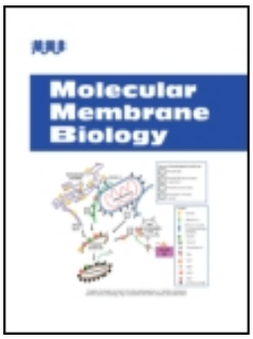


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# The prion protein and lipid rafts (Review)

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**Abstract**
Prions are the causative agent of the transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease in humans. In these prion diseases the normal cellular form of the prion protein ($PrP^C$) undergoes a post-translational conformational conversion to the infectious form ($PrP^{Sc}$). $PrP^C$ associates with cholesterol- and glycosphingolipid-rich lipid rafts through association of its glycosyl-phosphatidylinositol (GPI) anchor with saturated raft lipids and through interaction of its N-terminal region with an as yet unidentified raft associated molecule. $PrP^C$ resides in detergent-resistant domains that have different lipid and protein compositions to the domains occupied by another GPI-anchored protein, Thy-1. In some cells $PrP^C$ may endocytose through caveolae, but in neuronal cells, upon copper binding to the N-terminal octapeptide repeats, the protein translocates out of rafts into detergent-soluble regions of the plasma membrane prior to endocytosis through clathrin-coated pits. The current data suggest that the polybasic region at its N-terminus is required to engage $PrP^C$ with a transmembrane adaptor protein which in turn links with the clathrin endocytic machinery. $PrP^C$ associates in rafts with a variety of signalling molecules, including caveolin-1 and Fyn and Src tyrosine kinases. The clustering of $PrP^C$ triggers a range of signal transduction processes, including the recruitment of the neural cell adhesion molecule to rafts which in turn promotes neurite outgrowth. Lipid rafts appear to be involved in the conformational conversion of $PrP^C$ to $PrP^{Sc}$, possibly by providing a favourable environment for this process to occur and enabling disease progression.

**Keywords:** *Cholesterol, detergent-resistant membrane, glycosyl-phosphatidylinositol, lipid raft, prion protein*

## Introduction

Prions are the causative agent of the transmissible spongiform encephalopathies (TSEs). This group of diseases includes scrapie in sheep, bovine spongiform encephalopathy in cattle and Creutzfeldt-Jakob disease (CJD) in humans. In these diseases the cellular isoform of the prion protein ($PrP^C$) is post-translationally misfolded into the infectious scrapie isoform ($PrP^{Sc}$). $PrP^C$, encoded by the *Prpn* gene, is a cell surface glycosyl-phosphatidylinositol (GPI)-anchored protein expressed by a variety of cell types, but being particularly abundant in neurons. The protein consists of a flexible N-terminus, whose structure remains undetermined, and a C-terminal globular domain containing predominantly α-helical secondary structure [1]. According to the protein only hypothesis [2], an interaction between the pathogenic $PrP^{Sc}$ and endogenous $PrP^C$ is sufficient to cause the template-driven formation of more $PrP^{Sc}$.

Although $PrP^C$ is essential for the development of prion disease [3], the normal physiological function(s) of $PrP^C$ remains largely unknown. However, a growing number of studies implicate $PrP^C$ in the cellular resistance to oxidative stress [4], in cell signalling [5], in copper and zinc metabolism [6,7] and in synaptic transmission [8]. PrP is associated for much of its life cycle with cholesterol- and glycosphingolipid-rich lipid rafts and it is the purpose of this review to explore the role of lipid rafts in prion protein biology, both for the normal and disease-associated forms of the protein. In model membranes containing sphingomyelin, cholesterol and an unsaturated glycerophospholipid, the saturated lipids pack together in liquid ordered domains which are characterized by being insoluble in detergents, such as Triton X-100, at 4°C. Although extraction of membranes with cold non-ionic detergents has been extensively used to isolate 'rafts', a growing body of evidence suggests caution in identifying detergent-resistant membranes (DRMs) with

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rafts in cell membranes [9]. However, as extraction of membranes with detergents has been widely used to study the raft association of PrP and other proteins, for the purpose of this review we have equated DRMs with rafts in the membrane.

## Targeting of $PrP^C$ to lipid rafts

### *GPI anchor-dependent targeting*

$PrP^C$ is attached to the lipid bilayer via a GPI anchor which is added to the serine residue at position 231 (murine PrP numbering) [10] (Figure 1). The GPI anchor is added rapidly to the protein on translocation into the endoplasmic reticulum (ER), following the cleavage of the C-terminal GPI anchor addition signal sequence. Both cleavage of the polypeptide and addition of the preformed GPI anchor are carried out by a transamidase complex whose active site is on the lumenal side of the ER membrane. The GPI anchor consists of a core tetrasaccharide (three mannose residues and a glucosamine) linked through ethanolamine phosphate to Ser-231. The glucosamine residue is linked to the headgroup of phosphatidylinositol whose acyl chains contain the fully saturated stearic acid (C18:0) [10]. On transit to the cell surface a number of modifications and additions are made to the core GPI anchor structure, including the addition of sialic acid to one of the mannose residues [11]. This latter modification has only been documented in two other GPI-anchored proteins, porcine membrane dipeptidase [12] and human CD59 [13].

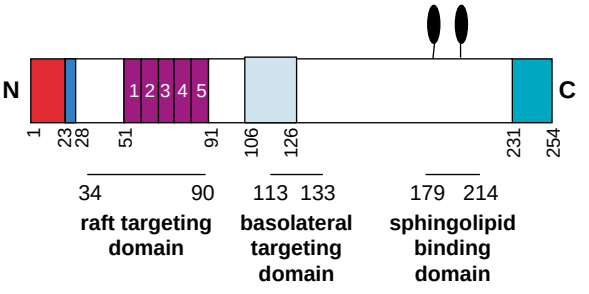


Figure 1. Schematic of $PrP^C$. Nascent murine $PrP^C$ is a protein of 254 amino acids. On translocation into the ER the N-terminal signal peptide (chequered box; red online) and the C-terminal GPI anchor addition signal (wavy line box; green online) are removed and the latter replaced with a GPI anchor. The polybasic region (black; blue online; residues 23–28) at the N-terminus of mature $PrP^C$, the copper-binding octapeptide repeats (stippled; purple online; residues 51-91), the central hydrophobic, neurotoxic domain (diagonal lines; light blue online; residues 106-126) and the two N-linked glycans (black lollipops; Asn-180 and Asn-196) are indicated. The positions of the raft targeting domain, the basolateral targeting domain and the sphingolipid binding domain (see text for details) are indicated below the protein. This figure is reproduced in colour in *Molecular Membrane Biology* online.

GPI anchors have the propensity to target proteins to lipid raft domains due to their saturated acyl chains preferentially associating with the saturated sphingolipids rather than the unsaturated glycerophospholipids [14,15]. In their seminal article, Brown and Rose [16] showed that GPI-anchored proteins become detergent-insoluble as they traverse the Golgi due to their association with detergent-insoluble, cholesterol-rich lipid rafts. The association of $PrP^C$ with rafts was assumed to occur in the Golgi, as several studies only detected the mature protein in DRMs [17–19]. Recently, however, it was reported that the immature diglycosylated precursor of $PrP^C$ associates with cholesterol-rich rafts in the ER [20]. This association was required to facilitate correct folding of the protein, as cholesterol depletion led to its misfolding. These authors suggested that association with cholesterol in the ER is required to target the immature $PrP^C$ to rafts or, perhaps by functioning as a lipochaperone within the ER, cholesterol directly affects the folding of $PrP^C$ [20].

The association of the GPI anchor with raft domains in the secretory pathway has been proposed to be the mechanism by which GPI anchored proteins are sorted and targeted to the apical surface of polarized epithelial cells [21], although for some GPI-anchored proteins it is the N-linked glycans, not the GPI anchor, that targets the protein to the apical surface [22]. Interestingly, when transfected into polarized Fischer rat thyroid (FRT) or Madin Darby canine kidney (MDCK) cells, $PrP^C$ was targeted to the basolateral membrane [23,24]. The basolateral targeting of $PrP^C$ was maintained after the cells were subjected to cholesterol depletion, indicating that the raft association of $PrP^C$ is not required for its exocytic transport [23]. Deletion mutagenesis studies have revealed that the internal hydrophobic domain of $PrP^C$ (residues 113–133; Figure 1) confers basolateral sorting in a dominant manner [24]. These data imply that the association of $PrP^C$ with rafts in the secretory pathway plays additional roles than simply membrane targeting.

### *Non GPI anchor-dependent targeting*

Several studies have provided evidence that PrP can associate with rafts by means other than through its GPI anchor. Our group has shown that the raft association of $PrP^C$ is not solely dependent upon its GPI anchor [25]. Using alternatively anchored forms of PrP expressed in human neuronal SH-SY5Y cells it was demonstrated that amino acid residues 23–90 of the flexible N-terminal domain (Figure 1) are necessary for this non-GPI-dependent raft association. Indeed, fusion of these residues to a

non-raft resident protein was sufficient to redirect the protein into DRMs. These data are supported by the observation that the binding of GPI-deficient PrP to sphingolipid-cholesterol-rich raft-like liposomes (SCRLs) was significantly decreased after deletion of residues 34–94 [26]. As all of the protein interacting partners of $PrP^C$ identified to date, such as the neural cell adhesion molecule (NCAM), stress inducible protein-1 and the 37 kDa/67 kDa laminin receptor, interact with $PrP^C$ C-terminal to residue 90 (for a review see [27]), the identity of the lipid raft interacting partner for $PrP^C$, be it protein or lipid, awaits determination.

Another lipid raft targeting motif identified within the prion protein is a sphingolipid binding domain [28] (Figure 1). This was identified through structural homology to a similar domain in the human immunodeficiency (HIV)-1 surface envelope protein gp120. It is also found in the Alzheimer amyloid-β peptide. Synthetic peptides derived from the predicted sphingolipid binding domains of these three proteins interacted with monomolecular films of galactosylceramide and sphingomyelin. Interestingly, the E200K mutation in PrP associated with some types of familial CJD occurs within this binding domain, which is within the disulphide-linked loop (Cys179–Cys214), and apparently interfered with sphingomyelin binding [28]. However, as full-length PrP containing the E200K mutation is still found in DRMs [29], the significance of this sphingolipid binding domain in the interaction of $PrP^C$ with rafts in cells is not clear.

## Distribution of $PrP^C$ in rafts

In studies comparing the membrane microdomains occupied by $PrP^C$ and another GPI-anchored protein, Thy-1, it was found that the two proteins displayed differential detergent solubility [30]. The sphingolipid-rafts containing $PrP^C$ were more detergent-soluble than those containing Thy-1, indicating that $PrP^C$ is likely to be present in less tightly ordered domains than Thy-1. Indeed, immunoaffinity purification studies suggested that domains containing $PrP^C$ border the edges of the more tightly ordered Thy-1 containing domains [30]. Alternative explanations are that $PrP^C$ is located at the boundary between two domains or that $PrP^C$ and Thy-1 reside in distinct domains with different compositions and properties. In a further study, the same group showed that the lipid composition of the $PrP^C$ and Thy-1 containing DRMs differed markedly [31]. The $PrP^C$ DRMs contained a larger proportion of unsaturated longer chain lipids than the Thy-1 domains. A higher proportion of unsaturated lipids would be expected to decrease the liquid order of the $PrP^C$ domains.

Doppel, the paralog of $PrP^C$, which is encoded by the *Prnd* gene some 16 kb downstream of the *Prnp* gene, is a protein of 179 amino acids that is expressed mainly in the testis and not in the brain of adult mice [32]. Doppel has sequence and structural similarity to the C-terminal half of PrP, but lacks the N-terminal $Cu^{2+}$-binding octapeptide repeats. It too is N-glycosylated and GPI anchored [32]. Doppel is also found in DRMs, although one study reported that at detergent conditions in which membrane rafts were intact, it did not co-immunoprecipitate with $PrP^C$, indicating that the two proteins are not present in the same raft domains [33], while another study showed that the two proteins share common membrane microdomains and internalization pathways [34].

A growing body of evidence is accumulating to indicate that rafts are heterogeneous in both protein and lipid composition, in their cellular localization, and, hence, in their biological function [35]. The limited data available for $PrP^C$ would be consistent with this, and thus any consideration of the association of $PrP^C$ with rafts and raft components almost certainly has to consider the heterogeneity of such structures.

## Endocytosis of $PrP^C$

Originally, it was proposed by Harris and co-workers, in experiments using chicken PrP (which has only approx. 30% sequence homology to mammalian PrPs) transfected into mouse neuroblastoma cells, that $PrP^C$ recycles between the cell surface and an endocytic compartment with a transit time of approximately 60 minutes [36]. The same group localized chicken $PrP^C$ to clathrin-coated pits and vesicles by electron microscopy [37]. However, it was subsequently shown that endogenous $PrP^C$ in murine neuronal N2a and Chinese Hamster Ovary (CHO) cells was localized in caveolae or morphologically similar caveolae-like domains [18,38,39]. Caveolae, flask-shaped invaginations of the plasma membrane that contain the coat protein caveolin-1, are involved in non-clathrin-dependent endocytosis [40]. As caveolae appear to be a subset of cholesterol-rich lipid rafts, depletion of cellular cholesterol disrupts their endocytosis [41]. In CHO cells that have endogenous caveolin-1, cryoimmunogold electron microscopy was used to show that at steady state $PrP^C$ was enriched in caveolae both at the plasma membrane and at the *trans*-Golgi network and in interconnecting chains of endocytic caveolae [42]. Furthermore, the cholesterol-binding agent filipin prevented the $Cu^{2+}$-induced endocytosis of

$PrP^C$ in both both neuronal and non-neuronal cells, leading to the conclusion that endocytosis is occurring through a caveolae-dependent mechanism [43]. However, this conclusion is confused by the fact that filipin is known also to induce the shedding of $PrP^C$ [43,44]. In addition, as most neuronal cells do not express caveolin-1 [45,46] and lack morphologically distinguishable caveolae [37], the role of caveolae in the endocytosis of $PrP^C$ in the brain has to be questioned.

Clathrin-coated pits are the most well studied endocytic mechanism and allow the endocytosis of transmembrane proteins that are recruited to the pits by interaction of targeting motifs in their cytoplasmic tails with various accessory proteins, including the adaptor protein AP-2, on the cytoplasmic face of the membrane [47]. As the GPI-anchored $PrP^C$ does not contain transmembrane or cytoplasmic domains, it would have to associate with a transmembrane adaptor in order to engage the clathrin endocytic machinery [48]. There is a precedent for this; the GPI-anchored urokinase-type plasminogen activator (uPA)-receptor when bound to uPA is internalized via interaction with the transmembrane low-density lipoprotein receptor-related protein-1 [49]. However, the highly ordered nature of the saturated lipids in rafts will not permit the tight curvature of the membrane required for the formation of clathrin-coated pits [50,51]. Therefore, for a GPI-anchored protein to be internalized through clathrin-coated pits it probably has to first translocate out of the rafts into non-raft regions of the membrane.

Sunyach et al. [50] were the first to report this to occur during the endocytosis of endogenous $PrP^C$ in both primary sensory neurons and N2a cells. This was based on 50% of the $PrP^C$ in N2a cells being detergent soluble (where 99% of another GPI-anchored protein remained detergent insoluble) and the statistically significant co-localisation of $PrP^C$ with the transferrin receptor and the low density lipoprotein receptor (as shown by light and electron microscopy), two prototypical receptors endocytosed through clathrin-coated pits. A green fluoresecent protein (GFP)-tagged form of PrP was shown to internalize via a dynamin-dependent endocytic pathway, with the protein being targeted to the recycling endosomal compartment via Rab5-positive early endosomes [52]. The steady state distribution of GFP-GPI between the plasma membrane and early endosomes was not affected by a Rab5 mutant, implying that the endocytosis of GFP-PrP is different from other GPI-anchored proteins and is not determined predominantly by the GPI anchor [52].

$PrP^C$ contains four complete octapeptide repeats (PHGG(G/S)WGQ) in its N-terminal half that are capable of binding $Cu^{2+}$ ions [53,54] (Figure 1). $Cu^{2+}$ ions, at levels similar to those occurring naturally in the extracellular spaces of the brain, can stimulate the endocytosis of $PrP^C$ in neuronal cells [6,55]. Recently, we determined the mechanism involved in the $Cu^{2+}$-stimulated endocytosis of $PrP^C$ in neuronal cells [56] (Figure 2). Under basal conditions $PrP^C$ was associated with DRMs,

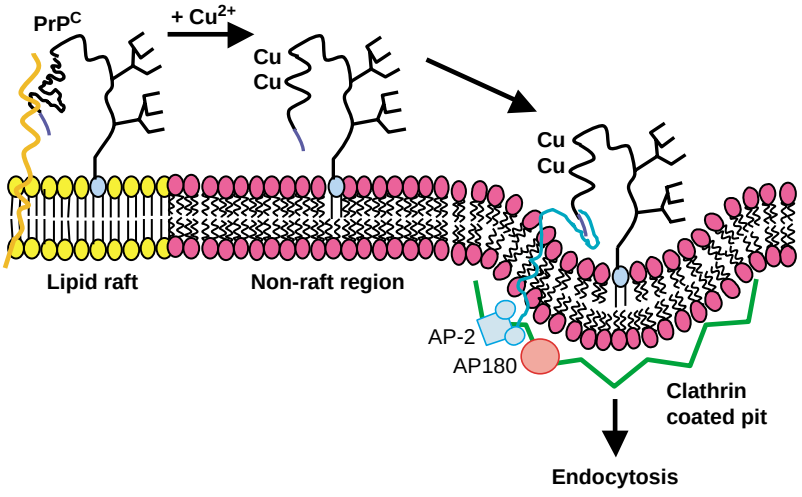


Figure 2. $PrP^C$ is localized in rafts but translocates out of them before being endocytosed through clathrin-coated pits. $PrP^C$ is attached to the exoplasmic leaflet of the plasma membrane via its GPI anchor and localises within detergent-insoluble rafts through interactions between its N-terminal region (residues 23-90) and a raft-resident protein (grey; orange online) or lipid. Upon $Cu^{2+}$ binding to the octapeptide repeats the protein undergoes a conformational change that dissociates it from the raft-resident partner and $PrP^C$ then moves laterally out of the rafts into detergent-soluble regions of the plasma membrane. The polybasic N-terminal region (blue online) then interacts with the ectodomain of a transmembrane protein (grey; turquoise online) that engages, via its cytoplasmic domain, with the adaptor protein AP-2 and the endocytic machinery of clathrin-coated pits. Modified from [56]. This figure is reproduced in colour in *Molecular Membrane Biology* online.

however, upon exposure of cells to $Cu^{2+}$ a proportion of $PrP^C$ moved into detergent-soluble regions of the plasma membrane. The subsequent internalization of $PrP^C$ could be specifically blocked by selectively inhibiting clathrin-mediated endocytosis. This was achieved using a tyrosine analogue, tyrphostin A23, that specifically disrupts interactions between transmembrane protein cytoplasmic tail coated-pit targeting motifs and AP-2 and with a dominant negative mutant of the clathrin assembly protein AP180 [56]. The deletion of large parts of the N-terminal region of PrP has revealed the critical importance of this region to its endocytosis [57–59]. More specifically, point mutations within the polybasic region (residues 23–28; Figure 1) disrupted the constitutive endocytosis of $PrP^C$ [50]. Using mutants of $PrP^C$ that either lacked the N-terminal polybasic region or the octapeptide repeats, we were able to show that copper binding, perhaps by driving a conformational change in the protein [60], is required to dissociate $PrP^C$ from lipid rafts, while the polybasic region is required to mediate the endocytosis of PrP, possibly through interaction with a transmembrane adaptor protein [56] (Figure 2). Although we and others have shown that $PrP^C$ in neuronal cells can translocate out of rafts and be internalized via the clathrin-endocytic machinery, the involvement of other raft-based endocytic mechanisms in the internalization of $PrP^C$ in other cells cannot be ruled out.

## $PrP^C$, lipid rafts and signal transduction

In many cells, including neurons, lipid rafts provide a platform for signal transduction processes [61]. Many-cell surface receptors and cytoplasmic signalling molecules, such as Src family kinases and trimeric and small GTPases, are concentrated in DRMs, and caveolin-1, acting as a scaffold protein, is essential in the regulation and formation of signalling complexes in rafts. For some years it has been known that cross-linking with antibodies of many diverse GPI-anchored proteins results in signal transduction that is characterized by: (i) transient elevation of cytoplasmic $Ca^{2+}$ concentrations; (ii) tyrosine phosphorylation of cellular substrates; and (iii) triggering of T cell proliferation and differentiation [62]. Therefore, in some ways, it was not too surprising when it was reported that antibody-mediated cross-linking of $PrP^C$ on the surface of differentiated murine 1C11 neuronal cells triggered a signal transduction cascade involving the caveolin-1-dependent coupling of $PrP^C$ to the intracellular tyrosine kinase Fyn [5]. More recently in the same differentiated 1C11 cells, it has been reported that $PrP^C$ via caveolin-1 modulates serotonin receptor coupling to G-proteins, thereby acting as a protagonist contributing to the homeostasis of serotoninergic neurons [63]. Further evidence for a role for $PrP^C$ in signal transduction came from the observation that $PrP^C$ interacts with the neuronal phosphoprotein synapsin Ib, the adaptor protein Grb2 and the prion interactor Pint1 [64].

$PrP^C$ has been shown also to be a component of the multimolecular signalling complex involved in T cell activation. $PrP^C$ associates with gangliosides in DRMs from neural and lymphocytic cells [65], and in T cells co-immunoprecipitates with Fyn and, after T cell activation, with the phosphorylation protein ZAP-70 [66]. Antibody cross-linking of $PrP^C$ on T cells resulted in its coclustering with the caveolin-like raft proteins flotillins-1 and -2 (reggie-2 and -1) in polarized caps [67]. Several signalling molecules, including Thy-1, CD3/TCR and LAT, were also directed to the cap and the cross-linking of $PrP^C$ provoked mitogen-activated protein (MAP) kinase activation and a brief elevation of the intracellular $Ca^{2+}$ concentration [67].

The activation of intracellular signals implies the existence of an extracellular ligand(s) capable of triggering activation of $PrP^C$. One candidate as such a ligand is the stress-inducible protein 1 that has been shown to bind to cell surface $PrP^C$ and induce neuroprotective signals via a cAMP/protein kinase A-dependent pathway that rescues cells from apoptosis [68,69]. Another candidate is the neural cell adhesion molecule (NCAM) which has been reported recently to interact directly both in *cis* (on the same cell membrane) and in *trans* (on neighbouring membranes) with $PrP^C$ at the neuronal cell surface [70]. $PrP^C$ was shown to promote the recruitment of transmembrane isoforms of NCAM to rafts where the latter activates Fyn kinase and enhances neurite outgrowth [70].

However, questions remain as to how the GPI-anchored $PrP^C$, present on the extracellular face of the plasma membrane can directly interact with signalling proteins on the cytosolic face. One suggestion is that transmembrane forms of PrP could link directly to such cytosolic proteins [64]. Another possibility is that $PrP^C$ links to such cytoplasmic signalling proteins via one or more transmembrane adaptors. Interestingly, antibodies against clathrin reduced, but did not ablate, the level of Fyn activation upon $PrP^C$ cross-linking, implicating clathrin in mediating, in part, the signal transduction from $PrP^C$ [5]. Whether this is due to a proportion of $PrP^C$ engaging with a transmembrane adaptor protein within clathrin-coated pits (see above) remains to be determined. Clustering of $PrP^C$ with antibodies on the surface of GT1-7 neurohypothalamic cells resulted in a rapid and transient

phosphorylation of the MAP kinases extracellular receptor kinases 1 and 2 (ERK1/2) and the microtubule-destabilizing protein stathmin [71], the latter protein being phosphorylated on Ser-16. Phosphorylation of stathmin on Ser-16 is induced upon activation of the transmembrane tyrosine kinase epidermal growth factor receptor (EGFR) and these authors were able to show that a specific EGFR inhibitor blocked both signalling pathways, indicating a recruitment and transactivation of EGFR upon antibody-mediated $PrP^C$ clustering [71]. This suggests that EGFR could be a functional transmembrane partner of $PrP^C$. Interestingly, prior to ligand binding to EGFR in quiescent fibroblasts, a significant portion (65%) of the receptor is in the low density plasma membrane fractions that contain caveolae and non-caveolae rafts [72]. After ligand binding, activated receptors rapidly move from this membrane fraction to non-raft regions of the plasma membrane where they are internalized by clathrin-coated pits [73]. Other candidates as signal transduction transmembrane adaptors linking $PrP^C$ to the cytosolic signalling proteins are the transmembrane isoforms of NCAM [70] (see above).

Although binding of stress-inducible protein 1 to $PrP^C$ induced neuroprotective signals [69] and $PrP^C$ activates phosphatidylinositol 3-kinase that plays a pivotal role in cell survival [74], another study reported that cross-linking of $PrP^C$ *in vivo* with specific monoclonal antibodies triggered rapid and extensive apoptosis in hippocampal and cerebellar neurons [75]. From these and other studies it is not clear whether different ligands, possibly in different cell types, could promote alternative signalling pathways, or whether the localization of $PrP^C$ in different rafts or in raft and non-raft domains of the membrane could trigger different cellular responses. As yet no data have been presented to show whether the localization of $PrP^C$ in rafts is critical for these two opposing responses.

One mechanism that could account for these apparently contradictory results would be if $PrP^C$ resides in multiple subtypes of raft that differ in their protein (and lipid) compositions or if under basal conditions $PrP^C$ in one type of raft is segregated from particular signalling molecules which reside in another raft. Upon ligand binding or antibody cross-linking the $PrP^C$ containing rafts fuse with the rafts containing a particular subset of signalling molecules and the appropriate signal transduction cascade and cellular response is triggered. In relation to $PrP^C$, evidence for the existence of different subtypes of rafts comes from the observation that in cerebellar granule cells DRMs containing most of $PrP^C$, GAP-43 and protein kinase C can be separated from those DRMs containing Fyn and MARCKS [76] and that $PrP^C$ and Thy-1 are present in different DRMs [30] (see above). Moreover, in resting human T cells $PrP^C$ was predominantly localized to non-raft regions of the plasma membrane but upon antibody cross-linking was recruited into rafts where it activated the Src tyrosine kinase [77]. This mechanism would be consistent with the emerging view that rafts may normally be relatively small, containing one or only a few protein molecules preferentially surrounded by a small group of lipids, so-called 'lipid shells' [78]. Upon cell stimulation or antibody cross-linking these small domains fuse together to form larger platforms [79]. The protein composition, and hence biological properties, of these larger, induced platforms will depend on which particular domains fuse in response to a particular stimulus.

## Role of lipid rafts in the conversion of $PrP^C$ to $PrP^{Sc}$

Lipid rafts appear to play a critical role in the conformational conversion of $PrP^C$ to $PrP^{Sc}$ [80]. Depletion of cellular cholesterol with the HMG CoA reductase inhibitor lovastatin, the polyene antibiotic filipin or the squalene synthase inhibitor squalestatin, diminished the formation of $PrP^{Sc}$ [17,43,81] and removing $PrP^C$ from rafts by replacing its GPI anchor addition signal with the transmembrane and cytosolic domains from non-raft proteins, prevented the formation of $PrP^{Sc}$ [17,39]. $PrP^{Sc}$ is present in DRMs, although it did not cofractionate with $PrP^C$ on a Nycodenz density gradient suggesting that the two forms of the protein may be located in distinct DRMs of different densities and composition [18,19].

The conversion of $PrP^C$-like proteinase K-sensitive PrP (PrP-sen) to $PrP^{Sc}$-like proteinase K-resistant PrP (PrP-res) by exogenous PrP-res in a cell-free system has provided further insight into the role of rafts and the GPI anchor on $PrP^C$ in the conversion process. In this cell-free conversion assay, raft-bound PrP-sen resisted conversion to PrP-res unless the PrP-sen was released from rafts by phospholipase C digestion or the PrP-sen was inserted into contiguous membranes with the source PrP-res by polyethylene glycol fusion [26,82]. Somewhat surprisingly, removal of the GPI anchor from the PrP-sen led to its conversion to PrP-res without phospholipase or polyethylene glycol treatment. These observations led Caughey and coworkers [82] to conclude that generation of new $PrP^{Sc}$ during TSE infection requires: (i) removal of $PrP^C$ from target cells, (ii) an exchange of membranes between cells, or (iii) insertion of incoming $PrP^{Sc}$ into the raft domains of recipient cells. A more recent study using a modified version of the protein

misfolding cyclic amplification (PMCA) cell-free conversion assay also observed that membrane attachment is not required for PrP-sen to convert efficiently into PrP-res [83]. However, incubation of N2a cells with filipin, which induces the shedding of $PrP^C$, inhibited the formation of PrP-res raising the possibility that the release of $PrP^C$ from the plasma membrane may decrease the amount of $PrP^C$ available for conversion to $PrP^{Sc}$ [43].

*In vitro* structural studies in model raft membranes using recombinant Syrian hamster PrP (residues 90–231) showed that when refolded into 'α-PrP', a conformation containing predominantly α-helical secondary structure, the α-helix content increased on binding of the protein to raft-like membranes and that this form of the protein was protected from aggregation and fibrillization [84]. In contrast, refolded 'β-PrP', which has predominantly β-sheet secondary structure, was converted into amyloid fibrils on binding to raft-like membranes [85]. α-PrP was found to bind, with decreasing affinity, to palmitoyloleoylphosphatidylglycerol, dipalmitoylphosphatidylcholine and raft-like membranes, suggesting that the majority of PrP may exist preferentially outside of lipid rafts in the steady state [86]. However, caution should be taken when interpreting the results of studies using recombinant bacterially-expressed truncated forms of PrP that do not contain any of the mammalian cell post-tanslational modifications, including the GPI anchor.

Evidence that $PrP^C$ and $PrP^{Sc}$ need to be membrane-bound in order for conversion to occur came from a study in which infected Scrapie mouse brain (SMB) cells were co-cultured with uninfected target cells [87]. This study showed that cell contact was required for efficient conversion of $PrP^C$ on the target cells. Both $PrP^C$ and $PrP^{Sc}$ are released into the extracellular environment in association with exosomes, membranous vesicles that are secreted upon fusion of multivesicular endosomes with the plasma membrane [88]. As these exosomes bearing $PrP^{Sc}$ are infectious, they may represent a mechanism by which $PrP^{Sc}$ is exchanged between membranes and enable the spread of $PrP^{Sc}$ throughout the organism [88]. As shown for other GPI-anchored proteins, $PrP^C$ can also be transferred efficiently between cells, a process that is dependent on an intact GPI anchor [89].

A recent study using scrapie-infected transgenic mice expressing PrP lacking the GPI anchor, reported that abnormal PrP-res was deposited as amyloid plaques, rather then the usual nonamyloid form of PrP-res, which, although able to induce brain damage reminiscent of Alzheimer's disease, had minimal clinical manifestations [90]. In addition, combined expression of anchorless and wild-type PrP produced accelerated clinical scrapie [90]. These observations imply that the GPI anchor plays a critical role in the presentation and progression of prion disease, and that there is a link between the cell surface topology of $PrP^{Sc}$ and prion disease pathogenesis. By disengaging $PrP^C$ from the cell surface, Chesebro and colleagues effectively uncoupled clinical disease from $PrP^{Sc}$ formation [91]. The available data can be accommodated in the following model (Figure 3). For conversion and disease progression, the incoming $PrP^{Sc}$ has to be inserted into a contiguous membrane with $PrP^C$. Rafts provide a favourable environment for conformational conversion of $PrP^C$ to $PrP^{Sc}$, by concentrating the proteins within confined regions of the membrane, by aligning them in a way that promotes their interaction or by providing accessory molecules that are required for formation of $PrP^{Sc}$ [20,80]. The conversion to $PrP^{Sc}$ may affect signalling events involving $PrP^C$, leading to the removal of neuroprotective signals and/or to the initiation of neurotoxic signals [91] (Figure 3). Soluble $PrP^C$ devoid of its membrane attachment can be converted into $PrP^{Sc}$, possibly more efficiently than membrane-bound $PrP^C$, but lack of membrane-anchorage prevents

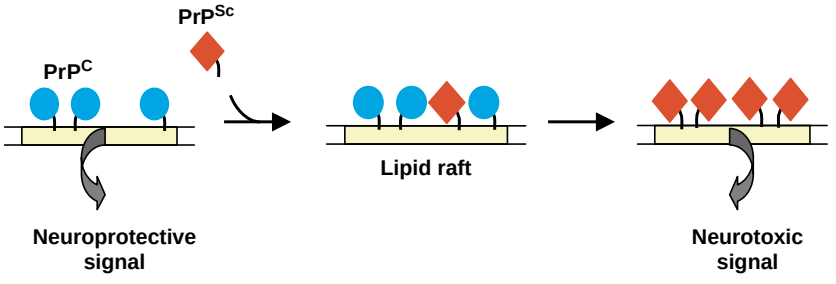


Figure 3. A model for the role of lipid rafts in the conversion of $PrP^C$ to $PrP^{Sc}$ and subsequent disease progression. $PrP^C$ is attached to the membrane via its GPI anchor and upon clustering in lipid rafts transduces neuroprotective signals into the cell. Infectious $PrP^{Sc}$ inserts into the target cell membrane alongside the $PrP^C$ in the rafts. Conversion of the $PrP^C$ to $PrP^{Sc}$ may affect signalling events involving $PrP^C$, leading to neurotoxicity and cell death. Reproduced with permission from [91]. This figure is reproduced in colour in *Molecular Membrane Biology* online.

disease progression as it fails to disrupt signal transduction processes.

Interestingly, during scrapie infection, although PrP-res accumulated in DRMs from retinas and optic nerves of mice, the $PrP^C$ interacting proteins caveolin-1 and synaptophysin were redistributed to detergent-soluble fractions [92]. This alteration of the distribution of caveolin-1, synaptophysin and possibly other cytoplasmic signalling proteins upon prion replication could provide a mechanism by which signal transduction processes emanating from PrP on the surface of infected cells could be altered. Such alterations in signal transduction from $PrP^C$ may in turn contribute to disease pathogenesis. However, it remains to be seen what effect prion infection and the conversion of $PrP^C$ to $PrP^{Sc}$ has on serotoninergic functions, neurite outgrowth and other $PrP^C$-dependent functions.

## Conclusions

It is clear that lipid rafts play a key role in both the normal and the pathological functioning of PrP. The association of $PrP^C$ with rafts is not a static event but should be considered as a dynamic process, with the protein exiting and entering rafts, and with the rafts containing $PrP^C$ fusing with other domains. Although $PrP^C$ probably interacts with rafts primarily via its GPI anchor, a region in its N-terminus is also important for regulating its raft association through interaction with an as yet unidentified raft component. Transmembrane adaptor proteins, both within rafts and in non-raft regions of the membrane, play important roles in the signal transduction and endocytosis of $PrP^C$, respectively. Signal transduction from $PrP^C$ may be enhanced by the clustering of small raft domains containing $PrP^C$, with other domains containing particular signalling proteins, although the precise molecular mechanisms by which clustering of $PrP^C$ triggers a variety of cellular responses requires further work. Finally, rafts are critically involved in the conformational conversion of $PrP^C$ to $PrP^{Sc}$, by providing a favourable environment for this process to occur and enabling disease progression. Whether conversion to $PrP^{Sc}$ alters signal transduction processes emanating from $PrP^C$ in rafts remains to be clarified.

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## References

[1] Zahn R, Liu A, Luhrs T, Riek R, von Schroetter C, Lopez Garcia F, Billeter M, Calzolai L, Wider G, Wuthrich K. NMR solution structure of the human prion protein. Proc Natl Acad Sci USA 2000;97:145–150.

[2] Prusiner SB. Prions. Proc Natl Acad Sci USA 1998;95: 13363–13383.

[3] Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell 1993;73:1339–1347.

[4] Milhavet O, Lehmann S. Oxidative stress and the prion protein in transmissible spongiform encephalopathies. Brain Res Brain Res Rev 2002;38:328–339.

[5] Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM, Kellermann O. Signal transduction through prion protein. Science 2000;289:1925–1928.

[6] Pauly PC, Harris DA. Copper stimulates endocytosis of the prion protein. J Biol Chem 1998;273:33107–33110.

[7] Watt NT, Hooper NM. The prion protein and neuronal zinc homeostasis. Trends Biochem Sci 2003;28:406–410.

[8] Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR, Jefferys JG. Prion protein is necessary for normal synaptic function. Nature 1994;370:295–297.

[9] Lichtenberg D, Goni FM, Heerklotz H. Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem Sci 2005;30:430–436.

[10] Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 1987;51:229–240.

[11] Stahl N, Baldwin MA, Hecker R, Pan K-M, Burlingame AL, Prusiner SB. Glycosylinositol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. Biochemistry 1992;31:5043–5053.

[12] Brewis IA, Ferguson MAJ, Mehlert A, Turner AJ, Hooper NM. Structures of the glycosyl-phosphatidylinositol anchors of porcine and human membrane dipeptidase. Interspecies comparison of the glycan core structures and further structural studies on the porcine anchor. J Biol Chem 1995;270:22946–22956.

[13] Meri S, Lehto T, Sutton CW, Tyynela J, Baumann M. Structural composition and functional characterization of soluble CD59: heterogeneity of the oligosaccharide and glycophosphoinositol (GPI) anchor revealed by laser-desorption mass spectrometric analysis. Biochem J 1996;316: 923–935.

[14] Brown DA, London E. Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 1998;14:111–136.

[15] Brown DA, London E. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J Biol Chem 2000;275:17221–17224.

[16] Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 1992;68:533–544.

[17] Taraboulos A, Scott M, Semenov A, Avraham D, Laszlo L, Prusiner SB. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J Cell Biol 1995; 129:121–132.

[18] Vey M, Pilkuhn S, Wille H, Nixon R, DeArmond SJ, Smart EJ, Anderson RGW, Taraboulos A, Prusiner SB. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci USA 1996;93:14945–14949.

[19] Naslavsky N, Stein R, Yanai A, Friedlander G, Taraboulos A. Characterization of detergent-insoluble complexes con-

taining the cellular prion protein and its scrapie isoform. J Biol Chem 1997;272:6324–6331.
[20] Sarnataro D, Campana V, Paladino S, Stornaiuolo M, Nitsch L, Zurzolo C. PrP(C) association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. Mol Biol Cell 2004;15:4031–4042.
[21] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569–572.
[22] Pang S, Urquhart P, Hooper NM. N-Glycans, not the GPI anchor, mediate the apical targeting of a naturally glycosylated, GPI-anchored protein in polarised epithelial cells. J Cell Sci 2004;117:5079–5086.
[23] Sarnataro D, Paladino S, Campana V, Grassi J, Nitsch L, Zurzolo C. PrPC is sorted to the basolateral membrane of epithelial cells independently of its association with rafts. Traffic 2002;3:810–821.
[24] Uelhoff A, Tatzelt J, Aguzzi A, Winklhofer KF, Haass C. A Pathogenic PrP Mutation and Doppel Interfere with Polarized Sorting of the Prion Protein. J Biol Chem 2005;280: 5137–5140.
[25] Walmsley AR, Zeng F, Hooper NM. The N-terminal region of the prion protein ectodomain contains a lipid raft targeting determinant. J Biol Chem 2003;278:37241–37248.
[26] Baron GS, Caughey B. Effect of glycosylphosphatidylinositol anchor-dependent and -independent prion protein association with model raft membranes on conversion to the protease-resistant isoform. J Biol Chem 2003;278:14883–14892.
[27] Lee KS, Linden R, Prado MA, Brentani RR, Martins VR. Towards cellular receptors for prions. Rev Med Virol 2003;13:399–408.
[28] Mahfoud R, Garmy N, Maresca M, Yahi N, Puigserver A, Fantini J. Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. J Biol Chem 2002;277:11292–11296.
[29] Rosenmann H, Talmor G, Halimi M, Yanai A, Gabizon R, Meiner Z. Prion protein with an E200K mutation displays properties similar to those of the cellular isoform PrP(C). J Neurochem 2001;76:1654–1662.
[30] Madore N, Smith KL, Graham CH, Jen A, Brady K, Hall S, Morris R. Functionally different GPI proteins are organised in different domains on the neuronal surface. EMBO J 1999;19:6917–6926.
[31] Brugger B, Graham C, Leibrecht I, Mombelli E, Jen A, Wieland F, Morris R. The membrane domains occupied by glycosylphosphatidylinositol-anchored prion protein and Thy-1 differ in lipid composition. J Biol Chem 2004;279: 7530–7536.
[32] Moore RC, Lee IY, Silverman GL, Harrison PM, Strome R, Heinrich C, Karunaratne A, Pasternak SH, Chishti MA, Liang Y, Mastrangelo P, Wang K, Smit AF, Katamine S, Carlson GA, Cohen FE, Prusiner SB, Melton DW, Tremblay P, Hood LE, Westaway D. Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel. J Mol Biol 1999;292:797–817.
[33] Shaked Y, Hijazi N, Gabizon R. Doppel and PrP(C) do not share the same membrane microenvironment. FEBS Lett 2002;530:85–88.
[34] Massimino ML, Ballarin C, Bertoli A, Casonato S, Genovesi S, Negro A, Sorgato MC. Human Doppel and prion protein share common membrane microdomains and internalization pathways. Int J Biochem Cell Biol 2004;36:2016–2031.
[35] Pike LJ. Lipid rafts: heterogeneity on the high seas. Biochem J 2004;378:281–292.
[36] Shyng S-L, Huber MT, Harris DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J Biol Chem 1993;268: 15922–15928.
[37] Shyng SL, Heuser JE, Harris DA. A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. J Cell Biol 1994;125:1239–1250.
[38] Harmey JH, Doyle D, Brown V, Rogers MS. The cellular isoform of the prion protein, PrPc, is associated with caveolae in mouse neuroblastoma (N2a) cells. Biochem Biophys Res Commun 1995;210:753–759.
[39] Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scapie isoform. Proc Natl Acad Sci USA 1997;94:2333–2338.
[40] Anderson RGW. The caveolae membrane system. Annu Rev Biochem 1998;67:199–225.
[41] Parpal S, Karlsson M, Thorn H, Stralfors P. Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J Biol Chem 2001;276:9670–9678.
[42] Peters PJ, Mironov A, Jr,, Peretz D, van Donselaar E, Leclerc E, Erpel S, DeArmond SJ, Burton DR, Williamson RA, Vey M, Prusiner SB. Trafficking of prion proteins through a caveolae-mediated endosomal pathway. J Cell Biol 2003;162:703–717.
[43] Marella M, Lehmann S, Grassi J, Chabry J. Filipin prevents pathological prion protein accumulation by reducing endocytosis and inducing cellular PrP release. J Biol Chem 2002;277:25457–25464.
[44] Parkin ET, Watt NT, Turner AJ, Hooper NM. Dual mechanisms for shedding of the cellular prion protein. J Biol Chem 2004;279:11170–11178.
[45] Gorodinsky A, Harris DA. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. J Cell Biol 1995;129:619–627.
[46] Parkin ET, Hussain I, Turner AJ, Hooper NM. The amyloid precursor protein is not enriched in caveolae-like, detergent-insoluble membrane microdomains. J Neurochem 1997; 69:2179–2188.
[47] Kirchhausen T. Clathrin. Annu Rev Biochem 2000;69:699–727.
[48] Harris DA. Cellular biology of prion diseases. Clin Microbiol Rev 1999;12:429–444.
[49] Conese M, Nykjaer A, Petersen CM, Cremona O, Pardi R, Andreasen PA, Gliemann J, Christensen EI, Blasi F. Alpha-2 Macroglobulin receptor/Ldl receptor-related protein(Lrp)-dependent internalization of the urokinase receptor. J Cell Biol 1995;131:1609–1622.
[50] Sunyach C, Jen A, Deng J, Fitzgerald KT, Frobert Y, Grassi J, McCaffrey MW, Morris R. The mechanism of internalization of glycosylphosphatidylinositol-anchored prion protein. EMBO J 2003;22:3591–3601.
[51] Nichols B. Caveosomes and endocytosis of lipid rafts. J Cell Sci 2003;116:4707–4714.
[52] Magalhaes AC, Silva JA, Lee KS, Martins VR, Prado VF, Ferguson SS, Gomez MV, Brentani RR, Prado MA. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J Biol Chem 2002;277:33311–33318.
[53] Stockel J, Safar J, Wallacen AC, Cohen FE, Prusiner SB. Prion protein selectively binds copper(II) ions. Biochemistry 1998;37:7185–7193.
[54] Viles JH, Cohen FE, Prusiner SB, Goodin DB, Wright PE, Dyson HJ. Copper binding to the prion protein: structural

implications of four identical cooperative binding sites. Proc Natl Acad Sci USA 1999;96:2042–2047.

[55] Perera WSS, Hooper NM. Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat region. Curr Biol 2001;11:519–523.

[56] Taylor DR, Watt NT, Perera WSS, Hooper NM. Assigning functions to distinct regions of the N-terminus of the prion protein that are involved in its copper-stimulated, clathrin-dependent endocytosis. J Cell Sci 2005;118:5141–5153.

[57] Shyng S-L, Moulder KL, Lesko A, Harris DA. The N-terminal domain of a glycolipid-anchored prion protein is essential for its endocytosis via clathrin-coated pits. J Biol Chem 1995;270:14793–14800.

[58] Kiachopoulos S, Heske J, Tatzelt J, Winklhofer KF. Misfolding of the prion protein at the plasma membrane induces endocytosis, intracellular retention and degradation. Traffic 2004;5:426–436.

[59] Nunziante M, Gilch S, Schatzl HM. Essential role of the prion protein N terminus in subcellular trafficking and half-life of cellular prion protein. J Biol Chem 2003;278:3726–3734.

[60] Zahn R. The octapeptide repeats in mammalian prion protein constitute a pH-dependent folding and aggregation site. J Mol Biol 2003;334:477–488.

[61] Tsui-Pierchala BA, Encinas M, Milbrandt J, Johnson EM, Jr. Lipid rafts in neuronal signaling and function. Trends Neurosci 2002;25:412–417.

[62] Horejsi V, Drbal K, Cebecauer M, Cerny J, Brdicka T, Angelisova P, Stockinger H. GPI-microdomains: a role in signalling via immunoreceptors. Immunology Today 1999;20:356–361.

[63] Mouillet-Richard S, Pietri M, Schneider B, Vidal C, Mutel V, Launay J-M, Kellermann O. Modulation of serotonergic receptor signaling and cross-talk by prion protein. J Biol Chem 2005;280:4592–4601.

[64] Spielhaupter C, Schatzl HM. PrPC Directly interacts with proteins involved in signaling pathways. J Biol Chem 2001;276:44604–44612.

[65] Mattei V, Garofalo T, Misasi R, Gizzi C, Mascellino MT, Dolo V, Pontieri GM, Sorice M, Pavan A. Association of cellular prion protein with gangliosides in plasma membrane microdomains of neural and lymphocytic cells. Neurochem Res 2002;27:743–749.

[66] Mattei V, Garofalo T, Misasi R, Circella A, Manganelli V, Lucania G, Pavan A, Sorice M. Prion protein is a component of the multimolecular signaling complex involved in T cell activation. FEBS Lett 2004;560:14–18.

[67] Stuermer CA, Langhorst MF, Wiechers MF, Legler DF, Von Hanwehr SH, Guse AH, Plattner H. PrPc capping in T cells promotes its association with the lipid raft proteins reggie-1 and reggie-2 and leads to signal transduction. FASEB J 2004;18:1731–1733.

[68] Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, de Oliveira E, Jachieri SG, Burlingame A, Huang L, Linden R, Brentani RR, Martins VR. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. EMBO J 2002;21:3307–3316.

[69] Chiarini LB, Freitas AR, Zanata SM, Brentani RR, Martins VR, Linden R. Cellular prion protein transduces neuroprotective signals. EMBO J 2002;21:3317–3326.

[70] Santuccione A, Sytnyk V, Leshchyns'ka I, Schachner M. Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. J Cell Biol 2005;169:341–354.

[71] Monnet C, Gavard J, Mege RM, Sobel A. Clustering of cellular prion protein induces ERK1/2 and stathmin phosphorylation in GT1-7 neuronal cells. FEBS Lett 2004; 576:114–118.

[72] Mineo C, James GL, Smart EJ, Anderson RGW. Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. J Biol Chem 1996;271: 11930–11935.

[73] Mineo C, Gill GN, Anderson RG. Regulated migration of epidermal growth factor receptor from caveolae. J Biol Chem 1999;274:30636–30643.

[74] Vassallo N, Herms J, Behrens C, Krebs B, Saeki K, Onodera T, Windl O, Kretzschmar HA. Activation of phosphatidylinositol 3-kinase by cellular prion protein and its role in cell survival. Biochem Biophys Res Commun 2005;332:75–82.

[75] Solforosi L, Criado JR, McGavern DB, Wirz S, Sanchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, Masliah E, Gilden D, Oldstone MB, Conti B, Williamson RA. Cross-linking cellular prion protein triggers neuronal apoptosis in vivo. Science 2004;303:1514–1516.

[76] Botto L, Masserini M, Cassetti A, Palestini P. Immunoseparation of prion protein-enriched domains from other detergent-resistant membrane fractions, isolated from neuronal cells. FEBS Lett 2004;557:143–147.

[77] Hugel B, Martinez MC, Kunzelmann C, Blattler T, Aguzzi A, Freyssinet JM. Modulation of signal transduction through the cellular prion protein is linked to its incorporation in lipid rafts. Cell Mol Life Sci 2004;61:2998–3007.

[78] Anderson RG, Jacobson K. A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. Science 2002;296:1821–1825.

[79] Mayor S, Rao M. Rafts: scale-dependent, active lipid organization at the cell surface. Traffic 2004;5:231–240.

[80] Campana V, Sarnataro D, Zurzolo C. The highways and byways of prion protein trafficking. Trends Cell Biol 2005;15:102–111.

[81] Bate C, Salmona M, Diomede L, Williams A. Squalestatin cures prion-infected neurons and protects against prion neurotoxicity. J Biol Chem 2004;279:14983–14990.

[82] Baron GS, Wehrly K, Dorward DW, Chesebro B, Caughey B. Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP(Sc)) into contiguous membranes. EMBO J 2002;21: 1031–1040.

[83] Nishina K, Deleault NR, Lucassen RW, Supattapone S. In vitro prion protein conversion in detergent-solubilized membranes. Biochemistry 2004;43:2613–2621.

[84] Sanghera N, Pinheiro TJ. Binding of prion protein to lipid membranes and implications for prion conversion. J Mol Biol 2002;315:1241–1256.

[85] Kazlauskaite J, Sanghera N, Sylvester I, Venien-Bryan C, Pinheiro TJ. Structural changes of the prion protein in lipid membranes leading to aggregation and fibrillization. Biochemistry 2003;42:3295–3304.

[86] Critchley P, Kazlauskaite J, Eason R, Pinheiro TJ. Binding of prion proteins to lipid membranes. Biochem Biophys Res Commun 2004;313:559–567.

[87] Kanu N, Imokawa Y, Drechsel DN, Williamson RA, Birkett CR, Bostock CJ, Brockes JP. Transfer of scrapie prion infectivity by cell contact in culture. Curr Biol 2002;12: 523–530.

[88] Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H, Raposo G. Cells release prions in association with exosomes. Proc Natl Acad Sci USA 2004;101:9683–9688.

[89] Liu T, Li R, Pan T, Liu D, Petersen RB, Wong BS, Gambetti P, Sy MS. Intercellular transfer of the cellular prion protein. J Biol Chem 2002;277:47671–47678.

[90] Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 2005;308:1435–1439.

[91] Aguzzi A. Cell biology. Prion toxicity: all sail and no anchor. Science 2005;308:1420–1421.

[92] Russelakis-Carneiro M, Hetz C, Maundrell K, Soto C. Prion replication alters the distribution of synaptophysin and caveolin 1 in neuronal lipid rafts. Am J Pathol 2004;165: 1839–1848.

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