JOHN ESSIGMANN: Hi, I'm John Essigman. I'm one of the instructors in 5.07. I teach with JoAnne Stubbe and Bogdan Fedeles, you've probably seen in earlier videos of this type. Today we're going to talk about the pentose phosphate pathway. Professionally, I'm a toxicologist. The pentose phosphate pathway is one that is very central to the people who work in my field, specifically because the pentose phosphate pathway provides us with one of the reagents that we need in order to be able to combat or counter the effects of oxidative stress. Oxidative stress is involved in many pathologies.

One of the reasons that the pentose phosphate pathway is challenging to teach is because it's a pathway that interacts very, very closely with other pathways-- for example, glycolysis, the energy-generating pathway in gluconeogenesis, a pathway by which, in some organs, you're able to synthesize glucose from non-carbohydrate precursors. The fact that these tend to be so closely related makes it difficult to see the distinct features of the pentose phosphate pathway. So let me show you the pentose phosphate pathway and how it interacts with these other pathways.

First of all, let me start with the roles of the pentose phosphate pathway. As I see it, there are three. The first is this is the cell's principle source of an NADPH, which is our reductive cofactor. It's used for the synthesis of such things as lipids and other carbohydrates, and so on. So this is how the reducing equivalent, the source of electrons that we use in order to be able to synthesize complex molecules.

The second major role of the pentose phosphate pathway is that it's our biosynthetic source of ribose. We have to make ribose for things like ATP. We have to make it for the nucleotides that are in our nucleic acids. This is where it comes from. What we're also going to see is that when we eat foods that contain ribose-- for example, ATP and nucleotides-- this is how they enter the mainstream of metabolism so that we can utilize them as raw material or energy material.

And the third thing about the pathway is that some of the intermediates you'll see in what we

call this carbon scrambling phase are critical for the synthesis of certain biomolecules. This is where the aromatic residues come from in our aromatic amino acids, such as tyrosine.

So the pentose phosphate pathway is very important because it provides us with our reducing equivalents. It provides us with a biosynthetic source of ribose for nucleotides. And it provides us with building blocks for more complicated molecules.

Because this pathway is associated with biosynthesis, in terms of its providing reducing equivalents, what we'll find is that the pathway is very highly expressed in tissues that make a lot of lipid. Lipid is our bioenergetically most expensive biosynthetic process. Requires a lot of NADPH. If the cell is dividing, what it's going to do is make membranes. And that's a lot of lipid biosynthesis.

This pathway, the pentose phosphate pathway, not surprisingly, is upregulated when we have to make a lot of membranes-- for example, in a cell that's growing. And that means, for example, that this could be a target for anti-cancer drugs. In other words, if you wanted to find a pathway to block in order to stop the provision of the resources, the reductive resources for growth, this would be one that would be looked at-- in fact, is being looked at by the modern pharmaceutical industry.

Over here toward the center of the top panel is what's often considered to be a little bit confusing, but it's really actually quite straightforward, which is the fact that this pathway can run in two modes. I said earlier that the pentose phosphate pathway is an offshoot of other pathways, which makes it a little bit difficult for some people to understand. So in this line right across here that I'm just moving across, that's going from glucose to pyruvate as we go left to right. And that's, of course, glycolysis. If we went from pyruvate to glucose-- that is, going from right to left-- that's gluconeogenesis.

So gluconeogenesis, for example, might be a situation in a diabetic. For example, I'm a diabetic, and when I go to sleep at night, I'm not eating, and what my liver is doing is it's saying, OK, you haven't eaten for a while. Your blood sugar is dropping. And my gluconeogenesis pathways start to manufacturing-- in my case, unfortunately, too much. From non-carbohydrate precursors, they move the carbon from right to left and put out a lot of glucose into my blood. That's one of the uses of the gluconeogenic pathway.

Similarly, if a Doberman Pinscher, which has become commonly seen in the 5.07 course so far, starts to chase me down the street, what I'm going to want to do is I'm going to want to

take and break down my glucose as quickly as possible to bring it toward pyruvate to get fast ATP that I can use to power my muscles to be able to run away. So that's the gluconeogenesis and the glycolysis pathways.

Now imagine that you've got a railroad track and that you've got a side track off of it. And that's what the pentose phosphate pathway is. It is a track that comes off of glycolysis or gluconeogenesis, stuff happens, and then you can re-enter the mainstream of the train line.

The interesting thing is that you can basically go in either direction at certain parts of this pathway. What this really means is we have common components in both the pentose phosphate pathway and along this main line of gluconeogenesis/glycolysis. And the common components we're going to see are, first, glucose 6-phosphate; second, fructose 6-phosphate; and third, glyceraldehyde 3-phosphate, or GAP. So these are the common features of all three pathways.

OK, so I'm going to give you a little bit of an overview first and show you how this side track works. Now, the pathway can be run in the oxidative mode exclusively. And what we'll see is-- and again, the details will come in a few minutes-- that this glucose 6-phosphate is going to be oxidized in order to generate-- and oxidation, of course, is when electrons are removed from the substrate. And they're going to be put into an NADP to form NADPH, which is our reductive cofactor. So this is an oxidative arm.

Now, one of the consequences of this oxidation is going to be-- we're going to be losing a carbon from the six-carbon glucose 6-phosphate and we're going to be forming a pentose, specifically ribulose 5-phosphate. It's going to be easily interconverted with other pentoses, five-carbon sugars.

And then I want you to notice that from here on, everything is reversible in the pathway. Loss of CO_2 typically is irreversible, so this first arm, the first oxidative arm, is irreversible. So if we go from glucose 6-phosphate to pentoses, that's a one-way street.

The pentoses, if we are in the process, say, of cell division, these can be used to make nucleotides. And this is how we make pentoses for nucleic acids. If we don't need the pentoses, then the carbon from the pentoses will continue along the pathway and will go into this complicated carbon-scrambling network, which we see underlined with a squiggly line over here in the center. And the scrambling will result in the pentoses being restructured into

fructose 6-phosphate, glyceraldehyde 3-phosphate, so the carbon can then continue along the pathway.

So I can imagine the pathway going like this. A cell-- let's say it's in a situation where it is growing and it needs NADPH, and it needs pentoses for making nucleotides. So it takes and it gets up to the glucose 6-phosphate step. And so rather than progressing in glycolysis, it takes, as you'll see, a right-hand turn. Make the NADPH that's going to be needed for making those membranes of the new cell. Getting pentoses, which are going to convert into nucleotides. And if you don't use all of the pentoses, then what you want to do is you don't want any waste material, so you go through the carbon-scrambling network, and the remainder of the material will go back as fructose 6-phosphate and glyceraldehyde 3-phosphate. So that's what we'll call the full pentose phosphate pathway, with the oxidative and the non-oxidative portions distinctly shown. Everything's oxidative up to the pentoses generation, and then it's non-oxidative from pentoses through the fructose 6-phosphate and glyceraldehyde 3-phosphate.

Now in the Case B over here-- we call it the purely non-oxidative mode. Let's say you're in a situation where you're not growing, but you do need, for example, ATP, and you need pentoses for that. That's where the back door comes in. We're going to be taking, in this case out of probably gluconeogenesis-- remember, gluconeogenesis is the flow of carbon from right to left in this pathway. So it could be amino acids and things from the tricarboxylic acid, or TCA cycle, are flowing carbon in this direction. And it goes up into the gluconeogenic pathway, and then the molecule repositories fill up for a glyceraldehyde 3-phosphate and fructose 6-phosphate.

If I were to block gluconeogenesis right here, then carbon would flow in this direction, would go through the backdoor of the pathway. The carbon-scrambling phase would result ultimately in pentoses. So this would be a biosynthetic route to pentoses. Again, that's a situation in which we do not need NADPH. Perhaps we just need pentoses.

OK, so now let me get into the details. In the center of this piece of paper, what you'll see is the stoichiometry of the pathway. In most of 5.07 so far, we have seen that we work with a single molecule of glucose working its way to two molecules of pyruvate, and so on. Here the tricarboxylic geometry works out best, or it's easiest to teach and to appreciate, if you start with three molecules of glucose. And we'll see why in a few minutes.

So what we'll do is-- I'm going to do is take three molecules of glucose 6-phosphate. Now

looking up at the top here-- so this is glucose to glucose 6-phosphate. This is the entry portion right here, with glucose 6-phosphate, into the oxidative arm of the pathway. So that's 18 carbons. 3 times 6 is 18.

We're going to lose three CO2s. That's going to leave us 15 carbons left. And those 15 carbons are going to be distributed among two fructose 6-phosphates and glyceraldehyde 3-phosphate. So here's the fructose 6-phosphate. Here's the glyceraldehyde 3-phosphate. That's the overall stoichiometry of the pathway.

So now what I'm going to do is go through the chemical details of the pathway, down here at the bottom and on the next page. We've got glucose going to glucose 6-phosphate. And I draw three lines there because I'll say this glucose 6-phosphate is the abbreviation that I use for the structure that's shown right here. So this is glucose 6-phosphate with the proper stereochemistry.

Now the hydroxyl group on the glucose-- we don't really know what its stereochemistry is, but we've got it up or in beta in this drawing. The first step is for the enzyme glucose 6-phosphate dehydrogenase, G6PDH, to attack the one carbon. The electrons go down to make a keto functionality. We lose hydride from the one carbon, which is used then to reduce NADP to NADPH. So that's our first oxidation step.

I want you to notice that this is a one-way arrow. Typically at the top of a pathway-- and in this case, it's at the very top-- these are the steps that are thermodynamically irreversible. These are also the steps where you principally effect regulation of the pathway. And as I said earlier, we're going to be running three molecules of glucose 6-phosphate through this step.

The next molecule in the pathway is this one. This is a lactone. A lactone is a cyclic ester. So this is an ester, and you can see it's cyclic. The next step involves the addition of water by lactonase in order to open up this six-membered ring to give you this linear structure that we'll see here. And this is basically 6-phosphoglucuronic acid.

The next step will involve the oxidation of the 1, 2, 3 carbon. I always try to find places in any pathway where we've seen the same chemistry before but with different molecules. This step right here, specifically working with lactonase and the enzyme 6 phosphogluconate dehydrogenase-- I'd like you to look back at the tricarboxylic acid cycle, specifically at the enzyme isocitrate dehydrogenase. While some of the specifics are different, basically what you'll see is the overall concept is the same for what's happening in the next couple of steps.

So let me now get back to the drawing.

So what we have here is the 1, 2, 3 carbon is vulnerable to what's going to be an oxidation event. So a base will remove the hydrogen from the 3-hydroxyl group. The electrons that were holding the hydrogen to the oxygen will now move down to the position between the oxygen and the 3 carbon. And then the hydride on the 3 carbon will be transferred to NADP to make NADPH. So this is the second of two oxidation steps.

That gives us this ring open structure here. And just as happens in the isocitrate dehydrogenase step, what we've done is we've generated a beta ketoacid. Whenever you see a beta ketoacid, you really always want to think that that's prone to either enzymatic or spontaneous decarboxylation. What you can see is the electrons can go like this and like this and like this, and that will result in the cleavage of the bond connecting carbon 1 and carbon 2. So now the new carbon 1 will be the what was carbon 2, which is the carbon that has this little purple ball on it. And we're going to lose the terminal, or 1 carbon, as CO2. The CO2 will have the triangle, which up until this point has defined our 1 carbon.

Now I just want to mention a couple of things. One is that we'll look at-- I put on-- like here's a dark ball. Here's a purple ball, and here's a black triangle. These could be-- for example, experimentally, you could use a radioactive chemical, a radioactive atom, in order to help you trace the reaction as you go from precursor to product. I'm using these just to help you see the relationship of a precursor and a product. And what we see here is that the 1 carbon is lost from the beta ketoacid as CO2 and according to the kind of chemistry that we've seen many times for decarboxylation.

Now, this is going to give us a five-carbon product. We've lost the CO2. And that is ribulose 5-phosphate. And for this, I'm going to go on to the next page.

On the previous page, we saw the loss of CO2 from the 1 carbon of the beta ketoacid. And this gives us this linear structure, five-carbon ribulose 5-phosphate. I've drawn it this way so you can see it from the perspective of what was a six-carbon molecule that's lost one carbon. But the more formal chemical way to draw this would be as the linear chain of five carbons starting with the new 1 carbon, which is the alcohol, with a purple circle. The next carbon would have the carbonyl group, which I'll use a blue box. And all the way down to what is now the five-carbon-- what was originally the six-carbon-- which has the dark black dot.

Now because we're processing three molecules of hexose and now pentose, where we've got

three molecules of ribulose 5-phosphate, and the two enzymes that are going to process this are ribulose 5-phosphate isomerase and ribulose 5-phosphate epimerase. Now, just because of the relative activities of these enzymes, we're going to see that about one-third of the molecules will be processed by the isomerase and two-thirds will be processed by the epimerase.

Now, let me tell you a little bit about what isomerization is, versus epimerization. Bogdan and others have told you about the importance of enediol or diolate intermediates in biochemistry. The section of the molecule in the ribulose 5-phosphate that I'm circling here, if you just take and form an enediolate here, what you can do is you can move the carbonyl functionality from the 2 carbon up to the 1 carbon. And that's what happens when you convert ribulose 5-phosphate into ribose 5-phosphate. So it's a simple enediol conversion.

Very similarly, I could have made the enediol between the 2 and the 3 carbon. And if I did that, I'll form an enediol. And depending on how I put the hydrogen back on that I take off, I can change the stereochemistry at the 3 carbon. In other words, I can form the epimer at the 3 carbon. So I can go from this epimer on the top to where the hydroxyl group is pointed to the right, to this epimer on the bottom, where the hydroxyl group is pointed to the left.

This molecule, the epimer of ribulose 5-phosphate, where the hydroxyl on 3 is pointed to the left, is called xylulose 5-phosphate. As I said, this enzyme, this epimerase enzyme, is a little more active than the isomerase enzyme. So we get about two molecules of the xylulose 5-phosphate for every molecule that we process-- again, by enediolate-type intermediates to form the ribose 5-phosphate. So this gives us a-- ribulose 5-phosphate is now converted into one molecule of ribose 5-phosphate and two molecules of xylulose 5-phosphate.

I'm going to give you a little bit of a mechanistic interlude here to remind you that some of the chemistry I'm going to be showing you with the enzyme transketolase is very reminiscent of chemistry we have seen several times in 5.07, specifically the enzymes pyruvate dehydrogenase, pyruvate decarboxylase, and alpha-ketoglutarate dehydrogenase.

Now, the molecule that I have right here in this little mechanistic interlude box is one of the ones we were just talking about. Specifically, this is xylulose 5-phosphate. You see the epimer with the 3-hydroxyl off on the left.

This carbonyl group is exactly the type of carbonyl that's attackable by thiamine

pyrophosphate, TPP. Let me remind you a little bit about why TPP can be a nucleophile to attack this carbonyl group. TPP, when you look at the whole cofactor, it has attached up here an aminopyridine. And the amino group can be in the imino tautomer, and that imino tautomer can help pull off this hydrogen, lowering the PK.

So the PK of this is actually something in the order of 18 or 19, which is sufficient to make this carbon a good nucleophile to be able to attack the carbonyl that's on our, in this case, xylulose 5-phosphate. That attack will result in the formation of an initial adduct in which the vitamin, the TPP, is covalently associated with the xylulose 5-phosphate. You can see that the keto group has been converted into a hydroxyl group. So we've now got a dihydroxy molecule at this point.

And just as we've seen with the other enzymes I mentioned earlier-- for example, the pyruvate dehydrogenase-- the delocalization of electrons through this system makes it possible to be able to remove this hydrogen. These electrons move to this position. These electrons move up here. And that means that the bond connecting the bottom three carbons of the xylulose 5-phosphate is broken, and that gives you-- if you look at the chemistry-- glyceraldehyde 3-phosphate, the very common biochemical intermediate. And what's left over is the vitamin TPP attached to two-carbon unit. And it's called dihydroxyethyl TPP. Hydroxy, hydroxy, this is an ethyl group, and it's an anion, the TPP anion.

So I abbreviate that minus, and then with a box, C2-TPP. It's a chemically reactive nucleophilic intermediate that we're going to be able to use to do powerful chemistry.

The enzyme transketolase, which I'm going to talk about from the standpoint of medical importance somewhat later, enables the removal of the top two carbons, via the chemistry I just showed you. It's a kind of a chemical decapitation to remove the C2-TPP in order to liberate it to be able to be used as a chemical reagent.

So both of our molecules of xylulose 5-phosphate are going to be susceptible to this chemistry, and that means we're going to get two molecules of this C2-TPP anion-- one here and one here-- and two molecules of glyceraldehyde 3-phosphate-- here and here. OK?

What are we going to do with the C2-TPP? Well, I want to draw your attention back up here to the ribulose 5-phosphate. Remember, I told you that it's possible, by way of an enediolate intermediate, with this isomerase enzyme, to move the carbonyl group from the 2 carbon up to the 1 carbon. And that produces ribose 5-phosphate. One of the things that I just want to point

out here is ribose 5-phosphate was one of the things that's one of the goals of the pathway. So I could, for example, stop the pathway here and shunt off this ribose 5-phosphate to be able to make nucleotides.

But this carbonyl is exactly the kind of functional group that the C2-TPP looks for, for chemical reaction. So I can plug in, just like a LEGO, the C2-TPP on the five-carbon ribose 5-phosphate. What that does is gives me-- 5 plus 2 is 7-- sedoheptulose 7-phosphate. 1, 2, 3, 4, 5, 6, 7. Sedoheptulose 7-phosphate. And just because of the way the chemistry works, we've basically pushed out the hydroxyl group at this position off on the left, on the number 3 carbon.

OK, so now we have a seven-carbon molecule. And again, this is another biosynthetic opportunity. As I said earlier, ribose 5-phosphate can go off to make ribonucleotides. If you look up the pathway of synthesis of the aromatic amino acid tyrosine, what you'll see is that basically this is the biosynthetic intermediate that will go off to produce that aromatic amino acid. OK, so this is sedoheptulose 7-phosphate.

Now if I look at the top three carbons here, you've got basically an alcohol, a carbonyl, and an alcohol. This looks almost identical to the carbon 1, 2, and 3 that you see in the aldolase reaction back in glycolysis. In other words, the linear form of-- in that case, it's fructose 1,6-bisphosphate-- in the linear form looks very much like this.

And what that means is that the aldolase enzyme, with its amino group at its active site, can attack this carbonyl group to form a protonated imine that will-- in the process of the chemistry, you can break the bond that connects carbon 3 to carbon 4 of this molecule. So the enzyme transaldolase that does this chemistry is very similar to the aldolase enzyme that we have looked at in the glycolysis pathway. This is the splitting, in this case, of a seven-carbon compound into a three-carbon and a four-carbon, whereas in the case of glycolysis, it was splitting a six-carbon compound into two three-carbon compounds.

This intermediate that I'm encircling here with the imine is very similar to the dihydroxyacetone phosphate precursor. So I'll call it a DHA-like fragment-- two methanol groups with a methyl imine in the middle.

So what we've done is I've taken the top three carbons of the sedoheptulose 7-phosphate and made it into something that looks a lot like carbons 1, 2, and 3 from the aldolase step of the glycolysis pathway. So it's a dihydroxyacetone-like phosphate. I'm just going to put that off to the side for a minute.

The bottom four carbons, carbons 4, 5, 6, and 7 of the sedoheptulose 7-phosphate, are something we haven't actually seen in 5.07 before. But if we look at what the structure is, it's going to be an aldehyde-- one, two, three, four-carbon aldehyde. And this is erythrose 4-phosphate, E4P.

Now, that in itself is not a valuable molecule. But it turns out that we can make it into a valuable molecule very easily. Remember back when we were talking about this C2-TPP electrophilic molecule that can put two carbons onto anything that has an aldehyde at the end? Well, erythrose 4-phosphate has an aldehyde at the end. If we do the chemistry and we plug this C2 fragment onto the erythrose 4-phosphate fragment up at the aldehyde, you get a six-carbon compound that is fructose 6-phosphate. So this is the source of fructose 6-phosphate that can then become part of the gluconeogenesis or glycolysis pathways.

So we haven't wasted anything. We've taken a kind of a trash molecule, the erythrose 4-phosphate, that we don't have a lot of other uses for, and we've converted into something that we can use for energy production, just as an example.

Now what I'd like to do is come back up to this dihydroxyacetone-like fragment that I talked about a little bit ago. In the glycolysis pathway, we start with the hexose glucose, and eventually, after about four or five steps, we get to another hexose, fructose 1,6-bisphosphate. Then the aldolase step takes and splits that in half into, on the one hand, a dihydroxyacetone-like fragment and, on the other hand, a glyceraldehyde 3-phosphate fragment. And the dihydroxyacetone-like fragment is covalently connected by way of its middle carbon, the carbonyl, to the enzyme aldolase.

When we look at what's happening in the transaldolase reaction, it's really the same thing. We've taken the seven-carbon molecule, in this case, and we've split off this three-carbon intermediate. Again, it's chemically just about identical to what happened in glycolysis, when we pulled apart the six-carbon sugar and we've now got this thing on the right which we can do something with.

The dihydroxyacetone phosphate is covalently connected to the enzyme transaldolase. It's a transaldolase. It can do the aldolase reaction, the splitting, but then it can take and it can transfer very easily. What are we going to transfer it to? Well the dihydroxyacetone phosphate chemically can be transferred very easily to glyceraldehyde 3-phosphate.

So I'm going to find a way now to connect this dihydroxyacetone-like fragment to a glyceraldehyde 3-phosphate. And I find one right here. Let me go back a little bit and see where it came from.

I had the xylulose 5-phosphate. I knocked off the top two carbons. I used those in order to make the sedoheptulose 7-phosphate. My residue was the three-carbon molecule glyceraldehyde 3-phosphate. But again, this could be a waste product, but instead of using it as a waste product, what I can do is to take the glyceraldehyde 3-phosphate that is a waste product of the transketolase reaction down here, and I can utilize it by combination with the dihydroxyacetone phosphate from up here, the transaldolase reaction. Transaldolase has the transfer ability to bring these molecules together covalently, plug them together, 3 and 3, to make 6. And that's your second molecule of fructose 6-phosphate.

While I've still got this sheet with the whole pathway on it, I'm going to give you a little bit of a summary. I took three molecules of glucose 6-phosphate, oxidized it twice, generated two molecules for each molecule of glucose 6-phosphate. I got two molecules of NADPH, the reducing equivalents.

The pathway up to that point created a beta ketoacid. Therefore there was this decarboxylation event. So what I ended up with is three five-carbon molecules, ribulose 5-phosphate. The ribulose 5-phosphate, depending upon the balance of activities of the next two enzymes, will partition at about a 2-to-1 ratio into xylulose 5-phosphate and ribose 5-phosphate.

Then I explained how the xylulose 5-phosphate had this carbonyl at the second position, and that looked like the carbonyl in a lot of other biochemical reactions we've seen earlier in 5.07. And it's vulnerable to attack by the ylid carbanion of TPP, thiamine pyrophosphate.

Applying that transketolase enzyme to xylulose 5-phosphate split off the top two carbons as this electrophilic chemical reagent that I call C2-TPP. The bottom three carbons became glyceraldehyde 3-phosphate. The C2-TPP got added onto ribose 5-phosphate, which is one of the molecules that was made from the early-on ribulose 5-phosphate. That made the seven-carbon molecule sedoheptulose 7-phosphate. And again, I said that that's a precursor to other things, like aromatic amino acids, if you need them.

The sedoheptulose 7-phosphate, however, has a structure that makes it amenable to having its top three carbons taken off by an aldolase-like enzyme, transaldolase. What makes this

aldolase special is it will take the three carbons off as a protein three-carbon adduct and allow that three carbons to be added onto one of the glyceraldehyde 3-phosphates that was generated in the transketolase reaction. 3 plus 3 is 6, and that becomes your fructose 6-phosphate.

Overall, what happens is you put in three glucose 6-phosphates, you lose some CO2s, and you produce two fructose 6-phosphates and one leftover glyceraldehyde 3-phosphate. And these are in the mainstream of glycolysis and gluconeogenesis.

So that's the details of the pathway. We start with a six-carbon compound, three molecules of it, and we generate three molecules of CO2. We're going to generate six molecules of NADPH. And at the beginning of the page that we're looking at here, we have three molecules of a five-carbon compound, this ribulose 5-phosphate. And again, at this point, the carbon-scrambling phase takes over. And at the end, we end up with taking the 15 carbons of the three 5 carbon ribulose 5-phosphates, and scrambling them into two six-carbon fructose 6-phosphates and one three-carbon glyceraldehyde 3-phosphate.

We've now looked at the pentose phosphate pathway from two levels. Let's say initially, what I see right here is the pathway from, let's say, the 10,000-foot level. We see there's the mainstream of metabolism of glucose to pyruvate-- that's glycolysis-- or pyruvate to glucose, gluconeogenesis. And we have seen how in the pentose phosphate pathway, we go from glucose 6-phosphate, we do some oxidation-- that's why you get the NADPH. You lose some CO2. You get the pentoses. I've introduced you to ribose, ribulose, and xylulose. Then in order to get these pentoses back into mainstream metabolism, we do this complicated carbon-scrambling routine, and then get back to fructose 6-phosphate and GAP to be able to continue in mainstream metabolism. Nothing is wasted. There's the 10,000-foot level.

And then I went, down here at the bottom, to sort of the 2-foot level, all the little details of the chemistry. So I've taught you this, the pentose phosphate pathway, in the beginning at the 10,000-foot level, and then got you down to the 2-foot level with all of the details of the chemical mechanisms. And our course, Biological Chemistry 5.07, is a chemistry course, so that's very appropriate.

But 5.07 is also 25.07. 20 is the Biological Engineering department. And the engineers actually think a lot about, let's say, mathematical equations more than chemical equations. And they think about schematics, written out like you'd look at an electrical schematic. So because the

Biological Chemistry group we have is quite large, we have kind of an ending to this pathway that appeals, I think, to more the engineering mindset.

So for the engineers, I'm going to move from the two-foot level up to about the 100-foot level. And I'm going to present it as a schematic. The shorthand that I use involves these little boxes. Each of these is a molecule.

And the abbreviation is in the top-- G6P, glucose 6-phosphate. It has six carbons. That'll help me keep track of how many carbons are there. And there are three molecules of it. So we've got 6 times 3, or 18 carbons, that we start with.

In the oxidative phase, we've seen that each molecule gives rise to two NADPHs. 2 times 3 is 6. So we've got 6 NADPHs. There are three molecules that are starting, and remember that beta ketoacid that's formed? We have three of those, and they're going to carboxylate. So I get rid of three CO2s. So the broken line indicates the boundary between the irreversible and the reversible parts of the reaction.

This gives us ribulose 5-phosphate. That's our initial product entering the pathway. It's a five-carbon molecule. There are three molecules of it, total of 15 carbons.

Depending upon the activities of the epimerase in the bottom-- E-- or the isomerase-- I-- on the top, you either get more or less of the X5P, xylulose 5-phosphate, or ribose 5-phosphate, R5P. And as I said, typically the epimerase wins out by about 2-to-1 over the isomerase.

So we've got a simple isomerization to take the ribulose 5-phosphate to ribose 5-phosphate. It's a C5 molecule. You get one of those. So we've got five of our carbons over here.

If we look at the epimerase reactions, we get two boxes, one for each molecule of xylulose 5-phosphate. Each of them are C51. So those are our two molecules.

Now let's take our first molecule of xylulose 5-phosphate, the one in the top X5P box. And again, keep in mind that these arrowheads are going both ways. Because the carbon can flow both ways in these reactions. These are equilibrium reactions.

We can take the top two carbons off of the X5P. This is the thiamine pyrophosphate reaction. And what that does is it gives us that chemically reactive C2-TPP fragment. Carbon 2, two carbons, one of those. OK? And the bottom half is glyceraldehyde 3-phosphate-- a three-carbon molecule, one of those. The ribose 5-phosphate, the five-carbon aldehyde, can have

the C2-TPP add to it's one carbon. 2 carbons plus 5 carbons give 7. Sedoheptulose 7-phosphate is the product-- one molecule of those. Transaldolase can split off the top three carbons to make that dihydroacetone-like fragment. This is the one that's covalently attached by this imine-like bond to the triose sugar. Three-carbon sugar-- one molecule of that.

And after you do this transaldolase reaction and you've taken off the top three carbons, you've got a residue of four carbons. So that's the erythrose 4-phosphate, C4, one molecule. Now, the transaldolase reaction can do a transfer. So you'll take this DHA, dihydroxyacetone-like fragment, and combine it with glyceraldehyde 3-phosphate that it got from the molecule 1 of xylulose 5-phosphate. So we can follow the schematic. Xylulose 5-phosphate gives us this gap molecule, glyceraldehyde 3phosphate. It can combine with the molecule, the DHA-like fragment, to form for our first molecule of fructose 6-phosphate, which will then go off into glycolysis or gluconeogenesis.

Now, the residue of this was this erythrose 4-phosphate product. What can we do with it? For again, this is an aldehyde. It can react with a C2 fragment from the C2-TPP. So where we get that C2-TPP is from our second molecule of xylulose 5-phosphate. Transketolase takes off the top two carbons and plunks it onto the erythrose 4-phosphate to form our second molecule of two of fructose 6-phosphate. C6, one molecule. And what's left over after all is said and done is this fragment right here, glyceraldehyde 3-phosphate. C3 molecule, one molecule.

So if I did this correctly, we should have 15 carbons over here-- 6, 6, and 3-- and we do. So these are the molecules that will flow into glycolysis or gluconeogenesis. Now because most of these arrows are double-headed, you can also imagine that fructose 6-phosphate and glyceraldehyde 3-phosphate, in the mainstream metabolism, in glycolysis and gluconeogenesis, can backflow right to left and end up with these pentoses.

So for example, if I wanted to make ribose 5-phosphate in order to make ATP, and I didn't need any reducing equivalents, all I would do is run all of this in reverse, get up to this point, ribose 5-phosphate, and then take this off to make nucleotides. On the other hand, if I'd like to make just NADPH for biosynthesis, what I would do is the following. I'd start with my glucose-6 phosphate. Early on, yield my 6 NADPHs. Go from left to right across the pathway, all the way to produce fructose 6-phosphate and GAP. And then what I'd do is I'd use gluconeogenesis in order to convert fructose 6-phosphate and GAP back into glucose 6-phosphate that then would be able to re-enter the pathway once again.

Now, what I want to do is to show you that, I want to go back to the original drawing that I had, to show you how that would work. So what I just said was that if I were in a situation where all I wanted was NADPH, I could do the following.

I could enter the carbon from glucose 6-phosphate, make the pentoses, get my NADPH, and then take the carbon and flush it back through the scrambling phase to produce fructose 6-phosphate and GAP. Now here's the important part, is I can now, through gluconeogenesis, move the carbon back up to glucose 6-phosphate and do it again, and again, and again. In other words, recursively, I can metabolize all of the carbon from glucose 6-phosphate. All six carbons can eventually become converted into CO2 with copious amounts of NADPH.

And that's part of the power of this pathway. It's very versatile. You can use it to make reducing equivalents. You can use it to make sugars. You can use it to make sedoheptulose 7-phosphate to make aromatic amino acids. It's a very powerful pathway.

The top part of this picture shows us the schematic of the pathway. And I think with the schematic up there, I think this is a good time to make some summary comments. So one thing I mentioned at the very beginning is that we need NADPH in order to finance biosynthesis of lipids-- in particular for making membranes. So these are needed for growth. So I think now you can see why this pathway is turned on in cells that are making new tissue, that are making new cells.

If you run it in the oxidative mode-- that's the one that I just described, where you purely use it to make NADPH-- you can oxidize all the carbons of your glucose 6-phosphate into CO2. That's the scenario that I just ran.

I didn't emphasize enough that if, for example, you eat food that contains nucleic acid, which of course we all do, that nucleic acid contains ribose. That ribose, we want to be able to do something with. And what happens is it enters right here into the pathway. In other words, if I wanted to take that ribose and if I wanted to make energy out of it, the ribose would go from left to right, and I would end up with fructose 6-phosphate, glyceraldehyde 3-phosphate. And then I could use that, putting it through glycolysis and then the TCA cycle to make energy.

Point 4 is one that I mentioned earlier. I'm a toxicologist, and point 4 shows how NADPH helps us defend against oxidative stress. And I have this little cartoon at the bottom. Free radicals are a consequence of living in an aerobic environment. We breathe oxygen. It can be incompletely reduced to produce things like superoxide and hydrogen peroxide and even

hydroxyl radical. These are chemically very dangerous, and they'll form peroxides and other molecules that can cause disease.

So we have protective systems based on a variety of small molecules in the cell. One of them is called glutathione. Millimolar concentrations of glutathione exist in our liver cells, and they exist in order to be able to do the kind of chemistry I'm going to show you right here. So a peroxide that I'm pointing to, ROOH, is dangerous because it can give rise to these free radicals that can be damaging to us.

What we can do is take two molecules of this sulfur-containing glutathione-- it's a tripeptide-- and they will give up their H dot dot, their hydrides, in order to be able to basically break this ROOH into ROH and alcohol and HOH, water-- in other words, relatively harmless products.

Now, glutathione, the RSH-- sometimes called GSH-- sacrifices itself and becomes this oxidized form, RSSR. Now, the reaction that does this first part's called glutathione peroxidase. In order to restore our cellular stores of reduced glutathione, RSH, we have to take the oxidized form, RSSR, and we have to reduce it. That's where the NADPH comes in. NADPH is the cofactor used by glutathione reductase. And what it does is puts in the reducing equivalents to basically convert oxidized glutathione back to what we want, which is the reduced glutathione, that can then go on to protect us against free radicals.

NADPH has a very special role in protecting any cell from oxidative stress. It has a very special role, however, when you deal with red blood cells. Red blood cells lack a nucleus. They lack a mitochondrion. They're pretty much entirely cytosol.

Cytosol has a glycolysis pathway. That's where they get their energy. And the pentose phosphate pathway, which is where they get the reducing equivalents that are necessary to help maintain the red blood cells' integrity-- if you think about it, you know, a red blood cell's carrying a lot oxygen. Oxygen itself can be very damaging. Red blood cells are damaged, and you really need to have these reducing equivalents available in order to maintain the structural and functional integrity of a red blood cell.

So anything that disrupts the NADPH pool in a red blood cell ultimately causes a kind of anemia, because that means the red blood cell can't persist as long as it would otherwise. People who have defects in NADPH metabolism-- for example, a person who might have a defect or a sluggish first enzyme in the pathway, the glucose 6-phosphate dehydrogenase-- the red blood cells, because of this NADPH problem, don't last as long.

Clinically, it's observed that these people have somewhat of a resistance against diseases such as malaria. That might be because the red blood cells don't last long enough for the organism that causes malaria, Plasmodium falciparum, to be able to complete its life cycle. So sometimes a genetic defect can actually provide an asset. And in some places in the world where people have evolved in the presence of the malaria Plasmodium organism, they have defects in the pathway that are maintained, from generation to generation, in order to be resistant to the environment.

The sixth point that I want to bring up with regard to this pathway has to do with the enzyme at the top of the pathway, once again, the glucose 6-phosphate dehydrogenase. I mentioned it at the very beginning, but I want to reiterate that enzymes that are at the top of a pathway where there is thermodynamically reversible steps, they are places where you have the ability to control entry into the pathway.

So these are the rate-determining steps. And in this case, the presence of NAD+-- that is, the product-- determines the rate of passage through the pathway. So for example, if a cell has been doing a lot of biosynthesis, the NADP+ levels go up. That interacts allosterically in order to be able to increase the throughput of the pathway. So you increase the amount of NADPH biosynthesis to be able to let it match the biosynthetic needs associated with doing a lot of lipid biosynthesis. So this is the regulated step.

The last thing I'm going to say about the pentose phosphate pathway is that it's strikingly similar to the pathway called the Calvin cycle, which are the dark reactions of photosynthesis. Photosynthesis involves the capture of one CO2 into a five-carbon scaffold to make a six-carbon compound. Then that six-carbon compounds splits into three-carbon compounds.

It turns out if you work with enough molecules of five-carbon compound, what you're able to do is go through the carbon-scrambling phase and work all your way back to the hexose glucose. The carbon-scrambling intermediates are almost identical chemically to the ones that we have just looked at in the pentose phosphate pathway. So if you look at the Calvin cycle, you'll see three-carbon and four-carbon and five-carbon and six-carbon and even seven-carbon intermediates, identical.

So nature, again, didn't reinvent the wheel twice. What it did was at some point in time, it took the pentose phosphate pathway and it converted it into the photosynthetic dark reactions, or

perhaps vice versa. They have different purposes. The photosynthetic pathway involves capturing carbon in order to make hexoses that we can use to store for energy, future energy use, or to make hexoses you could use to make cellulose, if you're a plant.

On the other hand, the pentose phosphate pathway-- again, the same chemistry. But in this case, the chemistry is all aimed at taking the hexose, the six-carbon compound, and using it to make reducing equivalents that we can use for biosynthesis, or to protect us from oxidative stress, or to make riboses that we need when we're growing, in order to be able to make nucleic acids, nucleotides, ATP, and so on, that we need.

The pentose phosphate pathway may seem a little bit difficult to learn. But I think it's very worthwhile. It is something that provides us with amazing versatility. It gives us compounds that are chemically reactive at the level of two carbons, three carbons, four carbons, five carbons, six carbons, and seven carbons. You can take these pieces and you can make a wide array of molecules that are absolutely essential for life.

Moreover, it provides us with reducing equivalents, which we need to grow-- biosynthesis. Moreover, those reducing equivalents also help protect us from oxidative stress. That helps keep us alive and healthy longer.

So the pentose phosphate pathway is complicated because it does so many different things. But any one of those things, I think, in itself makes it worthwhile learning all of this complicated chemistry. So that's the pentose phosphate pathway from the 10,000-foot level down to the 2-foot level. Incredibly versatile pathway, and I hope that this has helped you understand it. Thank you very much.