

Trapping the hOGG1:nucleosomal DNA complex

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Reactive oxygen species (ROS) in the body can damage DNA. If a guanine base is oxidized by ROS, the result is a conversion to 7,8-dihydro-oxo2'-deoxyguanosine (8-oxoG). This base can be incorrectly paired with an adenine during DNA replication which can lead to mutation, cancer, and cell aging.

In the body, the 8-oxoG base is removed by human 8-Oxoguanine DNA Glycosylase 1 (hOGG1), thereby preventing mutation to the cell's DNA.

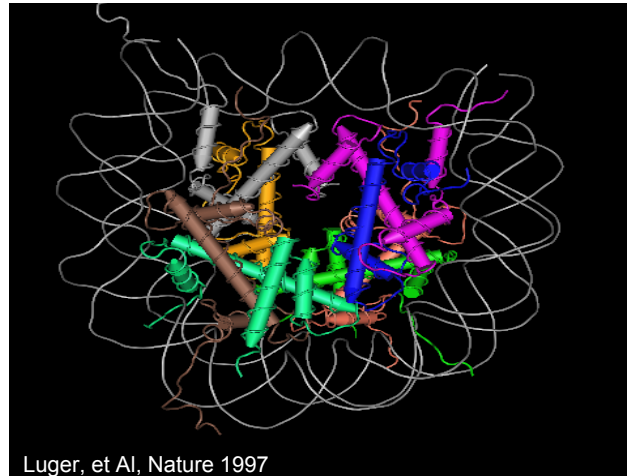
I am continuing the senior thesis work of Fang-Yuan Chang (HMC '07), studying how hOGG1 accesses and removes the 8-oxoG from compacted DNA. In vivo, DNA is nucleosomal, tightly wrapped around core histone particles for protection and expressive purposes.

The DNA was created via PCR, with a primer containing the 8-oxoG in a known location. The core histone particles were purified from fly embryos and the nucleosomes were reconstituted using double salt dialysis.

In order to study the access and excision of 8-oxoG, hOGG1 was trapped onto the nucleosome via the conversion of an imine bond to an amine bond in the presence of sodium borohydride.

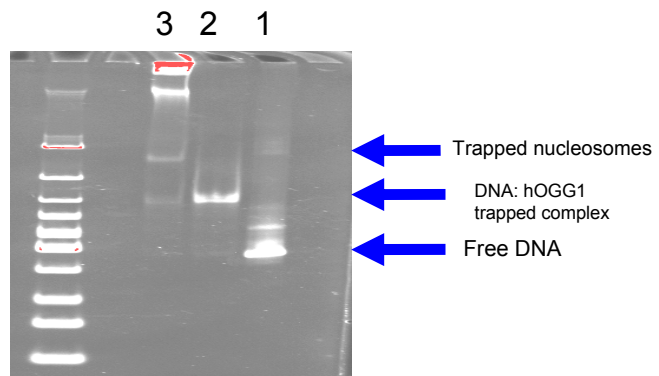
This trapped complex will be analyzed using restriction enzyme analysis. At various sites, the rates of excision for the trapped complex can be compared to the rate of excision for the free nucleosomes. A faster rate for the trapped complex means that at that the site is more freely accessible.

By mapping the relative rates of excision, we can visualize how the DNA conformation changes when hOGG1 accesses its target lesion.



In vivo, DNA is wrapped around core histone particles. Shown here is the crystal structure of a 146 base pair strand of DNA wrapped 1.75 times around a histone octamer.

In this experiment, I created an 8-oxoG containing 174 bp double-stranded DNA sequence wrapped around a core histone octamer.



On a PAGE gel:

Lane 1 is the purified PCR product, containing the free 174 bp DNA

Lane 2 is the trapping reaction, resulting from the incubation of hOGG1, DNA, and sodium borohydride

Lane 3 is the product of reconstitution of the DNA:hOGG1 complex onto core histone particles
We were able to successfully make each of our desired products

Several models were proposed for how hOGG1 accesses the nucleosomal DNA, including the formation of a bulge in the DNA. Our restriction analysis should indicate which model, if any, is correct.

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