Biochemistry 401 Lecture 34
Today we're going to talk about nucleotide catabolism. We're also going to discuss DNA structure, including A, B, and Z DNA, and we'll talk about histones and higher-order structures. So let's get started.

The breakdown of nucleotides relies on three major classes of enzymes. These are nucleotidases, nucleoside phosphorylases, and phosphoribomutases. The nucleotidases breakdown nucleotides to nucleosides, by removing the phosphate groups. Nucleoside phosphorylase removes the base from the nucleoside to yield a free base, plus ribose 1-phosphate. Phosphoribomutase isomerizes ribose 1-phosphate to ribose 5-phosphate, which is an intermediate in the production of PRPP. And so ribose 5-phosphate can be recycled to make more nucleotides.

Once the base is released, it can be broken down.
Now we're going to look at the catabolism of purines. The strategy is this. First convert both AMP and GMP into a common intermediate, xanthine, and then convert that into urate for excretion in the urine. We're going to start with a nucleotidase, that's going to cleave the phosphate group from the nucleotide, AMP, to form adenosine, the nucleoside. We're then going to release the amino group. We're going to use adenosine deaminase to do this, and we end up with free ammonium, and inosine. And the next thing that we're going to do is we're going to use a nucleoside phosphorylase to remove the base, hypoxanthine, from ribose 1-phosphate. We're then going to use a very important enzyme called xanthine oxidase. This is going to introduce another ketone group in hypoxanthine to make xanthine. There's one less oxygen in hypoxanthine than there is in xanthine, and that's a good way to remember the difference between these two intermediates, and so what we're going to do is we're going to add oxygen and water; we're going to make peroxide, and we're going to make xanthine. This is a common intermediate in the breakdown of both GMP and AMP. We're going to use xanthine oxidase again and this time we're going to make another ketone group in another position. We're going to end up with peroxide again, and uric acid. This is going to become deprotonated to form urate, which is the conjugate base.

And so, for humans, this is where it stops, with urate. Most mammals turn uric acid into allantoin, and then this is released in the urine. Fish break down allantoin even further, and release ammonia into the water.

But for humans and great apes, the catabolism of purines stops at urate. This is because we've lost the uricase gene, and so we cannot catabolize urate to form allentoin, and for this reason we excrete urate in the urine.

Now the thing is, urate circulates in the bloodstream at fairly high concentrations. Humans have an even higher concentration of urate than other primates, and this is thought to have given us an evolutionary advantage. This is because urate is a very potent antioxidant. In fact, it's just about as powerful as ascorbate, vitamin C, and so it's thought that perhaps the loss of uricase counters our loss of the ability to make vitamin C.

Urate is present in high concentration in the blood and it's really close to saturating concentrations. It can actually precipitate out as sodium urate crystals. These are shown in polarized light here, and they look like little needles. You can imagine if these precipitated out of the blood, into joints or into the kidneys, that it would be very painful, and this is true. This causes a painful condition called gout and persons who have overly high concentrations of urate can also have kidney stones.

Treatments for gout are to change the diet, to decrease the amount of foods taken in that have high purine concentrations. Believe it or not, one of the first things to go is beer. And shellfish. And spinach. Gout can also be treated with medicine. Now allopurinol is used as a competitive

inhibitor of xanthine oxidase. It inhibits in two places, both in the production of xanthine, and in the production of uric acid, and so in this way it blocks both the production of uric acid from AMP, and also from GMP.

Another problem that's associated with the catabolism of purines, is something that's called severe combined immunodeficiency syndrome, and this is caused by a defect in adenosine deaminase, and so the deamination of adenosine to make inosine is deficient, and for this reason there's a roadblock. Adenosine will build up, and AMP will build up.

Because of the ADA deficiency, the road from AMP and adenosine to inosine is blocked. Therefore, these intermediates, AMP and adenosine, will feed into the

production of ATP. Now we know that ATP is a positive regulator of ribonucleotide reductase, and because we have an overabundance of AMP and adenosine, above normal levels, these will be used in order to make dATP, and because of the rise in levels of dATP, this will inhibit ribonucleotide reductase, and will inhibit the production of deoxyribonucleotides as a whole, and this has a profound effect on one of the organs in our body, especially. This is the thymus.

The thymus is an organ that sits in the middle of the chest, just beneath the breastbone. It encircles part of the trachea. This is an organ that is responsible for the maturation of thymic cells, T cells, which are a primary cell involved in the immune response. As you can see from this slide, at birth, the thymus is about 15 g and it increases in size from birth to puberty, more than doubling in fact. Then there is a general decrease in size until, by 70 years of age, it's only about 5 g. It just about disappears. The mass, in fact, of the thymus at this point, is mostly adipose and connective tissue.

Now thymic tissue is especially sensitive to decreases in adenosine deaminase activity, and this is because the thymus cells generally have a great deal of adenosine deaminase activity. In fact, if you look at the activity of adenosine deaminase per milligram of protein, the levels of adenosine deaminase activity in human tissues taken from surgeries and postmortems on children that were up to and including one-year-old, as you can see, the greatest nanomole of adenosine activity per milligram of protein is seen in the thymus. By far and away, this is true. As we saw, the thymus should increase in size in children. This is why a loss of ADA hits the thymus hard. It causes severe combined immunodeficiency syndrome. Nearly half of all cases of SCID, in fact, are caused by ADA deficiency. This is because the maturing thymic cells, T cells, in children are especially sensitive to ADA deficit. This is because cell division is decreased because of a lack of deoxyribonucleotides for replication, and also for DNA repair. The incidence of apoptosis, program cell death, is increased. For this reason, these children have a compromised immune system, and are highly susceptible to infection. In the tissue preparation shown in blue, we see mouse thymus. On the left we see normal thymic tissue, and on the right we see thymic tissue in a mouse homozygous negative for ADA. Because adenosine deaminase is deficient, there is a reduction in the number of cells in this tissue, and an increase in apoptosis. Those cells that are apoptotic are shown in red, and you can see that there's an increase in apoptosis in the ADA-deficient thymus.