1. Interaction of a regulatory protein with DNA to prevent transcription is termed __repression__.

2. A(n) __operon__ is a group of genes that are controlled together but are not located together on the bacterial chromosome.

3. The __rho__ protein terminates transcription for about half of all E. Coli mRNAs.

4. The _poly A tail_ decreases in length as a mRNA molecule is translated repeatedly.

5. __enhancers_ are DNA sequences that can act over long distances and in either orientation to control transcription.

6. An untranslated region upstream of an initiation codon in mRNA is called a _5'UTR_.

7. During splicing of hnRNA a U1 snRNP binds to the __5' end__, and a U2 snrnp binds to the __branch point__.

8. In an interphase nucleus, dark-staining chromosomal material is called __heterochromatin__; light-staining material is called __euchromatin__.

9. A gene is a unit of DNA encoding one polypeptide chain.

10. Ultraviolet-damage analysis depends on the production of __T-T dimers__ during UV irradiation.

Multiple Choice. Choose the best answer or answers for each of the following:

1. The consensus sequence for poly(A) addition is
   a. the site of poly(A) tail addition.
   b. AAUAAA.
   c. downstream of the cleavage site.
   d. none of the above

2. Histone mRNAs lack
   a. poly(A) tails.
   b. introns.
   c. a 3’UTR.
   d. all of the above

3. Splice sites in pre-mRNA are marked by two universally conserved sequences contained
   a. in the middle of the intron.
   b. at the ends of the exons.
   c. at the ends of the introns.
   d. none of the above
4. microRNAs play a key role in which of the following?
   a. translational repression
   b. viral RNA degradation
   c. RNA interference
   d. all of the above

5. Which of the following synthesizes the phospholipids that form the plasma membrane?
   a. the rough endoplasmic reticulum
   b. the plasma membrane
   c. the Golgi apparatus
   d. the smooth endoplasmic reticulum

6. If a cellular homogenate were subjected to differential centrifugation, which of the following would be expected to pellet first?
   a. the endoplasmic reticulum
   b. mitochondria
   c. the cytosol
   d. nuclei

7. An example of the use of HAT medium to select cells of biological importance is the
   a. isolation of cells that produce monoclonal antibodies.
   b. isolation of cells that have undergone spontaneous transformation.
   c. isolation of cells with polarized epithelia.
   d. isolation of somatic cell hybrids.

8. Movement of phospholipids from one leaflet to the other
   a. occurs routinely.
   b. requires cholesterol.
   c. requires flippases.
   d. is impossible.

9. Cholesterol mixes with phospholipids in a biomembrane because cholesterol molecules are
   a. amphipathic.
   b. steroid derivatives.
   c. entirely hydrophobic.
   d. phospholipid derivatives.

10. Peripheral membrane proteins
    a. contain many hydrophobic amino acid residues.
    b. contain membrane spanning domains.
    c. have covalently attached lipid or fatty acid anchors.
    d. may noncovalently interact with phospholipid heads.

11. The __________________________ are transmembrane proteins.
    a. lipid-anchored membrane proteins
    b. integral membrane proteins
    c. peripheral membrane proteins
    d. extracellular matrix proteins
12. Porins
   a. are peripheral membrane proteins.
   b. contain no hydrophobic amino acid residues.
   c. have many hydrophobic α-helical regions.
   d. allow small water soluble molecules to pass through a membrane.

13. The major site of lipid synthesis in eukaryotic cells is the
   a. nucleus.
   b. endoplasmic reticulum (ER).
   c. peroxisome.
   d. mitochondria.

14. Type I membrane proteins have all of the following properties except
   a. cleavable signal sequence.
   b. internal signal-anchor sequence.
   c. internal stop-transfer sequence.
   d. N-out, C-in topology.

15. In N-glycosylation of proteins in the ER lumen, _____ sugar(s) are added to the nascent chain at once.
   a. 1   c. 7
   b. 3   d. 14

16. All the following proteins interact with exposed amino acids during protein folding in the ER except
   a. BiP.
   b. calnexin.
   c. PDI.
   d. prolyl isomerase.

17. Sorting of protein to mitochondria and chloroplasts is
   a. cotranslational.
   b. post-translational.
   c. pretranslational.
   d. quasitranslational.

18. Tom/Tim and Toc/Tic protein complexes are involved in
   a. post-receptor recognition events in the cytosolic folding of proteins prior to import into mitochondria or chloroplasts.
   b. pre-proteasomal steps in tagging aged proteins for degradation.
   c. protein translocation into mitochondria and chloroplasts, respectively.
   d. resetting biological clocks following rounds of intense protein synthesis.

19. Protein sequences for targeting to mitochondria or chloroplasts are located at
   a. the C-terminus of the precursor protein.
   b. amino acid position 173 in most mitochondrial and chloroplast proteins.
   c. the N-terminus of the precursor protein.
   d. b or c

20. Many peroxisomal matrix proteins are imported as
   a. folded proteins.
   b. nascent chains in the process of completing their elongation.
   c. protein fragments that are spliced together within the peroxisome.
   d. unfolded proteins
21. Which type of RNA participates in nuclear export of mRNA?
   a. snRNA
   b. hnRNA
   c. tRNA
   d. rRNA

22. The nuclear pore complex allows for
   a. passive diffusion of smaller molecules.
   b. import of proteins.
   c. active transport of very large molecules.
   d. all of the above

23. TATA boxes are used for initiation of transcription of
   a. 45S pre-rRNA
   b. 5S-rRNA
   c. tRNA
   d. protein-coding genes that are rapidly transcribed.
   e. genes that are transcribed at low rates.

17. Both prokaryote and eukaryotes exhibit the following characteristics:
   a. modification of RNA polymerase by additional protein factors.
   b. upstream location of TATA sequences.
   c. splicing of transcripts.
   d. interaction of transcripts with nonribosomal proteins to prevent tangling.
   e. presence of leader sequences in RNA

18. Indicate the order in which the following steps in the production of a mature mRNA occur (1=earliest; 4=latest)
   a. Initiation of transcription __1________
   b. Addition of 5' cap ____2_______
   c. splicing ____4__________
   d. addition of poly A tail __3______.

19. Eukaryotic genomic DNA containing the B-globin gene can be cloned into plasmid that replicate in E. coli, but the globin polypeptide is not expressed from this plasmid in E. coli. Why? How can expression of eukaryotic genes in bacteria be accomplished?

   Because there is no promoter sequence in the cloned B-globin gene to initiate the synthesis of mRNA in the prokaryote that is recognized by the bacterial polymerase. Attaching a prokaryote promoter to the 5' end of the B-globin gene would permit the gene to be transcribed and the protein to be produced. Of course it would not be the correct protein since the genomic DNA would have the introns in the transcript. Using a c-DNA construct would solve that problem.

20. Which features of mRNA are coded in DNA and which are the result of processing of hnRNA?

<table>
<thead>
<tr>
<th>Coded</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Amino acid sequence</td>
<td>a. exon-intron removal</td>
</tr>
<tr>
<td>b. Exon-intron boundaries</td>
<td>b. capping</td>
</tr>
<tr>
<td>c. Cap site</td>
<td>c. poly-A tail</td>
</tr>
<tr>
<td>d. Poly A tail site</td>
<td>d. alternative splicing</td>
</tr>
<tr>
<td>e. Alternative splice sites</td>
<td>e. other post-transcriptional modifications</td>
</tr>
</tbody>
</table>
21. a) Draw the structure of a simple phospholipid and identify the basic building blocks (low molecular weight precursors) which make up the structure.

Glycerol, phosphate, two long-chain fatty acids, a substituent group on the phosphate (ethanolamine, serine, choline, glycerol, inositol, etc).

b) Why is a phospholipid called an amphipathic molecule?

Because it has hydrophobic and hydrophilic parts.

c) Considering such a simple phospholipid in a lipid bilayer, what kind of motions can this molecule execute?

Can rotate around axis and move laterally within a single leaflet of the bilayer; can be flipped to other leaflet by “flippase”

d) What do we mean by "flip-flop" from one leaflet to the other? Does it happen spontaneously?

p-lipid can traverse from one leaflet to the other with the help of an enzyme, the flippase.

e) Explain in one or two sentences how lipid bilayers in real biological membranes are asymmetrical?

A lipid bilayer consists of two leaflets, with the hydrophobic tails facing each other and forming the hydrophobic interior. The composition of the two lipid leaflets is usually different with respect to individual types of lipid molecules.

f) The typical thickness of a lipid bilayer in a biological membrane is (select one of the following) i) 0.7 - 1.0 nm ii) 7 - 10 nm iii) 70 - 100 nm 7 - 10 nm (imagine two carbon chains, of 16 -20 carbons, arranged end to end: at least 32 - 40 C-C bonds of ~1.5 Angstroms, ie a total of >30 - 100 Angstroms, or 10 nm)

22. Two proteins are found to be associated with the plasma membrane of an animal cell. Both of them have been shown to be made on free polysomes (as opposed to polysomes associated with the ER or microsomal vesicles).

a) Explain two ways in which such a protein can be associated with a membrane

a) a protein made on free polysomes is not integrated into the membrane to be associated with the membrane, it has to become attached to an integral membrane protein ==> peripheral membrane protein OR, it can be modified by a covalent attachment (amide bond) of either a fatty acid (side chain myristyl- ) at the N-terminal, or a farnesyl group via a thioether linkage to a cysteine at the C terminal

b) How can one distinguish between the two types of attachment by a simple procedure?

b) a peripheral membrane protein can be solubilized at high salt or extreme conditions of pH a protein anchored by a myristyl or farnesyl chain must be solubilized by a detergent

c) If the intact cell is treated with trypsin prior to isolation of the plasma membrane, would you expect these two proteins to have suffered any damage? Explain briefly.

c) both of these proteins would be associated with the interior of the plasma membrane, and hence untouched by trypsin applied on the outside
23. An integral membrane protein can often be recognized nowadays by a detailed examination of its amino acid sequence (e.g., from cloning a gene in a genetic complementation assay). What kind of special features would make you reasonably convinced that a newly identified protein is an integral membrane protein?

The nucleotide sequence from the gene or from a cDNA would allow us to deduce the complete amino acid sequence for the protein. An integral membrane protein might be expected to show two features: i) a sequence of ~20 hydrophobic amino acids at the N-terminal serving as a signal sequence for binding to the SRP, and to be inserted into the RER membrane ii) in addition, we need to have at least one internal segment of the polypeptide containing a stretch of ~20 amino acids with hydrophobic side chains; these form the alpha-helical transmembrane segment of the IMP; if more than one such stretch of aa is found, the integral membrane protein may have multiple transmembrane segments.

24. Green fluorescent protein (GFP) from the marine invertebrate Aequorea victoria has become a popular "reporter" protein to study protein targeting in eukaryotic cells. By genetic engineering it is possible to make a chimeric gene for this protein such that the sequence SKL (serine-lysine-leucine) constitutes the C-terminal. One can make large quantities of this modified protein in bacteria and purify it. Small volumes of concentrated solutions of this protein can be microinjected into human cells from normal individuals and from patients suffering from Zellweger syndrome. When viewed in the fluorescence microscope one finds after a suitable time interval diffuse staining in the Zellweger cells, and "punctate" staining in cells from normal individuals. Explain.

a) In normal cells this GFP with the SKL sequence will be targeted to the peroxisome, and staining will be punctate, i.e., reflect the concentration of this protein in vesicles in cells from Zellweger patients. The uptake of this normal protein into peroxisomes does not occur, because they lack an essential component for transport of these proteins into the peroxisome. The GFP would be diffusely distributed in the cytosol.

25. a) Describe a very simple experiment which demonstrates that mitochondrial protein import can be post-translational.

a) one can translate a mitochondrial protein in vitro in the absence of any mitochondria (making it radioactive in the process by the inclusion of a radioactive amino acid in the translation mix); this protein can be digested when exposed to trypsin. If isolated, energized mitochondria (from yeast cells, for example) are added after protein synthesis is complete, the protein will be taken up into the mitochondria. This is shown by the treatment with trypsin after some elapsed time for import. The protein will be protected against digestion, (is inside the mitochondria). However, the lysis of mitochondria with detergent will again free a trypsin-sensitive peptide. Other organelles importing proteins that are complete are: peroxisomes, nuclei.

b) What is the role of chaperones in the translocation of a protein from the cytosol to a biologically active form in the mitochondrial matrix?

b) we require: hsp70 protein (chaperone) in the cytosol to denature the protein to be imported and remove secondary and tertiary structure the mitochondrial targeting sequence on the protein has to be recognized by a receptor on the outer membrane a membrane complex has to be assembled to make an aqueous channel through the outer and inner membrane (at the points of close contact between these two membranes) on the inside, the unfolded peptide is first bound again by a mitochondrial hsp70 protein, but later released to the hsp60 (chaperonin complex) which refolds the peptide into a biologically active secondary and tertiary structure.

26. The light-harvesting complex protein (LHCP) is an example of a chloroplast protein that is
synthesized in the cytosol and then imported and subsequently processed to a mature form. It is found in thylakoid membranes of the chloroplast.

In an experiment designed to explore the forces governing translocation of preLHCP, the in vitro translocation of [3H] preLHCP to isolated chloroplasts was measured. After an appropriate period of incubation, the chloroplasts were lysed and a fraction containing both thylakoid and envelope proteins was prepared and analyzed by SDS-PAGE autoradiography, as depicted in lane 2 of the figure below. Lane 1 is a control incubation of labeled preLHCP in the absence of chloroplasts. Following incubation, samples of the thylakoid membranes were treated with NaOH or a protease and then analyzed, as depicted in lane 3 (NaOH treatment), lane 4 (thermolysin treatment), and lane 5 (trypsin treatment).

a. What does the difference in the migration patterns in lanes 1 and 2 in the figure below indicate about preLHCP? Why is this a critical part of the experiment?

It is necessary to demonstrate that the processing of preLHCP is normal in the in vitro system. This is apparently the case as indicated by the conversion of the preLHCP to LHCP by the in vitro prep (lane 2)

b. Why were NaOH and proteases used in this experiment?

If the LHCP is in the stroma or is only partially integrated into the thylakoid membrane, the NAOH or protease should markedly alter the mobility of the resultant protein. However, if the LHCP is fully integrated into the thylakoid membrane it should be largely resistant to these treatments.

c. In the figure above, what does the difference in the migration patterns in lanes 4 and 5 compared with the pattern in lane 2 suggest about the location of LHCP in the thylakoid membrane?

Part of the LHCP protein is evidently exposed to the action of the protease in the stroma, so the protein is not fully integrated into the membrane, but must protrude into the stroma to some degree. As a result, the digested remnant of LHCP is smaller and consequently migrates farther.

d. In other experiments, purified thylakoid membranes, prepared on a sucrose gradient, were incubated with labeled preLHCP in the presence and absence of ATP, stroma, and protease. After an appropriate time, the samples were analyzed by SDS-PAGE autoradiography.

The resulting gel profiles are depicted below. What conclusions can be drawn from these data about the
effects of ATP and stroma on binding of preLHCP to thylakoid membranes?

The results tell us that the presence of ATP and stroma is required for conversion of the preLHCP to LHCP and sequestration of the LHCP in the thylakoid where it can be protected from the action of the protease. If either ATP or stroma is omitted, the protease, if present, digests the protein, and in any case the preLHCP is not converted to LHCP.

27. The signal recognition particle (SRP) consists of six polypeptide subunits (9, 14, 19, 54, 68, and 72 kDa), which are organized into three different functional entities surrounding a 7S-RNA molecule. After protein synthesis is initiated in the cytoplasm, the 54-kDa subunit of the SRP binds to the signal sequence shortly after it is synthesized on the ribosome. This SRP-ribosome unit then associates with the SRP receptor (docking protein) on the endoplasmic reticulum where synthesis continues and the newly synthesized protein is translocated across the endoplasmic reticulum.

In experiments designed to sort out the factors necessary to promote translocation, a complete cell-free translational system was incubated with an mRNA encoding a secretory protein of 40-kDa when fully modified,[35S]methionine to monitor protein synthesis, and various preparations containing different factors, as indicated in the table below. GMP-PNP and AMP-PNP are nonhydrolyzable analogs of GTP and ATP, respectively. After each of the preparations was incubated with the translational system in appropriate buffers, the sample was incubated with a protease (proteinase K). Then all proteins were precipitated, denatured, and separated on an SDS gel. Autoradiography of the gel revealed the pattern shown below. Each lane of the autoradiogram is labeled with the corresponding preparation number.
a. What is the significance of the discrete bands in lanes 1, 4, and 5 of the autoradiogram and of the diffuse bands in the other lanes?

Proteinase K cannot enter the ER (i.e. microsomal fraction), thus the digestion only affects proteins that are not successfully sequestered within the microsomes. Thus lanes 2, 3, 6, 7 were not successfully introduced into the ER.

b. What can you conclude from this experiment about the factors required for protein translocation and the mechanisms involved in this process?

ATP or GTP are required for successful sequestration (translocation into ER) according to results of lanes 1 and 6. But the failure of the experiment in lane 7 rules out ATP alone. Comparing the results in lane 3, 4 tells us ATP is hydrolyzed, but GTP is not, so GTP must be acting in a catalytic sense whereas ATP is providing the energy for translocation. In lane 2 the addition of the chelating agent EDTA stops the translocation, probably by sequestering Mg++ and thus preventing ATP hydrolysis required for motivating the translocation. Lane 5 is most interesting. Here the additional time apparently permitted the cleavage of the nascent translocated protein into the mature protein and the signal peptide (small fragment at bottom)

<table>
<thead>
<tr>
<th>Preparation number</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SRP-ribosome complex + microsomes + ATP + GTP</td>
</tr>
<tr>
<td>2</td>
<td>SRP-ribosome complex + microsomes + ATP + GTP + 5mM EDTA</td>
</tr>
<tr>
<td>3</td>
<td>SRP-ribosome complex + microsomes + AMP-PNP + GTP</td>
</tr>
<tr>
<td>4</td>
<td>SRP-ribosome complex + microsomes + ATP + GMP-PNP</td>
</tr>
<tr>
<td>5</td>
<td>SRP-ribosome complex + microsomes + ATP + GTP + more time for incubation</td>
</tr>
<tr>
<td>6</td>
<td>SRP-ribosome complex + microsomes</td>
</tr>
<tr>
<td>7</td>
<td>SRP-ribosome complex + microsomes + ATP</td>
</tr>
</tbody>
</table>
The figure below schematically depicts proteins containing various types of signal and topogenic sequences. Predict the arrangement of each type of protein shown in the figure with respect to the endoplasmic reticulum membrane and lumen.

(a) Ends up in ER lumen (Type I)
(b) Ends up in ER membrane with amino terminus in cytosol. (Type III)
(c) Like b but opposite direction (Type II)
(d) Amino terminus in ER carboxyl in cytosol with three membrane passes (Type IV)
(e) Both ends in cytosol with four membrane passes. (Type IV)