Overview and Integration of Cellular Metabolism

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Lecture 50: Nucleotide Metabolism – I (Purine Metabolism)

Hello, we are back to our lecture series on Overview and Integration of Cellular Metabolism and we will start with a new metabolism that is nucleotide metabolism, alright. So, we will be covering what do we mean by nucleotides. So, we will be briefly covering a nucleotide chemistry, we will be discussing in this class about de novo synthesis of purine, we will be discussing how the step is regulated, we will be discussing purine salvage pathway as well as about purine analogs, alright. So, this purines and pyrimidines actually when we refer to nucleotide the constituent molecules are purines and pyrimidines, right. So, what are they? They are actually nitrogen containing heterocyclic compound very important, right. So, nitrogen containing heterocyclic compound whose rings contain carbon and nitrogen, right.

Generally when we are discussing an aromatic ring we generally think of carbon, right. So, carbon, nitrogen both are present, right. The planar structure of purine and pyrimidine facilitates their close association of stacking which standardises or stabilises double standard DNA means what? These purines and pyrimidines are actually leading to formation of compounds nucleotides which are polymerising to form the unit of light that is DNA, right. They are also referred to as nitrogenous bases, ok.

So, these things whenever you are asking I mean discussing about DNA, purine, pyrimidine, maybe nitrogenous bases only one thing should come to your mind that is the question is being asked about nucleotide metabolism, ok. So, looking at the structure this is how they look like, right. In this class we will be focusing on purines, right. Upcoming classes we will be discussing purine, pyrimidine I mean disorders of purines and then we will be moving on to pyrimidine. So, pyrimidines actually the structure wise they are simpler, they have got one ring, ok.

Whereas, the purines they are actually two rings there is one hexagonal ring and there is one pentagonal ring. It can be represented the this pentagon may be present in this side, ok. It may be represent on this side as well, right. But the numbering you should be

careful, alright. The hexagonal nitrogen the ring in the six sided ring the nitrogen starts as the first one and ultimately it ends with the nitrogen in the pentagon, ok fine.

So, what are the types of purines that are that you should be knowing adenine, guanine, xanthine and hypoxanthine, alright. These are metabolically important purines. Of course, there are multiple other purine as well. For example, theobromine, theophylline and any other naturally offering proteins that are present in caffeine, coffee, cocoa, right. We are not discussing entire nucleotide chemistry to start with, but the very basics which will help you to understand the metabolism of nucleotides, right.

So, what are the function of nucleotide? Till now you already have come across nucleotides even if you are not knowing it, right. Number one DNA RNA we all know, right. So, provision of energy, right in the form of ATP, GTP. What I mean what does energy help? These energy help in muscle contraction, active transport, ion ingredients, multiple things. So, wherever there is an active energy transport we talk about ATP and GTP and this A and G are adenine and guanine those are nothing, but nucleotides, right.

Again we have come across these names NAD, NADH, FAD, FMN, right. So, nicotinamide, adenine, dinucleotide, phosphate, adenine, right. Flavin adenine dinucleotide, flavin mononucleotide and even coenzyme A also contains adenosine, right. So, very important you have come across these names multiple times and all of these are having nucleotide as the main players, right. Activated intermediates you have read about UDP glucose, UDP galactose, UDP glucoronic acid S adenosyl methionine wherever these names are coming again nucleotides are playing an important role.

Apart from that they are active phosphate don't know signal transduction second second messengers, right very important role and also by adenylation, uridylation multiple enzymes are activated. So, you have read even if you are not told about biological function just simply by recalling many functions you can already give specific examples, right I am sure about it, right. So, you should be able to recall where what are the areas where nucleotide is involved in a metabolism, right. Now, looking at the specific unit of a DNA and RNA, unit of DNA is actually nucleotide. So, what is a nucleotide? A nucleotide is a purine or pyrimidine base, ok those are nucleobases adenine guanine those are nitrogenous bases or nucleobases.

When they combine with a pentose sugar, ok the sugar may be ribose or deoxyribose, right. So, a ribose is how you know glucose looks like in hexagon ribose is a pentose sugar it has got 5 carbons, right and what is deoxyribose? In case of deoxyribose in the 2 prime position there is a H. Now, what is 2 prime, 1 prime you can see there is a prime symbol. This is to avoid a confusion where the purine base the ring of the purine base is

named as 1, 2, 3, 4, 5, 2, 9 and the carbon on of the ring of the purine base is the pentose sugar are named as 1 dash, 2 dash, 3 dash, right. So, a nucleobase along with a pentose sugar forms nucleoside, right and when a phosphate group is attached to it, it becomes a nucleotide.

So, nitrogenous base, nitrogenous base plus phosphate in pentose sugar becomes nucleoside and a nucleoside plus a phosphate becomes a nucleotide, alright. So, this fundamental concept should be very clear and here you can see some example of bases their corresponding nucleosides and their corresponding nucleotide, alright. So, these are the bases of DNA and RNA. You can choose to memorize this chart because something I mean anything from here may be combined as an MCQ question when it comes to all except type or even choosing the best answer type of MCQs. For example, in case of DNA the base is guanine, the nucleoside is deoxyguanosine and the nucleotide is deoxyguanilate and then ultimately it can be monophosphate, diphosphate or triphosphate depending on the number of phosphate bonds.

So, what is ATP? ATP is definitely a nucleotide, alright. So, once AMP is formed one phosphate group it can be at I mean phosphorylated to form ADP and then ATP, alright. So, now that we know what does purine and pyrimidine look like we have a basic idea about the nucleotide chemistry, we shall now discuss how this nucleobases are actually synthesized, ok. So, our topic of discussion today is regarding purine synthesis. So, purine can be synthesized in two ways, I mean this whole double ring structure can be synthesized in two ways.

Number one is known as de novo synthesis. De novo synthesis means you have heard the term de novo synthesis where one example is during de novo synthesis of fatty acid, alright. There are multiple example of de novo synthesis. So, de novo synthesis of purine like all other de novo synthesis it happens in our body in C 2 from very basic metabolites. All primary components are assembled to form a new molecule just like this new car is being assembled from multiple new components.

What is this purines have been seen to be formed from other pathway also that is known as salvage pathway which is nothing, but recycle of existing nucleotides. So, pre existing nucleotides if are present they may be recirculate in such a way under action of several enzymes. So, that purine same purine is formed it can be compared to a situation where multiple parts from a junkyard is assembled to form a functional car, right both will help you to drive. So, two major roots number one de novo synthesis main component is activated ribose in the form of PRPP phosphoribosyl pyrophosphate we will be discussing, right after the amino acid ATP CO 2 all of them there are many more multiple intermediates are there which forms this I mean core purine molecule, right.

Whereas, in case of salvage pathway we need the base and then we need the PRPP that can actually lead to directly formation of the nucleotide nucleotide meaning the nitrogenous base the phosphate group as well as the pentose sugar, alright.

So, when we are considered with de novo synthesis de novo synthesis we should always know what are the sources and this is a very common MCQ or an image based questions, right. So, you can see this is the number you can take a snapshot of this and you can actually number the carbon of your own, right on your own this is the first nitrogen, alright this is the second carbon and it goes on and on where this is the ninth, right and this is the eighth. So, you can do it, right you can actually easily do it I told you the hexagon and pentagon can be represented in either sides. Needless to say what are the sources the sources are aspartate, carbon dioxide in form of bicarbonate, right from the body, glycine, formyl tetrahydrofolate that is formate and glutamine, right. We already discussed the inter molecule of glycine is actually incorporated in this molecule, right.

So, over here we can see the components that are donating the first nitrogen is formed the amine of aspartate, the second and eighth carbon are form formate in the form of formyl tetrahydrofolate acid, formyl THF I told you during one carbon metabolism the folate components are getting inter converted into each other and the N 3 and N 9 nitrogen are coming from glutamine C 4, C 5 and C N 7, seventh nitrogen is coming from glycine and the sixth carbon is also coming from bicarbonate iron, ok. I am sorry this would be 2, this is 6, ok. So, we are now discussing how this steps are happening, alright. You might find it a bit difficult remember because there are multiple big names that you need to remember in sequence, ok. The first step or the preparatory step it is also called step 0, why? We will discuss.

Is formation of PRPP, right. I told you PRPP is nothing, but activated ribose 5 phosphate because ultimately it is ribose 5, if we already have got ribose 5 phosphate the entire purine nucleus can be loaded on ribose 5 phosphate and then we can get a nucleotide, alright is that easy to remember. So, ribose 5 phosphate plus ATP the enzyme is PRPP synthase or ribose phosphate pyrophosphokinase same thing it forms phosphoribosyl pyrophosphate and a molecule of AMP. So, this is the step 0. Remembering structure not important, I am just showing the structure so that you can conceptually understand the proceedings.

So, why we call it a step 0? We call it a step 0 because formation of PRPP is not exclusive in de novo synthesis of purine. What I mean is PRPP is also needed in the pyrimidine nucleotide synthesis, it is also in salvage pathway. So, PRPP is not considered a step in the de novo synthesis. Therefore, it is actually the starting molecule, right just like CPS of urea, right. You have you can correlate the analogy where it was

the starting molecule, but it was not directly participate the enzyme carbamoyl phosphate synthase 1 was not directly a part of the urea cycle.

Similarly, here PRPP synthesis not the not included in the purine synthesis pathway, however it is a preparatory step or step 0. So, what is the first step? The first step is actually combination of glutamine with PRPP to form glutamic acid or glutamate and 5 phosphoribosyl 1 amine or PRA, alright. Phosphoribosyl amine specifically 5 phosphoribosyl 1 amine. So, this is the structure over here you see the phosphate group was already present, right. It has already gone out in the form of PPI, this 2 phosphate goes out in form of PPI and the nitrogen of glutamine gets attached, alright.

Here this the entire ribose 5 phosphate has been shown, right and this is the NH 2 group that is attached to the ribose 5 phosphate, alright. You know in any amino acid the amine group can be present as NH 3 plus and CaO minus or NH 2 and CaO H. So, it is one and the same. So, this is the first step by formation of PRA, right and ribose 5 phosphate is first loaded with the amine group and ultimately the purine ring will be assembled on ribose 5 phosphate which we already discussed. So, if you just visualize this step the further steps will be much easier for you to understand.

Next we move to the second step. So, now, you can see basically the 2 rings will be formed one after another, right. So, what happens the next step is actually glycine incorporation, right. So, what happens over here the product that is formed is glycinamide ribonucleotide abbreviated as GAR, the enzyme glycinamide ribonucleotides synthetase because ATP is involved and one glycine molecule is incorporated. So, what is happening this NH 2 was already start present to start with this is the entire glycine molecule that has been incorporated. So, 2 carbon and 1 nitrogen from glycine is already in, right this is step 2.

In the next step what happens if you if you remember what were the donors then you can easily remember these steps also, right. So, next what is happening a formyl group is being introduced formyl group means an aldehyde group or CHO. So, formyl transferase or transformylase glycinamide ribonucleotide transformylase it forms formylglycinamide ribonucleotide. Who is the formyl donor? Of course, N 5, N 10, methylene tetrahydrofolate 1 carbon donor. This is a 1 molecule from 1 carbon pool it is donating 1 carbon into the formation of FGR or formylglycinamide ribonucleotides simply one CHO group is attached this is step 3.

Next what will happen? Who is the next? That is formation of this there is no keto group in purine, right the basic nucleotide. So, there is an NH group. So, this glutamine actually comes in, right and the reason whenever NH group is being transferred NH

group is also referred as amide group, right. So, the enzyme is amidotransferase, right and formylglycinamide ribonucleotide becomes formylglycinamideine ribonucleotide which is abbreviated as M, ok. So, this is the step where the OH group is converted to an NH group, ok.

Basically, keto acid is becoming an amine form, right. Next, what happens? There is a ring closure, right. So, all the 5 components of the pentagon are being attributed. So, what will happen? With the help of a cyclase enzyme there is a ring closure and FGM is transformed into aminoimidazole ribonucleotide. So, now, we have got a pentagon loaded on ribose 5 phosphate.

So, we are half way. So, we need another step few steps by which the entire 6 molecule ring can be formed, ok. So, let us see what happens? A carboxyl group is introduced, ok by simple. We know carbon dioxide. Carbon dioxide actually the bicarbonate actually dissociates to form carbon dioxide and water by combining with H plus ions. So, this donor can be found as bicarbonate in multiple text book.

Basically, a COO is coming in and it is forming 5 amino 4 carboxy aminoimidazole ribonucleotide which is abbreviated as ACIR. Aminoimidazole ribonucleotide is abbreviated as AIR, ok ACIR. This step 6. So, again we have started forming another ring over here, ok. So, next step what is happening? An aspartic acid is coming in, right.

Again ATP is utilized, the enzyme is synthetized and the previous intermediate that is 5 amino 4 carboxy aminoimidazole ribonucleotide is converted to N-succinyl 5-amineimidazole carboxy amyl ribonucleotide which is abbreviated as SAICAR or SICAR, right. Now, do you remember in urea cycle where there was a synthetase enzyme where aspartate came in argininosuccinate synthetase, if you have recalled it very good because what happens in the next step is very similar to that of urea cycle. Whenever aspartic acid is coming in synthetase, the next enzyme is over there was lyase and fumarate was going out, same thing is happening over here. So, aspartate has donated its nitrogen and now it has left the remaining part has left by fumarate and it ultimately converts to 5 aminoimidazole 4 carboxy ribonucleotide, the succinyl group is gone and this is the final product which is abbreviated as AICAR.

This is step 9. We are left with what was the next donor? Again a formyl group. So, same formyl donor, formyl transfer is a transformylase, N 10 formyl tetrahydrofolic acid. It donates a CHO molecule, right and it forms just formylated AICAR or N formyl aminimidazole 4 carboxyamide ribonucleotide, alright. Again this is a 1 carbon exchange in which there is a distribution of I mean incorporation of 1 formyl group. Can you tell me what is the next step? If you have paid attention, the next step is basically the ring

closure, right.

So, all of these things have already been done. So, ultimately finally, is the enzyme is known as IMP cyclohydrolase or IMP synthase and we get the final product at the end of de novo purine synthesis which is inosine monophosphate, alright, inosine monophosphate. Now, let me tell you in majority of cases, you may not choose to remember the various intermediates. You can conceptually remember if you are actually writing all the structures down, it is very easy for you to remember, but even then the name of the intermediates might be difficult for you to remember. These are changes that are happening. These are the donors in the fifth step and the tenth step there are ring closure and in the eighth step there is no extra incorporation of any donor because fumarate is moved, right.

These are the abbreviated names of the products that are written over here and if you are finding it difficult, there is a mnemonic by which you can actually remember this penguins go fishing, flipping and amazingly swim and float in water, right. So, this is how I chose to remember, right. This is specifically how I created it. You will not find it any textbook, trust me and you may remember it in any way you like, but let me tell you the things that we will be discussing next that is the later part of this video that is purine salvage pathway that is actually more important compared to the intermediate products of de novo synthesis pathway, but what you need to remember about de novo synthesis pathway is it how it is regulated. Anyway we are not done with our purines of interest because we needed AMP, GMP because those are the adenine and guanine are the most important purines, right, but we see that they are not directly synthesized.

So, the first intermediate is IMP, right and AMP and GMP are actually formed from IMP, ok. Anyway one thing that you need to know is the processes or the enzymes of purine synthesis there are 10 steps, right and in prokaryote each reaction has been seen to be catalyzed by a different polypeptide, alright. There is no problem in that. However, in eukaryotes like many other such multi enzyme polyiodate for example, in fatty acid synthesis we saw that a single polypeptide was coding for multiple enzymes. Similarly over here there are basically three polypeptides that are catalyzing multichannel reaction, multi catalyst reaction step 3, 4, 6, 7 and 8 and step 9 and 10 that list of phenomena that is known as substrate channeling.

So, what is substrate channeling? It is actually the process of direct transfer of an intermediate between active site of one enzyme and another enzyme. So, that a sequential reaction can be done in a biosynthetic pathway that will minimize the time required for substrate to go from one enzyme to another basically, right. The active site can be located in either separate domain in a multifunctional complex or separate

subunit, but what is important is these enzymes are a single polypeptide. So, again these numbers can be formulated as MCQ question you need to choose the right combination that which enzymes are actually acting together as a single polypeptide, right. So, just as we discussed after IMP is formed it is converted to AMP and GMP.

If we look at the conversion of IMP to AMP this is often referred to as step 11, right because up actually the first 10 steps which are actually controlled by multi subunit channeling reaction are catalyzed by only first 10 reactions. So, what happens adenylosuccinate synthetase active phosphate group is involved it is GTP it forms adenylosuccinate and ultimately adenylosuccinate lies in the again common theme just like purine synthesis just like urea cycle whenever aspartate comes in under a succinate in the synthetase enzyme a fumarate generally goes out if the motive is just to donate one amide moiety, alright. So, aspartate comes in fumarate goes out the names are also same synthetase analyze and ultimately we get adenosine monophosphate, ok or AMP. Whenever we when we are converting IMP to GMP first it is acted upon by a dehydrogenous enzyme which forms xanthosine monophosphate and ultimately by GMP synthetase, ok it is forming GMP, alright synthetase, alright because there is an active high energy phosphate group that is ATP is being donated. Now the beauty of it is when we are synthesizing AMP we are requiring synthesis of adenosine is requiring synthesis of guanine and here synthesis of guanosine is requiring synthesis of adenine.

So, this will come in handy. So, ultimately AMP will be converted to GMP ADP and ATP. So, basically AMP synthesis needs GTP or ATP synthesis needs GTP and GTP synthesis will need ATP because guanosine monophosphate will also be converted to GDP and GTP, alright. This goes to formation of ATP. So, ATP formation needs GTP and GTP formation needs ATP this thing you should keep in mind where why because it is the regulation of purine synthesis which is very important and this is the committed step in de novo synthesis is actually the first step that is catalyzed by amidotransferase the first reaction where PAR was formed and it is being actually inhibited by both AMP and GMP. So, whenever it is a kind of feedback inhibition when there are excess AMP and GMP in circulation they will inhibit their own formation, alright.

Also there is formation as I told you formation of AMP from IMP requires GTP and similarly formation of GMP requires ATP there is a reciprocal control of production. So, if one is low another will not be produced and if one is high then another will be produced, right. So, thus AMP and GMP inhibit their own formation by feedback inhibition and they also inhibit formation of IMP from HGPRT, right. HGPRT is an enzyme of purine salvage pathway, right. So, this actually regulation will also be applied when we are discussing the purine salvage pathway.

Main thing is ATP and GTP are reciprocally controlling each other and also the first committed step as well as HGPRT enzyme. So, above the level of IMP production, alright. So, above this level the generally it is independent as high energy control and there is forward activation by PRPP. If there is excess PRPP there will be excess formation once PRPP is formed there will be excess formation of purine and pyrimidine. However, below the level of IMP we saw there is reciprocal control ATP is controlling GTP and GTP is controlling ATP.

So, overall the total amount of pyranucleoside nucleotide is controlled and relative amount of GTP and ATP are also controlled. Now, we move on to a relatively more important, but less complicated pathway that is purine salvage pathways. I told you one that is nucleotides are actually available we do not need to go through 10 steps to synthesize a ring, but ultimately the nucleo I mean the bases are available in the nucleotides are actually getting attached I mean formed from the bases directly who is helping in again PRPP. So, this pathway ensures recycling of purines formed by degradation of nucleotides. So, when nucleotides are degraded actually the phosphates are gone the sugar is gone and nucleobases are remaining.

So, they can be actually reused here also PRPP is the starting material therefore, the level of PRPP actually controls both the de novo and the purine salvage pathway and hence this pathway are closely related. We should note that the de novo synthesis does not happen in all organs specially in brain de novo pathway is not operating over there since this purine salvage pathway is very important becomes very essential because still the demand for IMP, GMP and AMP can be met because all of these reactions are reversible and ultimately MP, GMP, IMP do not need to be synthesized and re synthesized de novo. So, very important once we are already having some nucleotides via de novo synthesis they can be easily interchanged among itself to form the corresponding nucleotides and what are the enzymes? Hypoxanthine is converted to IMP by hypoxanthine guanine phosphoribosyl transferase HGPRT this is the enzyme which both IMP and GMP were inhibiting right. Next guanine is converted to GMP by again same enzyme hypoxanthine guanine phosphoribosyl transferase and adenine can be converted to AMP via the enzyme adenine phosphoribosyl transferase or APRT. So, basically knowing three reaction we know about purine salvage pathway.

We do you know the regulation yes we also know the regulation that both AMP and GMP will inhibit the enzyme HGPRT right. So, if there is excess AMP and GMP we do not need purine salvage pathway as well, but if there is a dearth of these compounds in nucleotides purine salvage pathway will be in motion. Now the last part of this discussion are purine analogs right how this purine analogs are actually behaving as anti cancer drugs basically we saw how the purines look like. Mine are simple modification

of these purines will lead to a very structurally similar nucleotide that will be initially falsely recognized by the system or the nucleotide synthesizing system and then these purine this altered purine nucleotides are actually purine bases are incorporated in the DNA and RNA. And once they are incorporated the further DNA and RNA propagation cell division cannot occur and thus the cell division is arrested and thus these purine analogs act as anti cancer drug.

So, specifically we can see 6-marcaptopurine whenever we are hearing the word marcapto it means replace sulphur replacement right. So, 6-marcaptopurine inhibits formation of IMP conversion of IMP to GMP and AMP. So, it is dividing the cell of all these intermediates similarly cytarabinocytosine arabinocyte where the ribose is replaced by arabinose it kills the cells by getting arrested by arresting the cell dividing cells in the S phase. Folate antagonist for example, methotrexate is an altered nucleotide inhibitor of dihydrofolate reductase hampers with one carbon metabolism. So, that this one carbon groups are not available and we have seen in many synthetic reaction one carbon reaction are as almost essential therefore, all these drugs azacirine again glutamine antagonist inhibit steps 1 and 4.

So, all of them by inhibiting nucleotide synthesis ultimately inhibits synthesis of DNA and RNA and thus cancer cells who have got an increased turnover cannot be produced and thus they act as anti cancer drug. There are many other examples, but I have quoted a few. So, to conclude we have discussed the nucleotide chemistry we have discussed the overview of nucleotide chemistry we have discussed what are the 10 rather 11 steps of de novo synthesis of purine. We have discussed what are the components from which a purine ring is synthesized we have discussed the purine salvage pathway we have discussed the regulation of both of them and we have also discussed the application of purine analogs in medicine these are my references and I thank you for your kind attention. Thank you.