

- (8) b. Diagram the steps used in nonreplicative transposition.

See Lodish, Fig. 9-17.

1. Transposase nicks BOTH DNA strands at BOTH ends of Tn or IS in donor DNA.
2. Transposase nicks one DNA strand at each end of 5-10 bp Direct Repeat in target DNA so as to yield staggered nicks in target DNA
3. Transposase ligates released Tn or IS from donor to ssDNA overhangs in target DNA, yielding gapped DNA on either side of Tn or IS
4. Transposase ligates ends of donor DNA together: direct repeats remain in donor
5. DNA Pol I fills in the gaps in the target DNA, thereby duplicating the direct repeats in the target DNA on either side of the Tn or IS
6. Ligase seals the nicks.

- (4) c. Briefly describe how transposition of a retrotransposon is similar to replication of a retrovirus.

Compare Lodish, Fig. 9-14, with Lodish, Fig. 9-24

Transposition of a retrotransposon occurs via an RNA intermediate. Hence, the first step is similar to gene expression from the DNA of the retrotransposon. Conversion of the RNA intermediate into double-stranded DNA is, however, for viral retrotransposons identical to replication of a retrovirus (i.e., the steps whereby the RNA genome of the retrovirus are converted into the dsDNA provirus).

- (4) d. What novel gene expression mechanism is present in expressing retroviral genes?

The retroviral gag, pol, and env "genes" are all expressed as polyproteins. These polyproteins are then cleaved and matured into the final polypeptide chains. Each of these three "genes" is in fact several genes.

12 pts 7. In the Gross et al article, sigma-32 protein was purified and shown to be the product of the htpR gene.

- (6) a. Briefly describe the assay used for sigma-32 activity during its purification.

This was the Run-Off RNA transcription assay described in Fig 2. In this assay, RNA transcription is permitted to occur in vitro from some promoter, using linear DNA as a template (here, the linearized DNA were SacI or PvuI fragments). RNA transcription proceeds from the promoter to the end of the DNA and then "runs off" the DNA end. In a well-designed experiment, this will yield an RNA of specific length from each linear DNA template. In vitro RNA transcription is permitted to occur in presence of radioactive rNTP precursor, yielding radioactive RNA product, whose size is determined via PAGE (PolyAcrylamide Gel Electrophoresis) and autoradiography.

- (6) b. Design an experiment using PCR that would show that sigma-32 protein was the product of the htpR gene, assuming you have purified the sigma-32 protein.

Given that you have purified Sigma-32 protein, do a reverse genetics experiment:

1. Determine the sequence of part of the protein.
2. Create two mixed probe DNA molecules, one for each strand.
3. PCR amplify between these two probes, using E. coli DNA as template.
4. Use the PCR amplified DNA to isolate the E. coli DNA containing the sigma-32 gene.
5. Determine the sequence of this gene and show that it is identical to that of the htpR gene.

- (4) c. What is the structure and function of telomerase?

Telomerase is a ribonucleoprotein: RNA component and protein component.
Its function is to synthesize the first (T,G)-rich DNA strand found in the telomeric regions at the ends of chromosomes in a eukaryotic organism.
It does this by using the RNA as template for a reverse transcription type of polymerization step, coupled with a slippage mechanism to synthesize this DNA strand.

16 pts 5. Generalized Recombination:

- (2) a. Name TWO major cellular processes in which Strand Assimilation is important.

Generalized Recombination

Post-replication Recombination-mediated DNA repair

Sister Chromatid Exchange

Heteroduplex DNA often is created during generalized recombination.

- (4) b. What is heteroduplex DNA?

Heteroduplex DNA is dsDNA in which the sequence of the two strands is not precisely complementary (NOT identical, but complementary).

- (2) c. What step in generalized recombination creates heteroduplex DNA, and what E. coli protein does this?

The branch migration step, catalyzed by RuvAB.

- (4) d. Repair of heteroduplex DNA can lead to segregation ratios of 5:3. Briefly describe or diagram how this can occur.

See Soft Reserve notes, p. 55. In meiosis, after 2 stages of segregation, have four chromosomes. After gen recomb and branch migration between two of these, get a heteroduplex region encompassing allele D on one strand and d on the other strand:

D:D D:d d:D d:d

Let mismatch repair now recognize the mismatch in the first recombinant D:d and repair it, using the D strand as template:

D:D D:D d:D d:d

Segregation will now yield a 5:3 ratio of the D:d alleles.

- (4) e. What is the difference between generalized and site-specific recombination? Give an example of site-specific recombination.

Generalized Recombination can occur between any two DNA molecules of nearly identical sequence. Site-Specific Recombination events require specific sequences for recombination to occur.

Examples of Site-specific recombination:

Lambda integration at the bacterial B-O-B' site.

22 pts 6. Genetic Mobile Elements:

- (6) a. Draw the structure of a typical procaryotic composite transposon. Include rough lengths.

See Lodish, Fig 9-19

1. IS elements (500-2000 bp) at either end, each with Inverted Repeats (~ 50 bp) at both ends, one at least encoding a Transposase gene
2. DNA in middle, encoding at least one antibiotic-resistance gene and perhaps a Resolvase, yielding a total length for the Tn of 2-7 kb.

30 pts 3. DNA Repair:

(6) a. Briefly describe how the SOS response is turned on. Include protein names.

Products from repair of damaged DNA, probably short oligodeoxynucleotides, activate RecA protein: RecA → RecA*. RecA* activates protease activity indigenous to LexA and UmuD repressors, which then self-destruct. Genes under control of these repressors are then expressed; this is the SOS response. Those under LexA control (din genes) include *lexA* and *recA* themselves, as well as *uvrAB*, *suaA*, and others. Those under control of UmuD include *umuB* and *umuC*, which control UV mutagenesis.

(6) b. What is meant by Error Free DNA repair? What DNA repair system is Error Prone and why?

Error Free DNA repair is DNA synthesis associated with DNA repair that is high fidelity DNA synthesis, i.e. the mistake rate is very low. Hence no mutagenesis.

Both the SOS repair system and the Post-replication Recombination-mediated repair system are Error Prone due to induction of the UV mutagenesis system involving the *umuB* and *umuC* gene products.

(10) c. Diagram the steps that occur in *E. coli* Uracil BASE excision repair.

Name an enzyme that can catalyze each enzyme-mediated step.

See Soft Reserves key to this exam for diagram.

1. Uracil glycosylase recognizes Uracil, breaks glycosidic bond, removes base, leaving "hole" in DNA BUT with intact P-sugar backbone
2. AP nuclease nicks backbone on one or other or both sides of "hole" in DNA
3. Helicase or Exonuclease removes DNA at "hole" including the AP DNA
4. Polymerase I fills in gap or does nick translation
5. DNA ligase seals nick.

(4) d. What is XP and how is it related to human excision repair?

XP is the human disease Xeroderma pigmentosum. It is associated with mutations at several genetic loci, each of which appears to encode a protein which catalyzes one of the steps in human excision repair.

See Journal Articles 2, the first one by Sancar.

(4) e. What is transcription-coupled repair and why is it important to cells?

Transcription-coupled repair is the repair system that leads to specific repair of damage in the DNA strand serving as template within genes that are being expressed. This is important to cells because this is precisely the DNA that is most important to the cells, namely, the DNA, and strand of DNA, being expressed into protein and gene products.

14 pts 4. Telomeres

(4) a. What two major functions do telomeres perform in eukaryotic chromosomes?

1. Telomeres solve the DNA replication problem of how to synthesize both leading and lagging strands completely to the end of a linear DNA molecule, given the properties of DNA polymerases and semi-discontinuous modes of DNA replication.
2. Telomeres provide a structure for the ends of linear chromosomes that protects them from degradation and nucleolytic attack.

(6) b. What are the TWO major structural features of telomeric DNA?

1. Telomeres are direct repeats of an organism-specific short sequence that is T,G-rich in one strand and A,C-rich in the other strand.
2. Telomeres enjoy an interesting 4-stranded "quartet" structure of guanines interacting together via Hoogsteen interactions at the end of the telomere.

36 pts 1. DNA replication:

- (4) a. Regulation of DNA replication occurs at initiation and not at elongation. How then is more DNA synthesized per unit time?

Replication forks move at the same rate of about 50000 nucs/sec for bacterial growth rates of about 20 min to about 60 min. To synthesize more DNA per unit time, initiation events occur more often. Initiation of a second round of replication will occur PRIOR to completion of a first round of replication. One then has chromosomes with as many as 6 replication forks per chromosome moving simultaneously around the chromosome.

- (6) b. Briefly describe how methylation state of GATC sites is important in regulation of initiation of E. coli DNA replication.

Upon initiation, GATC sites in oriC are hemimethylated (A in parental strand GATC is methylated, A in daughter strand is not methylated). Part of the initiation "eclipse" (time between initiation events) is accounted for by the fact that one or more of the oriC GATC sites must become fully methylated before the daughter oriC origin can be used for an initiation event.

- (12) c. Diagram the biochemical steps that occur at a BACTERIAL origin to initiate replication at the two replication forks. Include protein names at each of the key steps.

See Soft Reserves key and Lodish, Fig. 10-9.

1. DnaA binds 9-mers at oriC.
2. With ATP, DnaA opens up 13-mers.
3. Primosome (DnaB, DnaC) bring Primase (DnaG) to PAS (Primosome Assembly Site)
4. Leading strand of Fork 1 synthesized by HoloPolIII
5. New PAS exposed on lagging strand; first O.frag made (HoloPolIII)
6. O.frag becomes Leading strand of Fork 2 as it passes through oriC
7. Fork 2 moving; first O.frag of lagging strand of Fork 2 comes back to oriC
8. PolI removes RNA, fills in gap, ligase seals ... to mature O.frag (see below)

- (14) d. Briefly describe or diagram the steps that occur in Lagging Strand DNA replication. Name an appropriate enzyme or protein for each step.

1. Primosome (DnaB, DnaC) brings Primase (DnaG) to PAS (Primosome Assembly Site)
2. Primase synthesizes primer RNA
3. HoloPolIII synthesizes O.frag DNA from RNA primer.
4. PolI, using nick translation, simultaneously removes RNA tail of one O.frag and extends the head of the adjacent O.frag, thereby replacing RNA with DNA.
5. DNA ligase joins O.frag together.

10 pts 2. CsCl gradients:

- (4) a. Briefly describe why DNA forms a band in a CsCl gradient.

As the CsCl gradient forms, DNA molecules at the bottom of the tube are like "wood in water" (density is less than that of the CsCl) and these DNA molecules tend to float. Conversely, those at the top of the tube are like a "stone in water" and tend to sink. The result is a band at the CsCl density equal to that of the DNA Cs salt.

- (6) b. CsCl gradients permit separation of molecules or complexes made before some given time from those made after this time. Briefly explain why and how this is true.

The "time" point in question is that at which a density shift is performed in growth of cells, e.g. from heavy growth medium to light growth medium.

Separation of molecules or complexes made before this time vs those made after this time, together with examination of combinations of those made before and those made after, e.g. the two strands of DNA in HL DNA, can be done using CsCl gradients, which separates molecular species on the basis of their buoyant density, or density in CsCl, NOT on the basis of molecular weight or size.