Endoproteolytic cleavage as a molecular switch regulating and diversifying prion protein function

The prion protein (PrP), through misfolding, is widely known for its causative role in prion diseases, which are transmissible neurodegenerative diseases of humans and animals. There is still no defined function assigned to PrP, especially in the central nervous system, despite many studies in this area. Proposed functions are protein and include signal transduction, neuroprotection, neurogenesis, neuritogenesis, metal-ion homeostasis, memory formation and consolidation, as well as circadian rhythms (Nicolas et al., 2009). Part of the difficulty in assigning a specific function to PrP could perhaps be that it does not have one single function. Instead it might be able to perform many functions and influence various pathways depending upon contextual post-translational modification.

PrP has been shown to undergo various post-translational modifications including glycosylphosphatidylinositol (GPI)-anchor attachment at its C-terminus, N-linked glycosylation at either or both of two locations, phosphorylation, metal ion co-ordination at no less than six sites, secretory cleavage close to the GPI-anchor and endoproteolytic cleavage at three or more sites (Haigh et al., 2010). Whilst all of these modifications may change mature PrP in ways that potentially alter its function or site of function, endoproteolytic cleavage creates new peptides with distinct features that are likely to contribute to the diversity of functions reported for this protein.

Alpha-(α-) and beta-(β-) cleavages of PrP were first characterized as constitutive processing events in both normal and diseased human brain twenty years ago (Chen et al., 1995). The cleavage sites and resulting fragments are shown schematically in Figure 1. The C-terminal fragments persist at detectable levels in cells post-cleavage, whereas the N-terminal fragments are likely secreted or released from cells and are detectible in the culture media. PrP cleavage differs depending upon cell type. In certain cells PrP can be over 50% α-cleaved suggesting that this processing may be part of its normal functioning (Lewis et al., 2009). The different properties and fates of the cleavage fragments further support that this cleavage is unlikely to represent a mere degradation step in the turnover of PrP but instead produces functional proteins. Indeed the different ratios across cell types may reflect the different functions of CNS cells. The β-cleavage event has traditionally been thought to be pathogenic as the relative amounts of cognate fragments are increased during prion disease (Chen et al., 1995). However, a cellular inability to undergo β-cleavage was found to result in a heightened susceptibility to cellular stress, which indicated that the produced N2/C2 fragments were also likely to be functional (Watt et al., 2005). For both α- and β-cleavages the specific site of cleavage is “raged” with these cleavages located either side of a charged cluster domain, defining which new peptide contains this basic domain.

Recently a further cleavage event has been extensively characterized (Lewis et al., 2015). Referred to as “gamma-cleavage”, this event occurs in the C-terminal structured domain and therefore produces fragments with very different features to both the α- and β-cleavages. A functional significance is yet to be assigned to this processing event but its presence in multiple cells and tissues, and in disease, suggests that the fragments produced are likely to exert cellular effects distinct to those produced by the other PrP constitutive processing events.

Our prior research has shown that the N2 fragment (and shorter fragments thereof that include the far N-terminal residues) displays an anti-oxidant, neuroprotective function in response to the mild stress of serum starvation (Haigh et al., 2009a). This function required the N-terminal amino acids to be intact, including the structure conferred on the first charged cluster domain (residues 23–38) by the two proline residues at positions 26 and 28. Anti-oxidant function was further influenced by the octarepeat region and its copper-saturation, requiring a minimum of two copper molecules available for co-ordination into this site. N2 interaction with the cell surface required intact lipid rafts and heparan sulphate containing proteoglycans and if these were absent transduction of the protective effect was abolished. It was later shown that the cell surface engagement of N2 was also influenced by copper binding, directing the N2 internalisation pathway, which in turn permitted the specific activation of MEK1 in the absence of MEK2 or ERK1/2 activation (Haigh et al., 2015a). The outcome of the MEK1 activation was lower lysosomal and mitochondrial reactive oxygen species production. Therefore, two post-translational modifications of PrP, specifically β-cleavage and metal ion co-ordination, appear to co-operatively regulate and orchestrate N2 signalling, underscoring that the various permutations of post-translational modifications may determine PrP functional modulation. Further illustrating this, combinations of post-translational PrP modifications is not restricted to influencing MEK1 but has also been shown to alter other signalling pathways. For example, copper ion binding alters α-cleavage profiles as a function of membrane fluidity and lipid raft integrity and this correlates with downstream activation of the ERK1/2, p38 and JNK signalling pathways (Haigh et al., 2009b).

Like the N2 cleavage fragment, N1 has also been shown to exert neuroprotective functions, counteracting staurosporine toxicity and hypoxia by reducing caspase-3 activation through modulating p53 protein levels and activity (Guillot-Sestier et al., 2009). Of interest, despite acting on different pathways, both N1 and N2 bind anionic synthetic lipid membranes at least as efficiently as the α-cleavage fragments but not N1 and N2 bind anionic synthetic lipid membranes at least as efficiently as the α-cleavage fragments. Another function that potentially alters its function or site of function, endoproteolytic cleavage creates new peptides with distinct features that are likely to contribute to the diversity of functions reported for this protein.

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regulated, precise and controlled events for specific ends. Cleavage profiles differ across different cell types (Lewis et al., 2009) and even so far suggests functional post-translational modifications of PrP are likely to differ and be context specific. The precise cellular locations of the cleavage events and of the resulting fragments, whether processing occurs at the cell surface and whether N-terminal fragments act in cis or trans, the enzymes controlling these events, the membrane micro-milieu, and dynamic rather than absolute cleavage levels may all potentially impact functional consequences. Until the various nuances of combined post-translational modifications of PrP coupled with cleavage events in different contexts and in different tissues are fully elucidated, PrP appears destined to remain an enigmatic “actor” playing in many apparent functional roles.

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