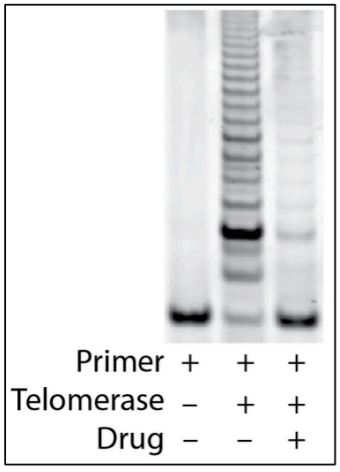
Question 2 – DNA Replication and DNA repair (25 pts).

2.1 Do the following statements apply to Bacteria, Eukaryotes, Both Bacteria and Eukaryotes, or Neither Bacteria nor Eukaryotes?
Write "Bacteria", "Eukaryotes", "Both", or "Neither" as appropriate in the boxes below (1.5 pts each).

Bacteria	Incomplete transcripts are used as emergency primers to restart DNA replication.
Neither	Polymerases synthesize DNA in the 3' to 5' direction on the lagging strand.
Eukaryotes	The leading and lagging strand are synthesized by different polymerases.
Both	Sliding clamps are used to increase processivity in lagging strand synthesis.
Bacteria	DNA replication proceeds bidirectionally from a single origin site.
Both	Sliding clamps are loaded onto RNA:DNA hybrids by clamp loader complexes.
Eukaryotes	The reverse transcriptase activity of telomerase extends chromosome 3' ends.
Bacteria	DNA methylation is used to distinguish old from new DNA in mismatch repair.
Neither	Nucleosomes are removed from DNA behind the replication fork.
Both	Initiation of DNA replication requires the melting of double-stranded DNA.

2.2-2.4: Scientists are trying to understand the mechanism of action of a new drug that shows promise as an anticancer agent. They discover that this drug directly impacts telomerase, which they assessed by measuring the ability of purified human telomerase to extend a 24 nt-long telomeric repeat primer in the presence (+) and absence (-) of the drug, with the products assessed using autoradiography. The results are shown on the right.



2.2: What are the bands that appear upon the addition of telomerase to the reaction? Justify your answer briefly (3 pts).

Telomeric repeats added to the 3' end of the primer → larger products appear above the template

2.3: What is the impact of the drug on telomerase activity? Justify your answer briefly (3 pts).

The drug seems to inhibit the activity of telomerase, as the intensity of the larger bands is lower compared to the no-drug condition

2.4: The drug is a nucleic acid molecule that contains an exact copy of human telomeric repeats. Scientists discover the drug is acting via Watson-Crick base pairing with something in the reaction.

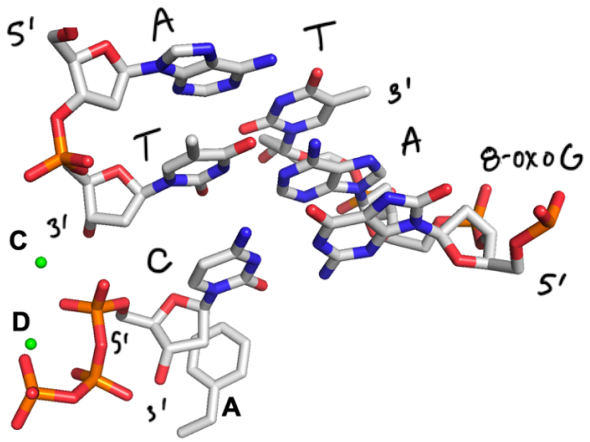
What is the drug most likely binding to in the reaction? (2 pts)

The template for telomeric repeat synthesis in hTR (telomerase RNA)

Why does this binding of the drug influence the polymerase activity of telomerase? (2 pts)

It acts as a competitive inhibitor that blocks binding of the telomeric repeats to telomerase

Question 2 – 16pts. This figure shows the structure of the active site of an enzyme in complex with its nucleic acid substrates. Green spheres marked C and D are two divalent cations.



A – Write the sequence of the nucleic acids and the nucleotides you can see in the space below in the 5'-3' direction. Position the free nucleotide(s) approximately where they are relative to the nucleic acids. Make sure you also identify any potentially modified or damaged nucleotides. You can use abbreviations. 3pts

5' dA dT 5'-dCTP-3'
3'. dT dA 8-oxoG 5'

B – What is the general biochemical reaction catalyzed in this active site? – 2pts

DNA polymerization

C– Propose a function for amino acid A in the active site of the enzyme. Justify your answer based on the position of this amino acid relative to the other molecules found – 3pts.

A= phenylalanine. It is very close to the 2' carbon atom of the incoming dCTP. It likely acts as a steric gate to prevent riboNTPs from entering the active site and getting incorporated into the DNA (riboNTPs have a 2'OH and would not fit in the active site).

D- Briefly indicate **ONE (and only one)** role for the divalent cation marked C in the mechanism catalyzed by the enzyme. 2pts.

→ Lowers the pKa of the 3'OH
OR → stabilizes the deprotonated 3'O-
OR → it aligns / bridges the two substrates of this reaction (3'OH and alpha phosphorus)

E – Explain how the interaction observed between two of the nucleotides found in this structure is different from the interaction that you would have expected based on the materials studied in class. 2pts.

8-oxoG is in an anti conformation here though it prefers to be in the syn conformation.
Here, it makes a normal Watson-Crick interaction with the incoming dCTP. Typically 8-oxoG will be in the anti conformation and make a Hoogsteen base pair with A.

F – Based on the substrates present in the active site, propose a specific function or role for this enzyme in DNA metabolism. 4pts.

This enzyme incorporates a dC opposite to an 8-oxoG. It prevents the typical misincorporation of A opposite to 8-oxoG (that's what we'd expect for a typical replicative DNA polymerase). This is a translesion or bypass DNA polymerase that prevents 8-oxoG from blocking replication and also prevents the mutagenic impact of 8-oxoG.