BIOL 4620 Exam 3 3514 Apr 8, 2014 Name [redacted]

PLEASE WRITE LEGIBLY in ball point pen.
IF THE GRADER CANNOT READ YOUR ANSWER IT WILL BE MARKED WRONG.

1-20. Multiple choice questions 1pt each. Please write the letter for the correct answer in the space provided.

1. A Kozak's Rules
   (a) define the sequence context required for a start codon to be recognized by a eukaryotic ribosome
   b. are important during ~~transcription~~
   c. are helpful for identifying the location of ~~introns~~ within eukaryotic genes
   d. determine how many A nucleotides will be added to an mRNA to form the ~~poly-A tail~~

2. A Which of the following statements about riboswitches is ***FALSE***?
   (a) Riboswitches are made from rRNAs.
   b. Riboswitches can bind metabolites. ✓
   c. Riboswitches can block the production of mRNAs.
   d. Riboswitches can control the ~~translation~~ of mRNAs ✓

3. B Dicer and RISC complexes are **NOT** involved in:
   a. Generating 20-30nt siRNA from ds RNA ✓
   (b.) ~~Transcriptional~~ gene silencing in response to dsRNA
   c. Modulation of viral translation in response to dsRNA
   d. Modulation of translation of specific mRNA via cellular miRNA ✓

4. B Detachment of binding proteins from the iron-response element in the 3' UTR of the transferrin receptor mRNA results in _____.
   a. increased rate of production of transferrin receptor mRNA
   (b.) degradation of transferrin receptor mRNA
   c. increased rate of translation of transferrin receptor mRNA
   d. decreased rate of translation of ferritin

5. A The branch point A residue involved in lariat formation is part of the
   (a.) intron
   b. exons
   c. 3' untranslated region
   d. 5' untranslated region

6. D Loss of the poly-A tail associated with eukaryotic mRNAs results in:
   a. rapid translation of the transcript.
   b. elongation of the transcript.
   c. decreased translation initiation on the transcript.
   (d.) rapid degradation of the transcript.

7. A Which of the following is ***NOT*** true of RNA processing?
   a. Exons are cut out before mRNA leaves the nucleus.
   b. Nucleotides may be added at both ends of the RNA.
   c. Ribozymes may function in RNA splicing.
   d. RNA splicing can be catalyzed by spliceosomes.

8. C Eukaryotic cells are able to carefully regulate level of transcription in specific genes via
   a. Histone phosphorylation
   b. Transcription attenuation
   c. Controlled activation of transcription factors
   d. Histone methylation

9. B The consensus splice site for intron splicing contains only a few highly conserved sequences. Nearly invariant sequences found in the mRNA are:
   a. GU-AG. These sequences are found at the 5' and 3' ends of the intron (respectively)
   b. GU-AG. These sequences are found at the 5' and 3' ends of the exon (respectively)
   c. GU-AG. These sequences are found both ends to the intron and mark the exon - intron border
   d. GU-AG. These sequences are found in the 5' and 3' UTR

10. B U1 snRNA initiates intron splicing by binding to:
   a. the central part of the first exon
   b. the 5' splice site of the intron
   c. the branch sequence of the intron
   d. the 3' splice site of the intron

11. B As a general rule, alternative splicing involving different 5' sites may be influenced by:
   a. formation of secondary structures that contains several domains formed by base-paired stems and single-stranded loops.
   b. proteins in the spliceosome assembly that either stimulate or repress the usage of one of the possible sites for splicing.
   c. a single type of spliced mRNA formed when an interrupted gene is transcribed into an RNA.
   d. the type of RNA ligase that functions in the reaction

12. C Isoaccepting tRNAs are
   a. found only in eukaryotes
   b. found only in bacterian
   c. aminoacylated by the same tRNA synthetase
   d. are charged with isomerized amino acids

13. D RNA editing in mammalian cells
   a. usually requires the addition of U residues via base pairing with guide RNA
   b. usually involves a single base change uridine to pseudouridine
   c. usually involves a single base change via a deacetylase
   d. usually involves a single base change via a deaminase

14. B The "near universality" of the genetic code suggests that
   a. all organisms are basically the same
   b. the genetic code arose early on in evolution of life
   c. any changes in codon meaning would be disruptive
   d. the third position of a codon has no use

15. B When translating secretory or membrane proteins, ribosomes are directed to the endoplasmic reticulum membrane by
   a. moving through a specialized channel of the nucleus.
   b. a signal sequence of RNA that precedes the start codon of the message. ✓
   c. a specific characteristic of the ribosome itself, which distinguishes free ribosomes from bound ribosomes.
   d. a signal-recognition particle that brings ribosomes to a receptor protein in the ER membrane.

16. B Which of the following is **NOT** a function of molecular chaperones in protein folding?
   a. Molecular chaperones can stabilize partially folded proteins and prevent them from aggregating with other proteins ✓
   b. Molecular chaperones specify the tertiary structure of a protein
   c. Molecular chaperones assist protein in finding their correct structure
   d. Molecular chaperones can shield and protect exposed hydrophobic regions of proteins ✓

17. D All of the statements are TRUE regarding the proteasome **EXCEPT**
   a. The proteasome is a structure comprised of two caps at both ends of a hollow cylinder through which proteins enter
   b. Proteolytic degradation by proteasomes generates short peptides approximately 4-10 amino acids in length.
   c. In bacteria, molecular recognition sequences on N- and C- termini target proteins for degradation by the proteasome
   d. Proteins do not need to be unfolded to enter the proteasome, but they must be bound to chaperone

18. C Insertion of Seleno-Cys-tRNA at certain UGA codons requires a downstream stem-loop (SECIS)
   a. And a SelB/ EF-Tu complex
   b. And a SelB/ EF-G complex
   c. And SelB
   d. And EF-Tu

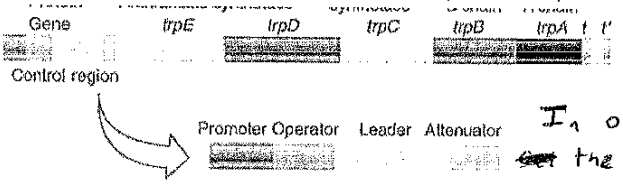
19. C Which of the following statements about disulfide bond formation is FALSE?
   a. Disulfide bonds do not form under reducing environments.
   b. Disulfide bonding stabilizes the structure of proteins. ✓
   c. Disulfide bonding occurs spontaneously by the oxidation of pairs of cysteine side chains on the protein when the protein enters the ER.
   d. Disulfide bonds form in the oxidizing environment of the ER lumen via protein disulfide isomerases.

20. A Two common features of programmed frameshifting are _____.
   a. slippery sequence and ribosome delay
   b. slippery sequence and less frequency than errors at nonprogrammed sites
   c. very high efficiency and ribosome delay
   d. occurs more often than nonprogrammed mutation and slippery sequence

21 (4pts) Cro and lambda repressor (cI) can bind at 3 sites adjacent to the PL promoter and 3 sites adjacent to the PR promoter. Why does Cro binding to OR3 lead to lysis

When Cro binds to O_R3, CI can no longer make a [redacted] oligiomer which is needed to maintain lysogeny. [redacted] Since Cro represses CI formation (which promotes lysogeny) then the lytic genes will be transcribed leading to cell lysis.

22. (4pts) In E. coli, the tryptophan biosynthetic operon is regulated by the Trp repressor and by attenuation. Describe the regulatory events that are necessary for expression of the Trp operon.



In order for [redacted] expression of Trp operon, the ribosome must stall when translating the Leader sequence so that the attenuator sequences 3 and 4 can unbase pair and remove the attenuator hair pin. The ribosome will stall if the levels of tryptophan in the cell are low because the leader sequence encodes two trp amino acids into the synthesized protein.

So if trp levels are low, the Trp operon will be expressed.

23 (4pts) Describe the mechanism by which **nuclear receptor** (e.g.thyroid hormone receptor – TR-RXR) only are able to activate transcription when in the hormone bound state.

When [redacted] nuclear receptors such as TR-RXR bind to the nucleus they are able to recruit other proteins that regulate transcription. In the Unbound state, TR-RXR recruits proteins such as HDAC which deactivates genes by deacetylation of histones. TR-RXR can only activate when bound by thyroid hormone because thyroid hormone changes TR-RXR such that it ~~recruits~~ recruits activator proteins instead of repressors.

13

24. (2pts) Sir proteins are involved in maintenance of the HMLalpha and HMRa mating type cassettes in yeast. Sir2 is a histone deacetylase. How does Sir2 affect expression of the mating type cassette genes?

Sir 2 lowers the expression of the mating type cassette genes because deacetylated histones are more tightly bound to the DNA which inhibits the ability of RNA polymerases to bind to DNA. 2

25. (2 pts) Identify two functions of the 5' cap of eukaryotic mRNAs

- The 5' cap protects mRNA from degradation by 5' to 3' exonucleases.
- The 5' cap allows cap binding proteins to properly recruit the 30S subunit of a ribosome to start translation. 2

26. (4pts) Explain how the correct 5' and 3' splice sites are recognized by the cell splicing apparatus using exon or intron definition.

In intron definition U1 binds to the GA site at the 3' end of the intron. Then U2AF binds at the GA site at the 5' end of the intron. The U2AF and U1 can identify or ignore certain splice sites depending on the presence of exon promotors (such as SR proteins) or exon inhibitors. Then U1 and U2 interact across an intron in order to exclude it from the final transcript. 4

27. (2pts) What is the role of guide RNAs in the pan-editing of some Trypanosome mitochondrial mRNA?

guide RNAs base pair with parts of the mitochondrial mRNA and then serve as a template as to whether U's should be added to the mRNA (if there are extra A's and G's in the guide) or removed from the mRNA (if there U doesn't have a bp partner) 2

28 (2pts) What is the role of SR proteins in RNA processing?

SR proteins generally promote the inclusion of exons in an mRNA molecule. The extent of phosphorylation of serine and arginine determine the binding affinity of SR proteins. 2

29. (2pts) What is the functional role of bacterial 16S rRNA in translation?

The role of bacteral 16S rRNA helps identify the Shine-Delgarno Sequence in the mRNA transcript such that the small ribosomal subunit is guided to the start codon. 2

30. (2pts) What is the functional role of bacterial 23S rRNA in translation?

23S rRNA is a catalytic rRNA (ribozyme) that helps incorporate amino acids into the growing polypeptide chain. 2

15

31. (4pts) Compare and contrast **translation initiation** in bacteria and in eukaryotes. Identify 2 significant similarities and 2 significant differences.

4

translation initiation similarities

- Both processes involve the binding of the small ribosomal subunit to the mRNA transcript with later recruitment of the large subunit.
- Both processes involve the use of a modified methionine tRNA

differences

- In eukaryotes, the start codon is identified first by binding to the 5' cap and then scanning for the Kozak consensus, while in bacteria, the start codon is identified by the 16S rRNA subunit interaction with Shine-Delgarno sequence.
- In eukaryotes the start tRNA is incorporated into the small subunit P site before attachment to mRNA while in bacteria, the tRNA-met is incorporated after start codon is identified.

32. (4 pts) Describe the major events that take place during bacterial translation termination.

4

In bacteria when a stop codon is reached it is recognized by class I release factors which enter the A site that hydrolyze the protein product away from the tRNA. Then ribosomal recycling factors enter the A site bound by GTP and hydrolyze GTP to break apart the ribosomal subunits to be used again.

33. (4pts) How is the initiator methionine tRNA distinct from the elongator methionine tRNA?

3

In bacteria the initiator methionine tRNA has a formylated methionine that is distinct from the normal methionine used in elongation.

34 (2pts) eIF4A has helicase activity. What role does this have in eukaryotic translation initiation?

2

In eukaryotic initiation, mRNA can form secondary structures by base pairing with itself. These base pairings need to be removed for the small submit to scan the mRNA properly.

35 (4pts) How can proteolytic cleavage yield functional protein products? Include an example in your response.

4

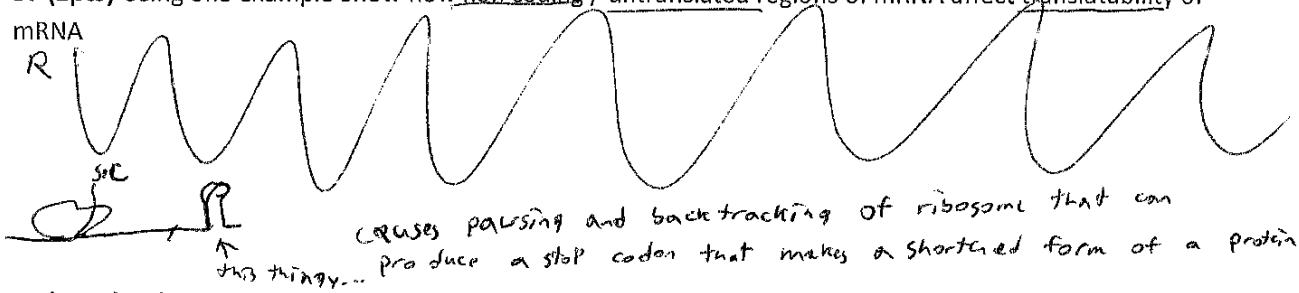
Proteolytic cleavage can remove peptides that are involved in regulation of a peptide such as leader sequences which are nessecsbary for regulation but not for the functional structure. For example prepro insulin needs to have its localization sequence cleaved and an inter protein sequence cleaved before it can become active insulin.

17

36. (2pts) Why is it important that aminoacyl tRNA-EF-Tu-GTP, EF-G and class-1 release factors all have similar 3 dimensional conformations?

2 All three of these factors need to be able to enter the A site of a ribosome.

37 (2pts) Using one example show how non coding / untranslated regions of mRNA affect translatability of mRNA



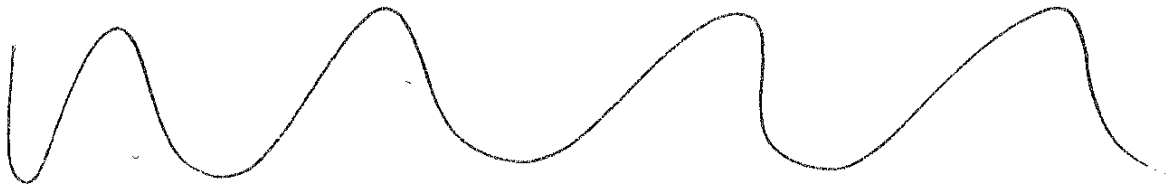
1 causes pausing and backtracking of ribosome that can produce a stop codon that makes a shortened form of a protein.

38 (2 pts) What are microarrays and how are they used to assess gene expression?

2 Microarrays are chips that are spotted with 1000's of gene sequences that correspond to an organisms ORF's. Then cDNA from mRNA transcripts can be washed on the chip to hybridize with the spotted gene sequences. This shows that if a cDNA hybridizes at a particular point, then that mRNA must have been expressed in the original cell.

39 (4 pts) Schena etal used microarrays to monitor expression of about 1000 human genes. What did the results demonstrate regarding the transcription response to heat shock vs transcription response to phorbol ester treatment?

3 These results demonstrated first that cells respond differently to heat shock and phorbol ester by transcribing different parts of the genome. Second Schena etal proved the concept that we could look at transcriptional responses to different conditions to identify mRNA's that are involved in different biological pathways.



40 (4 pts) Identify 2 major differences that distinguish O-Glc NAc (N-acetyl glucosamine) modification from typical O-linked glycosylation.

2 In ~~N-linked~~ O-GlcNAc modification, ~~S~~ [redacted] polysaccharides are added to Nitrogen containing arginine whereas O-linked glycosylation ~~occurs~~ occur on amino acids with alcohol groups.

A second major difference - - - 7

10

41 - 45. 2pts each.

D 41) In an experimental situation, a student researcher inserts an mRNA molecule into a eukaryotic cell after he has removed the 5' cap and poly-A tail from the mRNA. Which of the following would you expect him to find?

A) The mRNA molecule could not exit the nucleus to be translated.
B) The cell recognizes the absence of the tail and polyadenylates the mRNA molecule in the cytoplasm.
C) The mRNA molecule would be translocated to the nucleus for capping and polyadenylation
(D) The mRNA molecule is digested by exonucleases since it is no longer protected at the 5' and 3' ends.
E) The mRNA molecule attaches to a ribosome and is translated, but more slowly.

B 42) A mutant bacterial cell has a defective aminoacyl synthetase that attaches a lysine to tRNAs with the anticodon AAA instead of the normal phenylalanine. The consequence of this for the cell will be that

A) None of the proteins in the cell will contain phenylalanine.
(B) Proteins in the cell will include lysine instead of phenylalanine at amino acid positions specified by the codon UUU.
C) The cell will compensate for the defect by attaching phenylalanine to tRNAs with lysine-specifying anticodons.
D) The ribosome will skip a codon every time a UUU is encountered.
E) None of the options will occur; the cell will recognize the error and destroy the tRNA.

A 43) Chloramphenicol binds in the active site of the large ribosomal subunit and inhibits peptidyl transferase activity in the 23S rRNA. Why is this inhibition restricted to bacterial translation?

(A) In eukaryotes, a large ribosomal protein (L7) provides peptidyl transferase activity.
B) Eukaryotic ribosomes have distinct rRNA and r-proteins and chloramphenicol does not bind the 60S ribosomal subunit.
C) In eukaryotes, the small ribosomal ~~protein (S1) provides~~ the peptidyl transferase activity.
D) Chloramphenical binds to the 40S ribosomal subunit in eukaryotes, it does not block access to the peptidyl transferase active site.
E) Chloramphenicol binds the ricin/sarcin loop which is distant from the peptidyl transferase active site.

B 44) Which of the following is not a mechanism employed by repressor proteins to decrease transcription of a specific gene?

A) The repressor binds to the activation domain of an activator, eliminating its ability to increase transcription. ✓
(B) The repressor binds to DNA-binding domain of an activator, eliminating its ability to associate with enhancer.
C) The repressor binds to a DNA sequence in an enhancer, eliminating access to sequence by activator. ✓
D) The repressor associates with a promoter element, blocking RNA polymerase from binding promoter element. ✓
E) The repressor binds to RNA polymerase II, blocking its ability to associate with promoter element.

B 45) A researcher isolates a mutant EF-G. The mutation allows proper folding of the protein and binding of GTP but does not allow GTP hydrolysis. At what stage would translation be blocked?

A) Small subunit associates with ribosome binding site but intact ribosome cannot be formed.
B) The first peptide bond is formed but translocation cannot occur. ✓
C) ~~Amino acyl tRNA brought to A site but peptide bond cannot form.~~
D) Amino acyl tRNA cannot enter A site.
E) ~~Uncharged tRNA remains in~~ A site.

46-50 All of the following statements are false. Explain why (2pts each)

46. The ubiquitination of the initiation factor eIF-2 results in a repression of global translation initiation in eukaryotic cells

Ubiquitination of eIF-2 would target it for degradation, resulting in global inhibition ~~of~~ translation initiation.

47. O-linked glycosylation involves modification of the side chains of aspartic acid.

N-linked glycosylation involves modification of arginine.

~~Asparagine side chain modification.~~

48 Nuclear localization signals are cleaved after protein enters the nucleus

Nuclear localization signals remain attached to proteins because ~~they~~ they may need to be used again.

49. In heterochromatin, methylated DNA is bound by methylated DNA binding proteins that recruit transcription activators.

In heterochromatin, methylated ~~DNA is bound by~~ DNA is bound by methylated DNA binding proteins that inhibit transcription.

50. snoRNAs direct tRNA processing and base modification

snoRNAs direct rRNA processing and base modifications

Bonus: (2pts)

Cycloheximide blocks the peptidyl transferase center of the 60S ribosome and, therefore, can prevent viral translation. Why can't cycloheximide be used as an anti-viral therapy?

Cycloheximide can't be used as anti-viral therapy because it would also prevent normal genes that the host needs to survive from being transcribed.