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PROFESSOR: So last time, we discussed glycolysis as a pathway. And I just want to remind everybody that, like all pathways, glycolysis has to be favorable. That is, delta G across the entire pathway has to be less than 0. We spent a lot of time discussing the regulation of glycolysis.

And I just want to revisit this quickly. And remember, we discussed that the steps that are regulated are those with the biggest, quote, unquote, bio-energetic cost. That is, the biggest drop in delta G is as molecules would proceed across the pathway. And those are shown here-- the hexokinase step, the phosphofructokinase step, and the pyruvate kinase step of the pathway.

Now this is largely true of most pathways that we will consider, in that these regulated steps are often those with the biggest bio-energetic cost. And the reason for that is that it makes a lot of sense. Once you go through those steps, it's very difficult to go back.

Also, you'll note that these are the steps that regulate entrance and exit from the pathway. And this also is something we'll commonly see and other pathways and also makes some sense. Just to remind everybody, I drew up here a skeletonized version of glycolysis and the most important regulatory features of it.

Just to remind people that initially, hexokinase is regulated by glucose-6 phosphate. Glucose-6 phosphate negatively regulates hexokinase. And that's because if the glucose-6 phosphate levels in the cell go up such that it can't be shunted to glycogen-- something we'll talk about today-- or be sent through glycolysis, it makes sense not to trap more glucose.

Once glucose-6 phosphate enters the rest of the glycolytic pathway, the key step of regulation is phosphofructokinase. It, of course, will have positive feedback, saying, let's put carbon into the pathway. If energy charge low, AMP is high, activates it. This molecule fructose 2,6-bisphosphate that we talked about earlier today-- we'll mention again today-- can be involved in some other signaling inputs to allosterically regulate the enzyme.

FBP can feed forward to activate pyruvate kinase. You want to match input with output. And, of course, if PEP builds up, you want to slow it down. If a downstream product-- citrate-- builds up, you want to slow it down. And of course, a major output of glycolysis is ATP. And if ATP is high, no reason to continue oxidizing glucose carbons.

Now this regulation we discussed is often done at the allosteric level by controlling enzyme rates. That can be via a small molecule binding, as I've shown up here. And it could also be via signaling. And that's really a place where fructose 2,6-bisphosphate as a combination of signaling, and allosteric small molecules comes into play. That is, you can have signaling that controls the enzymes that generate fructose 2,6-bisphosphate, thereby creating an additional molecule that can act as allosteric regulator of the enzyme PFK to generate FBP and commit carbon to glycolysis.

Now we closed the discussion last time beginning to discuss how it is that organisms can make glucose. Obviously, this glucose carbon that is being oxidized to get ATP has to come from somewhere. We mentioned that plants make glucose during the day when they have plenty of excess energy from light from the sun but ther use that glucose to do constant metabolism to maintain their ATP levels at night. And we as animals obviously take advantage of this by eating the glucose that ultimately was created by plants using the energy of the sun.

We began to discuss an example of where us, as organisms, also make glucose. Of course, this is not net production of glucose. But for instance, if our muscle cells are working hard, lactate can build up. That lactate is excreted into the blood. That lactate can then be used by the liver to regenerate glucose. And one of the major jobs of the liver is to maintain a constant level of blood glucose to continue to supply fuel for various organs, including the muscle to be able to carry out the work that it needs to do.

And so this whole process is called the Cori cycle. And a major job of the liver is thus generating glucose to maintain blood glucose levels. And the pathways used to do this is a pathway that we began to talk about last time called gluconeogenesis.

And it's really a discussion of gluconeogenesis. That is, how can one build a pathway in plants, in the liver, wherever, as a way to generate glucose from something like lactate? Now, of course, we know from last time, lactate dehydrogenase can, obviously, it's a redox reaction to interconvert with pyruvate. And what gluconeogenesis really is it's a pathway that cells use to convert pyruvate or really anything else that can come into glycolysis-- and example being lactate. This, of course, can happen by the lactate dehydrogenase reaction. And then once it has that pyruvate, turn that pyruvate into glucose. That's really what we're talking about when we're discussing gluconeogenesis.

Now how can we turn pyruvate into glucose? Well, we just discussed the whole pathway where we could turn glucose into pyruvate-- glycolysis. And so this is really the reverse of glycolysis. I said many times that all enzymes are reversible. So why can't we just run the pathway in the opposite direction?

Well, hopefully, if you've been paying attention for the last several lectures, you realize there's two problems with doing that. The first is that, remember, why we're doing metabolism in the first place is to maintain a high ATP:ADP ratio under conditions so that the cell can use that ATP:ADP ratio to carry out otherwise unfavorable processes. And so thus, at a very high level, cells can't stop catabolism. Glycolysis or whatever has to continue to maintain ATP in the right range.

And so now, if we're going to do something anabolic-- that is, build something, turn it into glucose, this has to happen in the background where catabolism has still happened. And so at least you need some kind of way to control both and prevent this from being some kind of futile cycle.

Second, even if that ATP level is super high in the cell, it has extra energy, there is still a thermodynamic problem with just reversing glycolysis. And that is, remember, delta G has to be less than 0 for any pathway to work. And so it then stands to reason that if glucose oxidation to pyruvate, if this reaction is favored, as it is in glycolysis, that delta G is less than 0.

Well, under the conditions where that's occurring, if we want to then say, well, what about the reverse? Let's switch the signs-- pyruvate to glucose. We know that delta G is the same as it was in the opposite direction but, now, changing the signs from positive to negative or negative positive. And so, thus, delta G, for the reverse reaction, must be greater than 0 and, therefore, must be spontaneous-- is not spontaneous. And therefore says that we need to have a different pathway simply for energetic reasons if we're going to go in the opposite direction.

Now oxidizing glucose releases energy. That's how we keep the ATP:ADP ratio high. It stands to reason then that we are going to have to put energy in if we're going to run in the opposite direction. And so if we're going to build a pathway from pyruvate to glucose, you can guess, we're going to have to add couple that pathway to ATP-ADP hydrolysis-- that is, couple it to something favorable to now allow this otherwise unfavorable reaction to happen.

Now this energy, of course, in plants can come from the sun. We'll discuss that in great detail when we get to photosynthesis. However, for the sake of you and I, I guess, what it says is, if you think about this, if you release energy from glycolysis, it makes sense then you have to add energy if you're going to do gluconeogenesis.

But remember, to satisfy all the laws of thermodynamics, the net energy loss to the universe is going to be-- must exist, which means that it is going to cost more energy to make glucose than you're going to get back from breaking it down. That just, hopefully, fits with your intuition. And it actually, I guess, is good news, because when are we going to do anabolism?

When are we going to store all these excess calories as glucose? Well, we're going to do it if we overeat. And if we overeat, and it costs us energy in the form of calories to actually store that energy for later, I guess, it means that when we overeat, we don't have things quite as bad as it could have been. All right.

Now let's get back to the how one can actually do this. So if we want to think, what is a pathway that we can build to get from pyruvate back to glucose? Well, we know we're going to have to burn some ATP to do it. But what are the issues with simply reversing glycolysis?

Well, we can come back here and say, well, what was driving glycolysis in the first place? And it was these big steps of free energy change acts of hexokinase, phosphofructokinase, and pyruvate kinase. Where as many of these other reactions, at least under these standard conditions that are estimated to exist in cells, are relatively close to equilibrium. So there's really no problem with reversing glycolysis across here or across there.

The real issue comes from getting around the hexokinase to phosphofructokinase and the pyruvate kinase step. These are the places where the most energy is released, if you will, when you're going through glycolysis. So those are the steps we're going to have to add energy in order to reverse build a pathway that goes in the opposite direction and allows you to start with pyruvate and end up with glucose.

And so what we will find is that gluconeogenesis and glycolysis use many of the same enzymes. However, not all of the enzymes are the same because we need different enzymes if we're going to get around this hexokinase, phosphofructokinase, and pyruvate kinase steps of the pathway. And so for gluconeogenesis, we essentially need four new enzymes in order to get past those places. So we're going to need a new enzyme called glucose 6-phosphatase. And glucose 6-phosphatase is going to allow us to get from glucose 6-phosphate and turn it back into glucose.

All right. We're going to need an enzyme fructose 1,6-bisphosphatase, which is going to allow us to take FBP and turn it back into fructose 6-phosphate. And then we're going to need two enzymes to get around the pyruvate kinase step. These are two enzymes called PEPCK and PC. That stands for phosphoenolpyruvate carboxykinase and pyruvate carboxylase. I'll discuss these in great detail in a minute. And these are going to allow us to get from pyruvate back to PEP.

Also, essentially, I'll add them up here to this diagram. So to get back from pyruvate to PEP, two enzymes-- pyruvate carboxylase and phosphoenolpyruvate carboxykinase to get from fructose 1,6-bisphosphate, fructose 6-phosphate, an enzyme called FBPase. And to get from glucose 6-phosphate back to glucose, an enzyme called glucose 6-phosphatase.

OK. Now, of course, if you think back, we're also going to need to balance electrons in the pathway. Remember, glucose to pyruvate is an oxidation reaction. That is, we're generating NADH. That means to go in the reverse direction, we need a source of NADH to reverse that GAPDH reaction. I just want to point out that you're only doing gluconeogenesis if cells have plenty of energy.

What does plenty of energy mean? Well, that means the ATP:ADP ratio is high because you're doing lots of metabolism. But if you're doing lots of metabolism, and the ATP:ADP ratio's high, that also means the NADH/NAD+ ratio is high. And so you can think of the NAD regeneration by gluconeogenesis as being, for all intents and purposes, an alternative to fermentation, if you will, as a way to keep catabolism going. That is, you're only doing this if you have plenty of NADH around. And so dealing with that electron balance is less of an issue.

OK. Now let's discuss briefly how some of these enzymes work. So glucose 6-phosphate and fructose 1,6-bisphosphatase are very straightforward. Effectively, all they are is simply removing a phosphate. That's very favorable. Remember, when we did those reactions of hexokinase phosphofructokinase reactions and glycolysis, that, we had to couple otherwise unfavorable things-- adding a phosphate group to ATP hydrolysis. This is the reverse of that. And so the reverse of that, delta G is going to be less than 0. Delta G less than 0 means we just have a phosphatase there, just like you might have on a kinase enzyme. That's very straightforward.

But there's a much bigger issue to get around this other step, this pyruvate kinase step. Remember, equilibrium strongly favored PEP to pyruvate conversion. That was one of the reasons why we could use that step to maintain a high ATP:ADP ratio in cells. Well, now, the issue becomes, how can we trap that phosphate back onto pyruvate while generating phosphoenolpyruvate?

How one does that is an issue. Even if the ATP:ADP ratio is high, it's never high enough to reverse pyruvate kinase on its own. And so we need a new pathway with two steps in it. That's why there's two enzymes-- PC and PEPCK-- that are there to create a pathway where pyruvate to PEP now becomes favorable. That is, where delta G 0 prime is less than 0. That is, the equilibrium favors to the right. And under cellular conditions, this pathway can actually happen such that cells can net make glucose.

Now these are two steps, both of which require ATP. And so you effectively get one ATP from PEP to pyruvate conversion. It costs you to ADPs or two ADP equivalents to go back from pyruvate to PEP, which makes sense. You should have to spend more energy to go upstream than you get back coming downstream. All right. Here's how this pathway works.

So this is pyruvate, the first reaction catalyzed by PC, which stands for pyruvate carboxylase. Couples ATP hydrolysis to addition of a CO2 that generates this intermediate, which is called oxaloacetate, often abbreviated OAA for Oxaloacetic Acid.

That oxaloacetate is then decarboxylated. That is, the CO2 that is lost is removed-- that was added is removed. This reaction is coupled to GTP and is catalyzed by this enzyme, PEPCK PEP carboxykinase and uses that phosphate from GTP to trap pyruvate in the enol form as PEP or phosphoenolpyruvate. great.

So once you've regenerated PEP, now that PEP gets to this flat part, if we go over here to our delta G place. So now it can easily run back across this flat part of the curve. That is, go through enolase phosphoglycerate mutase, phosphoglycerate kinase, GAPDH, taking advantage of the high NADH/NAD+ ratio that would exist when you're doing gluconeogenesis-- triosephosphate isomerase, aldolase.

This generates a DHAP and a glyceraldehyde 3-phosphate that you can then combine to give a fructose 1,6-bisphosphate. Now we get back here to now we have this steep part there. But rather than going through FBP, we can just have a phosphatase, fructose 1,6-bisphosphatase that releases the phosphate. Now, we have fructose 6-phosphate.

That fructose 6-phosphate can then go through glucose 6-phosphate isomerase and generate glucose 6-phosphate. That now gets back to this other steep hill. So this is relatively flat. Now, you have this steep hill around hexokinase. But rather than coupling it to adding to ATP-ADP hydrolysis, you just remove the phosphate with glucose 6-phosphatase.

And in the end, now we have a pathway using these four extra enzymes that give us a way to start at pyruvate and run the opposite direction and generate glucose. And you can do so in a way that is thermodynamically favorable. In other words, we can start with 2 pyruvate and use 4 ATP-- so and ATP to turn pyruvate twice into oxaloacetate.

So that's two. And then another ATP at phosphoglycerate kinase to go backwards there to go from-- to run that reaction backwards, plus 2 GTP. Those 2 GTP are effectively the equivalent of an ATP. So this would be 6 ATP equivalents. Why are the GTPs equivalent of ATP? I just want to point out that cells have enzymes that basically can interconvert ATP and GDP or, really, any nucleoside triphosphate and diphosphate to GTP plus ADP.

This has a delta G 0 prime that is effectively 0. And so cells basically match their ATP to ADP ratios with any other ratios of nucleoside triphosphates and diphosphates because of this reaction. And so that's why the two GTP are roughly an ATP equivalent. So we'll just call it 6 ATP plus 2 NADH to reverse the GAPDH step. That gives us 6 ADP plus 2 NAD+ plus 6 inorganic phosphate plus a glucose.

The delta G 0 prime for this entire coupled pathway is on the order of negative 9 kcals per mol, with the delta G less than 0, equilibrium lies to the right. Net synthesis of glucose is favorable. And so if we just think about this in ATP terms, remember, when we discussed glycolysis, you harvest net two ATP from conversion of glucose into two pyruvate.

If we reverse the reaction doing gluconeogenesis, in ATP terms, we have to invest six ATP to turn that pyruvate back into glucose. Makes sense. We didn't make a perpetual motion machine. Both pathways are favorable. But it costs more energy to make a glucose than we can get out by burning the glucose. I should say, the energy isn't just the ATP. There's also this NADH.

That NADH that's produced from glycolysis also is storing energy, if you will, from oxidation and an NADH/NAD+ ratio, and you're consuming that going in the opposite direction. You only do gluconeogenesis if you have a high ATP:ADP ratio, of course, and a NADH/NAH+ plus ratio-- that is, if you have it under the right conditions to actually run this pathway and have it make sense for the cell.

All right. Now I want to discuss, briefly, how these enzymes in gluconeogenesis work. I'm not going to spend time on fructose 1,6-bisphosphatase or glucose 6-phosphatase. As I said before, this is just hydrolysis of that phosphate-alcohol bond. That's relatively straightforward. But I do want to spend time on pyruvate carboxylase and PEP carboxykinase because this introduces a new reaction, carboxylation.

But it also introduces a new concept in metabolism that is actually quite important in eukaryotic cells. And that is this issue of so-called compartmentalized metabolism. And, of course, you've learned in introductory biology that a big difference between eukaryotes and prokaryotes is eukaryotes have all of these organelles-- that is, these membrane-bound structures within cells that carry out various functions.

So one reason that it's useful to have these different membrane-bound organelles is it creates different compartments within the cell. What does that mean? Well, if you have different compartments, that means you can create different conditions within each compartment. And that's very important for metabolism.

Well, why is that important for metabolism? Well, because, remember, whether or not reactions are favorable depend on delta G, which, of course, is dependent on the equilibrium constant, which is related to delta G 0 prime but also to the ratio of the products and reactants within that compartment. And so by having different compartments, you can have different ratios of metabolites-- say, a different ratio of ATP to ATP or a different ratio of NADH to NAD+. Having those different ratios means that because delta G is proportional to the ratio of the reactants over the products-- I'm sorry-- products over reactants, by having different ratios of things like ATP and ADP, NAD, NADH, you can make different reactions more or less favorable depending on which compartment in the cell that they're located.

Now it turns out that glycolysis and most of gluconeogenesis takes place in the cytosol of eukaryotic cells. The cytosol is basically the space inside the cell that's not inside another organelle. However, pyruvate carboxylase is a reaction that takes place in mitochondria because mitochondria have a particularly high ATP:ADP ratio.

And it turns out, this helps favor the pyruvate carboxylase reaction. And so we'll talk a lot about reactions in the site cytosol versus the mitochondria. But basically, here's glucose to PEP. Whether you do that in one direction by glycolysis or the other direction by gluconeogenesis, that happens in the cytosol as does turning that PEP into pyruvate.

But if you're going to reverse the reaction, eukaryotes take advantage of a different compartment, the mitochondria, with a high ATP to ADP ratio. And basically, in the mitochondria, turn that pyruvate into oxaloacetic acid-- that is, carry out the pyruvate carboxylase reaction. And then that pyruvate carboxylase reaction generates oxaloacetic acid, and then PEPCK can, either in the mitochondria or in the cytosol, generate PEP.

And so here's a place where one can run one reaction-- PEP to pyruvate of t make ATP in the cytosol-- but a different reaction-- pyruvate carboxylase to turn pyruvate into oxaloacetic acid that would then be used for PEPCK to turn that back into PEP and have that take place in the mitochondria.

I want to point out, just so for MCAT exams and things like that, PEPCK is classically defined as a cytosolic activity, at least in terms of gluconeogenesis. Although, there is a mitochondrial isoform of PEPCK. And it's at least somewhat debated. There's some evidence that, at least, in some tissues, that might be important for gluconeogenesis as well. And so that's why I draw it as PEPCK using oxaloacetate to PEP either in the mitochondria or in the cytosol because that's an area of active investigation right now. All right.

Let's start by talking about how does pyruvate carboxylase work? This is an example of a carboxylase reaction, that is, adding a CO2 group to a molecule to make a carbon-carbon bond. That purple CO2 is added to the CH3 of pyruvate of to make oxaloacetate. And how this happens, in many cases, uses a cofactor. And that cofactor is called biotin. Biotin, like many cofactors, is a vitamin. And this provides a useful functional group. In this case, enzymes that use biotin use ATP to basically drive CO2 addition to biotin. And then that CO2 on the biotin is then activated to be transferred to another molecule in a carboxylation reaction.

All right. So what does biotin look like? I will draw it for you. So this, here, would be biotin. I've drawn it in the enolate form of biotin. I'll draw it in a different way in a second. Biotin is typically bound to a lysine molecule in the active site of an enzyme that uses this. And so this, here, is basically a peptide bond between the terminal amino group, the epsilon amino group of the side chain of a lysine in a residue that basically makes a peptide bond to link biotin into the active site of the enzyme that's using it.

Biotin is often drawn in the keto form. And the active part of biotin is basically this top part of the molecule. And so to draw it to see differences between the enolate and keto form, I just want to show that quickly. I drew it in the enolate form because it's easier to see, I think, how it-- so this, here, would be the keto form where biotin is drawn most commonly.

And basically, the reaction, the way biotin picks up a CO2 is as follows. And that is CO2 can exist particularly under basic conditions as CO2 or as-- this is bicarbonate. OK. So bicarbonate-- turns out, some enzymes use CO2 directly. Some enzymes use bicarbonate. It turns out that pyruvate carboxylase uses bicarbonate. So biotin bound in the active site of pyruvate carboxylase will utilize bicarbonate in the following way. And I'll just point out that the pH of the mitochondria is also more basic, which also helps this pyruvate carboxylation reaction.

All right. So what happens is this bicarbonate is phosphorylated by ATP. This phospho intermediate can then react with this active site here of biotin to release the phosphate. And now, you now have this activated CO2 group that's attached to the biotin. Here's pyruvate drawn in the enol form. You can look back in your notes about how to interconvert it between keto and the enol form. I've shown that several times.

And then this, then, can end up adding this CO2 to the end of pyruvate to generate oxaloacetic acid plus regenerate the biotin cofactor in the right form. It'll be the keto form, which can then get back to the enol form. And it's ready to do another catalytic cycle. And so, basically, pyruvate carboxylase uses ATP to produce this carboxylate of biotin. And then uses that to add the CO2 to pyruvate to generate oxaloacetic acid.

Now I want to point out that oxaloacetic acid is both an alpha keto acid like pyruvate was. And it's a beta keto acid. And so this ketone is alpha to that carboxylic acid. And it's beta to that carboxylic acid. So it's both an alpha and a beta keto acid. And it turns out that the way PEPCK works is it takes advantage of the favorable decarbonization of a beta keto acid, which we talked about last time, along with GTP to phosphorylate pyruvate and trap it in the enol form.

And so this is how PEPCK works. So I'm going to draw GTP in this very stylized way-- so guanine with three phosphate groups. There's oxaloacetate. That decarboxylates-- takes advantage of this being a beta keto acid to decarboxylate and add the phosphate. And that's how we can generate PEP. OK. Great. And so that's how you can build a different pathway that uses ATP and GTP for energy input in order to run glycolysis in the opposite direction-- gluconeogenesis.

All right. So how is gluconeogenesis regulated? Well, just like the principles we talked about in glycolysis, it works in a way that makes sense. And so you can guess that the steps that are going to be regulated are exactly the ones that you might guess. They were the same steps that were regulated running glycolysis. It's going to be these big changes where energy changes occur across the pathway, which are also the entrance and exit of the pathway. And basically, regulation has to be reciprocal.

So in glycolysis, you want to increase glycolysis under conditions where you need to produce ATP. And you want to decrease glycolysis if you have enough ATP or enough of another downstream product, such as citrate. All right. Well, gluconeogenesis is going to be exactly the opposite. You certainly don't want to do gluconeogenesis if you need energy release. If you need ATP, you don't want to run gluconeogenesis.

However, you want to increase gluconeogenesis, if the cell has ATP excess, you also want to do it if you have excess of other products like citrate because why go through the trouble of sending things down glycolysis if you have nowhere to put it. You might as well, instead, make glucose or shunt that glucose off to produce glycogen.

And so let me just add that regulation here. And so major regulators of gluconeogenesis are as follows. And so high levels of citrate is going to stimulate fructose 1,6-bisphosphatase. So high levels of citrate will inhibit things coming through glycolysis and activate gluconeogenesis by acting at fructose 1,6-bisphosphatase to match the energy considerations.

If you have high levels of AMP, energy charge is low. You want to stimulate glycolysis at phosphofructokinase. Similarly, that's going to inhibit gluconeogenesis at FBPase. And it turns out, high levels of ADP-- I'm sorry-- yeah, high levels of ADP, low energy charge, also inhibits PEPCK because you also don't want to try to generate PEP under those conditions. Great.

All right. Now in animals, we also want to regulate glucose catabolism and production under signaling control. And this gets back to this other topic we talked about, such as the liver's job being to maintain a constant level of blood sugars. Most of you are very familiar with blood sugar control through diabetes. You've probably heard that this is under hormonal control, that the job of insulin is to lower blood glucose. And you also have hormones like epinephrine or glucagon whose job is to raise blood glucose.

Why do you want to raise blood glucose? Well, if you have some kind of fight-or-flight response-- you see that lion, and you need to run away, your body has this adrenaline rush. That's what epinephrine is. That basically wants to supply more energy to your muscles, so that you can run away effectively.

So it turns out, both insulin and glucose act on lots of tissues in the body. I'm going to focus on what they do in the liver. And that's because in the liver, this is a major organ that regulates blood glucose levels. So if you have excess glucose around, what do you want to do? You want to stimulate glucose uptake into cells, its metabolism, and its storage. And so insulin with high blood sugar basically wants to stimulate, taking glucose into the liver and storing it as glycogen.

If you want to run away from the lion, well, now you have epinephrine around. You want to make sure that as you're consuming that glucose in your blood that you're continually making more. And so you want the epinephrine to stimulate release of glucose either from gluconeogenesis or from the breakdown of glycogen. And both of these hormones act-- insulin and epinephrine-- act at a level where they can regulate enzymes of glycolysis and gluconeogenesis as well as entry and exit of glucose monomers in and out of glycogen.

And so this is an example of how fructose 2,6-bisphosphate works. And so remember you learned from Professor Yaffe that epinephrine acts via cyclic AMP signaling, which turns on a kinase-- protein kinase A. And that protein kinase A can regulate enzymes that produce or break down, basically, these enzymes that produce or break down fructose 2,6-bisphosphatase which, in turn, regulates PFK activities such that you can match your need to burn that glucose versus doing gluconeogenesis in the case of the liver, so you can do enough gluconeogenesis in order to have glucose around for the body to use in that fight-or-flight response being driven by epinephrine.

Now a major effective insulin and epinephrine signaling, though, is actually on release or storage of glucose molecules in glycogen. And I alluded last time in talking about the regulation hexokinase that glucose 6-phosphate is basically the entry point to get glucose units in and out of glycogen. And I want to discuss how you add and subtract those glucose units into storage polymers. Now I'm going to discuss it in the context of glycogen storage in humans. But remember, plants actually store things also as glucose polymers. The chemistry is very similar. But, obviously, the regulation is very different.

Now you'll hopefully recall from past lectures that these storage polymers of glucose-- glycogen in humans, starch in plants-- are basically these alpha 1,4 linkages of glucose molecules. Remember, there was a non-reducing end and a reducing end of the molecule. So starch was a straight-chain polymer. Glycogen had these alpha 1,6 branch points that made this branch polymer, where there's lots of non-reducing ends and a single reducing end. And each of these non-reducing ends is a polymer of glucose molecules with this alpha 1,4 linkage that, at the branch point, has this alpha 1,6 linkage to make this long-chain branched polymer.

Now when we discussed this at the time, I mentioned that this is useful because you have all these non-reducing ends that can be used to add or subtract glucose monomers. And that's great. This is a nice compact form of storage with lots of places to put or remove glucose from. You either store it quickly, insulin driving glucose storage-- or remove it quickly, epinephrine driving release of glucose from the glycogen.

And so if we're going to have a way to add glucose polymers or break them down, those are two separate pathways, just like glycolysis and gluconeogenesis-- reciprocal activities, but we need two pathways to do it because delta G has to be less than 0 for each pathway to work. Also, we have to be able to regulate these separately because we don't want to have a futile cycle.

Now the way you add and subtract glucose polymers to glycogen acts through glucose 6-phosphate being first converted to glucose 1-phosphate. So this, here, is alpha glucose 6-phosphate. Remember, it's alpha because I drew that hydroxyl at the one position pointing down. This, obviously, can enter glycolysis and come in from glucose via hexokinase. All right.

This is first converted via a mutase reaction to move the phosphate from the 6 to the 1 position of glucose. So this, here, is glucose 1-phosphate. The way the mutase reaction works is exactly the same analogous mechanism that I explained for phosphoglycerate mutase in glycolysis-- moves the phosphate in this case from the 6 to the 1 position of glucose.

And then once you have that glucose 1-phosphate, that can be added to a non-reducing end of a glycogen polymer using an enzyme called Glycogen Synthase, GS. So that'll give me a glycogen molecule with one additional monomer added to the non-reducing end of the polymer. And you release that glucose back off via a different pathway, a different enzyme called phosphorylase. All right.

Now the activity of these enzymes-- glycogen synthase and phosphorylase-- that is, the pathway to add glucose 1-phosphate glucose monomer to the polymer versus remove it from the polymer is under hormonal signaling control in animals. And basically, that hormonal signaling works as follows.

So phosphorylase can be phosphorylated or dephosphorylated via a signaling enzyme, an enzyme like phosphorylase kinase, which is downstream of PKA, which is downstream of epinephrine signaling. OK.

And so when phosphorylase is phosphorylated by phosphorylase kinase-- that has, has a phosphate group added is a signaling cascade-- it is in the active state. And when it's dephosphorylated, it's in the inactive state. Well, glycogen synthase also is subject to regulation by protein phosphorylation on the enzyme by a kinase. Except this has the opposite relationship.

So when it is in the phosphorylated state, it is inactive. But it is in the non-phosphorylated state, it is active. And so protein kinase A, which is downstream of epinephrine, can both activate phosphorylation of phosphorylase and glycogen synthase. And that makes sense. You want to release glycogen glucose monomers from glycogen. You turn on, by phosphorylation, the pathway to release them. And you turn off the pathway to store them.

Another kinase that phosphorylates to glycogen synthase is a kinase called GSK or Glycogen Synthase Kinase. This kinase is inhibited by insulin signaling. And so this is a negative of a negative. So effectively, a negative of a negative will keep it in the inactive state.

And so insulin, by inhibiting the ability of GSK to put glycogen synthase in the inactive state, will make glycogen synthase active. And when you have high insulin around, that actives glycogen synthesis, and you store glucose monomers as glycogen. Get them out of the blood. OK. Now let's discuss the chemistry that allows us to do this glycogen synthase and phosphorylates reactions.

Now as we go through this, what you will see is that when we break down the polymer, energy is released. So that's going to be breaking down a polymer. That's the right direction of entropy. And so that's going to be released. And storing it is going to require energy. That is, you're going to have to have some energy input to build a polymer. But nature actually does this in a way that is actually quite efficient.

First, let's talk about how you store glucose 1-phosphate units by adding them to glycogen. So this uses energy input from UTP, which is an ATP equivalent for exactly the reason I described earlier. That is, because ATP plus UDP, like any other interconversion of nucleoside triphosphates and diphosphates is very close to equilibrium.

So the UTP to UDP ratio should be similar to the ATP the ADP ratio. And so UTP is really an ATP equivalent. But nature, for whatever reason, decided to use UTP here. And it generates a nucleoside sugar called UDP glucose. And so let me show you what that is. So here is glucose 1-phosphate. I will draw a stylized version of UTP here.

And so this molecule with the UDP added to the glucose is UDP glucose. This will also generate a pyrophosphate. And that pyrophosphate can be hydrolyzed to generate two inorganic phosphates, effectively, like building any other polymer, by doing this downstream-- keeping that product of the first reaction low helps pull that reaction forward for polymer synthesis. This UDP glucose can now react with the non-reducing end.

So this, here, will be the non-reducing end of the polymer. That's the OH group at the 4 position, at the non-producing end. And so that then generates that alpha 1,4 bond to add to the polymer. So here, we have glycogen n plus 1 plus releases UDP.

And so the net to add a glucose 1-phosphate to the non-reducing end of the growing glycogen polymer is net conversion of a UTP to a UDP. So that is one ATP equivalent to add glucose 1-phosphate to the end of the polymer. Now, of course, you had it to also have an ATP from hexokinase to capture that glucose 6-phosphate to begin with. But effectively, this is two ADP molecules to add a glucose to glycogen. All right.

Now if we're generating starch, that's all we have to do, just build a long polymer. But if we're making glycogen, remember, glycogen has all of these branch points on them as well. And to do the branch points, you basically have to make this alpha 1,6 bond, which then gives you two new non-reducing ends that you can add more subunits to.

The way nature does this is as follows. And so if I just draw this here in a stylized way, it turns out, once you get to about seven units here in the growing polymer-- so this is the reducing end, and this is the non-reducing end of the polymer. And so the polymer is growing by adding things to that end.

Once you get about seven, there's an enzyme that will basically cleave this, and move it and make a new alpha 1,6 bond. OK. Here we go. We have our reducing and our non-reducing end and basically transfers these 7 units over. And now, we have two non-reducing ends that we can continue to grow the polymer from.

You can think of this like the airplane analogy. When you load an airplane, at least the way that's most efficient, the people who are sitting in the back of the plane, they get on first. But then, they're the last people off the plane because you add basically to these non-reducing ends, and then you subtract from those non-reducing ends. And so the first glucose added, the one at the reducing end, is the last one that is going to be removed. All right.

Now phosphorylase is the enzyme that allows you to do the opposite-- that is, to break that alpha 1,4 bond and release monomers. And it's called phosphorylase because it uses phosphate to break that alpha 1,4 bond, taking advantage of the fact that breaking down the polymer is favorable and actually releasing the glucose from it again is a glucose 1-phosphate.

And so at the non-reducing end of the molecule-- so this, here, would be the non-reducing end of the glycogen polymer. You basically have a phosphate molecule that takes that off, so you have a glycogen n minus 1 plus a glucose 1 phosphate, which can now undergo a mutase reaction back to glucose 6-phosphate that could then be released from the cell if it's a liver, or it could be burned in glycolysis.

Now, obviously, if you're doing breakdown, that is, your chewing back from non-reducing ends, eventually, you're going to hit a situation where you come to where there's just a nub here with an alpha 1,6 bond there. That is, chew it back till you hit this single monomer with a non-reducing end here to this alpha 1,6 break point. Turns out, you just cleave off that nub, if you will.

And so that nub is then just released as free glucose. So other than release of that nub, what it means is that you actually basically get no ATP to actually break down in the phosphorylase reaction. So the net cost to store a glucose 1-phosphate molecule is one ATP via the UTP to UDP conversion in the glycogen synthase reaction. And you get that one glucose 1-phosphate back. And so two ATPs to put a glucose tin glycogen.

And then you get it directly back out as a glucose 1-phosphate. That's a phosphorylated glucose that can then enter glycolysis. So that's one less phosphate you need to spend on glycolysis. And so it's incredibly efficient to store glucose in that way. And so it's actually slightly greater than one ATP to store it as glycogen because you lose in ATP with that nub removal at the branch polymer. But for all intents and purposes, it costs one ATP to store your glucose as glycogen, which is pretty amazing.

All right. Now before I leave the topic of sugar storage, I want to make some comments on some sugars that are stored in forms other than glucose. And want to talk about these because these sugars are, of course, important parts of our diet. And so as we've mentioned in prior lectures, two of these sugars are sucrose and lactose. So remember, sucrose is a disaccharide of glucose plus fructose. And lactose is a disaccharide of glucose plus galactose. So those are storage forms of sugar-- sucrose in the case of plants, lactose in the case of milk made by mammals.

And so to break those sugars down, basically, you break the disaccharide. Now, you have the glucose molecules you know how to handle. That's glycolysis. But I want to spend a little bit of time how you deal with the fructose and the galactose that you get out from these sugars because this actually relates to some things that I'm sure you've all read about in the news.

For instance, fructose as a sugar in our diet is actually quite controversial. Lots of stuff out there that says fructose was cooked up in the devil's kitchen and is this evil thing. There's actually others who argue that, oh, fructose is the same as glucose, the same number of calories. There's both sides of the debate. Both sides are actually making factual claims. And I want you to understand the biochemistry, so you can actually judge for yourself who's right in those claims. All right.

So let's start with talking about how you metabolize fructose. And so fructose is first captured by phosphorylation to generate fructose 1-phosphate. This is carried out by an enzyme called ketohexokinase. Ketohexokinase is an enzyme this present in your gut and your liver. And so fructose in the diet, from breaking down sucrose or from high-fructose corn syrup, whatever, is captured with ketohexokinase to make fructose 1-phosphate. Turns out that phosphofructokinase, our old friend from glycolysis, is less efficient than ketohexokinase but can also carry out this reaction. And remember, what is it do in glycolysis? It adds a phosphate to the 1 position of fructose 6-phosphate. Well, it can also add a phosphate to the 1 position of fructose give you fructose 1-phosphate.

OK. Once you have this fructose 1-phosphate, which, remember, is not in glycolysis, it can be a substrate for the glycolytic enzyme aldolase. What does aldolase do in glycolysis? It splits FBP into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Well, here, you only have a phosphate on the 1 position. And so if you look back at how aldolase works, what you'll see is that if aldolase acts on fructose 1-phosphate, you'll generate a dihydroxyacetone phosphate. That phosphate will go to the dihydroxyacetone half, which, of course, is perfectly good substrate for glycolysis.

The other product of aldolase acting on fructose 1-phosphate is just glyceraldehyde without the phosphate on it. And then there's another enzyme that will phosphorylate glyceraldehyde to generate glyceraldehyde 3-phosphate, which can then also, of course, enter glycolysis. And so fructose is a hexose just like glucose. You generate two trioses-- DHAP and glyceraldehyde 3-phosphate.

That costs you two ATP to do it, exactly the same cost is what you spent to get glucose to make DHAP and glyceraldehyde 3-phosphate. And so people are absolutely right when they say metabolizing fructose has exactly the same caloric cost as metabolizing glucose-- two ATPs to get it into the trioses. And then whatever you get out of it, you get out of it two ATPs, four ATPs-- so net two ATPs if you just do fermentation, more if you do complete oxidation. But the bottom line is that from that standpoint, you get exactly the same number of calories from burning glucose versus fructose.

However, I want to point out that the regulation will not be the same. And that's because in this fructose metabolism, you will notice that there was no glucose 6-phosphate and no FBP generated. That means you are avoiding all of the feedbacks that control how much glucose you send to glycolysis-- first to glycogen. Remember, that's what glucose 6-phosphate does, acting on trapping sugar. And more importantly, you're actually not using phosphofructokinase in a way to fit with the rest of the feedback regulation. I also show that here on this slide. It's basically a better version of what I drew up here.

And I now want to point out this storage of citrate and this feedback regulation of citrate. So we'll talk about the specifics of this more in other lectures. But it turns out, citrate is the precursor for how one stores carbon as fat. And so if you're putting fructose glucose into the system and citrate is too high, it'll shut off phosphofructokinase and stop carbon coming down the system, shunting it instead to glycogen or just saying don't give me any more glucose at all.

If I'm doing the same thing with fructose, those feedbacks don't exist. Citrate doesn't feed back on any of the enzymes that are regulated for fructose metabolism. And so the idea is carbon keeps getting dumped into citrate, which ultimately ends up as fat. And this really underlies, at least on a theoretical basis, why there may be a connection between fructose and obesity or metabolic syndrome.

I've posted articles on Stellar, one from *The Popular Press,* one from a scientific journal that discusses the controversy, discusses this. But now, at least, you have the background to draw some of your own conclusions. OK. That's fructose metabolism. What about galactose metabolism?

Well, galactose metabolism interfaces with, effectively, glycogen metabolism or, at least, glucose 1-phosphate-- or sorry, UDP glucose and does so in a way that is a little bit non-intuitive. But it's actually very important to understand galactose metabolism, at least for those of you who are looking to go to medical school.

It turns out that galactose metabolism and issues with metabolizing galactose are what underlie a set of rare genetic diseases leading to a set of conditions called galactosemias. I guess, if you're a pediatrician, you may encounter these. If you're not, these are quite rare. Although med schools love to ask about these, and certainly, they're very popular on things like board exams. So it's something you should know about. And so I just want to briefly introduce galactose metabolism to you.

So this is galactose. Like all sugars, galactose is initially trapped by phosphorylation. It's phosphorylated on the 1 position to trap it, just like fructose. And so this is galactose 1-phosphate. Remember, galactose is an epimer of glucose. So it differs from glucose here by the stereochemistry of the hydroxyl group at this 4 carbon. And so the way galactose, once it's trapped as galactose 1-phosphate is metabolized is it will react with UDP glucose.

So here is UDP glucose from glycogen metabolism drawn in a very stylized way. That will generate a UDP galactose and a glucose 1-phosphate, which, of course, can enter glycolysis, can be stored as glycogen, et cetera. This UDP galactose can then be turned into UDP glucose via an epimerase reaction. And so remember, the epimerase reaction needs to change the stereochemistry of the hydroxyl group at this 4 position, such that it's pointing up in galactose or pointing down in glucose.

The way epimerases work is that they require a cofactor. That cofactor is NAD. and they work in the following way. And so if I just draw, here's the 4 position of galactose, the 4 carbon of galactose, with the hydroxyl group pointing up, an epimerase can basically remove a hydride from the bottom face of the molecule, transfer those two electrons to NAD to generate NADH.

Now, you get this lactone intermediate at the 4 carbon. And then you can have two electrons from an NADH can be added to the opposite face of the molecule that regenerates an NAD+. And now, we've effectively changed the stereochemistry at the 4 carbon to now be pointing such the hydroxyl points down, and that's glucose. And so the way epimerases work is they basically remove and add electrons from different faces of the pyranose ring. And that changes the stereochemistry of which direction the hydroxyl group is pointing.

And so effectively, this is how we metabolize all kinds of different sugars, either putting them in glycogen or other starch, some other storage carbohydrate that we can get them later, and then use them in glycolysis as a way to drive glucose oxidation as a way to release energy and keep ATP high in the cell. All right.

Now I want to shift topics and begin to discuss how we can oxidize pyruvate from glycolysis to make CO_2 as an alternative to how we can release energy from carbohydrate oxidation rather than just use fermentation. Now you'll see that the enzymes and ways that we do this is going to also be how we make citrate. And so this isn't just about releasing energy. It's also about storing energy because you'll see, citrate is a precursor for storage as fat.

The reactions downstream of pyruvate oxidation also make lots of other useful intermediates that can be used to synthesize other things cells need-- amino acids, nucleic acids. And so this pathways we're going to discuss are going to come up over and over again throughout the course. Now to facilitate this discussion, I'm really going to at least initially focus on catabolism.

And so just to remind you, if we do glycolysis by turning glucose pyruvate, this involves oxidation. So we need to dispose of those electrons somewhere. In other words, that NADH has to be regenerated NAD. Fermentation of pyruvate into something like lactate is a way to do that and allows net ATP production from glycolysis without the need for any oxygen.

However, if we're going to completely oxidize that pyruvate CO_2 we're going to generate many more electrons as waste that we have to put somewhere. Oxygen is a particularly good electron acceptor. And so we can reduce oxygen to water as a way to deal with that electron waste, and that allows us to, just burning wood, release lots of energy from glucose by transferring those electrons ultimately to oxygen. And of course, that same process-- oxygen to water-- if we're using the pyruvate to fully oxidize it, also is necessary to regenerate the NAD to run glycolysis.

Now ultimately, the series of reactions that allow chemically conversion of pyruvate to CO_2 is called the TCA cycle for try Tricarboxylic Acid Cycle or sometimes referred to as the Krebs cycle and also is referred to as the citric acid cycle-- so TCA, Tricarboxylic Acid Cycle, Krebs cycle, citric acid cycle-- all synonyms for the same thing.

What these cycles are is basically a way to take two carbon units. And those two carbon units can come from the oxidation of pyruvate or from the oxidation of lots of other fuels. And those two carbon units are combined with four carbon units. You'll see oxaloacetate is the four-carbon unit-- oxaloacetate gluconeogenesis. That oxaloacetate could come from the pyruvate-carboxylase reaction. Or it could come, as you'll see, from the TCA cycle itself.

This generates a six-carbon molecule, which is citrate. Citrate is a tricarboxylic acid, hence the citric acid cycle or the tricarboxylic acid cycle. And then those six carbon units are reoxidized, releasing two CO_2 molecules, back to four-carbon oxaloacetate. And hence, this is a cycle. Now this cycle was initially described by Hans Krebs, who predicted the cycle largely based on chemistry-- one of the most amazing feats in the discovery of metabolism. Turned out, he was right. And thus, it's also referred to as his name, the Krebs cycle.

Now lots of oxidation is happening in this cycle. You're oxidizing the molecule, releasing CO_2. That means lots of energy is released. Those electrons ultimately will be transferred oxygen. And so we can use this to make lots of ATP and do lots of other work.

Where are these reactions all occur is in the mitochondria, specifically in the matrix of the mitochondria. Remember, the mitochondria, from cell biology, is a double-membrane organelle. The innermost part of the mitochondria is referred to as the matrix. And these have conditions that favor pyruvate carboxylase reaction, as I talked about, but also favor other oxidation reactions, such as those that occur in the TCA cycle.

And so next time, what I will start with is by being more explicit about what chemistry is happening to allow these two carbon units to combine with oxaloacetate 4-carbon to generate citrate and go over the reactions that then allow you to run a cycle to regenerate oxaloacetate, that is, run the TCA cycle as a way to oxidize carbon. Thanks.