Biochemistry 465 **Your Name:** Grading Key

Biological Information Processing **Prof. Jason Kahn**

Final Exam (170 points total) **May 15, 2006**

You have 120 minutes for this exam.

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

Explanations should be concise and clear. I have given you more space than you should need, but just in case there is a blank page at the end.

You do not need a calculator for this exam, and no other study aids or materials are permitted. N=36

Generous partial credit will be given, *i.e.*, if you don't know, guess. Gardner, Nguyen → +15m

Honor Pledge: At the end of the exam time, please write out the following sentence and sign it, or talk to me about it:

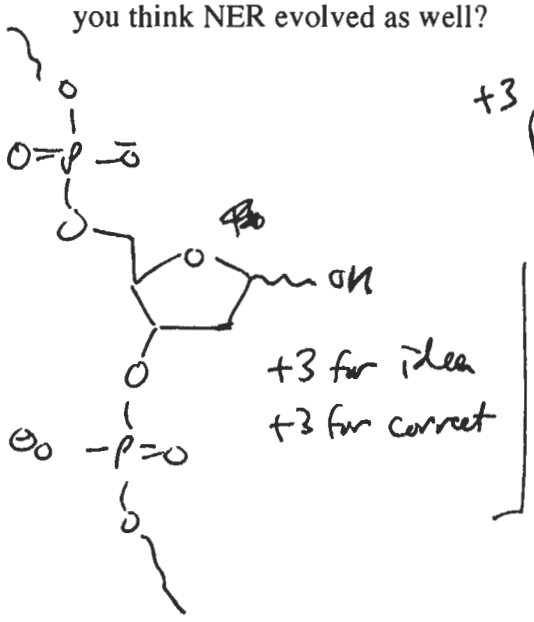
"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

Viewing 10-11 a.m. Weds? 2507

Poster evals → Garofalo, Oliver

1. DNA repair (36 pts):

(a; 15 pts) Draw the structure of an abasic site in DNA. This is the common intermediate for what type of DNA repair? Given that this type of repair exists, and that it's much simpler than NER, why do you think NER evolved as well?



+3 for idea
+3 for correct

+3 Base excision repair - BER

+3 BER recognizes each lesion specifically.
+3 NER is much more versatile - can recognizes xenobiotics via helix distortion / flexibility / dynamics
+1 for TCR

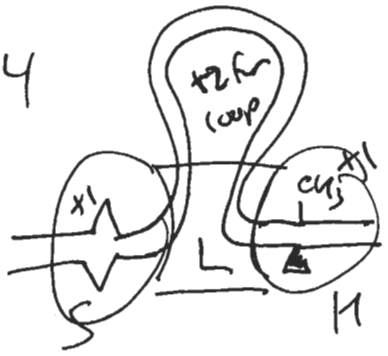
Score for the page_____

(b; 6 pts) What is the source of the information needed for error-free DNA repair via (1) direct repair, (2) NER, and (3) DSB repair? (Very short phrase each)

+2 each
1. It's built in to the lesion-
2. The other strand of the DNA
3. Another copy of the ~~ds~~ dsDNA, like a sister chromatid.

(c; 15 pts) Sketch the MutSHL complex on DNA just after incision, and indicate the function of each protein. We argued that through-solution looping does not make sense as a mechanism for establishing the MutSHL complex, that tracking makes more sense. What is the reasoning? (Note that while there's some experimental evidence for tracking, this is not a done deal.)

+4



S: mismatch recognition +2
L: matchmaker / ATPase +2
H: hemimethylation-specific nuclease +2

+3

- System needs to know which direction to travel to remove the daughter strand DNA. A through-space loop could form with MutH in either orientation.

+2

Speed - +2/5

Score for the page_____

2. Translation (~~36~~ 38 pts):

(a; 9 pts) In kinetic proofreading during aa-tRNA selection on the ribosome, there is a branched pathway for acceptance vs. rejection. What is the acceptance branch called and what happens during acceptance? What is the rejection branch?

+3 acceptance = accomodation

+3 tRNA 3' end moves into place for peptidyl transfer

for rejection = dissociation of aa-tRNA after GTP

+3 hydrolysis

The existence of tradeoffs among speed, energy cost, and fidelity is a general theme of biological information processing. Translation offers an example. Streptomycin is an antibiotic that inhibits growth by markedly increasing the error rate of tRNA selection in translation. The mechanism is complex but the bottom line is that ~~kinetic proofreading becomes much less effective at rejecting near-cognate tRNAs.~~ tRNA's is much slower.

(b; 6 pts) Why would an increased error rate for protein biosynthesis hurt the cell (not a trick question)? What name did we give the corresponding effect of inaccurate DNA polymerases?

+3 – a lot of bad protein would be made

+3 – the error catastrophe / hyper mutators

"mutagenesis" +1

(c; 8 pts) Streptomycin-resistant (SmR) ribosomes are hyperaccurate, i.e. they make fewer mistakes than wild type ribosomes. Recall that hyperaccurate DNA polymerases (antimutators) have very

+3 active 3'→5' exonucleases (fill in the blank). Analogously, how might the EF-Tu•GTP•aa-tRNA kinetic proofreading mechanism above be altered to make a hyperaccurate ribosome?

– Rejection of tRNA could be ~~catalyzed~~ faster, to

+5 increase the chance that a near-cognate tRNA won't have time to accommodate. Increased rate of rejection maximizes fidelity.

(other possible answers)

faster GTPase → fine-basis idea is less time allowed for accommodation

Score for the page_____

(d; 9 pts) The SmR ribosome is not the wild type: in other words, hyperaccuracy is actually ~~not selected for~~ Selected against in the absence of the antibiotic. Give two possible reasons, one based on economy and one based on speed; we will not consider the possibility that the errors per se can be useful. Based on general themes from the course, is the argument from economy or the argument from speed more likely to be correct?

what you know about ~~tRNA~~ ribosome abundance and n

+3 Economy - increased fidelity wastes GTP

+3 Speed - overall rate of translation will be decreased because rate of rejection of correct tRNA ↑

+3 Speed - ribosomes are rate limiting for growth | +1

×2 fidelity is expensive.

6

(e; ~~4~~ pts) Some SmR mutants are actually streptomycin-dependent (SmD), i.e. they die in the absence of the antibiotic. Why might this happen?

+3 | balancing of the two effects - the drug ~~speeds up incorporation~~ ~~fidelity~~ st apparently stabilizes tRNA on the ribosome, the ribosome lets it go - should balance to a state that translates

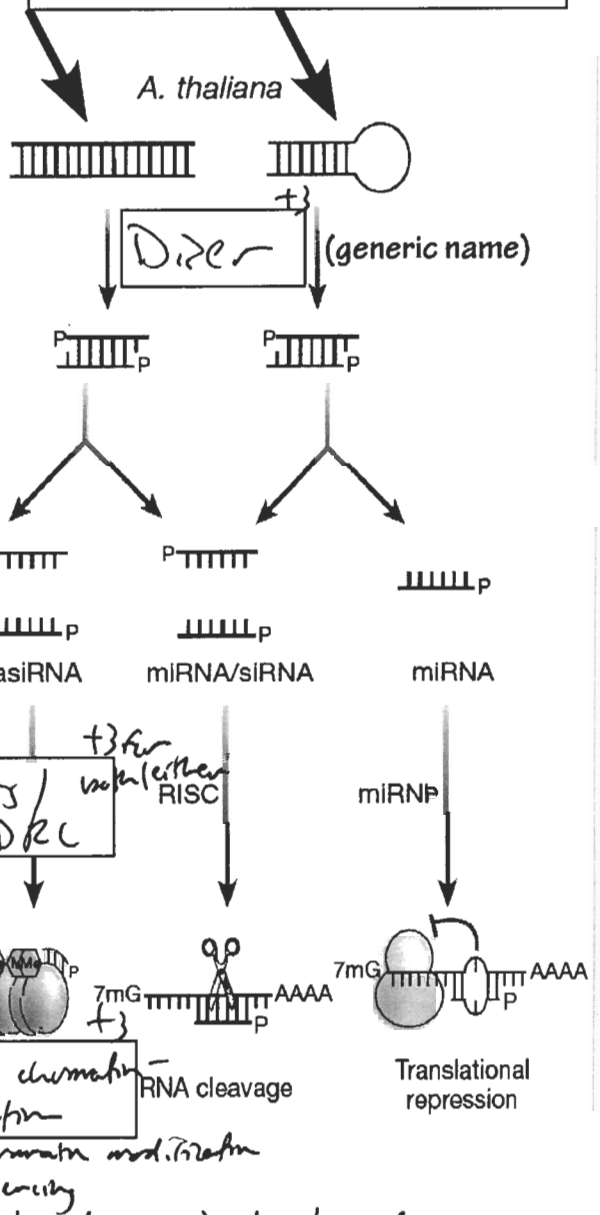
+3 | rapidly enough to live. In the absence of drug, translation is so slow they can't live.

Score for the page_____

3. RNA Biology (~~42~~ 48 pts):

(a; 15 pts) Fill in the boxes in the schematic at the right of three possible outcomes of RNAi in plants.

Two possible sources?: exogenous ds RNA +6 / endogenous miRNA txn



(b; ~~8~~ 6 pts) How might RNAi have evolved? Hint: administering long dsRNA molecules to mammalian cells sets off the ~~interferon response.~~ inflammatory response.

+3 Could have been an anti-viral response to destroy ds RNA viral invaders.

Interferon +3
immune, inflammation +2

(c; 9 pts) What are two of the main attractions for oligonucleotide-based therapeutics as opposed to e.g. small-molecule inhibitors of enzymes? What is one serious difficulty with administering oligonucleotides to patients?

+3 — Can knock out protein completely instead of just + activity — longer-lasting effect?

+3 — Any target can be approached with a similar mechanism

+3 — Using the body's own machinery to amplify signal.

+3 — They are big, charged molecules — hard to get into the cell, subject to nuclease activity

Score for the page_____

(d; 10 pts) RNA has been suggested as the primordial self-perpetuating macromolecule, because it can combine information carrying and catalytic function. In the last few billion years, RNA has specialized mainly in transesterifications of one kind or another. Why has DNA taken over information storage? Why has protein taken over most catalytic functions? Why does RNA retain primacy in nucleic acid transactions like splicing?

+3 – DNA is more stable, can have larger genomes

+3 – Proteins have much more versatile functional groups

+4 – RNA is good at base pairing to recognize substrates, and/or frozen accident, and/or good at moving substrate around

3. Eukaryotic Transcription (27 pts):

We've seen that eukaryotic transcription involves combinatorial regulation by sets of transcription factors, and that chromatin remodeling activities are recruited as part of the process.

(a; 6 pts) Wouldn't it be simpler to have a single transcription factor dedicated to each gene? Give two simple reasons hasn't life evolved this way.

+3 – Would need one TF per gene – and who would regulate the regulators?

+3 – This would not allow for integration of signals.

Score for the page_____

(b; 12 pts) In general, give two ways in which one transcription factors can potentiate the activity of another, and two ways in which one can repress the other. Some possible activate/repress ~~pairs~~ rely on similar mechanisms, that's okay.

Activate: 1. Cooperative binding
2. Recruitment of chromatin remodeling activity that allows binding of the other one
3. Formation of a common recruitment interface
4. Help form loop

Repress 1. Block binding of the other factor
2. Remodel chromatin to block access
3. Disruption of common interface
4. Alter DNA structure to separate partners

(c; 9 pts) How can SWI/SNF ATPases either activate or repress transcription depending on the gene in question? How might they end up repressing some genes even if locally they could only act to activate transcription?

What do SWI/SNP ATPases do?

+3 "Fluidize" nucleosomes - move them around

+3 - they can either reveal or cover the binding site for a transcription factor

+3 - they could activate a TF repressor of target genes

Score for the page_____

4. Connections and Miscellaneous (29 pts)

(a; 24 pts) We have seen several examples of potentially dangerous macromolecules with cryptic activities that are delivered or activated by molecular matchmakers. Describe two examples: for each, list (1) the molecule with the cryptic activity, (2) the ~~(b; 5 pts)~~ nature of the activity (i.e. a restriction enzyme, which is not otherwise an answer I am looking for, would be described as "cuts DNA at a palindromic recognition site"), (3) the partner that loads/activates the molecule from (1), and (4) one reason that molecule from (1) doesn't just do everything itself without help.

1. MutH	U6 sn RNP	Uvr ~~B~~C
2. nuclease	RNA ~~cutter~~ cleavage/splicing	nuclease
3. MutL	U4/U5/U6 from RNP	Uvr (A2)B

4. Would n't want nuclease activity running around the cell, or indiscriminate splicing

(b; 5 pts) Suggest an improvement I could make in the coverage of a topic of your choice.

more up to date with MutSHL-

Page	Score
1	
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8	
Total	

Score for the page_____