The following content is provided under a Creative Commons license. Your support will help MIT OpenCourseWare continue to offer high quality educational resources for free. To make a donation or view additional materials from hundreds of MIT courses, visit MIT OpenCourseWare at ocw.mit.edu.

ELIZABETH NOLAN: Today we should be completing the translation cycle. And the next topic that will come up is use of antibiotics as tools to study the ribosome and translation. So just a recap from last time, we went over the delivery of aminoacyl--tRNAs by EF-TU and looked at this model for understanding how that happens, OK?

So recall, we discussed the initial binding of the ternary complex of EF-TU GTP and the aminoacyl-tRNA. And that's codon independent. When a codon/anticodon match occurs, we have a push in the forward direction. EF-TU's a GTPase. There's activation of the GTP center. So conformational changes, GTP hydrolysis. So we have EF-TU and the GTP bound form. There's conformational change, and ultimately accommodation of this tRNA in the A site. And that allows for peptide bond formation.

So where we left off last time was discussing the conformational changes that occur in the decoding center and also in the GTP center of EF-TU. And just to highlight, I mentioned that there are conformational changes within the 16S rRNA, in particular three nucleotides that occur when it's a cognate codon/anticodon interaction. And these are just shown here. And effectively what we're looking at in these three panels are the 16S rRNA in the absence of the tRNA, in the presence of the tRNA, but the tRNA is removed from this image for simplicity, and then with the tRNA bound.

So some of the easiest changes to see here are with A1492 and A1493. So if we look in the absence of tRNA, they're pointing down the bases. And here as a result of tRNA binding in the A site, we see that A1492 and 1493 are flipped, flipped up. OK. And if we look here, you can see how these are interacting with the bound tRNA.

OK. So this conformational change helps to accelerate the forward steps. So that's in the decoding center. And then also just remember 70 angstroms away in the GTP center of EF-TU, there's conformational change of these hydrophobic residues that are thought to be a hydrophobic gate that allows histamine 84 to activate a water molecule for attack in GTP

hydrolysis.

So at this stage we're finally ready to have a peptide bond formed by the ribosome. And so we need to think about that mechanism and then what happens after. I'll say, so effectively what we have in the P site is the tRNA with some growing peptide chain.

[WRITING ON BOARD]

And then we have the aminoacyl-tRNA in the A site.

[WRITING ON BOARD]

And so what happens effectively, we have attack from here, release such that we end up with a P site with a deacylated tRNA. And in the A site we now have the peptidyl-tRNA that has grown by one amino acid monomer.

[WRITING ON BOARD]

Here, OK? OK. And so this is the N- terminal end of the protein or the polypeptide, and here's the C terminal end here. So thinking about this mechanism and having nucleophilic attack from this alpha amino group of the aminoacyl-tRNA in the A site, what do we need to think about? Is there anything surprising or unusual?

AUDIENCE: Think about protonation state.

ELIZABETH NOLAN: Yeah, right. Exactly. We need to think about the pKa. So typically do we think about an alpha amino group being protonated or deprotonated, that physiological pH. Yeah. Protonated we typically think about an H3+, not an H2 here. So what does that tell us? There has to be a general base somewhere that deprotonates this alpha amino group, such that we have this species that can attack, and then can imagine just formation and collapse of a tetrahedral intermediate here.

So what is the mechanism of catalysis? OK. Our room's possessed. So what is the mechanism of catalysis here? What do we know? So we know from looking at the structure that the ribosome is a ribozyme. So no proteins in the catalytic center.

What else do we know? There's no metal ions there and there's no covalent catalysis. So

really what is a paradigm here? We have a paradigm of conformational change and effectively we have substrate positioning. You can imagine there's some protons shuttling in an electrostatic network that allows this to happen. And so as soon as this aminoacyl-tRNA enters the A site, we have formulation of this peptide bond.

So what needs to happen next-- and once the screen gets fixed, we'll look at an actual depiction of these players in the PTC. What needs to happen after the peptide bond forms-- and we have now this peptidyl tRNA in the A site is that before the next round of elongation effectively we need to reset, and the mRNA and the tRNAs need to move relative to the ribosome. OK. So effectively we need to get this deacylated tRNA to the E site, and we need to get this peptidyl tRNA to the P site such that the A site is empty. Is it not going down? Pardon? OK, that's fine.

So this process is called translocation here, and effectively we can just consider the three sites. We have the E site, the P site, and the A site.

[WRITING ON BOARD]

OK. And in this process another elongation factor, this time elongation factor G is involved. And the outcome is that we end up with the deacylated tRNA in the E site, the peptidyl tRNA in the P site. OK. And then the A site is empty such that the next aminoacyl-tRNA can come in. OK. So immediately after peptide bond formation, and this is the state after the process called translocation.

So EFG is also a GTPase here. And effectively what happens is that EFG bound to GTP binds near the A site and GTP hydrolysis occurs. Bless you. OK. And as a result of GTP hydrolysis, there's conformational change. OK. And this results in translocation and then EFG is released.

And in thinking about translocation we think about two steps. OK. And so the first step is something called formation of hybrid states, and then the second step is the actual movement, the mRNA and tRNA relative to the ribosome here.

So we'll take a look at this. So here's just an overview of peptide bond formation backtracking a little bit, and then back to here. Thinking about confirmations and what this looks like, what we're seeing here in this depiction is the P site tRNA in green, we have the A site tRNA in this red color, and then we see the 23S rRNA shaded in light blue in the back. OK. So here's A76, here is an attached amino acid, and we see the nucleophile here, and attack there. OK. So

substrate positioning within this active site.

Here, as I talked about this translocation process, we're now at this stage as shown with a depiction of the ribosome. So this is where we're going using EFG in complex with GTP to allow the translocation process to occur. Here is another cartoon depiction. And so if we begin after peptide bond formation, we see the incoming EFG in complex with GTP. And if we look in this cartoon, it shows EFG binding near the A site. So it's not binding exactly in the A site, but nearby.

OK. There's GTP hydrolysis. We now have EFG in the GTP bound form. OK. And now we see translocation, and that these tRNAs have moved. And after this step, EFG is released. And at this stage the ribosome is ready for its next round of the translation cycle.

So let's take a look at what we know about the structure of EFG. So this is a really beautiful example of molecular mimicry, and EFG is shown here. And if we compare EFG to the complex of EF-TU with tRNA like the ternary complex shown here, what we see is they look very similar, and this domain IV of EFG resembles the tRNA quite well. So just looking at that we can begin to think maybe its domain IV that's coming in near that A site to have some interactions and cause this translocation event.

Here is just a comparison of the ribosomes with either EF-TU and tRNA bound, which we've seen before, and here we see EFG in this red color. So quite similar, but are they the same? And the answer is no. So quite recently a crystal structure was obtained with EFG bound to the ribosome, and it was determined that in this structure EFG is bound in the post translational state.

So what do we see? If we take a look here, we have a tRNA in the E site, a tRNA in the P site, and here in red we see EFG. And if you take a look, recall that ribosomal protein L12 that was involved in recruiting the ternary complex, we're seeing that there's some interaction here with EFG in that protein as well.

If we look in this panel B-- so we look at a close up view near the A site, what's happening? Here's a P site tRNA, here is the mRNA, and here is EFG bound, and it's domain IV that's sticking its way in. OK. And so what are we seeing here? Basically these tRNAs have moved at this stage and we still have EFG bound here.

So is EFG interacting with the A site codon of the mRNA based on this view?

AUDIENCE: Not really.

ELIZABETH NOLAN: Yeah. No. So not really. So it's not interacting in the same way as the tRNA. That's the take home here. They look the same, but the details are different here. OK.

So what about these hybrid states? They have two different views, and the slides for looking at this-- effectively what the hybrid states are-- OK. These describe basically the orientation of the tRNAs. So effectively they can be P/E or A/P here. And the P/E state talks about having the anticodon end in P and the deacylated three prime end in E. OK. So here we're referring to this tRNA that ultimately needs to get ejected from the ribosome.

And A/P we have the anticodon end in A and the peptidyl three prime end in P for the tRNA with the growing peptide chain. So effectively these hybrid states are describing the movement of the three prime ends of the tRNAs with respect to the 50S subunit. And that's shown in cartoon form on the slide here.

OK. So effectively if we take a look, we have accommodation, so the aminoacyl-tRNA we see there's formation of a peptide bond. So there's some color coding here, and then look, rather than having these tRNAs straight up and down, we see that the three prime ends have shifted. So here we have the P anticodon end in P, three prime end in E, here AP with anticodon end in A, and peptidyl three prime end in P.

OK. So first the three prime ends move, and then what do we see after the help of EFG? The five prime ends move and the A site is empty and able to take the next aminoacyl-tRNA. Here's just another view of the process for you to look at here. Yes?

AUDIENCE: It's kind of from a few slides back, if that's OK.

ELIZABETH NOLAN: That's OK.

AUDIENCE: So on EFG where you have that anti-codon looking blue, there's no hydrogen bond interacting with the transcript. Is that the take away?

ELIZABETH NOLAN: Can we say that based on what I'm showing you on this slide?

AUDIENCE: I don't know. It just looks like it's around there, but I can't tell what the actual hydrogen bond

interaction part.

ELIZABETH NOLAN: So those details are outside of the scope. EFG will interact with that mRNA, the peptidyl tRNA, but it's interacting differently than a standard aminoacyl-tRNA. So it's not really interacting with the codon at this level of depiction. We're not seeing individual bonds or hydrogen bonds. So we can't make a conclusion about that based on this depiction here.

AUDIENCE: What's the resolution of this structure? Is it high resolution or is it--

ELIZABETH NOLAN: Yeah. That's another issue here. I don't recall the resolution, but they're not great. So if you have a four angstrom resolution structure, for instance, is that type of information even available versus the resolution of maybe 1.5 or 1? Yeah. This resolution I don't recall, but that's a very good point to bring up. I don't think it's high enough to know that would be my guess here. Pardon?

Oh, the resolution? So rewinding back to recitation last week. So crystal structures have a resolution. And so what does one angstrom resolution versus two versus four allow us to see? Oh. Oh. The question is, are there hydrogen bonding interactions between EFG, and say, the mRNA? Well, there's definitely going to be hydrogens because you're going to have C-H bonds or C-N bonds. But--

AUDIENCE: I can't see any of them, at least from this picture. And also, is this picture actually a picture or is it just like a [INAUDIBLE]?

ELIZABETH NOLAN: This is from the crystal structure.

AUDIENCE: But it's not itself a crystal structure. They take out all the hydrogens, wouldn't you, and you wouldn't see anything, right?

ELIZABETH NOLAN: OK. So this is from the crystal structure and you can make choices as to what information you put in your depiction, whether or not you're going to show certain residues say, or just the backbone there.

AUDIENCE: I'm not sure what we are expecting to see, but don't see. We will get the heteroatom distances. Yeah. And so the question is, how much error is there, and if there's one error, you can't tell where the analide regions are.

AUDIENCE: So we're looking at distances like between potential hydrogen bonding sites as our measure--

ELIZABETH NOLAN: Or heteroatoms because you may not be able to see that hydrogen. But you can know something like, oh, if this heteroatom and that heteroatom are so many angstroms apart, is it likely that there's a hydrogen bonding interaction or not based on knowledge of bond distances here? So one thing I'll note and will come up as we're discussing antibiotics, I said nothing about how they've obtained the structure.

And that's just something to keep in mind. And this also gets to the question of resolution, and what can you see? But they had tremendous difficulties getting this structure, and they had to use a mutant ribosome, and they strategically used an antibiotic to stall the ribosome here. So many, many attempts to get crystals that are even good enough to get some information here.

OK. So back to these hybrid states and the formation of the hybrid states. Something important to know about-- and this is something I have a lot of time seeing in any cartoon that's presented is that the 30S subunit undergoes some conformational change called ratcheting. And effectively the ribosome can exist at this stage in either an unratcheted or ratcheted state and EFG selects from one over the other. So EFG will bind this ratcheted ribosome. And effectively what that terminology is describing is a small rotation of about 6 degrees of the 30S relative to the 50S in one direction. So the ribosome will be going between unratcheted and ratcheted.

EFG can bind the ratcheted form. OK. And after that occurs, they'll be GTP hydrolysis on these translocation events here. So an awful lot is going on to get that one peptide bond formed and the ribosome ready to do it again.

Where we're going to go at this stage is a brief discussion of the termination process in translation and the players that come up there. So effectively the elongation cycle is going to continue until a stop codon enters the A site. That's making the assumption some unforeseen circumstance hasn't happened to this ribosome. It hasn't stalled or prematurely stopped translation.

So what happens when a stop codon enters the A site? OK. Again, we have translation factors. These translation factors are release factors that recognize the stop codon, and they have the responsibility of cleaving the polypeptide chain from the P site tRNA.

And so there are two different classes of release factors. We have class 1, which are release

factors 1 and 2. Release factor 1 and release factor 2 each recognize they're in stop codons. So, for instance, RF1 recognizes UAA and UAG. Whereas RF2 recognizes UAA and UGA here. There's a class 3 release factor RF3. This one is a GTPase and it has the job of accelerating dissociation of RF1 or RF2 after peptide release.

So we'll look a little bit at structure and then one schematic for how this may all happen. So similar to EFG and the ternary complex of EF-TU GTP and the tRNA, we have another example of molecular mimicry with these release factors. And so initially when release factor 1 was crystallized, the structure shown here was obtained. So this was the protein crystallized in the absence of the ribosome, and it was a little difficult to reconcile this structure with function immediately. And then in later work RF1 was crystallized bound to the ribosome. And that structure is shown here.

And so if we compare the left to the right or what's described as the closed to the open version of RF1, what we see is that there's a pretty substantial change in conformation when we're looking at RF1 on the ribosome. And if we use a little imagination we can think about RF1 resembling a tRNA.

OK. We have this region here that's sticking out. And if we look at an overlay of RF1, so this structure of the ribosome bound structure in a tRNA, what do we see? So we have the tRNA, we have the anticodon end down here, we have the CCA end of the tRNA up here. And so what do we see? In terms of RF1, we have this PVT motif down here and we have this GGQ motif up here for that. And so this motif is important for hydrolysis of the peptidyl tRNA. And that's where it is.

In terms of a schematic for termination as a way to thinking about this-- so here we have our ribosome that's then translating and now there's a stop codon in the A site. So here comes a release factor, either 1 or 2. It recognizes this stop and binds. So there's hydrolysis of this linkage-- and should think about that chemistry to what's happening. --peptide release.

So what's shown in this depiction is that RF3 comes in and it was in GTP bound form. It binds in the region of the A site. There's some exchange. We have GTP coming in here and then some additional steps that involve GTP hydrolysis by RF3 involvement of the ribosome recycling factor. And we see that our friend EFG comes into play again here along with initiation factor 3.

So some of these other translation factors seem to play a role in this termination cycle. And

really, again, it's a question of looking at the data that's presented to you and interpreting that data and drawing some conclusions. So there's still a number of questions about this process and the ribosome recycling that remain.

So if we look about this slide and where we've come in this discussion of translation effectively, all of the pieces are shown here for prokaryotes. So this is just a map to work your way through when studying the system. But we have initiation, we have elongation, and then this process of peptide release and ribosome recycling. OK. And so throughout this we're seeing the action of GTPases. So the power of GTP hydrolysis is needed. Conversion of chemical to mechanical energy.

There's a lot of conformational change that's happening. The slides I've shown you don't do that justice but it's something to think about and keep in mind, and that this ribosome is amazingly dynamic. And so that is what's going to lead us into the next subtopic related to the ribosome, which is thinking about how have some of these observations been made? So how is it that we've obtained structural insights into the ribosome at different steps along this translation cycle?

And just as for consideration, there's a little excerpt from a paper I like. So this was in 2010. So shortly after the Nobel Prize was awarded. And so there's a number of perspectives, retrospectives in the literature. And in this one called the Ribosome Comes Alive, Joachim Frank is talking about these pioneering work of the X-ray structure.

And just in yellow here, he's stating, those who might have expected that the atomic resolution structure of this massive RNA protein complex would itself offer immediate insight into the mechanism of translation were thoroughly disappointed. And in fact the mechanism proposed from some of this early study ended up not being the correct mechanism here. There's a note about that on an earlier slide where the peptide bond formation step is shown.

So what does he say? "I'd like to compare this situation to a visit to Earth by a martian who wants to understand how an automobile works." OK. So we can all think about flipping up the hood of our car and what do we see? "She looks under the hood of a parked car, perhaps even takes the engine apart, but still has no clue. It's clear she'll have much better luck if she's able to see that engine in motion." And so that's been a major goal in terms of thinking about the ribosome as well as other micro-molecular machines. How can you actually see these in motion and see the dynamics and conformational changes here? Really critical.

So the question I pose is, is it possible to see the ribosome stopped at various points in translation cycle? And if so, how? So maybe we can't see the dynamics continually, but can we sort of park it at different steps? And the answer to that is yes. And basically a huge part of our understanding of this 70S ribosome does come from crystal structures, and researchers have been able to trap the ribosome at various points in the translation cycle using small molecules. And these small molecules are antibiotics.

So where we're going to focus on for the rest of today and probably the beginning of Monday is thinking about the use as antibiotics. So small molecules that inhibit bacterial growth as tools for studying ribosome function. So a few questions related to that. First of all, what types of antibiotics target the ribosome? Where do they bind to the ribosome, and how can we use them experimentally? And also something just to think about, we have a crisis in the clinic in terms of a lack of new antibiotics and emerging antibiotic resistance. So how can fundamental understanding of the ribosome help in terms of therapeutic development?

And this came up in a bit of a different context in seminar on Monday for anyone that was at Biological Chemistry Seminar. So we had Professor Matt Disney with us who was looking at small molecules to target RNA's. And one question that can come from that is, are there unknown molecules out there that might target the ribosome in different ways from the examples we currently have?

I was super excited this morning to learn about a new book. So if any of you are interested in antibiotics, Professor Chris Walsh and Professor Tim Wencewicz at St. Louis have written a new book looking at antibiotics from a very chemocentric perspective here, and our friends on the cover. So I suspect be a wonderful read if you're curious.

So let's take a look as a segue into thinking about these at the structure I just showed you a VFG bound to the ribosome. So we talked about how EFG is helping in the translocation process, and we saw the structure, and I told you in passing that this structure was very difficult for the researchers to obtain. And at the end of the day, they needed to use a mutant ribosome for reasons I won't go into. It's not relevant for this discussion. And also, a natural product that has antibacterial activity shown here.

And so this small molecule binds EFG and it binds to EFG when EFG is bound to the ribosome. And moreover, it binds to EFG after GTP hydrolysis occurs. OK. So the result is that this natural product can be used to trap the ribosome in this post translocational state where

EFG is still bound. So it's hydrolyzed GTP. There's been movement of the mRNA and tRNAs, but EFG cannot dissociate as a result of use of this small molecule.

So you can begin to imagine how including this molecule or maybe other antibiotics that stop the ribosome at different steps can be used to obtain crystals and crystal structures here. And furthermore, they can also be used in a number of biochemical studies-- and we'll look at an example of that in the context of this lecture. --and also in recitations and problem sets.

So where do antibiotics bind to the ribosome, and how many of them are out there that can bind the ribosome here? There's many options. So many antibiotics target the ribosome. And if we just look at a 30S subunit and a 50S subunit and take a handful of antibiotics that target the ribosome and see what we know about where they bind, we can make maps like these ones here and we can consider larger lists. You're not responsible for these details at all. Just the take home message is that there's many options and an extensive toolkit. Yeah?

AUDIENCE: Are the eukaryotic and prokaryotic ribosomes similar enough that most of these antibiotics also affect the eukaryotic ribosomes?

ELIZABETH NOLAN: Yeah. So that's a great question and something to think about. So that will depend on the molecule. There are many differences between the prokaryotic and eukaryotic ribosomes. Some will bind both. There is an example thiostrepton I believe that's quite specific, not for eukaryotic ribosomes. I mean, that's something also to think about. If they interact, are they interacting in the same way? And if they do inhibit the ribosome, is it by the same mechanism? And you can imagine implications related to therapeutic development in terms of that exact issue. Yeah?

AUDIENCE: Do we have like a lot more ribosomes than prokaryotes? Is that also [INAUDIBLE] to have a lot more [INAUDIBLE] a lot more antibiotic [INAUDIBLE] that for us too? Do you know what I mean?

ELIZABETH NOLAN: Yeah. I mean I think that's a little outside of the scope of our discussion because how do you get to counting ribosomes? Is that per cell or organism by organism and microbiome versus person? Is there another question?

AUDIENCE: The ratio of the number of ribosomes we have to the number of proteins that we need to be producing, eukaryotic cells are more complex--

ELIZABETH NOLAN: Eukaryotic cells are definitely more complex right there. So what I say is overall case by case basis. So what about structures? Here are just some examples. Structures are highly variable and the ways in which these molecules can inhibit translation are highly variable. These are some examples that you may have come up with some of these in terms of laboratory work or maybe even been prescribed.

So, for instance, chloroamphenicol This molecule here binds the 30S, prevents peptidyl transfer. Tetracycline binds the 50s and blocks accommodation. Gentamicin binds the 30S, causes premature termination. Erythromycin is a macrolide that for a long time has been thought to block exit of the polypeptide because it binds in the exit tunnel. But there's some new recent work suggesting a revision to that mechanism here.

What are some general observations we can make? And keep in mind, there's always exceptions to the rule. So most of the antibiotics targeting the ribosome that we know about interact with the RNA, but, of course, some can interact with proteins. And we just saw an example of that with EFG. These antibiotics primarily target the decoding center and peptidyl transfer A center. Which makes sense if you're thinking about inhibiting translation. But, again, there's some exceptions.

So thiostrepton interacts with the ribosomal protein that's not in that region. Magnesium might be necessary for antibiotic binding. So this is something to think about if using antibiotics in experiments. And just related to the earlier question, a given antibiotic may bind ribosomes of different species differently there. And so what are the consequences of that is something to think about.

So here we're just looking at an overview of various antibiotics bound to the 50S. So this is taken from multiple different structures and the ribosome itself has been removed. But imagine that the A site tRNA is around here, here P site tRNA. What do we see? We have antibiotics called puromycins that are down by the A site. Here we have chloroamphenicol bound. Here we have the macrolides here.

And just as an example of an antibiotic binding to the exit tunnel-- bless you. --here we're looking at the 50S. We have a P site tRNA, and here we have a nascent polypeptide coming through the exit tunnel, and here we have some examples from structural information about erythromycin, chloroamphenicol bound in this region here.

So we're going to look at a puromycin as a case study for using antibiotics as a tool in a

biochemical experiment. So the first thing that we need to think about is the chemical structure of puromycin and how that structure relates to its ability to inhibit translation. So these puromycins are molecules that cause chain termination. And we'll look at an example of a structure.

[WRITING ON BOARD]

So basically just want to use a little imagination when looking at this structure. What do we see? So what is this small molecule mimicking? Yeah. So what do we have up here? We have something that's adenosine like. Not exactly the same structure. But this may be similar to A76 of the three prime end of the tRNA. We have these methyl groups rather than an H2, but similar.

What's going on down here? Pardon? Yes. It's similar to one amino acid or a peptide. So we have something here that's amino acid like. So if we're thinking about this as a mimic of the three prime end of the tRNA with the amino acid bound, what's fundamentally different here that's going to result in different chemistry happening?

So how are the amino acids attached to the three prime end of the tRNA? What kind of linkage? Yeah. We have an ester in the normal circumstance. And what do we have here? Here we have an amide. So this is non-hydrolyzable. And what else do we have? Right here we have a nucleophile for the P site ester.

So what can happen? Imagine that puromycin somehow can enter the A site. There's a nucleophile that will allow for chain transfer, such that the peptide that's on the P site tRNA gets transferred. But then what? OK. We're stuck because of this MI bond here. So effectively chain termination.

OK. And so what's known is this molecule and its analogs can bind to the 50s A site. And that's something kind of incredible to think about. We talked about this machinery EF-TU that's needed to deliver the aminoacyl-tRNA. Puromycin can get there on its own. Which means maybe for an experiment, that is easier to do if you're going to use this in your experiment.

Moreover, people have synthesized more complex versions. Just an example is shown here. C-pmn where we also have C75 of the tRNA mimicked here. And so this is just an overview of elongation, and then effectively chain termination happening after thinking about having a

puromycin in the A site, a peptidyl tRNA, or some other molecule in the P site and the chemistry that occurs here.

So we'll think about and close with one experiment that's been done using puromycin. And we won't have time to go through all of it in the last few minutes of today, but I'll just introduce the problem and we'll continue with the experiment next time. OK. And so what we're going to think about is a translation factor that hasn't come up yet in class. And this is elongation factor P. OK. And for a long time its function was unclear.

[WRITING ON BOARD]

OK. And so over the years this translation factor was implicated in a variety of cellular processes, but there wasn't any clear answer in terms of really what is its role? And so about two years ago there were two back to back papers-- one of these papers by Rodina and co-workers. So they're the authors of the paper being studied in recitation this week. --published work reporting on why EFP is important for translation.

So prior to their work there were some preliminary studies indicating that somehow this elongation factor helps to modulate and accelerate peptide bond formation. But the questions are, when? So under what circumstances does EFP accelerate peptide bond formation? And then you can think kind of a follow up of that, how? And so we'll look at some experiments that were designed and performed using puromycin as a tool to address this question. And that's where we'll start on Monday.