JOHN ESSIGMANN: Let's take a look at storyboard number 10. Back in earlier sessions, we talked about the detail of glycolysis. One of the points that I emphasized is the fact that it's necessary to maintain redox neutrality in the cytoplasm of a mammalian cell. It's also necessary to maintain redox neutrality and prokaryotic cells, not just eukaryotic cells.

At the glyceraldehyde 3-phosphate dehydrogenase step of glycolysis, NAD+ was consumed and converted to NADH. That means we have to find a way to convert NADH back to NAD+ in order to make glycolysis a continuous process.

Back in lectures three and four, I said that there were three ways to convert the NADH back to NAD+ These were alcoholic fermentation in anaerobic cells. Homolactic fermentation, again, in an anaerobic environment. And the third is respiration, which occurs in the presence of oxygen.

I'm going to loop back now and revisit this topic. I want to spotlight three general strategies that cells use to achieve redox neutrality in the cytoplasm. The first is lactate dehydrogenase. The second is called the glycerol-3-phosphate shuttle. And the third is called the malate-aspartate shuttle.

Panel A of this figure shows the cytoplasm working in concert with the mitochondrion. You can see depicted on the left, the pathway of glycolysis. In the middle, pyruvate dehydrogenase. And to the right, the citric acid cycle or TCA cycle, which we just covered.

There are two important boundary conditions for the discussion we're about to have. The first is that oxaloacetate is, as I mentioned earlier, present only in very small concentrations within the cell and especially in the mitochondrion. As a consequence, the mitochondrion does not have a transporter to allow it to escape. In other words, its concentration is preserved at about one micromolar inside the mitochondrion.

The second boundary condition concerns the fact that NAD+ and NADH, as well as NADP+ and NADPH cannot go directly across the mitochondrial membrane. So in other words, there

are two separate pools of this nucleotide co-factor. One in the cytoplasm. One in the mitochondrion. I'll come back to the importance of the two pools in just a few minutes.

Let's look first, here in panel A, at the mechanism by which lactate dehydrogenase achieves redox neutrality in the cytoplasm. We've already covered this, so this is a bit of a review. Note that you see NAD+ getting converted to NADH in the cytoplasm. That's at that GAPDH or glyceraldehyde 3-phosphate dehydrogenase step.

The hatched lines that you see represent the flow of electrons. In other words, electrons flow from glucose and they end up in NADH. Then the lactate dehydrogenase enzyme transfers the electrons from NADH into lactate. So these electrons from glucose are involved in the reduction of the ketone functionality of pyruvate into the alcohol functionality of lactate. And that's where the electrons stay.

The other product of this reaction, as you'll see, NAD+ which is now available to enable the oxidation of the next molecule of glucose. What happens to the lactate that's produced? In a working muscle cell, that lactate will escape from the cell, go into the blood, and then go to the liver or another organ that's capable of doing the pathway of gluconeogenesis.

As I've mentioned in the past, gluconeogenesis is a pathway by which non carbohydrate precursors, such as lactate, are built back up into glucose. Keep that working muscle scenario in mind, because I'm going to come back to it later when I talk about physiological responses to stress, such as what I'll call the fight and flight scenario. That's all I'm going to say for now about the LDH shuttle. That is, lactate dehydrogenase shuttle in panel A, which is the first of the three pathways by which redox neutrality is maintained in the cytoplasm.

The second pathway to retain redox neutrality is the glycerol-3-phosphate shuttle. This pathway is particularly active in the brain and in skeletal muscle. Once again, follow the hatched lines to follow the path of electrons as they go from glucose. And ultimately, in this case, they're going to end up being deposited into oxygen to form water.

Starting at the top, you see in NAD+ being reduced to NADH at the glyceraldehyde 3-phosphate dehydrogenase step, GAPDH. Next, we're going to temporarily borrow a molecule of dihydroxyacetone phosphate, DHAP. DHAP is a ketone. And what we're going to do is deposit the electrons from NADH into the ketone functionality to make the alcohol, glycerol-3-phosphate.

The source of the electrons was NADH. And now you've accomplished your chemical goal, which was to restore the NAD+ pool -- the cytoplasm, but we borrowed a molecule of dihydroxyacetone phosphate. And we've somehow got to get that back. Let me point out, at this point, that the reduction of DHAP to glycerol-3-phosphate was accomplished by the cytoplasmic form of the enzyme glycerol-3-phosphate dehydrogenase, which catalyzed step 2 on the storyboarded.

We're going to deal more with coenzyme q in the next lecture. But for now, it's a molecule, specifically a quinone, that's easily reduced to its hydroquinone form, called QH2. The structures of q, in QH2, are shown in the box. QH2 is in the mitochondrial membrane. In glycerol-3-phosphate dehydrogenase the mitochondrial version of it is present in the outer part of the mitochondrial inner membrane.

Like NADH and FADH2, QH2, the hydroquinone, is what I've called a mobile electron carrier. QH2 is going to allow the electrons that started out in glucose or in any LDH of the gap DH step, to flow through the electron transport chain, which we'll come to in the next lecture. And flow into oxygen, which is reduced to form water.

This terminal reduction is shown in step five. Effectively, in the glycerol-3-phosphate shuttle we're using oxygen in order to oxidize NADH back to NAD+ And once again, maintaining a constant supply of NAD+ is necessary in order to make glycolysis a continuous process.

Panel C shows a third strategy for maintaining redox neutrality in the cytoplasm. This is called the malate-aspartate shuttle. and this pathway is operative in heart, liver, and kidney. To the left we see the production of NADH, just as we did in the previous two small pathways.

At step one, let's assume that there's a molecule of oxaloacetate present as part of the cytoplasmic pool of organic acids. Oxaloacetate or OA is ketone. And the cytoplasmic form of the enzyme malate dehydrogenase, working in the reverse direction from the one that we see operative in the TCA cycle is able to reduce the oxaloacetate to malate.

We just reduce to ketone OA to an alcohol malate. In step three, the accumulating malate is transported by a malate transporter into the mitochondrial matrix, which is, of course, the location of the TCA cycle. At this point, we're going to be using one of the steps of the TCA cycle.

Specifically, we're going to use malate dehydrogenase, the mitochondrial version of the

enzyme this time, to convert malate to oxaloacetate. That reaction is an oxidation. We use the mitochondrial pool of NAD+ to carry out that oxidation. In effect, we're using the electrons that came in from malate to reduce NAD+ to end NADH in the mitochondrion.

Now, take a careful look at step four. Looking to the left you see the hatched lines go all the way back to glucose, which was the source of the electrons. To the right, the hatched lines by step five, go to the electron transport chain all the way to oxygen.

We haven't done the electron transport chain as yet. So you're just going to have to trust me for a little while. There's an enzyme, NADH dehydrogenase, in the mitochondrial inner membrane that will take the electrons from NADH and eventually regenerate the NAD+ in the mitochondrial matrix.

In step five, we're taking the electrons from the NADH produced by malate dehydrogenase and entering those electrons into the electron transport chain. Then, in a manner that's quite similar to what we did in the previous shuttle, the glycerol-3-phosphate shuttle, those electrons are going to be transferred to oxygen to make water.

Before I go on, let's review a little bit. Between step one and step two in the cytoplasm, we deposited electrons into oxaloacetate to make malate. That step restored in NAD+ levels in the cytoplasm, which is what we wanted to accomplish. However, we've consumed a molecule of oxaloacetate. And as I've mentioned before, the cell has to try to preserve the concentration of this very precious molecule. We now have to find a way to restore oxaloacetate that we borrowed in step one.

Now let's look back at step four, where malate was converted to oxaloacetate in the mitochondrial matrix. Because the molecule of malate came from the cytoplasm, this is a net increase in the mitochondrial matrix of one unit of malate and, ultimately, one unit of oxaloacetate.

We need to find a way to get that molecule of oxaloacetate back out into the cytoplasm, in order to make the shuttle a continuous one. In the co-factor section of 5.07, JoAnne taught us about the ways that pyridoxal phosphate and pyridoxamine work, in order to put amino groups into organic acids, such as oxaloacetate. And that's what's going to happen in this case.

Oxaloacetate is converted into its amino acid homolog, aspartic acid. Why did we do this emanation reaction? Well, there's no way to get oxaloacetate directly out of the mitochondria

because there's no transporter for it. But there is a good transporter, the apartheid transporter, that will take aspartic acid out into the cytoplasm.

So the oxaloacetate is converted, temporarily, into aspartic acid in the mitochondrion. And that aspartic acid then slips out through its transporter to the cytoplasm. Once in the cytoplasm, there's a similar pyridoxal mediated mechanism to deaminate the aspartate to regenerate the cytoplasmic molecule of acetate that we borrowed at step one a few minutes ago.

In panel D I summarize. That we've looked at three different small pathways that enable the cytoplasm of the cell to always have enough NAD+ to oxidize glucose to pyruvate. These pathways are first, the lactate dehydrogenase system. Second, the glycerol-3-phosphate shuttle. And third, the malate departed shuttle.