[SQUEAKING]

[RUSTLING]

[CLICKING]

MATTHEW VANDER HEIDEN: OK. Today we will start our discussion of-- with a discussion of glycolysis as a pathway and how we can use favorable oxidation of glucose to enable phosphate addition while at the same time producing useful intermediates that enable us to do ATP synthase-- ATP synthesis and maintain a high ATP-ADP ratio in cells.

Now I drew up here the entire glycolytic pathway, just like you saw at the end of the last lecture, and I want to go through it here step by step. And so the first step in glycolysis, as we already talked about, is we have glucose. This here is, of course, alpha-D-Glucose in the-- I drew it in the alpha form. If the OH was pointing up, that would be the beta form, just some quick review. I'll drop the alpha and beta and the stereochemistry denotion going forward.

This glucose molecule, as we discussed previously, is first trapped in cells by being phosphorylated by the enzyme hexokinase, sometimes abbreviated HK, to give glucose 6-phosphate. Not shown here, this glucose 6-phosphate then undergoes an isomerase reaction via the open chain form. Again, we discussed this reaction when we discussed sugars.

And so this would be catalyzed by glucose phosphate isomerase, and takes it from the aldose to the ketose, and now you have the fructose-- the ketose fructose 6-phosphate shown here in the furanose form. This fructose 6-phosphate is then phosphorylated a second time by an enzyme called phosphofructokinase, typically abbreviated PVK. It's phosphorylated on the 1 position to give fructose 1,6-bisphosphate, shown here.

That fructose 1,6-bisphosphate can then be cleaved via the enzyme aldolase. Cleavage takes place in the open chain form which I drew up here in the corner. Basically splitting the molecule in half to form two trioses, one of which is the ketose dihydroxyacetone phosphate, basically the top half of the molecule. The other is the bottom half of the molecule, the aldose glyceraldehyde 3-phosphate.

These two trioses can be interconverted, the aldose and the ketose via on isomerase. The enzyme that does that is called trioses phosphate isomerase. And moving forward basically takes the dihydroxyacetone phosphate that comes from the top half and can turn all the carbon from fructose 1,6-bisphosphate into two glyceraldehyde 3-phosphates.

That glyceraldehyde 3-phosphate, as we discussed last time, is the substrate for the GAPDH reaction, the glyceraldehyde 3-phosphate dehydrogenase, GAPDH. which, as we discussed last time, couples oxidation of this aldehyde to this acid with phosphate addition. That oxidation reaction, the electrons have to go somewhere, that's where the cofactor NAD comes in. Picks up two electrons, generates NADH, and in the process, this enzyme generates this molecule, 1,3-bisphosphoglycerate or 1,3-BPG.

Remember, that 1,3-BPG molecule was one of the useful intermediates whereby we could undergo favorable synthesis of ATP even at the high ATP-ADP ratio found in cells, and that's because we can transfer the phosphate from this acid group onto ADP. That gives us an ATP and the product 3-phosphoglycerate 3-PG carried out by the enzyme phosphoglycerate kinase.

That 3-PG molecule can then undergo what's referred to as a mutase reaction. And so the phosphate is moved from the 3 to the 2 position of glycerate. So 3-phosphoglycerate to 2-phosphoglycerate, carried out by phosphoglycerate mutase, often abbreviated PGAM. This 2-phosphoglycerate can then undergo a dehydration to-- by an enzyme enolase to give the molecule phosphoenolpyruvate. . Phosphoenolpyruvate was the second of those useful intermediates that allow ATP synthesis despite the high ATP-ADP ratio in cells, and that reaction, as we already discussed, is carried out by pyruvate kinase and allows you to generate another ATP.

Now, as I drew out the glycolytic pathway here, I also listed the delta G0 prime for every single step in the reaction. Remember, this is informative as to the equilibrium for each of these individual reactions. You'll note that for many of the steps, that delta G0 prime is positive. Remember what that means. That means that equilibrium favors the left. That is the reverse direction of the entire pathway.

However, if you add all of these up, the delta G0 prime for the entire pathway-- so delta G0 prime for the entire pathway, glucose to pyruvate adds up to negative 6.9 kcals per mole. And so that negative 6.9 means that for the entire pathway, equilibrium favors the right. So glucose to pyruvate, the right direction. And that is why this can work as an overall pathway even though some of the individual steps, equilibrium may favor the opposite direction.

Now you'll notice that there's several favorable steps where delta G0 prime is quite negative. Pyruvate kinase, phosphoglycerate kinase, hexokinase, phosphofructokinase. As we talked about before, these are able to pull the less favorable steps upstream forward by keeping concentrations of products such as 1,3-bisphosphoglycerate low, thus allowing this net reaction to move forward.

You'll notice I drew three steps here as irreversible-- hexokinase, phosphofructokinase, and pyruvate kinase. Remember, these are all reactions in our-- there's no such thing as an irreversible reaction. There are some conditions where the reverse reaction, of course, can happen. Although remember, this-- when we discussed irreversible in metabolism, what we're referring to is irreversible under physiological conditions-- that is, conditions found in cells.

You'll notice that those three steps are three of the four steps that have very negative delta G0 prime. And so you would expect that if we were going to reverse those reactions, you would need some kind of energy input, and you'll see later that these are the control points or also regulated steps in this pathway. And there are also the sites, just to give you a preview, that we're going to have to deal with if we're going to try to synthesize glucose, gluconeogenesis, the reverse of glycolysis.

Now of course, these delta G0 primes are very informative of what happens at equilibrium, but of course, what actually happens in a cell or in any condition, remember, is dependent on the equilibrium constant, but also on the conditions. Remember, it's delta G, delta G equals delta G0 prime plus RT times the log of the products over the reactants. And so delta G is what really matters for any individual reactions, and what's shown here on this slide is basically a approximate delta G0 change across the glycolytic pathway based on someone's approximation of the conditions found in cells.

Now we can see and looking at this that there's really three big drops in delta G across the pathway. Here's the hexokinase reaction, here's the phosphofructokinase reaction, here's the pyruvate kinase reaction. And so it makes sense that these would be, then, the irreversible steps because there is what is actually pulling the pathway forward.

It also makes sense that one would exert control at each of these steps, because this is what drives the flux of the pathway forward, but it's also where it's difficult to go back. Once you get here on this flat part of the curve energetically it doesn't matter so much which direction you go along the curve, but to try to climb back up each of these hills becomes difficult. And so we'll revisit this later in the lecture, but this is basically where the control points will end up happening in this pathway.

Now, I want to note a couple other features about this pathway, and that is that we had to invest some energy early. You see you cost an ATP at the hexokinase and the phosphofructokinase step and then you harvest that ATP much later at the phosphoglycerate kinase and the pyruvate kinase steps.

The textbook typically will split glycolysis into what they refer to as two stages, an investment stage and a harvesting stage, and that basically relates to that fact. And that's because you have a glucose molecule that then you use to generate two trioses. And then each of those trioses can be used to generate a pyruvate molecule.

And so it costs 2 ATP to generate those to trioses, but then each triose generated allows you to recapture 2 ATPs. And so what that means, then, is that the net output from glycolysis is 2 ATPs. And so glucose going to two glyceraldehyde 3-phosphates costs you, whereas using those two glyceraldehyde 3-phosphates to generate two pyruvates allows you to recover 4 ATP for a net of plus 2 ATP molecules produced per glucose metabolized.

Now if we add up the entire stoichiometry of what's going on here, we can also draw it another way, and that is we can basically have glucose plus 2 ATP molecules plus 2 inorganic phosphates, goes to 2 pyruvates plus 2 ATP, and that's because we're able to incorporate 2 phosphate molecules here at the GAPDH step.

Now that's great, but if you're paying attention, you notice that we're missing one other thing in this stoichiometry, and that's that cofactor NAD that was also required to maintain electron balance across the GAPDH step. And so remember that for these pathways to work, we have to balance all things that are going in with all things are going out-- we can't create or destroy matter. And so those electrons have to be dealt with. When we discussed the GAPDH reaction last time, we needed that cofactor NAD in order to act as an acceptor for the electrons to allow that oxidative phosphorylation, if you will, of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.

And so in order for this pathway to work, we need a way to regenerate that NADH back to an NAD in order to make electron balance. In other words, we have to deal with the fact that we've generated-- or that we required 2 NAD+ to run this pathway and generate 2 NADH. And so we need a way to get rid of those electrons from NADH to regenerate that NAD and allow the pathway to be balanced.

Now it should be clear that this has to happen stoichiometrically for every single glucose molecule that goes through glycolysis. That is, we have to regenerate 2 NAD plusses for every glucose that we're going to convert into 2 pyruvates. And so for this NADH reoxidation to occur, we need a place to transfer those two electron pairs.

And so just like any oxidation and reduction reaction, if we're going to oxidize NADH back to NAD+, something else has to be reduced, and that thing has to be reduced stoichiometrically with every single pyruvate that's produced from glucose and glycolysis. And effectively you will see that this is the role of fermentation. It's an ability for us to solve this redox problem, that we have to dispose of those electrons in the oxidation at the GAPDH step in order to deal with this electron waste of the pathway.

Now, the useful way to do this is that we're generating a pyruvate stoichiometrically. And so we can use that stoichiometrically, that product pyruvate by reducing it to form the waste product, and that's effectively what fermentation is. It's turning that pyruvate into ethanol or lactate, which is reducing that pyruvate, picking up the electron waste from the NADH, and allowing you to regenerate the NAD that's required to run the GAPDH step.

And so I'll just illustrate this. The simplest fermentation reaction that we can do is the run that's done by animals, and that's to generate lactate. And so to just illustrate this explicitly, here is-- so this here would be the reduced form of NAD, NADH. So this here this-- this is the nicotinamide group. Remember, it's hooked up to an ADP ribose to give nicotinamide adenine dinucleotide in the reduced form.

So this can transfer electrons to pyruvate. Here's pyruvate. So as we showed last time, we can take 2 electrons from NADH reduced. That gives us this hydride ion, which can generate lactate. Plus, what we're left with is the oxidized form of the cofactor NAD+.

And now this NAD+ can be reduced back to NADH at the GAPDH step, and the electrons from NADH, as it's being reoxidized to NAD, can go and take pyruvate and reduce it to lactate. So NAD gets reoxidized to NAD. Pyruvate now gets-- the ketone gets reduced to the alcohol as you go from pyruvate to lactate.

Opposite of what's going on in the GAPDH step. There, the aldehyde is oxidized to the acid, while NAD+ oxidized goes-- is reduced to NADH. Here, NADH is reoxidized to NAD while taking the pyruvate and re-reducing it to lactate. This, by adding here, allows us now to regain balance across the entire pathway. This step is catalyzed by the enzyme lactate dehydrogenase, often abbreviated LDH. And that allows regenerating the NAD+ that's needed for the GAPDH step, which allows us to deal with the stoichiometry problem of the entire pathway.

And so what fermentation does for cells is allows electron disposal by taking the product of the pathway pyruvate, reducing it stoichiometrically to form a reduced product-- as shown here, lactate, and that allows you to maintain electron balance across the entire pathway. And so this entire fermentation pathway, glucose to lactate, now allows the generation of the net generation of 2 ATP and run this pathway in cells so cells can maintain a high ATP-ADP ratio by fermenting glucose into lactate.

Now, if you're paying attention, this also now begins to describe why it is that oxygen is such a useful molecule for metabolism and actually why it's quite key to supporting the bioenergetics of cells. And so recall, oxygen was there when we burned wood and we used that example. And why oxygen is so important for burning wood, why it's important for doing glucose catabolism in our cells is that oxygen is a really great electron acceptor.

That is, I can draw out oxygen can take 2 electrons that are generated as NADH waste, if you want to call it that, plus protons, and this generates water. And so an alternative to fermentation in order to allow glucose oxidation is rather than do fermentation, is to transfer those electrons to oxygen to run the pathway another way.

In other words, if we write glucose to pyruvate and realize that this is an oxidation reaction, and so therefore, generates electron waste in the form of NADH that has to be recycled to NAD, we can do so by putting those reoxidizing NADH, means something else has to be reduced. This something else could be oxygen as a good electron acceptor to be reduced to water and find an alternative place to put those electrons. Or, if oxygen is not present, now we can do fermentation, turning pyruvate into lactate.

It should be clear that if we want to completely oxidize glucose, as in burning wood-- that is, turn it-- all the carbon and glucose into the most oxidized form of carbon, CO2, this also requires places to put electrons. That is, we could generate more NADH. And of course, each of those electrons have to be dealt with. And the final home for those electrons needs to be something that's a good electron acceptor like oxygen, allowing you to continually reoxidize your NADH by reducing oxygen to water.

So, in the absence of fermentation, oxygen can be used to maintain electron balance, and of course, much more energy is going to be released if we completely burn glucose than if we only partially oxidize glucose. And this is why oxygen allows more energy to be released, or, as you probably learned in high school, more ATP to be produced from glucose metabolism.

Now the details of how all of this works are much more complicated than what I have drawn. Obviously fermentation happens the way I drew it, but all the other details about the role of oxygen and how it fits in to these pathways is more complicated than what I've drawn, and we'll cover this in the upcoming lectures.

But the key concept is here, and that is the relationship between oxygen and fermentation is really what's illustrated here. These are two different ways that cells can use to dispose of the electron waste that's produced from carbon oxidation and glucose metabolism. Those electrons have to go somewhere. They can go to the product of glycolysis, say pyruvate to lactate, by lactate dehydrogenase, reduce the pyruvate to lactate, fermentation, or they can go somewhere else. A great electron acceptor like oxygen which allows these pathways to happen while saving the pyruvate to do something else, like be completely oxidized to CO2.

Now it should be clear here, then, that one of the things that's special about fermentation is that absolutely no oxygen is required to do this, and this is part of the reason why this pathway is so ancient. Fermentation evolved in the pre-oxygen atmosphere, and that really is why it's such an ancient pathway. Only when oxygen levels rose in the atmosphere due to photosynthesis that enough oxygen was around that then other oxidative pathways could evolve as an alternative to fermentation.

Now, you'll note that the original description of fermentation by Pasteur was ethanol, not lactate. We'll cover the details of ethanol in a minute, but I just want to point out that making ethanol instead of lactate is simply an alternative product to dispose of pyruvate as a more reduced product. So ethanol is a more reduced product than pyruvate in that ethanol generation is an additional adaptation that allows you to dispose of the electron waste while also generating a molecule that's toxic, and therefore, allow organisms that make ethanol to better kill off their neighbors and their environment and compete for resources. But the overall concept is identical. Ethanol is really an alternative to lactate and fermentation but solves exactly the same problem.

I want to talk about ethanol metabolism in more detail, but before we do that first I want to go through and just describe at a very high level some of the chemistry that allows all these reactions in glycolysis to work, because you'll see that the chemistry itself is not all that complicated even though we can draw it and it seems like somewhat overwhelming when drawn out as a whole pathway.

Now I want to point out, we've already covered the chemistry for a lot of these steps. First of all, there's two dehydrogenase reactions. There's GAPDH and there's lactate dehydrogenase. Here's the mechanism for lactate dehydrogenase, it's a hydride transfer. The same thing happened at GAPDH with the additional details of adding the phosphate. We covered that previously in an earlier lecture.

Note, we also covered before the chemistry of pyruvate kinase and phosphoglycerate kinase. In addition, there's two more kinase reactions that exist here, hexokinase and phosphofructokinase. These are really just straightforward phosphotransfer reactions using ATP to transfer a phosphate. Very straightforward, exactly what Professor Yaffe has already talked to you about, about how one does phosphotransfer for kinase reactions on proteins.

Now, two other steps are isomerase reactions, triose phosphatase isomerase and glucose phosphate isomerase. We discussed this chemistry back when we did the sugar lectures. This is just acting on the open chain form of the carbohydrate and allows you to interconvert between the ketose and the aldose as I already drew during that lecture.

And so that just leaves a few steps left to discuss that we can cover briefly. The first one that we'll talk about is this enolase reaction, which is simply a dehydration. I'll draw it here just to be explicit as to what's going on. So here is 2-phosphoglycerate. Simply dehydration to remove water, and that gives us phosphoenolpyruvate, PEP. The next step I want to discuss, phosphoglycerate mutase, is an example of a mutase reaction that is, remember, it's moving the phosphate from the 3 to the 2 position of glycerate.

This is a class of reactions. It has somewhat of an interesting mechanism. And basically precedes by a phosphorylated enzyme intermediate. In the case of phosphoglycerate mutase, this is a histidine, and so there is a histidine with a nitrogen on it in the active site. And effectively, this nitrogen can pick up a phosphate and be primed via a reaction involving a molecule you've seen already from your discussion of hemoglobin with Professor Yaffe, and that's 2,3-bisphosphoglycerate.

And so again, here's-- this is glycerate phosphorylated on the 2 and 3 position. So this is 2,3-bisphosphoglycerate. And effectively, this molecule can bind in the active site and pick up a phosphate from either of the 2 or the 3 position. I drew it here is picking it up from the 2 position. That would, of course, generate a 3-phosphoglycerate. But more importantly, it ends up with the enzyme having this phosphorylated intermediate in the active site.

Once it has that phosphorylated intermediate in the active site, now it's ready to catalyze the mutase reaction. So I'll draw here first, this here would be 3-phosphoglycerate. Color the phosphate so you can see what's going on.

This would basically, in the active site of the enzyme, pick up the phosphate from the active site and generate transiently this 2,3-bisphosphoglycerate intermediate, and then it can retransfer the phosphate from the other position back on to the active site of the enzyme so it's ready to carry out another catalytic cycle, and in the process, move the phosphate effectively from the 3 position to the 2 position.

So you'll see that the phosphate is not actually being-- the same phosphate is not being moved within the same molecule, it's being ping-ponged back and forth off of this phosphate enzyme intermediate, and basically this is how cells catalyze various mutase reactions to move phosphates between hydroxyl groups.

And so you have to prime this once with a separately-generated 2,3-bisphosphoglycerate, but once you have that phosphorylated enzyme in the active site, it can now continually ping-pong that phosphate around to effectively convert 3-phosphoglycerate to 2-phosphoglycerate, or an example of another mutase reaction, move the phosphate between two other hydroxyl groups on the same molecule. OK.

Now the final reaction that we haven't talked about is this one, the aldolase reaction, which is a really key step in glycolysis because it splits that carbon-carbon bond to take the hexose-- fructose 1,6-bisphosphate, and split it into two trioses, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which ultimately allows you to make this pathway work.

And so I'll take you quickly through how aldolase works. So aldolase also has this amine in the active site. So here's FBP drawn in the open chain form. So you end up with this intermediate in the active site, which can undergo this chemistry to split this into the-- remove the lower half of the molecule.

So you can see here, this generates this lower half of the molecule, glyceraldehyde 3-phosphate, as well as the top half, which can regenerate the active site, as well as the other product, dihydroxyacetone phosphate.

And so very briefly, that's effectively the chemistry that allows aldolase to carry out this important reaction in glycolysis, splitting that fructose 1,6-bisphosphate into the aldose, glyceraldehyde 3-phosphate, and the ketose dihydroxyacetone phosphate. OK. So that is effectively the chemistry that allows you to turn glucose into pyruvate, as well as pyruvate into lactate, but what about making ethanol? How does that work?

Now ethanol is an alternative fermentative product. And so the reactions to make ethanol is basically this. So here's pyruvate. First thing that happens is we lose this CO_2. Without a change in oxidation state at the second carbon at the ketone in pyruvate. And so this ketone stays the same oxidation state, it's now an aldehyde.

This is a molecule called acetaldehyde. And now we can pick up those two electrons from NADH and reduce this aldehyde to the alcohol, reoxidizing that NADH back to NAD+ and generating ethyl alcohol or ethanol.

So, the fermentative part is turning the acetaldehyde into the ethanol because that allows you to dispose of those two electrons, reduce the aldehyde to an alcohol while reoxidizing the NADH to an NAD+, which is what allows glycolysis to continue so that NAD+ is available for the GAPDH step.

How this reaction works is-- I just drew it. It's exactly how the lactate dehydrogenase works. However, what we also now need to discuss is how you carry out this reaction, this carboxylation to take this CO2 off of pyruvate and make acetaldehyde. Now decarbonization reactions are really common reactions in metabolism. They end up being important for lots of pathways. Obviously if we're going to completely oxidize glucose into CO2, we have to remove CO2 for a molecule, and here's the first one-- first one that we're going to see for how that happens.

Now there's two general mechanisms that metabolism uses for decarboxylation reactions, and I want to introduce the concept here of both of them. Now, the first one here is that these are referred to as decarboxylation reactions that occur in the context of either an alpha keto acid or a beta keto acid.

Now, what do I mean by that? Well, pyruvate is an alpha keto acid, and that's because here's a acid group. The ketone is alpha to the carboxylic acid. This would be in contrast to this molecule. So I'll just draw with a generic R group. Where here, the ketone is beta to the carboxylic acid-- alpha, beta.

So pyruvate is an alpha keto acid. This here is a generic beta keto acid. Now I want to point out that decarboxylations of bait ketone acids are very favorable, and they're favorable for the following reason. And that's because if I remove this CO2 from a beta keto acid, I'm left with this molecule, which hopefully you'll recognize as an enol. And just like we talked about for the pyruvate kinase reaction, enols much prefer to rearrange into the keto form. And so decarbonization of a beta keto acid generates an enol which will rearrange to the ketone. This becomes very favorable, and so it becomes favorable to decarboxylate a beta keto acid.

Now, we can't use that exact same chemistry to decarboxylate an alpha keto acid. However, we can help that process, effectively mimicking the same thing that happens with beta keto acid, by instead introducing a cofactor.

So I introduced the concept of cofactors when we talked about NAD and NADH. And remember, cofactors are molecules that provide functional groups that help facilitate the chemistry of various reactions. And so there's a cofactor that facilitates the chemistry that allows decarboxylation of alpha keto acids, and that cofactor is abbreviated TPP+, which stands for the thiamine pyrophosphate. TPP+, which is derived from the vitamin thiamine, sometimes also referred to as the vitamin B1. And so this is what TPP+ looks like.

OK. So like many of our vitamins or cofactors, it's a complex molecule. So this is vitamin B1 or thiamine, and the cofactor TPP+ is basically the same molecule with a pyrophosphate group added on the end, TPP+. Now, to see what's going on, we're going to focus here just on this part, this reactive part of the molecule, which is right here, which for simplicity, I will just draw like this. So this here is just this basically part of the molecule here, that's the reactive part of TPP+.

And what makes this cofactor useful is that this basically can exist in this stabilized carbanion form. This stabilized carbanion can react with alpha keto acid like pyruvate, shown here. And then this intermediate now becomes favorable for decarboxylation of the CO2 that's alpha to the ketone.

And then here, we can regenerate the active TPP+ that we started with plus the other product, acetaldehyde. And then that acetaldehyde can then be reduced, regenerating NAD+ and giving alcohol for fermentation. OK.

Now incidentally, this is how you produce alcohol. How you metabolize alcohol is effectively reversing that last step and further oxidizing the acetaldehyde. And so this is ethanol. So the way ethanol is metabolized. And so the famous alcohol dehydrogenase enzyme basically does that reverse reaction, generates 2 electrons as a hydride ion. They get transferred to NAD+. That NAD+ gets reduced to NADH as this alcohol in ethanol is oxidized to the aldehyde in acetaldehyde. And this can then be further oxidized, that aldehyde to the acid.

So here again, two electrons from the hydride iron can reduce NAD+ to NADH. And that generates, in the process, oxidizes the aldehyde to the acid. In this case, this is acetate. The acid form it would be acetic acid, also known as vinegar. And so effectively, the way you turn alcohol into vinegar is by the microorganisms oxidizing the alcohol they produce into acetic acid or vinegar.

Now note, this is-- alcohol metabolism is two oxidation reactions. So we talked about last time, oxidation reactions are generally favorable, therefore energy is released. This is why alcohol has calories and it's impossible to make diet alcohol, because it will-- just no way around this.

A couple other things about this, as I mentioned. This enzyme catalyzes. This is the famous alcohol dehydrogenase. This enzyme is an aldehyde dehydrogenase. The issue is is that this aldehyde hydrogenate is a less efficient enzyme than alcohol dehydrogenase, thus it can become rate-limiting for alcohol metabolism. And so if you overwhelm the system by drinking too much, effectively this enzyme can't keep up with this enzyme, you generate excess of the toxic acetaldehyde product. Acetaldehyde product is toxic, and this is effectively what generates hangovers.

It's also the case that there are individuals with polymorphisms of this aldehyde dehydrogenase. This was, I believe, discussed in Professor Yaffe's lectures on enzyme kinetics that have different catalytic efficiency at this step, and the people who have those polymorphisms can't metabolize alcohol as well. They tend to get flushing and whatnot because they build up this toxic acetaldehyde, and in fact, can be quite dangerous if they drink too much.

Finally, this also illustrates effectively why when commercially you want to generate alcohol, like in beer or a wine, you need to keep oxygen levels low. Why is that? Because, well, the microbes will read their ethanol. It's a perfectly good source of calories for them as well because of these reactions. But they generate all this electron waste in the process.

Those electrons have to go somewhere. Where do they go? Well, oxygen is a great electron acceptor. And so to metabolize this ethanol, they need to put those electrons somewhere. They need to put them on oxygen. And so oxygen is required to carry out this ethanol metabolism by the microorganisms. And so by keeping oxygen out of your fermentation of alcohol in commercial beer and wine production, it prevents the formation of aldehyde and acetic acid products which would be undesirable in your finished drink product. OK.

Now this whole discussion also begins to suggest one big way in which glycolysis itself is regulated, and that's effectively by the availability of oxygen. And so to be explicit about this, I want to draw the relationship between fermentation, glycolysis, and oxygen in terms of maintaining electron balance across these pathways.

And so glucose is, of course, a major fuel for us. It's a major sugar in our blood. Yeast use a lot of glucose from the grapes in their environment. Plants, of course, make glucose as a storage carbohydrate. It's-- we discussed, starch is a polymer of glucose that uses glucose to survive the night. And effectively what they do is turn that glucose into two pyruvate molecules which can be used to generate net 2 ATP as we discussed. But creates this problem that it also generates NADH that needs to be recycled back to NAD+.

In the absence of oxygen, you can ferment that pyruvate into lactate or alcohol or some other product that allows you to regenerate NAD+. Or-- and this is a pathway that requires no oxygen. However, that pyruvate can be further oxidized to CO2, but that's going to generate even more electron waste. That electron waste also has to be dealt with, and that requires oxygen or some other place to put those electrons, oxygen to water being a major driver of further oxidative metabolism.

Now the beauty of fermentation as a pathway is that no oxygen is needed, but the trade-off is that it's much less efficient. You only get 2 ATP per mole of glucose that's-- 2 moles of ATP per of glucose that's fermented. You can imagine that a lot more energy is released-- if you can further oxidized glucose, you can generate, therefore, a lot more ATP if you use a complete oxidation of glucose, but this requires oxygen.

And so a major way that glycolysis and certainly fermentation is regulated is that low oxygen is what promotes fermentation, whereas high oxygen suppresses fermentation, and that makes sense. It's intuitive. You get a lot more energy. If you completely oxidize your glucose, you can more efficiently get energy from oxidation of available reduced glucose carbon if you do it by oxidative pathways, and so therefore, in the presence of plenty of oxygen, you don't need to do fermentation.

Whereas if you outstrip oxygen supply, as, say, might happen if your muscles are exercising, burning more ATP than can be kept up with by oxygen delivery from the blood, now you switch over to a more affirmative metabolism, something like lactic acid builds up that can then be dealt with later.

Now, this fact that high oxygen can suppress fermentation is experimentally true in many organisms in many contexts. It's likely because the high ATP-ADP that's produced with oxidative glucose metabolism can directly affect some steps or deplete the NAD that's required to drive glycolysis. Exactly the mechanism that causes this is somewhat debated and likely depends on context and conditions. But it also sets out at least a way for us to begin discussing, how is it at a level of individual enzymes or reactions might something like high ATP-ADP ratio effect flux or flow through a pathway?

All right. Well, let's just start by talking about what are the two things that could affect flux or flow through the pathway? Well, remember, thermodynamics, delta G, is what determines whether a pathway happens at all. And because delta G is proportional to things like the ATP-ADP ratio, you can imagine if this ratio gets too high, some of the steps might no longer be favorable to occur. That's certainly one possibility.

But the other way that regulation can be done is actually kinetic, and this is because even though enzymes don't determine whether a reaction can happen, at least thermodynamically, they oftentimes do determine the rate of the reaction, and when we're talking about rate and flow through-- flux through pathways, flow through pathways, now rate can become very important.

And so the kinetics of enzymes, exactly what you talked-- heard about with Professor Yaffe, properties like V max or Km of an enzyme, which, of course, can be affected by allostery, and Professor Yaffe covered in great detail, these also, you can imagine, can affect how a pathway is regulated. And of course, things like ATP, ADP, and AMP as small molecules can be good allosteric affectors of some enzymes.

And so let's now talk about, if we're going to regulate steps in a pathway, which steps should actually be regulated. And so shown here is, again, coming back to our delta G change map across glycolysis, as we alluded to earlier, the steps where it's hard to go back is this hexokinase step, this phosphofructokinase step, and this pyruvate kinase step, because these are where there's the big change in delta G that now makes it hard to reverse those steps.

And it's exactly those steps that are regulated, because that makes sense. If this delta G is thermodynamically driving flux through the pathway, changing the rate of the enzymes that catalyze those steps is also going to change the rate at which the pathway as a whole can happen. Now as a result, it's these steps, hexokinase, phosphofructokinase, pyruvate kinase, that are often referred to as the rate-limiting steps of glycolysis.

Personally, I don't like referring to memorizing things like these are the rate-limiting steps of the pathway, because in reality, what step of any pathway is going to depend on the context. However, often this concept of rate limitation when people refer to pathways really is coming from this energetic argument about which steps have the biggest drop in delta G, and it's important for you to understand that, not just memorize what steps are rate-limiting.

Now I also want to point out that these steps actually are in interesting places in glycolysis. So this is basically entry into the pathway, and this is exit from the pathway. And this makes sense, too. You don't want to start committing carbon into a pathway unless you're actually going to need it, and you also need to match output to input.

And what you'll see is that many steps in pathways that are regulated, in addition to being the ones with the biggest delta G changes, are also often the entrance and exit from the pathway, which, of course, is the case in glycolysis, but also in most pathways it makes a lot of sense just from logical considerations.

All right. Now if we're going to control things that the level of enzymes and rate, how can we control rate of a reaction? That is, how can we control enzyme kinetics? Well, if you remember from Professor Yaffe's lectures, you can increase the rate of a reaction-- that is, catalyzed by an enzyme-- by increasing the Vmax of that enzyme; or, if the substrate concentration is close to Km, by lowering the Km of that enzyme.

How can you decrease the rate? Of course, you can do the opposite. You can decrease the Vmax; or, if the substrate concentration is close to Km, you can increase the Km. So how can I change these things? How can I alter Vmax or Km of an individual enzyme? Well, there's a number of ways I can do this.

One is is I can make more enzyme. What will making more enzyme do? Well, effectively that will increase the Vmax of an enzyme. Or if I get rid of enzyme, that will decrease the Vmax of an enzyme. I can use a different version of the same enzyme. That is, have an enzyme like pyruvate kinase but have it come in a bunch of different varieties, have different genes encode different pyruvate kinase isoforms such that those isoforms have different Vmax and Km relationships with respect to substrate, and effectively, by using a different version of the enzyme, a so-called isoform of an enzyme, you can now have different properties.

Now you'll note that the change enzyme kinetics this way basically is new transcription translation. That's fairly long on the timescale of needing to adapt to metabolism, but that can work. But sometimes a lion's attacking you, you need to run away quickly, you may not have time to make more enzyme. You also need direct control of the enzymes, and this is where allosteric regulation of enzyme function comes into play.

Allosteric regulation can be a result of signaling. Phosphorylate, acetylate an enzyme, that can change its properties. Or you can have metabolite binding. Bind ATP, bind AMP into some allosteric site that can change Vmax or Km and operate on a short time scale to allow metabolism to adapt in a more acute setting.

I want to say that time scales matter for real biology if you want to understand physiology and how it applies to how metabolism and physiology relate to biology that you'll encounter in other contexts. You realize that some responses need to be acute. Those allow you to adapt to conditions quickly, be able to get enough ATP to run away from the lion, et cetera. But you also need adaptations that work on longer time scales, because it's things like this that allow you to enact programs that allow you to adapt long-term to whatever new conditions a cell may face in its environment.

All right. Now, before I get into talking exactly how these things actually work to regulate glycolysis, I want to discuss a few details about regulation of pathways in general. And while we talked about these with respect to glycolysis, these points will end up coming up over and over again and really apply to other pathways.

Now I may not explicitly point this out, but the same considerations for how glycolysis works, how it's regulated has to be true for all metabolic pathways. And remember, any pathway is just variations on relatively few chemical-- chemistry reactions that are repurposed in a way to build some other thing that's useful to the cell, and you'll see this over and over again.

Now, it has to be true for every pathway that it has to be thermodynamically favorable, and that means either that the pathway itself, glucose to pyruvate, is thermodynamically favorable, or it's coupled to some kind of energy input, like ATP hydrolysis that allows the pathway to be favorable.

Every pathway is going to be built in a way that intermediates are generated along the way that allow that pathway to accomplish its goals. In the case of glycolysis it was the two steps that allowed us to synthesize and incorporate phosphate despite the high ATP-ADP ratio in cells. And in all cases this has to obey the laws of thermodynamics. That is, we have to obey constant conservation of mass. That means we have to account for all carbon, all electrons, delta G has to be less than 0 across the entire pathway.

You'll see that the regulated steps will often be those with the largest thermodynamic considerations, like the big drops across hexokinase, phosphofructokinase, and pyruvate kinase. They'll often be at the entrance and exits to pathway because you want to regulate, you don't want to commit something to a pathway if you're not going to use it, and you want to match input and output. And all of this is going to have regulation that acts on both short and long time scales so it allows it to match physiology.

All right. Now with that in mind, now let's discuss how we can regulate glycolysis using both short and long time scales as-- using really short time scales, allosteric regulation of glycolysis in a way that makes it most useful to cells. All right. So the first step is uptake of glucose into the cell, which in animals, like us, is passive, but can still be regulated by glucose transporters, often regulated glute proteins. And it's really these glucose transporters that can often determine whether glucose is actually taken up into cells.

A famous example of this is FDG PET scanning, which is used to measure glucose uptake into tissues in humans, where basically a hydroxyl group is replaced with a positron-emitting fluorine atom, fluorodeoxyglucose, that is taken up and phosphorylated in cells, and it's this lighting up of this on a positron-emitting scan you can tell that which tissues in the body take up glucose and that's a property of cancer, and so it is often used as a way to detect or stage cancer.

Another example of this is insulin. Basically one of the major actions of insulin on your tissues is that when glucose is high, your body makes more insulin. That causes glucose transporters to be put on the surface of your muscle and your fat, which allows that glucose now to get into those cells and effectively allows those cells to dispose of the glucose in the blood and keep glucose in the right physiological range. And so uptake of glucose, at least in animals, is one important step that is really controlled largely by whether or not there is a transporter on the surface.

All right. Once that's trapped as-- sorry, once it's in the cell as glucose, hexokinase then traps that molecule as glucose 6-phosphate. And it turns out the glucose 6-phosphate is an inhibitor of hexokinase. Why is that? Well, we will see in the next lecture that glucose 6-phosphate can be turned into glycogen in the case of mammals or starch in the case of plants. And so glucose 6-phosphate, if you're going to store glucose, you can ship it off into glycogen, or you could commit it further down into glycolysis. But if you don't need it for a glycolysis and your stores are full, you'll build up glucose 6-phosphate, you want to inhibit hexokinase to keep things from flowing through the system.

The next step that's regulated is phosphofructokinase. So phosphofructokinase, of course, makes FTP. The step downstream of that that's regulated is pyruvate kinase, PEP to pyruvate, and it turns out that FTP will stimulate pyruvate kinase, whereas PEP will inhibit phosphofructokinase. Makes sense. You want to match input to output. If you have a lot of FTP, activate the enzyme to dispose of it. If you're building up PEP, tell you to stop sending molecules down the pipeline.

It turns out, PFK is actually the major regulatory step of glycolysis, because this is really what commits that carbon to going through glycolysis as opposed to upstream of that, it could be stored or not. So it's really that step that's a commitment step. And so it's also regulated by some of the outputs of the pathway.

And so some of those outputs are ATP downstream of pyruvate, which we'll talk about later in the course. There's some other molecules. Alanine, citrate. It turns out that high levels of citrate will also inhibit PFK. High levels of ATP will inhibit phosphofructokinase. Whereas high levels of AMP will activate phosphofructokinase.

Makes sense. A major output of glycolysis is to keep the ATP-ADP ratio high, the energy charge high in the cell. If the energy charge is very high, high ATP, shut off phosphofructokinase, don't need to do more glycolysis. If the energy charge low, high AMP, activate phosphofructokinase. If production of other molecules downstream like citrate are high, come back and stop sending things into the pathway. Turns out, alanine can inhibit pyruvate kinase, another step downstream.

Great. This is effectively general regulation, allosteric regulation of how glycolysis works. And what I wrote is was generally true across different tissues, but of course, these regulations can be tweaked to be more or less important to match physiology and function. Now I know many of you are going to take the MCAT. And while I would never want to teach just to take a test, a couple of things that you should remember for that test is phosphofructokinase is a major regulator. Its big allosteric regulators are AMP, as well as ATP and citrate.

Whereas the other steps, hexokinase, you should know that it's regulated by-- negatively regulated by glucose 6-phosphate, and pyruvate kinase is positively regulated by FBP. Now one other point that I want to mention, just because it will come up sometimes on MCAT exams, is that there's an additional detail about how PFK is allosterically regulated, and that's because there's this side reaction where fructose 6-phosphate can be phosphorylated on the 2 rather than the 1 position to make this molecule, fructose 2,6-bisphosphate. This is a reaction that is catalyzed by bifunctional enzyme called PFK-2/FBPase.

So basically phosphofructokinase on the 2 position, FBPase, we're moving it from the 2 position which allows you to regulate the levels of fructose 2,6-bisphosphate. It turns out that PFK in glycolysis, which, of course, generates FBP, is positively regulated by fructose 2,6-bisphosphate. And the production of fructose 2,6-bisphosphate is something that can be inhibited by ATP, inhibited by phosphoenolpyruvate, and activated by ATP.

So exactly what is shown here is allosteric regulation. Just some of this allosteric regulation is via this side enzyme to make a separate product, fructose 2,6-bisphosphate, and there's control theory considerations for why that might happen. All right. So at this point, we've learned how we can start with glucose, oxidase that glucose via a pathway that now allows us to get the energy that cells need to maintain ATP-ADP ratio in a good physiological range despite the high ATP-ADP ratio in cells.

However, it should also be clear that in order for this to work, we need a way to actually get that glucose from somewhere to begin with. You probably learned in high school that plants use photosynthesis as a way to get energy from the sun. Well, the synthesis part of photosynthesis is generating the sugar that those plants can then use to burn and keep ATP-ADP high at night and survive the night until the sun comes up again when they can again use energy from the sun directly to get energy.

We as animals evolve the way that we have to eat the plants. We have to get the glucose that the plants make, but of course, we also use-- generate-- have the ability to generate some glucose, and this is best illustrated from muscle physiology. And that is, we alluded to earlier, that if we're running away from the lion, our muscles might outstrip the supply of oxygen in the blood and basically generate a whole lot of lactate.

Well, that lactate is perfectly good fuel. Just like the yeast, can oxidize or we can reoxidize ethanol. We can also use lactate as a fuel, but it turns out, a big job of our liver is to take that lactate from the blood, as well as other molecules, and regenerate glucose so that our blood glucose remains in a constant level.

This cycling of glucose and lactate across the body between the muscle and the liver is sometimes referred to as the Cori cycle and involves a clear set of reactions to turn that lactate back into glucose, a process called gluconeogenesis. And unfortunately we're out of time today, but next time I will start off the lecture by going through how it is that gluconeogenesis works to turn something like lactate back into glucose so that organisms have the glucose to begin with to run glycolysis. Thank you.