Biochemistry 401 lecture 31. Today we're going to talk about protein degradation. We'll begin with dietary proteins, and then work on to cellular proteins. We're then going to talk about amino acid catabolism. This includes the urea cycle. We're then going to finish with inborn errors of amino acids metabolism.

The digestion of dietary proteins begins in the stomach. This occurs through the action of an enzyme called pepsin.

Pepsin is originally synthesized as an inactive zymogen called pepsinogen. Pepsinogen includes a 44 amino acid portion that's stuffed into the enzyme active site, and so in order for this enzyme to be activated, 44 amino acids must be clipped away. This happens when the pepsinogen hits the acidic environment of the stomach.

The low pH allows that protein to unfold just a little bit, because there are amino acids that become protonated, and so the salt bridges that were there – the ionic bonds – are no longer there. And so the enzyme opens up just enough for these 44 amino acids to become vulnerable to proteolysis.

And so pepsinogen autoproteolyzes, and the 44 amino acids are gone, and the pepsinogen becomes activated to pepsin.

Which of these amino acids has an R-group that you might expect to go from a non-protonated to a protonated state following a shift from pH 7.3 (what you would find inside a cell) to about pH 2. This is about the pH of the interior of the stomach. So which of these: lysine, glutamine, aspartate, alanine, or tyrosine? That's right, it's aspartate. There are actually aspartates in the active site that are responsible for the catalytic activity of this enzyme. And so when aspartate becomes protonated, it's no longer going to bind to a lysine. Therefore, the protein can open up just a little bit to become vulnerable to proteolysis, and so this 44-amino acid segment that is present in the active site is going to be cleaved away to activate this enzyme.

Now the thing with pepsin is, it is a random cleaver. It just goes along, and it chops up proteins randomly, non-specifically, and so there's not an amino acid sequence that it favors. It just goes after proteins and proteolyzes them.

The thing is, once this pepsin reaches the duodenum, the duodenum is a different place – It's the first part of the small intestines – you have bile coming in.

Now bile doesn't just have products from the liver coming in, not just bile salts; it also has pancreatic juices, it's called. These contain proteases, also,

that are secreted from the pancreas as zymogens, and then, once they get to the duodenum, they are activated.

Bile also contains bicarbonate, and the bicarbonate is going to raise the pH of those stomach contents that are coming from the stomach into this first part of the small intestines, and so this bile is going to increase the pH of the food as it travels into the duodenum. This means that pepsin is going to be deactivated. It does not do well in a high pH environment, and so it's going to become deactivated. This is really good for the proteases that are coming in from the pancreas. Now they're not going to be randomly cleaved by this pepsin. Awesome. Once pancreatic zymogens get to the small intestine, there is a protein on the outer surface of the enterocytes. So we have the small intestine, okay? Let's say this is this is the surface of my small intestine, and we're going to have a protein that's on the surface that is called enteropeptidase.

This is a transmembrane protein that has a protease activity that extends into the lumen of the small intestine. So, when the zymogens come in from pancreas, you first start off with trypsinogen. Now trypsinogen is the zymogen. You know it's a zymogen, because it has "ogen" at the end. These zymogens are either going to have "ogen" at the end or "pro" at the beginning. That's how you know. So here comes trypsinogen. It's going to encounter enteropeptidase. Enteropeptidase is going to cleave off the inhibitory portions of trypsinogen to make it into trypsin. Now trypsin is going to go along and activate all of those zymogens that come in from the pancreas. So this is – like –chymotrypsinogen, this is proelastase

– and you can see right here in the diagram that there is a cascade. Trypsinogen is going to be activated by enteropeptidase. It's going to become trypsin, and it's going to activate the proteases in this cascade. Now the thing is, is that these proteases are specific cleavers. They hydrolyze the peptide bond at specific amino acid sequences. Why? Because these particular sequences fit into the active sites of these enzymes. This has to do with the architecture of the active site itself in these proteases. Active site specificity. Let's take a look at that.

For instance, trypsin has an aspartate in the active site, whereas elastase has a couple of valines in the active site. And it is the nature of the active site that determines its specificity. Each of these proteases, chymotrypsin, trypsin, and elastase, cleave the peptide bond after a specific amino acid that fits into the active site. Can you pair these proteases up with their target amino acid? Because trypsin has a negatively-charged amino acid, aspartate, in its active site, it's going to be specific for positively-charged amino acids, like lysine and arginine. Chymotrypsin has a large active site without any bulky hydrophobic residues, without any charged residues in it, so it is going to cleave after large hydrophobic amino acids, like phenylalanine or tyrosine, or even tryptophan. Elastase likes to cleave after an alanine or a valine. Do you have to remember these specificities off the top of your head? No, but if I give you the active site, you'd have to be able to match that up with the type of amino acid that it would cleave after. And so once these

proteins are cleaved by these proteases – both from pepsin and pancreatic proteases – they're taken in to the enterocytes, the epithelial cells lining the gut (the small intestine) and then they're broken down even more by peptidases that are in the enterocytes. Finally, these are shuttled in the bloodstream as amino acids. What can we do with amino acids? Well, we can use them to make other

proteins, right? We can also use them to make other metabolites that we need. But

another thing we can do, is we can break those down, take the amino group off, and then we have a carbon skeleton, right? And so we can use that carbon skeleton in

anabolic reactions, and for energy. So now we're going to turn to the breakdown of endogenous proteins. There are times that we have to break these down. Why? Well, one

of the ways we control the activity of enzymes, is by cleaving them – getting rid of them –and reducing their concentration in the cell. So we can regulate enzyme activity by getting rid of some of them. Another thing we have to worry about is

proteins that are misfolded. Now these can either be proteins that never made it when they were being translated, they just came off the ribosome a little wonky and they never folded right. Chaperone couldn't help them, and so they're going to be degraded. Same thing for proteins that have gotten damaged in the course of their lifetime, and now they can't fold properly. They can also be degraded. if they can't be refolded by a chaperone, they get degraded. Another

thing that's important to know is that proteins have characteristic half-lives, right? They have sequences at their end that caused them to either be really stable proteins and hang around for a long time, or to be unstable proteins and have a lifespan of only a certain amount of time, and this is an intrinsic property of the protein itself, caused by its sequence. Another reason we would degrade proteins is so that we can use those proteins for fuel. We can break

down proteins and get the amino acids and then catabolize those in order to gain energy as we feed those carbon skeletons into the TCA cycle. And as we said before we can also use these amino acids for anabolic precursors. So let's look at how proteins are degraded in the cell. As you can imagine, it's something we have to be really careful of. You want to make sure that we degrade the right protein at the right time. Otherwise, we can get in big trouble, right? Okay, let's take a look at that.

So we talked about ubiquitination before. This is the addition of ubiquitin proteins to a protein that's being degraded by the

proteasome. The ubiquitination happens through the action of three enzymes – E1 activates the ubiquitin, E2 binds to the ubiquitin, and E3 transfers the ubiquitin to the protein. E2./E3 are very specific for specific targets. They have an architecture that is complementary to a certain protein shape and causes its ubiquitination, and its transfer to the proteasome. The thing is, is

we don't just have one ubiquitin that's attached, but we'll have a whole string of them. You know, at least four or more, that are attached to a protein in