JOANNE STUBBE: So what I want to do is finish up purines today and talk about some interesting aspects of purine metabolism. I hope I'm going to be able to get through. I've given you handouts for pyrimidines and deoxynucleotide biosynthesis as well.

The pyrimidines are pretty straightforward, much simpler than the purines. And so I think if I have time today, depending on when I get finished, I might talk a little bit about deoxynucleotide metabolism, since both Drennan's lab and my lab, both work in that area. So it would be good for you guys to know what's going on in the department and it's central to nucleotide metabolism.

We started out-- we were drawing this. This is my notes that I tried to reproduce for you to look at. And I'm not going to read. So this was a big overview slide where we're going. And so central to everything.

This wasn't in the original packet, but I will put this up. I'll try to get a better version of that. But PRPP is central. And we are talking about de novo purine bio-synthesis, but again, not only is de novo important, so is salvage. It depends on the cell type.

You know, if you have cancer cells that are rapidly growing or B cells and T cells, de novo becomes really important. In other types of cells almost everything is salvage. And so I have that PRPP, at least in the purine case-- and I'll show you an example of that in a few minutes-- goes directly-- can make your nucleotide directly. That's a salvage pathway.

And we'll see that the de novo pathway, which is what I was describing at the end-- and you've already seen this in recitation from last week-- is 10 steps to get to IMP. But then you need to get to GMP and AMP. And I showed you how all of this branches off with the cofactor folate between purines and pyrimidines. And in the end, we need both purines and pyrimidines.

We need it in the nucleotide levels. So two hydroxyls, the two prime three prime cis hydroxyls, which in the diphosphate stage, that's also unusual. Most of the time you don't see high levels of diphosphates inside the cell, either they're monophosphates or triphosphates.

So part of the complexity I think of nucleotide metabolism is figuring out where the kinases are and the phosotases are. And you'll notice that I've avoided that. And that's because every organism is different and every cell type is different, and the regulation is a little bit different. But I think it's important to realize that to make deoxynucleotides, which are required for DNA replication and repair, is done at the diphosphate level. So you make deoxynucleotides, but they still have to be converted to deoxy NTPs for DNA.

And over here you need to again, make NTPs for RNA. So that's sort of the big picture. We have a purine pathway de novo. We're not going to be able to talk about pyrimidines, but the salvage pathway with pyrimidines is extremely important.

It's a major target of cancer therapeutics now. I think only in the last few years has it been realized that in many cancers you have both pathways going on. And it turns out now with isotopic labeling and mass spec, metabolomics is coming into its own. So you can tell actually by feeding the cells this is all done in tissue culture. But you can tell by feeding the cells whether the deoxynucleotides came from de novo or whether they came from salvage.

And so we're getting a really different view of nucleotide metabolism. And as I said in the very beginning, I think the next decade we're going to understand a lot more about how all these things interact and the kinases and phosatases that put the nucleotides into the correct phosphorylation state. So that's key to everything and it's complicated.

So what I want to do now is briefly talk about the purine pathway. So we can look at the biology. I'm not going to write this down, because most of you already know this. So we'll just go through it again.

Purine nucleotides are central to everything. So knowing where they come from and how you control them is really pretty important. And we don't understand that much. So I mean, NTPs and dNTPs are central to our genetic material. So we need to get them and we need to control them.

If these levels become imbalanced, you have mutater phenotypes in DNA replication. And so fidelity of deoxynucleotide DNA replication is really important and regulated by ribonucelotide reductases.

Building blocks for cofactors. We've seen flavins. We've seen NAD. We've seen CoA. None of

this is an accident.

Adenine can self-assemble from cyanide formate in the prebiotic world. And so that's why they're central to everything. So they're in a lot of the cofactors we've already talked about is they're not necessarily the business end, but they've got the phosphates and the adenines stuck on to end, which presumably helps in some way for binding.

We're using GDP and ATP everywhere in the course of this semester. You've seen it in your macromolecular machines that you've talked about, especially in the first part of the course with the translational and protein folding and protein degradation all require energy ATP. We will see in today's pathway and today's lecture on purine biosynthesis de novo, it turns out 5 out of the 10 enzymes use ATP. So we'll see.

And what you will hopefully now will know is what ATP does. I'm going to show you two examples. But you see the same thing really over and over and over again. So this should be sort of-- you might not know whether it uses ATP to get at the gamma position-- chemistry at the gamma position, or at the alpha position but the chemistry is the same over and over again. And so that part hopefully is part of your repertoire now, about thinking about the role of the ATP on primary metabolic pathways.

And we've also seen in the last-- in the reactive oxygen species section, we are signaling by many mechanisms, signaling by phosphorylation is all over the place. And a lot of people are trying to understand. And I think one of the futures is how do you integrate signaling and primary metabolic pathways? And we're almost there.

I decided-- I wrote a lecture on this and decided-- it's really still very phenomenological. But all of these key regulators and signaling linked to purines and pyrimidines in some way. I think the linkages aren't totally clear, in my opinion.

So why else do we care about purines? When I was your age, purine and nucleotide metabolism was front and center. Why? Because people were successful at making drugs based on these molecules. The central role it plays in replication and repair has made them successful targets at many different levels.

Here, this has both purines and pyramidines, but I'll just pick out a few. This guy acyclovir is what we use as an anti-herpes medicine. In fact, I think I've taken it.

Here, mercaptopurine cures childhood leukemia. Clofarabine is something that's been studied

my lab. It's a drug that-- it's not particularly effective, but it's used clinically against certain hematological cancers. And so these are all anti-metabolites not focused on signaling, which is what everybody is focused on.

In reality, I think the success-- if there is success against cancer-- is going to be mixing the two. I think you need combinations of metabolic inhibitors. They're toxic. So is everything. But somehow figuring out how to use multiple approaches to avoid the resistance problem, which is a really important problem.

And to combine the two once we understand the interconnections better, I think is where it will be to get successful, more successful. Therapeutics, but ultimately what we would like to do is catch it in the bud, rather than waiting to try to treat something where it's completely out of control.

So I'll just show you one of my favorite ladies, Gertrude Elion. She worked at Burroughs Wellcome for many, many years. She went to Hunter College, as did many-- in New York City-- as did many outstanding women scientists.

And she was involved at Burroughs Wellcome in discovery of mercaptopurines, acyclovir treating herpes, and AZT. She made several contributions. Never had a PhD. So anyhow--

So what I also wanted to show you, we're going to talk about de novo pathways. I just want to show you this is a typical-- in the case of the purines-- salvage pathway. So what does that mean?

You get the bases, the nucleic acid bases from your diet. Or you're breaking down your DNA and your RNA. You have nucleic acid bases. Or you have nucleosides.

So can you take those and make them into the right components to do RNA biosynthesis and DNA replication, make ATP, et cetera. And so here's an example of hypoxanthine reacting with our central phosphoribosyl pyrophosphate, which I had in the original slide that I talked about last time to make, in this case, the nucleotide. And why is this interesting?

It's interesting because it turns out that many parasites like in malaria don't have any purines. So where do they get their purines from to replicate the DNA? They have to use salvage. So the salvage pathways have-- for treatment of those things-- have become front and center.

Can you make specific inhibitors of phosphoribosyl pyrophosphate reaction with the bases?

And we're pretty good at that actually. Vern Schramm's lab has done some beautiful work. And there's a lot of things in clinical trial targeting salvage pathways.

So again, there's something different about the metabolism of us and whatever is invading us. That's not true in cancer. So cancer is a much tougher problem, because you get normal cells as well. It's a question of what the therapeutic index is.

So that's all I want to say in the introduction to the biology. And then I want to talk about one cofactor. And then I'm going to talk about the pathway itself.

So there's one cofactor, which I sort of told you I was going there in the first place. Let me break this down over here. So the one cofactor that I wanted to talk about is folate. Let me also show you.

You don't have to sit and look at this. But I'm going to show you it's all written out. So you don't have to bob up and down. It's all written out on the handout. So this is folate.

And let me just point out a few things. This is going to be the business end of the molecule. So I want you to know where the business end of the molecule is. I don't expect you to remember the structures.

But what does this sort of look like? Anybody? This is the kind of chemistry-- I mean, I think there's a bunch of heterocyclic chemistry that you find in biology that most of you haven't been exposed to. And it's not intuitive what the most reactive positions are. This cofactor is much simpler than flavins, which we very briefly talked about before.

So this has a polyglutamate on the end. So this is folate. And what you really need to know is that this is 5, 6, 7, 8. And this is 10. So the active part of this cofactor is here.

So everything's going to happen at either N5-- if you have a copy of this, you can just circle N5, N10 and N5. That's where all the chemistry is going to happen. And it turns out the way this cofactor works-- so this is 1, 2, 3, 4. And so this is 4a, and this is 10a, 8a. It sort of looks like flavins.

And it sort of looks like pterins. And pterins actually can undergo redox chemistry under certain sets of conditions. These molecules are only involved in one carbon transfer.

So the major focus is one carbon transfers. And it can do it in the methyl state, in the aldehyde

state, or it can do it in the acid state. So all three oxidation states from one carbon transfers.

And so then how does it do it? And the chemistry actually is fairly simple compared to the chemistry that we've looked at before. And we looked at a little bit at hemes. We looked at a little bit at flavins. This is much simpler.

And so what we're after in the end-- and I'll show you how we get there-- so here's N5 methyl. And we'll see this is tetrahydrofolate. So this ring is completely reduced. And so this is tetrahydrofolate.

And this can undergo oxidation and reduction. And that becomes very important in the pyrimidine pathway to form thymidine, which is a major target of fluorouracil, which is a drug that's still used clinically. Anyhow, this is the reduced state here. So this is where the tetrahydro is.

So both of these can be oxidized. And that would be folate. So you can make dihydrofolate, folate, and tetrahydrofolate. And the oxidations occur here and here.

And we're not going to look at that, because we're not going to have time to look at pyrimidine metabolism. But the dihydrofolate plays an important role. It's the target of methotrexate. If you have rheumatoid arthritis, you take methotrexate is one of the drugs that people take nowadays.

So what's unusual about this-- and this is key to the purine pathway, it's also key to the pyrimidine pathway-- that's why folate have been central. People made folates for decades. Even when I was your age, people were making folates for treatment therapies in cancer.

And it's been successful. In fact, and if you've gone to Princeton's chemistry department, the whole department was funded on an anti-folate that Ted Taylor made 25 or 30 years ago. And they've tried it again under different conditions, and it's now being used clinically.

So how does this work? So we have this oxidation state. We have this oxidation state. And then we'll see that this can ring open.

And so this would be the aldehyde state. And this can hydrolyze. And that would be the acid state. So I'm going to show you in a second, I'm going to walk you through where those different states came from. So methyl state, aldehyde state, acid state.

So there's the model, because I like to have the windows open, you probably can't see the model very well up there now. But you can pull it up on your computer if you want. I'm going to write out the model. So we start out over here with tetrahydrofolate. So this is tetrahydrofolate.

And we have nothing here, which you notice was we could have something at N5, something at N10. We'll see the methyl group is always at N5. It could be at either, chemically, but it's always at N5. We'll see the aldehyde group is always at N10. It could be either chemically, but it's not.

So these are going to be the key stages. And here we have no carbons. So somehow we have to get the carbons into the molecules. So we start out with this molecule, tetrahydrofolate.

So what happens is you can start out here, and use formate. So formate is going to be the source of the one carbon in this case. So the names in this pathway are again, horrible, just like the purine pathway. And on the next slide I've written out the names. So it turns out that one enzyme can do three of these activities.

So this is one of the enzymes. And so this is activity one. And it attaches a formate, so they call it a formate ligase. The names again, in my opinion, are horrible.

But what it allows you to do is-- so what I'm going to draw out now is not the whole structure. I'm just going to focus on the business end of the molecule over here, and skip this ring over here. But that ring is there, and is key to making all of this work. So I'm just going to do this like that. And so what can happen is that you can formulate and form.

And so this is now N10 formal tetrahydrofolate. So this is N10, and we'll call this R. So that's the first step. That's the enzyme. The same enzyme catalyzes the next step.

And what you can picture happening here, if you watch me, is this nitrogen is juxtaposed to this imid. So can attack to form a tetrahedral intermediate and then lose a molecule of water. So that's called a cyclohydrolase. So this guy is attacking. And then you have loss of water.

And this is a cyclohydrolase. So this is the same enzyme. So this is two. This is one. But they're both on the same polypeptide.

So there are three of these on one polypeptide. You've seen that before in recitation last week. And so now what you've formed is-- and again, this is a cyclohydrolase-- now what you're formed is this structure.

So we've lost a molecule of water. So you can draw NR. And so if you hydrolyze this, you can get back to the aldehyde stage. So if water adds here, this is an iminium system. Water can add, it can collapse, it can ring open, it can ring close.

So the chemistry here-- we're going to see some really similar chemistry actually, because we can use N10 formal tetrahydrofolate in two steps in the purine pathway. So this chemistry I'm drawing right now is related to the pathway in general. And so this is called 5, 10 methylidine-- the names again, are horrible-- tetrahydrofolate.

And then the third enzyme in this pathway is a dehydrogenase, so DH. And so what you can imagine you could do here is we have an iminium system. And NAD pH is the reductive. So you can reduce this down to methylene tetrahydrofolate. So this can be converted to an NADP.

So this is the dehydrogenase. We've seen that used over and over again. This is the same enzyme. So this is also MTHFD.

And I've given you the nomenclature on the next slide. So if you want to look at-- So this is tetrahydrofolate whatever. So it has of formal ligase, it has a cyclohydrolase, and it has a D hydrogenase all on one enzyme. And so what do you generate then? You generate-- so this is methylene tetrahydrofolate.

And this is the key player in pyrimidine biosynthesis, which we are going to talk about. And it's an enzyme called thymidylate synthase, which makes thymidine, which is a major target for drugs in the treatment of cancer.

So now you can even take this a step further and reduce this further. We're still now here. If you ring open this, you're at the aldehyde stage. You can reduce the aldehyde stage down to the methyl group. And that's then getting us into the methyl state, the aldehyde state, and the acid state.

So I think when you sit down and look at this, it looks complicated at first. It's really not that complicated. So this can just ring open. And conceivably, it could ring open in either direction. It depends on the enzyme that's catalyzing it.

But we always get N5 methyl tetrahydrofolate. That's what's used inside the cell. People don't find N10 methyl tetrahydrofolate, but chemically, that could happen.

So what happens? This is now a new enzyme. And again, it's a dehydrogenase. So NADPH is going to NADP.

So this is a new enzyme. I'm not going to write out the name. But this then reduces this to N5 methyl tetrahydrofolate.

So what we've done then is, in the pathway I've drawn out here is, where do we get the one carbon from? Here, we got it from the formate. And we can change the oxidation states to get all three of these oxidation states, depending on what we need to do with it. You have to have the right enzymes and the right complexes to be able to make this all work.

Now many of you might not recall this, but in the Benkovic paper you read for recitation last week, one of the controls with this tri-functional protein. And it does not exist in the purinosome. Benkovic's been interested never in these enzymes, and channeling of reactive intermediates in these systems. This does not exist in the purinosome.

So then the question is how do you get back? And so there are three methylating agents inside the cell in a biology. Does anybody know what the other two are? So this is unusual, N5 methyl. So this is N5, this is again, N10.

STUDENT: [INAUDIBLE].

JOANNE STUBBE: So S-adenosyl methionine is probably the most prevalent. What's another one?

STUDENT: Methylcobalamin.

JOANNE STUBBE: Yeah. So methylcobalamin. So S-adenosyl methionine

is the universal methylating agent inside the cell. And then you also have-- I'm not going to draw the structure out. We're not going to talk about it, but methylcobalamin. And there's a single enzyme that uses all three of these methyl groups. And if I had another five lectures, I would talk about this enzyme.

This was studied extensively by Rowena Matthews' lab, who was one of Cathy's mentors. And then Cathy was involved in getting the first structures many years ago with the little pieces. So it's one of these enzymes. It's huge.

And it's got to juggle these three methyl groups to do the chemistry. It's really sort of

fascinating. And so what it does is it takes homocysteine-- so this is homocysteine-- and converts it to methionine.

I'm not going to draw the structure. So you methylate it. So you're going to methylate that cysteine. And then you're back to tetrahydrofolate.

So there's another important reaction that I just want to put in here is that there's another way to go from tetrahydrofolate to this one, which is methylene tetrahydrofolate. And so in addition to being able to put on the one carbon with formate, does anybody have any idea what another major way-- it's probably the major way of doing one carbon transfers from metabolic labeling experiments? It comes from an amino acid. What amino acid could you use?

So somehow we want to get from here to here. This is also a major target of therapeutics. Anybody got any ideas? We need to get one carbon out of an amino acid. What did you say?

STUDENT: Thymine?

JOANNE STUBBE: Thymine? That's not an amino acid.

STUDENT: Thiamine.

JOANNE STUBBE: Oh, thiamine.

STUDENT: Methionine.

JOANNE STUBBE: Oh, methionine. No. See, I guess I'm deaf. OK, I didn't hear you. No, that's, not it.

So I'm not going to spend a lot of time, but serine-- so this is ours-- I'll draw this out, because I think this is really important. This can be converted into formaldehyde. Does anybody know what the cofactor would be that would do that? And then what you end up with is glycine. So this is the major way-- serine is a major one carbon donor.

So seramine is going to generate the formaldehyde equivalent, which then can get picked up here and make methylene tetrahydrofolate. Anybody have any idea of how you would convert serine into glycine? You do learn about this cofactor. What is the cofactor that works on all amino acids, if you want to do something to it? There's only one.

STUDENT: PLP.

JOANNE STUBBE: PLP, yeah. So this isn't unusual-- PLP is sort of an amazing cofactor. It can do alpha

decarboxylations, racemizations. It can do aldol reactions.

And then it activates the beta positions so you can do beta eliminations replacements. It can do probably 10 or 15 different reactions. This one is unusual in that what you're doing is you're doing an aldol reaction. So you're cleaving that bond, and a reverse aldol reaction in this case.

And then the other thing is if you want to link this into pyrimidines, you have dihydrofolate. So this is dihydrofolate. And that's a major player in pyrimidine metabolism to make thymidine. I'm not going to have time to talk about this.

But folate is a central player in both purine and pyrimidine metabolism. And people have spent a lot of time thinking about it. And I think the chemistry of interconversions, once you sit and walk through this yourself, start over here and see if you can draw out the mechanisms. It's the same mechanisms we've seen over and over again, in addition to a carbonyl and loss of water.

So that was the introductory part. And really what I want to do now is-- we can put that up here for those who still want to stare at it-- what I want to do now is talk about the pathway. And what I want to do is write out the pathway, and then use a PowerPoint to talk about a few features of the pathway that I think are the most interesting, and that you can make generalizations to other pathways, like, what is the role of glutamine? That's universally conserved. What is the role of ATP?

And we're going to see the roles you see in the purine pathway are used in many metabolic pathways. So those are the ones I decided to focus on. So what I want to do is go step by step and just make a few comments.

And then I'm going to use a PowerPoint over here so you can see what I have written down. I'm going to write down a few things.

So that's the nomenclature. There's the pathway. We will start there.

So I told you that the first step in this pathway is we start with phosphoribosyl pyrophosphate. That's central to a lot of things. It's chemically very unstable. It falls apart. It's hard to isolate. And the first step in this pathway-- we talked about this briefly in recitation-- is to make phosphoribosylamine.

So the interesting thing about this pathway-- so is you start out-- and again, the nomenclature,

I've written out. On the exam, you probably will have something about purines there. I will give you the pathway, and I will give you all the names and the enzymes.

So you don't need to memorize that. I'm probably the only one that knows the names, because I've worked on it. Very confusing.

So what's unique, again, and we've already mentioned this, is you start out with ribosyl phosphate. And what you're going to do-- and this is what we're going to walk through-- is that the first thing you do is you build up the imidazole moeity of your purine. So using sort of basic metabolites in ATP-- there are five steps out of the 10 that use ATP-- you make this amino imidazole ribonucelotide.

And then what you do again, step by step, is convert this into the pyrimidine moiety. So you make your purine. So that's a step, one step at a time. And this was unraveled using metabolic labeling experiments.

So the first enzyme I'll spend a little bit of time on, because I think it's a paradigm for many enzymes in metabolism in general, where do you get ammonia from most of the time? The major source of ammonia is glutamine. So that's something that you see in this pathway.

So glutamine-- you all know glutamine has this part in this side chain-- is going to glutamate. And so you form glutamic acid. And the ammonia from the amid is going to interact with phosphoribosyl pyrophosphate, which is always bound to magnesium to form phosphoribosylamine.

And so I'm now going to start being sloppier. Instead of writing phosphate here, I'm going to have a phosphorus with a circle around it. That means we always have the five prime phosphate.

And furthermore, what I'm going to do is replace all of this with an R group, ribosylphosphate is present at every single step in the pathway. And in fact, one of the reasons I thought this pathway was interesting, every enzyme in the pathway has to have a binding site for ribosylphosphate. Well, have any of you ever thought about how metabolic pathways evolved? Where does it come from? You have these really complicated pathways.

Where do you start? How do you think about that? Well, this might be a fantastic place to look at that. Why? Because you might have a ribosyl binding site for everything.

So maybe it starts with something that binds ribosylphosphate. Anyhow, this is an unusual pathway in that you have something. You have a really good handle on to hang on to.

And as we already talked about-- so this, we're going to call R-- what's unusual, there are a couple things I want to say about this. But we already talked about this a little in terms of channeling and this question of why you would ever want to have clustering enzymes. And that's because the half-life of this is about 10 seconds at 37 degrees. So it took a lot of effort to see this thing.

I mean, you couldn't see it by normal methods. People inferred its presence because Buchanan actually was able to see the next intermediate in the pathway and inferred the existence of this. And many of these intermediates in the pathway, which is why Benkovic focused on this, are chemically unstable. Let's see if I have one of these. I don't have it in this pathway.

Anyhow, I'll show you another one, which has a half-life of five seconds or something like that, that took forever for people to identify it, because when you try to work it up as a chemist, it falls apart. And I would say this is something any of you get into metabolomics, people are looking for metabolites now. There's one metabolite that people have found quite frequently, and it seems to be involved in regulation of glycolysis. It's this one. See, where am I?

Aminoimidazole ribo-- this one. And that's because it's stable. And the lot of the other ones are not very stable. So I wouldn't be surprised if you ended up finding a lot more metabolites that are playing a central role in regulating enzymes in primary metabolism, because where does the serine come from? Does anybody know where the serine comes from that plays a key role in making this folate analog?

Anybody have any idea? So serine is three phosphoglyceric acid in the glycolysis pathway. It's actually very straightforward to write a mechanism of how you get there. Intimately links the glycolysis pathway to purine metabolism.

And we'll also see here of course, this is folate, but we also need glycine. That's the next step in small molecule in this pathway. It needs glycine. So everything is integrated. Once you see-- you sort of see the big picture and have central pictures of primary metabolism, everything becomes much more integrated.

So how does this happen? So what I want to do is I want to talk a little bit about this enzyme.

So here, let me just talk about this this. So if we call this Pur F, just so we have a name, Pur F is called an amidotransferase.

And what it's going to do is it's going to take glutamine-- and it turns out these enzymes have a domain. They always have multiple domains. And the domain that uses the glutamine can be the same. There are actually two different convergent evolutions of glutamine binding domains that do the same chemistry.

So what you do is-- we've seen this again many, many times-- so you form a covalent intermediate, which then hydrolyzes to glutamate regenerating ESH. And what happened during this reaction, you generate ammonia. So the goal of these amidotransferases in general, in many, many metabolic pathways, is to generate ammonia.

And so to me, what's striking about this is the way nature evolved these metabolic enzymes that generate ammonia. And so what you see in a cartoon view-- so we are always going to have all of these enzymes. They may be a single polypeptide. They may be two polypeptides, but they all have a glutaminase domain. So the glutaminase is just generating the ammonia.

But what do we have? We start out with phosphoribosyl pyrophosphate. So once we generate the ammonia, what can happen? You can now by-- it turns out by dissociated mechanism-- displace a pyrophosphate to form phosphoribosylamine. So all of these kinds of reactions involve dissociative rather than associative transition states.

That's not important. But what it what's amazing about this is that PRPP, in this case, binds to one domain, and the glutamine binds to this second domain. So ammonia, what would happen to ammonia if it went out into solution?

STUDENT: Protonated.

JOANNE STUBBE: Yeah. Gets protonated really rapidly, becomes unreactive. I don't know why nature designed this. But what you see with all these enzymes is she makes a tunnel across the domain interface that's about 25 to 40 angstroms long. So the ammonia this released never gets out into solutions.

This is another example of channeling a reactive intermediate, which we talked about as potentially a reason for channeling in the purine pathway. So there's a tunnel. And the tunnel can be 25-- we have a number of structures in the ammonia channels.

And I have no idea-- I mean, this surprised the heck out of me. I thought the way nature would hold on to this is by hanging on to not the covalent intermediate, but the preceding tetrahedral intermediate. And then when the white substrate was there, release it and then bind it, sitting right next to it. But nature, in all designs, has done this thing where you have this channel. And here is an example of Pur F.

This is the glutaminase domain up here. And here is where the phosphoribosyl pyrophosphate binds down here. You can't see the channel, but this is work of Jan Smith a number of years ago, was the first one that showed the channel in this pathway. So that's common.

And we're going to look at another glutamine requiring enzyme in this pathway. It's the fourth enzyme in the pathway. Also is a channel. Again, it's distinct. It all does this glutaminase covalent intermediate, but the structure of the glutaminase domain is distinct.

So what's the next enzyme in the pathway? So the next enzyme in the pathway again, is a paradigm for many, many unsungs and primary metabolic pathways. And if you look at the structure, let's just go back to the pathway.

If you look at this pathway, what you now want to do-- so we keep the ribose 5-phosphate all the way through the whole thing. That's the scaffold. Now what are you going to add? You're going to add glycine. So here is your phosphoribosylamine. And you're going to add glycine.

How do you inactivate an amino acid? You've seen activation of amino acids now many times. What are the two ways you can activate amino acids?

STUDENT: [INAUDIBLE].

JOANNE STUBBE: So either adenylate or phosphorylate. So that's a paradigm that you see over and over again in nature. This enzyme uses ATP.

This is one of the five enzymes. And it forms inorganic phosphate. So you're phosphorylating, not adenylating. And so I'll show you what the mechanism is up there. You've already seen this mechanism, but the idea is you phosphorylate this.

You're going to form the phosphoanhydride. And then the phosphoanhydride can react with the amino group. And kinetically-- this is something that's one of my students working on a long time ago-- there was evidence that this intermediate, which is chemically unstable, could channel between the two proteins. So you don't generate this own solution where it can fall

apart and it can anomerize. It gets transferred directly.

In fact, in the early days when we invented the first biochemistry labs at MIT, they used this system. I really pushed them to the limit, because they were dealing with the substrate. They had a very short half-life Anyhow, they learned a lot from the exercise.

So what you're going to have then is ribos-- I'm just going to call it R. And so here's our glycine. Whoops. Guess I'd better get the structure right.

So this is from lysine. So what we will see is that this is another-- we're not getting very far-- but this is a member of the ATP grasp superfamily of enzymes. They all do the same chemistry. So let me just move forward a little bit. I'm not going to draw this out.

You guys have seen this chemistry many times. So what's happening in this chemistry is you have a carboxylate. ATP phosphorylates it, and then you attack by a nucleophile, in this case, the nucleophile is the amino group of phosphoribosylamine.

So what I just want you to see here if you look at this, there are four enzymes that are involved in purine metabolism that all have the same structure. They all have ATP grasp structures. They all go through phosoanhydride intermediates. And you can, from bioinformatics, pick these structures out. So this is again, an example.

Once people defined-- there's almost a no sequence homology between these proteins-- but by knowing this chemistry, you can actually pick out that these are going to be family members. And if you know if they are organized in bacteria and operons, you can even guess at the substrate. And then you can test this model that they go through phosoanhydride intermediates.

And I'm over, but the next step in this pathway-- the next step in this pathway-- we're going to come back. And what are we going to use? We're going to use N10 formal tetrahydrofolate. That's why I went through this. We're going to put a formal group here.

And again, the chemistry is just the same. Go home and think about the chemistry of how you generate all the different oxidation states of the carbon. And then I think you can see the chemistry in this pathway actually is pretty simple, once a few basic reactions. So the ATP grasp family is interesting. The amidotransferase and the channel is interesting as being general in metabolism.