The central event in the pathogenesis of prion diseases, a group of fatal, transmissible neurodegenerative disorders including Creutzfeldt-Jakob disease (CJD) in humans, is the conversion of the normal or cellular prion protein (PrPC) into the abnormal or scrapie isoform (PrPSc). The basis of the PrPC to PrPSc conversion is thought to involve the diminution of α-helical domains accompanied by the increase of β structures within the PrP molecule. Consequently, treatment of PrPSc with proteinase K (PK) generates a large PK-resistant C-terminal core fragment termed PrP27–30 that in human prion diseases has a gel mobility of 19–21 kDa for the unglycosylated form, and a ragged N terminus between residues 78 and 103. PrP27–30 is considered the pathogenic and infectious core of PrPSc. Here we report the identification of two novel PK-resistant, but much smaller C-terminal fragments of PrP (PrP-CTF12/13) in brains of subjects with sporadic CJD. PrP-CTF 12/13 migrate at 12 and 13 kDa and have the N terminus that begins at residues 162/167 and 154/156, respectively. Therefore, PrP-CTF12/13 are 64–76 amino acids N-terminally shorter than PrP27–30 and are about half of the size of PrP27–30. PrP-CTF12/13 are likely to originate from a subpopulation of PrPSc distinct from that which generates PrP27–30. The finding of PrP-CTF12/13 in CJD brains widens the heterogeneity of the PK-resistant PrP fragments associated with prion diseases and may provide useful insights toward the understanding of the PrPSc structure and its formation.

Prion diseases consist of a group of fatal and transmissible neurodegenerative disorders including scrapie and bovine spongiform encephalopathy in animals, and Creutzfeldt-Jakob disease (CJD),1 fatal familial insomnia (FFI), and Gerstmann-Straussler-Scheinker disease (GSS) in humans (1, 2). Human prion diseases can be sporadic, familial, or acquired by infection. Most of them are characterized by the deposition of an abnormal prion protein, PrPSc, in brain. PrPSc derives from its host-encoded normal cellular isoform PrP that is predominately expressed in brain but also at lower levels in many other tissues (3–5). Mature human PrPC contains 209 amino acids encompassing residues 23–231, a disulfide bridge between residues 179 and 214, two consensus sites for N-linked glycans at residues 181 and 197, and it is attached to cell membranes via a C-terminal glycosylphosphatidylinositol (GPI) anchor (6–12). Although PrPC and PrPSc have an identical primary structure, they have distinct physiochemical properties. PrPC exists as a detergent-soluble monomer and is readily degraded by proteinase K (PK), whereas PrPSc forms detergent-insoluble aggregates and shows high resistance to PK digestion (6, 13–17). Following treatment with PK, PrPSc generates a protease-resistant core, referred to as PrP27–30 that is N-terminally truncated at the N terminus between residues 78 and 103 (6, 18). It is thought that the PrPC to PrPSc conversion involves the decrease of α-helical domains accompanied by the increase of β structures in the midportion of the PrP molecule (19–21). However, the precise location and extent of these structural changes within the PrP molecule and therefore, the tertiary and quaternary structures of PrPSc, are largely a matter of speculation.

Full-length PrPSc and PrP27–30 are the only known components of the naturally occurring infectious agent causing prion diseases (1) and are thought to be the primary cause of the histological changes in brains of subjects with prion diseases (22). However, the role of PrPSc in the pathogenesis of these changes is poorly understood. This issue is further compounded by the presence of other derivatives of PrPSc in human and animal prion diseases, particularly the 7–8-kDa-truncated PrP fragments found in GSS (23–26) and the 16- and 7-kDa C-terminal fragments in scrapie-infected hamsters (27, 28).

We have now characterized two novel C-terminal fragments of PrP in brains of patients with sporadic CJD (sCJD). These PrP fragments migrate at about 12 and 13 kDa on Tris-Tricine gradient gel, and have the N terminus that begins at residues 162/167 and 154/156, respectively, as determined by automated Edman degradation. They are resistant to PK digestion and derive from both glycosylated and unglycosylated forms. We propose that these fragments are distinct from PrP27–30. They are resistant to PK digestion and derive from both glycosylated and unglycosylated forms. We propose that these fragments are distinct from PrP27–30.
and derive from a subpopulation of full-length as well as N-terminally truncated PrPSc carrying a different conformation.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Proteinase K (PK) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. Urea, CHAPS, iodoacetamide, n-dithiothreitol (DTT), and immobilized pH gradient (IPG) strips (pH 3–10, 11 cm long) were from Bio-Rad. Ammonium pH 3–10 and reagents for enhanced chemiluminescence were from Amersham Biosciences. Magnetic beads (Dynabeads M-280, tosyl-activated) were from Dynal Co. (Oslo, Norway). Several well-characterized anti-PrP antibodies, including rabbit anti-C antisera (gift from the supplier with minor modifications using PROTEIN IEF cell (Bio-Rad) and terminated by the addition of 3 mM phenylmethylsulfonyl fluoride. The supernatant protein sequencing by automated Edman degradation was performed by a protocol described previously (18), at the ProSeq Micro sequencing Facility (Boxford, MA) with an Applied Biosystems 477A Protein Sequencer.

**N-terminal Protein Sequencing**—Purified proteins were separated by 10–20% mini-Tris-Scine SDS-PAGE gradient gels (Novex pre-cast gel, Invitrogen), transferred onto Problott membranes (Applied Biosystems, Foster City, CA), and visualized by Coomassie Blue staining. N-terminal protein sequencing by automated Edman degradation was performed by a protocol described previously (18), at the ProSeq Microsequencing Facility (Boxford, MA) with an Applied Biosystems 477A Protein Sequencer.

**RESULTS**

**PK-resistant C-terminal Fragments of Human PrPSc**

**PK-resistant C-terminal Fragments of Human PrPSc**

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**PK-resistant C-terminal Fragments of Human PrPSc**
The study of the brain distribution of PK-resistant PrP fragments was carried out in 14 cases showed that PrP-CTF12/13 generally were better represented in the neocortex than in the hippocampal formation and subcortical structures such as putamen, thalamus, and cerebellum.

**PrP-CTF12/13 Are Truncated Glycosforms of PrPSc**—Because PrP contains two consensus sites for N-linked glycans at residues 181 and 197 that contributes to its heterogeneity (6, 11, 12), deglycosylation by PNGase F is often used to simplify the gel migration pattern and to reveal the size of the PrP backbone. As shown in Fig. 2, PrP glycosforms were readily detectable in control brains, which upon deglycosylation, shifted mainly to a 27 kDa band corresponding to the full-length PrP and an 18 kDa band corresponding to an N-terminally truncated PrP fragment with an N terminus starting at residue 111/112, as characterized previously (29). They were invariably sensitive to PK digestion as expected for normal PrPSc. PrP-CTF12/13 in the molecular mass range of 12 and 13 kDa were not detected in control brains with or without deglycosylation. PrP-CTF12/13 were barely detectable in untreated brain homogenates from sCJD subjects. However, PK treatment and removal of glycans with PNGase F independently resulted in a substantial increase in amount of PrP-CTF12/13 with the same gel mobility of 12 and 13 kDa as that observed following treatment with both PK and PNGase F (Fig. 2). These findings indicate that PrP-CTF12/13, like PrP27–30 (16, 29, 32), are primarily generated by PK treatment of both glycosylated and unglycosylated PrPSc forms. PrP-CTF12/13 are unlikely to be generated during post mortem autolysis because they are present with the same characteristics in sCJD brain tissue obtained at biopsy, in which autolysis is not expected to play a role (data not shown).

**PrP-CTF12/13 Are Part of the C-terminal Domain**—PrP-CTF12/13 immunoreacted only with anti-C antibody (29) against the C terminus of PrP (residues 220–231), but not with 3F4 antibody (30) recognizing an epitope located in the mid-PrP region (residues 109–112) (Fig. 3). This finding suggests that PrP-CTF12/13 are generated by truncation of PrP at sites C-terminal to residue 112. Amino acid sequencing by automated Edman degradation showed that the N terminus of the PrP-CTF12 mainly started at residue 162 and occasionally at residue 167, while PrP-CTF13 preferentially began at residue 154 and infrequently at residue 156 (Table II). Therefore, PrP-CTF12/13 are made of the C-terminal domain of PrP spanning from residues 162/167 to 231 and residues 154/156 to 231, respectively.

**Two-dimensional Immunoblotting of PrP-CTF12/13**—We further examined PK-treated, detergent-insoluble fraction (P2) from sCJD brains using two-dimensional gel electrophoresis which separates proteins not only on the basis of the relative mass but also of net electrical charges (Fig. 4). A broad set of spots with molecular mass of 20–30 kDa and pH 5.2–8.0 that...
immunoreacted with both anti-C antibody (Fig. 4A) and 3F4 (Fig. 4C), corresponded to glycoforms of PrP27–30 (29, 41, 42). Two additional sets of spots were detected by anti-C antibody (Fig. 4A) but not by the 3F4 antibody (Fig. 4C). A distinct set of 4–6 spots with a pl within pH 5.0–6.5 and a molecular mass of 12–13 kDa is likely to represent the unglycosylated form of PrP-CTF12/13 readily detectable on SDS-PAGE gels. Another set of spots with more acidic pl values within pH 4.5–6.0 and a mass of ~20–21 kDa is likely to represent the glycosylated species of the PrP-CTF12/13 (Fig. 4A). To strengthen this conclusion, the PK-treated P2 fraction of sCJD brains was deglycosylated by PNGase F. After this treatment, the heterogeneity of PrP spots was greatly reduced. A group of 4–5 PrP spots with a mobility of 19–20 kDa and pl between pH of 6.5–8.5 were detected by both anti-C (Fig. 4B) and 3F4 (Fig. 4D). These spots matched the deglycosylated PrP27–30 reported in previous studies (41, 42). Another group of 4–6 spots displaying a gel mobility of 12–13 kDa and a pl within pH of 5–6.5 was detected only by anti-C (Fig. 4B), and corresponded to the pl of the unglycosylated PrP-CTF12/13. Therefore, the latter group is likely to derive from both the original unglycosylated PrP-CTF12/13 species and those generated following deglycosylation. The multiple spots present even after deglycosylation in both PrP27–30 and PrPCTF12/13 are likely due to the heterogeneity of the GPI moiety covalently linked to the C terminus (29, 41, 42). No major differences were observed between the two-dimensional gel profiles of PrPCTF12/13 from CJD cases with PrPSc types 1 and 2. However, detailed studies are needed to exclude minor variations.

We computed the expected molecular weight and pl of unglycosylated PrP-CTF12/13 using the ProtParam program of the SWISS-PROT (ca.expasy.org/tools/protparam.html). The

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**TABLE II**

**N-terminal sequencing of PrP-CTF12/13**

| Size  | N-terminal sequence | Starting site | N’ |
|-------|---------------------|---------------|----|
| 13 kDa| MHRYPNQVYY (major)  | 154           | 5  |
|       | RYPNNQVVYP (minor)  | 156           | 2  |
| 12 kDa| YYPYMDEYSN (major)  | 162           | 10 |
|       | DEYSNQNFV (minor)   | 167           | 3  |

* A major sequence was unequivocally determined from all experiments, while a minor sequence was observed in some experiments with yields about 20–30% that of the major sequence.

* PrP residue number at the N-terminal according to Kretzschmar et al. (7) (Swiss-Prot accession number: P04156).

* Number of determinations.

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**FIG. 3. Antibody mapping of PrP-CTF12/13.** Brain homogenates from sCJD subjects were either untreated or treated with PK and PNGase F. PrP bands were detected on immunoblots using either anti-C antibody (upper panel) or 3F4 antibody (lower panel). In all preparations, PrP-CTF12/13 were detected only by anti-C but not by 3F4. Therefore, PrP-CTF12/13 are N-terminally truncated PrP fragments. Molecular mass markers are shown in kDa.

**FIG. 4. Two-dimensional immunoblots of PrP-CTF12/13 and PrP27-30.** PK-treated P2 fraction from a sCJD case was either untreated or deglycosylated with PNGase F. Proteins were separated by two-dimensional gel electrophoresis. PrP was detected on immunoblots using either anti-C antibody or 3F4 antibody. A and B, two-dimensional immunoblots probed with anti-C antibody. Without deglycosylation (A), the spots corresponding to glycoforms of PrP27–30 migrated in a broad region from molecular mass 30 to 19 kDa and with pl between 5.2 and 8.0. The spots corresponding to PK-resistant PrP-CTF12/13 displayed pl between 5.0 and 6.5, while additional spots with pl within pH 4.5–6.0 and a mass of ~20–21 kDa are likely to represent the glycosylated form of PrP-CTF12/13. After deglycosylation by PNGase F (B), two groups of PrP spots were detected, representing unglycosylated PrP27–30 and PrP-CTF12/13 species, respectively. C and D, two-dimensional immunoblots probed with 3F4 antibody. In both untreated (C) and PNGase F-treated (D) samples, spots corresponding to glycosylated and unglycosylated PrP-CTF12/13 were not detected by 3F4 antibody. However, spots corresponding to glycoforms of PrP27–30 (C) and those representing deglycosylated PrP27–30 (D) were readily detected by 3F4 antibody. The positions of pl gradient and molecular mass markers (in kDa) are indicated on the bottom and on the left, respectively.
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We have identified two novel C-terminal fragments of PrP, PrP-CTF12/13, in human brains affected by different subtypes of sporadic CJD. The PrP-CTF12/13 are present along with the binding specifically and with high affinity to GPI-anchored proteins, which was then detected with a specific antibody (38). The PrP fragments detected by the anti-C antibody in a PK- and PNGase F-treated P2 fraction enriched in PrP-CTF12/13 co-migrated with the fragments detected by the monoclonal antibody to proaerolysin (Fig. 5B). This suggests that PrP-CTF12/13 fragments contain the C-terminal GPI anchor.

PrP-CTF12/13 Can Be Derived from PK Digestion and PNGase F Deglycosylation of Both Full-length and Truncated PrP Forms in Vitro—Previous studies from other and our laboratories have demonstrated that the untreated detergent-insoluble fraction (P2) prepared from CJD brains contains not only full-length PrP but also forms that are truncated at different sites of the N terminus (29, 41, 44). To determine if PrP-CTF12/13 derive from either full-length or truncated PrP, the full-length PrP molecules were separated from truncated forms by immunoprecipitation with 8B4, an antibody to the N terminus of PrP (31). The separated full-length and truncated PrP molecules in the SB4 immunoprecipitate and supernatant following immunoprecipitation with SB4, an antibody to the N terminus of PrP (31). The separated full-length and truncated PrP molecules in the SB4 immunoprecipitate and supernatant following immunoprecipitation, respectively, were then subjected to PK digestion and PNGase F deglycosylation. As shown in Fig. 6, full-length, but not truncated PrP was observed in immunoprecipitate while truncated PrP forms including PrP-CTF12/13 were present in supernatant only. After treatments with PK and PNGase F, PrP27–30 was found in both immunoprecipitate and supernatant in similar amounts, suggesting that PrP-CTF12/13 appear to be more abundant in the supernatant than in the full-length PrP fraction (immunoprecipitate).

DISCUSSION

We have identified two novel C-terminal fragments of PrP, PrP-CTF12/13, in human brains affected by different subtypes of sporadic CJD. The PrP-CTF12/13 are present along with the anchorless, we carried out sandwich blotting using proaerolysin, a toxin

Protein sequences containing C-terminal 78 and 70 amino acids matching those of PrPCTF12/13 were assigned molecular masses of 9.4 and 8.4 kDa, which, adding −4 kDa for the GPI anchor, become 13.4 and 12.4 kDa, comparable to the 13 and 12 kDa determined by the gel migration. Theoretical pIs of PrP-CTF12/13 are 5.2–5.8, not very different from the pIs indicated by two-dimensional electrophoresis. The subtle difference in pI (−0.5) could derive from the heterogeneity of the GPI anchors. These data confirm that the PrP-CTF-S are C-terminal fragments of PrPSc, i.e., they result from PK cleavages located much more toward the C terminus than the cleavages that generate PrP27–30.

**Fig. 5.** Characterization of GPI anchor in PrP-CTF 12/13. A, immunoblot of PIPLC-treated PrP-CTF12/13. Brain homogenates (10% w/v) were prepared in 1% Triton X-114 in PBS at 4 °C. Following the removal of cellular debris, phase separation was initiated at 37 °C for samples were incubated at 4 °C overnight in the presence (+) or absence (−) of PIPLC. PrP in the detergent (D) and aqueous (A) phases was determined by SDS-PAGE and immunoblotting with anti-C antibody. B, sandwich blotting of PrP-CTF12/13 with proaerolysin. PrP enriched in P2 fraction from CJD brains was digested with PK and was then deglycosylated by PNGase F. Proteins were separated on Tris-Tricine SDS-PAGE (10–17% gradient gel). The blot was incubated with proaerolysin at 0.5 μg/ml in PBS for 1 h at room temperature. Proaerolysin bound to GPI-anchored proteins were detected with immunoblotting with an anti-proaerolysin monoclonal antibody. The position of PrP-CTF12/13 is indicated by an asterisk (*).
PK-resistant C-terminal Fragments of Human PrP<sub>Sc</sub>

![Diagram of human PrP<sub>C</sub> and PK-resistant PrP fragments observed in human prion diseases.](image)

The NMR-derived but linearized human PrP<sup>C</sup> structure comprises an unstructured N-terminal domain and a globular C-terminal domain that contains three α-helices (α1–3) and two short β-strands (β1–2) (47). Post-translational modifications include a disulfide bridge (S–S) between residues 179 and 214, two consensus sites for N-linked glycans at residues 181 and 197 and a GPI anchor at the C terminus. PrP<sub>27–30</sub> is a well characterized PK-resistant PrP fragment present in most human prion diseases. It is generated by PK cleavage between residues 74 and 103 of human PrP<sup>Sc</sup> in sCJD producing a highly ragged N terminus (18). PrP<sub>7–8</sub> has to date only been observed in subjects with GSS and comprises a group of PK-resistant fragments spanning residues 74/90 to 146/153 (23–26). PrP-CTF12/13, identified in this study, are PK-resistant and contain both glycosylated and unglycosylated forms, but it remains to be determined whether they retain the disulfide bridge and the α-helix 2 and 3 structures.

**Well-known PK-resistant PrP fragments commonly referred to as PrP<sub>27–30</sub> (16).** Like PrP<sub>27–30</sub>, PrP-CTF12/13 are primarily generated by in vitro limited proteolysis and represent a PK-resistant C-terminal core of PrP<sup>Sc</sup> that includes glycosylated and unglycosylated forms. The glycosylated forms of PrP-CTF12/13 have a gel mobility of ~20–21 kDa, whereas the two unglycosylated forms migrate to ~12 kDa and ~13 kDa and are generated by cleavages at residues 154/156 and 162/167, respectively. The identification of PrP-CTF12/13 widens the heterogeneity of PK-resistant fragments of PrP associated with human prion diseases. It also raises questions concerning 1) the nature and mode of formation of these fragments, 2) how these fragments can be accommodated in current models of PrP<sub>C</sub> to PrP<sup>Sc</sup> conversion, and 3) the role that they may play in the pathogenesis of CJD.

**PK-resistant Fragments in Human Prion Diseases—**The discovery of PrP-CTF12/13 brings to a total of three groups of PrP fragments resistant to PK that are associated with human prion diseases (Fig. 7). The most common and best known is PrP<sub>27–30</sub> (16). It is the only PK-resistant, disease-associated species that has been proven to retain infectivity. It includes a large number of fragments of different size due to the highly ragged N terminus that spans from residues 78–103 (18). PrP<sub>27–30</sub> appears to have an intact C terminus identical to that of PrP<sup>P</sup> including the presence of the disulfide bridge (6–12). The site of cleavage of the full-length PrP<sup>Sc</sup> by PK and, therefore, the size of the PK-resistant PrP<sub>27–30</sub>, is influenced by the PrP genotype at codon 129 of the PrP gene, the location of a common methionine/valine (M/V) polymorphism (18). Our previous studies have shown that there are two major types of PrP<sub>27–30</sub> in human prion diseases. In PrP<sub>27–30</sub> type 1, the N terminus commonly starts at residue 82 but this type also includes several secondary species with the N terminus starting between residues 78 and 97. In contrast, the N terminus of PrP<sub>27–30</sub> type 2 commonly starts at residue 97 with secondary sites between residues 92 and 103 (18). Furthermore, PrP<sub>27–30</sub> types 1 and 2 co-distribute with distinct disease phenotypes (18, 32) and are conserved upon transmission to receptive animals (45).

The second family of PK-resistant PrP fragments includes much smaller species with relative molecular mass of 7–8 kDa (PrP<sub>7–8</sub>) (Fig. 7). PrP<sub>7–8</sub> has only been observed in subjects associated with mutations in the PrP gene linked to GSS, a group of familial prion diseases characterized by the presence of PrP amyloid deposits. PrP<sub>7–8</sub> is an internal fragment that varies in size according to the PrP gene mutation (23–26), but overall spans from residues 74–90 to residues 146–153 (25). PrP<sub>7–8</sub> is present in PK-untreated GSS brain preparations but increases in quantity following PK-treatment. Furthermore, it is expressed exclusively by the mutated allele (23–26). Therefore, it is likely that in vivo PrP<sub>7–8</sub> represents a PK-resistant region of PrP, possibly generated by an abnormal PrP processing pathway linked to GSS-causing mutations. The infectivity of PrP<sub>7–8</sub> has not been carefully assessed. However, a PrP synthetic peptide that spanned residues 89–143 and contained a GSS mutation accelerated or possibly triggered a GSS-like condition following inoculation to transgenic mice carrying the same GSS mutation but not to wild-type mice (46). Furthermore, before inoculation the synthetic PrP peptide had to be refolded in a β-structure-rich conformation, therefore mimicking the naturally occurring PrP<sub>7–8</sub>. These data suggest that PrP<sub>7–8</sub> can propagate the prion disease only when an appropriate PrP gene mutation is present in both donor and recipient, arguing that while PrP<sub>27–30</sub> can recruit wild type PrP, PrP<sub>7–8</sub> requires the presence of a GSS mutation.

PrP-CTF12/13, the third group of PK-resistant PrP fragments as reported in the present study, are different from PrP<sub>27–30</sub> not only in size but also because they do not show any effect of the PrP genotype at codon 129 on their PK cleavage site and correlation with the disease phenotype. They are
also quite different from PrP7–8 as for the size, the region of the PrP they embody and the lack of obligatory relation with PrP gene mutations (Fig. 7). Pertain to the above discussion on human prion diseases, it is of interest to note that a 16 kDa C-terminal domain spanning from PrP residues 115 to 217 (27) and a 7 kDa fragment derived from the extreme PrP C terminus (28) have been found in PK-treated preparations of scrapie-infected hamsters.

**Possible Modes of Formation of PrP-CTF12/13**—An interesting feature of PrP-CTF12/13 is that their N terminus matches the C terminus of PrP7–8 and the combination of these two fragments corresponds to PrP27–30 (Fig. 7). This finding raises the question as to whether upon PK treatment PrP-CTF12/13 are generated by two separate cleavages of the full-length PrPSc that would generate both PrP27–8 and PrP-CTF12/13. Alternatively, they may originate from another species of abnormal PrP that carries a PK-resistant core located closer to the C terminus than that of PrP27–30. Our failure to consistently detect PrP7–8 in preparations containing PrP-CTF12/13 makes the former possibility unlikely. Therefore, in vivo PrP-CTF12/13 may represent the C-terminal region of a subpopulation of PrPSc in which the PK-resistant core is displaced 64–76 residues toward the C terminus when compared with that of the PrPSc that generate PrP27–30. Since the detergent-insoluble fraction enriched in PK-resistant PrP contains both full-length and N-terminally truncated PrP forms (29), we reasoned that PrP-CTF12/13 might be the PK-resistant core of these truncated PrP forms while PrP27–30 is the PK-resistant core for the full-length forms. However, PrP-CTF12/13 could be recovered from both full-length and truncated PrP forms following PK- and PNGase treatments, although more appeared to be recovered from the truncated than from full-length forms. Therefore, generation of the PrP-CTF12/13 is not strictly related to the size of the PrPSc. If indeed PrP-CTF12/13 represent the C-terminal region of a subpopulation of PrPSc in which the PK-resistant core is displaced toward the C terminus, this “PrPSc” subpopulation must have a tertiary structure different from that of the PrPSc generating PrP27–30. This conclusion raises the question of how PrP-CTF12/13 fit in the current models of PrP27–30 formation. Structural NMR studies (47) have shown that the N-terminal region (residues 23–125) of human PrPSc is flexible and unstructured, while the C-terminal domain contains 3 α-helices (α-helix 1, residues 144–154; α-helix 2, residues 173–194; and α-helix 3, residues 200–228) and 2 short β-strands (β-strand 1, residues 128–131 and β-strand 2, residues 161–164) (Fig. 7). Although the tertiary structure of PrPSc is still unknown, a common model holds that the PK-resistant core of PrPSc corresponding to PrP27–30, is formed by conversion of the α-helix 1 to β structure, while the secondary and tertiary structures of the C terminus are preserved (48). The C-terminal end of the β structure domain is unknown but it is tentatively placed in the vicinity of residue 170, i.e. at the beginning of α-helix 2 (49, 50). The formation of PrP-CTF12/13 does not fit in this model. If the formation of a β structure followed by oligomerization, which protects the C terminus, is the mechanism that also provides PK resistance to PrP-CTF12/13, the newly formed β structure must start between residues 154–167, i.e. near the N terminus of the α-helix 2. This suggests that other PrP regions closer to the C terminus than that of PrP27–30 may trigger the formation of β structure. The limited available space between the N terminus of the putative β structure domain in PrP-CTF12/13 and the α-helix 2 raises the question of whether the PrP-CTF12/13 C-terminal region maintains the original structure or is also converted to β structure. Based on the evidence that the disulfide bridge joining α-helices 2 and 3 is present and is required for infectivity, the prevailing hypothesis is that the original globular structure of the PrP173–228 C-terminal domain is maintained in PrP27–30. However, a model in which the tertiary structure of PrP27–30 differs from that of PrPSc has also been proposed (49). Finally, PrP-CTF12/13 might derive from PrP through the activation of an alternative metabolic pathway. Increase in PK-resistant PrP has been recently demonstrated in cell culture when the proteasome activity is impaired (51).

**Role of PrP-CTF12/13 in the Pathogenesis of CJD**—It would certainly be of interest to know whether PrP-CTF12/13 are capable of propagating prion diseases and whether they are neurotoxic. One can speculate that if in vivo PrP-CTF12/13 are part of a subpopulation of PrPSc in which the β structure conformation is located in region immediately C-terminal of residues 154–167, this “PrPSc variant might be infectious. This possibility is supported by two sets of data obtained with PrP carrying various deletions, referred to as miniprions. One set showed that a redacted PrP (PrP106) lacking the 141–176 region can propagate the prion disease suggesting that this region is not required for infectivity (52). This finding is relevant because according to current models the PrP141–176 region is believed to have at least part of the β structure, which seems also to be largely absent in PrP-CTF12/13. The second set of experiments argues that miniprions lacking most of, or all, the C-terminal PrP region, which makes up the bulk of the PrP-CTF12/13, cannot transmit the prion disease, although they are highly toxic (53). Whether PrP-CTF12/13 are infectious and/or toxic in PK-treated PrP preparations as PrP27–30 remains to be determined. If they were infectious, they would further reduce the size of the PrPSc region required for infectivity.

In conclusion, the finding of the PrP-CTF12/13 present in sCJD-affected brains paves the way to further studies of nature, formation, and pathogenic role of prions. The data reported here raise the possibility that a novel subpopulation of PrPSc carrying a conformation different from that of the classical PrPSc is present in prion diseases.

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