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A 54-kDa normal cellular protein may be the precursor of the scrapie agent protease-resistant protein

(slow infection/spongiform encephalopathy/amyloid)

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ABSTRACT **Scrapie is the best understood of the transmissible spongiform encephalopathies. These neurologic disorders include the human diseases kuru and Creutzfeldt–Jakob disease and are caused by pathogens with unique biological and molecular properties. One major protein, protease-resistant protein (PrP)-27–30, is present in fractions isolated from scrapie-infected hamster brain that contain highly purified scrapie agent. PrP-27–30 appears to be the major protein component of the hamster scrapie agent. An antiserum generated to electrophoretically purified hamster scrapie PrP- 27–30 identified higher molecular weight proteins in immunoblots of homogenates of uninfected hamster and mouse brains. Antibodies to hamster and mouse scrapie agent proteins were obtained by immunoaffinity purification of this antiserum. These antibodies to hamster and mouse PrPs recognized a 54-kDa protein present in uninfected brain homogenates. Antibodies immunoaffinity purified from this antiserum using whole immunoblots of normal brain antigens also identified the 54-kDa protein and PrPs. Our findings demonstrate that scrapie agent proteins share epitopes with normal proteins and suggest that the 54-kDa protein is the normal protein precursor of the scrapie agent PrPs.**

The scrapie agent is the best characterized member of a class of unusual infectious pathogens that cause the spongiform encephalopathies of animals (scrapie, transmissible mink encephalopathy, chronic wasting disease) and humans (kuru, Creutzfeldt–Jakob disease, and Gerstmann–Sträussler syndrome) (1–3). The scrapie agent exhibits unusual physical and biochemical properties that distinguish it from viroids and appear to distinguish it from viruses (1, 2, 4). The terms "slow virus", "virino", and "prion" are used to denote this class of infectious agents (2, 5, 6).

Inactivation of the scrapie agent in brain homogenates by physical and chemical treatments have been cited to support a virus model (7, 8). However, these experiments do not differentiate between a virus model and a protein-only model. The existence of scrapie agent strains, suggested by apparent biological differences, also is used to support the virus model (9, 10). The only candidate for a scrapie agent-specific nucleic acid identified to date is a 4.3S RNA present in membrane fractions containing the partially purified scrapie agent (11, 12). This nucleic acid molecule is not of sufficient length to be a viral genome or to code for protease-resistant protein (PrP)-27–30 and has not been demonstrated to be required for infectivity.

PrP-27–30 (apparent molecular size of 27–30 kDa) is the major macromolecule that purifies with the infectious particle from hamster brain (13). A substantial body of data supports the hypothesis that PrP-27–30 is the major protein component of the hamster scrapie agent (13–19). Three similar PrPs purify with the scrapie agent from infected mouse brain (16, 17). These murine (M) scrapie PrPs (M-PrPs) share antigenic determinants with the hamster scrapie protein, PrP-27–30 (17).

A long-standing debate has focused on whether the scrapie agent-specific protein(s) is coded for by the host genome or by a nucleic acid contained within the infectious particle (2, 4, 7, 8). The scrapie agent is unusually resistant to procedures that inactivate nucleic acids (4, 20–24). Even with substantially purified preparations of the infectious agent, a scrapie agent genome has not been identified. Using synthetic oligonucleotide probes complementary to the N-terminal portion of PrP-27–30, Oesch *et al.* (25) and Chesebro *et al.* (26) succeeded in cloning major portions of the genes encoding hamster PrP-27–30 and the M-PrP(s). These clones identified mRNAs [2.1–2.5 kilobases (kb)] in RNA blots prepared from scrapie-infected hamster and mouse brains. Southern blot analysis revealed a single compact gene in both normal and infected brain as well as in DNA from other normal mouse tissue and human placenta (25). PrP mRNAs were found in a variety of uninfected and infected tissues at similar concentrations and in one neuroblastoma cell line (26). The *PrP* gene was not detected in suspensions of purified scrapie agent by slot blot hybridization using the cDNA clone as the probe (25). In conjunction with the absence of a detectable specific immune response in infected hosts, these data provide evidence that the genes coding for PrP-27–30 and M-PrPs reside in the respective host genomes. We now report additional evidence linking PrP-27–30 and M-PrPs to normal cellular proteins.

MATERIALS AND METHODS

Source and Purification of the Scrapie Agent and PrPs. Scrapie agents 263K and ME7 were propagated in LVG/LAK hamsters and C57BL mice (Charles River Breeding Colony, Lakeview, NJ), respectively. Animals were inoculated intracerebrally and sacrificed when clinically affected with scrapie. Substantially purified scrapie agent proteins were prepared by a modification of published procedures (17–19). A suspension of scrapie-infected brains (10% wt/vol) was made by high-speed homogenization in a solution of 10% (wt/vol) Sarkosyl, 10 mM NaH_2PO_4, pH 7.2. The suspension was sedimented at 17,000 rpm in a 45 Ti rotor for 30 min at 20°C. The supernatant was saved, and the pellet was rehomogenized and sedimented as described above. The supernatants were combined and sedimented at 56,000 rpm and 20°C for 2.5 hr in the 60 Ti rotor. The resulting pellet was sonicated in Tris-buffered saline (TBS, 10 mM Tris·HCl/133 mM NaCl, pH 7.4), containing 10% (wt/vol) NaCl and 1% (wt/vol) Sarkosyl, and the suspension was centrifuged in a 60 Ti rotor at 56,000 rpm for 3.5 hr. The pellet was resuspended,

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Abbreviations: PrP, protease-resistant protein; M, murine; kb, kilobase(s).

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stirred overnight at 37°C in TBS, 10% (wt/vol) NaCl, 1% (wt/vol) Sarkosyl, and centrifuged for 15 min in a table-top microcentrifuge. The pellets were resuspended in TBS, digested with micrococcal nuclease (1 μg/ml) for 1 hr at 37°C, followed by digestion with proteinase K (5 μg/ml) for 1 hr at 37°C, and then centrifuged again in the microcentrifuge. The final pellets were suspended in 0.1% Sarkosyl.

Preparation of Scrapie-Infected and Normal Brain Homogenates. Scrapie-infected or normal age-matched hamster and mouse brains were homogenized to give a 10% (wt/vol) suspension in 100 mM Tris·HCl, 5 mM EDTA, 10 mM dithiothreitol, 5 mM phenylmethylsulfonyl fluoride, 4% (wt/vol) $NaDodSO_4$, pH 8.9. The brain homogenates from infected and uninfected animals were immediately heated at 100°C for 5 min. Following addition of dithiothreitol to 1 M, aliquots were diluted with 3 vol of $NaDodSO_4$/PAGE sample buffer (16, 29) and boiled again.

Production of Scrapie PrP-27–30 Antiserum and Purification of Antibodies. The antiserum used in these studies has been described (27–30). Briefly, a rabbit was immunized with proteinase K-digested and $NaDodSO_4$/PAGE-purified scrapie PrP-27–30 emulsified in complete Freund's adjuvant. The primary immunization was given by injection into the popliteal lymph nodes and subcutaneous sites. A subsequent booster injection of proteinase K-digested, $NaDodSO_4$/PAGE-purified PrP-27–30 in incomplete Freund's adjuvant was given subcutaneously. The antiserum used in these studies was obtained prior to the third injection of antigen.

Specific antibodies were affinity purified from this antiserum using a modification of published methods (31–33). Purified hamster PrP, M-PrPs, or normal brain homogenates were prepared as described above. The $NaDodSO_4$/PAGE profiles of these fractions are shown in Fig. 1*a* (lanes 1, 2, 5, and 6). The proteins were separated by $NaDodSO_4$/PAGE and electrophoretically transferred to nitrocellulose sheets (35, 36). The sheets were incubated in blocking buffer [phosphate-buffered saline (PBS, 140 mM NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3 mM KCl, 3 mM NaN_3), pH 7.4, 0.05% Tween 20, 2 mg of bovine serum albumin per ml] for 1 hr at room temperature. Nitrocellulose sheets were incubated separately at 22°C for 1 hr in the rabbit anti-hamster PrP-27–30 serum diluted 1:500 in blocking buffer. The nitrocellulose sheets were washed with five changes of blocking buffer, and the bound antibodies were eluted from whole immunoblots with 0.1 M glycine·HCl (pH 2.2), 20 mM $Mg(OAc)_2$, and 50 mM KCl by incubating at 37°C for 90 min. The antibody binding and elution steps were repeated, the eluates were combined, and bovine serum albumin was added to a final concentration of 2 mg/ml. Physiologic pH was restored by overnight dialysis against PBS/0.05% Tween 20 at 4°C. The eluted, purified antibodies were used to develop immunoblots as described below.

Polyacrylamide Gel Electrophoresis and Immunoblotting. Protein separation was accomplished by electrophoresis through 15% polyacrylamide gels as described by Laemmli (37). After electrophoresis the proteins were transferred to nitrocellulose sheets by overnight electroblotting in a Trans-Blot apparatus (Bio-Rad) containing 25 mM Tris·HCl (pH 8.3), 192 mM glycine, and 20% (vol/vol) methanol (35, 36). Nitrocellulose sheets were incubated for 1 hr at room temperature in blocking buffer followed by a 2-hr incubation at 37°C with a 1:1000 dilution of rabbit anti-PrP serum. After a 30-min wash with five changes of blocking buffer, sheets were incubated for 1 hr at room temperature in a 1:1000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG (Tago, Burlingame, CA) in blocking buffer. Sheets were washed five times in blocking buffer and twice in PBS/Tween 20, and immunoreactive bands were detected by development in 0.01% diaminobenzidine (Sigma)/0.005% H_2O_2 in PBS/Tween 20, pH 7.4.

RESULTS

A rabbit antiserum to gel-purified PrP-27–30 was used in all of the experiments reported here. This antiserum identifies PrPs in gradient fractions containing the highly purified scrapie agent (27, 28). The gradient fractions were prepared by a method that uses detergent extraction, proteinase K and micrococcal nuclease digestions, and discontinuous sucrose gradient sedimentation (13, 15, 38). The antiserum does not recognize proteins found in comparable fractions prepared from normal brains by this method, which includes proteinase K digestion (27). The antiserum does not identify the major cytoskeletal proteins, and antisera to the cytoskeletal proteins do not crossreact with PrPs (28). Using a related antiserum, Oesch *et al.* (25) demonstrated the presence of a 33- to 35-kDa protein in normal uninfected hamster brain. The results presented here extend their observations by identifying a larger normal protein in both uninfected hamster and murine brains and show that antibodies purified against normal proteins recognize PrPs.

Shared antigenic sites on PrP-27–30 and M-PrPs were demonstrated in immunoblots of purified scrapie agent fractions (Fig. 1*b*, lanes 1 and 2). Immunospecific staining of higher apparent molecular weight proteins was observed in the purified fractions, as reported (27, 28, 30). Size heterogeneous proteins having apparent molecular sizes of 43–56 kDa are present in substantially purified scrapie agent preparations (14, 16, 38, 39). These proteins are less concentrated than the lower molecular size PrPs, but share physical and chemical properties with them, including protease resistance and antigenic crossreactivity (10, 27, 30). It is unknown whether these higher molecular weight proteins are precursors of PrP-27–30 or aggregates of lower molecular weight proteins that are not completely dissociated.

Immunoblots of scrapie-infected brain homogenates (hamster and mouse) developed with the anti-PrP-27–30 serum

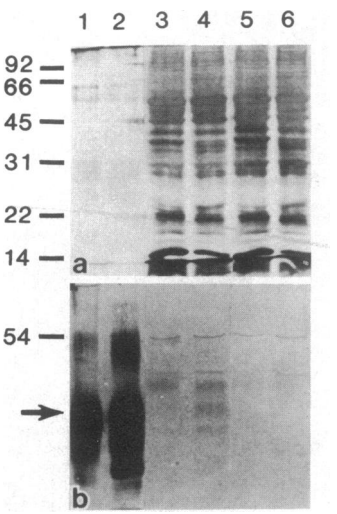

FIG. 1. Analysis of purified PrPs and homogenates of scrapie-infected and normal brain by gel electrophoresis and immunoblotting. Substantially purified scrapie agent proteins from hamster (lane 1) and mouse (lane 2) were denatured by boiling in $NaDodSO_4$/PAGE sample buffer. Infected hamster (lane 3) and mouse (lane 4) brains were homogenized to give a 10% (wt/vol) suspension that was boiled in $NaDodSO_4$/PAGE sample buffer and electrophoresed. Uninfected hamster (lane 5) and mouse (lane 6) brain homogenates were prepared in the same manner from age-matched animals. After electrophoresis into a 15% polyacrylamide gel, the proteins were either (*a*) silver stained by the method of Merril *et al.* (34) or (*b*) transferred to nitrocellulose sheets and immunoblots developed using the anti-PrP-27–30 serum. Arrow denotes position of PrP-27–30. Molecular sizes in kDa are indicated.

revealed two discrete proteins with apparent molecular size of 54 and 41 kDa, and a size-heterogeneous protein of 35–38 kDa (Fig. 1*b*, lanes 3 and 4). In the mouse, two additional size-heterogeneous proteins were visualized having apparent molecular sizes of 29–32 kDa and 25–26 kDa (Fig. 1*b*, lane 4). The 54-kDa, 41-kDa and 35- to 38-kDa proteins also were detected in homogenates of uninfected hamster and mouse brains (Fig. 1*b*, lanes 5 and 6). None of the homogenates had been digested with proteinase K and, therefore, protease-sensitive proteins sharing antigenic sites with PrPs were preserved. Preimmune serum showed no reaction with any of the purified PrPs or with the proteins in scrapie-infected and normal brain homogenates depicted in Fig. 1*a* and *b*.

The immunoaffinity technique described above was used to separate antibodies specific for PrP. Briefly, either substantially purified hamster PrP-27–30 or M-PrPs were separated by $NaDodSO_4$/PAGE and transferred to nitrocellulose. A 1:500 dilution of the anti-PrP-27–30 serum was incubated with the PrPs immobilized on the nitrocellulose paper. After washing to remove nonspecifically bound antibodies, specific antibodies were eluted at low pH. Antibodies eluted from hamster PrPs identified PrP-27–30 as well as M-PrPs (Fig. 2*a*, lanes 1 and 2). They also recognized the 54-kDa protein in both infected and normal brain homogenates (Fig. 2*a*, lanes 3–6). Identical results were obtained with antibodies eluted from M-PrPs (Fig. 2*b*, lanes 1–6). In each case the staining intensity was less than that seen in Fig. 1*b* for all identified proteins. Weak reactivity with the 25- to 26-kDa, 29- to 32-kDa, 35- to 38-kDa, and 41-kDa proteins identified in Fig. 1*b* (lanes 3 and 4) was observed in some experiments. These results corroborated the observation that PrPs share antigenic determinants with normal proteins.

The presence of common epitopes on PrPs and normal brain proteins suggested that it would be possible to separate from this antiserum antibodies specific to normal proteins that would also recognize the scrapie-specific proteins. Therefore, we separated proteins in aliquots of normal brain homogenates by $NaDodSO_4$/PAGE and transferred them to nitrocellulose. The nitrocellulose-bound proteins were incubated with the anti-PrP-27–30 serum as described above. Antibodies that bound to the proteins in total brain homogenates from normal hamsters or mice were eluted and used to develop immunoblots (Fig. 3). The purified antibodies identified PrPs in scrapie-enriched fractions (Fig. 3, lanes 1 and 2) and only one protein (54 kDa) in normal brain homogenates (Fig. 3, lanes 3 and 4) and scrapie brain homogenates (not shown). The 54-kDa protein band appeared to be identical to the protein band identified in Figs. 1 and 2. The staining of the PrP and 54-kDa bands was less intense with the antibodies purified with normal proteins than that seen with the primary antiserum or with antibodies specific for PrPs. These observations could be explained by the fact that fewer crossreacting proteins were present in the aliquots of the normal brain homogenates compared to the purified PrP fractions. Therefore, the specific antibodies eluted from the normal proteins were less concentrated. We expect that a higher concentration of these specific antibodies would also detect the 41-kDa and 35- to 38-kDa proteins.

Several control experiments confirmed the specificity of the antibody purification method (31–33). Nitrocellulose transfers were prepared from $NaDodSO_4$/PAGE gels containing proteinase K or sample buffer alone. The nitrocellulose sheets were incubated in blocking buffer and the anti-PrP-27–30 serum as described above. Following the elution and dialysis steps, the solutions were used to develop immunoblots of normal brain homogenates and purified PrPs. Normal brain proteins and PrPs were not labeled with antibodies. Antibodies eluted from the proteinase K-containing nitrocellulose sheet identified this protease on a separate immunoblot. This confirms the observation that the primary antiserum contains some antibodies to proteinase K (27, 28).

Oesch *et al.* (25) identified a 33- to 35-kDa protein in normal brain homogenates using an antiserum to PrP-27–30 and immunoblot development with 4-chloro-1-naphthol. A series of experiments suggests why they detected only this protein

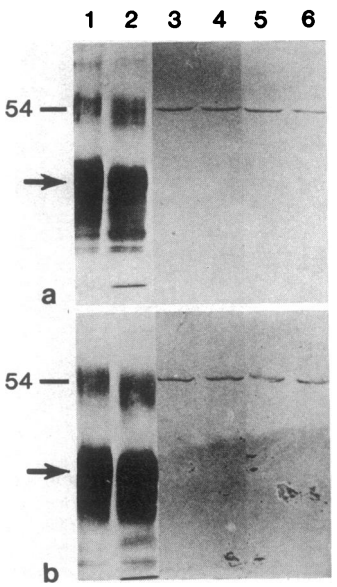

FIG. 2. (*a*) Immunoblot developed using antibodies purified by elution from immunoblots of hamster PrPs. (*b*) Immunoblot developed using antibodies purified by elution from whole immunoblots of M-PrPs. Lanes 1 and 2: purified hamster and mouse scrapie PrPs. Lanes 3 and 4: homogenates of scrapie-infected hamster and mouse brains. Lanes 5 and 6: homogenates of uninfected hamster and mouse brains. The immunoblots were photographed with backlighting to enhance contrast. Arrow indicates the position of PrP-27–30.

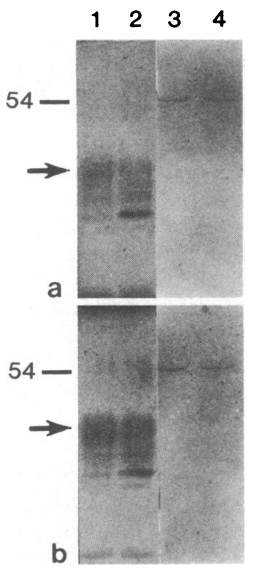

FIG. 3. Immunoblots of purified PrPs and normal brain homogenates developed with antibodies purified by elution from whole immunoblots of normal brain proteins. (*a*) Immunoblot developed using antibodies eluted from immunoblots of normal hamster brain proteins. (*b*) Immunoblot developed using antibodies eluted from immunoblots of normal mouse brain proteins. Lanes 1 and 2: purified hamster and mouse scrapie PrPs. Lanes 3 and 4: homogenates of uninfected hamster and mouse brains. Immunoblots of lanes 3 and 4 were produced by an overnight incubation at room temperature with the purified antibodies. The immunoblots were photographed with backlighting to enhance contrast. Arrow indicates the position of PrP-27–30.

and not the 54-kDa protein we identified. When brain homogenates were prepared by their method and the immunoblots were developed with 4-chloro-1-naphthol, only a faint diffuse band (≈35- to 38-kDa) was detected (data not shown). The 54-kDa protein was readily detected when immunoblots containing equivalent samples from the same homogenates were developed with diaminobenzidine. A third detection method that used ^{125}I-labeled second antibody and autoradiographic exposure of the immunoblots also decorated the 54-kDa protein convincingly. Thus, immunoblot development with 4-chloro-1-naphthol appears to be less sensitive in this system.

Other experiments suggest that the 54-kDa protein is present in approximately equal amounts in infected brains from hamsters and mice at various times during the course of incubation and disease. The ≈35- to 38-kDa proteins appear to accumulate late in the disease.

DISCUSSION

Our results indicate that 54-kDa proteins in normal hamster and murine brains share antigenic determinants with the major structural protein of the scrapie agent. The 54-kDa normal proteins as well as PrPs purified from mouse and hamster brains were identified by an antiserum made to $NaDodSO_4$/PAGE-purified PrP-27–30. Antibodies, affinity purified from this antiserum by binding to and elution from either immobilized PrPs or proteins from homogenates prepared from normal brain, identified the same normal and scrapie-agent proteins. The original antiserum was made to proteins in proteinase K-digested sucrose gradient fractions containing highly purified scrapie agent. This antiserum does not react with normal proteins found in analogous fractions from uninfected normal brain (27). Therefore, it is unlikely that the purified antibodies prepared by elution from PrPs are contaminated with antibodies to unrelated normal cellular proteins. On the other hand, it is not possible to state absolutely which protein(s) in normal brain homogenates were responsible for purification of antibodies that identified PrPs and the 54-kDa protein. As the 54-kDa protein is the protein consistently identified in normal brain homogenates, it seems likely to be the major protein responsible for the purification of these antibodies when whole brain homogenate immunoblots are used. The 41-kDa and 35- to 38-kDa normal proteins identified by the antiserum (Fig. 1*b*, lanes 3 and 4) and occasionally by the purified antibodies probably contributed to the purification of the antibodies.

The identical molecular size and shared epitopes of the 54-kDa normal proteins in hamsters and mice imply that a common or related gene codes for these proteins. The homology between these normal proteins is in parallel with scrapie-specific PrPs in hamsters and mice; these also share nearly identical molecular weights and common antigenic sites (refs. 16 and 17 and unpublished results). Additionally, Creutzfeldt–Jakob disease-specific protease-resistant proteins in both the experimental murine model and the naturally occurring human disease share physical properties and antigenic determinants with scrapie-specific PrPs (29, 30). PrPs possess unusual resistance to proteases (16, 17) and charge heterogeneity due at least in part to glycosylation (39). We believe the resistance to degradation is an important property leading to the accumulation of these agent-specific proteins during the course of disease. In preliminary experiments we have evidence that the 54-kDa protein is not protease resistant. The extent to which the 54-kDa protein is glycosylated is not known.

It is possible that the shared antigenic sites on PrPs and the 54-kDa proteins represent, at least in part, structural similarities due to posttranslational modification. PrP-27–30 is a sialoglycoprotein (39), and attached oligosaccharides may provide some of the related antigenic determinants. It seems unlikely that the major shared epitopes are oligosaccharides because in both hamster and mouse brains one predominant protein with an apparent molecular size of 54 kDa was identified. Given the limited structural variation of oligosaccharides (40, 41), we would expect many other glycoproteins to crossreact. Importantly, our antiserum reacts as strongly with a deglycosylated form of PrP-27–30 as it does with the glycosylated form (unpublished data). The molecular size of the deglycosylated protein is apparently 18–19 kDa. This size corresponds to the peptide portion of this protein as predicted from the sequence of a cDNA clone (25). The data suggest that the primary antiserum contains antibodies specific for the peptide portion of PrPs and that the 54-kDa protein and the PrPs share a common primary structure.

Two other proteins, 41 kDa and 35–38 kDa, were identified by the primary antiserum but were not visible consistently on immunoblots developed with the purified antibodies. Whether this results from decreased concentration of the purified antibodies or lower affinity of these antibodies for these proteins has not been determined. We believe that the 33- to 35-kDa protein reported by others (25) is probably the same protein that we calculate as having a molecular size of 35–38 kDa. The exact relationship between the 54-kDa protein and the smaller proteins is not known. From our data we predict that the 54-kDa protein is the primary translation product of the normal gene encoding these proteins. Lower molecular size normal proteins, including the 41-kDa and 35- to 38-kDa proteins, which are antigenically related, might be produced by posttranslational modifications.

Two papers reported the identification of the host genes that code for the scrapie agent proteins (PrPs) in both hamster and mouse (25, 26). Those results support the findings reported here. The two groups of investigators identified mRNAs of 2.1 or 2.5 kb using cDNA clones representing PrP-27–30. Oesch *et al.* (25) predict that the peptide portion of the precursor to PrP-27–30 should be ≈26 kDa, assuming a 2.1-kb mRNA, which contains a ≈1.2-kb noncoding region. However, if the mRNA is 2.5 kb in length, as measured by Chesebro *et al.* (26), the peptide portion of the precursor could be ≈43 kDa in mass. Although the cDNA clone of Oesch *et al.* has been shown to contain a 1.2-kb noncoding region, it has not been shown that the actual mRNA coding for the precursor protein contains this noncoding segment. Since we do not know if the 54-kDa protein is a glycoprotein, it is not possible to say what contribution oligosaccharides would make to its apparent molecular size.

Scrapie PrP-27–30 fulfills the generally accepted criteria for an amyloid protein (38, 42, 43). Scrapie agent preparations contain fibrous structures described as scrapie-associated fibrils (44–46) or prion rods (38). Ultrastructurally, these fibrils resemble amyloid fibrils and have been shown by immunoelectron microscopy to be composed of PrP-27–30 molecules (28). Light microscopy has shown that aggregates of these structures stain with Congo red and exhibit green birefringence under polarized light (38), a characteristic of amyloid. All amyloid proteins that are well characterized are derived from normal protein precursors (42, 43, 47). It remains to be determined whether the 54-kDa proteins described in this report are precursors of the scrapie amyloid proteins.

In situ, antibodies to PrP-27–30 identify accumulations of the protein in discrete, subependymal structures that resemble amyloid plaques morphologically as well as histochemically (27, 48). Amyloid plaques are found in a limited number of central nervous system disorders. They are abundant in Alzheimer disease, and similar structures are commonly found in kuru and Creutzfeldt–Jakob disease, and in many animals with scrapie (1, 3, 49–51). Alzheimer disease also shares with these transmissible diseases the absence of

the classic signs of infection—inflammation and a specific immune response. Although an infectious etiology has been postulated for Alzheimer disease, transmission has not been demonstrated (3, 52, 53).

A model in which a normal cellular gene codes for the major scrapie agent protein, PrP, helps explain the lack of any detectable specific immune response in infected animals. The presence of a normal gene product, putatively identified as the 54-kDa protein, which is metabolized in a healthy cell to one or more lower molecular weight forms would account for the presence of antigenically related proteins in brain homogenates of uninfected animals. The modification of these proteins to protease-resistant, nondegradable forms (PrPs) in scrapie-infected cells would explain the accumulation of PrPs in the brains of diseased animals. Exposure of a susceptible host to the scrapie agent would initiate abnormal conversion of the precursor protein to the modified forms. Whether the essential component in transmitting the disease is the modified protein alone or this protein in association with a small regulatory nucleic acid is not known. It is conceivable that some sporadic cases of the spongiform encephalopathies result not from transmission but through the action of one or more unknown factors that disrupt normal protein metabolism. In these cases, abnormal processing and impaired degradation of the precursor protein also result in slow accumulation of the modified proteins. A related mechanism could explain amyloid formation in both normal aged individuals and Alzheimer disease patients, although the specific amyloidogenic precursor protein is probably different.

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