Biochemistry 401 Lecture 36
Today we're going to talk about what can go wrong, and how to fix it. Safeguarding the genome. We're going to talk about mutations and repair of those mutations, and then we're going to talk about recombination.

Some things do not belong together, such as cytosine and adenine and guanine and thymine, but sometimes mistakes happen, and we're going to go over some of the things that can cause mistakes to happen, that can give rise to mutations.

And so when problems occur with mismatching and mutations, generally speaking, it's all about that base, hydrogen bond donors and acceptors. Normally deoxyadenosine binds to deoxythymidine, because of the patterns of hydrogen bond donors and acceptors, and the same with deoxyguanosine and deoxycytidine. These patterns of hydrogen bond donors and acceptors are complementary, and so they bind to one another efficiently, but when these patterns of hydrogen bond donors and acceptors are changed, that's when problems occur. In this diagram, we'll take a look at deoxyadenosine, and you can see that there's an amino group at position two. This amino group has two hydrogen bond donors and one of them makes contact with the hydrogen bond acceptor, oxygen, in deoxythymidine. It's a complementary pairing. The same thing for that nitrogen at position one, it's a hydrogen bond acceptor, and so it accepts the hydrogen bond from the nitrogen at position three in deoxythymidine. That is a hydrogen bond donor, and so again, that is complementary. But when you change these patterns, when you introduce a hydrogen bond donor where it doesn't belong, or when you take one away, this is what causes problems.

Some of the factors that change hydrogen bonding in deoxynucleotides are listed here. There's tautomerization, *syn-* versus *anti-* position of the base, relative to the sugar; water mediation - this is when three's a crowd, and the water molecule gets in between the two bases and forms its own hydrogen bonds between the bases. That can be bad news. Then there's oxidative deamination; alkyation; oxidative damage by reactive oxygen species; there's dimerization, and there's intercalation. That's a pretty big list, but let's look at them one at a time.

Sometimes deoxynucleotide bases can adopt alternate conformations by tautomerization, and the change of conformation that we see here in adenine is caused by a migration of the hydrogen shown in red, and a double bond. There

was a double bond originally, between the nitrogen at position one and the carbon at position six, as shown in the diagram in the upper right. This double bond has now formed an imino tautomer. Because of this, the nitrogen at position one is no longer a hydrogen bond acceptor, it's a hydrogen bond donor, and for this reason, alanine in the tautomeric form can bind with cytosine, because the pattern of hydrogen bond acceptor and donor is complementary.

And so tautomerization is the migration of a hydrogen atom or proton and concomitant switch of a single bond and an adjacent double bond.
In this diagram we see the predominant forms on the left and the more rare forms on the right, and in each case, whether we're going from an amino group to an imino group in adenine and cytosine, or from a keto group to an enol group, in guanine and thymine, these changes in configuration cause differences in hydrogen bonding patterns. Now, are you going to have to know the difference between a regular adenine and its tautomer? No you're not going to have to know those structural differences, but you do need to know the concept, and you do need to know what tautomerization is and why it can cause mutations in the DNA. You also do need to know that the common forms for deoxyribonucleotide bases are in the amino form, and the keto form, and the rare forms are the imino and enol forms.

Base pairing can also occur because of differences in hydrogen bonding between the *syn*- versus *anti*- conformations of the bases, with respect to the sugars. Now purines, once in a while, can be in the *syn*-conformation, but most of the time purines are in the *anti*-conformation. This is normal. Pyrimidines, on the other hand, are always in the *anti*-position. In the diagram above, we see *syn*-guanosine and *anti*-guanosine. You can see that in the *anti*-position, the bases are extending toward the center of what would be a DNA helix, whereas in the *syn*- position, the base lies over the ribose sugar. In the panels on the bottom, on the left, we can see the normal hydrogen bonding between guanine and cytidine, and on the right, we can see the abnormal bonding between adenosine, which has flipped into the *syn*-conformation, and guanosine, which is in the normal *anti*-configuration. In these configurations, the patterns of hydrogen bond donors and acceptors are such that adenine can bind with guanine.

Base pairings can also be disturbed when water gets into the center. Here we see a water molecule in the center between a thymine and a cytidine and this is

shown by the purple arrow. This disturbs the hydrogen bonding that would normally form, and so thymine can now bind with cytidine, rather than with adenine.

Chemical mutagens can also cause changes in hydrogen bonding patterns. For instance, an alkylating agent that adds a methyl group to the ketone in guanine can prevent the hydrogen bonding that would normally occur at that oxygen. So we've lost a hydrogen bond acceptor, and because of this alkylation, there's steric hindrance for the formation of a CG-base pair, and for this reason, O6-methyl-guanine will sometimes pair with thymine.

One of the most well known alkylating cleaning is aflatoxin. Aflatoxin is a toxin that's produced by a fungus that attacks peanuts. This aflatoxin, on its own, can intercalate between base pairs, but an enzyme in our liver does a really nasty trick. When cytochrome P450 tries to detoxify aflatoxin B1, it actually forms an epoxide, which is a highly reactive DNA-modifying agent. So this activated aflatoxin cannot only intercalate between base pairs, that the epoxide can form an aflatoxin-DNA adduct, and this is what we see in the lower left of this diagram. And so alkylating agents add carbon-containing molecules, as adducts, where they shouldn't be.

Reactive oxygen species can also attack DNA bases. In this case, we've oxidized the carbon at position eight, to form 8 oxo-guanine. In so doing, we've added another hydrogen bond donor at nitrogen seven, and so this base is a perfect complement to adenine.

Now another thing that can happen is the loss of hydrogen bond donors. This happens through the loss of an amino group. Chemical mutagens such as nitrous acid can cause oxidative deamination. Cytosine will lose an amino group to become uracil. We've seen this deamination before, and now, because we have a hydrogen bond acceptor, instead of a hydrogen bond donor, uracil can bind with adenine. Now adenine itself can become deaminated, to form hypo-xanthine. If you'll recall, hypo-xanthine is the nitrogenous base that's found in inosine. Hypoxanthine can bind to cytosine, because now there's a hydrogen bond acceptor at that ketone group, and hydrogen bond donor, so for this reason, inosine can bind to cytosine.