Identifying Key Components of the PrPC-PrPSc Replicative Interface

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In prion disease, direct interaction between the cellular prion protein (PrPC) and its misfolded disease-associated conformer PrPSc is a crucial, although poorly understood step promoting the formation of nascent PrPSc and prion infectivity. Recently, we hypothesized that three regions of PrP (corresponding to amino acid residues 23–33, 98–110, and 136–158) interacting specifically and robustly with PrPSc likely represent peptidic components of one flank of the prion replicative interface. In this study, we created epitope-tagged mouse PrPC molecules in which the PrP sequences 23–33, 98–110, and 136–158 were modified. These novel PrP molecules were individually expressed in the prion-infected neuroblastoma cell line (ScN2a) and the conversion of each mutated mouse PrPC substrate to PrPSc compared with that of the epitope-tagged wild-type mouse PrPC. Mutations within PrP 98–110, substituting all 4 wild-type lysine residues with alanine residues, prevented conversion to PrPSc. Furthermore, when residues within PrP 136–140 were collectively scrambled, changed to alanines, or amino acids at positions 136, 137, and 139 individually replaced by alanine, conversion to PrPSc was similarly halted. However, other PrP molecules containing mutations within regions 23–33 and 101–104 were able to readily convert to PrPSc. These results suggest that PrP sequence comprising residues 98–110 and 136–140 not only participates in the specific binding interaction between PrPC and PrPSc, but also in the process leading to conversion of PrPSc-sequestered PrPC into its disease-associated form.

Prion diseases such as Creutzfeldt-Jakob disease (CJD) in human, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in cervids, and scrapie in sheep, are a group of closely related fatal neurodegenerative conditions characterized by the change in the conformation of normal cellular prion protein (PrPC) into a pathogenic conformer (PrPSc) (1, 2). According to the protein-only hypothesis, PrPSc is the essential causative agent of prion disease and transmission (1–6). Once generated, PrPSc acts as a conformational template promoting, perhaps with the aid of an additional cellular cofactor(s) (7–10), the conversion of PrPC into nascent PrPSc (11, 12). Despite the fact that PrPC and PrPSc have the same amino acid sequence, they differ from each other in several aspects. Contrary to PrPC, PrPSc is insoluble with non-ionic detergents, partially resistant to proteinase K (PK) digestion, and presents an increased content of β-sheet structure (13–16). The molecular mechanisms involved in the process of conversion of PrPC to PrPSc are still poorly understood; however it is evident that direct interaction between the cellular and the abnormal forms of PrP is a crucial step in the pathway toward formation of additional PrPSc from PrPC (12).

It will be very important to gain more knowledge about PrPC-PrPSc interaction and conversion at the molecular level in the quest to develop disease models that can accommodate important, but poorly understood features of prions, such as the species barrier to prion transmission and the prion strain phenomenon, as well as strategies to create new therapeutic drugs. Using a large panel of recombinant antibody molecules displaying overlapping PrP grafts, we recently carried out a comprehensive and systematic molecular analysis of regions of PrPC that can specifically interact with disease-associated misfolded PrP but not with endogenous PrPC. This study identified three distinct regions of PrPC that bind tightly to PrPSc represented by PrP sequences 23–33, 98–110, and 136–158 (17, 18). Additional investigations indicated that the reactivity of PrP regions 23–33 and 98–110 with PrPSc is in large part conferred by positively charged residues in these sequences. Within the 136–158 PrPSc binding motif, peptide sequences composed of amino acids 136–140 and 149–158 were found to be of particular importance for the binding interaction with PrPSc. To further study the role of PrP sequences 23–33, 98–110, and 136–158 in PrPSc recognition and prion replication, a number of different epitope-tagged mouse PrPSc molecules were created in which these particular regions were modified. Within PrP sequences...
23–33 and 98–110, mutants were generated in which positively charged residues were changed to alanine. Furthermore, within PrP 136–158, mutants were prepared in which the native sequence within segments 136–140 and 149–158 was altered. Each of these novel full-length PrP molecules was individually expressed in the prion-infected neuroblastoma cell line (ScN2a). Conversion of the various mutated mouse PrPSc substrates to PrPSc were measured and compared with the conversion of equivalently expressed epitope tagged wild-type (wt) mouse PrPSc. Here we report that PrP mutant in which the lysine residues within the PrP sequence 98–110 were substituted with alanine residues, and PrP mutants in which the original residues in position 136–140 (RPMIH) were changed to alanine residues or scrambled (HIMPR), did not convert to PrPSc. When residues in position 136–140 were individually substituted with alanine, mutants 136 (Arg to Ala), 137 (Pro to Ala), and 139 (Ile to Ala) also did not convert to PrPSc.

EXPERIMENTAL PROCEDURES

Generation of PrP Mutants—The epitope-tagged mouse PrP mutants coding sequences were generated by overlap extension PCR and cloned into the pCB6 vector. The template for the PCR reactions was the vector pCB6 containing the wild-type mouse PrP sequence with the 3F4 epitope. A total of eleven mutants were generated. One in which the lysine and arginine residues present within the PrP sequence 23–33 were substituted with alanine residues. Two mutants within the region 101–110 were generated. One in which lysine residues in positions 101–104 were exchanged with alanines, and one in which all the lysines in this region (101, 104, 106, and 110) were substituted with alanines. For this last mutant, since the epitope for the antibody 3F4 was altered (19), the FLAG peptide (DYKDDDDK) was added in the loop between the β2 and the α2 structures, residues 168 (Gln) and 169 (Tyr). 136–140 mutants were produced in which the original residues (RPMIH) were changed to alanine or scrambled (HIMPR). Single mutants 136–140 in which the original residues were changed one by one to alanine, were also created. A 149–158 scrambled (YPYYRREYMN) mutant was also generated. All the PrP mutants we created are listed in Table 1. The nucleotide sequences of all constructs were confirmed by sequencing. The sequences of the oligonucleotide PCR pairs employed to create the original residues were changed one by one to alanine, were also created. Two mutants within the region 101–110 were generated. One in which lysine residues in positions 101–104 were exchanged with alanines, and one in which all the lysines in this region (101, 104, 106, and 110) were substituted with alanines. For this last mutant, since the epitope for the antibody 3F4 was altered (19), the FLAG peptide (DYKD- DDDK) was added in the loop between the β2 and the α2 structures, residues 168 (Gln) and 169 (Tyr). 136–140 mutants were produced in which the original residues (RPMIH) were changed to alanine or scrambled (HIMPR). Single mutants 136–140 in which the original residues were changed one by one to alanine, were also created. A 149–158 scrambled (YPYYRREYMN) mutant was also generated. All the PrP mutants we created are listed in Table 1. The nucleotide sequences of all constructs were confirmed by sequencing. The sequences of the oligonucleotide PCR pairs employed to generate individual PrP mutants are provided as supplemental information.

ScN2a Cell Line Transfection—The expression of mouse PrP mutant proteins was performed by transient transfection of ScN2a cells in OPTI-MEM medium (Invitrogen, Carlsbad, CA) with the pCB6+ vector containing the specific nucleotide sequences for the PrP mutants. In general, 8 μg of plasmid DNA and 20 μl of Lipofectamine 2000 ( Invitrogen) were used to transfect 2 × 106 cells in 60-mm dishes. Transfected cells were incubated at 37 °C in a humidified atmosphere of 5% CO2. Cells were harvested 48h post-transfection, lysed in 500 μl of lysis buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, and 0.5% sodium deoxycholate), and cleared by centrifugation at 1000 × g for 1 min. Total protein concentration was measured on the cleared lysate by bicinchoninic acid assay (BCA, Pierce). 700 μg of total protein was digested with proteinase K (1:50 ratio, PK:total protein) for 1 h at 37 °C. Proteolytic digestion was stopped by the addition of phenylmethylsulfonyl fluoride to a final concentration of 2 mM. The digested protein and 100 μg of undigested protein were individually precipitated with chloroform-methanol. The protein pellets were resuspended in 20 μl of loading buffer (150 mM Tris-HCl, pH 6.8, 6% SDS, 0.3% bromphenol blue, and 30% glycerol) and heated at 100 °C for 5 min.

Western Blots—Protein samples were prepared as described above, run on 12% SDS-PAGE gels, and transferred onto polyvinylidene difluoride membranes. Membranes were blocked with 5% (w/v) non-fat dry milk in TBS containing 0.1% Tween 20 (TBST) for 1 h at room temperature and blotted PrP was detected with IgG D13 or IgG D18 (total PrP) and IgG 3F4 or anti-FLAG antibody (transfected PrP) at 1 μg/ml. After five washes in TBST, blotted PrP was detected by incubation for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-human or anti-mouse IgGs (Jackson Immuno, West Grove, PA) diluted 1:10,000 in blocking buffer. Membranes were then washed five times in TBST and developed with enhanced pico or femto chemiluminescence reagent (ECL, Pierce) onto film.

Flow Cytometry—for surface staining of PrP mutants, transfected N2a cells were washed twice in FACS buffer. One million cells were incubated with antibody 3F4 (dilution 1:200, 5 μg/ml) or mouse anti-V5 (isotype, dilution 1:200, 5 μg/ml) for 45 min at 4 °C. Cells were then washed three times in FACS buffer and incubated for 30 min at 4 °C with R-Phycocerythrin-conjugated goat anti-mouse IgG (Jackson Immuno, West Grove, PA) diluted 1:200 in FACS buffer. Cells were then washed three times in FACS buffer, fixed with freshly prepared 4% (v/v) paraformaldehyde and analyzed using a FACS Canto (Becton Dickinson). The FACS data were then processed using the software FlowJo (TreeStar Inc.).

Immunoprecipitation—N2a cells were transfected as described above, harvested 48 h post-transfection, lysed in 500 μl of TBS containing 1% Triton X-100 and cleared by centrifugation at 1000 × g for 1 min. Total protein concentration was measured on the cleared lysate by bicinchoninic acid assay (BCA, Pierce). 100 μg of total protein preparations were immunoprecipitated with 10 μg/ml of IgG 6H4 or anti-V5 antibody (control) in a final volume of 500 μl for 2 h at room temperature. Tosyl-activated paramagnetic beads (Dynal/Invitrogen) coupled to polyclonal goat anti-mouse IgG F(ab’)2 were added to the antibody-cell homogenate mixture and incubated overnight at 4 °C. Beads were then washed nine times with TBS/ Triton X-100 1% and once with TBS. Pelleted beads were resuspended in 20 μl of loading buffer (150 mM Tris-HCl, pH 6.8, 6% SDS, 0.3% bromphenol blue, 30% glycerol), and heated at 100 °C for 5 min. Protein samples were then subjected to Western blot as described above.

Nomenclature—The numbering of amino acid residues corresponds to that of Syrian hamster PrP throughout.

RESULTS

Generation of Mouse PrP Mutants—Recently, we have identified three regions of the prion protein that specifically interact with PrPSc. These regions are represented by the amino acid sequences spanning between PrP residues 23–27, 98–110, and
We also showed, by mutating specific residues of these sequences, that the PrPSc-specific reactivity of regions 23–27 and 98–110 was conferred by the positive charged residues present in these sequences, whereas the binding to PrPSc of the peptide 136–158 was determined by two segments, 136–140 and 149–158.

Based on our previous biochemical observations, we now decided to determine empirically in a cell-based model,
whether mutation of key residues within PrPC can abolish or inhibit prion replication. Thus, a total number of eleven PrP mutant proteins was created. The complete list of these mutants is reported in Table 1.

Residues within PrP Sequences 98–110 and 136–140 Are Crucial for Prion Conversion—The PrP mutants we generated were individually evaluated by transient transfection in ScN2a cells for their ability to convert to PrPSc. In order to distinguish between the endogenous PrP and the proteins expressed through transfection, the PrP mutant constructs, as well as wt-PrP construct, were engineered with the hamster-specific 3F4 monoclonal antibody epitope (methionine substitutions at amino acid residues 108 and 111), known not to interfere with the conversion process (20). At 48 h post-transfection, cells were harvested, lysed, and analyzed by Western blot for the presence of PK-resistant PrPSc. The same amount of total protein was used for each sample. To ensure relatively equivalent loading of each PrP mutant, total PrP (endogenous plus recombinantly expressed), was detected by antibody D13 (Fig. 1, panel A) or antibody D18 (Fig. 1, panels C and E). As shown in Fig. 1, panel B, the mutant 23–27, in which the lysine residues in positions 23, 24, 27, and the arginine residue in position 25 were changed to alanine, converted to PK-resistant PrPSc as efficiently as wt-PrP. In contrast, the mutants in which the original residues in position 136–140 were changed to alanine or scrambled, were no longer able to convert to PrPSc. For the region 98–110 two mutants were generated; one in which only lysine residues in positions 101 and 104 were changed to alanine and another one in which all the lysine residues in this region, (101/104/106/110), were changed to alanine residues. For this last mutant, in which the epitope for 3F4 was altered, a FLAG peptide was inserted between PrP structures β2 and α2 and the transfected protein detected with an anti-FLAG antibody. The substitution of only two lysine residues in this region was not enough to prevent conversion of PrPSc to PrPSc (Fig. 1, panel D); however, when all the lysine residues were modified, conversion was impaired (Fig. 1, panel F). These results clearly indicate that regions 98–110 and 136–140 are important not only for PrP–PrPSc interaction, as we previously described, but also for the mechanism of conversion, at least in the cell model we employed. The analysis of the second PrPSc binding motif within the PrP region 136–158, represented by the sequence spanning between residues 149–158, did not allow for definitive conclusions. When the mutant for this region (149–158 scrambled) was subjected to Western blot, it was immediately apparent that the biochemical profile of this protein was different from the wild-type or the other PrP mutants (data not shown). This region forms part of the helix-1 of PrP (residues 144–154) and as others already reported (21) is of particular importance for the post-translational modifications and cellular trafficking of PrP. In that study, the authors showed in a N2a cell model that deletion of the helix-1 resulted in the lack of complex glycosylation, generation of high mannos glycoform of PrP as well as prevention of the GPI anchor attachment and secretion of the protein into the cell culture medium. Based on this knowledge, we decided to carry out no further analysis of this mutant. Furthermore, a detailed analysis of the role that individual charged amino acids within the helix-1 play in the conversion of PrPC to PrPSc has been reported, identifying key residues critical for prion conversion (22).

Key Residues of the PrP Region 136–140—As described above, when the original PrP residues 136–140 were changed to alanines or scrambled, PrPSc was no longer converted to PrPSc. To identify if any individual amino acid in this region plays a crucial role in the PrPSc–PrPSc conversion, five novel PrP constructs were generated in which the original residues 136–140 were changed one by one to alanine. The new constructs were tested in ScN2a cells as described above. As shown in Fig. 2, panel B, when the residue in position 136 was changed from the original arginine to an alanine, conversion of PrPSc to PrPSc was dramatically reduced, whereas, when residues in position 137 and 139 were changed individually from the original proline and isoleucine to alanine, conversion was completely abolished. In contrast, when residues 138 and 140 (methionine and histidine) were modified to alanine, conversion was efficiently maintained. In our previous study, in which we biochemically identified the regions of PrPSc–PrPSc conversion, we also mapped the residues in positions 136–140 important for the interaction with PrPSc one by one (18). Partially in contrast with the results we are reporting here, the earlier study found a reduced reactivity of this region of PrP with PrPSc when residues 136, 138, and 140, but not residues 137 and 139, were individually substituted with alanine. To exclude any possible experimental error, the constructs encoding the 136–140 PrP-grafted antibodies we used in our previous study were resequenced, retransfected into 293 cells, and the expressed IgGs repurified. The binding experiment was repeated with identical results (data not shown). The imperfect agreement between these cellular and biochemical data sets may derive from the 2-step nature of PrPSc formation; initial contact between the PrPSc and PrPSc, followed by conversion of PrPSc to PrPSc (23, 24). In this
model, contact between the 2 PrP conformers need not necessarily lead to conformational conversion and formation of nascent PrP\textsubscript{Sc}. Thus, even if residues in positions 137 and 139 are changed to alanine and the interaction between PrP\textsuperscript{C} and PrP\textsubscript{Sc} is maintained, conversion may not proceed. Our data is in agreement with results reported by Horiuchi et al. (25). In this study, the authors provided evidence that the sequence specificity of PrP\textsubscript{Sc} formation in their model was determined more by the conversion to protease resistance than by the initial binding step.

**136–140 Mutants Are Expressed on the Cell Surface, and the Helix-1 Is Conserved**—The PrP region 136–140 identified as being involved in PrP\textsuperscript{C} interaction with PrP\textsubscript{Sc} and conversion is localized immediately before the helix-1 of the protein (residues 144–154). As noted above, this region of PrP regulates its cellular trafficking and post-translational modifications. To exclude that the lack of conversion observed for some of the 136–140 mutants was due to an alteration of the structure of the helix-1 and of the consequent transport and folding of the protein, we performed the following experiments. First, a cell surface staining and flow cytometric (FACS) analysis was performed to evaluate the presence of the mutant proteins as well as wt-PrP on the cell membrane. Even though, the subcellular site for the formation of PrP\textsubscript{Sc} is unknown, there is supporting evidence that at least the initial interaction between PrP\textsuperscript{C} and PrP\textsubscript{Sc} takes place on the cell surface (12, 26–31). If the mutants generated were not present on the cell membrane, this would in all probability preclude the conversion process. The 136–140

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**FIGURE 3.** 136–140/PrP mutants that do not convert into PrP\textsubscript{Sc} and PrP with the FLAG peptide between β2 and α2, are present on the cells surface of N2a cells. Single cell suspensions of N2a cells transiently transfected with 3F4-tagged wt-PrP and 136–140/PrP mutants were analyzed by flow cytometry for presence of PrP on the cell surface. Profiles shown and the histogram indicate that all the 136–140 mutants are present on the cell surface at similar levels of the wt-protein (panel A). Single cell suspensions of N2a cells transiently transfected with 3F4-tagged wt- and 3F4 + FLAG-tagged wt-PrP were analyzed by flow cytometry for the presence of PrP on the cell surface. Profiles shown and the histogram indicate the presence of PrP on the cell surface for both samples (panel B).
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FIGURE 4. 136–140 single PrP mutants and the 136–140 scrambled PrP mutant show normal reactivity to the PrP-specific antibody 6H4, whereas the 136–140A/PrP mutant, in which the original residues are all changed to alanine, shows reduced reactivity, possibly indicating an alteration of the helix-1 of PrP (37). PK-treated and not-treatment lysates prepared from ScN2a cells transiently transfected with 3F4-tagged wt-PrP and 136–140/PrP mutants were incubated with the antibody 6H4, recognizing the helix 1 of the prion protein, residues 144–154. The complex 6H4-PrP was captured onto paramagnetic beads coupled to an anti-mouse IgG reagent, and any precipitated wt-PrP or PrP mutants were detected via Western blot by the PrP-specific antibody D13 (panels B and E/2) or 3F4 (panels D and F/2). Total and transfected PrP were as well detected by antibody D13 (panels A and E/1) and antibody 3F4 (panels C–F/1), employing Western blots. The reactivity of antibody 6H4 was reduced only for the 136–140 A PrP mutant (panel D, lanes 2, panel F/2 lane 3), whereas regular reactivity for the other 136–140/PrP mutants (panel D, lanes 3–7, panel F/2, lane 4) is shown.

The PrP Protein Carrying the FLAG Peptide Is Expressed on the Cell Surface—To exclude that insertion of the FLAG peptide between the β2 and the α2 structures of PrP could interfere with the regular transport of PrP and its exposure on the cell surface, we performed a FACS analysis in which wt-PrP and wt-PrP carrying the FLAG peptide were stained with the antibody 3F4. As shown in Fig. 3, panel B, the protein with the FLAG peptide is expressed on the cell surface at a similar level as the protein not containing such a peptide. In this experiment, we did not include the staining of the 101–110 mutant, since the epitope for 3F4 is altered, and the antibody is no longer able to recognize it (see Fig. 1, panel D).

The conversion of the cellular prion protein into its pathogenic and infectious form, PrPSc, represents the central event characterizing transmissible spongiform encephalopathies of humans and animals. The interaction between PrPC and PrPSc appears to be a key step in the process leading to the final conversion into PrPSc. However, the molecular events leading to the formation of PrPSc, i.e. how PrPC and PrPSc interact with each other, how PrPC is modified to PrPSc and where PrPSc is formed in the cells, are still open questions.

In this study we focused on three regions of PrPC known to bind to PrPSc. These regions are represented by the amino acid sequences spanning residues 23–33, 98–110, and 136–158 (18). Initially, we generated PrPC mutants for these regions and expressed them in ScN2a cells to study the potential for the mutants to be converted into PK-resistant PrPSc. The first screening of the mutants showed that conversion of PrPC into PrPSc was abolished when the four lysine residues present in the PrP sequence 98–110 were all substituted with alanines. Conversely, mutations introduced in the region 23–33, in which positively charged amino acids were replaced with alanines, did not have any effect upon the conversion into PrPSc. This PrPC mutant could be converted to PK-resistant PrPSc equally well as wild-type PrP. Conversion was also still possible for another mutant of the 98–110 region in which only two lysine residues (positions 101 and 104) were changed to alanine. When the original amino acids in position 136–140 were collectively changed to alanine residues or scrambled, once again conversion to PrPSc was prevented. When a more detailed analysis for the region 136–140 was carried out, residues 136, 137, and 139

mutants and wt-PrP were individually transfected in N2a cells as described above. At 48h post-transfection, cells were harvested, stained with antibody 3F4, and analyzed by FACS. For the isotype control, wt-PrP was also stained with an anti-V5 antibody. As determined by FACS analysis (Fig. 3, panel A), all the 136–140 mutants were present on the cell surface at similar levels to that of the wt protein, the ones that were still able to convert to PrPSc (138A and 140A) as well as the ones that did not convert (136–140A, 136–140 scramble, 136A, 137A, and 139A). Next, we wanted to verify that the helix-1 in the 136–140 mutants was maintained. Therefore, immunoprecipitation experiments were performed using the antibody 6H4 that specifically recognizes helix-1 of PrP, followed by Western blot detection of total PrP (using antibody D13) and transfected PrP (using antibody 3F4). The results showed that only for the mutant 136–140A, the reactivity of the capture antibody 6H4 was significantly reduced, whereas for all the other mutants it was maintained (Fig. 4, panels D and F/2). These results might indicate that the helix-1 is partially altered in the mutant 136–140A. Nevertheless, this eventual modification does not seem to interfere with the regular trafficking and biochemical profile of this PrP mutant. As a control for the immunoprecipitation experiment, antibody D13 was employed (Fig. 4, panels B and E/2).
individually appeared to be critical for the conversion process. Residues 136 and 137, which consist of an arginine and a proline, respectively, appear to be conserved among several species (32), whereas residue 139 (isoleucine) has been linked to the existence of a species prion transmission barrier between mouse and hamster, wherein a methionine is present in this position (20, 33, 34).

The two-step model of PrPSc formation, in which binding between PrPC and PrPSc and subsequent conversion of PrPC to PrPSc are considered two distinct events, has provided some insight into the details of the prion conversion mechanism (23–25). In the context of our results, the different mutations introduced in PrP that prevent prion conversion, could potentially interfere at different levels in the process of PrPSc formation; that is interaction of the two different conformers and/or conversion of PrPC to PrPSc after the PrPC–PrPSc complex is formed. Based on the findings reported here and on the PrP peptide–PrPSc in vitro binding assay of our earlier studies (18), we speculate it is likely that the mutations introduced in the 101–110 region of PrP impede the interaction between PrPC–PrPSc rather than the following conversion step. Indeed, the binding study showed that when two or more lysines within this region were changed to alanine, the binding of the peptide 101–110 to PrPSc was completely lost. That conversion of the 101–104 mutant, in which alanine residues substitute for lysine residues at position 101 and 104, proceeds in the cellular model, likely indicates that interaction between full-length PrPC and PrPSc can be maintained by regions of PrPC outside 101 and 104. However, the additional loss of lysine residues at positions 106 and 110 appears sufficient to perturb the PrPC–PrPSc interaction. These results are consistent with studies in which deletion of the N-terminal segment of the protein, residues 34–123, diminished the conversion efficiency and altered the conformation of PrPSc produced, possibly by influencing the interactions required for the conversion process (35, 36). This effect was already detected when the deletion involved only residues 34–94, but was more pronounced when it was extended to residue 113, indicating an individual role in the observed effects of residues spanning positions 94–113.

Examining PrP region 136–140, we hypothesize that the individual mutations at positions 137 and 139, which impair PrPSc formation, influence more the conversion process itself rather than the interaction between PrPC and PrPSc. Our previous PrP peptide–PrPSc binding study (18) showed indeed that when residues 137 and 139 were individually mutated to alanine, the binding of the sequence 136–158 to PrPSc was maintained. In the context of the present study, these findings suggest that even though binding of PrPC to PrPSc can still occur when the protein is mutated in positions 137 and 139, the subsequent conversion to PrPSc is impaired. Thus, residues 137 and 139 could potentially participate directly in the conversion of PrPSc–complexed PrPC, or, alternatively, play a key role in binding to auxiliary factors critical to conformational conversion of sequestered PrPC.

In contrast, the dramatic reduction in PrPSc–conversion observed when residue 136 was mutated likely results from its key role in promoting interaction between PrPC and PrPSc. The previous binding study (18) showed that when this residue was changed to alanine, the interaction of sequence 136–158 with PrPSc was significantly reduced. Our findings suggest that residues in positions 138 and 140 do not play an important role in the conversion of PrPC to PrPSc. These data contrast with the importance of these residues in promoting recognition of PrPSc by a 136–158 PrP-grafted antibody (18). This discrepancy may result from the ability of other regions of the full-length PrPC molecule to stabilize the PrPSc interaction or could arise from differences between the conformation adopted by the PrP 136–158 sequence within PrPC or within the antibody.

In summary, this study identifies two regions of PrPC that are critical for prion conversion in ScN2a cells. To further explore these findings, we intend to generate transgenic mouse models carrying the mutations we identify as critical for prion conversion. Importantly, these two regions may represent suitable molecular targets for the generation of small molecules or peptide inhibitors of prion replication.

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