Prion Protein Glycosylation Is Sensitive to Redox Change*

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The conversion of soluble prion protein into an insoluble, pathogenic, protease-resistant isoform is a key event in the development of prion diseases. Although the mechanism by which the conversion engenders a pathogenic event is unclear, there is increasing evidence to suggest that this may depend on the function of the prion protein in preventing oxidative damage. Therefore, in this study, we assessed the interrelationship between redox-sensitive cysteine, glycosylation, and prion metabolism. Cells were treated with a thioreductant, dithiothreitol, to assess the effect of the cellular redox status on the synthesis of the prion protein. This change in redox balance affected the glycosylation of the prion protein, resulting in the sole production of glycosylated forms. The role of the single disulfide bridge in mediating this effect within the prion protein was confirmed by mutating the cysteine residues involved in its formation. These data suggest that conditions that increase the rate of formation of the disulfide bridge favor formation of the unglycosylated prion protein. Thus, since the presence of glycans on the prion protein is protective against its pathogenic conversion, a change in the redox status of the cell would increase the risk of developing a prion disease by favoring the production of the unglycosylated form.

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*This work was supported by National Institutes of Health Grants AG08155 and AG08992 and by the Britton Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: PrP C, cellular prion protein; PrP SC, abnormal, protease-resistant prion protein; PrP, prion protein; DTT, dithiothreitol; ER, endoplasmic reticulum; PI-PLC, phosphoinositol-specific phospholipase C; PDI, protein-disulfide isomerase.

Conversion of the cellular prion protein (PrP C) to an abnormally conformed, aggregated, protease-resistant isoform (PrP SC) is a cardinal feature of prion diseases. In humans, PrP C comprises 209 amino acids, a disulfide bridge between residues 179 and 214, a glycosylphosphatidylinositol anchor, and two sites of non-obligatory N-linked glycosylation at amino acids 181 and 197 (1–5). The normal function of PrP C remains unclear, although roles in synaptic function, regulation of circadian rhythms, and copper transport have been proposed. Suppression of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The fact that copper is a key redox transition metal and the observation that PrPC regulates the formation of PrPSc led us to consider a possible interrelationship between the two. The prion protein is present at the cell surface as three species that differ in their degree of glycosylation. The unglycosylated (5%) and monoglycosylated (25%) isoforms of the prion protein are minor cell-surface components, whereas the diglycosylated form is dominant (70%) (2, 3, 10).

In this study, we examined the effect of the cellular redox balance on the ratio of the three PrP C glycoforms. The importance of PrP C glycoforms in the development of prion pathology was suggested in scrapie and confirmed in the human disease (10–12). The ratio between the glycoforms differs between PrP C and PrP SC suggesting that glycosylation can modulate the conversion of PrP C into PrP SC as was observed in vitro (13).

We found that treating cells overexpressing PrP C with the reducing agent DTT resulted in the production of almost entirely diglycosylated PrP C that reaches the cell surface. In contrast, although elimination of the disulfide bridge by mutagenesis of a single cysteine residue also resulted in the exclusive synthesis of diglycosylated PrP C, the protein was retained in the ER. Removal of both cysteine residues resulted in diglycosylated PrP C that reached the cell surface, but failed to recycle. These findings demonstrate a relationship between PrP C processing and redox balance and suggest a mechanism whereby oxidative stress could play a role in prion disease pathogenesis.

EXPERIMENTAL PROCEDURES

Cloning and Production of Cell Lines—M-17 human neuroblastoma cells were transfected with the episomal vector CEP48 containing a prion coding sequence (3). The inserted prion protein coding sequence, under the control of the cytomegalovirus promoter, was either normal or mutated at cysteine 179 or 214 or both. We created mutant PrP using oligonucleotide-directed mutagenesis (3) with the primer 5'-GCAG- GAGCAGATGCGCATACCCAG-3' (Cys-to-Ala mutation at codon 214), where boldface indicates the mutant codon. The primers were used together to produce the double mutant.

Transfected cells were grown as bulk selected hydrogymcin-resistant cultures in Opti-MEM with 5% calf serum supplement (iron-enriched; Life Technologies, Inc.) and 500 μg/ml hydrogymcin B (Calbiochem). For each experiment, cells were removed from the flask with trypsin and counted, and identical numbers of cells (3.4 × 10⁵ or 9 × 10⁵) were seeded onto 6- or 10-cm plates, respectively, and grown overnight to ~95% confluence.

Pulse-Chase—Cells were washed and then preincubated for 1 h at 37 °C in methionine-deficient minimum Eagle’s medium (ICN Pharmaceuticals Inc., Costa Mesa, CA) to deplete the endogenous pool. For the DTT experiments, the plates were also preincubated for 5 min in methionine-deficient minimum Eagle’s medium supplemented with 2 μM DTT (Sigma). This concentration was empirically determined to minimize inhibition of protein synthesis. Cells were then pulsed for the appropriate time with 0.5 μCi of Trasyl™-label (ICN Pharmaceuticals Inc.) with or without DTT in methionine-deficient minimum Eagle’s medium. A 0–120-min chase in Opti-MEM, with or without DTT, followed. For the DTT-treated cells, 10 min before lysis, iodoacetic acid was added to a final concentration of 20 mM to prevent oxidation. Iodoacetic acid was also added to the lysis buffer. Cells were then washed once with Opti-MEM and incubated in Opti-MEM ± PI-PLC (59 ng/ml) for the last 30 min of the chase at 37 °C. The medium was...
collected, centrifuged at 290 × g for 10 min to remove any cells, and processed for immunoprecipitation. The cells were washed with phosphate-buffered saline, pH 7.4, and lysed for immunoprecipitation.

**Cell Lysis and Immunoprecipitation—**Cells were lysed in 1 ml of 0.5% Nonidet P-40 and phosphate-buffered saline supplemented with 1 mM phenylmethylsulfonyl fluoride on ice. The cell lysates were centrifuged at 2100 × g for 10 min at 4 °C to remove nuclei and cell debris. The prion protein was immunoprecipitated with the appropriate antibody in 1% bovine serum albumin, 0.1% N-lauroyl sarcosine, and fresh 1 μM phenylmethylsulfonyl fluoride by rocking at 4 °C for 16 h. Protein A-Sepharose beads were used to collect the complexes. The beads were washed twice with ice-cold wash buffer (150 mM NaCl, 20 mM Tris, pH 7.8, and 0.1% N-lauroyl sarcosine with 0.1 mM phenylmethylsulfonyl fluoride), suspended in sample buffer (6% SDS, 5% β-mercaptoethanol, 4 mM EDTA, 20% glycerol, and 125 mM Tris, pH 6.8), and boiled for 5 min.

**Antibodies—**The following antibodies were used: 3F4, a monoclonal antibody that recognizes human PrP residues 109–112, provided by R. Rubenstein (14); S14, a mouse monoclonal antibody that reacts with the carboxy-terminal region of the prion protein (15); anti-calnexin, a polyclonal rabbit antibody against calnexin (Stressgen Biotech Corp., SPA-860); anti-calreticulin, a polyclonal rabbit antibody against calre- ticulin (Stressgen Biotech Corp., SPA-600); anti-KDEL, a monoclonal mouse antibody against a peptide containing the KDEL sequence that recognizes human PrP (Stressgen Biotech Corp., SPA-827); anti-grp94, a mono- clonal rat antibody against chicken grp94 (Stressgen Biotech Corp., SPA-850); anti-PDI, a monoclonal mouse antibody against PDI, pro- vided by Dr. M. E. Lamm (16); and anti-grp57, a monoclonal mouse antibody against ERp-57, generously provided by Dr. S. High (17).

**Endoglycosidase-H Treatment—**Proteins were precipitated using 4 volumes of methanol. Protein pellets were treated with Endoglycosi- dase-H (100 units; New England Biolabs Inc.) according to the manufacturer’s protocol.

**Double or Sequential Immunoprecipitation—**After a standard pulse-chase, the cells were lysed in 1% deionit and immunoprecipitated with anti-PDI, anti-grp57, anti-calnexin, anti-calreticulin, anti-KDEL, or anti-grp94 antibody. Following protein A-agarose recovery of the immune complexes, the beads were washed three times with 150 mM NaCl, 20 mM Tris, pH 7.8, and 0.2% Nonidet P-40 and once with phosphate-buffered saline. The immune complexes were released by boiling in 2% SDS and 0.1% β-mercaptoethanol. The supernatant was then immunoprecipitated with the 3F4 antibody to recover PrP that was present in complexes with the chaperones. The protein A-Sepha- rose recovery of the Prp3F4 complexes and washing of the beads were performed as described under “Pulse-Chase.” This protocol was also run in reverse, using the anti-PrP antibody first and then the anti-chaperone antibodies second, to confirm the findings.

**ImmunobLOTS—**Protein samples (whole cell lysates from ∼25,000 cells or double quantity for surface or secreted PrP) in sample buffer were separated on 14% SDS-polyacrylamide gels and transferred to Immobilon P (Millipore Corp.) for 2 h at 60 V. The membrane was blocked with 10% nonfat milk in Tris-buffered saline, pH 7.5, and probed with the appropriate antibody. The immunoreactivity was visual- ized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) on Kodak X-OMat film.

**SDS-Polyacrylamide Gel Electrophoresis and Fluorography—**Proteins were separated on 14% acrylamide gels (37.5:1 acrylamide/bisacrylamide). The gels were treated as described previously (3), dried, and exposed to film. Films were analyzed by densitometry using a PDI 4200 scanner. Student’s t-test was used for statistical analysis.

**RESULTS**

DTT has been used to inhibit oxidation and folding of pro- teins in the endoplasmic reticulum (18). Specifically, DTT has been used to inhibit the formation of disulfide bonds in newly synthesized proteins by altering the redox state of the cell (19). The single disulfide bridge in the prion protein surrounds the two sites of non- obligatory N-glycosylation. The influence of the disulfide bridge on the glycosylation of PrP<sup>Sc</sup> was demonstrated by treating cells with the reducing agent DTT. In the first experiment (Fig. 1A), cells expressing PrP<sup>Sc</sup> were labeled in the presence or absence of DTT and chased for 0–120 min, followed by immunoprecipitation with monoclonal antibody 3F4. Whereas untreated cells synthesized unglycosylated, monogly- cosylated, and diglycosylated forms, only the fully modified, diglycosylated form was observed in DTT-treated cells (compare lanes 4 and 8; note that the band seen after treatment with DTT (lane 5) at time 0 comigrated with the unmodified diglycosylated form (lane 1) at time 0 in the control). When samples were analyzed on SDS-polyacrylamide gels with or without β-mercaptoethanol in the sample buffer, under reduc- ing versus nonreducing conditions, the migration of the samples from the DTT-treated cells was unaltered, indicating that DTT treatment did in fact prevent disulfide bridge formation (data not shown). Thus, when cells were treated with DTT, both N-glycosylation sites were occupied, indicating that a redox change can alter core glycosylation of the prion protein.

Although treatment with DTT has been reported to lead to protein retention in the endoplasmic reticulum (19), we found that PrP<sup>Sc</sup> reached the cell surface under this condition (Fig. 1B) and could be released by cleavage of the glycosylphosphati- dylinositol anchor with the cell-impermeant enzyme PI-PLC. Whereas all three glycoforms (unglycosylated, monoglycosyl- ated, and diglycosylated) reached the cell surface in untreated cells, only the diglycosylated form was present at the cell surface after DTT treatment. From this experiment, we conclude that the change in redox state induced by DTT does not prevent prion protein transport to the cell surface.

To further demonstrate that the effects of DTT on glycosa- tion were mediated by the disulfide bridge in PrP<sup>Sc</sup>, we specifically mutated the cysteine residues involved in the disulfide bridge using site-directed mutagenesis. The constructs used in this study employed alanine replacement of cysteine at residue 179 alone (C179A (~Cys1)), residue 214 alone (C214A (~Cys1)), or residues 179 and 214 together (C179A/C214A (~Cys1+2)) (Fig. 2A). The effect of these replacements was assessed by immunoblotting (Fig. 2B). Mutation of either or both cysteine residues to alanine resulted in a shift to the production of only diglycosylated PrP<sup>Sc</sup>. In all cases, substitution of the cysteine residues resulted in the synthesis of an unmodified diglycosylated isoform, which differs from the corre- sponding modified glycoform of the control (Fig. 2B).

To assess the maturation of the glycans, we digested the mutant and control PrP<sup>Sc</sup> proteins with the enzyme Endoglyco- sidase-H, which cleaves only unmodified, high-mannose core glycans (20), and found that only the glycans of mutant PrP were released (Fig. 3). The same result was obtained when we digested the C214A and C179A/C214A mutants (data not shown). Unmodified, high-mannose glycans are indicative of proteins retained in the pre-medial Golgi region, probably in the ER.

Thus, whereas both DTT treatment and cysteine replacement resulted in almost exclusive production of diglycosylated PrP<sup>Sc</sup>, only treatment with DTT allowed transport to the cell surface, and mutation of each of the individual cysteine resi- dues blocked transport of the mutant protein. Therefore, the substitution of cysteine with alanine not only blocks the forma- tion of the disulfide bridge, but also results in the retention of
also carried out in which the anti-chaperone and 3F4 antibodies were reversed in order (data not shown). Immediately after a 10-min labeling period, we detected interaction of control PrP<sup>C</sup> with all the chaperones tested (Fig. 4B). At this time point, only immature forms of PrP<sup>C</sup> were present. After a 2-h chase, however, these interactions were no longer detectable (Fig. 4B). These data indicate that there is brief, transient interaction between PrP<sup>C</sup> and the major chaperones involved in ER folding and assembly. Thus, the naturally occurring prion protein appears to be processed in the conventional secretory pathway and is dependent on the known chaperones to achieve a properly folded state. However, with mutant PrP and the same battery of antibodies, it was apparent that C179A mutant PrP was principally retained in complexes with PDI and calnexin, both at the initial time point and after the 2-h chase (Fig. 4B). In contrast, the double mutant (C179A/C214A) demonstrated a stable interaction principally with grp94 (Fig. 4B).

Since the two mutant proteins were retained by different systems, we next investigated whether the two systems were equally efficient. The half-life of the double mutant appears to be shorter than those of both the control and the single mutant. After a 90-min chase, the double mutant appeared to be present in a smaller amount (27.2 ± 6.8%) than the single mutant (76 ± 5.3%) (Fig. 5). However, a significant fraction of C179A/C214A reached the cell surface or was secreted. In both locations, diglycosylated PrP was the major form present, as in the DTT experiment, confirming that the lack of the disulfide bridge leads to virtually complete occupancy of the two sites of glycosylation in PrP.

We examined whether the normal metabolism of the protein is maintained in the absence of the disulfide bridge since this post-translational modification has been highly conserved through evolution and PrP does not require the disulfide bridge to reach the cell surface, as demonstrated by the DTT experiment. After PrP is synthesized and transported to the cell surface, it reenters the cell via the endocytic pathway. At 60-min intervals, the protein is internalized into an endocytic compartment and then returned to the cell surface (23). During each passage, ~5% of the protein is cleaved at position 112, yielding an 18-kDa fragment (24), which then returns to the cell surface. As demonstrated above, the C179A/C214A mutant reached the cell surface. We wondered whether the mutant protein is recycled after reaching the surface. To test this hypothesis, we determined whether the mutant protein was able to produce the truncated 18-kDa fragment. The 18-kDa fragment was not detectable in cells after a 2-h chase (Fig. 6A) or at steady state on an immunoblot (Fig. 6B), indicating that this mutant is not recycled like normal PrP. Although we did not see any extra or different fragments, an alternative explanation of this finding is that the mutation could result in altered cleavage of PrP or in recycling without cleavage.

**DISCUSSION**

PrP is oxidized, folded, and modified by the non-obligatory addition of two N-linked glycans located within the span of the single disulfide bridge in the ER (2). These co/post-translational modifications are highly regulated, such that the sequences flanking the two cysteine residues, the distance between these two residues, and the position of the N-glycosylation sites relative to the two cysteine residues involved in the disulfide bond are evolutionarily conserved from mouse to man. PrP is transported through the secretory pathway and ultimately anchored to the cell surface by a glycosylphosphatidylinositol anchor (25). PrP is present at the cell surface as three species that differ in their degree of glycosylation. The unglycosylated (5%) and monoglycosylated (25%) isoforms of the prion protein are minor cell-surface compo-

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**FIG. 2.** Cysteine mutants. A, schematic diagram of the cysteine (C)-to-alanine (A) substitutions. Residues 181 and 197 are the sites of N-glycosylation. B, immunoblot of a control and the three mutants stained with the 3F4 antibody. None of the mutants could form a disulfide bridge. Note that all the mutants produced only diglycosylated immature PrP. The double mutant showed a small quantity of diglycosylated mature PrP. C, control cell line; C179A, C214A; C179A/C214A; -Cys1, -Cys2, C179A/C214A.

**FIG. 3.** Mutant PrP does not reach the cell surface and bears immature glycans. First and second lanes, PrP from total cell lysate; third and fourth lanes, PrP released from the cell surface; fifth and sixth lanes, PrP from total cell lysate treated with Endoglycosidase-H (EndoH). The 3F4 antibody was used in the immunoprecipitation. See the legend to Fig. 2 for definitions of abbreviations.

PrP in the cell. To investigate whether mutant PrP was retained in complexes with the chaperones responsible for disulfide bridge formation or protein folding (21, 22), we analyzed immunoprecipitated control and mutant PrP proteins under reducing or nonreducing conditions. The mutant lacking a single cysteine residue was involved in reduction-sensitive complexes, probably by virtue of the free cysteine (data not shown). However, this was not the sole retention mechanism since the addition of DTT during a pulse-chase experiment did not allow transport of mutant PrP to the cell surface (Fig. 4A).

We determined which chaperones were present in these complexes by employing two sets of double immunoprecipitation experiments. After pulse labeling cells followed by lysis in the presence of digitonin, we isolated chaperone-associated PrP<sup>C</sup> by immunoprecipitating with an anti-chaperone antibody (PDI, calnexin, calreticulin, BiP, or grp94) followed by immunoprecipitation with the 3F4 antibody (Fig. 4B). An experiment was carried out in which the anti-chaperone and 3F4 antibodies were reversed in order (data not shown). Immediately after a 10-min labeling period, we detected interaction of control PrP<sup>C</sup> with all the chaperones tested (Fig. 4B). At this time point, only immature forms of PrP<sup>C</sup> were present. After a 2-h chase, however, these interactions were no longer detectable (Fig. 4B). These data indicate that there is brief, transient interaction between PrP<sup>C</sup> and the major chaperones involved in ER folding and assembly. Thus, the naturally occurring prion protein appears to be processed in the conventional secretory pathway and is dependent on the known chaperones to achieve a properly folded state. However, with mutant PrP and the same battery of antibodies, it was apparent that C179A mutant PrP was principally retained in complexes with PDI and calnexin, both at the initial time point and after the 2-h chase (Fig. 4B). In contrast, the double mutant (C179A/C214A) demonstrated a stable interaction principally with grp94 (Fig. 4B).

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Glycosylation of proteins facilitates their folding by providing solubility-enhancing polar surface groups that prevent aggregation of folding intermediates as well as by enhancing interactions with ER enzymes and chaperones (26, 27). The two \( N \)-linked glycosylation sites in PrP have been preserved through 150 million years of evolution, and although the three glycoforms of PrP \( C \) are synthesized in essentially equal quantities in pulse-chase experiments (see Fig. 1, lane 1), the unglycosylated isof orm accounts for only 5% of cell-surface PrPC. This suggests that the regulated glycosylation of PrP has biological significance. Although not unprecedented, it is unusual for a glycoprotein to be expressed as multiple glycoforms. In this study, we found that the disulfide bridge, a second highly conserved element of PrPC, regulates the degree of core glycosylation and therefore the creation of multiple glycoforms. When disulfide bridge formation is blocked by the addition of DTT or by mutating the cysteine residues, PrP becomes completely diglycosylated. Since the addition of the core glycans and the formation of the disulfide bridge are believed to occur simultaneously, the multiple glycoforms of PrP must result from these two competitive processes.

Why is the disulfide bridge conserved? Transport of PrP \( C \) to the cell surface is not inhibited by lack of the disulfide bridge caused by DTT treatment, in contrast to other proteins (28). The transport of PrP in the presence of DTT and when both cysteine residues are mutated suggests that the disulfide bridge is not required for the prion protein to pass through the quality control check points. Other proteins, such as chromogranin B, which also contains a single disulfide bridge, are transported through the secretory pathway in the presence of DTT, although mis-sorting may occur (29, 30). The importance of the disulfide bridge in prion disease is underlined by the finding that the disulfide bridge is required for the conversion of PrPC\(^{C} \) to PrP\(^{Sc} \) (31). Another study suggested that the disulfide bridge might feature in the conversion of the \( \alpha \)-helical form to the \( \beta \)-sheeted form of the prion protein (32). What then is the role of the disulfide bridge under physiological conditions? Our results suggest that the normal metabolism, not only glycosylation but also recycling of PrPC, could be dependent on the presence of the disulfide bridge.

Although the lack of the disulfide bridge caused by the addition of DTT did not have an effect on PrP\(^{C} \) transport to the cell surface, PrP with a single cysteine was entirely retained in the endoplasmic reticulum. This observation is consistent with the effect of free thiols in ER retention (33). Our results indicate that retention of mutant PrP is mediated through the formation of stable complexes with the ER resident chaperones PDI and calnexin. The retention by a thiol-mediated mechanism, as shown for our single cysteine mutants, is associated
with a long half-life of mutant PrP and could cause accumulation of mutant PrP in the ER. Different retention mechanisms, as shown for our double mutant, conferring a shorter half-life to the protein would preclude this accumulation. Independent deletions of two α-helices in PrPΔC, which coincidentally cause the deletion of a single cysteine residue, have been made in transgenic mice. These mutations resulted in accumulation of mutant PrP and the early death of the animals overexpressing the protein (34). Our data suggest a mechanism in which the accumulation of mutant PrP is the result of retention in the ER mediated by the exposed thiol group rather than by deletion of the α-helices. In contrast, the pathogenic threonine-to-alanine mutation at residue 183 (35), which causes destabilization of mutant PrP (36), also results in retention in the ER, but not in a storage disease either in an animal model (37) or in human disease (35).

The present findings also provide a mechanism whereby the redox balance in a cell can affect the risk of developing a prion disease. The redox state of the cell can modify the rate of disulfide bridge formation in the prion protein. Disulfide bond formation in the prion protein has an inverse relationship with the addition of core glycans. Thus, under oxidizing conditions, the rate of disulfide bond formation would be increased, which would in turn favor the synthesis of unglycosylated PrP. The unglycosylated isoform converts more readily to a pathogenic form in a cell-free system (13). Moreover, in the human disease, there is preliminary evidence that unglycosylated PrP is more represented in brain regions with the lowest amount of PrPΔC accumulation and no pathology, which is thought to mirror the earliest stages of the disease process, suggesting that this is the first isoform to convert. Moreover, PrP lacking one or both of the glycosylation sites is more insoluble and has increased resistance to protease digestion (38). Therefore, the available evidence suggests that the unglycosylated form is the most pathogenic.

Although the pathogenic transformation of PrPΔC to PrPΔC is individually deleterious, it is a rare event, whereas the beneficial effect of a shift to the synthesis of unglycosylated PrP may be critical to cell survival. Furthermore, we cannot know whether the PrPΔC glycoforms have different properties that make the unglycosylated form beneficial under some conditions. For example, different glycoforms of a protein may be targeted to different cellular locations. In transgenic mice expressing PrPΔC where one or both of the glycosylation sites were destroyed, PrP within the central nervous system was distributed in aberrant neuroanatomic topologies (37).

Since one of the proposed functions of the prion protein is protection from oxidative stress (7), it is intriguing that redox state changes can affect the processing of PrPΔC. Perhaps the change in the glycoform ratio reflects a direct response by the cell to differing oxidative/reductive challenges and offers protection from those stresses. Until the role of the prion protein in the cell is elucidated, questions such as these will remain unanswered.

S. Capellari, unpublished data.

REFERENCES

1. Stahl, N., Borbelt, D. R., Hsiao, K., and Prusiner, S. B. (1987) Cell 51, 229–240
2. Caughey, B., Race, R. E., Ernst, D., Buchmeier, M. J., and Chesebro, B. (1989) J. Virol. 63, 175–181
3. Petersen, R. B., Parci, P., Richardson, S. L., Urig, C. B., and Gambetti, P. (1990) J. Biol. Chem. 271, 12661–12668
4. Hornshaw, M. F., McDermott, J. R., and Candy, J. M. (1995) Biochem. Biophys. Res. Commun. 207, 621–629
5. Brown, D. R., Schulz-Schaeffer, W. J., Schmidt, B., and Kretzschmar, H. A. (1997) Exp. Neurol. 146, 104–112
6. Brown, D. R., Qin, K., Herm, J. W., Madlun, A., Manson, J., Strome, R., Fraser, F. E., Kruck, T., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D., and Kretzschmar, H. (1997) Nature 390, 684–687
7. Brown, D. R., Schmidt, B., and Kretzschmar, H. A. (1997) Int. J. Dev. Neurosci. 15, 961–972
8. Rizzardini, M., Chiesa, R., Angeretti, N., Lucca, E., Salmona, M., Forloni, G., and Canton, L. (1997) J. Neurochem. 68, 715–720
9. Brown, D. R., Schmidt, B., and Kretzschmar, H. A. (1998) J. Neurochem. 70, 1686–1695
10. Monari, L., Chen, S. G., Brown, P., Parchi, P., Petersen, R. B., Mikol, J., Gray, F., Cortelli, P., Montagna, P., Ghezzi, B., Goldfarb, L. G., Gajdusek, D. C., Lugardey, E., Gambetti, P., and Antioilo-Gambetti, L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2839–2842
11. Parchi, P., Castellani, R., Capellari, S., Ghezzi, B., Young, K., Chen, S. G., Farlow, M., Dickson, D. W., Sima, A. A., Trojanowiski, J. Q., Petersen, R. B., and Gambetti, P. (1996) Ann. Neurol. 39, 767–777
12. Collinge, J., Sidle, K. C., Meads, J., Ironside, J., and Hill, A. F. (1996) Nature 383, 685–690
13. Kocisko, D. A., Come, J. H., Prida, S. A., Chesebro, B., Raymond, G. J., Lamsbury, P. T., and Caughey, B. (1994) Nature 370, 471–474
14. Kascak, R. J., Rubenstein, R., Mraz, P. A., Tonna-DeMasi, M., Fersko, R., Carp, R. I., Wiesniwecki, H. M., and Driringer, H. (1987) J. Virol. 61, 3688–3693
15. Zanusso, G., Liu, D., Ferrari, S., Hegyi, I., Yin, X., Aguzui, A., Hornemann, S., Liemann, S., Gockshuber, R., Manson, J. C., Brown, P., Petersen, R. B., Gambetti, P., and Sy, M. S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12142–12146
16. Kasetz, C. S., Rao, C. K., and Lamm, M. E. (1987) J. Virol. 62, 86–88
17. Braakman, I., Helenius, J., and Helenius, A. (1992) EMBO J. 11, 1717–1722
18. Tatu, U., Braakman, I., and Helenius, A. (1993) EMBO J. 12, 2151–2157
19. Tatu, U., and Helenius, A. (1997) J. Cell Biol. 136, 555–565
20. Pugli, A., Lyles, M. M., Noiva, R., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 11912–11915
21. Shyng, S. L., Huber, M. T., and Harris, D. A. (1993) J. Biol. Chem. 268, 15922–15929
22. Chen, S. G., Teplow, D. B., Parchi, P., Teller, J. K., Gambetti, P., and Antioilo-Gambetti, L. (1995) J. Biol. Chem. 270, 19173–19180
23. Allen, S., Naim, H. Y., and Buleid, N. J. (1995) J. Biol. Chem. 270, 4879–4884
24. Kromer, A., Grombik, M. M., Hutton, W. B., and Gerdes, H. H. (1998) J. Cell Biol. 140, 1331–1346
25. Grombik, M. M., Kromer, A., Salm, T., Hutton, W. B., and Gerdes, H. H. (1999) EMBO J. 18, 1059–1070
26. Herrmann, L. M., and Caughey, B. (1998) Neuroreport 9, 2457–2461
27. Jackson, G. S., Hosszu, L. L., Power, A., Hill, A. F., Kenney, J., Saibil, H., Craven, C. J., Waltho, J. P., Clarke, A. R., and Collinge, J. (1999) Science 283, 1335–1337
28. Isidoro, C., Magnoni, C., Demoz, M., Pizzagalli, A., Fra, A. M., and Saita, R. (1999) J. Biol. Chem. 274, 26138–26142
29. Muramatsu, T., DeArmond, S. J., Scott, M., Telling, G. C., Cohen, F. E., and Prusiner, S. B. (1997) Nat. Med. 3, 750–755
30. Nitrini, R., Rosemb erg, S., Passos-Bueno, M. R., da Silva, L. S., Lughi, P., Papadopoulos, M., Carrilho, P. M., Carameli, P., Albrecht, S., Zatz, M., and LeBlanc, A. (1997) Ann. Neurol. 42, 138–146
31. Liemann, S., and Glockshuber, R. (1999) Biochemistry 38, 3258–3267
32. DeArmond, S. J., Sanchez, H., Yehei, F., Qiu, Y., Ninchak-Casey, A., Dagett, V., Camerino, A. P., Cayetano, J., Rogers, M., Groth, D., Torechia, M., Tremblay, P., Scott, M. R., Cohen, F. E., and Prusiner, S. B. (1997) Neuron 19, 1337–1348
33. Lehmann, S., and Harris, D. A. (1997) J. Biol. Chem. 272, 21479–21487
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J. Biol. Chem. 1999, 274:34846-34850.
doi: 10.1074/jbc.274.49.34846

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