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: Where we'll spend the first part of today is finishing up where we left off last time with the experiments that were done to take a look at what types of polypeptides, the DNA, kDNA, J chaperone machinery interact with in E. coli.

And once we finish up that, we'll transition into module 3, which is protein degradation. And most of today will just be some general background about proteases and protein degradation. And then on Wednesday, we'll begin to look at the macromolecular machines that are involved in those processes.

OK. So last time we discussed DnaK, the chaperone, DnaJ, the co-chaperone. Right. So recall DnaJ went around and found some polypeptide in a non-native state and delivered it to DnaK, which can grip and hold on to the hydrophobic segments and somehow facilitate folding. And so where we left off were with experiments performed in a similar manner to what we saw for GroEL/GroES where pulse chase was done to label newly synthesized polypeptides. Right.

So during the pulse period with this radio label methionine, newly synthesized polypeptides are labeled. And that allows us to see specifically what newly synthesized polypeptides DnaK, J are interacting with without the background of everything else in the cell.

And why do we care about that? Imagine if we didn't somehow label to discriminate these newly synthesized polypeptides. Right. We can pull down many things in the precipitation, but we'd have no sense as to how long a given polypeptide existed in the cell.

So maybe it was newly synthesized. Maybe it had been around a long time and something happened to it such that it wasn't in a native fold and DnaK interacted with it. OK. So that's the key point with these pulse chase and this labeling for a short time period.

OK. So the researchers had an antibody to DnaK. They had to test its specificity as we discussed for GroEL/GroES. And then after immunoprecipitation, it's necessary to do the analysis. And so as I noted last time, in this particular study, the analysis were less

sophisticated than what we saw for GroEL/GroES so they just used one dimensional SDF page, which we're all pretty familiar with. And they didn't extend it to mass spec.

But with that said, there are a number of observations that are helpful that come from this study. So what we're going to do is examine their gels and see what conclusions we can come up with.

OK. So this is their experiment number one. And what they did was look at the soluble crude cell extracts that were generated in this pulse chase experiment. OK. And so what do we see in terms of how the data is presented? Right. We have two lanes on the left, one and two, that are basically total cytoplasmic proteins. And then the lanes three through six on the right are four samples that were immunoprecipitated with this anti-DnaK antibody.

OK. So something that was done in these experiments that was different than the GroEL/GroES work is that they use two different E. coli strains. So they used a wild type E. Coli strain. So that strain expresses DnaK. And they also used a mutant E. coli that is deficient in DnaK. So that's what this delta DnaK means. So they did some genetic manipulation and knocked out DnaK. OK. And as we learned in the introduction, these chaperone system is not essential.

So what do we see if we work through this gel? And first, we just want to go over what the data show and then why that's important here. So if we compare lanes one and two, what do lanes one and two tell us?

So these are the total cell lysates, soluble fraction, from either wild type E. Coli or delta DnaK. And why do we care to run these? So no immunoprecipitation. So I'll give you a start and then you all can contribute to the next ones. OK?

So what I would say looking at these two lanes is first it looks like the total amount of protein and the distribution of these proteins is similar for both the wild type E. Coli and the delta DnaK knockout. OK. There's proteins that are distributed across a wide range of molecular weights, from below 14 kilodaltons to upwards of 100. OK.

And why is this important to show? One, we want to see what the cell lysate looks like in the absence of immunoprecipitation. Right? And two, it's important to know whether or not knocking out DnaK has done anything to change those cells. And at least at the level here, it looks like in terms of the total cellular polypeptide pool, it's pretty much comparable in terms of

total protein.

So what happens now here where we have this immunoprecipitation? So we have four different lanes. We're going to focus on three, four, and five and ignore six. Basically, in three, we see immunoprecipitation from the wild type E. coli; in four, immunoprecipitation with wild type when the sample has been treated with SDS, so we need to think about why that was done and what that experiment shows; and then lane five in the delta DnaK knockout. So first of all, what does line three tell us? Kenny?

AUDIENCE: Can you get enrichment of higher molecular weight proteins? And as the note says that 15 times more protein was loaded into this well, so I think that's just so you can see the signal. But I think that just shows to prove that the higher molecular weight proteins are more enriched in that lane.

ELIZABETH NOLAN: Yeah. So are many proteins immunoprecipitated?

AUDIENCE: Yes.

ELIZABETH NOLAN: Yes. Right. We see many bands across a range of molecular weights. Right. And then as Kenny said, we're seeing much more intensity up here than down here. Right. So it looks like polypeptides in the range of about 20 to about 60 kilodaltons are enriched. So maybe there's some preference in that size range here. So that's a good observation.

What do we see in lane four? What happened for this sample? So effectively, the crude cell lysate, those extracts were treated with SDS.

AUDIENCE: They didn't bind anything?

ELIZABETH NOLAN: Yeah. So nothing bound. Right. What do we see here? Just one band for DnaK. So why didn't DnaK bind anything?

AUDIENCE: It's denatured by the SDS.

ELIZABETH NOLAN: Yeah. Not the page... which is the sodium dodecyl sulfate. Right. It's a denaturant and it will denature things. They don't need to be in a gel. If you add that to a sample, you'll have denaturation, right? So when these samples were denatured, DnaK didn't bind. Do we think that the SDS denatured DnaK itself? Right. So we do see one band here. Is that surprising?

AUDIENCE: No, because if the DnaK line is suggesting that that's the molecular weight of of DnaK, it's around there. And the antibody you're using to do immunoprecipitation... I mean, would likely probably sub the other bind.

ELIZABETH NOLAN: Yeah. So the antibody was still able to bind. Right. What kind of gel is this? Well, it's SDS page, but how is it being monitored?

AUDIENCE: It's a [INAUDIBLE] so it's a biodome radiogram.

ELIZABETH NOLAN: Yeah. We're looking at radioactivity too so just to keep that in mind for the ban. Right. That's what's allowing us to see. What about lane five?

AUDIENCE: It shows that in the delta DnaK line that nothing's pulled down by the immunoprecipitation.

ELIZABETH NOLAN: Right. So no DnaK. Nothing's pulled down. This is a very helpful control, because imagine if you did see bands, that would indicate that there's some lack of selectivity with this immunoprecipitation step. So I think that's quite a nice experiment they added into this piece of work here. OK.

So moving on to their next experiment, what happens during the chase if we look at different time points during the chase period? So this is similar again to what was done with the GroEL/GroES study.

So what we're looking at is a one DSDS page. We have the molecular weight marker. All of these are with the immunoprecipitation. And we're looking at times from below one minute up to 10 minutes. So the question is what do we see in these data here.

AUDIENCE: As time goes on, you see more and more concentrations of the smaller proteins.

ELIZABETH NOLAN: Yes. So we're seeing fewer proteins as time passes. Right. Let's start with the first time point here. Does that look to be in pretty good agreement to what we saw on the prior slide? Right. We see that there's a number of polypeptides that DnaK is interacting with. Right. And they're over a range of molecular weights.

Right. And then exactly as we just heard, as time progresses, what we see is, overall, there's fewer polypeptides. But it looks like there's fewer polypeptides of lower molecular weight here. So what does this suggest if you're going to interpret the data?

AUDIENCE: Probably that lower molecular rate peptides are folded more quickly.

ELIZABETH NOLAN: Yeah. So maybe the folding there is complete over this time or it's complete to a point that DnaK isn't needed anymore. Right here. What do you think about the DnaK band?

AUDIENCE: Quite constant.

AUDIENCE: Yeah.

ELIZABETH NOLAN: Quite constant. So does that make sense? Yeah, why does that make sense?

AUDIENCE: Because it's just the whole quantity is not changing. And it's not involving [INAUDIBLE]. Just like in the case of the less [INAUDIBLE] so when [INAUDIBLE].

ELIZABETH NOLAN: Yeah, right? So there was some newly-- what this indicates, right? There was some newly synthesized DnaK in those 15 seconds of the pulse. Right? And that has stuck around. And that's all precipitated to the same degree in each sample here. So what about this data here? And then, how helpful is this data? Right?

So effectively, what was done is, radioactivity was measured by liquid scintillation counting. OK, so they measured the total radioactivity in each sample prior to separation. And then, they've converted that to some arbitrary scale of proteins bound to DnaK. Right? So we see that, over time, the total radioactivity decreases and effectively comes to some sort of plateau.

So that's just some nice quantitation in terms of what we see here in the gel, right? It's quite easy to measure the total radioactivity in a sample. And you get a measure of that from liquid scintillation counting. And then, you can look at the gel, right? And they're in good agreement there. And as I said, this is completely arbitrary, what's on the y-axis.

So do these experiments tell us much about the specifics of DnaK function? So we see polypeptides being bound. We see over time that fewer are bound. What's actually happening in the cell?

AUDIENCE: I don't think we can conclude much from these. Only that we know that it interacts with the polypeptides for some amount of time.

ELIZABETH NOLAN: Yeah. Right. I agree. So is it acting as a foldase? Is it acting as an holdase-- an un-foldase? That's not clear from the data presented in these experiments. And I'd say overall, there are

studies that show different things, depending on the system there, for this. So that's where we're going to close with the chaperone systems. And where we're going to move into-- actually, one more comment, right, before closing on the chaperones.

What happens if certain ones are deleted? So just to reiterate, not all of these systems are required for cell viability of E. coli. It's only GroEL/GroES. Right? So you might ask if trigger factor or DnaK or DnaJ is deleted, what happens in the cell to keep things functioning properly? And just one observation. If trigger factor is deleted, OK, there's no growth phenotype. Is that surprising? Right?

That observation may depend on growth conditions. But say you're in some standard growth conditions. What's observed is that, in the absence of trigger factor, DnaK and J can basically compensate for that loss of function. OK? But then, if trigger factor and DnaK are deleted, at higher temperatures, that becomes lethal here. OK? The cells can't cope for that. But at lower temperature, GroEL/GroES can compensate for that loss of function.

OK. So we're on to protein degradation. There's some incredible macromolecular machines involved in this unit here. And we'll move on to that one come Wednesday. Just if we think about where we're going with lifecycle of a protein, right, we've gone from synthesis to folding. We've learned that misfolding can occur. OK? And at some point these polypeptides, whether they're folded or unfolded, need to be degraded. So they have some lifetime in the cell. OK?

And so we can think about proteases, so classical enzymes, like trypsin. And we can think about proteasomes, which are degradation chambers. And these players are really important because they have a role, big picture, in controlling the dynamics and lifetimes of all proteins and cells.

So what are some of our questions for this module? Why are proteins degraded? We just said a little bit about that. How are proteins degraded? And what types of proteases exist? We'll briefly today touch upon the general catalytic mechanism because that's important to have this background for thinking about the degradation chambers.

So what are the general mechanisms and what are the active site machineries? Protease inhibitors are really important at the lab bench, and they also have a big role in therapeutics. And so we'll talk about those a bit here. And then moving into protein degradation machines, we're going to look at ClpXP from E. coli as a case study. So we need to think about, what are the structures of these degradation machines, what are the mechanisms?

How do they differ in prokaryotes and eukaryotes? So after spring break, Joanne will spend some time talking about the eukaryotic proteasome there. We won't talk about it as much immediately here, but we'll come back to that later. And how are proteins that are destined for degradation by a proteasome tagged to get to that destination?

So here are our topics. An overview, which is where we'll focus today. And then, looking at ClpXP, and down the road, the 26S proteasome. So first, thinking about proteases. Some general points to get everyone up to speed. So because we all know proteases catalyze the hydrolysis of peptide bonds. So we can just think of some peptide and that peptide bond gets hydrolyzed to give us these products here.

Why do we need a protease? The bottom line is just that spontaneous hydrolysis of peptide bonds is very slow, right? So we can leave a protein or a polypeptide on the bench top. And maybe it will unfold. Maybe it will precipitate. But it's not going to have the peptide bonds being broken unless something else has been done to it, right? So we can think about a half life on the order of seven years.

And so proteases give tremendous rate accelerations on the order of 10 to the ninth. And we can just think about chemistry for a minute and what we might do as a chemist to hydrolyze a peptide bond. So hydrolysis is pH dependent. And so in chemistry we'll use acid or base to hydrolyze a peptide bond. And we can think about base catalyzed reactions, such as this one, where we have our OH-minus group attacking, or acid-catalyzed reactions, as this one here. OK?

So effectively we can just think about pH dependence of hydrolysis. Just if we have rate and we have pH. Something on the order of this, right? Where we have enhancements at low and high pH and a relative minimum at neutral pH here for that. And so these types of chemistry is going to come up in the context of the protease enzymes, depending on the type, as we'll see in a few slides.

So we can think about proteases as being irreversible biological switches, that these reactions are irreversible. And what does this mean from the standpoint of the cell? It means that the cell needs some way to handle and deal with these proteases, right, such that they don't cause unnecessary hydrolysis of polypeptides. That would be very deleterious to the cell, right, if a protease was running rampant and hydrolyzing proteins that it shouldn't here.

So what are some strategies that the cell can use? One, cells are quite good at controlling protease activity, both in terms of space and time. And there's a variety of different strategies, depending on the locale and the protease. So regulation is really key here. And some examples are provided here.

So one is that proteases will be stored as zymogens or inactivated precursors. And there'll have to be some event that activates this zymogen to give the active protease. Proteases can be stored in separate organelles here. So these might be zymogen granules or lysosomes. And sometimes they're stored with a protease inhibitor, as well.

And another strategy, which is really the strategy we're going to focus on as we move forward in this module, is that degradation chambers are used, such that you have this huge macromolecular machine where all of the protease activity is in the inside. And what this means is that somehow a condemned protein that needs to be degraded by this machine needs to be tagged. And there needs to be some mechanism to get it in the inside of the chamber. So effectively, degradation will limit access of the active sites to the rest of the cellular environment. So that's what we see in ClpXP and this 26S proteasome.

Just to note-- so just the other week in *C&E News,* there is a highlight of a pretty exciting paper. So I noted that proteases are of interest and important from therapeutic development. And here's a little excerpt about a molecule shown here that's found to hit the proteasome of malaria parasite. And so hopefully, by the end of this unit, if you go back and read this, you'll have some sense as to why is this a good inhibitor of the proteasome or a protease. And what's going on in terms of the proteasome machinery here. And how can we differentiate proteasomes from different organisms.

Back to some of the strategies. Just an example is zymogen activation, and thinking a little bit from the perspective of the organism. So here, we can think about the gut. We're in the small intestine. So there's the epithelium, the cells, these are crypts. And here's the lumen, so the space where the food goes through, et cetera. What do we see?

So inside the intestine, there's a protease named entarokinase. And it has a role of activating trypsinogen. So trypsinogen is a zymogen. It's produced by the pancreas. And the pancreas delivers trypsinogen and other things into the small intestine. And so once it reaches the small intestine where its activity is needed, it will be activated by the action of entarokinase to give trypsin.

OK? And then what can happen? Trypsin can also activate trypsinogen, and it will also activate chymotrypsinogen to give chymotrypsin. Right? So the net result here is protease activity in the intestinal lumen, which is the extracellular space here. And so they travel from the pancreas in a form that's inactive and then become active in the intestinal lumen there.

So as I said before, proteases are important. And if we think about this role in controlling dynamics and lifetimes of proteins and cells, what are some of those roles? And I guess I also point out this also-- they also can exist in the extracellular space. So if we think about homeostasis and how proteases can regulate homeostasis, just some examples. They can remove misfolded proteins or aggregated proteins. They can provide amino acids when needed, right?

So after destruction of a polypeptide, you have small fragments or amino acid monomers. And they can modulate many cellular functions. So just some examples. And this is to show the broad range. We can think about blood clotting, the generation of hormones, just digestion and recycling of amino acids. So energy harvesting, the cell cycle, control of the cell cycle, and even cell deaths. So thinking about apoptosis here.

And if we just select two of these cellular functions and how proteases play a role, what I have here is the maturation of insulin, a peptide hormone in the blood coagulation cascade. OK, so if we take a look, insulin is a really terrific molecule. And if you're looking from some trivia not shown here, it also binds zinc and forms an interesting oligomer. So if you're interested in metals, that's a good one.

But what do we see? We see that insulin is synthesized as a prepropeptide. And so in blue, we have a signal sequence. And then we have these chains here. And look, there's a bunch of cysteines, right? So there's action of a protease. And what do we see? The signal sequence is cleaved and at some point in this process, there's formation of disulfide bonds, right, in some regiospecific manner.

So this is pro insulin. And then what happens? There's another protease cleavage event that gives us the mature form of insulin. This grape chain here is removed. OK? So this is an example of a hormone being stored as an inactive precursor. And actually, there's many peptides that are stored as inactive precursors. And then some protease has to come and cleave a pro region.

So in my group, we're interested in a family of antibacterial peptides called defensins that are

in the intestine. And they have a pro region. And it's trypsin or another protease that comes along and has a cleavage event to release the active peptide. So not only limited to insulin here.

If we look at the blood coagulation cascade, we can imagine that we don't want blood to coagulate on whim, right? That'd be a huge problem. So proteases are required to allow coagulation to occur. And what we can see here is that prothrombin is converted to thrombin by a protease. And thrombin is a serine protease. And we'll hear more about serine proteases in a little bit. That converts fibrinogen to fibrin. And as a result, coagulation occurs. And that's important for wounds.

And there's many, many other examples. So if we think about types of proteases and mechanisms of catalysis, what I just would like you all to be aware of is that there's two general mechanisms. And we can think about four different mechanistic varieties within that.

And so we can divide these up by proteases that are involved in covalent catalysis. So there's formation of a covalent acylenzyme intermediate. And this is what we'll see for serine proteases, cysteine proteases, and threonine proteases. So examples here are quite relevant to this module as ClpXP is a serine protease. And as you'll see later on, the eukaryotic proteasome is an end terminal threonine protease.

The second general type are proteases that accelerate the direct attack of water on the substrate. OK, so this is non-covalent catalysis. And the types here are aspartyl proteases and zinc proteases. So there was a question a few lectures ago if there's metal-dependent proteases. And the answer is yes, zinc proteases. And we can also think about these from the standpoint of the acid and base catalyzed chemistry we saw before.

So just for some trivia. If we think about the human proteome-- 533 proteases. And this is a count here. So on the order of 200 serine proteases, 140 cysteine, around 190 metalloproteases, and 21 aspartyl proteases. So we have many of these enzymes to act at different places and points.

If we take a look at the active site machinery, what do we see? So here we have the serine proteases. They have a catalytic triad comprised of aspartate, a histidine and a serine here. Cysteine proteases-- we see a cysteine and a histidine. And so these are the ones involved in covalent catalysis.

Here we have the non-covalent catalysis. So the aspartic acid or aspartyl protease. We have two asp residues. And here we have an example of a zinc protease, where we see a single zinc ion coordinated by two histidines, and in this case, a glutamate and a bound water. OK?

So if we think about just the covalent versus non-covalent catalysis here. So when I get further along. So imagine we just have some dipeptide. What we find in these enzymes is that they have what's called an oxyanion hole here. And we can think about the enzyme allowing attack as such. So that or some nucleophile here.

So what do we get? We get a covalent acylenzyme intermediate. OK, we have the oxyanion hole. And these are the serine and the cysteine proteases here. OK? And we'll go through in more detail the mechanism in a minute.

If we think about non-covalent catalysis, and again, we have our dipeptide. We can just think about for a minute one of the metalloproteases, right? So in these cases, the protease is accelerating the direct attack by water. So I imagine we have some metal here that has water bound, right? What happens? Imagine we can de-proteinate the water molecule. And then there can be attack.

OK, so why does the metalloprotease allow this to occur? So what's happening when the water binds to the metal that will facilitate this? So we can think about the pKa of a water molecule, right? And what happens when a water molecule is bound to a metal, right? Say zinc. So how do we think about a metal?

AUDIENCE: A Lewis acid.

ELIZABETH NOLAN: Yeah. Right? We have a general Lewis acid here. Here. So what's going to be effect of the pKa of the bound water relative to unbound water?

AUDIENCE: It'll be more acidic.

ELIZABETH NOLAN: Right. We're going to lower the pKa of the bound water, which is going to help generate the nucleophile. Right? So that's how it's facilitating the direct attack here. OK, so what we're going to do is look at the serine protease example in a bit more detail.

AUDIENCE: Are the end termini de-pertinated? Or is it--

ELIZABETH I am just-- what would it be at physiological pH?

NOLAN:

AUDIENCE: NH3?

ELIZABETH NOLAN: The embryote NH3 plus. Right. So these are just showing a simple dipeptide that we have NH3 plus and O minus in terms of the acid ends there. OK. So just thinking about here, this covalent catalysis. So here's each protease type, the active site, and the nucleophile. So in the case of the serine proteases, the nucleophile is the serine side chain.

And I'm showing this because ClpXP-- ClpP protease-- uses serine protease chemistry here. So what we observe in this overview is a generally accepted mechanism. And we see formation and collapse of this covalent acylenzyme intermediate. So if we take a look here, we have a bound polypeptide. This is the oxyanion hole provided by these two NH. Here we see the aspartate, the histidine, and the serine. Right?

So what do we see happening here? First, there's formation of a tetrahedral intermediate. So there's an attack. OK? And here we have loss of the RNH2. And here what do we see? We see, basically, the histidine working on this water molecule. We have collapse of this acylenzyme intermediate, another tetrahedral intermediate, and release of the acid product here. OK?

So in thinking about this, we think about the histidine as being a general acid general base involved in general acid-base catalysis, a proton carrier. We see this oxyanion hole providing stabilization here and here. Right? We have this negative charge. And something that you need to think about are the pKas. If we think about just pKas of amino acids and how this chemistry is happening. Right?

So what is a little bit mysterious here, based on our knowledge of pKas of the catalytic triad? And they give some approximate values just for serine, histidine, and aspartate here.

AUDIENCE: Well, each proton abstraction is being done by something that should, theoretically, have a lower pKa. So you have an aspartate abstracting a proton from a histidine, which is abstracting a proton from serine. So there has to be a lot of perturbation of the system for that to happen.

ELIZABETH NOLAN: Yeah. Right. There needs to be a lot of perturbation to pKas for this to work, right? How easy is it to de-proteinate the serine by a typical histidine? Is that going to happen based on pKa?

AUDIENCE: No.

ELIZABETH NOLAN: Right? So there's something about this active site and the environment that's going to give perturbation of these values. If we just move beyond this cartoon form for a minute, and just look at the catalytic triad from chymotrypsin from a crystal structure. This is the orientation of the serine histidine and aspartate.

And something to keep in mind is that different serine proteases, or different proteases in general, have different substrate specificity's, which means they prefer to cut before or after a given type of amino acid, depending on the side chain. And this is just a cartoon depiction indicating that, here's the peptide, here's some side chain, and there's some recognition site here.

So there's a degree of substrate discrimination. For instance, trypsin likes to cut after arginine and lysine. But it will cut at other places, as well. Right? Chymotrypsin likes aromatic hydrophobic residues. Elastase likes small and uncharged residues here for that. So you may have seen diagrams or cartoons of specificity pockets for thinking about substrate discrimination amongst these proteases. And I guess what I would just say is, it's not so simple as those types of cartoons.

If we look at the structures of serine proteases, just to compare, what we see is that, for trypsin, elastase, and chymotrypsin, they have similar overall structure. So this is an overlay of the three enzymes. And the catalytic triad is shown in red. OK? So despite this similar overall structure, they have distinct substrate preferences. And it's just something to be aware of. So you're not responsible for the origins of this substrate discrimination.

And here's just a view showing more about the secondary structure of trypsin shown here. And in this case, there's an inhibitor bound. And I'll just note, in terms of the substrate preference, and these are the three types of activity we'll end up seeing within the eukaryotic proteasome. So if we just have some polypeptide. OK?

OK, and so imagine we're thinking about hydrolysis here. OK, so the C terminal end of this amino acid with our one side chain. We think about the enzyme and the identity of R1. For trypsin, it prefers to cut after arginine or lysine. OK, so a positive charge.

For chymotrypsin, phenylalanine, tyrosine, and also other ones like valine, leucine, and isoleucine. OK, so aromatic plus hydrophobic. And then elastase here. And we find that elastase prefers to cut after small. And if I'm boxing it, these are the ones we think is most

preferred, small and uncharged residues. OK?

So some discrimination based on side chain identity. So where we'll close the general background is just with a note on proteases and disease and protease inhibition. So if we consider various human diseases and proteases, there's many, many links. And many proteases are implicated in a variety of diseases and pathologies.

And so this is a table just to give you a sense of the breadth. What we see in terms of the class is that all of the classes are represented here. And that we see diseases ranging from cardiovascular problems, to cancer, et cetera, cystic fibrosis, inflammation here. OK? So as a result, there is quite a bit of interest in terms of the possibility of protease inhibitors as therapeutics.

And beyond that, they're also widely used in the lab. So how do these inhibitors work? Just as a general rule of thumb to think about, generally they react to form a covalent bond with the catalytic nucleophile. So for instance, for the serine proteases, they'll form some covalent bond with the active site serine residue.

And we can classify these inhibitors as being either reversible inhibitors or irreversible inhibitors. So as those names indicate, if it's a reversible inhibitor, that covalent linkage between the protease or the proteasome and the inhibitor can be broken down. And types of reversible inhibitors, for instance, use aldehydes as the reactive group.

So in contrast, the irreversible inhibitors form a covalent linkage that is not readily broken down with the catalytic nucleophile. And so irreversible inhibitors include vinylsulfones. And if you go back and look at that little excerpt from *C&E News* about this molecule that's inhibiting the proteasome of malaria, you'll see that it has a vinylsulfone on its terminus here. Epoxides are also employed.

OK, and generally, if we have inhibitors that block the function of a protease or a proteasome, they're going to interfere with many critical cellular functions right here. And just in terms of cancer, just some observations. So it's been found that proliferating cells are sensitive to proteasome inhibitors. And there's some proteasome inhibitors that can selectively induce apoptosis in proliferating cells.

And so cancer cells are proliferating, and there's interest in the use of these as anti-cancer drugs. So what I've included in these slides are some examples of inhibitors of each class, and

then the mechanisms. Here are just three molecules. So we have either reversible or irreversible inhibitors, right? And what is there to note looking at these molecules? They're all polypeptide-like. Right?

So there's amino acids or moieties that, with a little imagination, we can think about as being somewhat similar. And then we see these reactive groups on the terminus. So the aldehyde, for instance, the vinylsulfone. And so if you look at the structure of this molecule being used to inhibit the malaria proteasome, there's some clear similarities to these here.

In terms of mechanisms, we can think about these reversible inhibitors. So for instance, the chemistry with the peptide aldehyde. Here we're seeing the nucleophile of the eukaryotic proteasome, which is really interesting because it's an end terminal threonine. That's why we're seeing it drawn as such here. So we can have formation and collapse of this species here. Or in the case of the irreversible inhibitors, we have the vinylsulfone and the chemistry that happens here for that.

And so if you're interested in these, I encourage you to look at the mechanisms a bit more. And we'll see a little bit more on inhibitors being used experimentally as we go through the rest of this module. So where we'll start on Wednesday is looking at the structure of E. coli ClpXP, which is a degradation machine used to degrade certain condemned proteins there. OK?