Post-translational Import of the Prion Protein into the Endoplasmic Reticulum Interferes with Cell Viability

A CRITICAL ROLE FOR THE PUTATIVE TRANSMEMBRANE DOMAIN

Aberrant folding of the mammalian prion protein (PrP) is linked to prion diseases in humans and animals. We show that during post-translational targeting of PrP to the endoplasmic reticulum (ER) the putative transmembrane domain induces misfolding of PrP in the cytosol and interferes with its import into the ER. Unglycosylated and misfolded PrP with an uncleaved N-terminal signal sequence associates with ER membranes, and, moreover, decreases cell viability. PrP expressed in the cytosol lacking the N-terminal ER targeting sequence, also adopts a misfolded conformation; however, this has no adverse effect on cell growth. PrP processing, productive ER import, and cellular viability can be restored either by deleting the putative transmembrane domain or by using a N-terminal signal sequence specific for co-translational ER import. Our study reveals that the putative transmembrane domain features in the formation of misfolded PrP conformers and indicates that post-translational targeting of PrP to the ER can decrease cell viability.

Prion diseases in humans and animals are characterized by the accumulation of PrPSc, a partially protease-resistant isoform of the cellular prion protein PrPC. PrPSc is generated through a conformational transformation of PrPC and represents the major component of infectious prions (reviewed in Refs. 1–4). PrP1 is post-translationally modified by the attachment of two N-linked carbohydrate moieties (Asn180 and Asn196) (5–7) and a glycosylphosphatidylinositol (GPI) anchor at serine 231 (8) as well as by the formation of a disulfide bond between Cys178 and Cys213. Studies with recombinant PrP purified from bacteria revealed that the formation of the disulfide bond is essential for the native folding of PrP (9).

The co- and post-translational modifications of PrPC are initiated with the cleavage of the N-terminal signal peptide (aa 1–22) and the transfer of core glycans, whereas the nascent chain is still associated with the translocon. Shortly after the protein is fully translocated, the GPI anchor is attached to the acceptor amino acid close to the C terminus. The final maturation of PrPC includes the processing of the core glycans into complex-type glycans by a series of enzymatic reactions in the endoplasmic reticulum (ER) and Golgi compartment. Post-translational modifications, like N-linked glycosylation and GPI anchor attachment, are often used as diagnostic markers to monitor efficient import into the ER. In the case of PrP, however, we and others have shown that PrP devoid of a GPI anchor remains mainly unglycosylated but is imported efficiently into the ER and transported through the secretory pathway (10–14). It has been found that the only specific marker for ER import of PrP is a cleaved N-terminal signal sequence (10).3

Misfolding of PrPSc in the cytosol or in the ER can induce neurodegeneration in the absence of PrPSc. Neurotoxic properties of cytosolic PrP aggregates were observed after proteasomal inhibition in cultured cells or after the forced expression of cytosolic PrP in transgenic mice (15). Other studies revealed that a minor fraction of PrP can be synthesized as an integral membrane protein with two different topologies, termed either NtmPrP, with the N terminus facing the ER lumen, or CtmPrP, with the C terminus facing the ER lumen. Amino acids 112–135 of PrP were identified as a putative transmembrane domain (TM) (16), and mutations in the TM domain were found to alter the relative amount of CtmPrP and NtmPrP (17). Remarkably, the increased synthesis of CtmPrP has been shown to coincide with progressive neurodegeneration both in Gerstmann-Sträussler-Scheinker syndrome patients with an A117V mutation and in transgenic mice carrying a triple mutation within the putative TM domain (AV3) (17). Other studies on the putative TM domain indicated that the hydrophobic stretch of amino acids 106 to 126 has the propensity to form fibrils (18) and can induce cell death in cultured cells (19–22).

In mammalian cells, secretory proteins are usually translocated into the ER via the co-translational pathway, which requires the binding of the signal recognition particle (SRP) to the nascent protein. The SRP directs the targeting of the whole nascent chain-ribosome complex to its receptor (SRPR) on the ER membrane, resulting in the transfer of the growing protein to the translocation pore and a direct release into the ER lumen as soon as the synthesis has finished. An alternative post-translational import pathway has been described in mammalian cells for proteins of less than 75 amino acids in length; in this case the completely synthesized protein is targeted to the
ER independently of the SRP/SRP system (23, 24). In yeast cells, however, a variety of secretory proteins can be imported post-translationally. Whether a co- or post-translational translocation pathway is used in yeast cells seems to be determined mainly by the N-terminal signal sequence. Signal sequences with a hydrophobicity index (Kyte-Doolittle) higher than 2 are strictly dependent on the SRP/SRP pathway (25) (reviewed in refs. 26–30).

In this study we analyzed the biogenesis of mouse PrP and showed that the putative transmembrane domain induces the misfolding of PrP during post-translational targeting to the ER. As a consequence, unprocessed and misfolded PrP associates with ER membranes and interferes with cell viability.

EXPERIMENTAL PROCEDURES

Strains, Cells, Antibodies, and Reagents—Wild-type yeast (MATa, ura3, his4) was grown in rich medium containing 2% dextrose (YPD). Transformed cells were grown in synthetic complete medium (SCD, 2% dextrose, 0.7% yeast nitrogen base) supplemented with strain-specific nutrients at 30 °C. Solid media contained 2.3% agar (Difco). Mouse neuroblastoma (N2a) cells (31) were cultured as described previously (32). PrP was detected by the monoclonal anti-PrP antibody 3F4 (33) or the polyclonal anti-PrP antiseraum A7 (10). The anti-Gas1p antibody was kindly provided by R. Barz, and the anti-Gim2p antibody was described earlier (34). Vacular ATPase was detected with the monoclonal anti-V-ATPase antibody 10D7-AT-2B ( Molecular Probes). Renografin 76 was prepared by adjusting TE, pH 7.6, to 66% meglumine diatrizoate and 10% sodium diatrizoate. Chemicals were purchased from Sigma and United States Biochemical. Enzymes were purchased from New England Biolabs and Promega.

DNA Manipulations and Transformation—PrP constructs were cloned into pYES2 or pcDNA3.1 (In vivotrans) for expression in yeast and mammalian cells, respectively, using standard procedures. In yeast, expression was driven by the GAS1 promoter (bases −413 to −1). GAS1 sequences were amplified by PCR from pGQ1R14 (R. Barz). PrP sequences were amplified from pDNA3.1-3F4 (35), which contains the mouse PrP N-terminus noncovalently expressed in PrP-enhanced TM. For gPrP constructs, aa 1–140 and gPrP constructs, aa 1–231. For targeting into the yeast ER, aa 1–27 of PrP containing the endogenous mouse signal sequence were replaced by the ER signal sequences of Gas1p (gPrP constructs, aa 1–26) or Kre6p (kPrP constructs, aa 1–21). In gPrP and kPrP, asc 228–254 containing the mouse signal peptide sequence were replaced by the GAS1 signal peptide sequence (35).

RESULTS

PrP Targeted to the ER Is Unprocessed and Interferes with Yeast Growth—Our aim was to use the yeast model to specifically analyze the import of PrP into the ER. In yeast the hydrophobicity index of the N-terminal signal sequence determines whether a co- or post-translational translocation pathway is used (25). Therefore, we expressed PrP with its own signal peptide (pPrP) and also with the ER-targeting peptide of the endogenous GPI-anchored protein Gas1p (gPrP) (41, 42). Both signal sequences have a hydrophobicity index (Kyte-Doolittle) lower than 2, which suggests a post-translational targeting of gPrP and pPrP to the ER (25). gPrP contains the C-terminal Gas1p GPI anchor signal sequence, and further PrP constructs were generated lacking either the GPI anchor attachment signal (gPrP) or lacking both the ER and the GPI attachment signal (pPrP) or lacking both the ER and the GPI attachment signal (gPrP).

Expression of these PrP constructs was analyzed by Western blotting using the monoclonal anti-PrP antibody 3F4 (Fig. 1B). To monitor N-linked glycosylation of PrP, yeast extracts were treated with endo-β-N-acetylglucosaminidase H, which removes all asparagine-linked sugar moieties from yeast proteins (43). It appeared that neither the Gas1p nor the PrP signal sequence was able to mediate the import of PrP into the yeast ER. All PrP constructs remained unglycosylated. Moreover, the majority of gPrP (−95%), pPrP (100%), and gPrP (−50%) contained unglycosylated N-terminal signal sequences. In this context it is important to note that in mammalian cells PrP GPI is mainly unglycosylated, but in contrast to gPrP and pPrP expressed in yeast, it is N-terminally processed. In the same study we showed that PrP containing a nonfunctional C-terminal GPI anchor signal sequence is both N-terminally processed and core-glycosylated (10). Further biochemical analysis revealed that gPrP (Fig. 1D), gPrP, pPrP, and cyto-PrP (data not shown) were highly insoluble in detergent buffer and partially resistant to proteolytic digestion, corroborating earlier findings (44).
sylation, cell lysates were treated with... PrP cytosolic expression, both signal sequences were omitted (GPI). For the detection of PrP, yeast lysates expressing gPrP or the endogenous yeast protein Gas1p (gPrP/GPI). For cytosolic expression, both signal sequences were omitted (cyto-PrP). For the detection of N-linked glycosylation, cell lysates were treated with endoglycosidase H (Endo H +) prior to Western blot analysis with the anti-PrP antibody 3F4. C, expression of unprocessed PrP interferes with cell growth. Growth of cells expressing the indicated PrP constructs was monitored by serial dilutions of cells spotted onto agar plates (SCD-ura). To control for equal numbers of cells plated, the same dilutions were spotted on full medium plates (YPD, yeast extract-peptone-dextrose). D, PrPΔGPI adopts a partially proteinase K-resistant conformation. Yeast lysates expressing gPrPΔGPI were incubated with different amounts of PK as indicated. PrP was analyzed by Western blotting using the antibody 3F4. The arrow indicates a partially PK-resistant fragment corresponding to the C-terminal fragment of PrP (90–231).

In the course of these experiments we noticed a retarded growth of yeast cells expressing PrP with an uncleaved N-terminal signal sequence, be it gPrP, gPrPΔGPI, or pPrPΔGPI. To analyze the growth of PrP-expressing cells in more detail, yeast cultures were serially diluted and spotted onto selective agar plates (Fig. 1C, SCD-ura). Both gPrP and gPrPΔGPI showed a reduced growth compared with the vector control. Interestingly, decreased growth was specifically linked to the expression of PrP targeted to the ER and was also observed for pPrPΔGPI (data not shown). PrP expressed without a N-terminal signal peptide (cyto-PrP) had no adverse effects on cell growth.

These experiments revealed that expression of mouse PrP with a signal peptide specific for post-translational targeting to the ER generates N-terminally unprocessed misfolded PrP and interferes with yeast growth. Cytosolically expressed PrP lacking the signal peptide is misfolded as well; however, this has no adverse effects on yeast growth.

Deletion of the Putative Transmembrane Domain Restores Processing of PrP and Yeast Growth—Previous studies revealed that the ER import of PrP is modulated by an internal stretch of hydrophobic amino acids (aa112–135). Instead of being synthesized as a secreted protein, this domain can direct the formation of a transmembrane topology (45). Different studies indicate that this stretch of hydrophobic amino acids may have additional properties; short peptides comprising residues 106–126 formed fibrils in vitro (18) and induced cell death in cultured cells (19–22).

To address the role of the putative TM domain in PrP folding and ER import, several deletion mutants lacking the complete TM domain and also PrP-AV3 and PrP-G122P were included in our analysis. The triple A to V and G to P substitutions are located within the putative transmembrane domain (Fig. 2A). Although the AV3 mutation enhances the formation of Cterminal PrP, the G122P mutation abolishes the formation of any transmembrane topology, be it Cterminal PrP or Nterminal PrP (17, 46).

Western blot analysis revealed that all PrP mutants lacking the putative TM domain were glycosylated, indicated by an increased electrophoretic mobility after Endo H digestion (Fig. 2B, Endo H +). In addition, these glycosylated mutants were N-terminally processed. The mutants with amino acid substitutions in the putative TM domain, gPrP-G122P and gPrP-AV3, remained unprocessed and unglycosylated, similar to gPrP (data not shown), and interfered with yeast growth (Fig. 2C). However, cells expressing PrP mutants with a deleted putative TM domain grew at wild-type rates (Fig. 2C).

Our experiments revealed that PrP mutants lacking the putative TM domain were N-terminally processed and glycosylated, although they were targeted to the ER via the Gas1p signal peptide. In addition, these mutants did not interfere with yeast growth.

Unprocessed PrP Shows Prolonged Association with ER Membranes—PrP targeted to the ER via the PrP or Gas1p signal peptide (pPrP, gPrP) had biochemical properties similar to cyto-PrP and also seemed to remain in the cytosol. However, cyto-PrP did not interfere with yeast growth. To analyze the cellular localization of the different PrP mutants in more detail, we performed a Renografin density gradient centrifuga-
tion (39, 40). This analysis revealed a specific feature of PrP mutants that interfered with yeast growth.

As expected, cytosolic PrP remained in the bottom fractions together with the subunit Gim2p (34) of the cytosolic GimC complex (Fig. 3A). The glycosylated and N-terminally processed PrP mutant with a deleted TM domain (gPrP/H9004) was found in the same fractions as Vhp1p, the 100-kDa subunit of the vacuolar V-ATPase (47). Further analysis revealed that the other N-terminally processed and glycosylated PrP mutants analyzed, gPrP/H9004 TM and gPrP/H9004 28–140, were also exported from the ER and transported to the vacuole (data not shown). In contrast, the N-terminally unprocessed PrP mutant gPrPΔGPI, as well as gPrP, gPrP-AV3, and gPrP-G122P (data not shown), was membrane-associated and co-localized with the ER and plasma membrane fraction. Because gPrPΔGPI has no GPI anchor and was not secreted into the culture medium (data not shown), we assumed that it was associated with ER vesicles.

In summary, these data indicate that the PrP mutants characterized by an uncleaved N-terminal signal peptide have a unique feature; they show prolonged association with ER membranes.

In Mammalian Cells PrPΔGPI Is N-terminally Processed and Transported through the Secretory Pathway—Our analysis in yeast revealed that PrPΔGPI expression significantly interfered with cell growth. The question arose whether a similar phenotype could be observed in mammalian cells. PrPΔGPI was previously characterized by us and other groups, and adverse effects on the growth of mammalian cells have not been described (10–14). The phenotype of PrPΔGPI expressed in mouse neuroblastoma (N2a) cells is illustrated in Fig. 3B. Similar to gPrP or to pPrPΔGPI expressed in yeast, this PrP mutant is mainly unglycosylated. In contrast to the situation in yeast, however, in mammalian cells PrPΔGPI is N-terminally processed and transported through the secretory pathway (Fig. 3B and Ref. 10). To test whether the putative transmembrane domain also had an effect on the glycosylation of PrPΔGPI in mammalian cells we analyzed PrPΔTMΔGPI in N2a cells, and core glycosylation was shown to be restored (Fig. 3B).

Thus, in mammalian cells, PrPΔGPI, even though mainly unglycosylated, is transported through the secretory pathway and does not affect cell growth. These data suggest that the specific phenotype of gPrPΔGPI in yeast, such as an adverse effect on cell growth and a prolonged association with ER membranes.
membranes, is linked to the uncleaved signal peptide.

**Directing PrP to a Co-translational Import Pathway in Yeast Cells Restores Processing of PrP and Cell Growth**—In contrast to mammalian cells, in yeast many proteins destined for the secretory pathway can be imported post-translationally, i.e. independently of the signal recognition particle SRP and its receptor (SRPR). However, the import of a subset of yeast proteins is strictly dependent on SRP/SRPR. It has been shown previously that these proteins contain a hydrophobic signal sequence with a hydrophobicity index (Kyte-Doolittle) higher than 2 (25).

To address the possibility that the effect of PrP expression in yeast is due to a post-translational import pathway, we analyzed the biogenesis of PrP containing a N-terminal ER signal sequence specific for co-translational import. To do so, we replaced the moderately hydrophobic Gas1p ER signal of gPrP and gPrP\textsubscript{TM} with the extremely hydrophobic signal sequence of Kre5p (hydrophobicity index > 3) (48), generating kPrP and kPrP\textsubscript{TM}, respectively (Fig. 4A). The Western blot analysis revealed that kPrP was N-glycosylated and that the N-terminal signal sequence of kPrP\textsubscript{TM} was cleaved (Fig. 4B).

Remarkably, the expression of kPrP\textsubscript{TM} did not interfere with yeast growth (Fig. 4C). We then compared the subcellular localization of kPrP\textsubscript{TM} with that of gPrP\textsubscript{TM}. Indeed, PrP expressed with the Kre5p signal sequence was exported from the ER and found in the vacuole fraction (Fig. 4D). Interestingly, kPrP\textsubscript{TM} was highly insoluble in detergent and partially resistant to proteolytic digestion (data not shown), similar to gPrP and cyto-PrP expressed in yeast (Fig. 1) and PrP\textsubscript{GPI} expressed in N2a cells (Fig. 6B in Ref. 10).

Thus, PrP expressed with a signal sequence specific for co-translational translocation is imported into the yeast ER. N-terminally processed PrP molecules, whether or not they are glycosylated, have no adverse effects on cell growth.

**Post-translational Import of PrP\textsubscript{TM} in a Mammalian System**—Recent *in vitro* studies showed that PrP is not imported post-translationally into mammalian microsomal vesicles (49). Our experiments described above revealed that in yeast the deletion of the putative TM domain allowed the import and processing of PrP even when it was targeted to the ER via the post-translational pathway by the Gas1p signal (gPrP\textsubscript{TM}). Consequently, we asked whether the deletion of the putative TM domain would also enable the post-translational import of PrP\textsubscript{TM} in a mammalian system. To address this question we performed *in vitro* import experiments in rabbit reticulocyte lysate supplemented with dog pancreas microsomes. To specifically analyze post-translational import the translation was stopped with emetine (50) prior to the addition of microsomal membranes. The import of PrP was monitored by the appearance of core-glycosylated protein (Fig. 5, PrP, co). PrP with a deleted TM domain, however, was efficiently imported post-translationally (Fig. 5, PrP\textsubscript{TM}, post). Similarly, a PrP mutant with a larger N-terminal deletion including the TM domain was imported post-translationally (Fig. 5, PrP\textsubscript{TM}, post). In the context of a previous study (51) that described ER targeting of PrP via the C terminus, it is important to note that none of our PrP constructs analyzed for post-translational ER import contained the C-terminal GPI anchor signal sequence.

**DISCUSSION**

Aberrant conformations of the prion protein are a hallmark of prion diseases. However, the pathogenic mechanisms leading to neuronal cell death still remain enigmatic. By using a yeast model system we were able to characterize the features of misfolded PrP that specifically interfere with cell viability: an unclaved N-terminal signal sequence and a prolonged association with ER membranes. This unprocessed PrP was generated only in the presence of the putative transmembrane domain and only during post-translational targeting of PrP to the ER.

**The Putative Transmembrane Domain Interferes with Post-translational Import of PrP into the ER**—In initial experiments we expressed PrP with its own N-terminal signal peptide and also with the signal peptide of the endogenous yeast GPI-anchored protein Gas1p. Under both conditions full-length PrP and PrP\textsubscript{GPI} accumulated with unclesed signal peptides and
were not imported into the ER (Figs. 1 and 6). In our first approach to analyze this phenomenon mechanistically, we deleted the internal domains and could show that all PrP mutants lacking the putative transmembrane domain were N-terminally processed, received N-linked glycans, and were imported into the ER (Figs. 2 and 6). A different way to promote import of full-length PrP, or PrP/ΔGPI, into the yeast ER was to use the highly hydrophobic signal sequence of Kre5p (hydro-

**Fig. 4.** An extremely hydrophobic signal sequence restores processing and anterograde transport of PrP in yeast. A, the signal sequences of mouse PrP (ss-PrP), yeast Gas1p (ss-Gas1), and Kre5p (ss-Kre5) and their respective hydrophobicities. The hydrophobicities were calculated and plotted using Dnasis 1.2. B, the Kre5p signal sequence restores processing of PrP in yeast cells. PrP was expressed with the Kre5p signal (kPrP, kPrP/ΔGPI) and compared with PrP expressed with the N-terminal signal sequence of Gas1p signal (gPrP/ΔGPI). Proteins were analyzed as described for Fig. 1B. Note that kPrP receives N-linked glycans and that the N-terminal signal sequence is cleaved off from kPrP and kPrP/ΔGPI. C, expression of kPrP/ΔGPI does not interfere with yeast growth. A colony assay of yeast cells expressing kPrP/ΔGPI or gPrP/ΔGPI was performed as described for Fig. 1C. D, anterograde export of kPrP/ΔGPI from the yeast ER. Subcellular localization of kPrP/ΔGPI and gPrP/ΔGPI was analyzed as described for Fig. 3A. A as a marker for the vacuolar fraction Yph1p was used (vector).
phobicity index > 3), which is specific for co-translational import (Figs. 4 and 6). Based on these findings it seems most likely that the putative transmembrane domain specifically interferes with the post-translational import of PrP into the ER.

This model was supported by a mammalian in vitro ER import model system. Full-length PrP was not imported posttranslationally into dog pancreas microsomes (Fig. 5), corroborating earlier results (49). Efficient post-translational import was observed, however, for PrP constructs lacking the TM domain (Figs. 5 and 6).

How could the TM domain interfere with post-translational ER import? One possibility is that the TM domain induces a conformation in which the N-terminal signal peptide is buried. Thereby, the signal sequence would be functionally inactivated and post-translational targeting to the ER would be prevented. However, the fact that PrP expressed with a signal peptide was found in association with ER membranes, in contrast to cytosolic PrP, which does not have a signal peptide, points to a different scenario. Interference of the TM domain with post-translational translocation itself seems more plausible, suggesting that not the targeting to the ER membrane but the translocation process might be impaired. Two models, which are not mutually exclusive, could explain this phenomenon. First, during post-translational targeting the C-terminal domain of PrP might adopt a tight conformation incompatible with a subsequent translocation into the ER, analogous to the impaired post-translational import of dihydrofolate reductase into mitochondria when stabilized by methotrexate (52, 53). In
this context, it is important to note that after a limited proteolytic digestion the N terminus of misfolded PrP is rapidly degraded, whereas the C terminus adopts a partially PK-resistant core. Alternatively, the TM domain could interact with components present in the cytosol or in the ER membrane. Such an interaction, obviously not present during co-translational import, would directly interfere with the translocation process.

N-terminally Unprocessed PrP Associates with ER Membranes and Interferes with Cell Viability—Under physiological conditions in mammalian cells, PrP is post-translationally modified by the cleavage of the N-terminal signal peptide, the addition of two N-linked glycans, and a C-terminal GPI anchor. When we concentrated on the analysis of different PrPΔGPI constructs, we observed three different degrees of post-translational modifications. 1) pPrPΔGPI and gPrPΔGPI were neither N-terminally processed nor glycosylated. 2) kPrPΔGPI was also not glycosylated; however, the N-terminal signal peptide was cleaved. 3) gPrPΔGPI mutants devoid of the putative TM domain (gPrPΔTM, gPrPΔ28–140, gPrPΔ28–156) were both N-terminally processed and glycosylated. In a previous study our group analyzed the folding of PrP in the secretory pathway of mammalian cells and identified different PrP mutants, which were compromised in the post-translational modifications characteristic of PrPc. None of these mutants, however, contained an uncleaved signal peptide (10). In a different study we were also able to show that complex glycosylation of PrPc, a modification that does not occur in yeast cells, is dispensable for correct trafficking through the secretory pathway (54).

The retarded growth of yeast cells was specifically linked to the accumulation of PrP molecules falling in class 1, i.e. containing an uncleaved signal peptide (Fig. 6). Another feature, which was observed exclusively for these PrP constructs, was a prolonged association with ER membranes (Figs. 3 and 4).

How might the prolonged association of gPrP interfere with yeast growth? Previous studies proposed that impaired integrity of cellular membranes, because of a pore forming activity of the putative TM domain, could contribute to the pathological changes in prion diseases (55–57). In our yeast system, decreased cell growth was dependent on both the N-terminal signal peptide and the TM domain. Thus, it is conceivable that the signal peptide targets misfolded PrP to the ER membrane, where PrP might interact with a component of the ER membrane or the translocon, thereby impairing its function. A straightforward approach to test this hypothesis would be to perform co-immunoprecipitation experiments, but unfortunately the rapid aggregation of PrP interferes with such an approach. Alternatively, the yeast model offers the possibility to perform a suppressor screen to identify cellular components involved in the decreased growth of gPrP-expressing yeast cells.

PrP Misfolding in the Cytosol and Neurodegeneration—Our present study emphasizes the idea that aberrant PrP conformers in the cytosol and/or at the ER membrane have cytotoxic properties. In the mammalian system two PrP species, CmPrP and cytosolic PrP aggregates, have previously been shown to induce neurodegeneration independent of PrPc. The increased synthesis of CmPrP, as a result of mutations in the putative TM domain, is linked to inherited prion diseases in transgenic animals and humans (17, 46). Neurotoxicity and neurodegeneration linked to misfolded PrP in the cytosol was observed in cultured cells after treatment with proteasomal inhibitors and in transgenic mice after forced cytosolic PrP expression (15). In both models no infectious PrPc was found, similar to our PrP aggregates formed in yeast cells (data not shown). Indeed, it was suggested that the neurotoxic and the transmissible agent in prion disease might be distinct species (46).

Is there a possible relationship between the different toxic PrP species described thus far? In cell culture models CmPrP has not been shown to cause cell death, even though a particular PrP mutant was exclusively present as CmPrP (58). This might reflect a specific vulnerability of post-mitotic neurons under a prolonged pathogenesis in animals/humans. It is interesting, however, that both gPrP expressed in yeast and CmPrP contain unprocessed N-terminal signal peptides (49, 58).

In the cell culture model described by Ma et al. (15), cytosolic accumulation of PrP and cell death of N2a cells was induced by a proteasomal inhibitor. A recent report revealed that the cytosolic PrP molecules found after such a treatment contain an uncleaved signal peptide (59). Thus, the cytosolic species in the N2a cell culture model is very similar to gPrP, which interferes with yeast growth.

In contrast to the animal model described (15), we did not see any adverse effects of PrP expressed in the cytosol and lacking a N-terminal signal peptide. This might be explained by the fact that in transgenic mice PrP was expressed in the background of endogenous PrPc. This leaves the possibility that cytosolic PrP mediates its toxic effect by interfering with the import of endogenous PrP and/or the recruitment of the latter into cytosolic aggregates. Alternatively, the adverse effects of cytosolic PrP might be too moderate to be detected in fast dividing cells.

Our study is the first demonstration that PrP misfolding can interfere with the viability of non-mammalian cells. The use of the yeast system could help to define cellular pathways that are sensitive to the accumulation of misfolded PrP and might thereby lead to a better understanding of mammalian prion diseases.

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