EXAM PREPARATION

You have cloned and sequenced a new receptor protein that is found both in the plasma membrane and in the trans golgi membrane. Sequence analysis indicates there are 4 potential N-glycosylation sites in the protein (N). There is an N-terminal signal sequence ( ) and an internal hydrophobic region ( ) that predicted to be a stop transfer sequence. The predicted amino acid structure is shown below. The sizes of the various portions of the protein are shown below the diagram.

\[
\begin{array}{cccc}
  & N & N & N \\
3 \text{ KDa} & 30 \text{ KDa} & 3 \text{ KDa} & 50 \text{ KDa}
\end{array}
\]

You now want to test whether the structure you have predicted is correct so you perform the following experiment. You translate the mRNA for this protein in an \textit{in vitro} translation system in the presence or absence of microsomes. You treat half of each reaction with protease and run each reaction on an SDS PAGE gel. Each glycosylation adds about 2 KDa to the size of the protein. Predict the size of the protein bands that you would see in each lane of the protein gel from the list at the left.

| Microsomes | - | - | + | + | a. 94 KDa |
| Protease   | - | + | - | + | b. 89 KDa |
|            |   |   |   |   | c. 86 KDa |
|            |   |   |   |   | d. 85 KDa |
|            |   |   |   |   | e. 83 KDa |
|            |   |   |   |   | f. 59 KDa |
|            |   |   |   |   | g. 53 KDa |
|            |   |   |   |   | h. 35 KDa |
|            |   |   |   |   | i. 33 KDa |
|            |   |   |   |   | j. no band |

49. Lane I  __C___
50. Lane II ___J__
51. Lane III___D__
52. Lane IV___H__
Analytical Questions:

Answers to even numbered questions

From Chapter 8:
Question 2: The cells would be expected to secrete lysosomal enzymes into the medium. If the cells were incubated with $^{32}$P-phosphate, the label would not be expected to appear in lysosomal enzymes as it would in control cells. Golgi fractions prepared from the cells should lack the enzyme $N$-acetylglucosamine phosphotransferase, which transfers the phosphorylated sugar from UDP to a mannose receptor.

Question 12: Plasma membrane; SER; RER; lysosome; plant vacuole; plasma membrane; TGN (or other membranes of the biosynthetic pathway); cytosol.

Question 14: The surface of the RER would be smooth not unlike the SER

From Chapter 9:
Question 2: GTP, tubulin, MAPs

Question 6. Warm the preparation, add GTP, add EGTA, add more tubulin, add taxol. Chill the preparation, add colchicine or another depolymerizing agent, add $Ca^{2+}$, place the mixture under hydrostatic pressure.

Question 8: No. In fact, all the microtubules of the axon are oriented in the same direction; different motor proteins move vesicles in anterograde and retrograde directions.

Question 10: The heads, because they must all have a structure that enables them to move along the same type of microtubular track and all of them require a catalytic site that can hydrolyze ATP and use the energy to drive the conformational changes of the power stroke. In contrast, the structure of the tails would be expected to vary according to the type of cargo being transported.

Question 16: That virtually every residue in the protein is indispensable for either maintaining the protein's structure or carrying out its function and that the processes of cell contractility/motility operate by a similar molecular mechanism in all eukaryotic organisms.