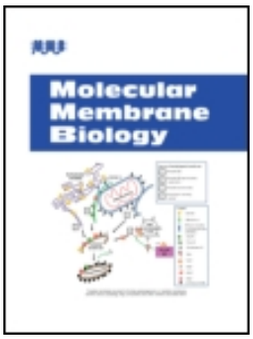

Molecular Membrane Biology

ISSN: 0968-7688 (Print) 1464-5203 (Online) Journal homepage: informahealthcare.com/journals/imbc20

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To cite this article: David R. Taylor & Nigel M. Hooper (2006) The prion protein and lipid rafts (Review), Molecular Membrane Biology, 23:1, 89-99, DOI: 10.1080/09687860500449994

To link to this article: https://doi.org/10.1080/09687860500449994

Molecular Membrane Biology, January–February 2006; 23(1): 89–99

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The prion protein and lipid rafts (Review)

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(Received 15 September 2005 and in revised form 27 October 2005)

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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ISSN 0968-7688 print/ISSN 1464-5203 online
DOI: 10.1080/09687860500449994

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].

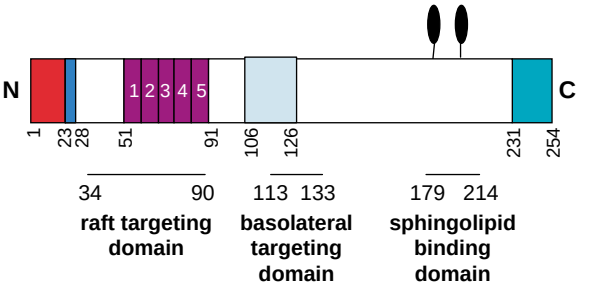

Figure 1. Schematic of PrP^C. Nascent murine PrP^C is a protein of 254 amino acids. On translocation into the ER the N-terminal signal peptide (chequered box; red online) and the C-terminal GPI anchor addition signal (wavy line box; green online) are removed and the latter replaced with a GPI anchor. 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,

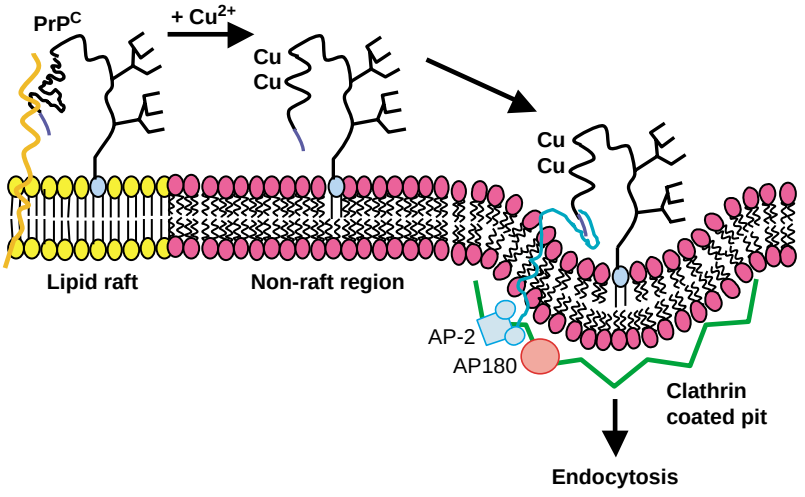

Figure 2. PrP^C is localized in rafts but translocates out of them before being endocytosed through clathrin-coated pits. PrP^C is attached to the exoplasmic leaflet of the plasma membrane via its GPI anchor and localises within detergent-insoluble rafts through interactions between its N-terminal region (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

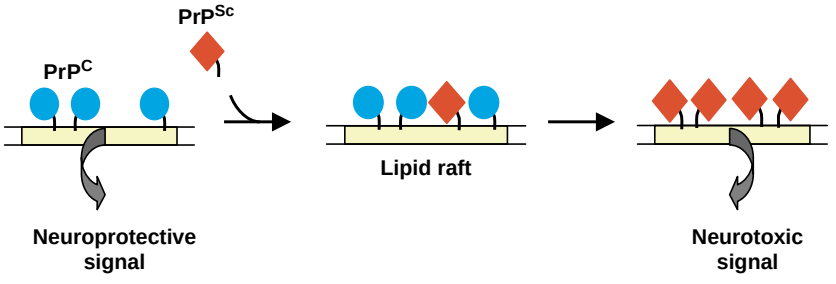

Figure 3. A model for the role of lipid rafts in the conversion of PrP^C to PrP^{Sc} and subsequent disease progression. PrP^C is attached to the membrane via its GPI anchor and upon clustering in lipid rafts transduces neuroprotective signals into the cell. Infectious PrP^{Sc} inserts into the target 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.

Acknowledgements

DRT was in receipt of a studentship from the Medical Research Council of Great Britain. Work on PrP in our laboratory is supported by grants from the Medical Research Council.

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This paper was first published online on prEview on 27 January 2006.