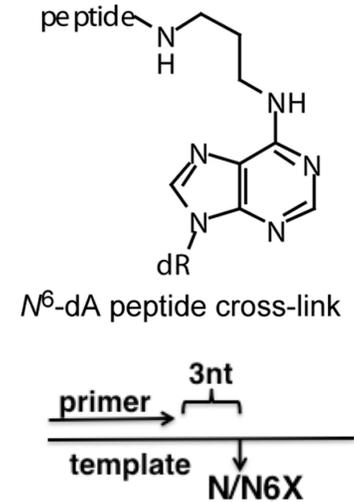


Discussion–Week 6 (DNA Repair) – Answer Key

Group 1. Biochemical studies have shown that amino groups of bases, such as the N6 of A can react with proteins bound in the major groove and form crosslinks like the one shown on the right (N6dA peptide cross-link = N6X).

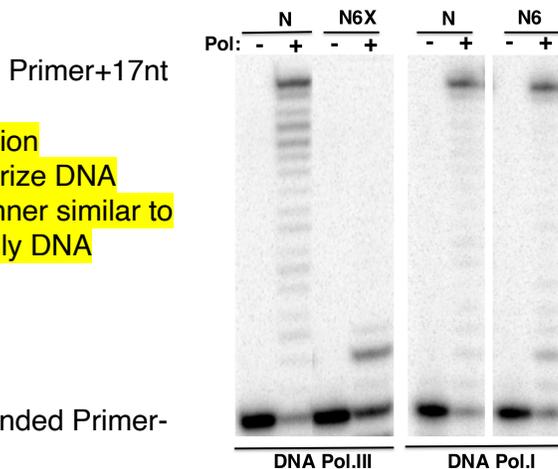
Primer extension experiments are performed using DNA Polymerase I or III (Pol.I or Pol.III) using a 5'-end radiolabeled primer and two different templates. Template N contains a normal dA located three nucleotides after the end of the duplex with the primer. Template N6X contains a N6dA peptide cross-link three nucleotide after the end of the duplex with the primer, as shown on the right. The length of the single stranded region of the template is 17 nt.



A - Describe the results obtained for DNA polymerase III and DNA polymerase I with the normal template (N) and the template containing N6X.

Both Pol.III and Pol.I can generate a full-length extension product using a normal template. However Pol.III cannot generate a full-length product when using the N6X template – it is blocked after 2 nt extension, when reaching the x-linked base. With Pol.I there is product visible at the same position but Pol.I is still able to generate a full-length product despite the x-linked base.

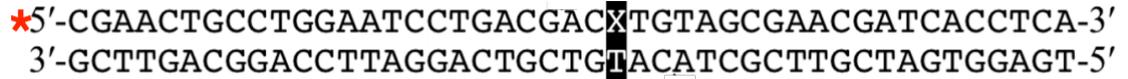
B- Based on these results, propose a function for DNA polymerase I in the process of replication that has not been covered in class. What protein in eukaryotic cells might fulfill a similar function?



Pol.I can act as a bypass or translesion polymerase, which is able to polymerize DNA containing damages. It acts in a manner similar to DNA polymerase eta or other Y-family DNA polymerases

Group 2

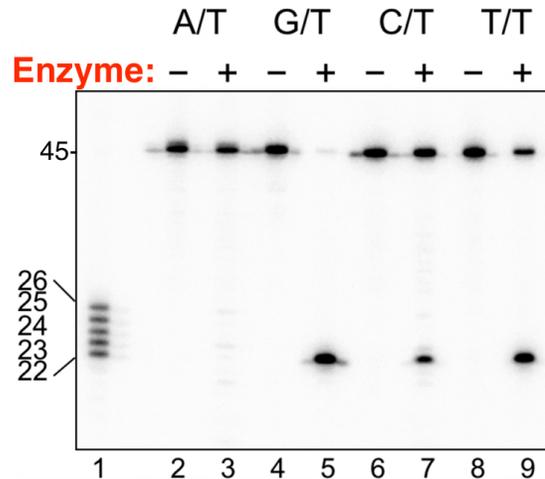
Scientists have purified an enzyme from an archaea. They incubate this protein with the substrate shown below, in the absence of any other nucleotides or nucleic acids.



The red

star indicates a radioactive label. They use 4 different versions of this substrate, which differ by the identity of the nucleotide at position X, as shown on the top of the gel.

After incubation with the enzyme (+), or without (-), the nucleic acids are fractionated according to their size and visualized by autoradiography such that only the radioactively labeled DNA fragments are visible. Numbers indicated on the left side indicate the sizes of DNA molecules; the DNA products shown on the left ranging from 22-26 nt is a size ladder to help determine the size of products obtained after incubation with the enzyme.



A – Based on the nature of the products obtained after incubation with the enzyme and how this enzyme acts on the different substrates, explain below what type of biochemical activity is catalyzed by this enzyme, and what its substrate preferences are.

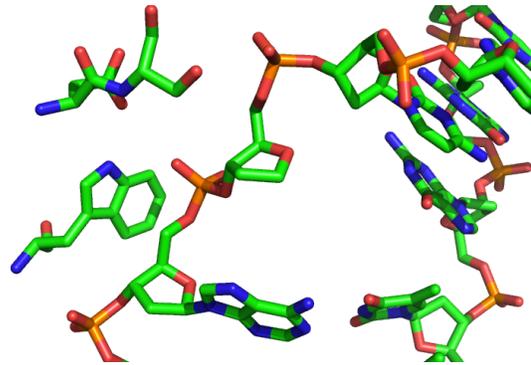
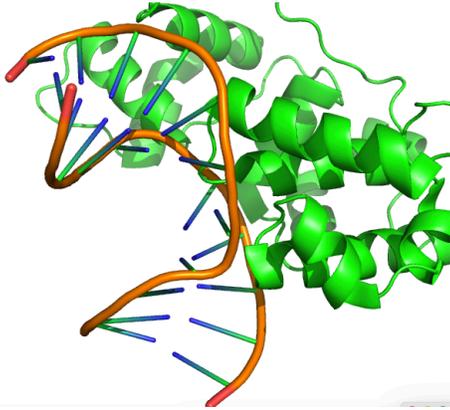
This enzyme cleaves substrates containing the G/T, C/T or T/T mismatches, but not a DNA with a normal base pair. The product generated is 21-22 nt long and contains the radiolabel, so it corresponds to the top strand cleaved immediately upstream the G, C or T mismatch. The activity is higher for a G/T mismatch than for pyrimidine-pyrimidine mismatches (C/T or T/T). So the enzyme has a substrate preference for purine/pyrimidine (R/Y) mismatches.

B- Explain below in what general DNA metabolism reaction/pathway this enzyme is expected to function; compare its activity to that of the enzymes known to function in the equivalent pathway in bacteria, and explain how it is similar and/or different.

This enzyme cleaves DNA containing R/Y mismatches, which are expected to happen when a replicative DNA polymerase makes a mistake. It is likely to be involved in mismatch repair, to promote cleavage in a strand containing a mistake and initiate the repair process. It is similar to MutH in its cleavage activity, but it is different in the sense that it cleaves the DNA directly near the site or mismatch, and not at a hemimethylated site like MutH.

Group3.

The structure of an enzyme that binds to DNA is shown below. The right side shows details of the interaction with the DNA near the active site of the enzyme.



A- Based on the picture shown on the left, what is the major consequence of the binding of this protein onto DNA?

There is a kink in the DNA in the center of the structure.

B – Based on the picture shown on the right, what is the major difference that this DNA exhibits compared to a normal DNA?)

It contains an abasic site on the left strand.

C– Predict the type of enzymatic activity that the protein would possess if the DNA bound to the enzyme in this structure corresponded to the enzyme's **substrate**. In which pathway would this activity be required?

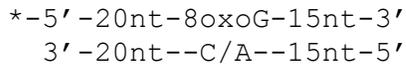
The substrate would be a DNA containing an abasic site opposite to a normal nucleotide (G). This would correspond to the situation after a glycosidase has cleaved the base of the nucleotide opposite to the G (or a spontaneous cleavage of the glycosidic bond, but it's less likely because it was likely a pyrimidine). An AP endonuclease involved in the base excision repair pathway would be the enzyme involved.

D– Predict the type of enzymatic activity that the protein would possess if the DNA bound to the enzyme corresponded to the **product** of the reaction catalyzed by the enzyme. In which pathway would this activity be required?

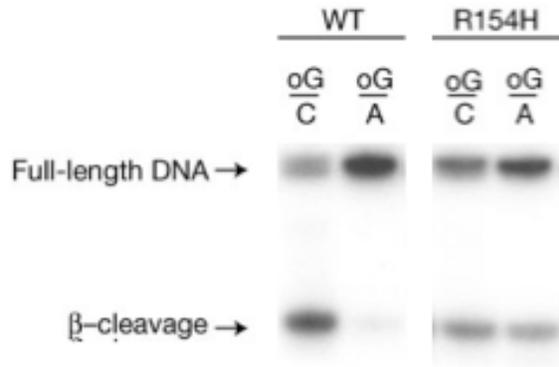
As stated above, a glycosidase/glycosylase enzyme would cleave a glycosidic bond and generate an abasic site. It could be the uracil glycosidase considering that the nucleotide opposite to the abasic site is a G – so the abasic nucleotide may have been a C deaminated into U. It would be involved in base excision repair.

Group4

Researchers study the function of residue R154 in OGG1. The interaction of this residue with a DNA containing an 8-oxoG residue is shown at the bottom of the page. Purified normal OGG1 (WT) and the R154H mutant OGG1 (R154H) are incubated with a radio-labeled DNA containing an oxoG (oG) residue paired opposite to either a C or an A in the complementary strand. The radiolabel (*) is in the strand containing the oxoG as shown below:



The reaction products are fractionated on a gel and the result is shown on the right. β -cleavage is a 20 nucleotide long DNA fragment.

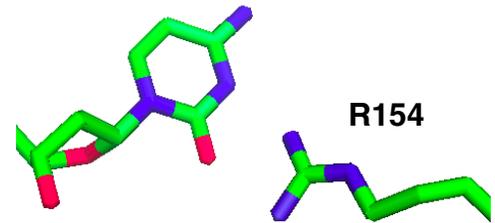


A – Explain what biochemical activity or activities of the OGG1 enzyme result in the production of the β -cleavage product when the WT enzyme is incubated with the oG/C product. Be specific and describe all the possible steps in the reaction.

We detect production of a cleavage fragment with the WT enzyme incubated with an 8oG/C substrate. The strand cleaved is the one that contains the 8oG. The reactions involved are (1) cleavage of the glycosidic bond linking the 8oG to the deoxyribose and (2) AP endonuclease that cleaves the DNA strand, since OGG1 has both activities.

B – Compare the biochemical activities detected for the WT and R154H OGG1 enzymes when they are incubated with the different substrates.

The WT enzyme cleaves the substrate with an 8oG/C duplex but not the substrate with a 8oG opposite to an A. The R154H cleaves both substrates with similar efficiencies – this enzyme has lost the ability to recognize 8oG groups that have not been replicated (8oG/C duplex) from 8oG that have already been replicated (8oG/A duplex).



C - Predict the genetic consequences of the R154H mutation of hOGG1 if this mutant enzyme is introduced in human cells. Be specific in your answer and as to what type of mutation events could be introduced or minimized.

This mutant enzyme would cleave 8oG bases that have already been replicated with an A opposite to it, unlike the mutant enzyme. It would increase the rate of G-C -> T-A mutations.