Lecture 1K. Nucleic acids: types, biological distribution and properties.

Study questions.

1. Independently replicating elements, called **plasmids**, can replicate indefinitely outside the host chromosome.

2. Guanine constitutes 20% of the base composition of the genome of a pathogenic bacterium. What percentage of the genome is adenine? **The 20% represents G's on both DNA strands of the genome. So C must also be 20%. This makes the GC % age 40. Therefore, the DNA is 60% AT and 30% A.**

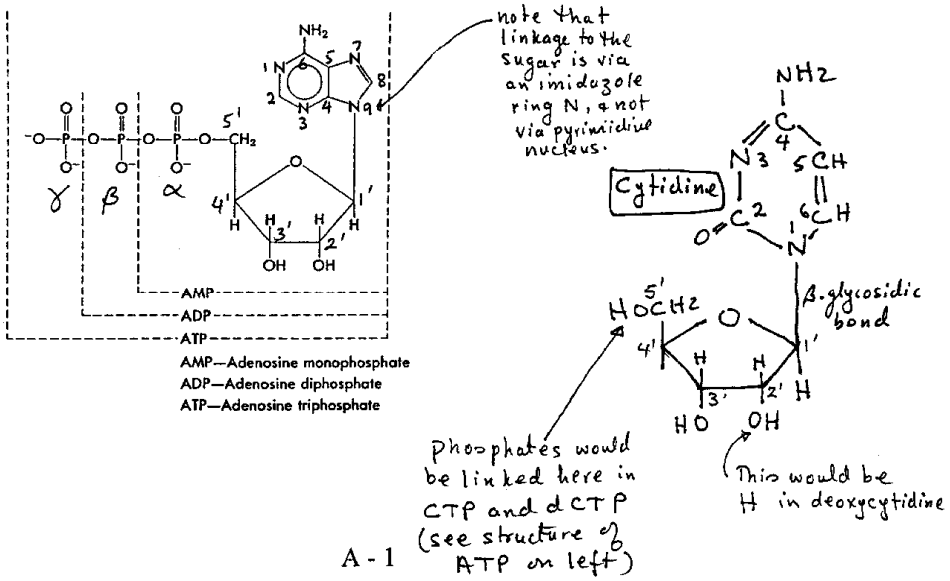
3. The base sequence of a segment of a human gene is 5' GACCTCATG 3' on one of its strands. What is the base sequence of the complementary strand? **5' CATGAGGTC 3'**

4. Thymine is present in which type of RNA? **Transfer RNA mostly.**

5. Draw the structures of:

   a) adenine. = **6 amino purine.**
   b) cytidine. = **riboside of cytosine (2 oxy 4 amino pyrimidine)**
Both are c) adenylic acid (adenosine monophosphate). = **AMP with PO4 on 5' C of ribose**
nucleotides d) dCTP. = **sugar is 2' deoxyribose and phosphates are α, β, γ from 5' C of sugar**

For each of these compounds number all carbon atoms and indicate if the compound is a purine or pyrimidine base, nucleoside, or nucleotide.



6. HIV is best described as: (**explanations for information** only).

a) a mycoplasma organism. - **Mycoplasma are prokaryotes.**
b) an archaebacterial virus. - **Archae are a distinct class of cells that resemble (but are not) prokaryotes.**
c) a prion virus. - **"Prions" are infectious proteins. No such thing as "prion virus".**
✓ d) a retrovirus.
e) a herpes - type virus
f) a rhinovirus. = **cold virus**
g) a bacteriophage.

7. The drug AZT, which is used in the treatment of AIDS patients, is an analogue of **thymidine** and replaces it during HIV replication.

(Thymidine is the deoxyriboside derivative of thymine.)

Lecture 2K. Structures and conformations of DNA and RNA.

Study questions.

1. Which of the following statements about the double-helical structure of DNA are correct?

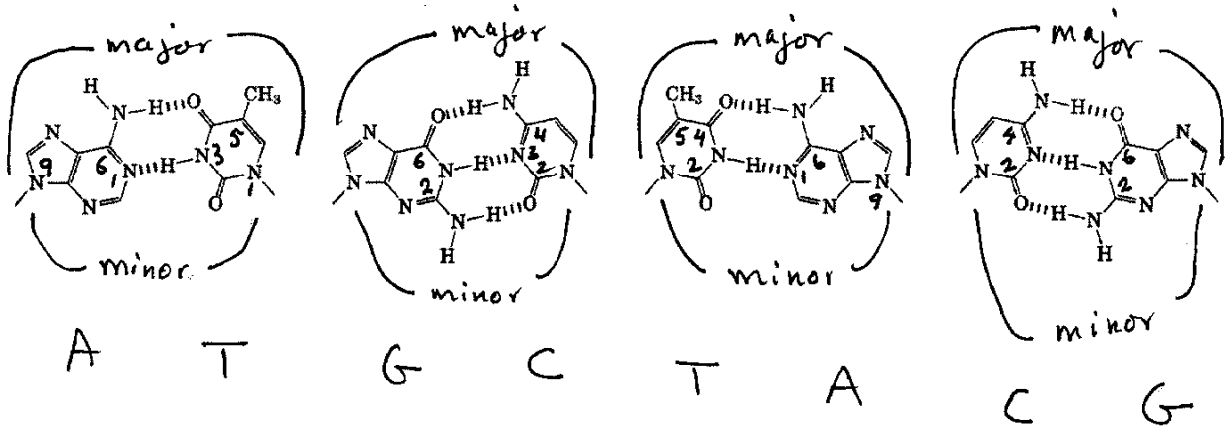
✓ **a)** It has adenine paired with thymine and cytosine paired with guanine.
✓ **b)** It can assume many different forms including A-DNA, B-DNA, and Z-DNA.
✓ **c)** In B-DNA, all the hydrogen-bonded base pairs lie in a plane perpendicular to the helix axis.
✓ **d)** It can be deformed by both smooth bends and kinks. **Not discussed in class.**
e) It is rigid and static.

2. The chart below shows the structures of all normal base pairings in B-DNA. Use the chart to answer the following questions:

a) Identify each purine and pyrimidine base and number its atoms.

b) Which hydrogen bond donors or acceptors of the base pairs are in the major groove?

c) Which hydrogen bond donors or acceptors of the base pairs are in the minor groove?

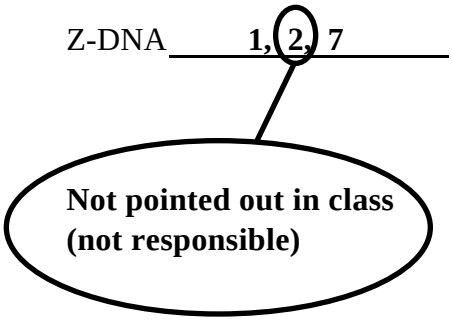


3. Match the features or characteristics in the right column with the type of DNA helix in the left column.

a) A-DNA **4, 6, 7**

b) B-DNA **3, 4, 5, 7**

c) Z-DNA **1, 2, 7**



1) Phosphates in the backbone are zigzagged.
2) Formation is favored by negative supercoiling.
3) Has a relatively wide and deep major groove.
4) Has a right-handed helix.
5) Has 10.4 base pairs per turn.
6) Has a structure similar to that of double-stranded RNA.
7) Strands in the helix have opposite polarities.

4. Which of the following nucleotide sequences might allow for the formation of Z-DNA? Explain your answer. **Alternating purine (esp G) - pyrimidine sequences.**

a) CCTAGTTA

b) TATATATA **Will, although less frequently than choice (c). dAMP is less likely to be *syn* than dGMP.**

c) CGTGTACA **Most likely to, because of G's tendency to exist in *syn* configuration.**

d) TGAATTCA

5. Suppose that a single-stranded circular DNA with the base composition 30% A, 20% T, 15% C, and 35% G serves as the template for the synthesis of a complementary strand by DNA polymerase.

a) Give the base composition of the complementary strand.

Strand #1	**0.3A**	**02.T**	**0.15C**	**0.35G**
Strand #2	**0.3T**	**0.2A**	**0.15G**	**0.35C**

b) Give the overall base composition of the resulting double-helical DNA.

50% AT, 50% GC

6 - 13. Answer questions 6 - 13 on page 619 of Devlin. Then, check your answers against Devlin's.

Answers are on p. 620 of Devlin. Regarding Nos. 7 and 8, you can expect that segments of DNA which are rich in AT base-pairs will melt (denature) at lower temperatures than segments rich in GC pairs. Diagrams B and C on p. 619 represent partially denatured DNA molecules.

Lecture 3K. DNA in chromosomes.

Study questions.

1. What do the antibiotics novobiocin and nalidixic acid inhibit and why are they useful in treating bacterial infections in humans?

   DNA gyrase, a type II topoisomerase that relaxes and introduces negative supercoils in bacterial DNA. By inhibiting DNA gyrase, these drugs inhibit bacterial DNA replication.

2. Which of the following statements about topoisomerases are correct?

✓ **a)** They alter the linking numbers of topoisomers. **Will not ask about "linking #" "twist" and "writhe" in exam.**

✓ **b)** They break and reseal phosphodiester bonds.

c) They require NAD^+ as a cofactor to supply the energy to drive the conversion of a supercoiled molecule to its relaxed form.

✓ **d)** They form covalent intermediates with their DNA substrates.

✓ **e)** They can, in the case of a particular topoisomerase, use ATP to form negatively supercoiled DNA from relaxed DNA in *E. coli*. **Refers particularly to DNA gyrase. ATP is required by all type II but not by Type I topoisomerases.**

4. **Topoisomerases** can be viewed as reversible nucleases that create either a transient single-strand break (type I) or a transient double-strand break (type II).

5. Topoisomerase I does not require ATP to break and to rejoin DNA strands because the energy of the phosphodiester bond is stored transiently in a phosphotyrosine linkage in the enzyme's active site. True or false? **TRUE.**

6 - 8. Answer questions 14 - 16 on page 619 of Devlin. Then, check your answers against Devlin's.

   Straightforward. You may be given a diagram like this and asked to identify components.

Lecture 4K. DNA damage and repair.

Study questions.

1. Which of the following statements about DNA polymerase I are correct?

✓ **a)** It adds deoxyribonucleotide units to the 3'-hydroxyl of a primer.
✓ **b)** It uses the template strand to select the deoxyribonucleotide unit to add to the growing DNA chain.
c) It contains a 3' → 5' nuclease that cleaves phosphodiester bonds to yield 3'-dNMPs and 3'-phosphate-terminated DNA. **It leaves 3' OH on DNA ends.**
✓ **d)** It contains two nuclease activities in the same polypeptide chain that contains the polymerase active site. **The 5' and 3' nuclease activities.**
✓ **e)** It can be cleaved with a protease into two fragments, each of which has a nuclease activity. **This alludes to the "Klenow fragment", which I did not discuss and will not ask on exam.**

2. What property of DNA allows the repair of some residues damaged through the action of mutagens? **Complementarity of 2 strands, when damage is on one strand, the other can be used as template for repair.**

3. How does the repair machinery of *E. coli* identify a DNA strand that has recently misincorporated a noncomplementary nucleotide during replication in order to repair it?

Via recognition of hemimethylated GATC sequences by the "methyl-directed" mismatch repair system".

4. Which of the following enzymes or processes can be involved in repairing DNA in *E. coli* damaged by uv light-induced formation of a thymine dimer?

✓ **a)** DNA ligase seals the newly synthesized strand to undamaged DNA to form the intact molecule.
✓ **b)** The UvrABC enzyme (excinuclease) hydrolyzes phosphodiester bonds on both sides of the thymine dimer.
✓ **c)** DNA polymerase I fills in the gap created by the removal of the oligonucleotide bearing the thymine dimer.
✓ **d)** The UvrABC enzyme recognizes a distortion in the DNA helix caused by the thymine dimer.
✓ **e)** A photoreactivating enzyme absorbs light and cleaves the thymine dimer to re-form two adjacent thymine residues. **This activity is lacking in humans.**

5. Patients with xeroderma pigmentosum develop skin cancers when exposed to sunlight because they have a deficiency in:

a) RNA primase.
b) DNA recombinase.
c) N-glycosylase.
✓ **d)** an enzyme of the excision repair pathway.
e) an enzyme of the mismatch-repair pathway.

6. The enzyme responsible for DNA synthesis in both replication and repair is called **DNA polymerase**.

7. A 5'-to-3' synthesis of DNA means that growth occurs by addition of dNTPs to the exposed 3'-OH group, with expulsion of inorganic pyrophosphate. True or false?

TRUE. dNTP's are hydrolyzed to dNMP and PPi in the process of being incorporated in DNA.

Lecture 5K. DNA replication.

Study questions.

1. Match the properties or functions in the right column with the DNA polymerase in the left column. Items to the right may be used more than once, and items to the left may have more than one of the choices from the right.

a)	DNA polymerase I **1, 2, 3, 5**	1)	Involved in replication.
		2)	Requires a primer and a template. **All DNA polymerases do.**
***b)**	DNA polymerase II **2, probably 3**	3)	Involved in DNA repair.
		4)	Makes most of the DNA during replication.
c)	DNA polymerase III **1, 2, 4**	5)	Removes the primer and fills in gaps during replication.

***DNA polymerase II is included in this question to emphasize that all DNA polymerases require priming.**

2. Which of the following statements about DNA replication in *E. coli* are correct?

✓ **a)** It occurs at a replication fork.
✓ **b)** It starts at a unique locus on the chromosome.
c) It proceeds with one replication fork per replicating molecule. ***E. coli* replication is bidirectional.**
✓ **d)** It is bidirectional.
e) It involves discontinuous synthesis on the leading strand. **Lagging strand synthesis is the one which is discontinuous.**
f) It uses RNA transiently as a template. **RNA is used as a primer not as template.**

3. Which of the following statements about DNA polymerase III holoenzyme from *E. coli* are correct?

✓ **a)** It elongates a growing DNA chain approximately 100 times faster than does DNA polymerase I. **Because of processivity.**
b) It associates with the parental template, adds a few nucleotides to the growing chain, and then dissociates before initiating another synthesis cycle. **Pol III holoenzyme is processive.**
✓ **c)** It maintains a high fidelity of replication, in part by acting in conjunction with a subunit containing a 3' → 5' exonuclease activity. **The "proofreading" or "editing" function.**

✓ **d)** When replicating DNA, it is a molecular assembly composed of at least 10 different kinds of subunits. **This assembly includes the "sliding clamp" and subunits that "load" the enzyme on DNA.**

4. Rifampicin, an antibiotic that inhibits bacterial RNA polymerase, inhibits *E. coli* DNA replication. Explain why. **RNAP is involved during initiation of DNA replication. (See Post-lectures comments.)**

5. Initiation of DNA synthesis on the lagging strand requires short **RNA primers** made by an enzyme called **Dna G protein or primase**, which uses ribonucleotide triphosphates as substrates.

6. The unwinding of the DNA helix at the replication fork is catalyzed by a **helicase**, which uses the energy from ATP hydrolysis to move unidirectionally along DNA.

7. **Ssb proteins**, which aid DNA unwinding, bind to single-stranded DNA in such a way that the bases are still available for templating reactions.

True or false?

8. DNA synthesis occurs in the 5'-to-3' direction on the leading strand and in the 3'-to-5' direction on the lagging strand. **FALSE. All DNA synthesis is 5'- to -3'.**

9. Single-strand binding proteins at the replication fork hold the two strands of DNA apart by covering the bases and thus preventing base-pairing. **FALSE. If they covered the bases, then new DNA cannot pair and the template would not be available. Most of the binding of these proteins is to the phosphate backbone.**

Lecture 6K. Mutation.

Study questions.

1. Which of the following nucleotide substitutions are transition mutations?

✓ **a)** G for A**, a purine for another purine.**
b) A for C
✓ **c)** C for T**, a pyrimidine for another pyrimidine.**
d) T for G

2. Which of the substitutions in question 1 are transversion mutations?

(b) and (d); purine for a pyrimidine or pyrimidine for a purine.

3. Explain why most nucleotides that have been misincorporated during DNA synthesis in *E. coli* do not lead to mutant progeny.

Because the methyl-directed mismatch repair pathway removes them.

4. Match the type of mutation or physiologic consequence in the right column with the appropriate mutagen in the left column.

See p. 624 Devlin a) 5-Bromouracil **2**

See p. 624 Devlin b) 2-Aminopurine **2**

See p. 629 Devlin c) Hydroxylamine **2**

d) Acridines **4, 3 (of 1-4 bases usually)**
These are aromatic dyes that intercalate in DNA, e.g., p. 632 Devlin.

See p. 629 Devlin e) Nitrous acid **2**

f) Ultraviolet light **5 (if lesion is unrepaired)**

See p. 633 Devlin g) Benza[a]pyrine **4, 3 (of 1 - 4 bases usually)**

1) Transversion. **There are no mutagens known that specifically cause transversions.**
2) Transition.
3) Insertion or deletion.
4) Translational frameshift.
5) Block in replication.

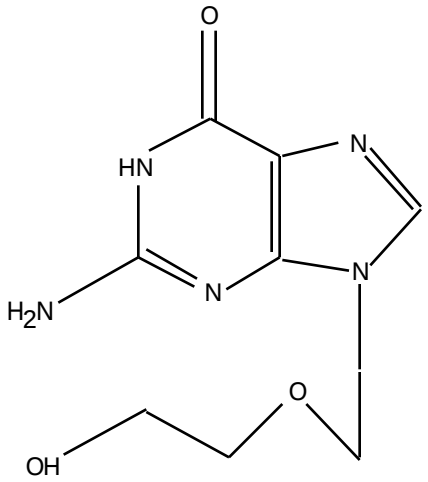
5. Explain how mutations in genes encoding proteins likely to be involved in DNA repair, such as those defective in xeroderma pigmentosum and hereditary nonpolyposis colorectal cancer, may contribute to the onset of cancer.

   The transformation of normal cells to cancer cells involves mutations in several genes. DNA repair deficiencies accelerate mutation frequencies towards the oncogenic state.

6. Eukaryotic DNA is highly methylated at the C-5 position of cytosine. The degree of methylation is inversely correlated with gene expression. Although the exact role of C-5 methylation in gene expression is not known, it is known that these C-5-methylated cytosines are a source of mutations. Explain why.

   Oxidative deamination of C-5-methylated cytosines leads to thymines in DNA. Unlike uracils (which are removed by Uracil N-glycosylase) thymines remain and ultimately what starts out as a G-C(Me) pair becomes an AT pair after replication.

7. [From lectures.] Acyclovir is an antiviral agent used to reduce the pain and promote the healing of herpes infections. Its structure is shown below.



Structure of acyclovir.

a) What nucleoside does this drug resemble? **Guanosine or deoxyguanosine.**
b) Why does the drug need to be administered in dephosphorylated form? **More soluble than phosphorylated form.**
c) Acyclovir has very few side effects because it inhibits DNA replication only in herpes-infected cells. This is because all herpes viruses encode a thymidine kinase gene that is able to activate the drug. What do you think this activating reaction is? **Phosphorylation of OH.**
d) Once acyclovir is activated, how does it inhibit DNA replication? **Used by viral DNA polymerase. Terminates DNA synthesis once incorporated.**
e) Why are cells uninfected by herpes virus relatively unaffected by acyclovir? **Acyclovir - TP is not used by host DNA polymerase.**
f) The herpes virus can become insensitive to acyclovir therapy by mutations in either of two genes. What might they be? **Thymidine kinase and DNA polymerase genes.**

8. If cytosine in DNA is deaminated, the resulting base is:

a) removed by an endonuclease.
b) removed by an exonuclease.
✓ **c)** removed by a glycosylase.
d) not removed, but ultimately replaced by cytosine in subsequent DNA replication.
e) not recognized by DNA polymerase, and thus inhibits DNA replication.

9. If DNA polymerase makes a mistake, thus creating a pair of incorrectly hydrogen-bonded bases, the mistake is corrected by a special **mismatch repair** system that uses methylation to distinguish new strands from old.

10. The enzyme that replicates the HIV virus is **reverse transcriptase**, which lacks **editing (3' - exonuclease)** activity and consequently has poor fidelity.

11. When acting on replicated DNA, the methyl-directed mismatch repair system in *E. coli* can distinguish the parental strand from the progeny strand as long as one or both are methylated, but not if both strands are unmethylated. True or false? **FALSE. Only hemimethylated (one but not the other strand is methylated) DNA is recognized by this repair system.**

Lecture 7K (1:00 - 3:00 p.m.; with demonstrations). Methods of DNA and RNA analysis.

Study questions.

1. **Restriction fragment length polymorphisms** (RFLPs) are medically useful in a growing number of arenas. Which statement best characterizes these RFLPs?

   a) Different restriction enzymes cut DNA at different palindromes.
✓ **b)** Differences in DNA sequences result in alterations in DNA sensitivity to individual restriction endonucleases.
   c) Restriction enzymes degrade different DNA samples into nucleotides at different rates.
   d) Loss of a restriction site by gene mutation would increase the number of restriction fragments.
   e) They are detected by Western blots.

2. The polymerase chain reaction (PCR) requires all of the following EXCEPT:

✓ **a)** highly purified and cloned DNA.
   b) a thermal cycling device.
   c) a supply of deoxyribonucleotide triphosphates.
   d) a heat stable DNA polymerase.
   e) synthetic oligonucleotide primers.

3. During the high temperature phase of the PCR technique:

   a) mRNA is denatured.
   b) proteins are denatured.
   c) annealing of mRNA and DNA is prevented.
✓ **d)** DNA duplexes melt to produce single-stranded DNA template.
   e) the primer oligonucleotides are inactivated.

4. *E. coli* DNA polymerase I is not suitable for use in the standard PCR protocol because...(give explanation). **It gets inactivated at the first heating of the reaction mix.**

5. Which of the following laboratory tests would be most useful in detecting an HIV infection?

   a) complete blood count.
✓ **b)** Northern blot.
✓ **c)** Western blot.
✓ **d)** Southern blot.
   e) Pap smear.

Provided these tests are done on cells prone to infection, e.g. T-lymphocytes or extracts of blood cells.

Lecture 8K. DNA recombination and transposition.

Study questions.

1. Which of the following statements about genetic rearrangements are correct?

✓ **a)** They generate new combinations of genes.
✓ **b)** They can move a segment of DNA from one chromosome to another.
✓ **c)** They are mediated by the breakage of DNA and the rejoining of the resulting fragments.
✓ **d)** They generate genome sequence variability, upon which natural selection can act. **Makes sense.**
✓ **e)** They can regulate gene expression. **Activates or inactivates genes.**
f) They always require extensive regions of sequence similarity between the interacting DNA molecules in order to juxtapose the recombining sites. **Only homologous recombination does.**

2. Place the following events in the order in which they would occur during homologous recombination between two DNA molecules: **Events will be taking place on both aligned chromosomes.**

a) Strand exchange occurs between duplexes via branch migration at the crossover point. **5**
b) A pair of strands with similar sequences is cleaved in each duplex. **2**
c) Two duplexes align at a region of sequence similarity. **1**
d) Each invading strand becomes covalently joined to its corresponding strand in the other duplex. **4**
e) Strands of the recombinant intermediate are cleaved at or near the crossover point and are joined to their corresponding strands in each duplex. **6**
f) The end of each single strand invades the other duplex and forms base pairs with its complementary strand. **3**

Don't worry if the way this question is asked is complicated. Exam questions will be much shorter and less complex.

3. Which of the following statements about the RecA protein of *E. coli* are correct?

a) It is an ATP-dependent nuclease that generates single-stranded DNA. **RecA protein lacks nuclease activity. Rec BCD is the nuclease.**
✓ **b)** It catalyzes an ATP-dependent strand-assimilation reaction in which a single-stranded DNA molecule associates with duplex DNA.
✓ **c)** It hydrolyzes ATP to promote branch migration.
✓ **d)** It binds to single-stranded DNA to form a filament.
✓ **e)** It facilitates the search of duplex DNA for regions with sequence similarity to the invading single-stranded DNA.

4. Homologous recombination <u>is likely</u> to require which of the following?

a) DnaB protein
✓ **b)** Topoisomerase I
✓ **c)** RecA protein
✓ **d)** RecBCD complex
✓ **e)** ATP
f) NAD^+
✓ **g)** Single-strand binding protein
h) DNA-dependent RNA polymerase
✓ **i)** DNA polymerase I
✓ **j)** DNA ligase
✓ **k)** dATP **One of the dNTPs needed for DNA polymerase I**

5. Which of the following statements about transposons is/are correct?

✓ **a)** They contain insertion sequences.
✓ **b)** They contain inverted terminal repeat sequences. **See Fig. 15.49 Devlin.**
✓ **c)** They contain one or more genes specifying one or more enzymes that catalyze the transposition event.
d) They require the *rec* gene products to complete their movements between or within genomes. **The *rec* gene products are required for homologous recombination.**
✓ **e)** They can lead to the duplication of short sequences of DNA in the recipient genome. **See Fig. 15.48 Devlin.**

6. Which of the following answers completes the sentence correctly? Site-specific recombination of V- to V-segment genes of immunoglobulins does not occur because [see p. 663, Devlin]: **Not required for exam.**

a) there is not an extensive sequence homology between them.
b) they are flanked by recognition sequences lacking a spacer sequence.
c) they lack an AT-rich nonamer sequence.
d) their recognition sequences, which include a 23-bp spacer sequence, cannot recombine with each other.
e) the recA enzyme does not bind to their palindromic heptamer sequences.

7. The kind of recombination displayed by transposons, sometimes called recA-independent recombination, has been regarded as having far greater evolutionary significance than does general, or recA-dependent, recombination. Why do you think this may be the case?

   Because it has the potential of transferring new genes into an evolving cell or organism.

8. Explain four different ways that an antibiotic resistance gene might move from the genomic DNA of one bacterium to the genomic DNA of another. **Conjugation, transduction, transformation, or by way of a transposable element.**

Fill in the blanks.

9. The **RecA protein** is required for chromosome pairing in *E. coli*; it binds to single-stranded DNA and promotes its pairing with homologous, double-stranded DNA.

10. A central intermediate in general recombination is the **Chi form**, which is also called a **Holliday structure**, after its discoverer.

True or false?

11. Transposases recognize sufficiently extensive sequences surrounding the integration sites so that the transposon does not become integrated into the middle of a gene, for gene disruption could be lethal to the cell. **FALSE. Transposons do disrupt genes because they can insert anywhere.**

12. The capacity of plasmids to replicate indefinitely without being part of a host chromosome distinguishes them from transposable elements. **TRUE. Transposable elements are considered "replication deficient" while plasmids and viruses are considered "replication proficient" or "replicons" (autonomously replicating units).**