JOHN ESSIGMANN: Let's take a look at storyboard 14, where I discuss the Q cycle. If you look back at my previous lecture in which I introduced the respiratory apparatus, you'll see that there are three points in the electron transport chain, at which protons are translocated across the mitochondrial inner membrane, into the intermembrane space.

The three sites are complex one, complex two, and complex four. If your entry point for the electrons is a pair of electrons at NADH, you'll translocate about 10 protons. This generates the electrochemical gradient that will be used in complex five, the F naught f1 synthase. And the energy released in proton translocation back into the cytoplasm, eventually will be used to synthesize ATP.

At this point, I'd like to take a look at the hypothetical mechanism by which proton translocation into the intermembrane space happens in complex three. At the outset, let me say that the mechanism here is different from the mechanism at complex one and complex four. And I'll also mention that other organisms have found other ways to solve this problem of translocating protons into the intermembrane space.

Let's look at panel A of storyboard 14. At the upper left, we see coenzyme Q in its quinone form. In this form, it's a taxadiene dione. It's one of our co-factors. And we've also referred to it in the past as a mobile electron carrier. In that regard, it falls into the same grouping of molecules, such as the NAD plus, NADH pair.

The squiggly line at the lower right corner of the quinone molecule depicts isoprene units. Actually, there's a series of about six to 10 of these isoprenes in the Q molecule. These probably facilitate association with hydrophobic regions, such as regions in membranes.

The structure of coenzyme Q allows it to pick up electrons one at a time from an electron donor. And deliver them one at a time to a place where the electrons are going to go. That is an electron recipient. This panel shows the step-by-step mechanism by which electrons, one at a time, can reduce the oxidized form of coenzyme Q. Called Q here, or the quinone, to its

fully reduced form QH2, also known as the hydroquinone.

These electrons come from complex one, or complex two, or another membrane bound entry point for electrons into respiration. The first electron converts the quinone Q to the semiquinone radical. The semiquinone will then pick up another proton and electron to form the fully reduced species, the hydroquinone, or QH2. The hydroquinone QH2 is fully loaded with electrons.

The fully reduced hydroquinone will next deliver its two electrons to complex three. The technical name of complex three is coenzyme Q, cytochrome c oxidoreductase. This name gives a hint that the electrons are going to pass from coenzyme Q in its reduced form, to an oxidized form of cytochrome c, which is also non-covalently associated with complex three.

Let's look at panel B. A single molecule of QH2, the hydroquinone, contains two electrons that are going to be transferred to complex three. One electron is going to reduce iron three to iron two, in a first molecule of cytochrome C.

And then the second electron is going to be used to reduce a second molecule of cytochrome c. It's convenient to break this overall reaction scheme into two parts. I'll call them cycle one and cycle two. In cycle one, the first molecule of cytochrome C is going to be reduced. And then in cycle two, the second molecule will be reduced.

I've further broken down the cycle into eight steps. At the upper left of complex three, you'll see a site that I've marked with an x. This is an iron cell for protein. This is going to be that docking site for QH2, the semiquinone. Sometimes it is called the Q delta site.

The reduced quinone, QH2, gives up two protons and one electron to form the semiquinone radical, Q.minus. The electron in step three goes over to reduce ferric ion to ferrous iron, that is iron three to iron two in cytochrome c.

As I mentioned earlier, cytochrome c is another one of our mobile electron carriers. It's going to float away and go off to complex four. At this point in step four B, the semiquinone radical is going to give up its second electron to cytochrome bL, which is a constituent of the complex three.

From the standpoint of coenzyme Q, at this point it's lost both of its electrons. It has been converted to the fully oxidized form Q, as depicted in step four A. The electron at cytochrome bL flows into cytochrome bH. And from there, it will subsequently flow into a molecule of the

parental diquinone Q. The one electron reduction of the diquinone Q, results in the formation of the semiquinone radical, once again.

This is at position Y-- the way I've drawn the complex three in panel B. Position Y is also called the Qi site. At this point, the semiquinone radical will just stay where it is for a few minutes. We'll pick it up again in the second half of the Q Cycle.

To summarize what happened in the first half of the Q Cycle, two protons have been translocated from the matrix into the intermembrane space. And one electron has been transferred to cytochrome c, which then translocates across the outer surface of the mitochondrial inner membrane to complex four.

At this point, please look at panel C. Now we're going to take a look at the second half of the Q Cycle. At the bottom of complex three in step 1, you'll see the orphaned semiquinone radical that we just created. Keep in mind that we are going to come back and use that radical in a couple of minutes. So bear with me.

At step two, we see a second molecule of the hydroquinone, QH2, inside the mitochondrial inner membrane. We're going to borrow this molecule for a short time. And then we're going to restore it. So this QH2 is, essentially, catalytic in the overall reaction scheme.

In step three, we see that this borrowed molecule of the hydroquinone, QH2, gives up two protons and one electron just as we had seen in the first half of the cycle. You will note that we are reducing a second molecule of cytochrome c, which is then going to go off to complex four. And we're also going to be producing a second molecule of coenzyme Q, the fully oxidized form of the co-factor.

In step four B, the semiquinone radical, just as happened in cycle one, will give up its electron to cytochrome bL. The electron will then go to cytochrome bH, and then will flow into the semiquinone radical that we had left over from the first cycle.

This radical exists at site Y, or as I called it before, the Qi site of complex three. The reduction of the semiquinone radical at step six is concomitant in step seven with the acquisition of two protons from the matrix. This series of reactions, ultimately, results in restoration of QH2, the semiquinone in the mitochondrial inner membrane.

At this point, we have restored the molecule of QH2, at step eight, that we had borrowed,

initially, at step two. The second cycle has also produced net Q, the oxidized form of the co-factor, which is now free to go on to complexes one and two, and to pick up more reducing equivalents.

Just looking at the second half of the Q Cycle, what we see is that we've translocated another two protons into the intermembrane space. And we have transferred a second electron to cytochrome c. So cycle one and cycle two, each, result in the translocation of two protons from the matrix to the intermembrane space. And each results in the transfer of one electron to cytochrome c.

Looking at the whole Q Cycle, that is cycle one and cycle two together, we get a pair of electrons that enter from QH2. Four protons are translocated into the intermembrane space. And two reduced molecules of cytochrome c, which then migrate to complex four, where they'll be later oxidized.

Let's turn to storyboard 15 and panel A. In panel A, I'm showing another way to look at the Q Cycle. I'll emphasize that this is not as chemically accurate as the two step process that I showed you previously. And I'm not going to discuss it here further. Nevertheless, I think that this presentation makes it relatively easy for you to see the overall stoichiometry of the Q Cycle.

Let's now turn to storyboard 15, panel B. In the way of a high-level review of electron transport so far, electrons from nutrient oxidation have been deposited into the electron transport chain. The transfer of electrons to oxygen resulted in energy that is used to power pumps-- pumps that pump protons into the intermembrane space. We've looked at the Q Cycle as one example, of several, of how protons are pumped.

It took energy to create this proton gradient. When we release the proton gradient by allowing the protons to flow through the ATP synthase, we're going to be able to use that energy in order to accomplish the otherwise energetically uphill phosphorylation of ADP into ATP.

Now let's look at storyboard 15, panel C. This panel shows the F naught F1 proton translocating ATP synthase, also known as the ATP synthase. To put things in perspective, at the top is the intermembrane space, which is where the protons have been translocated.

In the middle is the mitochondrial inner membrane. And at the bottom is the mitochondrial matrix. Because we've pumped protons into the intermembrane space, its pH is about three

quarters of a pH unit lower than the pH of the matrix of the mitochondria.

Proton flow is going to be regulated in response to physiologic needs. I'll talk about that regulation and how it occurs later. For now, however, let's look at protons flowing through the F naught F1 complex. Let's imagine that the broken line indicates a channel, through which protons will flow.

Other structural features include a shaft, which I've indicated as gamma, and a ring, in which the shaft is embedded, which I've indicated as the C-ring. When protons flow, both the shaft and the C-ring will move, as you'll see, in a clockwise rotation.

At the bottom of the overall apparatus are three alpha and three beta subunits. These are not going to rotate. That is, they're going to stay fixed in position while the C-ring and the gamma subunit rotate. The beta subunit contains a site x, which is going to be the active site for conversion of ADP plus inorganic phosphate to ATP.

I've drawn a little elbow on the bottom of the gamma subunit. Let's imagine that protons are flowing. And the flow makes that subunit spin around. And further, imagine that the elbow is bumping into the active sites-- that is, the x sites. There are three of the x sites, one on each of the b subunits at the bottom of the overall apparatus.

Imagine that the energy involved is translocated from the elbow to the active sites. And that's what's going to help us align ADP and Pi, to make the overall chemical reaction favorable. That is a hypothetical, but not far fetched way to think about the way that ATP is made.

Let's now look at panel D. The current view is that the active site oscillates among three different conformations during the time the protons are translocated. These three conformational states are referred to as O for open, L for loose, and T for tight.

In the open state, nothing is present at the active site x. The conversion of open to loose is concomitant with the binding of ADP and inorganic phosphate. As protons continue to flow, the loose site is converted into the tight state. The energy associated with this conformational change results in the alignment of the inorganic phosphate, such that the conditions are now favorable for it to phosphorylate ADP and form ATP.

Further proton flow results in the tight state being converted to the open state, which ejects the formed ATP out into the mitochondrial matrix. So at each cycle, of open to loose to tight, a molecule of ATP is made. And then it is ejected. This goes on again, and again, and again.

That's the overall mechanism by which ATP is synthesized.

At the high level, proton flow results in the movement as the turning of a rotor. It is a lot like a water wheel that might drive the rotation of a millstone, that grinds grain into flour. The rotation of the shaft provides energy to bump into the x sites, resulting in the conversion of a first subunit to the open conformation, another subunit to the loose state, and a third subunit to tight. Then the process starts over again, with each cycle producing one molecule of ATP.

As one final point-- a point that explains how this process is regulated. Proton flow results from the binding of ADP-- that is, ADP. This will become important in a few minutes when we talk about how the whole system is regulated.

Now let's look at storyboard 16. Let's look at all four panels, panels A through D. In the last part of this lecture, I'd like to talk about how respiration is coupled to the TCA cycle and glycolysis. This is really the first time in 5.07 that we're going to see the beauty of coordinated pathway regulation.

I'm going to use a physiological scenario in order, hopefully, to make it all make sense. In this scenario, imagine that you're being chased down the street by a dog. In order to start running away from the dog, we're going to need some ATP that's very quickly generated from glucose by glycolysis.

The TCA cycle is also going to be operative, along with a pathway we haven't seen so far, fatty acid oxidation. In both cases, the TCA cycle and fatty acid oxidation, reduced electron carriers are going to be produced. They're going to give up their electrons to the electron transport chain.

Ultimately, to reduce oxygen to water and with concomitant pumping of protons into the intermembrane space, resulting in the lowering of the pH-- that is, acidification of the intermembrane space. As I mentioned earlier, that flow of protons through the ATP synthase is triggered by the binding of ADP to the x site on the beta subunit of the enzyme complex.

As your muscles are working hard and you're running away from the dog, the concentration of ADP in the mitochondrial matrix is going to be increasing. Thus, proton flow is going to be initiated. As you run more and more, the protons are depleted from the intermembrane space.

It's not known exactly how this reduction in proton concentration results in accelerated

movement of electrons through the electron transport chain. It's tempting to speculate that one or more of the reductase centers, within the electron transfer chain, may be pH sensitive.

Let's imagine that that is the case. So let's imagine that the raising of pH-- that is, the decrease in hydrogen ion concentration in the intermembrane space, results in the facilitated flow of electrons from complex one or complex two, to oxygen.

Next, let's look to the far left at the bottom of panel D. As you draw more electrons into respiration, NADH is going to be converted to NAD plus. It's important, now, to remember that NADH feedback inhibits any step at which it's produced in the TCA cycle.

Thus, as you're running away from the dog, the NADH concentration drops. And that means that there's going to be less inhibition of the steps at which NADH is produced in the TCA cycle. Thus, looking at the big picture, the binding of increased concentrations of ADP to the ATP synthase, over on the far right, results in the activation of the TCA cycle and other metabolic pathways that will result in the delivery of more electrons through the electron transport chain, in order to allow you to continue to run away from the dog.

Next, let's imagine that you've been running for quite a while. And let's look at complex four, where oxygen is bound. Sooner or later you're going to be becoming oxygen limited. You're going to be panting heavily. This means that electron flow from a reduced electron carriers to oxygen is going to slow down.

As we become more hypoxic, respiration starts to fail, but hypoxia dramatically increases the levels or activities of the enzymes of glycolysis. Thus, glycolysis will switch over to becoming our principal ATP generation machine. As we increase the rate of glycolysis, the flux goes from glucose to pyruvate, but the pyruvate can't be oxidized because it can't enter respiration, because we don't have enough oxygen present in order to oxidize it.

So what happens is we activate lactate dehydrogenase to convert pyruvate to lactate. Conversion of pyruvate to lactate has two consequences. The first is that conversion results in the conversion of NADH to NAD plus. Remember, that this is the lactate shuttle.

And that NAD plus is now available to allow glyceraldehyde 3-phosphate dehydrogenase to continue processing molecules of glucose into ATP. A second consequence of activation of lactate dehydrogenase is the fact that lactate spills out into the blood, where it acidifies the blood. The pH drops.

Now let's think about what the consequences are when the pH of the blood drops. I want you to think back to the lectures in which JoAnne talked about cooperatively, the Bohr effect, and the affinity of oxygen for hemoglobin. JoAnne told us that protons are heterotropic allosteric effectors that, effectively, loosen the affinity of oxygen for hemoglobin.

So as you're running away from the dog, glycolysis becomes the main pathway. You produce a lot of lactate that goes into the blood. The pH drops. Oxygen lowers its affinity for hemoglobin, and thus, becomes more available for the pathway of respiration.

Oxygen is now back in the system and binds to complex four. So you kind of get what we could call a second wind that allows you to continue using respiration to run away from the dog. In other words, you've adapted to the stressful state and are now able to continue running.

Now let's put it all together. A dog starts chasing you. Your readily available energy reserves, and free glucose, as well as glycogen, will produce rapid ATP that will get you moving away from the dog. Additionally, respiration will be contributing lots of ATP to allow you to escape the dog.

And as you're running, ADP concentrations will go up inside your mitochondria. That will, basically, release the proton gradient, allowing the ATP synthase to work very quickly and very hard to make a lot more ATP. NADH concentrations in the mitochondria will drop with physical activities. This helps boot up the dehydrogenases of the TCA cycle. They become less inhibited.

Your TCA cycle will accelerate, in order to produce more reducing equivalents to power the electron transport chain. All of this will continue until your oxygen becomes limiting, then respiration starts to fail, but the hypoxic state turns up the activities of the enzymes of glycolysis, thus glycolysis accelerates and helps accommodate. But pyruvate, at the end of glycolysis, cannot be further oxidized because there's not enough oxygen. It has to be converted to lactate.

The lactate acidifies the blood. The Bohr effect causes the release of more oxygen into the blood, making it more available to complex four, in order to reboot the electron transport chain. Overall, this is a marvelously regulated physiological system that seems to have evolved in order to promote our survival.