Biochemistry 401 lecture 32.
Today we're going to continue our discussion of nitrogen containing biomolecules. We're going to talk about the incorporation of nitrogen into biomolecules. We're going to start with nitrogen fixation, and then go on to nitrogen assimilation, amino acid anabolic pathways, general synthetic strategies, and regulation. So let's get started.

The ultimate source of nitrogen for the planet is actually atmospheric nitrogen gas. Our atmosphere is about 80% nitrogen. The thing is, in order for this nitrogen to be used, it must be split and reduced to make ammonium. Most of the newly fixed nitrogen, about 60% is produced by archaea and bacteria. Other sources include lightning, ultraviolet radiation, and industry during the production of fertilizers. The plants take in nitrates and nitrites and they reduce them with electrons to reduce cofactors to make ammonium for synthetic reactions. Animals like us, ingest nitrogen-containing compounds and get our nitrogen supply that way.

Nitrogen fixation occurs in diazatrophic organisms like some bacteria and archaea. The term diazatrophic means nitrogen-fixing. These organisms can split and reduce atmospheric nitrogen, reducing it to ammonia. Now the triple nitrogen bond is difficult to break because of the instability of highly reactive, high-energy intermediates in the pathway. The general progression from N2 to ammonia is energetically downhill, it's just getting there that's difficult, because of the presence of high-energy intermediates. These reactions require special catalysts that are not found in higher organisms.

Nitrogen fixation is an energetically expensive process that requires 16 ATP per mole of nitrogen split and reduced. The overall progression is N2 +10 protons, 8 electrons yielding two molecules of ammonia and one molecule of hydrogen gas. Let's see how this happens.

This enzyme complex contains two halves, a reductase and a nitrogenase. The reductase transfers electrons from a donor protein, like ferredoxin to nitrogenase, and the nitrogenase splits and reduces the nitrogen. Now the passing of these electrons actually requires that the two halves of this complex come together, and this is accomplished through the hydrolysis of ATP. ATP hydrolysis causes a

conformational change that brings these two halves together, so that the electrons can be passed from reductase to nitrogenase.

In this diagram, we see reductase on the left in white, and the nitrogenase, in blue and green, on the right. The reductase has an iron sulfur cluster, and is called a Fe protein. The reductase contains an iron-sulfur cluster as well, but in addition contains an iron-sulfur-molybdenum cluster, and so nitrogenase is called a MoFe protein. The presence of molybdenum is unusual in an enzyme, and it is necessary for nitrogenase function.

Now the bacteria that carry this out can either be free-living bacteria, bacteria that live in the soil for instance, or can be symbiotic bacteria. Symbiotic bacteria include bacteria that infiltrate the roots of plants like legumes

and form root nodules. Here we see nodules. Root nodules like this are seen in such plants as beans and peas and alfalfa.

Now this whole process of incorporating nitrogen is very sensitive to oxygen, and so oxygen must be kept out of the system. The sequestering of oxygen ensures that side reactions, such as the production of water, do not occur,

Leghemoglobin sequesters oxygen. If you'll recall, leghemoglobin is a homolog of the beta subunit of hemoglobin and of myoglobin. The presence of this homolog means that the bacteria can still run the electron transport chain, without endangering the fixation process.

The enzymes that are involved in nitrogen flow can be a little confusing, so I have listed them here for you. We've already seen aspartate aminotransferase, and alanine aminotransferase. These catalyze reversible reactions. Glutamine synthetase catalyzes the irreversible formation of glutamine, whereas glutaminase catalyzes the irreversible loss of glutamine. Glutamine dehydrogenase we saw in the last lecture, and this is an enzyme that in animals goes in one direction predominantly, to lose ammonium. We use glutamate dehydrogenase to remove ammonium from glutamate, to yield alpha-ketoglutarate, and then we use the ammonium to make urea, and again this happens as we said, in the liver. Now in prokaryotes, especially those that are involved in nitrogen fixation, glutamate dehydrogenase goes in the reverse

direction, to assimilate ammonium to make glutamate. Lower organisms also have the enzyme glutamate synthetase. So let's look at this little more closely.

Here we see some of the major reactions that we're going to look at in this lecture, and some that we saw previously. Previously we saw glutamate dehydrogenase releasing ammonium from glutamate to make alpha-ketoglutarate. We also saw aspartate aminotransferase, and also alanine aminotransferase that transferred an amino group from an amino acid to alpha-ketoglutarate to make glutamate. Now we're going to look at some other reactions in which glutamine is made, and broken down. The irreversible formation of glutamine occurs via a synthetic reaction that requires ATP. This is catalyzed by glutamine synthetase. Glutaminase catalyzes the irreversible loss of glutamine to make glutamate, and ammonium is released. We're going to look at this little more closely.

Glutamate dehydrogenase in prokaryotes is involved in nitrogen assimilation. In the assimilation of nitrogen it's important that the L-amino acids are synthesized, and so the stereochemistry of this addition is important. The enzyme glutamate dehydrogenase catalyzes the reaction in which alpha-ketoglutarate plus ammonium yields a Shiff base intermediate, plus water. This Shiff base intermediate is then reduced to make glutamate. This is a synthetic reduction, and so in this case we're going to use NADPH. Now when we looked at the reverse reaction in eukaryotes in preparation for the urea cycle, we used NAD+ and that was an oxidative catabolism, and so in the reaction that you see here, which is a synthetic reaction we're actually going to use NADPH as a source of electrons, and the final product is glutamate and NADP+.

The reaction catalyzed by glutamine synthetase adds another amino group to glutamate. In order to get this done, glutamate is first phosphorylated to make an acyl-phosphate intermediate, and then this intermediate is amidated. We're going to release inorganic phosphate, and form an amide to make glutamine. Now glutamate synthetase catalyzes a similar reaction, only instead of glutamate as the initial substrate, glutamate synthetase uses alpha-ketoglutarate. As we go into further lectures, it's important to remember this process, phosphorylation first and then replacement of the phosphate group with an amino group. We're going to see this when we look at nucleotide synthesis. So remember this progression.