how transketolase and transaldolase work as enzymes.

And so as I said at the beginning of the course and have now alluded to several times, is that biochemistry is just nature repurposing the same chemistries, the same reactions, with slight variations over and over and over again to build that complexity. And I think that is very evident here.

So a lot of complexity in what we described, but it's really just using two enzymes-- transketolase and transaldolase. And transketolase and transaldolase basically use variations on chemistry that we've already seen to catalyze these reactions, which is almost certainly why it evolved this way, because you already have a reaction that catalyzes something. You tweak it. And now you can get these swaps and nature figures out a way to have it work.

And so what are those reactions? So transketolase, which, remember, catalyzes the 2-carbon movement. That's two carbons that are moving. It uses TPP+. So that's, remember, thiamine pyrophosphate. We saw it before.

It was involved in those decarbonization reactions of alpha-keto acids. We saw it in the pyruvate dehydrogenase reaction to turn pyruvate into acetyl-CoA. We saw it in ethanol metabolism to turn pyruvate into acetaldehyde. And we saw it in the TCA cycle to turn alpha-ketogluterate into succinate.

And in all of those cases, we decarboxylated-- that is broke a carbon-carbon bond of an alpha-ketoacid. And that used TPP+. And basically what happens in the transketolase reaction is TPP+ plus is also used to break a carbon-carbon bond. But this time, rather than releasing CO2, it's basically moving the 2-carbon unit over to another molecule, as I'll show in a minute.

Transaldolase, which, remember, moves three carbons, does not use TPP+. Instead, it uses an aldolase-like mechanism to move the three carbons. And so aldolase, you remember, if you think way back to when we described glycolysis, aldolase broke the carbon-carbon bond to take you from fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. And so that was done with an active site lysine. And transaldolase basically repurposed that reaction-- also has an active site lysine, breaks a carbon-carbon bond, but this time to transfer the 3-carbon unit from ketose donor to an aldose acceptor.

And when we go through the mechanism, you'll see it will be very reminiscent of, if you look back in your notes, how aldolase worked in glycolysis. So let's start with the mechanism of transketolase. So transit delays uses thiamine pyrophosphate. So remember this here is the active part of thiamine pyrophosphate, the stabilized carbanion.

So let's here just draw a generic ketose donor. So this intermediate is very similar to what we saw for the-- when we did decarboxilation of the alpha-ketoacid. Basically, this same mechanism would occur. But rather than release CO2, which gets released here instead is this shorter aldose with two carbons that are lost and remain here on the active site of the enzyme on thiamine pyrophosphate.

Now, this molecule here then needs to be transferred to a-- so here we have our aldose acceptor.

So that then will regenerate TPP+. And, of course, what we're left with is our former aldose, which now becomes a ketose that's two carbons longer. And so ketose donor, TPP+ breaks off the two carbons, releases that ketose two carbons shorter as an aldose. Those two carbons are then transferred to an aldose acceptor. And it becomes two carbons longer and becomes a ketose.

And so the net result is is that we take two carbons from the ketose donor give them to the aldose acceptor. The ketose gets shorter by two carbons to become a shorter aldose. And the former aldose acceptor gets longer by two carbons to become a ketose. And this is, basically, repurposing very similar chemistry to what we saw for the decarbonization of alpha-ketoacids.

So that's how transketolase works. This is how transaldolase works. And so this works to move, of course, three carbons. And so this is going to work much more like what we saw for the aldolase reaction in glycolysis.

So let's here use this here as our generic ketose donor. So this is going to be a donor of three carbons-- so active site lysine and transaldolase place.

So basically, this should look very familiar to you as the mechanism for how aldolase worked to break the carbon-carbon bond to divide fructose 1,6-bisphosphonate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, except, in this case, what we're left with is taking this ketose donor. And it releases an aldose that's three carbons shorter while retaining, down to the enzyme, the three carbons from the ketose donor, just like we saw for the aldolase reaction. Now, when we saw the aldolase reaction-- glycolysis, of course-- we just took this off the molecule. And that's how we ended up with glyceraldehyde 3-phosphate from the bottom and dihydroxyacetone on the top. The difference here is is now we're going to use this as a way to combine it, move this 3-carbon unit rather than just releasing it, transferred over to an aldose acceptor.

And then, once we re-take the 3-carbon unit and add it to the aldose acceptor, you'll see that then you release this, now, aldose three carbons longer as a ketose-- again, very similar mechanism, if you look back on your notes, to how aldolase worked in glycolysis. And so the net effective the transaldolase reaction is that we take three carbons from a ketose donor. We end up releasing an aldose that's three carbons shorter, take those three carbons, transfer them to an aldose acceptor, and now generate a ketose that is three carbons longer.

And so this ultimately moves to carbon units in the case of transketolase, three carbon units in the case of transaldolase. It evolved this way because it's repurposing existing chemistry to allow these two and three carbon swaps. That ultimately gives you the flexibility to build a cycle like the Calvin cycle, where one can interconvert 3-carbon sugars and 6-carbon sugars with 5-carbon sugars and ultimately make that cycle work. And so on the Calvin cycle this is useful because we can start with the 5-carbon ribulose-1,5-bisphosphate and net generate a hexose as a storage sugar, while regenerating our 5-carbon ribulose-1,5-bisphosphate.

But it has an additional benefit in that this is also a way to generate 5-carbon sugars. And so you can see that ribose 5-phosphate is one of the intermediates in the carbon cycle. And, of course, ribose is a useful sugar to generate nucleoside. So all the time you spent talking about RNA and DNA with Professor Yaffe, you saw lots of 5-carbon sugars, lots of pentoses, as the ribose in those backbones.

And so it's not just-- you know now, not just nucleosides. It's also part of lots of cofactors-- NAD, FAD, coenzyme A. All of those cofactors ended up having nucleosides in them-- and so lots of ribose needed by the cell in order to make nucleosides. And this is a way to, for at least photosynthetic organisms, the Calvin cycle, a way for them to make ribose to generate all those nucleosides.

Now, that's great if you're a photosynthetic organism, because you can run the Calvin cycle and do it in a way that you can store carbon for the night as some hexose that you can burn later when you don't have light to carry out the light reactions of photosynthesis. Or, if you want to grow, you can use it to make nucleosides and replicate your genome, or make RNA, or make ribosomes. And in fact, the Calvin cycle generating ribose is a way to get those. You'll see when we get to this in a few lectures that ribose 5-phosphate is the starting point to generate both purine and pyrimidine nucleotides.

However, this reactions, the Calvin cycle, is unique to photosynthetic organisms. But of course animals, including us-- are all nonphotosynthetic organisms-- also need to make ribose. And we also need a way to make NADPH. And we need a source of NADPH for the same reason plants needed it. And that is we need something to have an NADPH/NADP ratio that's useful to keep our glutathione reduced. That allows us to keep our cells in a reducing state, battle reactive oxygen species, help with reductive biosynthesis.

We all know that if we eat too much food, we can all store those excess calories as fat. Fat is reduced carbon. Making their reduced carbon in the fatty acids is going to require a source of electrons. All organisms, that source of electrons is going to be NADPH, which makes sense, because an NADPH/NADP ratio that's more reduced can favor reduction reactions. And in our next lecture, we will see how that's used to actually store carbon as fat when there's excess NADPH around.

Now, that makes a lot of sense. But obviously, as nonphotosynthetic organisms, we, as well as all non photosynthetic organisms, can't rely on photosynthesis in the Calvin cycle. And so we need another pathway that allows us to do both NADPH production as well as ribose production. And that's where we get to the pentose phosphate pathway.

And so the pentose phosphate pathway, the PPP-- also sometimes referred to as the pentose phosphate shunt-- is a major pathway that all organisms, photosynthetic and nonphotosynthetic, can use to generate NADPH, which is useful for cells as a source of reducing power, as well as ribose, which is going to be necessary if we're going to build nucleosides and cofactors that contain nucleosides sites. Now, how one runs the pentose phosphate pathway to get these two products is conceptually akin to the Calvin cycle. But it's a Calvin cycle in reverse.

And so if we look at what really happens in the Calvin cycle, we start with a 5-carbon sugar, we add CO_2, we reduce it with NADPH and ATP, and we end up generating a 6-carbon sugar. And so the pentose phosphate pathway is we're going to start with a 6-carbon sugar. We're going to oxidize to generate CO_2 and a 5-carbon sugar.

And if we oxidize, we're going to end up-- those electrons have to go somewhere. And so we can put those electrons and net generate NADPH. The Calvin cycle is, of course, doing reduction. Remember, reduced carbon is energy storage. Oxidizing carbon is energy release.

And so the Calvin cycle, we had to put energy in. That energy, of course, came from photosynthesis. That's the magic of us harvesting energy from the sun in order to do biology. And so the light products, the NADPH came from photosynthesis in order to net run the Calvin cycle. But just like we saw with glycolysis and gluconeogensis, if one direction of a pathway requires energy input, well the other direction is going to be favorable.

And so if we oxidize a 6-carbon sugar to make a 5-carbon sugar and CO_2, that intuitively, hopefully by now, should be favorable. And so we can use that energy release to charge up an NADPH to NDP ratio that now gives the cell a source of reducing power-- so energetically makes sense. Now, this pentose phosphate pathway is also sometimes called a shunt, because really the way this works is as a branch of glycolysis-- a shunt of glycolysis, if you will, because it's an alternative way to run glycolysis and get NADPH.

And so the glucose 6-phosphate-- remember, for glycolysis we can isomerize to give us fructose 6-phosphate. And then ultimately in glycolysis, we can generate glyceraldehyde 3-phosphate down to pyruvate, which can go to TCA cycle, et cetera, et cetera, et cetera. Of course, glucose is a 6-carbon sugar. Fructose is a 6-carbon sugar. glyceraldehyde 3-phosphate is a 3-carbon sugar-- aldose, ketose, aldose.

So if we take our glucose 6-phosphate and we send it into the pentose phosphate pathway, the pentose phosphate shunt, and rather than oxidize the glucose as we did in glycolysis, we oxidize it to release CO2 and generate a 5-carbon sugar, like a ribose 5-phosphate. Now we have a way to get 5-carbon sugars pentoses by oxidizing glucose 6-phosphate. Those electrons have to go somewhere. And so that ends up being a way to generate NADPH.

Once we get those 5-carbon sugars, that's great if we want to build nucleotides. But it turns out we could also run a bunch of transketolase and transaldolase reactions and interconvert 5-carbon sugars with 6-carbon sugars and 3-carbon sugars, just as we did in the Calvin cycle. And if we run it this way, you can see that I can now make this effectively an alternative version of glycolysis-- a shunt, if you will. Start with glucose 6-phosphate rather than going the traditional way. First, go to make 5-carbon sugars and then bring those carbons back into glycolysis that I can then further oxidize to pyruvate and oxidize in the TCA cycle, et cetera.

And so all of this-- so glycolysis happens and the cytosol, the pentose phosphate pathway happens in the cytosol. And really, this can be thought of as two separate phases. And so this glucose 6-phosphate going to a pentose, like ribose 5-phosphate, plus CO2, that is referred to as the oxidative pentose phosphate pathway-- the oxidative pentose phosphate pathway, because it's using oxidation of glucose carbon to generate ribose 5-phosphate and NADPH. Or, this swaps here between 5-carbon sugars and 6-carbon sugars and 3-carbon sugars-- very similar to what we saw in the Calvin cycle, which is really interconverting 6-carbon and 3-carbon sugars with 5-carbon sugars. So that is referred to as the nonoxidative pentose phosphate pathway.

And it makes sense. All those transketolase and transaldolase reactions that I just discussed with you in great detail-- no electron movement, no oxidation reduction, nonoxidative PPP. Similar to the Calvin cycle, it's converting pentoses with trioses and hexoses. So this should be very clear now that this is actually a really useful things for cells, because having this pentose phosphate pathway work in these two different ways, the oxidative than the nonoxidative, gives cells a lot of flexibility to make pentoses when they need ribose to make nucleosides, as well as NADPH when they need NADPH to deal with, say, oxidative stress. Suppose you take a drug, causes oxidative stress in your cells, pentose phosphate pathway can increase as a way to make more NADPH.

So if a cell needs NADPH because there's oxidative stress, it can run the oxidative pentose phosphate pathway if it also needs to make ribose great it has a source of pentoses to make ribose. However, if it just needs NADPH and not ribose, it and then take those ribose carbons, re-enter them into glycolysis. So running this shunt allows you to get NADPH as well as ATP while oxidizing glucose carbon.

However, if you need ribose and you don't need NADPH, well, forget the oxidative PPP, just take sugars from glycolysis and shunt them over via the nonoxidative PPP to make ribose. And so you can run this direction if you need NADPH. And you can run this direction if you just need ribose. So this is great because it gives cells a lot of flexibility. And so these two pathways, really, can work together. Or, they can work separately depending on what the cell needs, and can really be tuned in a way to allow the cell to get both ribose and NADPH and match it as demands.

And so in a lot of ways, it's probably better to think of them as two separate pathways-- a so-called oxidative PPP, which is really glucose 6-phosphate plus 2 NADP+, generating 2 NADPH plus pentose plus CO2. And a nonoxidative PPP, which is really interconverting 3 pentoses with 2 hexoses and 1 triose.

So 3 times 5 is 15 carbons. 2 times 6 is 12 carbons plus 3 carbons, 15 carbons. This reaction, oxidative PPP, is only going to be favorable in one direction.

You would need a separate pathway if you're going to run it the opposite direction. That doesn't happen in organisms. So that's, under physiological conditions, a one-way pathway. You can only go one direction through the oxidative PPP in cells, whereas a nonoxidative PPP is just this interconversion of trioses and hexoses with pentoses. This is very reversible, just like the Calvin cycle would be very reversible.

And so while they can work together as I described, it's really useful to think of them as separate pathways. And so let's go through each of them separately so you can see how they work. So let's start first here with the oxidative pentose phosphate pathway.

So this here, it would be alpha-glucose 6-phosphate.

So first step of the oxidative pentose phosphate pathway is we're going to oxidize the 1-carbon of glucose. That's, of course, going to have two electrons. So if we oxidize that carbon, something else has to be reduced. That hydride ion, those two electrons can go to NADP+ to make NADPH-- a reaction that we've now seen a million times. That oxidizes the alcohol, that carbon 1 to the ketone. And that gives us this lactone-- very similar to what we saw as the first step in the epimerase reaction, when we described that, to give us this molecule, 6-phosphogluconolactone.

Now, so, in essence, what this did, remember, is it oxidized-- remember, this 1-carbon was the aldehyde of glucose, this now becomes, on this lactose, it's actually an acid. And that becomes evident if we break open this ring here with water. That causes this molecule, 6-phosphogluconate.

Oh, I forgot to mention this first reaction here is catalyzed by this enzyme, G6PD-- glucose 6-phosphate dehydrogenase. Makes sense-- the oxidation reduction reaction, dehydrogenase, glucose 6-phosphate dehydrogenase. Just as an aside for those of you who are interested in medical school, turns out glucose 6-phosphate deficiency in humans is actually a quite common genetic disorder-- very common in Mediterranean regions because it is thought to have evolved because it confers some resistance to malaria. And so having less G6PD means you deal less well with oxidative stress. And as a result, some foods and drugs can cause increased symptoms in those patients, but also having that means that the cells are more resistant to malaria infection because the malaria ends up killing the cell before it can establish an infection because it causes some oxidative stress.

Back to the pathway-- so if we open up this lactone, what we have here is this molecule, 6-phosphor gluconate. It's basically glucose where the aldehyde has now been oxidized to the acid. That's how we generated NADPH. The next step is we're going to oxidize carbon 3.

So if we oxidize the alcohol on carbon 3, those electrons have to go somewhere. Put them on the net plus. That gives us an edge. This is catalyzed by 6 phophogluconate dehydrogenase-- generates an NADPH. And what ends up being generated is this intermediate.

If you look at this intermediate, this is a beta-keto acid. So here's our acid that is alpha beta to the ketone, so a beta-keto acid. Remember, decarboxylation of beta-keto acids is favorable-- same track that we saw before and the TCA cycle and other places. And so remember that generates this enol. And why it's so favorable is because the enol prefers to rearrange to the keto form.

And when we rearrange the keto form, this, of course, is the pentose ribulose 5-phosphate. And so ribose 5-phosphate, of course, we saw in the Calvin cycle. So we started with phosphorylation at the one position, ribulose-1,5-bisphosphate. So ribulose 5-phosphate can be produced by the oxidative pentose phosphate pathway glucose 6-phosphate. First oxidize the 1-carbon by G6PD. That generates 6--phosphogluconate. Oxidized the 3-carbon. That allows favorable decarboxylation to generate another NADPH and ribulose 5-phosphate.

Once you add that ribulose 5-phosphate, we can, of course, use an isomerase. And with an isomerase, we can generate ribose 5-phosphate, which is now available to be sent to do a nucleic acid synthesis. Or, we can use an epimerase. And if we use an epimerase, we can change the stereochemistry at this three position. And that gives me xylulose 5-phosphate, which was the ketose donor and acceptor for the transketolase, transaldolase reactions that were so useful for entering the nonoxidative pentose phosphate pathway or basically some version of the Calvin cycle.

And so we can get ribose 5-phosphate, xylulose 5-phosphate, ribulose 5-phosphate all as products of the oxidative pentose phosphate pathway, which will allow us to either generate ribose as a way to make nucleosides or have carbon in the right form that I can now run the oxidative pentose phosphate pathway and have it re-enter glycolysis. Great. So that's the oxidative pentose phosphate pathway.

Now, let's discuss the nonoxidative pentose phosphate pathway, which just like the Calvin cycle, is interconversion of 3-carbon and 6-carbon sugars with 5-carbon sugars. Basically it's the Calvin cycle, same idea-- transketolase, transaldolase. Obviously, it evolved from the Calvin cycle, because photosynthesis had to be first. So let me first put it in context, how it works. And then we can draw it in more detail.

So this here is glycolysis. If I run the oxidative pentose phosphate pathway, I can generate two NADPHs as well as a ribulose 5-phosphate. That ribulose 5-phosphate can be turned into ribose 5-phosphates and xylulose 5-phosphates-- aldose and ketose acceptors.

I can then use these 5-carbon sugars. If I do a transketolase reaction, I'll get a 3-carbon aldose and a 7-carbon ketose-- remember transketolase moves two carbons from the ketose to an aldose, giving you a shorter aldose and a longer ketose. Now, if I run a transaldolase reaction, I'll move three carbons. That gives me a 6-carbon ketose and a 4-carbon aldose.

If I run another transketolase reaction with this 4-carbon sugar with this 5-carbon sugar, I will then generate another 6-carbon ketose and a three carbon aldose. These six carbon are fructose phosphate and this 3-carbon aldose is glyceraldehyde 3-phosphate. And I can re-enter glycolysis.

The stoichiometry works up if I do this three times. So 3 times 5 equals 15 carbons. So three ribulose 5-phosphates from the oxidative PPP. If I turn them into one ribose 5-phosphate and two xylulose 5-phosphate, I can allocate one here and one here. These two can come together to give me one fructose 6-phosphate and one erythrose 4-phosphate. That erythrose 4-phosphate can use the other xylulose 5-phosphate to give me another fructose 6-phosphate and the glyceraldehyde 3-phosphate. So [INAUDIBLE] I have two hexoses and one triose coming from my three pentoses-- and so total here, again, of 15 carbons.

And so like the Calvin cycle, these transketolase and transaldolase reactions are all reversible. And so if I don't want to run the oxidative pentose phosphate pathway, I can start with fructose 6-phosphates and glyceraldehyde 3-phosphates from glycolysis and run it this direction to generate riboses in order to make nucleosides. Just to be explicit about this, hopefully this all makes sense after the long discussion we had of the Calvin cycle and how these reactions work. And you could work this out yourself.

But I'll close today by just showing this and how it works. I'll actually show it in the opposite direction. I described it here moving from products of the oxidative PPP running through the nonoxidative PPP to re-enter glycolysis. But I'm going to now draw it for you, starting with products of glycolysis, running the nonoxidative PPP in the other direction to generate pentoses just because this is so reversible, but also point out to you how this whole thing can work.

So here is a glyceraldehyde 3-phosphate from glycolysis. That's a aldose acceptor. Here's a fructose 6-phosphate from glycolysis. That can be a keto [INAUDIBLE]. And so if I run a transketolase reaction-- so remember, transketolase-- transfer two carbons from the ketose to the aldose.

What am I left with? Well, I'm left with a shorter aldose. And this is a erythrose 4-phosphate. And I'm left with a longer ketose.

There is a pentose xylulose 5-phosphate. Now I have my erythrose 4-phosphate and my fructose 6-phosphate. So if I now carry out a transaldolase reaction-- so I'm going to move three carbons now for my ketose donor, fructose 6-phosphate, to my aldose acceptor. And so what do I end up with?

So I end up with a-- this becomes 3 carbons shorter, so glyceraldehyde 3-phosphate. Move the three carbons from the ketose to the aldose acceptor. Now I end up with a longer ketose.

Now I have this 7-carbon sedoheptulose 7-phosphate. If I now, again, run a transketolase reaction-- so two carbons here transferred from the ketose to the aldose, what I end up with now is a 5-carbon xylulose 5-phosphate. And so aldose becomes a longer ketose by two carbons.

And our ketose becomes a shorter aldose by two carbons, which is a ribose 5-phosphate. I can now have two xylulose 5-phosphates and a ribose 5-phosphate. I can take my xylulose 5-phosphates and carry out an epimerase reaction. If I carry out an epimerase reaction, I, of course, get-- change the stereochemistry at the three position to get a ribulose 5-phosphate.

Or, I can carry out an isomerase reaction to take my ribose 5-phosphate to ribulose 5-phosphate. And so if you note that I started this time with products of glycolysis, glyceraldehyde 3-phosphate, fructose 6-phosphate, did a series of transketolase and transaldolase reactions, ultimately to generate two fructose 6-phosphates, one glyceraldehyde 3-phosphate-- so that's 6 plus 6 plus 3 equals 15 carbons. I get three pentoses out, a xylulose 5-phosphate, a ribose 5-phosphate, and a xylulose 5-phosphate, which I can then use epimerase or isomerase reactions to give me ribulose 5-phosphate, which, of course, all could be shuttled into ribose 5-phosphate, giving me the flexibility to start with products of glycolysis and net generate ribose 5-phosphate for nucleoside synthesis. If I run the oxidative pentose phosphate pathway, I end up with ribulose 5-phosphates.

I can take those ribulose 5-phosphates, turn them into xylulose 5-phosphates and ribose 5-phosphates, run the same series of transketolase and transaldolase reactions in reverse and get glyceraldehyde 3-phosphates and fructose 6-phosphates. Either direction, 15 carbons in, 15 carbons out, can start with products of the oxidative PPP and put them back in glycolysis or start with products of glycolysis and generate pentoses, such as ribose 5-phosphate for nucleoside synthesis, giving cells a lot of flexibility to take carbohydrates from glycolysis and make pentoses for nucleosides, as well as have a way to generate NADPH. I know there's a lot of carbons moving around and a lot of swapping, but hopefully after today how it works and why it works this way is a little bit clearer than if you just sit down and try to memorize these really confusing pathways.

Next time we'll talk about how one uses NADPH as a source of reducing power in all organisms in order to synthesize fatty acids. Thanks.