PROFESSOR: Let's talk about what was probably the first energy producing system that evolved. The thought is when the earth first formed and the first primitive organisms, perhaps resembling a present-day bacterium in some way came out, there were a lot of organic compounds that had been aided by lightning strikes and cosmic radiations triggering chemical reactions and so on. So there was food around, but they depleted those resources in the same way we're depleting the petroleum resources right now. If life was going to continue, somehow a way had to be found to make energy.

Glycolysis, it looks kind of complicated. It takes a molecule of sugar and then there are a series of 10 chemical reactions, each catalyzed by a separate enzyme that give two molecules of this, molecules of pyruvate, plus two ATPs, plus two NADHs. Which tells you there must have been some kind of oxidation step as part of this sequence of events, because electrons got taken off and got stashed on this NADH.

There are a couple of things that are important about this. One is its a pathway. It evolved probably 3.7 billion years ago or sometime, nobody really knows. But a long time ago. It's pretty much universal. Not perfectly so, but it's in bacteria, it's in yeast, it's in humans.

And another really important thing is that it evolved early in the evolution of earth, so it evolved when there was no oxygen around. So it's a way of making energy from glucose in the absence of oxygen. Which is a really important thing as you'll see as we go along.

You're not going to have to memorize this pathway. We'll give it to you if you need it. But you're going to need to understand its implications. And just let me point out a couple of things. You're going to see a sequence of 10 chemical transformations that in the end are going to end up with a couple of pyruvates being produced. And I'll try to explain to you why you should care about this.

There's a concept that you're familiar with, that if you want to make something and

you get a little start up company, what's the very first thing you have to do? You actually have to make an investment before you can get going. And you're out looking for venture capital things.

Well one of the odd things about this, here's probably the first sequence of reactions that arose on earth within some organism and enabled that organism to make energy out of glucose. And look, the first thing that happens. Trying to make ATP, the very first thing it does is it spends an ATP. And it takes glucose, and it makes glucose 6-phosphate. Go down a couple of steps. There's an enzyme that takes another molecule of ATP. And now you've got this point. You're at fructose with two phosphates on it. If this was your venture capital, we'd say, guys, how about some product? Stop spending, stop spending money.

But at this point then, this is a 6-carbon sugar. And it gets split into two 3-carbon compounds that are going back and forth. Oh I can see it. It's over there, OK. In equilibrium over here. And this particular 3-carbon compound then goes on to be oxidized. You get the production of NADH.

And at that point, this molecule has a lot of energy stored in it, and in the next transformation this cell is able to make two ATPs. And it gets back the initial investment. It goes all the way through the rest of the pathway. And the very last step, you get two more ATPs back. There's your net yield.

So what you get out of this are 4ATP+2NADH, and your investment was two ATPs. So your net 2ATP+NADH.

Why is this cell going and doing these initial investments? Well if we look at the changes in free energy associated with what's going on, there's glucose up in the upper left starting up there, and there's pyruvate down there. So you're going energetically downhill in the end. So this is a sequence of events that, in principle, you should be able to get some energy out of it.

But for reasons that may seem obscure to you at this point, before it gets to the point of making energy, it undergoes a set of transformations that's pushing the

reaction. It requires the reactants to go energetically uphill, i.e. in an unfavorable direction.

So what the cell does is, by coupling ATP hydrolysis to this step, it makes that reaction go. Here's another unfavorable one that makes that one go by coupling ATP hydrolysis to it. This is an uphill reaction, but look over here. This is an immensely favorable reaction that goes essentially to completion. It goes all the way.

So that means this product is just being continually taken out of the system, so the equilibrium is basically being pulled over the edge by the removal of that product. This is where the oxidation takes place. You get the NADH made right there. And it's finally down here where you've got to lose. This transformation gives you two ATPs and later there's another one.

Let me just give you a sense of why you get ATP at that step. The compound that you have at that point is 1, 3 diphosphoglycerate. Or sometimes this is called bis, is also used to describe this. But what is this compound? It's a 3-carbon compound. So glycerate is basically an oxidized version of glycerol that has been oxidized up to a carboxyl acid.

So this is a mixed anhydride between carboxyl acid and a phosphate ion. So that's a very reactive and unstable compound. And the other thing that the cell has succeeded in doing by all of these transformations is it's got these two phosphates with all their negative charges in. So this is a compound that would very much like to move to a lower energy. So you can get rid of this phosphate and move to an energy level, use that energy to make ATP. And there's a similar kind of logic that explains why you get energy out of the final step when you look at it.

So there's several points, I guess, to make out of this. One is its pathway. None of these reactions make any particular sense by themselves. You could have a cell that knew how to do one of them and it would gain nothing. Unless you wanted to use the product to make something. This thing only makes sense, these reactions only make sense in the context of this 10 step pathway. And each step in that

pathway we were looking at is catalyzed by a different enzyme. So for an organism to pull this off, the first one that did it had to collect in one cell all 10 of those enzymes. And probably there is the reason that this is such a complicated system.

If you were sitting as a designer you might be able to come up now with a more efficient way to get ATP out. But what happened evolutionarily was some bug somewhere got all of these things together, and now suddenly it could make energy. So it had a huge advantage over everybody else. And once it took over, that system took over, then it became universal. Whether it was the best that ever could be designed, it doesn't matter, because it had an evolutionary edge. And that's so, to some extent, we're looking at a living fossil, biochemical. But it's in bacteria, it's in yeast, and it's going on inside of our body.

Another principle that I think you can see here, which I've been trying to say, is in this case, the energy consuming reactions are driven by coupling them to the hydrolysis of ATP. The cell spends a bit of its energy money to get these intermediates, knowing that it's invest-- well not knowing, but at least conceptually anyway, knowing that it's going to get its investment back. And then the reactions that release energy are used to drive the synthesis of ATP. And you'll begin to see, we're going to just talk about some other aspects of this in just a minute.

So, what do you think? You're the first bug and you've got this and nobody else can do it, so you can start charging away. What do we need to do? We just let this thing cycle away? The stuff that I had up there, is it going to work? There's a problem. Anybody see what the problem is?

We're making two molecules of ATP and two molecules of NADH. Talk to the person beside you. Figure out why something else has to happen. Go ahead. See if you've got any ideas. We're going to keep doing this, over and over and over again.

AUDIENCE: [INAUDIBLE]. Where's the first ATP? Where's the first ATP?

PROFESSOR: OK, let's imagine for the-- I don't think this process could have invented ATP, it had to have been around, because many of the enzymes used it. What else is being

used in this thing though? Did I hear NAD? To make this thing work, I have to keep taking NADs out of my pocket and putting it in the reaction, or it isn't going to go anywhere. So this isn't such a great invention at the moment. We have to do something to get the NADH back to NAD+ so we can do another molecule of glucose.

You guys see? Do you see this? This is really, really an important consideration.

So in order for cells to make energy using glycolysis in the absence of oxygen, which is when it evolved, they have to do something with that NADH or it's only going to use up the few molecules of NAD+ in the cell, and then it stops.

And so there are two ways that nature's figured. Major ways nature had figured out how to do that. So here's a molecule of pyruvate. I got an extra. Something was nagging at me when I did this here. Sorry about that. It's always hard to see things when you're up at the board.

OK. Molecule of pyruvate. There's a couple of solutions that have been arrived at. One is to take NADH, 2NADH, this is 2H+. Convert, make these back into 2NAD+, and to take those electrons and put them on the pyruvate to give this molecule, which is lactic acid. So by parking the electrons there, the cell is able to recycle the NADH.

And lactic acid, we've run into that. That's why I showed you this picture of yogurt. The lactobacilli that make yogurt take the sugars that are present in milk and make them into lactic acid. And what's interesting in their case is they, even though there's oxygen around, they don't do respiration, which you'll see you can get more energy. They want it to get very acidic because that prevents their competitors from growing. And that's why you can leave yogurt sitting out on the tabletop and it'll be OK for quite a while. Whereas if you left some milk or something it'll go bad almost right away.

Here's another example of when we run into it. When we do hard aerobic exercise, when you're running or skating really hard, things you see in the Olympics all the

time, you deplete the oxygen supply in your blood when you do hard anaerobic exercise. And so the cells have the same problem of regenerating NADH. The way they solve it is they make the lactic acid. And that contributes to the sore muscles you feel after you've done hard anaerobic training.

The other way of handling this is to take the 2NADH plus two hydrogen ions to make it into acetaldehyde, two acetaldehydes. Plus two CO2s. Oops, excuse me. Let's do this first. And then take the 2NADH plus the 2H+. Convert this to 2NAD+, and what we get out of this are two molecules of ethanol plus two molecules of CO2.

Again, a process that's very similar to you, familiar to you, when I was showing you yeast growing. What yeast is doing is it's carrying out glycolysis and then it's taking those extra electrons, putting them on the pyruvate and making ethanol and carbon dioxide. I think there's a fermentation with what we call a fermentation with yeast. I think in that case they're making bourbon whiskey. Wine making, beer making, it's all the same thing. You have yeast, you're converting the sugars first to pyruvate, and then making ethanol and carboxylic acid.

So anyway. There's no energy gain out of this, but these are important processes. They're called fermentation. And they can happen when there's no oxygen around.

If you recall, there's a version of photosynthesis, what I called the second release of photosynthesis that began to evolve oxygen as a waste product. And then over the next ensuing millennia, the levels of oxygen slowly, slowly began to rise on earth.

And as oxygen levels got to higher levels, and recall the Cambrian period, which is down on the fourth blackboard down there. We were only still even there half a billion years ago. We were only about 5% the present oxygen levels. But as oxygen levels arose, new metabolic opportunities became available. And in particular, cells were able to get at that energy which is stored in NADH. In the absence of oxygen, NADH is just a nuisance. You've got to get rid of it. But as you'll see in a minute, you can do something interesting if you have oxygen around.

So just to look at this from a broad perspective, if we have glucose and we have all

these little steps going along to give the two pyruvate, if there's, in the absence of oxygen, they get 2 lactate or we can get 2 ethanol, 2CO2. And in both cases, 2ATP. 2ATP. These processes happening in the absence of oxygen to get rid of the, or at least not requiring oxygen in any case called fermentations.

However, when oxygen is available, it became possible to evolve a new system for handling these pyruvates. We go into a biochemical cycle known as the citric acid cycle. And I'll say a word about this in a minute. Plus something else that's known as oxidative phosphorylation. This is also referred to as the respiratory chain.

And what these two sets of processes together, enable the cell to take these two 3-carbon compounds and take them all the way down to six molecules of carbon dioxide, six molecules of water. And to make a net yield of 36 molecules of ATP.

So if you go by fermentation a molecule of sugar gives you two ATPs. If you go by glycolysis and then follow it by respiration, you get 36. So respiration using oxygen, 18 times more efficient than by glycolysis.

So in order to understand how this works though, we have to talk more about how you change from one form of energy to another. And it's interesting, although this process had to have evolved billions of years ago, it was only relatively recently that we understood the principal that was necessary for this kind of thing to happen. It's known as the Chemiosmotic Hypothesis. It was proposed by Peter Mitchell in 1961. He eventually got a Nobel Prize for it. It took quite a long time, it took more than 10 years for it to be accepted. In fact when I was in grad school in the mid '70s, people were still arguing whether this made sense or not.

So here's the way it works. And we have to consider first three different forms of chemical energy that can be all interconverted. One of them is familiar to you, we've been talking about it all along. It's a chemical bond. Energy can be stored in a high energy bond. And if we break it to get ADP, an inorganic phosphate, we can release energy.

However there's another way of storing energy as a concentration gradient. The

principal here would be to have a barrier, which in this case is the cell membrane, and to have a high concentration of whatever it is on one side, and a low concentration on the other side. And there's energy stored in that. If you give it a chance it'll get to be the same concentration on both sides. And the trick is to have whatever the substance is, is to have a protein in the membrane that can permit this thing to go across in a controlled fashion.

The third form is electrical potential. Again, the membrane actually acts as an insulator, and all cells, if this is the inside, and this is the outside, there's a gradient of hydrogen ions, so there are more hydrogen ions outside the cell than there are inside. So it creates an electrical potential. And these can't cross the membrane unless, guess what? There's a protein in the membrane that's able to permit their passage under controlled circumstances.

So there's basically three different forms of energy that can be interconverted. And Peter Mitchell's great insight, which I will say was not intuitive for many people, was the combination, so the combo of this proton concentration gradient plus the electrical potential, could be used to drive the synthesis of ATP.

And let me just say a couple of words. Because this may feel, how could this be? Could you really have energy? Well the potential across a cell is about 70 millivolts. May not seem all that much. But remember the membrane is about three nanometers thick. So that's about 200,000 volts per centimeter. High tension wires are 200,000 volts per mile or something. There's a lot of power in there.

And furthermore, let's see if I can bring this up. I've been showing you this little movie a couple of times. The bacteria with these little nanomotors are spinning those flagella, and we saw how there's this machinery that's a little nanomotor.

You know how it's powered? It's powered by the proton gradient. A proton trickles its way through this apparatus from the outside to the inside. It's coming down the gradient. That's the source of the power. And as I showed you, it's a pretty powerful motor. You can basically glue the propeller to a slide and it can twirl the bacteria all around.

In fact, one of my favorite demos is, years ago people took a bacterium, and they managed to pop it open. So all the cytoplasm, all of the stuff on the inside came out of the cell, and you just got buffer on the inside. But it had these flagella. So you had just shells of bacteria with nothing really inside them.

But, if you add a drop of acid to this media, now you've created a proton gradient with more protons on the outside than are on the inside, and guess what happens? The flagella motor starts working, and the bacteria start swimming, even though all the air, talk about dead man walking or something like that. It gives you an idea of the power that's in this combination of the proton gradient and the electric potential.

The combination of this is often referred to as the proton motive force. So here's the principle of how the cell is able to exploit that. And this is what underlies respiration.

There are two stages. Stage one, there's a membrane with some kind of membrane protein in it, which is actually a protein, functions as a proton pump. So it's a protein that's designed to be embedded into a membrane and to work there. This part here is the membrane itself. The proton gets transported from the inside to the outside when energy is put into this proton pump.

So in response to some energy producing event, the cell pumps protons from its inside to its outside, and this then establishes the proton gradient.

The second phase, then, is to take advantage of that proton gradient, and there's a different protein embedded in the membrane. It's known as an ATP synthase. And it permits a proton to come down the gradient, which you would want to do. But if that's all that happened, all you'd do is you'd just dissipate your gradient.

So the key here is that this proton is only allowed to come down the gradient to the energetically more favorable side if ADP and inorganic phosphate are bound to this ATP synthase. And the dropping of the proton down the gradient's passage through this ATP synthase, which is an energy favorable reaction, drives the synthesis of ATP. So much energy is basically given off with this, you can make an ATP and the thing will still go.

Now interestingly, this ATP synthase, which really lies at the heart of our energetics for how we function as human beings, is derived from it's crystal structure. But in fact, evolutionarily, it's related to that flagella motor. And as that proton comes down the gradient, or actually this is presented upside down, so there's the outside as it goes through in this direction, the ATP synthase, which is known as the F1F0 ATP synthase rotates. And probably this came first.

It's a little hard in this one because you don't have the flagella, so what scientists have done is they've been able to attach something like an actin filament onto this F1 ATP synthase, and show that as a proton passages the thing rotates.

So in all likelihood what happened in evolution was this came first, and then later the machinery got duplicated and evolved to become a nanomotor. And as I told you the other day, that apparatus for the flagella motor got evolved again into becoming a little syringe that bacteria like ursinia are able to use to pump or to squeeze proteins or squirt proteins from inside them into inside of a mammalian cell.

OK, well. Thanks to this work by Peter Mitchell then, we can now understand how cells were able to take advantage of that energy that was in the NADH. So this process is known as respiration. And basically it's taking the 2NADH. I'm supposed to see the physical therapist today, so I hope we're going to begin to make progress to lecturing on two feet sooner or later. Plus 2NAD+ plus two water.

So as I said earlier, NADH and protons, it's basically hydrogen. It's the equivalent of having hydrogen gas and adding oxygen, and we're burning the hydrogen gas down to water. So there's a lot to yield water. So there's a lot of energy potentially can be given off. That's the 50 kcals per mole.

Now if you recall when we talked about thermodynamics, so the NADH is up here, by the time we get down to the 2NAD+ plus the water, the two waters, energetically we're down here. And this is about a free energy changed of about 50 kcals per mole. In physiological terms, that's a huge amount of energy. And I think some of the textbooks compare it to letting a stick of dynamite off inside of a cell. So it's

really more than biology figured out how to handle this in a single step.

But do you remember that important principle about a thermodynamic property, when I had the little picture of the skier? It doesn't matter which pathway you take. You get the same amount of energy released whether you go down the black diamond slope or you go down the bunny slope.

So in fact, the way biology has learned, life has learned to control this amount of energy is basically taking the bunny slope. And so the energy drop occurs in a series of stages, where you have the transfer of two electrons to a lower state intermediate, transfer of two electrons to another one, transfer of two electrons to another one.

And where this connects with the stuff that I just told you, is as these two electrons are coming down, what's happening is a proton is being pumped from the inside to the outside. As it moves to the next lower energy state, another proton gets pumped from the inside, the outside. And the same thing happens here.

So at the end, you get the two hydrogens plus the half of an oxygen and we get a water molecule from these two electrons. But what's happened is these three protons have changed from inside to outside. That enables the cell to make three ATPs. So now instead of throwing away all that energy, losing the NADH as in the fermentations, the cell is extracting energy out of it by taking advantage of this principle of the proton gradient.

So the game changes if you're this evolutionary designer or something. If you were trying to design life from first principles now, you could take advantage of this. Well of course it doesn't happen that way. Experiments happen all the time in nature and something happens and sometimes it's very efficient, sometimes it isn't. But if it's there first it gets going.

In this case, the need now, or the opportunity was that if an organism could get more NADH out of that original molecule of glucose, it could make more energy than somebody else. And so the ultimate way to take a molecule of glucose is if you

burn it with, oxygen you end up with six carbon dioxides and water. You burn it all away.

So there's a system that, in essence, does that. It's known as the citric acid cycle. So you have the pyruvate that comes from glycolysis. And the way it's processed is first, one of the carboxyl group on the pyruvate is released, and this produces acetyl. You can look to see what CoA is. At the moment, it doesn't matter. What does matter is this is a 3-carbon compound. Acetyl, as you probably know, is a two-carbon compound.

And when you look in your textbooks at the citric acid cycle, you'll see this very confusing circle with lots of compounds and enzymes and stuff. But I want you just keep your eye on the ball here. If you'll notice, the compound over here is in the cycle, is four carbons. And what happens is this 2-carbon compound that was derived from pyruvate gets added to this to give a 6-carbon compound. And then that gets converted to a 5-carbon compound with a molecule of CO2 being given off.

That in turn gets converted to a 4-carbon compound with another molecule of CO2 given off. And then there's some molecular gymnastics here that change the nature of the four carbon compound a bit so you can get back into the cycle.

But look what's happened to those three carbons that were in the pyruvate. There's one of them, there's the other one, there's the other one. So this citric acid cycle produces, it actually makes some ATP, but it makes quite a bit of NADH. And it also makes another, one more reduced electron carrier. It's not NADH, it's another one that's used in the cell.

But anyway, the cell is then able to take all of this NADH and this electron carrier plus these to give you, what I'd said, the net yield you get from respiration. 36 ATPs from a single molecule of glucose. So sort of quite remarkable to some extent. We're looking at evolution, through, if you will, almost like looking at biochemical fossils and then when something works, it's a living fossil, we still find it in our cells.