MCB 110
First Midterm
SIX PAGES

NAME:

SID Number:

Question	Maximum Points	Your Points
I	27	
II	32	
III	35	
IV	33	
V	24	
	151	

This exam must be written in PEN if you want the option of a regrade.
DO NOT USE WHITE OUT. If you need a fresh page, ask for it during the exam.

Question I (27 points)

DNA binding proteins can favor a particular conformation of bound DNA in the protein-DNA complex. This structural stabilization of a particular DNA conformation is a crucial function of several DNA binding factors discussed in class. **For EACH of A-C below, answer EACH question 1-3.** Single-line length answers are sufficient, but provide enough detail to identify how the protein is distinct from other proteins discussed in class in its specificity of DNA structural stabilization.

1. What is the DNA structure stabilized by binding of the protein? Answer for only the initial DNA binding event. Include ALL biologically relevant features of the bound DNA.

2. Why is this DNA structural change an important biochemical activity of the protein? The answer should indicate why the structure formed by protein binding is important for the immediately subsequent reaction in the pathway.

3. DNA binding involves multiple protein subunits together, with subunits either preassembled into a multimer or assembled on the DNA by cooperative binding. Why is it necessary for the protein biological function that the structural change involves more than a single protein subunit's length of DNA binding site?

A. RecA

1. Unwound/stretched single-stranded DNA with a free 3'OH. [~18 nt/helix turn, but this is not necessary for full credit] [OK to *also* say that dsDNA is unwound, but not necessary]

2. Promotes homology search/strand exchange/base-pairing with another duplex.

3. Accurate homology search/stable strand exchange requires ~50 nt/a length greater than the binding site of a single RecA.

B. DnaA

1. Positive supercoil at *oriC*/dsDNA wrapping/ something similar gets full credit. Determining sequence specificity of origin binding is not a correct answer.

2. Favors melting/opening of adjacent dsDNA/AT-rich repeats// promotes formation of ssDNA to allow DnaB/helicase loading

3. More than a single DnaA is required to wrap sufficient dsDNA length to change topology

C. RuvA

1. Holliday junction/four-way dsDNA junction

2. Only Holliday junctions should assemble the branch migration machinery/two RuvB need to be loaded

3. There are four dsDNA segments that contribute to a Holliday junction/two RuvB need to be loaded

Question II (32 points)

A. (16 pts) Polymerases that share a feature can differ in other features. For the pair of enzymes listed in 1-2 below, indicate FOR EACH ENZYME the primer and template specificity as RNA AND/OR DNA - one feature will be shared, one will be different for each pair of enzymes.

1. Pol III and Pol delta

(a) Primer Pol III can use RNA or DNA, Pol delta can only use DNA

(b) Template single-stranded DNA

2. Telomerase and terminal deoxynucleotidyl transferase (TdT)

(a) Primer Telomerase and TdT both use single-stranded DNA primers

(b) Template Telomerase uses an RNA template, TdT uses no template

B. (16 pts) Numerous examples of DNA-associated proteins that slide on dsDNA were described in class. **For EACH of the proteins A-D below, answer EACH question 1-2.**

1. What are the biological requirements for loading on dsDNA? Explain with regard to any necessary DNA structure AND/OR any other protein required as a loading chaperone.

2. Is the protein an ATPase?

A. Ku

1. a dsDNA end [no accessory factor required, so if you indicate one, points subtracted]
2. No

B. Sliding clamp (*E. coli* beta or eukaryotic PCNA)

1. Clamp loader was the intended answer. Partial credit for indicating only ds/ss DNA junction, but in this case credit requires indicating 5' ssDNA overhang.
2. No

C. MutS/MutL complex

1. dsDNA. [Loading does not require a mismatch; the complex scans for mismatch after loading, so if your answer says "mismatch" no points given].
2. Yes

D. DnaB

1. Requires single-stranded DNA loading site and also DnaA and DnaC to catalyze loading
2. Yes

Question III (35 points)

Nucleases (more broadly defined as enzymes that nick or cut DNA) must have DNA sequence AND/OR structure specificity to limit their biochemical activity to suit their cellular function. **For EACH enzyme listed in a-g below, answer EACH question 1-3.**

1. (2 pts) What feature(s) of a DNA/RNA substrate are necessary for the action of this nuclease?
2. (1 pt) Is there a covalent protein-DNA intermediate in the reaction?
3. (2 pts) What number of strands (Watsons and/or Cricks) get cleaved in the biologically occurring reaction? Answer 1, 2 or 4. If the biological reaction has concerted action of more than one subunit of the nuclease (*e.g.* a protein dimer), answer for the biologically functional protein multimer.

(a) Type I topoisomerase

1. Lk unequal to Lko/linking number less than in the lowest energy DNA conformation
2. Yes
3. One

(b) Base excision repair glycosylase

1. Modified base in dsDNA
2. No
3. One

(c) Pol I RNA primer degradation activity

1. Free 5' end of RNA base-paired to DNA/Okazaki fragment 5' end
2. No
3. One

(d) Exonuclease processing activity required to allow RecA/Rad51 binding

1. dsDNA end/cleaved/cut
2. No
3. One

(e) Ruv C

1. Holliday junction/four-way dsDNA junction
2. No
3. Two

(f) Mut H

1. GATC sequence of dsDNA with a non-methylated strand [at least one non-methylated strand]
2. No
3. One

(g) Site-specific recombinase enzyme (like phage lambda integrase, for example)

1. Two dsDNA sequence-specific recognition sites
2. Yes
3. Four

Question IV (33 points)

For each of 1-3 below, give answers for A-C:

A. (3 pts) 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. (6 pts) 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. (2 pts) How many DNA strands have to have the phosphodiester backbone ligated as a consequence of *this* pathway of DNA repair? The answer here is one or two (*i.e.*, one or both strands of the Watson-Crick duplex).

1. Nucleotide excision repair

A. Pyrimidine/thymine/cyclobutane/intrastrand cross-link/dimer [or other suitable - e.g. non-BER]
Need to give DNA substrate not just that helix is distorted

B. UvrA/ UvrB or UvrA:B complex: recognize distortion
UvrC: excinuclease needed to include nuclease activity and formation of 12-bp fragment or cleaving on both 3' and 5' ends
NOT UvrD: not specific to pathway

C. One

2. SOS response (OK to answer protein state specific to SOS even if the protein is not specific)

A. Lesion/damage that stalls Pol III or replication fork
single-stranded DNA with damaged template
Any type of damage if mention of last resort to fix
NOT dsDNA break (this is recognized by other pathways first before this one, also must be processed before pathway recognition)

B. UmuC/ UmuD/ Pol V (Pol V not OK answer if UmuC or D is also listed): error prone polymerase
RecA* activation or stimulation of polymerase V (need to mention star otherwise not specific
Stalled polymerase or replication fork→ signal to activate pathway

C. One

3. Non-homologous end-joining (the NHEJ discussed as DNA repair, not VDJ recombination)

A. double-stranded DNA break

B. Ku/ DNA ligase IV/ anything creative that is clearly described: *e.g.* Ku bridging factor
Ku: bind dsDNA end/bridge ligase; DNA ligase IV: join phosphodiester backbones bridged by Ku

C. Two

Question V (24 points)

Two inverted repeat sequences could be subject to four different mechanisms of DNA rearrangement covered in class. For each of the recombination pathways below, indicate the fate of the DNA between the inverted repeats AND the fate of the flanking DNA in the biological process. Brief single-word answers will be sufficient if they are precisely descriptive.

1. One step of V(D)J recombination

Between inverted repeats: Excision
Flanking sequences: Sloppy joining

2. Site-specific recombination

Between inverted repeats: Excision/ inversion [either is a sufficient answer]
Flanking sequences: Precise joining [to re-create recombination site]

3. Transposon excision from the donor site (assume there is no target site integration)

Between inverted repeats: Excision
Flanking sequences: Sloppy joining

4. Inappropriate homologous recombination

Between inverted repeats: 50% chance of inversion, 50% chance of no change
Flanking sequences: Unaffected