[Frontiers in Bioscience 16, 151-168, January 1, 2011]

The role of lipid rafts in prion protein biology

Victoria Lewis [1,2], Nigel M. Hooper[2]

[1]Department of Pathology, University of Melbourne, Victoria 3010, Australia, [2]Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

TABLE OF CONTENTS

1. ABSTRACT

The conformational conversion of the cellular prion protein, PrP^C, to the misfolded isoform PrP^{Sc} is the central pathogenic event in the uniquely transmissible neurodegenerative prion diseases. As both PrP^C and PrP^{Sc} are associated with membranes, the nature of the membrane microenvironment may well play a significant role in both the conformational conversion process as well as the normal functions of PrP^C. Within the membrane are various microdomains, areas of distinct lipid and protein composition, the best studied of which are the cholesterol- and sphingolipid-rich lipid rafts. These domains are characterized biochemically by their relative resistance to solubilization in certain detergents at low temperature. In this article we review the evidence for the involvement of lipid rafts in the localization and trafficking of PrP^C, in the cellular signaling, neuroprotective and metal binding functions of PrP^C, and as sites for the conversion of PrP^C to PrP^{Sc} and in cell-to-cell prion transmission.

2. INTRODUCTION

The cellular prion protein, PrP^C, is a ubiquitously transcribed and expressed glycoprotein, found most abundantly in neuronal tissue (1-3), and localized predominantly at the cell surface (4). The primary physiological function of PrP^C has not yet been defined, although interestingly prion proteins from different mammalian species show high sequence identity (5), with many features conserved (6-10) (see Figure 1), implying the evolutionary and biological importance of this protein. Experimental evidence, particularly that pertaining to structural features of the prion protein, its subcellular localization and various binding partners, points to possible roles in cell signaling, neuroprotection and copper transport, amongst others. These aspects of prion protein biology and the significance of the localization of PrP^C to cholesterol-rich lipid rafts are reviewed in detail below.

Prion diseases are a group of fatal neurodegenerative diseases that occur naturally in humans

and various other mammalian species. Prion diseases are unique, in that they can have either a sporadic, inherited or acquired aetiology, and the transmissible causative agent appears to be a misfolded conformer (named PrP^{Sc}) of the normal cellular prion protein (11, 12). Prion diseases are transmissible both in nature and experimentally to other animals and to cultured cells *in vitro*, and are often characterized by the accumulation of a partially protease resistant prion protein conformer, known as PrP^{res}. In the context of prion infection, studies have confirmed the presence of PrP^{C} to be an absolute requirement for the development of disease (13-15). However, the role of the specific subcellular localization of each of the prion protein isoforms in prion diseases has not been conclusively established, and the possible implications of this are discussed herein.

3. LIPID RAFTS

The plasma membrane (PM) surrounding mammalian cells is a continuous lipid bilayer composed of hundreds of different sphingolipids, phospholipids and cholesterol. It provides a differentially permeable barrier for regulated passive and active transport of molecules into and out of the cell. Importantly, cellular lipid membranes are structurally dynamic, continually allowing adaptation in response to specific biological processes (16). Lipid rafts, also known as membrane rafts, are specific membrane microdomains composed primarily of liquid ordered cholesterol and sphingolipid, which laterally assemble to form small platforms of varying size that "float" within the more liquid disordered phospholipid-rich regions of the PM (17, 18). Rafts may first assemble in the Golgi complex after synthesis of sphingolipids (19), and can be found in other cellular membranes, including those of the endocytic pathway (20, 21).

3.1. Detergent resistant membranes

Cellular membranes, when extracted in non-ionic detergent buffers at low temperatures (e.g. 0-4^{o}C) can be fractionated into detergent soluble and insoluble components. Cellular phospholipids comprise the detergent soluble fraction, whereas the majority of sphingolipids and some cholesterol (i.e. the lipid components of rafts) remain in the insoluble fraction (22); thus leading to the term 'detergent resistant membranes' (DRMs). Extracted DRMs are also characterized by the capacity to 'float' to buoyant fractions when subject to density gradient fractionation. Interestingly, depending on the detergent used for DRM extraction, there can be substantial differences in the protein and lipid content of the isolated raft fraction (23). Rafts are therefore characterized biochemically by their resistance to solubilization in non-ionic detergents at low temperatures, although the end result of DRM extraction does not necessarily reflect the organization of membranes and the protein or lipid association *in vivo*.

The physical nature of rafts, namely the enrichment of sphingolipids with their high melting temperatures and saturated acyl chains when mixed with cholesterol, facilitates raft association with a specific group of membrane proteins; that is, proteins which prefer an ordered environment such as the cholesterol-rich liquid-ordered membrane phase (24). Glycosylphosphatidylinositol (GPI)-anchored proteins, some transmembrane proteins, and proteins acylated by myristate or palmitate all localize to rafts and can be recovered in DRMs (24-26).

3.2. Cellular rafts

Despite countless studies isolating DRMs from buoyant fractions of fractionated cells, there has been some debate as to whether membrane rafts actually exist in cells. That is, the presence of DRMs in fractionated cells is not absolute proof of *in vivo* raft domains. However, the decades of biochemical, microscopy, biophysical, cellular biological and proteomics studies have now provided enough evidence such that it is generally agreed that rafts are heterogeneous in nature, may exist as relatively stable or transient domains, and are very small, only 10-200nm in size (27). It also appears that small membrane rafts, upon stimulation or antibody cross-linking, may cluster together to form larger platforms, playing a role in bringing together proteins, for example those that are functionally related, so they can interact on the cell surface (18). Further, it is hypothesized that this clustering is specifically activated through protein-protein or protein-lipid interactions (28). One example of this clustering occurs in caveolae, a subset of rafts characterized as small surface invaginations or dimples which form when the raft associated protein caveolin is brought together by raft clustering, thus allowing polymerization of the caveolin, driving the dimpling process and resulting in endocytosis (18).

Due to the types of proteins identified as associated with DRMs, the formation of membrane rafts has been implicated in numerous cellular functions including signal transduction (29), membrane sorting and cell polarization (30), and cell fusion (31). As mentioned above, specific rafts may play a role in endocytic sorting. Not only does endocytosis occur through the caveolae subset of rafts, but in cells devoid of caveolin and morphological caveolae, there is still raft dependent endocytosis (reviewed in (32)). Further, there is evidence that for some proteins, endocytosis mediated through clathrin-coated pits may also be dependent on lipid-rafts (33-35). Lipid rafts also function in cellular signaling, whereby raft localization and/or clustering concentrates receptors to an environment of ligands, kinases and phosphatases which act in specific signaling cascades. Various proteins and receptors involved in cellular signaling have been localized to lipid rafts, and disruption of rafts, for example through cholesterol depletion or sequestration, impacts on signaling pathways (29). Rafts appear to be particularly important in immune (36) and central nervous system (37) cell signaling and sorting.

4. PRION PROTEIN LOCALIZATION AND TRAFFICKING

Following synthesis, immature PrP^{C} undergoes a number of post-translational modifications, and follows a cellular pathway similar to other cell surface or secreted proteins (38) (see Figure 2). More specifically, following synthesis into the endoplasmic reticulum (ER), PrP^{C}

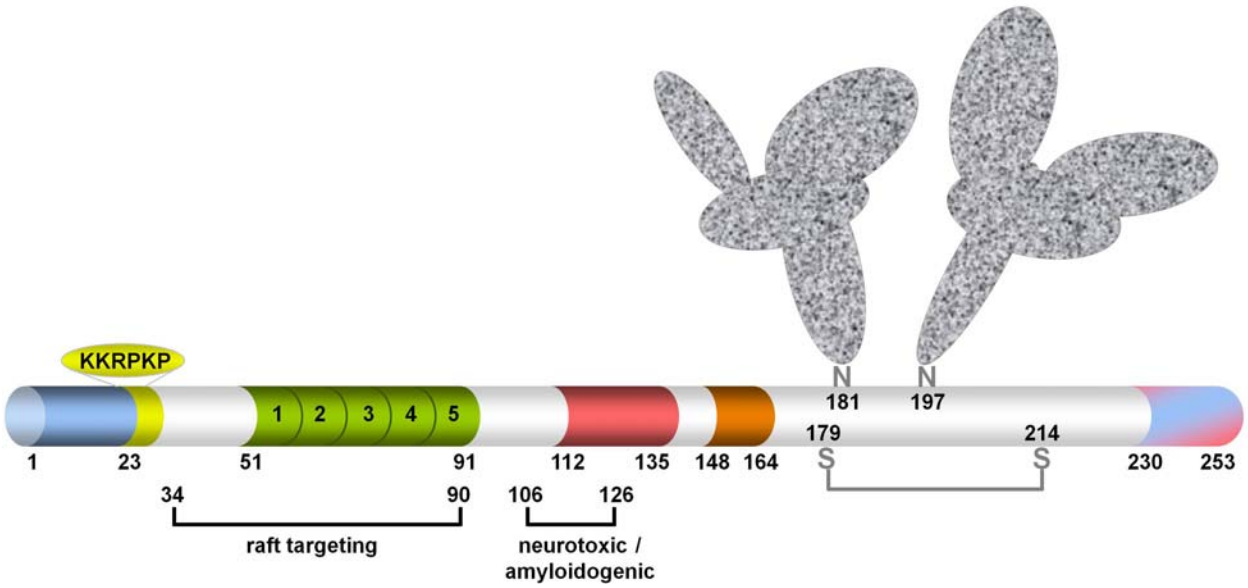

Figure 1. Mammalian PrP^C conserved features. Schematic representation of the mammalian prion protein (human PrP^C numbering); conserved N-terminal (blue) and C-terminal glycosylphosphatidylinositol anchor (blue and pink) signal sequences, six residue positively charged polybasic region (yellow), copper binding octapeptide repeat region (green), hydrophobic core (red), aromatic palindrome YYR motif (orange), asparagine-linked glycosylation sites (grey globular shapes), and an intramolecular disulphide bond (S-S) are highlighted. The N-terminal lipid-raft targeting domain (residues 34-90) and central neurotoxic / amyloidogenic domain (residues 106-126) are also indicated.

undergoes cleavage by signal peptidase to remove the N-terminal signal peptide, partial N-linked glycosylation with addition of simple sugars at two asparagine (N) residues in the C-terminal half of the protein (39), formation of a single intramolecular disulphide bond (40), and cleavage of the C-terminal signal peptide resulting in attachment of a GPI anchor (41, 42) (see Figure 1). PrP^C is then directed to the Golgi where the glycans are modified to more complex oligosaccharides (43). The majority of this mature PrP^C continues to the cell surface, retaining its GPI anchor. The specific membrane environment where PrP^C resides at the cell surface is likely to be important, not only in the normal biological functions of the protein, but also in the prion disease process, as it may influence any direct interactions of PrP^C with the disease associated isoform, PrP^{Sc}.

4.1. GPI anchor attachment to lipid rafts

When the C-terminus of a protein is lipidated via phosphoethanolamine attaching to an oligosaccharide-linked phosphatidylinositol lipid, it is said to possess a GPI anchor (44). Early studies of the cellular and disease associated prion proteins determined both to be attached to cellular membranes via a GPI anchor, which was sensitive to cleavage by phosphatidylinositol-specific phospholipase C (PIPLC) (4). A later study identified serine 231 as the site of phosphoethanolamine attachment, which links to a core tetrasaccharide, with the glucosamine component of the core linking to the headgroup of phosphatidylinositol (41) .

Like many other GPI-anchored proteins, PrP^C has been shown to associate at steady state levels predominantly with DRMs including lipid rafts and caveolae-like domains (CLD), the latter of which have a lipid composition similar to caveolae but lack the caveolin-1 protein (45-48). Due to the detection of mature PrP^C in these DRMs (i.e. after the N-linked glycans have been modified to more complex oligosaccharides), the localization of PrP^C to rafts is thought to occur as the protein traverses the Golgi. Additionally, there is evidence that immature PrP^C may localize to cholesterol-rich DRMs earlier in the secretory pathway, in the ER (49). The authors suggested that this may have implications for PrP^C biosynthesis and protein folding, as depletion of cholesterol slowed the maturation of PrP^C and led to an association (as assessed by co-immunoprecipitation) with various ER chaperones (49). However, whether this early association of PrP^C with DRMs was exclusively via a GPI-attachment or through another means, for example N-terminal targeting (see below), was not established. Importantly, PIPLC treatment of cultured cells results in the accumulation and detection of PrP^C in culture medium with a simultaneous loss of cell associated PrP^C (4), indicating that a large proportion of PrP^C is localized via its GPI anchor at the cell surface. Further, in some cell lines which express caveolin and have morphological caveolae, PrP^C has been detected at the cell surface localized within these raft domains (50-52).

4.2. N-terminal targeting to rafts

Although PrP^C associates with lipid rafts through a GPI anchor, a number of cell-free studies demonstrated that PrP^C could interact with sphingolipid- and cholesterol-rich model membranes in a GPI-independent manner (53-55), with one study identifying the flexible N-terminus of PrP as crucial to this interaction (54). Subsequent cell based

experiments utilizing cells expressing various PrP^C constructs mutated such that they were no longer GPI-anchored, confirmed that the N-terminus of PrP^C between residues 23-90 contains a raft targeting determinant (56). The far N-terminus of mature mammalian PrP^C is highly conserved (10), and contains a positively charged polybasic region between residues 23-28 (-KKRPKP-) immediately following the N-terminal signal peptide (Figure 1). This region is known to bind glycosaminoglycans (GAGs) (57, 58), the long polysaccharide chains covalently linked to the protein core of proteoglycans. Critically, in the context of lipid raft targeting, we determined recently that the major neuronal heparan sulphate proteoglycan, GPI-anchored glypican-1, was involved in the recruitment and stabilization of PrP^C in lipid rafts (59).

4.3. PrP^C endocytosis

Internalization of PM proteins can occur by distinct mechanisms, broadly described as clathrin-dependent and independent. Endocytosis through clathrin-coated pits, involves the assembly of clathrin, a large oligomeric protein, into lattice structures on the inner surface of the PM, assisting membrane invaginaton to form the coated pits. These 'pits' then have the ability to pinch off and form vesicles, which can traffic to and fuse with other intracellular organelles (60). Generally, this mechanism supports the internalization of transmembrane proteins, which localize in pits through association of their cytoplasmic domain with cytoplasmic clathrin-pit-accessory proteins (61). Somewhat surprisingly then, given the lack of a transmembrane or cytoplasmic domain in the majority of PrP^C topological variants, PrP^C has been shown to be present at the cell surface in these structures (62-64), and experimental evidence has shown that PrP^C does cycle between the plasma membrane and early endosomes via a clathrin coated pit dependent pathway (63-65). Importantly, there is evidence to suggest that for PrP^C to be internalized in this classical clathrin-mediated manner it must first translocate out of the lipid raft membrane region, which occurs upon Cu^{2+} binding to the protein (66). Then this non-raft localized PrP^C is rapidly endocytosed through an interaction involving the polybasic region of the N-terminus (65, 66) (Figure 1), with an adaptor protein. One such protein has been identified as the transmembrane low-density lipoprotein receptor-related protein-1 (LRP1) (67, 68), although others may exist.

As previously mentioned, PrP^C also localizes within caveolae and CLDs. Further, it is well established that in a clathrin-independent manner, caveolae can be endocytosed, and this has been shown to provide a mechanism for the internalization of PrP^C molecules and cycling to late endosomes and lysosomes (51). Previously it has been difficult to reconcile these different modes of PrP^C endocytosis, and it has been assumed that the manner of PrP^C internalization is largely influenced by the cell type studied. However, evidence that clathrin coated pit and lipid-raft mediated endocytosis are not necessarily mutually exclusive in the internalization of other proteins (33-35), lends to the possibility of a similar scenario for PrP^C internalization. Indeed, a recent study found that the capacity for PrP^C internalization was reduced with either lipid raft disruption or inhibition of clathrin-mediated endocytosis within a cell line, but was only completely abolished when cells were treated such as to simultaneously disrupt both pathways (69), indicating that even within a single cell there may be different pathways of PrP^C internalization. Figure 2 provides a summary of PrP^C endocytosis.

Evidence from kinetic studies suggests that PrP^C turnover in neuronal cell lines is rapid, with a half-life of approximately five hours (43, 70), and in primary cultures of neuronal and lymphoid cells, turnover is even more rapid (71). PrP^C can be degraded through the endosomal-lysosomal pathway (72) and approximately 10% of wild type PrP^C is subject to retrograde transport through endoplasmic reticulum associated degradation (ERAD), probably because it has been incorrectly processed, and is degraded via the proteasome (73). PrP^C has also been identified in the conditioned media of cultured cells, as well in human cerebrospinal fluid (74), providing evidence of PrP^C shedding from the cell surface into the extracellular space. This soluble extracellular PrP^C has been characterized as no longer having a GPI anchor, suggesting cleavage either within (by a phospholipase) or immediately adjacent to (by a protease) the GPI moiety (71, 75, 76). Recently, we demonstrated that PrP^C was shed from cells through the action of the zinc metalloprotease ADAM10, cleaving the Gly-Arg bond just upstream of the site of GPI attachment (77). Extracellular PrP^C has also been found in association with exosomes (78, 79), membrane bound vesicles contained within the 'lumen' of multivesicular bodies (MVB), expelled from the cell with the fusion of the MVB with the plasma membrane (80). As exosomes have a lipid composition, detergent insolubility and buoyant density which is similar to lipid rafts, and contain lipid raft marker proteins (reviewed in (81, 82)), it is possible that PrP^C secreted from the cell by this process, is fundamentally still lipid raft associated.

5. LIPID RAFTS AND PrP^C FUNCTION

The exact cellular function of the prion protein is presently unknown, although experimental evidence has suggested a number of potential roles. Surprisingly PrP^C knockout mice appeared normal, with no overt phenotypic abnormalities (83-85), suggesting that PrP^C could be functionally redundant. However, further more comprehensive analysis of PrP^C knockout mice and derived cell lines has implicated the cellular prion protein in a variety of biological functions.

5.1. PrP^C endoproteolytic processing

Like many structural and functional classes of proteins (86), PrP^C undergoes post-translational endoproteolytic cleavage. Endogenous N-terminally truncated PrP^C fragments have been identified in cultured cells, and importantly also *in vivo*. These PrP^C species remain glycosylated and GPI-anchored like the full-length protein (87, 88), and because of retention of the GPI anchor are most likely still membrane raft associated. The predominant cleavage, alpha-cleavage, occurs at residues 111 or 112 (human PrP^C sequence nomenclature) (87)

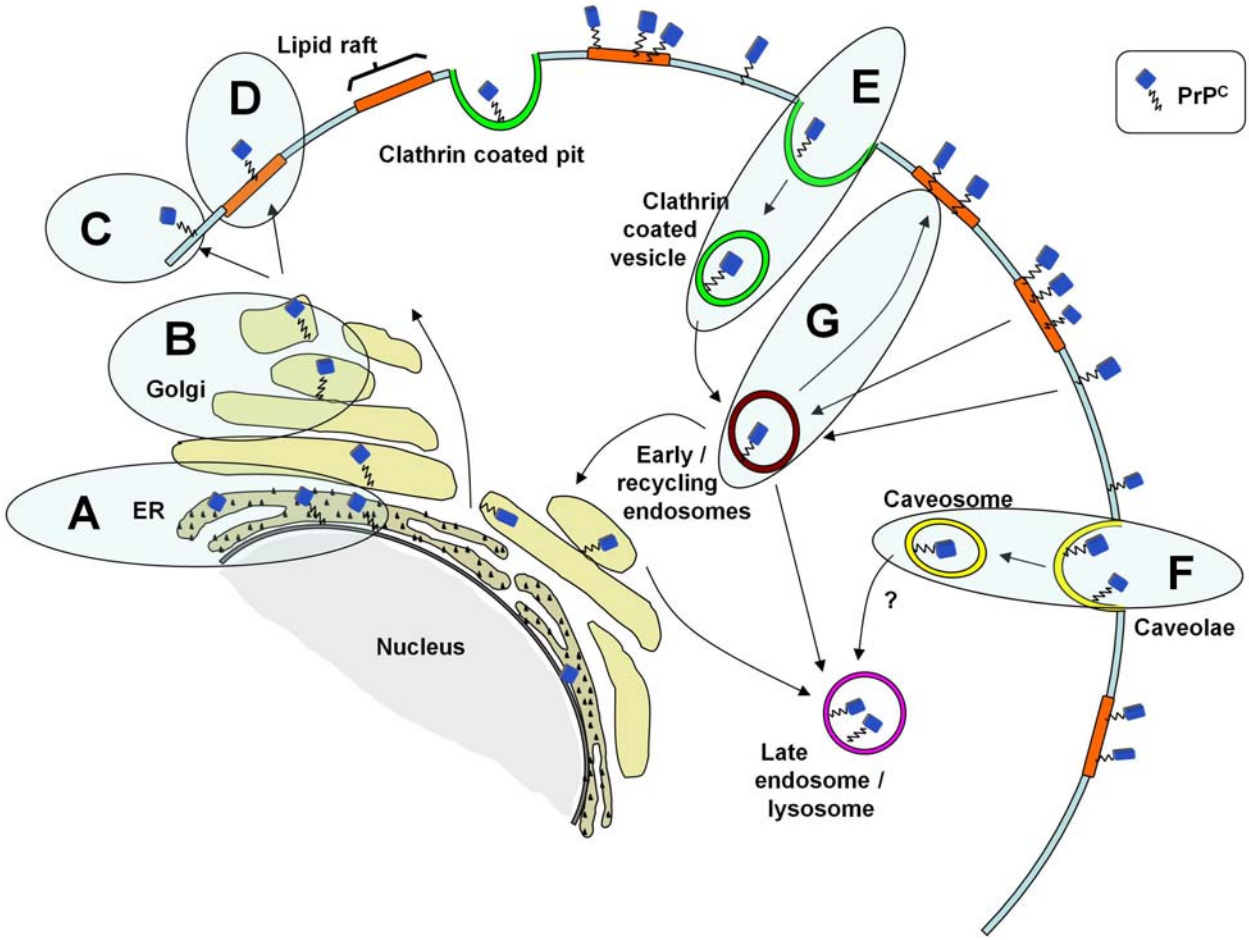

Figure 2. Trafficking and localization of the cellular PrP^C. PrP^C (blue square) is synthesized in the ER (A) and is trafficked through the Golgi (B) to the cell surface where it resides in the plasma membrane (C) predominantly in lipid raft domains (orange) (D). PrP^C endocytosis can be mediated via clathrin coated pits (green) (E) or caveolae (yellow) (F), and PrP^C can be transported back to the cell surface via recycling endosomes (brown) (G).

producing C1. PrP^C beta-cleavage occurs around the C-terminus of the octapeptide repeat domain (89, 90), producing C2, which is found at low levels in normal human (87, 88, 90) and cultured cells (89). The corresponding N-terminal fragments, N1 and N2, can also be detected, both in cell lysates and conditioned media (89, 91).

These endoproteolytic cleavages of PrP^C may result in membrane-bound and soluble fragments with biological functions distinct from the full-length protein and to each other. Furthermore, the specific subcellular localization of the different PrP^C N- and C-terminal species may influence these functions. As already mentioned, the N-terminus of PrP^C is required for its translocation out of lipid rafts and internalization via clathrin coated pits, therefore endoproteolytic cleavage to remove the N-terminus is likely to shift its trafficking pathways and mechanisms relative to the full length protein. This may be particularly relevant, given that often such a high percentage of PrP^C is N-terminally truncated (92), and that alpha-cleavage has recently been shown to occur before the protein reaches the cell surface, in a late stage of the secretory pathway, independent of PrP^C lipid raft localization (93). Conceivably this lack of the important N-terminal polybasic/raft targeting/co-factor binding domain in C1 as it traffics to the cell surface could result in localization to different membrane rafts relative to the full length protein, which may have subsequent implications.

There is some experimental evidence to suggest separate functions for the different PrP^C species. Some results indicate that the alpha-cleavage event produces biologically active fragments, with C1 being pro-apoptotic and N1 being protective against staurosporine treatment in a p53-dependent manner, whereas C2 and N2 are inert (94, 95). Conversely, it is known that PrP^C beta-cleavage occurs at the cell surface and is carried out by reactive oxygen species (ROS) (96, 97), and neuronal cells expressing PrP^C which has been mutated such that it is no longer subject to ROS-mediated beta-cleavage are more sensitive to oxidative stress (97), indicating that this cleavage event is a protective mechanism. In agreement with this, a separate study utilizing synthetic peptides representing the N2

fragment and its different domains found N2 was protective against oxidative stress when it contained an unmodified polybasic motif and was bound to Cu^{2+} (98). Interestingly, the protective effect appeared to be mediated through a proteoglycan binding partner found within, or at least requiring, intact lipid rafts.

5.2. Cellular signaling

The possibility of PrP^C involvement in cellular signaling was raised due to its ubiquitous expression and localization at the cell surface, especially in lipid raft domains which have long been recognized as signaling platforms (99). PrP^C is involved in signal transduction in T-lymphocytes, particularly in T-cell activation (100, 101), which is dependent on its lipid raft localization (102). Further, it has been shown that PrP^C cross-linking in lymphocytes leads to mitogen-activated protein kinase (MAPK) and consequently extracellular signal regulated kinase 1/2 (ERK1/2) activation (103). Similarly, antibody mediated clustering of cell surface PrP^C in neuroectodermal progenitor and differentiated cells, and other neuronal and non-neuronal cells resulted in ERK1/2 activation (104-106), which was shown to occur through nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase stimulation of ROS and in some cells a signaling cascade involving Fyn kinase (105, 106). Interestingly, a recent study highlighted a relationship between PrP^C alpha-cleavage with the MAPK/ERK signaling pathway, which was dependent on membrane raft integrity (107). Finally, PrP^C has been shown to bind synapsin Ib and Grb2, proteins both involved in neuronal signaling (108).

There are data which indicate that the PrP^C linked signaling and neuroprotection functions (see also sections 5.1 and 5.3) may be somewhat concomitant. Stress-inducible protein 1, which binds to PrP^C between residues 113-128, stimulated a PrP^C-dependent neuroprotective response in the neuroblastic layer of cultured retinal cells, through activation of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase pathway (109, 110). Further, there is the suggestion that the changes observed in signaling proteins in PrP^C knockout mice are a compensatory mechanism to maintain cell survival, related to the loss of the antioxidant function of PrP^C (111). However, conflicting results, showing antibody mediated cross-linking of PrP^C *in vivo* triggers apoptosis in hippocampal and cerebellar neurons (112), provokes questions as to whether alternative PrP^C membrane localizations (for example, in specific raft subsets), and therefore accessibility to different resident binding partners and ligands, can promote different signaling pathways.

5.3. Neuroprotection

As mentioned in section 5.1, PrP^C may also have a role as an antioxidant, which appears to be related to the beta-cleavage event and production of the N2 fragment. In addition to the cell based studies already mentioned, *in vivo* studies have shown similar findings. Mice devoid of PrP^C have an increased susceptibility to oxidative stress (111, 113, 114), and in PrP^C knockout mice (compared to wild type mice), the peripheral organs as well as the central nervous system showed marked increases in lipid peroxidation and protein oxidation, and decreased catalase activity (114), all indicators of oxidative stress. There is also evidence that the antioxidant and other related protective effects of PrP^C are more specific to its neuronal expression (111, 115). The prion protein has been found to behave in a superoxide dismutase (SOD)-like manner, dependent on copper binding to the octarepeat region, specifically during and not subsequent to, protein folding (116). Moreover, cytosolic SOD-1 activity was lower in PrP^C-deficient mice compared to wild-type mice, and presumably as a compensatory mechanism, the activities of 'other-SODs' were increased (111). However, there is conflicting evidence that shows PrP^C expression levels have no effect on SOD-1 activity in cultured cerebellar neurons and *in vivo* (117, 118), and that recombinant PrP does not possess any SOD-1 activity (119). Interestingly though, the murine prion protein gene, *Prnp*, shares a number of features with other mouse SOD genes (120), and is up-regulated as part of a cellular stress response (121, 122). Ultimately, as oxidative damage generated by ROS can be catalyzed by the presence of metals, putative anti-oxidant functions of PrP^C may relate to its metal binding affinities, likely influenced by protein conformation and localization within specific lipid environments, and interactions with local binding partners within these environments.

5.4. PrP^C and copper

PrP^C has two putative copper binding regions, one in the octapeptide repeat sequence (113, 123-126), and another in the neurotoxic and amyloidogenic region between residues 106 to 126 (human PrP^C numbering) (127, 128) (see Figure 1), implicating a possible role for PrP^C in copper metabolism, homeostasis or transport. As mentioned earlier, it is known that copper binding to PrP^C, dependent on an intact octarepeat region, results in lateral movement of PrP^C out of lipid rafts (66) and internalization of the protein (129, 130) through a clathrin-dependent route (63, 65). Therefore, PrP^C could function to internalize copper, which once liberated is available for use by other cupro-proteins. Alternatively, given the multitude of possible trafficking pathways and cellular environments where it can be found (see Figure 2), PrP^C could transport copper directly to or from specific intracellular proteins, cellular compartments or environments. In support of PrP^C functioning in copper transport is the finding that copper uptake and PrP^C expression levels correlate in mouse cerebellar cells (131). However, there is some evidence that argues against copper transport being a predominant function of PrP^C. This includes data indicating that in cell cultures with doxycycline-controlled levels of PrP^C expression that PrP^C does not traffic copper from the extracellular space into the cell at physiological concentrations (132), and the unaltered levels of ionic copper in the brains of mice with a range of PrP^C expression levels (117).

5.5. Other putative PrP^C functions

PrP^C has also been implicated in a variety of other physiological roles, including an involvement in normal synaptic function and long-term potentiation (133-139), and regulation of normal sleep patterns (111, 140,

141). Importantly we reported that PrP^C is involved in the regulation of the beta-secretase BACE-1 which is responsible for cleavage of the Alzheimer's disease (AD) amyloid precursor protein (APP) in the generation of the pathogenic amyloid-beta peptide (142). Raft-association of PrP^C was required for its inhibitory action on BACE-1, as neither a transmembrane-anchored construct that is no longer associated with rafts nor a soluble construct lacking any type of anchoring domain was effective. More recently it has been reported that oligomers of the amyloid-beta peptide bind to the charged cluster region (residues 95-110) of cell surface PrP^C and affect synaptic plasticity (143) and that expression of PrP^C is controlled by the APP intracellular domain, AICD (144). These studies all implicate PrP^C in the pathogenesis of AD (reviewed in (145). Although it might not seem possible for a single protein to have so many functions as described above, it may be that the specific biological role of PrP^C will differ, and be entirely dependent on, the cell type in which it is expressed, the precise localization within the cell, including the different plasma membrane environments, and/or the ratio or differential expression of any processed variants of the wild-type protein.

6. LIPID RAFTS IN PRION DISEASE

The expression of PrP^C is an absolute requirement for development of prion disease and a key event in prion diseases is the conversion of alpha-helical PrP^C into the beta-sheet disease-associated PrP^{Sc} isoforms. Experimental data supports that the conversion process is facilitated by another protein or proteins or other ancillary factor(s). Some studies have shown that PrP-lipid interactions can influence PrP conformation (53, 146, 147), therefore the PrP lipid environment has the potential to influence the PrP^C to PrP^{Sc} conversion process. One hypothesis states that potential molecular chaperones which might mediate PrP^C unfolding to allow, for example, biologically relevant endogenous cleavage, could have an inadvertent second function in the conversion process (148). There is evidence for the participation of a species-specific macromolecule, posited to be a protein (designated 'protein X') (149-151). There is also evidence to support the involvement of negatively charged macromolecules such as nucleic acids (152-156) or GAGs (157-159). Significantly, our recent finding that reducing levels of the GPI-anchored proteoglycan glypican-1 results in a decrease in the amount of protease resistant PrP^{Sc} detected in 22L-scrapie infected mouse neuroblastoma (ScN2a) cells (59) ties together some of this previous evidence. That is, glypican-1 may act as a co-factor in the conversion of PrP, perhaps by bringing together PrP^C and PrP^{Sc} isoforms as evidenced by the co-immunoprecipitation of glypican-1 with both isoforms (59). Although a similar study utilizing a different cell line (RML-scrapie infected mouse hypothalamic cells, ScGT1-1) observed the opposite effect, with reduction of glypican-1 increasing levels of PrP^{Sc} (160), these contrasting results may reflect differences due to cell line PrP^C and PrP^{Sc} strain specific interactions. This is not an unreasonable assumption due to the known varied expression and glycosylation profiles of PrP^C in different cell types (92, 161), along with strain specific PrP^{res} profiles, (162-164) which may reflect slightly different PrP^{Sc} conformations (165-168), and evidence that conversion is more favorable when PrP^C and the prion strain have similar glycosylation states (169, 170).

6.1. Sites of PrP^C to PrP^{Sc} conversion

There is much importance in trying to determine the site and mechanism of conversion of PrP^C to PrP^{Sc}, as inhibition of conversion is an obvious potential therapeutic option. Unlike PrP^C, the disease associated PrP^{Sc} conformers localize predominantly intracellularly, in late endosomes, multivesicular organelles, lysosomes or lysosome-like structures (171-177), perhaps in lipid raft domains within these organelles (48). Nevertheless, PrP^{res} has been shown to localize at the cell surface (177, 178), and importantly in plasma membrane (45) and endocytic pathway (48) DRMs. The mechanisms of internalization of PrP^{Sc} are not well understood, although recent evidence suggests the possibility of endocytosis through an association with clathrin coated pits (177). Due to the varied cellular localization of PrP^C and PrP^{Sc} / PrP^{res}, there are a number of different possible sites of conversion (summarized in Figure 3).

One plausible site of conversion is at the cell surface. This may be particularly true for cases of transmitted prion disease where a misfolded prion conformer would initially not be produced endogenously, notwithstanding the possibility of internalization of exogenously introduced PrP^{Sc} and conversion within a cellular compartment. However, two studies have shown that for conversion and propagation of infectivity to occur, the infectious agent does not necessarily need to be 'free', strongly suggesting initiation of conversion of PrP^C at the cell surface (179, 180). The first study utilized infectious prions adsorbed to steel wires as the infectious source *in vivo* and *in vitro*, and found infectious titers did not reduce upon multiple uses of the steel wires (179), suggesting that cellular infection required direct contact with the infectious prion, but did not depend on consumption or removal of the infectivity from the wire. Similarly, the second (*in vitro*) study found that glutaraldehyde fixation of infected cells prior to allowing direct surface contact with recipient cells did not abolish transfer of infection, although it did reduce efficiency (180), suggesting in this cell culture model there may be a trafficking pathway or intracellular compartment where conversion may be more efficient, or perhaps the fixation procedure adversely altered the structure or binding propensities (either to PrP^C or other essential co-factors) of the PrP^{Sc} on the donor cells.

For conversion and propagation at the cell surface, the specific membrane localization is likely to be important. As previously mentioned, PrP^C and PrP^{res} have been localized within DRMs, which presumably represent cellular lipid rafts, although interestingly these two PrP species are not necessarily in the same DRM (48). It remains possible then that PrP^C and PrP^{Sc}, and catalysts or participants in the conversion process, possibly other GPI-anchored proteins which are enriched in these membranes (such as glypican-1, see above) or other cellular factors which are recruited to rafts by ligand binding, may be

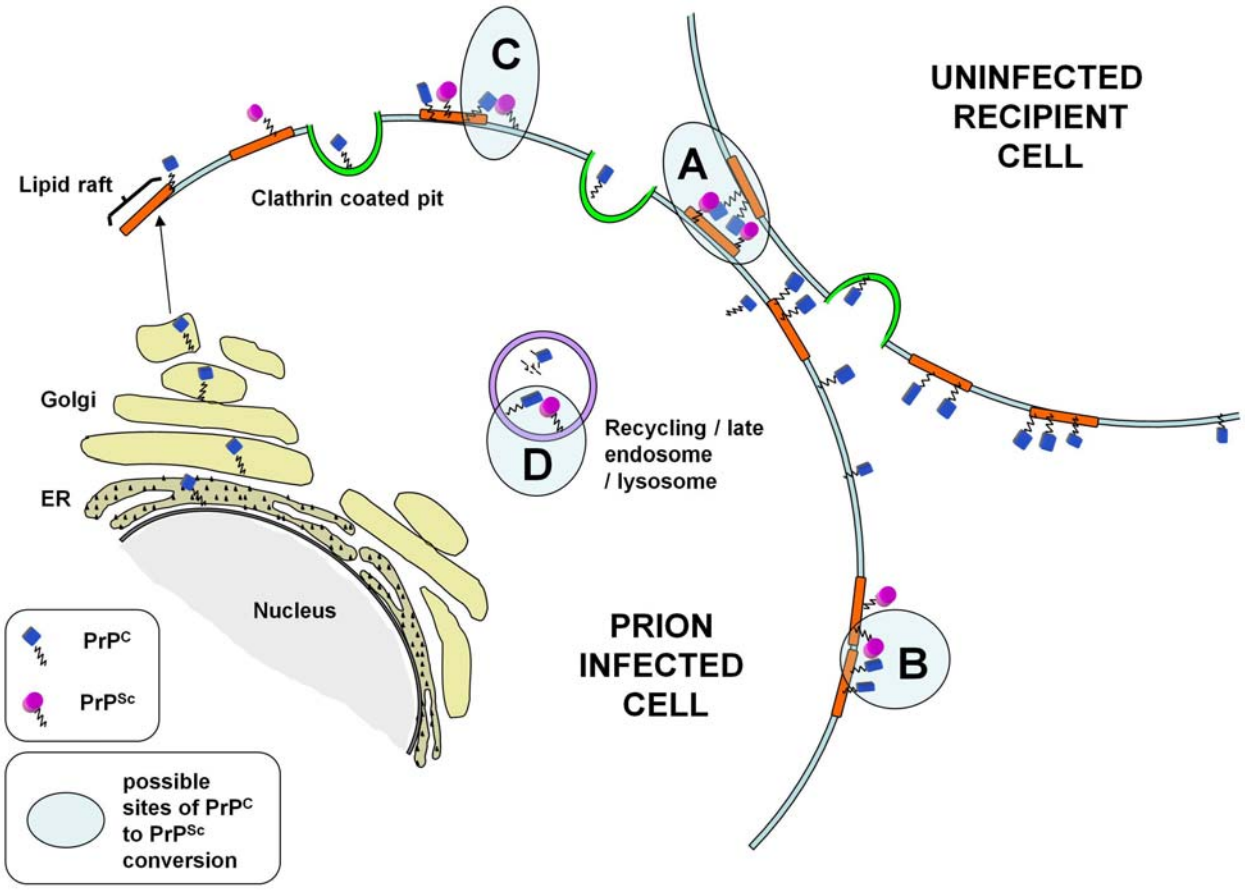

Figure 3. Possible sites of PrP^{C} to PrP^{Sc} conversion and propagation. (A) Initiation of conversion at the cell surface after direct contact between uninfected and infected cells. (B) Lipid raft clustering, enabling interaction of otherwise separated PrP^{C} and PrP^{Sc}. (C) Non-raft associated PrP^{Sc} promoting conversion of contiguous raft associated PrP^{C}. (D) Conversion of PrP^{C} to PrP^{Sc} in endocytic vesicles. Mode (A) is represented by lipid raft associated PrP, but could involve non-raft associated PrP. Mode (B) highlights conversion at the cell surface, but could occur intracellularly in vesicles known to contain raft-associated proteins.

brought together by a specific raft clustering event, thus allowing interaction and template directed conversion of PrP^{C} to PrP^{Sc} at the cell surface. Further, experimental evidence has shown that lovastatin, a compound known to diminish cholesterol levels and therefore potentially disorder cholesterol-rich domains, inhibited PrP^{Sc} formation (62). However, there are some conflicting results which indicate that PrP^{C} GPI-anchored within lipid rafts *in vitro* is resistant to conversion, with PrP^{C} needing to be free of its GPI anchor, or that there be movement of PrP^{C} and PrP^{res} into adjoining membranes, for efficient conversion (47). Moreover, when rafts were depleted of their sphingolipid component, the conversion rate of PrP^{C} was increased (181), suggesting at least in this model that disruption of rafts increases conversion, i.e. that raft localization protects against conversion. Interestingly, mice expressing GPI-anchorless PrP^{C} (in place of normal GPI-anchored PrP^{C}), which is secreted by cells (182) although appears to be able to associate with DRMs (183), are still susceptible to prion infection. Transgenic mice heterozygous for GPI-anchorless PrP^{C} produce GPI-anchorless PrP^{res} and harbour high titres of prion infectivity (182), whereas mice homozygous for the transgene and therefore expressing higher levels of GPI-anchorless PrP^{C} than the heterozygous mice, develop a unique clinical symptomology and neuropathology after prion infection which is directly related to the GPI-anchorless state of PrP^{C} (184). Furthermore, cultured cells expressing the same GPI-anchorless PrP^{C} permit conversion to GPI-anchorless PrP^{res} in the short-term but do not remain persistently infected and propagating PrP^{res} (185), with the authors suggesting that *in vivo* perhaps ongoing conversion leading to the distinctive disease is occurring extracellularly. Nevertheless, whichever scenario is considered, these results all underscore the influence of PrP^{C} and PrP^{Sc} localization on prion conversion and the pathogenesis of prion disease.

Raft-mediated conversion as described above could also occur intracellularly, as not only do lipid raft domains exist in vesicles of the endocytic pathway, but membrane rafts and raft clustering may be crucial to the endocytosis of PrP conformers. PrP^{C} has been localized to vesicles of the endosomal and lysosomal pathways, and PrP^{res} is known to accumulate in both late endosomes and lysosomes. Therefore, another location of conversion from

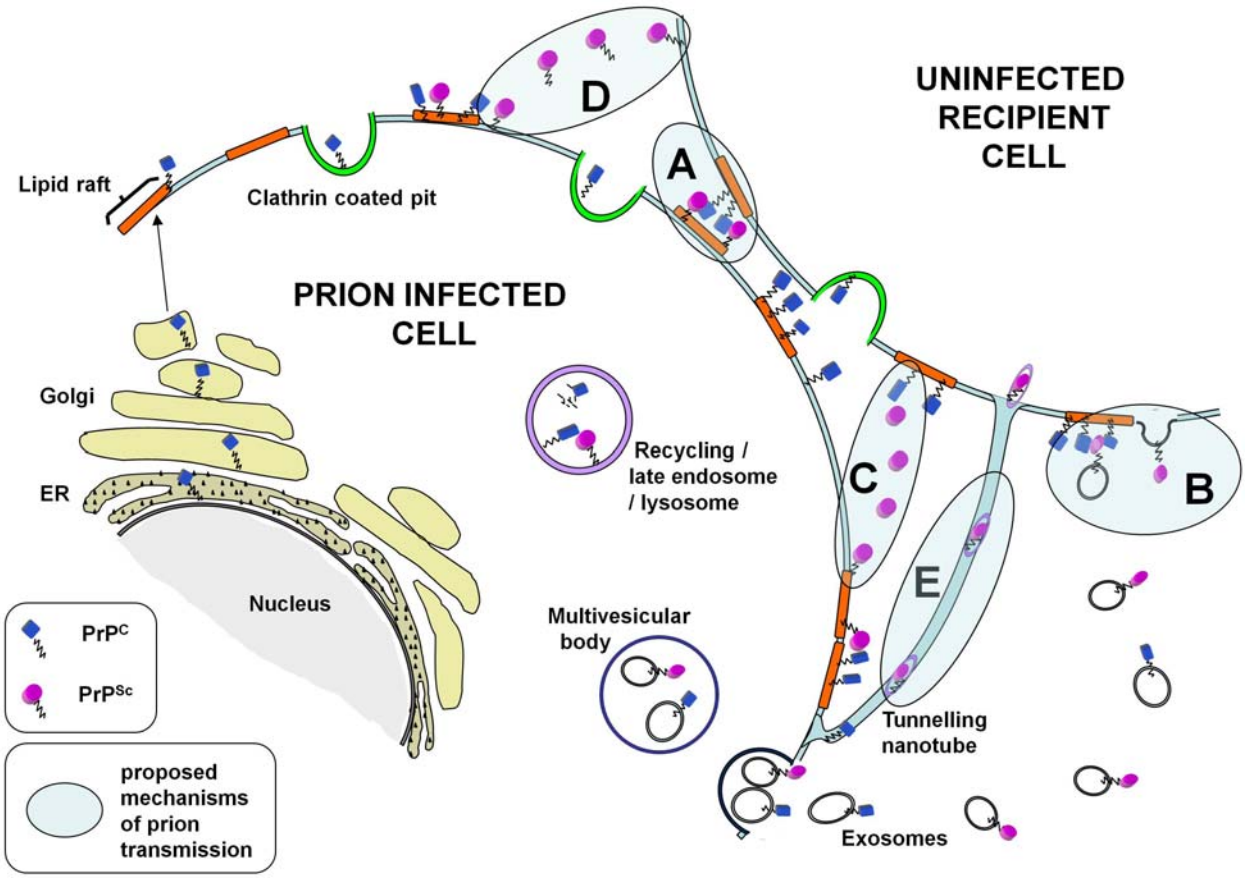

Figure 4. Proposed mechanisms of cell-to-cell spread of prion infectivity. (A) Prion transmission through direct cell-to-cell contact (conversion of recipient PrP^{C} without internalization of donor PrP^{Sc}). (B) Transmission of prions through exosomal PrP^{Sc} association; both a direct interaction of exosome-associated PrP^{Sc} with cell-associated PrP^{C} and incorporation of exosomal membrane with recipient cell membrane are represented. (C) C-terminal truncation of PrP^{Sc} allowing release from an infected cell and movement to an uninfected recipient cell. (D) "GPI-painting" mode of prion transfer. (E) PrP^{Sc} spread through tunnelling nanotubes, in association with small vesicles of lysosomal origin. Mode (A) is represented by lipid raft associated PrP, but could involve non-raft associated PrP. Mode (D) is depicted by transfer of cell surface PrP^{Sc}, but could potentially occur with exosomal PrP^{Sc}.

PrP^{C} to PrP^{Sc} is postulated to be shortly after internalization, during an endocytic process. Evidence for this was provided when researchers found that a more acidic pH, similar to that observed in late endosomes or lysosomes, promoted the conformational change of PrP^{C} to a more detergent insoluble (i.e. PrP^{Sc}-like) form (186) and changed recombinant PrP from predominantly alpha-helical to beta-sheet (187, 188). In addition, researchers have inhibited PrP^{res} production through lowering the culturing temperature to 18°C and thereby slowing the rate of PrP^{C} endocytosis (189). Similarly, others have shown through a different method of disrupting PrP^{C} traffic to the cell surface and therefore preventing normal passage through the endosomal / lysosomal pathway, that PrP^{res} production after exposure to scrapie was inhibited (72). Finally, through the use of selective inhibitors and siRNA treatments, researchers have found that at least a proportion of conversion occurs not in early or late endosomes, but more specifically in recycling endosomal compartments (190). However, these *in vitro* data do not completely eliminate the possibility of early stage conversion events, perhaps to intermediate protease sensitive molecules, occurring at the cell surface prior to internalization where the intermediate then acquires protease resistance and propagates further. Nevertheless, overall these data suggest that the conversion process may be most efficient in acidic environments, such as that provided by vesicles of the endosomal / lysosomal pathway, and therefore internalization of the PrP conformers might be crucial for ongoing propagation of prions.

6.2. Cell-to-cell prion transmission

Mechanisms of prion spread, including trafficking and release of disease associated PrP conformers from the cell have been proposed, and are summarized in Figure 4. As eluded to in section 6.1, one way in which the spread of prions may occur is through direct contact between cell surface PrP^{Sc} on an infected cell and PrP^{C} on a naïve cell, with initiation of conversion at the cell surface, or internalization of the donor cell PrP^{Sc}.

Linked to its intracellular endosomal or multivesicular body localization is the presence of PrP^{res} extracellularly, packaged in exosomes (78, 79), thereby secreted from the cell, and providing a mechanism of cell-to-cell spread of infectivity. In contrast tunnelling nanotubes (TNTs), actin containing membrane bridges which form between cells as a mode of communication and exchange, and allow movement of molecules along their surface and internally within small vesicles of mostly endosomal or lysosomal origin (191), were speculated to provide a means of prion transmission based on the co-localization of PrP^{Sc} with lysosomal marker positive small neuritic vesicles (192). More recently, TNTs were conclusively shown to support the traffic of PrP^{C} as well as exogenous and endogenous PrP^{Sc} between various cell types of both neuronal and non-neuronal origin (193). Interestingly this report found that under conditions where exosomal spread of prions was made impossible, efficient transmission of PrP^{Sc} (through TNTs) still occurred. Separate to vesicular release of disease associated PrP, approximately 15% of PrP^{res} purified from hamster brain was found to be C-terminally truncated to residue 228 (41). It remains possible that *in vivo* some of this truncation may arise from cleavage and release of plasma membrane associated PrP^{res}, perhaps through a phospholipase- or protease-like activity, providing another potential mechanism for cell-to-cell transmission. Transmission of infectious prions to recipient cells is also suggested to be possible via a process of "GPI-painting", whereby GPI-anchored PrP^{Sc} would transfer from one cell to another by re-inserting into the recipient plasma membrane whilst retaining normal function (47). A GPI-painting model of prion cell-to-cell transmission would therefore require the release of intact GPI-anchored PrP^{Sc} from the infected cell or possibly from exosomes, by a mechanism not yet identified. GPI-painting has been shown to occur *in vitro* (194) and *in vivo* (195) for proteins unrelated to PrP, and GPI-anchored proteins that re-insert into the plasma membrane can reorganize into membrane microdomains such as rafts (196).

The overall efficiency of cell-to-cell prion spread and therefore prion propagation would conceivably depend on the localization of PrP^{Sc} and the efficiency of these different proposed transmission mechanisms, and, similar to the possible sites of conversion and replication, are likely influenced by specific host cell-prion strain combinations.

7. CONCLUSIONS AND PERSPECTIVES

There is a large and increasing body of evidence implicating the membrane microenvironment, particularly cholesterol- and sphingolipid-rich lipid rafts, in both the normal cellular processing and functions of PrP^{C}, as well as its conformational conversion to the disease isoform. This field is not without controversy, due mainly to the limitation of techniques to study rafts *in situ*. The most widely used technique to study rafts involving their relative resistance to solubilization with detergents is not without its pitfalls but, together with other fractionation and imaging techniques, has clearly shown that rafts, or components therein, are critically involved in the function and conformational conversion of PrP^{C}. Not only because of its GPI anchor but also due to a raft targeting determinant in its N-terminal region, PrP^{C} associates with these membrane microdomains. The recent identification of glypican-1 as targeting PrP^{C} to rafts and facilitating conversion to PrP^{Sc} opens up new avenues for prion research and may, in part, explain the therapeutic effects of heparin and other GAG mimetics in prion disease. Further analysis of the molecules within rafts that PrP^{C} and PrP^{Sc} interact with will undoubtedly provide additional openings for prion biology researchers.

8. ACKNOWLEDGEMENTS

VL is supported by an Australian Government, National Health and Medical Research Council Training Fellowship #567123. We gratefully acknowledge the Medical Research Council of Great Britain and the Wellcome Trust for supporting our work on prion biology.

9. REFERENCES

1.P. E. Bendheim, H. R. Brown, R. D. Rudelli, L. J. Scala, N. L. Goller, G. Y. Wen, R. J. Kascsak, N. R. Cashman and D. C. Bolton: Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. *Neurology* 42(1), 149-56 (1992)

2.M. Horiuchi, N. Yamazaki, T. Ikeda, N. Ishiguro and M. Shinagawa: A cellular form of prion protein (PrPC) exists in many non-neuronal tissues of sheep. *J Gen Virol* 76 (Pt 10), 2583-7 (1995)

3.M. J. Ford, L. J. Burton, R. J. Morris and S. M. Hall: Selective expression of prion protein in peripheral tissues of the adult mouse. *Neuroscience* 113(1), 177-92 (2002)

4.N. Stahl, D. R. Borchelt, K. Hsiao and S. B. Prusiner: Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* 51(2), 229-40 (1987)

5.W. Goldmann: PrP gene and its association with spongiform encephalopathies. *Br Med Bull* 49(4), 839-59 (1993)

6.P. Mastrangelo and D. Westaway: Biology of the prion gene complex. *Biochem Cell Biol* 79(5), 613-28 (2001)

7.J. F. Bazan, R. J. Fletterick, M. P. McKinley and S. B. Prusiner: Predicted secondary structure and membrane topology of the scrapie prion protein. *Protein Eng* 1(2), 125-35 (1987)

8.E. Sulkowski: Aromatic palindrome motif in prion proteins. *Faseb J* 6(6), 2363 (1992)

9.E. Paramithiotis, M. Pinard, T. Lawton, S. LaBoissiere, V. L. Leathers, W. Q. Zou, L. A. Estey, J. Lamontagne, M. T. Lehto, L. H. Kondejewski, G. P. Francoeur, M. Papadopoulos, A. Haghighat, S. J. Spatz, M. Head, R. Will, J. Ironside, K. O'Rourke, Q. Tonelli, H. C. Ledebur, A. Chakrabartty and N. R. Cashman: A prion protein epitope

selective for the pathologically misfolded conformation. *Nat Med* 9(7), 893-9 (2003)

10.F. Wopfner, G. Weidenhofer, R. Schneider, A. von Brunn, S. Gilch, T. F. Schwarz, T. Werner and H. M. Schatzl: Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. *J Mol Biol* 289(5), 1163-78 (1999)

11.S. B. Prusiner, R. Gabizon and M. P. McKinley: On the biology of prions. *Acta Neuropathol* 72(4), 299-314 (1987)

12.K. M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z. Huang, R. J. Fletterick, F. E. Cohen and *et al.*: Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* 90(23), 10962-6 (1993)

13.H. Bueler, A. Aguzzi, A. Sailer, R. A. Greiner, P. Autenried, M. Aguet and C. Weissmann: Mice devoid of PrP are resistant to scrapie. *Cell* 73(7), 1339-47 (1993)

14.J. C. Manson, A. R. Clarke, P. A. McBride, I. McConnell and J. Hope: PrP gene dosage determines the timing but not the final intensity or distribution of lesions in scrapie pathology. *Neurodegeneration* 3(4), 331-40 (1994)

15.S. Sakaguchi, S. Katamine, K. Shigematsu, A. Nakatani, R. Moriuchi, N. Nishida, K. Kurokawa, R. Nakaoke, H. Sato, K. Jishage and *et al.*: Accumulation of proteinase K-resistant prion protein (PrP) is restricted by the expression level of normal PrP in mice inoculated with a mouse-adapted strain of the Creutzfeldt-Jakob disease agent. *J Virol* 69(12), 7586-92 (1995)

16.G. K. Radda: The dynamic properties of biological membranes. *Philos Trans R Soc Lond B Biol Sci* 272(915), 159-71 (1975)

17.H. Hering, C. C. Lin and M. Sheng: Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. *J Neurosci* 23(8), 3262-71 (2003)

18.K. Simons and R. Ehehalt: Cholesterol, lipid rafts, and disease. *J Clin Invest* 110(5), 597-603 (2002)

19.G. van Meer: Lipid traffic in animal cells. *Annu Rev Cell Biol* 5, 247-75 (1989)

20.J. Gruenberg: The endocytic pathway: a mosaic of domains. *Nat Rev Mol Cell Biol* 2(10), 721-30 (2001)

21.S. Mayor, S. Sabharanjak and F. R. Maxfield: Cholesterol-dependent retention of GPI-anchored proteins in endosomes. *EMBO J* 17(16), 4626-38 (1998)

22.D. A. Brown and J. K. Rose: Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* 68(3), 533-44 (1992)

23.S. Schuck, M. Honsho, K. Ekroos, A. Shevchenko and K. Simons: Resistance of cell membranes to different detergents. *Proc Natl Acad Sci U S A* 100(10), 5795-800 (2003)

24.D. A. Brown and E. London: Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol* 14, 111-36 (1998)

25.K. Simons and E. Ikonen: Functional rafts in cell membranes. *Nature* 387(6633), 569-72 (1997)

26.L. J. Pike: The challenge of lipid rafts. *J Lipid Res* 50 Suppl, S323-8 (2009)

27.L. J. Pike: Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J Lipid Res* 47(7), 1597-8 (2006)

28.D. Lingwood and K. Simons: Detergent resistance as a tool in membrane research. *Nat Protoc* 2(9), 2159-65 (2007)

29.K. Simons and D. Toomre: Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 1(1), 31-9 (2000)

30.S. Schuck and K. Simons: Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J Cell Sci* 117(Pt 25), 5955-64 (2004)

31.C. Salaun, D. J. James and L. H. Chamberlain: Lipid rafts and the regulation of exocytosis. *Traffic* 5(4), 255-64 (2004)

32.R. G. Parton and A. A. Richards: Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic* 4(11), 724-38 (2003)

33.K. Deinhardt, O. Berninghausen, H. J. Willison, C. R. Hopkins and G. Schiavo: Tetanus toxin is internalized by a sequential clathrin-dependent mechanism initiated within lipid microdomains and independent of epsin1. *J Cell Biol* 174(3), 459-71 (2006)

34.G. H. Hansen, S. M. Dalskov, C. R. Rasmussen, L. Immerdal, L. L. Niels-Christiansen and E. M. Danielsen: Cholera toxin entry into pig enterocytes occurs via a lipid raft- and clathrin-dependent mechanism. *Biochemistry* 44(3), 873-82 (2005)

35.R. Rollason, V. Korolchuk, C. Hamilton, P. Schu and G. Banting: Clathrin-mediated endocytosis of a lipid-raft-associated protein is mediated through a dual tyrosine motif. *J Cell Sci* 120(Pt 21), 3850-8 (2007)

36.M. Dykstra, A. Cherukuri, H. W. Sohn, S. J. Tzeng and S. K. Pierce: Location is everything: lipid rafts and immune cell signaling. *Annu Rev Immunol* 21, 457-81 (2003)

37.S. Maekawa, S. Iino and S. Miyata: Molecular characterization of the detergent-insoluble cholesterol-rich membrane microdomain (raft) of the central nervous system. *Biochim Biophys Acta* 1610(2), 261-70 (2003)

38.D. A. Harris: Trafficking, turnover and membrane topology of PrP. *Br Med Bull* 66, 71-85 (2003)

39.V. A. Lawson, S. J. Collins, C. L. Masters and A. F. Hill: Prion protein glycosylation. *J Neurochem* 93(4), 793-801 (2005)

40.E. Turk, D. B. Teplow, L. E. Hood and S. B. Prusiner: Purification and properties of the cellular and scrapie hamster prion proteins. *Eur J Biochem* 176(1), 21-30 (1988)

41.N. Stahl, M. A. Baldwin, A. L. Burlingame and S. B. Prusiner: Identification of glycoinositol phospholipid linked and truncated forms of the scrapie prion protein. *Biochemistry* 29(38), 8879-84 (1990)

42.M. A. Ferguson and A. F. Williams: Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Annu Rev Biochem* 57, 285-320 (1988)

43.B. Caughey, R. E. Race, D. Ernst, M. J. Buchmeier and B. Chesebro: Prion protein biosynthesis in scrapie-infected and uninfected neuroblastoma cells. *J Virol* 63(1), 175-81 (1989)

44.M. A. Baldwin: Analysis of glycosylphosphatidylinositol protein anchors: the prion protein. *Methods Enzymol* 405, 172-87 (2005)

45.M. Vey, S. Pilkuhn, H. Wille, R. Nixon, S. J. DeArmond, E. J. Smart, R. G. Anderson, A. Taraboulos and S. B. Prusiner: Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proc Natl Acad Sci U S A* 93(25), 14945-9 (1996)

46.A. Gorodinsky and D. A. Harris: Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. *J Cell Biol* 129(3), 619-27 (1995)

47.G. S. Baron, K. Wehrly, D. W. Dorward, B. Chesebro and B. Caughey: Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP(Sc)) into contiguous membranes. *Embo J* 21(5), 1031-40 (2002)

48.N. Naslavsky, R. Stein, A. Yanai, G. Friedlander and A. Taraboulos: Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. *J Biol Chem* 272(10), 6324-31 (1997)

49.D. Sarnataro, V. Campana, S. Paladino, M. Stornaiuolo, L. Nitsch and C. Zurzolo: PrP(C) association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. *Mol Biol Cell* 15(9), 4031-42 (2004)

50.J. H. Harmey, D. Doyle, V. Brown and M. S. Rogers: The cellular isoform of the prion protein, PrPc, is associated with caveolae in mouse neuroblastoma (N2a) cells. *Biochem Biophys Res Commun* 210(3), 753-9 (1995)

51.P. J. Peters, A. Mironov, Jr., D. Peretz, E. van Donselaar, E. Leclerc, S. Erpel, S. J. DeArmond, D. R. Burton, R. A. Williamson, M. Vey and S. B. Prusiner: Trafficking of prion proteins through a caveolae-mediated endosomal pathway. *J Cell Biol* 162(4), 703-17 (2003)

52.S. Mouillet-Richard, B. Schneider, E. Pradines, M. Pietri, M. Ermonval, J. Grassi, J. G. Richards, V. Mutel, J. M. Launay and O. Kellermann: Cellular prion protein signaling in serotonergic neuronal cells. *Ann N Y Acad Sci* 1096, 106-19 (2007)

53.N. Sanghera and T. J. Pinheiro: Binding of prion protein to lipid membranes and implications for prion conversion. *J Mol Biol* 315(5), 1241-56 (2002)

54.G. S. Baron and B. Caughey: Effect of glycosylphosphatidylinositol anchor-dependent and -independent prion protein association with model raft membranes on conversion to the protease-resistant isoform. *J Biol Chem* 278(17), 14883-92 (2003)

55.R. Mahfoud, N. Garmy, M. Maresca, N. Yahi, A. Puigserver and J. Fantini: Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. *J Biol Chem* 277(13), 11292-6 (2002)

56.A. R. Walmsley, F. Zeng and N. M. Hooper: The N-terminal region of the prion protein ectodomain contains a lipid raft targeting determinant. *J Biol Chem* 278(39), 37241-8 (2003)

57.T. Pan, B. S. Wong, T. Liu, R. Li, R. B. Petersen and M. S. Sy: Cell-surface prion protein interacts with glycosaminoglycans. *Biochem J* 368(Pt 1), 81-90 (2002)

58.R. G. Warner, C. Hundt, S. Weiss and J. E. Turnbull: Identification of the heparan sulfate binding sites in the cellular prion protein. *J Biol Chem* 277(21), 18421-30 (2002)

59.D. R. Taylor, I. J. Whitehouse and N. M. Hooper: Glypican-1 mediates both prion protein lipid raft association and disease isoform formation. *PLoS Pathog* 5(11), e1000666 (2009)

60.T. Kirchhausen: Clathrin. *Annu Rev Biochem* 69, 699-727 (2000)

61.J. S. Bonifacino and L. M. Traub: Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu Rev Biochem* 72, 395-447 (2003)

62.A. Taraboulos, M. Scott, A. Semenov, D. Avrahami, L. Laszlo, S. B. Prusiner and D. Avraham: Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. *J Cell Biol* 129(1), 121-32 (1995)

63.S. L. Shyng, J. E. Heuser and D. A. Harris: A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. *J Cell Biol* 125(6), 1239-50 (1994)

64.J. Laine, M. E. Marc, M. S. Sy and H. Axelrad: Cellular and subcellular morphological localization of normal prion protein in rodent cerebellum. *Eur J Neurosci* 14(1), 47-56 (2001)

65.C. Sunyach, A. Jen, J. Deng, K. T. Fitzgerald, Y. Frobert, J. Grassi, M. W. McCaffrey and R. Morris: The mechanism of internalization of glycosylphosphatidylinositol-anchored prion protein. *Embo J* 22(14), 3591-601 (2003)

66.D. R. Taylor, N. T. Watt, W. S. Perera and N. M. Hooper: 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* 118(Pt 21), 5141-53 (2005)

67.C. J. Parkyn, E. G. Vermeulen, R. C. Mootoosamy, C. Sunyach, C. Jacobsen, C. Oxvig, S. Moestrup, Q. Liu, G. Bu, A. Jen and R. J. Morris: LRP1 controls biosynthetic and endocytic trafficking of neuronal prion protein. *J Cell Sci* 121(Pt 6), 773-83 (2008)

68.D. R. Taylor and N. M. Hooper: The low-density lipoprotein receptor-related protein 1 (LRP1) mediates the endocytosis of the cellular prion protein. *Biochem J* 402(1), 17-23 (2007)

69.D. Sarnataro, A. Caputo, P. Casanova, C. Puri, S. Paladino, S. S. Tivodar, V. Campana, C. Tacchetti and C. Zurzolo: Lipid rafts and clathrin cooperate in the internalization of PrP in epithelial FRT cells. *PLoS One* 4(6), e5829 (2009)

70.D. R. Borchelt, M. Scott, A. Taraboulos, N. Stahl and S. B. Prusiner: Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. *J Cell Biol* 110(3), 743-52 (1990)

71.P. Parizek, C. Roeckl, J. Weber, E. Flechsig, A. Aguzzi and A. J. Raeber: Similar turnover and shedding of the cellular prion protein in primary lymphoid and neuronal cells. *J Biol Chem* 276(48), 44627-32 (2001)

72.V. Vetrugno, A. Cardinale, I. Filesi, S. Mattei, M. S. Sy, M. Pocchiari and S. Biocca: KDEL-tagged anti-prion intrabodies impair PrP lysosomal degradation and inhibit scrapie infectivity. *Biochem Biophys Res Commun* 338(4), 1791-7 (2005)

73.Y. Yedidia, L. Horonchik, S. Tzaban, A. Yanai and A. Taraboulos: Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. *Embo J* 20(19), 5383-91 (2001)

74.F. Tagliavini, F. Prelli, M. Porro, M. Salmona, O. Bugiani and B. Frangione: A soluble form of prion protein in human cerebrospinal fluid: implications for prion-related encephalopathies. *Biochem Biophys Res Commun* 184(3), 1398-404 (1992)

75.E. T. Parkin, N. T. Watt, A. J. Turner and N. M. Hooper: Dual mechanisms for shedding of the cellular prion protein. *J Biol Chem* 279(12), 11170-8 (2004)

76.D. R. Borchelt, M. Rogers, N. Stahl, G. Telling and S. B. Prusiner: Release of the cellular prion protein from cultured cells after loss of its glycoinositol phospholipid anchor. *Glycobiology* 3(4), 319-29 (1993)

77.D. R. Taylor, E. T. Parkin, S. L. Cocklin, J. R. Ault, A. E. Ashcroft, A. J. Turner and N. M. Hooper: Role of ADAMs in the ectodomain shedding and conformational conversion of the prion protein. *J Biol Chem* 284(34), 22590-600 (2009)

78.L. J. Vella, R. A. Sharples, V. A. Lawson, C. L. Masters, R. Cappai and A. F. Hill: Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *J Pathol* 211(5), 582-90 (2007)

79.B. Fevrier, D. Vilette, F. Archer, D. Loew, W. Faigle, M. Vidal, H. Laude and G. Raposo: Cells release prions in association with exosomes. *Proc Natl Acad Sci U S A* 101(26), 9683-8 (2004)

80.K. Denzer, M. J. Kleijmeer, H. F. Heijnen, W. Stoorvogel and H. J. Geuze: Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 113 Pt 19, 3365-74 (2000)

81.N. R. Smalheiser: Exosomal transfer of proteins and RNAs at synapses in the nervous system. *Biol Direct* 2, 35 (2007)

82.G. van Niel, I. Porto-Carreiro, S. Simoes and G. Raposo: Exosomes: a common pathway for a specialized function. *J Biochem* 140(1), 13-21 (2006)

83.H. Bueler, M. Fischer, Y. Lang, H. Bluethmann, H. P. Lipp, S. J. DeArmond, S. B. Prusiner, M. Aguet and C. Weissmann: Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 356(6370), 577-82 (1992)

84.J. C. Manson, A. R. Clarke, M. L. Hooper, L. Aitchison, I. McConnell and J. Hope: 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol Neurobiol* 8(2-3), 121-7 (1994)

85.H. P. Lipp, M. Stagliar-Bozicevic, M. Fischer and D. P. Wolfer: A 2-year longitudinal study of swimming navigation in mice devoid of the prion protein: no evidence for neurological anomalies or spatial learning impairments. *Behav Brain Res* 95(1), 47-54 (1998)

86.M. R. Ehlers and J. F. Riordan: Membrane proteins with soluble counterparts: role of proteolysis in the release of transmembrane proteins. *Biochemistry* 30(42), 10065-74 (1991)

87.S. G. Chen, D. B. Teplow, P. Parchi, J. K. Teller, P. Gambetti and L. Autilio-Gambetti: Truncated forms of the human prion protein in normal brain and in prion diseases. *J Biol Chem* 270(32), 19173-80. (1995)

88.T. Pan, R. Li, B. S. Wong, T. Liu, P. Gambetti and M. S. Sy: Heterogeneity of normal prion protein in two-

dimensional immunoblot: presence of various glycosylated and truncated forms. *J Neurochem* 81(5), 1092-101 (2002)
89.A. Mange, F. Beranger, K. Peoc'h, T. Onodera, Y. Frobert and S. Lehmann: Alpha- and beta- cleavages of the amino-terminus of the cellular prion protein. *Biol Cell* 96(2), 125-32 (2004)

90.A. Jimenez-Huete, P. M. Lievens, R. Vidal, P. Piccardo, B. Ghetti, F. Tagliavini, B. Frangione and F. Prelli: Endogenous proteolytic cleavage of normal and disease-associated isoforms of the human prion protein in neural and non-neural tissues. *Am J Pathol* 153(5), 1561-72. (1998)

91.B. Vincent, E. Paitel, Y. Frobert, S. Lehmann, J. Grassi and F. Checler: Phorbol ester-regulated cleavage of normal prion protein in HEK293 human cells and murine neurons. *J Biol Chem* 275(45), 35612-6 (2000)
92.V. Lewis, A. F. Hill, C. L. Haigh, G. M. Klug, C. L. Masters, V. A. Lawson and S. J. Collins: Increased Proportions of C1 Truncated Prion Protein Protect Against Cellular M1000 Prion Infection. *J Neuropathol Exp Neurol* 68(10), 1125-35 (2009)

93.A. R. Walmsley, N. T. Watt, D. R. Taylor, W. S. Perera and N. M. Hooper: alpha-cleavage of the prion protein occurs in a late compartment of the secretory pathway and is independent of lipid rafts. *Mol Cell Neurosci* 40(2), 242-8 (2009)

94.C. Sunyach, M. A. Cisse, C. A. da Costa, B. Vincent and F. Checler: The C-terminal products of cellular prion protein processing, C1 and C2, exert distinct influence on p53-dependent staurosporine-induced caspase-3 activation. *J Biol Chem* 282(3), 1956-63 (2007)

95.M. V. Guillot-Sestier, C. Sunyach, C. Druon, S. Scarzello and F. Checler: The alpha-secretase-derived N-terminal product of cellular prion, N1, displays neuroprotective function *in vitro* and *in vivo*. *J Biol Chem* 284(51), 35973-86 (2009)

96.H. E. McMahon, A. Mange, N. Nishida, C. Creminon, D. Casanova and S. Lehmann: Cleavage of the amino terminus of the prion protein by reactive oxygen species. *J Biol Chem* 276(3), 2286-91 (2001)

97.N. T. Watt, D. R. Taylor, A. Gillott, D. A. Thomas, W. S. Perera and N. M. Hooper: Reactive oxygen species-mediated beta-cleavage of the prion protein in the cellular response to oxidative stress. *J Biol Chem* 280(43), 35914-21 (2005)

98.C. L. Haigh, S. C. Drew, M. P. Boland, C. L. Masters, K. J. Barnham, V. A. Lawson and S. J. Collins: Dominant roles of the polybasic proline motif and copper in the PrP23-89-mediated stress protection response. *J Cell Sci* 122(Pt 10), 1518-28 (2009)

99.J. A. Allen, R. A. Halverson-Tamboli and M. M. Rasenick: Lipid raft microdomains and neurotransmitter signalling. *Nat Rev Neurosci* 8(2), 128-40 (2007)

100.N. A. Mabbott, K. L. Brown, J. Manson and M. E. Bruce: T-lymphocyte activation and the cellular form of the prion protein. *Immunology* 92(2), 161-5 (1997)

101.N. R. Cashman, R. Loertscher, J. Nalbantoglu, I. Shaw, R. J. Kascsak, D. C. Bolton and P. E. Bendheim: Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* 61(1), 185-92 (1990)

102.B. Hugel, M. C. Martinez, C. Kunzelmann, T. Blattler, A. Aguzzi and J. M. Freyssinet: Modulation of signal transduction through the cellular prion protein is linked to its incorporation in lipid rafts. *Cell Mol Life Sci* 61(23), 2998-3007 (2004)

103.C. A. Stuermer, M. F. Langhorst, M. F. Wiechers, D. F. Legler, S. H. Von Hanwehr, A. H. Guse and H. Plattner: 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* 18(14), 1731-3 (2004)

104.C. Monnet, J. Gavard, R. M. Mege and A. Sobel: Clustering of cellular prion protein induces ERK1/2 and stathmin phosphorylation in GT1-7 neuronal cells. *FEBS Lett* 576(1-2), 114-8 (2004)

105.S. Mouillet-Richard, M. Ermonval, C. Chebassier, J. L. Laplanche, S. Lehmann, J. M. Launay and O. Kellermann: Signal transduction through prion protein. *Science* 289(5486), 1925-8 (2000)

106.B. Schneider, V. Mutel, M. Pietri, M. Ermonval, S. Mouillet-Richard and O. Kellermann: NADPH oxidase and extracellular regulated kinases 1/2 are targets of prion protein signaling in neuronal and nonneuronal cells. *Proc Natl Acad Sci U S A* 100(23), 13326-31 (2003)

107.C. L. Haigh, V. A. Lewis, L. J. Vella, C. L. Masters, A. F. Hill, V. A. Lawson and S. J. Collins: PrPC-related signal transduction is influenced by copper, membrane integrity and the alpha cleavage site. *Cell Res* 19(9), 1062-78 (2009)

108.C. Spielhaupter and H. M. Schatzl: PrPC directly interacts with proteins involved in signaling pathways. *J Biol Chem* 276(48), 44604-12 (2001)

109.L. B. Chiarini, A. R. Freitas, S. M. Zanata, R. R. Brentani, V. R. Martins and R. Linden: Cellular prion protein transduces neuroprotective signals. *Embo J* 21(13), 3317-26 (2002)

110.S. M. Zanata, M. H. Lopes, A. F. Mercadante, G. N. Hajj, L. B. Chiarini, R. Nomizo, A. R. Freitas, A. L. Cabral, K. S. Lee, M. A. Juliano, E. de Oliveira, S. G. Jachieri, A. Burlingame, L. Huang, R. Linden, R. R. Brentani and V. R. Martins: Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J* 21(13), 3307-16 (2002)

111.D. R. Brown, R. S. Nicholas and L. Canevari: Lack of prion protein expression results in a neuronal phenotype sensitive to stress. *J Neurosci Res* 67(2), 211-24 (2002)

112.L. Solforosi, J. R. Criado, D. B. McGavern, S. Wirz, M. Sanchez-Alavez, S. Sugama, L. A. DeGiorgio, B. T. Volpe, E. Wiseman, G. Abalos, E. Masliah, D. Gilden, M. B. Oldstone, B. Conti and R. A. Williamson: Cross-linking cellular prion protein triggers neuronal apoptosis *in vivo*. *Science* 303(5663), 1514-6 (2004)

113.D. R. Brown, K. Qin, J. W. Herms, A. Madlung, J. Manson, R. Strome, P. E. Fraser, T. Kruck, A. von Bohlen, W. Schulz-Schaeffer, A. Giese, D. Westaway and H. Kretzschmar: The cellular prion protein binds copper *in vivo*. *Nature* 390(6661), 684-7 (1997)

114.F. Klamt, F. Dal-Pizzol, M. J. Conte da Frota, R. Walz, M. E. Andrades, E. G. da Silva, R. R. Brentani, I. Izquierdo and J. C. Fonseca Moreira: Imbalance of antioxidant defense in mice lacking cellular prion protein. *Free Radic Biol Med* 30(10), 1137-44 (2001)

115.K. J. Lee, A. Panzera, D. Rogawski, L. E. Greene and E. Eisenberg: Cellular prion protein (PrPC) protects neuronal cells from the effect of huntingtin aggregation. *J Cell Sci* 120(Pt 15), 2663-71 (2007)

116.D. R. Brown, B. S. Wong, F. Hafiz, C. Clive, S. J. Haswell and I. M. Jones: Normal prion protein has an activity like that of superoxide dismutase. *Biochem J* 344 Pt 1, 1-5 (1999)

117.D. J. Waggoner, B. Drisaldi, T. B. Bartnikas, R. L. Casareno, J. R. Prohaska, J. D. Gitlin and D. A. Harris: Brain copper content and cuproenzyme activity do not vary with prion protein expression level. *J Biol Chem* 275(11), 7455-8 (2000)

118.G. Hutter, F. L. Heppner and A. Aguzzi: No superoxide dismutase activity of cellular prion protein *in vivo*. *Biol Chem* 384(9), 1279-85 (2003)

119.S. Jones, M. Batchelor, D. Bhelt, A. R. Clarke, J. Collinge and G. S. Jackson: Recombinant prion protein does not possess SOD-1 activity. *Biochem J* 392(Pt 2), 309-12 (2005)

120.M. Premzl, M. Delbridge, J. E. Gready, P. Wilson, M. Johnson, J. Davis, E. Kuczek and J. A. Marshall Graves: The prion protein gene: identifying regulatory signals using marsupial sequence. *Gene* 349, 121-34 (2005)

121.V. Wang, T. C. Chuang, Y. D. Hsu, W. Y. Chou and M. C. Kao: Nitric oxide induces prion protein via MEK and p38 MAPK signaling. *Biochem Biophys Res Commun* 333(1), 95-100 (2005)

122.W. C. Shyu, S. Z. Lin, K. Saeki, A. Kubosaki, Y. Matsumoto, T. Onodera, M. F. Chiang, P. Thajeb and H. Li: Hyperbaric oxygen enhances the expression of prion protein and heat shock protein 70 in a mouse neuroblastoma cell line. *Cell Mol Neurobiol* 24(2), 257-68 (2004)

123.M. P. Hornshaw, J. R. McDermott and J. M. Candy: Copper binding to the N-terminal tandem repeat regions of mammalian and avian prion protein. *Biochem Biophys Res Commun* 207(2), 621-9 (1995)

124.M. P. Hornshaw, J. R. McDermott, J. M. Candy and J. H. Lakey: Copper binding to the N-terminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides. *Biochem Biophys Res Commun* 214(3), 993-9 (1995)

125.C. S. Burns, E. Aronoff-Spencer, C. M. Dunham, P. Lario, N. I. Avdievich, W. E. Antholine, M. M. Olmstead, A. Vrielink, G. J. Gerfen, J. Peisach, W. G. Scott and G. L. Millhauser: Molecular features of the copper binding sites in the octarepeat domain of the prion protein. *Biochemistry* 41(12), 3991-4001. (2002)

126.A. P. Garnett and J. H. Viles: Copper binding to the octarepeats of the prion protein. Affinity, specificity, folding, and cooperativity: insights from circular dichroism. *J Biol Chem* 278(9), 6795-802 (2003)

127.M. F. Jobling, X. Huang, L. R. Stewart, K. J. Barnham, C. Curtain, I. Volitakis, M. Perugini, A. R. White, R. A. Cherny, C. L. Masters, C. J. Barrow, S. J. Collins, A. I. Bush and R. Cappai: Copper and zinc binding modulates the aggregation and neurotoxic properties of the prion peptide PrP106-126. *Biochemistry* 40(27), 8073-84 (2001)

128.G. S. Jackson, I. Murray, L. L. Hosszu, N. Gibbs, J. P. Waltho, A. R. Clarke and J. Collinge: Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci U S A* 98(15), 8531-5 (2001)

129.P. C. Pauly and D. A. Harris: Copper stimulates endocytosis of the prion protein. *J Biol Chem* 273(50), 33107-10 (1998)

130.K. S. Lee, A. C. Magalhaes, S. M. Zanata, R. R. Brentani, V. R. Martins and M. A. Prado: Internalization of mammalian fluorescent cellular prion protein and N-terminal deletion mutants in living cells. *J Neurochem* 79(1), 79-87 (2001)

131.D. R. Brown: Prion protein expression aids cellular uptake and veratridine-induced release of copper. *J Neurosci Res* 58(5), 717-25 (1999)

132.W. Rachidi, D. Vilette, P. Guiraud, M. Arlotto, J. Riondel, H. Laude, S. Lehmann and A. Favier: Expression of prion protein increases cellular copper binding and antioxidant enzyme activities but not copper delivery. *J Biol Chem* 278(11), 9064-72 (2003)

133.J. W. Herms, T. Tings, S. Dunker and H. A. Kretzschmar: Prion protein affects Ca2+-activated K+ currents in cerebellar purkinje cells. *Neurobiol Dis* 8(2), 324-30 (2001)

134.S. B. Colling, J. Collinge and J. G. Jefferys: Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K+ currents. *Neurosci Lett* 209(1), 49-52 (1996)

135.J. Collinge, M. A. Whittington, K. C. Sidle, C. J. Smith, M. S. Palmer, A. R. Clarke and J. G. Jefferys: Prion

protein is necessary for normal synaptic function. *Nature* 370(6487), 295-7 (1994)

136.G. R. Mallucci, S. Ratte, E. A. Asante, J. Linehan, I. Gowland, J. G. Jefferys and J. Collinge: Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *Embo J* 21(3), 202-10 (2002)

137.J. R. Criado, M. Sanchez-Alavez, B. Conti, J. L. Giacchino, D. N. Wills, S. J. Henriksen, R. Race, J. C. Manson, B. Chesebro and M. B. Oldstone: Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. *Neurobiol Dis* 19(1-2), 255-65 (2005)

138.A. S. Coitinho, R. Roesler, V. R. Martins, R. R. Brentani and I. Izquierdo: Cellular prion protein ablation impairs behavior as a function of age. *Neuroreport* 14(10), 1375-9 (2003)

139.J. C. Manson, J. Hope, A. R. Clarke, A. Johnston, C. Black and N. MacLeod: PrP gene dosage and long term potentiation. *Neurodegeneration* 4(1), 113-4 (1995)

140.I. Tobler, T. Deboer and M. Fischer: Sleep and sleep regulation in normal and prion protein-deficient mice. *J Neurosci* 17(5), 1869-79 (1997)

141.I. Tobler, S. E. Gaus, T. Deboer, P. Achermann, M. Fischer, T. Rulicke, M. Moser, B. Oesch, P. A. McBride and J. C. Manson: Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* 380(6575), 639-42 (1996)

142.E. T. Parkin, N. T. Watt, I. Hussain, E. A. Eckman, C. B. Eckman, J. C. Manson, H. N. Baybutt, A. J. Turner and N. M. Hooper: Cellular prion protein regulates beta-secretase cleavage of the Alzheimer's amyloid precursor protein. *Proc Natl Acad Sci U S A* 104(26), 11062-7 (2007)

143.J. Lauren, D. A. Gimbel, H. B. Nygaard, J. W. Gilbert and S. M. Strittmatter: Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457(7233), 1128-32 (2009)

144.B. Vincent, C. Sunyach, H. D. Orzechowski, P. St George-Hyslop and F. Checler: p53-Dependent transcriptional control of cellular prion by presenilins. *J Neurosci* 29(20), 6752-60 (2009)

145.K. A. Kellett and N. M. Hooper: Prion protein and Alzheimer disease. *Prion* 3(4), 190-4 (2009)

146.J. Kazlauskaite, N. Sanghera, I. Sylvester, C. Venien-Bryan and T. J. Pinheiro: Structural changes of the prion protein in lipid membranes leading to aggregation and fibrillization. *Biochemistry* 42(11), 3295-304 (2003)

147.F. Wang, F. Yang, Y. Hu, X. Wang, C. Jin and J. Ma: Lipid interaction converts prion protein to a PrPSc-like proteinase K-resistant conformation under physiological conditions. *Biochemistry* 46(23), 7045-53 (2007)

148.N. S. Hachiya, M. Imagawa and K. Kaneko: The possible role of protein X, a putative auxiliary factor in pathological prion replication, in regulating a physiological endoproteolytic cleavage of cellular prion protein. *Med Hypotheses* 68(3), 670-3 (2007)

149.K. Kaneko, L. Zulianello, M. Scott, C. M. Cooper, A. C. Wallace, T. L. James, F. E. Cohen and S. B. Prusiner: Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc Natl Acad Sci U S A* 94(19), 10069-74. (1997)

150.G. C. Telling, M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F. E. Cohen, S. J. DeArmond and S. B. Prusiner: Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 83(1), 79-90 (1995)

151.F. Yehiely, P. Bamborough, M. Da Costa, B. J. Perry, G. Thinakaran, F. E. Cohen, G. A. Carlson and S. B. Prusiner: Identification of candidate proteins binding to prion protein. *Neurobiol Dis* 3(4), 339-55 (1997)

152.M. P. Gomes, T. A. Millen, P. S. Ferreira, E. S. N. L. Cunha, T. C. Vieira, M. S. Almeida, J. L. Silva and Y. Cordeiro: Prion protein complexed to N2a cellular RNAs through its N-terminal domain forms aggregates and is toxic to murine neuroblastoma cells. *J Biol Chem* (2008)

153.N. R. Deleault, R. W. Lucassen and S. Supattapone: RNA molecules stimulate prion protein conversion. *Nature* 425(6959), 717-20 (2003)

154.V. Adler, B. Zeiler, V. Kryukov, R. Kascsak, R. Rubenstein and A. Grossman: Small, highly structured RNAs participate in the conversion of human recombinant PrP(Sen) to PrP(Res) *in vitro*. *J Mol Biol* 332(1), 47-57 (2003)

155.J. C. Geoghegan, P. A. Valdes, N. R. Orem, N. R. Deleault, R. A. Williamson, B. T. Harris and S. Supattapone: Selective incorporation of polyanionic molecules into hamster prions. *J Biol Chem* 282(50), 36341-53 (2007)

156.M. Liu, S. Yu, J. Yang, X. Yin and D. Zhao: RNA and CuCl2 induced conformational changes of the recombinant ovine prion protein. *Mol Cell Biochem* 294(1-2), 197-203 (2007)

157.O. Schonberger, L. Horonchik, R. Gabizon, D. Papy-Garcia, D. Barritault and A. Taraboulos: Novel heparan mimetics potently inhibit the scrapie prion protein and its endocytosis. *Biochem Biophys Res Commun* 312(2), 473-9 (2003)

158.O. Ben-Zaken, S. Tzaban, Y. Tal, L. Horonchik, J. D. Esko, I. Vlodavsky and A. Taraboulos: Cellular heparan sulfate participates in the metabolism of prions. *J Biol Chem* 278(41), 40041-9 (2003)

159.T. Mayer-Sonnenfeld, M. Zeigler, M. Halimi, Y. Dayan, C. Herzog, C. I. Lasmezas and R. Gabizon: The metabolism of glycosaminoglycans is impaired in prion diseases. *Neurobiol Dis* 20(3), 738-43 (2005)

160.K. Lofgren, F. Cheng, L. A. Fransson, K. Bedecs and K. Mani: Involvement of glypican-1 autoprocessing in scrapie infection. *Eur J Neurosci* 28(5), 964-72 (2008)

161.C. Monnet, V. Marthiens, H. Enslen, Y. Frobert, A. Sobel and R. M. Mege: Heterogeneity and regulation of cellular prion protein glycoforms in neuronal cell lines. *Eur J Neurosci* 18(3), 542-8 (2003)

162.R. A. Bessen and R. F. Marsh: Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. *J Virol* 66(4), 2096-101 (1992)

163.R. A. Bessen and R. F. Marsh: Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol* 68(12), 7859-68 (1994)

164.R. A. Somerville, A. Chong, O. U. Mulqueen, C. R. Birkett, S. C. Wood and J. Hope: Biochemical typing of scrapie strains. *Nature* 386(6625), 564 (1997)

165.R. A. Bessen, D. A. Kocisko, G. J. Raymond, S. Nandan, P. T. Lansbury and B. Caughey: Non-genetic propagation of strain-specific properties of scrapie prion protein. *Nature* 375(6533), 698-700 (1995)

166.D. Peretz, R. A. Williamson, G. Legname, Y. Matsunaga, J. Vergara, D. R. Burton, S. J. DeArmond, S. B. Prusiner and M. R. Scott: A change in the conformation of prions accompanies the emergence of a new prion strain. *Neuron* 34(6), 921-32 (2002)

167.G. C. Telling, P. Parchi, S. J. DeArmond, P. Cortelli, P. Montagna, R. Gabizon, J. Mastrianni, E. Lugaresi, P. Gambetti and S. B. Prusiner: Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 274(5295), 2079-82 (1996)

168.B. Caughey, G. J. Raymond and R. A. Bessen: Strain-dependent differences in beta-sheet conformations of abnormal prion protein. *J Biol Chem* 273(48), 32230-5 (1998)

169.K. A. Nishina, N. R. Deleault, S. P. Mahal, I. Baskakov, T. Luhrs, R. Riek and S. Supattapone: The stoichiometry of host PrPC glycoforms modulates the efficiency of PrPSc formation *in vitro*. *Biochemistry* 45(47), 14129-39 (2006)

170.N. L. Tuzi, E. Cancellotti, H. Baybutt, L. Blackford, B. Bradford, C. Plinston, A. Coghill, P. Hart, P. Piccardo, R. M. Barron and J. C. Manson: Host PrP Glycosylation: A Major Factor Determining the Outcome of Prion Infection. In: *PLoS Biol*. (2008)

171.L. Laszlo, J. Lowe, T. Self, N. Kenward, M. Landon, T. McBride, C. Farquhar, I. McConnell, J. Brown, J. Hope and *et al.*: Lysosomes as key organelles in the pathogenesis of prion encephalopathies. *J Pathol* 166(4), 333-41 (1992)

172.B. Caughey, G. J. Raymond, D. Ernst and R. E. Race: N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): implications regarding the site of conversion of PrP to the protease-resistant state. *J Virol* 65(12), 6597-603 (1991)

173.M. P. McKinley, A. Taraboulos, L. Kenaga, D. Serban, A. Stieber, S. J. DeArmond, S. B. Prusiner and N. Gonatas: Ultrastructural localization of scrapie prion proteins in cytoplasmic vesicles of infected cultured cells. *Lab Invest* 65(6), 622-30 (1991)

174.J. E. Arnold, C. Tipler, L. Laszlo, J. Hope, M. Landon and R. J. Mayer: The abnormal isoform of the prion protein accumulates in late-endosome-like organelles in scrapie-infected mouse brain. *J Pathol* 176(4), 403-11 (1995)

175.V. Grigoriev, F. Escaig-Haye, N. Streichenberger, N. Kopp, J. Langeveld, P. Brown and J. G. Fournier: Submicroscopic immunodetection of PrP in the brain of a patient with a new-variant of Creutzfeldt-Jakob disease. *Neurosci Lett* 264(1-3), 57-60 (1999)

176.F. Pimpinelli, S. Lehmann and I. Maridonneau-Parini: The scrapie prion protein is present in flotillin-1-positive vesicles in central- but not peripheral-derived neuronal cell lines. *Eur J Neurosci* 21(8), 2063-72 (2005)

177.N. M. Veith, H. Plattner, C. A. Stuermer, W. J. Schulz-Schaeffer and A. Burkle: Immunolocalisation of PrPSc in scrapie-infected N2a mouse neuroblastoma cells by light and electron microscopy. *Eur J Cell Biol* 88(1), 45-63 (2009)

178.M. Kristiansen, P. Deriziotis, D. E. Dimcheff, G. S. Jackson, H. Ovaa, H. Naumann, A. R. Clarke, F. W. van Leeuwen, V. Menendez-Benito, N. P. Dantuma, J. L. Portis, J. Collinge and S. J. Tabrizi: Disease-associated prion protein oligomers inhibit the 26S proteasome. *Mol Cell* 26(2), 175-88 (2007)

179.E. Flechsig, I. Hegyi, M. Enari, P. Schwarz, J. Collinge and C. Weissmann: Transmission of scrapie by steel-surface-bound prions. *Mol Med* 7(10), 679-84 (2001)

180.N. Kanu, Y. Imokawa, D. N. Drechsel, R. A. Williamson, C. R. Birkett, C. J. Bostock and J. P. Brockes: Transfer of scrapie prion infectivity by cell contact in culture. *Curr Biol* 12(7), 523-30 (2002)

181.N. Naslavsky, H. Shmeeda, G. Friedlander, A. Yanai, A. H. Futerman, Y. Barenholz and A. Taraboulos: Sphingolipid depletion increases formation of the scrapie prion protein in neuroblastoma cells infected with prions. *J Biol Chem* 274(30), 20763-71 (1999)

182.B. Chesebro, M. Trifilo, R. Race, K. Meade-White, C. Teng, R. LaCasse, L. Raymond, C. Favara, G. Baron, S. Priola, B. Caughey, E. Masliah and M. Oldstone: Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science* 308(5727), 1435-9 (2005)

183.V. Campana, A. Caputo, D. Sarnataro, S. Paladino, S. Tivodar and C. Zurzolo: Characterization of the properties and trafficking of an anchorless form of the prion protein. *J Biol Chem* 282(31), 22747-56 (2007)

184.B. Chesebro, B. Race, K. Meade-White, R. Lacasse, R. Race, M. Klingeborn, J. Striebel, D. Dorward, G. McGovern and M. Jeffrey: Fatal transmissible amyloid encephalopathy: a new type of prion disease associated with lack of prion protein membrane anchoring. *PLoS Pathog* 6(3), e1000800 (2010)

185.K. L. McNally, A. E. Ward and S. A. Priola: Cells expressing anchorless prion protein are resistant to scrapie infection. *J Virol* 83(9), 4469-75 (2009)

186.W. Q. Zou and N. R. Cashman: Acidic pH and detergents enhance *in vitro* conversion of human brain PrPC to a PrPSc-like form. *J Biol Chem* 277(46), 43942-7 (2002)

187.S. Hornemann and R. Glockshuber: A scrapie-like unfolding intermediate of the prion protein domain PrP(121-231) induced by acidic pH. *Proc Natl Acad Sci U S A* 95(11), 6010-4 (1998)

188.W. Swietnicki, R. Petersen, P. Gambetti and W. K. Surewicz: pH-dependent stability and conformation of the recombinant human prion protein PrP(90-231). *J Biol Chem* 272(44), 27517-20 (1997)

189.D. R. Borchelt, A. Taraboulos and S. B. Prusiner: Evidence for synthesis of scrapie prion proteins in the endocytic pathway. *J Biol Chem* 267(23), 16188-99 (1992)

190.Z. Marijanovic, A. Caputo, V. Campana and C. Zurzolo: Identification of an intracellular site of prion conversion. *PLoS Pathog* 5(5), e1000426 (2009)

191.S. Gurke, J. F. Barroso and H. H. Gerdes: The art of cellular communication: tunneling nanotubes bridge the divide. *Histochem Cell Biol* 129(5), 539-50 (2008)

192.A. C. Magalhaes, G. S. Baron, K. S. Lee, O. Steele-Mortimer, D. Dorward, M. A. Prado and B. Caughey: Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. *J Neurosci* 25(21), 5207-16 (2005)

193.K. Gousset, E. Schiff, C. Langevin, Z. Marijanovic, A. Caputo, D. T. Browman, N. Chenouard, F. de Chaumont, A. Martino, J. Enninga, J. C. Olivo-Marin, D. Mannel and C. Zurzolo: Prions hijack tunnelling nanotubes for intercellular spread. *Nat Cell Biol* 11(3), 328-36 (2009)

194.D. F. Legler, M. A. Doucey, P. Schneider, L. Chapatte, F. C. Bender and C. Bron: Differential insertion of GPI-anchored GFPs into lipid rafts of live cells. *Faseb J* 19(1), 73-5 (2005)

195.D. L. Kooyman, G. W. Byrne, S. McClellan, D. Nielsen, M. Tone, H. Waldmann, T. M. Coffman, K. R. McCurry, J. L. Platt and J. S. Logan: *In vivo* transfer of GPI-linked complement restriction factors from erythrocytes to the endothelium. *Science* 269(5220), 89-92 (1995)

196.D. R. Premkumar, Y. Fukuoka, D. Sevlever, E. Brunschwig, T. L. Rosenberry, M. L. Tykocinski and M. E. Medof: Properties of exogenously added GPI-anchored proteins following their incorporation into cells. *J Cell Biochem* 82(2), 234-45 (2001)

Abbreviations: AD: Alzheimer's disease, AICD: amyloid precursor protein intracellular domain, APP: amyloid precursor protein, cAMP: cyclic adenosine monophosphate, CLD: caveolae-like domain, DRM: detergent resistant membrane, ER: endoplasmic reticulum, ERAD: endoplasmic reticulum associated degradation, ERK: extracellular signal regulated kinase, GAG: glycosaminoglycan, GPI: glycosylphosphatidylinositol, LRP1: low-density lipoprotein receptor–related protein-1, MAPK: mitogen-activated protein kinase, MVB: multivesicular body, NADPH: nicotinamide adenine dinucleotide phosphate, PIPLC: phosphatidylinositol-specific phospholipase C, PM: plasma membrane, *Prnp*: prion protein gene, PrP: prion protein, PrP^{C}: prion protein cellular isoform, PrP^{res}: prion protein protease resistant isoform, PrP^{Sc}: prion protein misfolded disease associated isoform, ROS: reactive oxygen species, SOD: superoxide dismutase, TNT: tunnelling nanotube

Key Words: Prion, Lipid Raft, Cholesterol, Sphingolipid, Caveolae, Review

Send correspondence to: Nigel M. Hooper, Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, LIGHT Laboratories, University of Leeds, Leeds LS2 9JT, UK, Tel: 44 113 343 3163, Fax: 44 113 343 5638, E-mail: n.m.hooper@leeds.ac.uk

http://www.bioscience.org/current/vol16.htm