9. It is well known that DNA polymerases synthesize DNA only in the 5′ to 3′ direction. Yet, at the replication fork, both strands of parental DNA are being replicated with the synthesis of new DNA. How is it possible that while one strand is being synthesized in the 5′ to 3′ direction, the other strand appears to be synthesized in the 3′ to 5′ direction? This apparent paradox is explained by

a. 3′ to 5′ DNA repair enzymes
b. 3′ to 5′ DNA polymerase
c. Okazaki fragments
d. Replication and immediate crossover of the leading strand
e. Lack of RNA primer on one of the strands

10. Given that the chromosomes of mammalian cells may be 20 times as large as those of *Escherichia coli*, how can replication of mammalian chromosomes be carried out in just a few minutes?

a. Eukaryotic DNA polymerases are extraordinarily fast compared with prokaryotic polymerases
b. The higher temperature of mammalian cells allows for an exponentially higher replication rate
c. Hundreds of replication forks work simultaneously on each piece of chromosomal DNA
d. A great many different RNA polymerases carry out replication simultaneously on chromosomal DNA
e. The presence of histones speeds up the rate of chromosomal DNA replication

20. Xeroderma pigmentosum (278700) is an inherited human skin disease that causes a variety of phenotypic changes in skin cells exposed to sunlight. The molecular basis of the disease appears to be

a. Rapid water loss caused by defects in the cell membrane permeability
b. The inactivation of temperature-sensitive transport enzymes in sunlight
c. The induction of a virulent provirus on ultraviolet exposure
d. The inability of the cells to synthesize carotenoid-type compounds
e. A defect in an excision-repair system that removes thymine dimers from DNA

22. Which of the following descriptions of DNA replication is not common to the synthesis of both leading and lagging strands?

a. RNA primer is synthesized
b. DNA polymerase III synthesizes DNA
c. Helicase (rep protein) continuously unwinds duplex DNA at the replication fork during synthesis
d. Nucleoside monophosphates are added in a 5′ to 3′ direction along the growing DNA chain
e. DNA ligase repeatedly joins the ends of DNA along the growing strand

23. Which of the following statements describing restriction endonucleases is true?

a. They always yield overhanging single-stranded ends
b. They recognize methylated DNA sequences
c. They recognize triplet repeats

d. They cleave both strands in duplex DNA
e. They always yield blunt ends

26. Which of the following enzymes can polymerize deoxyribonucleotides into DNA?
a. Primase
b. DNA ligase
c. DNA gyrase
d. RNA polymerase III
e. Reverse transcriptase

30. Which of the following enzymes can be described as a DNA-dependent RNA polymerase?
a. DNA ligase
b. Primase
c. DNA polymerase III
d. DNA polymerase I
e. Reverse transcriptase

35. The DNA sequence M, shown below, is the sense strand from a coding region known to be a mutational "hot spot" for a gene. It encodes amino acids 21 to 25. Given the genetic and amino acid codes CCC = proline (P), GCC = alanine (A), TTC = phenylalanine (F), and TAG = stop codon, which of the following sequences is a frame-shift mutation that causes termination of the encoded protein?
M 5'-CCC-CCT-AGG-TTC-AGG-3'
a. -CCA-CCT-AGG-TTC-AGG
b.-GCC-CCT-AGG-TTC-AGG
c.-CCA-CCC-TAG-GTT-CAG
d.-CCC-CTA-GGT-TCA-GG—
e. -CCC-CCT-AGG-AGG——

37. The hypothetical "stimulin" gene contains two exons that encode a protein of 100 amino acids. They are separated by an intron of 100 bp beginning after the codon for amino acid 10. Stimulin messenger RNA (mRNA) has 5′ and 3′ untranslated regions of 70 and 30 nucleotides, respectively. A complementary DNA (cDNA) made from mature stimulin RNA would have which of the following sizes?
a. 500 bp
b. 400 bp
c. 300 bp
d. 100 bp
e. 70 bp

39. In contrast to DNA polymerase, RNA polymerase

a. Fills in the gap between Okazaki fragments
b. Works only in a 5′ to 3′ direction
c. Edits as it synthesizes
d. Synthesizes RNA primer to initiate DNA synthesis
e. Adds nucleoside monophosphates to the growing polynucleotides

40. The removal of introns and subsequent self-splicing of adjacent exons occurs in some portions of primary ribosomal RNA transcripts. The splicing of introns in messenger RNA precursors is

a. RNA-catalyzed in the absence of protein
b. Self-splicing
c. Carried out by spliceosomes
d. Controlled by RNA polymerase
e. Regulated by RNA helicase

41. A promoter site on DNA

a. Transcribes repressor
b. Initiates transcription
c. Codes for RNA polymerase
d. Regulates termination
e. Translates specific proteins

43. An immigrant from eastern Europe is rushed into the emergency room with nausea, vomiting, diarrhea, and abdominal pain. His family indicates he has eaten wild mushrooms. They have brought a bag of fresh, uncooked mushrooms from a batch he had not yet prepared. You note the presence of *Amanita phalloides*, the death-cap mushroom. A liver biopsy indicates massive hepatic necrosis. Care is supportive. A major toxin of the death-cap mushroom is the hepatotoxic octapeptide α-amanitin, which inhibits

a. DNA primase
b. RNA nuclease
c. DNA ligase
d. RNA polymerase
e. RNA/DNA endonuclease

44. The consensus sequence 5′ TATAAAA 3′ found in eukaryotic genes is quite similar to a consensus sequence observed in prokaryotes. It is important as the

a. Only site of binding of RNA polymerase III
b. Promoter for all RNA polymerases
c. Termination site for RNA polymerase II
d. Major binding site of RNA polymerase I
e. First site of binding of a transcription factor for RNA polymerase II

45. The so-called caps of RNA molecules

a. Allow tRNA to be processed
b. Occur at the 3′ end of tRNA
c. Are composed of poly A
d. Are unique to eukaryotic mRNA
e. Allow correct translation of prokaryotic mRNA

48. Which one of the following statements correctly describes the synthesis of mammalian messenger RNA (mRNA)?

a. Each mRNA often encodes several different proteins
b. Several different genes may produce identical mRNA molecules
c. There is colinearity of the RNA sequence transcribed from a gene and the amino acid sequence of its encoded protein
d. The RNA sequence transcribed from a gene is virtually identical to the mRNA that exits from nucleus to cytoplasm
e. Mammalian mRNA undergoes minimal modification during its maturation

84. Which of the following best describes the negatively controlled lactose operon in *Escherichia coli*?

a. An inducer (lactose) binds to the operator, enhancing simultaneous transcription and translation of β-galactosidase (z), permease (y), and transacetylase (a) genes
b. An inducer (lactose) alters the repressor protein and uncovers the operator and promoter, allowing simultaneous transcription and translation of β-galactosidase (z), permease (y), and transacetylase (a) genes
c. The repressor (lactose) alters the operator protein and uncovers the promoter, allowing simultaneous transcription and translation of β-galactosidase (z), permease (y), and transacetylase (a) genes
d. The repressor (lactose) alters the catabolite repression protein and uncovers the operator and promoter, allowing simultaneous transcription and translation of β-galactosidase (z), permease (y), and transacetylase (a) genes
e. An inducer (lactose) alters the repressor protein, uncovers the β-galactosidase (z) operator, and allows transcription. The inducer also uncovers separate operators for the permease (y) and transacetylase (a) genes

85. The lactose operon is negatively controlled by the lactose repressor and positively controlled by which of the following?

a. Increased concentrations of glucose and cyclic AMP (cAMP)
b. Decreased concentrations of glucose and cAMP
c. Increased concentrations of glucose, decreased concentration of cAMP
d. Decreased concentrations of glucose, increased concentration of cAMP
e. Increased concentrations of glucose and adenosine triphosphate (ATP)

86. Which of the following regulators are said to act in "*cis*"?

a. The *lac* repressor and mammalian transcription factors
b. The *lac* repressor and the *lac* operator
c. The *lac* operator and mammalian enhancers
d. The *lac* operator and mammalian transcription factors
e. Mammalian transcription factors and enhancers

1. RNA molecules that exhibit catalytic activity are called

   (A) mRNAs
   (B) ribonucleases
   (C) ribosomes
   (D) ribozymes
   (E) ribonucleotides

14. Which of the following is true about a circular double-stranded DNA genome that is determined by chemical means to be 21 percent adenosine?

   (A) The genome is 10.5% guanosine.
   (B) The genome is 21% guanosine.
   (C) The genome is 29% guanosine.
   (D) The genome is 58% guanosine.
   (E) The base percent composition of guanosine in the genome cannot be determined from the information given.

56. A study is done on a mammalian cell line that has a doubling time of 24 hours. These cells are synchronized in G_1 and then labeled for 2 days with BrdU (an analog of thymidine that increases the density of DNA into which it is incorporated). At the end of the labeling period, chromosomal DNA is isolated from the cells and its density analyzed by equilibrium centrifugation in cesium chloride gradients. Which of the following patterns would be expected to be seen?
(H = heavy, L = light)

   (A) 100% H/H
   (B) 100% H/L
   (C) 50% H/H, 50% H/L
   (D) 50% H/H, 50% L/L
   (E) 25% H/H, 50% H/L, 25% L/L

50. A solution contains DNA polymerase I, Mg^{2+} salts of dATP, dGTP, dCTP, and dTTP, and an appropriate buffer. Which of the following DNA molecules would serve as a template for DNA synthesis when added to this solution?

(A) A single-stranded closed circle
(B) A single-stranded closed circle base-paired to a shorter linear strand with a 3′-terminal hydroxyl
(C) A single-stranded closed circle base-paired to a shorter linear strand with a 3′-terminal phosphate
(D) A double-stranded closed circle
(E) A blunt-ended, double-stranded linear molecule with a 3′-terminal hydroxyl at each end

105. Which of the following is NOT required for RecA-dependent recombination between two DNA molecules?

(A) Strand migration
(B) Ligation
(C) Mismatch repair
(D) Nuclease digestion
(E) DNA synthesis

113. The ribosome is involved in all of the following EXCEPT

(A) peptide bond formation
(B) aminoacylation of tRNA
(C) binding of protein factors during elongation
(D) binding of aminoacyl tRNA to mRNA
(E) binding of mRNA at an initiation codon

114. An *E. coli* strain lacking DNA polymerase I would be deficient in DNA

(A) repair
(B) methylation
(C) splicing
(D) degradation
(E) transcription

58. Which of the following best describes the function of the sigma subunit in the RNA polymerase of *E. coli* ?

(A) It is essential for elongation of the RNA transcript.
(B) It is essential for the recognition of and binding to the promoter sequence.
(C) It increases RNA polymerase binding to any DNA template.
(D) It is required for transcription termination.
(E) It keeps the core complex from dissociating.

71. DNA polymerase contains a lysine residue that is important for binding to DNA. Mutations were found that converted this lysine to either glutamate, glycine, valine, or arginine. Which mutations would be predicted to be the most and least harmful to the ability of the enzyme to bind DNA?

	Most	Least
(A)	Valine	Aspartate
(B)	Glycine	Arginine
(C)	Arginine	Glycine
(D)	Glutamate	Valine
(E)	Glutamate	Arginine

76. Which of the following is NOT a potential problem associated with expressing a eukaryotic, protein-coding nuclear gene in prokaryotic cells?

(A) Lack of an intron-splicing mechanism in prokaryotes
(B) Differences in the translation initiation codons used by eukaryotic cells and prokaryotic cells
(C) Susceptibility of the protein product to prokaryotic proteases
(D) Stability of mRNA in prokaryotic cells
(E) Differences in transcriptional signals between eukaryotic cells and prokaryotic cells

88. The recognition site of the restriction endonuclease *Ava*I is CPyCGPuG, where Py is any pyrimidine and Pu is any purine. What is the expected average distance, in nucleotide pairs, between *Ava*I cleavage sites in a random DNA sequence?

(A) 4,096
(B) 1,024
(C) 682
(D) 64
(E) 6

Tissue	Factor A	Protein Kinase Activity	Protein Phosphatase Activity
Muscle	+	–	–
Heart	+	+	–
Brain	+	–	+

92. Transcription of gene X is controlled by transcription factor A. Gene X is only transcribed when factor A is phosphorylated. Data on the tissue distribution of factor A and the activities of a protein kinase and a protein phosphatase specific for factor A are presented in the table above. Of these three tissues, gene X will be transcribed in

(A) muscle only
(B) heart only
(C) brain only
(D) brain and heart only
(E) muscle, heart, and brain

Questions 164-166

An experimenter generates a library of plasmids containing 10-15 kilobase (kb) inserts from the genome of a bacterium by partially digesting the bacterial genomic DNA with *Eco*RI and cloning the resulting fragments into the *Eco*RI site of a plasmid vector. The experimenter must then identify the plasmids containing the *purB* gene. To do this, 5 of the plasmids from the library were digested with *Eco*RI and the digests were separated by gel electrophoresis (Figure 1). In a second experiment, the same 5 plasmids were analyzed by PCR using primers derived from sequences internal to *purB* and electrophoresis was performed on the PCR products (Figure 2). Both gels were stained with ethidium bromide to visualize the DNA.

Figure 1. Electrophoresis of digests

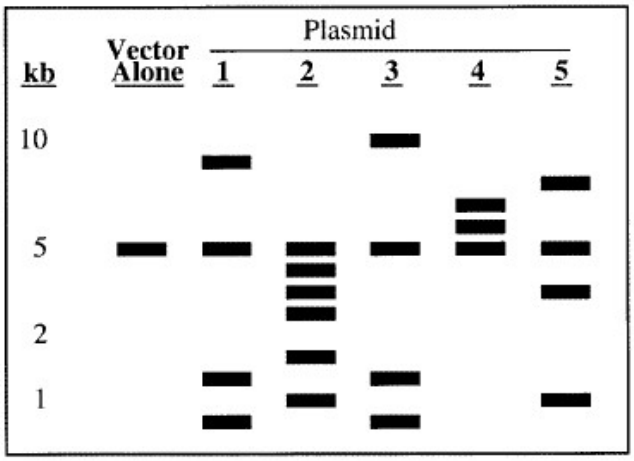
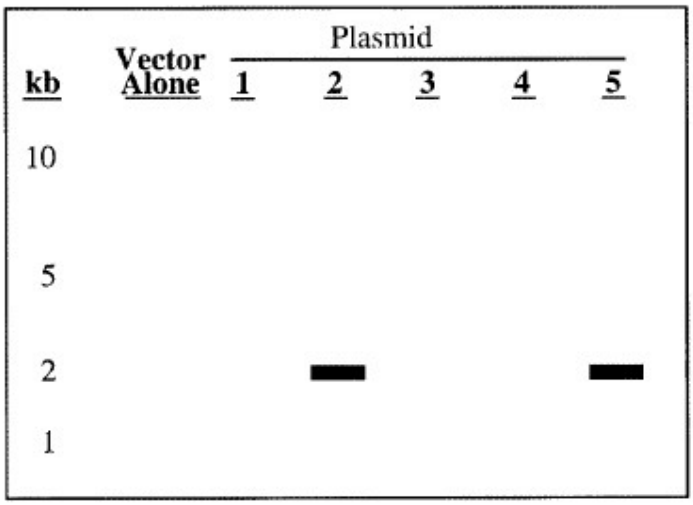


Figure 2. Electrophoresis of PCR products



164. The inserts in which of the following pairs of plasmids may overlap?

(A) 3 with 4 only
(B) 3 with 5 only
(C) 1 with 2 and 3 with 4 only
(D) 1 with 3 and 2 with 5 only
(E) All of the inserts may overlap.

165. Which of the following methods would NOT be a useful alternative to using PCR to determine which plasmids contain *purB* ?

(A) Testing for complementation of a *purB* auxotroph
(B) Sequencing the inserts
(C) Hybridizing the plasmids with a probe complementary to *purB*
(D) Mapping each plasmid with several restriction enzymes
(E) Footprinting with DNase

166. The part of *purB* complementary to the *purB* primers is contained in which of the following plasmids?

(A) 2 only
(B) 5 only
(C) 2 and 5 only
(D) 1, 3, and 4 only
(E) 1, 2, 3, 4, and 5

Questions 158-160

An *in vitro* system is used to study replication of a viral DNA genome that is a double-stranded, covalently closed circle. The location of the sites for the restriction endonuclease *Mbo*I on this DNA are shown in Figure 1. Replication reactions are carried out using viral DNA as a template, extracts of infected cells as a source of enzymes, and other exogenous nucleotides (dGTP, dCTP, dATP, dTTP, and ATP), all of which are labeled with ^{32}P. The reaction products are digested with *Mbo*I, analyzed by agarose gel electrophoresis, and visualized by autoradiography, producing the results shown in lane 1 of Figure 2. The same reaction is carried out in the presence of increasing concentrations of nonradioactive 2′, 3′-dideoxyGTP (ddGTP), and the results are shown in lanes 2-4. (Note: only full-length restriction fragments are shown.)

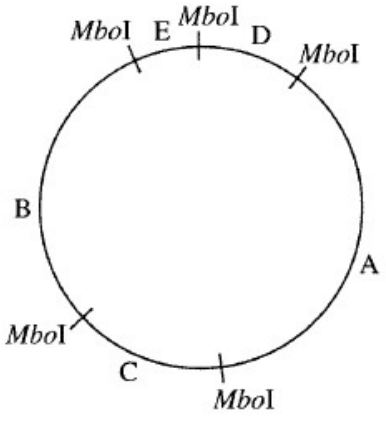


Figure 1

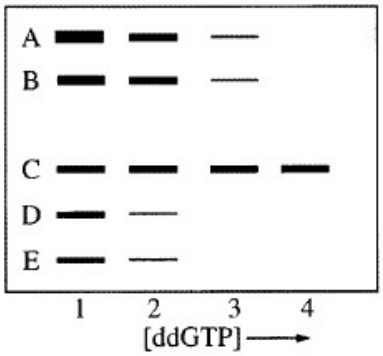


Figure 2

158. The specific activity of the DNA fragments in Figure 2 is defined as ^{32}P disintegrations per minute per microgram of DNA. Which of the following best describes the relative specific activities of the fragments in lane 1 ?

(A) Fragment A has the highest specific activity, followed by B, C, D and E.
(B) Fragment E has the highest specific activity, followed by D, C, B, and A.
(C) The specific activity depends on the order in which the fragments were replicated.
(D) All fragments have the same specific activity.
(E) The relative specific activities vary from experiment to experiment.

159. The data in Figure 2 indicate that the origin of replication of this DNA is in fragment

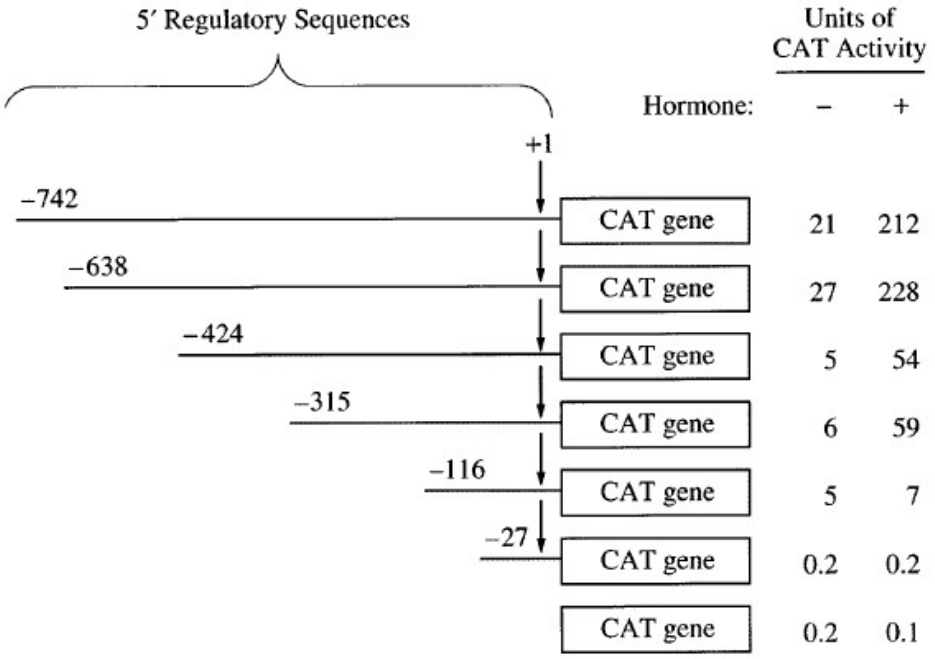
(A) A
(B) B
(C) C
(D) D
(E) E

160. The data in Figure 2 indicate that replication of this DNA is

(A) unidirectional
(B) bidirectional
(C) rolling circle
(D) conservative
(E) semiconservative

Questions 174-177

Researchers studying the regulation of a hormone-responsive gene isolated 750 base pairs of DNA immediately preceding the start site of transcription (+1). They demonstrated that if these sequences are cloned upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene and the DNA then introduced into mammalian cells, CAT enzyme activity increases in response to hormone treatment. To define the sequences involved in the regulation of this gene, they made a series of deletions containing various lengths of the 5′ regulatory sequences. They cloned these truncated DNA fragments upstream of the CAT gene as shown in the figure below, introduced the constructs into mammalian cells, and assayed for CAT enzyme activity in the absence (−) and presence (+) of hormone. The figure below gives the results of a representative experiment.



174. Assuming that there is a single hormone-responsive regulatory element in the gene, that element is located between

(A) −742 and −638
(B) −638 and −424
(C) −424 and −315
(D) −315 and −116
(E) −116 and −27

175. The maximal stimulation of CAT activity due to the addition of hormone is approximately

(A) 4-fold
(B) 10-fold
(C) 40-fold
(D) 100-fold
(E) 1,000-fold

176. Which of the following statements is NOT supported by the data?

(A) The bacterial CAT gene requires eukaryotic regulatory elements for significant expression in mammalian cells.
(B) Gene expression in the presence *versus* the absence of regulatory elements can differ by as much as a 1,000-fold.
(C) Hormone-independent regulatory elements lie downstream of −315.
(D) Hormone-independent regulatory elements lie upstream of −315.
(E) Regulatory elements probably do not lie upstream of −742.

177. A new construct was made that began at −742 and was identical to that shown in the figure except that the sequences between −424 and −315 were inverted. In this new construct, which of the following are closest to the expected CAT activities in the absence and presence, respectively, of hormone?

(A) 25 units/250 units
(B) 25 units/25 units
(C) 50 units/50 units
(D) 5 units/50 units
(E) 5 units/5 units

Questions 155-157

In a study of the biosynthesis of a particular secretory glycoprotein, the first step was to fractionate a crude RNA extract using an oligo-dT column. The RNA bound to the column was eluted and translated *in vitro* in the presence of [^{3}H]leucine and [^{14}C]mannose. An antibody specific for the secretory glycoprotein was added and the resulting immunoprecipitate was analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Distribution of radioactivity as a function of position in the gel was then analyzed as shown in Figure 1. The *in vitro* translation experiment was repeated in the presence of rough microsomes, which were then solubilized. Immunoprecipitation and electrophoresis were performed as in the previous experiment. The results are shown in Figure 2.

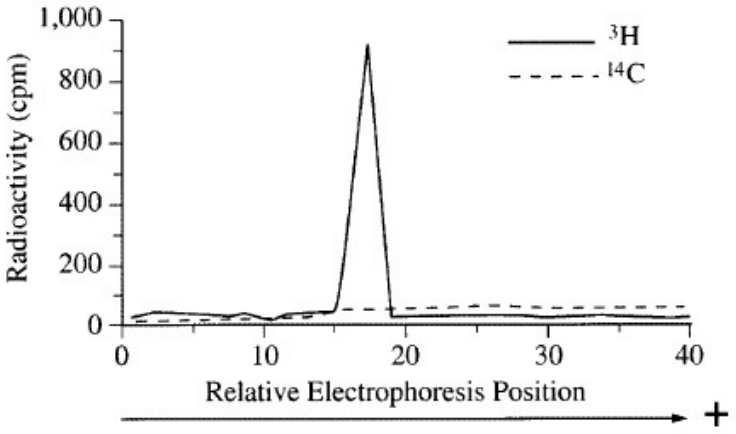


Figure 1
Without Microsomes

Figure 1
Without Microsomes

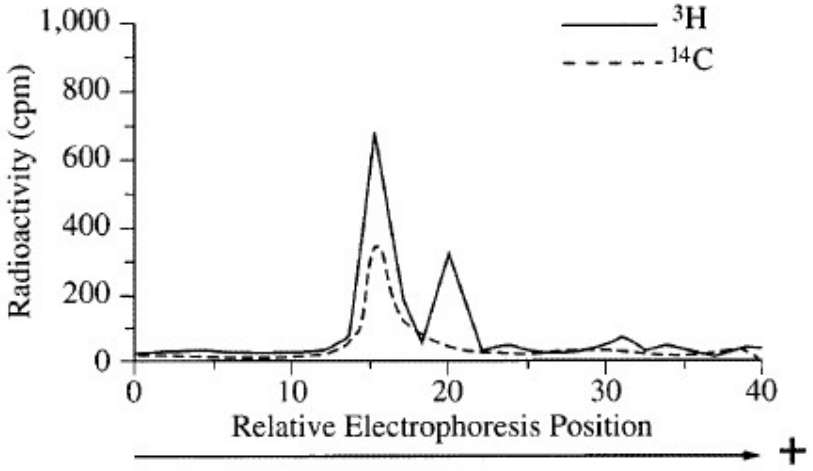


Figure 2
With Microsomes

155. Why is the oligo-dT column used in this experiment?

(A) Only hnRNA will bind to oligo-dT.
(B) Oligo-dT selects large RNA species.
(C) Ribosomal RNA is bound to the column.
(D) Intact, rather than partially hydrolyzed, RNA is retained on the column.
(E) Polyadenylated RNA is purified by the procedure.

156. The protein in the peak of ^{3}H at position 20 in Figure 2 is of lower monomeric molecular weight than the protein in Figure 1. This suggests that microsomes cause proteolysis but only one of the products of proteolysis appears on the gel in Figure 2. All of the following can account for the absence of a second product of proteolysis EXCEPT:

(A) It was not recognized by the antiserum.
(B) It contains no leucine or mannose residues.
(C) It was bound by, but not released from, the oligo-dT column.
(D) It was rapidly degraded.
(E) It was too small to be retained on this gel.

157. If glycosylation were blocked with tunicamycin in the experiment with microsomes, the resulting gel would display only ^{3}H-leucine labeling and most likely show which of the following characteristic profiles?

(A) The same as Figure 1
(B) Two peaks of radiolabel, one centered at position 15 and one centered at position 20
(C) Two peaks of radiolabel, one centered at position 20 and the other, twice the amplitude, centered at position 24
(D) A single peak of radiolabel centered at position 20
(E) A single peak of radiolabel centered at position 15

Questions 146-148

When normal human fibroblasts are cultured in medium containing calf serum, they divide with an average generation time of approximately 22 hours (M = 1 hr, G_1 = 10 hr, S = 6 hr, G_2 = 5 hr). To determine the effects of serum deprivation on cell cycle distribution, cells were incubated for 48 hours in medium with or without serum. At the end of this incubation, cells were harvested and stained with propidium iodide, which binds to DNA and fluoresces when exposed to ultraviolet light. The stained cells were analyzed for DNA content (fluorescence) in a flow cytometer. The results with serum are shown in Figure 1a. If deprived of serum, the cells stop proliferating and enter a quiescent state (Figure 1b).

In a second experiment, cells were deprived of serum for 48 hours and then treated either with serum alone or serum plus cycloheximide (CHX), an inhibitor of protein synthesis. At various times after treatment, RNA was isolated from the cells. Equal amounts of total cellular RNA from each sample were analyzed by gel electrophoresis and Northern blotting to detect the level of c-*fos* mRNA. The c-*fos* protein is involved in regulating cell proliferation. The results of this experiment are shown in Figure 2. ("–" indicates serum alone and "+" indicates serum plus CHX.)

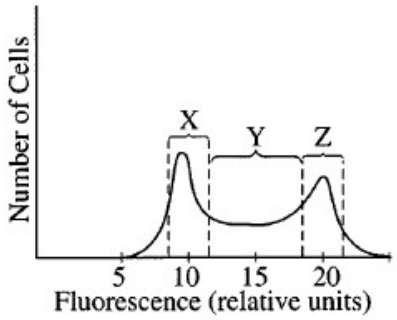


Figure 1a. With serum

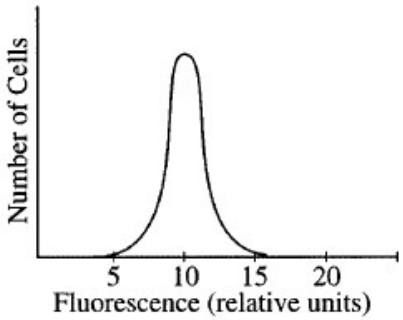


Figure 1b. Without serum

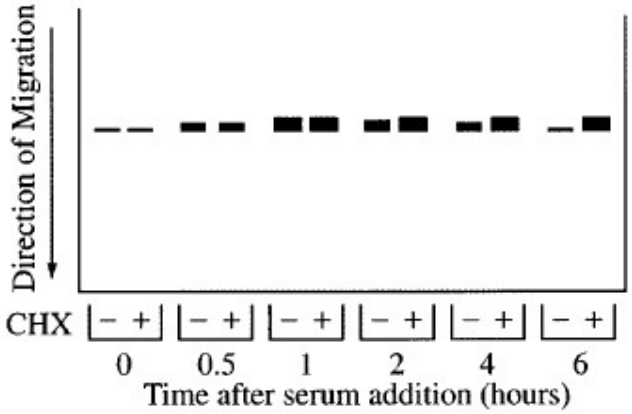


Figure 2. c-*fos* mRNA

146. In Figure 1a, the cells in the region labeled Y are in what stage of the cell cycle?

(A) G_1
(B) S
(C) G_2
(D) M
(E) G_0

147. Cells growing in the presence of serum were labeled for 3 hours with ^{3}H-thymidine and then analyzed by flow cytometry. Which of the following regions defined in Figure 1a will contain radioactive cells?

(A) X only
(B) Y only
(C) Z only
(D) Y and Z only
(E) X, Y, and Z

148. Based on the results shown in Figure 2, the differences in the amounts of c-*fos* mRNA in the presence *versus* the absence of cycloheximide at 2, 4, and 6 hours is best explained by which of the following?

(A) c-*fos* mRNA is degraded by an unstable nuclease.
(B) The c-*fos* promoter is regulated by an unstable transcriptional activator.
(C) The c-*fos* protein activates its own promoter.
(D) Splicing of c-*fos* pre-mRNA requires an unstable splicing factor.
(E) c-*fos* mRNA is degraded by cycloheximide-induced nuclease.