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BIOL 4620 Molecular Biology Exam 4 May 1, 2012 Name [redacted]

PLEASE WRITE LEGIBLY. **IF THE GRADER CANNOT READ YOUR ANSWER IT WILL BE MARKED WRONG.**
Multiple choice questions (20 pts/ 1 pt each) **Please write the letter for the correct answer in the appropriate space .** (circled answers will not receive credit)

C 1. If the *E coli* trp attenuator is deleted, how will expression of the trp operon be affected?
a. Expression will be eliminated.
b. The operon will be constitutively expressed.
c. Full expression of the operon will only occur in the absence of tryptophan.
d. Full expression of the operon will only occur in the presence of tryptophan.

A 2. Attenuation of gene expression in the trp operon in *B. subtilis* requires
a. translation of a leader sequence at the same time as RNA polymerase reaches terminator site
b. Binding of TRAP protein which leads to an increase in the degradation of trp mRNA
c. TRAP protein to bind mRNA when tryptophan levels are low
d. TRAP protein to bind mRNA when tryptophan levels are high

A 3. Riboswitches such as that in the GlmS mRNA are generally characterized by the presence of:
a. a long (>20bp) 5' untranslated region before the reading frame of the protein
b. a long (>20bp) 3' untranslated region after the reading frame of the protein
c. a short (<20bp) 5' untranslated region before the reading frame of the protein
d. a short (<20bp) 3' untranslated region after the reading frame of the protein

C 4. Which of the following describes RNA interference?
a. antisense RNA molecules block the transcription of mRNA molecules
b. double-stranded RNA molecules are bound by proteins that block their translation
c. double-stranded RNA molecules are cleaved into siRNA that target mRNA for degradation
d. short interfering RNA molecules bind to the ribosome to prevent translation of the viral mRNAs

15.2
19.2
19
+ 19.8
76.2

A 5. In eukaryotic chromosomes, replicons are:
a. activated independently once each during S phase
b. fired simultaneously once each during S phase
c. fired simultaneously twice each during S phase
d. activated independently twice each during S phase

B 6. Which of the following statements is **TRUE** about rolling circle replication?
a. Bacteria use rolling circle replication to produce many copies of their chromosome
b. Rolling circle replication can produce many copies of a genome with a single initiation of replication
c. the use of both strands of DNA allow rapid production of multiple copies of the genome
d. once initiated, rolling circle replication occurs bidirectionally around the genome

A 7. Which of the following statements is **TRUE**?
a. The eukaryotic equivalent of DnaG is DNA polymerase alpha/primase.
b. PCNA is the eukaryotic equivalent of DnaB
c. In eukaryotes, DNA polymerase I uses nick translation to replace RNA primer with DNA.
d. Eukaryotes use a single major replicating polymerase, DNA polymerase delta.

-1

B 8. The proofreading activity of a DNA polymerase is provided by:
a. 5'-3' exonuclease (b.) 3'-5' exonuclease c. nick translation d. 3'-5' synthetase

C 9. The activity of both gyrase and DnaB depends on _____.
a. methylation of the DNA (c.) ATP hydrolysis
b. the DNA polymerase activity d. the direction of DNA synthesis

C 10. The required elements for DNA ligase to seal a nick in double-stranded DNA are:
a. 5' and 3' phosphate ends adjacent to each and paired with another strand of DNA.
b. 5' and 3' OH ends adjacent to each other and paired with another strand of DNA.
(c.) A 5' phosphate and a 3' OH end adjacent to each other and paired with another strand of DNA.
d. A 5' OH and a 3' phosphate end adjacent to each other and paired with another strand of DNA.

D 11. What event typically initiates homologous DNA recombination?
a. A site-specific nick in one strand of duplex DNA
b. A site specific double strand break in duplex DNA
c. A random (not site specific) nick in one strand of duplex DNA
(d.) A random (not site specific) double strand break in duplex DNA

A 12. Which protein(s) promote branch migration of Holliday junctions in bacteria?
(a.) RuvAB, b. RuvC c. RecA d. RecBCD

A 13. During recombination single-strand invasion occurs _____.
(a.) when a free 3' end invades a region of a homologous duplex
b. when a free 5' end invades a region of a homologous duplex
c. which results in the generation of heteroduplex RNA
d. which results in the generation of a stem loop

C 14. Integration of Lambda DNA into the *E. coli* genome involves
a. homologous recombination
b. nonhomologous recombination
(c.) site-specific recombination between attB and attP
d. site-specific recombination between attL and attR

D 15. Which of the following describes nucleotide excision?
a. a region of double-stranded DNA containing damaged nucleotides is removed and replaced with new DNA
b. a single damaged nucleotide is removed and replaced with a new nucleotide
c. a single damaged base is removed and replaced with a new base
(d.) a region of single stranded DNA containing damaged nucleotides is removed and replaced with new DNA

A 16. Mismatches in DNA are usually repaired by
(a.) excision repair c. nonhomologous end joining
b. recombination repair d. photoreactivation

D 17. *Ecoli* dam mutants show
a. a decreased rate of spontaneous mutation b. an increased rate of deamination
c. a decreased rate of deamination (d.) an increased rate of spontaneous mutation

C 18. Which of the following is a feature common to an insertion sequence?
a. DNA coding for gyrase
b. poly A region
c. Short inverted terminal repeats
d. Short inverted repeats in the middle

C 19. How do cells minimize the potentially harmful effects of transposition?
a. immunoglobulin binds to the transposon-encoded proteins
b. transposon sequences are condensed into highly packed chromatin
c. transposon sequences are methylated
d. transposon proteins are targeted for degradation within proteasomes by ubiquitin

C 20. Which of the following are RNA transposons that lack long terminal repeats (LTRs) and but encode their own reverse transcriptases?
a. retroelements
b. endogenous retroviruses (ERVs)
c. long interspersed nuclear elements (LINES)
d. short interspersed nuclear elements (SINES)

21-28 (2 pts each) All of these statements are false. Explain why each statement is FALSE.

21. ORC complexes associate with the eukaryotic replication origin only during S phase.
False; ORC complexes (Cdc6 and Mcm) associate w/ eukaryotic replication origin as early as G1. Cdc6 and Mcm are actually displaced during S phase.

22. *E coli* replication origin is regulated by availability of nutrients in the media.
False; E. coli replication origin is regulated by cell mass (size) of the E. coli cell. -2 DNA methylation + sequestering factors

23. DNA polymerase I is the major replicating polymerase in *E coli.*
False; DNA polymerase III is the major replicating polymerase in E. coli.

24. All DNA damage results from the action of chemical or physical mutagen.
False; DNA damage can also result from keto-enol nucleotide base transitions, causing wrong bases to incorporate during replication ← this is not an example of damage resulting from the action of a chemical or physical mutagen. (which may not be fixed.)

25. The major role of homologous recombination in cells is to generate genetic diversity
False; The major role of homologous recombination in cells is to retrieve DNA sequences that may have been lost or damaged.

26. All RNA transposons (retrotransposons) move via conservative transposition
True; all RNA transposons move via a replicative method through reverse transcriptase in which there is no double-stranded break in the host.

27. In eukaryotic cells, Okazaki fragments are removed by DNA polymerase with 5' to 3' exonuclease activity
False; eukaryotic cells do not have an equivalent of DNA polymerase I w/ 5' to 3' exonuclease activity. Therefore, Okazaki fragments have to be removed using flap endonuclease and RNaseH.

28. Replication of the linear retroviral genome is primed by a protein that provides priming nucleotide
True; an example is that of an adenovirus genome, whose linear genome can be replicated b/c a protein provides a priming nucleotide.

-6

29. (2) Give one example of how noncoding sequences in mRNA can be important. Noncoding sequences such as transposons can insert themselves into random locations of the genome, altering + modifying the genome. This is a basis for evolution. riboswitch / regulation

30. (4) How is attenuation of the trp operon different in *Bacillus* vs *E coli*? In E. coli, In low levels of trp, TRAP protein will not bind to the mRNA (trp operon), causing an alternate secondary structure to form instead of a termination hair-pin, allowing it to be translated and causing more tryptophan to be synthesized. In E.coli, In Bacillus, attenuation of gene expression in the trp operon ~~is by~~ requires translation of leader sequence at the same time RNA polymerase reaches terminator site.

31. (2) How does methylation of GATC regulate initiation of replication in *E coli*? In order for replication to start again the ($^{5'}$GATC$^{3'}$ / $^{3'}$CTAG$^{5'}$) sequences must be fully methylated (both parent and daughter strand methylated).

32. (2) What is the role of Tus in *Ecoli* replication? Tus protein binds Ter sites (half-way from OrC sites in circular bacterial genomes), which terminate trap replication forks.

33. (2) What feature of the *Agrobacterium* Ti plasmid makes it attractive for use in the generating genetically modified plants? Agrobacterium Ti plasmid is an F+ plasmid which can conjugate with other F- plasmids in order to convert them to F+ plasmids as well.

34. (2) How does the yeast cell ensure that its genome is replicated completely only once per cell cycle?_____ licensing factor (Cdm 11) is degraded after ~~one~~ each replication cycle and only present again when yeast ~~cell~~ daughter cells enter G1 phase.

35. (2) *Ecoli* DNA replication takes 40 minutes. Human replication takes 6 hours (7.5X longer than *E coli*) yet human DNA genome contains 3000X more bases.
What is an important difference between *E. coli* and human replication that enables this? E. coli DNA replication corresponds to the cell growth/mass. Replication of its genome starts even b/f the division associated w/ the previous replication cycle. Human replication of genome occurs during the S phase, but cell does not divide until mitosis.

# of ORIs −1

36. (2) What could happen to a bacterial chromosome if it were not connected to the membrane? _____
Daughter bacterial cells can result in circular genome w/ missing Tra site, missing Ori C site, or both. In other words, bacterial chromosome would not be able to be replicated in a ~~way~~ proper way in which each daughter cell

✓

37. (2) Why do origins of replication contain a core of AT rich DNA? A core of AT rich DNA facilitates DNA melting. results in 1 ORC site and 2 Tra sites.

✓

38. (2) How are replication forks initiated at the E. coli origin of replication? DNA A (w/ ATP) (~~this~~ licensing factor) binds to origin, recruits DNA C (w ATP) → which helps load DNA B (helicase) activity). ~~DNA B unwinds~~ As DNA B starts to unwind DNA, gyrase helps relieve stress via its topoisomerase activity. SSB monomers help stabilize the single strands.

✓

39. (2) SSB does not have an enzymatic function. Why is it essential for replication? SSB monomers help stabilize the single DNA strands so that the won't reform the duplex DNA allowing the replication fork to pass through.

✓

40. (2) What is the function of Dna B in DNA replication? DNA B provides helicase activity (uses ATP) ~~to~~ to unwinds double-stranded DNA to single-stranded DNA, which can then serve as templates.

✓

41. (2) Why can't the DNA polymerase move continuously on both strands during replication? _____
DNA polymerase can move continuously on the leading strand b/c synthesis occurs from 5'→3' and as 3'-OH is available. However, a 3'-OH is ~~not~~ available in the lagging strand, and therefore DNA polymerase cannot move continuously b/c each Okazaki fragment needs a primer.

✓

−1

42. (2) How is priming of Okazaki fragments different in eukaryotes vs bacteria ?

In eukaryotes, priming of Okazaki fragments occurs via DNA polymerase α/primase, and in bacteria, priming of Okazaki fragments occurs via ~~DNA G~~ activity.

43. (2) Removal of the primer is especially important on the lagging strand. Why must the primer be removed?

The primer must be removed b/c it consists of RNA.

44. (2) Why is one clamp loader needed at the replication fork when there are two template strands being replicated? A clamp loader is needed only at the leading strand b/c DNA polymerase must be kept in place at this strand b/c it can continuously transcribe. However, at the lagging strand, DNA polymerase must constantly be removed after generation of each Okazaki fragment since priming for the next Okazaki fragment must take place.

45. (2) *E coli* DNA polymerase III and other high fidelity DNA polymerases have proofreading activity. What is DNA proofreading? DNA proofreading is 3'→5' exonuclease activity that excised a certain ~~base~~ base if it is not paired correctly.
End base already paired → polymerase wins
End base not paired yet → exonuclease activity wins.

46. (2) How is the expression of translesion DNA polymerases regulated in *Ecoli* cells? Translesion DNA polymerases (aka DNA polymerase ‡) will only replace DNA polymerase III if DNA polymerase III cannot continue to replicate b/c it encounters DNA damage/structural changes in DNA.

47. (2) Why are transcriptionally active genes preferentially repaired? transcriptionally active genes are preferentially repaired to avoid cell arrest (e.g. error-prone mechanism will insert random [not complementary] bases so that replication fork won't collapse / can be reassembled so that cell arrest can be avoided.

-2 RNA poly stalls → TFIIH recruits repair

48. (2) In *E coli* the A of the sequence GATC is methylated. What is the role of methylation in mismatch repair in *Ecoli*? After replication, the parent ~~GATC~~ adenine is methylated but the daughter adenine is not (hemimethylation). This allows mismatch repair mechanism to differentiate between parent and daughter strand and compare the two.

-2

49. (2) Deamination of cytosine yields uracil and a U-G base pair. How is this repaired in somatic cells? _____

During replication, U-G base pairs with A-G, which base pairs with T-C in another round. Thymine is preferentially excised to put guanine in its place. U-glycosylase

-1

50. (2) How are thymidine dimers repaired in *E coli* in daylight? In the pevence of light, E. coli code photolase (photoreactivation) → process that repairs thymidine dimers.

✓

51. (2) How are thymidine dimers repaired in humans in daylight? Humans have XP genes (XPA – XPG). These genes function in the identification of the thymidine dimers, cleavage of the thymidine dimers, and restoration of normal DNA.

✓

52. (2)If a replication error that creates an AG mismatch is not repaired before the next round of replication, what will be the sequences of the two daughter DNA strands after replication?_____

Parent: 5′ AG 3′ / 3′ CC 5′ Daughter: 5′ AG 3′ / 3′ TC 5′ and 5′ GG 3′ / 3′ CC 5′ ← replication error

Semi-conservative

✓

53. (2) What is the role of Ku protein in DNA repair? Ku proteins (Ku70/Ku80) function in non-homologous end junction repair where they line up broken ends, protect them from nuclease activity, and recruit other proteins so that they can be ligated together.

✓

54. (2) RecA, Rad51 and Dmc1 have equivalent roles in recombination. What is the role of these proteins? Bind single stranded 3′-OH strand that has been generated by double stranded break in a duplex DNA duplex; so that it can attack and displace a strand of another DNA duplex; in general formation of a Holliday junction.

✓

55. (2) Homologous recombination, homologous repair and gene conversion begin in a similar way. How are these processes initiated? All three are initiated by double-stranded break.

✓

-1

56. (2) How are recombinases such as lambda integrase or Cre recombinase similar to topoisomerase? _____

All three are capable of making double-strand breaks.

57. (2) Insertion of the transposon results in a direct repeat of the target site. How does this happen?_____

Transposase creates staggered cuts in target, transposon is inserted, and gaps are filled → generating direct repeat of target site.

58. (2) Can a DNA transposon with intact terminal repeats but mutant/inactive transposase ever transpose? Why or why not? Yes it can in the condition that an autonomous transposon is present in the genome that belongs to the same gene family as the DNA transposon in the question.

59. (2) Why are SINES in the human genome more likely to cause mutations by unequal homologous recombination than by transposition? B/C sines cannot ~~move from~~ generate copies of itself and move from one location to the next, they ~~generate~~ accumulate more mutations in the long run, with bases being inserted and ~~dele~~ deleted from the SINE sequence, being more likely to cause mutations by unequal homologous recombination.

Bonus (2pt)

Trypanosomes evade the immune system of infected hosts by switching the expression of variant surface glycoprotein (VSG).. What process is used for changing the identity of the expressed VSG?

Somatic recombination

→ gene conversion

+1

+1