Immunological Analysis of Host and Agent Effects on Creutzfeldt-Jakob Disease and Scrapie Prion Proteins

JEFFREY M. BOCKMAN* AND DAVID T. KINGSBURY

Department of Microbiology, the George Washington University Medical Center, Washington, DC 20037

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Creutzfeldt-Jakob disease (CJD) and scrapie are degenerative neurological diseases caused by unusual infectious pathogens. The term *prion* has been introduced to underscore the apparent distinctness of these agents from viruses and viroids. The only macromolecule shown to be associated with the infectious agent, the CJD or scrapie prion protein (PrP^{CJD} or PrP^{Sc}, respectively), is encoded by the same gene as a normal cellular protein. In several studies biochemical differences have been reported in PrP^{Sc}s derived from a common host species infected with different putative strains of the scrapie agent, suggesting agent-specific characteristics independent of the host. We analyzed various agent-host combinations by Western blotting of PrPs that were separated by size or charge. The profile of immunoreactive proteins for CJD prions isolated from mice, guinea pigs, and humans appeared distinct. Importantly, PrP^{CJD}s purified from a human brain and from the corresponding first-passage mouse brains were clearly distinguishable. PrP^{CJD}s isolated from CJD prions propagated in NAMRU or B10.Q mice, which are homozygous for a short-incubation-time gene; from the short-incubation-time backcross progeny of (B10.Q × I/LnJ)F₁ × B10.Q; or from NAMRU mice inoculated with I/LnJ prions were identical to each other but distinguishable from those of I/LnJ mice, which are homozygous for the long-incubation-time gene. The PrPs from human CJD and ovine scrapie propagated in the same mouse strain appeared the same, but they were distinct from the same isolate of scrapie passaged in hamsters. Lastly, PrP^{Sc}s purified from five different strains of scrapie propagated in C57BL mice were identical, including strains, ME7 and 139A, which were previously reported to be distinct. This evidence does not support, although it does not exclude, agent-mediated characteristics independent of host-mediated ones for scrapie and CJD.

Creutzfeldt-Jakob disease (CJD) of humans and scrapie of sheep and goats are degenerative neurological diseases which are experimentally transmissible. Like classical viral diseases, natural and experimental CJD and scrapie are caused by filterable, titratable agents which display a characteristic pathogenesis and histopathology (26). Unlike conventional viruses, however, the infectivity of these agents is extremely resistant to procedures which modify or degrade DNA and RNA, and attempts to isolate an agent-specific nucleic acid have been unsuccessful (5, 6, 48). The term *prion* has been introduced to underscore the distinctness of these agents from viruses and viroids (53).

To date, the only macromolecule associated with and inseparable from scrapie infectivity is the protease-resistant scrapie prion protein (PrP^{Sc}) (12, 44), which is encoded by a cellular gene (21, 48). In normal cells this same gene encodes an isoform, PrP^C (3, 48). While no chemical differences have as yet been directly demonstrated between PrP^{Sc} and PrP^C, the two proteins differ in their physical properties (47). The 33- to 35-kilodalton (kDa) PrP^{Sc} is converted by limited proteinase K digestion into a 27- to 30-kDa protein (PrP-27-30), while PrP^C is rapidly degraded. Both forms of PrP are found in membrane fractions. However, on disruption of the membranes by detergents, PrP^{Sc} aggregates into amyloid rods (46, 58), while PrP^C is solubilized. These two isoforms do not appear to be the result of differential expression or splicing, and it seems that a posttranslational event or conformational alteration is responsible (3, 48).

Although it has been suggested in several reports (14, 22, 42, 60) that PrP can be separated from infectivity and that PrP is a pathologic product of infection and not the agent

itself, most experimental evidence suggests that PrP^{Sc} is indeed an essential component of the infectious particle (12, 13, 18, 25, 44). It is hypothesized that the phenomenon of prion infectivity and replication is the direct or indirect conversion by PrP^{Sc} of PrP^C into this same conformationally or chemically modified isoform (10, 65). Within this paradigm, the genetic background of the host plays an integral role in the pathogenesis of the disease. Genetically controlled differences in the incubation periods of various hosts are evident (23, 39, 50), and the PrP gene itself, *Prn-p*, has been linked to and may be identical with one of the genes, *Prn-i*, that has major influence on the scrapie incubation period in mice (18).

That the prion contributes some information to the course of infection is clear, as exogenous introduction of the agent precipitates disease. The reported existence of strains of the scrapie agent (15) and mutation (16) indicates, however, that it has an independent genome, presumably nucleic acid (34). In support of the biological evidence for strains and the linking of PrP (referred to by these investigators as the scrapie-associated fibril protein) with scrapie pathogenesis are studies in which differences among strains have been reported at the molecular level (31, 32). Distinct biochemical and immunological characteristics of PrP for each scrapie strain were identified (31, 32).

In this study we report results of an analysis of different strains of CJD or scrapie propagated in the same host and the same strain of CJD or scrapie propagated in different hosts by immunoblotting of PrPs separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and nonequilibrium pH gradient electrophoresis (NEPHGE). If, in fact, strains are real and their biochemical differences are reflected by various and distinct patterns for

* Corresponding author.

their protease-resistant proteins, then two different strains in the same host should have distinguishable PrP patterns. The influence of the host would be revealed by the same strains in two different hosts, with the strains having distinguishable PrP patterns. If there were no host contribution, the same strain in two different hosts should have identical PrP patterns. Our data provide no biochemical or immunological evidence for strain-specific characteristics independent of the host of last passage, as reflected in PrPs.

MATERIALS AND METHODS

Source and propagation of CJD prions. The Fukuoka-1 (K.Fu.) isolate of the CJD agent (61, 62) adapted to mice (40) was propagated for purification pools in the NAMRU strain of randomly bred Swiss-Webster mice. Other strains of mice included C57BL, B10.Q and I/LnJ, as well as the F₁ and F₂ generations from crosses of these latter two strains. Scrapie adapted to mice (randomly bred Swiss) and hamsters (randomly bred LVG/LAK) was obtained from S. B. Prusiner (University of California, San Francisco). Strains of mouse-passaged (C57BL) scrapie 139A, ME7, 22C, 22L, and 79A and golden hamster-passaged scrapie 263K were provided by A. Dickinson and R. Kimberlin (AFRC and MRC Neuropathogenesis Unit, Edinburgh, United Kingdom). Guinea pig-adapted CJD was supplied by H. Amyx (National Institutes of Health, Bethesda, Md.). Human CJD tissue and the brains of the mice (CF1) to which these cases were transmitted were kindly supplied by J. Tateishi (Kyushu University, Fukuoka, Japan).

Purification of mouse and human prions. The protocol used for the purification of mouse and human prions (38) was derived from that used by Prusiner and colleagues (57) for the purification of the scrapie agent from hamster brains. Briefly, homogenates of infected brain (10% [wt/vol] in 320 mM sucrose) were clarified by two low-speed centrifugations and then extracted with anionic and nonionic detergents, precipitated by the addition of polyethylene glycol, and digested with micrococcal nuclease and then with 100 µg of proteinase K per ml for 8 h at 4°C. Subsequently, ammonium sulfate precipitates were suspended in detergents and layered on the top of a discontinuous sucrose gradient. The fractions collected at the 25 and 60% sucrose interface contained the highest concentration of CJD and scrapie prions. Protease resistance of PrP^{Sc} and PrP^{CJD} relative to other cellular proteins was demonstrated by further digestion with 50 µg of proteinase K per ml for 30 min at 37°C prior to separation by SDS-PAGE.

SDS-PAGE. Electrophoretic separation of proteins by SDS-PAGE was performed as described previously (8) on a discontinuous system as described by Laemmli (41), with a 12 or 15% separating gel on a Protean or Mini-Protein II apparatus (Bio-Rad Laboratories, Richmond, Calif.).

NEPHGE. Separation of charge isomers was performed by NEPHGE essentially as described previously (49), except that 1.5-mm-thick slab gels were cast in the Mini-Protein II apparatus (Bio-Rad). The Nonidet P-40 in these samples was added to a final concentration that was 8 times that of the anionic detergent in the sample (1). The gels were run at 400 V for 2 h, equilibrated in 1× SDS-PAGE sample buffer without bromophenol blue for 10 min, rinsed for 2 min in transfer buffer, and electroblotted.

Electrophoretic transfer. Following electrophoresis, proteins were transferred to 0.45-µm-pore-size nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) by either overnight electrotransfer in a Trans-Blot apparatus

(Bio-Rad) at 110 mA for 16-by-16-cm gels (64) or for 1 h at 100 V in a Mini Trans-Blot apparatus (Bio-Rad).

Immunoblot analysis. Rabbit antisera to gel-purified hamster scrapie PrP-27-30 (7) and the PrP^{Sc} carboxy terminus peptide P3 (2) were provided by R. Barry (University of California, San Francisco). Nitrocellulose membranes bearing electrotransferred proteins from SDS-polyacrylamide and nonequilibrium pH gradient gels (Western blots) were processed essentially as described previously with horseradish peroxidase-conjugated secondary antibody (8). If the secondary antibody was alkaline phosphatase conjugated (Bio-Rad), then the buffer was 20 mM Tris and 500 mM NaCl (pH 7.5) and the substrate was 5-bromo-4-chloro-3-indolyl phosphate and Nitro Blue Tetrazolium.

RESULTS

It has been demonstrated previously, by using a purification scheme that included proteinase K digestion, that CJD-infected human and murine brains contain proteins which are immunoreactive with antiserum to hamster scrapie PrP-27-30 (8, 9, 11, 43). It has subsequently become clear that the species of PrP^{Sc} and PrP^{CJD} with various molecular masses are generated by proteolytic cleavage of a 33- to 35-kDa precursor with proteinase K (47, 59). These fragments are relatively stable to further proteinase K digestion for up to 2 h at 37°C (44). The pattern of PrP-related fragments is characteristic for mice, hamsters, and humans under standardized digestion conditions. Alteration of the digestion conditions can influence the polypeptide profile; however, there seems to be a point at which fragmentation of the precursor protein into the lower-molecular-weight bands ceases for a given isolate, and thereafter, proteolysis causes equal degradation of the bands until their simultaneous disappearance (44; J. M. Bockman, unpublished data). In this study, all comparisons were undertaken with proteins that were digested to this point.

The proteinase K-generated immunoreactive PrPs from three different species, mouse, guinea pig, and human, infected with CJD prions are shown in Fig. 1A. The apparent molecular masses of the proteins were, for the mouse, 18 to 20, 22 to 24, 25 to 27, and 27.5 to 30 kDa; for the guinea pig, 25 to 27 and 29.5 to 31 kDa (there was also a faint band at 18 kDa); and for the human, 15 to 15.5, 21 to 22, and 23 to 25 kDa. These data alone do not allow us to conclude whether the observed differences are the result of sequence variation between species in the PrP gene or the result of modifications mediated by each of three different isolates or strains.

To address this issue, prions were purified from the brains of Japanese patients who were dying from CJD and from the CJD-infected brains of the corresponding first-passage mice to which the CJD agent was transmitted after a prolonged incubation period of over 300 days (10). The immunoblot in Fig. 1B, which was reacted with antiserum to a synthetic peptide (P3) corresponding to amino acid residues 220 to 233 of hamster PrP (2), reveals that the immunoreactive PrPs from the same isolate of CJD appeared to be distinct in two different hosts, the human and the mouse. That this distinctness directly reflects biochemical differences is demonstrated by the fact that a mouse antiserum which was produced against human PrP^{CJD}s and which was species specific (reacting only with human and not hamster or mouse PrP) detected PrPs in partially purified preparations from these Japanese brains but failed to detect the PrPs in analogous preparations from the corresponding first-passage mouse brains (10). These data are compatible with the

FIG. 1. Immunoblotting of PrP^{CJD}s from different host species. (A) Lanes 1 to 3, PrP^{CJD}s from partially purified fractions of mouse-adapted CJD (40, 62), guinea pig-adapted CJD, and human CJD (corresponding to a previously reported isolate [case 2 in reference 9]), respectively. Detection of PrP^{CJD}s with a 1:1,000 dilution of a rabbit polyclonal antiserum raised against hamster scrapie PrP-27-30. (B) PrP^{CJD} purified from the first-passage mice (strain CF1) (lane 1) to which a case of human CJD (lane 2, corresponding to a previously reported isolate [case 4 in reference 10]) was transmitted. PrP^{CJD}s were detected with a 1:1,000 dilution of rabbit antiserum to the synthetic PrP peptide P3 (2). Proteins were separated by SDS-PAGE. Apparent molecular sizes are given in kilodaltons.

transmission of human CJD to mice, resulting in the production of CJD prions composed of PrPs from the host of last passage, the mouse.

The effect of host passage on PrP was further illustrated by passaging of the Fukuoka-1 isolate of CJD into mice with short and long incubation times. Restriction fragment length polymorphism analysis distinguished six haplotypes for *Prn-p* (G. A. Carlson, P. A. Goodman, M. Lovett, B. A. Taylor, S. T. Marshall, M. Peterson-Torchia, D. Westaway, and S. B. Prusiner, Cell, in press). An *Xba*I restriction fragment length polymorphism distinguished most mice with short incubation periods from those with long incubation periods. These short-incubation-time mice were homozygous for the *Prn-p^a* allele, which was detected as a 3.8-kilobase-pair *Xba*I fragment by using a hamster PrP cDNA probe (18, 30). The only strains of mice tested to date which

FIG. 2. Immunoblotting of PrP^{CJD}s from different mouse strains with short incubation periods and the *Prn-p^a* allele and a mouse strain (I/LnJ) with a long incubation period and the *Prn-p^b* allele. (A) Lanes 1, 2, and 4, PrP^{CJD}s from the short-incubation-period and *Prn-p^a* mice B10.Q, NAMRU, and (B10.Q × I/LnJ)_{F₁} × B10.Q, respectively. The mice were inoculated with NAMRU CJD prions. Lane 3, NAMRU mice inoculated with I/LnJ CJD prions. (B) Lanes 1 and 2, PrP^{CJD}s purified from *Prn-p^a* NAMRU mice and *Prn-p^b* I/LnJ mice, respectively. Lanes contained approximately 1 µg of protein. PrP^{CJD}s were detected with a 1:500 dilution of rabbit anti-P3 peptide serum. Mice were identified as *Prn-p^a* or *Prn-p^b* by Southern blotting by using a hamster PrP cDNA probe to detect a *Xba*I restriction fragment length polymorphism, as described elsewhere (18). Proteins were separated by SDS-PAGE on a Protean I (A) or Mini-Protein II (B) apparatus. Apparent molecular sizes are given in kilodaltons.

have exceptionally long incubation times are *Prn-p^b*, which displays a 5.5-kilobase-pair *Xba*I restriction fragment. The mouse strains NAMRU and B10.Q have the *Prn-p^a* allele and CJD incubation times of <130 days, while I/LnJ mice have the *Prn-p^b* allele and CJD incubation times of >200 days (18, 39).

Partially purified PrPs (PrP-A) from NAMRU and B10.Q mice and from the homozygous *Prn-p^a* mice with short incubation times from the F₂ generation of (B10.Q × I/LnJ)_{F₁} × B10.Q were all identical by immunoblotting of SDS-polyacrylamide (Fig. 2A) and nonequilibrium pH gradient (data not shown) gels. In addition, the PrPs purified from NAMRU mice inoculated with I/LnJ prions appeared identical to the PrPs from the NAMRU, B10.Q, and F₂ mice, all of which were inoculated with NAMRU-propagated prions. I/LnJ prions inoculated into NAMRU mice had incubation periods of over 200 days at first passage, as opposed to the approximately 140 days for NAMRU CJD

FIG. 3. Immunoblotting of PrP^{CJD}s and PrP^{Sc}s propagated in the same host species. Lanes 1 to 4, PrP^{CJD}s in a purified fraction from CJD-infected mice, the equivalent fraction from normal mice, PrP^{Sc} from scrapie-infected mice, and PrP^{Sc} from scrapie-infected hamsters, respectively. PrP^{CJD}s and PrP^{Sc}s were detected with a 1:500 dilution of rabbit anti-P3 peptide serum. Proteins were separated by SDS-PAGE. Apparent molecular sizes are given in kilodaltons. Lanes contained approximately 1 μ g of protein.

prions inoculated into NAMRU mice (D. Kingsbury, unpublished data). Such prolongation is analogous to that observed with scrapie in C57BL and IM (VM) mice, in which an isolate such as 22A which has been propagated in the long-incubation-time mouse strain IM has, at first passage in the short-incubation-time strain C57BL, an incubation time that exceeds even that of the isolate in IM mice (19). Finally, the proteinase K-generated PrPs (PrP-B) from the I/LnJ mice had a recognizably different pattern of immunoreactive proteins from that of NAMRU mice, as determined with the P3 antiserum (Fig. 2B). The predicted sequences of the PrPs from the prototypic short-incubation-time mouse strain NZW and the long-incubation-time mouse strain I/LnJ have been shown to differ by two amino acids (65).

Complementary to data on the influence on PrP of passaging the same strain of CJD or scrapie in different hosts are data on the influence on PrP of different strains propagated in the same host. If passaging of scrapie and CJD across species barriers, that is, host adaptation, can select out advantageous mutations in the informational molecule associated with the agent (16), then CJD, a disease of humans, and scrapie, a disease of sheep and goats, could themselves be seen as different strains of the same agent when passaged in a common host. In fact, human CJD transmitted to goats is indistinguishable from natural caprine scrapie (28). The immunoreactive PrPs from the Chandler murine isolate of goat scrapie propagated in randomly bred Swiss mice (Rocky Mountain Laboratory) (13, 20) and the Fukuoka-1 murine isolate of human CJD propagated in the NAMRU strain of randomly bred Swiss-Webster mice (40) are shown in Fig. 3. The murine PrP^{CJD} and PrP^{Sc} were identical but were distinct from the Chandler hamster scrapie isolate passaged in randomly bred LVG/LAK hamsters (20, 36, 55).

Since the Chandler scrapie isolates have been reported to contain a mixture of strains (37), we obtained five different

FIG. 4. Immunoblotting of PrPs from five different reported strains of scrapie propagated in C57BL mice. (A) PrP^{Sc}s separated by SDS-PAGE (Mini-Protein II apparatus). Lane pairs 1 and 2, 3 and 4, 5 and 6, 7 and 8, and 9 and 10 represent PrP^{Sc} purified from mice inoculated with scrapie strains 139A, ME7, 79A, 22L, and 22C, respectively. The samples in the even-numbered lanes were subjected to an additional digestion with 50 μ g of proteinase K per ml at 37°C for 30 min. Apparent molecular sizes are given in kilodaltons. (B) PrP^{Sc} separated by slab NEPHGE on a Mini-Protein II apparatus (Bio-Rad). Lanes 1 to 5, Scrapie strains 22L, 22C, 139A, ME7, and 79A, respectively. The gel contained 2% pH 3.5 to 10.0 Ampholine (LKB Instruments, Inc., Rockville, Md.). Electrophoresis was from top to bottom, with the anode at the top. Proteins separated by SDS-PAGE and NEPHGE were electroblotted to nitrocellulose filters, and PrP^{Sc} was detected by a 1:500 dilution of rabbit anti-P3 peptide serum. Lanes contained approximately 10 μ g of protein.

strains of murine scrapie from A. Dickinson and R. Kimberlin: ME7, 139A, 79A, 22C, and 22L (15). These were propagated in female C57BL mice, and the scrapie prions were partially purified. Immunoblotting of SDS-PAGE-separated (Fig. 4A) or NEPHGE-separated (Fig. 4B) PrP^{Sc} from each strain with monospecific antiserum to the synthetic peptide P3 revealed identical patterns of immunoreactive proteins. Identical results were obtained with the polyclonal antiserum to hamster PrP-27-30 (data not shown). No differences were detected among the strains in the sensitivities of their PrPs to proteinase K, as judged by the intensities of their respective immunoreactive bands following a further digestion of the partially purified fractions with 50 μ g/ml for 30 min at 37°C.

DISCUSSION

In several recent studies an attempt has been made to correlate observed biochemical and immunological differences among PrP^{Sc}s with the apparent strains of scrapie from which the prions were derived and independent of the influence of the host of passage (31, 32). Such data constitute confirmation, at the molecular level, of the reported differ-

ences in biological properties among scrapie isolates and suggest agent-encoded information. While the evidence presented here does not exclude the possibility that there are agent-mediated characteristics, it does not support such a hypothesis.

When all five of the putative scrapie strains, 139A, ME7, 79A, 22C, and 22L, were propagated in C57BL mice, they appeared to be identical to one another both in terms of the profile of their immunoreactive proteins separated by molecular weight or charge, by using either antisera to PrP-27-30 or a synthetic peptide, as well as in terms of their sensitivity to proteinase K. In addition, the immunoreactive proteins from two different prion diseases, human CJD and caprine scrapie, appeared identical by one- and two-dimensional analysis when propagated in the mouse. Kascsak et al. (31) have presented data that demonstrate differences in the immunoreactive profiles of proteinase K-generated PrPs between scrapie strains 139A and ME7 in C57BL mice and scrapie strains 87V and ME7 in IM mice. In addition, 139A has been reported to be more susceptible to proteinase K than ME7 (32). The differences between 87V and ME7 in IM mice are the most distinct, and it would be of great value to analyze these scrapie strains; however, the distribution of certain scrapie strains such as 87V is highly restricted.

The reason for the apparently conflicting data, specifically those for scrapie strains 139A and ME7, is unclear. Since the several prion-specific immunoreactive species are generated by proteinase K from the 33- to 35-kDa precursor, the different conditions of proteinase K digestion used in the purification process may be relevant. Our protocol entailed digestion with 100 µg of proteinase K per ml for 8 h at 4°C versus the 50 µg/ml for 2 h at 37°C used by Kascsak et al. (31). Furthermore, our digested fractions contained the nonionic and anionic detergents Triton X-100 and Sarkosyl (CIBA-GEIGY Corp., Summit, N.J.), while the fractions of Kascsak et al. (31) contained only Sarkosyl. In a previous study (54) the effect of different detergent combinations on the protease sensitivity of the scrapie agent has been shown. It should be noted that while scrapie strains 139A and ME7 isolated from C57BL mice appeared distinct by the use of two different polyclonal sera (one to murine-propagated ME7 and one to hamster-propagated 263K) in the report by Kascsak et al. (31), in the same report 139A and ME7 appeared identical when antibodies were used that were affinity purified from these same sera by elution of the antibodies from the nitrocellulose-bound SDS-PAGE-separated scrapie-associated fibril proteins. Kascsak et al. (32) have previously shown by silver staining that 139A and ME7 are identical. This suggests strain-specific epitopes, with the antibodies detecting either changes in the primary structure of PrP or posttranslational modifications. Because it appears that differential expression or splicing is not responsible for the differences between PrP^C and PrP^{Sc} (3, 48), it is also likely that they do not contribute to any differences between strains, but that these differences, if any, would arise, as is presumed for the two PrP isoforms, by posttranslational mechanisms.

While we have no data that suggest strain-specific modification of PrP several different lines of evidence reflect the direct influence of the host of last passage on the structure of PrP. The molecular weight profiles of the immunoreactive PrPs generated by proteinase K from an isolate of human CJD and the corresponding first mouse passage of that same isolate were distinct. Their distinctness was dramatically revealed by the fact that murine species-specific antiserum to human CJD prions reacted only with the original human

PrP^{CJD} and not with the murine-passaged prions (10). Thus, if the infectious particle is composed of PrP, the CJD prions composed of human PrP^{CJD} molecules are changed to CJD prions composed of mouse PrP^{CJD} molecules on transmission of human CJD to mice. Results of our earlier report (10) on the existence of epitope differences on PrPs from different species have been confirmed recently by Kascsak et al. (33).

Passaging of the same isolate of CJD in different mouse strains which nonetheless shared the same short-incubation-time allele in the PrP gene revealed identical immunoreactive profiles for PrP. This included the first *Prn-p^a* mouse passage (NAMRU) of *Prn-p^b*-propagated (I/LnJ) CJD prions; the incubation times of these mice were longer than those of PrP-A prions inoculated into *Prn-p^a* mice (NAMRU CJD prions into NAMRU mice). This prolongation presumably reflects the constraints on the conversion of the cellular isoform into PrP-A molecules by the heterologous PrP-B protein (65). Similar results have been observed on transmission of human CJD to mice, with incubation periods of 300 to 500 days at first passage but times of approximately 120 days at second passage, with no further change on subsequent passage (63). This behavior is analogous to the species barrier effect reported by Pattison (51). Our data that show that human CJD prions are converted to mouse CJD prions at first passage in mice are consistent with these observations (10). It has been proposed that this phenomenon of the species barrier reflects the selection of mutants and a permanent change in the scrapie genome, presumably nucleic acid, although the host is thought to contribute components (PrP) which form a structural and functional part of the infectious agent (the donor species effect) (35). This is the virino concept proposed by Dickinson and Outram (24).

Lastly, the profiles of the immunoreactive PrPs, which were generated by proteinase K, of the Fukuoka-1 isolate of CJD propagated in *Prn-p^a* and *Prn-p^b* mice were distinguishable. On the basis of the nucleotide sequence, it is predicted that PrP^C and it is hypothesized that PrP^{Sc} and PrP^{CJD} have leucine and threonine residues at codons 108 and 189, respectively, in *Prn-p^a* mice and phenylalanine and valine residues, respectively, in *Prn-p^b* mice (65). PrP^C from *Prn-p^a* and *Prn-p^b* mice appeared identical by immunoblotting with polyclonal antiserum against PrP-27-30 (65). However, since these amino acid changes in the PrP gene were correlated with changes in scrapie pathogenesis, as reflected in scrapie incubation times, it is not unreasonable to expect that such changes could affect the conformation of the corresponding PrP^{Sc} or PrP^{CJD} or result in alternative posttranslational modifications. Such changes could alter the susceptibility to proteolysis, resulting in the observed differences between NAMRU and I/LnJ PrP^{CJD}. Surface hydrophobicity has been shown to be an important factor in the hydrolytic degradation of proteins (54). The agent of scrapie has been shown to display hydrophobic aggregation (45, 56), and conformational analysis of the predicted sequence of PrP has revealed several potential hydrophobic domains (4). It has been determined by immunoblotting that each of the several lower-molecular-weight species of PrP^{CJD} displays a dimer which is not dissociated or reassociated during SDS-PAGE (J. M. Bockman, unpublished data). In this regard it is interesting that the *Prn-p^b* mouse displayed a much more prominent oligomer (40 to 43 kDa) than the *Prn-p^a* mouse. Whether this is directly related to the change from the polar threonine to the nonpolar valine or to an altered posttranslational modification remains to be determined. Posttransla-

tional modifications such as myristoylation have been correlated with the acquisition of hydrophobicity (52).

Differences between the ME7 PrPs isolated from short-(C57BL) and long- (IM) incubation time mice were not observed by Kascak et al. (31). Scrapie strain ME7 has been referred to as a class I stability agent (15) by virtue of the fact that it seemingly retains its identity regardless of the host of prior passage; that is, ME7 passaged in long-incubation-time mice has been reported to have the same incubation period on introduction into short-incubation-time mice as ME7 passaged in short-incubation-time mice. Class II agents exhibit behavior analogous to that observed for the propagation of the I/LnJ-passaged Fukuoka-1 isolate of CJD in NAMRU mice and the transmission patterns of the CJD agent from Japanese patients to mice. Comparison of the effect of mouse genotype on PrP between scrapie strain ME7 and CJD isolate Fukuoka-1 may therefore not be valid if the biological differences between putative scrapie strains are real and are reflected in PrPs.

Alternatively, the data indicating that PrPs appear distinct in different hosts are not incompatible, by themselves, with the fact that PrP is a pathological product of infection induced by an as yet undefined infectious agent. Such a conclusion must be predicated on the ability to separate the native PrP from the infectious component. Several groups have presented evidence that seems to demonstrate this separation; this has led them to suggest that scrapie and CJD are examples of a virus-induced amyloidosis (14). The late appearance of the scrapie-associated fibril protein relative to the kinetics of infectivity (14, 22) and the apparent separation by lectin chromatography of half of the total amount of PrP from the infectious portion (42) are the two main pieces of evidence for the hypothesis that PrPs are not a component of the agent. Numerous lines of evidence, however, do in fact support the fact that PrPs are an essential component of the infectious particle (12, 13, 17, 18, 25, 44, 65), including the copurification of PrP with the infectivity, the presence of PrP before pathology in the brain and in the nonpathologic but infectious spleen, the proportionality of PrP concentration and the agent titer, chemical modification of PrP correlating with a decrease in titer, the linkage of the PrP gene and a gene controlling the incubation period, the partitioning of PrP and infectivity into different forms (membranes, rods, spheres, liposomes), and the production of PrP^{Sc} in scrapie-infected cells in culture. Furthermore, any correlation of the biophysical properties of PrP^{Sc} with the strain from which it was isolated (31, 32) could be interpreted as lending further support to the fact that PrP is part of the infectious agent; otherwise, one must consider strain-specific alterations in the pathological product of the infection. Although it has been reported that PrP^C and PrP^{Sc} have the same primary sequence and ionic charge distribution and may therefore have the same covalent structure (29), suggesting that PrP^{Sc} may be an abnormal by-product of infection, results of deglycosylation experiments (T. Haraguchi, D. Groth, R. A. Barry, et al., *Fed. Proc.* 46:1319, 1987 [abstract]) and further analysis of the ionic charge distribution (J. M. Bockman, unpublished data) suggest that the covalent structure of these two proteins are not identical, in accord with their different physical properties.

In conclusion, we suggest that at least some of the observed changes on passaging of scrapie isolates, which have been interpreted as selection from a mixture of strains or advantageous mutations, can be interpreted to be epigenetic (65). Our data revealed only host-specified alterations in PrP and were in contrast with the data of other investiga-

tors, who showed agent-specific alterations independent of the host of last passage. Interestingly, the model of a host-encoded but posttranslationally or conformationally altered isoform that induces the conversion of the host form to this altered isoform is strikingly similar to the model for scrapie as a protein agent proposed in 1967 by J. S. Griffith (27) for self-replication of a modified form of a normal cellular protein. More refined biochemical and biophysical analyses, particularly of the posttranslational modifications present on PrPs from different strains passaged in a common host, are necessary to resolve these issues on the relative informational contributions of the agent and host to the composition of the infectious particles of scrapie and CJD.

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