Biochemistry 465 **Your Name:** Key

Biological Information Processing **Prof. Jason Kahn**

Exam II (100 points total) **April 13, 2011**

N=64

You have 50 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.

You need a calculator for this exam, and no other study aids or materials are permitted.

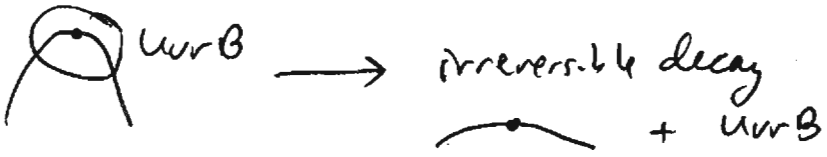
Generous partial credit will be given, *i.e.*, if you don't know, guess.

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."

1. DNA Repair (38 pts):

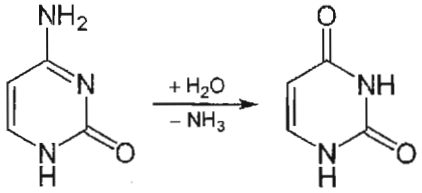
(a; 15 pts) The UvrB-DNA complex in nucleotide excision repair is metastable: its decay is irreversible in the absence of UvrA. Why didn't nature simply evolve a UvrB that could bind directly to DNA instead of evolving the complicated UvrA delivery mechanism? In your answer, discuss how the decay of metastable UvrB is analogous to exonucleolytic removal of a mismatched 3' end.



If UvrB could simply bind from solution, we would lose the initial checking step of damage recognition by UvrA. Also, the (+3)
stable time that the UvrB·DNA complex persists is an indication (+3)
of whether it is actually damaged. As with the 3'→5' exo, irreversible decay of an intermediate allows proofreading (+4)
without the possibility of "backflow", i.e. without creating a possibly dangerous intermediate that bypasses checking steps.
In the case of UvrB, the goal is to avoid unnecessary repair (+5)
of non-damaged DNA as much as possible.

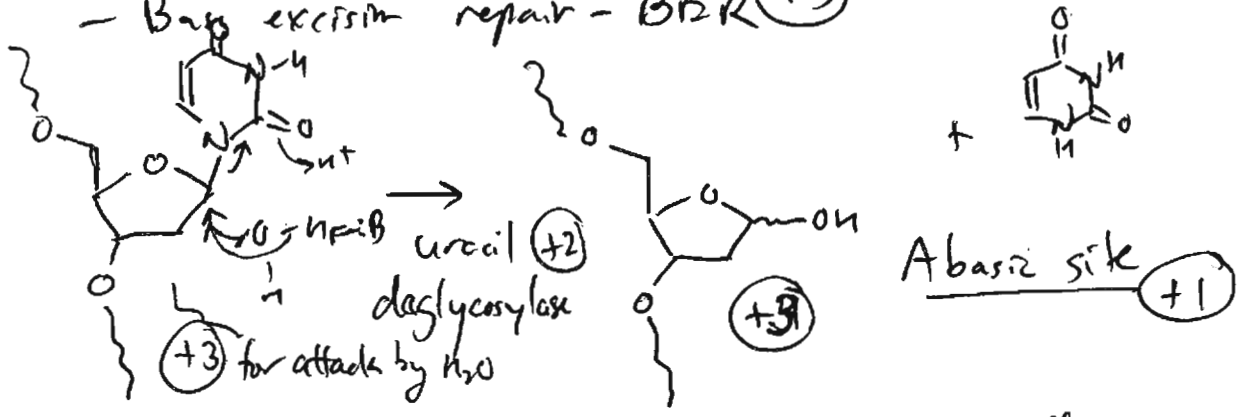
Score for the page 15

There are two differences between DNA and RNA. One is the sugar, the other is the presence of thymine in DNA instead of uracil in RNA. The evolution of thymine may have to do with DNA damage chemistry. It turns out that cytosine spontaneously deaminates to uracil in water:



(b; 15 pts) What kind of DNA repair replaces uracil in DNA with thymine (it's not direct reversal)? Draw the first reaction in the repair process. Why is the resulting product important for efficient of DNA repair in the cell?

– Base excision repair – BER (+3)



(+3) All BER funnels through abasic sites – allows for repair of N different lesions with only ~ N+5 enzymes, not 5N enzymes.

(c; 8 pts) What is the evolutionary advantage of using thymine instead of deoxyuracil, which would have the same protein coding capacity? Why is the modified base 5-methylcytosine associated with mutation even though it is not a miscoding lesion?

– If deoxyuracil were the DNA base, the cell could not distinguish a G·U pair that came from misincorporation of G opposite U from a G·U pair that came from deamination of C → repair would be wrong 50% of the time? (+5)

– 5-methyl C deaminates to T, so a G·T mismatch is the result. MM3 match repair or replication will give an A-T pair. (+3)

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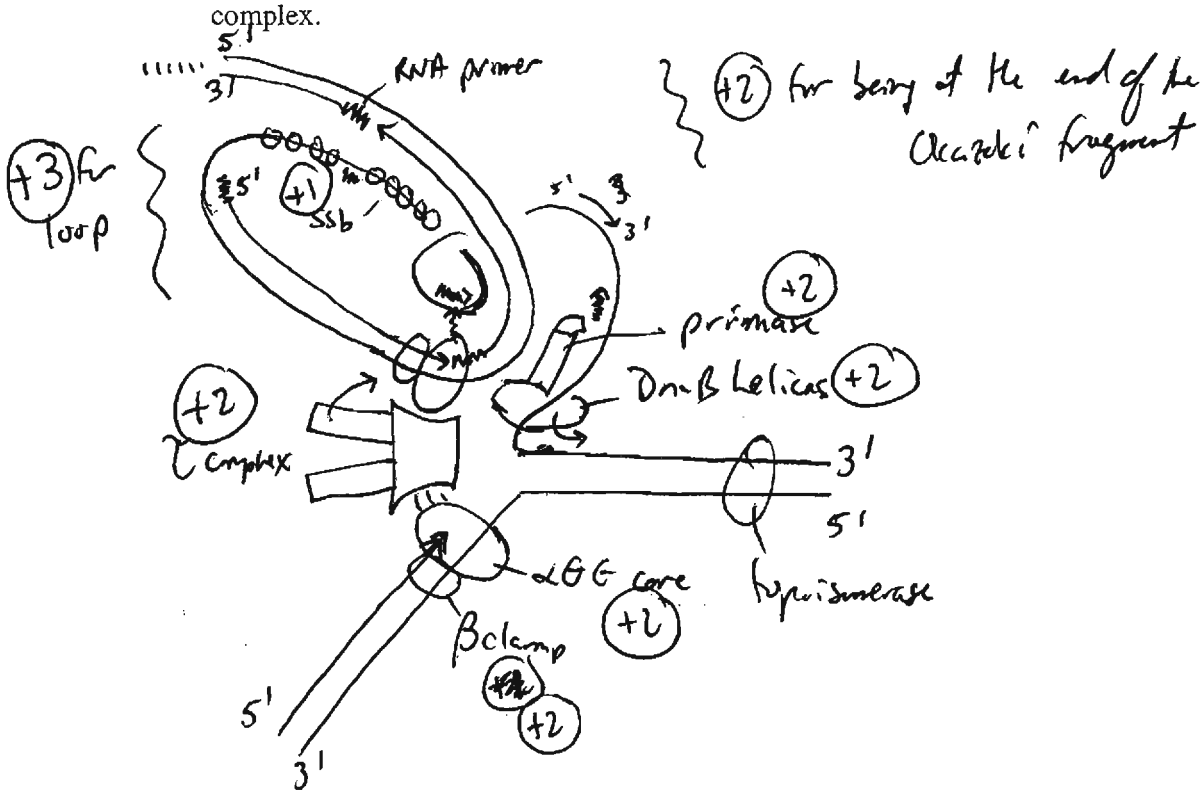
2. DNA Replication (37 pts):

(a; 15 pts) Sketch the trombone model for DNA replication by a dimeric DNA Polymerase III complex in *E. coli*, at the instant that the lagging strand polymerase completes an Okazaki fragment. Include in your picture the core polymerases, the sliding clamps, helicase, primase, SSB, and the tau complex.



(b; 3 pts) We mentioned that the tau complex $\tau_3\delta\delta'$ can support a triple polymerase replisome. Give one possible function we mentioned for the third polymerase.

(+3) – making two Okazaki fragments at the same time

or

(+3) – Holding a DNAP in reserve in case damage is encountered

Score for the page_____

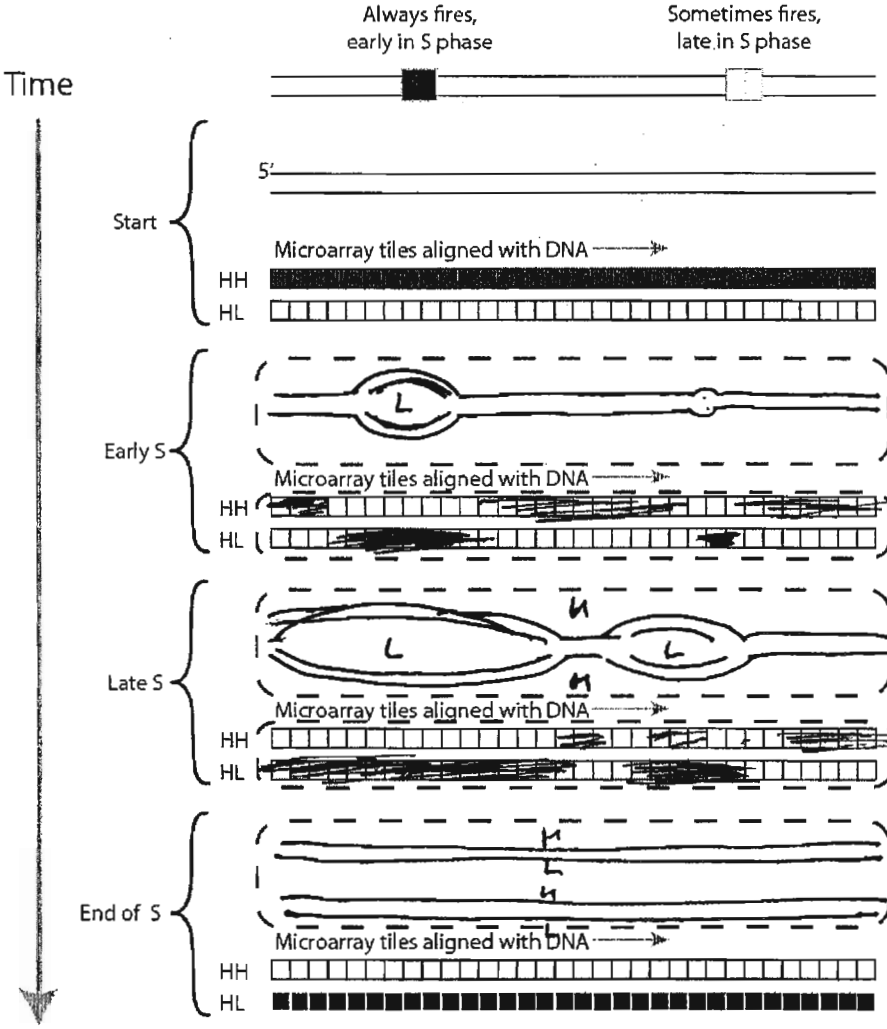
A clever combination of old and new methods was used in 2001 to map origins of replication in yeast. This is more difficult in eukaryotes than in bacteria because the origins do not behave identically in every cell: some fire and some don't. The authors' procedure was as follows: they

(1) grew cells in $^{15}N/^{13}C$ (Heavy) medium
(2) arrested their replication
(3) switched them to $^{14}N/^{12}C$ (Light) medium
(4) synchronized the yeast cell cycles so they all entered S(ynthesis) phase together
(5) fragmented the DNA at various times throughout S phase
(6) fractionated the DNA on a density gradient
(7) labeled the resulting HH and HL DNA fractions and hybridized each to a microarray with genomic DNA sequences on it.

(c; 9 pts) In the dashed areas of the diagram at the right, draw in the DNA that would be observed at each step, assuming that the origin on the left fired early and the one on the right fired late in S phase.

(d; 6 pts) Fill in the microarray stripes in the dashed areas, with the darkness of the microarray tiles indicating the intensity of the hybridization signal.

(e; 4 pts) Qualitatively explain how the results would change if the origin on the right did not fire.



+3 idea that origins are HL

+2 for reasonable spreading

+1 for darker HL

The average amount of HL

The HL would appear later in S-phase, only when the forks from nearby origins traversed the inactive origin

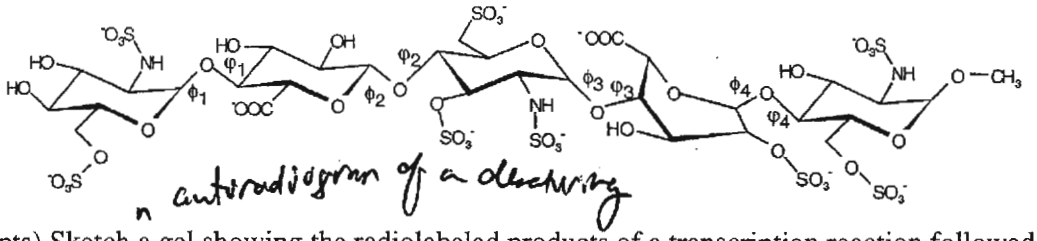
+4

Part C points

Score for the page 19/19

3. Transcription and Protein-DNA Recognition (25 pts):

The RPo open complex is much more stable than the RPc closed complex, which is disrupted by the sulfated polysaccharide anticoagulant heparin (recently in the news when contaminated batches caused severe allergic reactions in dialysis patients). Addition of heparin limits transcription reactions to a single "round," preventing re-initiation by RNA polymerase that has completed a transcript.



n antiradiogram of a decasaccharide

(a; 16 pts) Sketch a gel showing the radiolabeled products of a transcription reaction followed over time according to the scheme below, and identify the RNA products on the gel.

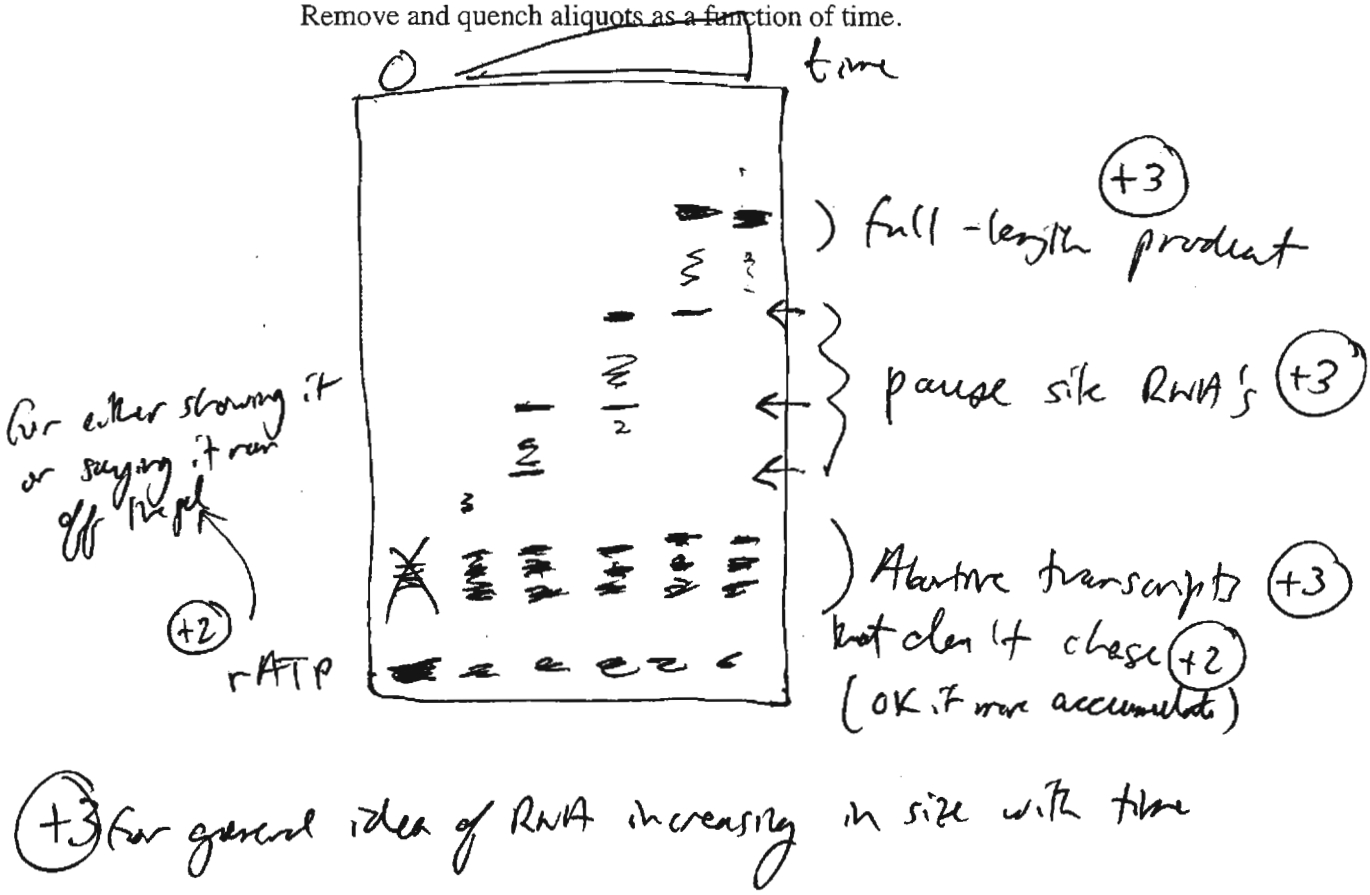
Mix RNAP + Promoter-containing DNA →

Allow RPo to form →

Add heparin to prevent transcription initiation by free RNAP →

Add triphosphates including [α-^{32}P]rATP & and the other three unlabeled rNTP's.

Remove and quench aliquots as a function of time.



(+3) for general idea of RNA increasing in size with time

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The σ^{54} sigma factor binds consensus sites centered at -24 and -12. One domain of σ^{54} bound to DNA is shown below.

(b; 6 pts) What kind of DNA binding domain is this, and how do you think it carries out sequence-specific recognition?

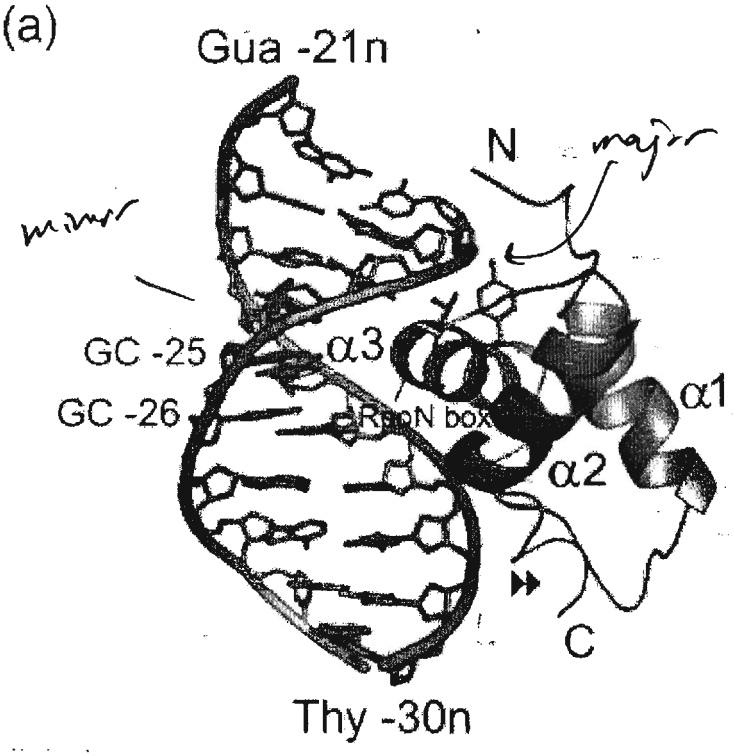
(+3) Helix-turn-helix (actually more a winged HTH)
or
α-helix in major groove

(+3) makes direct H-bonds to the major groove edge of the base pairs

(c; 3 pts) What is the function of σ^{54}?

(+3) - Programs E. coli to respond to nitrogen limitation

(a)



Page	Score
1	/15
2	/23
3	/18
4	/19
5	/16
6	/9
Total	/100

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