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Functional Depletion of Mahogunin by Cytosolically Exposed Prion Protein Contributes to Neurodegeneration

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SUMMARY

The pathways leading from aberrant Prion protein (PrP) metabolism to neurodegeneration are poorly understood. Some familial PrP mutants generate increased ^{Ctm}PrP, a transmembrane isoform associated with disease. In other disease situations, a potentially toxic cytosolic form (termed cyPrP) might be produced. However, the mechanisms by which ^{Ctm}PrP or cyPrP cause selective neuronal dysfunction are unknown. Here, we show that both ^{Ctm}PrP and cyPrP can interact with and disrupt the function of Mahogunin (Mgrn), a cytosolic ubiquitin ligase whose loss causes spongiform neurodegeneration. Cultured cells and transgenic mice expressing either ^{Ctm}PrP-producing mutants or cyPrP partially phenocopy Mgrn depletion, displaying aberrant lysosomal morphology and loss of Mgrn in selected brain regions. These effects were rescued by either Mgrn overexpression, competition for PrP-binding sites, or prevention of cytosolic PrP exposure. Thus, transient or partial exposure of PrP to the cytosol leads to inappropriate Mgrn sequestration that contributes to neuronal dysfunction and disease.

INTRODUCTION

Mammalian Prion protein (PrP) is a cell-surface GPI-linked glycoprotein implicated in several neurodegenerative diseases including scrapie, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker disease (Aguzzi et al., 2007; Collinge and Clarke, 2007). The most extensively studied aspect of these diseases is their transmissibility via an unusual agent (termed prion) composed largely, if not exclusively, of a misfolded isoform of PrP termed PrP^{Sc}. Prion propogation is thought to occur when PrP^{Sc} converts the normal cellular form of PrP (PrP^{C}) into additional copies of PrP^{Sc}. Although this explains how altered protein conformation can form the basis of disease transmission, relatively little is known about the pathways of cellular dysfunction that culminate in neurodegeneration in PrP-associated diseases.

Because PrP^{Sc} is highly insoluble and aggregation prone, it was long assumed that its accumulation in the central nervous system would be intrinsically harmful to neurons. However, this view appears to be overly simplistic since several experimental paradigms have partially or fully uncoupled PrP aggregate deposition from downstream neuropathology (Brandner et al., 1996; Mallucci et al., 2003; Chesebro et al., 2005). Conversely, several familial PrP mutations cause neurodegeneration with little or no generation of PrP^{Sc} or transmissible agent (Tateishi and Kitamoto, 1995; Tateishi et al., 1996; Chiesa et al., 2003). These and other observations suggest that neurodegeneration might involve different aspects of PrP metabolism beyond just PrP^{Sc} accumulation (Hetz and Soto, 2006; Chakrabarti et al., 2009), prompting investigation into other isoforms of PrP that might mediate neurotoxicity.

One minor isoform of PrP, termed ^{Ctm}PrP, spans the membrane once (at a hydrophobic domain [HD] from residues ~112–135) with the N-terminal domain exposed to the cytosol (Hegde et al., 1998). Remarkably, both a natural and several artificial mutants within the HD that lead to even modestly increased generation of ^{Ctm}PrP (between 5% and 20% of total PrP) cause neurodegeneration in transgenic mice (Hegde et al., 1998, 1999). Furthermore, several familial diseases in humans are associated with hydrophobicity-increasing mutations in the HD (e.g., A117V; Hsiao et al., 1991) that may increase ^{Ctm}PrP generation (Hegde et al., 1998). Indirect evidence in transgenic mice suggests that ^{Ctm}PrP levels might also be increased (or perhaps stabilized from degradation) upon PrP^{Sc} accumulation (Hegde et al., 1999). Thus, at least a subset of familial neurodegenerative diseases, and perhaps also PrP^{Sc}-mediated transmissible diseases, are associated with generation of ^{Ctm}PrP.

In separate studies, a small proportion of PrP was found to be degraded in the cytosol by the proteaseome (Yedidia et al., 2001; Ma and Lindquist, 2001). The observation that improving the efficiency of the PrP signal sequence markedly reduces the proportion of PrP degraded by the proteasome suggested that inefficient forward translocation into the ER is a major source of cyPrP (Rane et al., 2004). Interestingly, enforced cyPrP expression in transgenic mice caused neurodegeneration in a cell-type-selective manner (Ma et al., 2002). However, the relevance of this observation to either familial or transmissible disease caused by PrP has been unclear.

More recently, several observations have suggested an indirect means to potentially link cyPrP production to prion disease

pathogenesis. First, translocation of PrP into the ER is reduced during ER stress (Kang et al., 2006; Orsi et al., 2006), leading to increased cyPrP production. Second, ER stress appears to be an indirect consequence of prion infection and PrP^{Sc} accumulation (Hetz and Soto, 2006; Rane et al., 2008). Third, reduced PrP translocation at levels comparable to that seen during ER stress was sufficient to cause mild age-dependent neurologic dysfunction in transgenic mice despite essentially quantitative degradation of cyPrP (Rane et al., 2008). And finally, proteasome activity may decline with age (Dahlmann, 2007) and upon PrP^{Sc} accumulation (Kristiansen et al., 2007). Thus it is plausible that by the combined effects of a weak PrP signal sequence, reduced PrP translocation during ER stress, and reduced proteasome activity upon PrP^{Sc} accumulation, cyPrP is generated in sufficient amounts during prion disease to be a contributing factor in neurodegeneration (Rane et al., 2008).

And finally, PrP^{Sc} was shown to directly inhibit the proteasome in vitro (Kristiansen et al., 2007). Because proteasome activity was observed to be decreased with prion infection in cells and mice, it was proposed that cytosolic PrP^{Sc} inhibits the proteasome to cause neuronal death during disease pathogenesis. While it is not yet clear how PrP^{Sc} (normally formed in extracellular or endolysosomal compartments) could access the cytosol, its cytosolic mislocalization was a key point of this model. Thus, one theme that emerges from the above paradigms of neurodegeneration is the exposure of PrP to the cytosolic environment. Although only partially or very transiently exposed, this minor population of PrP could conceivably have adverse consequences for certain cells under certain conditions if it were to make inappropriate interactions with cellular factors whose functions become compromised. However, candidate interacting partners for cytosolic PrP are poorly studied and their roles in disease unknown.

In the context of this hypothesis, the cytosolic protein Mahogunin (Mgrn) is especially intriguing. Loss of Mgrn function was found to cause both the mahoganoid coat color phenotype and late-onset spongiform neurodegeneration in selected brain regions (He et al., 2003).The resemblance of Mgrn and prion disease pathology raised the possibility of a mechanistic relationship. However, a functional connection between Mgrn and PrP was not immediately apparent. Although Mgrn has E3 ubiquitin ligase activity, PrP is not a substrate in vitro and does not accumulate in vivo in the absence of Mgrn (He et al., 2003). Nevertheless, a growing appreciation that minor populations of PrP are either partially (in the case of ^{Ctm}PrP) or transiently (in the case of cyPrP) exposed to the cytosol during disease led us to consider the alternative hypothesis that cytosolically mislocalized forms of PrP might interact inappropriately with Mgrn to inhibit its function. This would phenocopy Mgrn depletion, leading to region-selective neurodegeneration. Here, we examine this hypothesis using in vitro, cell culture, and mouse models.

RESULTS

Experimental Logic

Under normal circumstances, the amounts of ^{Ctm}PrP and cyPrP are minor and often transient. Even mutations that favor production of these isoforms result in modest increases that, while relevant for disease over long time periods in certain cell types, nonetheless make analysis of potential protein-protein interactions daunting. To circumvent this problem, we initially used artificial systems that greatly exaggerate the abundance and stability of Mgrn and cytosolically exposed PrP to explore the possibility of an interaction between them. This strategy allowed the evaluation of potential interactions, mapping of interacting domains, characterization of downstream phenotypes, and detailed functional analysis in a robust experimental system. The physiologic relevance of the results from such exaggerated systems was validated subsequently in cellular and mouse models that more accurately reflect the disease state.

Interaction of Mgrn with Cytosolic PrP Aggregates

Expression of PrP in the cytosol leads to its rapid degradation by the ubiquitin-proteasome system. Degradation ensues regardless of whether cytosolic PrP is generated by mutation, by deletion of the signal, or by inhibitors of translocation (Ma et al., 2002; Kang et al., 2006). The very low steady-state levels of cytosolic PrP therefore make it difficult to assess a potential interaction with Mgrn in vivo without proteasome inhibitors that could have many indirect effects. To avoid this, we took advantage of the serendipitous observation that fluorescent protein (FP) tagged PrP lacking the N- and C-terminal signals is poorly degraded and artifactually forms aggregates in nearly all cells (Figure S1 available online). Such aggregates remained affixed in the cell upon selective release of freely diffusible cytosolic contents by digitonin-mediated semipermeabilization of the plasma membrane. We exploited these observations to develop an in vivo interaction assay based on coassociation of an FP-tagged test protein with FP-tagged cytosolic PrP aggregates (Figure 1A).

Coexpression of red fluorescent protein (RFP) with cyan fluorescent protein (CFP)-$PrP_{40–231}$ (CFP fused to residues 40–231 of PrP) followed by digitonin permeabilization led to a rapid and essentially complete loss of RFP signal (within ~2–5 min) from the nucleocytoplasmic compartment (Figures 1B and S1). By contrast, RFP-Mgrn was partially retained in the cell upon permeabilization, colocalizing precisely with aggregates formed by CFP-$PrP_{40–231}$ (Figures 1C and S1). Coaggregation was seen with PrP and Mgrn regardless of the FP tags used (we have used CFP, green fluorescent protein [GFP], and RFP in various combinations), in cells with widely varying expression levels of Mgrn (spanning at least 20-fold), and with aggregates of various sizes and morphology (unpublished data). Evidence for an interaction between RFP-Mgrn and CFP-$PrP_{40–231}$ could also be observed without permeabilization, especially in cells where the RFP-Mgrn was expressed at lower levels and the nonaggregated population did not confound the imaging (Figures 1C and S1). Furthermore, the observation that RFP-Mgrn was typically retained in coassociation with the aggregate over an hour after permeabilization (unpublished data) suggests that its sequestration was not rapidly reversible. Importantly, Mgrn sequestration was specific to PrP aggregates since aggregates formed by a GFP-tagged Huntingtin (Htt) fragment containing 103 glutamines failed to coassociate with RFP-Mgrn (Figure 1D). Thus, mislocalized PrP (artificially immobilized into aggregates in this case) can interact selectively with Mgrn in cultured cells.