

Biochemistry and Molecular Biology 400  
Second Examination  
Fall 2001  
October 15, 2001

Instructor: Hardison

This examination has 20 questions worth a total of 100 points. All are multiple choice, and **all count 5 points each**. Please answer these on the enclosed answer sheet. **BE SURE TO WRITE YOUR NAME, STUDENT NUMBER AND TEST FORM ON THE ANSWER SHEET AND ENCODE YOUR NUMBER!**

***THIS IS FORM B.***

PLEASE TURN IN YOUR ANSWER SHEET. You may keep the exam for review.

Some questions request you to choose all the correct statements from a list. Partial credit is given for choosing **some** of the correct answers, **maximal** credit is given for choosing **all** the correct statements, but **no** credit is given for a choice that includes **any incorrect** statements.

**Pick only one option per question.**

1. The mode of DNA replication in a novel organism was investigated in an experiment like that done by Meselson and Stahl. DNA was uniformly labeled with  $^{15}\text{N}$  (heavy isotope) by growth for several generations in  $[^{15}\text{N}] \text{NH}_4\text{Cl}$ . The organism was transferred to light medium containing  $[^{14}\text{N}] \text{NH}_4\text{Cl}$ , and DNA was isolated after 0, 1, 2 and 3 more generations of growth. The DNA was banded on a CsCl gradient to separate heavy ( $^{15}\text{N}$  labeled) from light ( $^{14}\text{N}$  labeled). At the beginning of the experiment (0 generations in light medium), all the DNA was heavy density (HH). After 1 generation, all the DNA banded at the hybrid density (HL). After the second and third generations, DNA continued to appear at the hybrid density (HL), but increasing amounts appeared at the light (LL) density. The mode of replication in this organism is

- a. conservative.
- b. semiconservative.
- c. a combination of conservative and nonconservative.
- d. dispersive (random).

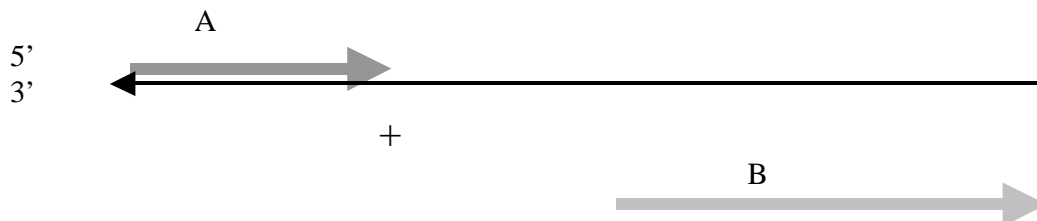
**b is correct.**

2. The following linear, gapped duplex was used as the substrate in a helicase assay. Strands A and B are both labeled, and they are different sizes. After incubation with a previously uncharacterized Enzyme Q plus ATP, you see that strand B has been displaced from the duplex, but fragment A is still hybridized to the bottom strand. The arrowheads point toward the 3' ends of the DNA strands.

Substrate:



Products:



What can you conclude about the activity of Enzyme Q?

- a. It is a helicase and tracks along single stranded DNA in a 5' to 3' direction.
- b. It is a helicase and tracks along single stranded DNA in a 3' to 5' direction.
- c. It is an ATP-dependent exonuclease.
- d. It is a topoisomerase.

**b is correct.**

3. Which statements about DNA polymerase III from *E. coli* are correct?

1. The (gamma) complex loads the sliding clamp onto the primer template junction.
2. The (alpha) subunit within the core catalyzes DNA chain elongation (i.e. incorporation of nucleotides into the growing DNA chain).
3. The (epsilon) subunit within the core is the proofreading 3' to 5' exonuclease
4. The (tau) subunit is the 5' to 3' exonuclease.
5. The (alpha epsilon theta) core has high processivity.

a. 1, 2, 3      b. 4, 5      c. 1, 3, 5      d. 2, 3      e. 2

**a. is correct, 3 points for d, 2 points for e**

4. Which subunits of the *E. coli* primosome are correctly matched with their activity?

1. PriA is a helicase that moves 3' to 5' along single-stranded DNA
2. DnaC forms a complex with DnaB when it is not bound to DNA.
3. DnaB is a helicase that moves 5' to 3' along single-stranded DNA.
4. DnaG synthesizes the primer

a. 4      b. 2, 4      c. 3, 4      d. 1, 2, 3      e. 1, 2, 3, 4

**e is correct, 3 for d, 2 for b, c; 1 point for a**

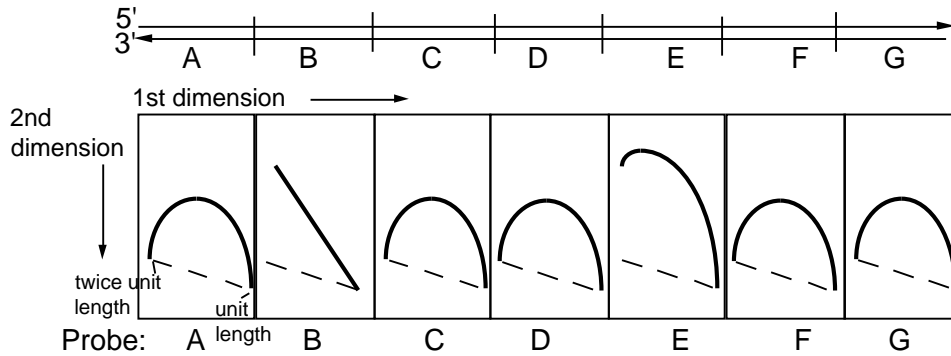
5. Which of the following pairs of eukaryotic and prokaryotic replication proteins carry out analogous functions?

1. PCNA,  $\gamma$  sliding clamp
2. DNA polymerase  $\delta$ , DNA polymerase I
3. RFA, SSB
4. DNA polymerase  $\epsilon$ , complex "clamp loader"

a. 1, 3      b. 2, 4      c. 1      d. 2      e. 1, 3, 4

**5 points for a, 3 points for c; 1 and 3 are correct**

For the next 2 questions, assume you are studying a region of a human chromosome with the restriction map shown below. Previous analysis has shown that replication is bidirectional in this region. You use the two-dimensional gels developed by Brewer and Fangman to map the origin(s). In this system, replicating molecules are cleaved with a restriction endonuclease and separated in two dimensions. The first dimension separates on the basis of size, and the second separates on the basis of shape (more pronounced deviations from linearity move slower in the second dimension). After blotting the DNA onto a membrane, you probe it with the restriction fragments and generate the pattern shown below the map. The dashed line denotes the diagonal expected if all molecules were linear.



6. Which of the following can be concluded from these data?

- a. Fragment A has an origin of replication and termination occurs in fragment G.
- b. Fragment B has an origin of replication and termination occurs in fragment E.
- c. Fragments A, C, D, F, and G each have an origin of replication and termination occurs in fragments B and E.
- d. Fragments C and D have origins of replication and termination occurs in fragment B.
- e. Fragment E has an origin of replication and termination occurs in fragment B.

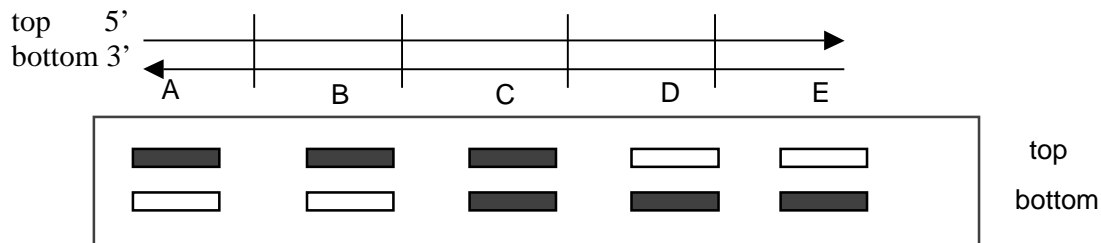
**e is correct**

7. In fragment F, **copying of which parental strand** requires the action of a primosome? Answer with the parental strand that is serving as the template for the primosome; top and bottom refer to the DNA strands shown in the restriction map.

- a. top
- b. bottom
- c. both
- d. Cannot determine from the information provided

**a (top strand) is correct. Fork movement through F is left to right, so the top parental strand serves as the template for discontinuous synthesis, and thus a primosome is needed for copying this strand.**

8. A replication direction assay was performed along a segment of a chromosome with restriction fragments A-E as diagrammed below. Replicating cells were treated with the drug emetine to inhibit lagging strand synthesis. DNA synthesized as the **leading strand** during the drug treatment was density labeled by incorporating 5-bromodeoxyuridine. The DNA was sheared and denatured, and the newly synthesized leading strand DNA was separated from the rest of the DNA by sedimentation equilibrium on Cs<sub>2</sub>SO<sub>4</sub> gradients. Samples of this preparation of newly synthesized leading strand DNA were spotted onto a membrane, and equal amounts were hybridized to radioactively labeled, separated strands of restriction fragments A-E. The results of hybridization of either the top or bottom strand of each restriction fragment to the newly synthesized, leading strand DNA is diagrammed below. A dark fill signifies strong hybridization; the strand used as the labeled probe is indicated to the right of the diagram.



What does this result tell you about the position of the replication origin(s) and/or termini, and the direction of replication?

- An origin is in fragment C, and replication proceeds bidirectionally.
- Origins are in fragments A and E, and replication proceeds from each toward the terminus in C.
- Origins are in fragments A, B, D and E, and replication proceeds from each toward the terminus in C.
- An origin is in fragment C and replication proceeds unidirectionally to the right.

**a is correct.**

**The origin is in fragment C and proceeds bidirectionally both left (leading strand is complementary to the top strand) and right (leading strand is complementary to the bottom strand).**

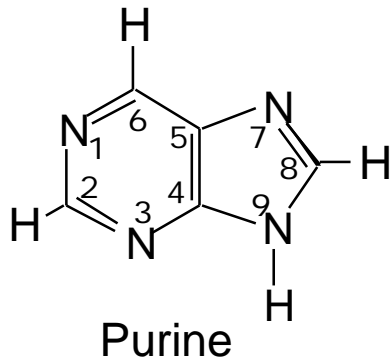
9. Consider a bacterium with a circular chromosome with one replication origin. It takes 45 min for bi-directional replication to copy its chromosome (the elongation time or C period) and 15 min from the end of DNA synthesis until the cell divides (the D period). How many replication forks are needed **per chromosome** to allow a culture of this bacterium to double in cell number every 30 min?

- 2 replication forks per chromosome
- Cycles between 6 and 2 replication forks per chromosome

- c. 6 replication forks per chromosome
- d. 12 replication forks per chromosome

**b is correct (5 pts) 3 pts for a and c.** Since the cells are dividing every 30 min, replication initiates every 30 min. The time to replicate the chromosome is 45 min. Consider initiation event  $n$ . After 30 min, initiation  $n+1$  occurs, giving 6 forks per chromosome: 4 from event  $n+1$  (2 for each origin) and 2 from event  $n$ . Then after 15 min, the forks from event  $n$  terminate, and there are 2 DNA molecules in the cell, each with 2 replication forks (from event  $n+1$ ). After 15 more min, the cell will divide, and initiation event  $n+2$  occurs. So immediately after cell division, the chromosome has 6 forks again: 4 from event  $n+2$  (2 for each origin) and 2 from event  $n+1$ . Thus the cells cycle between 6 forks and 2 forks.

**10.** Choose the correct abilities of the the N at position 1 and the N linked to C6 of adenine deoxynucleotides to donate or accept hydrogen bonds in the more prevalent *amino* tautomer and the less prevelant *imino* tautomer. The numbering system for purine bases is given below.



Option	<i>amino dA</i>		<i>imino dA</i>	
	N1	N linked to C6	N1	N linked to C6
a.	donor	acceptor	acceptor	donor
b.	donor	donor	acceptor	acceptor
c.	acceptor	acceptor	donor	donor
d.	acceptor	donor	donor	acceptor
e.	acceptor	donor	acceptor	donor

**d is correct.**

11. The following DNA segment is from the first exon of the *HRAS* gene. What will the sequence be if the C at position 24 (bottom strand) is oxidized by nitrous acid and then goes through 2 rounds of replication?

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                10           20           30
5'  TAAGCTGGTG  GTGGTGGGCG  CCGGCGGTGT
3'  ATTCGACCAC  CACCACCCGC  GGCCGCCACA

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Choose the one that will be found in the progeny:

- a. 5' TAAGCTGGTG GTGGTGGGCG CCGGCGGTGT  
3' ATTCGACCAC CACCACCCGC GGCUGCCACA
- b. 5' TAAGCTGGTG GTGGTGGGCG CCGACGGTGT  
3' ATTCGACCAC CACCACCCGC GGCTGCCACA
- c. 5' TAAGCTGGTG GTGGTGGGCG CCGGCGGTGT  
3' ATTCGACCAC CACCACCCGC GGCTGCCACA
- d. 5' TAAGCTGGTG GTGGTGGGCG CCGTCGGTGT  
3' ATTCGACCAC CACCACCCGC GGCAGCCACA

**b is correct (5 pts), 2 pts for a**

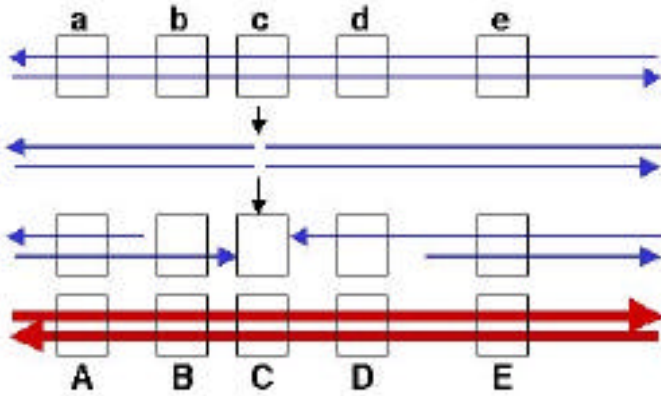
12. Which enzymes would be used **preferentially** to repair the damage caused by reaction of a C with nitrous acid?

1. Uracil-N-glycosylase
2. AP endonuclease
3. DNA polymerase I
4. Uvr ABC excinuclease
5. Mut HLS complex

- a. 1, 2
- b. 1, 2, 3
- c. 1, 2, 3, 4
- d. 2, 4, 5
- e. 1, 2, 3, 4, 5

**b is correct, 3 points for a; 1, 2, and 3 are correct**

For the next two problems, consider two DNA duplexes that undergo recombination by the double-strand break mechanism. The parental duplex indicated by thin black lines has recessive alleles for genes A, B, C, D, and E, denoted by the lower case letters. The other parental duplex shown as thick gray lines has dominant alleles, indicated by the upper case letters. The genes are indicated by boxes. The position of the initial double-strand DNA break (in gene C) and the extent of exonuclease cleavage during initiation of recombination are shown.



13. After strand invasion, repair synthesis and formation of Holliday junctions, what alleles are present in the intermediate prior to resolution? Assume no branch migration occurred, so that the Holliday junctions are at the points where exonucleolytic activity ended. In the following notation, A means allele A, a means allele a and A/a means a heteroduplex with one strand A and the other a.

Alleles for each gene

Option	Gene A	Gene B	Gene C	Gene D	Gene E
a.	a and A	b and B	c and C	d and D	e and E
b.	a and A	B and B/b	C and C	d/D and D	e and E
c.	a and A	b and B/b	c and c	d/D and d	e and E
d.	A and A	B and B	C and C	D and D	E and E
e.	a/A and A	B and B	c/C and c/C	d and D	e and E

**b is correct.**

14. If the recombination intermediate in the preceding problem were resolved horizontally at the left Holliday junction and vertically at the right Holliday junction, what will be the arrangement of the flanking markers (alleles at genes A and E) in the products? In the following, alleles on the same chromosome are connected by a line.

- a. A\_\_E and a\_\_e
- b. a\_\_e and a\_\_E
- c. A/a\_\_e and a/A\_\_E
- d. a\_\_E and A\_\_e

e. A/a\_\_\_E/e and a/A\_\_\_E/e

**d. is correct.**

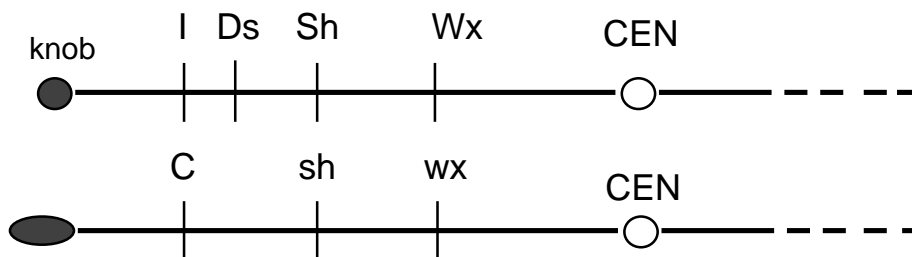
**15.** Which of the enzymes or enzyme complexes will generate a single-stranded DNA with an end close to a *chi* site (5'GCTGGTGG) to initiate recombination in *E. coli*?

- (1) RecA
- (2) RecBCD
- (3) RecE
- (4) RuvA and RuvB
- (5) RuvC

a. 1      b. 2      c. 2 and 3      d. 4 and 5      e. 1 and 4

**b. is correct; other options contain incorrect choices.**

**16.** Several loci on the short arm of chromosome 9 of maize affect the kernel. At the *C* locus, allele *C* generates a colored corn kernel, whereas the allele *I* (inhibitor) blocks *C* activity in a dominant fashion and generates colorless kernels. At the *shrunken* locus, the nonshrunken allele *Sh* is dominant to the shrunken allele *sh*. At the *waxy* locus, the dominant allele *Wx* generates starch in the endosperm, whereas the recessive allele *wx* has no detectable starch. *Ds* is a transposable element under control of *Ac*.



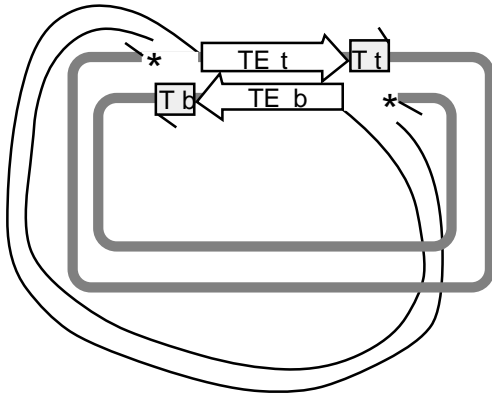
In a plant with the two chromosomes diagrammed above and an active *Ac* element in all kernels, what do you expect to see?

- a. The kernels are colorless, nonshrunken and have starch.
- b. Approximately equal numbers of three different phenotypes are seen: (i) colored, nonshrunken, with starch, (ii) colorless, shrunken, with starch, and (iii) colorless, nonshrunken, but no detectable starch.
- c. The kernels show a variegated phenotype with sectors that are colored and nonshrunken and have starch in the endosperm.

- d. The kernels show a variegated phenotype with sectors that are colored and shrunken and have no detectable starch in the endosperm.
- e. The kernels show a variegated phenotype with sectors that are colored and shrunken but still have starch in the endosperm.

**c. is correct.**

**17.** The following diagram shows the crossover intermediate in transposition. Transposase encoded by a transposable element (TE) has nicked on each side of the TE in the donor replicon (thin black lines) and made a staggered break in the recipient replicon (thick gray lines). The ends of the TE have been joined to the target (T) site in the recipient replicon. The strands of the replicons have been designated top (t) or bottom (b). The stars designate the 3' ends of the recipient DNA strands.



The action of an endonuclease at the 5' end of each strand of TE, followed by DNA polymerase and dNTPs to fill in the gaps (primed from the ends marked with stars), and finally DNA ligase (with ATP or NAD) leads to what product or result?

- a. A cointegrate with the donor and recipient replicons fused, joined by two copies of the TE.
- b. Resolution of the cointegrate, liberating with the donor and recipient replicons, each carrying one copy of the TE.
- c. Excision of the TE from the donor replicon and movement of the TE to the recipient replicon, with duplication of the target site.
- d. Excision of the TE from the original site and movement to the recipient replicon, with inversion of the target site.

**c. is correct**

**18.** Which of the following proteins or protein complexes catalyze hydrolysis of ATP?

- (1) epsilon subunit of DNA polymerase III

- (2) UvrD
- (3) MutHLS
- (4) RecBCD
- (5) RecA

a. 2                    b. 5                    c. 3, 4                    d. 2, 3, 4, 5                    e. 1, 2, 3, 4, 5

**d is correct, 2 points for a or b, 3 points for c.**

**19.** Which of the following proteins use hydrolysis of ATP to dissociate from a complex?

- (1) beta subunit of DNA polymerase III
- (2) gamma complex of DNA polymerase III
- (3) UvrA
- (4) DNA polymerase I

a. 3                    b. 2, 3                    c. 1, 2                    d. 1, 2, 3, 4                    e. 2

**b is correct, 4 points for a or e. The gamma complex hydrolyzes ATP to release the beta clamp, and UvrA dimers hydrolyze ATP to release from the damaged DNA.**

**20.** For which of the following proteins or complexes from *E. coli* are homologous or analogous functions found in mammals?

- (1) UvrAB
- (2) UvrBC
- (3) MutS
- (4) MutL
- (5) RecA

a. 2                    b. 1, 2                    c. 3, 4                    d. 1, 2, 3, 4                    e. 1, 2, 3, 4, 5

**e is correct, 2 points for a, 3 points for b or c, 4 pts for d.**