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

December 16, 2000 **Prof. Jason D. 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.

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 11 a.m. on Wednesday, December 19th in Chem 2507 (next to my office), or you can come by after the break to see the exam.

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

1. (25 pts) DNA Repair

The UvrABC system repairs bulky DNA adducts in *E. coli*.

(a; 8 pts) We discussed that DNA repair systems have special challenges relative to, say, hexokinase. What makes DNA repair a unique challenge for an enzyme system? What aspect of damaged DNA is likely to be recognized by the repair enzymes (we have a clear example in MutS)?

The $(UvrA)_2(UvrB)$ complex translocates on DNA and locates damage. UvrA then dissociates to leave a metastable UvrB•DNA complex.

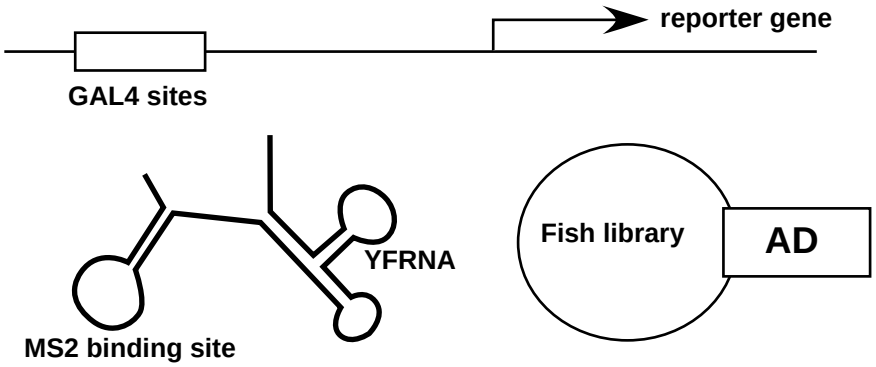
(b; 6 pts) UvrA is an example of what general kind of ATPase-dependent activity? Give another example from the course.

(c; 6 pts) We offered a rationale for why this complicated mechanism has evolved, vs. simply having UvrB bind DNA. Explain the role of UvrA in enhancing specificity, and why this is important.

(d; 5 pts) What common steps finish off all the DNA repair mechanisms we discussed in class, *i.e.* what happens once there is just a single-stranded gap on otherwise clean DNA? What makes DNA polymerase I a suitable repair polymerase for NER but not MMR?

2. (30 pts) Eukaryotic Transcription

Many variants of the yeast two-hybrid system have been developed. Among them is the "three-hybrid" system for analyzing protein-RNA interactions. Some of the components are sketched below. MS2 is a well-characterized RNA binding protein. AD = Activating Domain.



(a; 10 pts) What one additional component would you need in order to make a system for fishing for a protein that could bind Your Favorite domain of the RNA shown? Sketch the final activating complex.

(b; 8 pts) This system can give false positives, activation without YFRNA binding. Give two ways this could happen, and specify controls that identify them as false positives. (There is space on the next page too.)

Besides RNA polymerase II, eukaryotic transcription requires general transcription factors (GTFs), transcriptional activators, and often coactivators with chromatin remodeling activity.

(c; 6 pts) How are the functions of the GTF's, as a group, similar to those of the prokaryotic sigma subunits? Focus on promoter binding and initiation/escape.

(d; 6 pts) How do transcriptional activators and chromatin remodeling reciprocally affect each other? (What's the buzz word in eukaryotic transcription these days?)

3. (30 pts) Prokaryotic Transcription

(a; 10 pts) Sketch a cartoon for the prokaryotic ternary elongation complex, including DNA, RNA, dsDNA binding jaws, and RNA binding channel. Include 5′s and 3′s, and identify the template and non-template strand.

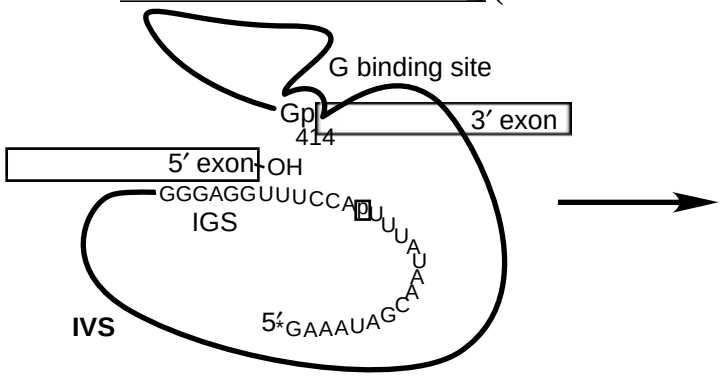
(b; 6 pts) Draw how the structure is believed to change upon transcribing a strong pause site or terminator.

(c; 10 pts) What is the best kinetic evidence for the existence of 1-dimensional rather than 3-dimensional search processes? How can sliding be experimentally distinguished from looping? What is the fundamental difference between sliding and tracking (e.g. as in the MutSHL system)?

(d; 4 pts) How are global changes in transcription patterns (e.g. heat shock) controlled in *E. coli* ?

4. (35 pts) RNA Splicing and Chemistry

(a; 15 pts) The self-splicing of the *Tetrahymena* 26S rRNA IVS is shown below, just before the second step of splicing. Show the product of the reaction. Then draw the conformation needed to form the "C-15" circular IVS (via reaction at the boxed phosphate), and the final circular product.



Conf. change

Circularize

(b; 16 pts) RNAs that (1) bind desired substrates, and then (2) catalyze reactions using them, can be isolated by selection-amplification. Lorsch and Szostak selected an RNA that can phosphorylate its own 5′ end using ATP as a phosphoryl donor (i.e. a kinase). This was done in two sequential selection-amplification protocols (two separate Nature papers corresponding to 1 and 2 above). Assuming you have a magical method for isolating 5′-phosphorylated RNA (see their papers for the real chemistry), sketch how the two selection-amplifications would be done.

(c; 4 pts) Why doesn't selection-amplification for a kinase from a completely random pool work?

5. (30 pts) Regulation of Transcription, and Feedback

DNA looping is believed to be important in both repression (especially in prokaryotes) and activation (especially in eukaryotes).

(a; 8 pts) What is the functional advantage of DNA looping by the Lac repressor tetramer (how does it contribute to more effective repression)? What is the in vivo evidence for this?

(b; 6 pts) What is the phenotype resulting from a Lac repressor mutant that can only form dimers? What kind of regulatory mutation might suppress the dimer phenotype?

(c; 10 pts) It has been observed that loop stability is much less important in activation loops than in repression loops, i.e. that changing the loop shape and size doesn't make much difference in eukaryotes. What level of operator occupancy is required for 2-fold repression of a gene, assuming that an occupied operator is 100% effective in repression? What level of occupancy is required for 5-fold activation of a gene that is basally transcribed 0.1% of the time, assuming that an occupied activator binding site leads to transcription 100% of the time? What does this say about different requirements for complex stability for activation vs. repression? Why don't eukaryotes need huge amounts of specific repressors?

(d; 6 pts) List one topic you would like to see dropped from the course, and one you would like to see added.

Do Not Write Below This Line

Score:	Question 1: _____	out of 25: DNA Repair
	Question 2: _____	out of 30: Eukaryotic Transcription
	Question 3: _____	out of 30: Prokaryotic Transcription
	Question 4: _____	out of 35: RNA Splicing and Chemistry
	Question 5: _____	out of 30: Regulation and Feedback
	Total: _____	**out of 150**