

22 pts 5. Homologous Recombination

(4) a. Define heteroduplex DNA.

Heteroduplex DNA is double-stranded DNA where one strand came from one DNA source and the second strand came from a different source. The two strands often have very close but not identical complementary sequences.

(4) b. How does heteroduplex DNA arise during homologous recombination?

Heteroduplex DNA arises during homologous recombination during the branch migration event. During this event, migration of the Holliday Junction produces DNA, one strand of which came from one sister chromatid, the other from the other sister chromatid; hence, heteroduplex DNA is formed.

(4) c. Briefly describe the reaction(s) that RecA protein catalyzes in homologous recombination.

RecA catalyzes TWO reactions:

1) strand displacement: displacement of the 5' end strand resulting from the initial nick in the DNA of one sister chromatid in the Meselson-Radding model

2) strand assimilation: uptake of this displaced strand into the DNA of the other sister chromatid, thereby forming a 'D-loop' structure.

(4) d. Briefly describe the function of RecA protein in the SOS response.

RecA functions in SOS in the "activated RecA" or RecA* form to catalyze the hydrolytic cleavage and hence inactivation of the repressors LexA and UmuD (and lambda CI if the cell is lysogenic for phage lambda). RecA* may either be a protease, or it may activate latent protease activities in these repressors ("self-destruct" model).

(6) e. Diagram how 3:1 segregants can arise from homologous recombination during meiosis.

Homologous recombination with alleles d and D in heteroduplex region => mismatch

	d:d	d:D	D:d	D:D
First Repair: d -> D ...	d:d	d:D	D:d	D:D
Second Repair: d -> D ...	d:d	D:D	D:D	D:D

Segregation then of: 6:2 = 3:1 for D:d segregants

10 pts 6. Telomeres:

(4) a. Telomerase is essentially absent in adult human cells. How is this detrimental to humans?

In the absence of telomerase, the telomeric DNA will get progressively shorter with each cell division and replication cycle. This will continue until the telomeres are gone, and then genes will begin to be lost. These cells then are mutated.

This absence of telomerase has been implicated in the aging process.

(6) b. Briefly describe the structure and function of Telomerase.

Telomerase is a ribonucleoprotein; it has both Protein and RNA components. The protein part catalyzes synthesis of the T,G-rich strand of telomeric DNA and the RNA serves as a C,A-rich template for this DNA synthesis. Telomerase is thus a reverse transcriptase, containing the RNA template for the reaction as part of the enzyme.

- (6) c. What is the function of the DnaN 'beta clamp' protein in *E. coli* DNA replication, and how does it work?

The DnaN beta clamp functions to hold the rest of HoloDNApolIII on the DNA during replication, thus greatly increasing processivity of the reaction.

It works as a homodimer, where each subunit is a crescent shaped protein that wraps around half of the DNA duplex. The dimer thus completely encircles the DNA (see Lodish, Fig. 10-13). The dimer interacts with HoloDNApolIII via protein-protein interactions, thereby holding HoloDNApolIII on the DNA.

- (6) d. What are the primary differences between the prokaryotic DNA 'replicase' and the eukaryotic DNA 'replicase'?

The prokaryotic DNA 'replicase' is HoloDNApolIII. This contains two copies of the core PolIII complex, each responsible for DNA synthesis, one on the leading strand, the other for the lagging strand. The lagging strand core has additional proteins, required for coordination during O.frag synthesis on the lagging strand. O.frag primer synthesis is catalyzed by a SEPARATE protein, DnaG primase.

The eukaryotic DNA 'replicase' is composed of TWO separate DNA polymerases, Pol-alpha for the lagging strand and Pol-delta for the leading strand. O.frag primer synthesis is catalyzed by the primase activity of Pol-alpha, NOT by a separate protein.

- (6) e. What are the two 'problems' and their solution in DNA replication that have to do with Okazaki fragments?

1. 3'→5' DNA synthesis problem - no known DNA polymerase can catalyze this reaction
Solution: discontinuous synthesis of O.frag in 5'→3' direction on lagging strand.

2. Primer for initiation of O.frag synthesis - DNA pols require a primer for DNA syn.
Solution: use of an RNA primer, catalyzed in *E. coli* by DnaG primase.

- (8) f. Diagram the structure of a yeast (*S.cerevisiae*) replication origin, and state the function of each element.

See Lodish, Fig. 10-8, and Journal Article 2, Fig. 2.

Also see key available from Soft Reserves or posted outside Smith's office, 5254 Muir Biology Building.

Four elements: see Lodish, Fig. 10-8, and J.Art.2, Fig. 2

A element - most conserved site - binds ORC proteins

B1 element - binds ORC (Origin Replication Complex) proteins

B2 element - an A,T-rich DUE (DNA Unwinding Element) site for origin unwinding

B3 element - binds transcription factor

8 pts 4. DNA Repair:

- (4) a. Briefly describe transcription-coupled repair.

Transcription-coupled repair is the selective repair of DNA damage found on the template strand (antisense strand) of the DNA used for transcription. This DNA is then DNA found in genes, the DNA used as template for synthesis of primary transcripts.

- (4) b. How does transcription-coupled repair in prokaryotes differ from that in eukaryotes?

In prokaryotes, RNA polymerase pauses at DNA damage and falls off as part of the transcription-coupled repair process. New transcription events then begin again on the repaired gene.

In eukaryotes, RNA polymerase II pauses at DNA damage in genes, but backs off away from the damage, permits the damage to be repaired, and then continues the transcription event. It does not fall off the DNA.

- (4) d. How does a retrovirus solve the problem of replication to the end of the linear genome?

Do NOT explain or diagram completely the replication mechanism for the proviral DNA.

Replication to the end of the linear genome is solved by use of TWO primers and by presence of a direct repeat at the ends of the linear genome, the R region.

Leading strand replication proceeds to the end of the RNA genome, the RNA is removed by RNaseH, circularization or joining of two replication intermediates occurs via R region hybridization, and replication goes to completion on the circularized or joined replication intermediate.

- 8 pts 2. Briefly describe how hemimethylated GATC sites function in each of the following two processes.

Do NOT describe the complete process for either of the two processes.

- (4) a. E. coli initiation of DNA replication.

Hemimethylated GATCs in *oriC* of E. coli, resulting from initiation, function to PREVENT a SECOND initiation event from that *oriC*, until the hemimethylated GATCs are converted into fully methylated GATCs by the DAM methylase.

- (4) b. E. coli mismatch repair

Hemimethylated GATCs function in mismatch repair to provide the strand discrimination for the nicking reaction of MthH, to insure that the daughter strand that received an incorrect base during DNA replication is the strand that gets repaired.

- 44 pts 3. DNA replication:

- (12) a. In a CsCl density shift experiment from heavy (squiggly line DNA) to light (straight line DNA) medium, diagram the product DNA for conservative, semiconservative, and dispersive (50% synthesis of each new DNA strand per generation) replication modes after 1) 1.0 generations and 2) 2.0 generations.

Conservative

Semiconservative

Dispersive

See key available from Soft Reserves or posted outside Smith's office, 5254 Muir Biology Building.

For dispersive, there are several possible answers which must satisfy the following:

1. total amount of squiggly line DNA remains constant: one chromosome worth
2. 50% of each parental DNA strand is dispersed (or distributed) to new daughter DNA per generation.

One possible correct answer is shown on the key.

- (6) b. A 4000 bp plasmid replicates unidirectionally from an Origin located 1000 bp from a unique HindIII site in the direction AWAY from the HindIII site, as shown. Diagram the replication intermediates seen via electron microscopy of isolated replicating plasmid DNA cut at the HindIII site.

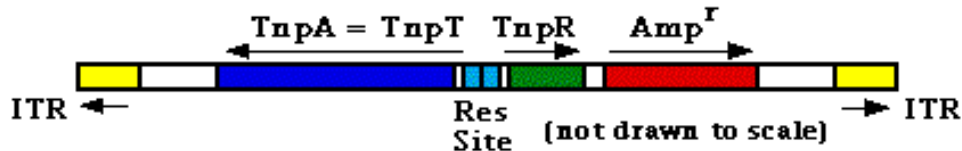
See Lodish, Fig. 10-6, and see key available from Soft Reserves or posted outside Smith's office, 5254 Muir Biology Building.

48 pts 1. Transposons etc:

a. Consider a noncomposite TnA transposon Tn13 encoding resistance to kanamycin that transposes via a replicative mode of transposition.

(12) 1) Draw the structure of Tn13 including the essential genetic elements. Label these elements.

See figure in Lecture Notes on Web, under Genetic Mobile Elements, at <http://www.biology.ucsd.edu/classes/bimm100.FA99/X.ISTns.html#XE>
Kan-resis here instead of Amp-resis



(10) 2) Briefly (few words) state the function of each of these elements.

ITR - Inverted Terminal Repeats: required for Transposase function

TnpA=TnpT - Transposase: catalyses transposition

TnpR - Resolvase / Repressor: 1) acts at Repressor at Promoters in Res site
2) Resolvase catalyses resolution of the Cointegrate formed in Rep Trans.

Res Site: site containing 3 binding sites for Resolvase and containing Promoters for TnpA and TnpR genes

Amp - Gene encoding Protein conferring antibiotic resistance (Ampicillin here)

(12) 3) Draw the mechanism of replicative transposition UP TO the point of formation of the cointegrate.

See Lodish, Fig. 9-18, and see key available in Soft Reserves and posted outside Smith's office door, 5254 Muir Biology Building

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(6) b. Briefly describe how each of the three types of transposable elements found in eukaryotes DIFFER from each other.

Transposon - similar to bacterial transposons, no RNA intermediate in transposition

Viral Retrotransposon - transposes via RNA intermediate, has genes homologous to those of retroviruses (not all are functional) - see Lodish, Fig 9-23

Nonviral Retrotransposon - transposes via RNA intermediate, does not have genes homologous to those of retroviruses - Lodish, Fig. 9-30

(4) c. In one sentence, briefly describe two mechanisms whereby a eukaryotic transposon can activate a protooncogene, thereby causing cancer.

See Lodish, Fig 26-24:

A eukaryotic transposon can activate protooncogenes by

1) insertion into the promoter of the protooncogene, or

2) by insertion into an enhancer controlling expression of the protooncogene.