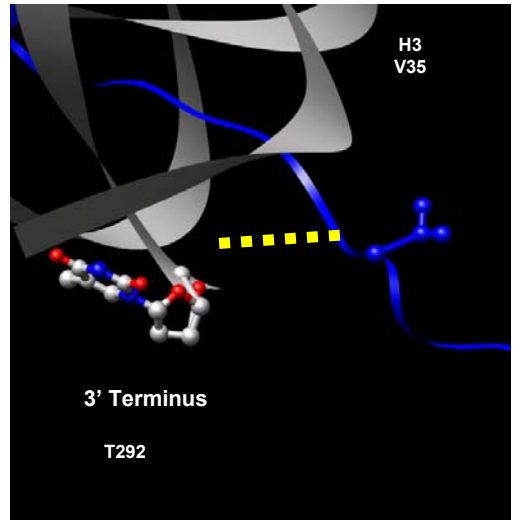


Crosslinking Histones and DNA to Prevent Transient Site Exposure

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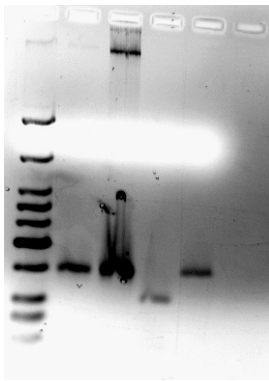
Background. Everyday, our DNA gets damaged. In each cell, we have repair enzymes that recognize and excise the damage. However, we don't really know how the DNA repair mechanism works at a nucleosomal level, when the DNA is wrapped around a histone octamer which is how it is found *in vivo*. Our goal is to test the transient site exposure model of DNA repair, where the DNA unwraps from the histone octamer to gain access to the damaged base pair.

Approach. To crosslink the DNA, we used a disulfide crosslink. Previous work had mapped out five possible crosslinking sites. We focused on the 3' end of the DNA this summer, which is pictured here. DNA with a

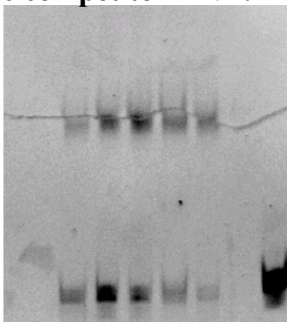


This shows the 3' end crosslinking site. The T292 basepair of DNA comes in close contact with H3 V35, making it an ideal crosslinking site.

The steps in synthesizing the thiol linker DNA: purified DNA, Crude product, Sty I digest, and ligated DNA.



Gel of reconstitution. This is varying concentration of non specific competitor DNA.



thiol linker as well as normal and mutant histones with one amino acid change to cysteine are synthesized. Recombinant histones, both wild and mutant histone octamers are folded and then reconstituted into nucleosomes with DNA. From there, the crosslink will be formed by an oxidation reaction.

Results. So far, mutant H3 V35C as well as all four of the wildtype histone proteins have been synthesized. About 12 mg of thiol linker DNA have been synthesized. The reconstitution protocol using native histones has also been optimized.

Future Work. Eventually, we want to reconstitute the mutant histone octamer and thiol linker DNA and crosslink them. We will eventually be performing restriction enzyme analysis to see how the crosslink affects DNA restriction.

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