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Review

Lipid rafts in health and disease

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Lipid rafts are sphingolipid- and cholesterol-rich domains of the plasma membrane which contain a variety of signalling and transport proteins. Different subtypes of lipid rafts can be distinguished according to their protein and lipid composition. Caveolae are types of rafts that are rich in proteins of the caveolin family (caveolin-1, -2 and -3) which present a distinct signalling platform. The importance of lipid raft signalling in the pathogenesis of a variety of conditions, such as Alzheimer's, Parkinson's, cardiovascular and prion diseases, systemic lupus erythematosus and HIV, has been elucidated over recent years and makes these specific membrane domains an interesting target for pharmacological approaches in the cure and prevention of these diseases. This Review analyses the importance of lipid raft proteins and lipids in health and disease, with a focus on the current state of knowledge.

Introduction

The traditional model of the plasma membrane as a homogeneous fluid lipid bilayer, as demonstrated by Singer and Nicholson (1972), has been extended in recent years, as it has become clear that the plasma membrane consists of thousands of different lipids and is a much more complex structure than previously thought. An early study by Yu et al. (1973) in erythrocytes indicated the existence of detergent-resistant sphingolipid-rich domains in the plasma membrane. Studies by van Meer et al. (1980, 1987) demonstrated an asymmetry in the distribution of phospholipids throughout the plasma membrane of erythrocytes, and an involvement of sphingolipids in post-Golgi membrane-lipid sorting in epithelial cells. Following these observations, Lisanti and colleagues (Lisanti et al., 1988; Lisanti and Rodriguez-Boulan, 1990) elucidated a link between sphingolipids and GPI (glycosylphosphatidylinositol)-anchored proteins for the first time by discovering a similar sorting pathway for fluorescently labelled GPI-linked proteins. These early discoveries were evident by localization of GPI-linked proteins to distinct cholesterol- and sphingolipid-rich microdomains in the plasma membrane (Zurzolo et al., 1994; Varma and Mayor, 1998) and the development of a method to isolate these domains by detergent extraction (Brown and Rose, 1992). The emerging idea of floating entities in the plasma membrane established the term 'raft'. This term recently received a comprehensive extended definition at the Keystone Symposium on Lipid Rafts and Cell Function: 'Membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes . . . ' (Pike, 2006).

Rafts have been shown to exist in both the extracellular, as well as the cytosolic, layer of the plasma membrane (Harder et al., 1998). The connection between both layers is yet unclear, but the spanning of long- and very-long-chain fatty acyl chains of sphingolipids through both layers, interacting with saturated acyl chains on phospholipids, appears likely to contribute to the involvement of both lipid layers (Rietveld and Simons, 1998). Primarily, two models have been developed to explain how lipid rafts accumulate and remain in the membrane as an entity. In one model, the headgroups of sphingolipids interact with each others amide and hydroxy/carboxy group, therefore holding sphingolipids together (Simons and Ikonen, 1997), while cholesterol fills the space between the

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Abbreviations used: AD, Alzheimer's disease; AICD, amyloid precursor protein intracellular domain; ApoAI, apolipoprotein AI; APP, amyloid precursor protein; Aβ, amyloid β-peptide; DISC, death-inducing signalling complex; DRM, detergent-resistant membrane; ER, oestrogen receptor; FAT, fatty acid translocase; FcR, Fc receptor; GPI, glycosylphosphatidylinositol; HDL, high-density lipoprotein; LCFA, long-chain fatty acid; LCK, lymphocyte-specific protein tyrosine kinase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; (e)NOS, (endothelial) nitric oxide synthase; PD, Parkinson's disease; PrP, prion-related protein; PrP^{C}, normal cellular PrP; PrP^{Sc}, abnormal disease-specific conformation of PrP; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; PUFA, polyunsaturated fatty acid; SLE, systemic lupus erythematosus.

bulky sphingolipid headgroups and is additionally kept in place by hydrogen bonds and van der Waals interactions between its 3-OH group and the sphingolipid amide groups (Filippov et al., 2006). A second model attributes the tight assembly in rafts to the interaction of mainly saturated acyl chains, which also favours cholesterol packing (London and Brown, 2000).

A major subclass of rafts are caveolae which are invaginations of the cell membrane characterized by the abundance of caveolin, a palmitoylated membrane protein that causes rafts to polymerize (Yamada, 1955). So far three isoforms of caveolin have been identified, caveolin-1, -2 and -3 (Okamoto et al., 1998), which are all transcribed from different genes. Caveolin-1, a 22 kDa protein, is the main component of caveolae and has been implicated in several signalling cascades, as it can be phosphorylated by Src kinases (Corley Mastick et al., 2001; Labrecque et al., 2004). The importance of this protein was established by the development of caveolin-1-knockout mouse models and the observation of impaired nitric oxide signalling and cardiovascular abnormalities in these mice (Drab et al., 2001; Razani et al., 2001). Caveolin-2 was first suspected only to support the function of caveolin-1, as caveolin-1 is required for the intracellular transport of caveolin-2. However, caveolin-2-knockout mice developed pulmonary malfunction, independently of caveolin-1 (Razani et al., 2002). Caveolin-3 (17 kDa) is a muscle-specific isoform which has been associated with the health and disease of cardiac myocytes and skeletal muscle (Song et al., 1996). Caveolin-3-knockout mice developed symptoms similar to those observed in muscular dystrophy (Galbiati et al., 2001), whereas transgenic mice overexpressing caveolin-3, as well as muscular dystrophy patients, appear to show impaired expression of dystrophin, a protein that is required for muscle cell structure and cytoskeleton integrity (Repetto et al., 1999; Sotgia et al., 2000). Caveolin-3-knockout mice also develop insulin resistance, which is probably related to the involvement of caveolin in insulin-stimulated glucose uptake (Oshikawa et al., 2004). Most of the functions of caveolins are currently unclear, but they have been proposed to act as cholesterol sensors and thereby regulate the number of lipid rafts in the cell membrane (Pol et al., 2001). They may also be involved in lipid trafficking by regulating the transport of lipids to and from lipid droplets within the cell (Pol et al., 2004). During recent years there has been a major focus on lipid rafts, mainly due to the localization of many membrane proteins in rafts, which stimulated theories of a possible involvement of lipid rafts in signal transduction [reviewed in Simons and Toomre (2000)]. This present Review aims to summarize the current knowledge on the importance of lipid rafts in health and disease [for a complementary review on lipids rafts and diseases, see Simons and Ehehalt (2002)].

Importance of lipid raft composition in health

As mentioned above, lipid raft domains are mainly characterized by a high content of sphingolipids and cholesterol. Furthermore, phosphatidylcholine, mainly with saturated acyl chains, is present, as well as small amounts of phosphatidylethanolamine and phosphatidylserine. The sphingolipids ganglioside 1 and 2 (GM1 and GM2) are commonly used as raft marker lipids. Palmitate is the predominant fatty acyl chain of the above-mentioned lipids, and unsaturated fatty acids are almost completely absent, probably due to the static problems arising from the bends in the acyl chain caused by unsaturation. Raft resident proteins are often GPI anchored; typical raft marker proteins include caveolins and flotillins.

Many different roles for these membrane domains have emerged as the existence of lipid rafts has become more and more accepted. The most widely studied function of rafts is to provide a distinct environment for signalling molecules and receptors, such as members of the tyrosine kinase Src family, G-proteins and various receptor proteins like the platelet receptor P2X (Vial and Evans, 2005), and therefore to allow a specific regulation of pathways related to these molecules at the plasma membrane. Recently, a role for lipid rafts in the transport of substrates, such as glucose and fatty acids, into the cell has emerged, implicating a localization of proteins associated with substrate transport to lipid rafts. The glucose transporter GLUT4 traffics from intracellular vesicles to the plasma membrane upon binding of insulin to the insulin receptor in skeletal muscle and adipose cells (Saltiel and Kahn, 2001). Although many downstream signalling pathways of the insulin receptor have been identified, the mechanism by which GLUT4 translocation is triggered

has yet to be clarified. Two main pathways have been shown to stimulate GLUT4 trafficking: one involves the insulin-response element and PI3K (phosphoinositide 3-kinase), and the other one signalling by the G-protein TC10, especially in adipocytes where TC10 localizes to lipid rafts and co-localizes with flotillin (Baumann et al., 2000; Chiang et al., 2001, 2006; Maffucci et al., 2003). In a recent study TC10 has also been linked to the exocyst, a protein complex that forms for exocytosis (Inoue et al., 2006). Proteins of this complex localize to lipid rafts upon insulin stimulation and trigger GLUT4 translocation and its temporary localization to lipid rafts in 3T3 adipocytes, indicating a transient presence of GLUT4 in these domains when glucose is present (Inoue et al., 2006). When extracellular glucose is low, GLUT4 transporters are internalized, possibly by a mechanism involving caveolae (Ros-Baro et al., 2001) and/or caveolin-1 (Shigematsu et al., 2003). In muscle cells, GLUT4 translocation has been associated with raft marker proteins, as GLUT4 co-localizes with flotillin-1 and is dependent on caveolin-3 (Fecchi et al., 2006). Mouse myotubes lacking either flotillin-1 or caveolin-3 show a significantly decreased insulin-stimulated uptake of glucose (Fecchi et al., 2006). A recent study has also demonstrated a co-localization of other members of the GLUT glucose transporter family, GLUT1 and GLUT3, as well as hexokinase, an important enzyme for glucose metabolism, with caveolin-1 (Rauch et al., 2006). These findings imply a regulating role of lipid rafts in insulin-stimulated glucose uptake, both in muscle and the adipose tissue.

The uptake of LCFAs (long-chain fatty acids) into adipose tissue may also be linked to lipid raft domains. Cholesterol loading of 3T3 adipocytes leads to an increase in caveolin-1 expression, as well as LCFA uptake, whereas overexpression of a caveolin-3-dominant mutant decreases LCFA transport in these cells (Pohl et al., 2004). FAT (fatty acid translocase)/CD36 is present inside and outside of DRMs (detergent-resistant membranes) isolated from adipocyte homogenates (Pohl et al., 2005), and is exclusively located in rafts in the plasma membrane, whereas the localization in detergent-soluble fractions seems to be mainly intracellularly (Pohl et al., 2004, 2005). Oleate preferably binds to FAT/CD36 located in rafts, and recent studies have shown a dependency of LCFA uptake on this localization (Pohl et al., 2004, 2005). These results demonstrate a correlation between lipid rafts and fatty acid uptake in adipocytes, and further support a role for rafts in substrate transport.

Lipid rafts in cardiovascular disease

Studies on a correlation between lipid rafts and cardiovascular disease have mainly focused on receptor-mediated signalling in endothelial cells of arteries and the heart muscle. In particular, binding of angiotensin II, a peptide that stimulates vasoconstriction and can therefore cause hypertension and pathological hypertrophy, to its receptor (angiotensin II receptor) results in the association of the receptor with lipid rafts in vascular smooth muscle cells (Zuo et al., 2005). The translocation of activated G-proteins to rafts upon receptor stimulation is also observed in the heart, as demonstrated in cardiac myocytes for adrenergic (Fujita et al., 2001; Steinberg 2004) and cholinergic (Feron et al., 1997) receptors. Furthermore, ion channels, such as the potassium channels Kv1, Kv2 and Kv4 in the heart, associate with both caveolae and caveolin-free rafts (Maguy et al., 2006). These channels maintain the membrane potential, and their disruption can lead to hypertension, ischaemia and heart failure (Maguy et al., 2006).

Caveolin-3 is present in caveolae of the heart and is up-regulated in cardiac hypertrophy (Kikuchi et al., 2005), but conversely, caveolin-3-knockout mice also develop cardiac hypertrophy and an increased activity of the MAPK (mitogen-activated protein kinase) pathway (Woodman et al., 2002). Therefore, it seems that a precise balance in caveolin-3 expression and caveolae formation is crucial for the regulation of signalling cascades in heart muscle.

NO (nitric oxide), which in contrast with angiotensin II promotes vasodilation and muscle relaxation, is synthesized in cardiac myocytes by the enzyme NOS (nitric oxide synthase), specifically the endothelial isoform, eNOS, which is localized to rafts in these cells. NO can inhibit the enzyme adenylate cyclase which produces cAMP, a signalling molecule that is involved in the regulation of muscle contractility. Disruption of rafts with cyclodextrin leads to impaired adenylate cyclase function (Ostrom et al., 2004). Furthermore, eNOS activity seems to be regulated by its interaction with caveolin-1 in the ischaemic heart (Der et al., 2006). These findings

further support the function of lipid rafts as signalling platforms.

Lipid rafts, especially of the caveolae type, may be involved in the pathogenesis of atherosclerosis, a disease of the blood vessels that is the main cause for cardiac disfunction in Western countries. The disease is caused by excessive cholesterol depositions in the arterial wall and subsequent uptake by macrophages (Shashkin et al., 2005). Cholesterol-loaded macrophages turn into foam cells and accumulate as plaques, which clot the arteries and lead to ischaemia and cardiac infarction (Shashkin et al., 2005). In healthy individuals, lipoproteins mediate the transport of cholesterol from the liver to tissues [LDLs (low-density lipoproteins)] and from the tissues back to the liver [HDLs (high-density lipoproteins)] (Ohashi et al., 2005) in the blood. In atherosclerosis, however, an excess in extracellular cholesterol leads to a saturation and therefore decreased uptake of LDL–cholesterol into tissues. The uptake of different LDL species, such as oxidized LDL and enzymically cleaved LDL, into macrophages requires the binding to the receptor CD36 and its localization to lipid rafts (Zeng et al., 2003), and LDL internalization induces formation of different raft domains in the macrophage plasma membrane (Grandl et al., 2006). ApoAI (apolipoprotein AI), the apolipoprotein of HDL, can mediate the uptake of cholesterol from LDL–cholesterol-loaded macrophages to avoid foam cell formation (Ohashi et al., 2005). The ApoAI interaction with macrophages (Gaus et al., 2004) and the cholesterol efflux from those cells (Gaus et al., 2001) is also lipid raft dependent and may alter their lipid raft composition (Drobnik et al., 2002). These findings implicate an involvement of lipid rafts in the pathogenesis of this complex disease.

Lipid rafts in apoptosis and carcinogenesis

Apoptosis is a programmed cell death whereby cells induce their own suicide as a vital step in development, differentiation and removal of excess cells, and thereby cancer prevention. Type I apoptosis is characterized by an extrinsic receptor-dependent pathway and requires the binding of a ligand to death receptors, such as Fas (CD95) or TNF (tumour necrosis factor), whereas type II apoptosis is an intrinsic pathway that involves cell organelles, such as mitochondria or the endoplasmic reticulum. Both pathways share downstream cascades involving caspases, proteolytic enzymes that initiate and execute apoptotic cell death.

The Fas receptor is a crucial mediator for apoptosis induction via different pathways, including DISC (death-inducing signalling complex). DISC requires the clustering of Fas trimers in the plasma membrane and the recruitment of intracellular signalling molecules, such as the FADD (Fas-associated death domain protein) and caspase-8 (Scaffidi et al., 1998; Gniadecki, 2004). Several downstream molecules of the Fas receptor seem to require its localization in lipid rafts, such as ROCK (Rho kinase) (Soderstrom et al., 2005), which is associated with caveolin-1 (Rashid-Doubell et al., 2006), and caspase-3 (Mandal et al., 2005). Fas trimerization and consequently apoptosis are increased in mouse WR/19L lymphoma cells expressing sphingomyelin (WR/Fas-SMS1) in contrast with the same cells lacking sphingomyelin synthase (WR/Fas-SM$^-$) (Miyaji et al., 2005). Furthermore, Fas stimulation is correlated with a greater ceramide content in lipid rafts of WR/Fas-SMS1 cells (Miyaji et al., 2005) and of neutrophils (Scheel-Toellner et al., 2004), where deletion of acid sphingomyelinase, the enzyme that catalyses ceramide synthesis from sphingomyelin, leads to a delayed apoptosis. As an involvement of ceramide in apoptosis has been proposed previously (Iwai et al., 2003; Taha et al., 2006), the localization of Fas in lipid rafts may not only facilitate signalling of the Fas receptor, but could also be directly related to the abundance of sphingomyelin, a precursor of ceramide, in raft domains. However, Fas can also be located outside of rafts in cells undergoing type II apoptosis where Fas only translocates into rafts after binding of the antigen CD28 (Legembre et al., 2005). Furthermore, Fas molecules can cluster in the membrane upon raft disruption and spontaneously induce a ligand-independent form of apoptosis (Gniadecki, 2004).

The localization of the ER (oestrogen receptor) to lipid rafts links these membrane domains to breast cancer, where the ER is constitutively expressed and is a marker for mammary carcinogenesis (Zhang et al., 2005). In MCF-7 human breast cancer cells the ER has been shown to co-localize with flotillin-2 (Marquez et al., 2006), and cholesterol depletion in these cells leads to impaired ERK1/2 (extracellular-signal-regulated kinase 1/2) signalling, which is a

common ER downstream pathway stimulated by oestrogen binding (Gilad et al., 2005). Caveolin-1 has been identified as a breast cancer suppressor (Bouras et al., 2004), as its overexpression significantly impairs cancer cell growth rate (Hino et al., 2003). Furthermore, caveolin-1-knockout mice show impaired mammary signalling cascades, as well as hyperactive tissue growth (Sotgia et al., 2006) and accelerated tumorigenesis and metastasis (Williams et al., 2004). In most types of breast cancer, caveolin-1 expression is decreased compared with healthy mammary cells, possibly due to insufficient methylation of the caveolin-1 promoter (Chen et al., 2004). The decreased expression of caveolin may promote the overexpression of the ER, as disruption of rafts with cyclodextrin and subsequent disappearance of caveolin-1 from the plasma membrane results in an increased expression of the ER in the human mammary MCF-10 cell line (Zhang et al., 2005). An exception from this pattern is inflammatory breast cancer where expression of both caveolin-1 and caveolin-2 is up-regulated (Van den Eynden et al., 2006). It is important to note that few of these studies have analysed lipid rafts in the respective cell lines and the observations are based on caveolin-1 expression; therefore, the tumour suppression has been linked solely to caveolin-1 and only indirectly to lipid rafts.

The CB1 receptor for anandamide, the human cannabinoid that may be another potential tumour suppressor in breast cancer, is also located in rafts of MCF-7 cells and inhibits proliferation of these cells, possibly dependent on cholesterol (Sarnataro et al., 2005). Interestingly, alteration of the raft lipid composition may also suppress tumour progression, as DHA (docosahexaenoic acid) has been demonstrated to induce apoptosis in the MDA-MB-231 breast cancer cell line by disrupting lipid rafts (Stillwell et al., 2005).

Caveolin-1 expression is altered in colon cancer, but in contrast with breast cancer caveolin-1 expression is up-regulated (Patlolla et al., 2004; Kim et al., 2006). This overexpression of caveolin-1 has been observed in several colon carcinoma cell lines (Patlolla et al., 2004), as well as in epithelial cells derived from colon cancer patients (Kim et al., 2006), and appears to be correlated with an increased growth of these cells (Patlolla et al., 2004). Furthermore, caveolin-1 expression is positively related with Akt expression, a protein kinase in the downstream signalling pathways of several cell surface receptors (Kim et al., 2006), and also increases the expression and membrane localization of cathepsin B and uPA (urokinase plasminogen activator), both caveolae-localized cell surface proteases, which may promote the invasiveness of colon cancer cells (Cavallo-Medved et al., 2005). The elevated caveolin-1 expression may be linked with an altered methylation of the caveolin-1 gene promoter (Lin et al., 2004), similar to the observations made in breast cancer cells (Chen et al., 2004).

In addition to altered caveolin-1 expression, the fatty acid composition of colon cancer cell membranes seems to differ from that of healthy colon cells, showing an increased proportion of saturated LCFAs, especially stearic acid, which may affect the formation of lipid rafts (Rakheja et al., 2005). Moreover, resident raft proteins of non-cancerous colon cells seem to be unable to localize to lipid rafts, which is especially important in the case of the death receptor Fas. As discussed above, this receptor requires localization to raft domains to promote its function, the initiation of apoptosis. If Fas cannot be distributed to rafts, apoptotic signalling is impaired and cell death does not occur, which may result in tumour growth and metastasis. Current pharmacological research focuses on compounds that promote redistribution of Fas to lipid rafts in order to destroy tumorigenic colon cells (Delmas et al., 2003; Lacour et al., 2004).

Lipid rafts and the immune system

An example of research on lipid rafts in relation to the immune system is on the mechanism by which PUFAs (polyunsaturated fatty acids) alter the immune response. Recent studies have demonstrated the incorporation of dietary PUFAs into membranes of T- and B-lymphocytes, and, subsequently, alterations in the lipid raft phospholipids of these cell types (Fan et al., 2003, 2004). These changes in membrane lipid composition have further been shown to affect localization of immunogenic receptors, such as IL-2 (interleukin 2) and FcR (Fc receptor), to lipid rafts (Li et al., 2005, 2006) and altered activation of their downstream mediators, such as PKCθ (protein kinase Cθ) and the transcription factors NF-κB (nuclear factor κB) and AP-1 (activator protein 1) (Fan et al., 2004). These findings imply a link between a PUFA-mediated immune response to altered

downstream signalling of raft-associated signalling molecules.

SLE (systemic lupus erythematosus) is characterized by abnormal signalling of T- and B-lymphocytes, and the subsequent increase of auto-antibodies against antigens in the cell nucleus. In normal T-cell signalling, antigen binding to the TCR (T-cell receptor) triggers intracellular phosphorylation cascades which involve Src kinases, PTKs (protein tyrosine kinases) and PTPs (protein tyrosine phosphatases). PTPs, such as CD45, regulate the phosphorylation of LCK (lymphocyte-specific protein tyrosine kinase), a crucial protein that controls both the active and inactive state of T-lymphocytes, which is located to the lipid raft domains of these cells (Jury et al., 2004). In activated T-cells of SLE patients, LCK expression is reduced and LCK and raft co-localization is decreased, whereas the LCK remaining in lipid rafts are mostly in the activated (phosphorylated) form (Jury et al., 2004). Inactivated SLE T-cells seem to have an increased GM1 content and an overall increased amount of lipid rafts in the plasma membrane of activated SLE T-cells (Krishnan et al., 2004; Pavon et al., 2006). Several proteins change their normal membrane distribution and are localized to lipid rafts in SLE T-cells, such as CD45, CD3 and FcRγ. CD3ζ, which is responsible for TCR capping and is present all over the membrane of healthy T-cells, localizes to rafts and displays an increased mobility and faster capping of TCR (Krishnan et al., 2004). Total CD3ζ content is reduced, possibly due to cleavage by caspase-3 (Krishnan et al., 2005). Interestingly, FcRγ is present in SLE T-cell rafts, which is a receptor protein in B-lymphocytes and not normally associated with rafts of T-cells (Krishnan et al., 2004). In B-cells of patients with SLE, a polymorphism of FcR, resulting in an $Ile^{232} \rightarrow$ Thr substitution, prevents the FcR localizing to lipid rafts (Floto et al., 2005; Kono et al., 2005).

HIV is a retrovirus that infects T-cells by budding to the plasma membrane and, subsequently, entry and reverse transcription and integration into the host genome. HIV entry into the host cell, as well as its infectivity, may be mediated by lipid rafts. Not only is one of the host cell receptors for HIV, CD4, localized to rafts (Popik et al., 2002), but also the disruption of host cell lipid rafts with cyclodextrin prevents HIV infection (Manes et al., 2000; Liao et al., 2003). Moreover, inhibition of sphingolipid synthesis of the virus particle itself makes it significantly less infectious, which could offer a new approach for HIV treatment (Brugger et al., 2006).

Lipid rafts in neurological disease

AD (Alzheimer's disease) is characterized by aggregation of the Aβ (amyloid β-peptide) and subsequent plaque formation, which leads to substantial changes in the morphology and function of the brain tissue. Symptoms of AD include memory loss (dementia) and changes in behaviour. Cleavage of the APP (amyloid precursor protein) by the enzyme β-secretase yields Aβ and the AICD (APP intracellular domain). The accumulation of Aβ has been observed to be related to cholesterol (Simons et al., 2001) and lipid rafts (Ehehalt et al., 2003). In a transgenic mouse model (Refolo et al., 2000) with double-mutant mice overexpressing both APP and presenilin-1 (McGowan et al., 1999), another protein involved in AD pathogenesis, a high-cholesterol diet led to a significant increase in Aβ formation. Furthermore, in cholesterol-depleted hippocampal neurons Aβ formation is inhibited (Simons et al., 1998), and β-secretase was localized to lipid rafts (Riddell et al., 2001). These findings imply that cholesterol-rich regions of hippocampal cell membranes are associated with increased Aβ formation, and that a lower cholesterol content of these membranes could lead to a decreased Aβ production. However, these findings are challenged by models in mice (Park et al., 2003) and AD patients (Abad-Rodriguez et al., 2004) where inhibition of cholesterol synthesis or a decreased membrane cholesterol content, in fact, leads to an increased formation of Aβ. These discrepancies may be in part explained by the fact that studies involving a decreased Aβ formation upon decreasing membrane cholesterol are almost entirely based on models where APP is overexpressed, whereas the studies with opposite results are based on animal or human models; therefore, the actual influence of membrane cholesterol on APP cleavage has to be elucidated in future work

Other lipid raft components, the gangliosides GM1 and GM2, have been associated with APP cleavage and plaque formation. In particular, GM1 is involved in Aβ formation, as well as Aβ processing (Zha et al., 2004), and has been shown to bind several Aβ derivatives (Ariga et al., 2001) which appears to induce conformational changes in Aβ, leading to

oligomerization and translocation of the peptide outside of rafts to phosphatidylcholine-rich membrane regions (Kakio et al., 2003). The concentrations of GM1 and GM2 are increased in the frontal cortex of AD brains (Molander-Melin et al., 2005), which may disrupt rafts and increase APP proteolysis and Aβ production, and therefore affect cell function. In a recent yeast two-hybrid study that involved screening human brain cDNA, an additional role of lipid rafts in AD pathogenesis was proposed, as the AICD was shown to interact with flotillin-1 (Chen et al., 2006). It has yet to be elucidated whether flotillin may recruit APP to lipid rafts and therefore initiate APP cleavage and Aβ action.

In PD (Parkinson's disease), neurons in the substantia nigra brain region producing dopamine, a hormone involved in the control of movement, are destroyed, which leads to a decrease in dopamine production and subsequent uncontrolled muscle contraction and movement (Tolosa et al., 2006), as well as cognitive disorders, such as dementia (Rippon and Marder, 2005), depression (Mentis and Delalot, 2005) and anxiety (Richard, 2005). α-Synuclein, a protein of unknown function which is present in both healthy and PD brains, has been implicated with PD pathogenesis (Eriksen et al., 2005). Point mutations in the α-synuclein gene on chromosome 4q21 have been identified in patients with inherited PD (Polymeropoulos et al., 1997), and an intracellular accumulation of α-synuclein in compartments called Lewy bodies occurs in neurons of PD brains (Liu et al., 2005). α-Synuclein has recently been linked to caveolin-1 expression in the B103 neuroblastoma cell line (Hashimoto et al., 2003). In this study, transfection of these cells with α-synuclein resulted in an up-regulation of the expression of caveolin-1 and a decreased signalling of the MAPK pathway, and, although α-synuclein did not directly co-immunoprecipitate with caveolin-1, it clearly seemed to alter its gene expression, implicating a possible link between PD and caveolae in neuronal cells (Hashimoto et al., 2003). Interestingly, flotillin-1 expression is also up-regulated in dopaminergic neurons of PD brains (Jacobowitz and Kallarakal, 2004). Another study (Fortin et al., 2004) specifically investigated the interaction of α-synuclein with lipid rafts using binding assays of the protein with isolated DRMs from HeLa cells. These experiments showed that α-synuclein co-localizes with CD55, a raft-associated protein, and appears attached to the plasma membrane regions using immunofluorescence staining and gradient fractionation (Fortin et al., 2004). In a similar study (Kubo et al., 2005), α-synuclein interaction with raft-like membrane structures was not impaired after proteolytic disruption of raft proteins, which implies that it binds to raft lipids, rather than to raft proteins. It is unclear how α-synuclein can interact with rafts, as it does not contain a transmembrane domain or any other known membrane-binding region. However, these results imply a potential involvement of lipid raft domains in the pathogenesis of PD.

Prion diseases present another type of neurological disease associated with lipid rafts. Prions are proteins, encoded by a gene on chromosome 20, which are present particularly in neurons of the central nervous system and in T-cells. Prion diseases are caused by a pathological transformation of PrP^{C} [normal cellular PrP (prion-related protein)] into a less soluble and less degradable form [PrP^{Sc} (abnormal disease-specific conformation of prion-related protein)] with altered function, leading to a disruption of neurons in the brain and development of a sponge-like brain morphology (Chakraborty et al., 2005). The most common prion diseases are scrapie, BSE (bovine spongiform encephalopathy) and CJD (Creutzfeld–Jakob disease), which are all fatal due to an irreversible loss of brain tissue (Johnson, 2005). The function of PrP^{C} is not yet clear, but it is known that it is a GPI-anchored protein, and both PrP^{C} and PrP^{Sc} associate with lipid rafts (Naslavsky et al., 1997), and the conversion of PrP^{C} into PrP^{Sc} also may occur in these membrane domains (Hooper, 2005). In a recent study in neuroblastoma cells, inhibition of cholesterol synthesis with mevinolin caused a dissociation of PrP^{C} from rafts and a decrease in cell surface expression, as well as an accumulation of PrP^{C} in the Golgi (Gilch et al., 2006). Cholesterol depletion leads to an impaired PrP localization in the plasma membrane, whereas this localization seems to be unaffected by depletion of sphingolipids (Sarnataro et al., 2004). The localization of PrP^{C} to rafts was found to be GPI dependent and caveolin-1 independent (Gilch et al., 2006), and the N-terminal domain of PrP is required for its interaction with rafts (Walmsley et al., 2003). However, although PrP^{C} and caveolin-1 do not co-localize, the pathogenic PrP^{Sc} triggers delocalization of caveolin-1, as well as synaptophysin,

a neuronal raft glycoprotein, from lipid rafts and may therefore indirectly affect caveolin-1 function and neuronal signalling (Russelakis-Carneiro et al., 2004). Stimulation of PrP^C with specific antibodies in T-cells leads to an increased signalling of Src kinases (Hugel et al., 2004) and MAPKs (Stuermer et al., 2004), as well as an increase in intracellular Ca^{2+} concentration which may trigger additional signalling cascades (Hugel et al., 2004; Stuermer et al., 2004). This downstream signalling of PrP is dependent on its localization to rafts (Hugel et al., 2004). Furthermore, antibody stimulation leads to capping of PrP^C and its co-localization with the raft marker proteins flotillin-1 and flotillin-2 (Stuermer et al., 2004). Interestingly, the rafts to which PrP localizes show certain unusual features, such as a high concentration in unsaturated fatty acids and hexosylceramide compared with other raft domains (Brugger et al., 2004). In addition, PrP has been shown to be internalized by clathrin-mediated endocytosis and not in a raft-dependent manner, which is very atypical for a raft resident protein (Taylor et al., 2005).

Conclusion

Sphingolipid- and cholesterol-rich membrane domains play an important role in both health and disease. Not only do they harbour a variety of important signalling proteins, but also mediate the transport of substrates, such as fatty acids and glucose, across the plasma membrane. Future studies may corroborate the involvement of these membrane domains in a variety of signalling pathways by further linking membrane receptors to lipid raft regions. It remains to be distinguished to what extent caveolins exhibit their function due to their localization to caveolae or as individually regulating proteins.

The study of lipid rafts has recently helped in explaining mechanisms of disease progression, and their involvement in a variety of conditions will further elucidate the importance of the plasma membrane as a signalling organelle.

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