A TOTAL OF SIX PAGES

ANSWER KEY *(answers in italics)*

Question	Maximum Points	Your Points
I	28	
II	41	
III	27	
IV	28	
V	26	
	150	

Please write your **name** and **student ID** number (if you have one) at the top of every page.

Note that this exam must be written in **INK** if you want the option of a regrade.

NAME: **Student ID#:**

Question I (28 points)

A. Proteins that bind to DNA directly have different types of DNA recognition specificity. Also, the protein-DNA interaction specificity is often combined with protein-protein interaction to recruit additional factors to the site of DNA recognition. **For each of (a) and (b) below, answer (i) and (ii):**

(i) What is protein that DIRECTLY recognizes the indicated DNA? What features of the DNA are important for recognition specificity (list any requirement for sequence or structure of the bound DNA)?

(ii) State one protein that is recruited to the DNA-bound factor listed in (i) by protein-protein interaction AND indicate the biochemical role of this protein (the type of protein activity is sufficient to answer at the level intended).

(a) Holliday junction

(i) *RuvA (don't need to say octamer). Four-way dsDNA junction.*

(ii) *RuvB (don't need to say two hexamers). Helicase (for branch migration).*
OR equally good answer RuvC (don't need to say dimer). (Endo)nuclease for HJ resolution.

(b) *E. coli* origin of DNA replication

(i) *DnaA (don't need to say multimer). Repeats of a specific sequence (don't need to say 9 bp repeats, but do need to say repeats and specific sequence).*

(ii) *DnaBC complex (full credit to say only DnaB or DnaC, if you say ONLY the correct biochemical role of the one factor that you listed). DnaB is a helicase. DnaC is loader for DnaB.*

B. Proteins can be loaded onto DNA, for example the *E. coli* beta sliding clamp, that then move from the site of initial recruitment. The beta sliding clamp can recruit several other proteins to DNA with specificity partly dependent on adjacent DNA structure and partly dependent on competition among the interacting proteins. List THREE protein factors/complexes that can bind to the *E. coli* beta sliding clamp as **(a), (b), and (c) below,** and for each **describe how it competes/cooperates** with one of the other factors that you have listed.

(a) *Clamp loader, OR Gamma complex. Competes for Pol III binding surface of the clamp.*

(b) *DNA Polymerase III, OR just Pol III. Competes for clamp loader binding surface or binds to the sliding clamp with pol V*

(c) *DNA Polymerase V, OR just Pol V, OR can say UmuCD or anything similar to that, or even "the SOS repair DNA polymerase" is OK for full credit. Shares the clamp with non-elongating Pol III OR can say only one clamp-interacting polymerase can elongate DNA OR Pol V elongation competes with Pol III elongation, or something similar.*

NAME: **Student ID#:**

Question II (41 points)

A. Nucleases have recognition specificity for DNA structure and/or sequence.
(a) List TWO **exonuclease activities** that **act on one, single strand of DNA that is PAIRED as a DNA duplex**. Indicate the polarity of the exonuclease activity and its biological function.
Note: Enzyme name is not needed

-- 3'-5' exo (acting at ds/ss junction 5' overhang), for polymerase proofreading
-- 5'-3' exo (acting at ss/ds junction 3' overhang), for RNA primer removal by Pol I
-- 5'-3' exo (acting at dsDNA break), for dsDNA break resection prior to strand exchange/ homologous recombination

(b) List THREE **endonucleases** that **act on one, single strand of DNA that is PAIRED as a DNA duplex**. Indicate all important features of substrate specificity of each endonuclease.
Only these answers are acceptable, nothing else

AP endonuclease, abasic site
MutH, GATC dsDNA sequence with at least one strand not methylated
UvrC, damaged DNA strand at damage site recognized by UvrAB
Topoisomerase type I (must say topo I or type I)

(c) List THREE **endonucleases** that act on a **structure other than a single-stranded or duplex DNA**. Indicate critical features of DNA structure specificity for the enzymes that you list.

RuvC, Holliday junction/4-way dsDNA junction
FEN1, 5' overhang ssDNA flap from junction of dsDNAs
RNase H, duplex of DNA and RNA strands
Hairpin nicking enzyme for V(D)J recombination, DNA hairpin end

B. Many enzymes use a cycle of ATP binding and hydrolysis to function. For the enzymes below, describe how ATP binding AND ATP hydrolysis affect enzyme properties.

(a) DnaA
ATP binding increases affinity for DNA, ATP hydrolysis (and product dissociation) reduce DNA binding affinity

(b) Gamma complex
ATP binding increases binding affinity for the sliding clamp (and the combined complex then binds DNA), ATP hydrolysis (and product dissociation) allow change in the conformation of the bound clamp (and the DNA-encircling clamp then promotes clamp loader dissociation)

(c) RecA
ATP binding increases affinity for DNA, ATP hydrolysis (and product dissociation) reduce DNA binding affinity

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Question III (27 points)

At a DNA replication fork, multiple enzyme activities are coordinated through physical interaction.

A. List FOUR physically associated ENZYMES at an *E. coli* replication fork.

(a) *leading strand Pol III*

(b) *lagging strand Pol III*

(c) *helicase OR DnaB*

(d) *primase OR DnaG*

(e) *clamp loader OR Gamma complex*

B. Other *E. coli* factors involved in replication are NOT physically associated with other proteins at the replication fork. List THREE such factors AND state the biochemical activity that is the contribution of each enzyme to complete genome replication

(a) *DNA ligase. Seals/repairs/joins phosphodiester backbone/Okazaki fragments.*

(b) *Topoisomerase. Reduces linking number between parental strands/removes positive supercoils introduced by helicase action/reduces strain against DNA melting/ something similar.*

(c) *Pol I (5'-3' exo, don't need to say that). Remove RNA primer. OR as a function for Pol I can say fill in DNA gap left by primer removal.*

NOTE: partial credit for separating out the two answers for DNA Pol I 5'-3' exo and pol activity.

(d) SSB OR single-stranded DNA binding protein. Ensures that template remains single-stranded rather than re-pairing or folding OR keeps bases exposed OR something similar to those statements.

NAME: **Student ID#:**

Question IV (28 points)

For each of 1-2 below, give answers for A-C:
A. What is a type of DNA damage that will be fixed by the listed type of DNA repair? Pick only one example of damage, but be as specific as necessary in description of the DNA substrate.
B. State two proteins **SPECIFIC for ONLY this repair pathway** and in one sentence describe the function/activity of each protein.
C. How much DNA will be synthesized during repair of the damage? To make it simple, choose between these options: 0, 1, 2-40, or more than 40 nt.

1. Base excision repair
A *abasic site OR uracil in DNA OR deaminated cytosine OR modified base recognized by glysolyase (NOTE that "modified base" alone is not sufficient as an answer)*

Bi *AP endonuclease, nicks (phosphodiester) backbone adjacent to missing base*

Bii *phosphodiesterase, removes sugar-phosphate unit (following AP endonuclease cleavage)*

NOTE: Also full credit for "DNA glycosylase" or UNG/UDG glycosylase or similar if the lesion wasn't listed as "abasic site"

C *1 nt*

2. Mismatch repair
A *two normal nucleotides paired incorrectly*

Bi *MutS: recognize/bind mispair*

Bii *MutH: nick/distinguish newly synthesized DNA strand (at hemimethylated GATC)*

Also full credit for MutL: linker between MutS and MutH
OR Dam methylase (DNA adenine methyltransferase, or anything similar): allow discrimination of parental DNA strand (or anything similar)

C *more than 40 nt*

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Question V (26 points)

A site-specific recombinase and a transposase each act as tetramers to "move" elements out of sites in a genome. For comparison below, consider the excision of an integrated lambda phage by site-specific recombination (SSR) *versus* the excision of a transposon by non-replicative DNA-only transposition - the mechanism covered in detail in class (TPN).

A. In a genome, the two sites recognized by the recombinase tetramer can have different relative orientations. Indicate the orientation of recombinase binding sites for the SSR AND TPN reactions described above and why the orientation is significant. ALSO indicate whether strand cleavages occur within or outside the region of sequence-specific recognition.

SSR orientation:
Direct. Only direct repeats will undergo excision by SSR (indirect repeats would undergo inversion, which would not release the phage genome to its own chromosome).

TPN orientation:
Indirect. Only cleavage at the outside edges of the indirect repeats will precisely release the intact transposon from donor DNA for its integration as a complete element at the target site.

SSR strand cleavage:
Within the binding site (the SSR reaction is completed entirely within the initial recombinase-DNA synpasis complex).

TPN strand cleavage:
Outside the binding site (the entire element and its inverted repeats moves with bound transposase).

B. Which strands are rejoined to a new partner by the recombinase enzyme itself? Which are later rejoined by DNA ligase and repair pathways?

SSR:
All four sites of strand cleavage are rejoined by the recombinase (four protein-DNA covalent bond intermediates).

TPN:
Two sites of strand cleavage are rejoined by the recombinase (only the element 3' ends are joined to the target-site 5' ends) so DNA ligase is required (after DNA repair synthesis) to join the element 5' ends to the target site (short direct repeats) Must indicate the polarity of the strands for full credit