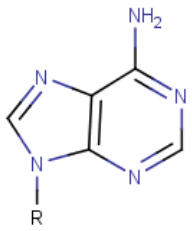
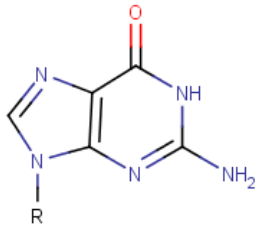
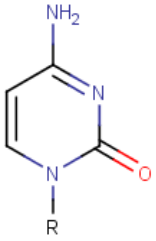
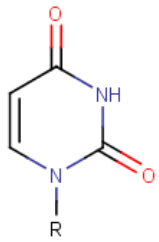
Name Key

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 ONE correct answer in each question. Part 2 is composed of several fill in the blank questions with answers that should be selected from the provided answer pool. Part 3 is composed of several more involved questions. Hints are always available for









Part 1. Clearly circle ONE answer per question.

1. Which of the following DNA sequences the anticodon for a 5'-GAC-3' codon?

5'-GAC-3' 5'-GTC-3' 5'-CAG-3'

2. Which of these are NOT important for RNA Polymerase interacting with DNA?

Shine-Delgarno Region Pribnow Box -10 Region -35 Region UP Elements

3. Self-complementary DNA sequences are not important for which of these transcription related processes?

Initiation Termination transcription factors rRNA post-transcriptional processing

4. Which of these proteins is not important for replication on the *leading* strand? Select all that apply.

SSB Helicase Pol I Pol III Primase

5. Which of these enzymes can use NADH as an energy source?

Pol I Pol III RNA Polymerase DNA Ligase SSB

6. SSB proteins make sequence specific interactions with ssDNA.

True False

7. Which of the following is not a DNA repair strategy?

BEER MMR NER SOS Response BER

8. Which arm of tRNA molecules contain the 5' end?

Variable Acceptor D TΨC Anticodon

9. Which arm(s) of tRNA molecules are the most involved in stabilizing the non-traditional RNA structure? Select all that apply.

Variable Acceptor D TΨC Anticodon

10. Which helix form is found in the active site of DNA and RNA Polymerases?

A-form B-form Z-form

11. Which step in translation is not energy dependent?

Decoding Translocation Transpeptidation

Part 2 – Matching Complete the following statements from the list of terms below – you may make any of these plural if necessary. You MAY deviate from the word-bank if you think there is another word that accurately fits into the blank. Each word is used only once.

Riboswitch, replication fork, aminoacyl, omega, thumb, DNA, RNA, A-form, B-form, Pol I, Pol III, MerR, processive, abortive, exonuclease, sigma, endonuclease, 3'→5', 5'→3', -10, -35, UP elements, nucleophile, electrophile, hand, thumb, fingers, palm, lariat, BER, NER, template, bridge, trigger, leading, lagging, terminal, phosphate, α, β, γ, right, left, carboxy, amino, transcription bubble, backbones, bidirectional, Shine-Delgarno, Pribnow, scrunching, AP sites, exons, introns, ribosome, okazaki

12. The "thumb" domain is responsible for processivity in DNA Polymerase.

13. The trigger helix is responsible for facilitating nucleotide sampling in RNA Polymerase.

14. DNA and RNA Polymerases always read the template strand in the 3'→5' direction.

15. aaRS enzymes catalyze the production of aminoacyl-tRNA molecules. The bond that is formed links the 3'-OH of the tRNA and the carboxy terminus of an amino acid.

16. BER is a repair strategy that corrects an error by removing only one nucleotide. In the process, a cytotoxic AP site is produced.

17. Okazaki fragments are produced on the lagging strand during replication.

18. Riboswitches are present at the 5' end of mRNA. The structure of these RNA molecules changes in response to a signaling molecule (e.g. TTP) and influence the ability of the mRNA to interact with the Shine-Delgarno sequence.

19. The Pribnow Box is a formal name for the -10 element of the promoter region.

20. Ejection of the Sigma factor from RNA Polymerase is necessary to avoid abortive initiation.

21. The spliceosome catalyzes the connecting of exons from eukaryotic mRNA. In the process, the lariat form of introns are produced.

22. The 5'→3' exonuclease activity of Pol I is critical for removal of RNA primers.

Part 3 – Not so short answer.

23. Why do most *bacterial* RNA molecules have a 5' triphosphate?

Because the 1st nucleotide added by RNA polymerase retains the 5' triphosphate.

What is one example of a bacterial RNA polymer that does **not** contain a 5' triphosphate? Why not?

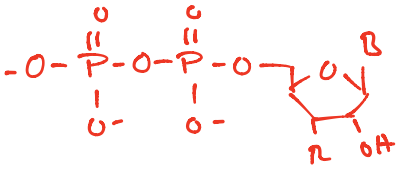
rRNA
tRNA. They are produced by post-transcriptional processing of long RNAs that contain ribosomal RNA + tRNA

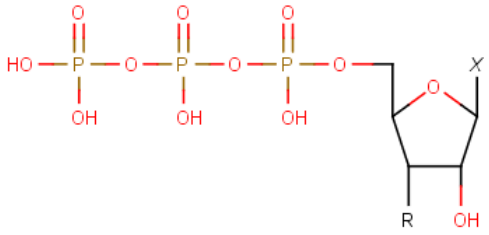
Mature eukaryotic mRNA contains a cap in place of the 5' triphosphate. What role does the 5' cap play in cellular processes?

recognition by initiation factors for translation

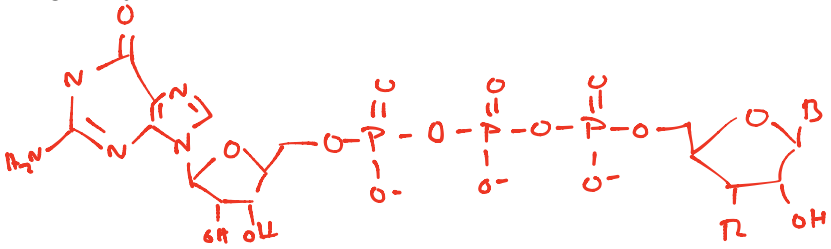
Draw the products for each of the 3 enzyme catalyzed steps involved in processing the 5' end of mRNA in eukaryotes. The image below gives you a starting point. Feel free to use the paper at the end of the exam if you need more space.

Triphosphatase

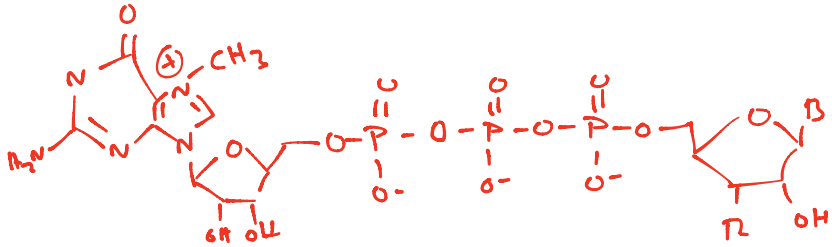




Capping Enzyme



Methyltransferase.



24. Describe the process of initiation for each of these process in ***prokaryotes.*** Include as much information as you feel is necessary.

Replication

DnaA recognizes DnaA boxes at the origin of replication (Ori)
- the DNA coils around the proteins (similar to how DNA coils around Histones)
- this causes the adjacent DNA to melt apart.
- the newly formed ssDNA is prevented from recoiling by SSB.
- DnaB (Helicase) binds to what will become the lagging strand
- the rest of the replication machinery is now recruited

Transcription

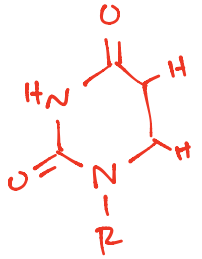
The holoenzyme RNA Polymerase (containing the sigma factor) binds to the promoter region of DNA. The interactions are via the -10 & -35 element and the sigma factor. The UP-elements can also interact with RNA Polymerase to enhance the interaction. Once the DNA / RNA Polymerase complex has formed, the transcription bubble forms by separating the templating strand from the non-templating. The first several nucleotides come in and begin polymerization. The non-templating strand is still locked in place, so strain is built up. This strain is relieved by ejecting the RNA chain (aborting initiation) or the sigma factor → processive elongation

Translation

IF3 is bound to the 30S ribosome, preventing it from interacting with the 50S.
IF-2 binds $tRNA_f^{met}$ and mRNA. This complex, in addition to IF1, then binds to the small subunit. The tRNA is partnered with the start codon on the mRNA and the Shine-Delgarno sequence base pairs with the 30S subunit to ensure the proper orientation/placement. GTP is hydrolyzed by IF-2 causing all IF proteins to dissociate from the small subunit. The large subunit comes in and forms the holoribosome.

25. Dihydrouridine (D) is a common base modification in tRNAs.

a. Draw the structure of this molecule (abbreviate the Ribose as R).



b. How does this modification modify the chemical properties of the molecule?

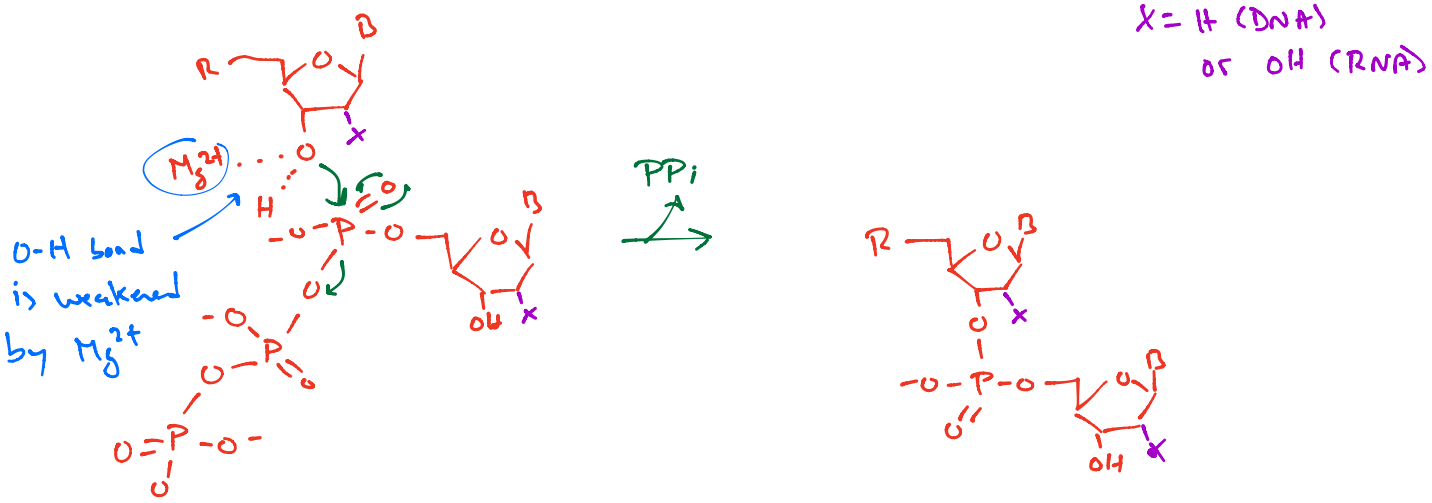
The base is no longer planar or fully aromatic

c. Based on your answer in part b, propose a reason (or consequence) for this base modification.

It will not be able to insert into the core of a helix because its not planar and has little π-stacking potential

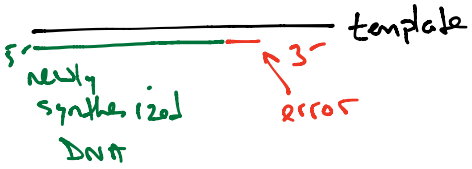
26. Please provide a chemical rational for why DNA and RNA polymerization is polarized (*i.e.* proceeds in a specific direction). A complete answer must include a simple mechanism. Make sure to include any relevant cofactors.

you need the 3' OH to be a nucleophile



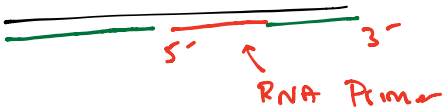
27. Please discuss the role of 3'→5' **and** 5'→3' exonuclease activities in the replication process. You are encouraged to use an image to clarify your answer.

3'→5' Proofreading



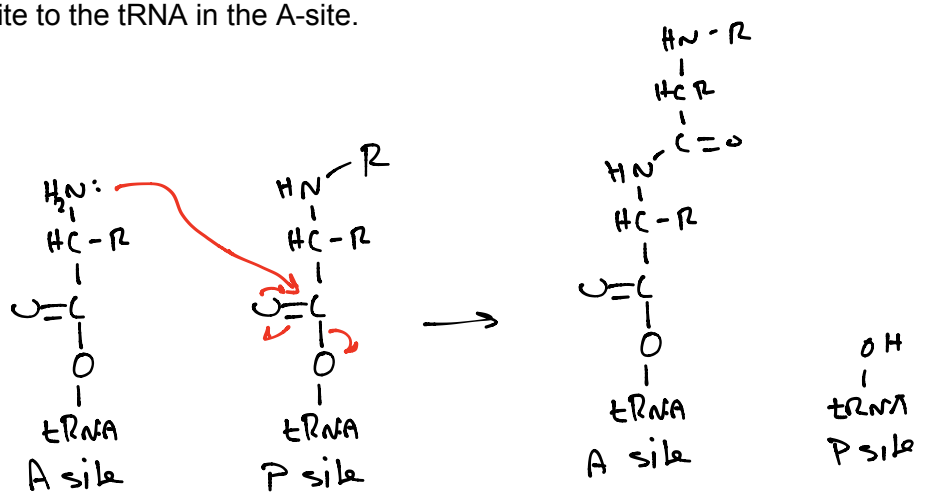
need to remove the mistake at the 3' end
direction of exonuclease
3'→5'

5'→3' Removing RNA Primers



To remove the primer, the exonuclease needs to start at the 5' end
5'→3'

28. Draw a reaction mechanism that shows how the growing peptide chain is transferred from the tRNA in the P-site to the tRNA in the A-site.



29. Describe one cellular strategy for:

Transcriptional Regulation: Lots of options.

one example: CAP

cAMP is produced in response to low glucose levels. CAP binds to cAMP and, in this form, will interact with the UP Elements and the α subunit of RNA Polymerase. This increases the affinity of RNAP for the promoter and results in increased transcription levels

Post-transcriptional Regulation:

again, lots of options. All deal with preventing mRNA from interacting with the ribosome. One example is riboswitches. These are part of the 5' end of mRNA. They have structures that respond to binding of some molecule. In one form, the Shine-Delgarno sequence is "hidden", so the mRNA can't bind to the ribosome