Midterm 2 Practice Questions

Part 1 - Who am I? In the blank provided, identify what each description is referring to.

____RNA polymerase II_____ is the enzyme which carries out transcription of protein coding genes in eukaryotes.

_____snRNA_____ small RNA molecules that are complexed with proteins to form the ribonucleoprotein particles involved in RNA splicing.

____Rho_____ is the enzyme which binds the rut sequence in prokaryotes to terminate transcription in a structure independent manner.

Sequence elements found after the transcription start site are _down___-stream of the promoter.

The DNA strand which codes for a gene is also known as the ____non-template____ strand during RNA transcription.

___aminoacyl-tRNA synthetase_____ is an enzyme which attaches the correct amino acid to a tRNA molecule to form the activated intermediates used in protein synthesis.

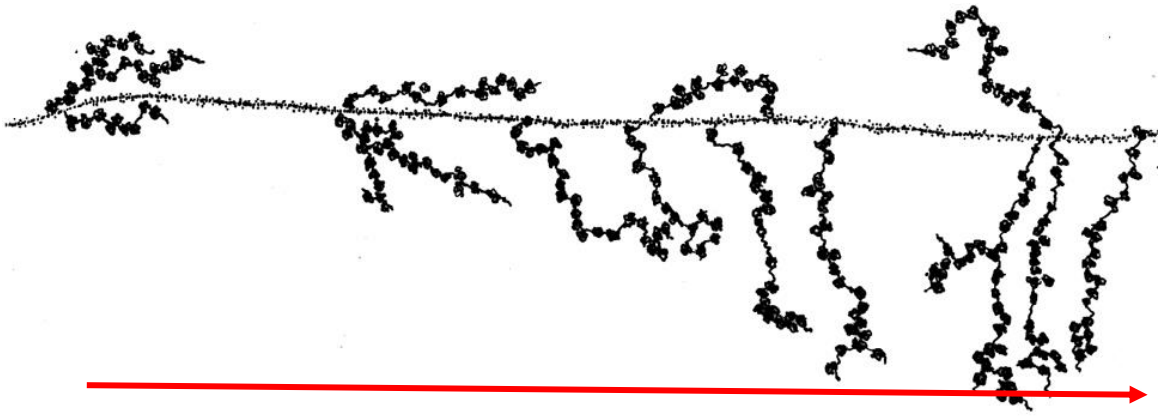
Part 2 - Short Answer Questions

1. Which statement about RNA is false?

A. RNA tends to be single-stranded, base pairing with different parts of its own strand, forming complex three-dimensional structure
B. In comparison with DNA, RNA has one hydroxyl less in the sugar moiety
C. RNA is a polymer built from nucleotides having the bases A,C, G and U
D. All of the above statements are true.

2. Match the following terms to the appropriate part of the central dogma by placing an "X" in the corresponding box. There is may be more than one answer for each term.

Term	Replication	Transcription	Translation
Mutations Occur	X		
Uses RNA Polymerase		X	
Uses DNA Polymerase	X		
Involves proofreading of the polymer produced	X		
Involves RNA Primers	X		
Produces RNA		X	
Produces DNA	X		
Produces Protein			X
Uses tRNA			X
Involves both strands of DNA	X		
Involves only one strand of DNA		X	
Doesn't use DNA			X
Uses amino acids			X
Involves mRNA		X	X
Involves Ribosomes			X



3. a) In the image above, a region of DNA containing a gene is undergoing transcription. Using an arrow, indicate which direction transcription is proceeding. (L to R)

b) Where would you expect to find RNA polymerase on this DNA sequence? At the base of each RNA molecule

4. A scientist observes that a cell has an RNA polymerase deficiency that prevents it from making proteins. Describe three additional observations that would together support the conclusion that a defect in RNA polymerase I activity, and not problems with the other polymerases, causes the defect.

To determine that an RNA polymerase I mutation or deficiency is causing the defect in protein production, the scientist would need to make observations that provide evidence that RNA polymerases II and III are working in the cell.

The observations eliminating RNA polymerase II as the defect could include:
- Transcription of mRNAs in the nucleus
- Presence of processed mRNAs in the cytoplasm

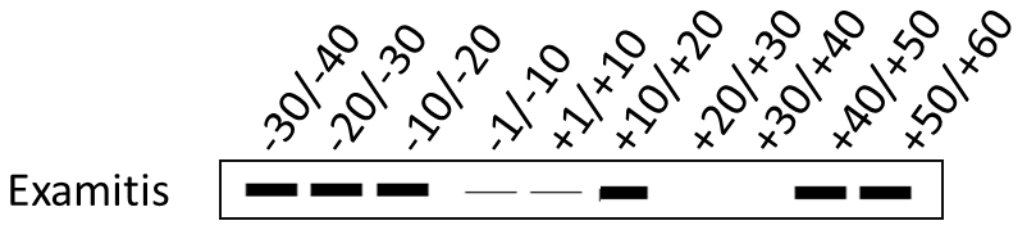
The observations eliminating RNA polymerase III could include:
- Isolation of small nuclear RNAs from the cell
- Isolation of microRNAs from the cell
- Transcription of 5S rRNA in the nucleus
- Presence of tRNAs in the cytoplasm

The observations implicating RNA polymerase I could include:
- A lack of functional ribosomes in the cytoplasm (RNA polymerase I or III)
- A lack of RNA polymerase I protein
- RNA polymerase I protein is non-functional

5.a) Briefly describe what is meant by the phrase 'a strong promoter'.

A strong promoter is one which can initiate the production of more transcripts per unit of time relative to a weaker promoter. Its sequence is likely closely matched to the consensus sequence for that organism/class of organisms.

b) As part of your honours project, you are investigating the promoter region of the examitis gene, a gene that causes profs to schedule too many midterms within the same week. You have developed constructs in which 10 bp segments within the promoter of interest have been replaced by a linker and expressed each of these constructs in a model system. The results depicting their level of expression are shown below.



i) Identify the promoter regions which play a role in regulating the expression of this gene and discuss their relative importance to one another.

+20 to +40 - required for transcription, expression is prevented when replaced by the linker

-10 to +10 – expression is reduced but not fully prevented when replaced by a linker – therefore less critical than +20 to +40 region but still important

ii) Name the core promoter element(s) you would predict are located within each of these regions and the general transcription factors that would bind to them during the initiation of transcription.

+20 to +40 region = downstream promoter element (DPE) – recognized by TFIID

-10 to +10 region = initiator motif (INR) – recognized by TFIID

6. Consider a pre-mRNA that consists of four exons and three introns arranged as follows.

5' – exon 1 – intron 1 – exon 2 - intron 2 – exon 3 - intron 3 –exon 4 - 3'

The sequence at the splice sites within each intron are as follows (5' followed by 3' splice site):

Intron 1 = GU and AG
Intron 2 = AU and AG
Intron 3 = AU and AC

a) Which of the introns is almost certainly going to be recognized by the spliceosome and why?

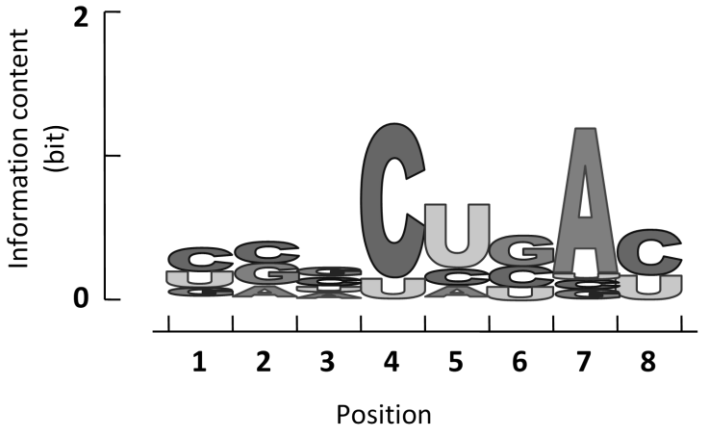
Intron 1 – because the sequences at its 5' and 3' boundaries are identical to the known consensus sequences at intron boundries for the major form of splicing

b) Considering only the mature mRNA(s) that do not retain introns, which of the following alternatively spliced mRNAs could arise if the pre-mRNA via the major form of spliceosome-mediated splicing?

i. **Exons 1, 2, 4 joined or exons 1, 3, 4 joined together**
ii. Exons 1, 2, 4 joined together
iii. Exons 1, 3, 4, joined together
iv. Exons 1, 2, 3 joined together or exons 1, 2, 3, 4 joined together

7. Your friend is studying the sequence conservation around the branch-point site on a subset of primary RNA transcripts in *Macaca mulatto* (rhesus macaque). She has sent you the following sequence logo of the region of interest but has forgotten to indicate the position of the branch-point nucleotide itself. Where would you predict that it is?

A. Position 5
B. Position 6
C. Position 7
D. Position 8
E. None of the above



8. Match the general transcription factors (TFIIB, TFIID, TFIIE, TFIIF, TFIIH) with the appropriate description. Note some TFs may be used more than once and others not at all.

__TFIIB___ Accurately positions RNA polymerase at the transcription start site via BRE elements

__TFIIH___ Unwinds DNA via its helicase activity

__TFIID___ Recognizes the TATA box

___TFIIF___ Associated with the RNA polymerase prior to its binding with the DNA

__TFIID___ Induces a bend in the promoter region of the associated DNA

9. Describe the process of translation initiation in eukaryotes. Be specific, make sure to name each of the key players and any energy sources used in the process.

- Met-tRNAi is loaded onto the small ribosomal subunit along with GTP bound eIF2
- The small ribosomal subunit then interacts with the 5' end of an mRNA via its cap and the initiation factors (eIF4E and eIF4G) associated with it
- The small subunit then scans for an AUG start codon
- Once a start codon is found:
  - GTP is hydrolyzed
  - the initiation factors dissociate
  - the large ribosomal subunit associates and elongation can proceed

10. In the table below identify the key differences (if there are any) between prokaryotic and eukaryotic organisms in each phase of translation.

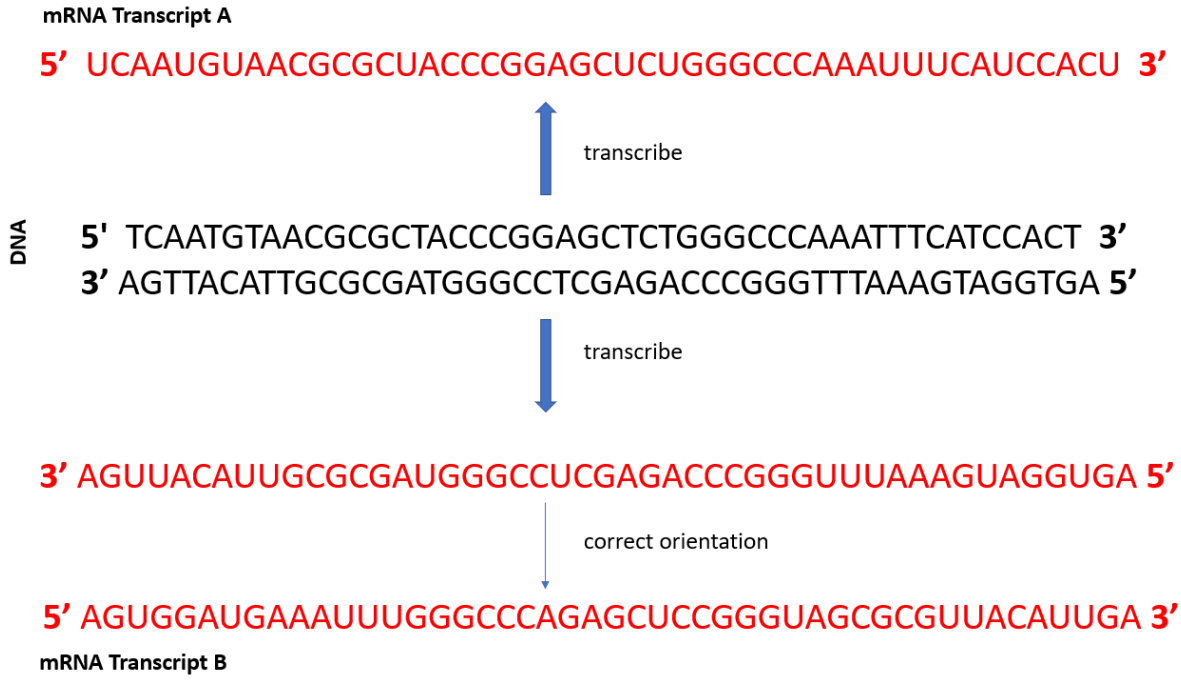
	Prokaryote vs Eukaryote
Initiation	Prokaryote – small ribosomal subunit recognizes the Shine-Delgarno sequence, positioning the ribosome over the corresponding AUG - different initiation factors (IF 1, 2, 3) - first AA incorporated is formylmethionine Eukaryote – small ribosomal subunit associates at the 5' end of the mRNA (recognizing the 5'cap/eIF4e/4g) and scans for an AUG, most likely selecting the 1st AUG, particularly if the preceding Kozak sequence matches the consensus seq
Elongation	Different elongation factors – EF-Tu and EF-G (in prokaryotes) and EF1/EF2 (in eukaryotes) – (however their overall function and the process of elongation is the same)
Termination	We did not discuss any differences

11. You have just sequenced the short segment of DNA below from a newly discovered strain of bacteria. Using the following prompts, analyze this DNA sequence to determine whether it could encode a protein.

5' TCAATGTAACGCGCTACCCGGAGCTCTGGGCCCAAATTTCATCCACT 3'

a) Transcribe this sequence into mRNA, indicating the 5' and 3' ends.

First write the complementary strand of DNA. Since you are given no indication as to which strand might serve as the coding strand, transcribe both.



b) Identify all of the possible reading frames in this sequence.
c) Identify the longest open reading frame (ORF) and write out the sequence of the protein it would produce (be sure to indicate the N and C termini).
d) Label the original DNA strands, indicated which would be the template and the non-template strand if the longest ORF were transcribed.

mRNA Transcript A

5’ UCAAUGUAACGCGCUACCCGGAGCUCUGGGCCCAAAUUUCAUCCACU 3’

5’ UCA AUG UAA CGC GCU ACC CGG AGC UCU GGG CCC AAA UUU CAU CCA CU 3’

M *

N terminus C terminus

5’ U CAA UGU AAC GCG CUA CCC GGA GCU CUG GGC CCA AAU UUC AUC CAC U 3’

No start codon – no ORF

5’ UC AAU GUA ACG CGC UAC CCG GAG CUC UGG GCC CAA AUU UCA UCC ACU 3’

No start codon – no ORF

mRNA Transcript B

5’ AGUGGAUGAAAUUUGGGCCCAGAGCUCCGGGUAGCGCGUUACAUUGA **3’**

5’ AGU GGA UGA AAU UUG GGC CCA GAG CUC CGG GUA GCG CGU UAC AUU GA **3’**

No start codon – no ORF

5’ A GUG GAU GAA AUU UGG GCC CAG AGC UCC GGG UAG CGC GUU ACA UUG A **3’**

No start codon – no ORF

5’ AG UGG AUG AAA UUU GGG CCC AGA GCU CCG GGU AGC GCG UUA CAU UGA **3’**

M K F G P R A P G S A L H *

N terminus

Longest ORF

C terminus

12. Shown below is a double-stranded bacterial (*E. coli*) DNA sequence coding for a hypothetical protein. Both strands are shown; the top strand reads 5’ to 3’ left to right, while the bottom strand reads 5’ to 3’ right to left. The nucleotides are numbered 1 to 100. NOTE: For this problem, transcription begins with and includes the red and underlined C/G (top strand/bottom strand) base pair and RNA polymerase proceeds from left to right along the DNA.

```
    1                   20                  40
5'-GTGTCCGTCTAATATTGTGAGATGTTATATCCCGCCGTCAACACCATCAA-3'
   ---------+---------+---------+---------+---------+
3'-CACAGGCAGATTATAACACTCTACAATATAGGGCGGCAGTTGTGGTAGTT-5'

              60                  80                  100
5'-ACAGGATAATCGCCTGCTGGGGCAAAGGCGGTGAAGGTAAAGGTGTTGCC-3'
   ---------+---------+---------+---------+---------+
3'-TGTCCTATTAGCGGACGACCCCGTTTCCGCCACTTCCATTTCCACAACGG-5'
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a) Which strand is used as a template for transcription, the top or the bottom? Bottom.

b) Where would the promoter be relative to the start of transcription? The promoter would be to the left/upstream of the transcription start site

c) What are the first 15 nucleotides of the resulting mRNA? Indicate the 5' and 3' ends of the mRNA. 5’ CUAAUAUUGUGAGAU 3’

d) What are the first 5 amino acids translated from the resulting mRNA? Indicate the amino (N) and carboxy (C) termini of the protein. N Met-Leu-Tyr-Pro-Ala C

e) Do the underlined nucleotides TAA (indicated in blue) encode a stop codon for the protein? Briefly explain your answer. No. The underlined TAA is not read as TAA because of the reading frame. The sequence GATAAT forms the codons: GAU AAU.

Consider the situations in parts (f-h) independently.
f) A mutation occurs which results in the insertion of an extra G/C (top strand/bottom strand) base- pair immediately after base pair 11 (shown in bold). What effect will this insertion mutation have on the mRNA transcript and resulting protein? The mRNA will be longer by one nucleotide, but because the insertion is prior to the start codon, the protein is unchanged.
g) A different mutation results in the substitution of the T/A base pair at position 30 (shown in bold and underlined) with a G/C base pair. How would this mutation affect the sequence of the protein that is produced? The codon UAU encoded Tyr, but now it is UAG, a stop codon. The protein is truncated.

h) A third mutation occurs which results in the substitution of the C/G base pair at position 42 (shown in bold italics) to a T/A base pair. How would this mutation affect the sequence of the protein that is produced?

The codon AAC which encoded Asn, now is AAU, which also encodes Asn. The protein is unchanged.