Overview and Integration of Cellular Metabolism

Prof. Arindam Ghosh

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Week 06

Lecture 30: Cholesterol Metabolism

Hello everyone, welcome back to the lecture series of Overview and Integration of Cellular Metabolism. So, we are going to discuss about the compounds which are formed from cholesterol, what is the fate of cholesterol and finally, few relevant clinical or applied significance with respect to cholesterol metabolism. So, before going into the metabolism or biosynthesis of cholesterol let us discuss a bit biochemistry of cholesterol that is cholesterol is as you can see in the structure that this is a 27 carbon compound it contains a sterile ring in it. So, this is our steroid nucleus or sterile ring sometimes we call it CPP ring cyclopentano perhydro phenanthrene ring. So, this is not a chemistry class. So, we are I am not going into much detail of this.

Then what is the importance of cholesterol metabolism I mean why we are very much intrigued with cholesterol metabolism. Now cholesterol remember even if we very frequently say the cholesterol is increased cholesterol is bad cholesterol should be reduced etcetera etcetera, but remember cholesterol is one very important and required product in our body and for our regular maintenance of human body actually. Why because one very important thing cellular cellular membrane. So, cholesterol is one important integral part of cell membrane then it is also required for nerve conduction because it imparts a insulate in an insulation effect over the nerve.

Then it has some biosynthetic property it access precursor for compounds like bile acid and bile salt then steroid hormones all the steroid hormones are actually synthesized from cholesterol starting from glucocorticoids all the glucocorticoids then androgens, estrogen they all are synthesized from cholesterol and one vitamin very important vitamin D 3 esterification of vitamin D 3 for that cholesterol is required. So, these are the important compounds or important functions of cholesterol for which cholesterol synthesis is very increasing metabolic topic we need to know. So, let us move to the synthesis of cholesterol. Now synthesis of cholesterol occurs almost in all the tissues, but the most important one is liver then intestine, skin adrenal cortex and in reproductive tissue. Every day daily around 1 gram of cholesterol is synthesized in adult cells then the

synthesizing enzymes are present in cytosol as well as microsomes.

So, again the biosynthesis of cholesterols occurs in with the help of cytosolic as well as microsomal enzyme. What is the precursor for cholesterol synthesis is acetyl coenzyme a remember acetyl coenzyme a is the precursor for all the carbons in cholesterol 27 carbons they all are actually derived from acetyl coenzyme a. And then one reducing equivalent is required that is NADPH and also the energy is provided by ATP. So, these are the basic points you need to remember for biosynthesis of cholesterol. Now cholesterol synthesis is one complex mechanism actually complex series of steps which finally, forms cholesterol, but we have divided the cholesterol synthesis in 4 important stages or phases rather.

So, the first phase or stage is formation of mevalonate formation of mevalonate from 3 acetyl coenzyme a or acetate units. Then that mevalonate is finally, forming isoprene unit or activated isoprene unit in stage 2. Now isoprene isoprene units are 5 carbons. So, remember mevalonate was 6 carbon compound from that 1 carbon is actually removed to form the 5 carbon isoprene unit that isoprene units are actually condensed to form squalene that is a 30 carbon compound cholesterol is 27 carbon compound. So, finally, and also cholesterol is one cyclical structure I told you there is a ring sterile ring or CPP ring is there, but all this up to squalene this these are all linear compound.

So, what we need is cyclization of the squalene to form the rings of sterile rings or steroid nucleus and there are further series of changes even after that sterile nucleus formation that is oxidation removal or migration of methyl groups to finally, form cholesterol which is the 27 carbon compound and in between that the ring structure is first forming in lanosterol. So, these are the 4 stages in biosynthesis of cholesterol. Now we will discuss these stages and what are their specific metabolic steps in under these phases. So, this is the overview that acetyl coenzyme A is a 2 carbon compound. So, they are forming mevalonate via HMG coenzyme A we will discuss HMG coenzyme A then mevalonate is the 6 carbon compound 6 from 6 carbon compound there is formation of isoprene unit.

Isoprene units are 5 carbon compounds these 5 carbon compounds are actually condensed to form 2 such 5 carbon compounds are condensed to form the activated 10 carbon compound then another 5 carbon compound is added to the 10 carbon compound to form C 15 compound. Finally, these 2 C 15s are condensed to form linear squalene then there is cyclization to form lanosterol. Now in this lanosterol this is the first structure of in the pathway of cholesterol synthesis this is the first cyclical structure and then further modification to form cholesterol. So, this is the gist of cholesterol synthesis fine. So, let us see one by one what happens that from acetyl coenzyme A first which is

formed is acetoacetyl coenzyme A.

So, there is condensation of 2 molecules of acetyl coenzyme A with the help of the enzyme acetoacetyl coenzyme A synthase remember this is synthase and synthase are those enzymes where ATP is required in synthase ATP is not required. So, here it is synthase acetoacetyl coenzyme A synthase sometimes we call it thiolase as well. So, there is formation of acetoacetyl coenzyme A from 2 molecules of acetyl coenzyme A then there is formation of HNG coenzyme A that is beta hydroxy H beta methyl glutaryl coenzyme A. So, this is our HNG coenzyme A and HNG coenzyme A is formed by condensing another acetyl coenzyme A to acetoacetyl coenzyme A. So, this is our 6 carbon compound and of course, there is a release of coenzyme A because here was one coenzyme A another coenzyme A was there.

So, one coenzyme A molecule is released and then HNG co A further undergoes reduction to form mevalonate. Now, the reducing equivalent you as I told you here is NADPH remember this is NADPH and also the coenzyme A is removed to form mevalonate once again mevalonate is 6 carbon compound then from this mevalonate isoprene units are formed. Now, isoprene units are 5 carbon compound. So, definitely there must be one decarboxylation one where carbon dioxide is released one carbon dioxide is released. Now, what happens during the formation of mevalo isoprene unit from mevalonate there is phosphorylation with ATP's and this phosphorylation happens for 3 times.

So, here you can see the first phosphorylation, second phosphorylation and third phosphorylation along with that there is and the last phosphorylation is followed by decarboxylation. So, what is formed the for the first intermediate is 3 phospho 5 pyrophospho mevalonate after 3 phosphorylation what we are getting is 3 phospho 5 pyrophospho mevalonate and here you can see one kinase is there one phosphotransferase is there and one decarboxylase is there fine. So, what we are getting after decarboxylation is the first isoprene unit that is isopentanil pyrophosphate isopentanil pyrophosphate. So, once again mevalonate undergoes 3 steps of phosphorylation with 3 molecules of ATP and also decarboxylation to form the first isoprene unit which is isopentanil pyrophosphate. Now, there are 2 types of isoprenoid units are formed one is isopentanil pyrophosphate and its isomer.

So, next step is isomerization of isopentanil pyrophosphate to form dimethyl allyl pyrophosphate this is another isoprene unit. So, from mevalonate we are getting 2 isoprene units one is isopentanil pyrophosphate another is dimethyl allyl pyrophosphate and both of them are required in cholesterol synthesis. So, after formation of this isoprene unit what will be there is condensation of this 5 carbon isoprene unit. So, first is

formation of the 10 carbon compound zanil pyrophosphate. Now, what happens there is condensation of one molecule of dimethyl allyl pyrophosphate and isopentanil pyrophosphate in head to tail fashion.

Now, head group is the part where phosphate pyrophosphate is attached. So, the head group of dimethyl allyl pyrophosphate and the tail part of isopentanil pyrophosphate are condensed to form zanil pyrophosphate which is a 10 carbon compound and definitely here you can say the pyrophosphate is released. Next there is another condensation these zanil pyrophosphate is once again condensed with another molecule of isopentanil pyrophosphate in head to tail fashion head of zanil pyrophosphate containing the pyrophosphate part and tail of isopentanil pyrophosphate is forming 15 carbon compound that is furnesile pyrophosphate furnesile pyrophosphate is a 15 carbon compound. So, what we are seeing here are 2 5 carbon compounds which are condensed to form the 10 carbon compounds zanil pyrophosphate zanil pyrophosphate is once again condensed with isopentanil pyrophosphate to form the 15 carbon compound furnesile pyrophosphate. Now, these two furnesile these furnesile pyrophosphate two such molecules are condensed to form 30 carbon compound squalene and these condensation occurs in head to head condensation.

So, basically what happens the both the pyrophosphate containing ends are actually condensed. So, you can see from both the ends two pyrophosphates are released and this is one NADPH dependent reaction where the enzyme is squalene synthase. So, what we are getting after condensation of the 5 carbon units is one 30 carbon compound squalene and this squalene remember this squalene is the linear structure. Now, squalene synthase what you need to remember need some cofactors cofactors like magnesium, manganese and cobalt and squalene synthase is one microsomal or smooth endoplasmic reticulum attached enzyme. So, at the end of the condensation of this 5 carbon isoprene units what we are getting is squalene squalene is one 30 carbon compound and remember this is a linear structure.

So, what we need is cyclization to form the sterol ring. So, next we are going to discuss about cyclization. So, squalene undergoes cyclization with the help of one monooxygenase which is known as squalene monooxygenase and this squalene monooxygenase once again is one NADPH dependent enzyme. Now, you can see here molecular oxygen is required. Now, that molecular oxygen one atom is attached to you can see at the end of the squalene structure forming squalene 2 3 epoxide and another atom of oxygen is actually forming water here you can see is utilized to form water.

So, squalene epoxide then undergoes cyclization by cyclase enzyme which forms a 30 carbon ring structure. So, this is the first ring structure that is known as lanosterol.

Lanosterol remember once again this is one very important question what is the first ring structure which is formed during the synthesis of cholesterol that is lanosterol and this is the first steroid compound. Now, lanosterol undergoes around 20 approximate 20 steps of reaction to form cholesterol. Now, all the steps are not till date even not explain it details, but there are few important steps which have been discussed vividly.

So, we are going to just highlight those structure. So, from lanosterol after multiple steps we are getting xymosterol from xymosterol we are getting desmosterol and finally, this desmosterol is forming cholesterol. Now, these modifications you might not remember, but for the information I will say that there are modifications like as I told methyl group addition in lanosterol leads to xymosterol formation. Then there is transfer or migration of double bonds in xymosterol to form desmosterol and finally, those the desmosterols they are those double bonds are actually reduced by NADPH to form cholesterol. So, this is how cholesterol is formed, but remember actually in between there are around 20 steps.

So, these are the important steps I have highlighted from lanosterol to cholesterol formation. So, once again to recapitulate that the precursor molecule is acetyl coenzyme A 3 such acetyl coenzyme A is condensed to form the 6 carbon compound mevalonate. Mevalonate forms the activated isoprene unit that is isopentanil pyrophosphate as well as its isomer dimethyl allyl pyrophosphate. These two are these two isoprene units are condensed to form 10 carbon compound geranil pyrophosphate, then 15 carbon compound, furnesyl pyrophosphate and finally, squalene 30 carbon compound. And these 30 carbon linear compound is actually undergo cyclisation to form lanosterol and lanosterol after around 20 steps from cholesterol.

Now, one very interesting thing about squalene is squalene was actually derived from sharks. So, the name came from the squalas term. And then also these compounds like furnesyl pyrophosphate, geranil pyrophosphate these isoprene units are actually the naturally occurring perfumes or aroma generating materials. So, like geranil was actually derived the name is derived from geraniol which is present in rose oils. Similarly furnesyl term is actually derived from furnesia acacia plant that is also one aroma generating plant.

So, these are the names are generated from names are given from where this compounds are actually initially derived. So, this is all about biosynthesis of cholesterol. Now, we are going to discuss the regulation of cholesterol. Now, remember once again regulation of cholesterol it is it has few long term regulation where we will discuss the synthesis of different enzyme or I mean the we are dealing with the numbers of enzymes as well as few short term regulation where we are talking about we will be talking about activation

or deactivation of enzyme. Now cholesterol meta I mean synthesis of cholesterol is regulated by hormones.

Hormones give signals for the regulation of cholesterol as well as the intracellular cholesterol content is one very important decisive factor for cholesterol synthesis. Now, the rate limiting step is the formation of mevalonate from HNG coenzyme A and the enzyme which is regulated or the regulatory enzyme in cholesterol synthesis is HNG coenzyme A reductase. Now, let us see how this long term regulation happens. So, long term regulation here we are talking about the synthesis decreased or increased synthesis of the regulatory enzyme that is HMG coenzyme A reductase. Now, remember transcriptional control is for cholesterol synthesis is not only for HNG coenzyme A reductase, but other enzymes are also regulated in this transcriptional control.

The most important one is HMG coenzyme A reductase obviously. So, to induce or regulate the transcription what we need is one transcription factor through which most of the long term regulation or transcriptional control imparted to the enzymes. Here the transcription factor is SREBP remember we already have discussed SREBP. Now, we are going to discuss in details how this SREBP is controlled by the intracellular cholesterol. Now, SREBP stands for Sterol Regulatory Element Binding Protein in many books or journals it is also represented as sterile responsive element binding protein.

And another adjunct protein is there that is SCAP protein the it is the full form of SCAP is SREBP Cleavage Activating Protein. So, SCAP protein is another adjunct protein. Now, the transcription factor is SREBP. Now, this transcription factor SREBP is a protein definitely. Now, it is amino terminal is a soluble part and this amino terminal imparts the actual transcriptional regulation activity, but the problem is this amino terminal is not free.

So, the whole SREBP molecule is actually attached to the membrane of endoplasmic reticulum. So, here you can see SREBP here it is attached with the endoplasmic reticulum. So, it is not freely movable or it is just cannot enters nucleus cannot bind with the DNA as it is what is needed is one regulation regulatory signal. Now, remember when there is adequate amount of cholesterol present in cell it is sensed by the SCAP protein. So, SCAP protein is actual sterile or cholesterol sensor here and this SCAP protein is attached with the SREBP.

So, SREBP and SCAP protein is they are attached together and is attached to the endoplasmic reticulum membrane. Now, when there is reduced cholesterol content in cell it is sensed by the SCAP protein. After sensing the cholesterol reduced cholesterol content what happens are two proteolytic cleavage. Now, in first proteolytic cleavage

this SCAP protein is separated from the SREBP. So, here you can see the SREBP is free from SCAP protein, but it is still attached to the endoplasmic reticulum.

The second proteolytic cleavage it breaks the amino terminal rather it detaches the amino terminal segment of SREBP from the membrane bound fraction. So, now, the amino terminal of SREBP is actually free to migrate inside the nucleus. Inside the nucleus it binds with the respective genes and those genes are actually of HMG coenzyme reductase synthesis or other cholesterol metabolizing enzymes related genes. So, now, the synthesis in is increased when there is cholesterol the cellular content of cholesterol is low. So, once again the low concentration of cholesterol is sensed by SCAP protein.

SCAP protein on sensing the cholesterol low content it undergoes two proteolytic cleavage to release the amino terminal domain of SREBP that amino terminal domain enters nucleus binds with the respective genes and causes increased synthesis of HMG coenzyme reductase which increases the synthesis of cholesterol. Now, when by this induction when the cholesterol content is finally, increasing that is also sense wise the SCAP protein. SCAP protein again reattaches with the SREBP fragment and SREBP now is not able to move inside the nucleus. So, the synthesis is now decreased. So, this is on demand cholesterol based on the available cholesterol concentration inside the cell.

Now, HMG coenzyme reductase enzyme itself can be itself can sense the signal of low or high cholesterol content. Now, HMG coenzyme reductase enzyme itself is one integral membrane protein and it is attached to the endoplasmic reticulum as I told it is the microsomal enzyme. So, this enzyme it is cytosolic domain is acting as the enzyme. So, this is imparting the catalytic effect whereas, the membrane domain this is our membrane domain it acts as the cholesterol sensor. The sensor is just like the SCAP protein it has the similar domain which is present on the SCAP protein.

So, basically what happens when there is high cholesterol content inside the cell it is sensed by the membrane bound domain of the HMG coenzyme reductase on sensing there is conformational change in the enzyme HMG coenzyme reductase such conformational changes makes this enzyme vulnerable for proteolytic degradation. So, HMG coenzyme reductase is actually proteolytically degraded. So, cholesterol synthesis is decreased. So, this is how HMG coenzyme reductase itself can sense the cholesterol content of the cell. Then there is short term regulation whereby where the enzymes are activated or deactivated following covalent modification.

Here the covalent modification is following phosphorylation dephosphorylation cycle the HNG coenzyme reductase enzyme is active when it is dephosphorylated and inactive

when it is phosphorylated and this phosphorylation dephosphorylation is triggered by hormones. So, cortisol or glucagon they causes the phosphorylation. So, basically they are decreasing the HNG coenzyme reductase activity. So, basically they are decreasing the cholesterol synthesis. Similarly, when there is excessive exercise that exercise there is breakdown of ATP.

ATP is the breakdown product of ATP is AMP. We already have discussed AMP acts as an activator of AMP kinase. This AMP kinase once again phosphorylate HMG coenzyme reductase and that causes inactivation of the enzyme. On the contrary insulin or thyroxine they activates HMG coenzyme reductase by activating the phosphatase or dephosphorylation and increases the synthesis of cholesterol. So, what we can see in regulation of cholesterol synthesis important hormones are there insulin and glucagon as well as the intracellular cholesterol content is also acting as a signal for cholesterol synthesis as well as receptor mediated uptake of cholesterol from the circulation. So, what happens if there is excess amount of cholesterol in circulation it forms in sorry if there is excess amount of cholesterol in cell it forms oxysterol.

Now, oxysterol inhibit the receptor mediated endocytosis of cholesterol. Similarly, if there is excess amount of cholesterol in cell it activates the ACAT enzyme acetyl coenzyme A acyltransferase or acyl coenzyme A acyltransferase that forms cholesterol esters. Similarly, AMPK or glucagon or cortisol they cause inactivation of HMG coenzyme A reductase or insulin activates insulin and thyroxine they activates the HMG coenzyme A reductase. So, these are the nutshell of regulation of cholesterol synthesis. Then the clinical or applied importance is there are multiple drugs which helps in lowering the intracellular cholesterol synthesis by inhibiting the enzyme HNG coenzyme A reductase.

Those drugs are statin group of drugs the compounds are like lovastatin, simvastatin like this. So, these are the statin group of drugs. Now, statin group of drugs acts as competitive inhibitor of HMG coenzyme A reductase. Basically, lovastatin in inactive form it is one lactone. Now, when ingested it is hydrolyzed to form the active form that is a beta hydroxy derivative of lovastatin.

This beta hydroxy derivative is actually competitively inhibiting HNG coenzyme A reductase thereby decreasing the intracellular synthesis of cholesterol. So, remember statin group of drugs are cholesterol lowering drugs. Then the fate of cholesterol in our body now cholesterol is supplied in our body either by diet or that is exogenous cholesterol or endogenous cholesterol which are circulating inside the body or synthesized in our body then de novo synthesis which is occurring in liver. So, these are the I mean the sources of cholesterol in our body. What are the fates of cholesterol in

body? Cholesterol is secreted in the form of VLDL.

So, this is our circulatory form of cholesterol. So, cholesterol is secreted from liver as VLDL it can be secreted as free cholesterol and is liberated in bile or the liver cholesterol can be converted to form bile acid or bile salts. Now, remember the free cholesterol which is released in bile it can be it is actually excreted to through feces. Now, this amount is around 1 gram per day and there is a bit of ring modification which is finally, forming coprostenol or cholesterol and that is influenced that is done by colonic bacterial activity. A part of this free cholesterol which is secreted in bile it can be absorbed in intestine and can be exported as chylomicron.

So, this is the fate of free cholesterol in bile. Remember cholesterol can be forming bile or cholesterol can be secreted as it is in bile. So, here we are talking about the free cholesterol. Now, by the part of bile synthesis of cholesterol a bit we need to know about that the bile is synthesized in liver cell definitely and there are few primary bile acids like two primary bile acid colic acid and keno deoxy colic acid. Then secondary bile acids like deoxy colic acid and litho colic acid a bit of the bile acid synthesis I am going to discuss.

So, this is formation of bile acid. Now, here you can see cholesterol undergoes hydroxylation in the 7th position of the cholesterol in 7 alpha hydroxylation and the enzyme is 7 alpha hydroxylase to form 7 alpha hydroxycholesterol. Then 7 alpha hydroxycholesterol without hydroxylation can form keno deoxycholyl colic acid or after hydroxylation in the 12th position can form colic acid. So, again the true two I mean two primary bile acids are formed from 7 alpha hydroxyl cholesterol. Colic acid is formed after 12 alpha hydroxylation which is an NADPH dependent enzyme. Then there is release of a 3 carbon compound in the form of propionyl coenzyme A that forms cholyl coenzyme A finally, and without 12 alpha hydroxylation here you can see there is no 12 hydroxylation no another hydroxylation is not there, but there is release of a 3 carbon compound propionyl coenzyme A.

Now, here you can see these 3 carbons are released as propionate then there is oxidation at the 24th carbon also 7 alpha hydroxylation as well as reduction of the double bonds. These are common for all the primary bile acid along with that one additional step is there for the colic acid that is 12 hydroxylation 12 alpha hydroxylation. So, what we are getting true two primary bile acids that is colic acid and keno deoxy colic acid. They are conjugated with torine and glycine to form toro-calc colic acid and glycolic acid or similarly toro-keno deoxy colic acid or glyco-keno deoxy colic acid. So, these are the primary bile acids and the their salt version are sodium or potassium toro-colate glyco-colate toro-keno deoxy colate or glyco-keno glyco-keno deoxy colate.

So, these are the primary bile acids. Now, primary bile acids when they are treated with colonic bacteria they form they undergoes decongigation and also dehydroxylation to form the secondary bile acids those are deoxy colic acid and lithocholic acid. So, these are our primary bile acid and secondary bile acid formation. Now remember bile salts keeps recycling in our body.

So, liver only synthesizes 0.2 to 0.6 gram per day bile salts, but their actions are very high. So, what is required is recycling. So, cholesterol what happens it forms bile acid these bile acids are stored in gallbladder and is released in intestine. Now, in intestine these undergoes reabsorption. So, reabsorption around 12 to 32 grams per day are bile salts are reabsorbed and return to liver for recycling.

So, there are around 95 percent of efficiency and the it the recycling is for around 6 to 8 times per day. Now, active these absorptions are basically in the intestine where active absorption occurs in ileum whereas, passive absorption occurs in jejunum. So, you can see if only this 0.2 to 0.6 grams of per day bile acids is synthesized in liver, but they are constantly recycling around 6 to 8 times and what is actually excluded through thesis is 0.

2 to 0.6 grams per day this is our loss which is actually synthesized here. So, this is the efficient bile salts enterohepatic circulation. So, these are the points which we have discussed today that cholesterol synthesis is mainly form from occurring from the precursor to the acid acetyl coenzyme a following a complex series of reaction and these synthesis is mostly regulated by hormonal control as well as intracellular concentration of cholesterol. So, this is all for today these are my references. Thank you all in the next class.