JOHN ESSIGMANN: We're now on storyboard 12, session 13. Let's take a look first at panel A. I mentioned earlier that respiration is the oxidative metabolism of all metabolic fuels, carbohydrates as well as lipids. If we were starting with a carbohydrate then respiration starts with the pyruvate dehydrogenase reaction and then proceeds into the TCA cycle, and then into electron transport and oxidative phosphorylation.

If the acetylcholine comes from fatty acid oxidation, which we'll cover later, then we do not have to do the pyruvate dehydrogenase step. Three molecules of NADH and one molecule of FADH2 are produced as the carriers of electrons from the intermediates in the TCA cycle from each input molecule of acetyl coenzyme A.

As I mentioned a number of times, we look at NADH and FADH2 as relatively mobile electron carriers. They're going to be picking up electrons from intermediates and biochemical pathways and then they bring those electrons into the mitochondrial inner membrane, where the reducing equivalents are passed along to the electron transport chain. Ultimately the electrons will end up being deposited into oxygen to make water.

Let's turn to panel B where I'm going to start laying out the big picture. In this small cartoon we see fuel being oxidized to carbon dioxide. The electrons are passed to either in NAD-plus, or FAD to form the reduced cofactor. The reduced co-factors find their way to the mitochondrial inner membrane and the electrons, or reducing equivalents, are passed to oxygen to make water.

This process is highly exergonic. And we'll do a model calculation in a minute to show just how energy yielding it is, resulting in the liberation of a lot of free energy. That energy is captured by the movement of protons from one side of the mitochondrial inner membrane to the other side of the membrane. In this process you're taking a low concentration of protons and creating a relatively high concentration of protons. That's an uphill process that's going to require an energy input, and the energy from nutrient oxidation is what powers this generation of an ion gradient.

Concentrating the protons in a small defined space is kind of like charging a battery. It took energy to create that high concentration of protons. We'll see that nature invented a way to channel the protons back through a device and a way to capture the energy released when the gradient is dissipated. That energy can be captured in various ways, enabling us to be able to do useful things with it.

For example, the energy could be used to accomplish the otherwise energy requiring process of putting a phosphate onto ADP to make ATP, and that way the energy is captured in a chemical bond.

Alternatively, let's think of a situation in which you may need to generate heat. You could allow the protons simply to flow back across the membrane through a channel. The energy of the proton gradient would then be released as heat. Thirdly, we can perhaps allow the protons to flow back through a device that creates rotary movement and that's how, for example, a flagellar motor can spin to move a bacterium from one place to another.

Let's now turn to panel C. We've seen earlier that oxidation can result in the production of NADH or FADH2. To begin let's consider NADH. In this panel you can see the NADH oxidized where the electrons flow-- and it doesn't matter what the path of the flow is-- all the way to oxygen. Thermodynamics gives us the tools to calculate how much energy you would get in this process.

JoAnne taught you a lot about thermodynamics in biological systems, And this is just a repeat of what she said. But what I'm going to do is put what she said into a concrete, practical example. I've drawn out at the bottom of panel C the overall reaction, showing electrons going from NADH to oxygen in the forward direction.

Now, of course, we could also think about the back reaction, where electrons would flow from water into NAD-plus to form NADH. At this point this is just an equation on a piece of paper and we don't know in which direction the reaction is overall favorable. That is, is it favorable in the forward direction as drawn, left to right, or in the reverse direction, right to left?

The reaction from left to right is the direction we usually think about in the context of nutrient oxidation. Interestingly, the reaction from right to left is photosynthesis. So both directions are biologically used, but we'll see that one of the directions will require substantial energy input in order to make it biologically useful.

Probably you can appreciate that photosynthesis is the energy requiring process, because we all know that sunlight is needed to make the process thermodynamically and kinetically favorable. We don't cover photosynthesis in 5.07, so let me say a few words about it. In photosynthesis nature takes electrons from water and uses them to reduce NADP-plus to NADPH. Note that I said NADP-plus, and not NAD-plus, but for the purposes here they are equivalent.

In the case of photosynthesis we know intuitively that the overall process is powered by light, so in going from right to left the process intuitively, once again, should require energy. By contrast, nutrient oxidation, where we go from left to right in this equation, should be a process that generates energy. But intuition aside, let's do the calculation. So the question is, in which direction is the equation at the bottom of panel C thermodynamically favorable-- left to right or right to left?

Let's take a look now at panel D. You'll see here that I have split the master equation into half reactions. I always write out the half reaction in the direction of reduction. For example, look at the second half reaction. It shows that half a mole of oxygen plus two protons, plus two electrons, go to water. Again, I've written out these reactions in the direction of reduction. It's just the way I do it.

Next I go to the redox chart, that is the table of redox potentials of electrochemical reactions, and I figure out what the standard energies are for each of these half reactions. As you can see, it's 0.32 volts, and, plus 0.82 volts respectively, for the two half reactions. Next I use a variant of the Nernst equation to calculate the free energy and ultimately the directionality of the reaction.

The equation I use is delta G naught prime equals N times Faraday's constant, times the difference in the reduction potentials of the two half reactions. To find delta E naught prime, we look at the reaction that I've written out, the overall reaction, and we looked at see which is the electron acceptor, in which is the electron donor the way the reaction was written. Then we subtract the reduction potential of the electron receptor from that of the electron donor.

The way the equation is written, left to right, oxygen is the electron acceptor. It's reduction potential is plus 0.82 volts, so delta E naught prime is plus 0.8 to minus a minus 0.32, or a total of plus 1.14 volts. In the Nernst equation, the number of electrons transferred in this case is two, and Faraday's constant is 96.4 kilojoules per mole times volts.

If you do the math, or as we say at MIT, plug and chug, what you find out is that the free energy change of the reaction that's written, the NADH oxidation reaction, is minus 220 kilojoules per mole. This number is negative and that means the reaction is favorable as drawn. That is, the reaction goes from left to right. The 220 kilojoules is the amount of energy that's available for the three purposes of the pathway. That is, ATP synthesis, heat generation, or movement.

If we were going to make ATP, we would divide 220 by 32, because we get about 32 kilojoules of energy by hydrolysis of ATP, and we can calculate that we're going to get something in the order of about 3 ATPs for every NADH that gives up two electrons to the electron transport chain. We could easily do the same oxidation reaction and study FADH2, but in this case, we would calculate that we would get actually less energy, because the oxidation potential of flavins, as compared to NAD, is different.

In that case, that is, FADH2 oxidation, you would get only about two ATPs per FADH2 oxidized. So the FADH2 produced in the succinate dehydrogenase step of the TCA cycle is less energy yielding than, for example, the oxidation of malate to oxaloacetate that occurs later in the pathway.

Let's turn now to storyboard 13. We now have an idea of the rough amount of energy that's going to be generated by nutrient oxidation. Next we're going to look a little bit more in detail at the mechanism by which the electrons are transported from the reduced substances that constitute our electron donors, NADH and FADH2, to molecular oxygen, or whatever the terminal electron acceptor is in the biological system under study.

As was seen, the electron transfer process that we've been studying liberates energy, and that energy is going to be used to power pumps that will transport protons from the matrix of the mitochondrian out into the intermembrane space between the mitochondrial inner and outer membranes. In the case of electron transport with the goal of ATP synthesis, the protons that are generated in the intermembrane space will be allowed to flow back through a device that mechanically couples motion-- that is the spinning of a shaft-- to drive conformational changes in enzymes that will allow the otherwise endergonic synthesis of ATP from ADP in inorganic phosphate. That machine is called the ATP synthase.

Let's look at panel A. This panel shows the details of the electron transport system. It looks a little bit complicated, but let's not lose sight of the fact that what it's all about is powering

pumps, pumps that pump protons. In the lower left of the panel we see the TCA cycle generating NADH and FADH2. The NADH approaches complex one of the mitochondrial inner membrane.

Complex one is in an NADH dehydrogenase enzyme. The enzyme has a flavin that accepts the electrons from NADH, passes them along to iron sulfur centers, and then to a variety of cytochromes. It moves the electrons up to the point where they're going to be transferred to coenzyme Q. In its oxidized form, coenzyme Q binds to complex one. The oxidized form of coenzyme Q will be reduced first to a semiquinone and then to a hydroquinone, which are located, as shown, inside the mitochondrial inner membrane.

We usually draw them as being free floating within the membrane, but that's probably inaccurate. These co-factors are probably bound to physical entities inside the mitochondrial inner membrane. The picture also shows FADH2 from the TCA cycle interacting with complex two, which is a flavin containing enzyme, and it will also transfer its electrons to the oxidized form of coenzyme Q, ultimately to create the reduced coenzyme QH2 which is the hydroquinone.

Just as a point of reference to the TCA cycle, complex two is also known as succinate dehydrogenase. We haven't done fatty acid oxidation yet, but there's a step in fatty acid oxidation in which an alkane is converted to an alkene. The electrons from that oxidation reaction go through a flavin protein called ETF-pre, for electron transferring flavor protein, and once again those electrons flow into coenzyme Q to form the reduced form of coenzyme Q.

Remember when we talked about the glycerol three phosphate shuttle? I mentioned that there's a mitochondrial membrane associated glycerol three phosphate dehydrogenase. You can see that enzyme at the top part of the inner membrane as I've drawn it. And once again, flavin in that enzyme will carry the electrons into coenzyme Q, generating reduced form of the co-factor QH2.

The left part of this picture pretty neatly shows how nutrient oxidation can channel the electrons into a common mobile electron carrier, coenzyme Q. Lots of different fuels give up their electrons to a common electron carrier. The hydroquinone QH2 will then go and interact with complex three of the electron transport chain. In complex three, electrons will be transported through a number of different electron relay stations and ultimately be picked up by cytochrome C, which is initially in its plus three oxidation state.

Cytochrome C is reduced by the single electron coming through complex three. In this process its iron is reduced to its plus two state. This electron on cytochrome C then migrates across the outer surface of the inner membrane of the mitochondrian to interact with complex four. When it interacts with complex four, the reduced form cytochrome C gives up its electron to a copper residue on the copper A subunit of complex four.

In the figure, this complex is called CuA. the more common name for complex four is cytochrome C oxidase. So cytochrome c oxidase oxidizes is the iron back to its iron three oxidation state, then cytochrome C migrates back to complex three where it's able to pick up another electron. We can think of cytochrome C as a mobile electron carrier that shuttles an electron along the inner surface of the mitochondrial inner membrane.

Looking back at complex four, or cytochrome C oxidase, the electrons are passed from cytochrome A to a series of other electron carriers, and ultimately they flow into molecular oxygen. Molecular oxygen is anchored on one side to heme A3 and on the other side to copper B.

Oxygen undergoes a four electron reduction and picks up four protons along the way to form two molecules of water. If the electrons started with NADH and ends up in oxygen to form water, you get 220 kilojoules of energy. And as I said, you get somewhat less of your electrons started out as a reduced flavin.

Now let's look at panel B. I want to say a few words at the outset about proton pumps, keeping in mind that the reason electrons were moved through the electron transport chain was to power these pumps.

In the lower left part of the figure, we can see complex one. The transit of electrons through complex one from NADH results in the transport of four protons from the mitochondrial matrix into the intermembrane space. That is, two electrons from NADH are transported, and coincident with that, four protons are pumped into the intermembrane space.

Later we'll see that complex three is the location of something called the Q cycle. I'm going to cover the proton pumping properties of the Q cycle in some detail in the next storyboard.

The passage of those two electrons through complex three results in the transport of an additional four protons into the intermembrane space.

Lastly, the transit of electrons through complex four, the cytochrome C oxidase, results in the transport of another two protons into the intermembrane space.

Now let's look at panel C. Adding things up, if we start with two electrons coming from NADH we transport about 10 protons into the intermembrane space. That's enough to make about three molecules of ATP. If our electrons start with FADH2 reduced flavin, we only transport about six protons. That's enough to make two ATPs.

If you remember, making ATP is only one of the things that we can do with the power of the proton gradient. I mentioned earlier that we can also use it to generate heat and motion.

Let me talk for a minute about why one would want to generate heat. Newborn babies are like small balls. They have a high surface to volume ratio. Their high surface to volume ratio makes heat loss a very significant reality, and, in fact, a problem for the newborn. They have, therefore, specialized mitochondria in their neck in an area called brown fat. The fat is brown because it is loaded with highly colored mitochondria.

These mitochondria have a protein that enables the protons pumped into the intermembrane space to flow back freely into the mitochondrial matrix with the generation of heat. That is, they don't make ATP in the brown fat, they make heat. This helps promote thermal regulation in the baby.

This is also the mechanism by which hibernating animals, such as a bear, can maintain body temperature during the winter when the bear is hibernating. This process overall is called uncoupling of electron transport, from the process that we're going to be looking at next, oxidative phosphorylation. Oxidative phosphorylation is the process by which we're going to be making ATP.

Uncoupling is also a process used by some flowers that have to come up through frozen ground in the springtime. For example, the skunk cabbage and the crocus are very good at uncoupling electron transport from oxidative phosphorylation. They let the protons in the proton gradient flow back across the mitochondrial inner membrane, and by doing so these plants are able to generate heat that will allow them to grow at subfreezing temperatures. In other words, the plant itself becomes a little heater.

At this point, let me give you a little bit of a preview of how we use this proton gradient in order to synthesize ATP. As we have seen, it took energy in order to create a gradient in which there

are protons in the intermembrane space, and protons are chemical entities, so we've created a chemical gradient. But also, we're putting a very high positive charge density in the small, enclosed intermembrane space. So we have also created an electrochemical gradient. It is the release of those gradients that empowers many of our vital processes.

For a final comment please see panel D. Peter Mitchell figured out how the energy in that electrochemical gradient could be released and converted into the energy it takes to make chemical bonds. Mitchell's conclusions serve as the basis of what is called the chemiosmotic coupling hypothesis, and we'll be looking at that in some detail later.