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Review

Lipid-binding proteins as stabilizers of membrane microdomains — possible physiological significance★✪

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Below the melting point temperature of lipids, artificial lipid membranes usually exist in the ordered gel phase. Above these temperatures lipid acyl chains become fluid and disordered (liquid-crystalline phase). Depending on the chemical composition of artificial membranes, phase separation may occur, leading to the formation of transient or stable membrane domains. A similar phase separation of lipids into ordered and disordered domains has been observed in natural membranes at physiological temperature range. Moreover, it has been reported that certain proteins prefer certain organization of lipids, as for example glycosylphosphatidylinositol-anchored proteins or Src family of tyrosine kinases. The aim of present review is to discuss the possibility that some lipid microdomains are induced or stabilized by lipid-binding proteins that under certain conditions, for example due to a rise of cytosolic Ca^{2+} or pH changes, may attach to the membrane surface, inducing clustering of lipid molecules and creation of ordered lipid microdomains. These domains may than attract other cytosolic proteins, either enzymes or regulatory proteins. It is, therefore, postulated that lipid microdomains play important roles within a cell, in signal transduction and enzymatic catalysis, and also in various pathological states, as Alzheimer's disease, anti-phosphatidylserine syndrome, or development of multidrug resistance of cancer cells.

★75th Anniversary of Membrane Lipid Bilayer Concept.

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Abbreviations: GPI, glycosylphosphatidylinositol; IP_3, inositol 1,4,5-trisphosphate; PH, pleckstrin homology; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PS, phosphatidylserine.

The general postulate of the "fluid mosaic model" for biological membrane structure and dynamics (Singer & Nicolson, 1972), stating that the basic structure of a biological membrane is the lipid bilayer, is still accepted. Undoubtfully, membrane lipids form amphipathic bilayers that surround cells and organelles and block the leakage of hydrophilic compounds, as well as provide the environment for amphipathic membrane proteins. However, the wide variety of lipids observed in biological membranes extends beyond the concept of a simple barrier function. For example, phospholipids alone display a tremendous variety of headgroups and acyl chain structures, and in addition, in cell membranes of Eukaryotes sphingolipids and sterols are present. One of the consequences of the chemical heterogeneity of lipids is the possibility of non-random mixing in the bilayer. Moreover, the last decade of investigations has clearly indicated that all biological membranes are not uniform in respect to chemical composition and distribution of lipids and proteins within both transversal and lateral planes. Additionally, experimental evidence has been provided that phase separations do occur in fluid membranes such as biological membranes, resulting in the formation of microscopic domains with various life-times (for a review see Brown & London, 1997, 1998, and a special issue of *Molecular and Membrane Biology* edited by Bergelson, Gawrisch, Ferretti & Blumenthal, 1995). It is suggested that these domains function in physiologically important cellular events, such as signal transduction (Pawson, 1995; Buday, 1999; Leevers *et al.,* 1999; Parent & Devreotes, 1999) or exocytosis (Mullem & Lee, 1997), as they can strongly affect membrane structure by concentrating interacting species in particular regions or by excluding diffusing molecules from other regions (Vaz & Almeida, 1993; Ediddin, 1997).

Lipid domains exist in a variety of forms and their formation can be caused by different mechanisms: lipid phase separation, protein barriers, and electrostatic interactions between membrane-associated components (Ediddin, 1992; McLaughlin & Aderem, 1995; Brown & London, 1998; Glomset, 1999). A variety of basic proteins or peptides bind to acidic phospholipids, and some of them are able to induce the formation of domains or affect phase separations. For example, clustering of phosphatidylserine (PS) molecules in large liposomes appears to be important in activating protein kinase C (PKC), and the appearance of PS-enriched microdomains regulates enzyme activity; these interactions are disrupted by high ionic strength and by a PKC inhibitor, polylysine (Yang & Glaser, 1995). Heterogeneity in the lateral distribution of lipids implies that some lipid molecules may be transiently segregated into domains, while others are staying out of these domains as disorganized lipids. It is suggested that long-range lateral order in membranes could extend for 100–200 nm or even on a larger scale (micrometers) (Welti & Glaser, 1994; Smart *et al.,* 1999).

LIPID DOMAINS IN BIOLOGICAL MEMBRANES

A membrane domain can be defined as any area in a membrane which differs in lipid and protein composition, and in physical properties, from other areas of the membrane (Fig. 1). Transmembrane asymmetry in lipid and protein composition between the two leaflets of the membrane is a general feature of all biological membranes, and obviously is crucial for cell functions, as well as cell death (Zachowski, 1993). However, the present review focuses on lipid domains existing in the lateral plane of the membrane. The term "lipid domain" encompasses many types of structures (Harder & Simons, 1997). They can range in size from tiny ones, comprising only several molecules, to very large ones, covering tens of square microns; also their life-time varies from nanoseconds to the life of a cell (Welti & Glaser, 1994; Ediddin, 1997; Brown

& London, 1998; Kurzchalia & Parton, 1999). Several types of lipid domains could potentially exist simultaneously in one membrane (Welti & Glaser, 1994). A unique type of microdomains are the tight junctions of polarized epithelial cells that regulate barrier function at mucosal surfaces and are considered as complexes of smaller microdomains; their assembly was found to be regulated by Ca^{2+} (Nusrat *et al.,* 2000).

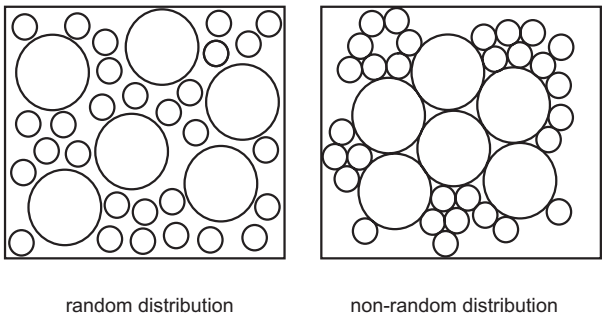

Figure 1. Lipid microdomains.

The co-existence within the membrane of regions with random (liquid-crystalline, unordered) and non-random (gel, ordered) structures as a consequence of phase separation in membranes comprising a mixture of various lipids, at a physiological temperature range; other explanations in the text.

What is the role of lipid domains? Domains rich in glycosphingolipids and cholesterol, the rafts, have been postulated to organize signal transduction complexes (since they concentrate signaling molecules) at the plasma membrane. They were also found to participate in the lateral recruitment of certain types of proteins, and in the polarized sorting of apical membrane proteins in epithelial cells (Brown, 1998; Smart *et al.,* 1999). Apart from their function in the transport of newly synthesized material to the cell surface, they may participate in clathrin-independent endocytosis (Harder & Simons, 1997). Lipids that form rafts exist in a state similar to the liquid-ordered phase. Furthermore, it has been proposed that proteins which prefer such ordered lipid environment will partition into these domains (Melkonian *et al.,* 1999; Brown & London, 2000); targeting of certain proteins to these domains could be mediated by lipid–lipid interactions between lipid-modified proteins and raft lipids. Indeed, it has been found that myristoylation and palmitoylation of the Gα subunit of heterotrimeric G proteins is necessary for the partitioning of Gα into rafts. Strikingly, partitioning of fatty-acylated Gα subunit was reduced by thioacylation of myristoylated Gα with *cis*-unsaturated fatty acids instead of palmitic acid. Therefore, structural differences between lipids that modify proteins may form a basis for the selectivity of protein targeting to rafts (Moffett *et al.,* 2000).

In order to probe the dynamics and size of lipid rafts in the membrane of living cells, local diffusion of single membrane proteins was measured using protein constructs with identical ectodomains and different membrane regions, and *vice versa*. With the aid of this experimental approach it has been demonstrated that when glycosylphosphatidylinositol (GPI)-anchored or transmembrane proteins become associated with rafts, their lateral diffusion is significantly reduced. On the other hand, by depletion of cholesterol one may accelerate the diffusion of raft-associated proteins (Pralle *et al.,* 2000), pointing to a crucial role of cholesterol in determining the physical properties of rafts (Brown, 1998).

A specialized type of rafts are caveolae. The major membrane proteins of caveolae, caveolins, are postulated to be involved in cholesterol-dependent regulation of specific signal transduction pathways, as well as in cholesterol traffic (Lavie *et al.,* 1999; Smart *et al.,* 1999). Additionally, in caveolae other proteins have also been identified, such as: PKCα, phospholipase D2, ectoapyrase CD39, endothelial nitric oxide synthase, receptors for epidermal and platelet-derived growth factors, Ras, Gα, phosphatidylinositol (PI) kinases, MDR1 (Ilangumaran *et al.,* 1999; Kurzchalia & Parton, 1999; Lavie *et al.,* 1999), amyloid precursor protein (Ikezu *et al.,* 1998), and annexins (Garver *et al.,* 1999).

Caveolae look under the microscope like small invaginations (50–100 nm in diameter) on the cell surface (Harder & Simons, 1997; Kurzchalia & Parton, 1999). In addition, it turned out that caveolae can also be flat within the plane of the plasma membrane or detached vesicles, or even can fuse to form grape-like structures and tubules significantly larger than 100 nm (Smart *et al.,* 1999). Caveolae are abundant in endothelia, muscle cells, adipocytes, and lung epithelial cells; similar structures were also found within the nervous system. Particularly in the nervous system these structures have been implicated not only in signal transduction, membrane structural organization, and cell adhesion, but also in pathologies such as Alzheimer's and prion diseases (Lavie *et al.,* 1999; Masserini *et al.,* 1999).

Caveolae are characterized by a unique lipid composition. In contrast to non-caveolar regions of the plasma membrane that are composed of phospholipids, caveolae are mainly composed of cholesterol and sphingolipids. They are resistant to detergent solubilization and are frequently called detergent-resistant membranes or low-density Triton-insoluble domains. Multiple independent approaches allowed numerous investigators to identify these microdomains in living cells (Smart *et al.,* 1999).

A different type of domains is formed by interactions between membrane-associated proteins and lipid-binding proteins (Kinnunen *et al.,* 1994; Bottomley *et al.,* 1998; Johnson & Cornell, 1999). For example, a membrane-associated positively charged peptide derived from the myristoylated alanine-rich C kinase substrate (MARCKS) has been shown to participate in the organization of domains rich in phosphatidylinositol 4,5-bisphosphate (PIP_2) through electrostatic interactions (Brown & London, 1998).

Targeting of proteins into microdomains is controlled by specific lipid composition and/or membrane protein receptors (Johnson & Cornell, 1999). In some cases, peripheral attachment of proteins to membranes is characterized by strong calcium dependence, e.g., for annexins (Nelsestuen & Ostrowski, 1999). Calcium sensitivity of membrane–protein interactions is assured by the presence of specific modules within the protein molecule (Ames *et al.,* 1997; Lohi & Lehto, 1998; Gelb *et al.,* 1999; Katan & Allen, 1999). Such domains include the "annexin fold" (Gerke & Moss, 1997; Lecat & Lafont, 1999; Nelsestuen & Ostrowski, 1999) and the C2 domain present in various protein families, for example in PKC isoforms (Nalefski & Falke, 1996; Mosior & Epand, 1997; Rizo & Südhof, 1998) and cytosolic phospholipase A_2 (PLA_2) (Perisic *et al.,* 1998). Other domains, as the pleckstrin homology (PH) domain, are responsible for charge–charge interactions of proteins with membranes enriched in PIP_2 (Harlan *et al.,* 1995; Lemmon *et al.,* 1996; Rebecchi & Scarlata, 1998). Moreover, experimental evidence has been presented that protein modular domains are not only responsible for specific interactions of proteins with lipids, but also for the co-ordinate disassembly of membrane domains (Fanning & Anderson, 1999).

In cell membranes domains created only by lipid–lipid interactions are small (< 10 nm). They can form because of very small differences in the energies of interaction between different species of phospholipids. Such domains can be also readily disrupted, therefore, they cannot grow larger. Larger domains can be observed as a result of interactions between lipids and solvent, detergent or protein molecules (Ediddin, 1997). The latter possibility is illustrated by the following example. It has been shown that crosslinking of IgE-$F_c(\varepsilon)$ receptor complexes on RBL-2H3 mast cells causes their association with lipid rafts, in a cholesterol-dependent process that precedes the initiation of signaling by these receptors. This is accompanied by co-redistribution of a GPI-anchored protein Thy-1, the Src family tyrosine kinase Lyn, and F-actin with IgE–$F_c(\varepsilon)$ receptor complexes in large patches at the plasma membrane. It was, therefore, postu-

lated that interactions between Lyn and IgE–$F_c(\varepsilon)$ receptor complexes are regulated by F-actin polymerization and segregation of GPI-anchored raft proteins (Holowka *et al.,* 2000).

ROLE OF LIPID-BINDING PROTEINS IN FORMATION OF LIPID DOMAINS

Annexins

Annexins are water-soluble, calcium-binding proteins which share common properties due to their homologous repeated core domains (approximately 72 amino-acid residues, forming five α-helices per domain). These domains bind anionic phospholipids in a Ca^{2+}-dependent manner (Raynal & Pollard, 1994). Annexins have been proposed to participate in calcium homeostasis (Frey *et al.,* 1999) and in the regulation of ion channel activities, and evidence is accumulating for their role in membrane traffic (Gerke & Moss, 1997; Bandorowicz-Pikuła & Pikuła, 1998). Their function is likely to be mediated by their interactions with other proteins such as the S100 proteins, C2 domain-containing molecules, G proteins, proteases, protein kinases, and cytoskeletal elements (Gerke & Moss, 1997; Kamal *et al.,* 1998). Also intracellular localization of annexins argues for their involvement in exocytosis and endocytosis (Bandorowicz-Pikuła & Pikuła, 1998; Bandorowicz-Pikuła *et al.,* 1999; Lecat & Lafont, 1999).

Several experiments show that the calcium-dependent binding to membranes is fast and may be reversed by the addition of calcium chelators (Swairjo & Seaton, 1994; Swairjo *et al.,* 1995; Benz & Hofmann, 1997). Upon binding to membranes, annexins are able to cluster acidic phospholipids, and reveal high binding energies (Bazzi & Nelsestuen, 1991a; 1991b). The large number of positive membrane contact sites for annexins is consistent with the reqiurement of many calcium ions for the binding (Nelsestuen & Ostrowski, 1999).

The membrane-bound forms of annexins were characterized by various techniques, as for example by neutron scattering of liposomes in the absence and presence of annexin V (Ravanat *et al.,* 1992). It has been found that in the presence of annexin V the radius of gyration of liposomes increases, consistent with the formation of a protein shell of 3.5 nm thickness around a liposome, with no insertion of the protein into the hydrophobic core of the membrane (Ravanat *et al.,* 1992). The interaction of the single tryptophan residue (Trp187) of annexin V with different positions in the spin-labelled acyl chain of lipids was investigated by measuring the quenching of its intrinsic fluorescence (Meers, 1990). The results indicated an interfacial location of Trp187 and a lack of membrane penetration (Meers, 1990).

The discovery that various annexins are able to form ion channels upon binding to membranes rose additional questions about the mechanism of interaction of these soluble proteins with membranes (Demange *et al.,* 1994; Burger *et al.,* 1994; Kaneko *et al.,* 1997; Hofmann *et al.,* 1998). Especially in the light of recent observations that at mildly acidic pH annexin V and annexin XII undergo structural transitions and become more hydrophobic; this allows these proteins to intercalate into the membrane hydrophobic core and form non-specific ion channels (Isas *et al.,* 2000).

Annexins are able to interact with specific membrane regions, e.g., the apical endocytic compartment in rat liver hepatocytes (Ortega *et al.,* 1998), and with specific membrane domains (Dillon *et al.,* 2000). It has been shown recently by Babiychuk *et al.* (1999) that upon smooth muscle cell stimulation, followed by a rise in intracellular Ca^{2+} concentration, annexin-dependent membrane-cytoskeleton complexes transiently appear. A detailed analysis of such complexes revealed that in smooth muscle cells annexin II and annexin VI may

interact with actomyosin and detergent-insoluble glycosphingolipid-enriched membrane domains. This interaction is preceded by the translocation of annexins from the cytoplasm to plasma membrane in a Ca^{2+}-dependent manner (Babiychuk *et al.,* 1999). A similar translocation to Triton-insoluble membrane domains has been observed for annexin II in chromaffin cells stimulated with nicotine (Sagot *et al.,* 1997). This property of annexins has been ascribed to their ability to bind actin (Hosoya *et al.,* 1992; Diakonowa *et al.,* 1997). This interaction is postulated to take place in specialized membrane domains, caveolae, where actin and myosin subfragment 1 have been indentified (Smart *et al.,* 1995; Schnitzer *et al.,* 1995), along with the caveolae-specific receptor for inositol 1,4,5-trisphosphate (IP_3) (Fujimoto *et al.,* 1992). Therefore, annexins may play a role in the organization of caveolae and also in the transmission of signals during muscle contraction *via* the annexins-cytoskeleton-microdomains complexes (Babiychuk *et al.,* 1999). In addition, it has been postulated that interactions of annexins with specific membrane domains may affect functioning of various integral membrane proteins (e.g., ion channels and enzymes) (Kaetzel *et al.,* 1995), as well as PKC isoforms (Dubois *et al.,* 1996) and phospholipases (Russo-Marie, 1999).

C2 domain-containing proteins

C2 domains of approximately 130 residues were first identified in PKC. Later they were found in a large number of eukaryotic signaling proteins that interact with cellular membranes and mediate a broad array of critical intracellular processes, including membrane trafficking, generation of lipid second messengers, activation of GTPases, and control of protein phosphorylation (Nalefski & Falke, 1996). As a group, C2 domains display the remarkable property of binding a variety of different ligands and substrates, including Ca^{2+}, phospholipids, inositol polyphosphates, and intracellular proteins. It is, however, worth stressing that C2 domains are not obligatory Ca^{2+}- and phospholipid-binding modules, and have diverged in the course of evolution into Ca^{2+}-dependent and Ca^{2+}-independent forms that interact with multiple targets (Rizo & Südhof, 1998).

In contrast to the "annexin fold", C2 domains are autonomously folding protein modules exhibiting β-sheet secondary structure. Multiple calcium ions bind as a cluster at the tip of a C2 domain, without inducing a large conformational change, and they work as electrostatic switches particularly useful for fast calcium-triggered reactions, such as neurotransmitter release (Rizo & Südhof, 1998). The crystal structure of cytosolic PLA_2 revealed that for this enzyme hydrophobic interactions are as important for membrane binding as electrostatic interactions (Perisic *et al.,* 1998).

Proteins containing C2 domains include enzymes involved in lipid metabolism (e.g. cytoplasmic PLA_2 of molecular mass 85 kDa, PLC isoforms, PI 3-kinases and PS decarboxylase) (Nalefski & Falke, 1996), and protein phosphorylation (e.g., isoforms of PKC) (Rizo & Südhof, 1998). To the C2 family of proteins belong also various proteins involved in membrane traffic (e.g., synaptotagmin, rabfilin, DOC2, Munc13, perforin) and in the regulation of GTPase activity (e.g., Ras-GAP, BUD2) (Nalefski & Falke, 1996; Rizo & Südhof, 1998). Recently, a group of proteins of uncertain functions, called copines, conserved from *Paramecium* to humans, has been identified to contain at their N-terminus two C2 domains (Creutz *et al.,* 1998).

Proteins containing PH domain

PH domains represent a third type, after the "annexin fold" and the C2 domain, of modular protein structures involved in mediating protein–lipid interactions. The PH domain was originally identified in pleckstrin and then in phospholipase C (PLC) isoforms, cyto-

plasmic PLA_2, guanine nucleotide-exchange factors, serine/threonine and tyrosine protein kinases, GTPases involved in vesicular traffic, cytoskeletal proteins (β-spectrin), adaptors, and inositol phosphatide-metabolizing enzymes (Lemmon *et al.,* 1996; Scaife & Margolis, 1997; Bottomley *et al.,* 1999; Katan & Allen, 1999).

The PH domain consists of approximately 100 amino-acid residues. Despite rather low sequence homology among PH modules identified in various proteins, their three-dimensional structure is remarkably conserved; minimal secondary structure elements are seven β-strands and one C-terminal α-helix (Rebecchi & Scarlata, 1998). Structural studies on the N-terminal PH domain of pleckstrin revealed a potential ability of this domain to interact with PIP_2 and probably with other phospholipids with negatively charged head portions (Harlan *et al.,* 1995). However, it has been shown that PH domains from PLCβ1 and PLCβ2 allow these proteins to interact strongly with surfaces composed only of neutral lipids (Wang *et al.,* 1999). These and other observations suggest that the view of PH domains as devices serving solely for the recruitment of proteins to the membrane is an oversimplification, since PH domains also trigger critical conformational changes of proteins, important in their function (Katan & Allen, 1999).

Other proteins

High calcium stoichiometry with respect to binding of peripheral proteins with membranes is a characteristic feature of pentraxins and vitamin K-dependent proteins. The biological function of pentraxins is not known. Pentraxins are divided into two subgroups: serum amyloid proteins and C-reactive proteins. Human serum amyloid protein has a high affinity for membranes that contain acidic phospholipids. Pentraxins normally exist as dimers of pentamers. The geometry and electrostatic asymmetry of the pentamer suggest that membrane-contact sites contain multiple calcium ions and are characterized by the presence of a highly electropositive surface (Nelsestuen & Ostrowski, 1999).

Vitamin K-binding proteins are characterized by the presence of 9–13 γ-carboxyglutamic acid residues in the N-terminal domain (residues 1–45). The high charge density of the N-terminus suggested that membrane contact occurs *via* a mechanism similar to that of annexins and pentraxins, however, recently it has been found that hydrophobic forces or a "specific binding site" are involved (Nelsestuen & Ostrowski, 1999).

Proteins associating with lipid rafts include also influenza virus hemagglutinin. This is the viral envelope glycoprotein that mediates fusion between the viral membrane and membranes of host cells. Hemagglutinin is a homotrimer; each subunit contains two disulfide-linked polypeptide chains, HA_1 and HA_2. Fusion experiments with recombinant hemagglutinin revealed that the GPI-anchored protein mediates "hemifusion" of membranes (Kemble *et al.,* 1994). By studing a synthetic peptide homologous to the transmembrane segment of hemagglutinin, it was found that the peptide is able to increase acyl chain order in lipid bilayers, which may be related to the preferential association of hemagglutinin with lipid rafts on the cell surface, and may be important for the hemagglutinin-catalyzed complete membrane fusion (Tatulian & Tamm, 2000).

CONCLUDING REMARKS

In this review evidence is presented indicating that lipid microdomains are induced and/or stabilized by specific interactions between lipid-binding proteins and lipid molecules (Fig. 2). Such interactions may evoke phase separation, lipid clustering and change the affinity of membrane components to one another. Some of these interactions are modu-

lated by calcium ions, due to the existence in protein molecules of specific calcium-sensing modules, e.g., the "annexin fold" (Gerke & Moss, 1997; Nelsestuen & Ostrowski, 1999) or the C2 domain (Nalefski & Falke, 1996), responsible for binding of proteins to PS molecules. Other modules, as the PH domains, are involved in binding of proteins to PI phosphates (Lemmon *et al.,* 1996; Rebecchi & Scarlata, 1998).

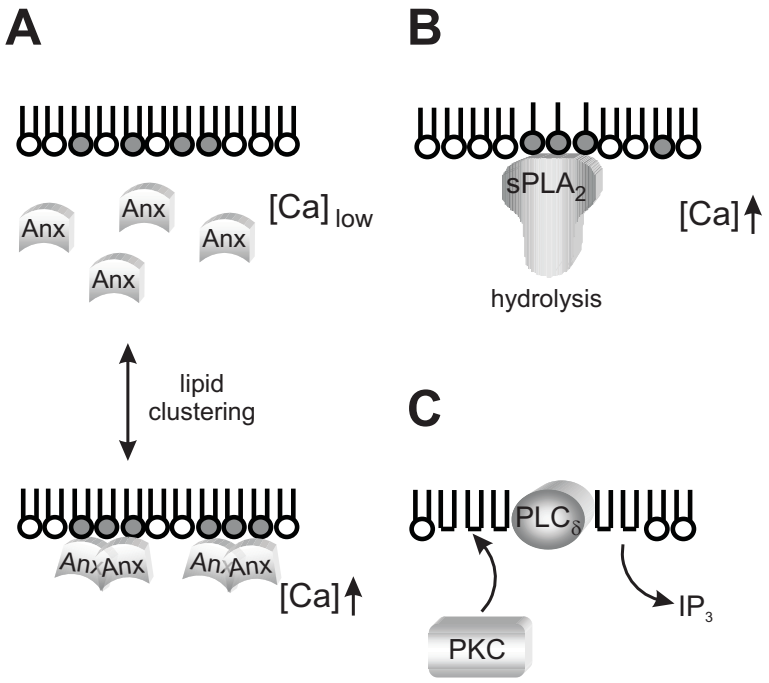

Figure 2. Lipid domains can be induced and/or stabilized by interfacial interactions with proteins.

A) Calcium-mediated interaction of annexin (Anx) molecules with acidic phospholipids (gray circles) that may induce clustering of these phospholipids and affect membrane lateral, as well as transversal, asymmetry. Annexins may provide some restrictions in lipid lateral diffusion or transversal flip-flop mobilities (Gerke & Moss, 1997). **B)** Lipid domains created by secreted PLA_2 ($sPLA_2$) performing multiple catalytic cycles at the membrane surface. Secreted PLA_2 is a small (about 14 kDa), Ca^{2+}-dependent enzyme that hydrolyses certain phospholipid classes. It is characterized by the presence of the so-called "interfacial-binding surface" that surrounds the catalytic site and seals the enzyme to the membrane (Gelb *et al.,* 1999). Secreted PLA_2 may exhibit specificity to anionic phospholipids; this may lead to the formation of a microdomain enriched in certain lysophospholipids. Such domain may change the local curvature of the membrane. **C)** Hydrolysis of PIP_2 molecules by PI phosphatide-specific PLCδ isoform creates a pool of diacylglycerol molecules ($\prod$) that provides an attachment site for certain PKC isoforms. In addition, a second messenger molecule (IP_3) is released (Rebecchi & Scarlata, 1998).

The attachment of lipid-binding proteins to membranes may play a role in the stabilization of preformed domains, functioning as receptor sites for other cytosolic proteins (secreted phospholipases and protein kinases), and cytoskeletal proteins. Disruption of physiological protein–lipid interactions within microdomains may lead to the development of various pathologies, e.g., pregnancy loss in anti-phospholipid syndrome (Rand, 1999) or abnormal processing of amyloid precursor protein in Alzheimer's disease (Ikezu *et al.,* 1998). Moreover, preferential distribution of cytosolic and integral membrane proteins within specific lipid domains points strongly to the role of these domains in providing appropriate environment for optimal functioning of these proteins. Last but not least, some microdomains may be created during enzymatic catalysis, as examplified by the digestion of phospholipids by PLC which provides diacylglycerol molecules being activators of PKC isoforms, while digestion by PLA_2 provides lysophospholipid molecules and free

fatty acids, both exhibiting detergent properties, therefore, influencing membrane fluidity.

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