

The following content is provided under a Creative Commons license. Your support will help MIT OpenCourseWare continue to offer high-quality educational resources for free. To make a donation or view additional materials from hundreds of MIT courses, visit MIT OpenCourseWare at ocw.mit.edu.

PROFESSOR:

Let's look now at storyboard 25, panels A and B. Most of the pathways we have studied so far in **5.07** have been catabolic-- that is, the breakdown of molecules, usually with the objective of producing energy rich molecules, such as ATP, or reducing equivalents that ultimately could be used for reductive biosynthesis.

Now, we're going to turn to anabolism, or biosynthesis. Biosynthetic pathways are going to require energy input, as well as reducing equivalents. Overall, biosynthesis is an endergonic process.

Looking at panel B, you can see that we're going to start with fatty acid and lipid biosynthesis. Fatty acids are made in the cytoplasm. The mitochondrion is going to play an important role, however. We'll see just how in a couple of minutes.

Let me say a couple of things about the mitochondrion. First, acetyl CoA, oxaloacetate and the NADP plus NADPH pair, as we have described above, cannot pass through the mitochondrial membrane. However, malate and citrate can. In fact, they can go in either direction. Keep these points in mind as we move ahead. Now let's look at panel c. We're going to break down fatty-acid biosynthesis into five steps.

The first step involves getting acetyl coenzyme A into the cytoplasm. As I just said, acetyl CoA cannot directly go past the mitochondrial membrane. To overcome this problem, nature, quote unquote, packages acetyl CoA as citrate, which can easily traverse the mitochondrial membrane. Hence, we put acetyl CoA into a molecule of citrate, bring citrate out into the cytoplasm, and then split off the acetyl CoA once again.

The second step involves maintaining oxaloacetate balance. We just transported a molecule of citrate out of the mitochondrial matrix, thus depleting the TCA cycle of one of its important intermediates. The levels of all TCA cycle intermediates are going to drop, including oxaloacetate. Now think about what's happened to the citrate in the cytoplasm. It was split into oxaloacetate and acetyl CoA. If we could send that on oxaloacetate back into the

mitochondrion, the TCA cycle would be replenished, once again. Step two will show how oxaloacetate finds its way back into the mitochondrial matrix.

The third step in fatty-acid biosynthesis involves the synthesis of malonyl-coenzyme A. This molecule is made by putting a carbon-dioxide molecule onto the methyl carbon of acetyl CoA using acetyl coenzyme A carboxylase-- an enzyme we looked at earlier in the carboxylase module of 5.07. malonyl-coenzyme A is the actual precursor to all, but two of the carbons in the fatty acid chain.

The fourth step in fatty-acid biosynthesis involves putting the malonyl-coenzyme A onto a very large protein on the fatty acid synthase complex. This large protein is called ACP, or acyl carrier protein.

The fifth step involves the actual reactions of the fatty acid synthase, sometimes called FAS. This is a large multi-protein complex. These are the enzymes by which we're going to see the stepwise addition of two carbon units, up until we get to the formation of a 16 carbon long fatty acid called palmitic acid. Palmitic acid, abbreviated 16:0, is the default fatty acid length made by the fatty acid synthase complex.

Let's look at panel D. After the five step synthesis of palmitic acid the C16 fatty acid can be elongated. It can have double bonds put into it, and it can be branched. We're not going to be looking into those reactions, but we shall be looking into the way that either two or three fatty acids will be placed onto a glycerol backbone in order to make either, a membrane lipid that is a diacylglyceride phosphate, or triacylglycerides. Triacylglycerides are our primary storage form of energy. We're not going to be looking into those reactions, but we shall be looking at the way that either two or three fatty acids can be placed onto a glycerol backbone in order to make either a membrane lipid that is a diacylglyceride phosphate, or triacylglycerides. Triacylglycerides are our primary storage forms of energy.

I'm not going to be going over another type of fatty-acid biosynthesis, called polyketide biosynthesis, but I'll encourage you to take a look at this pathway in the book. Polyketide biosynthesis is a very important source of a host of biologically active lipids.

Let's look at panel E. Before we look at a schematic of lipid biosynthesis, I'd like to introduce NADPH. NADPH is going to be the source of electrons that are used for reductive biosynthesis. NADPH is identical to NADH with the exception of the phosphate at the lower right of the molecule, as drawn in panel E. This phosphate is a molecular decoration that's

recognized by biosynthetic enzymes. That is, enzymes that are looking for a source of reducing equivalents. NADPH is the co-factor that provides those reducing equivalents.

The hydrides shown in the blue ellipse, at the top of the molecule, represents the reducing equivalents that were going to be used in biosynthesis. The two main pathways that result in the production of NADPH, for biosynthesis, are the pentose phosphate pathway, which I'm going to deal with in a separate lecture, and the malic enzyme, which I'm going to introduce in a few minutes.

Now turn to panel F. This schematic shows a high-level view of fatty-acid biosynthesis. I think it's useful to put fatty-acid biosynthesis in the context of one of our physiological scenarios. The scenario is eat sugar, get fat. Put more scientifically, this scenario is going to show how the sugar we eat can end up being converted, very efficiently, into fat. I think that, probably, most of us want to avoid getting fat. But keep in mind that converting sugar to fat is one way of converting energy into a very compact and mobile form.

Let's start with the glucose molecule over on the left of the panel. Starting with step one, follow the horizontal hatched line to the right. The glucose passes through glycolysis, produces pyruvate, pyruvate goes into the mitochondrion and is converted to acetyl CoA. Once again, following the hatched line, acetyl CoA condenses with oxaloacetate. This is the citrate synthase reaction to form citrate. Rather than progressing further into the TCA cycle where it would lose its carbons as carbon dioxide, the citrate at step two is exported by a transporter out into the cytoplasm.

Step three is catalyzed by an enzyme called ATP citrate lyase. This enzyme splits the citrate into acetyl CoA, which is what we want, and leave a residue-- a molecule of oxaloacetate. I'm not going to go through the mechanism of this enzyme, but I think it's worthwhile to look back at storyboard seven, look at the citrate synthase reaction and then imagine it running backwards. Now, it's not going to be exactly the reverse reaction as drawn, but at this point in 5.07 you should be able to look at the cosubstrates for the reaction, and be able to figure out how ATP citrate lyase liberates the oxaloacetate residue.

I'm going to come back to the oxaloacetate, which is labeled by a star, in a few minutes. Keep in mind that we have to find a way to return oxaloacetate to the mitochondrial matrix or else we won't have enough oxaloacetate to enable the next molecule of citrate to be formed from an incoming acetyl coenzyme A.

At step five we have acetyl coenzyme A in the cytoplasm, where we want it for fatty-acid biosynthesis. But we can also do other things with this molecule. In the last lecture, for example, I talked about ketone body formation. So look at the vertical arrow-- that is the one going up. You can see we can produce HMG CoA, hydroxy methyl glutaryl coenzyme A. Which, as I said in the last lecture, is a biosynthetic precursor to, for example, cholesterol, as well as ketone bodies. Then, follow the arrows through step 5A to the deposition of cholesterol into the plasma membrane. Keep in mind, cholesterol is a plasticizer of the membrane. Therefore, it's an essential molecule.

Now let's go back and pick up the acetyl CoA as it progresses through the fatty acid synthase, or FAS Reactions of 5B. Acetyl CoA is built up in eight steps to form the 16 carbon fatty-acid palmytate. Following the upward arrow, palmytate can go on to form a phospholipid, which will become an integral part of the membrane, or it can go horizontally, to the left, to form a triacylglyceride, which will become embedded in a lipid globule where it can serve as a storage depot for energy.

So at this point, through the series of reactions numbered 5A and 5B, we've made the membrane plasticizer, cholesterol, phospholipids, which are the main structural elements of a biological membrane, and a triacylglycerides, which is our principal energy storage molecule.

Now let's go back to the asterisked oxaloacetate, at step 3. This orphaned molecule of oxaloacetate has to be able to get back into the mitochondrion. There are two pathways by which this can happen-- 4A and 4B. Oxaloacetate is a ketone. It can be reduced by NADH to form the alcohol, malate. This is the reverse of the reaction that we're used to seeing in the TCA cycle. Nevertheless, this is indeed the thermodynamically favorable direction for this reaction. The malate dehydrogenase enzyme used, here, is closely related to the one that's in the mitochondrial matrix. Keep in mind, however, that this is the cytoplasmic version of the enzyme.

I mentioned earlier that malate can go in either direction across the mitochondrial membrane. In this case, path 4A, malate goes into the mitochondrion, becomes part of the mitochondrial malate pool, and then the mitochondrial malate dehydrogenase will convert it to oxaloacetate. And thus, we have restored the molecule of oxaloacetate that we borrowed from the mitochondrial matrix.

Path 4B represents an alternative way to return the oxaloacetate molecule to the mitochondrial

matrix. 4B involves the malic enzyme, ME, which uses NADP plus to oxidize malate back to oxaloacetate. In the process, NADP plus is reduced to NADPH, the biosynthetic precursor. This is one of the two major ways that a cell produces the NADPH that it needs for biosynthesis. The other way to make NADPH is the pentose phosphate pathway. It may seem kind of useless to have taken an oxaloacetate, and then, by using malate dehydrogenase convert that oxaloacetate to malate, and then, convert the malate back to oxaloacetate. But by doing this series of reactions, you are effectively converting NADH to an NADPH. And NADPH is desperately needed for biosynthesis, as we'll see. Lipid biosynthesis is very NADPH intensive.

Let's look at a couple of more details. Decyl acetate shown in brackets in step 4B exists on the malic enzyme. The enzyme facilitates the decarboxylation of that molecule. Remember, oxaloacetate is a beta keto acid. Decarboxylation by malic enzyme, at step 4B, produces pyruvate. Now we can follow the pyruvate, by the continuation of step 4B, into the mitochondrion.

The pyruvate becomes a substrate for pyruvate carboxylase, which we've looked at before. Pyruvate carboxylase will put a CO₂ back into pyruvate and convert it into oxaloacetate. Pyruvate carboxylase is an ATP requiring enzyme. Hence, path 4B has a finite energy requirement. It would seem as though this might be a problem. Nevertheless, when a cell is doing biosynthesis, it usually has a lot of energy around in the form of ATP. So this small investment is a good one, in order to, first of all, restore the oxaloacetate balance of the mitochondria, and secondly, keep in mind, that 4B also gives you an NADPH, which you need for biosynthesis, anyway.

Let's summarize this figure. At the upper left, we start with glucose, and by the hatched lines, that glucose is converted to citrate in the cytoplasm. Citrate then yields an acetyl CoA molecule that is the precursor to fatty acids and cholesterol. Step two dealt with the problem of getting oxaloacetate back into the mitochondrion. Remember, fatty-acid biosynthesis happens in the cytoplasm. We borrowed an oxaloacetate from the mitochondrion, hence, we have to get it back to where it belongs in the mitochondrial matrix. Paths 4A and 4B represent alternative pathways for getting the oxaloacetate back where it belongs. Once the oxaloacetate is back in the mitochondrion, then the next molecule of acetyl CoA can come in, get converted to citrate, and follow the hatched line path, ultimately resulting in synthesis of lipids.

Let's turn now to story board 26, panel A. At this point, we have a pool of acetyl coenzyme A in the cytoplasm. It's now ready to start making a fatty acid. In the carboxylase module, I talked about how acetyl CoA carboxylase is one of the carboxylases that took an active form of CO₂ and placed it onto the methyl carbon at the end of the acetyl coenzyme A molecule. The product of the carboxylase reaction is malonyl coenzyme A. This carboxylase is a biotin containing enzyme, and requires ATP. As I've said earlier, malonyl CoA is going to be the source of all, but two of the carbons that make up the fatty acid. The initial fatty acid that we're going to make is the C16 fatty acid, palmitate. Palmitate will be synthesized entirely on the fatty acid synthase.

Let's look at panel B. Fatty-acid synthesis in bacteria is carried out by a series of enzymes that work as discrete, or independent, units. Collectively, this ensemble of enzymes is called the fatty acid synthase complex. In a million cells, all of the enzymatic activities-- the same enzymatic activities-- are present in a single, very long peptide. At this point, I want to go over the chemistry behind each of the activities in the fatty acid synthase complex.

One of the peptides that I introduced, briefly, above is called ACP, or acyl carrier protein. It contains an assisting sulfhydryl residue that will attack the carbonyl group of malonyl CoA, forming a malonyl ACP adduct. Keep in mind that the product is still a thioester with all of the chemical properties you would find in acetyl coenzyme A. The enzymatic subunit that does this chemistry is called MAT, or mat, for malonyl acetyl transferase.

Another domain, shown at the bottom of the fatty acid synthase, has a cysteine residue that attacks the carbonyl group of acetyl CoA. This reaction is also catalyzed by MAT. Hence, in this case, the MAT subunit forms an acetyl thioester with the fatty acid synthase. At this point, we have a malonyl group at the-- let's call it, northern domain of the fatty acid synthase, as I've drawn it-- and an acetyl group at what I'll call, the southern domain.

Let's look at panel C. At this point, the fatty acid synthase is fully primed. That is, it is loaded with a malonyl group and acetyl group. Now we can start doing some chemistry to join these two groups together. The malonyl group has a beta keto acid functionality so can easily lose CO₂, and generate a nucleophile that will then go down and attack the carbonyl group of the acetyl residue that is on the southern domain of the enzyme. This reaction results in the acetyl group, from the southern domain, replacing the CO₂ moiety on the northern domain. That is, the carbonyl group from the southern domain ends up covalently attached to the CH₂ group, with the box over it, in the northern domain. The ketoacyl synthase, that is KS, domain of the

protein, does this reaction. The product of the reaction shows a beta keto acyl group attached to the northern domain. Keep in mind, that the beta keto acyl group is attached, specifically, to the ACL carrier protein.

The next step is catalyzed by the ketoacyl ACP reductase, or KR. In this case, NADPH will reduce the keto group that is next to the terminal carbon, forming an alcohol, specifically, beta hydroxy acyl carrier protein, or ACP. This beta hydroxyl four carbon compound is now primed for dehydration by a dehydratase, which removes water to form the trans-delta-2-enoyl ACP. This molecule is an alkyne with the double bond between the second and third carbons from the thioester.

Let's turn now to storyboard 27. The reaction continues with enoyl reductase, ER, which uses a second molecule of NADPH in order to saturate the double bond of the enoyl ACP. The product is a butyryl ACP at this point, it should be clear as to what we've done. We've taken two acetyl CoA molecules and fused them together. In the process we've done a number of reductions, and the product is a pure hydrocarbon. In this case, the four carbon butyryl coenzyme A.

In order for the cycle to repeat itself, we have to vacate the ACP, or northern site on fatty acid synthase, in order to make it available for the next molecule of malonyl CoA to come in. That's done by the enzyme, or subunit, called translocase, which simply moves the butyryl group from the northern site down to the southern site. The northern sulfhydryl on ACP, of the fatty acid synthase, is now available to connect with the next incoming molecule of malonyl coenzyme A. The biosynthetic cycle will now repeat itself six times in order to form the 16 carbon long hydrocarbon, palmitate. In the figure, its shown attached to the northern site of the fatty acid synthase.

Let's look back at the overall synthesis scheme. What you'll see is that the terminal two carbons, that is the two carbons furthest to the right in the molecule, came from acetyl CoA. But all the other carbons originally came from alanyl coenzyme A. The last step in the overall reaction scheme involves thioesterase-- transferring the thioester bound palmitate to a water molecule, forming palmitic acid, which is released.

Now let's turn to storyboard 28. As I mentioned earlier, the default length of a fatty acid is 16 carbons. That is palmitic acid. You can look in the book for pathways leading to elongation, desaturation, and branching of the parent fatty acid chain. I'd like to give you, however, a little

bit of detail on how membrane lipids are formed, as well as triacylglycerides.

Let's look at panel A. To make these higher order lipids, fatty acids will become connected to a three carbon glycerol backbone. The glycerol backbone is made from dihydroxyacetone phosphate, which is one of the intermediates in the glycolytic pathway. To make the lipid backbone, dihydroxyacetone phosphate is reduced by glycerol 3 phosphate dehydrogenase using NADH as the co-factor. The product of this reaction is glycerol 3 phosphate. An acyltransferase will then take a fatty acyl coenzyme A and place it on one of the hydroxyl groups of the glycerol backbone to form a monoacylglyceride phosphate.

Then, as shown in panel B, a second fatty acid group will be placed on the only available hydroxyl to form a diacylglyceride phosphate. This is otherwise known as a membrane phospholipid. If the biosynthetic objective is to make a triacylglyceride, which is of course our major storage form of energy, then a phosphatase will come in and hydrolyze off the phosphate from the three carbon of the diacylglyceride phosphate to form what's called a diacylglycerol. Then an acyltransferase, as above, will place a third fatty acid onto the glycerol backbone to form a triacylglyceride, or TAG. As I said above, this is our principal storage form of energy.