Biochemistry 674, Fall, 1996: Nucleic Acids **Prof. Jason Kahn**

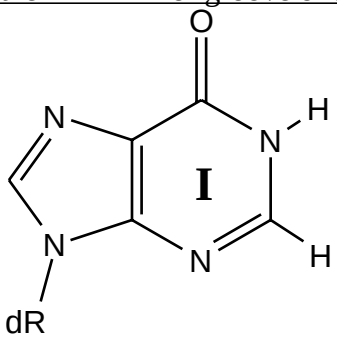
Final Exam: December 16, 1996 **Your Name Here:** _____

This exam has six questions worth various numbers of points, for a total of 200. Answer all six. You do not need a calculator. You have two hours. Keep your answers concise, but a blank page is provided at the end for extra space.

1. DNA Structure and Reactivity

a. (10) Draw a plausible structure for a non-Watson-Crick A•G base pair. What makes the four Watson-Crick base pairs special?

b. (12) The structure of inosine is given below. Design an experiment to use Watson-Crick inosine•cytosine base pairs in synthetic dsDNA oligonucleotides to distinguish whether a protein binds the DNA minor groove or the major groove?



c. (5) What does dimethyl sulfate do to DNA? What bond is thereby weakened? What is the name of the mechanism of the reaction which occurs after base hydrolysis and is used to cleave the DNA chain in sequencing and footprinting?

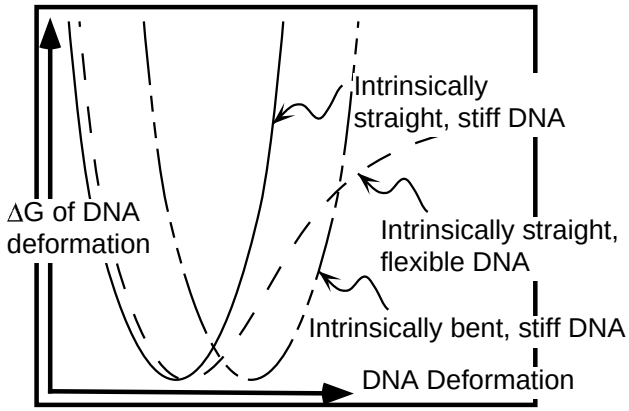
d. (8) What is the fundamental equation relating the topological and geometrical properties of closed-circular DNA? In terms of this equation, how can supercoiling provide a driving force for open complex formation in transcription?

2. Protein-DNA Interaction

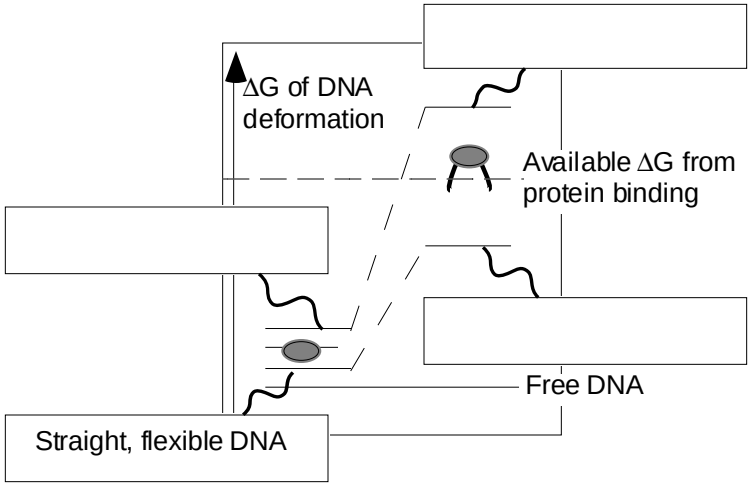
a. (10) Define direct and indirect readout in protein-DNA recognition.

b. (13) On the on the left graph below, the potential functions corresponding to intrinsically bent DNA and bendable DNA are labeled. Label the graph on the right to indicate whether the protein-bound DNA is bent or straight and whether it the DNA is stiff or flexible. Referring to both graphs, explain how a protein can discriminate among DNA sites according to DNA deformability. What is the cost the system must pay to achieve this increased specificity?

Deformed vs. Deformable DNA



Protein binding to intrinsically straight stiff vs. flexible DNA



c. (12) Briefly describe the modification-interference assay and draw the final gel.

3. Replication and the Cell Cycle

a. (9) What is the role of sliding clamps in chromosomal DNA replication? What ancillary machinery is needed for their use, and why?

b. (4) Name two cellular processes that p53 can activate or repress in response to DNA damage.

c. (8) What is the essential idea of "licensing models" in regards to the cell cycle?

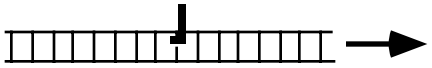
d. (9) Sketch the Bramhill and Kornberg model for the initiation of replication at *oriC* in *E. coli*.

4. Repair and Recombination

a. (10) What is transcription-repair coupling? What is the essential difference in terms of the transcription process between how this process works in prokaryotes and eukaryotes?

b. (3) What type of repair process removes 8-oxo-G from DNA (a three-letter answer would suffice)?

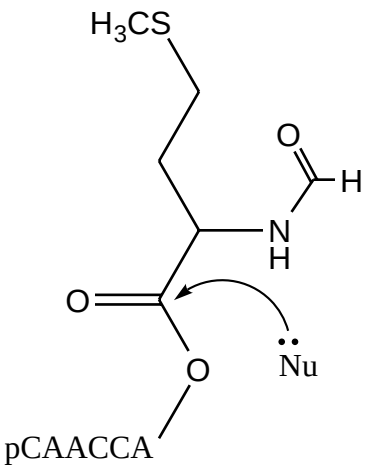
c. (10) Sketch the process of nucleotide excision repair of a bulky DNA adduct by Uvr(A)BC in prokaryotes. Don't worry about the exact locations of cut sites.



d. (7) Sketch the reactant and product DNA in the process of lambda phage integration into the *Escherichia coli* chromosome and list the proteins needed for integration; don't worry about the DNA shape or topology. How does the phage ensure irreversibility of integration or excision (one symbol)?

5. Ribozymes and RNA Splicing

The "fragment reaction" illustrated at the right has been used to model the peptidyl transferase step in translation.



a. (10) The fragment reaction may be catalyzed by de-proteinized 23S ribosomal RNA. How did this experiment and the demonstration of catalysis of this reaction by the Tetrahymena IVS expand the perceived scope of RNA catalysis? What is the nucleophile in the IVS-catalyzed reaction? What type of chemical reaction does RNA typically catalyze in our world?

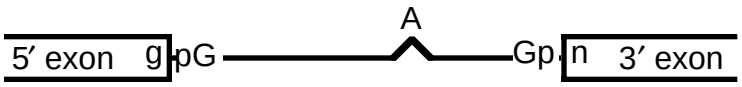
b. (5) What two main activities are necessary in RNA world → DNA world proposals, and are the subject of current searches for catalytic aptamers?

c. (9) Draw lines to match these questions and answers re pre-mRNA splicing:

The proposed catalytic center of spliceosome	U4
A possible RNA matchmaker	U2
snRNPs which each recognize both the 5′ and 3′ splice sites	U1,U2
The snRNPs responsible for splicing commitment.	U2/U6
snRNP's probably ejected before first catalytic step	U1,U4
snRNP responsible for branch point recognition	U1,U5

d. (4) Give some phylogenetic evidence which suggests that pre-mRNA splicing could be RNA-catalyzed.

e. (12) Sketch the covalent chemistry of pre-mRNA splicing, without drawing snRNPs etc., at the level of detail in the diagram given below of the pre-mRNA.



6. Transcription and Miscellaneous

a. (11) Imagine you are doing a yeast two-hybrid screen using a GAL4 DBD -YFP (DBD = DNA binding domain, YFP = your favorite protein) construct and a cDNA library-GAL4 AD (AD = activating domain) construct. Sketch how the experiment works and describe what it is used for. What typical properties of eukaryotic transactivating proteins and the process of transcription activation are necessary for the method to work? (Continue on next page if you need more space.)

b. (4) You isolate a clone which gives transcription activation, but the control experiment shows that it activates transcription even in the absence of GAL4 DBD - YFP. What have you probably isolated?

c. (5) What is the CTD, and what happens to it during the eukaryotic transcription cycle?

d. (10) List:
(1) Your favorite paper from the required reading list:

(2) The most useless paper of which you read at least half:

(3) The best lecture of the semester, if there was one:

(4) The worst lecture of the semester, if there was one:

(5) An area that you think should be covered in more depth:

Question	Score
1	/35
2	/35
3	/30
4	/30
5	/40
6	/30
Total	/200