

**Molecular Biology (BIOL 4320)**  
**Exam #2**  
**May 3, 2004**

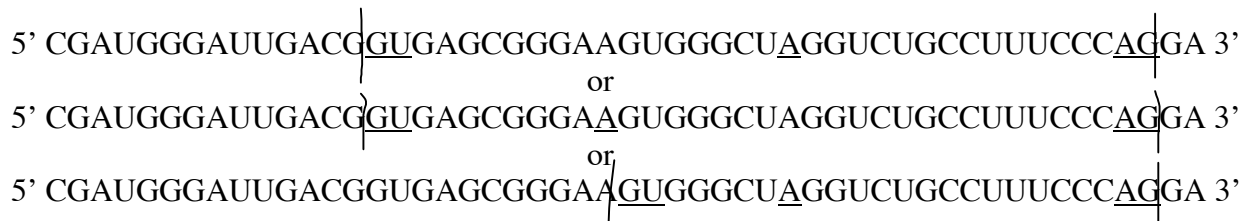
Name \_\_\_\_\_ SS# \_\_\_\_\_

This exam is worth a total of 100 points. The number of points each question is worth is shown in parentheses after the question number. Good luck!

1. (4) Histone acetylases acetylate lysine (amino acid) residues on the N-terminal tails of histones H3 and H4, resulting in transcriptional activation.

2. (2) Highly condensed DNA that is inaccessible to transcription factors is called heterochromatin.

3. (3) Identify the 5' splice site, the 3' splice site, and the branch point in the sequence below.



4. (3) During the first step in mRNA splicing, the hydroxyl group in the 2' \_\_\_\_\_ position of the branch point nucleotide attacks the phosphate bond 5' of the guanine (nucleotide) located at the 5' end of the intron, thereby forming a lariat structure.

5. (4) Describe a mutagenesis experiment that shows U1 RNA binds to the 5' splice site.
- 1) Mutagenize the 5' splice site of an intron so that it will not base pair with U1 RNA
  - 2) Test for splicing of this intron using Rnase protection after transfection into normal cells, and show that the intron does not get spliced.
  - 3) Make compensatory mutations in U1 so that it can base pair with the mutant intron 1 splice site.
  - 4) Test for splicing of this intron by Rnase protection upon transfection of a plasmid containing the mutant 5' splice site and the mutant U1 RNA, and show that the mutant intron is now spliced.
6. (5) List the order in which U RNAs are added to an intron during splicing and where they bind.
- 1) U1 binds first to the 5' splice site.
  - 2) U2 binds second to the branch point.
  - 3) U4/U6 and U5 come in to the complex as a group, with U6 binding to the 5' splice site, U4 not binding the mRNA and leaving the complex once U6 binds, and U5 binding the last base of exon 1 and the first base of exon 2.
7. (3) The yeast two-hybrid technique was used to identify proteins involved in what aspect of mRNA splicing?
- Commitment to splicing.
8. (3) During sex determination in *Drosophila*, briefly describe why Sex Lethal (SXL) protein is made in females, but not in males.
- In females, *sxl* mRNA is alternatively spliced in such a way that it skips an exon that contains a translation stop codon, thus resulting a functional SXL protein.
- In males, the exon containing the translation stop codon is not skipped, thus no functional SXL protein is made in males.
9. (3) During type 2 mRNA capping, GTP is added to the first nucleotide of an mRNA via a 5'-
- 5' triphosphate linkage, and methyl groups donated by
- S-adenosyl methionine are added to the 7' position of the guanine cap and the 2' position of the first two (number) nucleotides of the mRNA.

10. (4) If an mRNA loses its 5' cap, how will its properties differ from a capped mRNA?

- 1) An uncapped mRNA is less stable
- 2) An uncapped mRNA is translated less efficiently
- 3) Transport of an uncapped mRNA to the cytoplasm will be crippled
- 4) Splicing of intron 1 in an uncapped mRNA will be inefficient

11. (4) During polyadenylation, CPSF binds to a AAUAAA sequence and cooperates to promote CstF (factor) binding to the downstream GU/U rich region. Cleavage factors I and II then cleave the mRNA, and polyA polymerase adds an average of 200 adenines to the 3' end.

12. (2) An mRNA with many AUUUA sequences in its 3' UTR will be unstable.

13. (4) Indicate which of the following statements are true. Answer: c and d

- a) Transcription of transferrin receptor increases when iron is in short supply.
- b) Transferrin receptor protein levels increase when iron is abundant.
- c) There are multiple IREs in the 3'UTR of the transferrin receptor mRNA.
- d) High levels of iron remove IRE binding protein from transferrin receptor IREs.
- e) Transferrin receptor mRNA is destabilized when IRE binding protein is bound to an IRE.

14. (2) Write the consensus Kozak sequence below and label the translation initiation site.

CCRCCAUGG

15. (2) Phosphorylation of eIF4E increases its binding affinity for: Answer: b

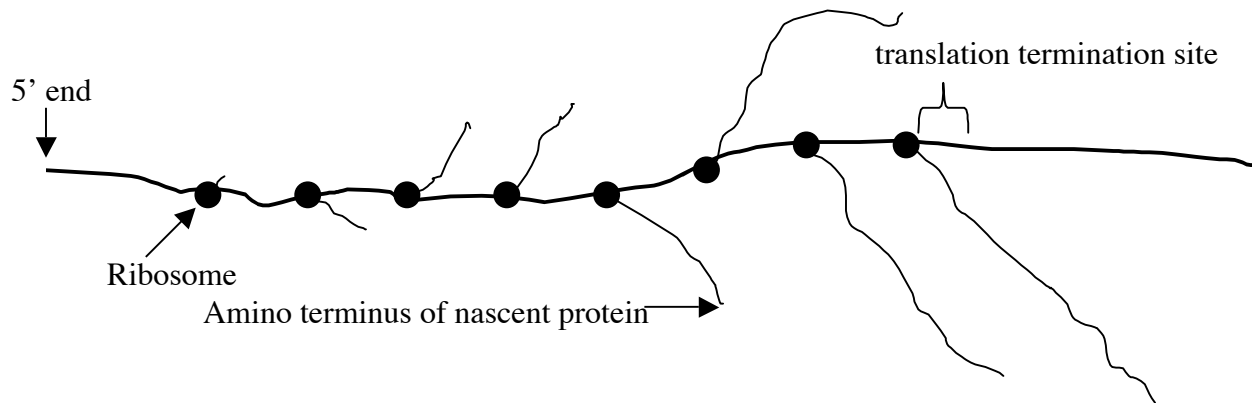
- a) GTP
- b) mRNA cap
- c) aminoacyl tRNA
- d) A site

16. (2) The function of eIF2 is to: Answer: c

- a) Promote binding of 60S subunit to the 48S complex
- b) Binds to the 5' cap of the mRNA
- c) Bind the initiating aminoacyl tRNA to the 40S subunit
- d) Unwind RNA
- e) Binds the 60S subunit to block premature 48S complex binding

17. (4) Indicate which of the following statements are true. Answer: b and d
- a) The stability of ferretin mRNA increases when iron is abundant.
  - b) Ferretin protein levels increase when iron is abundant.
  - c) There is a single IRE in the 3'UTR of ferretin mRNA.
  - d) High levels of iron remove IRE binding protein from the ferretin IRE.
  - e) Translation of ferretin mRNA increases when IRE binding protein is bound to an IRE.
18. (4) EF-Tu·GTP functions to bring an aminoacyl-tRNA to the A site during translation elongation. EF-Tu is released from the A site upon GTP hydrolysis, and EF-Tu·GTP is regenerated when EF-Ts exchanges GTP for GDP.
19. (2) A conserved adenine residue at position 2486 of the 23S rRNA catalyzes peptide bond formation.
20. (2) During translation, EF-G promotes mRNA translocation.
21. (4) Describe the roles of eRF1 and eRF3 during the process of translation termination
- 1) eRF1 binds to the ribosome A site upon recognizing any of the three stop codons
  - 2) eRF3 is a GTPase that binds to eRF1, and hydrolyzes GTP once it delivers eRF1 to the A site.
22. (6) Indicate whether the terms below describe a feature or constituent of the 50S or 30S ribosomal subunits.
- |                                |                                 |
|--------------------------------|---------------------------------|
| <u>30S</u> 16S rRNA            | <u>30S</u> Cleft                |
| <u>30S</u> Platform            | <u>50S</u> Peptidyl transferase |
| <u>50S</u> Peptide exit tunnel | <u>50S</u> 5S rRNA              |

23. (4) The mRNA below is being translated by polysomes. Please indicate the position of 1) the 5' end of the mRNA, 2) a ribosome, 3) the translation termination site, and 4) the amino terminus of a newly synthesized protein.



24. (4) Moving from 5' to 3' along a tRNA, the first structure encountered is the  D  loop, followed by the  anticodon  loop, then the  variable or TΨC  loop, and finally the  acceptor stem  at the 3' end.

25. (6) Name the two catalytic sites within an aminoacyl-tRNA synthetase and describe the substrates and the reaction that occurs in each site.

- 1) activation site – amino acids that are small enough enter the activation site and are adenylated
- 2) editing site – amino acids smaller than the correct amino acid enter the editing site and are deadenylated/hydrolyzed.

26. (3) Assuming an average insert size of 1 million base pairs, how many YAC clones would it take to make a library having a ten fold coverage of the human genome? Show your calculations.

Human genome = 3 billion bases

Ten fold coverage of the human genome would be 30 billion bases

30 billion bases divided by 1 million bases/YAC = 30,000 YACs in the library

27. (4) Name two advantages of using STSs as genetic markers compared to VNTRs or RFLPs.

- 1) STSs are evenly spaced throughout the genome, in contrast to VNTRs
- 2) Each STS has multiple alleles that are not rare in the population as opposed to RFLPs, which have only two alleles and one is typically very rare

28. (3) Describe the procedure that you would use to detect an STS.

1) Determine the DNA sequence around an STS so that unique complementary primers can be made

2) Isolate genomic DNA from many individuals and amplify the DNA from different individuals with the primers specific to this STS

3) Size separate the PCR products on a gel to identify the STS alleles from the individuals

29. (2) Name two reasons for gaps in the human genome sequence.

1) Unclonable sequences

2) Unsequenceable clones

30. (2) Expression of all genes in a genome can be measured simultaneously using

microarrays.