MIDTERM I

NAME:

Student ID Number:

Question	Maximum Points	Your Points
I	32	
II	33	
III	24	
IV	30	
V	31	
	150	

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

Question I (32 points)

Two DNA structures are shown below, which have double-stranded and single-stranded regions. Answer two questions: **A and B for Structure 1**, or **C and D for Structure 2**. The bacterial and eukaryotic names of a factor with the same function do NOT count as two different factors, but listing the same protein in different parts A,B,C,D is acceptable. There are more correct answers than you will need. Be sure that your answer is specific enough: a general category of proteins (such as "nuclease") is not a sufficient answer *unless* all members of that category of proteins have the same binding specificity, in which case the general category of proteins is a sufficient answer.

STRUCTURE 1

5'_____3'

3'_||||||||||__________5'

STRUCTURE 2

5'_____3'

3'__||||||||||5'

A. List TWO different factors that bind to the junction of double-stranded and single-stranded DNA shown in **Structure 1**. For EACH of the factors, explain why this particular junction structure is recognized in the factor's biological function.

clamp loader needs to load clamp at a polymerase substrate
polymerase substrate for dNTP addition/ 3'OH primer for DNA synthesis
[ok 5'-3' helicase/DnaB could thread on ssDNA moving 5'-3' to reach the junction]
3'-5' Pol I exonuclease activity
pol alpha; no points for primase (RNA-DNA junction not DNA structure as stated)

B. List TWO different factors that would bind directly to the single-stranded DNA shown in **Structure 1** equally as well as or better than the single-stranded DNA in **Structure 2**. For EACH of the factors, indicate why **Structure 1** is the preferred or equal binding substrate.

5'-3' helicase/DnaB could thread on ssDNA moving 5'-3'
SSB/single-stranded DNA binding protein/RPA binds ssDNA without need for other loader
RecA was acceptable, but no points for listing polymerases here (lots of people did) because they don't interact with ssDNA.

C. List TWO different factors that bind to the junction of double-stranded and single-stranded DNA shown in **Structure 2**. For EACH of the factors, explain why this particular junction structure is recognized in the factor's biological function.

BRCA2: binds the junction to load Rad51 in a 5'-3' direction on the ssDNA overhang
5'-3' exonuclease acting at a dsDNA break: end resection of dsDNA for 3' ssDNA overhang
Pol I [5'-3' exonuclease domain, but don't need to say that]: remove DNA from unligated 5' end
[ok 3'-5' helicase/MCM/CMG: could thread on ssDNA moving 3'-5' to reach the junction]

D. List TWO different factors that could bind directly to the single-stranded DNA shown in **Structure 2** equally as well as or better than the single-stranded DNA in **Structure 1**. For EACH of the factors, indicate why **Structure 2** is the preferred or equal binding substrate.

SSB/single-stranded DNA binding protein/RPA binds ssDNA without need for other loader
RecA (ok to say Rad51) assembles 5'-3' towards a free ssDNA 3' end
3'-5' helicase/MCM/CMG complex could thread on ssDNA moving 3'-5'

Question II (33 points)

A. (18 points) The DNA replication sliding clamp has numerous interaction partners. List the interaction partners with requirements detailed below, for a total of 6 interaction partners. There are more correct answers than you will need.

1. List TWO factors that are physically tethered to *E. coli* beta AT THE replication FORK

Pol III, gamma complex/clamp loader (gamma complex and clamp loader are the same answer)

2. List TWO factors that are physically tethered to eukaryotic PCNA AT THE replication FORK

Pol delta, Pol epsilon, RFC/clamp loader (RFC and clamp loader are the same answer)

3. List TWO factors that bind beta or PCNA that are NOT physically linked to the replication fork (your answers can be for one or the other sliding clamp or for both)

DNA ligase (or say DNA ligase I, the eukaryotic DNA replication ligase)
Pol V or say polymerase(s) involved in trans-lesion synthesis (TLS)/error-prone DNA repair
FEN1
DNA Pol I

B. (15 points) Helicases are targeted to their sites of activity by protein-protein interaction. For each helicase listed below, indicate the direct protein interaction partner of the helicase that recruits it to a specific sequence or structure of DNA (please list only DNA binding proteins or protein complexes). ALSO, indicate the DNA sequence or structure that the helicase is targeted to by this DNA binding protein partner.

1. UvrD
UvrAB (also ok to say one or the other - UvrA or UvrB)
substrate for nucleotide excision repair/distorted B-form DNA
OR
MutSL (also ok to say one or the other - MutS or MutL) [MutH is not an acceptable answer]
nick made by MutH (also ok to say a mispair/substrate for mismatch repair)

2. RuvB
RuvA (octamer, don't need to say that for full credit)
Holliday junction/four-way dsDNA junction

3. DnaB
DnaA [DnaC is not an acceptable answer]
oriC, 9 bp repeats/sequence-specific binding site of DnaA, origin of replication

Question III (24 points)

DNA binding proteins use diverse mechanisms to specialize their assembly with DNA.

A. (6 points) List TWO DNA binding proteins that use cooperative protein-protein as well as protein-DNA interaction to assemble on DNA. ALSO, state what DNA structural change the cooperative protein-protein interaction induces in the bound DNA.

DnaA	traps/wraps a positive supercoil [also melts adjacent ATrich DNA, not necessary to say for full credit]
RecA/Rad 51	untwists/stretches/reduces base (or base-pair) stacking

B. (6 points) List THREE proteins or protein complexes that in ATP-bound form have high DNA binding affinity but upon ATP hydrolysis have lower affinity for DNA. The bacterial and eukaryotic names of a protein with the same function do NOT count as two different proteins.

Clamp loader/gamma complex/RFC
DnaA
RecA/Rad51
based on extent of class discussion, ok to answer type II topoisomerase/topo II/topo IV

C. (12 points) List THREE nucleases that recognize DNA directly and act WITHOUT sequence OR DNA damage requirement - in other words, a "normal" structure of DNA in a cell. Make sure that you do NOT answer with a name of a nuclease that is recruited to DNA in part by protein-protein interaction. ALSO, indicate the DNA structure that the nuclease reacts in the cell.

type I topoisomerase/topo I	DNA with linking number different than Lko
type II topoisomerase/topo II/topo IV	respectively: DNA with linking number different than Lko/positive supercoil/catenane (catenated chromosomes)
RuvC	Holliday junction/four-way dsDNA junction
the 5'-3' exonuclease acting at a dsDNA break	dsDNA break

Question IV (30 points)

For EACH of 1-3 below, answer A, B, and C.

A. (3 points) What is a type of DNA damage that will be fixed by the DNA repair pathway? Pick only one example, but be as specific as necessary in description of the substrate.
B. (6 points) State two proteins SPECIFIC for ONLY this repair pathway and in one sentence describe the function/activity of each protein. If you don't remember the name of the protein, give its specificity of binding or activity in sufficient detail to identify it uniquely.
C. (1 point) 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 nucleotides.

1. Nucleotide excision repair (NER)

A. distorted B-form DNA, intrastrand pyrimidine dimer, product of TLS polymerase synthesis across a damaged template (second step of SOS response repair)

B.
UvrA binds damaged DNA (or say UvrAB complex)
UvrB binds damaged DNA (ok to say recruits UvrC or UvrD)
UvrC nicks damaged DNA strand [twice, don't need to say]
[UvrD is not an acceptable answer]

C. 2-40

2. Mismatch repair (MR)

A. incorrectly paired bases, insertion or deletion on one strand (indel)

B.
MutS recognizes mispair
MutL links MutS to MutH
MutH nicks newly synthesized strand [at GATC on unmethylated strand, don't need to say]
(ok to say Dam methylase 6-me-A modification)

C. more than 40

3. Base excision repair (BER)

A.
apurinic (or apyrimidinic) site, missing base
uracil in DNA, deaminated C, damaged base recognized by a glycosylase enzyme

B.
AP endonuclease nicks the backbone adjacent to a missing base
phosphodiesterase removes sugar-phosphate unit
ONLY if the second line in A has the answer you gave in A: (uracil) DNA glycosylase/enzyme that cleaves the glycosidic linkage of the damaged base

C. 1

Question V (31 points)

Consider homologous recombination (HR), site-specific recombination (SSR), non-replicative cut-and-paste transposition (TPN), and VDJ recombination.

A. (8 points) Compare HR to SSR for how strand exchange is initiated.
What determines the specificity of the two DNAs brought together for recombination?
What protein carries out the strand exchange?

FOR HR:
> 50 bp of perfect homology
RecA/Rad51

FOR SSR:
a specific sequence recognized by the recombinase/the recombinase binding sites
the site-specific recombinase enzyme [a tetramer, don't need to say for full credit]

B. (5 points) TPN and VDJ recombination differ in what the recombinase that is removing the transposon or transposon-like DNA does to the DONOR SITE (the host cell DNA flanking the transposon or the protein coding regions flanking a deleted DNA in VDJ recombination). Indicate the structure of the host DNA ends left after recombinase action (after ONLY the recombinase cleavage step(s), not what other cellular factors do subsequently).

FOR TPN: blunt ends

FOR VDJ: hairpin coding ends

C. (18 points) HR, SSR, and TPN differ in the entire recombination pathway requirements for DNA ligase, formation of a covalent protein-DNA intermediate, and whether cleavage reactions are done by hydrolysis and/or strand transfer (in other words, whether water is the only nucleophile or does a chemical group on a DNA strand provide a nucleophile to attack a bond). Fill in the grid below using only the letters N (no) and Y (yes) for a total of TWELVE answers.

	DNA ligase?	Covalent protein-DNA?	Some cleavage by hydrolysis?	Some reaction by strand transfer?
HR	Y	N	Y	N
SSR	N	Y	N	Y
TPN	Y	N	Y	Y