

CHAPTER

2

Cell Membrane Structures and Functions

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OUTLINE

Phospholipid Bilayers

Cells are bounded by proteins arrayed in lipid bilayers
Amphiphatic molecules can form bilayered lamellar structures spontaneously if they have an appropriate geometry

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Biological Membranes

The fluidity of lipid bilayers permits dynamic interactions among membrane proteins
The lipid compositions of plasma membranes, endoplasmic reticulum and golgi membranes are distinct

Neurons are specialized to integrate selected extracellular signals, both spatially and temporally. In addition to generating action potentials and, through synaptic activity, signaling other cells, structural modifications that may be as transient as ion channel gating or as long lasting as memory are initiated within neurons. Nearly all of this activity involves cell membranes, and many of these membrane functions are discussed in subsequent chapters. This chapter begins with brief discussions of the physical chemistry underlying the lipid and protein components of cell membranes (Figs. 2-1 to 2-4), proceeds to examine some aspects of membrane biochemistry relevant to neurons and their supporting cells, and concludes with discussion of some issues of cell membrane functions that are subjects of current investigations. To perform its unique functional role each neuron must regulate a host of intracellular activities that occur in axons and dendrites

distant from the cell nucleus. For example, both axonal guidance during development and remodeling of dendritic spines in response to local input involve many different complex control systems that are highly localized and largely autonomous (Fivaz & Meyer 2003).

PHOSPHOLIPID BILAYERS

Cells are bounded by proteins arrayed in lipid bilayers

The importance of lipids in membrane structure was established early in the 20th century when pioneering biophysicists established positive correlations between cell membrane

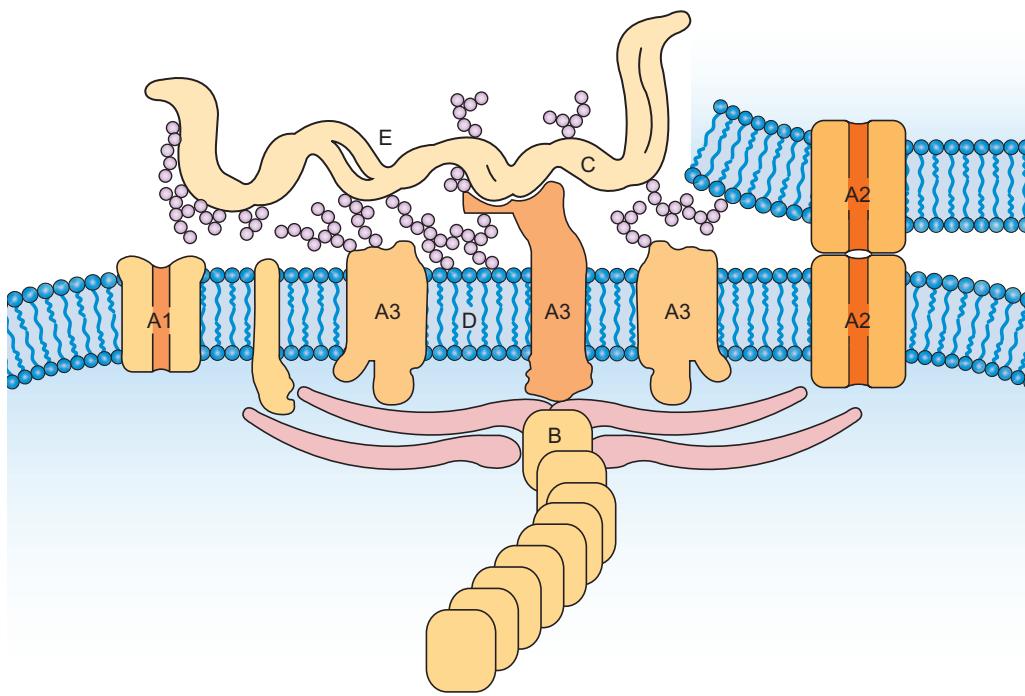


FIGURE 2-1 Overview of plasma membrane structure. Plasma membranes are distinguishable from other cellular membranes by the presence of both glycolipids and glycoproteins on their outer surfaces and the attachment of cytoskeletal proteins to their cytoplasmic surfaces. Interrelations among typical membrane components are depicted. Proteins that are inserted through the lipid bilayer (A_1 – A_3), termed ‘integral’ membrane proteins, are often glycosylated (dark orange circles), as are some bilayer lipids (D) and many components of the extracellular matrix (E). Many interactions at the extracellular surface are stabilized by hydrogen bonding among these glycosyl residues. Certain integral membrane proteins can interact by virtue of specific receptor sites with intracellular proteins (B), with extracellular components (C), and to form specific junctions with other cells (A_2). A host of integral membrane proteins mediates different signal-transduction and active-transport pathways.

permeabilities to small non-electrolytes and the oil/water partition coefficients of these molecules.

Contemporary measurements of the electrical impedance of cell suspensions suggested that cells are surrounded by a hydrocarbon barrier, which was first estimated to be about 3.3 nm thick. This was originally thought to be a lipid monolayer. Among the pioneering biophysical experiments were those that established that the ratio of the area of a monolayer formed from erythrocyte membrane lipids to the surface area of these cells is nearly two. These and other studies of the physical chemistry of lipids led to the concept of a continuous lipid bilayer as a major component of cell membranes. This concept received support from other approaches, including measurements of X-ray diffraction patterns of intact cell membranes. Forces acting between lipids and between lipids and proteins are primarily noncovalent, consisting of electrostatic, hydrogen-bonding, and van der Waals interactions. These are weak interactions relative to covalent bond formation, but they sum to produce very stable associations.

Ionic and polar parts of molecules exposed to water will become hydrated. Substances dissolve in a solvent only if their molecules interact with the solvent more strongly than with each other. In aqueous solution large molecules having two or more domain surfaces of differing polarity will form an internal hydrophobic phase and hydrate the more polar surfaces. Such molecules are termed amphipathic and include most biological lipids and proteins.

Amphipathic molecules can form bilayered lamellar structures spontaneously if they have an appropriate geometry

Most of the major cell membrane lipids have a polar head, most commonly a glycerophosphorylester moiety, and a hydrocarbon tail, usually consisting of two esterified fatty acids. Both domains have similar cross-sectional areas. Consequently, as the head groups interact with each other and with water, and the nonpolar tails aggregate with each other to form an internal phase, the similar cross-sections of the two phases can produce planar bilayers. Three principal phases with different structures are formed by phospholipids in the presence of water (Tanford, 1980) (Fig. 2-2). Although the lamellar, or bilayer, structure is generally found in cell membranes, the hexagonal phases may occur transiently during membrane shape transformations.

The importance of molecular geometry for bilayer stability is illustrated by the effect of phospholipase A2, a component of many venoms, on erythrocytes: this enzyme removes the C-2 fatty acid from phospholipids to produce lysophosphatides. Because of the ‘conical’ geometry of lysophosphatides, this process ultimately destabilizes bilayers relative to the hexagonal phase structures; this disrupts cell membranes and lyses the cells. Detergents are amphipathic molecules with the ability to transform lipid bilayers into water-soluble micelles. In contrast to the destabilizing effects of lysophosphatides and

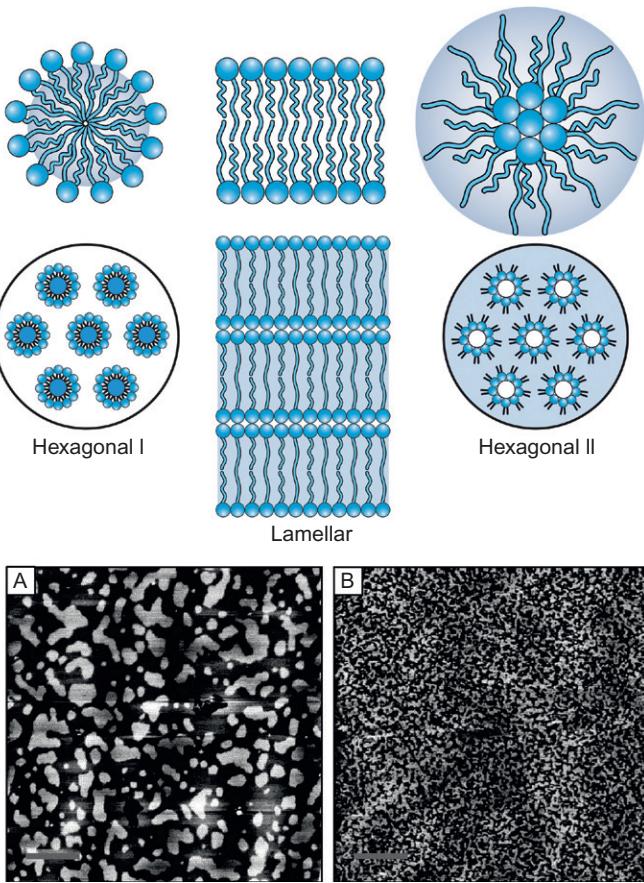


FIGURE 2-2 Top row: Complex lipids interact with water and with each other to form different states of aggregation, or ‘phases’, shown here schematically. Open circles represent the more polar head groups, and dark lines and areas represent nonpolar hydrocarbon chains. The phase structures are generally classified as illustrated in the middle row of the figure. The hexagonal I and lamellar phases can be dispersed in aqueous media to form the micellar structures shown in the top row. Hexagonal II phase lipids will form ‘reverse’ micelles in nonpolar solvents. The stability of lamellar relative to hexagonal structures depends on hydrocarbon chain lengths, presence of double bonds, relative sizes of polar head and hydrocarbon tail groups, and temperature. Bottom row: Atomic Force Microscopic images (6×6 mm, scale bar = 1 mm, Z-scale = 5 nm) showing (left) domains in bilayers of 1:1 sphingomyelin:dioleylphosphatidylcholine combined with 30% cholesterol and (right) domains of 1:1 dipalmitoylphosphatidylcholine:dioleylphosphatidylcholine combined with 30% cholesterol. Lighter areas are higher than darker areas. From (31) with permission. From (Van Duyl, Ganchev, Chupin, de Kruijff, & Killian, 2003) with permission.

other detergents, cholesterol stabilizes bilayers by intercalating at the interface between head and tail regions of phospholipids so as to satisfy the bulk requirements for a planar geometry. The multilamellar bilayer structures that form spontaneously on adding water to solid- or liquid-phase phospholipids can be dispersed to form vesicular structures called liposomes. These are often employed in studies of bilayer properties and may be combined with membrane proteins to reconstitute functional membrane systems. A valuable technique for studying the properties of proteins inserted into bilayers employs a single bilayer lamella, also termed a black lipid membrane, formed across a small aperture in a thin partition between

two aqueous compartments. Because pristine lipid bilayers have very low ion conductivities, the modifications of ion-conducting properties produced by membrane proteins can be measured with great sensitivity (Ch. 4). In aqueous systems, membrane lipids may exist in a gel-like solid state or as a two-dimensional liquid. In the case of pure phospholipids, these states interconvert at a well-defined transition temperature, T_c , which increases with alkyl chain length and decreases with introduction of alkyl chain unsaturation. In cell membranes, which have marked heterogeneity in both the polar and non-polar domains of the bilayer, this state is described as ‘liquid disordered’. The presence of sufficient sphingolipids, with saturated alkyl chains, and of cholesterol, which has a rigid planar structure, can cause a ‘liquid-ordered’ structure to separate laterally into microdomains that are in phase equilibrium with ‘liquid-disordered’ structures (Fig. 2-2, bottom row). Such microdomains, consisting of ‘lipid rafts’ enriched in cholesterol and sphingomyelin, may function in biomembranes to concentrate or localize certain membrane proteins, as discussed below.

Alkyl chain heterogeneities cause cell membrane bilayers to remain in the fluid state over a broad temperature range. This permits rapid lateral diffusion of membrane lipids and proteins within the plane of the bilayer. The lateral diffusion rate for an unconstrained phospholipid in a bilayer is of the order of $1\text{ mm}^2\text{s}^{-1}$; an integral membrane protein such as rhodopsin would diffuse $\approx 40\text{ nm}^2\text{s}^{-1}$.

MEMBRANE PROTEINS

Membrane integral proteins have transmembrane domains that insert directly into lipid bilayers

Transmembrane domains (TMDs) consist predominantly of nonpolar amino acid residues and may traverse the bilayer once or several times. High-resolution structural information is available for only a few integral membrane proteins, primarily because it is difficult to obtain membrane protein crystals that are adequate for X-ray diffraction measurements. TMDs usually consist of α helices. The peptide bond is intrinsically polar and can form internal hydrogen bonds between carbonyl oxygens and amide nitrogens, or either of these may be hydrated. Within the lipid bilayer, where water is essentially excluded, peptides usually adopt the α helical configuration that maximizes their internal hydrogen bonding. A length of helix of 18–21 amino acid residues is sufficient to span the usual width of a lipid bilayer (Fig. 2-3). Because the surface properties of a helix are determined by its side chains, a single helical segment that can insert into or through a bilayer will consist largely of hydrophobic residues. Integral membrane proteins with one transmembrane domain may have ‘soluble’ domains at either or both surfaces. An example of a monotopic protein, cytochrome b_5 , has a single hydrophobic segment that forms a hairpin loop, acting as an anchor to the cytoplasmic surface but probably not totally penetrating the bilayer. Bitopic proteins with a single transmembrane helix are more common. If oriented with the N-terminus extracytoplasmic, they are classified as type I or, if cytoplasmic, type II (Fig. 2-4). Bitopic membrane proteins are often involved in signal transduction,

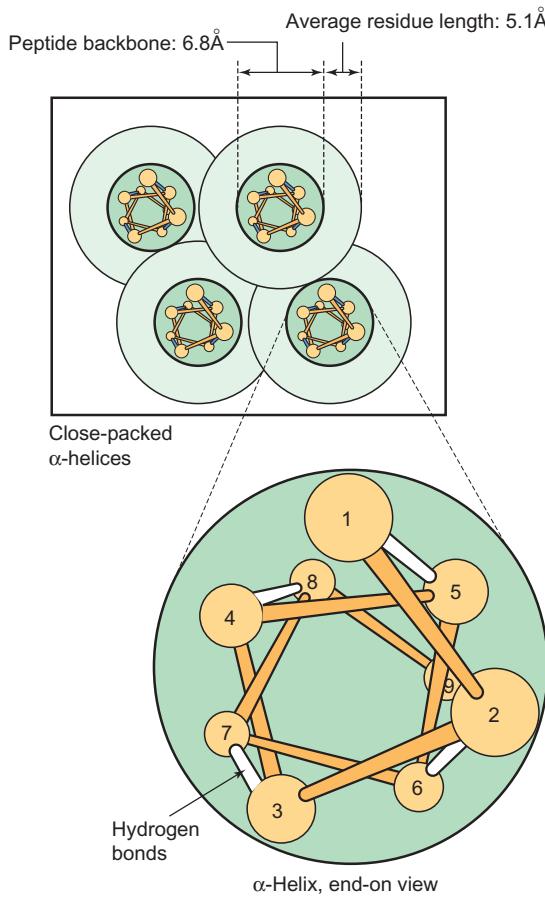


FIGURE 2-3 The transmembrane domains of integral membrane proteins are predominantly α -helices. This structure causes the amino acid side chains to project radially. When several parallel α -helices are closely packed, their side chains may intermesh as shown, or steric constraints may cause the formation of interchain channels. The outwardly directed residues must be predominantly hydrophobic to interact with the fatty acid chains of lipid bilayers. The bilayer is about 3 nm thick.

Each peptide residue extends an α -helix by 1.5 Å. Thus, although local modifications of the bilayer or interactions with other membrane polypeptides may alter this requirement, transmembrane segments usually require about 20 residues to span the bilayer. Integral membrane proteins are characterized by the presence of hydrophobic segments approximating this length.

as exemplified by receptor activated tyrosine kinases (Ch. 26); agonist occupation of an extra-cytoplasmic receptor domain can transmit structural changes via a single transmembrane helix to activate the latent kinase activity in a cytoplasmic domain. Ion channels, transporters, and many receptors are polytopic. Polar and helix-destabilizing residues are likely to occur within their transmembrane segments to form the requisite gates, channels, or binding domains. Transmembrane helices in polytopic proteins are usually closely packed. Examples of this are G-protein-coupled receptors (GPCRs; Ch. 21), and the sarcoplasmic Ca^{2+} pump (Ch. 3). Each peptide bond has a significant dipole moment, which is transmitted to the ends of a helix. This circumstance favors close packing of antiparallel

helices and is the observed disposition of helices in bacteriorhodopsin (Kimura et al., 1997). In oligomeric transmembrane proteins, intersubunit packing can encompass extramembranous guanylyl protein domains, and bilayer lipids.

Many transmembrane proteins that mediate intracellular signaling form complexes with both intra- and extracellular proteins

For example, neural cell adhesion molecules (NCAMs) are cell-surface glycoproteins (Ch. 9). The extracellular domains of NCAMs can activate fibroblast growth factor receptors when clustered by reaction with NCAM antibodies (Leshchynska et al., 2003) or by homotypic binding to domains of adjacent cells (see Fig. 9-3). Activation was found to sequester a complex of NCAM, β I spectrin and $\text{PKC}\beta 2$ into rafts, as defined by the operational criteria discussed above. Assembly of this complex has been implicated in neurite outgrowth. Disassembly of the NCAM/spectrin complex leads to perforation of the postsynaptic density and formation of postsynaptic endocytic zones (Puchkov et al., 2011).

Membrane associations can occur by selective protein binding to lipid head groups

One example is spectrin, which binds to phosphatidylinositol-4,5-bisphosphate by means of a pleckstrin-homology (PH) domain (Wang & Shaw, 1995) (Fig. 2-5) and also to phosphatidyl serine (An & Guo, 2004) (Fig. 2-6). Ca^{2+} influx initiates protein and membrane associations by several different mechanisms. Allosteric regulation of the hydrophobicity of protein-binding surfaces frequently occurs. One of the best-studied examples is the Ca^{2+} -dependent binding of calmodulin to other proteins (Ch. 24). Annexins are a family of proteins that exhibit Ca^{2+} -dependent associations with cell membranes through direct interaction with phospholipids. Conversely, interactions of annexins with phospholipids increase the affinities of the annexins for Ca^{2+} (Mollenhauer, 1997).

BIOLOGICAL MEMBRANES

The fluidity of lipid bilayers permits dynamic interactions among membrane proteins

For example, the interactions of a neurotransmitter or hormone with its receptor can dissociate a 'transducer' protein, which in turn will diffuse to interact with other effector proteins (Ch. 12). A given effector protein, such as adenylyl cyclase, may respond differently to different receptors because of mediation by different transducers. These dynamic interactions require rapid protein diffusion within the plane of the membrane bilayer. Receptor occupation can initiate extensive redistribution of membrane proteins, as exemplified by the clustering of membrane antigens consequent to binding bivalent antibodies (Poo, 1985). In contrast to these examples of lateral mobility, the surface distribution of integral membrane proteins can be fixed by interactions with other proteins.

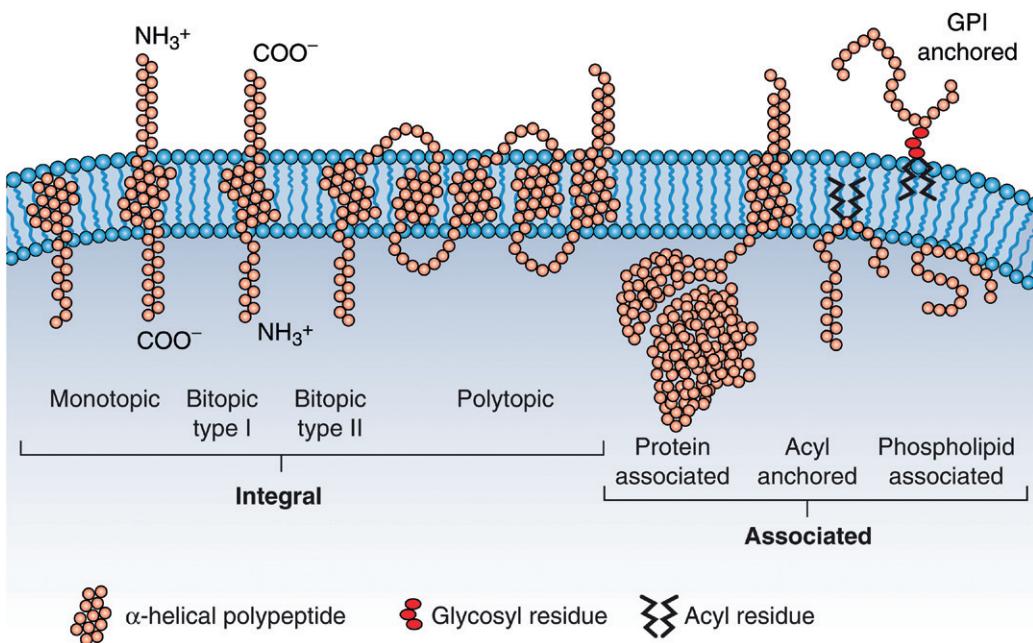


FIGURE 2-4 Left: Integral membrane proteins can be classified with respect to the orientation and complexity of their transmembrane segments. Right: Proteins may associate with membranes through several types of interactions with the bilayer lipids and with other integral membrane proteins. They also can be ‘anchored’ by integration into the bilayer of covalently bound lipids. GPI = glycosylphosphatidylinositol.

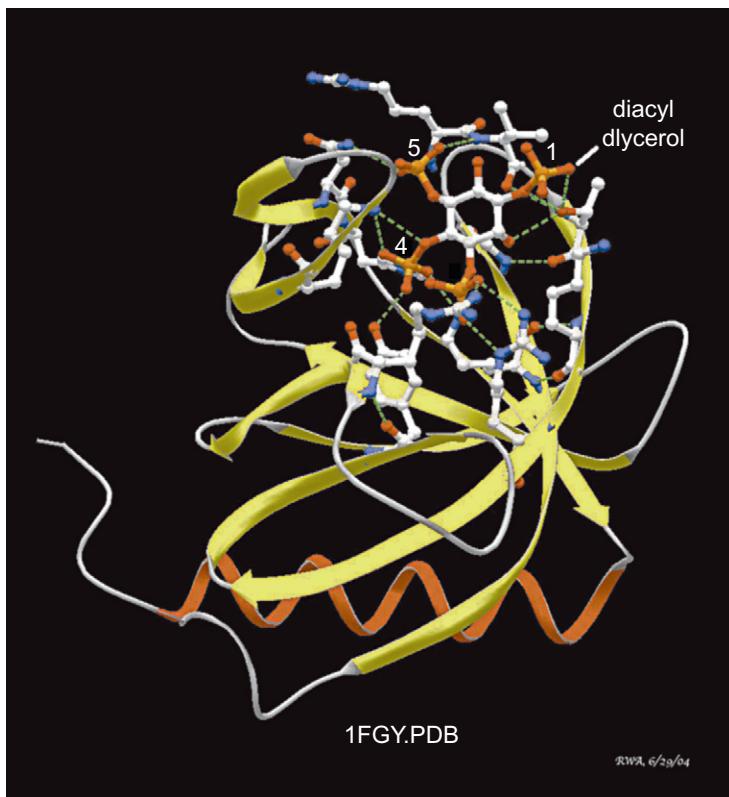


FIGURE 2-5 The pleckstrin homology (PH) domain of Grp1, a GDP-GTP exchange factor for Arf GTPases. Activation of Grp1 occurs when this domain binds to PI(3,4)P₂ or PI(3,4,5)P₃ produced in the inner leaflet of plasma membranes by a PI3-kinase (Ch. 21). This structure is shown complexed with inositol(1,3,4,5)-tetraphosphate as it was crystallized for X-ray diffraction, whereas diacyl glycerol would be esterified to the inositol-1-phosphate in the membrane-bound form. The amino-acid residues (ball-and-stick models) shown are those that approach the inositol tetraphosphate nearly enough to form hydrogen bonds (green dashes). The model was constructed from the Protein Data Bank coordinates 1fgy (Lietzke et al., 2000) using Deep View 3.7 (Guex et al., 2001).

Membranes may also be partitioned into local spatial domains consisting of networks of cytoskeletal and scaffolding proteins or lipid rafts. This partitioning may restrict the translational motion of enmeshed proteins and yet allow rapid rotational diffusion. Examples of such spatial localization include

restriction of Na,K pumps to the basolateral domains of most epithelial cells, Na⁺ channels to nodes of Ranvier, and nicotinic acetylcholine receptors to the postsynaptic membranes of neuromuscular junctions. Because membrane components participate in nearly every cell activity, their structures are

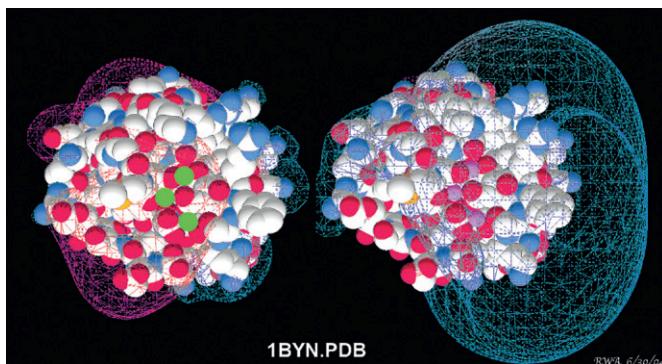


FIGURE 2-6 The C2 Ca^{2+} -dependent lipid-binding domain of **synaptotagmin**. Although binding calcium to this domain has little effect on its conformation, it produces a markedly increased affinity for negatively charged lipids such as phosphatidyl serine. The red and blue grids are isopotential lines over the molecular surface: red = negative, blue = positive potentials. On the left are the potentials in the absence of calcium (the green circles mark the empty Ca^{2+} binding sites. On the right are the grids after Ca^{2+} -binding, which intensifies the positive field and presumably increases the affinity for negatively charged phospholipids.

More sophisticated evaluations of C2 domains from different proteins show that in some cases calcium can drive the binding surface to a neutral potential, possibly favoring binding of zwitterionic phospholipids (Murray & Honig, 2002). Constructed with Deep View 3.7 from the Protein Data Bank coordinates 1byn (Guex et al., 2001).

also dynamic and far from the equilibrium states that are most readily understood in biophysical terms. Newly synthesized bilayer lipids are initially associated with endoplasmic reticulum (Chs. 3, 5); some lipids such as phospholipids initially insert into the luminal endoplasmic reticulum (ER) leaflet. Glycosylation of ceramides occurs as they transit the Golgi compartments, forming cerebrosides and gangliosides in the luminal leaflet. Thus, unlike model systems, the leaflets of ER membranes are asymmetric by virtue of their mode of biosynthesis.

The lipid compositions of plasma membranes, endoplasmic reticulum and golgi membranes are distinct

Plasma membranes, endoplasmic reticulum and Golgi membranes all have asymmetric distributions of lipids between cytoplasmic and exocyttoplasmic leaflets. Little is known of the intrinsic mechanisms that maintain the distinctions among these interacting membrane classes. As Golgi vesicles fuse into the plasma membrane their luminal surfaces become extracellular at the plasma membrane surface. The aminophospholipids phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) remain cytoplasmic, but most phosphatidyl choline (PC) is transported to the exoleaflet, perhaps by the ATP-dependent ABCA1 transporters that occur in both neurons and glia (see Chapter 5). Conditions that elevate cell Ca^{2+} , such as anoxia, can activate a Ca^{2+} -dependent ‘scramblase’ that catalyzes transverse PS movements. Exposure of PS extracellularly constitutes a phagocytotic signal to

microglia and other phagocytes that express surface receptors for PS (DeSimone, Ajmone-Cat, & Minghetti, 2004). An ATP-dependent aminophospholipid translocase activity in plasma membranes prevents this occurrence in healthy cells.

Cholesterol transport and regulation in the central nervous system is isolated from that of peripheral tissues

Blood-borne cholesterol is excluded from the CNS by the blood-brain barrier. Neurons express a form of cytochrome P-450, 46A, which oxidizes cholesterol to 24(S)-hydroxycholesterol (Lund et al., 2003) and may oxidize it further to 24,25- and 24,27-dihydroxy products (Mast et al., 2003). In other tissues hydroxylation of the alkyl side chain of cholesterol at C22 or C27 is known to produce products that diffuse out of cells into the plasma circulation. Although the rate of cholesterol turnover in mature brain is relatively low, 24-hydroxylation may be a principal efflux path to the liver because it is not further oxidized in the CNS (Dietschy & Turley, 2004). During brain development *de novo* cholesterol synthesis occurs at high rates at various stages in all types of brain cells. In human adults, brain cholesterol constitutes 23% of total body cholesterol, about 10 times higher than the average of all tissues. Much of this cholesterol derives from the oligodendrocyte plasma membrane component of myelin; about 80% is associated with myelin in mouse brain and a somewhat larger fraction in human brain. In contrast to its high content, the metabolic turnover of adult brain cholesterol is relatively low: for humans this rate is estimated to be 0.03% per day for brain cholesterol, compared to 0.7% per day for whole-body cholesterol (Dietschy & Turley, 2004).

In adult brain most cholesterol synthesis occurs in astrocytes

Apoprotein E (apoE) is the major apolipoprotein of the CNS, where it is secreted by astrocytes. In astrocyte cultures apoE appears in the media as cholesterol-rich particles of a size similar to peripheral HDL (5–12 nm) (Fig. 2-7). The ATP-dependent transporter ABCA1, expressed by both astrocytes and neurons, promotes the formation of the apoE-stabilized high-density lipoprotein (HDL)-sized particles from astrocytic cholesterol. Although the extracellular release of cholesterol is sometimes described as a passive ‘shedding’ process, in astrocytes it seems clear that cholesterol and phospholipid are mobilized from plasma membranes and that their transfer to extracellular apoE to form lipoprotein particles is facilitated by ABCA1 transporters. The mechanism for cholesterol transfer into neurons is less certain. There are seven members of the ‘low-density lipoprotein receptor’ (LDLR) family and they all are expressed either in developing or adult brain. They are all type I bitopic membrane receptors that uniformly incorporate a characteristic array of domains: they exhibit extracellularly a ligand-binding domain and between one and eight epidermal growth factor domains; intracellularly they exhibit one or two NPxY motifs that act both as phosphotyrosine-binding domains and endocytotic signals. Two LDLRs,

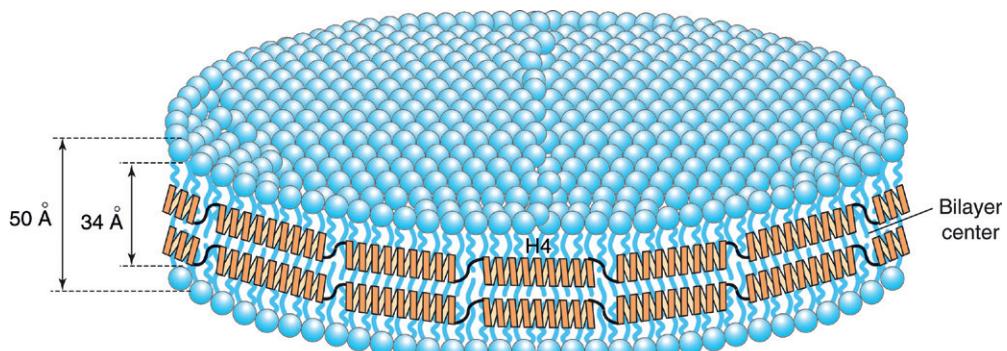


FIGURE 2-7 The ATP-dependent transporter ABCA1, expressed by both astrocytes and neurons, promotes the formation of the apoE-stabilized high-density lipoprotein, (HDL)-sized particles, from astrocytic cholesterol. This putative model of an apoE-stabilized HDL particle assumes that 2 molecules of apoE encircle a discoidal phospholipid bilayer with their helical axes oriented perpendicularly to the hydrocarbon chains. Adapted from Narayanaswami et al. (2004) with permission.

apoER2 and very low-density lipoprotein receptor (VLDLR), are apoE receptors expressed on neuron plasma membranes, and probably mediate HDL-lipid uptake into neurons. These same receptors interact with signaling ligands and adaptor proteins that mediate neuronal migration during brain development. They seem to have a role in adult brain, perhaps involving axoplasmic transport of essential components for synaptic remodeling (Chs. 7, 56), and are subject to damage in Alzheimer's disease (Ch. 46).

Although apoE HDL particles are formed by astrocytes *in vitro*, the brain contents of apoE knockout ($-/-$) were not found to differ in lipid content in comparison to those obtained from normal animals (Han, Cheng, Fryer, Fagan, & Holtzman, 2003). A probable explanation is that newly synthesized cholesterol can be transported from astrocyte ER to plasma membrane via an alternative route that employs caveolae to form apoA1-HDL (Ito et al., 2002). See Chapter 7 for a discussion of caveolae; these are endocytic structures enriched in cholesterol.

The astrocytic cholesterol supply to neurons is important for neuronal development and remodeling

This is supported by observations of neuronal cell cultures (Goritz et al., 2002). Pure cultures of rat retinal ganglion cells from 8-day-old rats, in the absence of glia or serum, will extend axons and form synapses (autapses) that display low-frequency postsynaptic currents. Co-culturing these neurons with glia from 2-day-old rats was observed to stimulate the formation of twice as many synapses and these exhibited about 12 times higher frequencies of postsynaptic activity. Astrocytes and oligodendrocytes, but not microglia, produced similar effects, as did replacement of the glia with glial-conditioned culture medium. ApoE was secreted by glia into their culture medium, but adding recombinant apoE to the retinal ganglion cell cultures did not stimulate the formation of synapses, nor did it increase their efficacy. However both of these effects were replicated by adding cholesterol to the retinal ganglion cell cultures (Fig. 2-8).

Other investigators have grown similar purified preparations of retinal ganglion cells on channel-inscribed plates that cause the axons to extend linearly and permit measurement of axon elongation rates (Hayashi et al., 2004). These axons will grow across siliconized barriers into side channels, which effectively isolates, externally, the axon membranes from the soma. Glial-conditioned medium was found to stimulate the axon elongation rates about 50% for several days when added to the side channels, but it had no effect if present only in the central compartments containing the neuronal soma. HDL lipoproteins, purified from glial conditioned medium, stimulated equally well, but unlike the effects on synapses in the previous study, neither pure apoE nor cholesterol could replace the lipoprotein in stimulating axon elongation. The problem of why cholesterol is sufficient to support synapse formation (Goritz et al., 2005) but not axon elongation remains unresolved.

The stimulations of synaptic activity and of axon elongation observed in these experiments were both inhibited by RAP, an inhibitor protein selective for members of the LDLR family. These inhibitions are most readily explained as resulting from blockade of apoE-lipoprotein uptake via neuronal LDL receptors. Developing neurons can synthesize cholesterol, but this capacity decreases as neurons mature. Under conditions of rapid extension of neurites, glial support may be necessary to meet the large energy demands of neuronal membrane biosynthesis (Ch. 11) and also to circumvent the limitations of endocytotic vesicle trafficking from neuronal soma to distal axons and dendrites (Ch. 8). Even in adult brain, continuous cholesterol synthesis is necessary for the remodeling of synapses that is now recognized to be part of information processing (Ch. 56).

Cholesterol and sphingolipids are synthesized and transported through the ER and Golgi systems (Bjorkhem & Meaney, 2004). However they are present at much higher levels in the outer leaflet of plasma membranes. Adding cholesterol and sphingomyelin to synthetic lipid bilayers can produce thicker 'liquid-ordered' membranes. This has led to proposals that bilayer thickness may be a factor in sorting integral membrane proteins between ER and Golgi. Liquid-ordered bilayers have an 'elastic' ability to adjust

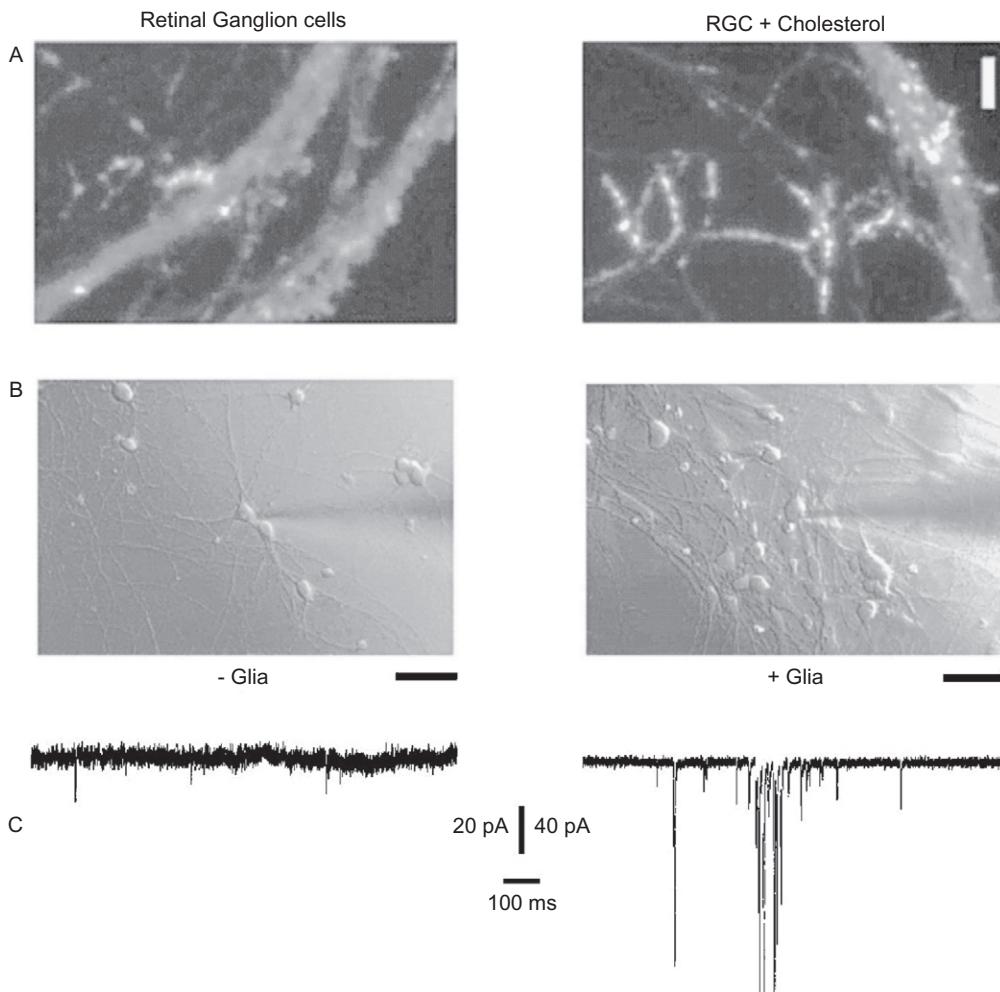


FIGURE 2-8 Cholesterol increases the number and release efficacy of synapses in single retinal ganglion cells. (A) Fluorescence micrographs of retinal ganglion cell synapses labeled by a synapsin I-specific antibody. The cells were cultured in the absence (left) or presence (right) of cholesterol. Scale bar = 5 nm. (Reproduced from (Mauch et al., 2001) with permission.) (B) Hoffman-modulation contrast micrographs of retinal ganglion cells cultured for 5 days in defined serum-free medium in the absence (left) or presence (right) of collicular glia. Scale bar = 50 nm. The density of neurons was similar in both cultures. (C) Spontaneous synaptic activity in these cells: whole-cell patch-clamp recordings of spontaneous currents (EPSCs) at a holding potential of -70 mV . (Reproduced from Pfrieger & Barres (1997) with permission.)

their thickness as they interact with TMDs. Alkyl chain associations with hydrophobic protein residues are energetically favorable relative to interactions with more polar surfaces. This adjustment has an energetic cost that depends on the extent of bilayer deformation (Lundbaek et al., 2003). Because many plasma membrane integral proteins (PMIPs) are polytopic, with TMDs of varying length, the alkyl chain heterogeneity in length and in unsaturation of lipid bilayers may also be factors in sorting proteins into different membranes. Mechanisms of selection of integral membrane proteins for transit through or retention by the Golgi system are largely unknown. Shortening the TMD of a plasma membrane protein was found to result in Golgi retention, (Sivasubramanian & Nayak, 1987) and the converse effect occurred on lengthening the TMD of a normally retained Golgi protein (Munro, 1995). The lengths of transmembrane domains for Golgi-retained proteins are usually less than for those directed to the plasma membrane.

The structure and roles of membrane microdomains (lipid rafts) in cell membranes are under intensive study but many aspects are still unresolved

Several lines of evidence indicate that membrane signaling occurs in dynamic subcompartments composed of cholesterol, sphingolipids and proteins (Lingwood & Simons, 2010; Simons & Gerl, 2010). Unlike in synthetic bilayers (Fig. 2-2), no way has been found to directly visualize rafts in biomembranes (Munro, 2003; Kusumi et al., 2004). Many investigators operationally define raft components as those membrane lipids and proteins (1) that remain insoluble after extraction with cold $\approx 1\%$ Triton X-100 detergent, (2) that are recovered as a low-density band that can be isolated by flotation centrifugation, and (3) whose presence in this fraction is reduced by cholesterol depletion.

Much of the plasma membrane cholesterol is removed by incubating cells with β -methylcyclodextrin for several hours. Cells remain viable after this treatment but the raft fraction is reduced. It is inferred that the depleted proteins are normally associated with cholesterol-dependent lipid rafts. Some, but not all, glycosylphosphatidylinositol (GPI)-anchored proteins are recovered in the fractions defined by this procedure. A proteomic study of the HeLa cell proteins in raft fractions, identified by these criteria and estimated by quantitative mass spectrometry, has identified 241 'authentic raft proteins' (Foster, de Hoog, & Mann, 2003). This analysis found that the 'raft proteins' most enriched relative to total membrane proteins belong to several classes of signaling protein: kinases and phosphatases, heterotrimeric G proteins, and small G proteins.

Most of the raft proteins identified in this way are not plasma membrane-insoluble proteins (PMIPs), but rather are proteins that associate with the cytoplasmic lipid leaflet or with cytoplasmic domains of PMIPs. The inability to visualize rafts in biological membranes by direct optical methods suggests that they are very small and/or very transient. This has been investigated by methods such as fluorescence recovery after photolysis (FRAP), which provides information about lateral diffusion, and fluorescence resonant energy transfer (FRET), which can detect 'molecular crowding' when separation distances are less than $\approx 1\text{ nm}$. These experiments have generally supported the existence of very small domains of restricted lateral diffusion and of protein clustering in living cell plasma membranes.

GPI-anchored proteins (GPI-APs) are synthesized in the cytoplasm, and their transport into the ER occurs during the process of acquiring a GPI anchor, which is ultimately sorted into the outer leaflet of plasma membranes (Murakami et al., 2003).

A FRET study of fluorophore-labeled GPI-APs in cultured cells (Sharma et al., 2002) concluded that most of the GPI-APs existed as monomers in these cells but a significant fraction, 20–40%, existed as very small, dense clusters that may be a signal for endocytosis. The study's authors also suggest that the size of these GPI-AP clusters may be controlled by processes that regulate plasma membrane cholesterol.

The membrane-associated small G proteins, H-Ras and K-Ras, have been studied with respect to their association with cytoplasmic leaflets. These two proteins have nearly identical structures and functions but different membrane anchors, membrane distributions, and effector responses. Application of the FRAP method to fluorescent constructs of H-Ras and K-Ras revealed that only H-Ras in its guanosine diphosphate (GDP)-bound form associates with cholesterol-dependent rafts (Niv et al., 2002).

In addition to fluorescence methods, another study (Parton & Hancock, 2004) employed a method to permit electron microscopic localization of Ras anchor domains on cytoplasmic membrane surfaces by immunogold labeling. The particle neighbor distances can be analyzed to obtain information about possible domain structure. Expressing H-Ras and K-Ras in baby hamster kidney cells, a nonrandom particle distribution was obtained from which the estimated mean raft size was $7.5\text{--}22\text{ nm}$ and about 35% of the membrane area consisted of rafts. The same technique applied to cells that had been incubated with β -cyclodextrin to reduce cholesterol

produced completely random distributions of H-Ras. This cholesterol dependence suggests some type of coupling of rafts in the inner and the outer membrane leaflets.

Mechanical functions of cells require interactions between integral membrane proteins and the cytoskeleton

These functions include organization of signaling cascades; formation of cell junctions; and regulation of cell shape, motility, and endo- and exocytosis. Several different families of membrane-associated proteins mediate specific interactions among integral membrane proteins, cytoskeletal proteins, and contractile proteins. Many of these linker proteins consist largely of various combinations of conserved protein-association domains, which often occur in multiple variant copies. In erythrocytes and most other cells, the major structural link of plasma membranes to the cytoskeleton is mediated by interactions between ankyrin and various integral membrane proteins, including Cl⁻/HCO₃⁻ antiporters, sodium ion pumps, and voltage-dependent sodium ion channels. Ankyrin also binds to the $\approx 100\text{ nm}$, rod-shaped, antiparallel heterodimers of spectrin and thus secures the cytoskeleton to the plasma membrane.

Spectrin dimers self-associate to form tetramers and further to form a polygonal network parallel to the plasma membrane (Fig. 2-9D). Neurons contain both spectrin I, also termed erythroid spectrin, and spectrin II, also termed fodrin. Spectrin II is found throughout neurons, including axons, and binds to microtubules, whereas spectrin I occurs only in the soma and dendrites. This spectrin network further binds to actin microfilaments and to numerous other ligands. These associations are probably dynamic. For example, phosphorylation of ankyrin can alter its affinity for spectrin. The functions of the multiple protein-interaction domains of both spectrin and ankyrin have been as yet only partially defined (see Ch. 6).

The spectrin–ankyrin network comprises a general form of membrane-organizing cytoskeleton within which a variety of membrane–cytoskeletal specializations are interspersed

Many of these are concerned with cell-cell or cell-matrix interactions (Ch. 6). The several morphological types of cell-cell junctions are associated with junction-specific structural and linking proteins. For example, tight junctions, also termed *zona occludens*, are constructed of the integral membrane protein occludin, which binds the linking proteins ZO-1 and ZO-2 (Ito et al., 2002). These linking proteins are members of a large family, termed membrane-associated guanylyl kinase homologs (MAGUKs). The general structure of this family has, distributed from the N-terminus to the C-terminus, one or more PDZ-binding domains, a src-homology-3 (SH3) domain (see Ch. 26) and a guanylyl kinase homolog domain. Other members of the PDZ family are expressed in neurons at postsynaptic densities. One of these, PSD-95, contains two N-terminal PDZ domains that can bind to a motif, $-\text{E-S/T-D-V}-$, which occurs in N-methyl-D aspartate (NMDA) receptors. Multimeric clusters of these receptors or channels can be formed through

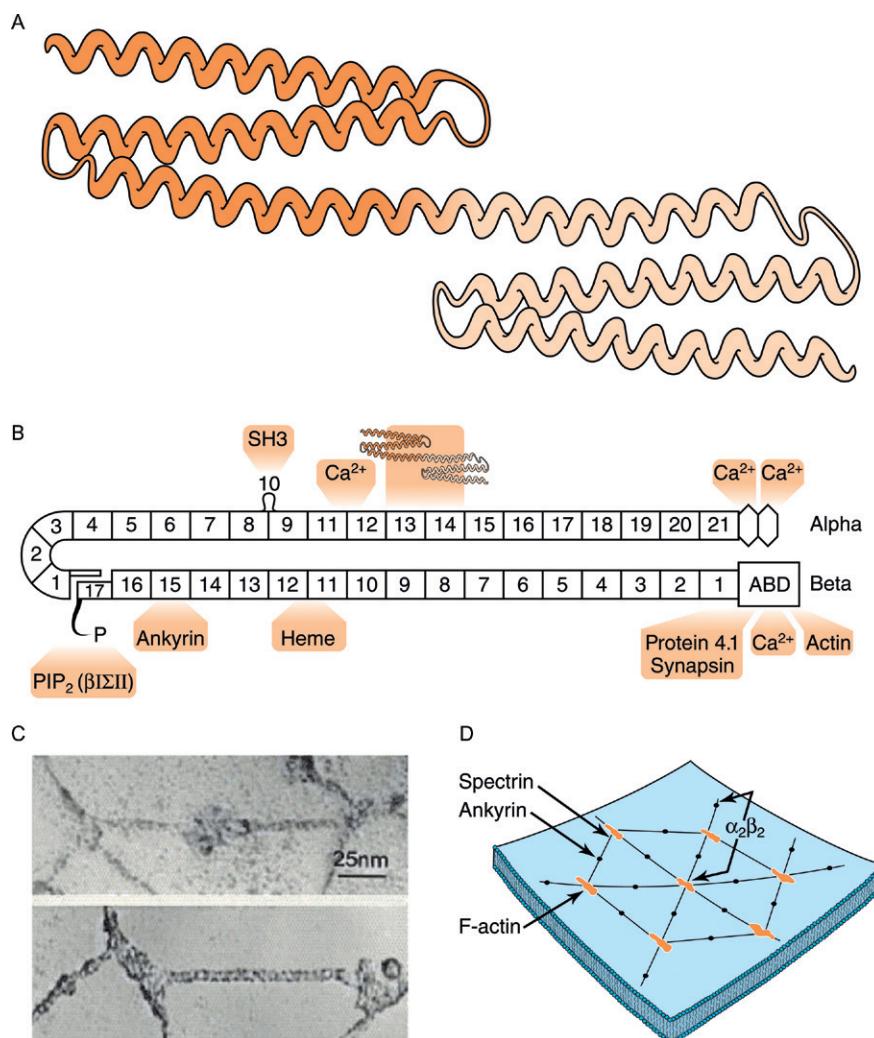


FIGURE 2-9 The ankyrin-spectrin lattice. (A) Structural model of a spectrin repeat unit based on the crystal structure of a dimer of the 14th repeat unit of *Drosophila* spectrin (Adapted from (Yan et al., 1993), with permission). (B) Cartoon of the domain structure of a spectrin dimer. Many of the repeat units constitute binding domains with different specificities. Some have been identified and are labeled here. ABD = actin-binding domain; PIP₂ = phosphatidylinositol-4,5-biphosphate domain—occurs only on the βIΣII isoform; SH3 = src-homology-3 domain. (Adapted from (Ursitti et al., 1996), with permission.) (C) Electron micrographs of rotary-shadowed spectrin tetramers. Note the periodic substructure of spectrin filaments and the putative site of a complex with an ankyrin molecule (top, center). (Courtesy of J. Ursitti). (D) Schematic organization of the spectrin-ankyrin cytoskeleton on the cytoplasmic surface of neurons $\alpha_2\beta_2$ are the spectrin subunits depicted in (B). (Redrawn from Goodman et al., 1995, with permission.)

disulfide cross-linking between cysteines of the N-terminal domains of PSD-95 molecules (Hueh et al., 1997). Different PDZ domains within a single linker protein can display different peptide motif selectivities. Accordingly, it has been suggested that a given linker protein may simultaneously bind to multiple different channels and receptors to produce complex clusters at various postsynaptic sites.

Interaction of rafts with the cytoskeleton is suggested by the results of video microscopy

Brightfield video microscopy can record movements of single 40-nm gold particles on the outer surface of cells in culture. Such particles, if coated with an appropriate binding protein,

can bind to a component of the cell surface. In the experiments to be described, gold particles with fluorescein antibody were attached to fluorescein-derivatized dioleylphosphatidyl ethanolamine under conditions designed to produce beads with predominantly single molecules attached. These were introduced into cells on coverslip cultures, and the movements of these particles were recorded with digital video and analyzed (Fig. 2-10) (Murase et al., 2004). Fig. 2-10A shows the video trace of a single particle's movement over 10 seconds, which appears random and unstructured when recorded at 33-ms intervals. The same particle movements, when recorded at 110 µs intervals (300 times faster), reveals a path consisting of random hops from one 'confinement compartment' to another, usually at less than 33-ms intervals and thus only detectable at the higher speeds. Additional experiments were designed to examine

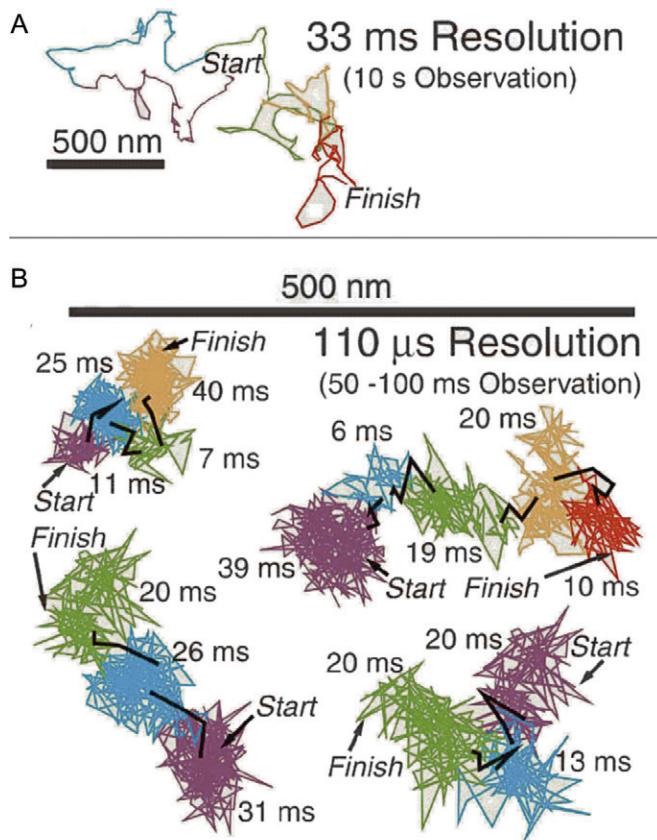


FIGURE 2-10 Tracking a gold particle attached to a single molecule of phosphatidylethanolamine. What appears to be simple Brownian diffusion at a time resolution of 33ms per video frame (**A**) is revealed to consist of fast 'hop diffusion' by recording 300 times faster (**B**) at 110 μ s per video frame. In (**A**) each color represents 60 frames = 2 seconds. In (**B**) each color indicates an apparent period of confinement within a compartment and black indicates intercompartmental hops. The residency time for each compartment is indicated. The hypothetical explanations are illustrated in part (**C**) and discussed in the text. Adapted from Murase et al. (2004).

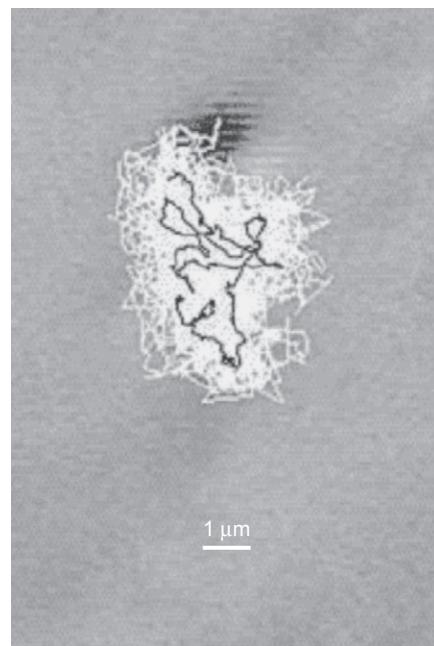


FIGURE 2-11 Video-enhanced Differential Interference Contrast (DIC) images of gold-labeled μ -opioid GPCR on the surface of a GPCR-transfected fibroblast. The white trace is the trajectory of one particle over 2 minutes at 25 frames/s. The black trace is the mean square displacement of the particle as a function of time. Reproduced from Figure 1 of Daumas et al. (2003), with permission.

the generality of this phenomenon in different cell types and to establish the structure of the confinement compartments. Compartmentalization was detected in all of the eight mammalian cell types examined with sizes \approx 32–230nm diameter. Prior treatment of the cells with the actin depolymerizing agent cytochalasin on average increased the compartment size approximately twofold. Treatment with an actin-stabilizing agent had little effect on the compartment size but increased the median residency time approximately sevenfold. Both the size and the actin dependence suggest that the actin–spectrin network may be the source of the compartments. Another research group has applied gold particle tracking to measure movements of the μ -opioid GPCR on the surface of GPCR-transfected fibroblasts (Daumas et al., 2003). They describe the pattern observed in Fig. 2-11 as a ‘walking defined diffusion mode’. More than 90% of the observed particles displayed this pattern, which consists of rapid diffusion within a ‘domain area’ (with a mean size of about 150nm) combined with a much slower (\approx 10-fold) drift of the whole domain. These authors interpret their data as the natural result of restrictions imposed on the free diffusion of the labeled receptor by encounters with other transmembrane proteins in the bilayer. However, they consider that their data are incompatible with the hop-and-skip model based on spectrin mesh confinement.

SPECTRIN AND LIPID RAFT MEMBRANE COMPONENTS PARTICIPATE IN THE PATHOLOGY OF BRAIN INJURY

George J. Siegel

Spectrin: Trauma that results in the disruption of cell membrane molecular structure may involve separation of ankyrin from its linkage to membrane proteins and the spectrin network subjacent to the membrane. After membrane disruption, the spectrin may undergo proteolysis by calpain and caspase, which are excessively stimulated by unregulated influxes of Ca^{2+} through neurotransmitter- and voltage-activated channels. The disrupted membrane organization involves various proteins, including ion channels, transporters, receptors, cell adhesion molecules and scaffolding proteins (Bennett & Healy 2008). In various types of trauma, including mechanical, ischemic, hemorrhagic and toxic, spectrin breakdown products are found in the CSF, and the amount is correlated with the extent of brain trauma (Gold et al., 2009). Calpain proteolysis of the axon initial segment cytoskeletal proteins ankyrin and spectrin leads to loss of ion channels and loss of polarity in neurons, which can be prevented by inhibition of calpain (Schafer et al., 2009). Beta-III spectrin is highly expressed in cerebellar Purkinje cells where a critical function is stabilization of the EAAT4 glutamate transporter (see Ch. 17). Mutations in this spectrin gene are the cause of spinocerebellar atrophy type 5, which has been described in an 11-generation kindred descending from President Abraham Lincoln's grandparents and two other families (Ikeda et al., 2006). Mutations in the spectrin and ankyrin families are causes of hereditary spherocytosis and long QT (ankyrin B or sick sinus) syndrome [1].

Lipid rafts are membrane microdomains enriched in sphingolipids and cholesterol, and they contain regulatory proteins, including certain enzymes, receptors, and signaling and transport proteins. Alterations in the composition of membrane lipids associated with lipid rafts occur as a function of aging and neurodegeneration (Schengrund 2010; Rushworth & Hooper 2010), with possible changes in the function of proteins in the microdomains. Lipid rafts are where the active γ -secretase complexes are located. These complexes are needed to form β -amyloid from amyloid precursor protein (APP) and for the accumulation of amyloid-ganglioside complexes in Alzheimer's disease [6] (see also Ch. 46). Lipid rafts are also loci for conformational conversion of cellular prion to infective prion molecules (see Ch. 50) (Taylor & Hooper 2007) and for phosphorylation of α -synuclein, which accumulates in Parkinson's disease (see Chs. 47, 49) (Zabrocki et al., 2008). Entry of HIV (human immunodeficiency virus) and perhaps other viruses into cells involves interaction of the viruses with receptors in lipid rafts (Carter et al., 2009). Thus, lipid raft components are targets for research into potential therapies for neurodegenerative and viral diseases and for prevention of infection (Cheng et al., 2007).

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