Cytosolic Prion Protein Is Not Toxic and Protects against Bax-mediated Cell Death in Human Primary Neurons*

Recently, it was observed that reverse-translocated cytosolic PrP and PrP expressed in the cytosol induce rapid death in neurons (Ma, J., Wollmann, R., and Lindquist, S. (2002) Science 298, 1781-1785). In this study, we investigated whether accumulation of prion protein (PrP) in the cytosol is toxic to human neurons in primary culture. We show that in these neurons, a single PrP isoform lacking signal peptide accumulates in the cytosol of neurons treated with epoxomicin, a specific proteasome inhibitor. Therefore, endogenously expressed PrP is subject to the endoplasmic reticulum-associated degradation (ERAD) pathway and is degraded by the proteasome in human primary neurons. In contrast to its toxicity in N2a cells, reverse-translocated PrP (ERAD-PrP) is not toxic even when neurons are microinjected with cDNA constructs to overexpress either wild-type PrP or mutant PrP1D78N. We found that ERAD-PrP in human neurons remains detergent-soluble and proteinase K-sensitive, in contrast to its detergent-insoluble and proteinase K-resistant state in N2a cells. Furthermore, not only is microinjection of a cDNA construct expressing CyPrP not toxic, it protects these neurons against Bax-mediated cell death. We conclude that in human neurons, ERAD-PrP is not converted naturally into a form reminiscent of scrapie PrP and that PrP located in the cytosol retains its protective function against Bax. Thus, it is unlikely that simple accumulation of PrP in the cytosol can cause neurodegeneration in prion diseases.

The prion protein (PrP) is mostly expressed as a secreted glycoprotein that remains attached to the cell surface through a glycophosphatidylinositol anchor. On occasion, PrP also accumulates as a transmembrane type I or type II glycoprotein. Furthermore, wild-type and mutant PrPs, Y145stop and Q217R, respectively, generate intracellular PrP in the presence of proteasome inhibitors, indicating that the normally secreted PrP is subject to the degradative pathway termed endoplasmic reticulum associated protein degradation (ERAD), possibly to eliminate misfolded PrP molecules (2-5). This form of PrP is termed ERAD-PrP. However, immunodetection of PrP with an intact N-terminal signal peptide in transfected cells treated with a proteasome inhibitor raises the possibility that a fraction of the overexpressed PrP (SP-PrP) is synthesized in the cytosol instead of going through the secretory pathway.

The presence of PrP in the cytosol (ERAD-PrP or SP-PrP) suggests that deregulation of the proteasome during aging could result in the accumulation of cytotoxic PrP molecules (7). Indeed, PrP that accumulates in the cytosol of mouse neuroblastoma N2a cells, Chinese hamster ovary cells, and African green monkey kidney COS-1 cells forms insoluble aggregates partially resistant to proteinase K, which is reminiscent of PrP scrapie (PrPSC) (4, 5, 8). Furthermore, accumulation of PrP in the presence of proteasome inhibitors is toxic to N2a cells but not to COS-1 or Swiss mouse embry0 NIH3T3 fibroblasts, suggesting that cytosolic PrP is toxic only to neuronal cell types (9). In vivo, expression of a transgene encoding a form of PrP that lacks both the N- and C-terminal signal peptides and is exclusively expressed in the cytosol (CyPrP) induces a massive loss of granule cerebellar neurons and gliosis (9). Because CyPrP is expressed at a very low level in the transgenic model, it is assumed that CyPrP is a very toxic isoform that is responsible for the initiation of neurodegeneration in prion diseases and that it contributes to the production of the protease-resistant PrPSc (9).

Because of the importance of these findings on the development of prion diseases, we studied the effect of PrP accumulation in the cytosol of human primary neurons. Human neurons are a more appropriate model to address the toxicity of PrP in relation to prion diseases in human, as they may behave differently than cell lines because they are terminally differentiated, polarized, and have a very long life span. We show that in human primary neurons, endogenously expressed PrP accumulates in the cytosol when cells are treated with epoxomicin, a specific irreversible inhibitor of the proteasome. The protein does not possess an N-terminal signal peptide and is, therefore, likely to arise from the ERAD pathway. However, in contrast to N2a cells, ERAD-PrP in the cytosol of human neurons remains proteinase K-sensitive and detergent-soluble and is not toxic. Moreover, overexpression of WTPrP, PrP1D78N, or CyPrP from microinjected cDNA constructs is not toxic in human neurons, nor is CyPrP expression toxic in two human neuroblastoma cell lines, BE(2)-M17 and SK-N-SH, treated with epoxomicin. In addition, we demonstrate that CyPrP expression protects hu-
man neurons against Bax-mediated cell death, similar to what we previously observed with WtPrP (10). We conclude that accumulation of PrP in the cytosol of neurons is not sufficient for neurotoxicity.

EXPERIMENTAL PROCEDURES

Clones—Cloning of human PrP, PrP-D178N, and Bax in pCEPβ (Invitrogen) was described previously (10). CyPrP (residues 23–231) was PCR-amplified using forward primer 5'-taagaatggcgccgctggaagcgcggcaagctgtgag-3’ and reverse primer 5’-ggcgatctacctagctacctagcttcttgtaaag-3’ and cloned between the NorI and BamHI sites of pCEPβ. The pCEPβ-enhanced green fluorescent protein (EGFP)-Bax construct was obtained by PCR amplification of Bax with 5'-'ggcgatctacctagctacctagcttcttgtaaag-3’ and 5'-ggcgatctacctagctacctagcttcttgtaaag-3’, and the PCR product was cloned in the Xhol/HindIII sites of pCPEβ-EGFP. pCEPβ-EGFP was created by transferring EGFP from EGFP-C1 (Clontech) into the Nhel/BamHI restriction sites of pCPEβ.

Cell Culture, Microinjections, Transfection, and Cell Death—Human primary neurons were cultured as described (11). Microinjections and cell death determination were described previously (12). Briefly, neurons were microinjected with 25 pl containing 0.75 pg of DNA and 2.5 pg of dextran Texas Red (DTR) in phosphate-buffered saline. Two hundred neurons from at least three independent neuronal preparations were injected. Cells were fixed in 4% sucrose and 0.1% paraformaldehyde in phosphate-buffered saline; cell death was assessed by TUNEL according to the manufacturer’s protocol (In Situ Cell Death Detection Kit (AP), Roche Applied Science). The percentage of cell death was determined as the number of DTR and TUNEL double-positive over the total number of DTR-positive neurons. All cells were obtained from American Type Culture Collection (Manassas, VA). Human neuroblastoma cells BE(2)-M17 and SK-N-SH were maintained in OptiMEM plus 10% fetal bovine serum and minimum essential medium plus 5% bovine calf serum, respectively (Invitrogen). N2a mouse neuroblastoma cells were maintained in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum (Invitrogen). Transfections were carried out in 6-well plates using ExGen 500 and 4 μg of DNA according to the manufacturer’s protocol (MBI, Burlington, Ontario, Canada). For detection of SP-PrP, BE(2)-M17 cells were transfected with 10 μg of DNA, as described previously (6). Nuclei were visualized after staining 20 min with 1 μg/ml Hoechst 33342 (Sigma) 24 h after transfection.

Subcellular Fractionation and PrP Detection—Subcellular fractions were prepared as described previously (13) with some modification. Approximately 72 × 10^6 cells were washed three times with ice-cold phosphate-buffered saline buffer and homogenized with 20 strokes in a Potters-Elvehjem homogenizer (homogenization buffer: 8% sucrose (w/v), 20 mM HCl-Tricine, pH 7.8, 1 mM EDTA). After a first spin at 2000 × g for 10 min to eliminate unbroken cells and nuclei, the supernatant was centrifuged at 100,000 × g for 30 min. Supernatant and pellet from the 100,000 × g centrifugation represent the cytosolic and membrane fractions, respectively. Proteins of each fraction were solubilized in loading buffer (0.5% SDS (w/v), 1.25% 2-mercaptoethanol (v/v), 4% glycerol (v/v), 0.01% bromphenol blue (w/v), 15 mM Tris-HCl, pH 6.8), and 100 μg of protein was separated in a 15% SDS-PAGE. PrP was detected by Western blotting using the 3F4 monoclonal antibody (50,000 dilution) and an antibody directed against PrP N-terminal signal peptide (anti SP-PrP, 1:1000 dilution, kindly provided by Dr. D. A. Harris, Washington University School of Medicine). For Bcl2 and tau detection, a polyclonal N19 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and a monoclonal T-5530 antibody (Sigma) were used, respectively. Analysis of PrP Aggregation and Proteinase K Digestion—Culture media and cell lysates were replaced with media containing 5 μg/ml brefeldin A (Sigma) and either 0.25 μg/ml (cell lines) or 1 μg/ml (neurons) epoxomycin (BioMol, Plymouth Meeting, PA) 24 h after transfection. After 16 h of treatment, cells in 6-well plates were lysed with 0.2 ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.5% Triton X-100 (v/v), and 0.5% sodium deoxycholate (w/v)) for 20 min at 4 °C. After centrifugation at 15,000 × g for 10 min at 4 °C, soluble PrP was present in the supernatant, and aggregated PrP was present in the pellet. Proteins from the pellet were resuspended in 0.2 ml of lysis buffer. Proteins in both supernatants and pellets were precipitated with 4 volumes of methanol and analyzed by Western blotting. Resistance to proteinase K was assessed by incubating cell lysates with 10 μg/ml proteinase K for 30 min at 37 °C.

Statistical Evaluation—The significance of variance was analyzed with analysis of variance followed by post-hoc Scheffe’s test using StatView (SAS Institute Inc., Cary, NC). A p < 0.05 was taken as a significant difference.

RESULTS

To assess whether PrP is subject to the ERAD pathway in human neurons, we treated cells with both brefeldin A (BFA) and epoxomycin. BFA blocks the transport of secreted proteins beyond the endoplasmic reticulum (ER)-cis-Golgi compartment (14) and is expected to increase the amount of PrP diverted to the ERAD. Epoxomycin is a specific and irreversible inhibitor of the proteasome (15) and is expected to allow PrP accumulation in the cytosome. Cells were homogenized, and the presence of PrP was analyzed by Western blotting of proteins from crude extracts, cytosol, and membrane subcellular fractions (Fig. 1A).

![Fig. 1. Accumulation of PrP in the cytosol of human neurons is not toxic.](image-url)
In untreated cells, PrP was mainly detected in membranes but not in the cytosol. Treatment of cells with BFA + epoxomicin resulted in the synthesis of immature PrP. Moreover, a large proportion of PrP was present in the cytosol. The presence of Bcl2 in membranes but not in the cytosol and the detection of tau protein isoforms mainly in the cytosol show that relatively pure subcellular fractions were used in this experiment. The apparent molecular mass of PrP in the cytosol was slightly decreased (Fig. 1A). To determine if the signal peptide was removed from PrP, we used an antibody directed against the N-terminal signal peptide of PrP (6) (Fig. 1A). The SP-PrP antibody did not detect PrP in the cytosol, suggesting the form of PrP that accumulated in the presence of epoxomicin and BFA was indeed reverse-translocated. Interestingly, no SP-PrP was detected in the crude lysate of neurons. Therefore, either the signal peptide was efficiently removed from endogenously expressed PrP protein, or the amount of SP-PrP was below the limit of detection. The specificity of the SP-PrP antibody was verified in BE(2)-M17 cells transfected with 10 μg of WtPrP cDNA (Fig. 1B), as described previously (6). The antibody detects a form of PrP that contains the SP in WtPrP-transfected BE(2)-M17 cells. In contrast, neither fractionated cytosolic PrP nor PrP in crude extracts from control or BFA + epoxomycintreated human neurons were detected with the SP-antibody. The expression of PrP was verified by reprobing the blot with the 3F4 antibody (Fig. 1B).

To determine whether the presence of ERAD-PrP in the cytosol of human neurons is as toxic as it is in neuroblastoma N2a cells (9), cell death in untreated or BFA + epoxomicin-treated neurons was assessed by TUNEL assay. Accumulation of PrP in the cytosol not toxic in human neurons, but it also protects against Bax-mediated cytotoxicity.

Cytosolic PrP Is Neuroprotective in Human Primary Neurons

To address more precisely the cytotoxicity of PrP in the cytosol of human neurons, a cytosolic form of PrP (CyPrP, amino acids 23–231) that contains neither the N-terminal ER-targeting peptide nor the C-terminal signal peptide required for the addition of a glycosphatidylinositol anchor was cloned. This construct expressed stable levels of CyPrP in neuroblastoma BE(2)-M17 cells that lack endogenous PrP expression (Fig. 1D, inset). Microinjection of CyPrP cDNA did not induce neuronal cell death even 72 h after injection (Fig. 1D). In contrast, N2a cells are susceptible to CyPrP expression within 24 h of transfection, as shown by Ma, Wollmann, and Lindquist (9) (Fig. 1E). Moreover, attempts to select stable N2a cells expressing CyPrP failed (not shown). This finding prompted us to test the cytotoxicity of CyPrP in other human neuroblastoma cell lines. In contrast to what we observed in N2a murine neuroblastoma cells, expression of CyPrP was toxic to neither BE(2)-M17 nor SK-N-SH cells (Fig. 1E). These cells are all competent to undergo apoptosis, as verified by overexpression of Bax. Therefore, not all neuronal cells are susceptible to CyPrP-mediated toxicity.

When it accumulates in the cytosol of N2a cells, ERAD-PrP/SP-PrP is converted to a detergent-insoluble and proteinase K-resistant form reminiscent of the transmissible PrPSc (8). Therefore, we tested the detergent solubility and proteinase K resistance of PrP in primary neurons or transiently transfected N2a, BE(2)-M17, or SK-N-SH cell lines in the absence and presence of BFA + epoxomicin (Fig. 2A). In the absence of BFA + epoxomicin, PrP is completely detergent soluble in all cell types. However, in the presence of BFA + epoxomicin, a small amount of PrP resisted detergent solubility in N2a cells but not in primary neurons and BE(2)-M17 or SK-N-SH cells (Fig. 2A).

To assess whether CyPrP retains its neuroprotective function against Bax in human neurons, we co-microinjected a Bax-expressing construct with CyPrP. Microinjection of Bax cDNA killed about 45% of neurons within 24 h (Fig. 3A). As observed previously, co-expression of WtPrP completely protects against Bax-mediated cell death. Co-injection of CyPrP cDNA also protects against Bax-mediated cell death. To ensure that WtPrP and CyPrP do not inhibit Bax expression, an EGFP-Bax fusion protein cDNA previously shown to behave like WtBax (16) was cloned and microinjected with WtPrP or CyPrP constructs. In the absence of WtPrP and CyPrP co-injection, EGFP was detected in only 25 of the 100 cells injected, indicating that the fusion Bax-EGFP protein may be very toxic and that dying neurons either degrade EGFP-Bax more efficiently or have a lower synthesis rate (Fig. 3B).

In untreated cells, PrP was mainly detected in membranes but not in the cytosol. Treatment of cells with BFA + epoxomicin resulted in the synthesis of immature PrP. Moreover, a large proportion of PrP was present in the cytosol. The presence of Bcl2 in membranes but not in the cytosol and the detection of tau protein isoforms mainly in the cytosol show that relatively pure subcellular fractions were used in this experiment. The apparent molecular mass of PrP in the cytosol was slightly decreased (Fig. 1A). To determine if the signal peptide was removed from PrP, we used an antibody directed against the N-terminal signal peptide of PrP (6) (Fig. 1A). The SP-PrP antibody did not detect PrP in the cytosol, suggesting the form of PrP that accumulated in the presence of epoxomicin and BFA was indeed reverse-translocated. Interestingly, no SP-PrP was detected in the crude lysate of neurons. Therefore, either the signal peptide was efficiently removed from endogenously expressed PrP protein, or the amount of SP-PrP was below the limit of detection. The specificity of the SP-PrP antibody was verified in BE(2)-M17 cells transfected with 10 μg of WtPrP cDNA (Fig. 1B), as described previously (6). The antibody detects a form of PrP that contains the SP in WtPrP-transfected BE(2)-M17 cells. In contrast, neither fractionated cytosolic PrP nor PrP in crude extracts from control or BFA + epoxomycintreated human neurons were detected with the SP-antibody. The expression of PrP was verified by reprobing the blot with the 3F4 antibody (Fig. 1B).

To determine whether the presence of ERAD-PrP in the cytosol of human neurons is as toxic as it is in neuroblastoma N2a cells (9), cell death in untreated or BFA + epoxomicin-treated neurons was assessed by TUNEL assay. Accumulation of PrP in the cytosol does not induce cell death (Fig. 1C, Ctl). To test whether toxicity may occur only in conditions of overexpression of PrP, neurons were microinjected with a WtPrP cDNA high expression construct before the incubation with BFA + epoxomicin. Again, no toxicity was observed (Fig. 1C). Furthermore, microinjection of a construct expressing mutant D178NPrP known to enhance PrP trafficking through the ERAD pathway (5) also does not induce toxicity. However, neurons remain sensitive to Bax overexpression in the absence or presence of BFA + epoxomicin.

To address more precisely the cytotoxicity of PrP in the cytosol of human neurons, a cytosolic form of PrP (CyPrP, amino acids 23–231) that contains neither the N-terminal ER-targeting peptide nor the C-terminal signal peptide required for the addition of a glycosphatidylinositol anchor was cloned. This construct expressed stable levels of CyPrP in neuroblastoma BE(2)-M17 cells that lack endogenous PrP expression (Fig. 1D, inset). Microinjection of CyPrP cDNA did not induce neuronal cell death even 72 h after injection (Fig. 1D). In contrast, N2a cells are susceptible to CyPrP expression within 24 h of transfection, as shown by Ma, Wollmann, and Lindquist (9) (Fig. 1E). Moreover, attempts to select stable N2a cells expressing CyPrP failed (not shown). This finding prompted us to test the cytotoxicity of CyPrP in other human neuroblastoma cell lines. In contrast to what we observed in N2a murine neuroblastoma cells, expression of CyPrP was toxic to neither BE(2)-M17 nor SK-N-SH cells (Fig. 1E). These cells are all competent to undergo apoptosis, as verified by overexpression of Bax. Therefore, not all neuronal cells are susceptible to CyPrP-mediated toxicity.

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Similarly, PrP became partially resistant to proteinase K in N2a but not in primary neurons and BE(2)-M17 or SK-N-SH cells treated with BFA + epoxomicin (Fig. 2B). These results show that the conversion of ERAD-PrP does not occur significantly in human primary neurons or neuroblastoma cell lines.

To assess whether CyPrP retains its neuroprotective function against Bax in human neurons, we co-microinjected a Bax-expressing construct with CyPrP. Microinjection of Bax cDNA killed about 45% of neurons within 24 h (Fig. 3A). As observed previously, co-expression of WtPrP completely protects against Bax-mediated cell death. Co-injection of CyPrP cDNA also protects against Bax-mediated cell death. To ensure that WtPrP and CyPrP do not inhibit Bax expression, an EGFP-Bax fusion protein cDNA previously shown to behave like WtBax (16) was cloned and microinjected with WtPrP or CyPrP constructs. In the absence of WtPrP and CyPrP co-injection, EGFP was detected in only 25 of the 100 cells injected, indicating that the fusion Bax-EGFP protein may be very toxic and that dying neurons either degrade EGFP-Bax more efficiently or have a lower synthesis rate (Fig. 3B). However, in the presence of WtPrP or CyPrP, 70–80 of 100 cells injected showed EGFP fluorescence, indicating that WtPrP or CyPrP do not inhibit Bax expression but inhibit Bax-mediated cell death. Therefore, not only is expression of PrP in the cytosol not toxic in human neurons, but it also protects against Bax-mediated cytotoxicity.
WtPrP and Bax

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WtPrP or pCEP4

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as expected in a retro-translocated protein. These results indicate that prior to proteasomal degradation, PrP is first translocated into the ER where the signal peptides are cleaved and immature glycosylation occurs. Then, after undergoing deglycosylation, PrP can be reverse-translocated into the cytosol, leading to the appearance of ERAD-PrP.

Converson of Cytosolically Located PrP into a PrPSc-like Isoform Is Limited to Certain Neuronal Cell Types—When ERAD-PrP/SP-PrP accumulates in the cytosol of N2a cells, it converts into a form reminiscent of PrPSc and is toxic (Ref. 8 and this study). Therefore, it was important to determine the state and the toxicity of ERAD-PrP in human neurons. Here, we show evidence that in human primary neurons and in two human neuroblastoma cell lines, BE(2)-M17 and SK-N-SH, ERAD-PrP does not convert into a PrPSc-like form. Several hypotheses could explain why ERAD-PrP conversion seems to be cell type-dependent. First, overexpression of PrP in transfected cells may induce PrP aggregation and resistance to proteasomal inhibition. Overexpression-induced protein aggregation in transfected cells has already been reported for α-synuclein (involved in Parkinson’s disease) (18) and tau (involved in tauropathies in transfected cells) (17). As mentioned by Drisaldi et al. (6), proteasome inhibitors may cause a significant increase in PrP expression from the cytomegalovirus promoter, leading to accumulation of large amounts of PrP in cells. The hypothesis that PrP overexpression leads to PrP aggregation in human neurons is supported because they only express endogenous levels of PrP and there is no PrP conversion. However, SK-N-SH cells consistently accumulate more ERAD-PrP than N2a cells, yet they do not produce detergent-insoluble and protease K-resistant PrP (Fig. 2A). Second, intracellular redox conditions could affect conversion of PrP into a PrPSc-like form. We have not tested this hypothesis in human neurons and N2a cells, but evidence obtained in yeast and mammalian cells treated with a reducing agent show that cellular PrP is converted into a detergent-insoluble and protease K-resistant form (9, 19). Therefore, it is plausible that the reducing conditions in the cytosol vary in different cell types and affect conversion of PrP. Interestingly, recombinant PrP under reducing conditions also forms insoluble aggregates in vitro (20). Third, other cellular parameters such as pH may be involved in PrP conversion. Acidic pH favors PrP conversion in vitro, and some in vivo studies suggest that PrP conversion occurs in intracellular acidic compartments (21–24). Interestingly, human neurons maintain stable pH, as evidenced by phenol red pH indicator in culture medium, whereas the pH in the culture medium of N2a cells becomes acidic rapidly. Finally, because ERAD-PrP does not convert into a scrapie-like conformation in all cells, it is possible that some cytosolic factors allow or prevent PrP conversion. Chaperones GroEL, a close homolog of mammalian Hsp60, and Hsp104 promote the conversion of PrP into PrPSc-like protein in vitro (25–27).

**DISCUSSION**

In this paper, we show that endogenously expressed PrP is subject to the ERAD pathway in human primary neurons and that ERAD-PrP does not convert into a PrPSc-like state in the presence of a proteasome inhibitor. In the cytosol, neither ERAD-PrP nor transfected CyPrP is toxic. Moreover, CyPrP inhibits Bax-mediated cell death.

**PrP Goes through the ERAD Pathway in Human Neurons—** Here, we show conclusively that endogenously expressed PrP goes through the ERAD pathway in primary human neurons. Previous studies used immunofluorescence in transfected Chinese hamster ovary, COS, N2a, NIH3T3, and undifferentiated rat pheochromocytoma PC12 cell lines to detect PrP in the cytosol of cells treated with proteasome inhibitors (4–6). However, immunofluorescence experiments cannot exclude the possibility that PrP is associated with cytoplasmic structures or organelles. Our observation (obtained by subcellular fractionation) that PrP is detected in the cytosol strongly supports the presence of soluble cytosolic PrP in human neurons. Moreover, expression of PrP under the control of a strong promoter such as cytomegalovirus might artificially produce PrP molecules bearing the N-terminal signal peptide (SP-PrP) that accumulate in the presence of proteasome inhibitors (6). In human neurons, ERAD-PrP is made from endogenously expressed PrP, and the N-terminal signal peptide is removed from the protein because an antibody directed against this peptide does not detect PrP in the cytosol of cells treated with epoxomicin. ERAD-PrP is detected in a purified cytosolic fraction but not in a crude extract, indicating that the protein is present at a relatively low level. The shift in molecular weight of ERAD-PrP implies that the molecule has lost its immature glycosylation, as expected in a retro-translocated protein. These results indicate that prior to proteasomal degradation, PrP is first translocated into the ER where the signal peptides are cleaved and immature glycosylation occurs. Then, after undergoing deglycosylation, PrP can be reverse-translocated into the cytosol, leading to the appearance of ERAD-PrP.
conversion into a detergent-insoluble and proteinase K-resistant form. To address this question, agents that can prevent ERAD-PrP conversion in N2a cells will need to be discovered and tested. However, the toxicity of detergent-soluble and proteinase K-sensitive CyPrP in N2a but not NIH3T3 cells and the absence of detectable PrP \(^{34}\) in cytoplasmic PrP in N2a cells but not NIH3T3 cells suggests that PrP may interact directly with Bax/.

The ERAD pathway (2–5) that both WT PrP and CyPrP inhibit Bax killing (Ref. 10 and this study) and that four identical N-terminal PrP octapeptide repeats share similarity with the Bcl2 homology domain 2 (39) raise the possibility that, in a manner similar to Bcl2, PrP may interact directly with Bax. However, Bax and PrP did not interact in a yeast two-hybrid system (40). Moreover, PrP is present mainly at the cell surface and in luminal vesicular sites (ER, Golgi, endosomes), whereas only a small proportion is retro-translocated (10% of nascent PrP molecules in a cell culture model) (4). Further studies are required to understand how PrP prevents Bax-mediated cell death. In summary, we have demonstrated that not only is CyPrP not toxic in human primary neurons, it also protects these cells against the major neuronal proapoptotic protein, Bax. Therefore, based on the observation that expression of CyPrP in transgenic mice leads to neurodegeneration, it seems premature to conclude that reverse-translocation of PrP in the cytosol represents a step in the development of prion diseases in humans. This study also highlights the importance of using, in addition to cell lines and animal models, cultured human primary neurons as a cell model of prion diseases.

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