MCB 110
First Midterm Sp06
SIX PAGES

NAME:

SID Number:

Question	Maximum Points	Your Points
I	25	
II	33	
III	32	
IV	30	
V	30	
	150	

FINAL SCORE:

This exam must be written in PEN if you want the option of a regrade.

Question I (25 points)

(A) Topoisomerases and restriction endonucleases

Cleavage of the DNA phosphodiester backbone can occur in several ways. List FIVE separate distinctions between a type I topoisomerase and a restriction endonuclease (HINT: compare substrate sequence specificity, type(s) of protein-DNA interaction, reaction features)

Type I topoisomerase	Restriction endonuclease

(B) ATPases

Enzymes that bind and hydrolyze ATP use this activity to accomplish different types of molecular work. For each enzyme below, list the specificity of its interaction with substrate (enzyme is recruited by what DNA and/or protein structure?). Indicate the reason why its reaction cycle needs to be 'powered' by use of ATP (what is the energetically unfavorable event?).

(1) Eukaryotic RF-C/ *E. coli* gamma complex

enzyme is recruited by what DNA and/or protein structure:

what is the energetically unfavorable event:

(2) DnaA

enzyme is recruited by what DNA and/or protein structure:

what is the energetically unfavorable event:

(3) DnaB

enzyme is recruited by what DNA and/or protein structure:

what is the energetically unfavorable event:

Question II (33 points)

(A) *E. coli* DNA polymerase I and DNA polymerase III:

Separately list for EACH protein ALL of the associated exonuclease activities discussed in class.
DNA polymerase I:

DNA polymerase III:

Separately list a cellular function for each polymerase.
DNA polymerase I:

DNA polymerase III:

Separately list what primer(s) each polymerase uses in the course of this normal cellular function.
DNA polymerase I:

DNA polymerase III:

State for each polymerase whether single-stranded DNA binding protein would activate or inhibit.
DNA polymerase I:

DNA polymerase III:

(B) Human DNA polymerase alpha and DNA polymerase delta:

Separately list for EACH protein ALL of the associated exonuclease activities discussed in class.
DNA polymerase alpha:

DNA polymerase delta:

Separately list a cellular function for each polymerase.
DNA polymerase alpha:

DNA polymerase delta:

Separately list what primer(s) each polymerase uses in the course of this normal cellular function.
DNA polymerase alpha:

DNA polymerase delta:

Indicate one other DNA replication accessory protein that interacts specifically with ONLY polymerase alpha and one that interacts specifically with ONLY polymerase delta.
DNA polymerase alpha:

DNA polymerase delta:

Question III (32 points)

For each of 1-2 below, give answers for A, B, and C.

A. The type of repair system likely to fix the error (pick only one, even if more are possible).

B. An enzymatic activity or binding factor UNIQUE among the repair reactions described in class to the system of repair in (A), AND the role of this protein in the repair reaction.

C. The amount of DNA resynthesized during the repair (choose 0, 1-8, 9-29, or more than 30 nt).

1. Deamination of 5-methyl-cytosine <u>in eukaryotic cells</u>:

A.

B.

C.

2. O^6-methylguanosine <u>in *E. coli*</u>:

A.

B.

C.

For 1-2 below, indicate ONE DNA repair pathway that could repair the damage. For each, list TWO proteins required for that repair pathway AND indicate the function of each protein in the repair process. Do not list DNA polymerases.

1. DNA break

<u>Repair pathway:</u>

Protein 1:
Its function:

Protein 2:
Its function:

2. Intrastrand thymidine dimer

<u>Repair pathway:</u>

Protein 1:
Its function:

Protein 2:
Its function:

Question IV (30 points)

(A) *E. coli* RecA

(1) When RecA binds DNA, the favorable energy of binding is used to force DNA to adopt a particular structure. State an important change in DNA structure in a RecA filament **AND** explain how this change in structure promotes strand exchange.

(2) RecA protein binds to DNA cooperatively. Define cooperative binding **AND** explain how this biochemical property of RecA promotes strand exhange.

(3) To exchange 50 nt, what is the minimum number of RecA molecules assembled on DNA?

(B) Eukaryotic RAD51 has some properties similar to *E. coli* RecA.

(1) RAD51 needs help assembling onto DNA during the repair of a double-stranded DNA break. First, double-stranded DNA must become single-stranded. How is this accomplished?

(2) RAD51 binding to this single-stranded DNA is aided by the protein BRCA2.

a. What is the biochemical function of BRCA2?

b. What is its DNA binding specificity?

Question V (30 points)

SITE-SPECIFIC RECOMBINATION
(A) What kind of cut does recombinase make in **donor** DNA: Single- or double- stranded?

(B) What kind of cut does recombinase make in **target** DNA: Single- or double- stranded?

DNA-ONLY TRANSPOSITION (assuming that the genome contains one transposon at any time)
(A) What kind of cut does transposase make in **donor** DNA: Single- or double- stranded?

If double-stranded: is the cut even, 3' overhang, or 5' overhang?

(B) What kind of cut does transposase make in **target** DNA: Single- or double- stranded?

If double-stranded: is the cut even, 3' overhang, or 5' overhang?

LTR RETROELEMENT TRANSPOSITION
(A) Describe the length and boundaries of the RNA intermediate relative to the integrated transposon.

(B) What kind of cut does integrase make in the **target** DNA: Single- or double- stranded?

If double-stranded: is the cut even, 3' overhang, or 5' overhang?