10 Biomembrane Structure

Review the Concepts

1. The spontaneous assembly of phospholipid molecules into a lipid bilayer creates a sheetlike structure that is two molecules thick. Each layer is arranged so that the polar head groups of the phospholipids are exposed to the aqueous environment on one side of the bilayer and the hydrocarbon tails associate with the tails of the other layer to create a hydrophobic core. In cross section, the bilayer structure thus consists of a hydrophobic core bordered by polar head groups. When stained with osmium tetroxide, which binds strongly to polar head groups, and viewed in cross section, the bilayer looks like a railroad track with a light center bounded on each side by a thin dark line.

2. The three main types of lipid molecules in biomembranes are phosphoglycerides, sphingolipids, and steroids. All are amphipathic molecules having a polar head group and a hydrophobic tail, but the three types differ in chemical structure, abundance and function.

3. Cytosolic proteins are anchored to the plasma membrane by acylation or prenylation. In the case of acylation, an N-terminal glycine residue of a protein is covalently attached to the 14-carbon fatty acid myristate (myristoylation) or a cysteine residue in a protein is attached to the 16-carbon fatty acid palmitate (palmitoylation). Prenylation occurs when the –SH group on a cysteine residue at or near the C-terminus of the protein is bound through a thioether bond to either a farnesyl or a geranylgeranyl (prenyl) group. Cell-surface proteins and heavily glycosylated proteoglycans are present on the exoplasmic face of the membrane and are linked there by a glycophosphatidylinositol (GPI) anchor.

4. Lipid bilayers are considered to be two-dimensional fluids because lipid molecules (and proteins if present) are able to rotate along their long axes and move laterally within each leaflet. Such movements are driven by thermal energy, and may be quantified by measuring fluorescence recovery after photobleaching, the (FRAP) technique. In this technique, specific membrane lipids or proteins are labeled with a fluorescent reagent, and then a laser is used to irreversibly bleach a small area of the membrane surface. The extent and rate at which fluorescence recovers in the bleached area, as fluorescent molecules diffuse back into the bleach zone and bleached molecules diffuse outward, can be measured. The extent of recovery is proportional to the fraction of labeled molecules that are mobile, and the rate of recovery is used to calculate a diffusion coefficient, which is a measure of the molecule's rate of diffusion within the bilayer. The degree of fluidity depends on factors such as temperature, the length and saturation of the fatty acid chain portion of phospholipids, and the presence/absence of specific lipids such as cholesterol.

5a. Membrane phospholipids are synthesized at the interface between the cytosolic leaflet of the endoplasmic reticulum (ER) and the cytosol. Water-soluble, small molecules are synthesized and activated in the cytosol. Membrane bound enzymes of the ER then link these small molecules to create larger, hydrophobic membrane phospholipids.

5b. Membrane phospholipids can be flipped from the cytosolic leaflet of the ER membrane to the exoplasmic leaflet. This process, mediated by flippases, results in the incorporation of newly synthesized phospholipids into both leaflets.

5c. Phospholipids can be moved from their site of synthesis to other membranes, e.g., to the plasma membrane. Some of this transport is by vesicles. Some is due to direct contact between membranes. Small, soluble lipid-transfer proteins also mediate transfer. On the whole, the mechanism of phospholipid transfer between membranes is not well understood.

6. Fatty acids have very low solubility inside an aqueous-rich intracellular environment. Therefore, they associate with fatty-acid-binding proteins (FABPs), which are cytosolic proteins that contain a hydrophobic pocket or barrel, lined by β sheets. This pocket provides a haven for the long-chain fatty acid, where it interacts in a noncovalent fashion with the FABP.

7. The common fatty-acid chains in glycerophospholipids include myristate, palmitdate, stearate, oleate, linoleate, and arachidonate. These fatty acids differ in carbon atom number by multiples of 2 because they are elongated by the addition of 2 carbon units. For example, the acetyl group of acetyl CoA is a 2-carbon moiety.

8. The key regulated enzyme in cholesterol biosynthesis is HMG (b-hydroxy-b-methylglutaryl)-CoA reductase. This enzyme catalyzes the rate-controlling step in cholesterol biosynthesis. The enzyme is subject to negative feedback regulation by cholesterol. In fact, the cholesterol biosynthetic pathway was the first biosynthetic pathway shown to exhibit this type of end-product regulation. As the cellular cholesterol level rises, the need to synthesize additional cholesterol goes down. The expression and enzymatic activity of HMG-CoA reductase is suppressed. HMG-CoA reductase has eight transmembrane segments and, of these, five compose the sterol-sensing domain. Sterol sensing by this domain triggers the rapid, ubiquitin-dependent proteasomal degradation of HMG-CoA reductase. Homologous domains are found in other proteins such as SCAP (SREBP cleavage-activating protein) and Niemann-Pick C1 (NPC1) protein, which take part in cholesterol transport and regulation.

9. Several aspects of cholesterol metabolism lead to the conclusion that cholesterol is a multifunctional lipid. Cholesterol is a metabolic precursor to steroid hormones critical for intercellular signaling, vitamin D, bile acids (which help emulsify dietary fats for digestion and absorption in the intestine), stored cholesterol esters, and sterol modified proteins such as Hedgehog.

10. Most phospholipids and cholesterol membrane-to-membrane transport in cells is not by Golgi-mediated vesicular transport. One line of evidence for this is the effect of chemical and mutational inhibition of the classical secretory pathway. Either fails to prevent cholesterol or phospholipids transport between membranes, although they do disrupt the transport of proteins and Golgi-derived sphingolipids. Membrane lipids produced in the ER can not move to the mitochondria by classic secretory transport vesicles. No vesicles budding from the ER have been found to fuse with mitochondria. Other mechanisms are thought to exist. However, presently these are poorly defined (see Figure 10-27). They include direct membrane-membrane contact and small, soluble lipid-transfer proteins.

11. The amphipathic nature of phospholipid molecules (a hydrophilic head and hydrophobic tail) allows these molecules to self-assemble spontaneously into closed bilayer structures when in an aqueous environment. The phospholipid bilayer provides a barrier with selective permeability that restricts the movement of hydrophilic molecules and macromolecules in and out of the compartment. The different types of proteins present on the two faces of the bilayer contribute to the distinctive functions of each compartment's interior and exterior, and control the movement of selected hydrophilic molecules and macromolecules across the bilayer.

12. Membrane-associated proteins may be classified as integral membrane proteins, lipid-anchored

membrane proteins, or peripheral membrane proteins. Integral membrane proteins pass through the lipid bilayer and are therefore composed of three domains: a cytosolic domain exposed on the cytosolic face of the bilayer, a exoplasmic domain exposed on the exoplasmic face of the bilayer, and a membrane-spanning domain, which passes through the bilayer and connects the cytosolic and exoplasmic domains. Lipid-anchored membrane proteins have one or more covalently attached lipid molecules, which embed in one leaflet of the membrane and thereby anchor the protein to one face of the bilayer. Peripheral proteins associate with the lipid bilayer through interactions with either integral membrane proteins or with phospholipid heads on one face of the bilayer.

13. Since biomembranes form closed compartments, one face of the bilayer is automatically exposed to the interior of the compartment while the other is exposed to the exterior of the compartment. Each face therefore interacts with different environments and performs different functions. The different functions are in turn directly dependent on the specific molecular composition of each face. For example, different types of phospholipids and lipid-anchored membrane proteins are typically present on the two faces. In addition, different domains of integral proteins are exposed on each face of the bilayer. Finally, in the case of the plasma membrane, the lipids and proteins of the exoplasmic face are often modified with carbohydrates.

Analyze the Data

a. In the liposomes, there are no constraints on the diffusion of GFP-XR in the plane of the bilayer. Accordingly, when GFP-XR in a small zone of the liposome is bleached, diffusion of the bleached molecules out of and of unbleached molecules from the surrounding region into the bleached zone will result in recovery of the fluorescence in that zone. In the cell, however, no recovery is observed, and these observations suggest that, in vivo, the lateral diffusion of GFP-XR is constrained.

b. XR in the cell membrane is not immobile as is the XR adhered to the microscope slide. Therefore, the lack of fluorescence recovery observed for XR in the cells is not the result of XR's being immobilized but rather the result of XR's being confined within a small domain.

c. In the cells, FRET is observed between GFP-XR and YFP-XR, as indicated by the data showing that yellow light is emitted when GFP is excited. Accordingly, these data suggest that XR molecules are clustered together. XR appears not to be randomly distributed in the plasma membrane as would be expected if the protein were free to diffuse. In liposomes, FRET has not occurred between XR molecules, as no yellow fluorescence is detected. Therefore, XR is not clustered in liposomes and is free to diffuse, as was also suggested by the FRAP and single particle tracking data above.