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99/100

BIOL 4620 Molecular Biology
Exam 3 Apr 3,2012

Name [redacted]

PLEASE WRITE LEGIBLY. **IF THE GRADER CANNOT READ YOUR ANSWER IT WILL BE MARKED WRONG.**

1-18. Multiple choice. (18 pts/ 1 pt each). Enter the letter of choice that best answers question in the space provided. (circled sentences will NOT receive credit)

B ✓ 1. Association of DNA with core histones
A. is stable and occurs only in a sequence specific manner.
(B.) requires energy to alter structure and exclude nucleosomes at promoter.
C rapidly changes in response to an increased concentration of sequence DNA binding protein.
D. occurs completely at random.

C ✓ 2. Methylation of H3 at K9 or K27 _____
A. occurs in extremely active regions of chromatin.
B. is randomly distributed throughout euchromatin and heterochromatin.
(C) is found in heterochromatin and regions that are not expressed.
D. requires ATP-dependent chromatin remodeling complexes.

A ✓ 3. Which of the following statements about the process of silencing a region of chromatin is FALSE?
(A.) Deacetylation of H3K9 occurs after methylation of the lysine. ✶
✓B. Once methylated, H3K9 recruits HP1.
✓C. HP1 recruits DNA methyltransferases.
✓D. Methylation of H3K9 causes methylation of DNA at CpG islands.

C ✓ 4. Changes in chromatin independent of DNA sequence that persist through multiple cell divisions are called
A. translational effects. B. remodeling effects. (C.) epigenetic effects. ~~D.~~ prions.

A ✓ 5. The HP1 protein plays a key role in formation of heterochromatin in mammals by:
(A.) binding to methylated histone H3.
~~B.~~ binding to methylated histone H5.
~~C.~~ binding to acetylated histone H1.
~~D.~~ binding to acetylated histone H2B.

D ✓ 6. Imprinting is a term used to describe:
~~A.~~ the maternal and paternal specific acetylation pattern on histones, which influences chromatin activity.
B. the maternal and paternal specific pattern of methyl groups on histones, which influences chromatin activity.
~~C.~~ the maternal and paternal specific pattern of acetyl groups on DNA, which influences activity of an allele.
(D.) the maternal and paternal specific pattern of methyl groups on DNA, which influences activity of an allele.

B ✓ 7. What is the FIRST step involved in transesterification during RNA splicing?
~~A.~~ the 2'–OH of the exon attacks the bond at the 3' splice site.
(B.) the 2'–OH of the invariant branchpoint A attacks bond at the 5' splice site.
~~C.~~ Exons are joined and linear intron is released.
~~D.~~ The intron is released upon cleavage at the 3' splice site.

C ✓ 8. Splice sites in pre-mRNA are marked by two universally conserved sequences contained
~~A.~~ at the ends of the exons.
~~B.~~ In the middle of the exon.
(C.) at the ends of the introns.
~~D.~~ In the middle of the intron.

B 9. U1 snRNA is required for mRNA splicing. What is its role?
A. single stranded region in 5' end of U1 snRNA forms complementary base pairs with 3' splice site of the intron.
B. single stranded region in 5' end of U1 snRNA forms complementary base pairs with 5' splice site of the intron.
C. single stranded region in 5' end of U1 snRNA forms complementary base pairs with branch site of the intron an the unbasepairid A nucleotide becomes branchpoint of lariat.
D. Single stranded region of U1 snRNA binds consensus sequences in the exon adjacent to the 5' splice site.

D 10. The U2 snRNA base pairs with:
A. a sequence spanning the first exon–intron boundary.
B. the 3' splice site of the intron.
C. a sequence spanning the intron–second exon boundary.
D. the branch sequence within the intron.

B 11. 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 that either stimulate or repress the usage of one of the possible sites for splicing.
C. a protein encoded in intron that directs modification of bases and results in suppression of splice sites usage.
D. the type of RNA ligase that functions in the reaction.

D 12. In the Drosophila sex determination pathway
A. development of male flies requires default splicing of Sxl and alternative splicing of Tra/Tra2.
B. development of female flies requires default splicing of Sxl and default splicing of Tra/Tra2.
C. development of male flies requires alternative splicing of Sxl and Tra/Tra 2.
D. development of female flies requires alternative splicing of Sxl and Tra/Tra2.

A 13. Maturation of pre rRNA includes
A. specific cleavages to yield mature sized rRNA and base modifications.
B. nucleolar intron splicing and base modifications.
C. autocatalytic intron splicing and base modifications.
D. base modifications only.

B 14. The maturation of the tRNA requires the removal of intron sequences via mechanism that
A. involves cleavage and ligation via a ribozyme.
B. results in cleavage and release of linear intron followed by ligation of 2 halves of tRNA.
C. results in two transesterifications and release of circular intron.
D. results in two transesterifications and release of linear intron.

C 15. Cellular protein synthesis proceeds in which direction?
A. 5' to 3' B. 3' to 5' C. amino to carboxyl terminus D. carboxyl to amino terminus

C 16. Each aminoacyl tRNA synthetase
A. is specific for a certain anticodon, and would normally recognize several different amino acids.
B. is specific for a specific variable loop of the tRNA, and would normally recognize several different amino acids.
C. is specific for an certain amino acid, and would normally recognize several tRNAs with different anticodons.
D. Is specific for the D-loop of a tRNA, and would normally recognize several amino acids.

B 17. What is the basis of interaction between the ribosome and an mRNA molecule in bacteria?

A. Specific protein-RNA interactions between ribosomal proteins and the mRNA.
B. Specific base pairing between the 3' end of the 16S rRNA in the 30S subunit and a conserved sequence in the mRNA.
C. Specific base paring between the 5' end of the 16S rRNA in the 30S subunit and a conserved sequence in the mRNA.
D. Specific interaction between ribosomal proteins and a binding protein that associates with the 5' end of the mRNA.

A 18. What is the general mechanism for initiation of translation in eukaryotes?

A. The small subunit of the ribosome binds to the 5' cap of the mRNA and scans the mRNA for the initiation codon.
B. The large subunit of the ribosome binds to the 5' cap of the mRNA and scans the mRNA for the initiation codon.
C. The intact ribosome binds to the initiation codon on the mRNA molecule.
D. The small subunit binds to the ribosome binding site on the mRNA molecule.

19-28. (2pts each) For each of the following statements, indicate whether the statement is TRUE or FALSE. If the statement is TRUE, explain. If a statement is FALSE, correct the statement or explain why it is false.

19. Polycomb (Pc-G) proteins recognize the repression established by homeotic transcription factors then maintain the repression through subsequent cell divisions.
True; Polycomb (PC-G) proteins are not involved in the initiation but the maintainence of already established heterochromatin states. Polycomb proteins are recruited by the homeotic transcription factors. Repression is maintained in daughter cells as well.

20. During X inactivation, Xist RNA is expressed from the active X and coats the inactive X chromosome.
False; During X inactivation, Xist RNA is expressed and stabalized from the inactive X chromosome to be and then is stabalized so that it can coat the inactive X chromosome.

21. Some mammalian genes are imprinted; maternal and paternal alleles have the same DNA sequences but have different methylation patterns.
True; Different methylation patterns on both maternal and paternal alleles cause certain maternal alleles to be expressed while the same paternal alleles are not expressed and vice versa.

22. All 5' splice site are unique but all 3' splice sites are functionally equivalent.
False; All 5' splice sites and 3' splice sites are conserved and therefore functionally equivalent. e.g. of intron splice site in majority of eukaryotic genes: 5'-GU....AG-3'

23. In trans-splicing, the order of exons within an RNA transcript is rearranged to yield a different mRNA sequence.
False; In trans-splicing, exons from different pre-RNA transcripts are litigated together. In the case of cis-splicing, the order of exons within an RNA transcript is never rearranged.

24. The required sequence elements needed for group I intron splicing form a characteristic secondary structure based on conserved internal inverted repeats.
False, the only required elements for group I intron splicing are a guanosine base (GMP, GDP, or GTP) b/c a 3'-OH factor is needed for first transesterification reaction, a monocationic factor, and a dicationic factor.

25. The sequence AAUAAA is a signal for cleavage to generate a 3' end of mRNA to which polyA polymerase adds non template A residues.
True; in eukaryotes, the sequence AAUAAA binds to cleavage and polyadenylation recognition factor which along with poly A endocleave activity and poly A polymerase, generates a 3' end of mRNA by cleavage at downstream GU repeat site and allows poly A polymerase to add non-template A residues.

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26. snoRNAs have a role in tRNA base modification.

False; snoRNAs have a role in rRNA base modifications ✓

27. Guide RNAs provide the template for deamination of specific bases in trypanosome mRNA.

False; ~~True;~~ Guide RNAs provide the template for insertion (majority of time) and deletion of uridine bases in trypanosome bacteria, resulting in frame shift mutations.

28. Translation elongation requires energy from ATP hydrolysis.

~~True;~~ False; translation elongation requires energy from GTP hydrolysis (~~EG GTP → EG GDP~~) (EF-Tu GTP → EF-Tu GDP)

29. (2pts) What would be the effect on transcription of addition an HDAC inhibitor to a cell?_____

HDAC → histone deacetylase → responsible for gene activation. In the case that a certain gene (DNA sequence) is acetylated and an HDAC inhibitor it present, ~~the~~ there will be active ✓ transcription.

30. (4pts) Why does it make sense that chromatin remodelers do not recognize promoters directly, but rather are recruited by site-specific factors? Chromatin remodelers are recruited by site-specific factors such as certain transcription factors and activators. If chromatin remodelers had affinity for/ recognized promoters directly, then all genes in a genome would be activated even when they are not needed by an organism, causing waste of energy and other consequences detrimental consequences as cancer, etc. ✓

31. (4pts) HP1 recognizes H3 methylated on lysine 9 but NOT H3 methylated on lysine 4. Why is this specificity critical? HP1 protein is a Chromodomain protein that is associated with heterochromatin formation in mammals. H3 methylation on lysine 9 signals for gene deactivation. On the other hand, H3 methylation on lysine 4 signals for activation. ~~If~~ Without HP1 specificity, it ~~can~~ may bind to H3 methylation on lysine 4, leading to gene deactivation rather than expected activation. ✓

32. (4pts) Eukaryotic DNA is methylated at the C of a CG doublet. This requires both a maintenance methylase and a de novo methylase.

What is the role of the maintenance methylase? Maintenance methylase methylates a double stranded DNA sequence at the C of a CG doublet, in which one strand has already been methylated.

What is the role of the de novo methylase? De novo methylase methylates a double-stranded DNA sequence at the C of a CG doublet that has not been methylated ~~at~~ yet at either strand. ✓

Makes sure parent methylation patterns are followed and are inherited.

33. (4pts) Explain how the correct 5' and 3' splice sites are recognized by the cell splicing apparatus.

Correct 5' and 3' splice sites are recognized by the cell splicing apparatus because the end of introns to be spliced have a conserved 5'- GU...AG-3' sequence that is directly recognized by cell splicing apparatus, along with the nucleotide A that forms a branch (pre-spliceosomal A) when bound by U2 snRNP. (specifically U1 snRNP binds 5' intron splice site will U2AF65 binds 3' splice site in intron splicing).

34. (2pts) Why are exon junction complexes important?

Exon junction complexes are important b/c they have a protein called REF associated with them that helps RNA transport out of the nucleus through the nuclear pore into the cytoplasm. REF proteins initially bind the splicing apparatus and after splicing, associate with exon junction complexes.

35. (4pts) The sexual development of female Drosophila results from a cascade of alternative splicing events.

Functional Tra is an SR protein which is produced only in female flies. What is an SR protein?_____

Serine - arginine rich protein that binds ESE sequences

Give one function of an SR protein in splicing involved when introns are too long or splice sites are weak.

binds ESE sequences and connects U1 snRNP to U2AF65-35 (brings together 5' end of intron closer to 3' end of intron so that splicing can take place)

36. (4pts) Compare processes of **trans-splicing** and **pre-mRNA splicing**. Your answer should include at least one similarity and one difference between these processes.

Similarity: trans-splicing and pre-mRNA splicing both splice out introns and ligate exons together.

Difference: trans-splicing ligates exons of different RNA transcripts together pre-mRNA splicing is cis-splicing in the sense that it ligates exons belonging to a single mRNA together.

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37. (4pts) Compare the processes of **self—splicing** (group1/group II introns) and **pre-mRNA splicing**. Your answer should include at least one similarity and one difference.

Similarity: Both self-splicing and pre-mRNA splicing strive to undergo 2 transesterification reactions. ✓

Difference: Self-splicing do not process does not need the assistance of ~~ez~~ additional proteins in vivo.

On the other hand, pre-mRNA splicing needs small nucleoler ribonuclear proteins such as U1, U2, U4, U5, and U6.

38. (4pts) Distinguish between **tRNA splicing** and **pre- mRNA splicing** indicating at least two differences between the processes.

tRNA splicing: does not require a spliceosome, requires cleavage of ~~tRNAs~~ unusual 5' and 3' ends by RNAase P. ✓

pre-mRNA splicing: require a spliceosome. Spliceosome apparates with snRNPs as U1, U2, U4, U5, U6, recognition of conserved intron sequence 5'-GU.... AG 3'

39. (2pts) What is the role of snoRNAs in the processing of rRNAs?

A snoRNA base pairs with a sequence of rRNA that contains the target sequence to generate a structure that is substrate for modification. 1

40. (2pts) How do eukaryotic 40S ribosomal subunits find the initiation codon of an mRNA?

40S ribosomal subunit (small subunit) binds to the 5' end of the mRNA (specifically the 5' cap of the mRNA) and scans the mRNA for the initiation codon travelling in the 5'->3' direction. ✓

41. (4pts) During bacterial translation, how are AUG and GUG initiation codons distinguished from AUG and GUG codons downstream in an ORF? (codon table is on page 8)

The intitiater tRNA in bacterial translation whose anticodon recognizes the AUG and GUG initiation codon is formylated (fmet-tRNA). ✓ Two initiation codons AUG and GUG are recognized b/c of the wobble position of the 1 base of the anticodon. downstream AUG and GUG codons are not recognized by (fmet-tRNA) but regular (met-tRNA).

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42.(6pts) A researcher isolates mutant variants of the bacterial translation factors **IF-2, EF-Tu, EF-G.** In each case 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 by each mutant protein? (be specific)

defective IF2: Translation would be blocked during initiation b/c of defective IF2-GTP complex associated with ~~tR~~ initiator tRNA that enters the P site of ribosome can not be hydrolyzed, in which case the large subunit cannot bind. ✓

defective EF-Tu: With [illegible] defective EF-TU-GTP hydrolysis, elongation cannot procede b/c peptidyl transferase activity cannot transfer nascent polypeptide to aminoacyl tRNA.

defective EF-G ~~With~~ [illegible] defective EF-G GTP hydrolysis ~~elongation (and thus termination)~~ prevents termination ~~cannot occur~~ b/c no energy will be supplied for ribosome [illegible] to release tRNAs at P and E sites and for ribosome subunits to disassociate ~~→ move 3 codons towards 3' end of mRNA, inhibiting peptidyl-tRNA to move to E site, aminoacyl-tRNA to move to P site, and therefore inhibiting~~ inhibiting ~~vacation of A site to accept another aminoacyl tRNA or release factor~~

Such that A site at stop codon of mRNA

43. (2pts) Why do class-1 release factors, aminoacyl tRNA-EF-Tu and EF-G all have similar 3 dimensional conformations?

Class 1 release factors, aminoacyl tRNA-EF-TU and EF-G all have ~~the~~ similar 3 dimensional conformations b/c they must all bind to the same A site on the ribosomal subunit. ✓

44. (4pts) What are the functional roles of bacterial 16S and 23S rRNAs in translation?

16S rRNA forms the foundation of 30S subunit by base-pairing with Shine-Delgarno sequence in bacteria (ribosome ~~reco~~ binding site) and aids in allowing ribosome bind to the correct site ~~in preparation for tra~~ during initiation. ✓

23S rRNA is a ribozyme because it is involve in peptidyl transferase activity during the elongation step of translation or rather it is involved in peptide bond formation between ~~two amino acids~~, amino acid at P site and amino acid at A site.

45. (2pts) The human genome encodes 48 species of tRNA that are able to read 61 codons. How is this possible?

tRNAs, b/c of ~~the shape~~ their loop-shaped anti-codon stems allows flexibility at their first anticodon base, which can base-pair with more than 1 third position bases of a codon. This is ~~a~~ called wobbling.

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46. (2pts) What features of a tRNA allow its unique recognition by an aminoacyl tRNA synthetase?

→ The distinguisher sequence located between acceptor stem and 3' end of tRNA.
→ At least one base of the anticodon.

47 (2pts) How is selenocysteine incorporated at certain UGA codons?

Selenocysteine incorporation at certain UGA codons requires the gene cluster sel A-D. Sel C encodes the tRNA with anticodon 3'-ACU-5' and tRNA aminoacyl tRNA synthetase. Sel A and D modify serine amino acid on tRNA so that selenocysteine becomes incorporated into at UGA codon when selenocysteines. Sel B encodes and alternate EF-Tu so translation of elongation with selenocysteine can continue.

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC		UCC		UAC		UGC	
UUA	Leu	UCA		UAA	STOP	UGA	STOP
UUG		UCG		UAG		UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC		CCC		CAC		CGC	
CUA		CCA		CAA	Gln	CGA	
CUG		CCG		CAG		CGG	
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC		ACC		AAC		AGC	
AUA		ACA		AAA	Lys	AGA	Arg
AUG	Met	ACG		AAG		AGG	
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC		GCC		GAC		GGC	
GUA		GCA		GAA	Glu	GGA	
GUG		GCG		GAG		GGG	

Bonus (2pts)
Some viroids and virusoids encode endonuclease activity. Why are these of interest to genetic engineering?

Viroids and virusoids encode endonuclease activity as a result of the function of their hammerhead secondary structures. Viroids and virusoid mRNA does not contain 5' caps, allowing hammerhead endonuclease activity to function on viroid and virusoid mRNA but not on eukaryotic mRNA b/c it contains a 5' cap.