(1) State how you could demonstrate this based on sequence information present in the DELETED DNA segment in normal skin.
Deleted DNA segment should NOT be bordered by inverted repeat (transposase binding site).
(2) State how you could demonstrate this based on sequence information from the JOINED DNA region in albino skin.
Joined site should have NOT have a short direct repeat (created by transposon insertion).
B. (6 pts) **How could you demonstrate from sequence information that the event did NOT occur by homologous recombination?**
Region surrounding the deleted segment borders in the normal color skin should have LESS THAN 50 bp of perfect homology.
C. (14 pts) **You claim that the event occurred by site-specific recombination. (1) State what you expect to find in the sequences of normal AND albino skin.**
Normal skin: two copies of a specific sequence overlapping the borders of the segment to be deleted. Albino skin: one copy of the specific sequence joining the flanks of the deleted segment.
(2) Describe an experiment to demonstrate the presence of an appropriate site-specific recombinase enzyme activity in cell extracts. Be sure to use any controls necessary to demonstrate that the activity derives from a recombinase specific for the rearrangement detected in the patient family.
Use a plasmid with the specific sequence noted in C) AND a plasmid without this sequence. Demonstrate that plasmids with the specific sequence but not plasmids without it can be joined to make multimer-sized plasmid in the presence of extract. You could also include a control showing that no ATP is required.

Question I (32 points)

For each of 1-8, give answers to A, B and C:

A. (2 pts) For each enzyme or enzyme complex listed below, note the important feature(s) of its intended DNA substrates.

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

C. (1 pt) Does the enzymatic activity require ATP? Answer Yes or No.

	A	B	C
1. UvrB	Distorted B-form DNA (can be caused by TT dimer) bound by UVRA	No	No
2. UmuD'C	ds DNA with interstrand linkage, with stalled replication fork and bound by RecA	No	No
3. Type I topoisomerase	~~dsDNA~~	Yes	No
4. DNA ligase	binds ~~dsDNA~~ ssDNA w/ phosphodiester linkage missing between two adjacent sugars of the 2 nucleotides. One base has monophosphate (P), while another has 3'OH	Yes	Yes
5. Restriction enzyme	at dsDNA where there is no methylated base: 6MeA or 5MeG.	No	No
6. Integrase	specific sequences on two dsDNA. The two have common region normally called O, in between two specific sequence	Yes	No
7. Gamma (γ) complex	at the dsDNA/ssDNA junction	No	Yes
8. Uracil DNA glycosylase	ssDNA w/ a base replaced by Uracil.	No	No

Question II (30 points)

A. (12 pts) Helicases are specialized for their diverse cellular roles. For each of the three helicases listed below, answer (1) cellular function of this helicase; (2) a DNA-bound factor that is importanting for recruiting this helicase; (3) ideal helicase processivity: select <100 bp //100-10,000 bp// >10,000 bp

	(1)	(2)	(3)
UvrD	Unpairs ds DNA during nucleotide excision repair,	UvrA	<100 bp
RuvB	Acts during branch migration to make heteroduplex	~~RuvC~~ RuvA	100 ~ 10,000 bp
DnaB	Unpairs ds DNA during DNA replication	DnaA	> 10,000 bp

12/12

B. (9 pts) Nucleases are specialized for their diverse cellular roles. For each of the three nuclease tasks listed below, describe the nuclease responsible with (1) any important polarity or strand-specificity (2) exo- or endo-nucleolytic.

	(1)	(2)
Removal of RNA primers in E. coli	Recognize RNA primer, 5'→3'	exo.
Base excision repair	Recognize lesion such as ~~methylated~~ O6MeG. 3'→5'	endo
Processing of DNA breaks in eukaryotes prior to repair	No strand specificity 5'→3'	exo.

7/9

C. (9 pts) E. coli DNA has "hot-spots" for homologous recombination. One mechanism for initiating strand exchange at these hot-spots would be to displace single-stranded DNA with a free 3' end.

(1) Suggest how an endonuclease could recognize a particular sequence of dsDNA and nick one of the two DNA strands in specific (note: methylation is known not to play a role – only the sequence itself matters).

There could be a TT dimer in one of the strand and that will cause nick at that strand.

(2) At this nick, if the helicase loads on the intact strand (opposite the nick), what polarity should it have to create a free 3' end? 3'→5'

0/9

Question III (33 points)

Initiation of genomic DNA replication at a cellular origin is highly regulated. To overcome this regulation, bacteriophages and animal viruses have evolved unique schemes for starting replication of their genomes. Some features of chromosomal replication are shared and some are bypassed in these simple genome replication systems. For each of A-C, answer questions 1-2:

1. (3 pts) Do you expect a functional coupling of two replicative DNA polymerases? **Why?**
2. (8 pts) List two factors (e.g. enzyme activities or DNA binding proteins) that should be encoded by the phage or virus genome to substitute for factors of host cell replication machinery. For each, **explain why** the substitution is necessary.

A. Some linear, double-stranded phage and virus genomes replicate using a serine side chain from terminus protein as primer.

+2 1) Yes, Pol I will extend a few bases after the primer (<10bp) and Pol III will have to come in to extend longer bp

+8 2). a) Helicase → the priming mechanism by phage/virus might not be able to couple w/ helicase used by cells, but ds DNA have to unpar. for replication.

b) ~~Pol I~~ : Pol I: the priming mechanism or helicase might not be able to couple w/ polymerase used by cells.

B. Some circular, single-stranded phage genomes replicate by rolling circle: a complementary DNA strand is made, circularized, and then used as template for continuous synthesis of many tandem copies of the single-stranded genome.

+3 1) ~~Yes, Pol I has to extend a few bp and Pol III can come in.~~ No, DNA will be synthesized w/ leading strand.

+8 2). a). Primase → DNA synthesize needs a primer and the phage might not be able to use primase by cells to make the first few bp.

b) β-dimer (clamping for processivity): To keep pol III on the ss DNA during replication. ~~Because~~ Phage might not be able to use cell's clamping mechanism, which normally link w/ helicase.

C. Some circular, double-stranded phage and virus genomes have specific origin sequences that function like chromosomal origins under different regulation.

+3 1) Yes, one polymerase extend lagging strand, while another extend leading strand.

+7 2) a). DnaA = The phage ~~have~~ has to recognize the specific origin, and needs enzyme that resembles DnaA

b) helicase, DNA has to unpur for replication to occur.

24

Question IV (27 points)

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

A. (3 pts) Give an example of specific 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 to make sure that this particular DNA repair pathway and NO OTHER ONE is optimal.

B. (4 pts) State one protein **SPECIFIC for ONLY this repair pathway** and in one sentence describe the function/activity of the protein.

C. (2 pts) How much DNA will be synthesized during the repair? To make it simple, choose between these options: 0, 1, 2-100, or more than 100 nt.

1. **Base excision repair**

3 A A base thats replaced by Uracil. There is damage only on one strand, no direct repair enzyme can fix this.

4 B glycosylase → It breaks the bond between Uracil and sugar

2 C 1.

2. **SOS response**

3 A Intrastrand linkage caused stalled replication fork in E-Coli. SOS response will make polymerase able to bypass lesion and new DNA can still be made. There is damage on both strands so cant use any of the single strand damage repair methods.

9 B Umu D - acts like a polymerase, but can skip the lesion area and add bp across lesion.

0 C 2 ~ 100

3. **Direct repair**

3 A O6MeG. The G is methylated at wrong place and direct repair is most efficient b/c it requires 0 DNA synthesis

3 B methyl transferase - Remove the methyl group on the guanine.....

2 C 0

Question V (28 points)

You have earned a prestigious medical research fellowship. In your fellowship studies, you are investigating the molecular basis for mosaic skin pattern in a patient family. You discover that cells from patches of albino (no pigment) skin are missing a region of DNA at one locus that is present all cells in normal-color skin. With your comprehensive knowledge of DNA rearrangement mechanisms, you set out to characterize the basis for DNA loss.

A. (8 pts) How could you demonstrate from sequence information that the event did NOT occur by transposition? Your answer should have two parts.

(1) State how you could demonstrate this based on sequence information present in the DELETED DNA segment in normal skin.

2/4 Transposons have to have two direct repeats. The deleted DNA segment in normal skin will not be flanked by the direct repeat true but what would be present if it left

(2) State how you could demonstrate this based on sequence information from the JOINED DNA region in albino skin.

~~Non duplicative transposition will have to inverted repeat~~ Non-duplicative transposition will have two direct repeat regions close together adjacent to each other after transposon leaves. This would be present

6/8/9/14

B. (6 pts) How could you demonstrate from sequence information that the event did NOT occur by homologous recombination?

5/6 For a segment to be excised by homologous recombination, the sequence has to be > 30 bp. If the missing segment is < 30 bp, this is not homologous recombination

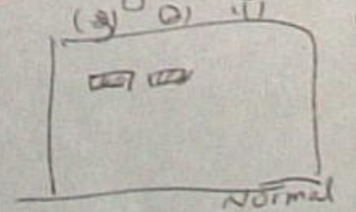
C. (14 pts) You claim that the event occurred by site-specific recombination.

(1) State what you expect to find in the sequences of normal AND albino skin.

4/7 To excised by site specific recombination, there has to be 2 site specific sequence right after and right before the missing sequence. Normal chromosome have OK the 2 direct repeats, but chromosomes of albino patient will: The will be gone. Also, there will be common region within the site-specific sequence

(2) Describe an experiment to demonstrate the presence of an appropriate site-specific recombinase enzyme activity in cell extracts. Be sure to use any controls necessary to demonstrate that the activity derives from a recombinase specific for the rearrangement detected in the patient family.

3/7 We can obtain the cell extracts from both normal and albino patients. We can also isolate the site specific recombinase enzyme. Then we run all 3 in SDS-PAGE, Western blot w/ antibodies specific for recombinase enzyme. the gel will appear:



(1) = cell extract from normal people

(2) = cell extract from albino patient

(3) = site-specific recombinase enzyme.

The control is the normal people's extract

FO3 Midterm Answers

Question I (32 points)

A. (2 pts) Note the important feature(s) of its intended DNA substrates.
B. (1 pt) Is there a covalent protein-DNA intermediate?
C. (1 pt) Does the enzymatic activity require ATP?
Grading note: dsDNA is same as B-form DNA and is OK to draw in all cases

1. UvrB

A) the damaged strand (+1) of dsDNA bound by UvrA (+1)
(also ok: DNA damaged on one strand; NER substrate)
B) No
C) No

2. UmuD'C

A) primer annealed to damaged template (+1) at stalled replication fork (+1)
B) No
C) No

3. Type I topoisomerase

A) Lk unequal to Lko (+2)
B) Yes
C) No

4. DNA ligase

A) 5' phosphate and 3' OH (+1 only for both) of nicked dsDNA (+1 for ds)
B) No
C) Yes

5. Restriction enzyme

A) specific sequence (+1) of dsDNA (+1)
B) No
C) No

6. Integrase

A) two copies (+1) of specific dsDNA sequence (+1)
B) Yes
C) No

7. Gamma (γ) complex

A) primer-template duplex (OK to draw this; only +1 for ssDNA template alone)
B) No
C) Yes (enzyme activity is to load sliding clamp)

8. Uracil DNA glycosylase

A) Uracil base (+1) in dsDNA (+1)
B) No
C) No

Question II (30 points)

A. (12 pts) Answer (1) cellular function of this helicase (+2); (2) a DNA-bound factor that is importanting for recruiting this helicase (+1); (3) ideal helicase processivity: select <100 bp //100-10,000 bp// >10,000 bp (+1)

UvrD

(1) unwind (or displace) damaged DNA strand in nucleotide excision repair (NER)
(2) UvrA (UvrB,C are ok, perhaps not ideal as they might block loading)
(3) <100 bp

RuvB

(1) Homologous recombination
(2) RuvA
(3) 100-10,000 bp

DnaB

(1) genome replication
(2) DnaA (note: not DnaC, because not DNA-bound)
(3) >10,000 bp

B. (9 pts) Describe the nuclease responsible with (1) any important polarity or strand-specificity (2) exo- or endo-nucleolytic.

Removal of RNA primers in E. coli

(1) cuts RNA primer in RNA-DNA duplex (+1) 5'-3' polarity (+1)
(2) exo (+1)

Base excision repair

(1) cuts dsDNA backbone on one strand (+1) at an abasic OR apurinic OR apyrimidinic OR AP site (+1)
(2) endo (+1)

Processing of DNA breaks in eukaryotes prior to repair

(1) cuts 5'-3' polarity (+2)
(2) exo (+1)

C. **Suggest how an endonuclease could recognize a particular sequence of dsDNA and nick one of the two DNA strands in specific (note: only the sequence itself matters).**

Proteins can recognize undistorted dsDNA in a manner that is sequence-specific by interaction of *side chains with base-pairs in the major groove*.(+3) If sense and antisense *strands have different sequence* (+2), one strand will be positioned differently than the other.

At this nick, if the helicase loads on the intact strand (opposite the nick), what polarity should it have to create a free 3' end?

(+4) 5' to 3'

Question III (33 points)

1. (3 pts) Do you expect a functional coupling of two replicative DNA polymerases? Why?
2. (8 pts) List two factors (e.g. enzyme activities or DNA binding proteins) that should be encoded by the phage or virus genome to substitute for factors of host cell replication machinery. For each, explain why the substitution is necessary.

A. Some linear, double-stranded phage and virus genomes replicate using a serine side chain from terminus protein as primer.

(1) No, because no lagging strand synthesis can occur with terminal priming.
(2) Terminal protein substitutes primase, DNA polymerase substitutes cellular one to allow elongation from terminal protein.

B. Some circular, single-stranded phage genomes replicate by rolling circle: a complementary DNA strand is made, circularized, and then used as template for continuous synthesis of many tandem copies of the single-stranded genome.
(1) No, because the product is a single-stranded genome.
(2) Large number of possible answers are possible but not necessary: specific primase, genome circularizing enzyme, DNA polymerase that strand-displaces, DnaB loader, etc.
C. Some circular, double-stranded phage and virus genomes have specific origin sequences that function like chromosomal origins under different regulation.
(1) Yes, by analogy to cellular origin function.
(2) Substitutes for functions of DnaA and DnaC, to mark origin and help load helicase.

Question IV (27 points)
A. (3 pts) Give an example of specific 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 to make sure that this particular DNA repair pathway and NO OTHER ONE is optimal.
B. (4 pts) State one protein SPECIFIC for ONLY this repair pathway and in one sentence describe the function/activity of the protein.
C. (2 pts) How much DNA will be synthesized during the repair? To make it simple, choose between these options: 0, 1, 2-100, or more than 100 nt.

1. Base excision repair
A) AP site OR uracil OR base substitution recognized by a specific glycosylase
B) AP endonuclease (nicks DNA backbone) OR uracil DNA glycosylase (removes damaged base)
C) 1

2. SOS response
A) interstrand cross-link OR damage to both strands OR damage that stalls replicative polymerase
B) UmuD OR UmuC (act as polymerase) OR LexA (note: not SSB, RecA, Pol III)
C) more than 100

3. Direct repair
A) O6methylG OR thymine dimer OR damage recognized by specific damage transferase (note: methylation is not a sufficient answer)
B) O6methylG transferase (transfers damage from DNA to enzyme) or photolyase (reverses thymine dimer intrastrand cross-link)
C) 0

Question V (28 points)
You have earned a prestigious medical research fellowship. In your fellowship studies, you are investigating the molecular basis for mosaic skin pattern in a patient family. You discover that cells from patches of albino (no pigment) skin are missing a region of DNA at one locus that is present all cells in normal-color skin. With your comprehensive knowledge of DNA rearrangement mechanisms, you set out to characterize the basis for DNA loss.
A. (8 pts) How could you demonstrate from sequence information that the event did NOT occur by transposition? Your answer should have two parts.

MCB 110
First Midterm
October 2, 2003
THERE ARE FIVE QUESTIONS (SIX PAGES)

NAME: Mike Liu

ID Number: 14723745

Question	Maximum Points	Your Points