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# Subcellular distribution of the prion protein in sickness and in health

Susan F. Godsave[a,*], Peter J. Peters[a,b], Holger Wille[c,**]

[a] Department of Cell Biology II, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands
[b] Institute of Nanoscopy, University of Maastricht, Universiteitssingel 50, 6229 ER Maastricht, Netherlands[1]
[c] Department of Biochemistry and Centre for Prions and Protein Folding Diseases, University of Alberta, 204 Brain and Aging Research Building, Edmonton, Alberta T6G 2M8, Canada

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ABSTRACT

The cellular prion protein ($PrP^{C}$) is an ubiquitously expressed glycoprotein that is most abundant in the central nervous system. It is thought to play a role in many cellular processes, including neuroprotection, but may also contribute to Alzheimer's disease and some cancers. However, it is best known for its central role in the prion diseases, such as Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), and scrapie. These protein misfolding diseases can be sporadic, acquired, or genetic and are caused by refolding of endogenous $PrP^{C}$ into a beta sheet-rich, pathogenic form, $PrP^{Sc}$. Once prions are present in the central nervous system, they increase and spread during a long incubation period that is followed by a relatively short clinical disease phase, ending in death. PrP molecules can be broadly categorized as either 'good' (cellular) $PrP^{C}$ or 'bad' (scrapie prion-type) $PrP^{Sc}$, but both populations are heterogeneous and different forms of $PrP^{C}$ may influence various cellular activities. Both $PrP^{C}$ and $PrP^{Sc}$ are localized predominantly at the cell surface, with the C-terminus attached to the plasma membrane via a glycosyl-phosphatidylinositol (GPI) anchor and both can exist in cleaved forms. $PrP^{C}$ also has cytosolic and transmembrane forms, and $PrP^{Sc}$ is known to exist in a variety of conformations and aggregation states. Here, we discuss the roles of different PrP isoforms in sickness and in health, and show the subcellular distributions of several forms of PrP that are particularly relevant for $PrP^{C}$ to $PrP^{Sc}$ conversion and prion-induced pathology in the hippocampus.



## 1. $PrP^{C}$-in health

### 1.1. Cell surface $PrP^{C}$ may influence many cellular activities

Mature $PrP^{C}$ is a secreted, 208–209 amino acid protein, tethered to the cell membrane with a GPI anchor (Stahl et al., 1993). $PrP^{C}$ has an alpha helix-rich C-terminal globular domain, containing two asparagine-linked glycosylation sites and an intramolecular disulphide bond. It has a hydrophobic central region and a relatively unstructured N-terminal domain, containing five repeats of a copper-binding octapeptide (reviewed in Colby and Prusiner, 2011) (Fig. 1b).

In normal tissues, $PrP^{C}$ is localized predominantly on plasma membranes, as illustrated by cryo-immunogold electron microscopy (EM) of brain (Godsave et al., 2008; Mironov et al., 2003) (Fig. 2). Here, it appears to follow the standard biosynthetic trafficking pathway for GPI-anchored glycoproteins and is then transported to axons and dendrites in the neuropil, with no apparent preferential localization at synapses. It is internalized via endosomes and recycled back to the plasma membrane and can be detected by cryo-immunogold electron microscopy in small diameter vesicles resembling early endocytic or recycling vesicles. It is also found, less frequently, in late endosomal structures (Godsave et al., 2008; Mironov et al., 2003), where degradation of membrane-associated $PrP^{C}$ occurs. For a review of $PrP^{C}$ trafficking, see Campana et al. (2005).

GPI-anchored $PrP^{C}$ is often associated with cholesterol- and sphingolipid-rich membrane 'rafts' which can serve as organizing centres for signalling complexes that may influence a variety of cellular activities (reviewed in Campana et al., 2005). GPI-anchored $PrP^{C}$ does not span the membrane and cannot transduce signals into

*Abbreviations:* BSE, bovine spongiform encephalopathy; cAMP, cyclic adenosine monophosphate; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; EM, electron microscopy; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GPI, glycosyl-phosphatidylinositol; *PRNP*, human prion protein gene; PrP, prion protein; $PrP^{C}$, cellular form of PrP; $PrP^{Sc}$, prion form of PrP; SDS PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; UPR, unfolded protein response.

* Corresponding author.
** Corresponding author. Tel.: +17802481712.
*E-mail addresses:* s.godsave@outlook.com (S.F. Godsave), wille@ualberta.ca (H. Wille).
[1] Present address.

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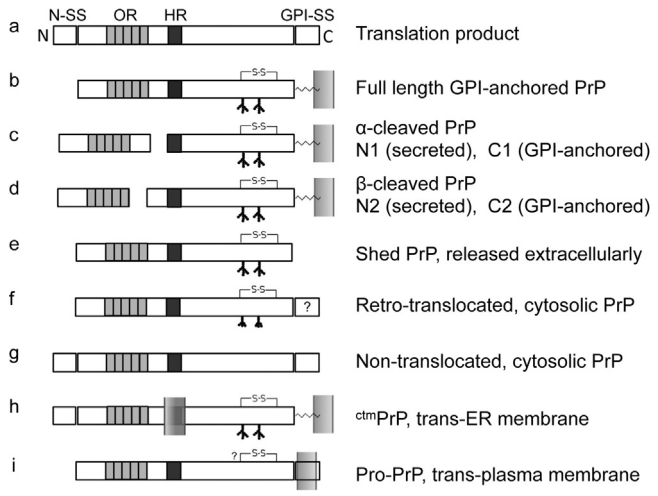


**Fig. 1.** $PrP^C$ forms described in the text. Translation of the human *PRNP* gene results in a pre-pro form with N-terminal signal sequence for secretion (N-SS, residues 1–22) and signal sequence for GPI anchor addition (GPI-SS, residues 232–253) (a). The N-SS and/or GPI-SS are retained in some post-translationally modified forms of PrP (f–i). Octarepeat, metal-binding sites are shown as grey rectangles (OR, residues 59–90), and the hydrophobic region (HR, residues 112–133) as a black rectangle. The presence of a GPI anchor is illustrated in lower images by a zigzag line and a membrane by a large grey rectangle. In mature $PrP^C$ there is a single disulphide bond between residues 179 and 214 and glycosylation sites at residues 181 and 197, which may be variably filled. Non-translocated PrP and pro-PrP are reported to be unglycosylated (g and i). Glycosylation in retro-translocated PrP is thought to be immature (f). A question mark (?) indicates uncertainty about the presence of a structure. References are provided in the text.

the cytosol, but it has many binding partners and has been proposed to play a role in the assembly of signalling complexes. Among its cell surface binding partners are stress inducible protein 1 (STI1) (Lopes et al., 2005; Zanata et al., 2002), the 37 kDa Laminin Receptor Precursor/67 kDa Laminin Receptor (37LRP/67LR) (Gauczynski et al., 2001), neural cell adhesion molecule (NCAM) (Santuccione et al., 2005; Schmitt-Ulms et al., 2001), laminin (Graner et al., 2000) and vitronectin (Hajj et al., 2007), as well as glycosaminoglycans (Caughey et al., 1994; Pan et al., 2002) and copper (Brown et al., 1997; Jones et al., 2004) (reviewed in Linden et al., 2012; Martins et al., 2010). Several studies have further indicated that $PrP^C$ is involved in signalling via the cAMP-dependent protein kinase A and ERK1/2 pathways, thereby influencing neural survival and neuritogenesis, and possibly memory formation (reviewed in Didonna, 2013; Martins et al., 2010). $PrP^C$ is also reported to regulate the proliferation of haematopoietic stem cells, human embryonic stem cells and neural precursors (Lee and Baskakov, 2010; Santos et al., 2011; Steele et al., 2007c).

A major function of $PrP^C$ appears to be protection of neurons from oxidative stress and apoptosis. Mice in which the prion gene has been knocked out are viable and resistant to prion infection (Bueler et al., 1993), but have subtle abnormalities consistent with a neuroprotective function for $PrP^C$, including enhanced susceptibility to ischaemia. Other reported effects include abnormalities in sleep, brain copper levels, neuronal excitability and memory (Steele et al., 2007c). In zebrafish, which have two PrP genes, PrP-2 appears to have similar functions to mammalian $PrP^C$ (Fleisch et al., 2013). By contrast, loss of *PrP-1* function is embryonic lethal, causing defects in cell-to-cell adhesion and gastrulation arrest (Malaga-Trillo et al., 2009).

### 1.2. Release of cytoprotective fragments by $PrP^C$ cleavage and shedding

A physiological cleavage of membrane-associated $PrP^C$ after amino acid 110 or 111 (humans) releases a soluble N-terminal fragment (N1) and creates a GPI-anchored C-terminal fragment (C1) (Chen et al., 1995; Harris et al., 1993) (Fig. 1c). This so-called alpha cleavage occurs adjacent to the central hydrophobic region of PrP that is necessary for $PrP^C$ to $PrP^{Sc}$ conversion, and transgenic mice expressing only the C1 region of PrP are resistant to infectious prion disease (Lewis et al., 2009; Westergard et al., 2011). Proteolytic cleavage may be a mechanism for regulating $PrP^C$ activity, since the N-terminal portion contains the binding sites for a variety of ligands (Martins et al., 2010). Cytoprotective activities have been described for N1. Addition of recombinant N1 to cultured cortical neurons reduced staurosporine-induced caspase-3 activation in a p53-depedent manner and N1 was also able to protect cells from amyloid β-induced neurotoxicity (Guillot-Sestier et al., 2009, 2012). The function of C1 seems less clear (see Section 2.3).

Another type of cleavage, termed beta cleavage, has been found to occur at around amino acid 90 of $PrP^C$ (Mange et al., 2004) (Fig. 1d). This can be mediated by reactive oxygen species and is associated with greater cell viability in response to oxidative stress (McMahon et al., 2001; Watt et al., 2005). $PrP^{Sc}$ can undergo cleavage at a similar position (see Section 4.1).

Cleavage of $PrP^C$ close to the GPI anchor has also been described, causing the release of soluble, almost full length $PrP^C$ from the membrane (Borchelt et al., 1993; Stahl et al., 1990; Wik et al., 2012) (Fig. 1e). This form can be found in cerebrospinal fluid (CSF) and blood and might act as a soluble trophic factor. 'Shedding' may also be a mechanism for regulating PrP levels on the cell surface (Borchelt et al., 1993; Wik et al., 2012; reviewed in Altmeppen et al., 2012).

### 1.3. Cytosolic PrP and protection against apoptosis

$PrP^C$ may enter the cytosol as a result of retro-translocation from the ER (Ashok and Hegde, 2008; Yedidia et al., 2001) (Fig. 1f), and it has been reported that this form of $PrP^C$ is able to protect human primary neurons from Bax-mediated apoptosis. Similar cytoprotection has also been found for other cell types, but is not universal (Lin et al., 2008; Roucou et al., 2003, 2004). Cytosolic $PrP^C$ has been reported in subpopulations of neurons in normal mouse brain although its significance in these cells is still unclear (Mironov et al., 2003).

$PrP^C$ may also enter the cytosol by avoiding translocation into the endoplasmic reticulum (ER) (Drisaldi et al., 2003; Orsi et al., 2006) (Fig. 1g). A small proportion of newly synthesized $PrP^C$ may normally 'leak' into the cytosol in this way, due to inherent inefficiencies of the signal sequence for secretion (Levine et al., 2005). There are ER stress response elements in the gene encoding PrP (*PRNP*) (Dery et al., 2013) and unglycosylated, cytosolic $PrP^C$ is increased by ER stress (Orsi et al., 2006). This may be due to pre-emptive quality control preventing translocation of newly formed $PrP^C$ molecules into the ER (Rane et al., 2008). $PrP^C$ that enters the cytosol is normally rapidly degraded, but if this occurs inefficiently, deleterious aggregates may form (Section 2.4).

An alternative, shorter transcript from the prion gene with a completely different amino acid sequence also exists (termed Alt-PrP). This is constitutively co-expressed with $PrP^C$, localizes to mitochondria and is up-regulated by ER stress (Vanderperre et al., 2011). Its function remains to be established.

## 2. $PrP^C$ – in sickness

Various $PrP^C$-dependent activities have been described, sometimes apparently conflicting, due to differences in binding partners and/or in post-translational processing and trafficking, and some of its activities can be deleterious. For example, $PrP^C$ is undoubtedly critical for prion disease, not only as a substrate for prion