Introduction to Biomolecules
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Lecture-30
Fatty Acid Biosynthesis

So, let us continue fatty acid metabolism, so today what we are going to do is we are going to do the opposite of what we saw in the last class, last class breaking down 16 carbon, free fatty acid, 2 acetyl CoA and then how that can go through TCA cycle. So, today what we are going to see is how we join acetyl CoA molecules to make a 16 carbon fatty acid. So, fatty acid biosynthesis, this is what we are going to see.

So, as we have seen at the very beginning introduction to metabolism the catabolic pathways and anabolic pathways are not exactly the opposite of one another. So, at least one reaction will be different and thermodynamically favourable in a given direction, therefore when catabolic pathway is active in a cell you do not have anabolic pathway simultaneously active and vice versa.

So, the same applies here too, so here you are not going to have a situation where the 4 steps of beta oxidation are not going to be exactly in the reverse direction and in this particular ways it starts with the very first step. In beta oxidation one of the main regulations is the transport of the fatty acyl CoA into mitochondria by other carnitine shuttle, so the acyl carnitine; carnitine transporter.

So, that step is a critical step there, because once it enters into mitochondria it is destined for beta oxidation. So, here the activation of acetyl-CoA by means of carboxylating it, a carbon dioxide group is added to the acetyl-CoA molecule converting into malonyl CoA and that step is the critical step or the committed step for fatty acid biosynthesis and malonyl CoA is not involved in beta oxidation.

So, only when you have an odd number of carbon atoms in a free fatty acid, so the last step generates a profile CoA 3 carbon molecule and that is carboxylated to make methyl malonyl CoA and then an epimerase and a mutase converts that into succinyl CoA. So, there you saw the word malonyl, but there has a methylmalonyl and that becomes succinyl CoA. So, here

intentionally malonyl CoA is produced from acetyl CoA by carboxylation. So, that is the first step.

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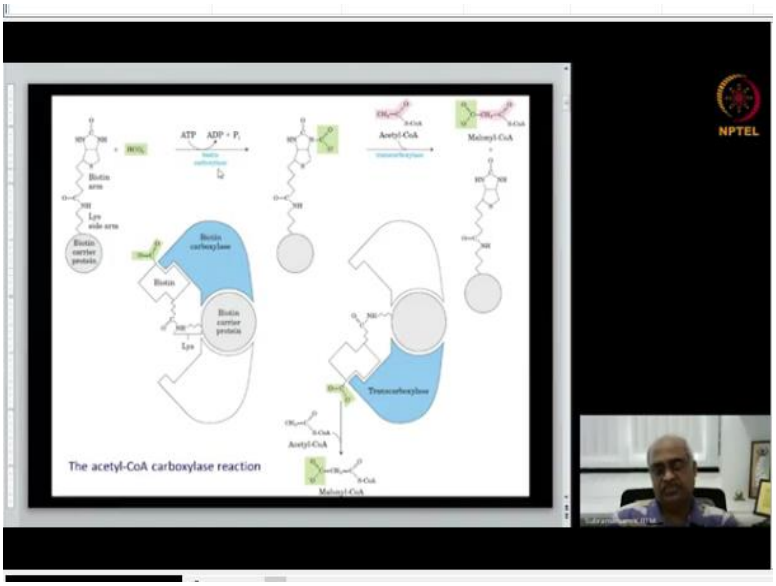

So and there we have our familiar enzyme, we have seen this carboxylase already twice. So, here the substrate is acetyl-CoA and that is going to be carboxylated so the enzyme is acetyl-CoA carboxylase. So, we saw this first as pyruvate carboxylase that is where we encountered biotin a B complex vitamin for the first time. So, there we saw that biotin temporarily on its nitrogen.

So, here carries carbon dioxide and its long arm extended by this enzymes lysine side chain where it is an amide bond with this epsilon amino group of glycine helps as a tether and switch from one active site to another active site and generates oxaloacetate. So, that is the example we saw. That is required for gluconeogenesis as well as an anaplerotic reaction meaning replenishing the intermediates of TCA cycles.

So, there we encountered this first time and there we went through the mechanism. So, it just to recapitulate or understanding of the mechanism we will see that again here in the next slide and the second time we encountered this enzyme was yesterday the propanol CoA when beta oxidation of odd number carbon fat, free fatty acids, end up producing at the very terminal part of it this propanol CoA, there it is carboxylated to produce a d-methylmalonyl CoA. So, there we saw propanol CoA carboxylase.

So, today we are seeing it using acetyl-CoA substrate, so this is acetyl-CoA carboxylase. So, the reaction mechanism everything is identical to the other 2 enzymes. So, here again first an activated carbon dioxide formed by phosphorylating this carboxyl group of bicarbonic acid. So, the bicarbonate becomes phosphorylated by the first active site of the biotin carboxylase. So, that is going to involve the ADP hydrolysis and from there then it is transferred that activated phosphoryl or carboxy phosphate.

So, this one in anhydride linkage with the phosphate from ATP. So, this is telling you the summary reaction it is not telling you the intermediate which I am telling and that will be there shown in the next slide. And from the carboxy phosphate the carboxyl group is carried on this nitrogen. So, this is done at active site 1. So, this is a 3 subunit protein you have a biotin carrier protein whose lysine side chain has this biotin tether and at this first active site you have this carboxylation of the biotin then it flips to the next active site.

So, this is the summary reaction in the next active site which is the trans-carboxylase activity. There this is transferred to the acetyl-CoA generating malonyl CoA. So, malonyl CoA is quite simple you have 2 carboxylic acid connected by a methylene group in between. So, this is a dicarboxylic acid. So, this is the overall reaction, first carboxylation of the biotin group using a carbonic acid or bicarbonate ion and that generates this carboxylated biotin, then that is transferred by the trans carboxylase to acetyl-CoA to make malonyl CoA and then the free biotin attached to the biotin carrier protein.

So, this is a cartoon representation of the active site there. So, this is one active site the carboxylase site and then it swings so that is the advantage of this tether, to the other active site where it is transferred to the acetyl group generating the malonyl CoA. So, this is the reaction of acetyl-CoA carboxylase.

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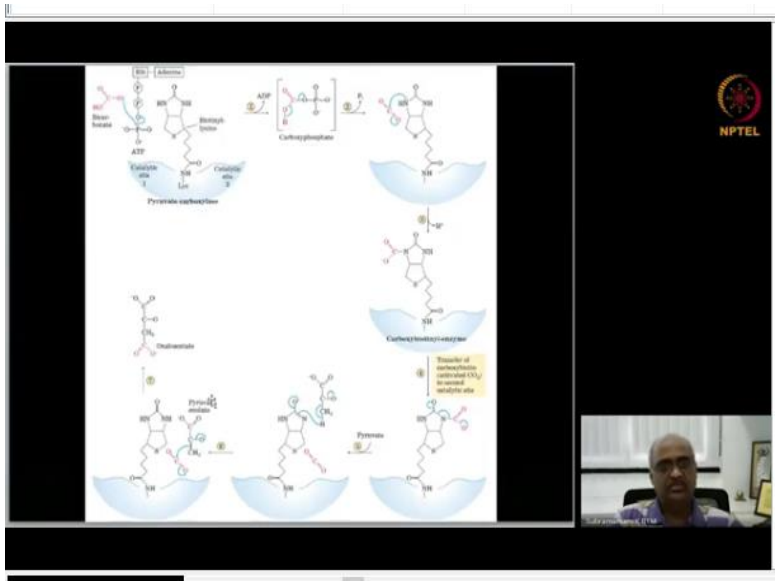

So, the mechanism is shown here, which is the same slide that I showed you when we learnt about anaplerotic reactions and gluconeogenesis when we were learning TCA cycle. So, here you have this bicarbonate ion becoming an anhydride with this terminal phosphate group of ATP forming this carboxy phosphate releasing ADP. So, from the carboxy phosphate then due to this nucleophilic attack by this nitrogen you have the inorganic phosphate released.

And then you have the carbon dioxide temporarily carried on this nitrogen. So, then again shift of electrons as shown by these arrows generate the free carbon dioxide by then this tether also swings to the other active site. This transcarboxylase site and there an abstraction of proton by this nitrogen from pyruvate leads to an enolate intermediate. So, this is the enol group here.

And third sets for this again another nucleophilic attack leading to formation of this oxaloacetate this carboxylated pyruvate and releasing the enzyme back. So, this very same mechanism is what happens here again. So, here again it is the same reaction ending up carboxylating the acetyl CoA. So, why this complex carboxylating acetyl-CoA will become clear when we go to the next step and when we complete the fatty acid biosynthesis.

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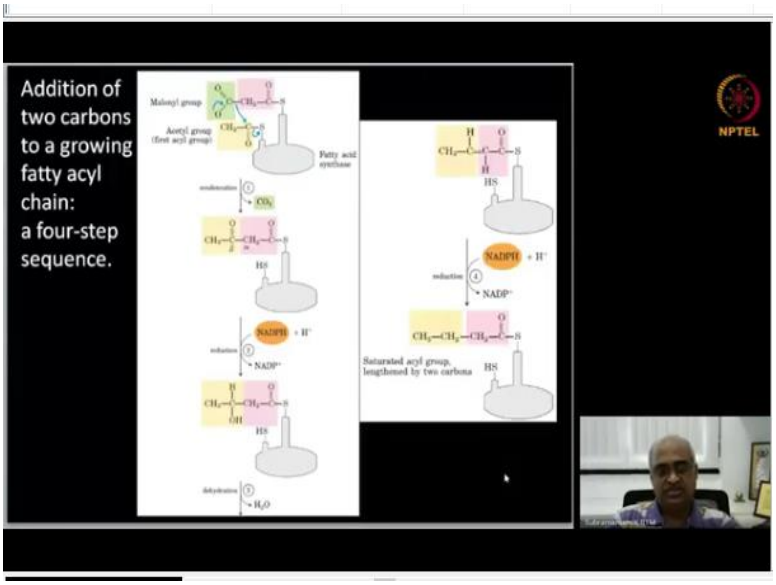

So, that is sort of summarized here and then we get into individual steps, this is again 4 steps it is quite simple, it is not very complicated. So, we will see that right here why that malonyl group is important? So, essentially for fatty acid biosynthesis the substrates are acetyl-CoA. So, acetyl-CoA 2 carbons at a time gets added just like the way beta oxidation broke the fatty acid chain the aliphatic chain 2 carbons at a time.

This carboxylation to generate malonyl group is actually making it an active molecule for this reaction. So, remember the carbon-carbon single bond the methylene bond in the aliphatic chain is very stable. So, this is what I told you at the beginning of beta oxidation and to make it amenable for breaking we had that dehydration then removal of the hydrogen atom first not removed of water it is a hydrogen atom, hydrogen molecule essentially.

Making a carbon-carbon double bond then that was converted by hydration to CHOH and again another dehydrogenation there generated a C double bond O. So, we created a carbonyl group like what you see here. So, that is how that carbon-carbon stable bond has been converted into a labile which is labile for cleavage. So, similarly the reverse reaction therefore is going to be endergonic and it is not going to be easy to make the carbon-carbon bond.

So, you need energy to facilitate that or you attach a carbon dioxide here. So, now when we see this electron flow diagram here that becomes obvious but before that let me start with the first step. So, this acetyl-CoA joining the 2 carbonate that I am joining is catalyzed by an

enzyme complex called fatty acid synthase. This enzyme has 7 active sites; in vertebrates a single polypeptide chain carries all the 7 active sites.

And in bacteria like Escherichia coli you have 7 polypeptides each one having 1 active site and very early eukaryotes like yeast have 2 polypeptides, 4 activity in 1 polypeptide and 3 activities in the other one. So, we will see at in detail this enzyme complex in subsequent slides. Let us focus on the first step. So, the first step actually in the enzyme on the enzyme 2 sulfhydryl groups get covalently attached to the 2 reactants.

So, the first one we just saw the acetyl-CoA getting carboxylated to malonyl CoA that malonyl CoA is now attached to the sulfhydryl group in thioester, contrast this with beta oxidation, in beta oxidation this energization was within coenzyme is a thiol group, it was fatty acyl CoA, instead here to the enzymes side chain a cysteine side chain is where it is attached.

So, that is how this thioester is made and at another group you have a another thiol there the acetyl CoA is attached, instead the coenzyme versions here they are on the enzymes thiol group. So, that is how this energized form of this carboxyl groups are attached and these 2 sites are very close facilitating the reaction between these 2 molecules. Now you can see the once these are brought together like this.

This is the first step like joining the 2; we are not even considering that as one of the 4 steps, these are preparatory steps, the acetyl CoA carboxylase reaction as well as joining the product of that reaction that is malonyl CoA and joining of another acetyl CoA to another active site. So, these 3 are preparative steps. The preparative steps namely the first one is acetyl CoA carboxylation.

And the second is the product of that; that is malonyl CoA attaching to the enzyme as a thioester that is the second step malonyl CoA transferase activity. Then acetyl CoA transacetylase activity why is this called transacetylase will become clear when we go through the repetitive cycles of this reaction and the third one attaches the acetyl-CoA. So, after these 3 preparative steps are done now the actual synthesis reactions take place.

And that is facilitated by this decarboxylation. So, instead of having a methyl group here having a carbonyl carbon enables the synthesis. So, this creates a partial positive charge here and this is slightly acidic or it can readily donate proton and all of that enables this reaction leading to the removal of carbon dioxide. So, the carbon dioxide you look at the color here, look at the green.

It is the same bicarbonate ion that entered into biotin and then becoming the malonyl part of this acetyl CoA that same carbon dioxide leaves right away, it was only transiently here. So, this carbon dioxide does not come from one of the acetyl CoAs, this is the carbon dioxide that was temporarily attached and removed. And the sole purpose is to make this reaction happen, just bringing the right groups for the reaction.

Essentially this carbonyl group and as a result this being a carbon ion and this being partially positively charged that enables the cleavage of this bond and shifting of this acetyl-CoA to the CH 2 forming this beta-keto structure on the one of the thiol groups. So, this remains attached, so the first acetyl-CoA which actually became malonyl CoA that is this pink one and this new one is the one that is transferred to that.

So, the remaining steps are more like the reversal of beta oxidation. So, here dehydrogenase removed and made a double bond, so now you do the opposite reduction taking energy from NADPH. So, this NADPH production in mammals we did not see that is the hexose phosphate HMP shunt or pentose phosphate pathway. So, we did not look at that; that is a pathway of carbohydrate metabolism that generates pentose sugars required for nucleotides formation.

And another product of that is the production of the NADPH and in plants of course in photosynthesis we saw NADPH being produced. So, there that is not an issue. So, here that HMP shunt or pentose phosphate pathway, so it is called with 2 different names they mean the same pathway. That pathway is an alternative pathway to glycolysis and that generates a pentose sugars as end product instead of pyruvate.

And another product of that is NADPH and the NADPH is used by this enzyme to reduce and then you have this beta hydroxy structure. Then the third step is again another dehydration leading to double bond there and the next step is a reduction again and that reduction

generates a fully saturated methylene group here. So, now the acetyl CoA is now extended by 2 carbons.

So, these are the 4 steps, essentially the second, third and fourth are chemically the reversal although catalyzed by a different enzyme complex, chemically exact reverse of beta oxidation. So, only the first step differs by this activation of acetyl-CoA by converting into malonyl CoA and then a decarboxylation. So, this decarboxylation facilitates the condensation reaction, then reduction, dehydration reduction.

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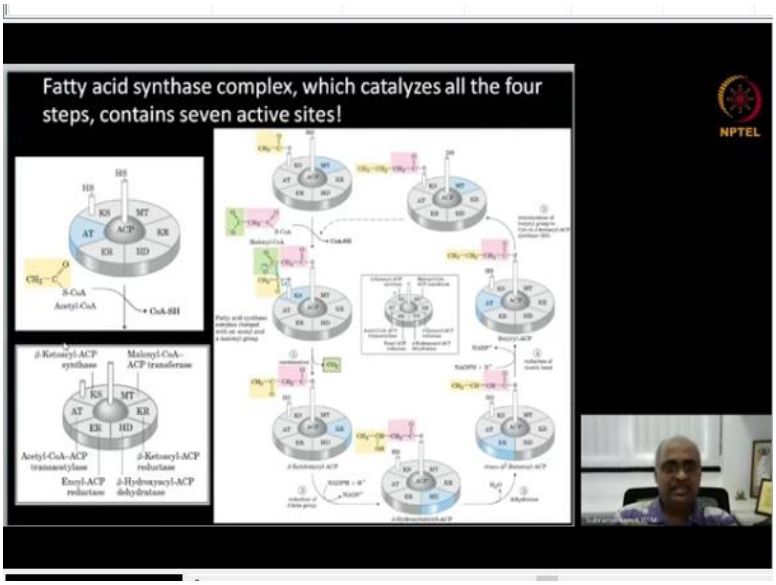

So, now we will see the enzyme in all its glory. So, this is the 7 active site containing fatty acids synthase enzyme and a central to this is this acetyl carrier protein ACP, because it carries the initially the malonyl CoA and later acyl CoA extend, so it is acyl carrier protein as well ACP. So and you have other activities and they are all labeled in this diagram.

So, you have an acetyl-CoA ACP transacetylase, so that is the one that is going to charge one of the thiol groups with the acetyl-CoA and then the other one here malonyl CoA acyl carrier protein transferase that charges the other thiol group that is the ACP's thiol group this one with malonyl CoA. So, this one transfers to this synthase beta ketoacyl ACP synthase because that structure is beta keto.

So, this is the beta keto group and so keto acyl synthase that is why it is called KS acetyl CoA transacetylase that is AT, so then each of the other ones we will see as we go through the this reaction one by one. So, this diagram is just providing you the expansion for the abbreviation

of the 7 active sites. So, the first steps each one is just before the step the enzyme that is going to catalyze that is highlighted in blue.

So, the first step is acetyl CoA ACP transacetylase that charges this thiol group with acetyl-CoA. So, that is the first step, then the next step malonyl CoA transferase, so this thiol group is going to be charged with the malonyl CoA, so malonyl CoA product of the acetyl CoA carboxylase. So, that is charged then this beta keto acyl CoA synthesis, meaning it is going to synthesize that molecule this beta keto thing condensation.

So, the condensation reaction is what we saw in good detail in the previous slide, at the very beginning 2 slides ago. So, that is the condensation releasing the carbon dioxide. So, remember this carbon dioxide is not coming from the acetyl-CoA molecules; it is bicarbonate carbon dioxide that was temporarily attached to acetyl-CoA, just to facilitate this condensation reaction.

So, next one is beta keto acyl CoA reductase. So, that is going to reduce that of this double bond and you have this beta hydroxy butyryl ACP. So, at this first cycle it is butyryl because it is 4 carbons. So, later it will become beta hydroxy acyl as it extends. Then you have a dehydration enzyme, so that is going to remove water molecule here generating a double bond and that is going to be reduced by this ketoacyl reductase and that gives you the chain extended.

Now you have this acetyl-CoA transacetylase enzyme transferring this from this ACP thiol group to this synthase is ketosis synthase is a thiol group. So, like this, so this is the reason it is called transacetylase and then the first step again malonyl CoA transferase is going to malonyl CoA ACP transferase. So, that is going to bring a new malonyl CoA into that site.

So, now in the second cycle this is not acetyl CoA instead it is butyryl group, it is a butyrate. So, if you hydrolyze this is the butyrate, butyric acid and like this it keeps cycling and you need to remember the new carbons that are being added come into this growing chain in the form of malonyl CoA and so the addition is near the carboxyl group not at the methyl group. So, that is made possible by this transfer, from here it is transferred to this and the new incoming malonyl CoA comes to this ACP's thiol group, of transfer this is free to accept the malonyl CoA and then you have that.

So, this is how the fatty acid synthesis proceeds and for reasons that have not yet been figured out when the chain length reaches 16 it releases the chain out, it does not continue to keep going through the cycle. So, we do not know how that is sensed and stopped, some of the plant tissues make even shorter ones 8 carbon to 12 carbon ones also, the E-coli enzyme and other most of the animal enzymes release after 16 carbon then a separate set of reactions which we will not go through because every little detailed reaction of biochemistry is not essential for an in introductory course.

So, this is the main part of fatty acid synthesis, so I am not going to go into elongation phase. So, after the required number of cycles I guess 7 cycles you get the 16 carbon, palmitic acid and the palmitoyl CoA and that is cleaved and released as palmitic acid. So, this is again recapitulation of the second cycle that is after we have gone through it, this whatever we just quickly saw the same thing is shown here in good detail.

The same key for the enzymes here and after that of AT reaction it transferred from this ACP thiol group, the synthase is thiol group then a new malonyl CoA comes and then you have again a condensation and say you have the next beta keto acyl group, it is the same reaction is just shown separately in magnified image.

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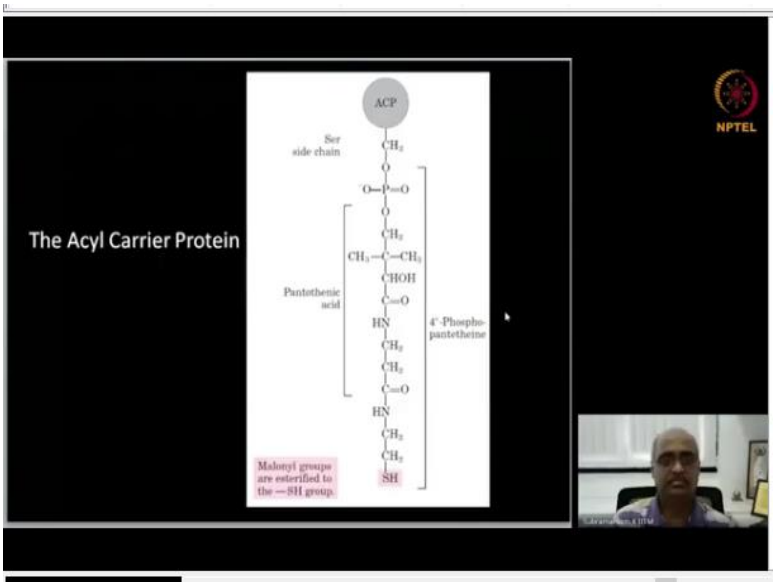

One of the main heroes of this reaction is acyl carrier protein that I mentioned but did not elaborate . So, if you remember coenzyme A structure, so we have a pantothenic acid and a mercapto group attached to it mercaptoethylamine providing the SH group, very similar thing

here this acyl carrier proteins carries this pantothenic acid moiety and at the end of it you have this sulfhydryl group.

So, this is a 4 pantetheine that is the structure, this whole thing, from here phosphopantetheine. So, this is the pantetheine moiety and so that as this sulfhydryl group. So, this is the one that carries the malonyl CoA and this arm helps in swinging from one active site to the other active site, you see here, so you need this long arm helps it to reach all the active sites here.

So, essentially if you imagine mitochondria, cytoplasm and think if your imagination can get into nano scale you actually see a lot of molecular motors working there, swinging their arms here and there and this interesting like a hydroelectric turbine kind of enzyme that ATP synthase rotational catalysis. If you think of rotational catalysis pyruvate dehydrogenase complex where you have the dehydrator like oil group and these carboxylation reactions where this biotin is involved.

You see lot of what happening like a construction site all the time, things swinging from here to there, things rotating, all happening at nano scale, you are not even consciously thinking of it, but this is what is making your existence and your activities possible.

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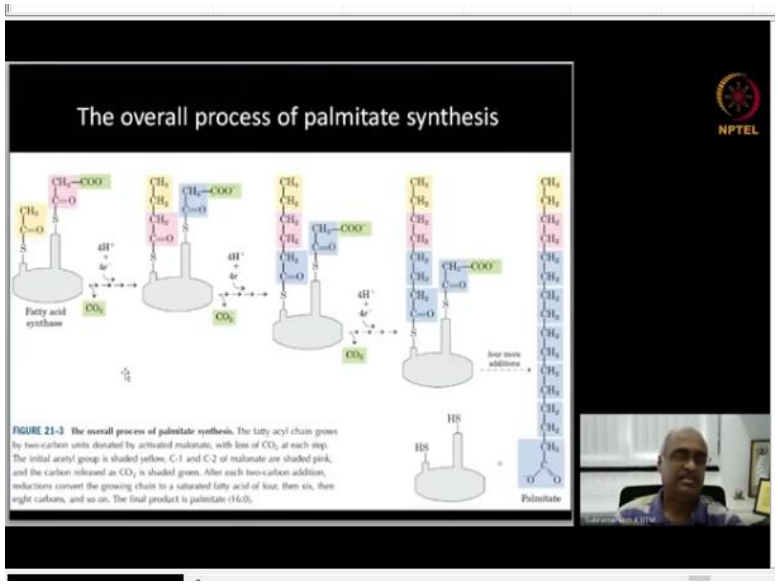

So, this is summary of the entire series of reactions to make the palmitate. So, we saw this first step after the preparatory steps. Now remember by this step we have gone through 3 reactions, this carboxylation loading on the ACP's thiol group, loading on the synthase thiol

group, then condensation, reduction, dehydration reduction. Then you get this and then you go through this multiple cycles. So, that is 1, 2, 3, and then 4 more then you end up getting 16 carbon palmitate.

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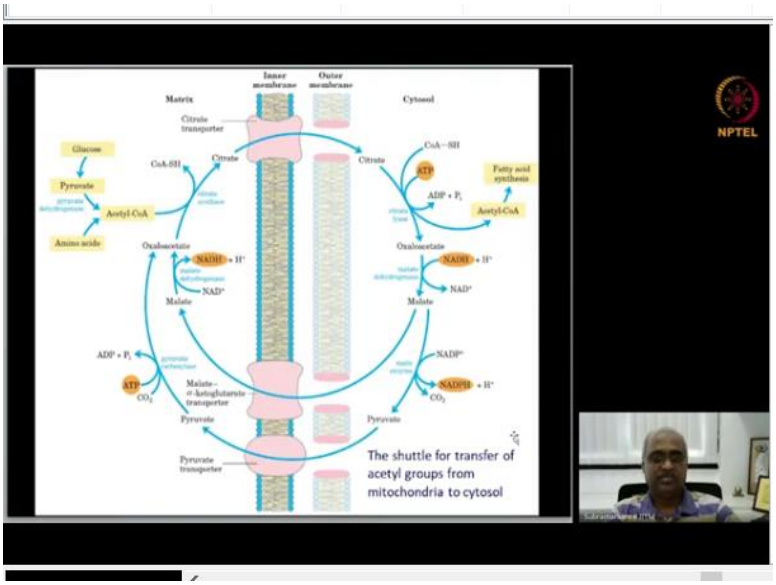

So, now that we have understood the 4 reactions plus the 3 preparative, let us say 7 reactions that make fatty acid biosynthesis possible. Now let us look at where the raw materials are coming and how that whole thing is the shop floor operation is handled. That is what is shown in this slide. So, it is not very complex, it just looks complex but if you begin in the right place and look at it, it is not difficult to us.

So, while beta oxidation TCA cycle pyruvate dehydrogenase complex, all of them are in mitochondria. So, therefore acetyl-CoA is produced in the mitochondria. And the fatty acid biosynthesis almost all synthetic reactions they happen in the cytoplasm which means the acetyl-CoA which is the raw material for fatty acid biosynthesis must be transported from mitochondria to cytoplasm. So, this circular diagram explains how that is done.

So, the acetyl CoA produced from variety of sources, not the one produced from beta oxidation, yesterday we saw these 2 are. If one is operational another one is shut off, if malonyl CoA is available then you are not going to transport any fatty acid into mitochondria to produce acetyl-CoA. So, here when we start off with acetyl-CoA this acetyl-CoA is either coming from carbohydrate or from proteins and this acetyl-CoA cannot cross the 2 membranes and that is why this complex cycle.

So, it goes through TCA cycle to become citrate the very first reaction and we have a transporter for citrate and the citrate transporter transports across the 2 membranes into the cytoplasm and there a reversal like step, reversal of the citrate synthase reaction citrate lyase which will require ATP cleaves and generates acetyl-CoA and oxaloacetate and this acetyl-CoA now goes to fatty acid synthesis.

So, essentially acetyl CoA cannot cross the membrane, it crosses in the form of citrate by entering into TCA cycle and then that citrate coming out instead of going through the TCA cycle and here if the reaction is reversed by a different enzyme citrate lyase generating acetyl-CoA and oxaloacetate. And again this oxaloacetate does not have a transporter to go out and therefore by reversal of the last step of TCA cycle malate dehydrogenase.

That generated oxaloacetate from malate. Now in the reversal consuming energy, they are reducing equivalent here, NADH gets oxidized. So, you get malate and malate has 2 ways of getting back to mitochondria, one malate is directly transported by malate alpha ketoglutarate transporter or it may be converted into pyruvate by malic enzyme, another source of NADPH. So, one I showed you this HMP shunt or also known as pentose phosphate pathway that produces NADPH.

And another source is this mallet being converted into pyruvate by malic enzyme. So, that reduces NADP to NADPH and carbon dioxide is released there and so either as malate or pyruvate it returns to the mitochondria. So, this is how acetyl-CoA is shuttled out from mitochondria to cytoplasm and made available for fatty acid synthesis. The simple thing you need to remember is acetyl-CoA comes out from mitochondria in the form of citrate.

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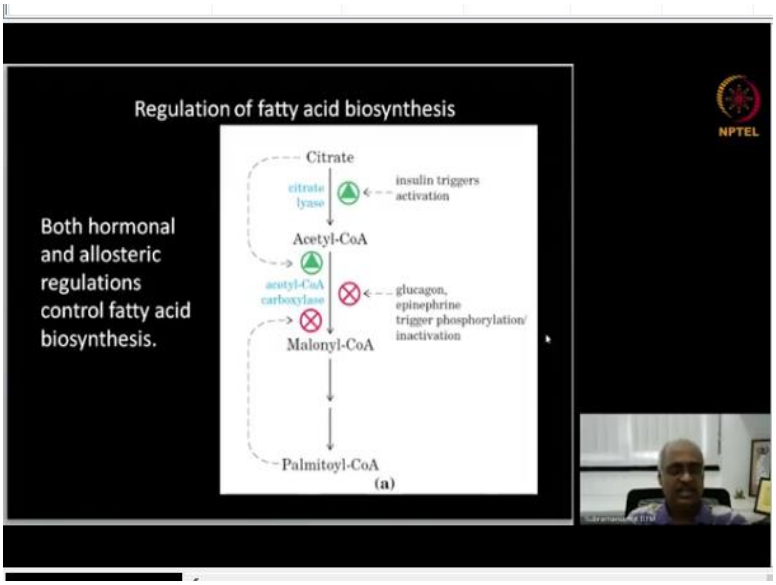

So, the last thing in this is how is this pathway regulated? Again it is a regulation although have been elaborate a set of experiments over a long period by a lot of labs to figure out the regulation. But it simply follows common sense the products become feedback, inhibitors, substrates become activators. So, that is the basic theme. So, whenever you have citrate accumulation and that citrate acts as a positive effector on ACC the acetyl-CoA carboxylase.

Yesterday we saw this and I told you this enzyme you have not encountered yet because it is part of the fatty acid biosynthesis, but today we have seen it in all its glory and this is this famous ACC and this is the first step I told you malonyl CoA means you are committing to making fatty acid and that gets activated when the substrate is available. Citrate means like a camouflaged form of acetyl-CoA we just saw that in the previous slide.

So, citrate activates, if you have plenty of citrate meaning you have a lot of carbohydrate becoming pyruvate, becoming acetyl-CoA and TCA cycle is filled with its intermediates and therefore that stimulates this and that can now use the acetyl-CoA that is transported in the form of citrate into the cytoplasm and it can convert into malonyl CoA. So, citrate as a positive regulator is the new information we are learning today.

So, yesterday we saw this glucagon, epinephrine and this phosphorylation, dephosphorylation regulation of ACC. The new information added today is citrate as a positive regulator and how that makes sense based on what we have learnt in the fatty acid biosynthetic pathway steps as well as the mitochondria to cytoplasmic shuttle of the acetyl groups and the second

information that we added today is the end product palmitoyl CoA acts as a negative allosteric effector of this ACC enzyme.

And the third new information today is insulin which is secreted when you have high glucose, when you have high glucose then only you will have high citrate. So, this one promotes citrate lyase in the cytoplasm. This is the one that is going to cleave citrate and generate acetyl-CoA in the cytoplasm. So, that one activates the citrate lyase and this fourth point we have seen yesterday.

So, acetyl-CoA carboxylase therefore is subject to both covalent modification that is dephosphorylation stimulated by insulin and phosphorylation stimulated by glucagon and epinephrine respectively activates and deactivates. That is the covalent modification and now we have seen allosteric effectors, positive effect by citrate, negative by palmitoyl CoA. So, this is a key enzyme and therefore it is multiple levels of control meaning multiple molecules regulated.

But 2 important distinct mechanisms, one is allosteric regulation and another one is covalent modification. So, this is how both the hormonal and allosteric regulation control fatty acid biosynthesis. Because it is key, this regulation optimum regulation of this is critical to maintain constant body mass for more than 40 years of adult life in homosapiens without regardless of how much food intake comes in and how much physical activity you have, in a healthy situation it maintains a constant body weight as an adult for more than 40 years.

And that sort of thing requires these kinds of very fine controls, we cannot leave lipid metabolism only looking at these aliphatic molecules, free fatty acids and we are not looking at triaxial glycerol formation, but steroids require our attention. So, therefore we will look at biosynthesis of cholesterol the very first central steroid molecule from which other steroids are made. So, cholesterol biosynthesis we will discuss on Monday that is next process, if you have questions.