BCHM 674: Nucleic Acids **Your Name:** _____

Final Exam, Dec, 17, 2004 **Prof. Jason Kahn**

You have 120 minutes for this exam, which is worth 150 points. Thus you get about the same "points per minute" as for the 80 minute exams.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

No study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Explanations should be concise and clear. I have given you more space than you should need.

There will be a viewing from 10 a.m. to 12 noon on Wednesday, December 22.

I will email the reflector the moment the grades are submitted, hopefully by Monday or so.

Honor Pledge: At the end of the exam time, please write out the following sentence and sign it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. Transcription (40 pts):

(a; 12 pts) Briefly describe two functions each for the α and σ subunits in *E. coli* RNA polymerase.

Score _____

(b; 14 pts) Sketch the nucleic acid components of a transcription bubble and a replication fork (with 5′s and 3′s). Rationalize why RNA polymerase is inherently processive whereas DNA polymerases are not.

(d; 14 pts) Briefly describe general mechanisms for the following connections in eukaryotic transcription:

What is the general effect of chromatin on the level of transcription?

How can transcriptional activators control chromatin structure?

How can chromatin remodelers affect transcription factor binding?

How can TFIID affect chromatin structure?

How can TFIID respond to chromatin structure?

Score

2. Transcriptional regulation (25 pts):

(a; 12 pts) Give brief explanations for the following aspects of lac operon regulation:

How do the secondary operators enhance the efficiency of repression by Lac repressor?

Lac repressor with bound inducer is still quite a respectable DNA binding protein. How is it then that the inducer causes Lac repressor to leave the promoter?

Why has the system evolved so that transcription of the operon is low except in the presence of CAP•cAMP?

How does CAP•cAMP activate transcription?

Score

(b; 13 pts) Sketch and describe the activation of transcription by NtrC, focusing on how it differs from the activation of $E\sigma^{70}$.

3. Repair and connections among processes (50 pts):

(a; 12 pts) Sketch the mechanism that leads to rapid repair of DNA damage on the transcribed strand of active genes. You may conclude with the recruitment of UvrA, no need to show the rest of NER. Why does it make sense that this type of damage is repaired first?

Score

(b; 10 pts) The model proposed by Park et al. for Mfd-mediated rescue/release is that Mfd forces RNAP to move forward and that the RNAP then either elongates or is dissociated. This should remind you of our model for proofreading in DNA polymerases. What is the kinetic partitioning involved in the Mfd reaction? Where does irreversibility come from? How is the speed of transcriptional elongation modulated in their experiments?

(c; 15 pts) Mismatch repair is responsible for correcting replication errors. In the *E. coli* system that we discussed, what are the functions of MutS, MutH, and MutL? How is the daughter strand identified? How does the system "know" which direction to exonucleolytically remove starting from the nick?

Score

(d; 6 pts) We speculated on how eukaryotes could identify daughter strands in strand-specific mismatch repair. What are two possible mechanisms?

(e; 7 pts) Sketch how a DNA nick can lead to a double-strand break upon replication. Why do RecBCD mutants have decreased viability?

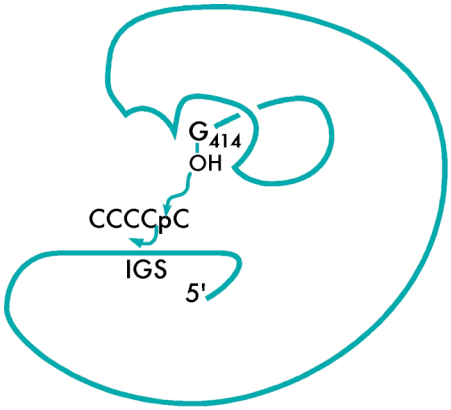
4. RNA biology (25 pts):

(a; 6 pts) Describe two advantages of RNAi-mediated repression over more traditional methods of knocking down gene expression.

Score

(b; 5 pts) Long stretches of ribosome-free RNA trigger termination in prokaryotes and mRNA decay in eukaryotes. Why (not how; it's a short answer)?

(c; 10 pts) The diagram below shows one step of an enzymatic reaction carried out by the *Tetrahymena* ribozyme. Assuming a two-metal ion mechanism, sketch the chemistry at the active site.



(d; 4 pts) Why is RNA an attractive candidate for the macromolecular ancestor of life as we know it?

Score