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

Final Exam, Dec. 20, 2005 **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 min exams. Improvement will be considered for final grades.

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 Friday, December 23.

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; 18 pts) Sketch how it is that a σ factor can specify the promoter sequence recognized by *E. coli* RNA polymerase even though isolated full-length σ factors do not bind DNA. Give an example of an alternative σ factor in *E. coli* and its function. What do eukaryotes use to carry out the functions of σ factors?

Score _____

(b; 22 pts total) (i-12 pts) Sketch the ternary elongation complex during normal elongation; for this sketch only, label 5′ and 3′, label the template and nontemplate DNA strands, and indicate the direction of transcription. (ii-4 pts) Sketch the TEC upon hitting a pause-inducing hairpin. (iii-6 pts) Sketch the TEC after backtracking. Indicate what part of the RNA transcript is used for continued synthesis if transcript cleavage occurs in the backtracked complex.

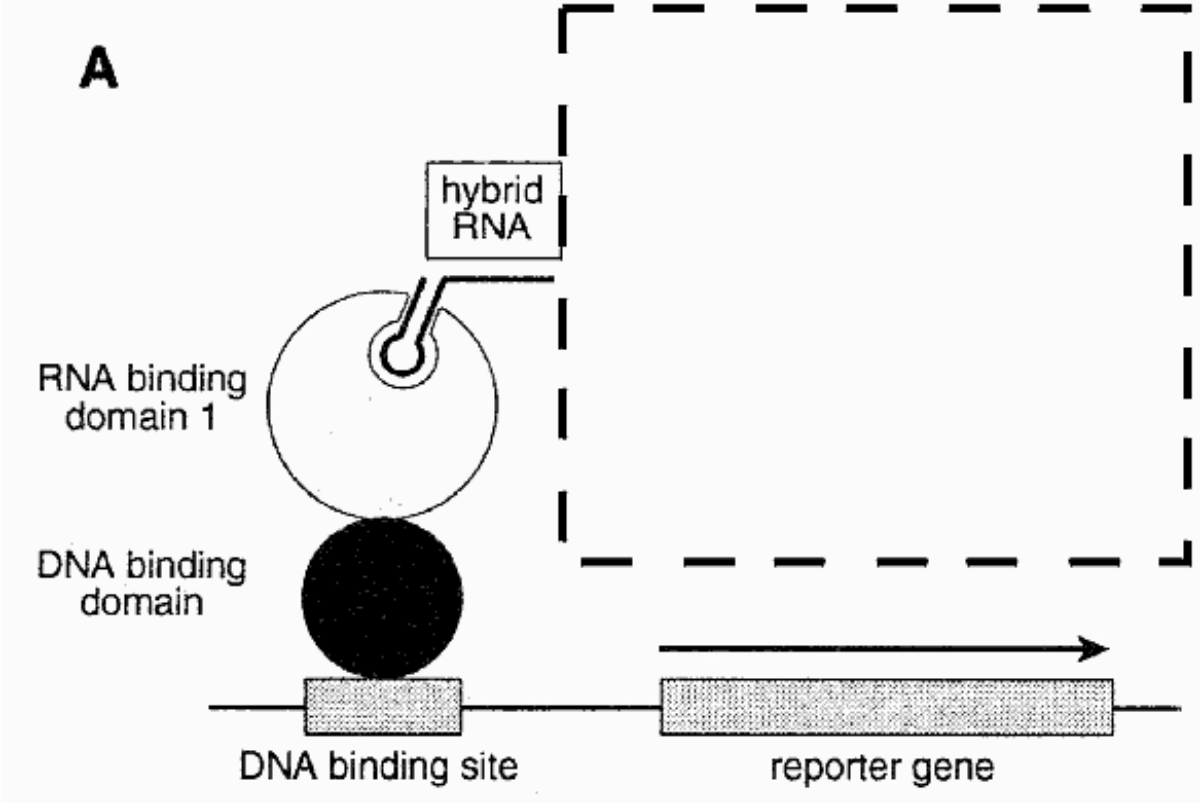
Score

2. Transcriptional regulation (45 pts):

(a; 17 pts) Sketch the processing reactions and give the names of the complexes that carry out RNAi-mediated knockdown of gene expression, starting with a long dsRNA.

Score

(b; 10 pts) The yeast two-hybrid method has been elaborated in may ways. For example, the yeast three-hybrid method allows for screening of RNA-protein interactions. The sketch below shows parts of some components of the system. "RNA binding domain 1" is from the phage MS2 coat protein, which binds the hairpin part of the hybrid RNA as shown. Sketch what goes into the box to complete a system for analyzing YFR (your favorite RNA)-protein binding.



(c; 6 pts) As with the two-hybrid method, the three-hybrid system is vulnerable to possible false positive and false negative artifacts. Describe one of each below.

Score

(d; 12 pts) In their classic 1999 paper, Cosma, Tanaka, and Nasmyth use ChIP to study in vivo occupancy of the *HO* endonuclease promoter. One of the main points of the paper is the use of this cell-cycle regulated gene as a good model system for following the order of events as a promoter is activated. Give a very brief (one or two sentences) summary of their key results. Why was it important to use a cell-cycle related gene, i.e. why couldn't the authors just do their experiments with an unsynchronized population of cells? Suggest another way to look at the order of events at a promoter.

Score

3. Repair and connections among processes (38 pts):

(a; 22 pts) Throughout the class, we have emphasized that high fidelity in biological information processing requires energy dissipation (irreversible checking steps), and that this is usually done via kinetic partitioning pathways. Sketch how dissipative kinetic partitioning is done for NER (the Uvr(A)BC excinuclease) and in tRNA selection during translation (EF-Tu). What would the consequences of "backflow" be for each process?

Score

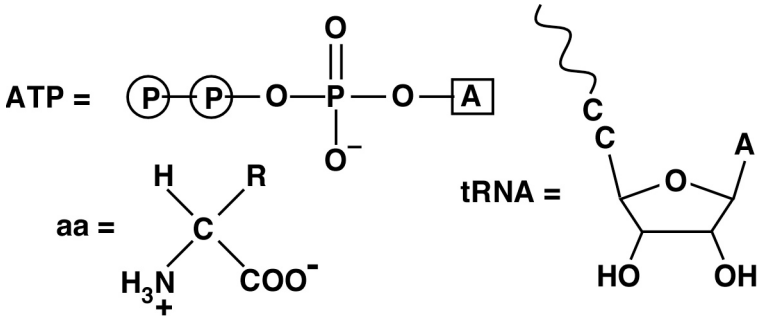
(b; 16 pts) Briefly discuss the functions of TFIIH in the processes of (1) transcription initiation, (2) promoter escape, and (3) DNA repair. Speculate on why DNA repair and transcription share this important component.

4. Translation (27 pts):

(a; 12 pts) What are the respective functions of the 50S and the 30S ribosomal subunits in translation? What is the most straightforward evidence that the ribosome is a ribozyme? What general class of "clock proteins" includes EF-Tu and EF-G?

Score

(b; 15 pts) Draw out the chemical reactions for amino acid activation, tRNA charging and peptidyl transfer. You can use the shorthand representations below:



Score