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ESSIGMANN:**

The second carbohydrate biosynthetic pathway we're going to look at is called gluconeogenesis. Gluconeogenesis is the synthesis of glucose from noncarbohydrate precursors. Let's look at Panel A of this storyboard, Storyboard 31.

There are some organs in the body that require glucose as their metabolic fuel, yet they don't have the capacity to make it, or to make much of it. Examples are the brain, renal medulla, red blood cells, and the testes. To give an idea of the scale of the problem the body faces, we only have about 200 grams of glucose or glycogen stored away in our body, and the brain alone uses more than half of that each day, so we don't have much in the way of a carbohydrate reserve.

The body compensates by having certain organs, specifically the liver and renal cortex, act as specialized manufacturing centers to produce and export glucose. The chemical raw materials they use include organic acids such as lactate, some amino acids, and the three-carbon residues from odd-chain fatty acids that enter catabolism. To get an idea of how gluconeogenesis works, let's take a look at the box here at the bottom of Panel A.

You see a muscle working very hard, as evidenced by the fact that it's secreting lactate out into the blood. The lactate travels from the working muscle by the blood to the liver, where the lactate is taken in. The pathway of gluconeogenesis, with energy and reducing equivalent input, rebuilds glucose from that lactate precursor. The glucose is then sent back out into the blood, returns to the muscle, enabling the muscle to continue hard work. As I've described earlier in 5.07, this functional relationship between a muscle and the liver is called the Cori Cycle.

Let's look at Panel B. Depending on how you look at it, there are seven or possibly eight noncarbohydrate precursors to glucose in gluconeogenesis. They're listed in this panel. And I've indicated these precursors by numbers 1 through 8.

The first seven are not carbohydrates, so they qualify as noncarbohydrate precursors as fits

the definition for a gluconeogenic compound. The eighth is actually a carbohydrate. It's ribose. I'm semi-illegally squeezing it in here because ribose acts as a major gluconeogenesis precursor by way of the Pentose Phosphate Pathway, or PPP.

We haven't covered the Pentose Phosphate Pathway yet, but we'll see, when we do cover it, exactly how this pathway works to manufacture glucose. In other words, ribose from the diet can be converted into glucose by way of the Pentose Phosphate Pathway coupled to gluconeogenesis. A little bit later in this lecture, we'll see how each of these precursors-- that is, numbers 1 through 8-- are converted to glucose by the pathway of gluconeogenesis.

The best way to start thinking about gluconeogenesis is to think of it as glycolysis running in reverse. As shown here in Panel C, glycolysis is conversion of glucose, ultimately, to pyruvate. As we know, that pathway goes with a net generation of ATP. That is, it is exergonic.

Accordingly. If we go from pyruvate back to glucose, we know that in order to make that reverse pathway exergonic, we're going to need to have energy input. As we learned earlier, there are 10 steps in glycolysis going from glucose to pyruvate. Three of those steps are thermodynamically irreversible. Those are the steps that commit molecules to flow from left to right, as drawn in this panel.

The thermodynamically irreversible steps of glycolysis are first, hexokinase/glucokinase, that is, the conversion of glucose to glucose 6-phosphate. The second irreversible step is phosphofructokinase 1, the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. The third irreversible step is pyruvate kinase, the conversion of phosphoenolpyruvate to pyruvate.

We have to find a way in gluconeogenesis to bypass these three thermodynamically irreversible steps. The enzymes that were invented to circumvent the three thermodynamically irreversible steps of glycolysis are shown in green asterisks in this panel. For example, glucose 6-phosphatase hydrolyzes the sixth phosphate from glucose 6-phosphate, converting it to glucose. A second asterisked enzyme of gluconeogenesis is fructose 1,6-bisphosphatase, which converts fructose 1,6-bisphosphate by hydrolysis to fructose 6-phosphate.

The third irreversible step that we need to bypass is pyruvate kinase. Two enzymes were invented to enable this bypass. Pyruvate carboxylase, which we have studied before in several contexts in 5.07, and a new enzyme, phosphoenolpyruvate carboxykinase, or PEPCK. Pyruvate, carboxylase, and PEPCK work in a partnership with several other enzymes, actually

in a mini-pathway, to bypass the pyruvate kinase step of glycolysis, and hence, enable gluconeogenesis.

Lastly, at the right of this panel is a large inverted L. This is meant to symbolize the 8 precursors to gluconeogenesis. These precursors will enter the gluconeogenesis pathway, and flow by the hatched arrow lines all the way up to glucose. This network of reactions allows the conversion of molecules, such as the amino acid glutamate, all the way to glucose.

Now we're going to turn to Storyboard 32. Let's look at Panel A. And let's start out by looking at the mechanisms by which the four key enzymes of gluconeogenesis works.

The first two enzymes, glucose 6-phosphatase and glucose 1,6-bisphosphatase, carry out simple phosphate-ester hydrolyses as shown. The third enzyme is pyruvate carboxylase. We looked at this enzyme mechanistically back in the fatty acid catabolism lectures, when I taught about the mechanisms by which CO₂ can be captured and added to an organic acid.

Pyruvate carboxylase will convert pyruvate to oxaloacetate. Hence, it's in an anaplerotic enzyme that helps maintain the levels of TCA intermediates. In the cases we've looked at so far, PC helps maintain specifically oxaloacetate levels. Refer back to the lecture on carboxylases to see how this enzyme works from a mechanistic perspective.

Now let's look at Panel B. The last and fourth key enzyme I want to discuss in gluconeogenesis is phosphoenolpyruvate carboxykinase, or PEPCK. We haven't seen an enzyme that works like this before, so let me go through its mechanism in some detail.

PEPCK is going to convert oxaloacetate to phosphoenolpyruvate. The enzyme is active in the liver and renal cortex under physiological conditions that mandate gluconeogenesis be turned on. The enzyme takes oxaloacetate, which, as we know, is a beta keto acid. Thus, it's prone to decarboxylation.

When CO₂ is removed from oxaloacetate, you get a transient pyruvate enolate, which is an anion. The oxygen anion of the pyruvate enolate will attack the gamma phosphate, that is, the terminal phosphate of GTP, and thus, the pyruvate enolate will become phosphorylated. The product of phosphorylation is phosphoenolpyruvate, or PEP. Hence, the concerted action of two enzymes, pyruvate carboxylase and PEPCK, enables reversal of the otherwise irreversible PK step, the pyruvate kinase step, and allows the cell to do gluconeogenesis.

Let's look now at Panel C. Now that we've seen the identities of the eight precursors to glucose in gluconeogenesis, and we've done a little bit of learning about the mechanisms of some of the key enzymes in the pathway, we can turn our attention to a network analysis of the overall gluconeogenic pathway. The pathway in this panel may seem a bit daunting, because it's pretty much everything we've learned about metabolism so far in 5.07.

So let's go through it slowly in little pieces. Let's start over on the left, where the glucose molecule is situated in a squiggly box. Note that the glucose in the squiggly box is being sent out of the cell-- that is, it's going in the opposite direction from the direction in which we have dealt with glucose so far in metabolism.

Usually, we think of glucose as coming into the cell and entering metabolism. This is a liver cell or renal cortex cell that's in the process of manufacturing glucose from those eight precursors, the ones I mentioned above, and sending the manufactured glucose out into the blood. In this metabolic map, the hatch lines-- that is, the lines that look like railroad tracks-- represent that gluconeogenic pathways.

Now let's start walking through the metabolic map. Slightly to the right of the middle of the diagram, you see the amino acid alanine and the three-carbon organic acid lactate. Lactate is precursor number one in my scheme. The numbering, again, is in Panel B of the previous storyboard. And alanine is precursor number two.

Let's start with lactate. Lactate would typically come into the liver from the blood, perhaps from a working muscle. Think about the Cori Cycle for a moment. Lactate dehydrogenase will convert the lactate into pyruvate in the liver or in the renal cortex, in the other gluconeogenic organ.

Let's look next at alanine. Alanine, gluconeogenic precursor number two, is, of course, an amino acid. And a PLP-mediated reaction will convert it to pyruvate. That reaction involves specifically the loss of alanine's amino group.

Now imagine the resulting pyruvate, that is, from either lactate or alanine, translocating to the right into the mitochondrion, where it's carboxylated by pyruvate carboxylase into oxaloacetate. This is the reaction mechanism we looked at a short time ago. Now remember that malate dehydrogenase, or MDH, thermodynamically favors the formation of malate from oxaloacetate.

So our two molecules navigating the gluconeogenic pathway-- that is, precursors one and two, transit from oxaloacetate into malate. Malate is in the mitochondrion at this point. In the next step, it exits the mitochondrion and goes into the cytosol.

As I've mentioned before, the transporter for malate works in both directions. Malate, now transported into the cytosol, can be converted back to oxaloacetate by the cytoplasmic version of malate dehydrogenase. The oxaloacetate thus produced in the cytosol goes upward, following the vertical arrow. Lastly, PEPCK, phosphoenolpyruvate carboxykinase, converts the oxaloacetate into cytoplasmic phosphoenolpyruvate.

Let's pause here for a minute and review. Look at where phosphoenolpyruvate is on the metabolic map, and then look to the right. On the right, you'll see pyruvate on the rightward side of the one-way arrow.

You will also see lactate and alanine, molecules one and two, respectively, having their carbons flow into the pool of pyruvate. The pyruvate pool, which starts in the cytosol, gets shuttled into the mitochondrion, where pyruvate carboxylase converts the molecules derived from pyruvate-- that is, lactate and alanine-- into mitochondrial oxaloacetate. Then the mitochondrial pool is converted from oxaloacetate into malate, which exits the mitochondrion.

And the molecules now represent a malate pool that's in the cytosol. That malate pool, which began as pyruvate, alanine, and lactate, is converted to oxaloacetate. And lastly, our new enzyme, PEPCK, converts the oxaloacetate into cytoplasmic phosphoenolpyruvate.

All that this was done in order to bypass the one-way step between phosphoenolpyruvate and pyruvate. In other words, I can go from phosphoenolpyruvate to pyruvate quite easily by pyruvate kinase. But because this reaction is so strongly exothermic, I cannot go the other way. The reverse step is irreversible.

The mini-network that we've just navigated with all of its steps, some of which require ATP, was done in order to bypass the irreversible pyruvate kinase reaction. We've just worked very hard to move atoms from pyruvate, alanine, and lactate into the cytosol, where those atoms are now packaged as PEP, or phosphoenolpyruvate. Now, let's focus on phosphoenolpyruvate, and let it flow backward through the reverse of glycolysis all the way up to the next irreversible step at fructose 1,6-bisphosphate.

All of the steps between phosphoenolpyruvate and fructose 1,6-bisphosphate are common to

both gluconeogenesis and glycolysis. Fructose 1,6-bisphosphate cannot be converted to fructose 6-phosphate by reversal of the PFK 1, phosphofructokinase 1, enzymatic step. That step is too thermodynamically irreversible.

Consequently, Nature invented fructose 1,6-bisphosphatase. We saw the mechanism of that enzyme earlier in the lecture. It allows the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate.

Then the molecule continues upstream to form glucose 6-phosphate. And once again, a gluconeogenic-specific enzyme, glucose 6-phosphatase, will convert glucose 6-phosphate into glucose. Lastly, the manufactured glucose is exported from the cell by the glucose transporter.

So while it was a long passage, the carbon atoms from alanine and lactate have traveled all the way to glucose. And then the glucose has been sent out of the cell. That is, their carbons are now incorporated into the molecules of glucose that go off from the liver and renal cortex to organs that need glucose to meet their energy needs, or their needs for the glucose skeleton for other biochemical purposes.

Gluconeogenic precursor three is glutamate. This is the amino acid equivalent of the organic acid alpha ketoglutarate. So look at alpha ketoglutarate at about 3 o'clock on the TCA cycle. You'll see glutamate precursor three being de-aminated by a PLP-mediated process.

This converts glutamate into alpha ketoglutarate. Most of the carbons of the alpha ketoglutarate will then move clockwise around the TCA cycle, and end up at about 9 o'clock as malate. Then, as we saw for precursors one and two, the malate will be exported from the mitochondrion. And next, most of its atoms will find their way back to glucose, just as we saw with alanine and lactate earlier.

Precursor four is aspartic acid. Aspartic acid is the amino acid equivalent of oxaloacetate. Aspartate is de-aminated into oxaloacetate, which enters the gluconeogenic pathway at about 10 o'clock on the TCA cycle.

At this point, the oxaloacetate formed from aspartate joins the oxaloacetate made from alanine and lactate, precursors one and two, and continues all the way back to glucose.

Gluconeogenic precursor five comes from odd-chained fatty acids, which are depicted way over on the right of the metabolic map. As we have seen, odd-chain fatty acids are catabolized into three- carbon compounds, such as propionyl coenzyme A. One of the carboxylases,

propionyl coenzyme A carboxylase, will add a carbon to this three-carbon molecule.

Then methylmalonyl-coenzyme A mutase and its partner, epimerase, will complete conversion of the three-carbon propionyl-CoA to the four-carbon product succinyl-CoA, which will then integrate into the TCA cycle. Succinyl-coenzyme A enters at about five o'clock on the TCA cycle. The carbons then flow clockwise to malate. Just as we've seen before, most of those carbons will flow all the way back to be built into glucose.

Gluconeogenic precursors indicated by number six are the amino acids, isoleucine, methionine, and valine. These are, once again, way over on the right-hand side of the figure. These amino acids are also converted to propionyl-CoA. And just as with the odd-chain fatty acids, they will be converted into succinyl-coenzyme A. Then their atoms follow the pathway all the way back to glucose, as we have seen now several times.

Slightly to the left of center, you'll see gluconeogenic precursor number seven, which is glycerol. Glycerol is a tri-alcohol produced from the metabolism of complex lipids. It derives from the three-carbon backbone that holds fatty acids and phosphates by ester linkages.

Glycerol is first phosphorylated to glycerol 3-phosphate. And then that product is oxidized to dihydroxyacetone phosphate. We've seen this chemistry before. From DHAP, or dihydroxyacetone phosphate, the molecules flow leftward on the chart to fructose 1,6-bisphosphate, and then they continue on through gluconeogenesis to glucose.

Lastly, precursor number eight is ribose. We mainly obtain ribose from our diet. In the next lecture, I'm going to cover the pentose phosphate pathway.

In this pathway, ribose from the diet will enter what I call the "carbon scrambling phase" of the pentose phosphate pathway. Ribose goes into the carbon scrambling phase of the pathway, and GAP, glyceraldehyde 3-phosphate and fructose 6-phosphate come out. Both of these molecules, GAP and fructose 6-phosphate, will flow from right to left all the way to glucose in the pathway that I've drawn out in Panel C. Hence, ribose is a good gluconeogenic precursor.

Let me summarize the functional dimensions of the gluconeogenesis pathway from a high altitude. Imagine that overnight, as you sleep, you're not consuming any food. Consequently, at some point, your blood sugar is going to drop.

Your gluconeogenic organs, the liver and renal cortex, will sense this drop in blood sugar, and turn on the hatched-line pathways we've seen above, in order to take readily available

noncarbohydrate precursors, such as lactate, alanine, ribose, and so on, and convert those noncarbohydrate precursors into glucose. The liver and renal cortex will then send that glucose out into the bloodstream to client organs, for example, the brain, red blood cells, and renal medulla. Ultimately, these client organs will be able to rely on a constant level of glucose. That, in a nutshell, is the role of the gluconeogenic pathway.