

MCB 110
 First Midterm
SIX PAGES

NAME:

SID Number:

Question	Maximum Points	Your Points
I	36	
II	30	
III	24	
IV	33	
V	27	
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	150	

Question I (36 points)

For each of 1-4, give answers to A, B and C:

A. (3 pts) For each enzyme below, note the important feature(s) of its intended DNA substrates.

B. (1 pt) Is there a covalent protein-DNA intermediate? Yes or No.

C. (1 pt) Does the enzyme's function require energy input, e.g. ATP hydrolysis? Yes or No.

	A	B	C
1. type I topoisomerase	Lk unequal to Lk_0	Yes	No
2. DNA ligase	DNA nick with 5' PO ₄ , 3'OH	No	Yes
3. integrase	2 copies of sequence-specific binding site	Yes	No
4. uracil DNA glycosylase	uracil base in dsDNA	No	No

For each of 1-4:

(4 pts each) What are this protein's properties of DNA binding or loading? Indicate all relevant structural requirements including specificity for single and/or double stranded DNA and sequence specificity.

- transposase
Binds sequence-specifically to transposon end inverted repeats
Binds sequence non-specifically to target DNA
- gamma complex
Binds to a dsDNA-ssDNA junction with a recessed 3'OH
(or say a 5' overhang)
- UvrA
Binds to dsDNA with damage that distorts B-form DNA
(or say bulky adduct, etc.)
- RuvB
Binds to Holliday junction (or say 4-way duplex junction) that is held by RuvA

Question II (30 points)

A. (5 pts each) Nucleases are specialized for diverse cellular roles. For each of the three nuclease tasks listed below, describe the nuclease responsible by indicating any important feature of nucleic acid strand specificity and whether activity is exo- or endo- nucleolytic.

1. Removal of RNA primers in *E. coli*

DNA pol I 5'-3' exonuclease: duplexes with a strand having an exposed 5' end, substrate strand can be RNA or DNA

2. Base excision repair

Nicks dsDNA endonucleolytically, at the site of a missing or altered base, on the damaged strand

3. Resolution of a Holliday junction

RuvC recognizes a Holliday junction/4-way dsDNA junction/DNA bound by RuvA and endonucleolytically nicks two strands of the same polarity (either the Watson strands or Crick strands)

B. (5 pts each) Several cases of cooperative protein assembly on DNA were described in class. List THREE examples of cooperative binding of a protein and explain why cooperativity is important for protein function.

1. SSB/RPA must coat ssDNA to prepare for its use as template by Pol III/Pol delta or epsilon (ok to say "protects ssDNA")

2. DnaA must wrap/fold/hold oriC DNA to promote unpairing of adjacent AT-rich region: could also answer that cooperative binding increases specificity of recognition of oriC

3. RecA must make a filament long enough to catalyze exchange of enough ssDNA to generate a stable pairing with one strand of the invaded duplex

Question III (24 points)

Genomic DNA replication is highly regulated. To overcome this regulation, bacteriophages and animal viruses evolved diverse schemes for bypassing particular host DNA replication requirements.

For each of A-C:

List TWO *E. coli* proteins required for chromosome replication that would not be required for replication of the phage or virus. Also indicate why these *E. coli* proteins are no longer required.

A. Some linear phage and virus genomes replicate using a serine side chain from a protein bound to the chromosome end as primer.

1. Primase, not required due to priming from terminal protein
2. DnaA (DnaC accepted as an answer, but not the best), linear genome and terminal protein priming remove requirement for loading of DnaB onto melted duplex at internal site within dsDNA circular chromosome

B. Some circular, single-stranded phages replicate by making a genome-complementary single-stranded circular DNA and using this as a template for synthesis of the single-stranded phage genome.

1. DnaB, not required due to single-stranded template
2. DnaA (or DnaC) no duplex DNA at origin, no need to load DnaB

C. T7 phage encodes its own DNA polymerase for genome replication. This DNA polymerase uses a completely different mechanism for processivity: it borrows an unrelated *E. coli* host protein that binds to T7 DNA polymerase to close off the top of the polymerase active site cleft, trapping a bound primer-template duplex in the cleft.

1. DNA pol III, not required due to T7 phage's own genome replicative polymerase
2. beta/sliding clamp, not required due to different mode of processivity
3. gamma complex/sliding clamp loader, not required due lack of requirement for sliding clamp

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. (3 pts each) State two proteins **SPECIFIC for ONLY this repair pathway** and in one sentence describe the function/activity of each protein.

C. (2 pts) How much DNA will be synthesized to during repair of the damage? To make it simple, choose between these options: 0, 1, 2-40, or more than 40 nt.

1. Nucleotide excision repair

A damage on one strand that distorts B-form DNA structure

B UvrA, B, C, D: recognize damage, catalyze endonucleolytic cleavage of damaged strand, remove damaged strand by helicase activity

Note that DNA pol I is not a valid answer, because it would be used for BER as well.

C 2-40

2. Mismatch repair

A mispairing of normal bases

B MutS, recognizes mispair

MutH, nicks damaged strand

MutL, activates MutH when MutS bound nearby

OK to indicate a helicase specialized for this process, or the Dam methylase

C more than 40 nt

3. SOS response

A damaged template ahead of replication fork

B UmuC polymerase subunit for repair of faulty template

UmuD (or D') polymerase subunit for repair of faulty template

note that RecA, Pol III, SSB, etc. are not valid due to use for ds break repair as well

(RecA* is OK, or parts of the signalling pathway)

C 2-40 (but more than 40 can be given full credit as well)

Question V (27 points)

For each of 1-3 below, answer questions A-C:

- A. (4 points) Are there specific DNA sequence/structure requirements for this reaction? If so, what are they and what is the role of the sequence/structure?
- B. (2 points) Does this complete process require ATP (at the level of detail covered in class)? Yes or No.
- C. (3 points) Describe the change in donor and target sequences.

1. Strand exchange by RecA

- A. No DNA sequence requirement, but requires HOMOLOGOUS donor and target. Donor should have a least 50 nt of ssDNA with a free 3' OH.
- B. yes
- C. Exchange of pairing by strands with perfect complementarity

2. Site-specific recombination by an invertase

- A. Inverted sequence-specific recognition sites. Bind site-specific recombinase and exchange parental for recombinant ends (small region of strand exchange)
- B. no
- C. Recognition sites are regenerated, but intervening sequence is inverted

3. Non-replicative transposition

- A. Inverted sequence-specific recognition sites on the donor and non-specific target DNA. The former bind transposase to initiate strand cleavages and the later receive insertion of transposon.
- B. yes (for donor DNA repair, not for transposase itself)
- C. Donor loses transposon, target gets transposon with target site duplication