JOHN ESSIGMANN: We're still on storyboard 7. We're on panel C. Panel C is where I introduced the TCA cycle. As I mentioned earlier, respiration consists of three stages. The first is the pyruvate dehydrogenase reaction, which we just covered, taking pyruvate to acetyl-CoA. The second is the TCA cycle, or tricarboxylic acid cycle taking that acetyl-CoA and basically oxidizing it in order to generate CO2 but also to produce more reducing equivalents in the form of mobile electron carriers. And then the third step of respiration is taking those reducing equivalents to the mitochondrial inner membrane, where the molecules containing those reducing equivalents are oxidized. And then, the electrons from that oxidation reaction are used to power proton pumps that ultimately will generate the proton gradient that can be used for generation of ATP or for generation of movement or for other things.

The tricarboxylic acid cycle, which is sometimes called the Krebs cycle, takes acetyl-CoA from several different sources. One of those sources is glycolysis to pyruvate and pyruvate to acetyl-CoA, as we've just seen. And the second source of acetyl-CoA is from fatty acid oxidation. We'll come to that pathway somewhat later. Again, looking at panel C, the TCA cycle starts with the reaction of acetyl coenzyme A, a 2-carbon compound with oxaloacetate, a 4-carbon compound, to form the 6-carbon product citrate. Citrate will lose 2 carbons as carbon dioxide, and in the process, there'll be a series of oxidation steps that generate three NADHes one FADH2, and one either GTP or ATP by substrate-level phosphorylation.

In terms of the banking system of the cell, the NADHes that are generated in the mitochondrion are exchangeable for about three ATPs. FADH2 is exchangeable for about two ATPs. So if you look up the total number of nucleotide triphosphate, or NTP, equivalents that can be produced in the TCA cycle, you'll get about 12 ATPs for each 2-carbon unit of acetyl-CoA that's oxidized. As a detailed point, I want to mention that the two carbons that entered the TCA cycle as acetyl-CoA are not exactly the same two carbons that come out as CO2 in that cycle. The carbon dioxides from the input acetyl-CoA will emerge in later turns of the TCA cycle.

Now we're going to look at panel D. In panel D, we start looking at the details of the TCA cycle. JoAnne explained why nature uses thioesters. The sulfur allows enolization stabilization of a carbanion at carbon 2, the carbon that's distal to the coenzyme A functionality. The carbanion is then able to attack the number 2 carbon, carbonyl, of oxaloacetate in the reaction catalyzed by citrate synthase. An intermediate is formed, citroyl coenzyme A, which loses its coenzyme A moiety by hydrolysis in a very thermodynamically irreversible step, resulting in the product citrate. This step is at the top of the pathway, and as is usually the case, this highly exothermic step makes the pathway, overall, irreversible.

Chemically, citrate synthase does a mixed aldol-Claisen ester condensation. The product of the citrate synthase reaction, citrate, is a tertiary alcohol, and tertiary alcohols are relatively difficult to oxidize. The next enzyme in the pathway, aconitase, which is shown in storyboard 8, panel A, removes a water molecule and then adds a different water molecule back to rearrange the hydroxyl group, making a secondary alcohol, which is much easier to oxidize. Looking again at panel A, the hydroxyl group of high isocitrate is oxidized to a ketone, with the transfer of hydride to NAD+ to make NADH. This reaction is catalyzed by the enzyme isocitrate dehydrogenase, ICDH. The intermediate in this reaction is oxalosuccinate, which is a beta-keto acid. As we know from what JoAnne taught us, beta-keto acids are prone to spontaneous decarboxylation.

So the second half of the isocitrate dehydrogenase reaction involves the loss of carbon dioxide in what's usually considered to be an irreversible step. I do want to point out, however, that under certain circumstances, you can re-add the carbon dioxide in order to make the reaction go in the other direction.

After the ICDH reactions, which generated NADH and resulted in loss of CO2, the product is alpha-ketoglutarate, a 5-carbon keto acid. Take a look at the structure of alpha-ketoglutarate in the upper right-hand portion of storyboard 8, panel A. If you hold your finger over the top 2 carbons of alpha-ketoglutarate, you'll notice that the residue is pyruvate. So pyruvate plus an acetyl functionality equals, effectively, alpha-ketoglutarate.

Now take a look back at the mechanism by which pyruvate is oxidized by pyruvate dehydrogenase. It's going to be a very similar mechanism for the oxidation of alpha-ketoglutarate. The product of the alpha-ketoglutarate dehydrogenase reaction is succinyl-CoA. Again, if you look at the structure of this molecule, succinyl-CoA, it's actually an acetyl-CoA with an acetyl group put onto one end.

Now let's go back and look at the alpha-ketoglutarate from a different perspective. I want to make a point here in that alpha-ketoglutarate is an alpha-keto acid, and if we were to replace its keto group with an amino group, you'd convert this keto acid into the amino acid glutamic acid. As with most enzymatic reactions involving such nitrogen functionalities, a pyridoxal pyridoxamine phosphate cofactor will be needed to interconvert the alpha-ketoglutarate and glutamic acid. So glutamic acid can serve as a source of alpha-ketoglutarate if the cell is starved for TCA cycle intermediates. Alternatively, alpha-ketoglutarate can be a source for glutamic acid when a cell may need amino acids for protein biosynthesis.

Now, let's turn to panel B, where we'll start with succinyl coenzyme A. Looking at the molecule of succinyl-CoA, note that I've labeled each of the atoms with a symbol. The triangle and square were from the original acetyl-CoA molecule that came in at the top of the pathway. The enzyme that processes succinyl-CoA is succinyl coenzyme A synthetase. As JoAnne taught us, synthetases are enzymes that typically need a nucleotide. In this case, the nucleotide involved is GDP in mammalian systems or ADP in bacterial systems. In the traditional clockwise direction of the TCA cycle, they are phosphorylated to form GTP or ATP, respectively.

The molecule that you get after the phosphorylation reaction in the hydrolysis of the coenzyme A is succinate, a 4-carbon compound, which is also a dicarboxylic acid. This molecule is perfectly symmetrical and can tumble in three-dimensional space. The next enzyme in the pathway, succinate dehydrogenase, cannot distinguish one arm of its substrate, succinate, from the other. So if there were a radio label in the acetyl-CoA at the beginning of the pathway, that radio label would become scrambled at this point, uniformly distributed among the two carbons of the two arms. From this point onward in the TCA cycle, you will note that the label denoted as the triangle and box is scrambled, as indicated by triangle divided by 2 or box divided by 2.

The next enzyme in the pathway is succinate dehydrogenase. This is the only membrane-bound enzyme in the TCA cycle. It is a dehydrogenase, and it uses flavin as a cofactor to help remove electrons from the succinate substrate. Flavin picks up electrons from succinate, converting FAD to FADH2 in the mitochondrial membrane. The product of the reaction is the alkene fumarate. The enzyme fumarase adds water to fumarate to form the alcohol product malate. The hydroxyl group of the alcohol malate is primed for oxidation by the next enzyme in the pathway, malate dehydrogenase, or MDH. MDH oxidizes malate, which is an alcohol, to a

ketone. The ketone product is oxaloacetate. The hydride removed from malate is transferred to NAD+ to form NADH. As I mentioned, the product of the overall reaction is oxaloacetate, and its ketone functionality is now primed for attack by the next molecule of acetyl-CoA entering the TCA cycle.

As a final point, I've mentioned several times that oxaloacetate is present at a very low concentration, only in the micromolar range, inside the mitochondrion of a mammalian cell. So it's always at rather limiting concentration. The cell has to work very hard to preserve enough of the oxaloacetate to enable the next cycle of the TCA cycle, that is the acquisition of the next acetyl coenzyme A group. One of the ways that the cell can generate oxaloacetate is by deamination, or transamination of aspartic acid to the keto acid oxaloacetate. We typically have plenty of aspartic acid and this PLP-mediated reaction helps to maintain a critical level of oxaloacetate.

I want to return to storyboard 7 to make some comments about the importance of prochirality in some enzymatic reactions. This short interlude will help explain how to track a radio label in a TCA cycle intermediate as that intermediate progresses through the TCA cycle. As you will note, at first glance, the label does some unexpected things. But at the end of the day, the fact that the label does surprising things helped early biochemists figure out mechanistically how several enzymes work in concert during the linear steps of a pathway.

We're going to look at storyboards 7, 8, and 9, starting with the chemical reaction at the bottom right of storyboard 7, panel D. This is the chemical reaction that's catalyzed by citrate synthase. You'll notice that I've highlighted the two carbons with either a triangle or a box. As we have seen, the nucleophile on acetyl-CoA attacks the electropositive carbon of the carbonyl functionality of oxaloacetate. The carbonyl functionality is a flat sp2 hybridized center. So if this were a typical organic chemical reaction, the electrophile could come in from either the top or the bottom, and you'd get two different stereochemistries in the product. That is the citrate at the very bottom would have equally labeled acetyl arms at the top and bottom of the molecule as I've drawn it. You would have a delta divided by 2 for the blue methylene group, and a box or square divided by 2 for the red carboxylate group in each of the two acetyl arms. Again, these arms are at the bottom and top of the molecule as drawn in the lower right of panel D. You'll note, however, that only the top acetyl group of citrate has the labels. Initially, the observation that only the top arm acquired label was a puzzle to early biochemists.

One way to think about the citrate synthase reaction is to think about the oxaloacetate laying

on the surface of the citrate synthase enzyme. Now imagine that the enzyme precludes, or blocks, access to the carbonyl from the bottom and allows access only to the top, giving rise to only one stereochemical outcome, the one that I've shown in the citrate to the right.

Now move ahead to the storyboard number 9, panel B. This panel shows a more cartoon-like representation of the molecule of citrate. You can see the acetyl arm on the top, the pro-S arm, as having the labels. And the pro-R arm, the one that came from oxaloacetate, at the bottom, is label free. So while the pro-R and pro-S arms are chemically identical, they're going to be handled by the next enzyme in the series, aconitase, as being chemically different from one another. All biochemistry is going to be occurring on the pro-R arm, that is the arm that came in from oxaloacetate and not the arm that came in for acid 2 with acetyl coenzyme A.

In the bottom right of panel B, I've sketched out an imaginary active site for the aconitase enzyme. I show a base picking up a proton from the pro-R arm of the citrate molecule. And you can see the elimination of the water molecule from the 3 carbon. So despite the fact that the citrate molecule is chemically symmetrical, aconitase, the next enzyme in the reaction series, is able to distinguish between the pro-R and the pro-S arms.

At this point, I want you to look back at storyboard 8, panel A. Look once again at the citrate that is at the upper left-hand corner of storyboard number 8, and let's imagine that the aconitase chemistry has happened on the pro-S arm, that is the one in the box. Keep in mind that these experiments have shown that this does not happen. This is just a hypothetical scenario. In this hypothetical case, the hydroxyl group would end up on the number 2 carbon, the one with the blue triangle. You have to draw it out, but if you traced this molecule, in which the chemistry happened on the pro-S arm, all the way around to alpha-ketoglutarate, you would find out that the alpha-ketoglutarate dehydrogenase would liberate CO_2 from the carboxylate that has the red box on it.

This is in contrast to the molecule succinate that appears on storyboard 8, panel B. Succinate is another symmetrical molecule, but for some reason, succinate dehydrogenase cannot distinguish between the two acetyl arms. Because the enzyme is unable to distinguish the two arms, in this case, unlike that of aconitase, the radio label would become scrambled. The reason that I'm belaboring this point is because one of the ways that biochemists work out the chemical reactions involved in a biochemical pathway is by putting in some kind of labeled molecule. It could be a radio label or a heavy isotope. And then they trace the position of the label in the molecule as you move from molecule to molecule along the pathway. So label

tracer studies are ones that are absolutely central to all of biochemistry. And as I mentioned earlier, you'll get lots of experience in the problem sets in using labels to work out the details of a pathway.

Before leaving the TCA cycle, there are a couple of big picture points that I want to make. You put in two carbons as acetyl-CoA, deposit them into oxaloacetate to form citrate, a 6-carbon compound, and then in the cycle, you lose 2 carbons as carbon dioxide. That means that there's no loss or gain of carbon in this cycle. If you had just one molecule of oxaloacetate, you'd be able to complete the TCA cycle. What happens, however, when the cell is in an, let's say, "energy needed" situation, where it needs to buff up this cycle, that is increase the number of molecules cycling to be able to accommodate the processing of more and more molecules of acetyl-CoA? Those molecules of acetyl-CoA might flood in from carbohydrate metabolism or, as we'll see later, from catabolism of lipids.

Let's look at panel A of storyboard 11. As shown in this figure, one way to accomplish increasing the carbon content of the TCA cycle is to take an amino acid, such as glutamate, and remove its amino group to form alpha-ketoglutarate. Note that if you increase the concentration of any one molecule in the TCA cycle, for example, alpha-ketoglutarate, you're effectively increasing the concentrations of all molecules in the cycle. Because it's a cycle, many of the molecules are in equilibrium with one another. Going clockwise around the TCA cycle from alpha-ketoglutarate, we come to succinyl-CoA. Succinyl-CoA is an entry point into the TCA cycle from odd-chain fatty acids in certain amino acids, such as methionine. So these molecules can give rise to succinyl-CoA that itself will then increase the concentration of all molecules in the TCA cycle. Our third primary input point is at oxaloacetate. It involves aspartic acid being deaminated into oxaloacetate. This is a very common way to increase the amount of oxaloacetate available for the TCA cycle. This reaction can dramatically increase the rate of processing of molecules by the TCA cycle.

Finally, there's an enzyme we'll look at later called pyruvate carboxylase, or PC, which can take pyruvate in the mitochondrion, add CO_2 to it, and form oxaloacetate. We're going to come to this enzyme later when we talk about carboxylase enzymes as a class. This is an important enzyme in the pathway of gluconeogenesis.

So overall, I think you can see that there are several ways that a cell can increase the overall concentration of the intermediates of the TCA cycle. Increasing any one intermediate increases all of them. And that will increase the rate by which acetyl-CoA molecules can be

processed ultimately to generate energy. The general word describing this buffing up of the TCA cycle is anapleurosis, which comes from the Greek word "filling up."