OK. Today we're going to get into some stuff where we're kind of peering way back in evolution about how life first learned to make energy. But before we do that I just want to finish up talking a little bit more about enzymes, the biological catalysts that are critical for life to exist and about how energy is stored. I want to clarify a point that clearly confused a couple of you in the last lecture. So the Gibbs free energy that we talked about can tell us that a reaction could go and, in this case, would actually release energy if it occurred, if these reactants were converted to these products. But the problem for most reactions is that in order for the reaction to take place there's a state in the middle, some chemical state known as the transition state which is energetically less favorable than either the reactants or the products. And if A and B are going to convert to C and D they have to probably start coming together in some kind of way, and that becomes energetically unfavorable. And this gives this activation energy -- -- or delta G00. And if the cell wants chemical reactions to take place at 25 degrees Centigrade aqueous solution it has to do something about that, and so it employs biological catalysts. And what a catalyst does, as you've heard in chemistry, is it lowers the activation energy in some way so that the molecules have enough energy just within their normal energy distribution at that temperature to get over the hump. These biological catalysts come in two flavors. As I said there are enzymes which are made of protein with all of those amino acids, the side chains that we talked about when the thing folds up in 3-dimensional space form a little chemical environment that enables that activation energy to be lowered. There are also few -- Not so many. But we now know that there are catalysts made out of RNA. These are called ribozymes. There are not so many of them but they're important. Now, the characteristics of these that are important is the specificity -- Each enzyme or ribozyme is highly specific for a given reaction. So that means the reaction probably will barely go unless that enzyme or, in some cases, ribozyme is present. And so that's really the secret to how cells control all of these many, many, many hundreds or thousands of chemical reactions that take place that are necessary for life. Because what they need to do, and if they want to control whether reaction takes place or not is they control the availability or the activity of an enzyme. And when we talk about gene regulation you'll see, for example, one way a cell might do it is to not even bother to make the enzyme unless it wants a particular reaction to take place. Or it could take an enzyme that's there and put little bells and whistles on it that make it more active or less active. And we'll see an example of that pretty soon. That is the secret to how cells are then able to regulate metabolism. And these biological catalysts use a whole variety of different molecular mechanisms, although all of them follow this principle of what they're trying to do is lower the activation energy. So I'll just give you an example. I showed you of how one particular enzyme does it just in sort of cartoon form. I gave you the example of glutamate being converted to glutamine. Now, both of those are amino acids that are critical for making proteins. The cell has to make both of them. But as I showed you converting glutamate to glutamine is energetically unfavorable. It's got a delta G plus 7. And then I showed you if you had an ATP going to ADP at the same time you could actually drive the whole reaction forward because there was a net gain. But how is that actually accomplished? And it's the enzyme that carries this out. And I'll just show you, as I say, in sort of cartoon form. The way the enzyme works it has one binding pocket for glutamic acid or glutamate. It fits in here. It makes lots of specialized contacts, all those sort of molecular interactions we're talking about. And it also binds this molecule adenine triphosphate or ATP which is an adenine, a ribose and then three phosphates joined together. And it makes interactions along here that enable it to bind very specifically. Now, by providing all this binding energy for ATP and for glutamic acid what the enzyme has done is positioned the carboxyl group of glutamic acid right next to the last phosphate on the ATP. This enables this to form a bond here which liberates ADP and leaves you now with the glutamic acid with a phosphate on. That reaction goes forward because you broke the bond of ATP, but this is still a pretty unhappy molecule. It's got a lot of oxygens at very close proximity. So the enzyme has another binding pocket that's absolutely specific for ammonia. It won't fit water which is very close, which is a good thing because that would just reverse the process. Ammonia gets in there and then it attacks here and liberates the phosphate. And that then gives you glutamine and the inorganic phosphate. So the enzyme has provided this binding surface that makes the reactions go under biological conditions. But it's also managed in the same process to have it go by a mechanism in which it sort of temporarily captured that energy that's in the ATP bond and then used it to drive the rest of the reaction. I mean it's the magic of how all of this developed. It's really amazing but that's how every single biochemical step in your body takes place. Virtually of them require an enzyme that in some way is highly tuned to do just the one single reaction. As I said, the principle of how these enzymes work is they lower the activation energy. And the way they do that in general is they provide a binding pocket that resembles the transition state. So as things approach here then it fits best into the pocket and therefore you get some energy back and kind of lowered the energy hump that's necessary to go over. And here's a reaction I'll be showing you in today's lecture. It's going to involve the transfer of a phosphate to a glucose. And the first thing that happens is this enzyme interacts with ATP and takes one of the phosphates and attaches it to one of its aspartic acid carboxyl groups. So you've got actually a mixed in hydride if you know chemistry. But again it's captured that phosphate. This is a very unstable bond. And so if you break it you will release energy. And what the enzyme does is it allows the hydroxyl of here to come and attack this phosphate, and that then releases the aspartate of the enzyme and you end up affecting the transfer of the phosphate that began life on ATP. And now it ends up on the glucose. But, as you can see here, phosphate interacts with four atoms. But as this hydroxyl comes in it has to attack the phosphate. And somewhere in the middle there's an intermediate where all of these things are interacting. And some crystallographers actually managed to capture that in a crystal structure. And here you can see this is the oxygen coming from the sugar, this is the oxygen of the aspartate and here is the phosphate where it's now, as the attack is taking place the thing is sort of pushed out, and it's caught right at that transition state. And that's what the enzyme is providing a binding pocket for and thereby lowering the activation energy. It's a really beautiful piece of structural work. The second thing then I want to clarify was this molecule ATP which is, as I say, like energy money for the cell. When there's a

thing then I want to clarify was this molecule ATP. Whereas, as I say, like energy money for the cell. When there's a reaction where it can extract energy it tries to make ATP. And when there's a reaction that doesn't want to go it will somehow figure out a way to spend that energy and make the reaction go forward. And the molecule, just to put it again, because it's a pretty important one in biology. That's adenine which you already saw when we talked about nucleic acids. And it's got three phosphates like this. You can see it's probably a pretty unhappy molecule because it's got all of these oxygens stuck together. And if you break this bond then you release some energy. So you could think of it in this kind of way. That if we have ADP, which is adenosine diphosphate plus inorganic phosphate, and ATP is here. And if you were to break the bond and make it back into ADP and inorganic phosphate then you would have gone energetically downhill. But in order to make this you could think of it as taking an inorganic phosphate ion and this ADP, if you start pushing them together the negative charges are going to repel and you kind of go up an energy hill. But if you ever get them close enough then they start to share electrons and they fall into this sort of energy well. And this is what ATP is. And so it's sort of like taking a spring and pushing it together. And then when you form the bond it's like you put a little hook on it. And now you've got this spring that's compressed. And it's stable, it won't do anything, but there's energy stored in there that you can use. And it's the same principle in terms of how the cell stores energy within ATP. And this energy is stored -- -- if you think of it in bundles of about 12 kilocalories per mole. That's about how much energy is released under physiological conditions when you hydrolyze that bond. So hydrolyzing ATP to give ADP plus inorganic phosphate will have a delta G of minus 12 kilocalories per mole under physiological conditions. Now something in terms of evolution, which I know a number of you said you were interested in, here's a really interesting thing. This is the main energy storage molecule for the cell, but you've heard about it before because adenosine, that's the nucleotide that we find in RNA. And, in fact, ATP is also the precursor, as we'll learn, for making RNA. And one of the things that puzzled scientists for many years is how did life ever get started in the first place? There seemed to be a chicken and an egg issue that proteins did the work and DNA stored the information and RNA was kind of a messenger in between, and we'll talk a lot about that in between. So how could you ever get life started? So the current thinking is that sometime, if you remember in that first lecture, we had about 4. billion years ago the first organism, something like today's bacterium showed up here about maybe 3.8 billion years ago. That somewhere in between there was what people are now thinking of as an Ã¬RNA worldÃ® where RNA managed to act as a ribosome and catalyzed chemical reactions, but it also had the capacity to store information. But it's sort of intriguing, although no one has proven that. It's just a hypothesis. We also see that the major energy storage molecule found in all living things is also a building block of RNA. It certainly sort of fits with that kind of idea. Now, there's one other kind of reaction I'm going to have to tell you about. Penny will talk quite a bit about this when you're thinking about how organisms make living. But this is a set of reactions known as Ã¬redox reactionsÃ®. So the loss of one or more electron(s) is called an oxidation. And the gain of one or more electron(s) is called a reduction. If you're going to take away an electron somebody else has to get it. So these things always happen together. And therefore they're given the term redox reactions where electron(s) from somebody goes to somebody else. So somebody gets oxidized and somebody gets reduced in the same reaction. And you can think of them as a transfer of hydrogen atoms, not hydrogon ions. And the most familiar kind of sequence that you will see over and over again in biology is the sequence you go from, let's say, a methyl group to an alcohol with a hydroxyl to an aldehyde or a ketone with the double bond oxygen to a carboxyl group. You go one more step then it's CO2. So going in this direction it's an oxidation. If it's going in that direction the molecules are getting reduced. Just the same way that the cell and life have molecules that store energy in ATP, they also have an important molecule that stores electrons. And that molecule is known as NAD or nicotinamide adenine dinucleotide. NAD+. And its structure is, let's say a ribose, a five carbon sugar. And it's got this entity on it. This is in your book so don't worry if you don't get the structure down. There's a positive charge on the nitrogen here. And it's joined through a diphosphate linkage to, guess what? Another molecule of adenosine. Here we find again a piece of a thing we find in RNA is now part of this system for storing electrons. And the way this works is if you have two hydrogen atoms transferred to here then this entity right here goes to this plus a hydrogen ion. And this we would know is NADH. I left out an oxygen here. Somebody picked it up. [LAUGHTER] Just too excited by the annual Valentine's Day visit here. I wish the rest of you had a song for you, too, but we didn't have time to set that up. So there's an important thing here, too, because actually a lot of energy is stored in there. This is a bundle of energy in this molecule that's actually about 50 kilocalories per mole. And especially when we get to next week's lecture you'll see how cells go about extracting the energy out of that and making that energy into ATP, which is sort of a universal currency the cells can spend. Now, somebody asked about memorizing all of these structures I mean really, As Julia says in her thing, we're trying to get you to focus on the concepts here. You won't have to memorize the structure of everything. It would be helpful if you recognized that glutamine and glutamate are of the 20 amino acids, but we'll give you the structures and we'll give you the structures of something like NADH if you needed to do something with it. But the important thing is to remember that energy is stored in that high energy bond of ATP, that electrons are stored in this NADH, and they can be used in reactions that oxidize or reduce. NAD and NADH can be used in reactions that remove or give electrons to biomolecules. Now, the same thing goes for what I'm about to tell you now because one of the first things that had to happen as life evolved was there had to be some mechanism of getting energy made. And the reaction I'm going to tell you about is called glycolysis. And it's a way of taking a molecule of glucose through a whole series of biochemical transformations and to end up yielding -- -- two molecules of something that's known as pyruvate. And it also makes two molecules of ATP and two molecules of NADH. So it's a way that was invented in evolution of making ATP by carrying out a chemical transformation. And this is basically the same chemical transformation that we've been talking about that Lavoisier and Pasteur studied except that, as I'll show you, you do a little bit to convert it either to lactate or to ethanol. I'll get to that in a few minutes. Remember the point, also just to remind you, the reason I gave you that historical thing is because what it turned out when people started out to study something, was winemaking of great interest to French scientists, was what they actually learned was how cells made energy. And, in fact, here we're looking at a sort of

was what they actually learned was how cells made energy. And, in fact, here we're looking at a sort of biochemical fossil in a way because this pathway of glycolysis, which you'll see is kind of awkward. It's got ten different biochemical steps, it needs ten different enzymes, and what the cells got out of it is two molecules of ATP. But this system developed apparently way, way back in evolution before life forms got into these various Kingdoms because it's in virtually in every living creature no matter what it is and it's essentially biochemically identical. Now, it's possible we could go back nowadays and devise a better method, but once that something like that gets fixed in evolution, if something mutates to try and change it most of the time it's a disadvantage. And so if something gets locked in, and this is true of many, many of these very complicated biochemical pathways. So you won't have to remember all these structures I'm going to put on the board, but try and stay with me because I want to sort of show you one of these. This is probably the most ancient of these pathways. And it's still in all of us. It's in the bacteria in our guts. It's in the plants in the field. If you go out in the open ocean organisms still can carry out glycolysis. So one thing, though, I want to try and put it in this way, if I came to you and said I've got the greatest idea. This is going to be how we're going to make energy and evolution as part of this entrepreneurship, I think you'd be right to be skeptical so I'll probably sort of tell you in that way. So I've already shown you how to write glucose in a linear form, although I then told you that most of the time in solution it's cyclized into a pyranose ring, a six membered ring. But for the moment we can think of glucose as a stick. And I'll get you to just focus on the one position, the two position and the six position in that linear thing. If you look back at your notes you can see what the full structure of glucose looks like. But this is how the process of glycolysis starts. This is if your body is going to take a molecule of glucose and make energy out of it, this is the first thing it does. It takes an ATP. It converts it to an ADP. It puts the phosphate down here to give glucose-6- phosphate. That's the only thing that changes. Isn't this just like most young entrepreneurs? Give them some venture capital. The first thing they do is spend it, buy a nice potted plant for the company they're building. It doesn't seem to be, if you want to make energy, starting out here spending energy is the first thing that the cell is doing. It's using up an ADP, although the overall goal is to make ADP. It then does a little shuffle, reverses the position of the double bond and the hydroxyl. This is an energetically something without much cost, but this sugar is different because this is now fructose-6-phosphate. It's got a little bit different arrangement of the double bond and the hydroxyl, but energetically it's pretty much the same thing. Then the next thing that happens the cells spends another molecule of ATP. It gives now -- -- fructose-1, , this is the sixth position, the one position to two position, 1,6-diphosphate. It doesn't look like we're on our way to make energy yet. Cells invested two molecules of ATP and what it's done is it's got this glucose transformed to fructose 1, -disphopshate. Well, what happens now then is the cell splits this into two three carbon units. There were six carbons in glucose. Yeah? Well, it's a linear molecule. There's a phosphate here and a separate phosphate down there. They should be. Yeah. I'm probably dropping charges and hydroxyls, OK? But check your book if you notice something like that. So what we get -- What the cell gets out of this then are two three carbon units, one of which is this -- -- known as dihydroxyacetone phosphate. And you can find these names in your book. You don't have to, as I say, remember the structures. What I've done is basically taken this molecule and I've flipped it over so that the phosphate will be down. And you'll see why I've done that in a second. And from the bottom half of the molecule then we get -- This is glycereldahyde-3-phosphate. So this is three carbons. This is three carbons. This was six carbons. So the cell has split it into these three carbon units that are very similarly related except where the double bond is. And there's an enzyme that actually catalyzes the conversion of those two. It's a catalytically perfect enzyme that goes. It's just limited by the rate of diffusion. And it can do something of the order of ten to the eighth molecules a second. It's a really, really efficient catalyst. So what happens then is this, since these are in equilibrium the cell is going to now start to pull these -- This. But these will be converted into that and will be able to get here. So we're going to follow the fait then of these -- -- two glycaraldahyde-3-phosphate molecules. Excuse me. Sorry. OK. Now, at this point the cell is at the aldehyde stage. And it's going to carry out an oxidation reaction. So it's going to take a couple of electrons away from here, and it's going to therefore be carrying out an oxidation. If the molecule is getting oxidized something else has to be reduced. What's going to get reduced is NAD+. We'll need two molecules of that because we've got two molecules of glyceraldehydes phosphate. So we end up with two molecules of NADH plus a hydrogen ion. And this is an energetically favorable reaction. So the cell is able to sneak a phosphate in and make a molecule and still have the reaction go forward, have a molecule that's not very stable, but it can make it because the overall thing goes forward. And there are two of these. And what we have now is 1,3-phosphoglycerate. What the cell has basically managed to do is to get two phosphate groups very, very close together. So you're probably getting, hopefully, the concept that if you stick a bunch of negative charges together and hold them together that molecules, if you break one of those bonds are going to go energetically downhill. And you can do work. And the way it does that then is in breaking this bond it uses it to make two molecules of ATP. So you've now got, this is up at the acid level or a carboxyl group. And we've got three phosphoglycerates. So at least from the point of view of this as a plan for making energy, we've now managed to get back those two ATPs we invested. So up until now we've got our, the venture capital money we put in has be recovered, and we've got a couple of molecules of NADH out of it. But what the cell now does is finish, to carry out some more steps that let it make a couple more molecules of ATP. So the first step then is a kind of just a switcheroo between where this hydroxyl is and this phosphate is. So it brings the phosphate up to here. As you might guess this is energetically not much of a change. However, what it does now is it enables the cell to eliminate a molecule of water from here so we get two molecules of water come out because we had all along here we're carrying on two molecules from up there because we have two of these three carbon units. Then the molecule that we then get here -- -- is this molecule which is known as phosphoenolpyruvate. And several of you are saying you don't remember much from chemistry. So this is a keto group, which I know you were introduced to. But it's in an equilibrium with what's termed an enol form where you have an OH here, a double bond like that, and that's known as an enol. Now, this is energetically greatly disfavored. So normally most of the time you find something in a keto form, but occasionally you find it in an enol form. And what's happened here really is the cell has trapped what would like to be a keto at this position in an

form. And what's happened here really is the cell has trapped what would like to be a keto at this position in an enol form. Again, this is a very energetically unstable molecule. You've got all these oxygens together, two of these, and so the cell is once again able to take ADP and make two molecules of ATP. And we end up with -- -- two molecules of pyruvate. And extraordinary amount of work. What do we get out of it? Well, we've got a total of four ATPs now plus two NADHs. What did we invest? Two ATPs. So the net yield from this reaction is two ATPs plus two NADHs. So strange as it seems this was one of the first sequences of biochemical steps that were put together in a pathway that we're capable of letting an organism generate molecules of ATP, or sort of form of energy money, but metabolizing something it could find like a molecule of sugar. There are two enzymatic steps. That means that there has to be a separate enzyme for every step in the pathway. Now, the ATPs, as I said, have energy in bundles of about 12 kilocalories per mole. There's a lot of energy here in NADH. And in the next lecture I'm going to talk to you about respiration, which is something you're aware of. You know we respire, but chemically what that we'll see means is basically it's a way of extracting the energy that's in the NADH by transferring electrons to oxygen. And that's a wonderful way to make energy. It's far more efficient than this ancient pathway, but at the time life started there wasn't any oxygen in the environment. And, in fact, it didn't reach, as I said, I think it was something like 20% of today's levels until we were about a half a billion years or so ago in evolution. So organisms had to learn to make energy without oxygen being around. And this was the way that they did it. And it was such a success in evolution that our bodies do, the bacteria in our gut do it and just virtually all living forms. So it's sort of a biochemical fossil but it was so successful it took hold. It's sort of like legs. Those appeared in evolution. And there are all sorts of organisms now that use legs, and they've evolved into wings and everything, but it's all the same basic idea. You could imagine a life form that started with wheels. And maybe if it had been the first thing to do maybe there'd be some sort of organisms with wheels, but legs were such a success at some point that that's what got used and then evolution made various embellishments on it. But there is a problem here. I don't know if anybody can see what it is. If I'm going to be able to use ATP to make energy and I want to keep generating more and more molecules of ATP so I can build stuff, I cannot give those electrons in NADH to oxygen. So what would happen if I just kept running this system? Anybody see what the problem would be? Yeah. You'd run out of NADH, exactly. We need to somehow recycle that NAD so it can take place. If we could give it to oxygen, oxygen as I've told you in respiratory, that would be cool. But organisms didn't have that option. And so they worked out ways of doing things with pyruvate. And this is where you'll see this coming together with what we talked about the other day. So let's take those two molecules of pyruvate. And there are basically two strategies, two major strategies you find in nature. One is to take the two NADHs plus two hydrogen ions and convert it to two molecules of NAD+ so that regenerates it. And what do you get if you do that? You end up with molecule. Two molecules of that which I introduced you to the other day. That's lactic acid or lactate, the organisms that make yogurt carry out. That's what they do. That's why yogurt goes sour. What the organisms are doing when they're making the yogurt that you had for lunch, I love those pictures. I found them on the Web and put them in. What they're doing really is they're getting rid of that NADH so that they can do another cycle and make more energy. Now, I mentioned that this happens to us, too. And this happens in athletic events where you exercise really, really hard, you know, like sprints or speed skating or something like that. Because what happens is you're exercising so hard that you use up the oxygen in your muscles faster than your bloodstream can bring you more. So what you're doing is you're making your muscles go anaerobic. It's like you're going back way, way in evolution when there's was no oxygen around. And your muscles have to keep working, so what they do, since there's no oxygen around, they stick it on pyruvate and you get lactic acid in your muscles. So if you go out for the track team in the spring and you haven't exercised and you run a whole lot of sprints and, God, your muscles are so sore, they're all full of lactic acid. So you don't have to worry about it accumulating in your muscles from eating yogurt, but it does show up in this kind of way. And the other thing then that the way nature has found to recycle these NADHs is to it this way, to carry out a transformation where you get two molecules of carbon dioxide and two molecules of acetaldehyde. And this can be converted to the two molecules of carbon dioxide and two molecules of ethanol. So this is the fermentation that we talked about. And so when those yeasts that we saw growing the other day are busy metabolizing sugar into ethanol and carbon dioxide, the reason they're doing it is they need to get energy to carry out all the biosynthetic reactions that they need to make more biomaterial. But what's happening to the whole system is that you're generating carbon dioxide and making stuff into ethanol. So it doesn't matter if people are making wine or beer or something they're going to distil to make whiskey or brandy or something. It's all the basic thing. The yeast take the sugars, make it into carbon dioxide and to ethanol. But when you're making bread you're only really interested in the carbon dioxide because those little bubbles then expand when you heat it up and that's what makes bread rise. And that was an open fermentation, as you can guess, like in making wine. People like to have a closed system so that, for example, a lactic acid bacteria doesn't get in and turn your whole set of grapes into something that would be sour. Sour wine. So that's where we'll stop today, the most ancient of these energy-producing things. Again, you don't have to memorize all this, but I think, hopefully if you think about, you'll see some really, really important concepts that are critical to understanding how life works. OK? See you on Wednesday. Happy Valentine's Day.