[SQUEAKING]

[RUSTLING]

[CLICKING]

PROFESSOR: Today I want to discuss nucleic acid metabolism. But before getting to that, I want to take a step back and talk a bit about what we closed with last time, which was discussing serine, glycine metabolism and how they can be used to generate one-carbon units that are for one-carbon transfer reactions. And these are one-carbon transfer reactions moving carbon that's more reduced than CO2.

You'll remember, if we're going to do a carboxylation reaction, transfer of CO2, that often uses biotin, whereas if you're transferring a one-carbon unit that's more reduced than CO2, it comes from these folate species. And we described some of the different folate species-- N5-methyl-THF, which can donate a fully reduced one-carbon unit, a methyl group, to SAM, SAM being the universal methyl donor for most methylation reactions in cells.

You can also have folates carry more oxidized one-carbon units, N5, N10-methylene-THF and N10-formyl-THF. And those will be very important today as we discuss one-carbon transfer reactions and nucleotide synthesis.

Now, I recognize that folate metabolism can be very confusing. The structures are complex. The names are long and convoluted and hard to incorporate. But if you can think of it as just transferring one-carbon units of a different oxidation state, hopefully that will help you better understand what's going on in folate reactions.

And so really, all it is, remember, serine has three carbons. Glycine has two carbons. And so when you convert serine to glycine, you end up generating a one-carbon unit.

As I showed you in the lecture last time, this one-carbon unit is in the formaldehyde oxidation state. And so ultimately, that's generated. That's donated to a folate species to generate this carrier, the N5, N10-methylene-THF, which you can really think of as a carrier for one-carbon units in the formaldehyde oxidation state.

Now, those can undergo an oxidation reaction. And so if we oxidize that carbon from the aldehyde to the acid, now we end up getting the N10-formyl-THF. All right, so that's carrying the one-carbon unit as the acid. Or we can reduce that one-carbon unit to the methyl group. And that's carrying it as the N5-methyl-THF, so carrying this as a methyl group, more reduced carbon than where we started.

And so try to cut through all the names and whatnot and just realize that it's simply transfers of carbon in different oxidation states. And the relevant ones today is going to be N10-formyl and N5, N10-methylene-THF because it turns out generating those oxidized one-carbon units, formate groups, et cetera, is going to be important for purine as well as thymine synthesis.

OK, so our topic today is really to discuss how we generate nucleic acids, how we break down purines and pyrimidines or purines. And this is really the final topic here in the course. In some ways, it's a bit fitting because it really incorporates lots of aspects of metabolism that we've been discussing over the course of the semester, all come together in nucleotide metabolism.

Now, nucleotide metabolism itself is a very large topic, probably deserves more than one 90 minute lecture. But unfortunately, the time is what it is. And we just don't have time to cover every single detail. However, we can go pretty fast and at least give you a flavor of what's going on because as I've alluded to, this draws to lots of metabolism.

I drew here just a very skeletonized view of glycolysis, the TCA cycle, the pentose phosphate pathway, couple aspects of amino acid synthesis, folate metabolism, and drew boxes around all the precursors that are necessary to generate various nucleotides. And you can see it's really drawn from across these metabolic pathways.

As we go through these, you'll see that you actually know a lot. And this synthesizes, really, what we've learned from lots of other pathways coming together into understanding how cells make purines and pyrimidines. Now down here, I've color coded all the different-- so here's a pyrimidine.

Here's a purine, the ring structure that you're probably familiar with from these purines and pyrimidines-- and color coded where it is that each of these atoms come from, where they're drawn from across metabolism. And again, it's a nice way to sort of synthesize lots of the pathways that we've discussed in class.

Now ribose also is going to come from ribose 5-phosphate. That's, of course, a product of the pentose phosphate pathway, oxidative or non-oxidative, depending on how the cell wants to use those pathways-- the Calvin cycle and photosynthesis, lots of ways to get ribose 5-phosphate. That generates the ribose. And then there's a lot of complexity that goes into how one builds these complex base structures.

Now, we just want to start here and show you on the slide here, here's a general purine base. Here's a general pyrimidine base. Kind of a stupid mnemonic that's always helped me that maybe will help you if you need to remember which of these is the purine and which of these is the pyrimidine as you go through, take MCAT exams or something like that-- so the purine ring looks kind of like a pope hat.

And the pope is pure. And so it's kind of stupid, but it's one way to remember that this pope hat-looking structure is the purine. And the other one is the pyrimidine.

Of course, you're familiar with purines and pyrimidines because these generate the various bases that are used for RNA and DNA, so adenine and guanine, the two purine bases in RNA and DNA, and then cytosine and uracil in RNA, and cytosine and thymine in DNA are the pyrimidine bases in RNA and DNA.

Now, these are, of course, not the only possible purines and pyrimidines. They're not the only purines and pyrimidines that nature cares about. Here's a few other ones shown here. And so here is uric acid.

Uric acid, as we've discussed, is the way birds excrete nitrogen. We'll discuss later today that it's the cause of gout, a very common human condition. There's also lots of other pharmacologically active molecules among the purines and pyrimidines. So here's caffeine, certainly a very important molecule for many of us to get going in the morning.

There's things like adenine. So adenine plus the ribose sugar is a molecule called adenosine. Adenosine is a very important signaling molecule in our bodies. If you go to medical school, you'll use adenosine to figure out people's heart rhythms.

There's also lots of drugs that come from purines and pyrimidines. So here's an example here. This is the drug 5-fluorouracil, a widely used cancer drug. And so it's an analog of a pyrimidine. Lots of anti-cancer drugs as well as antimicrobials end up being drugs that interfere with nucleotide metabolism and often are analogs of purines and pyrimidines.

Now the first general comment I want to make is that in cells, the free bases of these things are much less useful than the nucleosides and the nucleotides. And so just to remind you of a little bit of nomenclature is that these purine and pyrimidine bases are usually attached to ribose and a nucleoside. So a nucleoside is basically the base, purine and pyrimidine base, plus ribose, but no phosphate.

All right, so adenosine would be adenine stuck to ribose, but no phosphates on it. That's adenosine. And then you have your nucleotides. And the nucleotides are the base plus the ribose plus phosphate. And so it can be nucleotide monophosphate, diphosphate, triphosphate, AMP, ADP, ATP-- examples of nucleotides.

Now, there are two major ways that cells build nucleotides. And that is they can make them de novo. So de novo is building them from scratch. Or you'll see that they can so-called salvage the bases.

And this is, you might imagine, that if as animals, we eat other organisms. We take in their RNA and DNA. We get some of these purine and pyrimidine bases that these can be salvaged-- that is, added back to ribose to generate nucleosides and nucleotides.

And you'll see, as we go through how you do de novo synthesis, that making these things is complex. It's energetically expensive. And so, of course, salvaging prevents you having to expend this energy and effort in order to generate them de novo.

I also want to remind you because it's evident in the fact that uric acid is a way that birds and reptiles can excrete nitrogen is that just back to our prior discussion, that in the world, in the biosphere, nitrogen is a precious commodity. Remember, it has to be fixed. It's not infinitely available.

Relatively few organisms can fix nitrogen into its useable form. And so salvaging premade bases is salvaging nitrogen in a usable form. And so evolutionarily, for a lot of organisms, that's also a beneficial thing to do.

All right, now another last sort of introductory comment about why this lecture ends up being important and having some appreciation of these pathways is that certainly if you go to medical school, you will come across all kinds of things having to do with the metabolism of today's lecture. And that's because it turns out that making nucleosides and making nucleotides is limiting for proliferation in all kinds of biological contexts.

And this is really back to what I alluded to earlier. A lot of our anti-cancer drugs, they also end up being anti-inflammatory. So things to treat inflammatory diseases and cancer oftentimes are things that block steps in nucleotide metabolism. Lots of antimicrobials or antibiotics also attack some of the same pathways, but are more specific for bacterial rather than human enzymes.

And so really, these drugs converge on pathways that either produce or salvage nucleotides. And it's good to have some appreciation of where these things are acting because this ends up being really important for all kinds of aspects of human medicine, as well as understanding other bits of biology.

All right, now either de novo synthesis of purine and pyrimidine nucleotides or salvage of those things, of purine and pyrimidine nucleotides, needs to add the base somehow to a ribose. And that starts with generating a molecule called PRPP, also abbreviated up there in my general overview. This stands for polyribo-- sorry, stands for phosphoribose pyrophosphate, PRPP, phosphoribose pyrophosphate.

So what is that? Well, this is, if we draw here our ribose sugar and we draw it in the alpha-furanose form, so this would be alpha-5-ribose-phosphate so-- sorry, alpha-ribose 5-phosphate.

OK, so that's ribose 5-phosphate. That's generated from pentose phosphate pathway, generated from the Calvin cycle, whatever. This is really starting point, discuss lots of ways we can generate that through different pathways.

Well, if we take ATP and convert it to AMP-- that is, transfer two phosphates, pyrophosphorylate the one position-- now we end up with this molecule. OK, so this molecule here would be PRPP, so phosphoribose pyrophosphate. And PRPP can pick up a nitrogen, either the nitrogen from the base if it's the salvage pathway or whatever nitrogen-containing molecule is going to be the first step in de novo synthesis, which we'll get to later, and basically releases the pyrophosphate.

That can be cleaved to two inorganic phosphates, pull the reaction forward. And what one ends up with is basically this nitrogen added either as the nucleoside monophosphate or some precursor to nitrogen-containing precursor to the nucleoside monophosphate that we can then build the purine or pyrimidine base on top.

And so the dogma is that de novo synthesis of nucleotides is important for new proliferation of cells or a salvage is important for maintenance of cells. This is a huge oversimplification. It's effectively untrue as far as I'm concerned.

Lots of drugs that affect salvage can affect diseases involved in proliferation and whatnot. But nonetheless, you should just realize that these early steps-- that is, adding PRPP-- is important either to salvage things or to do de novo synthesis.

Now today, given our limited amount of time, I'm really going to focus on de novo synthesis of purines and pyrimidines. I will discuss a little bit about purine catabolism. Mainly this is because these are the most topics where there's something to say about biochemistry and metabolism that builds off a lot of the themes that we've covered in the class.

They also focus on lots of aspects of relevant medicine. And so those of you who go to medical school will see this again. And I want you to appreciate it a little bit.

And particularly, some aspects of salvage are important in biology and medicine. But really, my goal today is not for you to understand absolutely every chemical step that goes on, but really to appreciate how these things happen and hopefully synthesize some of what you've learned over the course of the semester.

All right, so let's talk first about how you make purines, purine synthesis. So purine synthesis, you should know your MIT history. So the purine synthesis pathway, the de novo-- that is building purines from scratch-- was largely worked out by a guy by the name of Jack Buchanan.

He partially did this at MIT. He basically was recruited from elsewhere, came to MIT. And when he was at MIT, also was the person who really modernized the MIT biology department to be what it is today.

So prior to Jack Buchanan, biology department was much more about ecology and things like that. And really, he moved the MIT biology department into the molecular era. And so for the MIT students, it's good for you to appreciate some of our history.

All right, now purine synthesis-- as I said, I'm going to go through all the steps of purine synthesis. You'll see it's complicated, all right? I will show you some of the chemistry. I don't have time to go into great detail or explain all of the steps in great detail. But again, my goal here is for you to appreciate how purines are made, not necessarily memorize all the steps in the pathway.

All right, so purine synthesis starts with phosphoribose pyrophosphate, OK? We're going to build on the ribose base. And the first step is taking glutamine and converting it to glutamate. So what is that transferring? That's transferring a nitrogen.

Remember, the difference between glutamine and glutamate, you can pick that up with glutamine synthase, is that was one way to put an ammonia onto glutamate to make glutamine. And so this ends up carrying out the reaction that I showed you earlier and adds a nitrogen to the ribose sugar, giving you this compound.

All right, the next step is to take a glycine molecule. And that glycine molecule gets phosphorylated with ATP. So phosphorylate the carboxylic acid on the glycine molecule, and then this will combine with the prior molecule, releasing phosphate and generating this molecule, which I'm now going to just draw phosphoribose as like that because drawing ribose over and over again will become painful.

And that generates this intermediate, which is called glycinamide ribonucleotide. I'm not going to write out nucleotide. And you will see that many of the steps in purine synthesis end up having these abbreviations.

This one is called GAR for glycinamide ribonucleotide. And you'll see, as we get these long complex names, why one uses these abbreviations like GAR, which are actually used quite commonly.

All right, the next step is we're going to add a one-carbon unit, a formate group. So remember, the formate carrier comes from N10-formyl-THF. And so-- eraser-- going to come from N10-formyl-THF, all right?

So that's going to take the one-carbon unit off to regenerate THF, which, of course, can pick up the one-carbon unit again from serine and glycine, and then undergo an oxidation reaction to get the formate group, the N10-formyl-THF. And that generates this intermediate, which is called FGAR for formyl GAR, so formylglycinamide ribonucleotide, or FGAR.

All right, the next step is we're going to add a nitro-- basically swap this oxygen here on this carbonyl to a nitrogen, so a C double bond to a nitrogen bond. And that requires-- so that's going to come from glutamine again. And it requires ATP.

And it's somewhat of a complex reaction. And I think it's-- again, I will show you very briefly how this works. If you don't follow all of it, don't worry about it. But basically, if we just draw the middle part here of the molecule, I don't want to draw out the whole thing.

So basically, this is phosphorylation here. So the phosphate from ATP transferred here to give this intermediate. That will lose a water, giving this intermediate. Then the ammonia from glutamine to glutamate come here, release the phosphate.

And then typically, this is drawn where we just rearrange the double bond. And one ends up with this intermediate, which is called FGAM.

So this is this intermediate, which is FGAM for formyl glycinamide ribonucleotide, so FGAM. All right, makes sense? All right, the next reaction is also a little bit complex and maybe hard to see.

Again, I will draw it out. If you don't follow all of it, don't worry about it. But it's nice for those of you who want to follow it to see what's happening. So again, now we're going to take another ATP, hydrolyze it to ADP plus Pi, release of water.

And in the end, what we're going to generate is we're going to close this ring to generate-- so we're going to close that ring, so make a bond from this nitrogen to this carbon, close this ring. This generates this intermediate called AIR, or amino ribo-- sorry, aminoimidazole ribonucleotide. Don't worry about the long names. Everyone would just call it AIR.

So how does this happen? Well, it happens as follows. So first step is phosphorylation of-- so ATP will phosphorylate the aldehyde to give this intermediate.

Water's lost. OK, now you have this nitrogen down here, can-- losing the phosphate. OK, so that will end up closing the ring on that side of the molecule.

And then all I've done is I've just removed the electrons here so that rather than being a carbon-nitrogen double bond, it's now a carbon-carbon double bond. And that generates FGAM to AIR, costing an ATP-- again, one of the more complicated reactions to see. Again, if you're into how the reactions work, hopefully I gave you enough information to understand it. If you're not, just realize that this is the next step.

OK, the next step is a reaction that's very similar to one that we saw in the urea cycle. And that is we are going to add-- oh, sorry. The next step is one that-- sorry. Before we do that step, the next step is one where we're going to add a CO_2 group to this carbon.

So that's a carboxylation reaction. You would typically think of a carboxylation reaction to involve biotin. This is one that doesn't. It's an exception to that rule, does not involve biotin. But it does involve ATP.

OK, so remember, the way we did this before is we would have phosphorylation of our bicarbonate. So use ATP to phosphorylate bicarbonate. And so if we just draw this part of the molecule here, displacing the phosphate, and then what we end up with is-- OK, we end up with that.

And if I just rearrange so that the double bond is here instead of there, then we end up with this next intermediate, which is CAIR or carboxy-AIR, so CAIR or carboxy-AIR.

All right, now we get to the step that's very similar to the one that we saw in the urea cycle where we're going to use aspartate to fumerate conversion to add a nitrogen to this carboxylic acid. And so you'll remember that way we did that in the urea cycle is we used ATP to AMP conversion to end up adding that aspartate group.

In this case, it's just ATP to ADP plus Pi. OK, so that's going to phosphorylate this acid group. OK, so I'm not going to draw the whole thing. Phosphorylate that, and then we'll have an aspartate molecule.

OK, so this is the amino acid aspartate. OK, and one gets-- didn't leave myself enough room, but that's an aspartate molecule bound up there to that carboxylic acid, just like we saw before.

This is a molecule called SAICAR, which is succinyl-amino-imidazole-carboxy-amide ribonucleotide. OK, definitely everyone calls it SAICAR. Now just as we saw then from the urea cycle, we are now going to take off a fumerate.

OK, so this is reaction that you've seen before. OK, just like we saw in the urea cycle, so that reaction, lose the fumerate. And what you're left with is basically just the nitrogen left behind.

OK, and you get this intermediate, which is referred to as AICAR, so amino-imidazole-carboxy-amide ribonucleotide, so minus the succinate group, AICAR.

OK, next step is we're going to use a formate group and add it to this nitrogen. So that's straightforward. So if we draw out here our formate group from N10-formyl-THF, so that will be our N10-formyl-THF, generate THF.

And then we get-- OK, let me get that formate group added there. And so this is FAICAR, or formyl AICAR, FAICAR.

All right, next step is we're going to close the ring. And so close the ring, that's going to give this. OK, so that part of the molecule where the ring closes, it just rearrange like that to lose water.

And that is going to generate our purine ring the way you're used to seeing it drawn. And so this is the purine IMP for inosine monophosphate. And inosine monophosphate is the precursor for all purines.

And so if you look at this, what you see is that in the end, there is two nitrogens that come from ammonia, from glutamine to glutamate, from the ammonia released from glutamine to glutamate, one nitrogen here that comes from glycine, and that nitrogen there that comes from aspartate. Two of the carbons, these two, come from the formate group, from N10-formyl-THF.

Another carbon up here comes from CO_2. And the remaining two carbons here come from a glycine molecule, with, of course, the ribose phosphate from PRPP.

And so if you go through, what you'll find is that it took two ATP to generate the PRPP from ribose phosphate-- so starting with a ribose phosphate, two ATP to generate the PRPP, and then five additional ATPs. So if you go up and look through all the steps, there are five steps that require ATP, so quite a lot of ATP to generate this purine ring, inosine monophosphate.

Now, inosine is not found in RNA or DNA. And so this has to be, of course, turned into adenine and guanine. And so how does that happen? Well, I'll show you. And so turning it, inosine monophosphate into adenine, basically involves putting a nitrogen here instead of this double bond to oxygen.

And so that occurs via a very similar-- in fact, the same reaction, that we saw to take CAIR and generate SAICAR, all right? And so it was basically using aspartate to transfer that nitrogen. I don't have time to go through the whole mechanism. But it's basically what I already showed you.

And so you take GTP plus acetate, get GDP plus Pi. Note, you use guanine triphosphate, so GTP rather than ATP, to end up generating the adenine nucleotide. All right, it's going to release water. Water was also released in that reaction.

And what you end up with is this intermediate. So I'm not going to draw the whole structure for the sake of time. But this here would be the top here of the purine ring. So this would have a-- so that's going to add an aspartate molecule up there. And then from that, we're going to release fumerate.

OK, so fumerate comes off. And what we're left with is-- OK, and this here is adenosine monophosphate, AMP. And, of course, I can just take two phosphorylation events and get ATP.

Basically, phospho transfer ATP to make ADP, and then, ultimately, ADP to make ATP via glycolysis, oxidative phosphorylation, whatever. And so that's how you generate adenine nucleotides.

Generate guanine nucleotides, what has to happen is that we need to put a nitrogen here. OK, so putting a nitrogen on this carbon, first thing that happens is we add water across that double bond. OK, so that's this part here of the molecule, added water across that double bond.

Now we're going to oxidize this carbon-nitrogen bond, seen this a million times-- generates a hydride ion. What's that hydride ion-- or sorry, oxidize this alcohol to the ketone, so generates a hydride ion. Those electrons go to NAD+, generate an NADH, oxidation reaction. And that gives us this.

OK, and then the next step is that we're going to take that ketone that was generated here, and we're going to turn it into, basically, replace that oxygen with a nitrogen. And so that's exactly the reaction that we saw when we went here from FGAR to FGAM. All right, so if I go from same reaction that I used to go from FGAR to FGAM, so this is ATP goes to ADP plus Pi.

All right, nitrogen comes here from glutamine to glutamate, same reaction that occurs there. Water comes off. What I end up doing is I replace this. I'll end up getting this structure instead. And then I can just rearrange the double bond to be on the ring rather than to the nitrogen outside the ring. And that generates-- OK, and so this is GMP.

Notice that the ATP was used to make GMP. GTP was used to make AMP. So use the opposite purine to make each reaction. I know I went through these quickly, just don't have time to go into it in more detail.

But it's really just repurposing similar reactions that are involved in purine synthesis to end up changing this inosine monophosphate into AMP or into GMP. And I guess for consistency, I should have drawn that in yellow because the nitrogen comes from the glutamine.

OK, so that is purine synthesis. That's how you get AMP and GMP. If you're going to break these things down, of course, you're going to use separate pathways. Why are you going to use separate pathways? Well, because as we've heard over and over again in the class, for thermodynamic reasons, delta G has to be less than 0 for any pathway to work, can't be less than 0 in both directions.

One direction needs energy input. I had energy input to make these purine nucleotides. Opposite direction don't need energy to make it. I don't have time to get into all the details of purine breakdown. Although, here are some things. I just summarize it here for you on this slide.

Basically, you start from AMP. You take the phosphate off. You end up generating adenosine. You end up going to inosine, remove the ribose. Then you get another base. And the base on inosine monophosphate, when it's just the base, so the nucleoside and nucleotide is inosine, when you get rid of the ribose, now the base is called hypoxanthine.

It's a little bit of a confusing thing. But just recognize that hypoxanthine is the base that is found on inosine or inosine monophosphate, all right? And so just to summarize what I showed you what was on the slide, so to break down AMP, you'd remove the phosphate. Now you end up with the nucleoside adenosine.

So that's adenine plus the ribose. Take off the nitrogen to move back to the nucleoside inosine. Now we can remove the ribose from inosine. What we're left with is the base, which is hypoxanthine. OK, so I'll draw out hypoxanthine for you here.

OK, so hypoxanthine, you'll see is basically exactly what we drew out for inosine monophosphate. It's just the base from inosine monophosphate is hypoxanthine. Now if we oxidize this carbon-nitrogen bond, which is carried out by a complex oxidation reaction, requiring oxygen and water and producing hydrogen peroxide by an enzyme called xanthine oxidase.

OK, so the enzyme xanthine oxidase-- interesting enzyme-- has a molybdenum group. And in it, is a cofactor and iron and FAD-- a complex redox reaction we don't have time to get into-- ends up generating this purine base.

OK, this purine base is xanthine. Xanthine oxidase, same enzyme, can also use the same chemistry to oxidize this nitrogen-carbon bond. So this one here, when it was oxidized there, now we're going to oxidize this one here. And what does that generate?

That generates this compound, uric acid. And so for the birds and reptiles to excrete nitrogen, it would synthesize a purine like AMP, and then break it down to uric acid as a way to eliminate nitrogen, all right? Guanine GMP, lose a phosphate, you get the nucleoside guanosine, so guanine the base, plus the ribose with no phosphate. Take the ribose group off. Now we're left with guanine.

And then if we start with guanine, remove nitrogen from guanine, then we end up with xanthine. OK, so remember, there was additional oxidation step to make GMP that wasn't there in AMP. That means we get a more oxidized product back when we break it down.

So it's broken down to xanthine. And that can be acted on also to generate uric acid. And so you could also make GMP, and then break down the GMP to make uric acid as a way to excrete nitrogen.

Now uric acid is also medically important in humans. This is, of course, the thing that is the cause of gout. So gout is uric acid, is produced in excess, deposits and crystallizes in joints and causes a lot of pain.

And one way to treat gout is with a xanthine oxidase inhibitor. There's a drug called allopurinol that inhibits xanthine oxidase. And that ends up being a way to slow the production of uric acid and can treat patients with gout.

All right, now, if you break down purines and you want to recapture them, you can also use salvage. And so to save some time, here I drew out, basically, the salvage pathways for purines. And so you can start with adenine, or you can start with guanine. Or you can start with hypoxanthine.

And basically, these are simply added to PRPP. So if you take adenine, add it to PRPP, now you have AMP. If you take hypoxanthine, add it to PRPP, now you have IMP.

And you can use that IMP to then synthesize AMP, or not shown here, GMP, by the pathways I've already shown. Or if you have guanine, you can salvage that guanine, add it to PRPP, and then generate GMP. And so different enzymes do this.

This enzyme here, hypoxanthine-guanine phosphoribosyltransferase, which is how you take guanine or hypoxanthine and make IMP or GMP via salvage pathway is actually a very famous enzyme because it's part of an inborn area of metabolism called Lesch-Nyhan syndrome or hypoguanine phosphoribosyltransferase or HPRT.

So HPRT deficiency is what causes Lesch-Nyhan syndrome. And it's an inborn error of metabolism. You're deficient in this HGPRT enzyme, which means you can't salvage guanine or hypoxanthine.

Now I bring this up, not because we have time to talk about all of the inborn errors of metabolism, but mentioning this one really points out that we don't understand metabolism as well as we think we do. So if I ask you to predict what would be the consequences of an inability to salvage guanine and hypoxanthine, you might say, oh, well, that means that you're going to not be able to take these things up. Maybe you'll have excess guanine and hypoxanthine breakdown. And maybe you'll get more uric acid and get gout.

And that's absolutely the case. Patients with Lesch-Nyhan syndrome, HPRT deficiency, do indeed get gout. That makes sense. But the main problem they have is they have a self mutilation phenotype.

They have a mental retardation, a lot of neurological stuff. And no one can really explain why it is that one gets this complex neurological phenotype because you can't salvage guanine and hypoxanthine.

The salvage of purines, which, again, I show here on a slide, really that shows all of the different enzymes and ways here is that HPRT to salvage guanine and hypoxanthine to make IMP and GMP. There's actually quite complex enzymes that can allow you to basically interconvert all of these nucleosides with nucleotides as well as with the free purine bases.

Each of these enzymes has its own syndrome associated with it if there's humans that are deficient. Some of it causes immune system problems. There's the mental problems I talked about with HPRT deficiency.

Again, why certain enzymes and pathways are more important in specific cells and tissues, not so well understood. And really, what I want you to appreciate is that this is complicated and just realize that this happens in case you encounter it in your first future career and you want to come back and study it. OK.

All right, so we just went through the way that you can salvage purines or make purines de novo. And of course, that gives you ribonucleotides that can be used to generate RNA. If you're going to generate DNA, you then need to, instead of generating the ribonucleotides, end up with the deoxyribonucleoside triphosphates.

And what does that mean? So if we draw here, here's a-- for whatever base, adenine, guanine, whatever, basically, if we're going to generate a deoxyribonucleoside, we have to-- remember, this is the 2 position of ribose. And so we need to reduce this 2 position of ribose-- basically, reduce it from the alcohol to the saturated hydrocarbon to generate the deoxyribonucleoside.

This is carried out by an enzyme called ribonucleotide reductase. Makes sense-- we're doing a redox reaction, reducing that alcohol to saturated hydrocarbon, ribonucleotide reductase. Interesting mechanism, you can look it up if you're interested.

Ribonucleotide reductase, I drew it this way on purpose, always acts on the dNDP, so the nucleoside diphosphate-- sorry, on the nucleoside diphosphate to generate the deoxynucleoside-- sorry, deoxynucleotide diphosphate. So the nucleotide diphosphate is what ribonucleotide reductase acts on to generate the deoxynucleotide diphosphate. So you would take ADP and generate dADP, GDP, and generate dGDP. All right, and we'll come back to this in a minute for pyrimidines.

Before I get to that, I just want to quickly go through pyrimidine synthesis. And so pyrimidine synthesis is a lot simpler than purine synthesis. I also like to point out that pyrimidines are also much less abundant in cells than purines. So remember, ATP, 10 millimolar concentration in cells, 1 to 10 millimolar-- GTP, somewhere in the high hundreds of micromolar.

Purines are more-- sorry, pyrimidines are more tens of micromolar. And so orders of magnitude, less pyrimidines in cells than there are purines. And that's because GTP, ATP, those are important energy transduction molecules.

We saw a couple places where pyrimidines can be energy transduction molecules-- UTP, glucose, et cetera. But in general, most reactions use ATP and GTP as energy sources. And so whereas pyrimidines are primarily for building RNA and DNA, and so much less abundant in cells.

All right, so pyrimidine synthesis-- get my colors. So pyrimidine synthesis starts with carbamoyl phosphate. And so you'll remember carbamoyl phosphate from when we discussed the urea cycle. OK, so just as a quick reminder, so here's bicarbonate. I won't use colors to start, just for time.

So if we phosphorylate bicarbonate, we ended up getting this molecule, that bicarbonate. We can then have glutamine to glutamate conversion. That gives us-- releases an ammonia. That ammonia can release that phosphate.

So ATP to ADP plus phosphate, then gives us this molecule, which we can phosphorylate again to give us this molecule, which is carbamoyl phosphate. Nothing new here, exactly what we showed last time in the urea cycle. So carbamoyl phosphate will react with aspartate.

So here's aspartate, the amino acid aspartate. OK, and that generates this intermediate.

OK, so that generates that intermediate. This can now undergo ring closure like that. And then we end up with a molecule that looks like the familiar ring structure for pyrimidine shown here, OK?

I drew it this way because the next step, so this molecule is called dihydroorotate. And the next step is going to be to oxidize this carbon-carbon bond. If we oxidize this carbon-carbon bond, that gives us hydride ion-- oxidizing a carbon-carbon bond, just like we saw in lipid oxidation, just like we saw in succinate dehydrogenase.

This is an FAD as an electron acceptor. FAD is reduced to FADH2. This occurs as an alternative to complex II in the electron transport chain. So this would occur in the mitochondrial membrane and be a way to take dihydroorotate to orotate, carried out by an enzyme called dihydroorotate dehydrogenase or DHODH, which ends up forming this alternate electron transport chain, electrons ultimately ending up as oxygen.

And this generates the base pyrimidine, the IMP equivalent, if you will, of a pyrimidine, which is this molecule, which is this molecule called orotate. And then orotate will react with PRPP, generating a pyrophosphate, which can be cleaved to two inorganic phosphates, pull the reaction forward.

And we end up with this phosphoribose. And basically, this is going to add to this nitrogen right here. And so you're used to seeing it drawn if I add to that nitrogen, and now rotate the molecule in that direction.

And you get this molecule, which is orotate monophosphate, orotate monophosphate, which is the starting pyrimidine. And simply decarboxylating orotate monophosphate will give us the pyrimidine, uridine monophosphate or UMP. OK, and so UMP generated in that way.

Now to turn UMP into CTP, what happens is UMP is phosphorylated twice. So that's going to cost two ADP. That's going to generate a UTP. And then we're going to take that UTP, and carry out, again, the same reaction that you saw earlier for FGAR to FGAM, xanthine monophosphate to guanine monophosphate.

So I'm going to take glutamine to glutamate, transfer the ammonia, take ATP to ADP plus Pi. And basically what we're going to end up doing is changing that double bond oxygen, so double bond there, and an amino group on top. And that is how we generate the other major purine, cytidine triphosphate-- or another major pyrimidine, cytidine triphosphate.

So UTP to CTP reaction that we've already seen before gets us CTP. Now to get deoxycytidine, obviously, remember ribonucleotide reductase acts on the diphosphate, so release of phosphates. So now we have cytidine diphosphate. And now ribonucleotide reductase can generate deoxycytidine diphosphate.

Now remember, uracil is not used in DNA. Instead of uracil, we use thymidine. So there's no deoxyuracil, or you don't want to make deoxyuracil because that will incorporate into DNA. That's undesirable.

And so the way you make thymidine is you take dCDP and you further remove a phosphate from it. And now you get dCMP. And then you take the dCMP, and you convert it back to dUMP.

Converting it back to dUMP is basically just losing the nitrogen. We've seen that before when we broke down guanine-- same reaction, dCMP back to dUMP. And then dUMP is a substrate for an enzyme called thymidylate synthase, which will take uracil and add a one-carbon unit there, which is methyl group as D and generate thymidine.

So where does that come from? Well, we're going to add a methyl group. We could do that from the folate. Now you might guess that you'd use 5-methyl-THF for this, but you actually don't. Instead, what you use is this N5, N10-methyl methylene THF, which we got directly from serine to glycine conversion.

And so this then is going to add the methyl group to have dUMP. So we're going to add a methyl group there. And this is going to give me dTMP.

This is what thymidylate synthase does. But I've added a methyl group. Remember, this is a formaldehyde oxidation state. So if I'm going to add a methyl group, that means that one-carbon unit is getting reduced when it's being added. If it's being reduced, something else has to be oxidized.

And the thing that gets oxidized is the folate itself. So this is-- the tetrahydrofolate is oxidized to instead be a dihydrofolate, so double bond here. So that's oxidation. Now it becomes dihydrofolate.

And that dihydrofolate, of course, has to be re-reduced back to the tetrahydrofolate and pick up a one-carbon unit. And so this comes from NADPH to NADP+. So oxidze NADPH, reduce dihydrofolate back to tetrahydrofolate, pick up another one-carbon unit from serine to glycine conversion. And in the end, this is how you synthesize thymidine.

I went through this very quickly. But this is actually a very important reaction pharmacologically. This is an important target for antibiotics. So the antibiotic Bactrim, a very famous antibiotic, basically blocks this step called dihydrofolate reductase, dihydrofolate reductase, DHFR, basically taking dihydrofolate back to tetrahydrofolate before it can pick up a serine-glycine unit.

Effects on the bacteria-- very effective antibiotic. Also, antifolate chemotherapies-- methotrexate, inhibitor of dihydrofolate reductase, one of the first chemotherapies ever discovered, also works because it blocks thymidine synthesis by preventing your ability to recycle the dihydrofolate back to tetrahydrofolate and carry out thymidine synthesis. And so ends up being a pharmacologically, at least for human medicine, very important series of reactions.

All right, so for pyrimidine salvage, there's almost nothing to say about it. You just take the pyrimidine bases, and you add it to PRPP. And now you have your pyrimidine back. And so nothing more to say there. And really, what I want to do is close with a few comments about regulation of nucleoside and nucleotide metabolism.

And so obviously, if you're going to synthesize RNA and DNA, you need to have the right ratios of nucleotides. So if your RNA polymerase is making an RNA molecule and it doesn't have the right base, it can't keep going. And this is particularly a problem for synthesis because remember, and as you will learn or have learned in cell biology, you have to license your origins of replication before you start DNA replication.

And that prevents you from ever replicating the same amount of DNA twice, meaning you say, here's where you start. And once you start, you can't stop until you finish DNA replication. Well, this becomes a tremendous problem if you're trying to replicate your DNA and you run out of nucleosides or nucleotides.

And so there has to be complex mechanisms in place to make sure that cells always have the right levels of nucleotide triphosphates and deoxynucleotide triphosphates. And so there's complex feedbacks that we could give a whole lecture on. But for the purposes of today, I just want you to be familiar and know that they're here.

And they also all make sense. They fit exactly with what we've talked about in other pathways. And so shown here on the slide is the control of purine synthesis. And so this makes perfect sense.

So if you have plenty of purines, meaning you have a lot of AMP, GMP, IMP. Well, what do you do? Well, don't charge the ribose 5-phosphate to PRPP. Or don't carry out the first step in purine synthesis where you take that ammonia from glutamine and take PRPP and add it to the PRPP to begin purine synthesis. And so all of these purine monophosphates inhibit this early steps of purine synthesis, makes sense.

What if you have an imbalance in your AMP and GMP pools? Well, the way you prevent that, remember, we already discussed that to turn IMP into AMP, you use GTP as an energy phosphate donor. And to turn IMP into GMP, you use ATP as an energy phosphate donor.

So that's one way you regulate this. But the other thing is if you have enough AMP, well, don't try to make AMP. And if you have enough GMP, don't try to make GMP. And so there's feedbacks there as well that add to balancing the AMP and GMP pools.

What about pyrimidines? Well, pyrimidines-- simpler synthesis, simpler regulation. If you have enough pyrimidines, enough CTP, don't do the first step where you take aspartate and carbamoyl phosphate and generate that first step here in pyrimidine synthesis. dNTPs, obviously, as I said, is particularly critical because once you start replicating your DNA, you always have to have the right balance of deoxyribonucleotides.

And so ribonucleotide reductase, that enzyme up there that basically balances and synthesizes your deoxyribonucleotides, it has incredibly complex feedback mechanisms that one could spend a whole lecture talking about. But effectively, it has various feedback mechanisms with allosteric sites and other feedbacks that are still, honestly, being worked out, that, in the end, is a way that helps you balance to make sure you have the right levels of all the different deoxyribonucleotides in the end.

All right, if you're interested more in the feedbacks of ribonucleotide reductase, these are covered well in your textbook. Any of the topics that we didn't have time to cover that might become relevant for your future endeavors in biology, medicine, metabolism, whatever, all of those are well covered.

And hopefully, if you could absorb some of the concepts that I tried to impart to you about how metabolism works, you can understand those pathways better. Hope you enjoyed 7.05. Hope you enjoyed learning about metabolism. And thank you very much for your attention.