Prion protein (PrP) is synthesized at the membrane of the endoplasmic reticulum (ER) in three different topological forms as follows: a fully translocated one ("secPrP") and two with opposite orientations in the membrane (NtmPrP and CtmPrP). We asked whether other signal sequences exist in the PrP, other than the N-terminal signal sequence, that contribute to its topological diversity. In vitro translocation assays showed that PrP lacking its N-terminal signal sequence could still translocate into ER microsomes, although at reduced efficiency. Deletion of each of the two hydrophobic regions in PrP revealed that the C-terminally located hydrophobic region (TM2) can function as second signal sequence in PrP. Translocation mediated by the TM2 alone can occur post-translationally and yields mainly CtmPrP, which is implicated in some forms of neurodegeneration in prion diseases. We conclude that, in vitro, PrP can insert into ER membranes co- and post-translationally and can use two different signal sequences. We propose that the unusually complex topology of PrP results from the differential utilization of two signal sequences in PrP.

Prion diseases such as scrapie in sheep, bovine spongiform encephalopathy in cattle, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler-Scheinker disease in man are fatal neurodegenerative diseases that can have an infectious, sporadic, or familial origin. They are characterized by the intracellular accumulation of PrPSc, an abnormally folded, aggregated version of the normal, host-encoded cellular prion protein, PrPc (1-3). The molecular mechanisms leading to the structural changes in PrP are still unclear.

At steady state in normal brain the 35-kDa glycoprotein PrP is anchored in the plasma membrane by a C-terminal glycosylphosphatidylinositol (GPI) moiety exposing the polypeptide to the extracellular face of the plasma membrane (4, 5). During early stages of biogenesis at the ER membrane, PrP can adopt any one of the three topological forms (4, 5). During this period, PrP can target the protein to the ER membrane and mediate membrane insertion. We deleted the SS alone or in combination with the other hydrophobic regions and tested the resulting components at the translocation site mediate insertion of CtmPrP as opposed to secPrP and NtmPrP (7).

In vitro translocation assays the three topological PrP forms were produced in different amounts, with the secPrP and NtmPrP forms being about equally abundant (40–50%), and the CtmPrP form representing about 10% of translocated PrP (5). Disease-associated mutants within PrP affected the proportion of these three topological forms in vitro but also in transgenic animals expressing these mutant forms of PrP (5). These findings suggest that early stages in the biogenesis of PrP may be important for understanding the generation of some variant forms of PrP that lead to neurodegeneration.

Formation of the different topological forms of PrP could involve different signal sequences in PrP. The N-terminal SS obviously mediates translocation of the N-terminal region in secPrP and NtmPrP. For generating CtmPrP the SS may not be engaged, and the TM1 or TM2 may then function as internal topogenic sequences. To test this hypothesis, we analyzed whether in the absence of SS other hydrophobic regions in PrP can target the protein to the ER membrane and mediate membrane insertion. We deleted the SS alone or in combination with the other hydrophobic regions and tested the resulting PrP mutants for membrane insertion and translocation in a cell-free assay.

We have found that PrP contains, in addition to the SS, a second potential targeting signal at its C terminus, TM2. Targeting by TM2 occurs post-translationally and leads to the preferential formation of the CtmPrP form.

**EXPERIMENTAL PROCEDURES**

**Materials**—General chemicals were from Merck or Sigma. Restriction enzymes were from Roche Molecular Biochemicals. [°35S]methionine was from Amersham Pharmacia Biotech; pGEM-3Z was from Promega; and protein A-Sepharose was from Amersham Pharmacia Biotech. The monoclonal antisera 3F4, which specifically recognizes amino acid residues 108–111 in PrP (8), was from Senetek PLC. The polyclonal antisera directed against preprolactin was made against the peptide encoding amino acid residues 68–80 of preprolactin. The monoclonal antibody 3E5 recognizes residues 51–89 in PrP (9).

**Plasmids and Transcription**—The plasmid pGEM-3ZK-PrP3F4, codon for mouse PrP tagged with the epitope for the monoclonal antibody 3F4, under the control of the SP6 promoter was generated by inserting the AadII/SacI fragment harboring the PrP-coding region derived from pUC-PrP (10) into the SnaI/SacI sites of pGEM-3Z. The 3F4 epitope

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The preprolactin cDNA was linearized with in vitro transcription using the pGEM-3Z vector and SP6 RNA polymerase. The mRNA encoding preprolactin was synthesized by using the ExSite™ polymerase chain reaction-based site-directed mutation to remove the PrP stop codon. The mRNA encoding preprolactin was used for translation in the presence of a double-stranded oligonucleotide encompassing the deletion.

The deletion of the TM1 region (codons 112–121) was generated by using the ExSite™ polymerase chain reaction-based site-directed mutagenesis kit (Stratagene). Primers and reaction conditions were selected as recommended in the manufacturers' instructions.

By using pGEM-3ZK-PrP3F4, codons 3–22 were removed to yield pGEM-3ZK-PrP3F4-ΔSS. This was achieved by cleavage of pGEM-3ZK-PrP3F4 with BamHI and SgrAI and subsequent re-ligation in the presence of the relevant plasmids were amplified by polymerase chain reaction using the ExSite™ polymerase chain reaction-based site-directed mutagenesis kit (Stratagene) yielding plasmid pGEM-3ZK-PrP3F4-ΔSS-TM1. All plasmids were amplified in Escherichia coli and isolated with the QiAprep DNA purification kit (Machery Nagel). The complete PrP-coding regions of all constructs were checked by DNA sequencing.

To obtain mRNAs encoding the respective PrP chains, the coding regions of the relevant plasmids were amplified by polymerase chain reaction using Pfu DNA polymerase (Stratagene) and transcribed with SP6 RNA polymerase (11, 12). To express PrP mutants with a deleted TM2 region, a 3′ primer encoding an artificial stop codon at codon 231 was used. In all other cases, the 3′ primer used encoded the endogenous PrP stop codon. The mRNA encoding preprolactin was synthesized by in vitro transcription using the pGEM-3Z vector and SP6 RNA polymerase. The preprolactin cDNA was linearized with EcoRI.

Translation—mRNAs were translated *in vitro* in 10 μl of nuclelease-treated reticulocyte lysate (Promega) in the presence of microsomal membranes (RM) (13) prepared from dog pancreas and [35S]methionine as described previously (14). Translations were incubated at 32 °C for 60 min. Translation reactions that were not incubated with proteinase K were centrifuged at 27,000 g for 15 min at 4 °C. Alternatively, protease accessibility assays were performed as described below. The pelleted rough microsomes were solubilized in Tris-HCl (100 mM, pH 7.5) containing 1% SDS and then processed for immunoprecipitation (see below).

**Assay for Post-translational Translocation**—mRNAs were translated *in vitro* in 10 μl of nuclelease-treated reticulocyte lysate at 32 °C for 30 min. Puromycin (final concentration 1.25 mM) was added, and the reaction was further incubated for 15 min at 32 °C. Where indicated, a further incubation with apyrase (Sigma, final concentration 50 milliunits/μl) was performed for 10 min at 32 °C. To test for post-translational translocation activity, RM were added, and the incubation was continued for 15 min. When a cotranslational assay was performed in parallel to the post-translational assay, translation was also carried out for 30 min at 32 °C. The resulting translocation products were analyzed by protease accessibility assays followed by immunoprecipitation analysis with the monoclonal antibody described below.

**Protease Accessibility Assays**—When protease accessibility of translocation products was tested, proteinase K (500 μg/ml) and Triton X-100 (1%, w/v) were added as indicated. After incubation for 60 min on ice, phenylmethylsulfonyl fluoride was added to 1 mg/ml, and membranes were further incubated on ice for 5 min prior to analysis by immunoprecipitation as described below.

After quantification of the PhosphorImager results, the numbers obtained for each of the three protein fragments representing the three topological forms of PrP were corrected for methionine content and then normalized to the amount of precursor produced in the absence of RM.

**Immunoprecipitation and Endo H Treatment**—For immunoprecipitation, proteins were denatured in 78 μl of Tris-HCl (100 mM, pH 7.5) containing 1% SDS for 5 min at 95 °C. The proteins were then solubilized in 500 μl of buffer containing 20 mM HepsKOH, pH 7.6, 100 mM NaCl, 5 mM MgCl2, 0.5% (w/v) Triton X-100, 0.2 mg/ml phenylmethylsulfonyl fluoride, and 10 μg/ml chymostatin, leupeptin, aprotinin, and pepstatin A. A precleaving step was performed by incubating the samples for 1 h at 4 °C with 30 μl of protein A-Sepharose (Amersham Pharmacia Biotech) equilibrated in IP buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2 mM EDTA, 0.2% (w/v) Triton X-100). After removal of the protein A-Sepharose beads by centrifugation, the supernatants were supplemented with the relevant antibodies and incubated overnight at 4 °C. Antigen-antibody complexes were adsorbed to protein A-Sepharose and recovered by centrifugation. The Sepharose beads were washed twice with 1 ml of IP buffer, twice with 1 ml of IP buffer containing 0.25 M NaCl, and twice with 1 ml of 10 mM Tris-HCl, pH 7.5 (15). For Endo H treatment, washed Sepharose beads were resuspended in 50 μl of 50 mM sodium citrate, pH 5.5, supplemented with 2 μl (2000 units) of Endo H (New England Biolabs). After incubation for 1 h at 37 °C, the beads were washed with 1 ml of 10 mM Tris-HCl, pH 7.5. The washed protein A-Sepharose beads were resuspended in 40 μl of SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 5 mM EDTA, 2% 2-mercaptoethanol, 5% glycercol and 2% SDS), incubated for 10 min at 95 °C, and analyzed on 13.5% acrylamide gels (16). [35S]Methionine-labeled proteins were visualized by using a Fuji PhosphorImager BAS1000 and quantified with Fuji MacBas Ver 2.0 software.

**RESULTS**

**Membrane Insertion of PrP Lacking Its Signal Sequence (PrP-ΔSS)**—To investigate whether there are other ER targeting signals in PrP in addition to the N-terminal SS, we deleted amino acids 3–22 comprising essentially the SS. Membrane insertion of the resulting PrP-ΔSS (outlined in Fig. 1A) was then studied using an *in vitro* translation system (reticulocyte lysate) supplemented with pancreatic, ER-derived rough microsomes (RM). To determine the topologies of the resulting translocation products, nontranslocated portions of the proteins were digested by the addition of protease. The fragments remaining after proteolysis were identified by immunoprecipitation using antibodies to two PrP epitopes. Proteins were then separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and [35S]-labeled proteins visualized by phosphorimaging.

Translation of wild-type PrP in the absence of RM led to the formation of one major band (~27 kDa) representing the uncleaved precursor form of PrP (pPrP) (Fig. 1B, lane 1). In the presence of RM the SS of pPrP was cleaved and glycosylation of PrP at both N-glycosylation sites occurred, resulting in the formation of PrP with two glycans (PrP-2g) (Fig. 1B, lane 2), as has been demonstrated previously (5). Proteolysis and Endo H treatment of translocation reactions confirmed the production of three different topological forms characterized by the protection of the glycosylated, full-length PrP (~30 kDa), the glycosylated Ctm fragment (CtmPrP), and the unglycosylated Ntm fragment (NtmPrP) (Fig. 1B, lane 3) schematically depicted in Fig. 1D. Endo H treatment confirmed that the Ctm fragment and the fully translocated PrP were glycosylated, whereas the Ntm fragment was not (Fig. 1B, compare lanes 3 and 7). The slightly slower migration of PrP after Endo H treatment as compared with the unglycosylated protein (Fig. 1B, lanes 6 and 7) is due to the cleavage specificity of the glycosidase, leaving one N-acetylgalactosamine attached to each asparagine. The identities of the Ntm and Ctm fragments were confirmed by differential immunoprecipitations with the 3F4 and 3B5 antibodies (Fig. 1B, lanes 3 and 4) (9). The monoclonal antibody 3F4 is directed against an epitope located N-terminal to TM1 (Fig. 1A) and recognizes PrP fragments of all three topological forms after translocation/proteolysis assays (5). The monoclonal antibody 3B5 recognizes an epitope in the N-terminal part of PrP (amino acid residues 51–89) (Fig. 1A) (9). Proteinase K treatment after solubilization of the membranes with Triton X-100 resulted in complete digestion of all proteins, demonstrating that protease protection was indeed due to translocation into ER microsomes (Fig. 1B, lanes 5 and 9).

Translation of mRNA coding for PrP-ΔSS in the absence of RM led to the production of one major product with a mass of ~26 kDa, PrP-ΔSS (Fig. 1C, lane 1). Translation in the presence of RM resulted in the formation of an additional product with a mass of ~31 kDa. This form disappeared after Endo H digestion suggesting it to be the doubly glycosylated form, PrP-ΔSS-2g (Fig. 1C, lane 2). As expected, no SS-cleared form was observed (Fig. 1C, compare lanes 1 and 2). After proteinase K treatment and subsequent immunoprecipitation with antibody 3F4, two major fragments were immunoprecipitated, the C-terminal, glycosylated PrP fragment (Ctm-2g, ~25 kDa) and the N-terminal fragment (Ntm, ~13 kDa) (Fig. 1C, lane 3). A small amount of fully translocated, di-glycosylated PrP-ΔSS-2g was also produced, as is evident from the presence of a fragment that is not reduced in size after proteinase K digestion (Fig. 1C, lane 3). The Ntm fragment was also immunoprecipitated with the 3B5 antibody (Fig. 1C, lanes 3 and 4).
and 9). Endo H treatment resulted in a 6-kDa reduction in size of the Ctm-2g fragment confirming its glycosylation (Fig. 1
C, lanes 3 and 8).

Thus the same translocated fragments are seen with pPrP and PrP-ΔSS, although with different efficiencies and different proportions. The overall translocation efficiency of PrP-ΔSS was reduced as compared with pPrP. 7.1% of total PrP-ΔSS synthesized became membrane-inserted or translocated as compared with 84.2% of pPrP (Fig. 1E). Differences are also seen with respect to the proportion of membrane spanning and translocated forms. The absolute amount of CtmPrP was almost identical in translocation reactions with pPrP and PrP-ΔSS (pPrP 55.1%, PrP-ΔSS 54.8%, Fig. 1E). In contrast, the efficiency of formation of the other two forms was drastically reduced (for NtmPrP, pPrP 537.7%, PrP-ΔSS 51.6%; and for secPrP, pPrP 541.4%, PrP-ΔSS 50.7%).

**TM2 Can Function as a Second ER Targeting Signal**—The fact that PrP-ΔSS was membrane-inserted suggested that a
The second ER targeting signal must exist in PrP. Both the internal TM1 region and the C-terminal TM2 region are of hydrophobic nature and therefore could be potential targeting signals (17, 18). To identify the second targeting signal sequence, we deleted the core region of TM1 (amino acid residues 112–121) in addition to the SS resulting in PrP-SS-TM1 (Fig. 2A). Translocation assays followed by protease digestion and immunoprecipitation with the 3F4-antibody were performed as described above. In the presence of RM a glycosylated form, PrP-SS-TM1–2g, accumulated, that was protected against protease digestion (Fig. 2B, lanes 7 and 8). Glycosylation of this protein was demonstrated by the reduction in size after treatment with Endo H (Fig. 2B, lane 9). Thus deletion of TM1 did not abolish translocation. No accumulation of either Ntm or Ctm fragments occurred as in the case of PrP-SS (Fig. 2B, lanes 3 and 4) consistent with TM1 being required for the generation of membrane-spanning PrP forms.

To see whether the second targeting signal is localized to the C-terminal hydrophobic region TM2, translocation of PrP-SS-TM2 (outlined in Fig. 2A) was analyzed by protease protection experiments followed by immunoprecipitations. PrP-SS-TM2 did not result in any glycosylated forms nor were protease-protected fragments produced (Fig. 2C, lane 3). This indicates that in the absence of an N-terminal signal sequence the TM2 is required for ER targeting and translocation.

**Membrane Insertion/Translocation of pPrP-ΔTM2**—To test whether TM2 also contributes to membrane insertion when an SS is present, we deleted TM2 from pPrP (outlined in Fig. 3A). Translation of mRNA coding for pPrP-ΔTM2 resulted in the production of one major protein species, pPrP-ΔTM2 (Fig. 3B, lane 1). When the translation was performed in the presence of RM, three protein species could be observed. These are the...
signal sequence cleaved forms with different degrees of glycosylation, namely non- (PrP-ΔTM2), mono- (PrP-ΔTM2–1g), and di-glycosylated (PrP-ΔTM2–2g) (Fig. 3B, lane 2). Endo H digestion confirmed that the increase in molecular weight was indeed due to glycosylation (Fig. 3B, compare lane 2 and 7). The glycosylation efficiency of pPrP-ΔTM2 was significantly reduced compared with pPrP, leading to the formation of about equal amounts of unglycosylated, mono-glycosylated, and di-glycosylated forms. Most of the glycosylated PrP-ΔTM2 resisted proteinase K digestion indicating complete translocation. Some N^tmPrP was produced, as is evident from the presence of the Ntm fragment after proteinase K digestion (Fig. 3B, lanes 4 and 5 and 9 and 10). After Endo H treatment, a very small amount of the Ctm fragment representing C^cmPrP could be observed (Fig. 3B, lane 9). When compared with pPrP, the amount of PrP-ΔTM2 inserted in the C^cmPrP orientation was significantly reduced by about 75% (see also Fig. 3C for the absolute values). A less pronounced reduction of about 50% was observed for N^tmPrP. In contrast, the efficiency of full translocation was slightly increased (pPrP-ΔTM2 = 50.0%, pPrP = 41.4%).

Thus the absence of TM2 in pPrP affects the proportion of C^cmPrP and, to a lesser extent, also of N^tmPrP.

Post-translational Targeting by TM2—The fact that the second ER targeting signal is located at the extreme C terminus of PrP makes it likely to function post-translationally because it would not emerge from the ribosome before translation is terminated. To test this we added RM after PrP-ΔSS-ΔTM1 had been synthesized and any nascent chains had been released from ribosomes with puromycin. A proportion of PrP-ΔSS-ΔTM1 became fully translocated and twice glycosylated (PrP-ΔSS-ΔTM1–2g) as evidenced by proteinase K (Fig. 4A, lane 6) and Endo H treatment (Fig. 4A, lane 9), respectively. The efficiency of post-translational translocation of PrP-ΔSS-ΔTM1 was 20.8% of its cotranslational translocation efficiency (Fig. 4A, lanes 3 and 6). Post-translational translocation was inhibited by aprotinin treatment, as shown by the lack of proteinase-protected PrP-ΔSS-ΔTM1–2g (Fig. 4A, lane 12), strongly indicating that it is ATP-dependent. ATP-dependent post-translational translocation was also observed for PrP-ASS (data not shown). When the same post-translational assay was performed with preprolactin, no translocation occurred in contrast to efficient cotranslational translocation of preprolactin (Fig. 4B, compare lanes 3 and 6). When pPrP and pPrP-ΔTM2 were tested for post-translational translocation, only very small amounts of translocated, proteinase K-protected and -glycosylated PrP could be detected (as shown for pPrP-ΔTM2 in Fig. 5, lane 4, post-translational translocation efficiency: 3.3% of its cotranslational translocation efficiency, lane 2). In contrast to the SS-deleted PrP forms, translocation of pPrP and pPrP-ΔTM2 was not inhibited by aprotinin treatment (shown for pPrP-ΔTM2 in Fig. 5, lane 6). Post-translational translocation assays performed with both PrP-ΔSS-ΔTM1 and pPrP-ΔTM2 showed the production of protein fragments of low molecular weight that were resistant to proteinase K digestion even in the presence of Triton X-100. These could potentially be derived from abnormally aggregated PrP.

DISCUSSION

The generation of different topological forms of PrP in the ER suggests the presence of different signal sequences in PrP and the alternative use of these signals. To identify additional signal sequences in PrP, we have deleted the N-terminal signal sequence and analyzed the resulting mutant protein for the presence of a second signal sequence. We have identified the C-terminal hydrophobic region, TM2, as a signal sequence that can mediate post-translational membrane insertion. Membrane insertion mainly occurred in the Ctm orientation. When the TM2 was deleted from PrP the amount of C^cmPrP was drastically reduced. We conclude from our data that the TM2 of PrP contributes significantly to the formation of the Ctm form of PrP.
CtmPrP exposes the N terminus on the cytoplasmic side and spans the membrane at the internal hydrophobic region, TM1. During biosynthesis, the C-terminal TM2 region is inserted into the membrane and then cleaved and replaced by a glycolipid membrane anchor (19). A C-terminal hydrophobic region is also found in the so-called “tail-anchored proteins.” However, in this case the entire N-terminal part of these proteins is exposed to the cytoplasmic side, and only the small C-terminal tail is inserted into the membrane (20, 21). It remains to be seen what sequence characteristics determine the orientation of proteins that are inserted into the membrane by C-terminal hydrophobic regions.

How could an N- and C-terminal signal sequence cooperate in the generation of alternative topologies? Our data suggest that the N-terminal (cotranslational) signal sequence of PrP is “inefficient,” allowing some molecules to be fully synthesized and then post-translationally translocated by using the TM2 as signal sequence. Cotranslational translocation requires that a cytoplasmic ribonucleoprotein particle, the signal recognition particle (SRP), efficiently interacts with the signal sequence of the nascent polypeptide resulting in an arrest of translation. Subsequently, the complex of ribosome, nascent polypeptide and SRP contacts the SRP receptor at the ER membrane leading to the insertion of the nascent polypeptide into the membrane (31–33). We propose that multiple topologies of PrP are generated by the differential use of the two PrP signal sequences. Inefficient use of a signal sequence has been demonstrated previously for both membrane as well as secretory proteins. Functional consequence is the expression of topologically different proteins produced from the same mRNA. In the case of polytopic membrane proteins, the facultative insertion of a transmembrane region can lead to the formation of alternative membrane topologies of a single protein. One such example is the large envelope protein (L protein) of duck hepatitis B virus. Its transmembrane region I is inserted into the membrane with an efficiency of about 50% (34). This results in cytosolically or lumenally located preS domain. On the cytosolic
side preS has a role in capsid binding during virus budding, whereas in the opposite orientation it is required for receptor binding during virus infection. Another example is the generation of a secreted and a cytosolic form of the plasminogen activator inhibitor-2. In this case, the signal sequence functions inefficiently at two steps as follows: SRP-mediated targeting to the ER membrane and the subsequent formation of a committed translocation complex (35). In contrast to these proteins, where the differential utilization of a single signal sequence leads to the formation of two different topologies, we propose a model for PrP translocation where not only the N-terminal signal sequence is facultatively used but, in addition, another signal sequence at the C terminus is used post-translationally.

In pPrP, about 80% of polypeptides are targeted cotranslationally by the N-terminal SS yielding αcPrP and αnPrP. According to our model, the 5% polypeptides integrated in the αnPrP orientation would be derived from the 20% of chains not translocated cotranslationally. This implies that the translocation efficiency of TM2 in pPrP is about 25%, being 5-fold higher than in PrP-ASS where only 5% translocation efficiency by TM2 was achieved. Efficient post-translational translocation by TM2 therefore seems to be promoted by the presence of the N-terminal SS. Following successful SRP-mediated targeting to the ER, a proportion of PrP nascent chains may not engage in productive cotranslational translocation due to inefficient recognition of the SS by components of the translocon. PrP polypeptides that failed to translocate cotranslationally would then be a substrate for post-translational translocation by TM2.

It is very likely that the synthesis of the different topological forms of PrP varies in different cell types. In addition to regulation at the level of SRP binding to SS, changes of the translocation machinery, either by expression of different regulatory factors (36, 37) or by differential modification of certain translocon components (38), could influence the ratio of the different PrP forms. As this could be a mechanism for the functional regulation of PrP in different cell types or under varying physiological conditions, it is possible that incorrect regulation of the translocation machinery leading to the perturbation of the delicate balance of the different forms of PrP could lead to disease. Indeed, it has already been shown that a shift of the relative ratios toward increased expression of αcPrP resulting from changes in the PrP sequence itself can lead to neurodegenerative disease (5, 39). Transgenic mice expressing mutant forms of PrP showed increased levels of CtmPrP, the levels of which could be correlated with the propensity of each mutant form to induce neurodegeneration (39). However, it remains unclear what proportion of the CtmPrP production occurred coreg- or post-translationally.

The mechanism by which increased expression of CtmPrP leads to neurodegeneration is not yet known. In analogy to processing of the amyloid precursor protein in Alzheimer’s disease (40, 41), different cleavage products of PrP, either destroying or leaving intact the amyloidogenic region in TM1, have been identified (42). Very likely, if PrP is processed by potential secretases located at the ER membrane, then cleavage products could vary with different PrP topologies.

It will be interesting to see if a perturbation of the balance between SS- and TM2-mediated membrane insertion of PrP is related to neurodegeneration in certain forms of prion disease.

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