

Name Key

This exam is schedule for 75 minutes and I anticipate it to take the full time allotted. You are free to leave if you finish. The exam is split into two sections. Part 1 is multiple choice – select the most correct answer in each question. Part 2 is composed of several short answer questions.

Part 1. Clearly circle the most appropriate answer. (3 pts each).

1. What is the strongest driving force for DNA/RNA structure?
electrostatic interactions pi-stacking hydrogen bonding supercoiling
2. Which is **not** true about histone proteins?
A. Histones are very highly conserved in eukaryotes
B. Histone proteins form an octameric complex
C. Histone proteins wrap genomic DNA to relieve topological stress.
D. Histone proteins contain a high Glu/Asp content.
3. Adding a Writhe to supercoiled DNA _____ the overall topological stress (Linking Number)
Increases Decreases No effect
4. Which exonuclease direction is necessary for proofreading in DNA replication?
5' → 3' 3' → 5' either neither both
5. The *E. coli* genome contains multiple origins of replication.
True False
6. *E. coli* has multiple replication termination elements
True False
7. The mechanism of DNA ligase proceeds through a(n) _____ intermediate.
aminoacyl-AMP phosphodiester phosphoamide Schiff base
8. Which of these transcription factors is most likely responsible for responding to low glucose levels in *E. coli*?
AraC MerR CAP σ^{70}
9. The apo ribosome contains how many chains of RNA?
1 2 3 4 5
10. Which *E. coli* enzyme has 5'→3' exonuclease activity?
DNA Ligase Pol I Pol III aaRS RNA Polymerase

Part 2 – Short (and not-so-short) answer.

11. What are the three regions of DNA that promote RNA Polymerase binding? (2 pts)

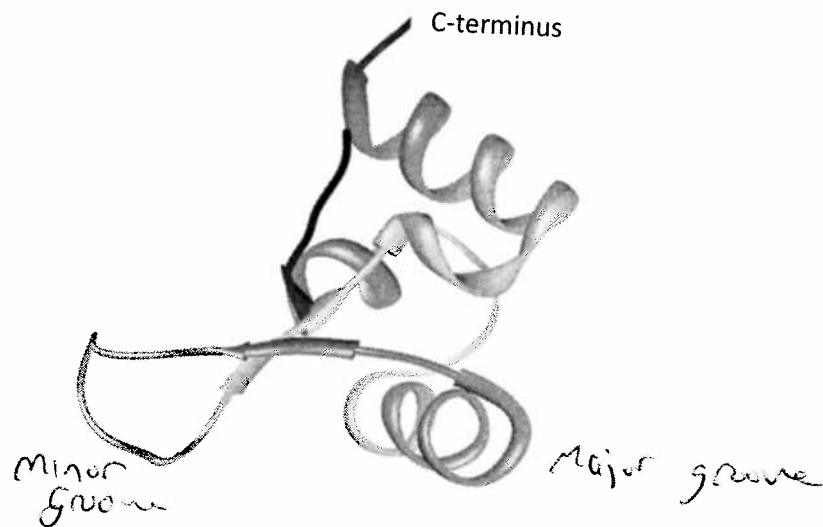
-10, -35, and the upstream elements (-40 → -60)

What subunit of RNA Polymerase contacts each of these regions? (3 pts)

α → UP elements

σ → -10 + -35

12. The Winged Helix-Turn-Helix motif is a common fold in DNA binding proteins. In the sketch below, identify which region of the protein will interact with the major groove and minor groove of DNA. (3 pts)



13. Why are palindromic DNA sequences important for transcriptional regulatory proteins? (2 pts)

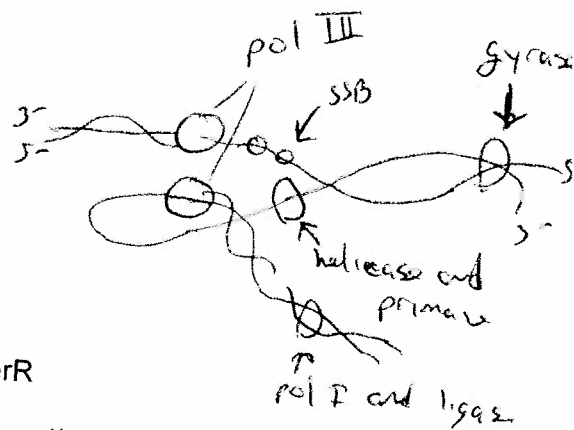
Since regulatory proteins are symmetrical dimers, they look for symmetrical sequences of DNA. This requires the DNA to be palindromic.

14. What is abortive initiation and why does it occur? (5 pts)

The release of small chunks of RNA from RNA Polymerase. They are formed because RNAP is not always able to transition to a processive conformation.

15. DNA replication in *E. coli* is a multi-enzyme task. List all enzymes that participate in this process and briefly describe the role they play. Feel free to include a sketch. (10 pts)

- Pol III → This is the replisome. Builds new DNA in the 5' → 3' direction on both the leading and lagging strand
- (B) Pol I → removes RNA primers from lagging strand
- (C) helicase → unwinds dsDNA @ replication fork
- (D) primase → makes RNA primers on lagging strand
- (E) Gyrase → topoisomerase that travels ahead of the replication fork to relieve superhelical stress
- (F) Ligase → seals nicks in lagging strand
- (G) SSB → binds to single stranded DNA to prevent damage and reannealing



16. Choose one of the following transcriptional regulators.

CAP

AraC

MerR

Describe how it influences the level of transcription for that operon (i.e. how does it work). Make sure to include any relevant information. (10 pts)

AraC → see notes

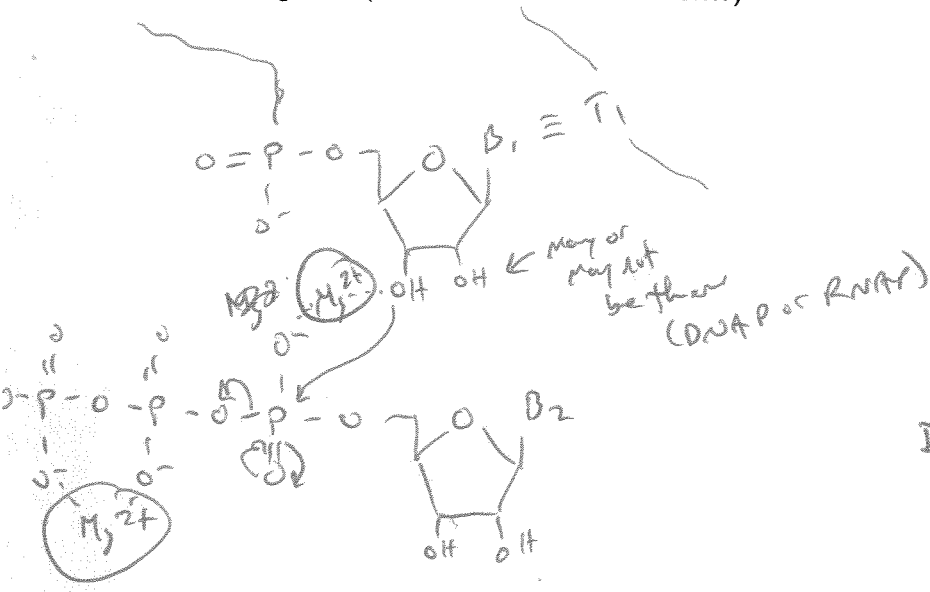
CAP

in the presence of cAMP, CAP binds to a region upstream of the -10 + -35 elements, with UP element core. It makes contact with the α subunit of RNAP and helps the α subunit bind to these regions. This increases the likelihood that RNAP binds to the promoter and activates transcription.

MerR

The MerR promoter's -10 + -35 elements are spaced such that they are on opposite sides of the dsDNA. This is not ok for σ binding. MerR binds to the DNA & in the presence of its substrate, bends and twists the DNA to put the -10 + -35 elements in appropriate proximity for σ factor binding.

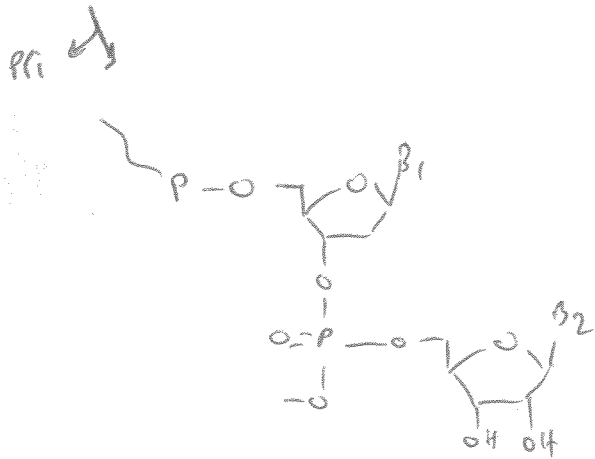
17. Sketch a mechanism describing a **single step** in the chain elongation process of DNA Polymerase or RNA Polymerase. Make sure to **include any necessary cofactors and indicate exactly why they are necessary**. Feel free to abbreviate non-essential parts of the nucleotides. (5 pts) For 2 bonus points, describe how your enzyme manages processive elongation (how does the ratchet work?)



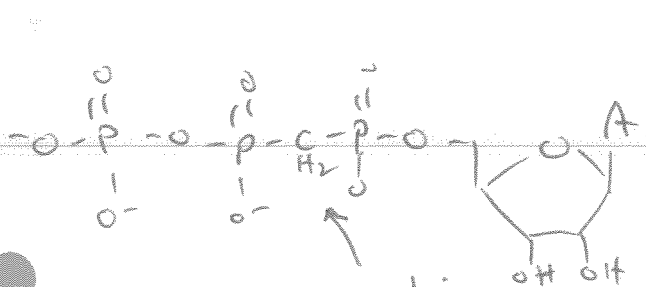
Mg^{2+} ions \rightarrow one stabilizes the pyrophosphate and the other activates $3' OH$ for attack

DNA Polymerase \rightarrow thumb domain is the ratchet that moves the template to promote processivity.

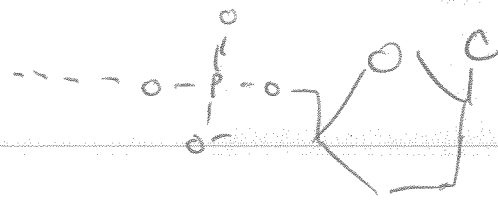
RNA Polymerase \rightarrow the bridge helix is the ratchet that moves the helix. It works together with the trigger helix to open/close the substrate channel.



18. When investigating the structures of DNA Polymerase and RNA Polymerase, we saw two different inhibitors that were used to trap the enzyme in its active conformation. **Draw one of these molecules and discuss why it effectively inhibits polymerization**. (5 pts)



AMPCPP
Cation in triphosphate is NOT hydrolyzable, so chain elongation is not possible



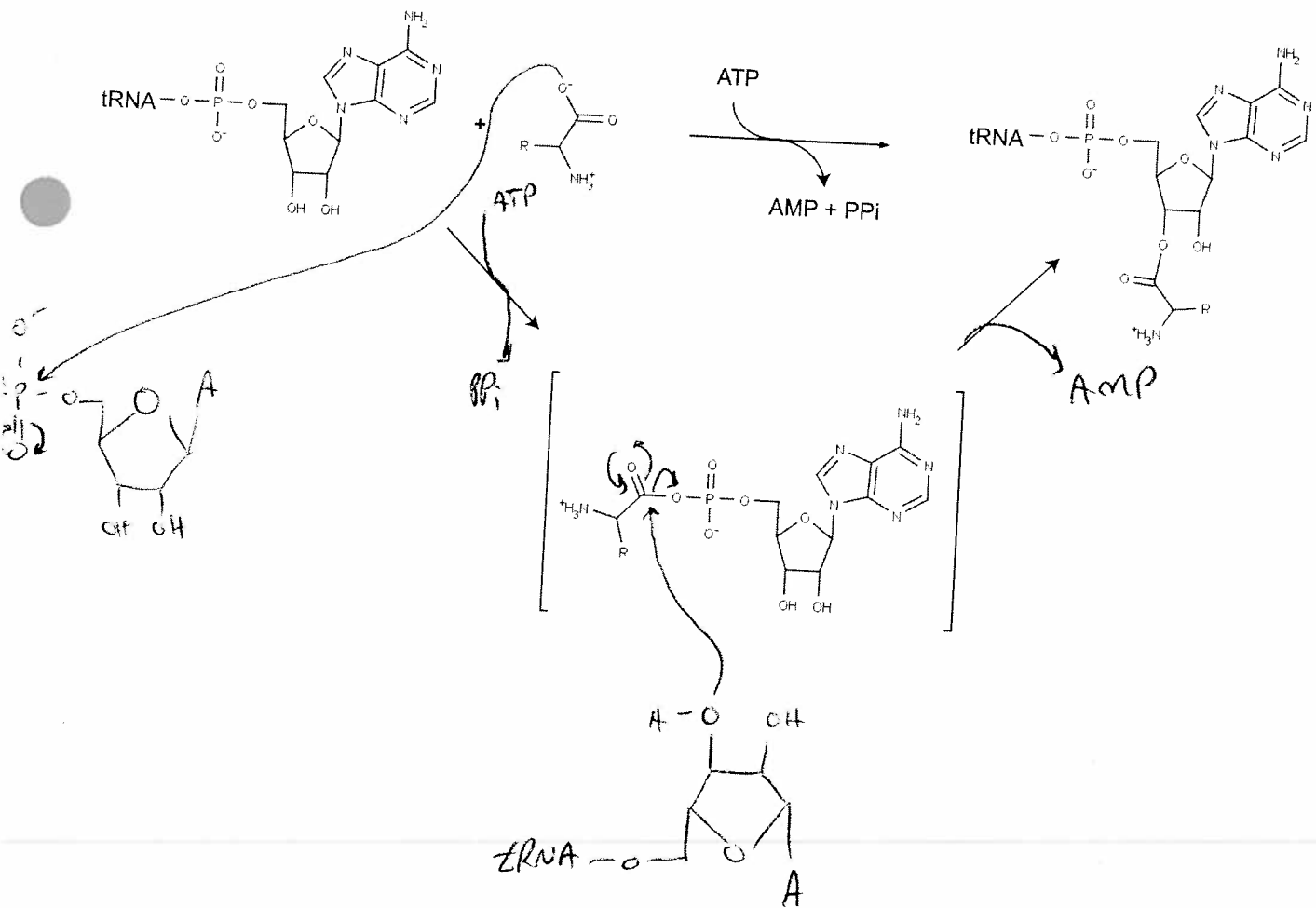
2',3'-dideoxy-CTP

No $3' OH$, so no possibility for nucleophilic attack

19. The 70S ribosome can bind 3 tRNA molecules at the A, P and E sites. What do these letters stand for and why is that name appropriate? (3 pts)

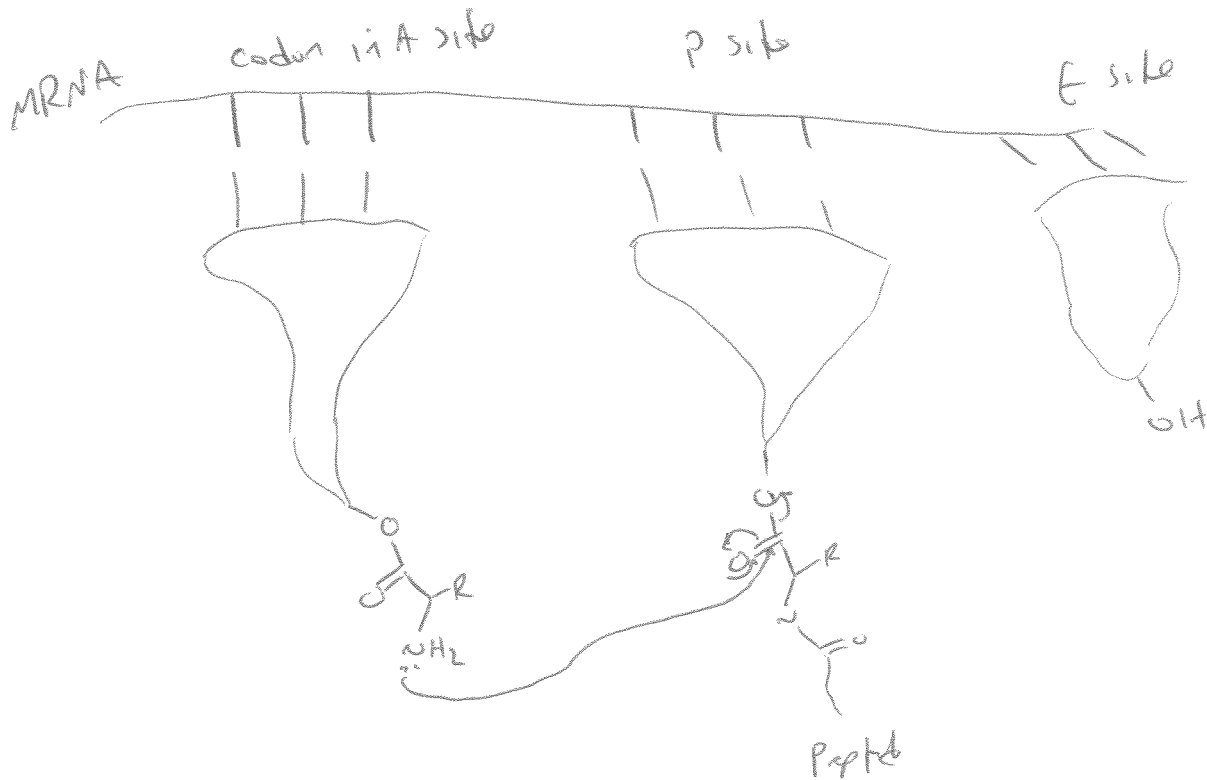
A → aminoacyl → this is where the new charged tRNA comes in
 P → peptidyl → the site where the growing peptide chain is attached to a tRNA
 E → Exit → where the uncharged tRNA is poised for exit

20. The image below shows the net reaction catalyzed by an aminoacyl tRNA Synthase (aaRS) as well as the aminoacyl-AMP intermediate. Please indicate electron flow showing the formation of the intermediate and the conversion to the final product. (10 pts)



21. Describe the process of chain elongation by the ribosome. In your description, please include a simple mechanism that shows how a peptide bond is formed. (12 pts)

For bonus points (5 possible), include the role of each elongation factor (EF-Tu, EF-Ts and EF-G)



The codons in the 3 sites of the ribosome have 3 appropriate tRNAs bound. The aminoacyl tRNA in the A site attacks the aminoacyl tRNA in the P-site. - this is how the peptide chain grows. EF-G then promotes the translocation of the tRNAs to the next site (A \rightarrow P, P \rightarrow E, E \rightarrow solvent).

The new tRNA comes into the A-site with the ~~aid~~^{aid} of EF-Tu. EF-Ts is important for making the EF-Tu / tRNA interaction.