

Biochemistry 465

Your Printed Name:_____

Section I and only

May 21, 1999

Your SS#:_____

Final Exam

Prof. Jason D. Kahn

Your Signature:_____

You have 120 minutes for this exam, which is worth 200 points. Thus you get more “points per minute” than for the midterm exams.

Though each question has several parts, all of the parts are completely independent of each other. In other words, don’t give up if you can’t answer part (a).

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 on Monday, May 24, from 3-4 p.m., in Chemistry 2507** (next to my office in the Biochemistry wing).

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

1. (40 pts) DNA Repair and Connections to other Processes

(a; 10 pts) Which DNA repair system repairs bulky adducts like BPDE bound to one strand of DNA? In *E. coli*, what proteins comprise the repair system in question? What makes DNA repair unusual in biochemistry in terms of enzyme specificity, relative to (for example) enzymes involved in intermediary metabolism?

(b; 12 pts) In prokaryotes, the template strand of actively transcribed genes is repaired more efficiently than DNA elsewhere in the genome. Why does this make biological sense? In general, what is the key operation that the cell generally does not want to do with damaged DNA? Transcription-repair coupling in eukaryotes is conceptually similar, with one important difference in regards to what happens to the RNAP. What is this difference, and why does it make sense?

(c; 8 pts) Sketch the pathway for base excision repair or methyl-directed mismatch repair or transcription-coupled repair in prokaryotes, whichever one is your favorite.

(d; 4 pts) Repair of inter-strand crosslinks in DNA requires recombination. Why (in principle, no need to draw a pathway)?

(e; 6 pts) Briefly, how does p53 act as a tumor suppressor?

2. (40 pts) Translation

(a; 11 pts) Draw the chemical steps in charging a tRNA with an amino acid. What class of enzymes perform this reaction? In what sense do they determine the fidelity of translation?

(b; 6 pts) What are the names and functions of the two ribosomal subunits in *E. coli*?

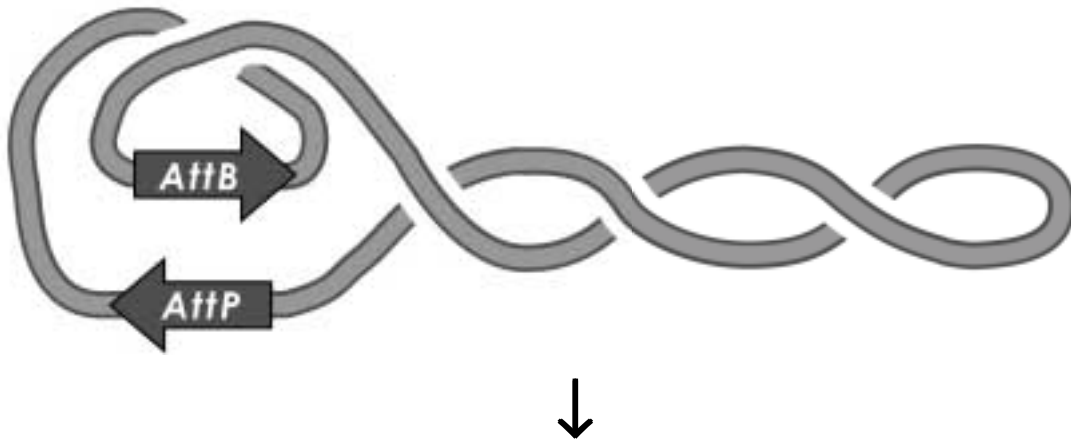
(c; 3 pts) Why is translation called translation? In other words, what is the connection between the vernacular and scientific uses of the word “translation?”

(d; 11 pts) What is the function of EF-Tu in translation? Sketch the reaction cycle involving EF-Tu. How does its GTPase activity provide kinetic proofreading?

(e; 9 pts) Give some of the evidence for the critical role of the 23S RNA as the catalyst for peptidyl transfer, as opposed to its being simply a scaffolding element.

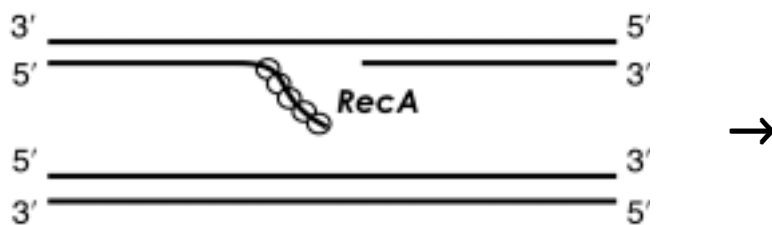
3. (35 pts) DNA Recombination and Evolution

(a; 15 pts) The sketch below shows the initial configuration for site-specific recombination between the *AttB* and *AttP* sites in a plasmid model system for lambda phage integration. Label the superhelical nodes with their signs. Draw the product of the recombination reaction catalyzed by IHF and Int, with its superhelical nodes. What is the linking number change in the reaction? What does the fact that there is a definite answer to this question tell us about the mechanism of site-specific recombination, and why does this make sense in terms of evolution of mobile DNA?



(b; 10 pts) Give two evolutionary rationales for the benefits of recombination. Why do transposable elements persist in our genomes (what phrase did we co-opt to describe them)?

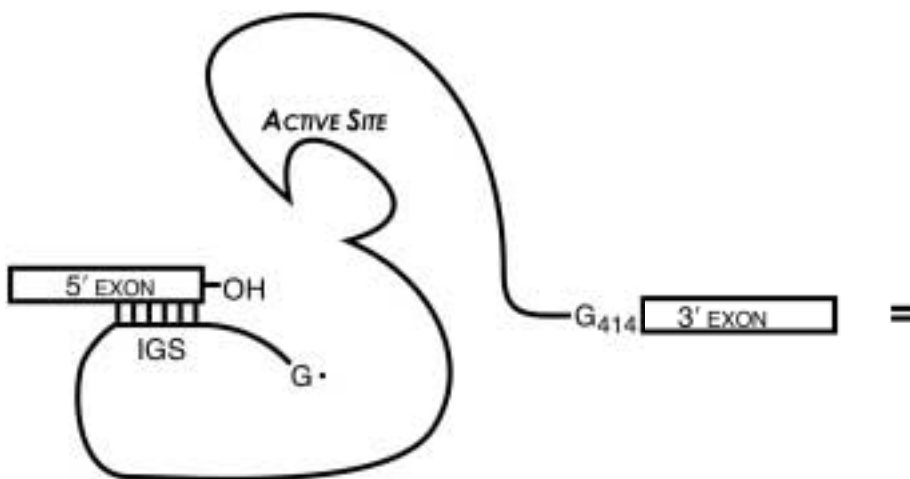
(c; 10 pts) Sketch the Holliday junction (in the crossed over representation) which would eventually result from invasion of the RecA coated single strand as indicated below. Sketch the product of branch migration by the RuvAB motor, and draw/explain how resolution by RuvC always results in a heteroduplex segment but may or may not exchange flanking genetic markers.



4. (40 pts) RNA Splicing and Chemistry

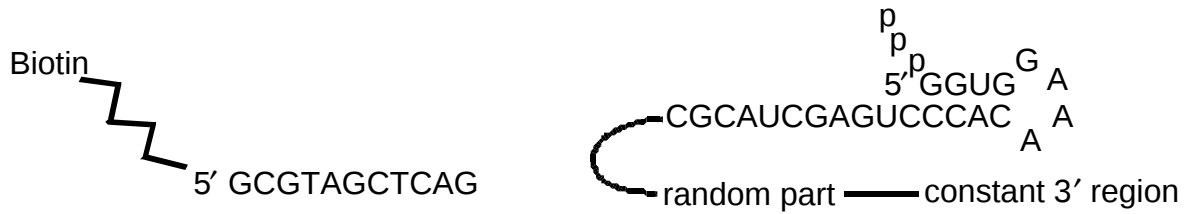
(a; 9 pts) We discussed the idea of paradigm shifts in science. How did the discovery of self-splicing of group I introns change a reigning paradigm in biochemistry, and how did this affect ideas on the origin of life (what makes RNA special)?

(b; 9 pts) The sketch below shows the group I intron after the first step of splicing. Redraw the molecule in the space to the right to prepare it for the second step, indicate the transesterification of the second step on your diagram, and show the products below.



(c; 6 pts) The spliceosome, which helps process pre-mRNA, is about the size of a ribosome and does simpler chemistry. Give two reasons that the ribosome is much better understood.

(d;16 pts) You are given (1) the DNA oligonucleotide on the left below. (2) a solid support which binds biotin very tightly, (3) in vitro transcription, (4) reverse transcription and PCR, and (5) RNase H. Sketch a selection-amplification scheme for isolating RNA sequences which have RNA ligase activity, from the pool of semi-random RNA on the right.



5. (45 pts) Regulation of Transcription and Pedagogy

(a; 12 pts) A genetic screen for *E. coli* unable to grow on lactose turned up a mutant lac repressor which cannot bind IPTG or other inducers but is otherwise functional. How could you test to make sure that the mutation was in lac repressor and not, for example, in β -galactosidase? It was found that the mutant phenotype could be suppressed by a change in the promoter region. What might this change be? What effect would the suppressor mutation have on lac operon expression in the absence of lactose or glucose, in an otherwise wild-type cell?

(b; 7 pts) What are the functions of the eukaryotic general transcription factor TFIID, which includes the TATA box binding protein? What protein provides a roughly analogous activity in prokaryotes (the protein in question has other activities as well)?

(c; 6 pts) Histone acetylases and deacetylases are important in eukaryotic transcription. Which activity is associated with transcriptional activation and which with repression, and why?

(d; 8 pts) DNA looping is the predominant mechanism for transcriptional activation in eukaryotes, whereas it is rare in prokaryotes. Discuss why this makes sense in light of the complexity of eukaryotic gene regulation and the size of eukaryotic genomes.

(e; 12 pts) Estimate the percentage of material covered in this course which you had seen before in other courses. What was the most boring lecture that you attended? What was the most confusing? What was the best?

Do Not Write Below This Line

Score:	Question 1: _____	out of 40: DNA Repair
	Question 2: _____	out of 40: Translation
	Question 3: _____	out of 35: Recombination
	Question 4: _____	out of 40: Splicing
	Question 5: _____	out of 45: Regulation and Feedback
Total:	_____	out of 200