Biochemistry and Molecular Biology 400 Instructor: Hardison

Second Examination

Fall 1998

October 19, 1998

This examination has 20 questions worth a total of 80 points. All are multiple choice, and all but one counts 4 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.

Questions that require choosing from a list of potentially correct responses will give partial credit for options that have some but not all correct response, full credit for options with all correct responses, but *no* credit for options with *any incorrect* response. For instance, if the problem is to choose all plants from the following list:

(1) tomato
(2) shoe
(3) oak tree
(4) turtle,

option a. 1 would get 2 points for the tomato,
option b. 1, 3 would get 5 points for the tomato and the oak tree,
but
option c. 1, 2 would get *no* credit, since a shoe is not plant, even though a tomato is a plant.

Pick only one option per question.

A3 = B1 =C8. (4 pts) 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}N (heavy isotope) by growth for several generations in [^{15}N] NH_4Cl. The organism was transferred to light medium containing [^{14}N] NH_4Cl, 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 from light (^{15}N from ^{14}N). At the beginning of the experiment (0 generations in light medium), all the DNA was heavy density (HH). After 1 generation, half of the DNA banded at the heavy density (HH) and half of the DNA banded at the light density (LL). After the second and third generations, the same amount of DNA appeared at the heavy density, and increasing amounts appeared at the light density. No DNA was observed at a hybrid density (HL, between the ^{15}N and ^{14}N-labeled DNA). The mode of replication in this organism is

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

a is correct.

A4 = B2 = C9. (4 pts) Which of the following enzymes or groups of enzymes from *E. coli* has a 3' to 5' exonuclease activity?

1. DNA polymerase III
2. PriA
3. DnaG
4. UvrABCD
5. RecBCD

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

c is correct, **2 pts for a**

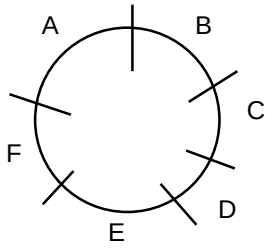
A5 =B3=C10. (4 pts) Which of the following enzymes or groups of enzymes from *E. coli* has an ATPase activity?

1. The gamma (γ) complex of DNA polymerase III
2. PriA
3. DnaG
4. UvrABCD
5. RecBCD

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

b is correct, 2 pts for d, 2 pts for e

For the next two problems, imagine that you have isolated a new virus with a double-stranded, circular DNA that is 6000 bp long. The restriction endonuclease *Hha*I cleaves the DNA as shown below to generate 6 fragments.



A pulse-labeling procedure was used to map the origin and terminus of replication. Infected cells were first allowed to incorporate [^{32}P] phosphate into the DNA for several hours to uniformly label the DNA, and then [^{3}H] thymidine was added for short periods of time (pulse labels), i.e. 4, 8 and 12 min. *Completed* viral DNA molecules were isolated, cut with *Hha*I, and separated on polyacrylamide gels. The amount of [^{32}P] and [^{3}H] in each fragment was determined for each period of pulse label, corrected for thymidine content and normalized so that fragment F has a ratio of 1. The results are tabulated below.

Relative amount of pulse label

Fragment	4 min	8 min	12 min
A	3.2	2.8	1.7
B	5.0	4.1	2.3
C	3.0	2.1	1.4
D	1.9	1.6	1.4
E	0	0.7	1.0
F	1.0	1.0	1.0

A1=B4=C6. (4 pts) Which *Hha*I fragments contain the origin and terminus of replication?

a. The origin is in fragment A and the terminus is in fragment C.
b. The origin is in fragment E and the terminus is in fragment F.
c. The origin is in fragment B and the terminus is in fragment E.
d. The origin is in fragment E and the terminus is in fragment B.

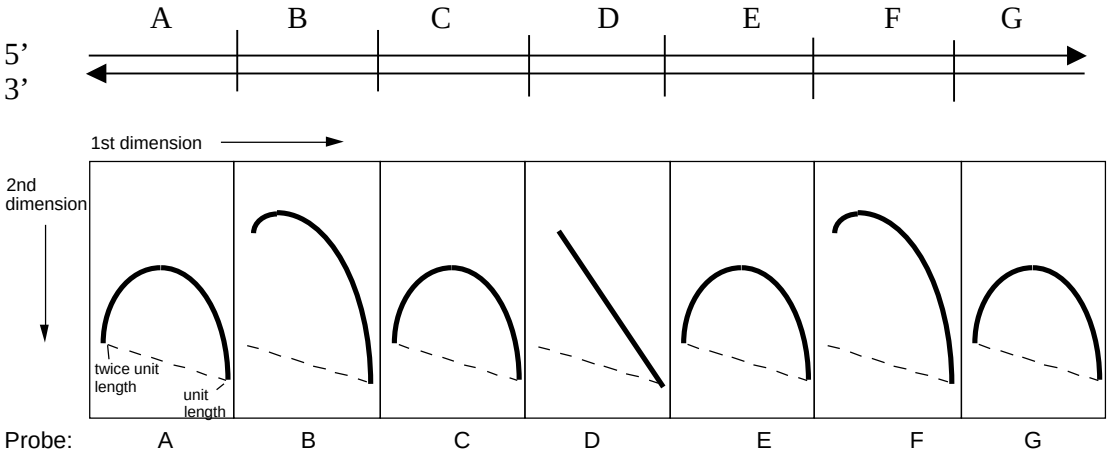
d is correct

A2=B5=C7. (4 pts) What is mode and direction(s) of replication?

a. Replication is unidirectional in a clockwise direction.
b. Replication is bidirectional.
c. Replication is unidirectional in a counter-clockwise direction.
d. Replication is unidirectional but the orientation cannot be determined from these data.

b is correct

For the next 3 questions, assume you are studying a region of a human chromosome with the restriction map shown below. Previous analyses have shown that it has more than one origin of replication and that replication is bidirectional from those origins. You use the two-dimensional gels developed by Brewer and Fangman to map the origins. 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 dotted line denotes the diagonal expected if all molecules were linear.



A8=B6=C1. (4 pts) Which DNA fragment(s) has/have an origin of replication?

a. A, C, E, G
b. B, D, F
c. B and F
d. D

c is correct.

A9=B7=C2. (3 pts) Which DNA fragment(s) contain(s) a terminus?

a. A, C, E, G
b. A, B, D, E, F, G
c. B and F
d. D

d is correct.

A10=B8=C3. (4 pts) In fragment E, **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 diagram.

a. top
b. bottom

c. both
d. Cannot determine from the information provided

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

A6=B9=C4. (4 pts) 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 A has been displaced from the duplex, but fragment B is still hybridized to the bottom strand. The arrowheads point toward the 3' ends of the DNA strands.

Substrate:

Products:

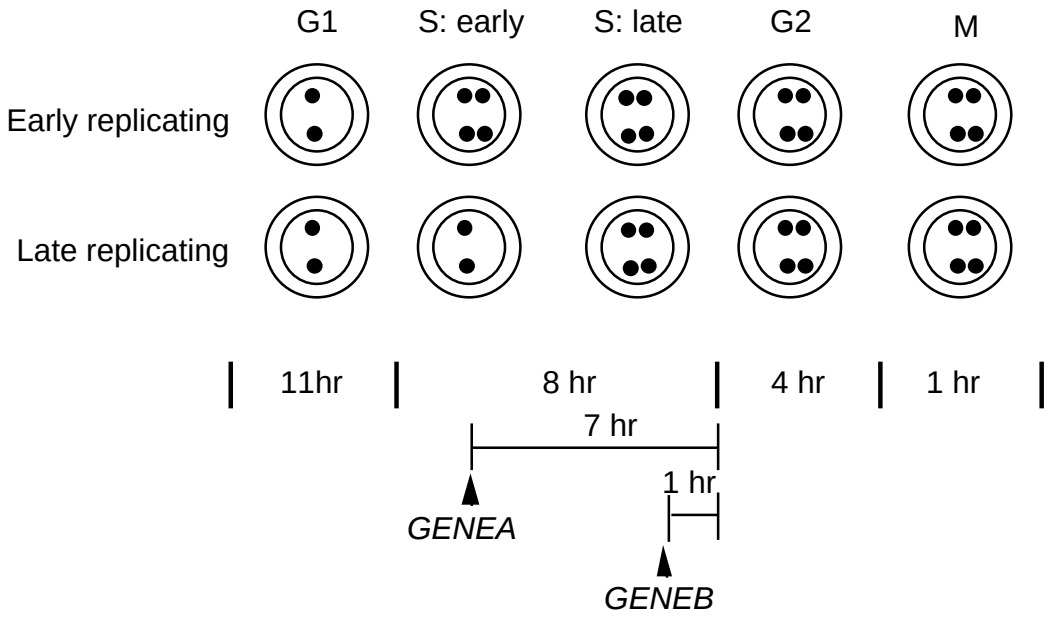
What can you conclude about the activity of Enzyme Q?

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 exouclease.
d. It is a topoisomerase.

a is correct.

A7=B10=C5. (4 pts) In many eukaryotes, actively transcribed genes are replicated early in S phase and inactive genes are replicated late. One assay to determine replication timing is *in situ* hybridization of cells with a gene-specific, fluorescent probe, followed by examination of the number of signals per nucleus. In diploid cells, an unreplicated gene will be seen as 2 fluorescent dots per nucleus, whereas a replicated gene will be seen as 4 dots. They look like 2 doublets, indicating that the replicated chromatids are close in the nucleus.

The types of patterns one can see at various stages of the cell cycle are shown below. Each dark dot is a fluorescent signal, the larger circle is the cell, and the smaller circle is the nucleus.



The fraction of cells in an asynchronous population with 2 dots or 4 dots is then tabulated. In an asynchronous population, the number of cells in each phase of the cell cycle is directly proportional to the length of that phase. If *GENEA* were replicated 1 hr after entry into S phase, and *GENEB* were replicated 1 hr before the end of S phase, what fraction of cells would show 4 dots (two doublets) for each? The length of each phase of the cell cycle is given in the figure, and the time of synthesis is shown by the vertical arrowhead. The time from synthesis of each gene until the beginning of G2 is shown above a horizontal line. Consider cells in M to have 4 dots (i.e., assume that the transition from 4 dots to 2 occurs at the M to G1 boundary).
The fraction of cells showing the indicated gene with 4 dots (two doublets) is:

a.	*GENEA*	0.5	*GENEB*	0.25
b.	*GENEA*	0.8	*GENEB*	0.6
c.	*GENEA*	0.67	*GENEB*	0.33
d.	*GENEA*	0.4	*GENEB*	0.8

a is correct.

A13=B11=C17. (4 pts) Which of the following statement(s) about NUCLEOTIDE EXCISION REPAIR in E. coli is/are correct?

(1) A complex composed of UvrA and UvrB recognizes a site of damage in DNA.
(2) A complex composed of MutH, MutL, and MutS recognizes the damage in DNA and excise it.
(3) A complex composed of UvrB and UvrC nicks on each side of the damage in DNA, in an ATP-dependent step.
(4) Uracil-N-glycosylase cleaves removes the base U from DNA.

Correct statement(s) is/are:

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

c is correct, 2 pts for a.

A14=B12=C18. (4 pts) Which of the following statement(s) about MISMATCH REPAIR in *E. coli* is/are correct?

(1) UmuC and UmuD will recognize a mismatch and cleave 5' to the G of the nearest methylated GATC motif ($G^{me}ATC$).
(2) MutS will recognize a mismatch in DNA.
(3) In a complex with MutS and MutL at the mismatch, an activated MutH cleaves 5' to the G of the nearest unmethylated GATC motif and 3' to the mismatch.
(4) UvrABC provides a helicase function to remove the patch of DNA with the mismatched nucleotide.
(5) Mutations in human genes homologous to some of those encoding mismatch repair enzymes in *E. coli* cause a common hereditary cancer.

Correct statement(s) is/are:

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

d is correct, 2 pts for b.

A11=B13=C19. (4 pts) For the following DNA segment, what will the sequence be if the G at position 14 (top strand) is methylated at the O^6 position by MNNG and then goes through 2 rounds of replication?

```
              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:

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a. 5'  TAAGCTGGTG GTGATGGGCG CCGGCGGTGT
   3'  ATTCGACCAC CACTACCCGC GGCCGCCACA

b. 5'  TAAGCTGGTG GTGCTGGGCG CCGGCGGTGT
   3'  ATTCGACCAC CACGACCCGC GGCCGCCACA

c. 5'  TAAGCTGGTG GTGTTGGGCG CCGGCGGTGT
   3'  ATTCGACCAC CACAACCCGC GGCCGCCACA

d. 5'  TAAGCTGGTG GTGGTGGGCG CCGGCGGTGT
   3'  ATTCGACCAC CACAACCCGC GGCCGCCACA
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a. is correct.

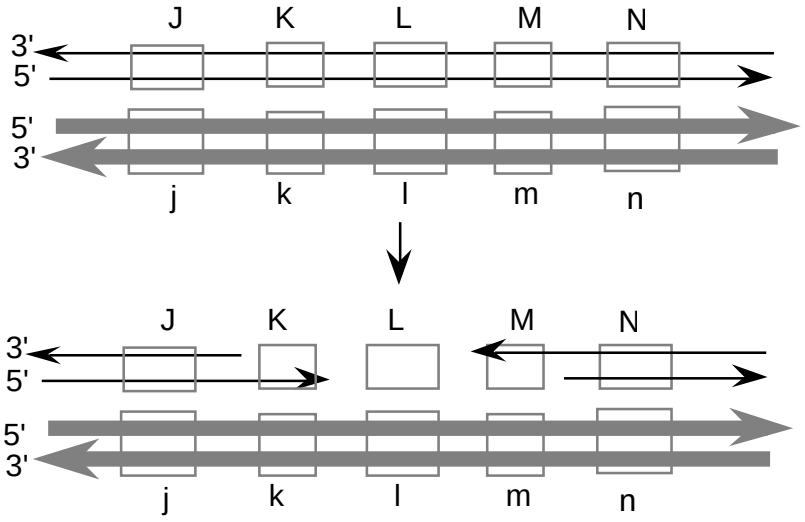
A12=B14=C20. (4 pts) Which of the following enzymes are in pathways that could be used to repair DNA containing a G methylated at the O^6 position?

(1) Methylpurine-N-glycosylase
(2) Photolyase
(3) UvrABCD
(4) AP endonuclease

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

c is correct, 2 pts for a, 2 pts for b

For the next two problems, consider two DNA duplexes that undergo recombination by the double-strand break mechanism in an *Ascomycete* fungus. The parental duplex indicated by thin black lines has dominant alleles for genes J, K, L, M, and N, and the parental duplex shown in thick gray lines has recessive alleles, indicated by the lower case letters. The genes are indicated by dotted boxes. The extent of exonuclease cleavage during initiation of recombination is also shown in the 2nd part of the figure.



A17=B15=C11. (4 pts) If there is no branch migration and the left Holliday junction is resolved horizontally and the right junction is resolved vertically, what will be products be? Answer in terms of the alleles for the flanking genes J and N.

a. J___N and j___n; i.e. the flanking markers are the same as on the parental duplexes.
b. J___N and j___N; i.e. the n allele has undergone gene conversion.
c. J/j___N and j/J___n; i.e. the J locus is in heteroduplex in both products.
d. J___n and j___N; i.e. the flanking markers have exchanged.

d. is correct.

18=B16=C12. (4 pts) Assume that the sister chromatids for each duplex did NOT undergo recombination, and that the products of meiosis undergo an additional round of replication in the process of post-meiotic segregation. What will the ratios of alleles be for loci K, L and M in the 8 spores of the ascus?

	Locus K	Locus L	Locus M
a.	4 K:4 k	3 L:5 l	4 M:4 m
b.	3 K:5 k	2 L:6 l	3 M:5 m
c.	1 K:2 k	0 L:2 l	1 M:2 m
d.	1 K:3 k	0 L:4 l	1 M:3 m

b is correct.

A15=B17=C13. (4 pts) Which of the statements about generating the single-stranded ends on DNA to initiate recombination in *E. coli* is/are correct?

(1) RecBCD generates single-stranded DNA segments with a chi site at the 5' end.
(2) RecBCD is a 5' to 3' exonuclease prior to encountering a chi site.
(3) RecBCD shifts the polarity of its exonucleolytic activity after encountering a chi site.
(4) *E. coli* mutants with no RecBCD activity cannot generate single-stranded DNA for recombination.

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

a is correct.

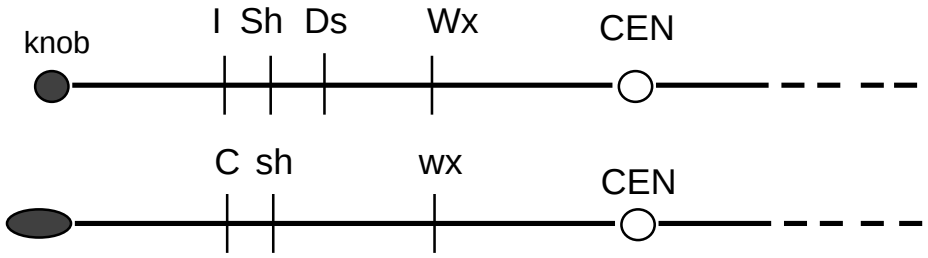
A16=B18=C14. (4 pts) Which of the following statements about RecA is/are correct?

(1) In the presence of ATP, RecA forms a filament on the DNA with 3 to 5 nucleotides of single stranded DNA per RecA monomer
(2) In a step utilizing ATP hydrolysis, RecA mediates assimilation of a single strand of DNA into a duplex, i.e. strand exchange.
(3) The polarity of RecA-mediated strand exchange is 3' to 5', relative to the invading single strand.
(4) .RecA binds to Holliday junctions.

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

c. is correct, 2 pts for a, 2 pts for b

A20=B19=C15. (4 pts) Several loci on the short arm of chromosome 9 of maize have been intensively studied. 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*.

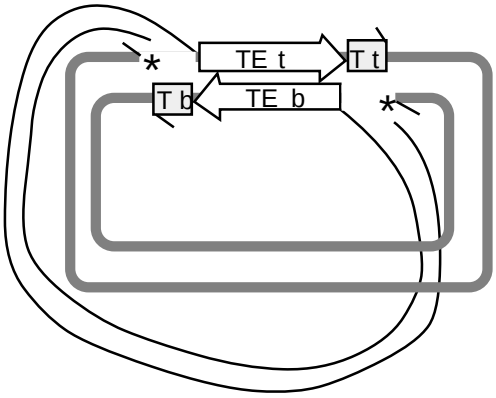


In a plant with the two chromosomes diagramed above and an active *Ac* element in all kernels, what do you expect to see?

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) noncolored, nonshrunken, but no detectable starch.
c. The kernels show a variegated phenotype with sectors that are colored and shrunken and have no detectable starch in the endosperm.
d. The kernels show a variegated phenotype with sectors that are colored and shrunken but still have starch in the endosperm.

d. is correct.

A19=B20=C16. (4 pts) 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 DNA polymerase plus dNTPs, primed at the positions indicated by stars, followed by ligase (with ATP or NAD) leads to what product or result?

a. Movement of the transposable element into the recipient replicon and separation from the donor.
b. A cointegrate structure containing two copies of the transposable element and a fusion of the donor and recipient replicons.
c. Nonreplicative transposition from the donor to the recipient replicon.
d. Formation of flanking direct repeats and two Holliday junctions.

b. is correct.