MCB 110
Spring 2017 Exam 1
SIX PAGES

NAME:

SID Number:

Question	Maximum Points	Your Points
I	28	
II	32	
III	32	
IV	30	
V	28	
	150	

PLEASE WRITE your NAME or SID number on each page.
This exam must be written in PEN if you want the option of a regrade.
DO NOT USE WHITE-OUT: ask for a clean page of exam to start over if you need it.

Question I (28 points)

A. (+16 points) Considering the many examples discussed in class, list 8 enzymes with a the stated nuclease specificities (give 2, 2, or 4 examples respectively, as requested below). Note that there are more correct answers than necessary to complete the list, but list ONLY the requested number of enzymes. Alternate names for the same nuclease activity are not separate answers.

1. Exonuclease activity that disrupts a phosphodiester bond on only one strand of a duplex
Give two answers:

Answers: 3'-5' proofreading, Pol I 5'-3', enzyme that resects 5'-3' from dsDNA break.
Also accepted: phosphodiesterase, Pol theta (from class content could be possible)

2. Endonuclease activity that acts on undamaged duplex DNA in a sequence-specific manner (other than restriction endonucleases – don't use those in your answers)
Give two answers:

Answers: Mut H, Transposase, recombinase (SSR), RAG1/2
Also accepted: Cas9,

3. Endonuclease activity that is structure-specific but not sequence-specific (OK to list a nuclease that needs to be recruited by another protein to its DNA cleavage site)
Give four answers:

Answers: Topo I, Topo II, Artemis, RuvC, UvrC
Also accepted: AP endonuclease, FEN1, Pol theta (from class content could be possible)

B. (+6 points) Would each of these proteins or protein complexes bind a 5' AND/OR 3' single-stranded overhang junction with duplex DNA? Use only these answers: 5', 3', both, neither.

1. Clamp loader 5'

2. BRCA2 3'

3. DnaB 5'

C. (+ 6) List three proteins or protein complexes that can slide on DNA without complete dissociation. Include at least one single-stranded and one double-stranded DNA binding factor. OK to include proteins that slide with or without ATP involvement.

Double-stranded DNA: MutS (or MutSL complex), PCNA (or beta)

Single-stranded DNA: SSB (or RPA), helicase (or helicase name)

NOT DnaA, RecA, Rad51

Question II (32 points)

A. (+16 points) In class, some DNA polymerases were described that did NOT act in canonical DNA replication (i.e. they do not synthesize primer or replicate an entire leading or lagging strand). Name FOUR, and for EACH indicate general cellular FUNCTION. You can count as separate answers an *E.coli* polymerase and a eukaryotic polymerase with same general cellular function.

Answers:
*Pol V (or Pol IV) -- error-prone repair/trans lesion synthesis/ rescue stalled replication fork/ SOS response (answer SOS only if stated Pol V)
*Pol theta (or mu or lambda) -- NHEJ
*TdT -- VDJ recombination diversity/ add dNTPs to antibody loci segments/ etc.
*Telomerase – telomeric repeat synthesis/ chromosome elongation/ etc.
Also accepted: Pol beta or Pol II -- DNA repair, Pol gamma –mitochondrial DNA replication
Part credit: Pol I (although it functions in Okazaki frag synthesis/primer removal which are not OK function answers, it also has functions in DNA repair)]

B. (+16 points) Diverse enzymes join DNA ends to (re)create an intact phosphodiester backbone. For each example below, answer the questions about the backbone ligation/repair reaction.

<u>1. *E. coli* DNA ligase</u>
(i) What are the 5' and 3' chemical groups required for DNA ends to be joined by ligase activity?
5' monophosphate, 3' OH
(ii) Describe the use of ATP.
ATP is hydrolyzed to <u>covalently link</u> AMP to the enzyme active site and then the <u>5' monophosphate to serve as a leaving group</u>

<u>2. Ligase IV</u>
(i) What is one of the accessory proteins or protein complexes that recruits this ligase to DNA?
Ku, Ku70, Ku80, DNA-PKcs
(ii) What is this ligase's specificity for DNA that is distinct from *E. coli* DNA ligase?
It can ligate strands at a break, not just a nick.

<u>3. Topoisomerase type I</u>
(i) By what number(s) and in what direction(s) will linking number be changed by this enzyme?
+1 or -1 AND towards Lko (both for full credit)
(ii) Why is ATP unnecessary for the reaction?
Covalent protein-DNA intermediate

<u>4. *E. coli* DNA gyrase (a type II topoisomerase that works ahead of the replication fork)</u>
(i) By what number(s) and in what direction(s) will linking number be changed by this enzyme?
-2 OR say + supercoil to – supercoil (change supercoil is part credit only)
(ii) Why does replication fork progression require DNA gyrase activity?
Parental strands must be separated for replication, requiring loss of twist (or related answer)

Question III (32 points)

A. (+20 points) Proteins bind DNA with sequence and structure specificity and impose changes in DNA conformation upon binding. For EACH factor listed below, answer THREE questions:

1. (+2) What is structure and/or sequence **of DNA** that protein will recognize for initial binding? Answer for the DNA BEFORE it is bound to the protein.
2. (+2) How is **DNA structure** changed by enzyme **binding**? Answer for protein-DNA complex, not anything subsequent to enzyme release of bound DNA.
3. (+1) What provides the favorability for change in DNA structure induced by protein binding?

(a) DNA adenosine methyltransferase (Dam1)

1. duplex DNA with GATC sequence (or say sequence-specific) [OK not to say hemimethylated]
2. base that will be modified is flipped out into the enzyme active site
3. protein side chain(s) insert into duplex to replace lost base-stacking

(b) A DNA helicase

1. single-stranded DNA (not sequence specific)
2. duplex is dissociated
3. ATP hydrolysis (induced enzyme conformational changes)

(c) DnaA

1. duplex DNA (9 base-pair) repeats OR *oriC*
2. duplex is wrapped around DnaA AND say as a positive supercoil
3. protein-protein interaction/ cooperative assembly/ filament formation (protein binds to ATP is not OK answer)

(d) RecA

1. single-stranded DNA (not sequence specific) with a free 3' OH
2. stretched/ undertwisted/ less stacked/ able to sample base-pairing interaction with duplex, etc.
3. protein-protein interaction/ cooperative assembly (protein binds to ATP is not OK answer)

B. (+12 points) DnaB loading at *oriC* requires two additional proteins. Describe all of the roles of those two proteins at *oriC* and the roles of ATP binding and hydrolysis for each.

1.
-(hexamer of) DnaC bound to ATP binds (hexamer of) DnaB and change its conformation such that it can load on ssDNA,
-DnaC binds DnaA to place DnaB on one strand of oriC ssDNA,
-ATP hydrolysis releases DnaC from DnaB.

2.
-(filament of) ATP-bound DnaA binds oriC,
-wraps dsDNA to favor melting of ds to ssDNA (in the AT rich repeat),
-interacts with DnaB of BC complex to load one DnaB hexamer,
-interacts with DnaC of BC complex to load the other DnaB hexamer.
-ATP hydrolysis releases DnaA from *oriC*.

Question IV (30 points)

For each of 1-5 below, give answers for A and B:

A. (+2 points) Give ONE example of a form of DNA damage that will be fixed by the listed type of DNA repair. Be as specific as necessary in description of the DNA substrate to make the substrate *most suited to this pathway compared to any other repair pathway covered in class*.

B. (+4 points) State one protein or its specialized enzyme activity (i.e. "nuclease" is not a sufficient answer) SPECIFIC for ONLY this repair pathway, AND in one sentence describe the function/activity of that protein.

1. Mismatch repair

A. mispair of normal bases in duplex; one strand with insertion or deletion relative to the other (say both underlined things for full credit)

B. MutS (sense mispair etc), MutL (link MutS to MutH, OK to say endonuclease), MutH nick unmethylated/ newly replicated DNA strand

2. Nucleotide excision repair

A. bulky lesion on one strand; base modification not recognized by BER, (say both underlined things for full credit)

B. UvrA or B or AB (recognizes damage), UvrC (endonuclease)
UvrD not an acceptable answer.

3. Base excision repair

A. uracil; anything recognized by a glycosylase; an abasic site; depurinated site

B. AP endonuclease (nick DNA backbone next to abasic site), uracil DNA glycoslyase (remove uracil base from DNA), phosphodiesterase (remove sugar-phosphate (at abasic site))

4. Homologous recombination

A. dsDNA break *with resected strand 5' ends* (OR say in *G2 phase*) (include italic for full credit)

B. RecA [is also in SOS response but in a different way], RuvA, RuvB, RuvC. BRCA2, Rad51. Strand exchange, Holliday junction binding/recruit RuvB, helicase for branch migration, HJ resolution/cleavage, nucleate Rad51 assembly, strand exchange, consecutively.

5. Classical NHEJ

A. dsDNA break *that is blunt or near-blunt* (OR say with *no homolog* OR say in *G1 phase*) (include italic for full credit)

B. Ku, Ku70, Ku80 (bind dsDNA ends); DNA-PKcs (recruit Ligase IV or bridge ends); Ligase IV (ligate strands at the break) [Artemis showed up again in VDJ recombination, but OK to answer][Not OK to say Pol theta]

Question V (28 points)

A. (+ 14 points) Strand exchange reactions in *E. coli* homologous recombination are mediated by many proteins including RecA and RuvB. For each protein, answer EACH of the THREE questions below.

1. What is the exchanged strand(s) length? As answers use only 0, 5, 50, or 1000 base-pairs.
2. What DNA structure does the protein bind? Include any important element of DNA structure AND any other DNA-interacting proteins required for recruitment.
3. Describe the role of protein ATP hydrolysis and its implication for strand base-pairing

(a) RecA
1. 50
2. single-stranded DNA with free 3’ OH
3. ATP hydrolysis by RecA allows its release from DNA. because strand exchange is passive, no mispairs are tolerated.

(b) RuvB
1. 1000
2. four-way dsDNA junction bound to RuvA/ Holliday junction bound by RuvA
3. ATP hydrolysis by RuvB helicase forces dsDNA unwinding and heteroduplex creation, so individual mispairs are tolerated.

B. Site-specific recombination
1. (+3 points) Each reaction has 100% probability of recombinant ends. How does the catalysis of SSR ensure this specificity? One sentence could be a sufficient answer.

Both Watson strands and both Crick strands are nicked and ends traded/ recombinase tetramer stays bound to initial site of strand exchange so that two sets of nicks can be made on different strands/ Holliday junction intermediate remains bound to recombinase/etc.

2. (+3 points) Why is there no requirement for DNA ligase? Only need one sentence to answer.

Covalent protein-DNA intermediates

C. Transposition
1. (+4 points) Transposase makes double-strand cuts at the edges of the transposon donor DNA. Are these cuts blunt (even on both strands), 3’ overhang, or 5’ overhang? What implication does this have for the transposon-excised donor DNA ends?

blunt. Donor site ends must be repaired by NHEJ.

2. (+4 points) Transposase makes a double-strand cut in the target DNA. Is the cut blunt, 3’ overhang, or 5’ overhang? What implication does this have for the transposon-flanking target DNA?

5’ overhang. Fill-in synthesis across the 5’ overhang creates target site direct repeat.