[2 pts] Feature(s) of DNA found in the Watson-Crick model of B-DNA include:

a) two antiparallel polynucleotide chains coiled in a helix around a common axis.
b) the pyrimidine and purine bases lie on the inside of the helix.
c) the bases are nearly perpendicular to the axis.
d) all of the above.
e) none of the above.

[5 pts] The protein enzymes listed below all function in interactions with DNA. The binding of these proteins to DNA are either independent or dependent on the nucleotide sequence of the DNA with which they interact. From what you have learned about the principles of protein/DNA interactions, classify each protein's basis of interactions with DNA as:

d (Dependent on the DNA nucleotide sequence) or
i (Independent of the DNA nucleotide sequence)

Write **d** or **i** in the space provided

__**d**__ *Sal* I restriction enzyme
__**i**__ Deoxyribonuclease I
__**i**__ DNA ligase
__**d**__ *Eco RV* restriction enzyme
__**i**__ *Thermus aquaticus (Taq)* DNA polymerase [for PCR]

[2 pts] **Circle the correct answer(s): B-DNA** occurs in cells most often in base paired regions of (mRNA, RNA-DNA hybrids, **DNA**, both RNA and DNA).

[2 pts each] ANSWER TRUE OR FALSE

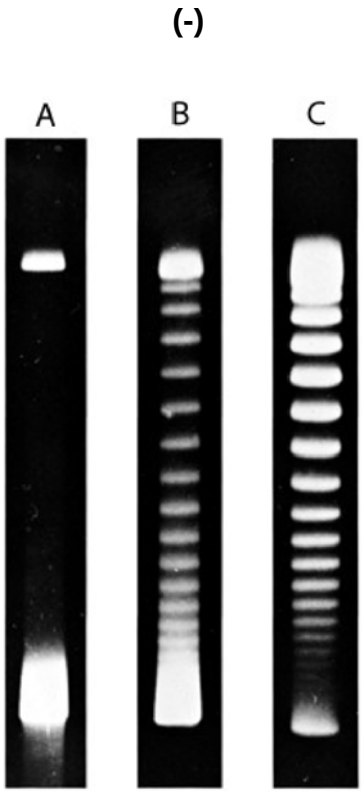
__**T**__ Proteins whose binding to DNA is dependent on the specific base sequence of the DNA generally form hydrogen bonds between amino acids and functional groups located in the major groove of the DNA B helix.

__**F**__ The exact 3-dimensional structure of DNA is independent of its base sequence.

__**F**__ Proteins whose binding to DNA is not dependent on specific base sequences generally form hydrogen bonds with the sugar-phosphate backbone of the DNA B helix.

[12 pts] **READ ALL PARTS OF THIS QUESTION BEFORE ANSWERING ANY OF IT**

Circular DNA from SV40 virus was isolated and subjected to agarose gel electrophoresis. The results are shown in **lane A** (the control) of the adjoining gel patterns. DNA migrates in this gel from top (-) to bottom (+)



[2 pts] On what basis does the DNA separate in agarose gel electrophoresis?

Answer: Answer: **<u>Topology</u>-(complex function of size and shape in this case)**

[4pts] How does the DNA in each band in **Lane A** differ?

Answer: **The sizes are all the same, but the DNA at the top (-) is relaxed (open circle) and the DNA at the bottom (+) is highly supercoiled DNA.**

The DNA was then incubated with topoisomerase I for 5 minutes and again analyzed by gel electrophoresis with the results shown in **lane B**.

[2 pts] What types of DNA do the various bands in **lane B** represent **(+)**

Answer: **Topoisomers (all the same size) with different degrees of supercoiling**

Another sample of DNA was incubated with topoisomerase I for 30 min and again analyzed as shown in **lane C**.

[4 pts] What is the reason that more of the DNA is in slower moving forms in **lane C?**

Answer: **The DNA is becoming progressively more unwound, or relaxed, with less and less supercoiling, and thus slower moving on the gel (because the DNA is more like open circles which are retarded in the gel matrix, relative to the supercoiled form of this DNA.**

[24 pts] 6) Match the molecules or structures in the left hand column with the function or features related to *E. coli* DNA replication in the right hand column. **[2 pts for each correct answer. Some answers are used more than once]**

PUT NUMBERS IN SPACES GIVEN

_9__ Proofreads most of the newly-synthesized DNA

__11__ Joins lagging strand pieces together

_8__ Is an RNA polymerase

__6__ Prevents unwound DNA from reforming base-paired helix

__3__ Is synthesized discontinuosly

__7__ Relieves stress introduced by positive supercoiling

__10__ Removes RNA primers

_7___ requires ATP to add negative supercoils to DNA

__2__ First place where primosome functions

_3___ Polarity of synthesis is opposite to replication fork movement

_5___ Unwinds DNA at replication fork

__9__ Synthesizes most of DNA during replication

1) Replication fork
2) origin of replication
3) Lagging strand
4) leading strand
5) DNA helicase
6) single-strand binding protein
7) DNA gyrase
8) Primase
9) DNA polymerse III holoenzyme (an asymmetric dimer)
10) DNA polymerase I
11) DNA ligase

[5 pts] How long would it take for *E. coli* DNA polymerase III to replicate the *E.coli* chromosome (4×10^6 base pairs) from 2 replication forks? **[Assume that Pol III can add 1,000 bases/second to each daughter strand being synthesized]**. Ignore removal of primers and filling gaps with Pol I.

Answer: 2(strands) x (4×10^6 bases/strand) x (1 sec/4×10^3 bases) x (1 min/60 sec) = 33 min
Hint: the Pol III dimer copies both strands at each fork = 4×10^3 bases/sec/chromosome)

[5 pts] If *E. coli* DNA polymerase I was needed to replicate all of the *E.coli* chromosome (4×10^6 base pairs) from 2 replication forks, how long would this replication take ? **[Assume that Pol I can add 20 bases/second to each daughter strand being synthesized]**. Ignore the need to remove primers and fill in gaps.

Answer: 2(strands) x (4×10^6 bases/strand) x (1 sec/20 bases x 4) x (1 min/60 sec) = 1,666 min

- Do Problems 5, 9 and 13 at end of Chapter 28.

Chapter 28 Mutations and DNA Repair:

[9 pts] 3) In the left column, write nine (9) correct letters of corresponding items in the right column.

_A,C,F_ Thymine dimer repair	A. DNA polymerase I
	B. MutHLS
_A,D,F_ Removal of uracil	C. uvrABC excinuclese
_B,E,F_ Mismatch repair	D. Uracil-DNA glycosidase
	E. DNA polymerase III
	F. DNA ligase

ANSWER TRUE OR FALSE

__**T**__ Mutations called transitions involve replacement of one pyrimidine base by the other.

__**T**_ Mutations called transversions involve replacement of a purine base by a pyrimidine base.

__**F**__ UV light causes formation of covalent links between thymine bases on opposite DNA strands.

__**T**__ A DNA repair system can distinguish thymine from uracil formed by deamination of cytosine.

__**F**__ Acridine dyes cause transversion mutations.

__**F**__ DNA repair enzymes probably interact with DNA by binding to specific base sequences.

__**F**__ A highly reactive, chemically-modified aflatoxin can cause mutations after linking to adenine in DNA.

Fill in the Blank Questions

___Z-DNA__ is a left-handed double helix.

DNA can serve as a ___template__ to direct synthesis of the complementary strand of DNA or RNA.

The small DNA pieces observed during DNA replication called _Okazaki____fragments have a short stretch of ____RNA_____ at the 5’ end .

Proteins that use ATP to melt (unwind) the DNA at specific sites are called __helicases___.

The ends of eukaryotic chromosomes are called __telomeres__.

An assay used to determine carcinogenic potential is the __Ames_____ test which measures frequency of ___reversion_____ of a mutant to a normal (wild-type) gene.

The topological state (degree of __supercoiling __) of DNA can be modified by the enzymes known as _topoisomerases_ __.

The primer for DNA synthesis is an RNA molecule formed by the enzyme primase .

The DNA strand that is replicated continuously is known as the leading strand.

DNA polymerase III is approximately 100 times faster than DNA polymerase I.

During DNA replication, the RNA primer pieces are removed by DNA polymerase I .

UV light causes damage to DNA by forming pyrimidine dimers .

The human, genetic skin disease, caused by a mutation in components of the human nucleotide-excision-repair pathway is called xeroderma pigmentosum .

Holliday junctions are intermediates in recombination pathways composed of four polynucleotide chains in a cross-like structure.

Multiple Choice Questions

How can the leading and lagging strands be synthesized in a coordinated fashion?

A) Specific enzymes control the size of the DNA opening.
B) Lagging-strand binding proteins inhibit leading-strand replication if the strands become disproportionate in size.
C) Pol III is a dimeric holoenzyme, and the looped lagging strand allows the enzyme to proceed in the same direction with each strand.
D) All of the above.
E) None of the above.

Common types of mutations include

A) the mismatch of bases in the DNA.
B) the deletion of one or more bases in the DNA.
C) the insertion of one or more bases in the DNA.
D) b and c.
E) a, b, and c.

Huntington's disease is caused by

A) pyrimidine dimers.
B) trinucleotide expansion.
C) suppressor mutants.
D) all of the above.
E) none of the above.

Afflatoxin B1 is an example of a(n)

A)intercalating chemical.
B)alkylating agent.
C) base analog.
D)all of the above.
E) none of the above.

Short-Answer Questions

Describe, in simple terms, some hallmark characteristic features of DNA structure.

Ans: DNA consists of two chains of polynucleotides, paired via hydrogen bonds, but running in opposite directions in a right-handed helical form around a central axis. The bases are found on the inside of the helix, with the phosphates and sugars on the outside. The bases are held together by hydrogen bonds: adenine and thymine by two hydrogen bonds, and guanine and cytosine by three hydrogen bonds. The bases are perpendicular to the axis.

Compare some major features of A- and B-DNA.

Ans: A-DNA is a more dehydrated form, observed at lower humidity. It is a right-hand helix of antiparallel strands, like B-DNA. However, it is wider and shorter, and the base pairs are tilted and less perpendicular to the central axis. In A-DNA the pitch is smaller, and the base pairs per turn slightly greater.

What features of real DNA did x-ray analysis of crystallized DNA reveal that are different from the original Watson-Crick model of DNA?

Ans: These studies indicted that deviations from the ôBö structure are common. The rotation of the helix, and placement of the bases, can vary. Frequently, the bases are not coplanar, but twisted like propeller blades.

How do DNA topoisomerases (Types I and II) change the state of supercoiling of DNA?

Ans: 1) One or both strands of DNA must be cleaved. 2) A segment of DNA is passed through the break. 3) The DNA break is resealed.

How are breaks sealed in discontinuous lagging strand DNA fragments that are formed during replication?

Ans: DNA ligase is the enzyme that seals the breaks by creating a phosphodiester bond between a 3' hydroxyl group and a 5' phosphate at the end of the other piece. It can only seal a break in a double-strand piece of DNA. It normally uses the energy provided by ATP, except in bacteria, where NAD+ serves as the AMP donor.

What is a processive polymerase enzyme?

Ans :Processive enzymes catalyze multiple reactions without dropping the polymer substrate.

How is the processivity of DNA polymerase III accomplished?

Ans: The structure of the enzyme complex allows the reaction processivity. The enzyme is a large complex, which forms a ring around the DNA polymer. A specific subunit leaves enough space for the newly assembled DNA to be formed properly, and acts as a sliding DNA clamp.

How are single-stranded regions of DNA maintained during replication?

Ans: Single-stranded binding proteins (SSB) bind to the single-stranded regions of DNA.

Describe the consequences of incorrect DNA replication or DNA damage.

Ans: The most serious consequences are cell death, cell transformation, and blockage of DNA replication. The changes in DNA can also be inherited and passed on to future generations.

Why is thymine used in DNA instead of uracil?

Ans: The methyl group on thymine allows it to be unique from deaminated cytosine. Deamination of cytosine forms uracil, and is a fairly common event. The uracil is recognized as foreign and is repaired.