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PROFESSOR: Today we're going to discuss bioenergetics, continue that discussion and use that as a transition into also beginning to see how that applies to understanding glycolysis, the pathway that allows glucose breakdown. So just as a reminder of a couple of the points that we discussed last time, so last time we discussed what makes reactions favorable.

And so remember, you could have any reaction, A to B-- A to B could be a single step. A to B could be an entire pathway, all steps in the pathway, that whether or not that reaction or pathway happens is determined by thermodynamics. It's not determined by enzymes. Enzymes are important, of course, to control the rate of reactions so they may allow things to happen that otherwise wouldn't happen. But whether the reaction happens or not is determined by free energy.

And so free energy, delta G, remember we discussed is the following relationship. So it's related to this constant delta G0 prime, which is related to the equilibrium constant for any pair of products and reactants as well as this formula rt times the log of the products over the reactants. And so remember this means that whether a reaction happens depends on the equilibrium constant, but it also depends on the actual conditions present. That is how much substrate and how much product is there.

And so what this means is that biology can come up with ways by keeping the product concentrations low to do things that may move in the opposite direction of what you would predict from equilibrium. And also by coupling these less favorable reactions to more favorable reactions, you can also allow biology to do unfavorable stuff. And this is, of course, where the role of ATP comes in. And we discussed this at the end of last time as well.

Of course, what's important then becomes the ratio of ATP to ADP. And to illustrate that, we use this example. We use the-- remember ATP hydrolysis has a delta G0 prime of minus 7.5 kcals per mole. That, of course, tells you this is about the equilibrium constant. That tells us the equilibrium lies to the right. We can couple it to this first reaction in glycolysis, which traps glucose in the cell by adding a phosphate group. Delta G0 prime for this is positive 3.3 kcals per mole. This positive number tells you that the equilibrium lies towards the left.

But when we couple these two reactions together, we add these two numbers together, and now we get a delta G0 prime of minus 4.2 kcals per mole, which are the coupled reaction. The equilibrium lies now again to the right. And that's how we can use ATP hydrolysis in order to carry out this unfavorable process, trapping the glucose in the cell.

Now how much glucose will be trapped? Well, of course, that will come from this reaction up here. And that's because whether or not a reaction occurs or not of course depends not on the equilibrium constant but on the actual conditions and the delta G of that reaction.

And so we can just plug that in for this system. And so we see delta G0 prime minus 4.2 kcals per mole. That says equilibrium lies to the right. But how far really depends on the conditions. And that's defined by this ratio of products over reactants. And you can imagine that this term here, as long as it's less than 4.2, you will favor glucose trapping inside the cell, whereas, of course, if it's greater than 4.2, then you'd favor-- the opposite direction would be favorable.

Now this is really critical because this really explains how it is that ATP actually works to help cells do various reactions. And so if an ATP-ADP ratio exists such that it can make delta G less than 0 for the reaction that it's coupled to, that is how it provides the energy that then allows unfavorable reactions to occur. And so please do not make the common mistake that many, many biologists make when they state, oh, ATP equals energy. There's a lot of ATP. The cell must have a lot of energy.

Remember it's the ratio of ATP to ADP that provides the energy. And so that means ATP can be sky high, but if ADP is equally high, there's no energy there. Consequently, ATP can be very low. But if ADP is extremely low, now you still have a high ATP to ADP ratio. And that is what provides the energy. And that's because this occurs regardless of concentration. It's because it's this ratio of ATP to ADP that ultimately says how much energy is there for the ATP to drive a reaction. In other words, delta G, or whatever the reaction is coupled to, is going to be proportional to the ratio of ADP to ATP. Great.

Now how much ATP do you need then? What ratio do you need to drive a reaction? Well, it's hard to say that in absolute terms because it's going to be defined by how that ratio is coupled to the ratio of whatever other reactants and products. It also tells you, remember, as we talked about before, we can switch the directions of these reactions and we just flip the signs. The directions here are arbitrary.

And so that means it also tells us how much energy we need to put in if we're going to charge up an ATP-ADP ratio. In other words, if we want to synthesize ATP, that is take ADP plus phosphate and make an ATP, well, what's this? What we just-- the exact opposite sign of that delta G0 prime is going to now equal plus 7.5 kcals per mole. And so that means that the equilibrium of this reaction is going to lie to the left just as that one lied to the right. And it's going to lie to the left by that much.

And if we want to know how much energy it then takes to make ATP, well, obviously, it's not going to happen because equilibrium favors this direction. It's not spontaneous in that direction. But at various conditions, we can know by plugging in our formula delta G equals delta G0 prime plus 7.5 kcals per mole plus RT times the log of ATP.

So if I have my ADP-- I'm sorry-- my ATP concentration sufficiently low relative to my ADP concentration, I can still make this reaction happen. Now that's not going to give you an ATP-ADP ratio that is actually consistent with being able to do anything else into the cell. And so but what it says is that if I am able to couple energy from nutrient metabolism to this reaction and overcome this positive 7.5 kcals per mole, that basically will allow me to now synthesize ATP and maintain an ATP-ADP ratio in the cell that allows cells to carry out other functions by the same rules that we talked about that would be unfavorable.

Now there's lots of implications here for metabolism, because it says how much input you need to synthesize ATP. How much energy you need to synthesize ATP is going to depend on how charged this ratio is. And how useful that ratio is to catalyze other reactions is also going to depend on how charged that ratio is.

And in essence, this is why we cannot store energy as ATP. This is why we didn't eat ATP for breakfast and instead ate cereal that has a bunch of glucose in it, because we can use that glucose to get ATP but the ATP itself is not something that we really can burn to get energy in a sustained way. In other words, the delta G of ATP synthesis, making ATP is going to be proportional to the log of the ratio of ATP over ADP for all the reasons I described.

The delta G of using ATP, ATP hydrolysis, using ATP to drive reactions is also proportional to RT times the log, in this case, of the ADP to ATP ratio. And so because you need to consume this ratio to get energy, and you need to put in energy to create this ratio, you are constant-- and how much is an exponential property-- you are constantly having to generate ATP in a way that allows you to then have a ratio that can then be consumed to fuel other reactions. And that's what's shown here on the slide.

So I just graphed here what the ATP-ADP ratio is relative to a theoretical delta G that's shown here as a linear scale, here on a log scale, that basically there's this logarithmic relationship and so you need an exponential increase in energy input to drive this ratio up higher in order to, quote unquote, "store" more energy that can be used for later.

And so this system is not one that you can use as an energy storage system and ultimately explains why cells have to do constant metabolism, have to constantly do some sort of metabolism to keep this ATP-ADP ratio charged for it to be in a useful range to have an ATP-ADP ratio that can be coupled to drive otherwise unfavorable reactions in our cells. So to say this another way, our cells need to constantly catabolize nutrients, burn glucose, burn some other fuel. They have to do this because they have to keep that ratio charged in a range where that can then support other cell functions, allow them to fight entropy and maintain order in biology.

We alluded last time if you stop making ATP, well, what's going to happen? Well, that ATP-ADP ratio the cell has is going to fuel whatever reactions it can do until suddenly that ratio falls below the point where it's useful to drive those unfavorable reactions. In fact, if you stop ATP production, you will consume that ATP-ADP ratio in seconds. And this is why if you poison ATP production, cells die very quickly.

Remember last time we mentioned you have a heart attack, blood flow stops going to a tissue. No more nutrients and oxygen. Cells die quickly. Cyanide poisoning does the same thing. Cells die quickly if you stop their ability to maintain this ATP-ADP ratio in the right range. And so remember the concentration, the absolute concentration of ATP for these reasons is not a good indicator of cell energy.

The correct indicator of cell energy is the ratio of ATP to ADP. This term is sometimes referred to as the energy charge. And so that can be the ATP to ADP ratio, or oftentimes more correctly is the ATP to AMP ratio.

Why the ATP to AMP ratio? Well, that's because once you understand this, you can now understand that cells have a failsafe mechanism to protect themselves if this ratio of ATP to ADP becomes too low. What is that mechanism? Well, you can carry out this reaction. Take two ATP molecules and turn them into transfer a phosphate to make an ATP plus an AMP.

Why is that a failsafe mechanism? Well, if they care about this, if the ATP-ADP ratio is what maintains your ability to drive these thermodynamically unfavorable reactions, if I sacrifice the denominator by removing an ATP and get an ATP out of it, I've now at least temporarily fixed that ratio. Now this is something that obviously cannot go on forever.

But in the short term, it does allow cells to protect that ratio. And in fact, what cells actually defend is this ATP-AMP ratio. And it's very, very difficult to change the ATP-AMP ratio in a cell and actually have it live. So it's really this ATP-AMP ratio that is most formally related to what would be called energy charge. But this is really why this is important is for all the reasons that I just described.

All right. So hopefully, it's clear to you now why cells have to keep ATP-ADP ratio in the right range, why this is useful for them to now carry out all of these unfavorable reactions-- fight entropy, stave off death. And they can even have a failsafe here and sacrifice ADP as a way to try to maintain that as a very last resort.

It also says why cells have to always do catabolism, because you always need the energy input to keep that ratio in the right range. However, you might imagine that, in real life, the messiness of biology, that there's lots of situations where the demand for ATP will suddenly increase.

Well, of course, maybe you can just metabolize more sugar, do more nutrient catabolism to try to keep that ratio high. But that takes time. And so you need ways for cells to quickly respond to then allow them to mobilize all of this extra thing to-- extra fuel to do that. For example, we're walking around our ancestors on the plains somewhere and a lion appears.

We don't have time to mobilize a bunch of new fuel. We have to be able to run away really quickly or the lion's going to eat us. And so cells have systems to suddenly respond to I need a lot more ATP demand to run away by having ways that one can buffer this ATP-ADP ratio, protect it in the short term, and allow cells time to ramp up nutrient metabolism in order to keep this high.

All right. Well, if we wanted to create such a buffer system, how would we actually do it? Well, obviously, we have to follow all the rules of thermodynamics that we discussed. And we have to do so in a way that actually illustrates how it is that very well how metabolism works. In other words, if suddenly the ATP-ADP ratio drops, if we have a sudden drop in ATP-ADP ratio because we have increased demand, needing to run away from a lion, how could we actually create a buffer system that protects this?

Well, to do it, we would need to couple ATP-ADP to some other product reactant pair, let's call them x and y, such that the ratio of y to x is coupled to the ratio of ATP to ADP. And you want it coupled in such a way that if that ratio goes down, this ratio can now protect ATP, basically favor ATP to ADP synthesis. However, when nutrients are plentiful and this ratio is really high, it then in turn can charge up this ratio so that it can buffer it later when the ATP-ADP ratio starts to fall.

Now the most famous example of this actually comes from muscle physiology. And it's best described in terms of muscles and how they quickly respond to increased demand, like that running away from a lion. However, it in fact applies to many, many cells, including most non-muscle cells in animals.

All right. And I should say that it turns out that if you turn off ATP synthesis in a muscle, people have estimated that your ATP-ADP ratio will fall into a range where it is no longer useful to do anything within a matter of seconds, some claim even less than a second. And so this system ends up being very important.

Now the system that muscle uses, and many other cells do as well, is something called the creatine/creatine phosphate system. Now if you guys are weightlifters or bodybuilders out there, you probably have heard of creatinine because it's a very popular supplement that is taken for weightlifting, something I obviously don't do a lot of. But that creatine/creatine phosphate is out there. I'll describe to you what it actually does for muscle cells. And you can decide for yourself if this is actually useful as a energy or weightlifting supplement.

So what is creatine/creatine phosphate? What does it look like? Well, it's basically looks like this. By the way, I will use this abbreviation often throughout this course, just a shorthand to draw a lot of these structures. So this here would be a carboxylic acid group drawn like this or like that, drawn to the carboxylic acid group just so you understand my shorthand.

OK. So this here is the molecule creatine. If I put a phosphate here, this now becomes creatine phosphate. Again, just to remind you, this P with a circle over it is a phosphate group. I'll draw this particular phosphate group because it's a nitrogen-phosphate bond, a phosphoramidate bond. This is actually a bond that is more labile than the PO bonds that we're used to seeing in the phosphodiesters like ATP, et cetera.

All right. So that's creatine and creatine phosphate. And if we draw out the reaction, creatine phosphate goes to creatine plus inorganic phosphate, so hydrolysis of creatine phosphate. This has a delta G0 prime of minus 10 kcals per mole. What does that mean? It's negative.

Tells us about the equilibrium constant. Equilibrium lies to the right. Remember ATP to ADP hydrolysis shown over there, that hydrolysis has a delta G0 prime of minus 7.5 kcals per mole. Equilibrium also lies to the right. This number is more negative. This equilibrium lies further to the right than ATP hydrolysis does.

So now what happens if we couple this reaction to the ATP hydrolysis reaction? So now we can do ATP plus creatine goes to creatine phosphate plus ADP. So we've coupled those two reactions together. So delta G0 prime, ATP to ADP is minus 7.5. Creatine phosphate to creatine in that direction is negative 10. In this direction, it would be positive 10.

And so as I've drawn the reaction, this delta G0 prime will be plus 2.5 kcals per mole. 10 minus 7.5 plus 2.5, which tells us then that this equilibrium now lies to the left. However, which direction this reaction actually goes is not determined only by the equilibrium. It's really determined by delta G. So we can calculate that. So delta G equals delta G0 prime plus RT log of, as we've drawn it, creatine phosphate over creatine and ADP over ATP.

So you can now imagine that this term here is the sum of those two. So it's plus 2.5. And so if this term is less than 2.5, what does that mean? Well, if it's-- I'm sorry. Less than negative 2.5, then delta G will be negative and you will favor creatine phosphate production. If this term is greater than negative 2.5, then delta G will be positive and you will favor ATP production.

Why is that important? Well, if you think about the conditions, how you could make this term less than 2.5 or less than negative 2.5 or greater than negative 2.5, basically, what's going to happen is that if the ATP-ADP ratio is high, lots of energy around, no problems charging up, lots of things to use, charge up this ATP/ADP to a very high ratio, well, now you will favor this direction, creatine phosphate synthesis.

However, if ATP/ADP begins to fall and is lower, well, now this term is going to flip and you'll now favor ATP production. And so basically, by charging up this creatine/creatine phosphate ratio, it now creates a situation where it can buffer that ATP/ADP ratio so that when times are good, you charge up the buffer. When times are bad, you then consume the buffer in a way that allows you to protect your ATP/ADP ratio for a short time.

And this is illustrated very nicely here in this slide that basically shows what happens in muscle physiology. And so this is just a theoretical curve that if you suddenly need to contract your muscle and you stop ATP production, well, ATP levels will fall. That ATP/ADP ratio more correctly will fall rapidly within the cell. Creatine phosphate will then now kick in to protect that ATP/ADP ratio as you can ramp up either anaerobic or aerobic metabolism to now buffer that ATP/ADP ratio and keep that muscle functioning.

Now I want to make one point because oftentimes biochemistry textbooks will refer to things like ATP or creatine phosphate as, quote unquote, "high energy compounds." And of course, I guess they are high energy because they have these labile phosphate-nitrogen or phosphate-oxygen bonds.

And because they're labile, hydrolysis is favorable. And to a certain extent, it's that hydrolysis that is providing energy. But remember it's not the metabolite or the bond in isolation that's really providing the energy. It's these ratios of ATP to ADP, creatine phosphate to creatine, that allow you to couple, for all the reasons we've spent now a lecture and a half discussing, two other reactions that really provides biological energy.

All right. Now we're ready to discuss how it is that we can use glucose oxidation, nutrient catabolism, to keep ATP/ADP high, that is in a useful range, where it can allow cells to do otherwise thermodynamically unfavorable things. So how could we make this happen?

Well, just like this example with creatine and creatine phosphate, if we want to use nutrient catabolism to do the same thing, we need to couple reactions of glucose oxidation, nutrient catabolism, that will allow us to synthesize ATP, ADP to ATP, and have that synthesis be favorable despite the high ATP/ADP ratio in cells.

So let's think about it. So what is this? Nutrient catabolism. What did we talk about last time? Oxidizing glucose to carbon and CO_2. So here's our reaction for that. So here's carbohydrate, glucose CH_2O_6 plus six O_2 goes to six CO_2 plus six H_2O. That's the chemical formula for burning wood.

So delta G0 prime for this is minus 686 kcals per mole. Quite a negative number. Equilibrium greatly favors the completely burned state, oxidized state, of that carbohydrate. And so you can imagine that this equilibrium is so favorable to the right, if we couple this reaction to ATP synthesis, you're clearly going to have plenty of energy there in order to maintain that ATP/ADP ratio in the right range to be compatible with cells using it to do other things.

All right. So how do we actually do that? Well, if we do it, we have to follow the same rules of thermodynamics and do the same things that we did before. Basically, we have to do this reaction in a way that we can draw out a similar type of equation as we did for creatine and creatine phosphate. And at the highest level, that is how nutrient catabolism will allow us to keep ATP/ADP ratio high even as ATP hydrolysis is constantly fueling all these other unfavorable processes in the cell.

Now if we did this all in one step, our burning wood example, lots of heat is generated. That's too much energy. Not so good for biology. But rather what biology does, as we alluded to last time, is that because what we talked about in the beginning, that all of the things that we discussed are true for individual reactions or many, many reactions, basically, if we turn A into B and we do it in one step, like burning wood, or we do it in 500 steps, the thermodynamic consequences are exactly the same.

And so what we need to do is do stepwise catabolism of glucose and take advantage of the fact that that delta G change is the same whether it's one step or many steps and in that stepwise fashion build in reactions where we create intermediate chemistries that allow us to do things like we did with creatine and creatine phosphate such that we can favor ATP synthesis when ATP/ADP ratio is in the physiological range.

And in essence, this is the magic of how biology does this and basically couples nutrient catabolism in order to provide energy. And so the pathway that we're going to discuss today and in the next lecture is something called glycolysis, which simply put is glucose lysis. It's the stepwise chemical oxidation of glucose, which we know from our burning wood example is favorable. And we're going to use this pathway that basically will be a pathway to allow the stepwise lysis of glucose, a favorable reaction, to individual steps that set it up in a way such that ADP to ATP synthesis is favored despite a physiological ATP/ADP ratio.

Now I want to point out that this really illustrates, back to that high energy compound thing, that ATP is not the only energy currency in the cell. In fact, glucose is much more energy for a cell than ATP. You get much more energy released from burning wood than you get from hydrolyzing ATP. And in essence, this is why we use glucose-- why nature has used glucose, and other molecules like it, as an energy storage device, because here is a relatively stable molecule, can sit around for a long time, and you can burn it when you need it to keep your ATP-ADP ratio high.

All right. So, at the level of reactions and pathways now, how can we use glucose oxidation and couple it to drive ATP synthesis? Well, hopefully it's very clear that we have to basically set up a situation like this creatine-creatine-phosphate system that we already discussed. And so I want to start by discussing, what are the reactions in glycolysis that ultimately behave like this and allow this favorable ADP-to-ATP synthesis? And then we will go ahead and consider how we can make those reactions work in the context of an entire pathway that allows glucose oxidation.

All right. Now, like creatine, phosphate, and creatine, both of the steps in glycolysis that favor ATP synthesis involve intermediates where you have phosphate on some molecule, plus ADP goes to ATP plus the phosphate coming off that molecule.

Now, if we go to our creatine-creatine-phosphate system, obviously this equilibrium needs to lie to the right. And so the first one of these is as follows. It involves this molecule, phosphoenolpyruvate, also referred to as PEP. You will see throughout the course that we love to give these little acronyms to our metabolites because it's hard to constantly say and write out phosphoenolpyruvate. Much easier to say PEP.

So phosphoenolpyruvate plus ADP goes to ATP. Plus, if I take the phosphate off of there, I get this molecule. This molecule is enolpyruvate. Enolpyruvate can be rearranged as follows. To make this molecule a ketone pyruvate.

You'll note-- hopefully remember from your organic chemistry classes that ketones like pyruvate can exist in two forms. They can exist in the ketone form or the enol form, rearranging via the chemistry that I've shown. The keto form is greatly favored over the enol form. However, by having a phosphate trap pyruvate the enol form-- phosphoenolpyruvate, when you remove the phosphate and it's wanting to exist in the keto form, greatly enhances the energetics of this-- basically makes the reaction, the equilibrium want to lie very far to the right.

And by the way of chemical tricks like this and others like it, you will see used over and over in biology. In fact, this is illustrated, if we asked what's delta G0 prime for this reaction, it's minus 15 kcals per mole. That tells us that the equilibrium of this reaction lies to the right. In fact, it lies far to the right, and in fact, this is-- you may remember ATP hydrolysis is negative 7.5 kcals per mole. So lies twice as far to the right as ATP hydrolysis does. And in fact, this minus 15 kcals per mole is the most favorable, at least in terms of individual reactions that at least I know of in metabolism.

All right. Well, if this equilibrium for this is PEP to pyruvate, this here is delta G0 prime for ATP-ADP. So if we want to know what's delta G0 prime for the entire reaction, well, if PEP to pyruvate is minus 15, ATP to ADP is minus 7.5. Well here, we're going in the opposite direction, so it'd be plus 7.5. So it's minus 15 plus 7.5, means that for the entire reaction, this is minus 7.5 kcals per mole.

Means that this is favorable, equilibrium lies to the right. If we drew out-- I'm not going to do it again because I've done it a lot, but if we redrew out this equation, replacing pyruvate and PEP in the correct places, there for creatine, phosphate, and creatine, you would see that there's lots of conditions within cells that will favor ATP synthesis even when ATP-ADP ratio is high.

All right. This reaction is catalyzed by an enzyme called pyruvate kinase. And pyruvate kinase is a classic example of a, quote-unquote, "irreversible reaction." However, I want to point out that the enzyme pyruvate kinase is actually named for the reverse reaction that's supposedly irreversible. Pyruvate kinase. If you take pyruvate and you act on the kinase as you learn from Professor Yaffe, that phosphorylates something. You phosphorylate pyruvate, you get phosphoenolpyruvate pyruvate kinase.

And so remember that absolutely no reaction is reversible. It's only reversed-- what they mean by irreversible when they say pyruvate kinase step in glycolysis is irreversible, is that it is effectively irreversible under the conditions that are compatible with life that exist in cells. Of course, if you have very low ADP and very high ATP, you can come up with a way to net synthesize PEP. You just can't drive the reverse reaction under the ATP-ADP and the PEP pyruvate ratios that physiologically exist in cells.

However, this is set up in a way such that you can favor ADP-to-ATP synthesis by converting PEP to pyruvate in a pathway, as we will see happens in glycolysis. All right. Now, the second reaction that I want to-- that allows ATP synthesis in glycolysis is catalyzed by an enzyme called phosphoglycerate kinase. And this carries out the following reaction.

OK. So this molecule is 1,3-Bisphosphoglycerate, abbreviated 1,3-BPG. And basically the phosphate is transferred from here to ADP, which gives you this molecule, 3-phosphoglycerate or 3-PG.

So this reaction, the delta G0 prime for the entire coupled reaction, both steps of the reaction, is minus 4.5 kcals per mole. Tells you about the equilibrium constant. Equilibrium lies to the right, doesn't lie as far to the right as the PEP plus ADP goes to ATP plus pyruvate reaction, but nonetheless, is one where there's lots of conditions that are favorable in cells to maintain a high ATP-ADP ratio, although we will see later that this reaction actually runs quite close to equilibrium in cells, so there's actually conditions where it can go in the opposite direction as well, and we'll get to that later in the course.

I want to point out one thing about this, one thing that makes this reaction favorable, as you notice here that this is a phosphate on an acid group, a so-called acid anhydride. And this is a pretty good phosphate donor. And so you want to lose that phosphate, which is one of the reasons why this reaction is favorable for ATP synthesis even at high ATP-ADP ratios.

All right. So these are the two reactions in glycolysis that can favor ATP synthesis at a high ATP-ADP ratio just like creatine and creatine phosphate. And so hopefully now it's become apparent to you that if during our pathway of glucose catabolism we can build a pathway that couples what we already know is favorable, glucose oxidation, to generate these intermediates, PEP and 1,3-Bisphosphoglycerate, lacerate we can then build a pathway where those reactions can make synthesis of ATP favorable even though there's a high ATP-ADP ratio in cells, and in fact, maintain that high ATP-ADP ratio in cells. And in essence, this is the logic or the goal, if you will, of glycolysis and why it's important to provide energy to cells. OK.

All right. Now, if we're going to make a pathway where glucose oxidation is coupled to making PEP and 1,3-Bisphosphoglycerate, it's obvious we need to add phosphate to the system. There's no phosphate molecule on glucose. Now already mentioned earlier-- and I just erased it-- that to trap glucose in the cell, the first step in glycolysis, you add phosphate.

But that phosphate donor is ATP. And so ATP being the phosphate donor doesn't help us here. If we're going to use phosphate transfer here, we need to have phosphate added into the system if this is going to work. We can't have ATP as our phosphate donor.

You also saw lots of reactions from Professor Yaffe that involved phosphate transfer reactions. But again, ATP-- or GTP is the phosphate donor. And so for this system to work, we need a way to get inorganic phosphate onto a carbohydrate or we can't make things like PEP or 1,3-Bisphosphoglycerate that will allow ATP synthesis.

Now we already saw earlier that additional phosphate to glucose is not favored. Remember, delta G0 prime of phosphorylating glucose was plus 3.3 kcals per mole. And it turns out that says equilibrium lies to the wrong side, so we can't net do that without ATP or energy input. And it turns out this is true for other sugars as well. And so you need energy input to add phosphate to the sugars.

Plenty of energy input from glucose oxidation, but we need a reaction where phosphate addition is coupled to another reaction that makes it favorable, and we have to do this without making ATP. And again, it illustrates, again, the biological energy is not just about ATP. At a deeper level It's something different.

And so to really discuss this and understand it, I need to introduce two concepts that are going to come up again over and over again in this class and across metabolism. The first of these is a very high-level bioenergetic concept, although it's really an energetic concept, and it comes back to what we already talked about.

So, we know burning wood is favorable. The CN H2O N plus oxygen going to CO2 plus water. That's burning wood, lots of energy released. Drew it up there for a single-glucose molecule and what the energy release the delta G0 prime is for that reaction.

Now I want to point out chemically what is going on in this reaction. Chemically what is happening for this to occur is that electrons from the carbon are being moved to the oxygen. You are changing the oxidation state of carbon and of oxygen in order to carry out this reaction. Oxygen is a better electron acceptor than carbon, and so transferring those electrons from the carbon to the oxygen is favorable. That is, in essence, what is going on in this very favorable reaction to burn wood.

Let me illustrate that a bit more explicitly here. So the carbon-- most of the carbons in carbohydrates are alcohols. So here, you can call this our carbohydrate, with the exception of the ketone or the aldehyde if it's a ketose or an aldose. All the other carbons in the sugar are alcohols. All the other bonds there.

And so if we oxidize that carbon-- that is, if we remove electrons from the carbon, how can we do that? So this here generates this H- hydride ion. This is, in essence, two electrons that we are removing from the carbon. That gives us a ketone. So if we oxidize the alcohol and we remove two electrons, we get the ketone. We could oxidize this ketone further. That will give us the carboxylic acid. If we oxidize it even further, now we get CO2.

So, moving in this direction, this is oxidation. Transferring electrons from the carbon to the oxygen to go in this oxidation direction, that is glucose oxidation is what's happening when we're burning wood. It's what happens when we burn glucose. Energy is released. This is largely what catabolism is about in biological systems.

Now then reasons, then, that if we're going to move in the opposite direction, so-called reduction, that is going in this direction. Reduction. That's largely energy storage or anabolism in metabolism. We are storing energy that we can burn later.

And of course, what's the more extreme version of this? Well, what if we add two more electrons to the carbon going in this direction? Well, what do we end up with? Well, now we end up with the saturated carbon. more reduced than the alcohol is the fully saturated carbon. You will see later that this is what fat is. You saw that in some of the earlier lectures. It's also what gasoline is. What's gasoline? It's fully saturated hydrocarbons that we then burn to CO2 and derive energy.

Just like burning wood, burning gasoline all in one step, oxidizing the gasoline gives you energy. There's more energy in gasoline. You can release more energy per weight from gasoline, from fully-saturated hydrocarbons than you can from wood. Same thing happening here. What has more calories in your food? Everyone knows fat has more calories per weight than sugar. It's because the more reduced our carbon is, the more energy it stores, and as we oxidize that carbon, that is what releases the energy.

And so energy transduction in biological systems-- and actually, in non-biological systems, as I just illustrated with the wood and gasoline example, but definitely in biological systems, is largely about oxidation and reduction reactions, and you are going to see this coming up over and over again.

Now, based on what I just said, the real magic, if you will, of glycolysis can be further refined to say that what glycolysis does is it couples the favorable oxidation of carbon with reactions that allow phosphate additions and production of intermediates, like PEP and 1,3-Bisphosphoglycerate, where synthesis of ATP is favored despite the high ATP-ADP ratio in cells.

If you think about it, that process then allows you to keep that ATP-ADP ratio in the range where it can then be useful for all the reasons we've been talking about now for two lectures. Allows the cell to couple that ATP-ADP ratio, that-- in ATP hydrolysis to otherwise carry out otherwise unfavorable reactions.

OK. So, based on that, arguably the key, or at least a very important reaction in glycolysis, is the oxidation step which happens to add the phosphate and also happens to make 1,3-Bisphosphoglycerate, which we saw earlier can be used to drive ATP synthesis. And so that reaction is the following.

So this molecule hopefully looks familiar to you from the carbohydrate lectures. This is D-glyceraldehyde 3-phosphate. I will abbreviate it frequently is G-3P for glyceraldehyde 3-phosphate, but it's D-glyceraldehyde 3-phosphate. Remember, it's a D, sugar, because the OH group is pointing to the right as we drew it this way. It's also an aldose because it's a aldehyde sugar, and it's phosphorylated on the 3 position-- remember the way we numbered sugars was starting at the carbonyl-- 1, 2, 3. This is carbon-3, phosphorylation on the 3 position. Glyceraldehyde 3-phosphate.

OK. So, this step generates 1,3-Bisphosphoglycerate. Well, to do this, there's actually two things that happened here. First we had to add the phosphate to this. It says two phosphates on it. bisphosphoglycerate, this has a single phosphate. So inorganic phosphate has to be added to generate that molecule.

By the way, adding that phosphate delta G0 prime is positive plus 1.5 kcals per mole. That means even equilibrium, this reaction would lie to the left, of course, because delta G0 prime is positive. However, remember the next step downstream of this is the one shown over here where we do ATP synthesis. That step is very favorable. So this is an example where metabolism has coupled a very favorable step to a step that's less favorable. That can keep the 1,3-BPG levels low and pull the entire system forward, the metabolic trick we discussed in the last lecture.

OK. You'll note the other thing that's happening here, other than adding that phosphate group, is that we've changed this aldehyde to a carboxylic acid. That is an oxidation reaction. Ketone to the acid is an oxidation reaction. I will illustrate over here this so you can see why it's an oxidation reaction.

So here's our aldehyde. If we take electrons from the carbon and that generates this hydride ion to electrons, what are we left with? Well, we're left with this intermediate that will never exist with a positive charge on the carbon. Water, which, of course, is abundant in biological systems. And then come-- OK. And it basically illustrates that as I go from the aldehyde to the acid, of course, I make a proton and a hydride ion, but really, I have changed the oxidation state of the carbon from the ketone to the acid state. Two electrons are lost there, is shown as that hydride ion. OK.

So, what does that mean? Well, that means that we have to maintain that balance in this reaction as well. So water is going to have to come in and a proton will be produced. But the key thing is we also generate two electrons, this hydride ion that have to go somewhere. Now, finding a place for those two electrons to go is what brings up the second high-level concept that I now need to spend some time discussing.

And this second high-level concept that I need to introduce is the role of cofactors, and cofactors also is effectively the role of vitamins and what role that these things play in metabolism. Most vitamins are used to support metabolic reactions, and we're going to learn all about what many of the vitamins that you guys are familiar with reading the side of your cereal box actually do throughout the course.

Now cofactors, as I said, often involve vitamins, are molecules that provide useful chemical groups that facilitate the reaction chemistries that are needed in metabolism. So they provide some useful chemical groups to facilitate some of the chemistries that we need to carry out metabolic reactions.

They are, in general, in small non-stoichiometric quantities in order to carry out this function. What do I mean by that? Meaning that you can imagine that the cell will convert lots of glyceraldehyde 3-phosphate to 1,3-Bisphosphoglycerate, but the enzyme, you don't need one enzyme for every time you convert that reaction. The same enzyme can do it over and over and over again. Well, it turns out, cofactors are much more like enzymes in that respect. They need to be recycled in a way that they can catalyze the turnover of reaction many, many, many times, and it'll be much clearer what I mean by this when we get into some of the details.

Now, the vitamin concept comes from the fact that often these cofactors or parts of the cofactors are not made by animals, not made by humans. If they're not made by us, but we need them to do our metabolism, well now we have to get them from the diet, and this is really the concept of vitamins. These are small things that we have to get in quantities from the diet, and it turns out they're very important to help us carry out the metabolism we do.

As an aside, the vitamin industry is a very big business. Lots of unfounded claims out there. I'm not going to get into-- that can get very political. I'm just going to say that just know that more is not always better, and the goal for this class is really to understand what some of these vitamins actually do.

OK. Now, the relevant cofactor for the reaction that we're talking about is a cofactor called nicotinamide adenine dinucleotide, which is always abbreviated NAD or NAD+. This stands for nicotinamide adenine dinucleotide. You see why we want to abbreviate that NAD.

And basically what nicotinamide adenine dinucleotide is is it's a factor that can solve our problem of where to put these electrons in this oxidation reaction. That is, you can accept two electrons from a reaction like this one here and cycle to another form of the cofactor called NADH.

All right. What is nicotinamide adenine dinucleotide? Well first, let's draw what it looks like. OK. Sorry it's squeeze down here a little bit. So this here is adenine. This group up here is nicotinamide.

So if you look at this, this is a dinucleotide. And so down here, this, of course, is, from here down, is AMP. That phosphate is now over here. This is a separate nucleotide, where rather than having one of the AGCTU bases that you're used to from RNA and DNA, nicotinamide is the base. And so it's this AMP plus this nicotinamide base is a monophosphate, or a nicotinamide adenine dinucleotide.

This, the nicotinamide group, is derived from a vitamin called niacin. And so from the side of your cereal box, this is what niacin does. It allows you to generate the nicotinamide group that's part of this cofactor, nicotinamide adenine dinucleotide. Now, nicotinamide adenine dinucleotide is useful because of what that nicotinamide group can do.

And so I'm not going to draw out the whole molecule, but I'll show you. So NAD+, which is the oxidized form of nicotinamide adenine dinucleotide, is as I drew it up there. So this here would be attached to the rest of the dinucleotide. So this is the oxidized form. I can have-- here's my hydride ion, which, remember, is two electrons.

If I carry out that chemistry to add those two electrons to the nicotinamide group, you now end up with the reduced form of nicotinamide adenine dinucleotide NADH. So the difference between NAD+ and NADH, it's the H, but it's really the two electrons that were added in adding that H. So it's oxidized form and reduced form.

So remember, I want to stress this again, at least in biology we can't create or destroy matter. You can do that in nuclear physics, but we can't do that in biology. And so that means that if we do an electron transfer like we did in this reaction where we oxidized the aldehyde in glyceraldehyde 3-phosphate to the acid in 1,3-Bisphosphoglycerate, those electrons have to be accounted for.

And so if one molecule is oxidized, another one has to be reduced. So redox reactions always-- that is, redox, oxidation-reduction reactions, always have to happen in pairs. And so over there we have the aldehyde in glyceraldehyde 3-phosphate is oxidized to form the acid in 1,3-Bisphosphoglycerate.

As part of that reaction, we can couple it such that the nicotinamide group, which is oxidized in NAD, can be reduced to the nicotinamide group that is found in NADH. So that means we can draw this in here, is that having that cofactor coupled to this reaction now allows us to fix the redox problem of this reaction.

Now this reaction here is catalyzed by an enzyme called glycerol 3-phosphate dehydrogenase. I'll draw that out in the second. Often abbreviated GAPDH, the loading control in all of your western blots, is the enzyme GAPDH, glyceraldehyde phosphate dehydrogenase. That is the enzyme that carries out that reaction.

Now notice, the name fits. It's a dehydrogenase, it's removing a hydrogen, removing two electrons as a hydride ion, but it's really-- what's important here is the electrons moving, not the hydrogen itself. So in that way it's a little bit misleading. And you will see that in general, dehydrogenases are classes of enzymes that catalyze oxidation-reduction reactions, and as a result, often involve NAD/NADH as cofactors because they facilitate those electron transfer reactions.

OK. So now we're able to describe how GAPDH works in great detail. And so the chemistry of GAPDH works as follows. And so in the active site of GAPDH, there's a cystine residue. That cystine residue has a sulfur shown here. Also bound near the active site is in NAD molecule to catalyze the reaction shown. That NAD has to be in the oxidized state, so it's NAD+.

And so glyceraldehyde 3-phosphate binds in the active site. There's glyceraldehyde 3-phosphate. By the way, I will show you how many reactions in metabolism work chemically. Obviously we don't have time in this class, an overview class to go into all the great chemical details of what's going on and exactly how everything works, but I'll at least try to give you a flavor of some of the chemistry that happens throughout the course.

All right. So once you have, then-- OK. So now you have this intermediate bound into the enzyme active site. You can now carry out the oxidation of that carbon. So these are two electrons being removed as a hydride ion. They can be transferred to NAD in the active site. That generates NADH by the exact chemistry that I just showed you. OK.

That NADH now needs to be exchanged out or the enzyme will not be able to catalyze the next series of reactions, and that NADH will then go somewhere else, and of course, has to be cycled back to NAD, but that doesn't happen in this reaction, per se. OK.

Next, this here, you'll notice, is that by the way this chemistry worked, we formed this thioester bond in the active site. This is another thing that you will see over and over again in metabolism. This sulfur in the thioester bond is a good leaving group, and so it's really setting up a situation that will now help with the phosphate addition. So here, I'll draw out inorganic phosphate. And that, of course, will give us the identical enzyme active site that we started with plus 1,3-Bisphosphoglycerate. And that 1,3-Bisphosphoglycerate can now be coupled to ATP synthesis to make 3-phosphoglycerate.

And so you can see, in essence, what this reaction has done is that it has coupled oxidation of the aldehyde to the acid, the aldehyde in 3-phosphate to the acid in 1,3-Bisphosphoglycerate, which can then do phosphorylation of ADP to make ATP. So in essence, this is oxidative phosphorylation.

Now I realize in high school or in other classes, oxidative phosphorylation means something different to you, and of course, we will discuss the process that is called oxidative phosphorylation in great detail later in this course. But I want to point out that the chemistry that's actually happening here in glycolysis is also oxidative phosphorylation. It's basically how you coupled this favorable carbon oxidation that's occurring at this GAPDH step in a way that allows you to synthesize ATP at a high ATP-ADP ratio, which in the end will allow cells to do otherwise unfavorable things.

OK. Now we're ready to discuss these reactions, the GAPDH reaction, the pyruvate kinase reaction, the phosphoglycerate kinase reaction, and how they fit into glycolysis as a metabolic pathway in a way that allows cells to use glucose metabolism-- that is, the breakdown of glucose, the burning of glucose as a way to get energy. And that pathway is, of course, glycolysis or glucose lysis that we mentioned earlier.

Glycolysis is a very ancient and ubiquitous pathway. It's used by essentially all life on Earth and probably has been for at least 2 to 3 billion years. Glycolysis, I guess, as a pathway was, quote-unquote, "discovered" mid-19th century by Louis Pasteur, and Louis Pasteur, of course, described life, microorganisms as being responsible for the process of fermentation. That is, conversion of glucose into alcohol, something many college students are interested in, as well as other organic acids.

And of course, fermentation to make alcohol or to store other types of organic acids has been used in food preservation for centuries, which is really why Pasteur's discoveries were so impactful at the time. Now even though Pasteur discovered this, he didn't really understand chemically how it worked, and it actually took decades to figure that out. And when it was finally figured out and described as the pathway called glycolysis, this was done by two chemists, two chemists in Germany, Embden and Meyerhof.

And basically what they did is they pieced together several enzymatic or chemical activities that have been purified from cell lysates mostly from yeast and used those to build a pathway that described chemically how you could start with glucose and chemically break down that glucose in a way that made sense in this fermentation pathway, and that is why glycolysis is sometimes called the Embden-Meyerhof pathway.

Now, glycolysis does not completely convert glucose to CO2. Instead, what glycolysis does is it converts glucose--so I will draw here in the pyranose form. It converts glucose by a number of steps into two molecules of the organic acid pyruvate, which, of course, was the product of that pyruvate kinase reaction that we showed earlier.

Note that the fermentation that Louis Pasteur described was further metabolism of that pyruvate into something else. In the case of yeast, it was ethanol or ethyl alcohol, or something else. The fermentative product in mammals is this organic acid lactate.

And what we will see next time is that this fermentation of pyruvate into one of these other molecules ends up being an alternative to the further oxidation of pyruvate to CO2 which requires oxygen, and the reason oxidizing pyruvate to CO2 requires oxygen is because ultimately we have to regenerate that NADH we made in glycolysis to allow that cofactor to cycle and glycolysis to keep moving.

Fermentation gives us an alternative way to do this, which we'll discuss in great detail in the next lecture. But first I want to focus on glycolysis and what we can describe next time as really how we can build a pathway to turn glucose into pyruvate. This glycolysis, this Embden-Meyerhof pathway, and some of the details of that pathway are shown here on this slide.

And this is where we'll start off the next lecture, discussing how each of these steps in glycolysis function in a way that allow a pathway to generate phosphoenolpyruvate, 1,3-Bisphosphoglycerate, incorporate this GAPDH reaction, and do so in a way that's overall energetically favorable to allow the cell to couple this oxidation of glucose to keeping ATP-ADP ratio in the right range to support other unfavorable functions in the cell. OK, thanks.