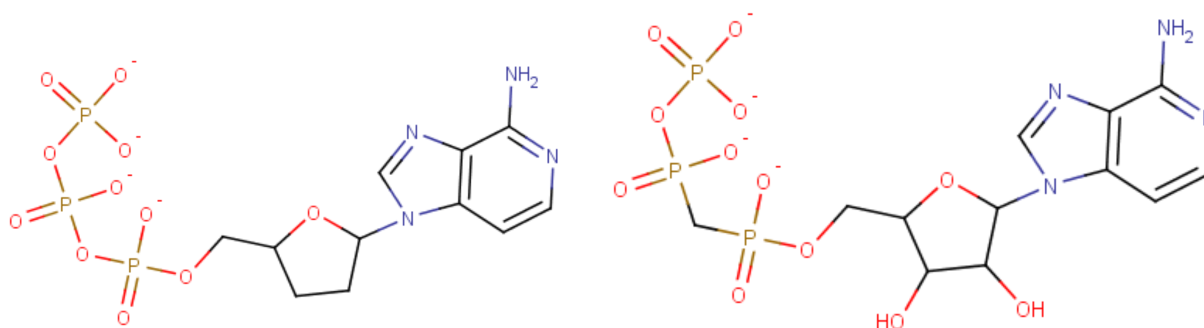
Problem Set 10 **(Due Nov. 18th)**

1. Discuss any upstream elements that are important for the initiation of transcription. Make sure to mention where these DNA sequences contact RNA Polymerase. These elements are only present upstream of select genes. What makes these genes particularly important/special? UP elements are AT rich sequences that are found ~40-60 nucleotides upstream of the transcription start site. They help to increase the affinity of the RNA Polymerase for the promoter by interacting with the alpha-subunit (note this is independent of the sigma factor). The genes that have UP elements tend to be transcribed at high levels (e.g. ribosomal RNA genes).

2. In our discussion of the active sites of DNA Polymerase and RNA Polymerase, the elongation reaction was inhibited by the presence of very specific NTP adducts. For each enzyme, draw the structure of this molecule and identify exactly what is present or absent that inhibits chain elongation. DNA Polymerase – 2'3'-dideoxyribose is used. It lacks the 3' hydroxyl to be a nucleophile. RNA Polymerase – The P-O-P bond between the α and β phosphate is converted to a carbon. This prevents PPi from leaving.



3. What is "abortive initiation" and why does it occur? This is when the RNA polymerase ejects a small (10-12 nt) RNA polymer instead of transitioning into a elongation competent conformation. The transition requires ejecting the sigma factor. The sigma factor is tightly bound to the -10 and -35 elements on the non-templating strand. To transition into the elongation conformation, this must be ejected, which requires the sigma factor breaking its contacts with the DNA and the RNA Polymerase. Sometimes, the polymerization reaction doesn't have enough momentum to get over the energy hurdle, so the RNA chain is ejected. Other times it does, and the sigma factor is ejected.

4. Explain the two ways that *E. coli* can terminate processive RNA polymerization. Rho-dependent, which uses the helicase, Rho, to melt apart the DNA/RNA hybrid present at the RNAP active site. This is an energy dependent process. The other way is Rho-independent, which relies on the formation of a stem loop and a weak AT rich DNA/RNA hybrid helix. A GC-rich palindromic RNA molecule is transcribed which leads to the formation of a stable stem loop that interacts with the RNAP. After the stem loop is made, an AT-rich sequence is transcribed – this is a weak helix and is easily torn away from the templating strand.

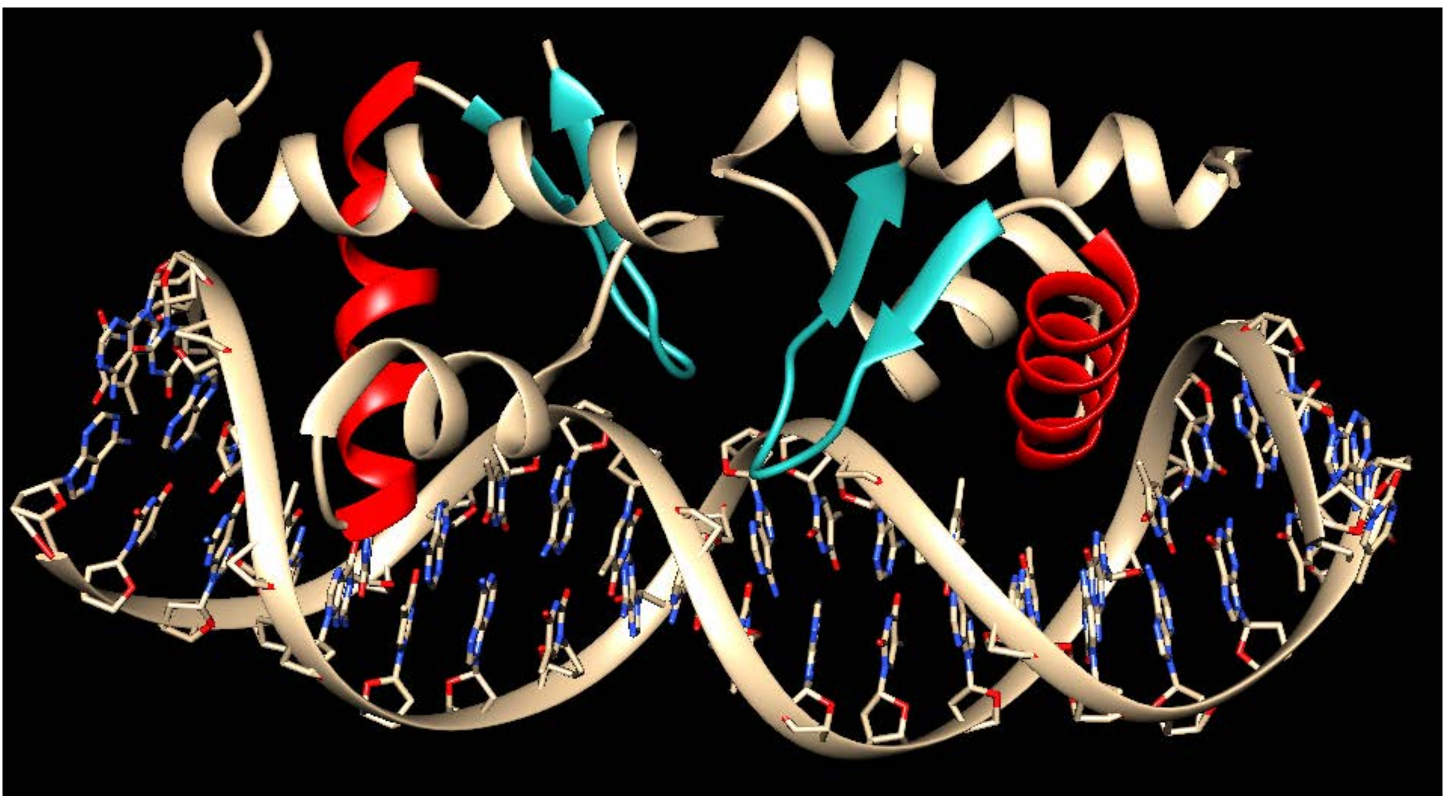
5. Describe the mechanism RNA Polymerase uses form NTP sampling and processive elongation. Make sure to include the role of the Bridge and Trigger helices. The trigger helix is in an open conformation such that NTPs can be sampled into the active site. One the correct NTP is present, the trigger helix switches to the closed conformation, stabilized by an interaction between an Arg on the helix and the β/γ phosphates of the NTP. The NMP is added to the growing RNA polymer and pyrophosphate is created. The trigger helix switches back to the open conformation, which allows the PPi to leave the

active site. The transition between closed→open also moves the bridge helix slightly, which allows the next templating base (Which is currently flipped out of the active site and under significant stress) to flip into the active site.

6. Describe how RecA and LexA work together to regulate the SOS response. RecA scans the DNA. When an error is found, it becomes activated and hydrolyzes the backbone of LexA. Upon proteolysis, LexA dissociates from the DNA and there is a vase upregulation of genes including DNA repair machinery.

7. Describe the BER process. Make sure to include any cellular machinery that is important. BER removes a single base and allows it to be repaired by Pol I and DNA ligase. DNA Glycosylase hydrolyzes the glycosidic bond between the deoxyribose and the damaged base – this results in an AP site (apurinic/apyrimidinic), which is quite toxic to the cell. As such, this site is never exposed; DNA Glycosylase hangs on to the AP site until AP Endonuclease comes along to remove the deoxyribose. Pol I and DNA Ligase can now repair the missing base.

8. Investigate the structure of LexA bound to DNA (pdbID 3JSO). This protein binds to the DNA using a standard winged helix turn helix (wHTH) structural motif. Prepare and image with the wHTH motifs colored differently than the rest of the protein. What part of the B-form DNA does the recognition helix interact with. How about the wings? The 2nd Helix of the HTH motif is interacting with the major groove of the DNA while both wings are inserted in the minor groove (this is actually an uncommon conformation of the wHTH – typically, the wings are not in the same minor groove)



9. Summarize the Nucleotide Excision Repair process. Use as much detail as you think appropriate. This process relies on UvrA, UvrB, UvrC, and UvrD. The UvrA/B complex finds an error in the DNA; it binds to this sequence. ATP is hydrolyzed, ejecting the A/A dimer and bending the DNA; this results in the

error being overly exposed; UvrC comes in and cleaves the DNA ~6 NT upstream and downstream of the error. The resulting oligonucleotide (~12mer) contains the error and is removed by the action of UvrD and ATP hydrolysis. Pol I and DNA Ligase come in and repairs the error.

10. UvrC contains 2 endonuclease domains. Each domain uses a divalent cation (typically Mg^{2+}) to promote catalysis. Propose a Mg^{2+} dependent mechanism for this reaction.