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Prion protein and RNA: a view from the cytoplasm

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Accumulation of cytoplasmic PrP results in the formation of large aggregates
4. Cytoplasmic PrP inhibits the assembly of stress granules and alters the normal distribution of mRNAs
5. Aggregation and high levels of cytoplasmic PrP are not essential for the inhibition of stress granules and mRNA aggregation
5.1. mRNA aggregation and SGs inhibition are independent from the aggregation of cytoplasmic PrP
5.2. mRNA aggregation and SGs inhibition occur at low levels of cytoplasmic PrP
6. The ribonucleoprotein particle generated upon expression of PrP in the cytoplasm is similar to chromatoid bodies
7. Summary and perspectives
8. Acknowledgements
9. References

1. ABSTRACT

Since it was posited that a cytoplasmic isoform of PrP may be involved in prion diseases, controversies about the isoform's biogenesis and function have emerged in the literature. While the existence of cytoplasmic PrP *in vivo* and in different cell cultures systems has now been well-established, whether it has specific activity remains unknown. This review outlines recent evidence about the molecular activity of cytoplasmic PrP. Cytoplasmic PrP inhibits a normal cellular stress response by preventing the assembly of protective mRNA stress granules and the synthesis of heat-shock protein 70 following environmental stress. Interference with the stress response correlates with the coalescence of mRNAs in a large cytoplasmic ribonucleoprotein particle. This particle shares similarities with the chromatoid body, a particle that organizes and controls RNA processing in mammalian germ cells as well as in neurons and stem cells from planarians with high regenerative abilities.

2. INTRODUCTION

Prion diseases are fatal neurodegenerative disorders including Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and scrapie in sheep and goats. The molecular feature of these disorders is the accumulation of abnormal prion protein conformers (PrP^{Sc}) derived from normal cellular host prion protein (PrP^{C}) (1). If PrP^{Sc} is directly responsible for the transmission of prion diseases, there is much evidence that argues against its direct neurotoxicity {(2-4). These recent observations have spawned debate about whether neuronal degeneration results from the loss of PrP^{C} function or from a still elusive neurotoxic isoform of PrP. Recently, the discovery of a cytoplasmic form of PrP has fuelled this debate (5-8).

Although PrP^{C} is primarily a plasma membrane GPI-anchored glycoprotein localized in specialized domains known as lipid rafts, the presence of PrP^{C} has also

been detected in the cytoplasm of different cell types in physiological conditions. PrP^C is present in the cytosol in a subpopulation of neurons in the hippocampus, neocortex, and thalamus of mice (9). PrP^C is abundantly expressed in the cytoplasm of beta-pancreatic cells in rats (10). Finally, PrP^C is also associated with sperm cytoplasmic droplets (11). All together, these results point to a possible role of PrP in the cytoplasm. This hypothesis seems to be supported by the observation that about 20% of PrP never translocates into the ER due to a particularly inefficient ER translocation signal (12). Furthermore, cytoplasmic PrP was identified as the PrP isoform responsible for its neuroprotective activity in human primary neurons (10).

Yet other evidence points to a noxious function of cytoplasmic PrP. Indeed, several pathological conditions result in an increase of cytoplasmic PrP levels, indicating that such an isoform may be involved in cellular toxicity. ER stress prevents nascent PrP molecules from being translocated into the ER (14, 15). Expression of a PrP variant with reduced translocation into the ER was sufficient to cause several pathological manifestations of PrP-mediated neurodegeneration (16). Evidence of cytoplasmic PrP aggregates was also found in the brain of prion-infected mice (17). Thus, prions may facilitate mistrafficking of PrP in the cytosol. Cytoplasmic PrP levels increase in response to hyperglycemia in beta-pancreatic cells from rats {(10). Hypoxia also induces the expression of a GPI-anchorless splice variant of PrP located in the cytosol of a human glioblastoma cell line (18). This cytosolic variant has also been detected in human brains and in non-neuronal tissues (18). Strong evidence for a pathological role of cytoplasmic PrP comes from *in vivo* studies in which the expression in transgenic mice of a recombinant PrP termed cyPrP without an ER translocation signal is neurotoxic and results host death (5). The toxic activity of cytoplasmic PrP is also conserved in transgenic *C. elegans*, expressing the cytosolic form of the mouse prion protein (19).

Cytoplasmic PrP is now a well-established isoform of PrP. Clearly, any function of cytoplasmic PrP in physiological or pathological conditions cannot be delineated without investigating its impact on cell physiology. This paper reviews recent experimental work that has led to the conclusion that PrP interferes with the cell stress response in the cytoplasm and induces the mistrafficking of several types of RNA molecules and concentrates them in a large ribonucleoprotein particle.

3. ACCUMULATION OF CYTOPLASMIC PrP RESULTS IN THE FORMATION OF LARGE AGGREGATES

PrP normally translocates into the ER and travels through the secretory pathway en route to the cell surface. Its appearance in the cytoplasm is therefore not trivial. Studies focused on the biogenesis and toxicity of cytoplasmic PrP led to the discovery of two possible pathways. The N-terminal signal peptide of PrP is relatively inefficient and the translocation of the protein through the ER membrane is regulated (12). An alternative pathway to the cytoplasm by retrotranslocation from the ER was also revealed in different cell lines and in primary neurons (6, 20, 21). These two mechanisms—insufficient translocation and retrotranslocation—are not mutually exclusive and their contribution to the accumulation of cytoplasmic PrP may vary depending on experimental conditions and cell types. These findings open the possibility that targeting of PrP to the cytoplasm is a controlled physiological mechanism and that PrP may also have a function in the cytosol. There are other examples in the literature of proteins having a function in two cellular locations (mostly mitochondrial and cytosolic), while recent data indicate that dual targeting of proteins appears to be more common than previously reported (22, 23). Proteins with functions in both the ER and cytoplasm have also been proposed (24).

Several strategies were developed to reconstitute the accumulation of cytoplasmic PrP in cultured cells, including the addition of drugs (such as cyclosporine and proteasome inhibitors) and the expression of a construct without N- and C-terminal signal peptides (7, 21, 25-27). In all of these studies, cytoplasmic PrP is insoluble in nonionic detergents and becomes partially resistant to proteinase K digestion. Some investigators have extended their studies to determine the molecular morphology of cytoplasmic PrP aggregates. Similar to other aggregation-prone proteins in eukaryotes, cytoplasmic PrP forms aggresomes (26, 27). Aggresomes are large perinuclear protein aggregates with specific features. Small aggregates form throughout the cytoplasm and are transported on microtubules to the centrosome where they generally concentrate in an inclusion body surrounded by a cage of the intermediate filament protein vimentin. Aggresome formation is mediated by dynein/dynactin-mediated microtubule-based transport of misfolded proteins to the centrosome and involves several regulators, including histone deacetylase 6 and the carboxy terminus of the Hsp70-interacting CHIP protein (28-30).

It should come as no surprise that PrP is able to form aggresomes in cultured cells. Many proteins implicated in several nonneurodegenerative and neurodegenerative diseases form aggresomes in cultured cells, spontaneously or in the presence of proteasomal inhibitors (31, 32). These proteins include huntingtin (Huntington's disease), alpha-synuclein (Parkinson's disease), superoxide dismutase (familial amyotrophic lateral sclerosis), cytokeratins (alcoholic liver disease), and cystic fibrosis transconductance regulator (cystic fibrosis). Thus, abnormal cellular metabolism of these proteins is a possible pathogenic mechanism in these diseases; the accumulation of such proteins in proteinaceous inclusions is actively being investigated. In addition, some components of the aggresomal pathway have become potential targets for therapy in neurodegenerative disorders (32).

Further experiments are required to address the issue of whether cytoplasmic PrP inevitably forms aggregates or if the protein remains soluble at low levels. This question is

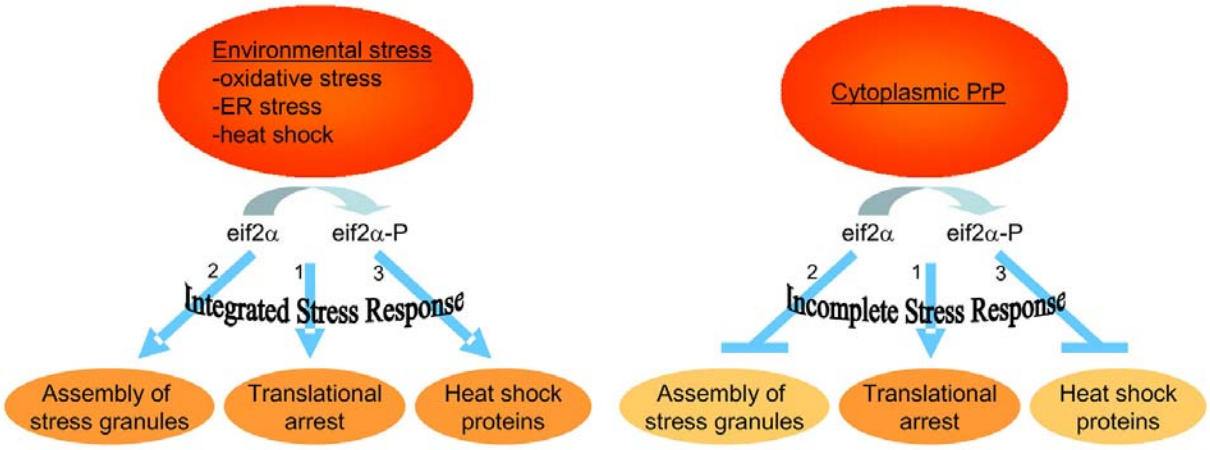

Figure 1. Cytoplasmic PrP inhibits the cell-stress response. Any environmental stress–including oxidative stress, ER stress, and heat shock–elicits an integrated cell-stress response initiated by eiF2a phosphorylation by one of four stress kinases. The cell-stress response normally involves three mechanisms: (1) inhibition of general protein synthesis, (2) assembly of stress granules (SGs) to store and protect mRNAs during the stress, and (3) synthesis of heat-shock proteins (Hsps). Cytoplasmic PrP induces a PKR-mediated phosphorylation of eiF2α and a general arrest of protein synthesis, but prevents the SG formation and Hsp synthesis. The cell stress response is incomplete, and cells are more sensitive to any subsequent environmental stress.

not trivial since levels and solubility may regulate cytoplasmic PrP function and toxicity.

4. CYTOPLASMIC PrP INHIBITS THE ASSEMBLY OF STRESS GRANULES AND ALTERS THE NORMAL DISTRIBUTION OF mRNAs

One question that we addressed was to test if cytoplasmic PrP induces cellular stress and elicits a cellular-stress response (Figure 1). Three mechanisms characterize the cell stress response. First, one of four kinases (RNA-dependent protein kinase PKR, PKR-like endoplasmic reticulum kinase (PERK), heme-regulated inhibitor (HRI), or general control non-derepressible-2 (GCN2) is activated and phosphorylates the translation initiation factor eIF2α at Ser51 (33). This phosphorylation converts eIF2 from a substrate to a competitive inhibitor of eIF2B, which is the guanine nucleotide exchange factor responsible for regenerating eIF2-GTP. This results in a limited availability of the ternary complex eIF2-GTPtRNA-Met for the assembly of the 43S preinitiation complex, and thus a reduced rate of translation initiation (34). Second, RNA granules—termed stress granules (SGs)—containing stalled translation initiation complexes are assembled in the cytoplasm (35). SG components may be divided into three groups: stalled initiation complexes still bound to mRNA, mRNA-binding proteins linked to translational silencing or mRNA stability, and RNA-binding proteins that regulate mRNA splicing, RNA editing, and RNA localization (36). Third, cells induce the synthesis of heat-shock proteins (Hsps) that participate in protein refolding, elimination of misfolded proteins, and apoptosis inhibition (37, 38).

We observed that PKR is activated in cells expressing a PrP cytoplasmic construct and that the eukaryotic initiation factor eiF2α is phosphorylated at position Ser51 (39). As expected, protein synthesis was also dramatically reduced in these cells. These observations are good indications that cytoplasmic PrP induces cell stress. The cell stress response was only partial, however, since no SG assembly and no synthesis of the major heat-shock protein Hsp70 were observed (Figure 1). Furthermore, cells did not display a complete response after an environmental stress such as oxidative stress, heat-shock treatment, or ER stress. Moreover, mRNAs did not accumulate in SGs and Hsp70 was not induced after these chemical insults. As a consequence, cells expressing cytoplasmic PrP were more sensitive to environmental stress (40). It is important to note that all these effects are PrP specific. Other proteins forming aggresomes did not induce or interfere with the cell stress response (39). Interestingly, scrapie-infected cells did not induce Hsp70 synthesis after oxidative stress or heat shock (40). This common characteristic between prion-infected cells and cells expressing cytoplasmic PrP is remarkable. It supports the idea that PrP aggresomes may be present in scrapie-infected animals and participate in neurotoxic mechanisms in prion diseases (17).

Cytoplasmic PrP is therefore an SG inhibitor. Many proteins independently promote the SG assembly and could be targeted by inhibition mechanisms (36). Viruses have developed such strategies to inhibit SG assembly. Indeed, cells use SGs as a defence process to limit the availability of translation factors and to interfere with viral infections. Some viruses avoid the cell-stress response by targeting cellular proteins involved in SG formation. The poliovirus 3C proteinase cleaves and inactivates the Ras-GAP SH3 domain-binding protein (G3BP) (41). The nonstructural rotavirus NSP3 protein is responsible for relocating the poly(A) binding protein in the nucleus (42). The nonstructural protein NS3 from two different

flaviviruses—West Nile virus and dengue virus—interact with T cell intracellular antigen-1 (TIA-1) and the related protein TIAR (43).

The mechanism by which cytoplasmic PrP inhibits SG assembly is novel: the protein induces mRNA aggregation (39). Experiments using oligo-dT probes and fluorescence *in situ* hybridization (FISH) demonstrated that cytoplasmic PrP induces the coalescence of mRNAs and coaggregation with the aggresome; trapped mRNAs cannot be recruited to SGs (39). Pull-down experiments in which PrP could be purified with and oligo-dT resin confirmed the interaction between the protein and polyA(+) RNA. Importantly, other proteins forming aggresomes did not trigger mRNA aggregation (39). These observations agree with sequence homology-based predictions and large numbers of data demonstrating that PrP has nucleic-acid binding activity *in vitro* (44-47). Yet further experiments described below indicate that the *in vivo* relationship between PrP and mRNA is more complex than simple interaction.

How cytoplasmic PrP inhibits SG assembly and heat-shock protein synthesis is significant, since these two mechanisms are essential neuroprotective pathways (48, 49). Hsp70, whose expression is inhibited by cytoplasmic PrP, has clear neuroprotective activities in cellular and animal models of Alzheimer's disease (50), Parkinson's disease (51), amyotrophic lateral sclerosis (52), and polyglutamine expansion diseases (53). In addition, cells must be able to display full stress response to environmental stress, including oxidative stress. Cytoplasmic PrP inhibition of the cell-stress response may be the mechanism that makes this isoform toxic *in vivo*.

5. AGGREGATION AND HIGH LEVELS OF CYTOPLASMIC PrP ARE NOT ESSENTIAL FOR THE INHIBITION OF STRESS GRANULES AND mRNA AGGREGATION

5.1 mRNA Aggregation and SGs inhibition are independent from the aggregation of cytoplasmic PrP

Several mutants were tested to determine what PrP region is responsible for mRNA aggregation (54). Since it was previously demonstrated that the structured C-terminal region of PrP contains the cytoplasmic aggregation determinant (27), it was expected that a mutant deleted from the C-terminal domain would not be able to induce mRNA aggregation. In contrast, although the N-terminal domain did not form aggresomes, it still induced mRNA coalescence into a perinuclear inclusion body with structural and functional characteristics similar to protein aggresomes. mRNA aggregates formed in cells expressing the N-terminal domain of cytoplasmic PrP concentrated at the centrosome were surrounded by a cage of vimentin protein and could not be assembled upon microtubule disruption (54). Conversely, a construct encoding the C-terminal domain of cytoplasmic PrP formed aggresomes but could not induce mRNA aggregation. Three important conclusions can be drawn from this experiment. First, aggregation of cytoplasmic PrP is not essential for mRNA aggregation; these two mechanisms are independent and can be separated. Second, the N-terminal domain of PrP causes the mRNA aggregation by a mechanism that remains to be discovered. Third, cytoplasmic PrP induces a new RNA organelle. This RNA organelle was termed PrP-RNP for PrP-induced ribonucleoprotein particle (54). These findings also have implications for aggresome formation. In fact, they demonstrate that the aggresomal pathway may be activated by a mechanism independent of protein aggregation and that aggresomes are not necessarily composed of misfolded proteins.

5.2 mRNA aggregation and SGs inhibition occur at low levels of cytoplasmic PrP

The relation between cytoplasmic PrP levels and mRNA aggregation is an important question. To address this issue, a mutant of PrP unable to accumulate in large amounts in the cytoplasm and generally forming a small numbers of microaggregates was expressed (Figure 2). This mutant—Y162A—is still able to generate the PrP-RNP. This result implies that mRNA aggregation is not an artefact of overexpression of cytoplasmic PrP. Interestingly, Y162A also prevented SG formation after environmental stress (unpublished). Thus, the absence of SGs in cells expressing cytoplasmic PrP likely results from mRNA aggregation. However, other mechanims cannot be excluded.

6. THE RIBONUCLEOPROTEIN PARTICLE GENERATED UPON EXPRESSION OF PrP IN THE CYTOPLASM IS SIMILAR TO CHROMATOID BODIES

In an effort to better characterize PrP-RNP, the presence of different classes of RNA was sought by FISH using specific probes. PrP-RNP contains rRNA 5S, tRNAs, small nuclear U1 RNA, and micro-RNAs, including miR-122a, miR-21, and let-7a (54). There is specificity in the RNA composition since cytoplasmic PrP did not modify the distribution of 18S and 28S rRNAs.

The only known large RNA organelle of similar composition described in the literature is the germ cell RNA granule, also called the chromatoid body. The chromatoid body constitutes a mechanism of centralizing the posttranscriptional processing and storage of various RNA species (55). Its specific function is still unknown, but it is predicted to give germ cells the ability to differentiate, while maintaining a totipotent genome (56). This RNA granule is also present in planarian neoblasts and neurons (57, 58). Neoblasts are stem cells responsible for the strong regeneration ability of planarians. It has been proposed that the chromatoid body plays an essential role in this mechanism.

Chromatoid bodies are ribonucleoprotein particles. They contain a specific set of proteins, such as the mRNA Dcp1a decapping enzyme; the DEAD box-family RNA helicase VASA/MVH; Dicer; a double-stranded RNAseIII essential for RNA interference and miRNA biogenesis; and Sm proteins that are essential core components of small nuclear ribonucleoprotein particles present in the spliceosome (56). Remarkably, Dcp1a, Dicer,

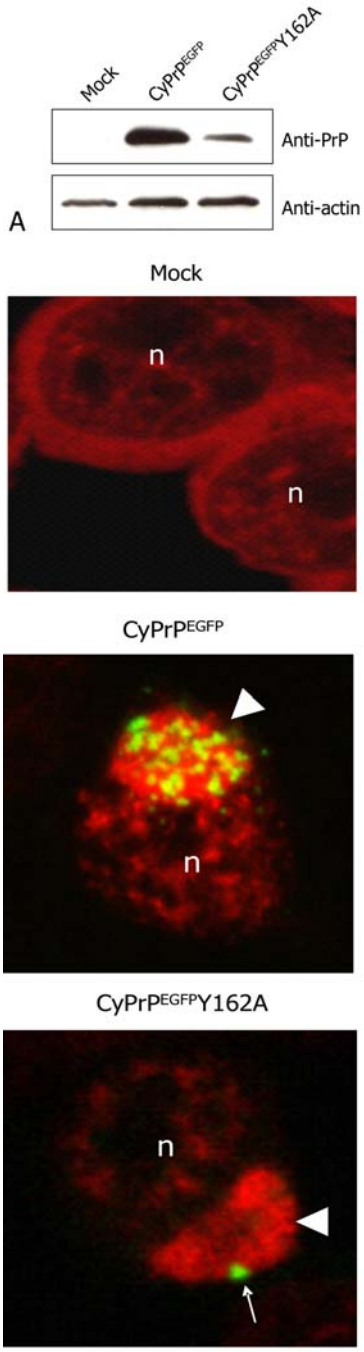

Figure 2. Independence between levels of cytoplasmic PrP and mRNA aggregation. Neuroblastoma N2a cells were transfected with empty vector (mock) or a construct encoding cytoplasmic PrP tagged with EGFP ($CyPrP^{EGFP}$) or cytoplasmic PrP tagged with EGFP with mutation Y162A ($CyPrP^{EGFP}Y162A$). A. Expression of the proteins was determined by western blot using a PrP-specific antibody and equal loading verified with actin antibodies. B. Distribution of cytoplasmic PrP and mRNA was observed by direct fluorescence and fluorescence *in situ* hybridization, respectively.

and Sm proteins B/B'/N also accumulate in the RNA granule induced by cytoplasmic PrP. VASA/MVH is specific to germ cells and we have determined the localization of DDX6, a more ubiquitous DEAD-box RNA helicase. DDX6 also accumulates in the RNA organelle.

One important feature of chromatoid bodies is their contacts with nuclear pore complexes (59). The connection between nuclear pore complexes and PrP-RNPs was confirmed by combining FISH with immunofluorescence using antibodies against FXFG repeat nucleoporins, which are positioned throughout the pore complexes (54). Furthermore, high levels of FXFG repeat nucleoporins were also detected in PrP-RNPs. Altogether, these results indicate that PrP-RNPs and chromatoid bodies share striking structural similarities (54).

7. SUMMARY AND PERSPECTIVES

Two major issues remain unanswered in the field of prion diseases: the normal function of PrP and the nature of the toxic molecule. Unless these issues are elucidated, it may be difficult to find efficient therapies for these disorders. In this context, all isoforms of PrP, including cytoplasmic PrP, should be thoroughly investigated. In the cytoplasm, cytoplasmic PrP spontaneously changes conformation and aggregates, with high levels of the protein resulting in aggresome assembly. This property is shared by numerous proteins implicated in several neurodegenerative diseases. Cytoplasmic PrP interferes with two important cellular mechanisms. First, it inhibits two components of the cell-stress response, SG assembly, and Hsp synthesis. Second, it induces the mistrafficking of RNA molecules and the formation of a large ribonucleoprotein particle—PrP-RNP—of unknown function.

Although our knowledge about the biogenesis and molecular activity of cytoplasmic PrP is growing, the main unresolved issue concerns the function of this PrP isoform. The relation between cytoplasmic PrP, RNA, and cell-stress response certainly deserves further investigation. The resemblance between PrP-RNPs and the chromatoid body is particularly striking. This large particle could play a role in organizing and processing RNA molecules to help maintain a totipotent genome. The view from the cytoplasm on PrP and RNA may bring significant clues on the normal function of PrP and its role in neuronal toxicity in prion diseases.

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9. REFERENCES

1. Prusiner, S.B.: Prions. *Proc. Natl. Acad. Sci. USA.* 95, 13363-13383 (1998)

2. Brandner, S., S. Isenmann, A. Raeber, M. Fischer, A. Sailer, Y. Kobayashi, S. Marino, C. Weissmann, & A.

Aguzzi: Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature.* 379, 339-343 (1996)

3. Hill, A.F., & J. Collinge: Subclinical prion infection. *Trends Microbiol.* 11, 578-584 (2003)

4. Mallucci, G., A. Dickinson, J. Linehan, P.C. Klohn, S. Brandner, & J. Collinge: Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science.* 302, 871-874 (2003)

5. Ma, J., R. Wollmann, & S. Lindquist: Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol. *Science.* 298, 1781-1785 (2002)

6. Roucou, X., Q. Guo, Y. Zhang, C.G. Goodyer, & A.C. LeBlanc: Cytosolic prion protein is not toxic and protects against Bax-mediated cell death in human primary neurons. *J Biol Chem.* 278, 40877-40881 (2003)

7. Fioriti, L., S. Dossena, L.R. Stewart, R.S. Stewart, D.A. Harris, G. Forloni, & R. Chiesa: Cytosolic prion protein (PrP) is not toxic in N2a cells and primary neurons expressing pathogenic PrP mutations. *J Biol Chem.* 280, 11320-11328 (2005)

8. Roucou, X., & A.C. LeBlanc: Cellular prion protein neuroprotective function: implications in prion diseases. *J Mol Med.* 83, 3-11 (2005)

9. Mironov, A.Jr., D. Latawiec, H. Wille, E. Bouzamondo-Bernstein, G. Legname, R.A. Williamson, D. Burton, S.J. DeArmond, S.B. Prusiner, & P.J. Peters: Cytosolic prion protein in neurons. *J. Neurosci.* 23, 7183–7193 (2003)

10. Strom, A., G.S. Wang, R. Reimer, D.T. Finegood, & F.W. Scott: Pronounced cytosolic aggregation of cellular prion protein in pancreatic beta-cells in response to hyperglycemia. *Lab Invest.* 87, 139-149 (2007)

11. Ecroyd, H., P. Sarradin, J.L. Dacheux, & J.L. Gatti: Compartmentalization of prion isoforms within the reproductive tract of the ram. *Biol Reprod.* 71, 993-1001 (2004)

12. Rane, N.S., J.L. Yonkovich, & R.S. Hegde: Protection from cytosolic prion protein toxicity by modulation of protein translocation. *EMBO J.* 23, 4550-4559 (2004)

13. Lin, D.T., J. Jodoin, M. Baril, C.G. Goodyer, & A.C. Leblanc: Cytosolic prion protein is the predominant anti-Bax prion protein form: exclusion of transmembrane and secreted prion protein forms in the anti-Bax function. *Biochim Biophys Acta.* 1783, 2001-2012 (2008)

14. Orsi, A., L. Fioriti, R. Chiesa, & R. Sitia: Conditions of endoplasmic reticulum stress favor the accumulation of cytosolic prion protein. *J. Biol. Chem.* 281, 30431-30438 (2006)

15. Kang, S.W., N.S. Rane, S.J. Kim, J.L. Garrison, J. Taunton, & R.S. Hegde: Substrate-specific translocational attenuation during ER stress defines a pre-emptive quality control pathway. *Cell.* 127, 999-1013 (2006)

16. Rane, N.S., S.W. Kang, O. Chakrabarti, L. Feigenbaum, & R.S. Hegde: Reduced translocation of nascent prion protein during ER stress contributes to neurodegeneration. *Dev Cell.* 15, 359-370 (2008)

17. Kristiansen, M., M.J. Messenger, P.C. Klohn, S. Brandner, J.D. Wadsworth, J. Collinge, & S.J. Tabrizi: Disease-related prion protein forms aggresomes in neuronal cells leading to caspase activation and apoptosis. *J. Biol. Chem.* 280, 38851-38861 (2005)

18. Kikuchi, Y., T. Kakeya, O. Nakajima, A. Sakai, K. Ikeda, N. Yamaguchi, T. Yamazaki, K.I. Tanamoto, H. Matsuda, J.I. Sawada, & K. Takatori: Hypoxia induces expression of a GPI-anchorless splice variant of the prion protein. *FEBS J.* 275, 2965-2976 (2008)

19. Park, K.W., & L. Li: Cytoplasmic expression of mouse prion protein causes severe toxicity in Caenorhabditis elegans. *Biochem Biophys Res Commun.* 372, 697-702 (2008)

20. Ma, J., & S. Lindquist: Wild-type PrP and a mutant associated with prion disease are subject to retrograde transport and proteasome degradation. *Proc Natl Acad Sci USA.* 98, 14955−14960 (2001)

21. Ma, J., & S. Lindquist: Conversion of PrP to a self-perpetuating PrPSc-like conformation in the cytosol. *Science.* 298, 1785−1788 (2002)

22. Karniely, S., & O. Pines: Single translation-dual destination: mechanisms of dual protein targeting in eukaryotes. *EMBO Rep.* 6, 420-425 (2005)

23. Regev-Rudzki, N., O. Yogev, & O. Pines: The mitochondrial targeting sequence tilts the balance between mitochondrial and cytosolic dual localization. *J Cell Sci.* 121, 2423-2431 (2008)

24. Hegde R.S., & V.R. Lingappa V.R.: Regulation of protein biogenesis at the endoplasmic reticulum membrane. *Trends Cell Biol.* 9, 132−137 (1999)

25. Yedidia, Y., L. Horonchik, S. Tzaban, A. Yanai, & A. Taraboulos: Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. *EMBO J.* 20, 5383-5391 (2001)

26. Cohen, E. & A. Taraboulos: Scrapie-like prion protein accumulates in aggresomes of cyclosporin A-treated cells. *EMBO J.* 22, 404-417 (2003)

27. Grenier, C., C. Bissonnette, L. Volkov, & X. Roucou: Molecular morphology and toxicity of cytoplasmic prion protein aggregates in neuronal and non-neuronal cells. *J Neurochem.* 97, 1456-1466 (2006)

28. Johnston JA, M.E. Illing, & R.R. Kopito: Cytoplasmic dynein/dynactin mediates the assembly of aggresomes. *Cell Motil Cytoskeleton.* 53, 26-38 (2002)

29. Kawaguchi, Y., J. Kovacs, A. McLaurin, J. Vance, A. Ito, & T. Yao: The Deacetylase HDAC6 Regulates Aggresome Formation and Cell Viability in Response to Misfolded Protein Stress. *Cell.* 115, 727-738 (2006)

30. Sha, Y., L. Pandit, S. Zeng, & N.T. Eissa NT: A Critical Role for CHIP in the Aggresome Pathway. *Mol Cell Biol.* 29, 116-128 (2008)

31. Garcia-Mata R., Y.S. Gao, & E. Sztul: Hassles with taking out the garbage: aggravating aggresomes. *Traffic.* 3, 388-396 (2002)

32. Olzmann, J.A., L. Li, & L.S. Chin: Aggresome formation and neurodegenerative diseases: therapeutic implications. *Curr Med Chem.* 15, 47-60 (2008)

33. Dever, T.E., A.C. Dar, and F. Sicheri: The eiF2α kinases, in : M.B. Mathews, N. Sonenberg, & J.W.B. Hershey (Eds.), Translational control in biology and medicine, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 319-343 (2007)

34. Hershey, J.W., & W.C. Merrick: Initiation of protein synthesis, in N. Sonenberg (Ed.), Translational control of gene expression, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 33-38 (2000)

35. Anderson, P. & N. Kedersha: Stressful initiations. *J. Cell Sci.* 115, 3227-3234 (2002).

36. Anderson P., & N. Kedersha: Stress granules: the Tao of RNA triage. *TIBS.* 33, 141-150 (2008)

37. Hartl, F.U.: Molecular chaperones in cellular protein folding. *Nature.* 381, 571-579 (1996)

38. Beere, H.M.: Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin. Invest.* 115, 2633-2639 (2005)

39. Goggin, K., S. Beaudoin, C. Grenier, A.A. Brown, & X. Roucou: Prion protein aggresomes are poly(A)+ ribonucleoprotein complexes that induce a PKR-mediated deficient cell stress response. *Biochim Biophys Acta.* 1783, 479-491 (2008)

40. Tatzelt, J., J. Zuo, R. Voellmy, M. Scott, U. Hartl, S.B. Prusiner, & W.J. Welch: Scrapie prions selectively modify the stress response in neuroblastoma cells. *Proc. Natl. Acad. Sci. USA.* 92, 2944-2948 (1995)

41. White, J.P., A.M. Cardenas, W.E. Marissen, & R.E. Lloyd: Inhibition of cytoplasmic mRNA stress granule formation by a viral proteinase. *Cell Host Microbe.* 2, 295-305 (2007)

42. Montero H., M. Rojas, C.F. Arias, & S. López: Rotavirus infection induces the phosphorylation of eIF2alpha but prevents the formation of stress granules. *J Virol.* 82, 1496-1504 (2008)

43. Emara, M.M., & M.A. Brinton: Interaction of TIA-1/TIAR with West Nile and dengue virus products in infected cells interferes with stress granule formation and processing body assembly. *Proc Natl Acad Sci USA.* 104, 9041-9046 (2007)

44. Radulescu, R.T., & C. Korth: Prion function and dysfunction: a structure-based scenario. *Med Hypotheses.* 46, 225-228 (1996)

45. Marc, D., R. Mercey, & F. Lantier: Scavenger, transducer, RNA chaperone? What ligands of the prion protein teach us about its function. *Cell Mol Life Sci.* 64, 815-829 (2007)

46. Silva, J.L., L.M. Lima, D. Foguel, & Y. Cordeiro: Intriguing nucleic-acid-binding features of mammalian prion protein. *Trends Biochem Sci.* 33, 132-140 (2008)

47. Nandi, P.K.: Conformational changes of prion protein and nucleic acid arising from their interaction and relation of the altered structures in causing prion disease. *Mini Rev Med Chem.* 8, 784-789 (2008)

48. Morimoto, R.I., & M.G. Santoro: Stress-inducible responses and heat shock proteins: new pharmacologic targets for cytoprotection. *Nature Biotechnol.* 16, 833–838 (1998)

49. Muchowski, P.J., & J.L. Wacker: Modulation of neurodegeneration by molecular chaperones. Nat *Rev Neurosci.* 6, 11-22 (2005)

50. Magrane, J., R.C. Smith, K. Walsh, & H.W. Querfurth: Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed β-amyloid in neurons. *J. Neurosci.* 24, 1700–1706 (2004)

51. Auluck, P.K., H.Y. Chan, J.Q. Trojanowski, V.M. Lee, & N.M. Bonini: Chaperone suppression of α-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science.* 295, 865–868 (2002).

52. Bruening, W., J. Roy, B. Giasson, D.A. Fligewicz, W.E. Mushynski, & H.D. Durham: Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. *J. Neurochem.* 72, 693–699 (1999)

53. Cummings, C.J., Y. Sun, P. Opal, B. Antalffy, R. Mestril, H.T. Orr, W.H. Dillmann, & H.Y. Zoghbi: Over-expression of inducible Hsp70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum. Mol. Genet.* 10, 1511–1518 (2001)

54. Beaudoin, S., B. Vanderperre, C. Grenier, I. Tremblay, F. Leduc, & X. Roucou: A large ribonucleoprotein particle induced by cytoplasmic PrP shares striking similarities with the chromatoid body, an RNA granule predicted to function in posttranscriptional gene regulation. *Biochim Biophys Acta.* In Press (2008)

55. Kotaja, N., & P. Sassone-Corsi: The chromatoid body: a germ-cell-specific RNA-processing centre. *Nat Rev Mol Cell Biol.* 8, 85-90 (2007)

56. Seydoux, G., & R.E. Braun: Pathway to totipotency: lessons from germ cells. *Cell.* 127, 891-904 (2006)

57. Shibata, N., Y. Umesono, H. Orii, T. Sakurai, K. Watanabe, & K. Agata: Expression of VASA(vas)-related genes in germline cells and totipotent somatic stem cells of planarians. *Dev Biol.* 206, 73-87 (1999)

58. Yoshida-Kashikawa, M., N. Shibata, K. Takechi, K. & K. Agata: DjCBC-1, a conserved DEAD box RNA helicase of the RCK/p54/Me31B family, is a component of RNA-protein complexes in planarian stem cells and neurons. *Dev Dyn.* 236, 3436-3450 (2007)

59. Parvinen, M.: The chromatoid body in spermatogenesis. *Int J Androl.* 28, 189-201 (2005)

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