Biochemistry 674, Nucleic Acids **Your Name:** _____

Final Exam, December 17, 1997 **Prof. Jason D. Kahn**

You have 2 hours for this 200 point exam (33% more points per minute than the hour exams). Thus, answers should still be concise. The questions are independent of each other.
You do not need a calculator.
An exam viewing date will be announced on the Web page.

1. (15) Draw the wobble base pair which results from C to U deamination in a GC base pair. What is the type of repair and what is the first step in repairing the lesion? How does the enzyme gain access to the uracil base? How does this pathway provide a rationale for the evolutionary development of thymine in DNA instead of uracil?

2. (8) $\Delta H°$ for base-pair formation is (circle one) positive/negative. $\Delta S°$ is positive/negative. Increasing $[Na^+]$ from 0.5 to 200 mM increases/decreases T_m. Why?

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3. (10) Draw the most stable secondary structure for the RNA below. Then add lines to indicate a possible pseudoknot interaction between a single-stranded region and the loop of a stem-loop.

RNA = 5′-GCGCGCAAUCUAGCGCGCAAAAUAGAUCCCCC-3′

4. (15) We saw a proposed two-metal-ion mechanism for transesterifications twice. What were the two systems? Sketch the transition state and describe briefly what the metal ions do to help catalyze the reactions.

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5. (10) Some thermophilic bacteria have been found to possess reverse gyrase activity, which uses ATP energy to introduce positive supercoils. Why might living at high temperature favor the evolution of such an activity? Hint: What is one possible role of negative supercoiling in *E. coli*?

6. (8) What item of recreational equipment describes the shape of TBP? What is believed to be the basis of DNA recognition by TBP?

7. (8) Draw a plausible arginine-guanine hydrogen bonding interaction.

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8. (10) How does the nitrocellulose filter-binding assay work? What are its main advantages and disadvantages relative to footprinting for quantitative studies?

9. (10) How does DNA methylation by the dam methylase (methylates A in 5′-GATC) provide a signal for mismatch repair and a "clock" for DNA replication?

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10. (10) Name two ways to remodel chromatin. How are the requisite enzymatic activities believed to be delivered to the promoter?

11. (12) Briefly describe the process of selection-amplification of an RNA that binds a ligand of interest. Why is this often done as a prequel to selecting an RNA catalyst? Why not just go for the catalyst directly?

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12. (15) Site-specific recombination reactions usually have definite linking number changes associated with the reaction (e.g. $\Delta Lk = +2$ for lambda phage insertion). In general, what does this tell us about the reaction mechanism (what would a random ΔLk suggest?). In the particular case of lambda phage insertion, based on the above ΔLk, why would you expect the reaction to be thermodynamically driven by negative supercoiling? In fact, this thermodynamic driving force is **not** believed to be the reason the reaction requires supercoiling (in the cell, kinetics is king). What **is** the actual probable reason for this requirement?

13. (10) What is one unusual general challenge faced by the nucleotide excision repair system in the modern age of novel DNA-adducting agents? (Hint: How does this enzyme system's job differ fundamentally from that of, say, ornithine decarboxylase?) What are the proteins in *E. coli* which carry out NER?

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14. (12) Sketch what happens when a replication fork encounters a nick or gap in the leading strand template DNA. What would RecBCD do to this "collapsed fork," assuming there was a nearby Chi site? Which repair system would be needed to repair the collapsed fork: direct reversal, BER, NER, MMR, or recombination-mediated repair?

15. (10) Transcription-coupled repair refers to the preferential repair of the transcribed strand of active genes. What is the heuristic rationale for this process? Why might eukaryotic RNA polymerases back up to allow repair and then continue, whereas prokaryotic polymerases terminate?

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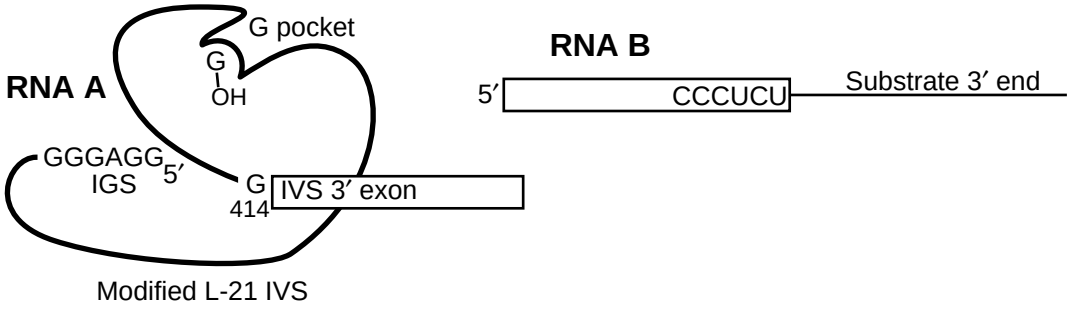
16. (22) Cech and Sullenger have adapted the *Tetrahymena* IVS for use in RNA repair using the modified IVS **A** below. The first step of group I self-splicing is attack of exogenous G on the 5′ splice site. Draw the analogous reaction in which **A** acts as an endoribonuclease on RNA **B** bound at the IGS. Draw the second step of splicing in this context, assuming that **A** refolds to bring G_{414} into the G pocket. Now, explain how this reaction could repair an aberrant RNA **B** (hint: what if the IVS 3′ exon and the substrate 3′ end were nearly the same?).



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17. (10) What do the functions of the U4 snRNP in pre-mRNA splicing and the UvrA protein in repair apparently have in common? Why is ATP hydrolysis associated with both their functions? What is the catchy phrase that describes this function?

18. (5) In the table below indicate whether in future years the course could benefit from more/the same/less emphasis on the items listed.

Topic/aspect	More	Perfect as is	Less
Biotechnology			
Philosophy of science			
Humor			
RNA			
Problem sets			
Math			
Basic molecular genetics			
Reading			
Computer modeling			
Handouts			

---------Additional space for clearly identified previous questions:----------

Page	Points
1	/23
2	/25
3	/26
4	/20
5	/22
6	/25
7	/22
8	/22
9	/15
Total	/200

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