ELIZABETH NOLAN: So last time, we were talking about these aminoacyl tRNA synthetases that are responsible for attaching amino acid monomers to the three prime end of tRNAs. And we were looking at the isoleucyl aminoacyl tRNA synthetase as an example, looking at experiments that were done to study mechanisms. So recall, we left off having discussed a two-step model, where there's an intermediate, an amino adenylate formed.

And then, in the second step, there's transfer of that amino acid to the tRNA by the aaRS. And so we looked at some data from steady-state kinetic experiments. Recall that a C14 radiolabel was used to watch transfer, and then we closed discussing an ATP-PPi exchange assay which gave evidence for formation of that amino adenylate intermediate. Right?

And then, lastly, we talked about use of a stopped-flow to do experiments that allow you to look at early points within a reaction. And so what we're going to do is to close these discussions of experiments and this aaRS mechanism is just look at one more experiment that was done to further probe the rate-determining step of this reaction using the stopped-flow. OK? And so this experiment pertains more to reaction kinetics, and the question is, let's monitor transfer of the amino acid to the tRNA by another method here.

These experiments were set up in two different ways depending on what components were mixed. And if you just rewind to Monday and recall the ATP-PPi exchange assay and the steps in that assay, in that we showed that the amino adenylate intermediate remained bound to the enzyme there. Recall then only PPi was released in that assay. And so in these experiments, the fact that the amino adenylate can remain bound was taken advantage of. And the researchers were actually able to have a preformed complex there, so basically starting after step two.

So in experiment one, how I'm going to show these is by drawing the two syringes and listing the components of each syringe. And this is a good way for setting up problems within the problem sets, thinking about stopped-flow experiments. So the question is what are we going

to mix? So we have syringe one and syringe two, and recall that these go to some mixer. So the two solutions can be rapidly mixed, and that's where the chemistry is going to happen.

So in experiment one, in syringe one, what we have is the purified complex. OK? So we have C-14 labeled isoleucine-AMP bound to the aminoacyl tRNA synthetase of a purified complex, here. And then in this other syringe two, what we have is the tRNA. OK? So imagine these are rapidly mixed. There'll be transfer of the radiolabeled isoleucine to the tRNA, and so formation of that aminoacyl tRNA can be monitored. OK?

In the second experiment, we have just theme in variation, and if you're interested in more details, the reference is provided in the slides. So again, in syringe two, we have the tRNA, and in syringe one, what will be combined are the components here. OK? So then, the question is, in each case, what do we see? And those data are presented here from the paper, and there's some additional details about the experimental setup.

So effectively, what we're looking at on the y-axis is the amount of tRNA that's been modified. So tRNA acylation measured by transfer of the radiolabel versus time. And in the black circles, we have the data from experiment one, shown here, and in the open circles, we have the data from experiment two. So what is the conclusion from these data? And this value here is not similar to something we've seen before in this system.

Both experimental setups are giving the same result. Right? Effectively, these data are superimposable, and they can be fit the same. So what does that tell us about the rate-determining step?

AUDIENCE: [INAUDIBLE] versus forming the intermediate.

ELIZABETH NOLAN: Yeah. Right. Aminoacylation of tRNA is the rate-determining step. So some of you suggested that in class on Monday. Right? So that's the case here. OK? So formation of the intermediate is much more rapid than acylation of the tRNA here.

So we've examined now the mechanism in terms of getting the amino acid onto the tRNA. What do we need to think about next here? So what we need to think about is fidelity. OK, and we've looked at the overall rate of error in protein biosynthesis, how often errors occur on the order of 10 to the 3.

So how is the correct amino acid loaded onto the correct tRNA? Each tRNA has an anticodon that is a cognate pair with a codon. And so different tRNAs need to have different amino acids

attached. OK, and what does that mean? That means, in general, there's a dedicated aminoacyl tRNA synthetase for each amino acid, in general here.

So how are amino acids with similar side chains differentiated by these enzymes? And is it possible for an incorrect amino acid to get loaded onto a tRNA? And if that happens, what are the consequences? So we're going to examine fidelity some here. And as background, an observation made, say from studies like that ATP-PPi exchange assay, is that some aminoacyl tRNA synthetases can activate multiple amino acids, so not only the one they're supposed to activate but also others.

So what does that mean? That means that the enzyme can bind and activate effectively the wrong amino acid, and if we think about fidelity, we can think about this as being a problem here. So what happens? What happens is that these enzymes have an editing function, and they're able to sense if a wrong amino acid is activated. And then they have a way to deal with it, and this is by hydrolysis. OK?

And so let's consider an example, for instance, just similar side chains. So if we just consider, for instance, valine, isoleucine, and threonine, these will be the players for our discussion. OK? They're different, but they're not too different. Right? Oops, sorry about this. We're missing a methyl.

Valine, an isoleucine, we have a difference of a methyl group. Threonine, we have this OH group. Right? And we can just ask the question, for instance, how is valine differentiated from isoleucine or threonine here? And so as an example, what's found is, if we consider our friend that we studied for the mechanism here, what we find is that this binds and activates isoleucine, as we saw, but it will also bind and activate valine here.

And effectively, if this happens, we have a mismatch, because the end result will be isoleucine-RS with valine AMP bound here. OK? And what's found is that the catalytic efficiency or Kcat over Km, in this case, is about 150-fold less than the native substrate. So that doesn't account for the 10 to the 3 error rate here. So we need more specificity. So what's going on?

So we're going to consider this editing function and a model that's often used to describe how these aaRS do editing is one of two sieves. These enzymes don't actually have a sieve. It's just a conceptual way to think about it.

So this double-sieve editing model involves a first sieve which is considered to be a course

one. So imagine if you have like a change sorter. It will let the quarters through as well as the and dimes and the pennies. There's some sort of discrimination of amino acids based on size, and then depending what gets through this first sieve or gate, there's a second sieve which is considered to be a fine one.

And this one can differentiate perhaps on the basis of size or maybe on hydrophilicity or hydrophobic of the side chain. So effectively, if an incorrect amino acid passes through this first sieve-- so in other words, if it binds to the enzyme and becomes activated-- hydrolytic editing will occur. OK? So think about hydrolysis in terms of having breakdown of these species. So if the incorrect amino acid passes through and is adenylated, there'll be hydrolysis.

So let's consider some examples so the first example here we can consider this guy and isoleucine and valine. So as I mentioned, this aaRS will activate both. So in this case, the first sieve can't differentiate isoleucine from valine. They have similar sizes according to this aaRS. But then what happens here in the second sieve, isoleucine is too big, and so there's no hydrolysis, and it moves on to form the desired charged tRNA.

In contrast, valine's a bit smaller. It passes through the sieve, and it ends up being hydrolyzed. So these aaRS also have an editing domain, and this editing domain, as we'll see in a few slides in a structure, is responsible for this hydrolysis, so stated here. Right? Different sites, so there's an aminoacylation site and an editing site here. So valine can reach the editing site, but isoleucine cannot.

So how do you predict? Just to keep in mind, every enzyme is different in terms of the model for discrimination and also when editing occurs. So you really need to look at the data when the data is presented to you to sort out how this works. Let's just look at another example with a cartoon depiction.

So this is for the valine RS, and we're going to consider the three amino acids here-- valine, threonine, and isoleucine. So in green, we have the first sieve, and this is based on size. So what do we see in this cartoon? So threonine and valine make it through, but isoleucine does not. It's rejected right away, so it's never activated. So if threonine and valine pass through, what happens?

We see each one is activated as the amino adenylate, and then what? Well, valine, we want to transfer the valine to the tRNA, so it can move on and help with protein synthesis. If

threonine's activated, and here we see that threonine is transferred to the tRNA as well, this is hydrolyzed by the editing site, in this case. So the threonine is removed from the tRNA with the anticodon for valine. Right, so think about the ester bonds that we saw last time in terms of the three prime end of the tRNA being modified and the chemistry that will happen there to result in hydrolysis of and release of the amino acid here.

So what that cartoon hints to is that the hydrolysis can occur at different steps. So we can have hydrolysis that is pre-transfer, which means the editing occurs before the tRNA is modified. Or we can have post-transfer editing which is what we saw in the prior slide, where the editing and hydrolysis occurs after the amino acid monomer is transferred to the tRNA. OK? And this schematic here depicts that, so what do we have?

We have the aaRS responsible for modifying tRNA for isoleucine, and we combine that with valine, the wrong amino acid, and ATP. What happens? So E is for enzyme. We have formulation of the amino adenylate intermediate. Here's the tRNA with the anticodon for isoleucine.

What happens? So we have this complex form in this depiction. Pre-transfer editing would occur at this stage, before the valine is transferred to the tRNA, and so what do we see? We see breakdown and these species. If the valine is transferred to the tRNA, we don't want this, because that would result in this reading of the genetic code.

Post-transfer editing, this species here is hydrolyzed. So whether pre or post-transfer editing occurs is going to depend on the aminoacyl tRNA synthetase, and some can use both mechanisms. That's what we're seeing here. OK? Some only use one, for instance, the valine RS only uses a post-transfer editing mechanism.

So when presented with the data, look at the data and see what species is being hydrolyzed. And if both are, how did the steady-state kinetics, for instance, compare? Just to take a look in the context of a structure of one of these aaRS. So the sites where aminoacylation occur and editing occur are separated by about 30 Angstroms, and that's shown here, where we have the aminoacylation site, and here we have the editing site. That's responsible for pre and/or post-transfer editing.

So in thinking about this and thinking about how one could leverage this 30 Angstrom separation and these two distinct sites in terms of experiments, what does that allow one to

do? So imagine if you want to ask, what are the consequences of having aaRS that have faulty editing function? And effectively, mischarged tRNAs or put the wrong amino acid on a tRNA. What does that mean for a cell? There's an opportunity to do that here.

So you could imagine mutating residues that are critical for editing function in the editing site. Such that you have an aaRS variant that can activate amino acids and transfer them to the tRNA but cannot edit when a mistake happens. Right? So you can imagine a site-directed mutagenesis, purifying the enzyme and doing some in vitro characterization to see how it behaves. And then you could also imagine translating this into a cellular context and asking say in cell culture what happens here?

So basically, what are the consequences of faulty editing? And these types of studies have been done. We're not going to look at them in detail. But just as an overview and some concepts that will come up within our folding section, what's been shown is that a single point mutation in an editing domain of one of these aminoacyl tRNA synthetases may have deleterious consequences. And we can imagine that these consequences could result from proteins or enzymes that gain a new function or don't do their correct function. Right?

So just imagine that some mischarged tRNAs, where mischarged means the wrong amino acid is attached, are around because of some mutant aaRS. And these tRNA that are mischarged can be delivered to the ribosome, which means that point mutations form within synthesized polypeptide chains. So there's some mixture where some of these proteins are native, and others are mutant, and what might happen here in terms of consequences?

So native protein will go on and do its job. Imagine there's some mutant protein here that's altered in some way, and these are just some examples of possible outcomes. So maybe there's a breakdown of some essential cellular process. Here, we have triggering of autoimmune-like responses, things that are not good.

What if these mutant proteins misfold? So they can't form their correct fold, and fold is important for function. Maybe there's aggregation. Maybe there's stress on the proteasome, ER response, unfolded protein response, cell death. So fidelity's important.

And just some things to think about as we close this section. We can consider error rates of various biological polymerizations, whether that be DNA replication, transcription, or translation, and they vary quite a bit here from this. And what the take-home can be by comparing these error rates is infrequent mistakes in decoding the mRNA are accepted as a

source of infidelity. So they do occur, and they occur more frequently than, say, an error in replicating the DNA, and that makes sense. Right? If an error occurs in DNA replication, there's a huge problem likely compared to an error in translation.

So some questions just to think about, answers aren't going to come up within the context of this course. But higher accuracy is important, but actually how much accuracy is enough? And there is a cost in terms of cellular energy for accuracy, and is it that the cell tunes its accuracy to some point that could be considered optimal, and are there benefits to translational infidelity? Right? So the prior slide showed negative consequences, but are there benefits? So that discussion, we'll close considering how the amino acids get attached to tRNAs, and so where we're moving to now is the elongation cycle.

AUDIENCE: So is there a specific part of the cytoplasm where the tRNAs and the amino acids come together, or does this happen everywhere?

ELIZABETH NOLAN: So I actually don't know, but I think of them as being everywhere in terms of the tRNAs. Because as we'll see in a few slides, EF-Tu, which is required for delivering the tRNAs to the ribosome, is highly abundant. At least, that's my thinking for prokaryotes. Do you have anything to say? The question was effectively are there certain regions of the cell where tRNAs get modified more than other regions?

JOANNE STUBBE: I don't know. In mammalian cells, they have weirdo complexes with tRNA synthases that they've been around forever. and I still think we don't really understand what the function is.

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: Can you speak a little bit louder?

ELIZABETH NOLAN: The question is, do we have information about say the distribution of tRNAs as being amino acid modified versus unmodified?

AUDIENCE: I think maybe we could [INAUDIBLE] I don't know.

ELIZABETH NOLAN: There's always a way, probably. Right? But I don't know what that distribution is either in terms of the percentage of tRNAs that are aminoacylated at any one given time. Yeah, just don't know. I think one key thing to think about as we come to the next part is that these tRNAs are bound by EF-Tu. So to think of them as in complex with a translation factor as opposed to

tRNAs floating around in the cytoplasm, so I think that that's a key point of focus.

So moving into elongation, what do we need to think about here? So we need to think about delivery of the amino acid tRNAs. How does the ribosome ensure that the correct aminoacyl tRNA is delivered? So we have the correct amino acid onto the tRNA, but we also have to get the correct amino acid to the ribosome.

How is peptide bond formation catalyzed? What is the method by which polypeptides leave the ribosome, and how is translation terminated here? So effectively, these are all questions we need to address in terms of thinking about how the ribosome translates the genetic code and synthesizes the polypeptide. So within the notes posted on Stellar, there's a number of pages of definitions, so terminology that comes up within these discussions of the ribosome to refer to.

And in terms of our translation overview slide, where we are now is here, in elongation. So we have the mRNA our 70S, and we're going to focus for the rest of today on thinking about EF-Tu, this elongation factor that's responsible for delivering the amino acid tRNAs to the ribosome here. So as an overview in terms of a cartoon, where are we going?

Here, we have our ribosome, and in this depiction, it has been translating. So we have a nascent polypeptide emerging through the exit tunnel of the 50S. So we see this peptidyl tRNA in the P-site, and we have this deacylated tRNA in the E-site. So what happens?

That A-site is empty, and for another round of elongation to occur, the aminoacyl tRNA needs to be delivered. And as we'll see today and in recitation this week, EF-Tu is responsible for that. So there's a ternary complex that forms between EF-Tu-GTP. So EF-Tu is a GTPase and the aminoacyl tRNA. And this ternary complex delivers the aminoacyl tRNA to the A-site. OK? This allows for peptide bond formation to occur in the catalytic center.

And then there's a process called translocation, in which the elongation factor-G in complex with GTP comes in and helps to reset the ribosome such that another aminoacyl tRNA can come in. So where we're going to focus for the rest of today is on this process here, thinking about EF-Tu and how that delivers amino acid attached to tRNAs to the A-site. OK, so just in our cartoon, where we left off, with initiation process, so we have that initiator tRNA in the P-site, and the A-site is empty. OK?

And one other thing I'll just show here, I mentioned when describing ribosome structure that

some ribosomal proteins have additional jobs. So it's not just that these proteins help with the overall structural integrity of the ribosome. And there's two ribosomal proteins, L7 and L12, and these are involved in recruitment of that ternary complex between EF-Tu, the GTP, and the aminoacyl tRNA. So now, we need to get the aminoacyl tRNA to the A-site, and this requires EF-Tu. And when we think about this, we always need to think about this ternary complex which is EF-Tu bound to the aminoacyl tRNA bound to GTP.

So a little bit about EF-Tu. So in E. coli, EF-Tu is the most abundant protein. So there's tons of EF-Tu. OK, approximately here, we have 100,000 copies per cell. So it's about 5% of total cellular protein.

And so, as I just said in response to a question about these tRNAs in the cells, we can think about this entire tRNA pool, or aminoacylated tRNA pool, as being sequestered by EF-Tu. So EF-Tu binds the aminoacyl tRNA, and it binds GTP to form the ternary complex. And this allows EF-Tu to deliver these amino acids attached to the tRNAs to the A-site, and it's a GTPase. And we need to think a lot about how this activity relates to its function and fidelity.

So here is a depiction of the structure of a ternary complex. So what we see is that we have a tRNA here, and here we have EF-Tu bound to the tRNA. So here is the anticodon loop, and if we consider this structure of the ternary complex bound to mRNA, what do we see? So we have an mRNA in green. OK, here's the tRNA, and the anticodon end, and here's EF-Tu.

And as I said, EF-Tu is a GTPase. Where is the GTPase center? That's up here. So this GTPase center of EF-Tu is quite far from the tRNA anticodon, down here. This distance is about 70 Angstroms.

And so this is something quite incredible to think about, because as we'll see, when there's codon recognition-- meaning this codon-anticodon interaction, that's a cognate pair-- GTP hydrolysis is stimulated. So how is that communicated over 70 Angstroms? If there's a recognition of that here between the mRNA and the tRNA anticodon, and GTP hydrolysis happens up here, how is that signaled over 70 Angstroms? Right? So clearly, there's going to be some conformational changes that occur that allow this GTPase activity to turn on.

Just another view, so here, again, we have the structure of the ternary complex bound to the mRNA, and here, we can look at the ternary complex bound to a 70S ribosome. So we have the ribosome in this orangey-gold color, the 50S the 30S. Here, we have the PTC and decoding site. The tRNA is in green, and EF-Tu is in this darker orange here, to place that in

the perspective of the 70S ribosome here. So conformational change is required to signal code on recognition to the GTPase center, and this is something that will be spoken about in quite some detail this week in recitation.

One other point of review before moving forward with delivery of the amino acid tRNA. We need to think about codon-anticodon interactions here for decoding. So we have cognate versus near-cognate versus non-cognate, and this is for the codon-anticodon interaction. OK, and so if we imagine we have some mRNA, and you need to think about the five prime and three prime ends with this. And then we have some tRNA, three prime, five prime, we need to ask how do these match?

So for instance here, if we have AAG, and we have positions one, two, three, from left to right of the mRNA, right here we have a cognate match. OK? So we have the AU match in positions one and two, and then wobble's allowed in position three, this GU here. So no, no interaction.

OK, just as another example here, imagine we have GAG, here. What we see is that there's only one match, meaning Watson-Crick base pairing, in position two. OK. Here, this GU, that's not a match based on Watson-Crick base pairing, and as a result, the ribosome is going to want to reject this tRNA, if this is what's happening in the A-site here. And then, we can just imagine some situation, where we have a tRNA and an mRNA where there's just no match. OK? No Watson-Crick base pairing here.

So what we need to ask is, as EF-Tu is delivering these aminoacyl tRNAs, what happens if it's a cognate match versus a near-cognate versus a non-cognate? How does the ribosome deal with the wrong tRNA entering the A-site? Right? So again, this is something important for fidelity, and these both need to be rejected.

So why are we reviewing this? We're reviewing this, because it's important in terms of what happens during initial binding of aminoacyl tRNAs to the ribosome. So we're going to go over some of this in words and then look at a cartoon that explains this process. And what we're focused on is delivery of the aminoacyl tRNA to the A-site.

So what happens first? OK. First, there needs to be an initial binding event, where the ternary complex binds to the ribosome. So initial binding, it binds to the 70S, and these ribosomal proteins are involved in the recruitment of the ternary complex. This initial binding event of the ternary complex to the ribosome is independent of the mRNA.

What happens next is that there's codon recognition. So we need to think about that tRNA entering the A-site, and there's some sort of sampling that occurs in the decoding center, so sampling of codon-anticodon pairs in the A-site, and so what happens? What happens if there's a cognate event or a non-cognate event? So if a cognate anticodon recognition event occurs, there's a series of steps that then happen.

So with a cognate codon-anticodon interaction, there will be a conformational change in EF-Tu, and this activates the GTPase center which allows for GTP hydrolysis. OK, and effectively this conformational change stabilizes the codon-anticodon interaction here, and that stabilization accelerates the GTP hydrolysis step. So this is all building towards a kinetic scheme. In terms of enhancements, what's found is that the rate of GTP hydrolysis by EF-Tu increases by about 5 times 10 to the 4th with cognate anticodon recognition in the A-site. So we have GTP hydrolysis, and then there's another conformational change.

So we have EF-Tu in its GDP-bound form, and effectively, EF-Tu will dissociate from the aminoacyl tRNA, and the aminoacyl tRNA will fully enter the A-site. OK so this process is called accommodation, and once that happens, peptide bond formation can occur. So this is the good scenario. The polypeptide can keep being made.

What if it's not a cognate? So what if a near-cognate tRNA is delivered to that A-site during this initial binding event which is independent of the mRNA? That's why this can occur. If it's a near-cognate anticodon, what we observe-- and this is all from experiments you'll be learning about this week-- the ternary complex rapidly dissociates from the ribosome. And what's found from kinetic measurements is that the dissociation of the ternary complex, when it's a near-cognate situation, is about 350-fold faster than cognate.

So let's look at this stepwise within a cartoon format. You'll see another depiction of this scheme in the recitation notes and in problem set two. So here, we have multiple steps in this overall process.

All of these steps have some rate that's been measured by multiple types of methods, and Joanne will be presenting this week on a lot of pre-steady-state kinetic analysis that were done to measure these rates here. And basically, the key point to keep in mind, and that I'd like to stress from what was just said on the prior slide, is that what you'll see throughout this is that conformational changes are coupled to these rapid chemical steps. And the chemical steps are irreversible, this GTP hydrolysis.

So what do we see? We begin with initial selection. Here, we have our ribosome, and there's a polypeptide being synthesized. Here's the ternary complex-- EF-Tu, GTP, and the aminoacyl tRNA. So there's an initial binding step that's governed by k1 in the forward direction and k minus 1 in the back direction, and said before, this is independent of the mRNA.

So what happens? The ternary complex binds the ribosome, there's sampling in the A-site of the anticodon, and then there is a step described as codon recognition with k2 and k minus 2. OK? In this scheme, if an arrow is colored, red arrow indicates the rate is greater for near-cognate than cognate. OK? Which means in the event here of a cognate pair, this is going to push forward in the forward direction. If it's near-cognate, this back step has a greater rate of about 350-fold. OK? So we're going to end up back here.

With cognate recognition, next, we have GTPase activation, again, forward and reverse. Green indicates the rate is greater for a cognate match than near-cognate. So if it's the correct anticodon, it's going to plow through to here. We have GTPase activation.

And then what happens down here? We have a GTP hydrolysis step. We have a conformational change in EF-Tu, and then what? We can have accommodation such that the tRNA was installed fully into the A-site and then rapid peptide bond formation or peptidyl transfer.

The ribosome has one last chance to correct a mistake. So you can imagine that after GTP hydrolysis, after the conformational change in EF-Tu and its dissociation, there's a last chance at rejection here. Realize that step is occurring at the expense of GTP here. So in thinking about how to deconvolute this model or how to design experiments to test this model, there's a lot that needs to be done. Right? A lot of rates that need to be measured, a lot of different species along the way with the ribosome. Right?

So how do you get a read out of each of these steps? That's what we'll be focused on in recitation this week and next here. So here are some more details on this initial binding process with some information related to the k1s and k minus 1s here. That's provided to help navigate the reading this week for recitation here.

So what happens in the GTPase center of EF-Tu? What are some of these conformational changes? And effectively, there are conformational changes in the decoding center that are critical on one hand. So that's not at the GTPase center, but first asking what's happening when the mRNA and tRNA codon interact? And then what's happening in the GTPase center

here?

So just to note, not shown in the slide in terms of the decoding center. OK, what we need to be focusing on are changes in the 16S RNA, and effectively, I'll just point out three of the positions. So we have A1492, A1493, and G530 of the 16S, here. And what we find is that these bases effectively change conformation with a cognate match. And they effectively flip and interact with that cognate anticodon to help stabilize the codon-anticodon interaction.

So this stabilizes the codon-anticodon interaction, and that stabilization accelerates the forward steps. So that results in this acceleration of GTP hydrolysis. So then the question is, what's happening in the GTPase center of EF-Tu? Because there has to be a change in conformation at that GTPase center 70 Angstroms away to allow for GTP hydrolysis to occur, and somehow, that all has to be signaled from here to there.

So what we're looking at here is an excerpt of the structures looking at this GTPase center, and so what do we see? Effectively, two residues, so isoleucine-60 and valine-20 have been described as a hydrophobic gate in the GTPase center. OK, and the idea is that if this gate is closed, it prevents a certain histidine residue, histidine-84, from activating a water molecule which then allows for the GTP to be hydrolyzed. OK, but if there's a change in conformation, and this gate opens, that chemistry can occur.

So what are we looking at here in these structures? Effectively here, we have the two hydrophobic residues of the gate, so valine-20, isoleucine-60, and here's that histidine-84 I told you about, and what is this, GTPCP? So what we have there is a nonhydrolizable GTP analog. These types of molecules are very helpful in terms of getting structural information, in terms of doing certain types of biochemical experiments. OK? So effectively, we can have an analog bound that cannot hydrolyze.

What are we looking at here? Here, we're looking at the, say, activated species, and what do we see? We see that this histidine has changed position. So here, it's flipped that way, here this way and here, what we see is a view with EF-Tu in the GTP-bound form. So the idea is that overall conformational changes that occur 70 Angstroms away, because of codon-anticodon recognition, effectively signal conformational changes in GTPase center that allow for GTP hydrolysis to occur and things to move in the forward direction there.

So that's where we'll close for today. On Friday, we'll continue moving forward in this

elongation cycle, and starting in recitation tomorrow, you'll look at experiments that allowed for this kinetic model to be analyzed and presented. You really need to come to recitation this week and read the paper.

JOANNE STUBBE: And you need to read the paper more than once. It's a complicated paper.

ELIZABETH NOLAN: That's on [INAUDIBLE]. It's a complicated paper which is why we have two weeks of recitation for it. There's a lot of methods, and I'll also point out that problem set three has very similar types of experiments, but it's looking at EFG instead of EF-Tu. So spending the time on this paper in the upcoming weeks is really important.