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
+1 EACH for total of 10	
Not sequence specific	**sequence specific**
Lk not at Lk_o	**any Lk or no Lk due to linear DNA**
Binds to restrain change in Lk to +/-1	**binds as typically symmetric dimer**
Covalent protein-DNA intermediate	**no protein-DNA intermediate**
One strand is cut: nick	**two strands are cut: break**
Backbone rejoined	**backbone not rejoined**
Catalytic tyrosine	**catalytic metal ions**
Etc.	

(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?).

+ 12 total

(1) Eukaryotic RF-C (the equivalent of the *E. coli* gamma complex)

binds ds/ss DNA junction MUST SAY with recessed 3' end/ 3'OH

must open the sliding clamp closed ring

(2) DnaA

cooperatively binds dsDNA at oriC/ 9bp repeats {not the A/T rich region}

ATP binding gives DnaA favorable DNA binding, while ATP hydrolysis allows loss of this favorable binding and therefore DnaA dissociation from DNA.

(3) DnaB

recruited to single-stranded/melted/unpaired origin DNA {oriC} by bound DnaA {optional to include: when DnaB is bound by DnaC}

uses ATP to {break hydrogen bonds in} converting dsDNA to ssDNA

Question II (33 points)

+2 each answer, except the very first which should be +1 for partial and +3 for total answer

(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: 3'-5' exonuclease AND 5'-3' exonuclease

DNA polymerase III: 3'-5' exonuclease

Separately list a cellular function for each polymerase.

Pol I remove RNA primers, BER, NER

Pol III genome replication/ mismatch repair/ homologous recombination or break repair

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

Pol I DNA primer

Pol III depending on answer above should indicate RNA primer/ DNA primer/ DNA primer

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

Pol I inhibit

Pol III activate

(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: NONE

DNA polymerase delta: 3'-5' exonuclease

Separately list a cellular function for each polymerase.

Pol alpha extend RNA primers (ok to say BER and NER but not really true)

Pol delta genome replication/ mismatch repair/ homologous recombination or break repair

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

Pol alpha RNA primer

Pol delta DNA primer

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

Pol alpha primase (partial credit for DnaG, the *E. coli* primase, which is not the same)

Pol delta sliding clamp (not clamp loader)

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).

Each A +3, B +2 and +2, C +1. Total of +16.

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

A. mismatch repair (T-G mispair)

B. MutS (recognizes damage), MutL (activates MutS); NOT MutH; processive helicase or exonuclease is an OK answer

C. more than 30 nt

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

A. direct repair

B. methylase/ suicide repair enzyme/fine to say 'that protein that … (transfers methyl group to its active site to remove it from base)

C. 0

For 1-2 below, indicate ONE DNA repair pathway that could repair the damage ***(+2 each).*** For each, list TWO proteins required for that repair pathway ***(+2 each)*** AND indicate the function of each protein in the repair process ***(+1 each).*** Do not list DNA polymerases. ***Total of +16***

1. DNA break

<u>Repair pathway 1: NHEJ</u>

Proteins: Ku (end binding protein protects/bridges two ends), DNA ligase IV or NHEJ-specific DNA ligase OK (joins ends bridged by Ku); not covered but correct would be DNA-PKcs (activates joining)

<u>Repair pathway 2: HR</u>

Proteins: RAD51 (strand exchange), BRCA2 (assembles RAD51), RPA or RFA (single-stranded DNA binding protein); also OK to say 5'-3' exonuclease (create ssDNA for strand exchange) or "RuvA/B/C equivalent in eukaryotes" (see functions listed below in SOS repair); other RAD proteins listed on slide but not discussed

2. Intrastrand thymidine dimer

<u>Repair pathway 1: NER (E. coli and eukaryotes)</u>

Proteins: UvrA (recognizes damage), UvrB or UvrC (endonucleases that nick damaged strand), UvrD (helicase); XP proteins with a similar distribution of functions

<u>Repair pathway 2: direct repair (E. coli)</u>

Protein: light activated reversal enzyme

<u>Repair pathway 3: SOS response (E. coli)</u>

Proteins: RecA (initiates damage signal), RuvA (binds Holliday junction), RuvB (Holliday junction helicase), RuvC (holiday junction endonuclease)/ true but told NOT to list polymerases: polV or UmuC, UmuD

Question IV (30 points)

(A) *E. coli* RecA ***Total of +15***

(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.

+3 and +3

underwound

decreased duplex stability allows base flipping and alternate base pairing

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

+3 and +3

'free' subunit prefers to bind a site with adjacent 'bound' subunit (or anything else rational)

coats DNA to allow continous region of strand exchange large enough for stable heteroduplex

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

+3

3 nt or bp per monomer/ 17

(B) Eukaryotic RAD51 has some properties similar to *E. coli* RecA. ***Total of +15***

(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?

+5

5'-3' exonuclease

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

a. What is the biochemical function of BRCA2?

+5

Promote RAD51 assembly in place of RPA

b. What is its DNA binding specificity?

+5

ds/ss DNA junction MUST SAY ALSO with recessed 5' end or overhang 3' end

Question V (30 points)

+3 each except for LTR (A) which gets +3 for 'shorter' partial answer and +6 for full answer
SITE-SPECIFIC RECOMBINATION
(A) What kind of cut does recombinase make in donor DNA: Single- or double- stranded?
ds

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

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?
ds

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

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

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

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

Shorter: Begins within the 5' LTR and ends within the 3' LTR

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

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