Biochemistry 674, Final Exam **Your Name:**_____

December 18, 1999 **Prof. Jason D. Kahn**

You have 120 minutes for this exam, which is worth 200 points. Thus you get more "points per minute" than for the 80 minute exams.

Though each question has several parts, many of the parts are completely independent of each other. In other words, don't give up if you can't answer part (a).

Explanations should be concise.

You will not need a calculator for this exam, and no other study aids or materials are permitted.

There will be a viewing at 9 a.m. on December 21st in Chem 2507 (next to my office), or you can come by after the break.

Final grades will be available only through MARS or at the viewing.

1. (40 pts) DNA Repair

(a; 12 pts) Write the structure of 8-oxo-guanosine. Draw a conformation which explains how it can act as a miscoding lesion. 8-oxoG is repaired by base excision repair. Sketch the first step of BER.

(b; 8 pts) Transcription-repair coupling in eukaryotes in part relies on the XPB and XPD subunits of the multisubunit TFIIH complex. Why does it make sense to use TFIIH for both transcription and repair, even though it does not travel with the RNAP (i.e. it's not a built-in repair subunit of the polymerase)? What functions does TFIIH carry out during transcription initiation?

(c; 11 pts) What is a molecular matchmaker (there are two critical aspects of the definition)? Discuss how UvrA typifies this kind of activity, and its function.

(d; 9 pts) Why does *E. coli* survive high doses of UV light best when it is subsequently grown in visible light and poor media?

2. (45 pts) Miscellaneous Review Material

(a; 10 pts) Sketch the prokaryotic DNA replication fork in all its glory, but in the traditional two-dimensional representation. Include the following: SSB, γ complex, τ dimer, Pol III cores, helicase, primase, β sliding clamp, topoisomerase, 5′ and 3′ ends, and identification of leading and lagging..

(b; 5 pts) Sketch the looped dimer model of the replication fork with just the DNA and the Pol III cores, half way through the synthesis of an Okazaki fragment.

Running a gel containing ethidium bromide allows separation of plasmid topoisomers, as on the gel below. Plasmid DNA as normally isolated is a mixture of several negatively supercoiled topoisomers (left lane below), and even after relaxation several topoisomers are present at equilibrium. Assume we also have managed to isolate a single topoisomer with $\Delta Lk = 0$ (right lane).

(c; 6 pts) Why does the nicked circle (NC) comigrate with the $\Delta Lk = -4$ topoisomer in the gel? (The point is that there is a minimum in mobility for $\Delta Lk < 0$, not the numerical value 4). What is the sign of the writhe <u>in the gel</u> of the $\Delta Lk = 0$ topoisomer?

(c; 15 pts) On the gel below, <u>sketch the distributions observed after the following treatments</u>:

(A) mixed pool + single hit amount of Topo I

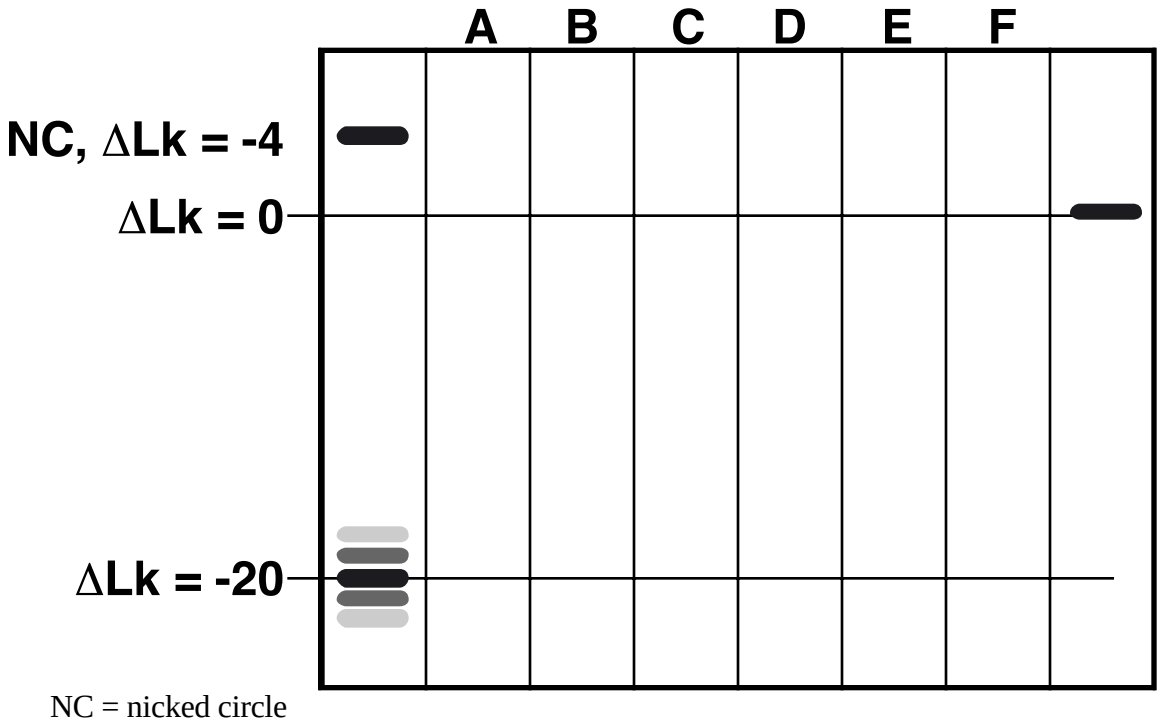
(B) pool + single-hit amount of Topo II (eukaryotic, can only relax)

(C) pool + excess Topo I

(D) pool + excess Topo II

(E) unique $\Delta Lk = 0$ topoisomer + excess Topo I

(F) unique $\Delta Lk = 0$ topoisomer + excess Topo II.



NC = nicked circle

(d; 9 pts) What is one important consequence of the binding of positively charged proteins to DNA? Do the electrostatics contribute enthalpy or entropy to the favorable free energy of binding, and why?

3. (35 pts) Transcription

(a; 15 pts) What are the functions of the α, the β/β′, and the σ subunits of *E. coli* RNA polymerase? How does the separability of sequence-specific DNA recognition and catalytic function enable global shifts in transcription patterns? How do eukaryotes carry out a similar separation of binding and catalysis?

(b; 12 pts) What are the four classes of transcriptional activity levels ascribed to eukaryotic DNA in chromatin? Describe one protein or activity which shifts the DNA from one class to another, and how it is believed to work.

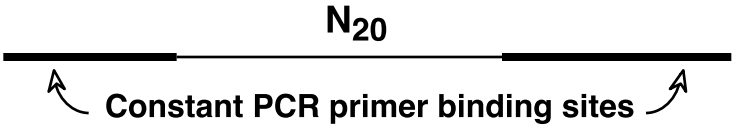
(c; 8 pts) How do prokaryotic and eukaryotic RNA polymerases solve the promoter escape problem? In other words, where does the energy come from to break the promoter-specific contacts?

4. (40 pts) RNA Splicing and Chemistry

(a; 9 pts) We discussed the idea that the Nobel prize is awarded to people who force the rewriting of textbooks. How did the discovery of self-splicing of group I introns change a reigning paradigm in biochemistry, and how did this affect ideas on the origin of life (what makes RNA special)? What is the ultimate goal of recent work on selection of novel RNA catalysts?

(b; 15 pts) Sketch the chemistry of splicing for self-splicing Group II introns. What general type of reaction does RNA catalyze in the modern world (with the possible exception of the ribosome)?

(c; 16 pts) We discussed selection-amplification of RNA's that bind desired substrates or catalyze reactions. Similar approaches can be applied to selecting dsDNA binding sites which are optimal for binding a protein of interest. Starting with the ssDNA pool below and a purified DNA binding protein, how would you isolate the optimal binding sequences? Based on what you know about the limiting factors in PCR amplification, include some mention of how you would make sure that you were selecting double-stranded, fully paired DNA molecules.



5. (40 pts) Regulation of Transcription and Feedback

(a; 6 pts) Sketch the structure believed to be important in the activation of the $E\sigma^{54}$ RNA polymerase by the NtrC transcriptional activator. Where would the bending protein IHF bind to modulate transcription activation?

(b; 15 pts) Describe how you could use DNA arrays to assess the transcriptional response to a change in nutrient availability (e.g. a shift from glucose to galactose). You need not go into detail about how the array is read. Why doesn't this approach necessarily identify the transcription factor(s) responsible for the changes in gene expression? Assuming you have the genomic sequence, how might you go after the earliest transcription factors using bioinformatics and biochemically?

(c; 10 pts) What effect would activation mutations in the CAP protein have on transcription of the lac operon? Mutations in which protein would be most likely to suppress these CAP mutations, and how does all this go together in the current model for transcriptional activation by CAP?

(d; 6 pts) If I had assigned a 15 page paper on a topic of your choice instead of having this final, what would your title have been? Would you have preferred a paper?

(e; 3 pts) What was your favorite nucleic acids/molecular biology related seminar on campus this semester?

Do Not Write Below This Line

Score:	Question 1: _____	out of 40: DNA Repair
	Question 2: _____	out of 40: Miscellaneous review material
	Question 3: _____	out of 40: Transcription
	Question 4: _____	out of 40: RNA Splicing and Chemistry
	Question 5: _____	out of 40: Regulation and Feedback
	Total: _____	**out of 200**