Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases

Jacques Fantini, Nicolas Garmy, Radhia Mahfoud and Nouara Yahi

The fluid mosaic model of the plasma membrane has evolved considerably since its original formulation 30 years ago. Membrane lipids do not form a homogeneous phase consisting of glycerophospholipids (GPLs) and cholesterol, but a mosaic of domains with unique biochemical compositions. Among these domains, those containing sphingolipids and cholesterol, referred to as membrane or lipid rafts, have received much attention in the past few years. Lipid rafts have unique physicochemical properties that direct their organisation into liquid-ordered phases floating in a liquid-crystalline ocean of GPLs. These domains are resistant to detergent solubilisation at 4°C and are destabilised by cholesterol- and sphingolipid-depleting agents. Lipid rafts have

Jacques Fantini (corresponding author)
Professor of Biochemistry, Laboratoire de Biochimie et Physicochimie des Membranes Biologiques, Institut Méditerranéen de Recherche en Nutrition, UMR-INRA 1111, Faculté des Sciences de St-Jérôme, 13331 Marseille cedex 20, France. Tel: +33 491 288 761; Fax: +33 491 288 440; E-mail: jacques.fantini@univ.u-3mrs.fr

Nicolas Garmy
PhD Student, Laboratoire de Biochimie et Physicochimie des Membranes Biologiques, Institut Méditerranéen de Recherche en Nutrition, UMR-INRA 1111, Faculté des Sciences de St-Jérôme, 13331 Marseille cedex 20, France. Tel: +33 491 282 754; Fax: +33 491 288 440; E-mail: n.garmy@caramail.com

Radhia Mahfoud
PhD Student, Laboratoire de Biochimie et Physicochimie des Membranes Biologiques, Institut Méditerranéen de Recherche en Nutrition, UMR-INRA 1111, Faculté des Sciences de St-Jérôme, 13331 Marseille cedex 20, France. Tel: +33 491 282 754; Fax: +33 491 288 440; E-mail: raddouda@caramail.com

Nouara Yahi
Assistant Professor, Laboratoire de Biochimie et Physicochimie des Membranes Biologiques, Institut Méditerranéen de Recherche en Nutrition, UMR-INRA 1111, Faculté des Sciences de St-Jérôme, 13331 Marseille cedex 20, France. Tel: +33 491 288 761; Fax: +33 491 288 440; E-mail: nouara.yahi@univ.u-3mrs.fr

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

been morphologically characterised as small membrane patches that are tens of nanometres in diameter. Cellular and/or exogenous proteins that interact with lipid rafts can use them as transport shuttles on the cell surface. Thus, rafts act as molecular sorting machines capable of co-ordinating the spatiotemporal organisation of signal transduction pathways within selected areas ('signalosomes') of the plasma membrane. In addition, rafts serve as a portal of entry for various pathogens and toxins, such as human immunodeficiency virus 1 (HIV-1). In the case of HIV-1, raft microdomains mediate the lateral assemblies and the conformational changes required for fusion of HIV-1 with the host cell. Lipid rafts are also preferential sites of formation for pathological forms of the prion protein (PrP^{Sc}) and of the β-amyloid peptide associated with Alzheimer's disease. The possibility of modulating raft homeostasis, using statins and synthetic sphingolipid analogues, offers new approaches for therapeutic interventions in raft-associated diseases.

In the traditional fluid mosaic model of biological membrane structures, bilayer lipids form a uniform and homogeneous fluid mixture (Ref. 1). Hence, membrane lipids have long been considered as a two-dimensional solvent phase for membrane proteins. This prevailing view has been considerably refined during the past decade in the light of physicochemical studies of membrane lipids (Ref. 2). Consider the case of cholesterol: in the fluid mosaic model, this sterol-based lipid was initially thought to act as a homogenising agent of the membrane matrix, which is composed of several molecular species of phospholipids. Since each of these phospholipids has a distinct gel–fluid transition temperature, cholesterol was assumed to favour the mixing of membrane lipids at the physiological temperature by minimising the differences between fluid and gel states of the glycerophospholipids (GPLs). This model was challenged when it appeared that cholesterol is not evenly distributed within membranes but is instead unevenly distributed into cholesterol-rich and cholesterol-poor domains (Ref. 3).

Biological membranes are now better described as a 'mosaic of lipid domains' rather than a homogeneous fluid mosaic. A growing body of evidence has shown that specialised lipid domains exist in membranes. Among these domains, those containing sphingolipids and cholesterol, referred to as lipid rafts (Ref. 4), have received much attention in the past few years (Refs 2, 3, 4, 5). In addition to a demonstrated role in signal transduction within the host cell, lipid rafts serve as portals of entry for various pathogens, including viruses, bacteria and their toxins. There is also increasing evidence that lipid rafts are involved in the conformational changes underlying the formation of amyloid plaques in Alzheimer's and prion diseases. The purpose of this review is to discuss the role of lipid rafts in cell biology and medicine on the basis of their specific biochemical composition and physicochemical properties.

Rafts as membrane phases: physicochemical basis

From a biochemical point of view, the organisation of lipids in a membrane can be predicted from the individual molecular structure of membrane lipids (Fig. 1) (Refs 2, 3, 4, 5, 6, 7). GPLs such as phosphatidylcholine (lecithin) are rich in kinked unsaturated acyl chains (where the carbon chain contains one or more double bonds), whereas sphingolipids such as sphingomyelin or glycosphingolipids (GSLs) contain saturated acyl chains (Ref. 2). In most sphingolipids, there is only one double bond, in the 'trans' configuration. This double bond is located between the fourth and fifth carbon atoms of the 18-carbon sphingoid base. By contrast, the acyl chain bound to carbon sn-2 of glycerol is always unsaturated with one or several double bonds in the 'cis' configuration. These structural features could explain the physicochemical properties of these lipids in biological membranes (Ref. 2).

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

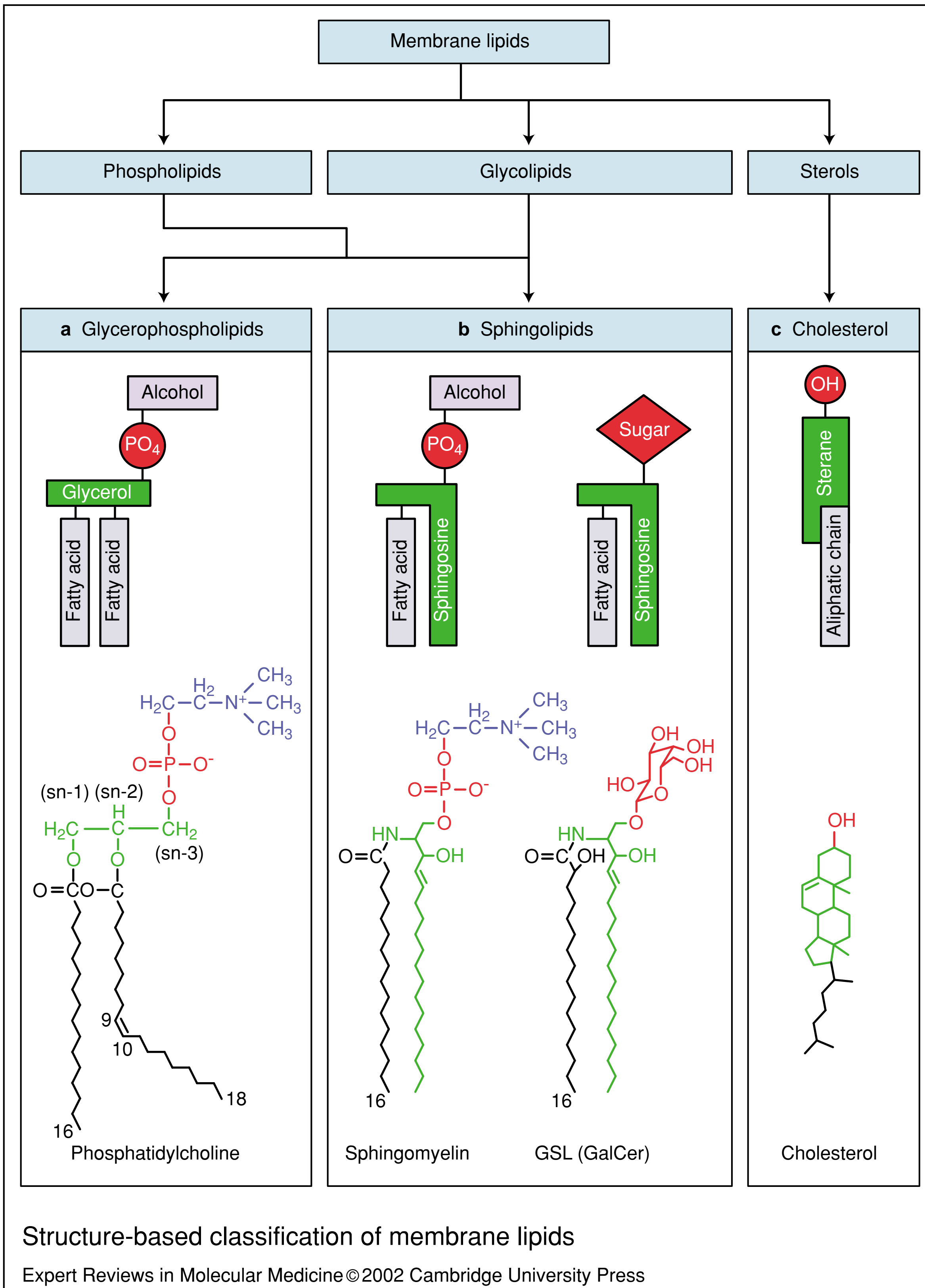

Figure 1. Structure-based classification of membrane lipids *(see next page for legend)* (fig001jfm).

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

Figure 1. Structure-based classification of membrane lipids. Plasma membrane lipids comprise phospholipids, glycolipids and sterols. More specifically, these lipids can be categorised as glycerophospholipids (GPLs), sphingolipids and cholesterol. (It should be noted that GPLs have been erroneously referred to as 'phospholipids' – this is incorrect as not all phospholipids contain glycerol.) (a) GPLs are the major component of membrane lipids. GPLs differ from each other with respect to polar head groups (the X group coming from an alcohol X-OH) whose OH group is esterified to the sn-3 carbon of glycerol. The main X-OH molecules are choline, ethanolamine, serine, glycerol and inositol. In GPLs, the fatty acid at sn-1 has a saturated chain with 16 or 18 carbon atoms. At sn-2, the fatty acid is generally longer (at least 18 carbon atoms) and is always unsaturated, with one or more *cis* double bonds (b) The phospholipids sphingomyelin (SM) and phosphatidylcholine (PC) share the same polar head group (i.e. phosphorylcholine) but differ in their hydrophobic moiety. The backbone structure of sphingolipids contains a sphingosine unit, and a saturated fatty acid with a long chain (up to 24 carbon atoms) is linked to the amino group of sphingosine via an amide linkage. This acyl chain is often 2-hydroxylated (as shown in the GalCer molecule). The acylated sphingosine is referred to as a ceramide. When a sugar or an oligosaccharide is linked by a β-glycosidic bond to the 1-OH group of ceramide, a glycosphingolipid (GSL) results. GSLs containing sialic acids in their carbohydrate moiety are called gangliosides. (c) The polar head group of cholesterol is a single OH group, whereas the hydrophobic moiety contains an iso-octyl carbon chain linked to a sterane-derived unit **(fig001jfm)**.

The saturated chains of sphingolipids allow them to pack tightly together through van der Waals interactions, forming a gel-like phase (Lβ) at the physiological temperature from which GPLs are excluded. In addition, sphingolipids may self-associate through hydrogen bonds between the hydroxyl (OH) groups of the sphingosine base and the α-OH group present in the fatty acid on many sphingolipids. In contrast, the kink structure of the polyunsaturated acyl chain in GPLs impedes straightening and tight packing of the chains, and at the physiological temperature GPLs are in a loosely packed disordered state usually referred to as the fluid liquid-crystalline (Lc) phase. Overall, sphingolipids have a much higher melting temperature (Tm) than that of GPLs [Tm is 83°C for GalCer purified from bovine brain (LIPIDAT ID#12501; http://www.lipidat.chemistry.ohio-state.edu/), but <0°C for natural lecithins]. Thus, the close association between sphingolipids can be quantified by a high Tm, representing the greater energy required for the gel–fluid transition.

The differential packing capability of sphingolipids and GPLs leads to phase separation in the membrane (Fig. 2). Below their Tm, pure GPLs are in a solid, gel-like phase (Lβ). In the presence of cholesterol, GPLs form a homogeneous phase with properties intermediate between gel and Lc phases: the fluid mosaic model of biological membranes is based on this physicochemical feature. At 37°C, sphingolipids form a quasi-solid gel phase (Lβ) characterised by a tight packing of carbon chains in a highly ordered way. Cholesterol interacts preferentially with sphingolipids and favours phase separation of GPLs and sphingolipids. Consequently, sphingolipids adopt an intermediate phase referred to as the liquid-ordered (Lo) phase (Ref. 3). In the Lo phase, the lipid acyl chains are tightly packed as in the gel phase, but have a higher degree of mobility owing to the intercalation of cholesterol molecules between sphingolipids. It has also been proposed that cholesterol might localise along the border between raft sphingolipids and GPLs. This organisation of cholesterol could create an energy-favourable transition area between Lo and Lc phases in the plasma membrane (Ref. 6).

To summarise, membrane lipids exist in different biophysical states controlled by several physicochemical parameters such as the temperature, presence of cholesterol and chemical nature of the hydrocarbon chains (Fig. 2). Because they are excluded from the Lc phase of GPLs, sphingolipids organise into specific, cholesterol-enriched entities referred to as plasma membrane microdomains or lipid rafts. These microdomains can be considered as small semi-rigid rafts floating in the more-liquid GPL-rich bulk of the plasma membrane. A schematic model of lipid organisation in the plasma membrane is proposed in Figure 3. This model, based on the work of Israelachvili et al. (Ref. 7), takes into account the shape of each membrane lipid and the coexistence of different lipid phases (Lc and Lo) within the membrane. It should be emphasised that this modern interpretation of membrane structure challenges the traditonal view (currently found in textbooks) that lipids and proteins are uniformly distributed in a homogenous bilayer.

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

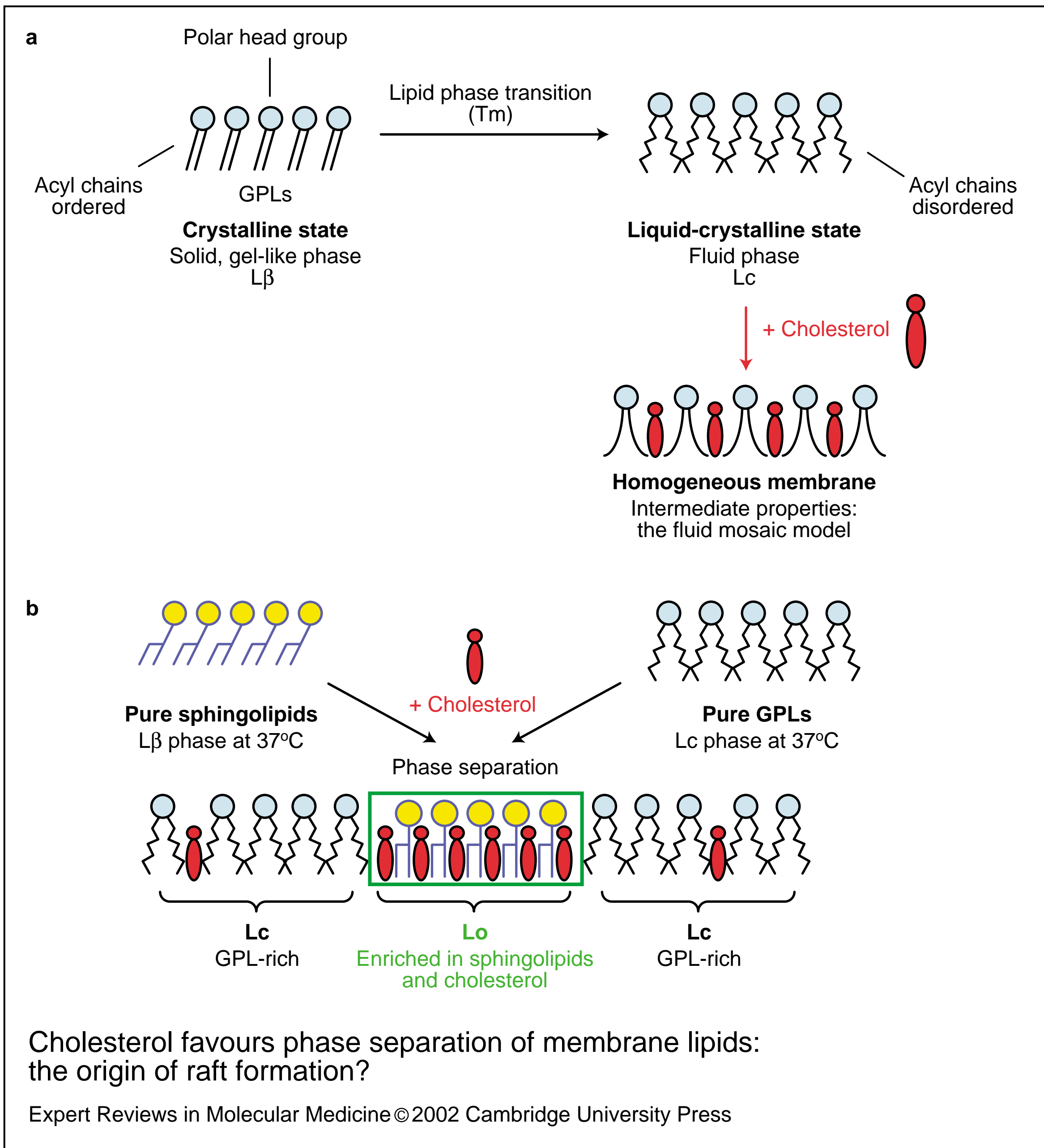

Figure 2. Cholesterol favours phase separation of membrane lipids: the origin of raft formation? (a) In bilayers of the glycerophospholipid (GPL) phosphatidylcholine below the Tm (melting temperature), the molecules can be packed such that the acyl chains are tilted with respect to the normal bilayer to form a crystalline, solid gel-like phase (Lβ), but at 37°C (above Tm) the bilayer converts to a liquid-crystalline, fluid phase (Lc; also sometimes referred to as Lα). The addition of cholesterol to pure GPLs abolishes the normal thermal transition between Lβ and Lc phases, giving membrane properties intermediate between the two phases. This well-known effect of cholesterol initially suggested that the slight differences in the Tm of various GPLs were 'corrected' by cholesterol, resulting in a homogeneous lipid phase at the physiological temperature. (b) In contrast to pure GPLs, pure sphingolipids form a gel phase (Lβ) at 37°C, with tight packing of the saturated chains. Cholesterol interacts preferentially (although not exclusively) with sphingolipids and favours the phase separation between sphingolipids and GPLs. In the plasma membrane, GPLs form a relatively cholesterol-poor Lc phase, whereas sphingolipids form a liquid-ordered (Lo) phase highly enriched in cholesterol. Rafts probably exist in a Lo phase or a state with similar properties **(fig002jfm)**.

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

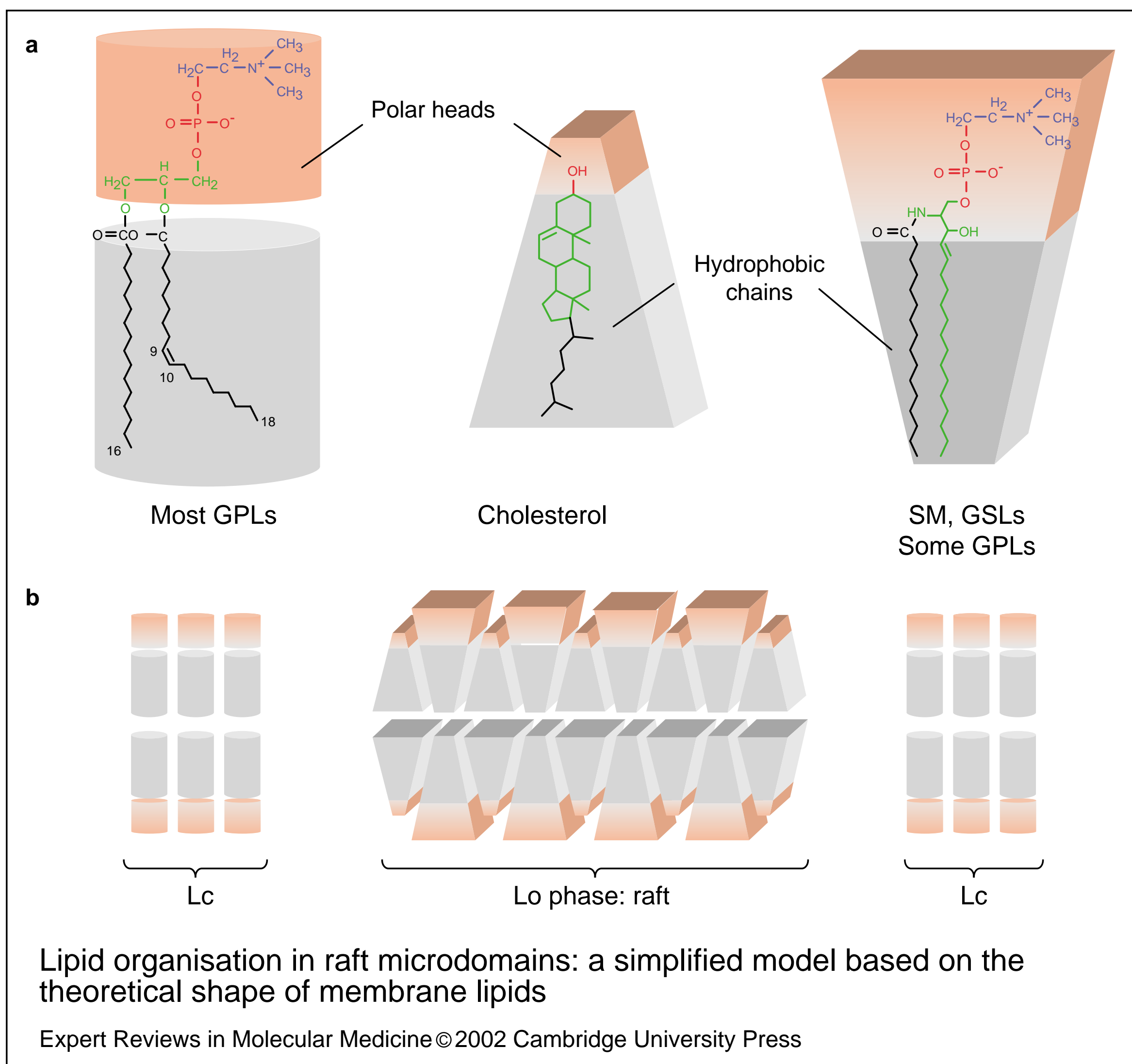

Figure 3. Lipid organisation in raft microdomains: a simplified model based on the theoretical shape of membrane lipids. The ability of membrane lipids to form the basic bilayer structure is the result of several properties, the most important of which is their amphipathic character. Amphipathic molecules have a polar, hydrophilic head group region and a non-polar, hydrophobic part. In aqueous solvents, amphipathic molecules naturally orientate themselves to ensure that the polar groups associate with water molecules, whereas the hydrophobic chains interact with each other so that a maximal number of water molecules are excluded from the hydrophobic phase. If the lipid is roughly cylindrical in dimension, biplanar leaflets will be the most thermodynamically stable configuration. (a) Glycerophospholipids (GPLs), which form the Lc phase of the plasma membrane (see Fig. 2), are indeed roughly cylindrical; however, cholesterol and sphingolipids [especially glycosphingolipids (GSLs)] have a pyramidal or cone-like shape. In sphingolipids the polar head group occupies a larger area than does the hydrophobic region (Ref. 4), whereas the converse is true for cholesterol. (b) Sphingolipids are almost exclusively found in the external (outer) leaflet of the plasma membrane, where – given the remarkable fit between the global shape of cholesterol and sphingolipids – any void between associated sphingolipids is thought to be filled by cholesterol functioning as a molecular spacer. The enrichment of cholesterol in Lo phase domains (see Fig. 2) is consistent with this model. A close interaction between cholesterol and sphingomyelin has been demonstrated in various reconstituted membrane systems. The tail-to-tail organisation of cholesterol in raft areas could rigidify the cytoplasmic (inner) leaflet of the plasma membrane, which is virtually devoid of sphingolipids but contains selected GPLs (e.g. phosphatidylinositol and phosphatidylethanolamine with saturated acyl chains) with physicochemical properties close to those of sphingomyelin. SM, sphingomyelin **(fig003jfm)**.

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

Analysis of lipid rafts in cell membranes: biochemical and morphological approaches

The characteristic partitioning of raft-associated lipids into ordered lipid phases renders them relatively insoluble in certain detergents such as Triton X-100 at 4°C (Refs 8, 9). Accordingly, rafts can be readily purified as detergent-insoluble membranes (DIMs) or detergent-resistant membranes (DRMs) by ultracentrifugation on sucrose density gradients. Under these conditions, the DRMs are recovered as molecular complexes from the buoyant fractions. The migration of DRMs to these low-density layers is consistent with the relatively high lipid content of these fractions. Indeed, morphological analysis of DRMs by transmission electron microscopy revealed the presence of small membrane vesicles (Ref. 8). Biochemical analysis of DRMs demonstrated a specific enrichment in GSLs, sphingomyelin and cholesterol. However, with the exception of phosphatidylinositol, the fractions are relatively poor in GPLs. In addition, DRM GPLs mainly contained saturated and monounsaturated, rather than polyunsaturated, acyl chains (Ref. 10), in agreement with the concept that acyl chain saturation favours raft association.

Taken together, these data strongly suggested that DRMs correspond to raft microdomains, and the Triton X-100 extraction procedure has become the most popular method of raft isolation from natural and artificial membranes. However, this method requires isolation at 4°C, a temperature that could artefactually increase or even induce raft formation in the plasma membrane, leading to the questioning of the existence of rafts by several investigators (Refs 11, 12). This controversial issue is still a matter of debate.

Morphological approaches have since been developed to study the in situ localisation of raft-associated lipids and proteins on the surface of intact cells as well as in membrane models (Refs 13, 14). For instance, the co-localisation of several raft proteins with the ganglioside GM1, a raft marker, has been demonstrated in various cell types by confocal microscopy. Modern microscopy techniques such as atomic force microscopy and fluorescence resonance energy transfer provided the first evidence for the existence of rafts in vivo, and allowed researchers to evaluate the size of membrane rafts as small patches approximately 50–70 nm in diameter (Refs 15, 16, 17). Chemical crosslinking confirmed that glycosylphosphatidylinositol (GPI)-anchored proteins are associated with membrane microdomains consisting of at least 15 molecules, which are much smaller than those observed after detergent extraction (Ref. 18). The improvement of solubilisation procedures will certainly help clarify the structure and dynamics of lipid rafts in the plasma membrane. For instance, the use of Brij 98 (a polyoxyethylene detergent with one double bond in the C_{18} aliphatic chain) has been used to prepare detergent-insoluble, raft-like microdomains at 37°C (Ref. 19). As pointed out by Edidin (Ref. 12), 'whichever detergent is used, it yields a snapshot of membrane composition that depends on the partition of lipids and proteins into detergent micelles. This snapshot does not report the organization of native membranes.' Although this opinion might be rather extreme, it is clear that a reliable method allowing raft isolation in the absence of detergent is urgently needed. Indeed, standard Triton X-100 extraction conditions might result in a higher cholesterol content together with an underestimation of arachidonic-acid-containing GPLs (Ref. 20). Nevertheless, this problem would be minimised with Brij 98, suggesting that the use of appropriate detergents will still be very relevant for raft isolation. (Ref. 19)

Another popular approach used to study the structure and function of rafts is to modulate their lipid composition. Molecules able to deplete cholesterol from the plasma membrane (such as β-methyl-cyclodextrin) have been widely used as raft-disrupting agents (Ref. 21). The integrity of lipid rafts can also be affected by metabolic inhibitors of sphingolipid biosynthesis [L-cycloserine, fumonisin B1, PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol)](Refs 22, 23). Nevertheless, one should keep in mind that lowering membrane cholesterol might induce large-scale effects that cannot be exclusively attributed to the dispersion of rafts (Ref. 12). For this reason, a combination of biochemical and morphological approaches is recommended to study the physiological functions of rafts and their role in various non-infectious and infectious pathologies.

The function of lipid rafts

It is widely held that lipid rafts are involved in signalling events (Ref. 24) and intracellular trafficking of proteins (including bacterial toxins)

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

and lipids (Ref. 25), as well as being preferential sites for host–pathogen/toxin interactions (Ref. 26). Rafts also appear to be involved in the generation of pathological forms of proteins associated with Alzheimer's and prion diseases (Refs 27, 28). These aspects of raft functions are discussed below.

Role of lipid rafts in signal transduction pathways

In addition to sphingolipids and cholesterol, specific proteins can be associated transiently or constitutively with lipid rafts. The presence of the cholesterol-binding protein caveolin within a Lo microdomain defines an ultrastructural differentiation of the plasma membrane called caveolae (Ref. 29), which were originally identified as local invaginations (50–100 nm diameter) of the plasma membrane in endothelial and epithelial cells. Caveolae can be described as a particular case of Lo domains of the plasma membrane – that is, caveolin-containing lipid rafts.

Lipid rafts and caveolae are particularly enriched in proteins that play pivotal roles in signal transduction. These include: (1) external proteins bound to the outer leaflet of the plasma membrane by a GPI anchor (e.g. the prion protein PrP); (2) transmembrane proteins (e.g. the IgE receptor FcεRI); and (3) acylated protein tyrosine kinases of the Src family (e.g. Lyn) bound to the inner leaflet of the membrane (Refs 30, 31). GPI proteins are anchored in the external leaflet of the plasma membrane by two saturated chains (1-alkyl-2-acyl-glycerol) that can tightly pack with raft lipids. Acylated proteins are anchored in the internal leaflet with two or more saturated acyl chains (generally myristate and palmitate) that interact preferentially with raft lipids. Although sphingolipids are usually not found in the cytoplasmic leaflet of the plasma membrane, specific GPLs such as phosphatidylserine and phosphatidylethanolamine with saturated chains might form Lo domains through interaction with long sphingolipid acyl chains of the outer monolayer (Fig. 3).

Because they can diffuse laterally in the plasma membrane, rafts act as floating shuttles that transport and bring together activated receptors and transducer molecules (Ref. 32). The 'coalescence' model of signal transduction can be illustrated by immune cell signalling. For instance, in quiescent mast cells, IgE receptors (FcεRI) and Lyn are localised outside membrane rafts. Upon binding of the antigen (Ag)–IgE complex to FcεRI, Lyn and FcεRI are recruited to specific rafts. The coalescence of these rafts induced by the multivalent antigen allows a physical interaction between FcεRI and Lyn, which triggers the signal transduction pathway: the co-compartmentation of FcεRI receptors with the raft-associated tyrosine kinase Lyn provides an adequate spatial proximity for phosphorylation of FcεRI on tyrosine-based activation motifs (ITAMs), and this triggers the signalling cascade that leads to release of mediators of the allergic response. Cholesterol deprivation experiments led to a clear decrease in the tyrosine phosphorylation step, in agreement with the involvement of rafts in the initiation of this signalling cascade (Ref. 32). In $CD4^+$ T cells, the main components of the T-cell receptor (TCR) signal initiation machinery (i.e. the TCR–CD3 complex, Lck ZAP-70 kinases and the CD4 co-receptor) appear to be constitutively partitioned into a subset of membrane rafts (Ref. 19). Thus, some signal transduction units can be pre-assembled in lipid rafts of quiescent cells, allowing rapid and efficient signal initiation upon activation. Overall, rafts can be described as molecular sorting machines capable of coordinating the spatiotemporal organisation of signal transduction pathways within selected areas ('signalosomes') of the plasma membrane.

Rafts as portals of entry for pathogens

A broad range of pathogens, including viruses, parasites, bacteria and their toxins, use lipid rafts to enter host cells as an infection strategy, utilising both cell-surface GPI-anchored proteins and raft lipids (GSL, sphingomyelin and cholesterol) as primary or accessory receptors. For instance, cholera toxin binds to ganglioside GM1, Shiga toxin binds to the neutral glycolipid Gb3, mycobacteria bind to cholesterol, and *Escherichia coli* strains expressing the adhesin FimH bind to the GPI-anchored protein CD48 (for recent reviews see Refs 26, 33, 34). This section briefly illustrates the various roles of membrane rafts in the pathogenesis of bacterial toxins.

Of particular interest is the interaction of tetanus and botulinum toxins with neural cells. These neurotoxins bind to several di- and tri-sialogangliosides (chiefly GD1a, GD1b and GT1b) on the surface of the presynaptic membrane (Ref. 35). However, the affinity of the toxins for these gangliosides is unexpectedly low, given the

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

high levels of toxicity achieved by subpicomolar concentrations of the toxins. An elegant theory, known as the 'double receptor' model, has been developed by Montecucco to explain this paradox (Ref. 36). In this model, the toxin binds to the negatively charged surface of presynaptic membranes through low-affinity interactions with high concentrations of ganglioside receptors, and then moves laterally to bind to a hypothetical protein receptor. Since the final binding affinity is the product of these two binding constants, a very high affinity is achieved. The identification of a 58 kDa protein from rat brain synaptosomes that binds botulinum and tetanus neurotoxin only in the presence of GT1b or GD1a strongly supports this model (Ref. 37). A further possibility discussed by Montecucco is that the binding to gangliosides induces a conformational rearrangement of the toxin structure that increases its affinity to the protein receptor. This model is particularly interesting because it illustrates the various properties of lipid rafts that are particularly useful to pathogens and their toxins (referred to as the 'invader', as viewed by the host): (1) the raft environment provides a high amount of low-affinity receptors that stabilise the invader on the cell surface; (2) the raft can deliver the invader to adequate high-affinity receptors; and (3) specific lipids in the raft environment might act as chaperones, inducing conformational changes in the invader structure in the vicinity of the high-affinity receptors. As discussed later, this model is surprisingly consistent with the fusion reaction that occurs during infection by human immunodeficiency virus 1 (HIV-1) (Ref. 38).

The interaction of cholera toxin with target cells illustrates another aspect of raft–toxin interactions. Cholera toxin comprises five identical B polypeptides that bind ganglioside GM1, and a single A subunit that contains the active A1 peptide that enters the cell and activates adenylyl cyclase (Ref. 39). The pentameric B-subunit specifically binds five GM1 molecules with high affinity. In this case, the main role of the raft is to concentrate the toxin receptor, ensuring a maximal binding capacity of the toxin to the cell surface (Ref. 26). Moreover, the pore-forming toxin aerolysin from *Aeromonas hydrophila* is also targeted to lipid rafts through multiple interactions with GPI-anchored proteins (Ref. 40). Indeed, most pore-forming toxins (e.g. *Vibrio cholera* cytolysin) use the concentration capacity of rafts for the oligomerisation step that is a prerequisite for channel formation (Ref. 26). In many cases, the raft components recognised by the toxin are major raft lipids such as cholesterol or sphingomyelin. In the case of Shiga toxin, the glycolipid receptor (Gb3) is important not only for providing cell-surface binding sites, but also for transporting the toxin into the endoplasmic reticulum (retrograde transport through the secretory pathway) (Refs 41, 42). In this respect, it is interesting to note that some pathogens and their toxins exploit normal cellular functions of lipid rafts (e.g. intracellular trafficking) to gain entry into host cells (Ref. 26). Finally, some bacterial toxins alter the localisation of tight junction proteins within raft-like domains; these cell–cell junctions normally seal adjacent epithelial cells together, in order to prevent the passage of dissolved molecules from one side to the other, and alteration results in a defect of the epithelial barrier function. This mechanism has been demonstrated for the exotoxins produced by *Clostridium difficile*, the aetiological agent of pseudomembranous colitis (Ref. 43).

Role of lipid rafts in HIV-1 infection and pathogenesis

HIV-1 binding and fusion

HIV-1 is an enveloped virus that fuses with the plasma membrane to deliver its genomic RNA into the host cells (Ref. 44). The fusion of HIV-1 with $CD4^+$ T cells is a highly controlled, fully automated and irreversible process. For this crucial event, the virus has its own 'harpoon', provided by the hydrophobic N-terminal part of gp41, the transmembrane (TM) glycoprotein of the viral envelope, which is known as the fusion peptide. Because of its hydrophobicity, the fusion peptide is initially buried in a pocket of the HIV-1 surface envelope glycoprotein (gp120), so that it is protected from the aqueous environment. Following a structural rearrangement of the viral envelope, the fusion peptide is suddenly ejected outside the viral spike where it has to face a highly polar aqueous environment. To minimise its energy, the fusion peptide penetrates into the plasma membrane of the target cell where it finds stabilising hydrophobic conditions. This thermodynamic interpretation of the fusion reaction has been called the 'viral mouse trap' model (Ref. 45). In this original formulation of the HIV-1 fusion paradigm, the role of membrane lipids was strikingly underestimated. Today, it is

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

widely admitted that lipid rafts play a major role in HIV-1 fusion, as demonstrated by a number of remarkably convergent studies from various laboratories (Refs 46, 47, 48, 49, 50, 51, 52).

The assembly of the HIV-1 fusion machinery (Ref. 38), which works essentially to unmask the fusion peptide, requires a high-affinity receptor (CD4), low-affinity gp120-binding GSLs (the ganglioside GM3 and the neutral GSL globotriaosylceramide Gb3), and a fusion co-factor referred to as a HIV-1 co-receptor (Fig. 4a). HIV-1 co-receptors identified to date include chemokine receptors (mainly CXCR4, CCR5, CCR3 and CCR2b) and a series of orphan receptors, all of which belong to the family of G-protein-coupled seven-transmembrane-domain receptors (Ref. 53). The binding of gp120 to GSLs is mediated by a disulphide-linked domain called the V3 loop, which corresponds to the principal neutralisation domain of gp120 and is clearly distinct from the CD4-binding region. In the first step of HIV-1 fusion with $CD4^+$ T cells, a trimolecular complex between gp120, CD4 and GSLs is formed in raft areas of the plasma membrane (Ref. 46). GSLs mediate several roles in this process: (1) they stabilise the virus on the cell surface (Fig. 4a); (2) they facilitate the migration of the CD4–gp120 complex to an appropriate co-receptor, thereby mediating the lateral assemblies required for the HIV-1 fusion machinery (Fig. 4b); and (3) they assist the conformational changes of gp120 (Fig. 4c) that eventually lead to the release of the fusion peptide outside the virus spike (Fig. 4d).

As discussed above for bacterial neurotoxins, the GSL patch functions as a raft that drags the CD4 receptor and takes aboard the viral particle (Fig. 4a and 4b). The stabilisation of the virus onto the GSL moving platform results from multiple low-affinity interactions between the V3 domain of gp120 and the carbohydrate moiety of GM3 and/or Gb3. The raft might then float on the cell surface until it finds an adequate co-receptor, the choice of which is driven by a molecular selection process based on V3–co-receptor interactions (Ref. 46). Subtle changes in the orientation of the V3 loop might be necessary to displace gp120 from the GSL to the co-receptor, as proposed in Fig. 4c. Finally, the GSLs might facilitate the final conformational changes of gp120 that lead to the separation (shedding) of gp120 from the viral spike and to the release of the fusion peptide (Ref. 48). The dispersion of the raft (Ref. 49), which occurs just before the beginning of the fusion reaction (Fig. 4c), might facilitate interaction of the co-receptor and CD4–gp120 complex. The model presented in Figure 4 has striking similarities with the 'double receptor' model proposed by Montecucco for bacterial neurotoxins. The paradigm might apply to other pathogens such as Ebola virus, which binds to a GPI-anchored protein (the folate receptor) and uses lipid rafts to enter host cells (Refs 54, 55).

Nature of the interaction between lipid rafts and HIV gp120

In the intestinal mucosa, lipid rafts have been shown to be involved in the transfer of infectious HIV-1 virions through intact intestinal epithelial cells (Ref. 56) and in the pathogenesis of HIV-1 enteropathy (Refs 57, 58). In both cases, the interactions of HIV-1 with intestinal lipid rafts are mediated by the GSL GalCer, a high-affinity receptor for gp120 that was initially discovered in neural cells (Ref. 59) and in the intestinal epithelium (Ref. 60). GalCer is recognised by the V3 loop of gp120, as demonstrated by various biochemical and physicochemical techniques (Refs 61, 62).

Overall, it is now clearly established that the V3 loop of gp120 is a sphingolipid-binding domain that mediates the attachment of HIV-1 to lipid rafts from various cell types. Indeed, V3-derived synthetic peptides bind to GSLs and inhibit HIV-1 infection in $CD4^-$ and $CD4^+$ cells (Ref. 63). Thus, both low/moderate-affinity (Gb3 and GM3, with K_d values ranging from 10^{-7} to 10^{-8} M) and high-affinity (GalCer, with a K_d of 10^{-9} M) GSL receptors are recognised by the gp120 V3 loop. The affinity between two ligands depends on the number of structured water molecules that are released to bulk solution as a result of the binding reaction (Ref. 64). The terminal galactose residue of GalCer, GM3 and Gb3 is involved in gp120 binding. In the case of GalCer, this sugar is in close interaction with the membrane, so that gp120 binding might result in the release of numerous water molecules ordered around the lipid–aqueous interface. In GM3 and Gb3, the galactose residue is distant from the membrane, so that fewer water molecules might be displaced by gp120. This might explain why synthetic soluble analogues of GalCer bind to the V3 domain of gp120 with high affinity and block HIV-1 infection of $CD4^+$ T cells (Ref. 61).

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

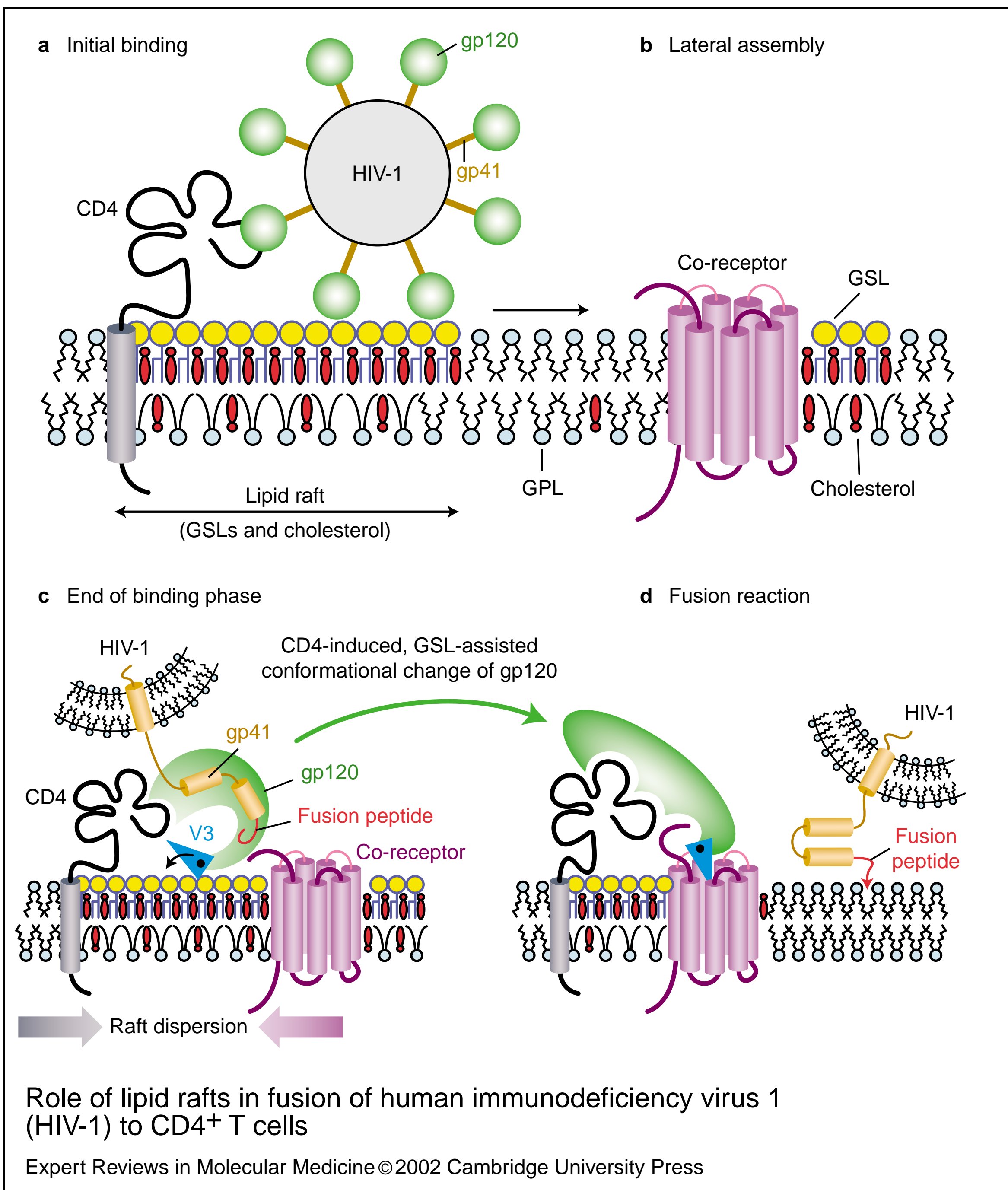

Figure 4. Role of lipid rafts in fusion of human immunodeficiency virus 1 (HIV-1) to CD4^{+} T cells *(see next page for legend)* (fig004jfm).

HIV-1 budding

Several reports suggest that HIV-1 buds from lipid rafts (Ref. 65), a property shared with other viruses such as measles, influenza and Ebola. The myristylation of capsid proteins ensures their localisation in condensed areas of the cytoplasmic leaflet of the plasma membrane. For instance, the myristylated p17 matrix protein of the HIV-1 capsid interacts with gp41, so that both proteins partition into the detergent-insoluble membrane fraction of HIV-1-infected cells. Clearly, the segregation of viral components in the raft (which

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

Figure 4. Role of lipid rafts in fusion of human immunodeficiency virus 1 (HIV-1) to CD4+ T cells. (a) Initial binding of HIV-1 to the host CD4+ T cell. CD4 is present in microdomains enriched in glycosphingolipids (GSLs) (e.g. GM3/Gb3) and cholesterol. The HIV-1 surface envelope glycoprotein gp120 binds to CD4, leaving its V3 domain available for secondary interactions with raft GSLs. At this stage, the HIV-1 transmembrane glycoprotein gp41 is still bound to gp120 in an inactive conformation. (b) Lateral assembly of the HIV-1 fusion complex. Once bound to CD4, the viral particle is conveyed to an appropriate co-receptor (e.g. chemokine receptors CXCR4 or CCR5 or other G-protein-coupled seven-transmembrane-domain receptors) by the GSL raft, which moves freely in the outer leaflet of the plasma membrane composed of other lipids such as glycerophospholipids (GPLs). (c) End of the binding phase. Following the primary interaction with CD4 in the raft environment, a conformational change in gp120 renders cryptic regions of the viral glycoprotein (including the V3 domain – shown as a hinged triangle) available for secondary interactions with an appropriate co-receptor. As seven-transmembrane domain receptors are almost flush with the cell membrane, binding of gp120 to the co-receptor moves the viral spike close to the target membrane. In addition, the raft begins to disperse, allowing close contact between the co-receptor and the CD4–gp120 complex. Additional conformational changes in the HIV-1 envelope glycoprotein trimer are necessary to unmask the fusion peptide at the N-terminus of the transmembrane glycoprotein gp41. These conformational changes are stimulated by GSLs, which act as lipid chaperones in the raft environment. (d) Beginning of the fusion reaction. The conformational change in gp120 is shown on the left; the conformational change in gp41, allowing the beginning of the fusion reaction, is shown on the right. Once the hydrophobic fusion peptide is ejected outside the viral spike, it faces a highly polar aqueous environment and consequently penetrates into the plasma membrane of the target cell, where it finds stabilising hydrophobic conditions. This irreversible process induces a close contact between the viral envelope and the plasma membrane, which fuse together, allowing the entry of the nucleocapsid in the cytoplasm of the target cell **(fig004jfm)**.

acts as an assembly platform) involves specific interactions with these myristylated proteins in a highly ordered process. The molecular associations between HIV-1 proteins and lipids form an electron-dense crescent-shaped complex on the inner leaflet of the plasma membrane during viral assembly (Ref. 44). The next step is the budding of HIV-1 from the infected cell, so that the host cell membrane becomes the new viral envelope. As a consequence, the HIV-1 envelope acquires host cell cholesterol, sphingolipids (sphingomyelin, GM1) and GPI-anchored proteins (Thy-1 and CD59).

Lipid rafts and prion propagation

Evidence for prion conversion in lipid rafts

Spongiform encephalopathies are an intriguing group of neurodegenerative diseases caused by an agent consisting exclusively of a protein usually referred to as a prion (from 'proteinaceous infectious only'). One of the hallmarks of prion diseases is the cerebral accumulation of a protease-resistant, misfolded isoform of the prion protein (PrP), the so-called PrP^{Sc} (for scrapie PrP), which is derived from the normal cell-surface glycoprotein PrP^{C} (for cellular PrP) (Ref. 66). (Scrapie is one of the major degenerative diseases caused by infectious prions in sheep.) PrP^{Sc} and PrP^{C} have the same amino acid sequence but differ in their conformation. Upon physical interaction with PrP^{Sc}, PrP^{C} is converted into PrP^{Sc}, inducing an endless chain reaction. The conformational changes associated with the PrP^{C} to PrP^{Sc} conversion consist of an α-helix to β-helix transformation (see discussion below).

PrP^{Sc} is partially resistant to proteolysis by proteinase K, a property that is widely used to discriminate between PrP^{C} (proteinase-K-sensitive; PrP^{sen}) and PrP^{Sc} (proteinase-K-resistant, PrP^{res}) (Ref. 66). Several lines of evidence suggest that rafts are a candidate site for the generation of PrP^{Sc} in infected cells: (1) like other GPI-anchored proteins, PrP is naturally enriched in lipid rafts (Ref. 67); (2) both PrP^{C} and PrP^{Sc} are recovered within DRMs (Ref. 68); (3) cholesterol depletion decreases the formation of PrP^{Sc} whereas sphingolipid depletion increases PrP^{Sc} (Refs 69, 70); and (4) infectious prion rods were found to contain the two sphingolipids GalCer and sphingomyelin (Ref. 71), suggesting that selected raft lipids might interact with normal and/or pathogenic prion proteins. Recently, a cell-free conversion reaction approximating physiological conditions was developed by Caughey and co-workers (Ref. 27). In this system, PrP^{C} is provided by DRMs prepared from neuroblastoma cells, and brain microsomes from scrapie-infected mice are used as a source of PrP^{Sc}. Under these conditions, the PrP^{C} to PrP^{Sc} conversion was observed only when PrP^{Sc} molecules were first inserted into host cell membranes (Fig. 5).

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

The importance of the membrane environment in the conversion reaction has been underscored by several studies. In particular, PrP^{C} can bind to raft-like membranes enriched in cholesterol and sphingomyelin (Ref. 72). This interaction appears to induce folding of the unstructured N-terminal domain of PrP^{C}, resulting in a protein with a higher content of α-helix compared with the structure of the protein in solution. These data suggest that the interaction of PrP^{C} with lipid rafts might stabilise the 'normal' conformation of PrP^{C}. These protective lipid–PrP^{C} interactions should be destabilised when exogenous PrP^{Sc} is inserted in the vicinity of PrP^{C} in the raft environment (Fig. 5). Since a chaperone activity appears essential to assist the conformational change of PrP (Ref. 73), it is likely that the conversion reaction involves a co-factor that might be either a raft-associated protein ('protein X', according to Prusiner; Ref. 66) or selected raft lipids. In this respect, there is a striking similarity between HIV-1 gp120 and PrP, since both proteins undergo major conformational changes in rafts. Indeed, a sphingolipid-binding domain that is structurally related to the V3 loop of gp120 has been characterised in PrP^{C} (Ref. 74). The V3-like domain of PrP consists of a helix-turn-helix motif formed by 33 of the 36 amino acid residues of a disulphide-linked loop (Cys179–Cys214). This loop includes the α2 and α3 helix of PrP^{C} (Fig. 6). In the V3 loop of HIV-1 gp120, the motif is a hairpin structure with only one α-helix corresponding to α3 in PrP. Interestingly, the V3-like motif of PrP contains His, Tyr and/or Phe residues that mediate binding to individual sugar rings of complex carbohydrates (Ref. 75).

Synthetic peptides derived from the predicted V3-like domain of PrP^{C} were found to interact with GalCer and sphingomyelin. Moreover, the V3-like domain of PrP^{C} includes the E200K mutation site associated with familial Creutzfeldt–Jakob disease (Fig. 6). This mutation abrogated sphingomyelin recognition, probably because of an electrostatic repulsion between the positive charges of the Lys residue and of the phosphorylcholine group of sphingomyelin. Taken together, these data strongly suggest that sphingolipids such as GalCer and/or sphingomyelin stabilise the non-pathological conformation of PrP^{C} in the lipid raft through specific interactions with the V3-like domain of PrP^{C}. When exogenous PrP^{Sc} is inserted in the target cell membrane, these low-affinity interactions are destabilised, allowing the formation of the PrP^{C}–PrP^{Sc}–co-factor complex (Refs 72, 73). The consequence of this autocatalytic process is the pathological formation of amyloid fibrils (prion rods), which accumulate in brain tissues.

A molecular model for prion conversion?

The structure of PrP^{Sc} has remained elusive for a long time: circular dichroism studies suggested an α-helix to β-sheet transition (Ref. 66), but the insolubility of PrP^{Sc} has thwarted attempts to investigate its structure by either X-ray crystallography or nuclear magnetic resonance spectroscopy. Recently, structural studies by electron crystallography suggested that PrP^{Sc} contains a parallel β-helix, and not an anti-parallel β-sheet as previously anticipated from molecular modelling studies (Ref. 76). Not only are parallel β-helices very stable, they are also particularly suitable for polymerisation, since β-helices provide hydrophobic surfaces (flat sheets) for lateral assembly into disk-like oligomers and filamentous assemblies (Ref. 77). The presence of aromatic residues (Phe and Tyr) in the V3 loop of gp120 and in the sphingolipid-binding domain of PrP is consistent with the establishment of a stacking interaction with the sugar head of raft GSLs (Ref. 74). As long as these aromatic residues are involved in GSL binding, they are not available for interacting with each other in a nascent β-helical structure.

Thus, raft GSLs might constitutively inhibit the formation of β-helical structures in PrP by locking the aromatic residues in the gel phase of the raft environment. Changing the physicochemical properties of the raft by modulating the raft composition could result in the dissocation of PrP^{C} from protective GSLs such as GalCer. At this stage, the presence of a convenient co-factor (PrP^{Sc}, protein X) in the raft would induce the formation of a β-helix in PrP. This would allow the dimerisation of PrP, a key step in the process of PrP^{C} to PrP^{Sc} conversion leading to the formation of amyloid fibrils (Ref. 78). Yet the situation in vivo is certainly more complex than this simple model since PrP^{Sc} can form different types of aggregates (Ref. 66), including: (1) fibrillar amyloid prion rods that are infectious; (2) ordered, non-fibrillar aggregates that are also infectious but are not amyloid; and (3) non-infectious amorphous aggregates with a fibrillar structure. The relationship between these

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

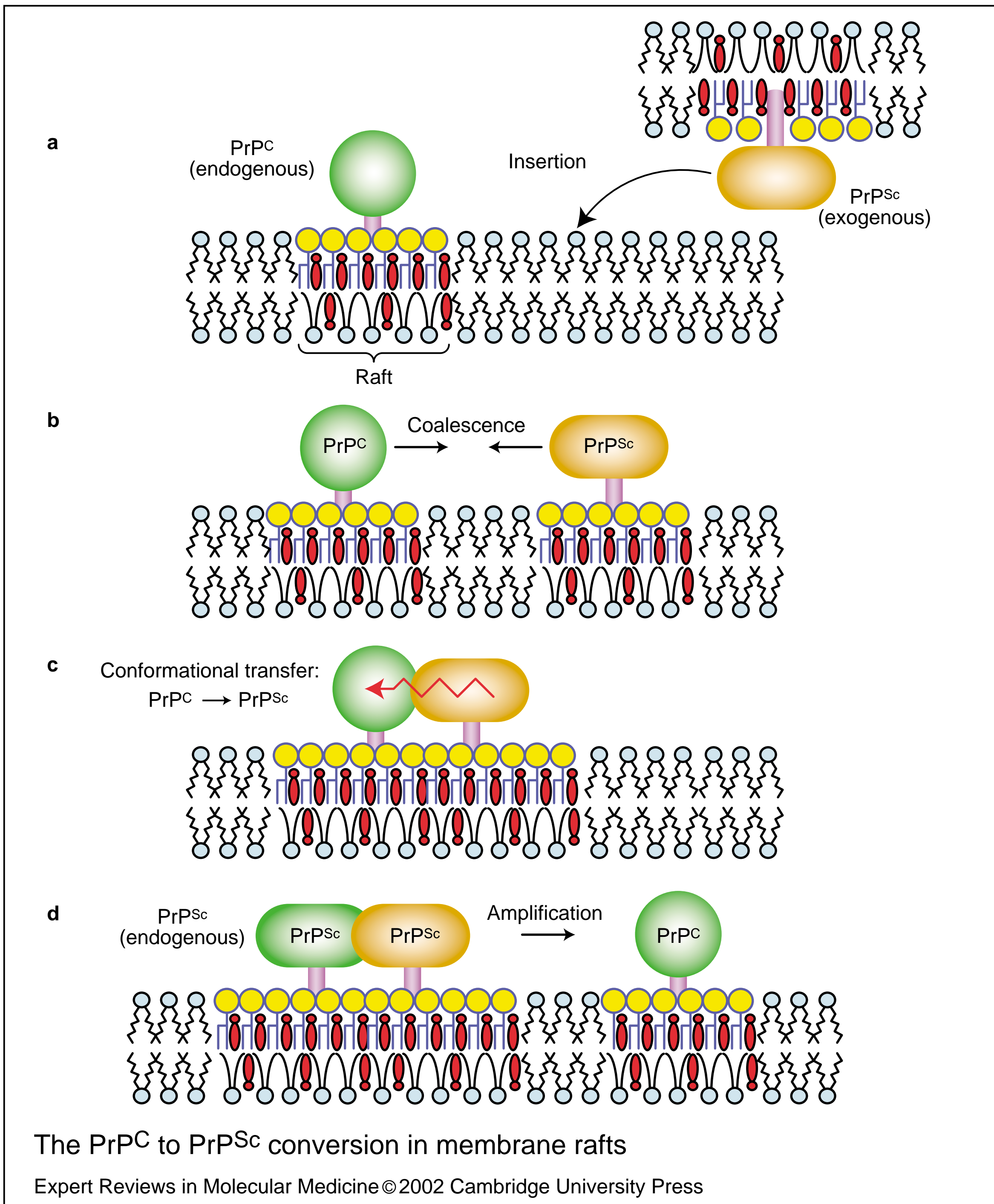

Figure 5. The PrP^{C} to PrP^{Sc} conversion in membrane rafts. A possible model for transmission of PrP^{Sc} from an infected cell to an uninfected cell. This model is based on recent data from the Caughey laboratory showing that the conversion of raft-associated prion proteins requires insertion of PrP^{Sc} into contiguous membranes (Ref. 27). (a) Infectious prions (either individual PrP^{Sc} molecules or small membrane vesicles enriched in PrP^{Sc}) are shed from the surface of an infected cell, and PrP^{Sc} is inserted into the plasma membrane of an uninfected cell. (b) At this stage, endogenous PrP^{C} and infectious PrP^{Sc} proteins are probably localised into distinct rafts of the recipient cell. The coalescence of these rafts will allow a close contact between PrP^{C} and PrP^{Sc}. (c) The PrP^{C} to PrP^{Sc} conversion occurs in membrane rafts. (d) The infection is propagated on the surface of the host cell **(fig005jfm)**.

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

different forms of PrP^{Sc} and prion infectivity, as well as the role of lipid rafts in each type of conversion, is unclear.

Lipid rafts and Alzheimer's disease

Amyloid fibril formation is one of the pathological hallmarks of Alzheimer's disease (Ref. 79). In this case, the fibrils form cerebrovascular senile plaques composed of the β-amyloid peptide (Aβ), a 39–42 residue fragment that is processed from a larger transmembrane protein known as the amyloid precursor protein (APP). Aβ plays a key role in the development of Alzheimer's disease, since all known inherited forms of the disease are associated with changes in Aβ processing and production. Aβ is produced from APP as a result of two sequential proteolytic cleavages involving: (1) a membrane-bound aspartyl protease (referred to as β-secretase); and (2) two homologous membrane proteases (presenilins 1 and 2, which probably correspond to the formerly described γ-secretase activities) (Ref. 80).

APP, β-secretase and presenilin 1 all reside in lipid rafts (Refs 80, 81). Thus, the production and accumulation of Aβ might occur primarily in these microdomains. Two raft lipids (cholesterol and GM1) bind to Aβ and might promote fibril formation (Refs 28, 80, 82). The molecular mechanism of amyloid fibril formation involves a major conformational change of Aβ, transforming an α-helix to a β-sheet or β-helix (Ref. 83). Membrane vesicles containing gangliosides such as GM1 bind to Aβ and induce an increased amount of α-helical structure at pH 7 and β-structure at pH 6 (Ref. 84). Taken together, these data support the view that raft GSLs can affect the conformation of Aβ.

A sphingolipid-binding domain similar to the V3-like domain of PrP has been identified in Aβ (Fig. 6), suggesting a common way by which HIV-1, prion and Alzheimer proteins interact with lipid rafts (Ref. 74). The molecular model proposed above to explain the role of raft lipids in the PrP^{C} to PrP^{Sc} conversion might also apply for Aβ. Amyloid formation proceeds by hydrophobic interactions among conformationally altered Aβ amyloidogenic intermediates. Short synthetic peptides, partially homologous to the Aβ region that undergoes abnormal conformational change, stabilise the normal conformation of Aβ. In a rat model of amyloidosis, these 'β-sheet breaker peptides' decreased the cerebral accumulation of Aβ and completely blocked the deposition of amyloid-like lesions (Ref. 83). The active peptides contain two Phe residues that interact in an antiparallel way with the central region of Aβ. It can be reasonably hypothesised that the sugar rings of GSLs could also stack against this region and modulate the conformational changes of Aβ. These important findings might serve as a paradigm for drug design to control amyloid formation process in Alzheimer's disease and other diseases involving changes in protein conformation.

Clinical implications/applications

The finding of a common sphingolipid-binding motif in HIV-1, prion and Alzheimer proteins underscores the role of lipid rafts in the pathogenesis of these diseases. Further studies are warranted to assess whether raft lipids act as chaperones implicated in the conformational change of PrP, as shown for HIV-1 gp120 (Ref. 48) and for the Alzheimer Aβ peptide (Ref. 84). The demonstration that raft lipids act as receptors for various exogenous proteins, convey these proteins to specific areas of the cell surface, and catalyse specific conformational changes in these proteins opens an exciting new field in molecular medicine (Ref. 85).

Therapeutic strategies targeting the structure and function of lipid rafts are rapidly emerging. Synthetic soluble analogues of GalCer bind to the V3 loop of gp120 and inhibit HIV-1 fusion (Refs 61, 86). Monovalent and polyvalent oligosaccharide derivatives (glycodendrimers) have been designed and are currently under evaluation as anti-adhesive therapies against viruses or bacterial toxins (Refs 87, 88). The development of specific β-sheet breaker peptides that bind to PrP and amyloid peptides and stabilise their normal conformation is a promising approach in the therapy of prion, Alzheimer's and other diseases caused by defective protein folding (Refs 83, 89). Cholesterol-lowering agents (statins) have been shown to reduce the incidence of Alzheimer's disease in humans (Ref. 80). In animal models, statin treatment decreased the levels of the β-amyloid peptide, in agreement with the key role of cholesterol in the formation of amyloid plaques. The recognition of the involvement of lipid rafts in various infectious and non-infectious pathologies will certainly offer, in the near future, new possibilites for therapeutic interventions.

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

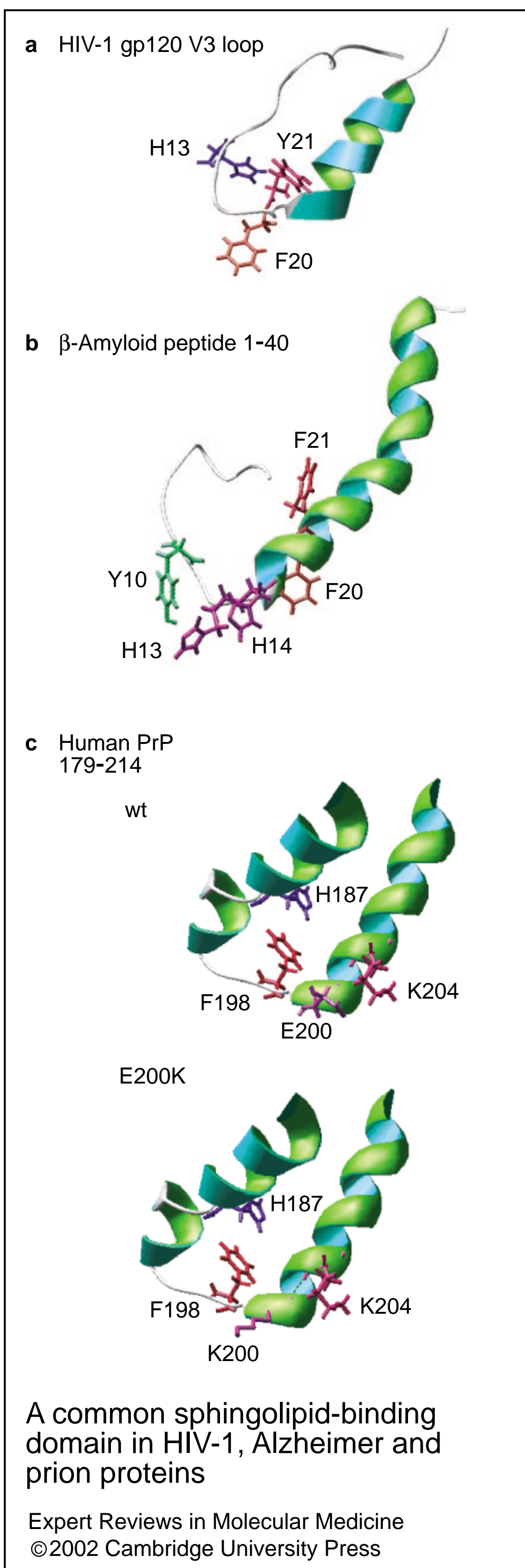

Figure 6. A common sphingolipid-binding domain in HIV-1, Alzheimer and prion proteins. The lateral chains of the residues of pathologically important proteins known to be involved in binding to glycosphingolipids and sphingomyelin in plasma membranes are shown. These data were generated with the CE program (http://cl.sdsc.edu/ce.html) and the results visualised with Swiss-PDB viewer (http://www.expasy.ch/swissmod/SWISS-MODEL.html). (a) The human immunodeficiency virus 1 gp120 V3 loop. (b) The β-amyloid peptide 1–40, involved in Alzheimer's disease. (c) Comparison of the wild-type (wt) human PrP with the E200K mutation, which is associated with familial Creutzfeldt–Jakob disease; this mutation might impair the association of the mutated PrP^{C} with raft lipids such as sphingomyelin through electrostatic repulsion between the positive charges of sphingomyelin and of the lysine (K) residue **(fig006jfm)**.

Research in progress and outstanding research questions

Despite intense research efforts, the molecular characterisation of lipid rafts on the surface of live cells is still in its infancy. One important issue is to understand how the external leaflet of the plasma membrane, containing GPI-anchored proteins, sphingolipids and cholesterol, is coupled to the internal leaflet into which acylated signal transducers insert. The exact size of rafts, and their lipid and protein composition, will not be accurately known until the development of new methods allowing the isolation of rafts without detergent.

In addition, the rules governing the interplay between protein association with and exclusion from lipid rafts are mostly unknown. Specifically, the molecular determinants controlling the interaction of HIV-1 gp120, PrP and Aβ with raft lipids need to better defined. Characterisation of the common sphingolipid-binding domain (V3-like domain) in these proteins has provided an attractive molecular basis for interaction with lipid rafts, but this domain might not be entirely responsible for their association with lipid rafts. Future studies will also help to clarify the molecular mechanisms involved in the chaperone activity of raft lipids. The conformational change of proteins induced by membrane lipids is a fundamental aspect of biological interactions that has been largely underestimated in the past and warrants thorough investigations.

The detailed description of raft–protein interactions at the molecular level will allow

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

the rational design of synthetic analogues of sphingolipids able to stabilise the non-pathological conformations of PrP and Alzheimer proteins. These analogues will have to be sufficiently soluble in water and, at the same time, be able to mimic the characteristic organisation of the corresponding sphingolipids in the raft. This can be achieved by polymerisation of the glycan motif in a multivalent analogue, or by self-association of the analogue in supramolecular structures (micelles, vesicles). The design of the aglycone part of GSL analogues will require special attention since: (1) the fatty acid composition of GSLs has a profound impact on the orientation of the sugar head (Ref. 90); and (2) cholesterol is known to modulate receptor activity (Ref. 91). In this respect, interesting results were recently obtained with adamantyl Gb3, a semi-synthetic soluble analogue of Gb3. In this analogue, which was originally designed to inhibit verotoxin binding to its glycolipid receptor Gb3 (Ref. 88), the fatty acid chain is replaced with a rigid globular hydrocarbon frame (adamantane). The presence of adamantane conferred specific physicochemical properties to the analogue, and eliminated the requirement of cholesterol for optimal Gb3–gp120 interaction (Ref. 92). In conclusion, the various biochemical and physicochemical approaches used to elucidate the structure and functions of lipid rafts will facilitate the design of new GSL analogues for future anti-adhesive and/or anti-amyloid therapies.

Acknowledgements and funding

We thank Eric Di Pasquale for critical reading of the manuscript, and the peer reviewers Dr Clifford Lingwood (Hospital for Sick Children, Toronto, Ontario, Canada) and Dr Hai-Tao He (Centre d'Immunologie de Marseille-Luminy, Marseille, France). Financial support from the Groupement d'Intérêt Scientifique 'Infections à Prions' is gratefully acknowledged.

References

1 Singer, S.J. and Nicolson, G.L. (1972) The fluid mosaic model of the structure of cell membranes. Science 175, 720-731, PubMed: 72101048

2 Brown, R.E. (1998) Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. J Cell Sci 111, 1-9, PubMed: 98055824

3 Brown, D.A. and London, E. (2000) Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J Biol Chem 275, 17221-17224, PubMed: 20298779

4 Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569-572, PubMed: 97320491

5 Brown, D.A. and London, E. (1998) Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 14, 111-136, PubMed: 99108929

6 Rietveld, A. and Simons, K. (1998) The differential miscibility of lipids as the basis for the formation of functional membrane rafts. Biochim Biophys Acta 1376, 467-479, PubMed: 99023786

7 Israelachvili, J.N., Marcelja, S. and Horn, R.G. (1980) Physical principles of membrane organization. Q Rev Biophys 13, 121-200, PubMed: 81200094

8 Brown, D.A. and Rose, J.K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 68, 533-544, PubMed: 92154668

9 London, E. and Brown, D.A. (2000) Insolubility of lipids in triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). Biochim Biophys Acta 1508, 182-195, PubMed: 20544799

10 Fridriksson, E.K. et al. (1999) Quantitative analysis of phospholipids in functionally important membrane domains from RBL-2H3 mast cells using tandem high-resolution mass spectrometry. Biochemistry 38, 8056-8063, PubMed: 99315181

11 Jacobson, K. and Dietrich, C. (1999) Looking at lipid rafts? Trends Cell Biol 9, 87-91, PubMed: 99217129

12 Edidin, M. (2001) Shrinking patches and slippery rafts: scales of domains in the plasma membrane. Trends Cell Biol 11, 492-496, PubMed: 21576838

13 Matko, J. and Szollosi, J. (2002) Landing of immune receptors and signal proteins on lipid rafts: a safe way to be spatio-temporally coordinated? Immunol Lett 82, 3-15, PubMed: 22003811

14 Rinia, H.A. and de Kruijff, B. (2001) Imaging domains in model membranes with atomic force microscopy. FEBS Lett 504, 194-199, PubMed: 21423575

15 Varma, R. and Mayor, S. (1998) GPI-anchored proteins are organized in submicron domains at the cell surface. Nature 394, 798-801, PubMed: 98389014

16 Pralle, A. et al. (2000) Sphingolipid-cholesterol

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

rafts diffuse as small entities in the plasma membrane of mammalian cells. J Cell Biol 148, 997-1008, PubMed: 20171434

17 Zacharias, D.A. et al. (2002) Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science 296, 913-916, PubMed: 21984700

18 Friedrichson, T. and Kurzchalia, T.V. (1998) Microdomains of GPI-anchored proteins in living cells revealed by crosslinking. Nature 394, 802-805, PubMed: 98389015

19 Drevot, P. et al. (2002) TCR signal initiation machinery is pre-assembled and activated in a subset of membrane rafts. Embo J 21, 1899-1908, PubMed: 21950335

20 Pike, L.J. et al. (2002) Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. Biochemistry 41, 2075-2088, PubMed: 21685765

21 Ilangumaran, S. and Hoessli, D.C. (1998) Effects of cholesterol depletion by cyclodextrin on the sphingolipid microdomains of the plasma membrane. Biochem J 335, 433-440, PubMed: 98437140

22 Merrill, A.H., Jr. et al. (2001) Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ Health Perspect 109 Suppl 2, 283-289, PubMed: 21259671

23 Shu, L. et al. (2000) Caveolar structure and protein sorting are maintained in NIH 3T3 cells independent of glycosphingolipid depletion. Arch Biochem Biophys 373, 83-90, PubMed: 20088598

24 Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1, 31-39, PubMed: 21306552

25 Ikonen, E. (2001) Roles of lipid rafts in membrane transport. Curr Opin Cell Biol 13, 470-477, PubMed: 21347352

26 van der Goot, F.G. and Harder, T. (2001) Raft membrane domains: from a liquid-ordered membrane phase to a site of pathogen attack. Semin Immunol 13, 89-97, PubMed: 21205654

27 Baron, G.S. et al. (2002) Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP(Sc)) into contiguous membranes. Embo J 21, 1031-1040, PubMed: 21856312

28 Kakio, A. et al. (2002) Interactions of amyloid beta-protein with various gangliosides in raft-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. Biochemistry 41, 7385-7390, PubMed: 22039829

29 Smart, E.J. et al. (1999) Caveolins, liquid-ordered domains, and signal transduction. Mol Cell Biol 19, 7289-7304, PubMed: 99454986

30 Benting, J. et al. (1999) Acyl and alkyl chain length of GPI-anchors is critical for raft association in vitro. FEBS Lett 462, 47-50, PubMed: 20047824

31 Hakomori, S.I. (2000) Cell adhesion/recognition and signal transduction through glycosphingolipid microdomain. Glycoconj J 17, 143-151, PubMed: 21069103

32 Prieschl, E.E. and Baumruker, T. (2000) Sphingolipids: second messengers, mediators and raft constituents in signaling. Immunol Today 21, 555-560, PubMed: 20547459

33 Norkin, L.C. (2001) Caveolae in the uptake and targeting of infectious agents and secreted toxins. Adv Drug Deliv Rev 49, 301-315, PubMed: 21435465

34 Shin, J.S. and Abraham, S.N. (2001) Caveolae as portals of entry for microbes. Microbes Infect 3, 755-761, PubMed: 21382756

35 Swaminathan, S. and Eswaramoorthy, S. (2000) Structural analysis of the catalytic and binding sites of Clostridium botulinum neurotoxin B. Nat Struct Biol 7, 693-699, PubMed: 20392549

36 Montecucco, C. (1986) How do tetanus and botulinum toxins bind to neuronal membranes? Trends Biochem Sci 11, 314-317

37 Nishiki, T. et al. (1996) The high-affinity binding of Clostridium botulinum type B neurotoxin to synaptotagmin II associated with gangliosides GT1b/GD1a. FEBS Lett 378, 253-257, PubMed: 96149443

38 Fantini, J. et al. (2000) Role of glycosphingolipid microdomains in CD4-dependent HIV-1 fusion. Glycoconj J 17, 199-204, PubMed: 21069109

39 Badizadegan, K. et al. (2000) Floating cholera toxin into epithelial cells: functional association with caveolae-like detergent-insoluble membrane microdomains. Int J Med Microbiol 290, 403-408, PubMed: 20562501

40 Abrami, L., Fivaz, M. and van der Goot, F.G. (2000) Adventures of a pore-forming toxin at the target cell surface. Trends Microbiol 8, 168-172, PubMed: 20219650

41 Lingwood, C.A. (1999) Glycolipid receptors for verotoxin and Helicobacter pylori: role in pathology. Biochim Biophys Acta 1455, 375-386, PubMed: 20035803

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

42 Falguieres, T. et al. (2001) Targeting of Shiga toxin B-subunit to retrograde transport route in association with detergent-resistant membranes. Mol Biol Cell 12, 2453-2468, PubMed: 21406009
43 Nusrat, A. et al. (2001) Clostridium difficile toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. Infect Immun 69, 1329-1336, PubMed: 21116939
44 Levy, J.A. (1993) Pathogenesis of human immunodeficiency virus infection. Microbiol Rev 57, 183-289, PubMed: 93218576
45 Binley, J. and Moore, J.P. (1997) HIV-cell fusion. The viral mousetrap. Nature 387, 346-348, PubMed: 97305945
46 Hammache, D. et al. (1999) Human erythrocyte glycosphingolipids as alternative cofactors for human immunodeficiency virus type 1 (HIV-1) entry: evidence for CD4-induced interactions between HIV-1 gp120 and reconstituted membrane microdomains of glycosphingolipids (Gb3 and GM3). J Virol 73, 5244-5248, PubMed: 99252226
47 Puri, A. et al. (1998) The neutral glycosphingolipid globotriaosylceramide promotes fusion mediated by a CD4-dependent CXCR4-utilizing HIV type 1 envelope glycoprotein. Proc Natl Acad Sci U S A 95, 14435-14440, PubMed: 99045702
48 Hug, P. et al. (2000) Glycosphingolipids promote entry of a broad range of human immunodeficiency virus type 1 isolates into cell lines expressing CD4, CXCR4, and/or CCR5. J Virol 74, 6377-6385, PubMed: 20323488
49 Kozak, S.L., Heard, J.M. and Kabat, D. (2002) Segregation of CD4 and CXCR4 into distinct lipid microdomains in T lymphocytes suggests a mechanism for membrane destabilization by human immunodeficiency virus. J Virol 76, 1802-1815, PubMed: 21657456
50 Manes, S. et al. (2000) Membrane raft microdomains mediate lateral assemblies required for HIV-1 infection. EMBO Rep 1, 190-196, PubMed: 21163502
51 Liao, Z. et al. (2001) Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1. AIDS Res Hum Retroviruses 17, 1009-1019, PubMed: 21377981
52 Popik, W., Alce, T.M. and Au, W.C. (2002) Human immunodeficiency virus type 1 uses lipid raft-colocalized CD4 and chemokine receptors for productive entry into CD4(+) T cells. J Virol 76, 4709-4722, PubMed: 21964416
53 Berger, E.A., Murphy, P.M. and Farber, J.M. (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 17, 657-700, PubMed: 99286823
54 Chan, S.Y. et al. (2001) Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses. Cell 106, 117-126, PubMed: 21354316
55 Bavari, S. et al. (2002) Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. J Exp Med 195, 593-602, PubMed: 21866506
56 Meng, G. et al. (2002) Primary intestinal epithelial cells selectively transfer R5 HIV-1 to CCR5+ cells. Nat Med 8, 150-156, PubMed: 21679718
57 Fantini, J. et al. (2000) Glycosphingolipid (GSL) microdomains as attachment platforms for host pathogens and their toxins on intestinal epithelial cells: activation of signal transduction pathways and perturbations of intestinal absorption and secretion. Glycoconj J 17, 173-179, PubMed: 21069106
58 Clayton, F. et al. (2001) Gp120-induced Bob/GPR15 activation: a possible cause of human immunodeficiency virus enteropathy. Am J Pathol 159, 1933-1939, PubMed: 21552804
59 Harouse, J.M. et al. (1991) Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science 253, 320-323, PubMed: 91313382
60 Yahi, N. et al. (1992) Galactosyl ceramide (or a closely related molecule) is the receptor for human immunodeficiency virus type 1 on human colon epithelial HT29 cells. J Virol 66, 4848-4854, PubMed: 92333666
61 Fantini, J. et al. (1997) Synthetic soluble analogs of galactosylceramide (GalCer) bind to the V3 domain of HIV-1 gp120 and inhibit HIV-1-induced fusion and entry. J Biol Chem 272, 7245-7252, PubMed: 97207285
62 Hammache, D. et al. (1998) Specific interaction of HIV-1 and HIV-2 surface envelope glycoproteins with monolayers of galactosylceramide and ganglioside GM3. J Biol Chem 273, 7967-7971, PubMed: 98192581
63 Delezay, O. et al. (1996) SPC3, a V3 loop-derived synthetic peptide inhibitor of HIV-1 infection, binds to cell surface glycosphingolipids. Biochemistry 35, 15663-15671, PubMed: 97121263
64 Israelachvili, J. and Wennerstrom, H. (1996) Role of hydration and water structure in biological

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

and colloidal interactions. Nature 379, 219-225, PubMed: 96138377
65 Campbell, S.M., Crowe, S.M. and Mak, J. (2001) Lipid rafts and HIV-1: from viral entry to assembly of progeny virions. J Clin Virol 22, 217-227, PubMed: 21448629
66 Prusiner, S.B. (1998) Prions. Proc Natl Acad Sci U S A 95, 13363-13383, PubMed: 99030576
67 Vey, M. et al. (1996) Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci U S A 93, 14945-14949, PubMed: 97121495
68 Naslavsky, N. et al. (1997) Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J Biol Chem 272, 6324-6331, PubMed: 97197802
69 Taraboulos, A. et al. (1995) Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J Cell Biol 129, 121-132, PubMed: 95213278
70 Naslavsky, N. et al. (1999) Sphingolipid depletion increases formation of the scrapie prion protein in neuroblastoma cells infected with prions. J Biol Chem 274, 20763-20771, PubMed: 99339997
71 Klein, T.R. et al. (1998) Prion rods contain small amounts of two host sphingolipids as revealed by thin-layer chromatography and mass spectrometry. Biol Chem 379, 655-666, PubMed: 98349803
72 Sanghera, N. and Pinheiro, T.J. (2002) Binding of prion protein to lipid membranes and implications for prion conversion. J Mol Biol 315, 1241-1256, PubMed: 21686313
73 Kaneko, K. et al. (1997) Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc Natl Acad Sci U S A 94, 10069-10074, PubMed: 97439818
74 Mahfoud, R. et al. (2002) Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. J Biol Chem 277, 11292-11296, PubMed: 21909538
75 Ling, H. et al. (1998) Structure of the shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb3. Biochemistry 37, 1777-1788, PubMed: 98153655
76 Wille, H. et al. (2002) Structural studies of the scrapie prion protein by electron crystallography. Proc Natl Acad Sci U S A 99, 3563-3568, PubMed: 21902002
77 Jenkins, J. and Pickersgill, R. (2001) The architecture of parallel beta-helices and related folds. Prog Biophys Mol Biol 77, 111-175, PubMed: 21615649
78 Knaus, K.J. et al. (2001) Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nat Struct Biol 8, 770-774, PubMed: 21415763
79 Dumery, L. et al. (2001) beta-Amyloid protein aggregation: its implication in the physiopathology of Alzheimer's disease. Pathol Biol (Paris) 49, 72-85, PubMed: 21164159
80 Golde, T.E. and Eckman, C.B. (2001) Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease. Drug Discov Today 6, 1049-1055
81 Riddell, D.R. et al. (2001) Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. Curr Biol 11, 1288-1293, PubMed: 21417181
82 Kakio, A. et al. (2001) Cholesterol-dependent formation of GM1 ganglioside-bound amyloid beta-protein, an endogenous seed for Alzheimer amyloid. J Biol Chem 276, 24985-24990, PubMed: 21326166
83 Soto, C. (1999) Plaque busters: strategies to inhibit amyloid formation in Alzheimer's disease. Mol Med Today 5, 343-350, PubMed: 99362835
84 McLaurin, J. et al. (1998) Structural transitions associated with the interaction of Alzheimer beta-amyloid peptides with gangliosides. J Biol Chem 273, 4506-4515, PubMed: 98136149
85 Sui, S.F. (2000) Membrane-induced conformational change of proteins. Adv Colloid Interface Sci 85, 257-267, PubMed: 20228970
86 Fantini, J. (2000) Synthetic soluble analogs of glycolipids for studies of virus-glycolipid interactions. Methods Enzymol 311, 626-638, PubMed: 20029092
87 Mulvey, G. et al. (2001) Glycan mimicry as a basis for novel anti-infective drugs. Biochimie 83, 841-847, PubMed: 21421094
88 Mylvaganam, M. and Lingwood, C.A. (1999) Adamantyl globotriaosyl ceramide: a monovalent soluble mimic which inhibits verotoxin binding to its glycolipid receptor. Biochem Biophys Res Commun 257, 391-394, PubMed: 99216290
89 Soto, C. et al. (2000) Reversion of prion protein conformational changes by synthetic beta-sheet breaker peptides. Lancet 355, 192-197, PubMed: 20137584
90 Mamelak, D. et al. (2001) Hsp70s contain a

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

specific sulfogalactolipid binding site. Differential aglycone influence on sulfogalactosyl ceramide binding by recombinant prokaryotic and eukaryotic hsp70 family members. Biochemistry 40, 3572-3582, PubMed: 21194687

91 Gimpl, G., Burger, K. and Fahrenholz, F. (1997) Cholesterol as modulator of receptor function. Biochemistry 36, 10959-10974, PubMed: 97428226

92 Mahfoud, R. et al. (2002) A novel soluble analog of the HIV-1 fusion cofactor, globotriaosylceramide (Gb(3)), eliminates the cholesterol requirement for high affinity gp120/Gb(3) interaction. J Lipid Res 43, 1670-1679, PubMed: 22251052

Further reading, resources and contacts

Recent reviews on intracellular sphingolipid transport, prion protein interactions, and the lipid shell hypothesis:

van Meer, G. and Holthuis, J.C. (2000) Sphingolipid transport in eukaryotic cells. Biochim Biophys Acta 1486, 145-170, PubMed: 20315743

Caughey, B. (2001) Interactions between prion protein isoforms: the kiss of death? Trends Biochem Sci 26, 235-242, PubMed: 21192795

Anderson, R.G. and Jacobson, K. (2002) A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. Science 296, 1821-1825, PubMed: 22048390

Related articles published recently in *Expert Reviews in Molecular Medicine*:

Jean C. Manson and Nadia L. Tuzi (2001) Transgenic models of the transmissible spongiform encephalopathies. Exp. Rev. Mol. Med. 11 May, http://www-ermm.cbcu.cam.ac.uk/01002952h.htm

Gunnar K. Gouras (2001) Current theories for the molecular and cellular pathogenesis of Alzheimer's disease. Exp. Rev. Mol. Med. 31 May, http://www-ermm.cbcu.cam.ac.uk/01003167h.htm

Qi Chen and David Schubert (2002) Presenilin-interacting proteins. Exp. Rev. Mol. Med. 21 August, http://www.expertreviews.org/02005008h.htm

Robert J. Salmond, Jeffrey A. Luross and Neil A. Williams (2002) Immune modulation by the cholera-like enterotoxins. Exp. Rev. Mol. Med. 1 October, http://www.expertreviews.org/02005057h.htm

Sophie Malagarie-Cazenave, Nathalie Andrieu-Abadie, Bruno Ségui, Valérie Gouazé, Claudine Tardy, Olivier Cuvillier and Thierry Levade (2002) Sphingolipid signalling: molecular basis and role in TNF-α-induced cell death. Exp. Rev. Mol. Med. 20 December, http://www.expertreviews.org/0200546X

The HIV Sequence Database includes review articles on the infectious cycle of HIV:

http://hiv-web.lanl.gov/content/hiv-db/mainpage.html

The Alzheimer Research Forum website includes research news on Alzheimer's disease and prion diseases:

http://www.alzforum.org

The Realistic Images of the Lipid Bilayer Membrane website, by Bill Wimley at Tulane University Health Sciences Center, New Orleans, LA, USA, compares the classical cartoon of a lipid bilayer membrane with a molecular dynamics image of a more-realistic lipid bilayer membrane:

http://www.tulane.edu/~biochem/faculty/facfigs/bilayer.htm

LIPIDAT is a relational database of thermodynamic and structural information on lipid mesophase and crystal polymorphic transitions:

http://www.lipidat.chemistry.ohio-state.edu/

(continued on next page)

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press

Further reading, resources and contacts *(continued)*

Tools for protein structure analysis can be found at The Protein Databank, SWISS-MODEL and a representative structure comparison website:

http://cl.sdsc.edu/ce.html
http://www.expasy.ch/swissmod/SWISS-MODEL.html
http://www.rcsb.org/pdb/searchlite.html

Features associated with this article

Figures

Figure 1. Structure-based classification of membrane lipids (fig001jfm).
Figure 2. Cholesterol favours phase separation of membrane lipids: the origin of raft formation? (fig002jfm)
Figure 3. Lipid organisation in raft microdomains: a simplified model based on the theoretical shape of membrane lipids (fig003jfm).
Figure 4. Role of lipid rafts in fusion of human immunodeficiency virus 1 (HIV-1) to $CD4^+$ T cells (fig004jfm).
Figure 5. The PrP^C to PrP^{Sc} conversion in membrane rafts (fig005jfm).
Figure 6. A common sphingolipid-binding domain in HIV-1, Alzheimer and prion proteins (fig006jfm).

Citation details for this article

Jacques Fantini, Nicolas Garmy, Radhia Mahfoud and Nouara Yahi (2002) Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Exp. Rev. Mol. Med. 20 December, http://www.expertreviews.org/02005392h.htm

Accession information: DOI: 10.1017/S1462399402005392; 20 December 2002
©2002 Cambridge University Press