**BC + ATP = BC-ATP**

**BC-ATP + HCO3- = BC-ATP-HCO3-**

**BC-ATP-HCO3- -> BC-Pi-HCO3- + ADP (irreversible because you break a high-energy phosphate bond)**

**BC-Pi-HCO3- + BCCP-Biotin = BC-Pi-HCO3- BCCP-Biotin**

**BC-Pi-HCO3- BCCP-Biotin -> BC + CO2- BCCP-Biotin + Pi (irreversible because you break a high-energy phosphate bond)**

CO2- BCCP-Biotin + CT = CT-CO2- BCCP-Biotin

And so on.

**David:**

**Model:**

* Biotin-dependent carboxylases require:
* 1) adenosine triphosphate.
* Enzymes that require adenosine triphosphate have this Mg2+ ion that's able to be chelated on one side by those aspartate residues, but on the other side, it sort of holds the ATP in there through electrostatic interactions.
* 2) Biotin
* It has a carboxylic acid derivative, which is an amide, and that nitrogen is the epsilon amine of a lysine residue.
* The biotin is in an amide linkage to a lysine residue in the active site.
* 3) Bicarbonate
* Biotin carboxylase is the domain that's going to carboxylate a nitrogen of the biotin.
* The transcarboxylase is going to transfer that carboxyl group, which was on that nitrogen and transfer onto Acetyl-CoA.
* The lysine is going to rotate and it will orient the biotin into the active site of the transcarboxylase.
* There is a base in the active site of these carboxylases that we don’t know what it is; it might be water.
* The enzyme is going to allow bicarbonate into the active site, and the base is going to deprotonate bicarbonate.
* At physiological pH, bicarbonate exists with one proton on it.
* So, the base deprotonates it, and you get this double bond rearrangement with these pi electrons coming out and doing a nucleophilic acyl substitution on the gamma phosphate of ATP.
* Now, in this step, you would generate a trigonal bipyramidal intermediate, but for the purpose of simplicity, we're eliminating that step, and we're just going to cause the collapsing of the trigonal bipyramidal intermediate back to its tetrahedral state with the loss of adenosine diphosphate.
* The base is now protonated, and it will stay that way through the end of the mechanism.
* We now have phosphorylated bicarbonate.
* Next we will generate carbon dioxide.
* So, the thing about biotin-dependent carboxylases is they're strange in the sense that they don't just use free carbon dioxide; they must synthesize it themselves, which is very strange.
* So, in this step, what's going to happen is these electrons are going to form a carbonyl right here, and that's going to expel the phosphate right here.
* And so, what you generate is this carbon dioxide right here, and this carbon dioxide is going to be used to attach to the biotin, and that will be the source of the carboxyl group on Acetyl-CoA to make Malonyl-CoA.
* And of course, in the process, we generate phosphate.
* However, in this state, it's PO43- right because none of the oxygens have protons on them, but that's going to change in the next step.
* One of these oxygens that's oriented closest to the nitrogen that's distal from this chain right here.
* The nitrogen that's furthest away, that's the one that's going to play a role in carboxylating.
* So, the phosphate is going to deprotonate that nitrogen, and that's going to force a double bond rearrangement that forces an oxygen to have a negative charge.
* So, we're going to form this sort of pseudo enolate except for the fact that it has a nitrogen.
* And the carbon dioxide is going to be oriented right next to that nitrogen, and so then the carbonyl bond is going to reform and this shift base electron system is going to come out and attack the carbonyl carbon of carbon dioxide, and you're going to generate a carboxylated biotin, and the carboxylated biotin we see right here.
* Now, keep in mind we also have this phosphate with a two-minus charge.
* In other words, it's monoprotic phosphate or dibasic phosphate, and then we also have, again, our carboxylated biotin.
* Recap: An unknown base in the active site is going to deprotonate bicarbonate, forcing nucleophilic attack on the gamma phosphate of ATP, forcing an acyl substitution into a trigonal bipyramidal intermediate with loss of adenosine diphosphate as the leaving group, and we get a phosphorylated bicarbonate. And what's going to happen now is you're going to generate carbon dioxide right here with the loss of phosphate, and then phosphate is going to deprotonate the nitrogen distal from this valeral chain right here of the biotin, and that's going to force a tautomerization, forcing an oxygen up here to have a negative charge that activates the pi electrons for nucleophilic attack.
* So, these electrons right here, the ones I'm going to circle, let me do it in red, these electrons right here are activated. Whenever this carbonyl bond reforms, these electrons that are part of this shift base can attack this carbon of carbon dioxide, and that's what ultimately carboxylates biotin.
* And one of the things about this is I won't go into a whole lot of detail here, but this carbon right here is especially activated electrophilically. And when it attacks this carbon, you're going to get a nucleophilic acyl substitution, so right now you have a trigonal planar carbon that's going to move up to a tetrahedral intermediate, and then you're going to have loss of the leaving group, which in most cases is just going to be the rest of this biotin skeleton.
* In this video, we're going to look at the transcarboxylase mechanism.
* What we're assuming at this point is that we've already generated carboxybiotin, or sometimes specifically it's n-carboxybiotin, because the carboxyl group is attached to a nitrogen.
* Now that we've generated that, the biotin, which is this right here, this is now carboxylated.
* It's going to rotate; it's going to rotate over here, and it's going to interact with the transcarboxylase domain.
* Now, there are functional residues in the active site here.
* One of them is going to be a lysine residue, and that's going to be situated basically above this carbonyl right here of the biotin, and then below it, there's an aspartate residue.
* The lysine exists at rest in the protonated state, as we would expect at physiological pH, and the aspartate exists in the deprotonated state.
* Both are going to be involved in Bronsted-Lowry acid-base proton transfers.
* In the first step, what we're going to do is we're going to tautomerize acetyl CoA.
* This critical aspartate residue in the active site is going to deprotonate this carbon of the acetyl-CoA, forcing tautomerization.
* Now, of course, in the process, we generate an enolate version of acetyl CoA, and as we know from our studies of carbonyl chemistry, enolates are terribly unstable; they're very reactive.
* There's a driving force to re-tautomerize the acetyl-CoA back to its carbonyl state.
* Instead of just simply doing a simple tautomerization back to a carbonyl, it's going to form the carbonyl, but you're also going to get nucleophilic attack from these pi electrons, and those pi electrons are going to attack the carboxyl group of the carboxybiotin.
* Now, some textbooks will just show this as a simple loss of a leaving group, but some purport that it goes through a nucleophilic acyl substitution-type mechanism, which is probably the most probable mechanism that it's going to go through, and we'll do that here.
* So, you would generate a tetrahedral intermediate here, but remember that that's short-lived; it'll quickly collapse back to the trigonal planar state.
* So, what's going to happen is, keep in mind that biotin, at least with the carboxyl group attached, had this amide linkage attached to it.
* Instead of just simply losing a leaving group, which would be biotin, we're going to tautomerize the amide.
* These electrons right here are going to come in and form the shift base, and we're going to cause nucleophilic attack of these pi electrons on the lysine residue.
* This is not an enol by any means; in fact, it's just the protonated tautomer of an amide.
* But there is a driving force to re-tautomerize, and that's done using the lysine and the aspartate.
* Keep in mind the aspartate's protonated because it deprotonated acetyl-CoA.
* What's going to happen now is the lysine, now in the deprotonated state, is going to re-abstract the proton from this tautomer of the amide, forcing carbonyl reformation, and then these shift-based electrons are going to come and re-abstract the proton from the aspartate residue.
* What that effectively does is it regenerates the protonated state of lysine and the deprotonated state of the aspartate, and along with it, we end up regenerating the resting state of our biotin.
* So, notice that our biotin now is in the state that it was at the very beginning of our mechanism.
* So, its re-reset biotin, and in the process, we end up generating this guy, which is malonyl-CoA.
* But when we had this guy right here, which is our enolate version of acetyl-CoA, when we reform the carbonyl and these electrons come out and attack this carboxyl carbon right here, we end up getting a nucleophilic acyl substitution.
* But in the process, that's where we generate the malonyl-CoA.
* As we mentioned in the previous video and in this video as well, the actual biotin, once it got carboxylated, rotated over to the transcarboxylase domain, and we just saw the mechanism that happens there.
* Well, that rotation towards the transcarboxylase domain is just done through changes in the enzyme conformation.
* But as soon as we take that carboxyl group right here, as soon as we take that off biotin and put it on acetyl-CoA, there's another change in confirmation which is going to reset the enzyme back to where the biotin is now in orientation with biotin carboxylase.
* So, there's an interplay between these two subunits.
* In one case, we start with the biotin interacting with biotin carboxylase.
* It gets carboxylated and changes in enzyme conformation rotate the biotin to interact with the transcarboxylase.
* Once the transcarboxylase catalyzes its reaction to form malonyl-CoA, the biotin loses carbon dioxide, in being attached to acetyl-CoA, and it rotates back to orient with biotin carboxylase, all driven by changes in enzyme conformation. And that's the interplay between these two subunits.
* What we're going to find is the two carbons that become incorporated into the fatty acid are this one right here that's part of the carbonyl and this one. Those are the only two carbons that are going to get incorporated into the fatty acid. This carboxyl group right here is just going to get lost as carbon dioxide. So, we use carbon dioxide to attach it to biotin, and then we're just going to lose carbon dioxide again.
* Really, the function of that carboxyl group is just to activate the acetyl-CoA.

Welcome back! We're currently in the fat biosynthesis playlist, and in this video, we're going to explore the biosynthesis of a molecule called Malonyl CoA. It's important to realize that Malonyl CoA is the molecule used to build fatty acids. Fatty acid biosynthesis is a process carried out by humans, all mammals, and indeed, all organisms. The building block from which they synthesize it is Acetyl CoA. But to build the fatty acid, the Acetyl CoA must be converted into a different molecule called Malonyl CoA. So, the molecule we're going to look at is Malonyl CoA. All Malonyl CoA is, is a carboxylated version of Acetyl CoA. So, if this is our Coenzyme A right here, this right here would be Acetyl CoA. But when we have Malonyl CoA, it's going to have this extra carboxylate group right here. So that molecule that I've shown is Malonyl CoA, and that's the molecule that we use to biosynthesize fats, specifically our fatty acids. Let's talk about what all biotin-dependent carboxylases require. Number one, they all require adenosine triphosphate. And one thing that sort of goes along with enzymes that require adenosine triphosphate is they're going to have this magnesium 2+ ion that's sort of chelated there in the active site, ordinarily by aspartate residues. And the magnesium, keep in mind, has a positive charge, and it's able to be chelated on one side by those aspartate residues, but on the other side, it sort of holds the ATP in there through electrostatic interactions. So that's one of the things that allows the ATP to stay in there. And as we'll find, later in the mechanism, we're going to produce an inorganic phosphate that will also be held in there due to electrostatic interactions with the magnesium ion. So, when we say ATP, we're generally also implying magnesium. And obviously, if you're a biotin-dependent carboxylase, you also require biotin. And the biotin that you highlight in light blue is the biotin. That's the biotin. And what you'll notice is that it has this carboxylic acid derivative right here, which is an amide, and that nitrogen is the epsilon amine of a lysine residue. So, this is a lysine residue. And of course, it comes off the carboxylase. In other words, you can say that the biotin is in an amide linkage to a lysine residue in the active site. That amid linkage, in other words, is a linkage between the epsilon amine of lysine in the active site and a carboxylate of the biotin itself. And there are enzymes called biotinylases or biotin ligases that will ultimately ligate the biotin onto the lysine residues. So that's done by a separate activity, and we won't consider that here. And the third thing that is required by all these enzymes is going to be bicarbonate. And the structure of bicarbonate is shown right here. And in fact, when we look at Malonyl CoA, this carbon right here, this is ultimately the carbon that's going to get donated to specific molecules. For instance, if we were looking at propionyl CoA carboxylate, that's the carbon, the carbon green, that's the carbon that forms the carboxy group on succinyl COA. In other words, you can say that these carboxylases are transferring this carboxyl group right here. So, this carbon that's in green, this is the carbon that's going to get donated to specific molecules. In the context of Acetyl CoA carboxylase, that carbon is ultimately going to come from this carbon of bicarbonate. So that carbon that I highlighted in green on bicarbonate, that's the same carbon that's here in Malonyl CoA. Notice, all Malonyl CoA is a carboxylated Acetyl CoA. The actual carboxylation of Acetyl COA will be in the next video. In this video, we're simply going to look at the carboxylation of biotin, and that's why we're going to see this video in other playlists as well. Now, what I want to mention at this point before we get into the organic mechanism of this is that not only do you have a biotin carboxylase part of this enzyme, but you also have a transcarboxylase. So, we can divide this type of enzyme into two domains. Number one, there's always going to be a biotin carboxy domain, so this constitutes domain one. And biotin carboxylase, as you'll often hear it, is the actual domain that's going to carboxylate this nitrogen right here of the biotin. That's the nitrogen that's going to originally get carboxylated through the mechanism. And that's done by biotin carboxylase. However, there's a second domain, D2, which is called the transcarboxylase. Transcarboxylase, and the transcarboxylase is going to transfer that carboxyl group, which was on this nitrogen right here, it's going to transfer that onto the molecule of choice. In the context of this enzyme, it would be Acetyl CoA, and we'll see that in the next video. Now, what's important to understand about these enzymes is you sort of have within this enzyme you have one activity right here, which is biotin carboxylase, and you have the second activity, which is the transcarboxylase. So, what you must keep in mind is that if you have your lysine residue right here, so here's your lysine residue, and then we'll say this is our biotin. So, here's my biotin co-enzyme right attached to the lysine. In the first state of the biotin, it's sort of associated with this biotin carboxylase, but you can sort of view the biotin carboxylase domain as being completely separated from the transcarboxylase domain. And so, what will happen is, in the first step, biotin carboxylase will carboxylate the biotin right on that nitrogen that I circle in orange, right? And then what's going to happen literally is the lysine is going to rotate and it will orient the biotin into the active site of the transcarboxylase. So, the biotin will no longer be in the active site of biotin carboxylase. The lysine literally rotates into the transcarboxylase active site. And so, what you'll find there is in purple here would be the biotin, and then of course, you'd have the carboxyl group on there, and you transfer the carboxyl group onto Acetyl CoA to make Malonyl CoA. I want to make that perfectly clear. The active sites of biotin carboxylase and transcarboxylase are different. Literally what the lysine functions to do is it ligates the biotin, and it literally rotates to orient the biotin with respect to the first D1 and then to D2. They're different active sites. Often what you'll see for abbreviation is biotin carboxylase will be BC and the transcarboxylase will be CT, often because it's called carboxyltransferase. I usually call it transcarboxylase though and some textbooks use that as well. Enough boring on that. Let's look at the mechanism because that's what’s most important about learning this enzyme. I'll do the mechanistic steps in green. There exists a base in the active site of these carboxylases that is unknown. We don't know exactly what the base is; it might very well be a water that's in the active site, but it's unknown. And the enzyme is going to allow bicarbonate into the active site, and the base is going to deprotonate bicarbonate. At physiological pH, bicarbonate exists with one proton on it. That's a function of the pKa of all those oxygens, so it's going to have a proton. So, the base deprotonates it, and you get this double bond rearrangement with these pi electrons coming out and doing a nucleophilic acyl substitution on the gamma phosphate of ATP. Now, in this step, you would generate a trigonal bipyramidal intermediate, but for the purpose of simplicity, we're eliminating that step, and we're just going to cause the collapsing of the trigonal bipyramidal intermediate back to its tetrahedral state with the loss of adenosine diphosphate, and we see that adenosine diphosphate right here. And so, what we've effectively done in this step is several things. Number one, we have this base that's now protonated. The base is protonated, and it will stay that way through the end of the mechanism. We haven't done anything to the biotin yet; that's going to come later. But what we've done now is we've phosphorylated bicarbonate. And what's going to happen now is we're going to generate carbon dioxide. So, the thing about biotin-dependent carboxylases is they're strange in the sense that they don't just use free carbon dioxide; they must synthesize it themselves, which is very strange. So, in this step, what's going to happen is these electrons are going to form a carbonyl right here, and that's going to expel the phosphate right here. And so, what you generate is this carbon dioxide right here, and this carbon dioxide is going to be used to attach to the biotin, and that will be the source of the carboxyl group on Acetyl-CoA to make Malonyl-CoA. And of course, in the process, we generate phosphate. However, in this state, it's PO43- right because none of the oxygens have protons on them, but that's going to change in the next step. One of these oxygens that's oriented closest to the nitrogen that's distal from this chain right here. Notice how with respect to at this chain, there's this nitrogen and there's this one, right? The one that's furthest away, right? The one that's furthest away, that's the one that's going to play a role in carboxylating. So, the phosphate is going to deprotonate that nitrogen, and that's going to force a double bond rearrangement that forces an oxygen to have a negative charge. And one thing you can sort of view it like is sort of like an enolate. If you had this molecule right here and this is just this is not the same thing, but you can sort of view it in a similar way. When you drew the mechanism of, for instance, an enolate tautomerization right, you had some base, right? You had some base, and the base deprotonated this carbon and that forced the double bond right here and the pi electrons on the oxygen. So, what you ended up with was an enolate. So whenever you drew the mechanism of nucleophilic attack like let's say you had an electrophile right here, okay, here's our electrophile, when you drew the mechanism you couldn't just draw these electrons right here attacking the electrophile right, you had to first show it in the tautomerization, then these electrons are going to kick back down and then you get nucleophilic attack on the electrophile, right? The same thing is going to be true here except for the fact that instead of having a carbon right here, we have a nitrogen. So, we're going to form this sort of pseudo enolate except for the fact that it has a nitrogen. And the carbon dioxide is going to be oriented right next to that nitrogen, and so then the carbonyl bond is going to reform and this shift base electron system is going to come out and attack the carbonyl carbon of carbon dioxide, and you're going to generate a carboxylated biotin, and the carboxylated biotin we see right here. Now, keep in mind we also have this phosphate with a two-minus charge. In other words, it's monoprotic phosphate or dibasic phosphate, and then we also have, again, our carboxylated biotin. This mechanistic step right here takes us through the end of what was the biotin carboxylase domain's mechanism. Remember that from the beginning we had two domains for this enzyme, we had a biotin carboxylase and a transcarboxylase. The biotin carboxylate mechanism is well-conserved, it's the exact same for every single biotin-dependent carboxylate. However, what's going to change is the transcarboxylase, and really the only thing about the transcarboxylase that changes are the substrate. For instance, if we were dealing with the enzyme pyruvate carboxylate transcarboxylase's substrate would be pyruvate. If it was propionyl-CoA carboxylate transcarboxylase's substrate would be propionyl-CoA. In this case, our substrate is going to be Acetyl-CoA, and we'll look at that mechanism in the next video. So, in the next video, we'll look at transcarboxylase, but right now let's do a very quick recap of what we saw. An unknown base in the active site is going to deprotonate bicarbonate, forcing nucleophilic attack on the gamma phosphate of ATP, forcing an acyl substitution into a trigonal bipyramidal intermediate with loss of adenosine diphosphate as the leaving group, and we get a phosphorylated bicarbonate. And what's going to happen now is you're going to generate carbon dioxide right here with the loss of phosphate, and then phosphate is going to deprotonate the nitrogen distal from this valeral chain right here of the biotin, and that's going to force a tautomerization, forcing an oxygen up here to have a negative charge that activates the pi electrons for nucleophilic attack. So, these electrons right here, the ones I'm going to circle, let me do it in red, these electrons right here are activated. Whenever this carbonyl bond reforms, these electrons that are part of this shift base can attack this carbon of carbon dioxide, and that's what ultimately carboxylates biotin. And one of the things about this is I won't go into a whole lot of detail here, but this carbon right here is especially activated electrophilically. So, if you have a nucleophile, and the nucleophile can come in several forms, but it's usually a carbon with a negative charge, the nucleophile can attack here. And when it attacks this carbon, you're going to get a nucleophilic acyl substitution, so right now you have a trigonal planar carbon that's going to move up to a tetrahedral intermediate, and then you're going to have loss of the leaving group, which in most cases is just going to be the rest of this biotin skeleton. And we're going to see that in the next video which is going to be on transcarboxylase. See you soon.

Welcome back! In the last video, we examined the mechanism of biotin carboxylase and found that this mechanism is utilized by many different carboxylases involving biotin, ATP, and bicarbonate. Furthermore, we found that biotin is ligated to a lysine residue in the active site. In one orientation, the biotin can interact with domain one, which is the biotin carboxylase. However, if you rotate the lysine residue along with the biotin, it could interact with a second enzyme called transcarboxylase. Both enzymes work together in the same complex. In one case, the biotin interacts with biotin carboxylase, but if you rotate it using the lysine residue, it can interact with transcarboxylase. In this video, we're going to look at the transcarboxylase mechanism. Also, another important thing about this mechanism is it's also well conserved. We saw that the biotin carboxylase mechanism is very well conserved; in fact, it's identical in pretty much every carboxylase that you'll find that works according to this mechanism. So too is the transcarboxylase. What we're assuming at this point is that we've already generated carboxybiotin, or sometimes specifically it's n-carboxybiotin, because the carboxyl group is attached to a nitrogen. Now that we've generated that, the biotin, which is this right here, this is now carboxylated. It's going to rotate; it's going to rotate over here, and it's going to interact with the transcarboxylase domain. That's where we left off in the last video; we saw how we carboxylated biotin. Now let's look and see how your carboxylate other molecules, and that's catalyzed by the transcarboxylase. Now, there are functional residues in the active site here. One of them is going to be a lysine residue, and that's going to be situated basically above this carbonyl right here of the biotin, and then below it, there's an aspartate residue. The lysine exists at rest in the protonated state, as we would expect at physiological pH, and the aspartate exists in the deprotonated state. Both are going to be involved in Bronsted-Lowry acid-base proton transfers. In the first step, what we're going to do is we're going to tautomerize acetyl CoA. This critical aspartate residue in the active site is going to deprotonate this carbon of the acetyl-CoA, forcing tautomerization. We note that tautomer of acetyl CoA right here. Now, of course, in the process, we generate an enolate version of acetyl CoA, and as we know from our studies of carbonyl chemistry, enolates are terribly unstable; they're very reactive. There's a driving force to re-tautomerize the acetyl-CoA back to its carbonyl state. Instead of just simply doing a simple tautomerization back to a carbonyl, it's going to form the carbonyl, but you're also going to get nucleophilic attack from these pi electrons, and those pi electrons are going to attack the carboxyl group of the carboxybiotin. Now, some textbooks will just show this as a simple loss of a leaving group, but some purport that it goes through a nucleophilic acyl substitution-type mechanism, which is probably the most probable mechanism that it's going to go through, and we'll do that here. So, you would generate a tetrahedral intermediate here, but remember that that's short-lived; it'll quickly collapse back to the trigonal planar state. So, what's going to happen is, keep in mind that biotin, at least with the carboxyl group attached, had this amide linkage attached to it. Instead of just simply losing a leaving group, which would be biotin, we're going to tautomerize the amide. These electrons right here are going to come in and form the shift base, and we're going to cause nucleophilic attack of these pi electrons on the lysine residue. What we end up generating is this molecule right here. So, what we generate is this guy right here. If you look at this part of the molecule, something should immediately strike you, and that's that it sort of looks like an enol. An enol would be in this form. So, you'd have a double bond right there, and then you'd have this oxygen that's protonated. This is an enol, and as we know, enols are very unstable, high energy. There's a driving force to re-tautomerize. This is not an enol by any means; in fact, it's just the protonated tautomer of an amide. But there is a driving force to re-tautomerize, and that's done using the lysine and the aspartate. Keep in mind the aspartate's protonated because it deprotonated acetyl-CoA. What's going to happen now is the lysine, now in the deprotonated state, is going to re-abstract the proton from this tautomer of the amide, forcing carbonyl reformation, and then these shift-based electrons are going to come and re-abstract the proton from the aspartate residue. What that effectively does is it regenerates the protonated state of lysine and the deprotonated state of the aspartate, and along with it, we end up regenerating the resting state of our biotin. So, notice that our biotin now is in the state that it was at the very beginning of our mechanism. So, its re-reset biotin, and in the process, we end up generating this guy, which is malonyl-CoA. One thing I want to point your attention to is the step that we did that in because we sort of glossed over it a little bit and focused on the biotin itself. But when we had this guy right here, which is our enolate version of acetyl-CoA, when we reform the carbonyl and these electrons come out and attack this carboxyl carbon right here, we end up getting a nucleophilic acyl substitution. But in the process, that's where we generate the malonyl-CoA. So, this carbon that I'm about to circle, let me do this in purple, this carbon right here, that's in purple. And you can even make the argument that that carbon in purple, keep in mind that that was the carbon that came from bicarbonate, from the initial step when we activated bicarbonate using ATP. That's the same carbon that came from bicarbonate. So, when we look at the initial step where we had this carbon that was part of bicarbonate, if we track that carbon through the end of this mechanism, that carbon is going to wind up as the carboxylate carbon of malonyl-CoA. So that's what this molecule is right here; this is malonyl-CoA. As we mentioned in the previous video and in this video as well, the actual biotin, once it got carboxylated, rotated over to the transcarboxylase domain, and we just saw the mechanism that happens there. Well, that rotation towards the transcarboxylase domain is just done through changes in the enzyme conformation. But as soon as we take that carboxyl group right here, as soon as we take that off biotin and put it on acetyl-CoA, there's another change in confirmation which is going to reset the enzyme back to where the biotin is now in orientation with biotin carboxylase. So, there's an interplay between these two subunits. In one case, we start with the biotin interacting with biotin carboxylase. It gets carboxylated and changes in enzyme conformation rotate the biotin to interact with the transcarboxylase. Once the transcarboxylase catalyzes its reaction to form malonyl-CoA, the biotin loses carbon dioxide, in being attached to acetyl-CoA, and it rotates back to orient with biotin carboxylase, all driven by changes in enzyme conformation. And that's the interplay between these two subunits. In another video, we'll look at the regulation of this enzyme, and as we'll find, it's an allosteric enzyme. One thing I want to mention just to leave you with before we go into any other videos is that this malonyl-CoA that we generated is an extremely important molecule. As we mentioned in the first video in this playlist, it's important because it's used in fatty acid biosynthesis. All fatty acids are going to require the use of malonyl-CoA to build them up from acetyl-CoA. So, in this step, we generated malonyl-CoA from acetyl-CoA, and then we're going to use those malonyl-CoA building blocks to generate fatty acids two carbons at a time. If you notice, there's three carbons in this section right here of malonyl-CoA. If we look at this section, there's three carbons. What we're going to find is the two carbons that become incorporated into the fatty acid are this one right here that's part of the carbonyl and this one. Those are the only two carbons that are going to get incorporated into the fatty acid. This carboxyl group right here is just going to get lost as carbon dioxide. So, we use carbon dioxide to attach it to biotin, and then we're just going to lose carbon dioxide again. Really, the function of that carboxyl group is just to activate the acetyl-CoA. When we look at the mechanism of fatty acid synthase, we'll see why that is. The enzyme system that's going to use malonyl-CoA as building blocks to make fatty acids is called fatty acid synthase, and this is a classic example of an enzyme that uses something called substrate channeling. It's a huge enzyme complex that's going to basically take malonyl-CoAs and condense them two carbons at a time into a fatty acid. We'll look at the mechanism of fatty acid synthase in another video. I think this gives you a good starting point. We've seen how we use a biotin-dependent carboxylase to generate n-carboxybiotin, and now we have this building block that we can make fatty acids from, and that's malonyl-CoA. Just bear that in mind, that the carbons that I highlighted in yellow, those are the ones that get incorporated into the fatty acid. The other one, which let me do that in this color, this kind of dark red, that's just going to get lost as carbon dioxide. When we look at the mechanism of fatty acid synthase, we'll see that. We'll do that in the next video. See you soon.