JOANNE STUBBE: What I want to do today is finish up module 7 on reactive oxygen species and then move on into the last module, which we are obviously not going to get completely through. We're going to be focused mostly on purines and maybe some pyrimidines. And I'll give you a big overview of what I think the things are you need to think about in nucleotide and deoxynucleotide metabolism as a starting point.

OK. So we've been talking about module 7 and, in this section, how you control reactive oxygen species for signaling. We were going through the generic overview. And at the end of the last lecture, this is the system we were talking about using epidermal growth factor receptor, which we've now looked at quite a bit as an example.

But what I wanted to point out is that it's not limited to epidermal growth factor receptor. So you have insulin growth factor receptor, nerve growth factor signaling, VEGF, IL-1, IL-4, et cetera.

And all of these things are all distinct. They all have different signaling cascades. But the generic approach that we've been looking at in the Kate Carroll paper is also, I think, applicable to these other systems.

And so what I wanted to do was just make one more point with this, and then what I'm going to do is summarize the general principles of post-translational modification by anything-- we're using post-translational modification by sulfenylation and then briefly come back to the methods used. But we spent a lot of time in recitations in 11 and 12 focused on methods, so I'm not going to spend very much time on that. It's also in your PowerPoint handouts.

So the key thing here is the general-- is we have EGF, OK, so that's Epidermal Growth Factor in the membrane. We have epidermal growth factor receptor, which you all know has to dimerize and you all know, at this stage, is a tyrosine kinase.

And the key thing we're going to be focused on is if we modify these proteins, what is the

biological consequence, OK? Do you have any biological consequence? And if you don't, it's probably just an artifact of the fact that cysteines react rapidly with hydrogen-- not rapidly, but they react with hydrogen peroxide at some level to give you modification.

So this is all, I'm just going to say, tyrosine kinase activity. We've already gone through that. And what happens is you activate the NOX proteins. And in this case, it's the NOX2 isozymes. And this is outside, and this is inside the cell.

And NOX2 can generate superoxide-- OK, so let's just put this in parentheses-- which can rapidly generate hydrogen peroxide. And so the issue is that the superoxide and all of the hydrogen peroxide needs to come from the outside of the cell to the inside of the cell. OK.

So we have hydrogen peroxide. And what is hydrogen peroxide doing? So the model is-- and this is what we've been focusing on-- that the hydrogen peroxide can modify the cysteine by sulfenylation, OK? So we can go from SH to SOH.

And in the case of the tyrosine kinase and in the paper you had to read, it turns out that tyrosine kinase by activity assays was more active. So it's phosphorylated. It's sulfenylated. That leads to higher activity. That means it's potentially biologically interesting.

And we also, in the Kate Carroll paper, looked not only at the activity, but we looked downstream at the signaling pathways, and we saw signaling as defined by phosphorylation events. We saw more signaling. So those are the kinds of peak criteria people are looking at for being biologically interesting.

Now, what we also have is a key control, and, in these cascades, like over there, we also have PTP. And that's Protein Tyrosine Phosphatase. And these proteins all have a cysteine at the active site. We talked about this before.

And the cysteine at the active site, what can it do? It can really sort of dephosphorylate the tyrosine kinase. And if you remove the phosphate, the activity is lowered. OK.

So again, you have something that activates, something that removes it. But what we also know-- so this is the active form, and this is the key in all these signaling events. And so what we also have-- so let me go over here, since I didn't leave quite enough room. So we have PTP that can also react with hydrogen peroxide to become sulfenylated. That's the inactive form.

So when it's in this state, basically, you put a roadblock in this pathway. So this is inactive. OK. And the Carroll paper spent a lot of time trying to define-- there are lots of protein tyrosine phosphatases inside the cell-- not anywhere near as many as kinases. So one protein tyrosine phosphatase services many proteins. But both of these guys are regulated by sulfenylation.

And there's one third thing, and so this is just giving us the big picture now. If you have hydrogen peroxide in the cell, I've already told you that there are enzymes that can degrade hydrogen peroxide-- peroxiredoxins. And so that removes the hydrogen peroxide, which then prevents these things from happening.

So you have peroxiredoxins, which I already talked about. And so the hydrogen peroxide concentration goes down. So that's another mechanism of control. OK.

So the take-home message is shown in this slide. It's shown in the papers you had to read. And there are many proteins that have some variation on this theme, and this is a really active area of research to look at this in more detail. OK. Yeah?

AUDIENCE: The tyrosine kinase activity, [INAUDIBLE] 160% or something. I was just wondering how they actually classified that as [INAUDIBLE].

JOANNE STUBBE: Active? So, I mean, in biology, that's a huge effect.

AUDIENCE: OK.

JOANNE STUBBE: So, I mean, to somebody that's doing something in the test tube, a factor of two is nothing. In biology, that's all it takes.

So the question is, is it enough? And you should always ask that question. And then you've got to look at the consequences, and you do more experiments. If you hadn't seen any effect, well, maybe you didn't have the right proteins in there, and you need five more proteins to assay, which would give you a bigger effect.

OK. So that's the issue with all of these problems. That particular experiment, if you go back and look at it, was done in crude extracts, OK. And the activity is extremely low. They had to use a luciferase assay to be able to measure this and amplify the signal, OK, which probably has a lot of issues with-- can have a lot of issues.

So if you're not happy with that, then you're going to have trouble in biology. So the question

is, what is the baseline? How much slop do you have? And then you have to do the experiments many, many times. It's all a question of statistics.

And then do you believe it? And does the rest of the community believe it? So that's a good question. But if that's what they saw, that's what they saw. And their interpretation was, based on this and other-- they did a lot of experiments in the paper, and that's why we chose that paper-- suggested this is a good working hypothesis.

So I'm one of these people, you always start out with the simplest working hypothesis. You do experiments. It always gets more complicated, always.

And then you expand it, or you change it. There's nothing wrong with that. That's what science is all about.

So NOX. We've been talking about NOX. We talked about it for the last couple lectures. And I had already told you that there were seven isozymes of NOX. OK.

We had focused on this guy in the phagosome. We now are focusing on NOX2 again. And this guy is also important, but this guy is not. That's the phagasome oxidase.

So you're changing the factors inside the cell that govern what happens. And so each one of these guys-- you Google it, you find another 100 papers on this. People are trying to understand the details of what's going on with these systems. OK.

So that sort of just shows you, again, with generic, it affects a lot of growth factors, or a lot of cytokines can use these signaling pathways that NOX is important in. Many of them, in the model, sulfenylation is also important. But in many cases, that remains to be established. OK.

So what I want to briefly do, then, is look at the general principles of regulation. OK. And I'm just going to briefly outline these.

And we've gone through each one of these examples in the two recitation sections. So I'm just sort of reviewing this and making a point.

So is post-translational modification important? OK. And I think your generation needs to think about this, because as the methods become more and more sophisticated, OK, we've got really amazing mass spec methods if you can figure out how to do them correctly.

Everything, almost any metabolite in the cells, can modify a protein. Acetyl-CoA, it acetylates

things. S-Adenosyl methionine, the universal methylating agent, methylates things. OK.

So you have hundreds of modifications on your protein, OK, and it is, in fact, related to, in part, I think, the metabolites interacting with the proteins not enzymatically. The question is, is it interesting? So I want you to think about that.

So that's why the general principle is, what are you going to use as a control? You can see it. Are you going to spend five years of your life chasing this? Or is it not interesting?

So you need to think about that question. It's not an easy question. And that's what everybody is into now. That's the future for the next five years.

So one of the things we see is that-- and I told you this 20 times-- it needs to be reversible. OK. So in our case, it doesn't matter whether it's phosphorylation, dephosphorylation, acetylation, deacetylation, methylation, lots of the methyl group. OK. Ubiquitination, deubiquitination. We seen many examples of this.

It needs to be reversible. In our case-- and this is related to, again, oxidative stress-- so this is forward-- how do you reverse this? So you need a reductant.

And this could be any one of a number of things. There are lots of reductants inside the cells. So I'm just going to say reductant. I've used thioredoxin here, but this has not been identified in the case of the NOX2 system in the epidermal growth factor receptor.

We've already looked at this. You can convert this back to the cysteine. This is reduced. Something else needs to be oxidized. OK.

So that's a basic thing that you need to think about. OK. The second thing, which I think is very important, and I think this is a general principle used in biology over and over and over again, is increasing the effective molarity. OK. And so we say increasing effective molarity.

And why is that important? Because if you have two things reacting-- here, we have the things reacting. Here, we have two things reacting, hydrogen peroxide and a protein. So if we can generate them right next to each other, the concentration is much higher. The rate of the reaction has to be faster. OK.

And so how do you increase the effective molarity in the case of the epidermal growth factor receptor? We looked at this. These guys were in the membrane, and NOX interacted with the

epidermal growth factor receptor by the immunoprecipitation experiments that we looked at in the recitation. So an example is, I'll just say, NOX dot EGF receptor immunoprecipitation. OK.

So another way that-- and we're talking about signaling-- nature has used over and over again is, lots of times, we have these little G proteins, GTPases. And these GTPases can be in the cytosol.

But a lot of the time, they do all of the signaling at the membrane. How do you get them to the membrane? Anybody got any idea?

So these things move around inside the cell. Localization becomes really key. How would you get a little, soluble G protein to the membrane?

AUDIENCE: Through post-translational modification, like a GPI anchor.

JOANNE STUBBE: Yeah, so you would put an anchor on it. And what do you use as anchors? You can use isoprenes. So farnesylated, geranylated are frequently used. You've seen that in the first module, module 5, of the second half. And you also can put a fatty acid on there. It's used over and over again.

So the prenylation reaction, people have been looking for the reversibility of that for a long time. And as far as I know, no one's found it. But the fatty acid, which is put on usually as a thioester or as an amide, you can hydrolyze it off.

So what you can do, then, is have-- let's just use fatty acid whatever. So you have CoA fatty acid. And so this gets modified. And then this goes to the membrane.

So what that does, then, is it takes it out of solution under a certain set of conditions. So you've modified your protein, just like we had by sulfenylation. And you bring it to wherever the proteins are it's interacting with. So you're increasing the effective molarity. OK.

So this happens all the time. Putting that into a big picture related to what I'm going to say next is, I think, incredibly important. So this is a general principle, but one that needs to be studied or described in a lot more detail.

So the third thing is the post-translational modification must have a biological phenotype. So this is the question that Shiva was just asking. If this is increased by whatever, 50%, is it interesting? Is it important? OK. So you need to do additional experiments if you don't think,

based on what you know about the system, that that's true.

And so in the case of the NOX system, what do we use? Remember, we talked about this. We did two things. We have increased activity of the tyrosine kinase. And then we also had increased downstream signaling.

And how did they look at that? We looked at that by phosphorylation. So we used antibodies to serine phosphate. OK. So by those two criteria, the NOX system in the Carroll paper was interesting.

And then the fourth thing that I think is also really important is relating to this one. Whatever the signaling agent is, if you have ways of removing it, you then decrease the signaling. OK.

And so this is frequently observed in many of these systems. So enzymes can modulate the concentrations of the signaling agent.

And the example I used up there with the NOX system-- so we're looking at hydrogen peroxide, and I'm not going to draw the structures out, because I've already done this before, the peroxiredoxins. We've gone through that two lectures ago. Something can remove that signaling agent. OK.

So to me, these are the key things you need to think about if you're looking at whether you think your post-translational modification is interesting or not. And a lot of people are doing that. So we see lots of modifications because of the power of mass spec. The question is, are they interesting?

And so finally, the only other thing I wanted to say here is in the last little section. And I'm not going to look at this in any detail, either. But if you look at methods-- so this is the last-- how do you look at this?

So what you saw in the Carroll case-- and again, it's not unique to the Carroll case-- is you need to develop a reagent that's specific for the post-translational modification. So number one, you need to develop a reagent specific for post-translational modification.

It needs to be specific. It needs to be fast. So the kinetics are important under physiological conditions. And it needs to be cell permeable. OK.

Because ultimately, with something like hydrogen peroxide or NO or many of the other

signaling agents, these guys are really reactive. And you crack open the cells, and you do things out, and you add more oxygen. You can change the levels of modification all over the place. So you really want to look at this contained within the cell under controlled growth conditions. And this is what the two papers we looked at by Carroll were focused on. OK.

So you have a reagent. Hopefully, you believe dimedone was a good reagent. OK. So I'm not going to-- but NOX for NOX, sulfenylation, we use dimedone. OK. We discussed this. We've discussed the mechanism.

And then what we looked at is MS analysis and how you had modified the reagent so it worked effectively inside the cells so you can enrich. And then use modern methods. We break down the protein into peptides and sequence doing this MS, MS. OK.

So I'm not going to talk about that more, because we had two whole recitations on these topics. OK. So that's what I wanted to say in this module on reactive oxygen species. Reactive oxygen species, I think, are front and center. You can't pick up any journals or even listen on the radio or newspapers, if you read newspapers, without seeing reactive oxygen, reactive nitrogen species. I think you now know what you need to think about.

And here's an example-- reactive oxygen species can modify cysteines. Cysteines, you've seen over and over and over again, play central roles in enzymatic reactions and control of signaling pathways. And I think the growth factor receptor is a good example of that, of the kinds of things you need to do to try to determine whether these modifications, which are everywhere, are really, in fact, real. OK.

So that's what I wanted you to get out of this little module. What I want to do now is move into the next module. And the next module, last module, module 8, is going to be on nucleotide metabolism.

How bad am I? Oh, good. I've got lots of time. All right. OK. So let me just erase something so we have some place to start.

So nucleotide metabolism is something that, in our introductory course, we don't talk about at all, because we just don't have time, and we just focus on glycolysis, sugar biosynthesis and degradation, fatty acid biosynthesis and degradation. But you all know, and I'll show you that, nucleotides are everywhere.

And so, in my opinion, nucleotides had their heyday when I was your age. Everybody and his

brother was focused on nucleotide metabolism. The data is really old. We learned how to make nucleotides back in those days.

But we didn't have any of the tools we have now. We used a T60 rather than an 800 megahertz machine to look at [INAUDIBLE]. I mean, you had to take the spectra 20 times to remove the spinning sidebands.

Anyhow, we didn't have any of the modern methods. But everything back in those days was correct, because people really cared about the truth back in those days, as opposed to publishing in *Nature, Cell,* and *Science.* OK. So that data, if you want reproducibility and you go back in the literature, is absolutely going to be reproducible. OK.

So I'm going to show you where we are. But I would say, in the next decade, it's going to be the era of nucleotides. But what we need is ways of looking at nucleotides inside the cell.

And I'll show you the complexity of this. But nucleotides are everywhere. They control everything. OK. And we really don't know that much about regulation. And to understand regulation, you need to be inside the cell.

I can tell you what all these enzymes do. I know a lot about the enzymes. But the question is, how do they work inside the cell? And how are they regulated?

So I'm going to try to give you sort of a picture of what the issues are and teach you something about pathways, because a purine pathway, to me, is sort of an amazing-- it's not erasing-- it's sort of an amazing pathway. And in fact, one of my heroes, when I first moved to MIT, is Jack Buchanan, whose picture is on the first slide. He was still here.

And I just remember talking to other people. He was older than me. I think he was probably 75. And he was just my hero. I mean, if you read his papers, it's totally mind boggling what the guy did with what he had. OK.

And everybody was dumping on him, because he had moved into the state of the art back in those days. OK. But if you took what he did in perspective, he'd done so much more than all the people that were dumping on him. It drove me nuts. So I used to have fights with everybody when I got here, telling everybody what a great scientist this guy was. And I'll try to point out why I think he was such a great scientist when we look at the pathway.

Anyhow, the purine biosynthetic pathway, we'll see, was elucidated in pigeons. He used to

catch the pigeons in the Boston Common. And then I'll tell you why. They have a different metabolism of excretion than humans do, and so you could feed the pigeons N15.

This was back in the 1940s, 1950s. You could feed them stable isotopically labeled nitrogen stuff. And we'll see purine's got nitrogens all over the place.

And then you isolate the poop and then characterized it. And that's how we unraveled the pathway. OK. All right. So where am I? All right. I just want to make sure I'm in order.

So reading. So what I've assigned you to read in 5.07, people haven't done nucleotide metabolism. So we put it online for the chapter on nucleotide metabolism from Voet and Voet. There's a lot of stuff in there that's not right, but it gives you sort of an overview.

And you can take it out of any book if you use Stryer or if you use whatever. You can use any book you want. It just sort of gives you a big picture. And the picture keeps changing, and the books don't keep up to date. OK. I gave you an article to read by Benkovic, which is a review not just focused on the papers that we've talked about and we'll talk about today in recitation.

And so what I want to do is, after introducing you to the nomenclature, I'm going to give you a general overview of nucleotide metabolism, focus a little bit on the biology of purines. Then we'll talk about the pathway and why I think the pathway is interesting.

And we were going to close with this section, which is what we're doing on today. One of the reasons I talked about this is because I think this idea of purinosomes, complexes of transiently interacting proteins, has captured people's attention for decades. And when this paper came out in 2008, it was one of the first examples where people thought they might have gotten evidence inside tissue culture cells-- so it's still in vitro-- to show that these transient interactions of pathways play another regulatory mechanism inside cells. OK.

So that's where we're going. OK. So nomenclature. OK. So many of you probably have seen this before if you took 7.05 instead of 5.07. I guess they taught in-- did they teach you in 5.07 nucleotides? Any of you have Ting and Klibanov? Didn't they teach you about nucleotide metabolism? I thought they taught about DNA replication.

AUDIENCE: They talked about DNA replication.

JOANNE STUBBE: Well, how can you talk about DNA replication without knowing what a nucleotide is? Sorry. All right. So anyhow, I'm not going to draw. I'm not going to draw these structures on the board.

But this is like the amino acids. I think you should know the nucleotides, OK? People hate me for the amino acid side chains, and the pKa is something else you can dislike me for.

But anyhow, these are the bases. The names are not so easy to remember. But, I mean, it's central to all of genetic material. So it's pretty darn important, no matter what kind of a biologist, biochemist you are.

So we're going to be looking at the purines-- adenine and guanine. So these are the bases-- thymine, cytosine, and uracil. OK.

And if you take the base and stick on a sugar-- OK, so this sugar is ribose-- you now have the nucleoside. OK. And this is in the introductory-- if you don't know this, you should read the first few pages of Voet and Voet, and they'll introduce you to this nomenclature again. But you can come back to your notes. So I've redone these notes again, and I will repost them again-- whoops-- putting in more detail, because I didn't really know what your backgrounds are.

So this is something that I think-- so we have adenosine, cytidine, guanosine, uridine. What about thymidine? Why don't I have that up there? So this is a take-home message from the next few lectures.

AUDIENCE: Because they're [INAUDIBLE].

JOANNE STUBBE: So these all have [INAUDIBLE], two prime, three prime sys hydroxyls. There is no ribothymidine. OK. You only have deoxy. OK.

So thymidine, some people write "deoxy." That's redundant. It is deoxy. Thymidine is deoxy.

So this hydroxyl is replaced with a hydrogen, OK, on thymidine. So that becomes really important in connecting nucleotide and deoxynucleotide metabolism, because you have to get from the nucleoside to the deoxynucleoside. And it's not straightforward. OK.

There are many, many steps. The metabolism is complicated. And I'll show you one of them. But every organism is slightly different. OK.

So one of the things I want you to remember is you have bases, and you have bases in the sugar. Those are the nucleosides. These are the bases.

And in DNA, you have T, or, as in RNA, you have U. So you need both uridine and you need

thymidine in DNA as the building blocks for DNA biosynthesis. OK.

And what we're going to do-- and this was, again, developed mostly from the work of Jack Buchanan's lab a long time ago. And you don't need to remember this. But what pigeons excrete is uric acid. And so this is the molecule they isolated from pigeon poop, OK, which allowed them to tell, ultimately-- which is the key to these isotopic labeling experiments-- the source of all of the different atoms in purines. OK.

And we're going to come back to this. But what I want you to see-- this is true in both purines and pyrimidines. And what we're focusing on, what we're going to be focusing on, is de novo purine biosynthesis. But what I'm going to also show you, of course, is you have salvage. So you can get purines from the diet your DNA breaks down, your RNA breaks down. So all of that stuff can then be used, as well.

And so it's a question of de novo, and it's a question of salvage. I think it's really underappreciated how important salvage pathways are. And now, with mass spec and isotopic labeling, we can actually figure that out fairly recently. And people interested in making chemotherapeutics are finding, really, sort of things nobody ever expected in terms of how much comes from salvage versus how much comes from de novo. OK.

And the salvage is easy to understand. I'll show you. That's chemically simple. The de novo is much more complicated. OK.

So anyhow, it's these labeling-- we'll come back to this in a minute. But I think this is important. All of these atoms come from simple building blocks. And you'll see that when we look at the pathway.

So glutamine. Glutamine is the major source of ammonia in all metabolic pathways. How does that happen? I'm going to show you. That will be one of the generic reactions I talk about, because the same approach is used over and over again by nature. And the nitrogens play a key role in these heterocyclic purines and pyrimidines.

Glycine. We'll see where glycine comes from. Aspartic acid, formate, and bicarbonate. OK. So you can't get much simpler than that. And most of you probably know these all self assemble, allowing you to maybe think about the evolution of this process. You can throw them all together, and you can get a purine out the other side with varying degrees of success. OK.

So that's a purine. So what I want to do now is sort of give you an overview. So I've introduce

you to the nomenclature and what the purines are going to be. But I want to give you an overview to nucleotide metabolism in general. OK.

There's a lot of stuff, so the way I'm going to do this is up and down. OK. So you need a piece of paper, if you're writing this down, that goes up and down. OK.

So what's central to everything is phosphoribosyl pyrophosphate. OK. So this is a central player. So this is PRPP.

And in your recitation and also in your handout, I've given you the horrible names that are involved with the purine pathway. If we have a test, I will give you the structures of the purine pathway. I don't expect you to remember the details of the purine pathway. It's complicated, and I'm not sure I would have designed it that way to start with. So it's not like it's so logical, like some of the other pathways, which are straightforward. OK.

So where do you think phosphoribosyl pyrophosphate comes from? Does anybody have any idea? What did you do learn from basic metabolism? This is something that's covered in most introductory courses. Where does PRPP most likely come from?

AUDIENCE: Out of the pentose phosphate pathway?

JOANNE STUBBE: Yeah, out of the pentose phosphate. So the two things that play a really critical role in nucleotide metabolism are the oxidative and non-oxidative pentose phosphate pathway where you form ribose biphosphate and NADPH. OK.

So over here, we have ribose biphosphate. And for phosphate, from now on, and for pyrophosphate, from now on, I'm going to abbreviate it so I don't have to draw the structures out. But the chargers are important, so you need to remember the structures that are charged.

So this is ribose biphosphate. 1 prime, 2 prime, 3 prime, 4 prime, 5 prime. OK.

Let me ask this question. Why do you think this is the major form of ribose inside the cell? I don't know if they teach you this or not, but I think it's important. Why is ribose always phosphorylated inside the cell?

AUDIENCE: To keep it in the cell?

JOANNE STUBBE: To keep it in the cell. But what happens if you don't phosphorylate it? Yeah, to keep it in the

cell. Phosphates keep things inside cells. What happens to that structure when it's not phosphorylated?

AUDIENCE: The [INAUDIBLE] it can open.

JOANNE STUBBE: So it can open. Is that what you see inside the cell?

AUDIENCE: No.

JOANNE STUBBE: No. What do you see? What kind of a sugar is this if you look at-- it's a five-membered ring sugar. What's that? Anybody remember that?

This is what happens. I digress, and then we don't get to finish the course. So it's a furanose. OK.

If you ring open this thing, then it can close. It either forms a five-membered ring or a six-membered ring. It forms the six-membered ring almost all the time. That's a pyranose.

So it's not in the right state. So then you have to have your enzymes. And there are enzymes that do this that can catalyze back into conversion into the ribose form.

So phosphorylation plays-- it keeps it inside the cell, which is incredibly important. But it also controls the state with which you want to deal in metabolic pathways. OK.

And we're going to be talking about-- hopefully, we'll get this far. I'm not sure. But if we start with glutamine, then it'll abbreviate like this. Bicarbonate and aspartate. OK.

So those are the same three things I just told you were involved in making the purine ring. These are also involved in making the pyrimidine ring. So what we're looking at now is de novo pyrimidine pathway. OK.

And what we'll see if we do this is that we have-- skipped a number. So there are four steps. And you make the molecule called orotate. OK.

And what we're doing now, which is going to be completely distinct from the purine pathway, is you make the base. OK. So you make your nucleotide base. Let me go back. You make your nucleotide base first. And then you're going to stick on the ribose biphosphate. In purine biosynthesis, you make the base on the ribose biphosphate. So the strategy is distinct. OK.

So here, we have no base on it. So what happens here is it interacts with the phosphoribosyl

pyrophosphate. We'll talk about this reaction, because it's a major way you use salvage proteins if you get a base. How do you put them back together to form the nucleoside?

It allows you to form OMP. OK. So OMP, we're not there yet. OMP, we'll see, can get converted. It loses CO2. We'll look a little bit. The chemistry in this pathway is really pretty simple.

So this is enzyme five. This is enzyme six. So you lose CO2, and you form UMP. OK.

So UMP is one of the nucleotides we need to actually make RNA. To make DNA, we need deoxythymidine. OK. And we also need deoxycytidine.

So this pathway does not give us cytidine. And so the way we go from UMP to the cytidine monophosphate is complicated. OK.

So you're going to see there's a couple little-- central to everything is sort of straightforward. But then you'll see it's going to be organism specific. And there's a lot of messing around you have to do with kinases and hydrolases to get you into the right stage to get you all of the building blocks required for RNA and DNA biosynthesis. OK.

So I'm going to go over here. And I'm going to say many steps. And we'll look at this to form CTP.

And this does not go through CMP. OK. So there are many steps here. And so let's just put a question mark there.

Also, we need to have TTP. And again, there are many steps. And we're going to have to figure out how to do this. OK.

So it's not simple to get from UMP to CMP or deoxy TMP. OK. So I'm just telling you where you're going to see the complexity in the end. OK.

So phosphoribosyl pyrophosphate is central in what it does. I'm not going to have enough room to do this. But anyhow, there are 10 steps.

And you've already seen this if you had recitation on Thursday. Or hopefully, you read the paper. This is the purine pathway de novo. OK.

And so what we're doing is we have the sugar. And so in every single step in the pathway,

what you're doing is you're building up the base. OK. So you're adding it. So that's why there are so many steps. And I showed you whatever on the first slide or maybe the second one where all of the pieces come from.

So again, let me just emphasize this. These all come from small building blocks. Let me do that over here. So you have glycine, bicarbonate, aspartic acid, and formate. OK.

So the other thing from PRPP is salvage. And the salvage pathways are really important when you're scarfing up bases that are provided by the diet or from breakdown of DNA and RNA. So you have the salvage pathways. OK. And so this can come from the diet or from nucleoside or tide, tide being having a phosphate on it break down.

And why is this important? It's important because many organisms like parasites, like malaria parasites, don't make purines. The only way they can get purines for anything is from salvage pathways. It's a major target focusing on anti-malarial and, in some cases, antiviral systems.

So here, we have 10 steps. And at the bottom of this, I'm not going to draw the structure out. We don't get to AMP and GMP, which is what we were looking at in the previous slide. We get to IMP. OK.

And then IMP, that's a branch point. IMP can get converted either to GMP and AMP. So those are the two purine nucleotides that we need as building blocks to make both RNA and DNA. So we end up over here with AMP and GMP. OK.

So when you get that down, there's one other thing I want to say up on the top board. And that's to introduce you to a co-factor that many of you probably haven't thought about before, which I plan to talk about. And that's folate.

So any of you think about chemo therapeutics, folates have been around for decades. And it's a major target, successful target, of drugs that are used clinically in the treatment of a wide range of cancers.

So folate, this is a key co-factor. And what I will show you is that it can do chemistry. It does one carbon transfer, so one carbon at a time. And what's really interesting about it, and I'll draw the mechanisms out-- it can transfer the methyl-- it can transfer one carbon in an all oxidation state in the methyl state, the aldehyde state, and the acid state.

So for example, in the purine pathway over here-- I'm just going to draw this out-- you need it

in this state, the acid state. OK. In this state, what you're going to see is you need it in this state. We'll come back and look at this again. Sorry. Methylenetetrahydrofolate, which is a key player.

So this is going to end up being the methyl group and thymidine. OK. The only interesting co-factor chemistry in the purine pathway is folate. And folate plays a central role in therapeutic design. OK.

So then we're down here, and we still haven't gotten finished. How are we doing? All right, I'm over.

So just let me say this. So now, you're into kinases. OK. And there are lots of different kinases. So the kinase story gets complicated, but it's extremely important.

So if you're going to make deoxynucleotides, you have to have it in the diphosphate stage. So there are kinases that can convert these guys and also the pyrimidines from over there into NDPs. OK. So we're going to have to think about kinases.

And in all organisms-- again, this is de novo-- deoxy NDPs are made by ribonucleotide reductases. OK. So this is the only way, de novo, that you could make deoxynucleotides. If you think about the substrates for DNA replication and repair, they need to be triphosphate. So again, you need kinases again.

So I'm going to stop here. I will finish off the last half to get this to go back together. And we will talk about folate metabolism, introductory and folate metabolism, so I don't sort of digress. And then we're going to look at the purine pathway and things that I think are interesting about the purine pathway.