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

Final Exam, Dec, 17, 2004 **Prof. Jason Kahn**

You have 120 minutes for this exam, which is worth ~~150~~ 140 oops points. Thus you get about the same "points per minute" as for the 80 minute exams. multiply all scores by 15/14 ! oops.

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

No study aids or materials are permitted.

N=21

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 Wednesday, December 22.

I will email the reflector the moment the grades are submitted, hopefully by Monday or so.

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; 12 pts) Briefly describe two functions each for the α and σ subunits in *E. coli* RNA polymerase. (+3 each)

α: - interacts with activators such as CAP → recruitment
- interacts with DNA upstream of -35 (UP elements)
- RNAP assembly

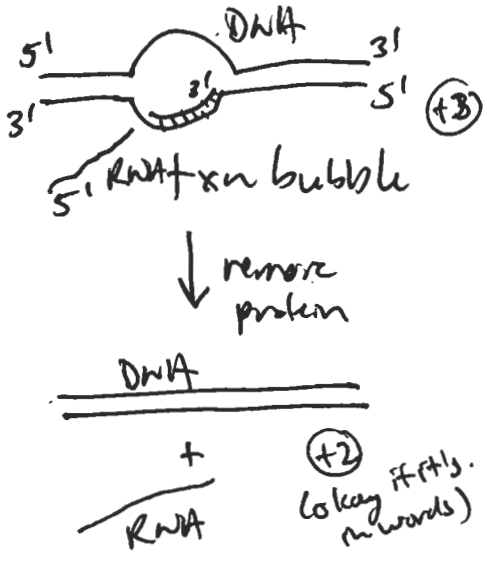
σ: - confers promoter specificity
- decreases non-specific DNA binding

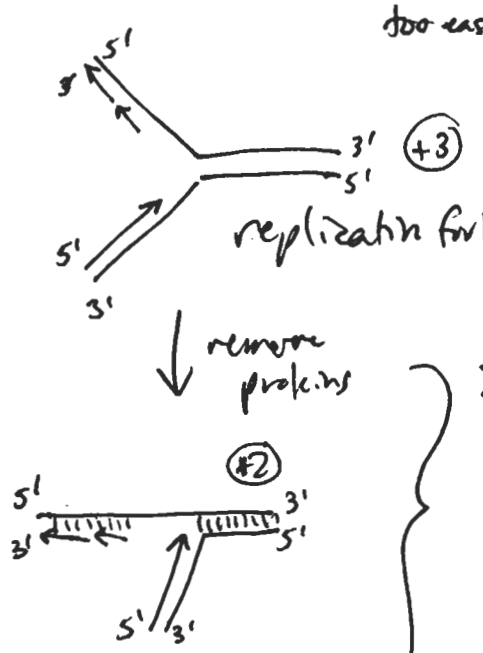
- σ^{54} interacts with NtrC
- redirects general transcriptional program

Score 12/12

(b; 14 pts) Sketch the nucleic acid components of a transcription bubble and a replication fork (with 5′s and 3′s). Rationalize why RNA polymerase is inherently processive whereas DNA polymerases are not.

too easy! [+9 for good pics and explanation about non-processive lagging strand]





+4 for idea

If RNAP dissociates, nucleic acid components collapse — there's nothing to come back to — for DNA there will still be a primer-template

(c; 14 pts) Briefly describe general mechanisms for the following connections in eukaryotic transcription:

What is the general effect of chromatin on the level of transcription?

+2 Chromatin is a general repressor. "Regulates" +1

How can transcriptional activators control chromatin structure?

+3 Activators can recruit HAT or SWI/SNF activity to "loosen" chromatin structure

How can chromatin remodelers affect transcription factor binding?

+3 HAT and SWI/SNF can reveal previously occluded sites (or block them) to control access by transcription factors and other proteins.

How can TFIID affect chromatin structure?

+3 TFIID has HAT, ubiquitinylation activities or bind + bend DNA +2

How can TFIID respond to chromatin structure?

+3 for either answer It has bromodomains that bind acetylated DNA; it binds TATA box only when it is accessible

Score 128

2. Transcriptional regulation (25 pts):

(a; 12 pts) Give brief explanations for the following aspects of lac operon regulation:
How do the secondary operators enhance the efficiency of repression by Lac repressor?

(+3) Secondary operators potentiate loop formation, which increases local [ ] of repressor in the neighborhood of the primary operator.

Lac repressor with bound inducer is still quite a respectable DNA binding protein. How is it then that the inducer causes Lac repressor to leave the promoter?

(+3) It's much less specific for the operator and therefore diffuses away into the huge amount of non-specific competitor available.

Why has the system evolved so that transcription of the operon is low except in the presence of CAP•cAMP?

(+3) The presence of CAP·cAMP is a sign of low glucose. If glucose is abundant, there is no need to metabolize lactose.

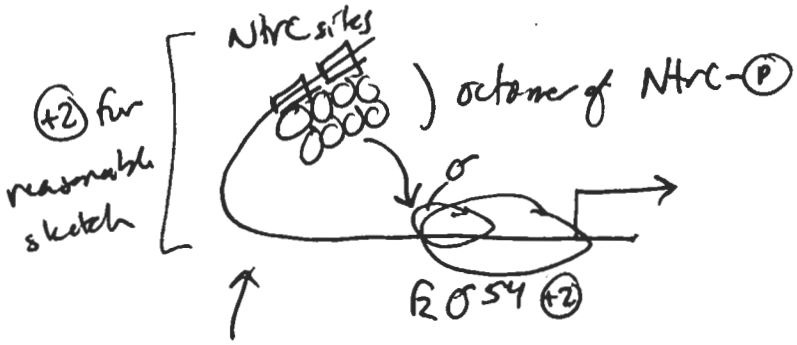
How does CAP•cAMP activate transcription?

(+3) Binds DNA, binds a contact patch on the α subunit. Therefore ↑ binding affinity of ~~repressor~~ RNAP for DNA, and also probably induces conformational change in α by helping it bind DNA.

+2 for mentioning recruitment only

Score 12/12

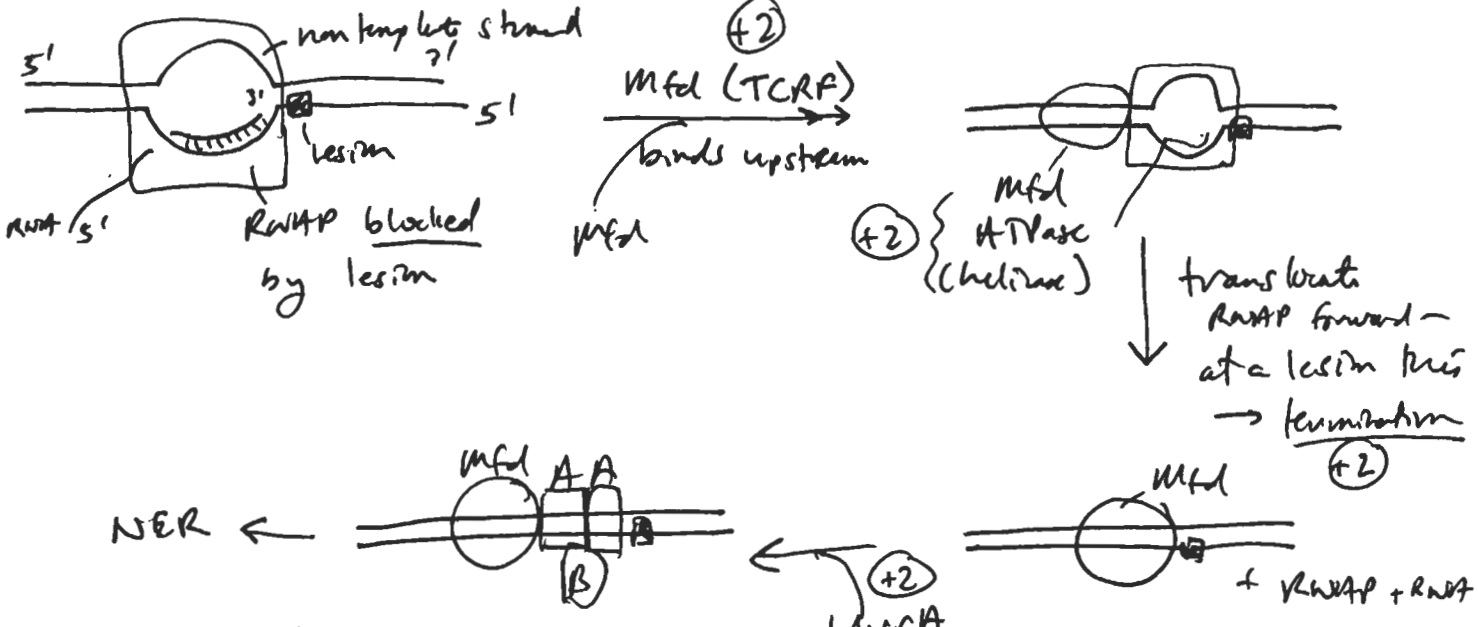
(b; 13 pts) Sketch and describe the activation of transcription by NtrC, focusing on how it differs from the activation of $E\sigma^{70}$.



+3 phosphorylated NtrC is an AAA+ protein. It binds upstream. Activates a stable but previously closed complex of σ^{54}, stimulates open complex formation.

3. Repair and connections among processes (50 pts):

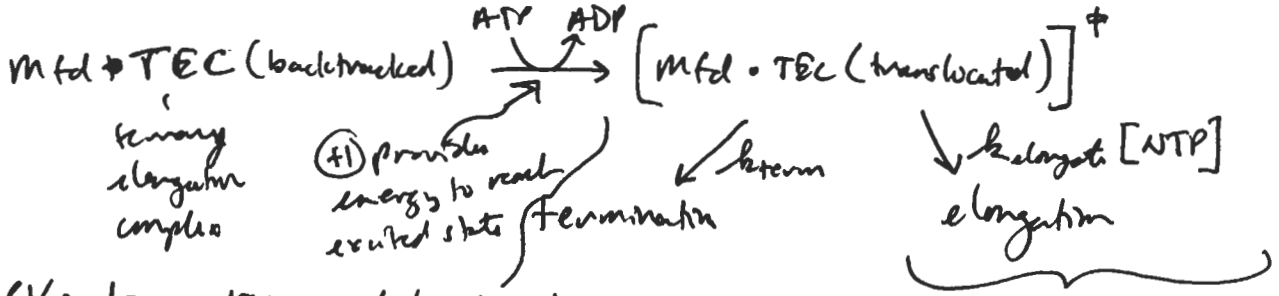
(a; 12 pts) Sketch the mechanism that leads to rapid repair of DNA damage on the transcribed strand of active genes. You may conclude with the recruitment of UvrA, no need to show the rest of NER. Why does it make sense that this type of damage is repaired first?



+4 The most critical damage is to DNA that is to be copied — until then it doesn't hurt anything. This mechanism allows rapid repair of active genes to give full-length protein.

Score 125

(b; 10 pts) The model proposed by Park et al. for Mfd-mediated rescue/release is that Mfd forces RNAP to move forward and that the RNAP then either elongates or is dissociated. This should remind you of our model for proofreading in DNA polymerases. What is the kinetic partitioning involved in the Mfd reaction? Where does irreversibility come from? How is the speed of transcriptional elongation modulated in their experiments?



(+3) – Kinetic partitioning of the breakdown of the translocated intermediate depends on the

(+2) rate of elongation – if it's slow, RNAP will terminate.

(+2) rate of elongation varies w/ [NTP] and damage
↳ in Park experiment (nonphysiological)

Similar to control of 3'→5' exo via slow extension of a mismatch.

(+2)(+2) Irreversible because translocated TEC breaks down irreversibly – or irrev. creation of metastable translocated state.

(c; 15 pts) Mismatch repair is responsible for correcting replication errors. In the *E. coli* system that we discussed, what are the functions of MutS, MutH, and MutL? How is the daughter strand identified? How does the system "know" which direction to exonucleolytically remove starting from the nick?

- MutS – recognizes mismatches
- MutL – translocates to bring MutS + MutH into contact
- MutH – nicks hemimethylated DNA at GATC sites

(+3) each

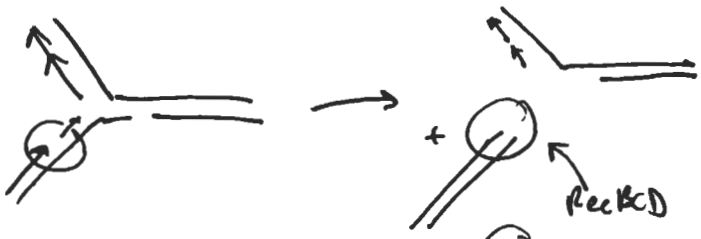
- Daughter strand identified because it is the unmethylated one.
- Exonucleotytic degradation moves toward MutL – direction established by translocation to extrude a loop.

Score 12.5

(d; 6 pts) We speculated on how eukaryotes could identify daughter strands in strand-specific mismatch repair. What are two possible mechanisms?

- Could recognize nicks in lagging strand (or 3' end of leading strand if DNAP gets out of the way) (+3)
- Could recognize the orientation of the β subunits released from the replication fork - the face that doesn't interact with DNA polymerase α β oriented toward the 5' end of the growing strand. (+3)

(e; 7 pts) Sketch how a DNA nick can lead to a double-strand break upon replication. Why do RecBCD mutants have decreased viability?



DNA polymerase runs off the end of the leading strand template. (+2)

(+3)

RecBCD is needed to repair collapsed replication forks - the breaks kill replicating cells otherwise. (+2)

4. RNA biology (25 pts):

(a; 6 pts) Describe two advantages of RNAi-mediated repression over more traditional methods of knocking down gene expression.

(+3) each

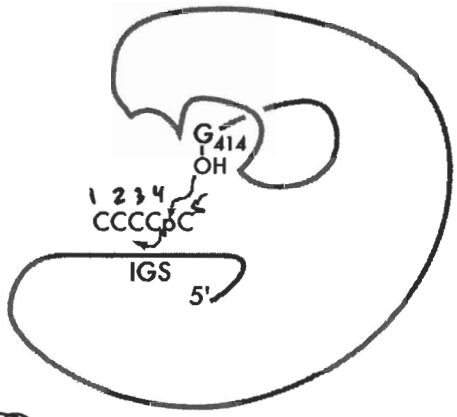
- Can be used to knock down essential genes that would be lethal otherwise.
- Much easier + faster than doing genetics
- More easily regulated
- Can identify synthetic lethal interactions using multiple ~~RNAi~~ siRNA's

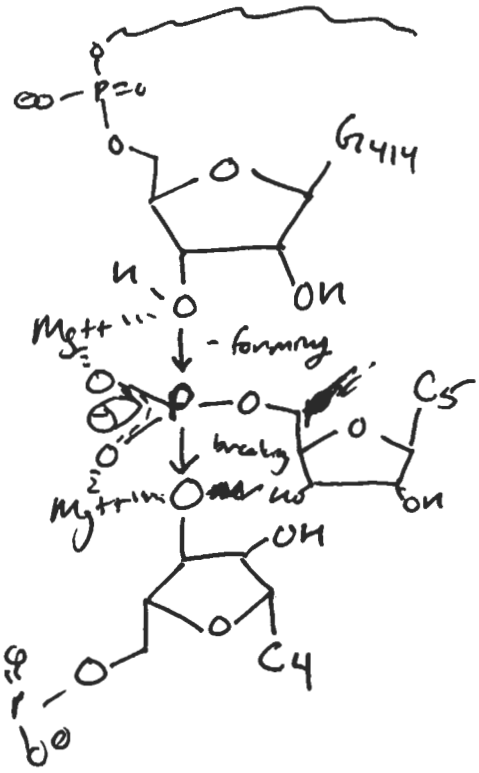
Score /19

(b; 5 pts) Long stretches of ribosome-free RNA trigger ρ-dependent termination in prokaryotes and mRNA decay in eukaryotes. Why (not how; it's a short answer)?

+3 (It's a sign of premature ~~transcript~~ termination of
+2 (translation. If the mRNA is defective it is destroyed rather than ~~allo~~ templating additional truncated protein.

(c; 10 pts) The diagram below shows one step of an enzymatic reaction carried out by the *Tetrahymena* ribozyme. Assuming a two-metal ion mechanism, sketch the chemistry at the active site.





(+3) for general idea
(+3) for arrangement of ligands at phosphorus
(+2) for metal ions chelated to O3's and phosphate oxygens C_1 C_2 C_3—O
(+2) for in-line attack + clarity

(d; 4 pts) Why is RNA an attractive candidate for the macromolecular ancestor of life as we know it?

(+4) – embodies both information and function and prototype reactions for a replicase have been ~~generated~~ observed.

Score 19