

Chem 465  
Biochemistry II

Multiple choice (4 points apiece):

1. In homologous recombination in *E. coli*, the protein that moves along a double-stranded DNA, unwinding the strands ahead of it and degrading them, is:

- A) chi.
- B) DNA ligase.
- C) RecA protein.
- D) RecBCD enzyme.
- E) RuvC protein (resolvase).

2. In homologous recombination in *E. coli*, the protein that assembles into long, helical filaments that coat a region of DNA is:

- A) DNA methylase.
- B) DNA polymerase.
- C) histone.
- D) RecA protein.
- E) RecBCD enzyme.

3. In homologous genetic recombination, RecA protein is involved in:

- A) formation of Holliday intermediates and branch migration.
- B) introduction of negative supercoils into the recombination products.
- C) nicking the two duplex DNA molecules to initiate the reaction.
- D) pairing a DNA strand from one duplex DNA molecule with sequences in another duplex, regardless of complementarity.
- E) resolution of the Holliday intermediate.

4. Which of the following statements about *E. coli* RNA polymerase (core enzyme) is *false*?

- A) In the absence of the  $\sigma$  subunit, core polymerase has little specificity for where initiation begins.
- B) The core enzyme contains several different subunits.
- C) The core enzyme has no polymerizing activity until the  $\sigma$  subunit is bound.
- D) The RNA chain grows in a  $5' \rightarrow 3'$  direction.
- E) The RNA product is complementary to the DNA template.

5. The sigma factor of *E. coli* RNA polymerase:

- A) associates with the promoter before binding core enzyme.
- B) combines with the core enzyme to confer specific binding to a promoter.
- C) is inseparable from the core enzyme.
- D) is required for termination of an RNA chain.
- E) will catalyze synthesis of RNA from both DNA template strands in the absence of the core enzyme.

6. The 5'-terminal cap structure of eukaryotic mRNAs is a(n):
- A) 7-methylcytosine joined to the mRNA via a 2',3'-cyclic linkage.
  - B) 7-methylguanosine joined to the mRNA via a 5' → 3' diphosphate linkage.
  - C) 7-methylguanosine joined to the mRNA via a 5' → 5' triphosphate linkage.
  - D) N<sup>6</sup>-methyladenosine joined to the mRNA via a 5' → 5' phosphodiester bond.
  - E) O<sup>6</sup>-methylguanosine joined to the mRNA via a 5' → 5' triphosphate linkage.
7. A branched ("lariat") structure is formed during:
- A) attachment of a 5' cap to mRNA.
  - B) attachment of poly(A) tails to mRNA.
  - C) processing of preribosomal RNA.
  - D) splicing of all classes of introns.
  - E) splicing of group II introns.
8. Compared with DNA polymerase, reverse transcriptase:
- A) does not require a primer to initiate synthesis.
  - B) introduces no errors into genetic material because it synthesizes RNA, not DNA.
  - C) makes fewer errors in synthesizing a complementary polynucleotide.
  - D) makes more errors because it lacks the 3' → 5' proofreading exonuclease activity.
  - E) synthesizes complementary strands in the opposite direction from 3' → 5'.

Essay questions - answer any 5

1. Explain how homologous genetic recombination takes place in *E. Coli*. Include in this explanation the names and functions of all the enzymes involved in this process. G

A figure like 25-33 is helpful. Homologous recombination starts when the RecBCD protein complex recognizes and binds to a broken end of DNA. The enzyme then uses ATP energy to degrade both strands of DNA back from the break until it encounters a chi sequence. At this point degradation of the 3' strand is slowed, but the 5' degradation continues. RecA monomers, then bind to the single strand DNA to make a DNA/protein filament. Now with the use of more ATP energy the homologous duplex DNA is bound to the DNA filament, and branch migration occurs resulting in a Holliday structure. Once the Holliday structure is formed other enzymes involved such as RuvAB, resolvase, DNA polymerase, RuvC generate full length full length unbranched repaired DNA

2. What is a transposon and how does it work

A transposon is a segment of DNA that can move or "jump" from one place location to another. The simplest transposon contains the gene for a transposase flanked on both the 3' and 5' end with insertion sequences that are recognized by the transposase.

In simple transposition the transposase first cuts the transposon and both ends excising it from the chromosome and leaving a double strand break behind. The transposase then find a new site in the DNA and introduces a staggered cut at this site. The transposon is then ligated to the staggered end of this cut and the gaps are filled in to integrate the transposon into this new location.

In replicative transposition the transposon is replicated during the transposition process leaving a copy in the original location as well as a copy in the new location.

3. Compare and contrast the structure, mechanism, and cellular role of DNA polymerase III with RNA polymerase II and with Reverse transcriptase.

DNA polymerase III Synthesize DNA from DNA - Complex of >10 subunits, usually only starts at the ori sequence, polymerizes both strand simultaneously in the 5'→3' direction, has 3'→5' exonuclease proof-reading capability, requires primer, highly processive (>500,000 bases)

RNA polymerase II - Synthesize RNA from DNA -Complex of 5 subunits, starts at many different promoters depending on the  $\sigma$  subunit, polymerizes only one strand in 5'→3' direction, does not have a 3'→5' exonuclease for proofreading, does not require a primer, it is processive, but is released as specific sequences.

Reverse Transcriptase - 1<sup>st</sup> Synthesizes DNA from RNA, then it degrades the original RNA then it synthesizes DNA from DNA using a piece of t-RNA as a primer, Complex of 2 subunits, only binds and replicates its own RNA, does not have a 3'→5' proofreading exonuclease, and is in fact, highly error prone. Processiveness not given.

4. Compare and contrast the processing of eukariotic mRNA, tRNA, and rRNA

mRNA transcribed by RNA polymerase II- Needs a 5' cap, a 3' polyA tail, and excision of introns by splicisomes

rRNA transcribed by RNA polymerase I- 18S, 5.8S and 28S rRNA all synthesized in one large piece. The RNA is then methylated at many sites, and the intervening RNA removed by specific endonucleases rather than by splicing

tRNA transcribed by RNA polymerase III - 40-50 distinct tRNA's all longer than actual active tRNA- residues removed from both 3' and 5' end by specific enzymes, sometimes a single intron is removed, 3' CCA is added, specific bases are modified.

5. In chapter 26 the author of this text speculates that there is a common origin between transposons, retroviruses, and introns. Explain what each of these things are and how they might be related.

*Retrovirus* - A virus that infects a cell with RNA that codes for a few viral proteins as well as a reverse transcriptase the will translate the infecting RNA into DNA, destroy the RNA, replicate the DNA, and then integrate the DNA into the host chromosome.

*Transposon* -DNA that codes for a transposase with terminal repeats at both ends, the transposase is an enzyme that can clip out the transposon sequence and insert it into a different location in the chromosome. In most eukariotic cells the transposons are actually retrotransposons that go through an RNA intermediate and the retrotransposase is a also a reverse transcriptase. Due to the similar mechanism it would appear that the above retrotransposons are retroviruses that lost the genes for their viral coat, so now simply move around withing the chromosome since they cannot leave the cell.

*Intron* - The simplest intron piece of RNA that can splice out an interior piece of itself. In some more complicated system the intron may itself code for a homing endonuclease/reverse transcriptase that will allow the RNA sequence to get integrated into the DNA at a new location. This then looks like a retrotransposon.

6. The death cap mushroom, *Amanita phalloides*, contains several dangerous substances, including the lethal  $\alpha$ -amanitin. This toxin blocks RNA elongation in consumers of the mushroom by binding to eukaryotic RNA polymerase II with very high affinity; it is deadly in concentrations as low as  $10^{-8}$ M. The initial reaction to ingestion of the mushroom is intestinal distress (caused by some of the other toxins). These symptoms disappear, but about 48 hours later, the mushroom-eater dies, usually from liver dysfunction. Speculate on why it takes this long for the  $\alpha$ -amanitin to kill.

The  $\alpha$ -amanitin probably shut down m-RNA synthesis in the liver very quickly after ingestion. However since the liver cells were healthy up to that point, there was a supply mRNA and of all the enzymes that the liver needed at that time. Over the next 48 hours, however, both the proteins and the mRNA to make the proteins is getting degraded so the proteins cannot be replaced because protein synthesis has halted due to a lack of mRNA to code for replacement protein. Eventually the liver cells begin to die, and with then the organism as a whole dies.