

Test Bank for Molecular Biology of The Cell 5th edition by Alberts

Chapter 10:Membrane Structure

Questions

10-1 You are interested in studying the composition of lipid bilayers and how they are maintained. You discover two uncharacterized phospholipids, which you call PLX and PLZ. You decide to characterize the behavior of PLX and PLZ by labeling the head group of each phospholipid. This label is stable when the lipid resides in the membrane's outer leaflet but unstable when the lipid resides in the membrane's inner leaflet. You incorporate labeled versions of PLX and PLZ into either the inside or the outside of the cell, and monitor the change in signal intensity of these lipids in the plasma membrane. Your data are presented in the graphs in Figure Q10-1.

Figure Q10-1

1. Where in the plasma membrane are PLX and PLZ normally located?
2. Are there flippases in the cell for either of these phospholipids? Why?

10-2 You are interested in studying lipid rafts. You have devised a method to create an artificial lipid bilayer that contains small patches of lipids that are straighter and longer than those found in the rest of the bilayer. How would you expect the fluidity of these small patches to compare with the rest of the lipid bilayer? Explain.

10-3 It is thought that excess lipids in the cell are packaged into lipid droplets in the endoplasmic reticulum. Explain why lipid droplets are surrounded by a

phospholipid monolayer and not a phospholipid bilayer, like other vesicles that bud from the endoplasmic reticulum.

10-4 Your friend has isolated plasma membranes and reassembled the membranes into small vesicles. Using fluorescently labeled lectin, he sees that some of his vesicles are fluorescently labeled and some are not. Recall that lectin binds to carbohydrates. Furthermore, his labeled lectin cannot permeate membranes.

1. Which population of vesicles has a surface similar to that of the cell? Why?
2. How do you explain the other population of vesicles?

10-5 Your friend is working on a protein that he calls p125, because of its molecular mass. He knows that p125 is a transmembrane protein with three membrane-spanning domains. It has been previously reported that p125 interacts with three proteins called p175, p80, and p50 (again, on the basis of their apparent sizes on an SDS polyacrylamide gel). These four proteins are thought to exist as a protein complex in the cell. To determine how these proteins interact with the membrane, you perform a set of experiments in which you first lyse the cells and save some of your lysate, which you run in the input lane (labeled "I" in Figure Q10-5). The lysate is then subjected to a low-speed centrifugation so that you separate out the membrane fraction (which ends up in the pellet, "P") from the cytoplasm (which is in the supernatant, "S"). You then wash the pellet from the first extraction with a high-salt wash that does not disrupt the lipid bilayer, and save a little bit to run on the gel. After the high-salt wash, you centrifuge the pellet again. Your results are illustrated on the gel in Figure Q10-5. From these data, explain the nature of the association of these proteins with cellular membranes.

Figure Q10-5

10-6 Examine the two hydropathy plots in Figure Q10-6.

Figure Q10-6

1. Which is a plot of a protein that contains α -helices that cross the membrane?
2. For the protein containing a transmembrane domain, how many transmembrane domains would you predict there are? Why?
3. The plots in Figure Q10-6 were created with a “window size” of 19. In creating a hydrophathy plot, a value is assigned to an amino acid based on how hydrophobic it is deemed to be. The window size determines the number of amino acids over which a hydrophobicity value is averaged. Once a hydrophobicity value is calculated for a particular window, a hydrophobicity value is then calculated starting at the next amino acid for the next window. Explain why a “window size” of 19 is more useful for calculating a hydrophobicity plot for examining transmembrane proteins as opposed to a smaller “window size” such as 5.

10-7 Is the following sentence true or false? Explain.

Peptide bonds are polar and thus must be covalently modified before proteins can be inserted into the membrane.

10-8 Your friend is examining the mobility of transmembrane proteins using FRAP.

1. Explain what FRAP stands for and how it is performed.
2. Figure Q10-8A and B depicts two typical graphs your friend has obtained from her FRAP studies. Which of these graphs would best represent the following types of proteins? Explain your reasoning.

(1) A transmembrane protein that is tethered to the extracellular matrix.

(2) A transmembrane protein that is highly mobile in the membrane.

1. Your friend has also obtained a third graph from her experiments, shown in Figure Q10-8C. Explain how you might get a reading such as this from a FRAP experiment examining transmembrane proteins.

Figure Q10-8

Answers

10-1

1. PLX could be either inside or outside the cell; these data do not allow you to distinguish between these two possibilities. PLZ is outside the cell, because PLZ is rapidly transferred to the outside of the cell and stabilized there.
2. PLZ probably has a flippase. Whether you incorporate it on the inside of the cell or the outside of the cell, it ends up outside the cell.

10-2 The membrane fluidity in these patches is probably less than the fluidity of the rest of the bilayer. The longer the tail is, the greater is the tendency of the hydrocarbon tails to interact, increasing hydrophobic interactions between lipids and making the membrane less fluid. The straighter the tail is (and therefore the fewer *cis* double bonds there are) the easier it is for the lipids to pack together. In addition, increased tail-tail hydrophobic interaction makes the membrane less fluid than regions that contain lipids with more *cis* double bonds because these bonds produce kinks in the hydrocarbon tails.

10-3 The contents of lipid droplets are hydrophobic and therefore interact with the nonpolar tails of phospholipids, whereas the exterior of the droplet is aqueous. Taken together, these properties mean that a monolayer will be optimal, with the phospholipid tails oriented toward the interior of the droplet. Other vesicles have aqueous contents and so require a polar head group facing inside as well as outside the vesicle.

10-4

1. The population of vesicles labeled with fluorescent lectin is most likely to have a surface similar to that of the cell. Lectins bind carbohydrates and the cell adds polysaccharides to the noncytosolic portion of membrane proteins. Because the noncytosolic side of a vesicle will eventually become the extracellular surface of the plasma membrane, it is equivalent to the surface of the cell.

2. The population of vesicles that do not label with fluorescent lectin are probably “inside-out” vesicles. These vesicles have their cytoplasmic face on the outside of the vesicle and thus the glycosylated proteins and lipids are within the vesicles, where they are unable to be labeled.

10-5 We know that p125 is a transmembrane domain protein. In addition, p50 seems to be tightly associated with the membrane. However, p175 is probably a peripheral membrane protein, because it does associate with the membrane fraction after the first extraction but is removed from the membrane after a treatment with high salt concentrations. This suggests that the association of p175 with the membrane is probably via protein–protein contacts with a membrane protein, because high salt concentrations will disrupt protein–protein interactions. In contrast, p80 is a protein that associates with the membrane only via its interaction with the complex, because in the initial purification it is in the cytoplasmic fraction; it is likely that p80 associates only weakly with the complex and thus does not co-sediment with the membrane fraction.

10-6

1. Plot A is a plot of a transmembrane domain protein.
2. The transmembrane protein plotted on graph A is likely to have seven transmembrane domains because there are seven regions that have a hydrophathy index greater than zero, indicating the existence of a stretch of hydrophobic amino acids that could cross the membrane.
3. A window score of 19 is more useful than a window score of 5 because it takes about 20 amino acids to cross the plasma membrane. A stretch of 5 hydrophobic amino acids surrounded by other hydrophilic amino acids would not be sufficient to span the membrane, and thus such a plot would be less informative.

10-7 The statement is false. Although it is true that peptide bonds are polar, the peptide bonds will form hydrogen bonds with themselves in either the α -helix or β -barrel structures that cross the membrane, and thus are shielded from the lipid bilayer. The residues that are exposed to the hydrophobic lipid bilayer are the R-groups of the amino acids.

10-8

1. FRAP stands for Fluorescence Recovery After Photobleaching and is a technique that measures the rate of diffusion of a fluorescently labeled protein. To perform this technique, a protein of interest is fluorescently labeled. The fluorescent molecules in a small area of the cell are then bleached with a laser beam, and the cell is observed over time to see whether unbleached fluorescently labeled molecules will diffuse into the bleached area.

2.

(1) Graph B represents a transmembrane protein that is tethered to the extracellular matrix. If a protein is tethered to the extracellular matrix, it will not be able to diffuse freely. Thus, after bleaching of the area of interest, there will be very little recovery of fluorescence seen, as shown in graph B.

(2) Graph A represents a transmembrane protein that is highly mobile in the membrane, as this protein diffuses back into the bleached area over time.

1. The graph in part C depicts the recovery of a protein that is diffusible, in that fluorescence does increase over time. However, the recovery does not return to the full level observed before the bleaching event. The simplest explanation for this kind of a recovery curve is that there are two populations of this protein: one population that is freely diffusible and one population that has a very small diffusion coefficient.

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