MIDTERM I

NAME:

Student ID Number:

Question	Maximum Points	Your Points
I	35	
II	29	
III	30	
IV	32	
V	24	
	150	

Please write your name/student ID number on each of the following five pages. This exam must be written in INK **without any white-out** if you want the option of a regrade.

Question I (35 points)

Different nucleases, acting in their biologically active form (could be a single subunit, dimer or tetramer), hydrolyze 1, 2 or 4 phosphodiester backbone linkage(s) in one round of enzyme biological reaction. FOR EACH nuclease that you list below (10 total), indicate whether it is an exonuclease or endonuclease and indicate all of the features of a biological substrate that recruit the nuclease (answers could include: double-stranded or single-stranded DNA, specific sequence or structure, 5' or 3' overhang, a particular DNA-bound protein) NOTE: there are more correct answers than necessary to complete the question.

1. One phosphodiester bond hydrolysis: (a -f)

Endonuclease activity that hydrolyzes 1 phosphodiester backbone:

RNase H	RNA:DNA duplex
FEN1 (5' flap endonuclease)	5' single-stranded overhang from a duplex
AP endonuclease	abasic site OR missing base
MutH	dsDNA GATC (say non-methylated strand for full credit)
Topoisomerase I/ Type I topo	dsDNA (say Lk unequal to Lk_o for full credit)
XP-F OR XP-G	distorted B-form DNA (bound by XP-A/D for full credit)
UvrC	[distorted] dsDNA bound by UvrAB

Credit not given for UvrC. In the context of the whole enzyme there are two cuts (one by N-terminal domain and one by C-terminal domain). Otherwise one could consider single domains or subunits of other enzymes discussed in class (restriction enzymes, Rag1/2) to make one cut. This is not how these enzymes perform their biological functions. Same applies for 2 cuts for site specific recombinase.

Credit given for phosphodiesterase (exonuclease), ExoVII, ExoX etc. If the student provided homologues of the same enzyme in different organism only one answer was counted. Did not accept multiple polymerases with the same function (3'-> 5' exo) as different nucleases. In addition DNA Polα is exonuclease deficient.

Exonuclease activity that hydrolyzes 1 phosphodiester backbone:

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

2. Two phosphodiester bond hydrolyses: (a-c)

Endonuclease activity that hydrolyzes 2 phosphodiester backbones:

Restriction endonuclease	specific sequence of dsDNA
Topoisomerase II OR Type II topo	supercoil OR two dsDNA
Topoisomerase IV	catenated/intertwined dsDNA/chromosomes
RuvC	Holliday junction OR 4-way duplex junction
RAG1/2	two signal sequence ends [don't need to say 12/23 spacer combo for full credit]
UvrC	distorted B-form DNA (bound by UvrAB for full credit)

3. Four phosphodiester bond hydrolyses: (a)

Endonuclease activity that hydrolyzes 4 phosphodiester backbones:

Site-specific recombinase two SSR binding sites

Transposase transposon inverted repeat ends (say two of them OR use the plural "ends" for full credit) [+/- also say target dsDNA]

[transposase can disrupt more than 4; given full credit here for answer of 4]

Question II (29 points)

A. (12 points) Enzymes that rejoin DNA strands to make an intact phosphodiester backbone have different requirements for ATP. Give examples of an enzyme for EACH of the three specificities listed below (4 total answers) and address the additional question asked.

1. The enzyme requires high-energy cofactor hydrolysis (ATP hydrolysis) because catalysis has an energetically unfavorable step. What is the initial structure of the 3' and 5' DNA ends joined?
(a) *DNA ligase. 5' monophosphate and 3' OH.*

2. The strand joining reaction does not require ATP hydrolysis because the broken phosphodiester bond energy has been conserved, but the enzyme uses ATP hydrolysis to bias the direction of the reaction. Give one example of why this bias could be necessary.
(a) *Type II topoisomerase/ Topo II/ Topo IV/ DNA gyrase.*
Remove + supercoil ahead of replication fork/remove Lk of parental strands/decatanate dsDNA chromosomes/change Lk in unfavorable direction/etc.

3. No role for ATP hydrolysis (at least none discussed in class). What is the high-energy bond that is exchanged for joined DNA strands?
(a) and (b)
-Type I topoisomerase/Topo I.
-Site-specific recombinase (OK to just say recombinase or lambda integrase).
-Transposase also OK answer.
[If you answer type II topoisomerase here, maybe OK based on logic from class but you must NOT have also give that same answer above!]
FOR ALL, answer: covalent protein-DNA intermediate/ tyrosine attached to DNA strand

B. (8 points) Different mechanisms can generate a single-stranded region of DNA. For 1-2 listed below, indicate (a) the enzyme involved and (b) how it creates single-stranded DNA from duplex and (c) a biological role of that activity.
1. RNA primer removal in *E. coli.*
Pol I. 5'-3' exonuclease activity. Removes RNA primers.

2. DNA unwinding at *E. coli oriC*
DnaA. Wraps DNA to favor melting of adjacent region. Initiation at origin of replication/oriC

C. (9 points) Homologous recombination requires strand exchange initiated by a single-stranded DNA 3' end coated with *E. coli* RecA or mammalian Rad51. For each question below, indicate any critical protein properties for both RecA and for Rad51. NOTE: these properties are mostly similar but have some differences as well.
1. What is required for protein assembly on DNA?
RecA requires free 3' end. Rad51 requires free 3' end and BRCA2.
2. What is required for protein disassembly from DNA?
for both: ATP hydrolysis
3. How does the protein favor strand exchange of bound DNA?
for both: stretch/unwind/extend/etc. ssDNA AND dsDNA to destabilize dsDNA base-pairing/allow base-pair trading/etc.

Question III (30 points)

A. (20 points) For the polymerases listed below, indicate the features of template(s) for the polymerase [indicate DNA, damaged DNA, and/or RNA], the primer(s) [indicate DNA and/or RNA], and a directly interacting protein required to recruit/engage/position the polymerase on its biological substrates [indicate "NONE" or the name of the interacting protein].

	Template:	Primer:	Interacting protein
1. Pol I	DNA	DNA	None
2. Pol III	DNA	DNA or RNA	beta/sliding clamp (OK SSB)
3. Pol V	damaged DNA	DNA	beta/sliding clamp (and/or RecA*/RecA)
4. Pol alpha	DNA	RNA (OK also DNA)	primase
5. Telomerase	RNA	DNA	None

B. (10 points) Helicases must be "loaded" to their sites of biological activity. Before engaging their DNA substrates, they are often held by chaperones. To be brought to a particular DNA substrate, they are often recruited by interaction with protein already bound to DNA. To begin DNA unwinding activity, conformational change is required. Considering all of the helicases discussed in class, fill in the blanks to give an example of each specificity described below including the name of the helicase and if relevant the other protein factor bound.

1. A helicase chaperone, ___*DnaC*_____, must pre-associate to ____*DnaB*_____ helicase to allow helicase loading on DNA.

2. _____DnaA___, the DNA-bound recruiter of __DnaB-DnaC/DnaB_, requires ATP hydrolysis to release from DNA before the helicase can unwind it.

3. Helicase activity of ____UvrD__ removes its DNA-bound recruiter, ___UvrA/B___ by displacement (hint: the displaced protein does NOT remove itself by ATP hydrolysis).

4. _____ RuvA__ is a DNA-bound recruiter of ___RuvB____ helicase that remains associated with the helicase while the helicase translocates itself and its bound recruiter along the DNA substrate.

Question IV (32 points)

A. (20 points) In eukaryotes, 5-methyl-cytidine is an intentional base modification of genomic DNA. Spontaneous deamination of cytidine and 5-methyl-cytidine produces DNA damage that must be repaired differently. (a) What is the type of DNA damage created by each deamination? (b) What pathway of DNA repair fixes the damage? (c) What is the DNA repair protein that recognizes the DNA damage initially? (d) Approximately what is the amount of DNA synthesized during repair: answer either 1, 10, or 1000 nt?

1. cytidine
(a)

(b)

(c)

(d)
cytidine to uridine: U is a wrong base for DNA, base excision repair/BER, UDG/UNG/DNA glycosylase, 1 nt

2. 5-methyl-cytidine
(a)

(b)

(c)

(d)
5-methyl-cytidine to thymidine: T-G mispair, mismatch repair/MR, MutS OR SL, 1000 nt

B. (12 points) In *E. coli*, 6-methyl-adenosine modification plays a role in DNA mismatch repair.

1. Describe how DNA methylation coordinates DNA mismatch repair with DNA replication to preferentially repair the newly synthesized DNA strand.

(+3) Post-synthesis lag in methylation of the newly synthesized strand allows discrimination.

2. What enzyme adds this DNA methylation? The name of the enzyme is not necessary for a complete answer, but if you don't give the name please indicate what sequence specificity it has.

(+3) Dam methylase/ DNA adenine methyltransferase/ modifies A in GATC dsDNA

3. What nuclease discriminates the newly synthesized strand? What prevents the nuclease from attacking the parental DNA strand?

(+3 each question) MutH. Can't cleave strand modified [by 6-me-A]

Question V (24 points)

Consider the excision of integrated lambda phage by site-specific recombination (SSR) *versus* the excision of a transposon by non-replicative cut-and-paste transposition (TPN).

A. (8 points) Indicate the orientation of recombinase binding sites (repeat sites) for the SSR and TPN reactions AND why the orientation is significant to the biological reaction.

SSR orientation:
Direct repeats. 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 repeats. Transposase will precisely rejoin both of the precise transposon 3' ends to target DNA (or anything similar and reasonable as an explanation).

B. (6 points) Indicate whether strand cleavages at the two paired recombinase binding sites occur within or outside the length of sequence-specific recognition for the direct or indirect repeats.

SSR strand cleavage inside or outside the paired recombinase binding sites:
Within the binding site (the SSR reaction is completed entirely within the initial recombinase-DNA complex).

TPN strand cleavage inside or outside the paired recombinase binding sites:
Outside the binding site (the entire element including its inverted repeats moves with bound transposase).

C. (10 points) Which strands are rejoined to a new partner by the recombinase enzyme itself? Which are later rejoined by other enzymes necessary to regenerating intact DNA?

SSR: (+4 points)
All four sites of strand cleavage are rejoined by the recombinase (four protein-DNA covalent bond intermediates).

TPN: (+6 points)
Two sites of strand cleavage are rejoined by the recombinase (only the element 3' ends are joined to the target-site 5' ends). DNA ligase is required to join the transposon 5' ends/polymerase-extended target DNA 3' ends. Also DNA donor site ends must be joined by DNA repair.