Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5610–5614, May 1996
Neurobiology

Two mutant prion proteins expressed in cultured cells acquire biochemical properties reminiscent of the scrapie isoform

(neurodegenerative/genetic/Creutzfeldt–Jakob disease/metabolism/posttranslational)

SYLVAIN LEHMANN AND DAVID A. HARRIS*

Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Gerald D. Fischbach, Harvard Medical School, Boston, MA, January 2, 1996 (received for review November 20, 1995)

ABSTRACT **Prion diseases are a group of fatal neurodegenerative disorders that are unique in being infectious, genetic, and sporadic in origin. Infectious cases are caused by prions, which are composed primarily of PrP^{Sc}, a posttranslationally modified isoform of the normal cellular prion protein PrP^{C}. Inherited cases are linked to insertional or point mutations in the host gene encoding PrP^{C}. To investigate the molecular mechanisms underlying inherited prion diseases, we have constructed stably transfected Chinese hamster ovary cells that express mouse PrPs homologous to two human PrPs associated with familial Creutzfeldt–Jakob disease. One mouse PrP molecule carries a Glu → Lys substitution at codon 199, and the other carries an insertion of six additional octapeptide repeats between codons 51 and 90. We find that both of these mutant PrPs display several biochemical hallmarks of PrP^{Sc} when synthesized in cell culture. Unlike wild-type PrP, the mutant proteins are detergent insoluble and are relatively resistant to digestion by proteinase K, yielding an N-terminally truncated core fragment of 27–30 kDa. Pulse-chase labeling experiments demonstrate that these properties are acquired posttranslationally, and are accompanied by increased metabolic stability of the protein. Our results provide the first evidence that a molecule with properties reminiscent of PrP^{Sc} can be generated *de novo* in cultured cells.**

Prion diseases are unusual neurodegenerative disorders of humans and animals that are characterized clinically by dementia and motor dysfunction, and neuropathologically by cerebral spongiosis and amyloidosis (1, 2). These diseases arise in one of three ways: (*i*) by infection, (*ii*) by inheritance, and (*iii*) sporadically. Infectious cases, exemplified by kuru and experimental scrapie, are thought to be caused by prions, which are composed primarily of PrP^{Sc}, a protease-resistant isoform of the normal cellular protein PrP^{C} (3, 4). Familial forms, including Gerstmann-Sträussler syndrome, fatal familial insomnia, and about 10% of the cases of Creutzfeldt–Jakob disease, are linked in an autosomal dominant fashion to insertional and point mutations in the gene that encodes PrP^{C} (5).

The central molecular event underlying all three forms of prion diseases is likely to be a posttranslational alteration of the PrP^{C} molecule that transforms it into PrP^{Sc} (6). The structural details of this transition are uncertain, but may involve conversion of α-helices into β-sheets in critical regions of the polypeptide chain (7–9). In infectious forms of the disease, the conversion is thought to be induced by a species-specific interaction between PrP^{Sc} in the inoculum and PrP^{C} in host cells, a model consistent with experiments on mice carrying ablated or heterologous PrP genes (10–12). The conversion is postulated to occur spontaneously in PrP^{C} molecules carrying disease-specific mutations, as suggested by the appearance of PrP^{Sc} in the brains of patients with inherited prion diseases (2, 13).

Elucidation of the molecular mechanisms underlying generation of PrP^{Sc} requires cell culture models where the process can be dissected and experimentally manipulated. Scrapie-infected neuroblastoma and hamster brain cells have provided useful models of infectious forms of prion disease (14–16). These permanently infected cell lines produce low levels of PrP^{Sc}, which is identifiable by its detergent insolubility and protease resistance, as well as by its infectivity in animal bioassays.

There has been no comparable cell culture model for inherited prion diseases. To develop such a system, we have expressed PrP molecules carrying disease-related mutations in cultured Chinese hamster ovary (CHO) cells, and analyzed the biochemical properties of the mutant proteins. We have previously shown that a mouse PrP (moPrP) molecule carrying six additional octapeptide repeats, an insertion homologous to one in human PrP associated with familial Creutzfeldt–Jakob disease, displays an abnormally tight association with the plasma membrane, as revealed by retention of the protein on the cell surface following enzymatic cleavage of its glycosylphosphatidylinositol anchor (17). We now significantly extend these observations by showing that this mutant protein, as well as a second moPrP carrying another Creutzfeldt–Jakob disease-related mutation, acquires biochemical properties of PrP^{Sc} in cultured CHO cells.

MATERIALS AND METHODS

PrP Constructs and Cell Lines. CHO cell lines expressing PG11 moPrP have been described (17). The E199K mutation was introduced into moPrP using recombinant PCR, and the resulting cDNA was cloned into the expression vector pBC12/CMV and stably transfected into CHO cells as described (17). A single subcloned line was analyzed for each construct, although similar results have been obtained for pools of transiently transfected cells (data not shown).

Assay of Detergent-Insolubility. Confluent cultures of CHO cells were labeled for 20 min in methionine-free MEM containing $Trans^{35}$-Label (ICN) (250 μCi/ml; 1,000 Ci/mmole), and chased for 3 h in Opti-MEM (GIBCO). Cells were then lysed at 4°C in a buffer that contained 150 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 50 mM Tris·HCl (pH 7.5), plus protease inhibitors (1 μg/ml pepstatin/1 μg/ml leupeptin/0.5 mM phenylmethylsulfonyl fluoride/2 mM EDTA). Lysates were centrifuged at 4°C, first at 16,000 × *g* in an Eppendorf microcentrifuge, and then at 265,000 × *g* in the TLA 100.3 rotor of a Beckman model Optima TL ultracentrifuge. Immunoprecipitation of moPrP in pellet and supernatant fractions was performed using antibody P45–66 (17) as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CHO, Chinese hamster ovary; PrP, prion protein; moPrP, mouse PrP; PrP^{C}, cellular isoform of the prion protein; PrP^{Sc}, scrapie isoform of the prion protein.

*To whom reprint requests should be addressed.

described (18), with the modification that immunocomplexes were collected using protein A-Sepharose. Samples were treated with N-glycosidase F before immunoprecipitation to produce a single band of deglycosylated PrP that could be more easily quantitated (18). Immunoprecipitated proteins were analyzed by SDS/PAGE and radioactive gels were quantitated using a PhosphorImager (Molecular Dynamics).

Assay of Protease Resistance. Proteins were methanol precipitated from metabolically labeled cell lysates, digested with proteinase K, and resuspended in detergent–lipid–protein complexes for immunoprecipitation of PrP, all as described (19). Immunoprecipitations used anti-ME7 antibody (a gift of Rick Kascsak, Institute for Basic Research, Staten Island, NY), which was raised against PrP 27–30 from scrapie-infected mouse brain; although this antibody reacts with both mouse and hamster PrP, CHO cells do not synthesize detectable levels of endogenous hamster PrP (data not shown), so that only recombinant moPrP is detected. Unlabeled lysates were treated with proteinase K, and proteins were precipitated with methanol before immunoblotting with either P45–66 or 3F4 (20) antibodies.

RESULTS AND DISCUSSION

We have constructed stably transfected CHO cells that express two moPrPs carrying mutations homologous to those that have been genetically linked to familial Creutzfeldt–Jakob disease (Fig. 1). PG11 moPrP contains six octapeptide repeats in addition to the normal five, and its human homologue has been described in a large English pedigree spanning seven generations (21–23) and, more recently, in two other families (24, 25). E199K moPrP carries a Glu → Lys substitution at codon 199, and its human homologue (E200K) has been identified in several families of Libyan-born Jews (26, 27), as well as in families originating in a number of other countries (27–29).

Three biochemical properties have been widely used as markers for PrP^{Sc}, the isoform that accumulates in the brains of humans and animals afflicted with genetic, infectious, and sporadic forms of prion disease (2). These include (*i*) aggregation or insolubility in nondenaturing detergents, (*ii*) relative resistance to protease digestion, and (*iii*) increased metabolic stability. We undertook to determine whether the mutant moPrPs expressed in CHO cells exhibited any of these properties.

To test detergent insolubility, we centrifuged detergent lysates of [^{35}S]methionine-labeled cells at 265,000 × *g* for 40 min, a protocol that sediments PrP^{Sc} but not PrP^{C} (30) (Fig. 2*A*). We observed that both mutant PrPs were significantly more insoluble than wild-type PrP, with ≈90% of the PG11 protein and ≈60% of the E199K protein pelleting, compared with ≈15% for the wild-type protein (Fig. 2*B*). The difference in sedimentation behavior between E199K than PG11 suggests that the two mutant proteins form aggregates of different sizes, and is reminiscent of the variation in the sedimentation properties of PrP^{Sc} derived from different strains of scrapie (31).

Digestion of PrP^{Sc} by proteinase K cleaves the molecule between amino acid residues 88 and 89, resulting in a protease-resistant fragment of 27–30 kDa (designated PrP 27–30); under the same conditions, PrP^{C} is totally degraded (4). To assess the protease resistance of moPrPs expressed by CHO cells, we digested proteins extracted from metabolically labeled cells with 3.3 μg/ml of proteinase K, and then isolated moPrP by immunoprecipitation (Fig. 3*A*). We observed that after 10 and 20 min of digestion, significant amounts of immunoreactive PG11 and E199K moPrP remained, while wild-type PrP was completely degraded (Fig. 3*B*). This result is not attributable to variations in the amount of protein substrate, because similar amounts (to within 20%) of both total protein and radiolabeled moPrP were present in all samples before digestion. In other experiments using clones of transfected cells displaying differing levels of moPrP expression, we have always

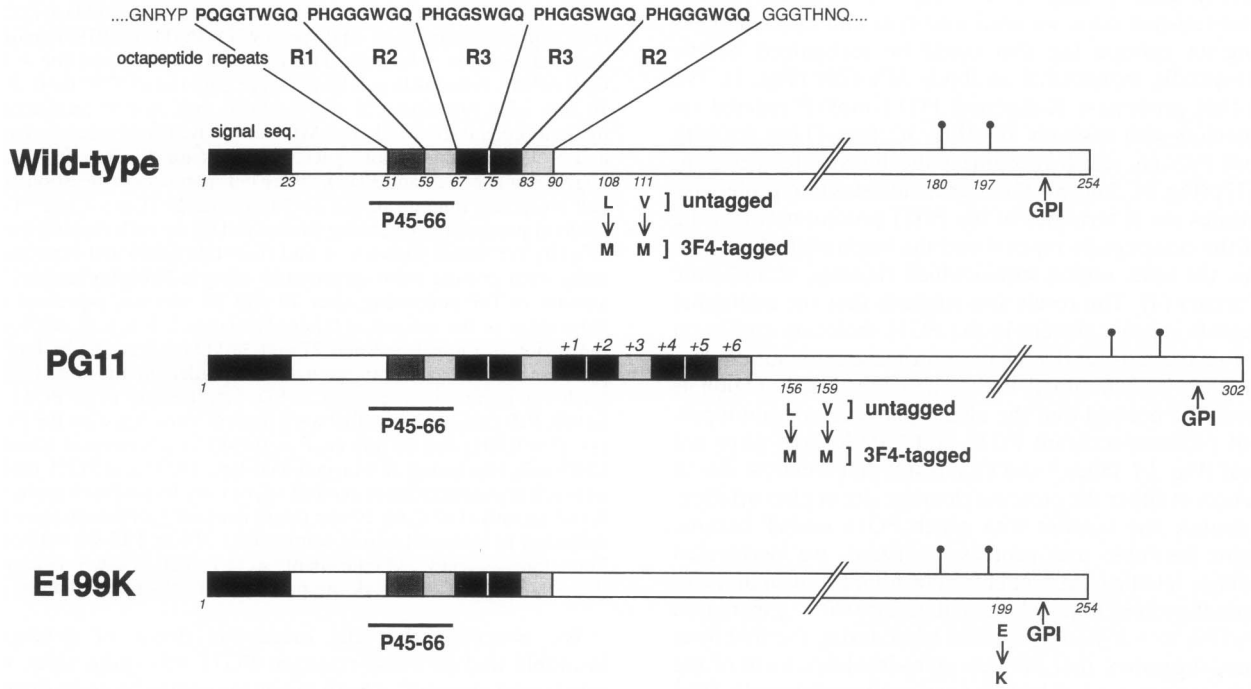

FIG. 1. Structures of moPrP constructs. Wild-type moPrP contains five octapeptide repeats, each displaying one of three related amino acid sequences (R1, R2, and R3). PG11 moPrP contains 11 octapeptide repeats, corresponding to two additional copies of the sequence R3-R3-R2 (blocks labeled +1 through +6). E199K moPrP contains a glu→lys substitution at position 199. The lollipop symbols indicate N-glycosylation sites, and the arrow labeled "GPI" indicates the site of attachment of the glycolipid anchor. One set of wild-type and PG11 constructs had methionine residues substituted at the two positions indicated, which allowed the proteins to be recognized by the monoclonal antibody 3F4 (20). The horizontal lines labeled "P45–66" indicate the position of the peptide used to raise a polyclonal antibody against moPrP, which does not cross-react with hamster PrP (17).

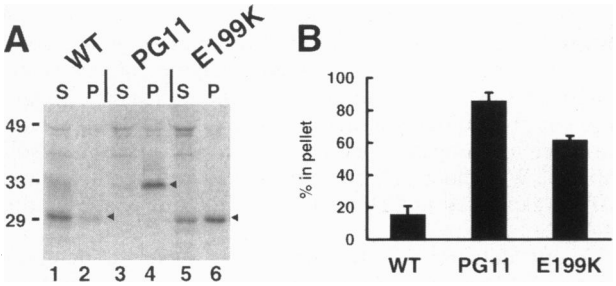

FIG. 2. PG11 and E199K moPrPs sediment at 265,000 × g. (*A*) Detergent lysates of metabolically labeled CHO cells expressing untagged wild-type (WT), PG11, or E199K moPrP were centrifuged at 16,000 × *g* for 5 min to remove nuclei and cell debris, and then recentrifuged at 265,000 × *g* for 40 min. moPrP in the pellet and supernatant fractions from the second centrifugation were immunoprecipitated using antibody P45–66. The arrowheads indicate PrP-specific bands, immunoprecipitation of which is blocked when the antibody is preincubated with the peptide immunogen (not shown). Molecular weight markers are in kilodaltons. (*B*) PrP bands from the experiment shown in *A* and from two additional experiments were quantitated using a PhosphorImager, and the distribution of each protein in the pellet and supernatant fractions from the 265,000 × *g* centrifugation was determined. Each bar represents the mean ± SD.

observed that the two mutant PrPs are more protease resistant than wild-type PrP. Although some forms of PrP^{Sc} from scrapie-infected brain (33) and neuroblastoma cells (19) can withstand digestion conditions that are harsher than those used here, we note that the protease resistance of PrP^{Sc} can vary considerably, depending on the scrapie strain from which it is derived (31) and on the cell type where it is synthesized (34).

The mutant proteins remaining after proteinase K digestion were reduced in size, with the bulk migrating as a broad band between 27 and 30 kDa, the same size as PrP 27–30 (Fig. 3*A*, brackets in lanes 5 and 8). To map the structure of this protease-resistant core, we used wild-type and PG11 moPrPs carrying an epitope tag that could be recognized by the species-specific monoclonal antibody 3F4 (20) (Fig. 1). We found that proteinase K-digested PG11 moPrP reacted on immunoblots with antibody 3F4 (Fig. 3*C*, lane 4) but not with antibody P45–66, which recognizes the R1 and R2 octapeptides (17) (Fig. 3*C*, lane 8). This result indicates that proteinase K truncates the N terminus of the PG11 protein between the end of the octapeptide repeats and the beginning of the 3F4 epitope, the same region within which cleavage of authentic PrP^{Sc} occurs (4). The result also suggests that the additional octapeptide repeats present in the PG11 molecule confer on the rest of the polypeptide chain a protease-resistant conformation that is maintained even when the repeat region is removed. We noticed that the electrophoretic migration patterns of protease-resistant PG11 and E199K PrPs were not identical (Fig. 3*A*, lanes 5 and 8), a result that could be due to differences in either the protease cleavage site or glycosylation.

To analyze the kinetics with which PG11 moPrP became detergent insoluble and protease resistant, we performed pulse-chase labeling experiments. We found that amount of the mutant protein that sedimented at 265,000 × *g* increased from ≈15% to ≈35% of the initial label during the first hour of chase, suggesting that the detergent-insoluble form of the protein was generated, at least in part, posttranslationally (Fig. 4 *A* and *B*). Consistent with this result, the amount of protease-resistant PG11 protein also increased during the chase period (Fig. 4 *D* and *E*), although the rise occurred more slowly, and the maximum amount of converted protein was lower (11% at 6 hr), indicating that not all detergent-insoluble protein is protease resistant under our experimental conditions.

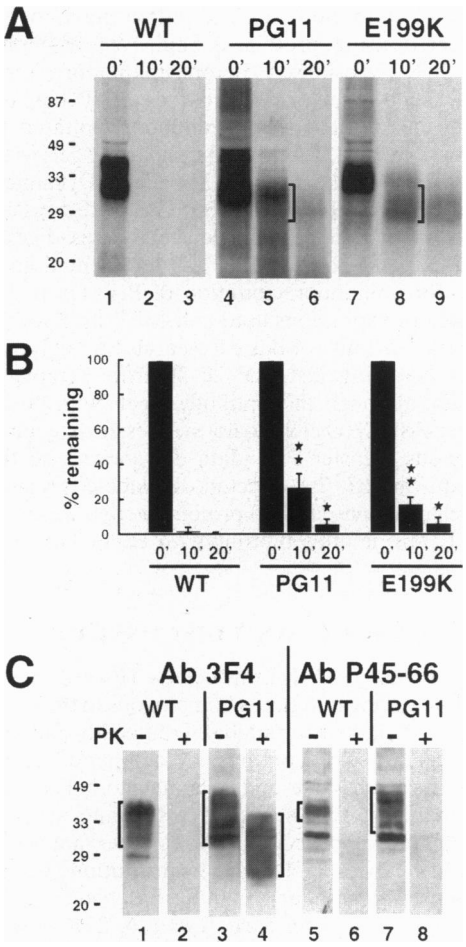

FIG. 3. PG11 and E199K moPrPs are more protease resistant than wild-type moPrP and produce a core fragment of 27–30 kDa. (*A*) CHO cells expressing untagged wild-type (WT), PG11, or E199K moPrPs were labeled for 3 hr with $Trans^{35}$-Label, and chased for 4 h in Opti-MEM. Proteins in cell lysates were digested at 37°C for 0, 10, or 20 min with proteinase K (3.3 μg/ml), and moPrP recovered by immunoprecipitation with anti-ME7 antibody. The brackets in lanes 5 and 8 indicate the major species of protease-digested PrP, which migrates between 27 and 30 kDa. Even before protease digestion, some PrP molecules migrate in the 27–30 kDa range (lanes 4 and 7) as a result of proteolytic processing carried out by the cells themselves (30, 32). (*B*) PrP bands shown in *A* and from two additional experiments using each protein were quantitated using a PhosphorImager. The amount of PrP remaining after 10 and 20 min was expressed as a percentage of the amount at 0 min. For lanes 2, 3, 5, 6, 8, and 9, only the region of the gel between 27 and 30 kDa, where the bulk of the protease-resistant PrP migrated, was included in the quantitation. Each bar represents the mean ± SD. Significantly more PG11 and E199K PrP than wild-type PrP were present after digestion for 10 min (**, $P < 0.001$) and 20 min (*, $P < 0.015$). (*C*) Detergent lysates of CHO cells expressing 3F4-tagged wild-type (WT) and PG11 moPrPs were left undigested (lanes marked −), or were treated with proteinase K (3.3 μg/ml) at 37°C for 10 min (lanes marked +). Proteins were then subjected to immunoblotting using either 3F4 or P45–66 antibodies. Four times as many cell equivalents were loaded in the + lanes as in the − lanes. Brackets mark the positions of PrP-specific bands.

We observed that the metabolic decay of detergent-insoluble and protease-resistant PG11 was quite slow, with substantial amounts of both forms remaining by 16 hr of chase (Fig. 4 *B* and *E*). Presumably, the enhanced stability of the these forms accounts for the slower decay of total PG11 protein ($t_{1/2}$ = 2.2 hr), in comparison to wild-type protein ($t_{1/2}$ = 1.2 hr) (Fig. 4*C*). The behavior of the detergent-insoluble and protease-resistant forms of PG11 moPrP are reminiscent of PrP^{Sc} synthesized in scrapie-infected neuroblas-

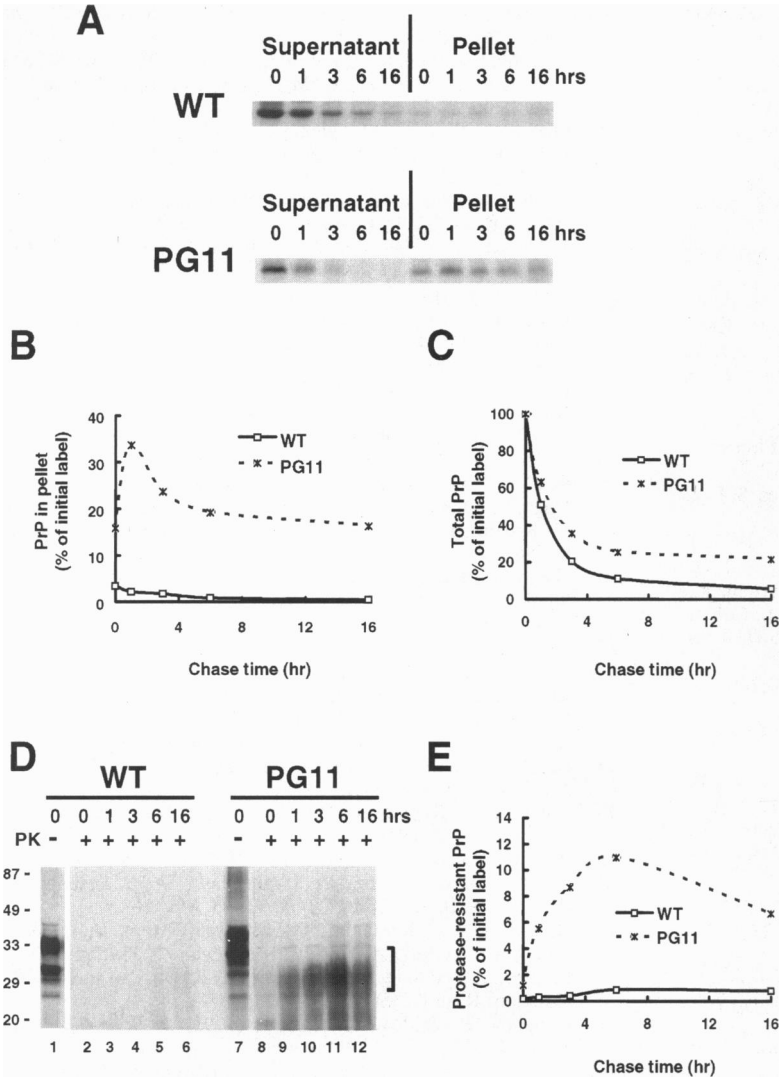

FIG. 4. Pulse-labeled molecules of PG11 moPrP become detergent insoluble and protease resistant during the chase period. (*A*) CHO cells expressing untagged wild-type (WT) or PG11 moPrP were labeled for 30 min with Trans^{35}-Label and chased for the indicated times in Opti-MEM. At the end of each chase period, cell lysates were centrifuged at 265,000 × *g*, and moPrP immunoprecipitated from supernatants and pellets as described in Fig. 2. Only the region of the gel containing the PrP bands is shown; an example of a complete gel is shown in Fig. 2*A*. (*B*) The PrP bands shown in *A* were quantitated using a PhosphorImager, and the amount of PrP in the pellet at each time point was plotted as a percentage of the total amount of radioactive PrP present at the end of the labeling period. The result shown is representative of two similar experiments. (*C*) The total amount of PrP at each time point (supernatant plus pellet, from *A*) was plotted as a percentage of the amount of radioactive PrP present at the end of the labeling period. The result shown is representative of four similar experiments. (*D*) CHO cells expressing untagged wild-type (WT) or PG11 moPrP were labeled for 45 min with Trans^{35}-Label, chased for the indicated times in Opti-MEM, and then lysed. Lanes 2–6 and 8–12: proteins in cell lysates were digested at 37°C for 10 min with proteinase K (3.3 μg/ml) and moPrP recovered by immunoprecipitation with anti-ME7 as described in Fig. 3. Lanes 1 and 7: Samples were not digested with proteinase K, and one-fourth the number of cell-equivalents as in the other lanes was loaded. The bracket indicates the position of PrP 27–30. (*E*) The PrP bands shown in *D* were quantitated using a PhosphorImager, and the amount of protease-resistant PrP each time point (M_r 27–30 kDa) was plotted as a percentage of the total amount of radioactive PrP present at the end of the labeling period. The result shown is representative of two similar experiments.

toma cells, which is stable for more than 24 hr after pulse-labeling (19, 35).

Although PrP^{Sc} is produced in brain tissue and in neuroblastoma and brain-derived cell lines after infection with scrapie prions (16, 19, 30, 33, 35, 36), our results, to our knowledge, are the first demonstration that a mutant PrP is able to acquire PrP^{Sc}-like properties *de novo*, in uninfected cultured cells. The similarities between the mutant PrPs expressed in CHO cells and authentic PrP^{Sc} include detergent-insolubility, relative protease-resistance, and slow metabolic generation and decay. Although the mutant proteins are more protease resistant than wild-type PrP, they are considerably less resistant than most forms of authentic PrP^{Sc}, a result that may indicate that they have undergone only partial conversion to the PrP^{Sc} state. However, the fact that two different disease-related mutations, one amino acid substitution and one insertion, produce molecules with PrP^{Sc}-like properties argues that the conversion phenomenon we have modeled in CHO cells is related to the pathogenic process that occurs in affected patients. Although there is one report that CHO cells cannot be infected with scrapie prions (37), the cellular factors that influence production of PrP^{Sc} in genetic and infectious manifestations may be different. Consistent with the results reported here, human beings carrying several different PrP mutations (13), including E200K (26, 29, 34), produce protease-resistant PrP in their brains. We are currently testing whether the mutant PrPs that we have expressed in CHO cells are infectious, although we note that rates of transmission to laboratory animals are considerably lower for familial prion diseases than for infectious and sporadic forms (13).

Although infectious forms of prion disease are initiated by exogenous PrP^{Sc}, whereas genetic forms involve mutation-induced alterations in the endogenous molecule, our data strongly support the idea that a critical posttranslational modification of the PrP polypeptide underlies both manifestations of the disease (6, 19, 35). We have found that both PG11 and E199K moPrP remain tightly associated with the plasma membrane, even after enzymatic cleavage of their glycosyl-phosphatidylinositol anchors (ref. 17; unpublished data). Therefore, it is attractive to hypothesize that an alteration in membrane topology or affinity is related to acquisition of PrP^{Sc}-like properties by these mutant proteins. The experimental system we describe here, in which mutant PrPs undergo conversion to a detergent-insoluble and protease-resistant state in cell culture, will make further exploration of the relevant molecular mechanisms possible.

Note added in proof. We have now demonstrated that two additional moPrP mutants (P101L and D177N) are detergent-insoluble and protease-resistant like PG11 and E199K, and that all four moPrPs remain associated with the plasma membrane after cleavage of their glycolipid anchors (38).

We thank Rick Kascsak for 3F4 and anti-ME7 antibodies and Jim Krause for providing CHO cells. We also acknowledge Krista Moulder for excellent technical assistance, as well as Gene Johnson, Carl Romano, Maurine Linder, and members of the Harris laboratory for

comments on an earlier draft of the manuscript. S.L. is the recipient of a Postdoctoral Fellowship for Physicians from the Howard Hughes Medical Institute, and of awards from Institut National de la Santé et de la Recherche Médicale and the Simone and Cino del Duca Foundation. This work was also supported by grants to D.A.H. from the Alzheimer's Association and the March of Dimes Birth Defects Foundation.

1. Gajdusek, D. C. (1990) in *Virology*, eds. Fields, B. N. & Knipe, D. M. (Raven, New York), 2nd Ed., pp. 2289–2323.
2. Prusiner, S. B. & DeArmond, S. J. (1994) *Annu. Rev. Neurosci.* **17,** 311–339.
3. Prusiner, S. B. (1982) *Science* **216,** 136–144.
4. Oesch, B., Westaway, D., Wälchli, M., McKinley, M. P., Kent, S. B. H., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L. E., Prusiner, S. B. & Weissmann, C. (1985) *Cell* **40,** 735–746.
5. Prusiner, S. B. & Hsiao, K. K. (1994) *Ann. Neurol.* **35,** 385–395.
6. Cohen, F. E., Pan, K.-M., Huang, Z., Baldwin, M., Fletterick, R. J. & Prusiner, S. B. (1994) *Science* **264,** 530–531.
7. Caughey, B. W., Dong, A., Bhat, K. S., Ernst, D., Hayes, S. F. & Caughey, W. S. (1991) *Biochemistry* **30,** 7672–7680.
8. Safar, J., Roller, P. P., Gajdusek, D. C. & Gibbs, C. J. (1993) *J. Biol. Chem.* **268,** 20276–20284.
9. Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z. W., Fletterick, R. J., Cohen, F. E. & Prusiner, S. B. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10962–10966.
10. Prusiner, S. B., Scott, M., Foster, D., Pan, K. M., Groth, D., Mirenda, C., Torchia, M., Yang, S. L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D. & DeArmond, S. J. (1990) *Cell* **63,** 673–686.
11. Büeler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. & Weissmann, C. (1993) *Cell* **73,** 1339–1347.
12. Kocisko, D. A., Priola, S. A., Raymond, G. J., Chesebro, B., Landsbury, P. T. & Caughey, B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3923–3927.
13. Brown, P., Gibbs, C. J., Rodgers, J. P., Asher, D. M., Sulima, M. P., Bacote, A., Goldfarb, L. G. & Gajdusek, D. C. (1994) *Ann. Neurol.* **35,** 513–529.
14. Butler, D. A., Scott, M. R. D., Bockman, J. M., Borchelt, D. R., Taraboulos, A., Hsiao, K. K., Kingsbury, D. T. & Prusiner, S. B. (1988) *J. Virol.* **62,** 1558–1564.
15. Race, R. E., Caughey, B., Graham, K., Ernst, D. & Chesebro, B. (1988) *J. Virol.* **62,** 2845–2849.
16. Taraboulos, A., Serban, D. & Prusiner, S. B. (1990) *J. Cell Biol.* **110,** 2117–2132.
17. Lehmann, S. & Harris, D. A. (1995) *J. Biol. Chem.* **270,** 24589–24597.
18. Harris, D. A., Huber, M. T., van Dijken, P., Shyng, S. L., Chait, B. T. & Wang, R. (1993) *Biochemistry* **32,** 1009–1016.
19. Borchelt, D. R., Taraboulos, A. & Prusiner, S. B. (1992) *J. Biol. Chem.* **267,** 16188–16199.
20. Bolton, D. C., Seligman, S. J., Bablanian, G., Windsor, D., Scala, L. J., Kim, K. S., Chen, C.-M. J., Kascsak, R. J. & Bendheim, P. E. (1991) *J. Virol.* **65,** 3667–3675.
21. Owen, F., Poulter, M., Shah, T., Collinge, J., Lofthouse, R., Baker, H., Ridley, R., McVey, J. & Crow, T. J. (1990) *Mol. Brain Res.* **7,** 273–276.
22. Poulter, M., Baker, H. F., Frith, C. D., Leach, M., Lofthouse, R., Ridley, R. M., Shah, T., Owen, F., Collinge, J., Brown, J., Hardy, J., Mullan, M. J., Harding, A. E., Bennett, C., Doshi, R. & Crow, T. J. (1992) *Brain* **115,** 675–685.
23. Collinge, J., Brown, J., Hardy, J., Mullan, M., Rossor, M. N., Baker, H., Crow, T. J., Lofthouse, R., Poulter, M., Ridley, R., Owen, F., Bennett, C., Dunn, G., Harding, A. E., Quinn, N., Doshi, B., Roberts, G. W., Honavar, M., Janota, I. & Lantos, P. L. (1992) *Brain* **115,** 685–710.
24. Nicholl, D., Windl, O., de Silva, R., Sawcer, S., Dempster, M., Ironside, J. W., Estibeiro, J. P., Yuill, G. M., Lathe, R. & Will, R. G. (1995) *J. Neurol. Neurosurg. Psychol.* **58,** 65–69.
25. Oda, T., Kitamoto, T., Tateishi, J., Mitsuhashi, T., Iwabuchi, K., Haga, C., Oguni, E., Kato, Y., Tominaga, I., Yanai, K., Kashima, H., Kogure, T., Hori, K. & Ogino, K. (1995) *Acta Neuropathol.* **90,** 80–86.
26. Hsiao, K., Meiner, Z., Kahana, E., Cass, C., Kahana, I., Avrahami, D., Scarlato, G., Abramsky, O., Prusiner, S. B. & Gabizon, R. (1991) *N. Engl. J. Med.* **324,** 1091–1097.
27. Goldfarb, L. G., Brown, P., Mitrova, E., Cervenakova, L., Goldin, L., Korczyn, A. D., Chapman, J., Galvez, S., Cartier, L., Rubenstein, R. & Gajdusek, D. C. (1991) *Eur. J. Epidemiol.* **7,** 477–486.
28. Collinge, J., Palmer, M. S., Campbell, T., Sidle, K. C. L., Carroll, D. & Harding, A. (1993) *Br. Med. J.* **306,** 301–302.
29. Inoue, I., Kitamoto, T., Doh-ura, K., Shii, H., Goto, I. & Tateishi, J. (1994) *Neurology* **44,** 299–301.
30. Caughey, B., Raymond, G. J., Ernst, D. & Race, R. E. (1991) *J. Virol.* **65,** 6597–6603.
31. Bessen, R. A. & Marsh, R. F. (1992) *J. Virol.* **66,** 2096–2101.
32. Taraboulos, A., Raeber, A. J., Borchelt, D. R., Serban, D. & Prusiner, S. B. (1992) *Mol. Biol. Cell* **3,** 851–863.
33. Bolton, D. C., Bendheim, P. E., Marmorstein, A. D. & Potempska, A. (1987) *Arch. Biochem. Biophys.* **258,** 579–590.
34. Meiner, Z., Halimi, M., Polakiewicz, R. D., Prusiner, S. B. & Gabizon, R. (1992) *Neurology* **42,** 1355–1360.
35. Caughey, B. & Raymond, G. J. (1991) *J. Biol. Chem.* **266,** 18217–18223.
36. Rogers, M., Yehiely, F., Scott, M. & Prusiner, S. B. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3182–3186.
37. Butler, D. A., Scott, M. R. D., Bockman, J. M., Borchelt, D.r., Taraboulos, A., Hsiao, K. K., Kingsbury, D. T. & Prusiner, S. B. (1988) *J. Virol.* **62,** 1558–1564.
38. Lehmann, S. & Harris, D. A. (1996) *J. Biol. Chem.* **271,** 1633–1637.