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# Biogenesis and Transmembrane Orientation of the Cellular Isoform of the Scrapie Prion Protein

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**Considerable evidence suggests that the scrapie prion protein (PrP) is a component of the infectious particle. We studied the biogenesis and transmembrane orientation of an integral-membrane form of PrP in a cell-free transcription-linked translation-coupled translocation system programmed with a full-length PrP cDNA cloned behind the SP6 promoter. Translation of SP6 transcripts of the cDNA or of native mRNA from either normal or infected hamster brain in the absence of dog pancreas membranes resulted in the synthesis of a single PrP immunoreactive polypeptide (each polypeptide was the same size; $M_r$, 28,000), as predicted from the known sequence of the coding region. In the cotranslational presence of membranes, two additional forms were observed. Using peptide antisera specific to sequences from the amino- or the carboxy-terminal domain of PrP together with proteinase K or endoglycosidase H digestion or both, we showed that one of these forms included an integrated and glycosylated form of PrP ($M_r$ = 33,000) which spans the bilayer twice, with domains of both the amino and carboxy termini in the extracytoplasmic space. By these criteria, the other form appeared to be an unglycosylated intermediate of similar transmembrane orientation. The PrP cell-free translation products did not display resistance to proteinase K digestion in the presence of nondenaturing detergents. These results suggest that the PrP cell-free translation products most closely resemble the normal cellular isoform of the protein, since its homolog from infected brain was proteinase K resistant. The implications of these findings for PrP structure and function are discussed.**

Scrapie is a degenerative neurological disease of sheep and goats (34), which can be transmitted to a variety of laboratory rodents (19). Because the unusual properties of the scrapie agent distinguish it from both viruses and viroids, the term prion was introduced (36). Three human diseases may also be caused by prions; these are Creutzfeldt-Jakob disease, kuru, and Gerstmann-Sträussler syndrome (6, 18, 20, 24, 30).

Purification of scrapie prions led to isolation of a sialoglycoprotein of a relative molecular weight of 27,000 to 30,000, called prion protein (PrP) 27-30 (7, 8, 37, 41). The protein, but no nucleic acid component, has been identified in highly purified preparations of prions. N-terminal amino acid sequencing of PrP 27-30 (39) led to the isolation of cDNA clones from scrapie-infected brains of hamsters (33) and mice (12) by using a mixture of synthetic oligonucleotides as a probe. Southern blots showed that PrP 27-30 is encoded by a cellular gene and not by the infectious prion particles (33).

Recent studies have shown that the PrP gene (*prnP*) in mice on chromosome 2 (R. S. Sparkes, M. Simon, V. H. Cohen, R. E. K. Fournier, J. Lem, I. Klisak, C. Heinzmann, C. Blatt, M. Lucero, T. Mohandas, S. J. DeArmond, D. Westaway, S. B. Prusiner, and L. P. Weiner, Proc. Natl. Acad. Sci. USA, in press) is linked to a gene (*prnI*) controlling the scrapie incubation time (11). The genetic linkage of PrP with the cardinal feature of scrapie infection, i.e., long incubation times, argues for a central role of a Prp 27-30 precursor ($Prp^{Sc}$) in the pathogenesis of this degenerative brain disorder.

Northern blots indicated that normal and scrapie-infected brains contain similar concentrations of poly(A)$^+$ mRNA encoding PrP or closely related species (12, 33). The finding of PrP mRNA in uninfected hamster brain led to the discovery of a normal cellular isoform of PrP, termed $PrP^C$ or PrP 33-35$^C$, and the demonstration that PrP 27-30 is derived from a precursor, designated $Prp^{Sc}$ or Prp 33-35$^{Sc}$ (2, 31, 33), in scrapie-infected brains. These studies also showed that $PrP^C$ is completely degraded during proteinase K digestion, in contrast to the behavior of PrP 27-30. Subsequent studies have suggested that both the cellular and scrapie PrP isoforms are membrane bound (31). In contrast to $PrP^C$, which is solubilized by nondenaturing detergents, $PrP^{Sc}$ as well as PrP 27-30 polymerizes into amyloid rods when membranes from scrapie-infected brains are extracted with detergent (31). Thus, the rods found in highly purified preparations of scrapie prions are an artifact of detergent extraction (41). These observations are of particular interest, since many earlier studies on the scrapie agent stressed its association with membranes (32, 44) and its apparent hydrophobicity (40, 42).

To determine whether PrP is an integral membrane protein and, if so, to characterize its transmembrane orientation, we studied its biogenesis in a wheat germ cell-free system programmed with SP6 transcripts of the full-length cDNA or with native mRNA from normal or infected hamster brain. We conclude that at least one form of PrP is an integral membrane protein spanning the bilayer twice, with both amino- and carboxy-terminal domains in the extracytoplasmic space. Moreover, cell-free translation products from the cloned PrP cDNA transcripts were indistinguishable in size as well as transmembrane orientation from those of normal or infected-brain mRNA. Neither the translation products

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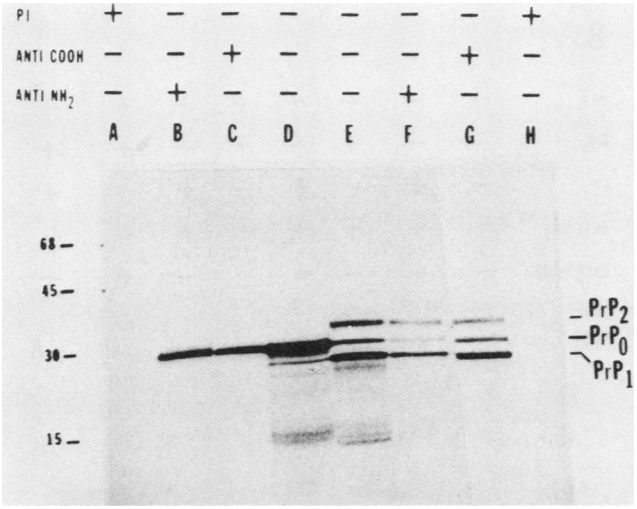


FIG. 1. Translation and immunoprecipitation of products encoded by plasmid pSP PrP. Transcription-linked translation was performed in a wheat germ cell-free system with (lanes E to H) and without (lanes A to D) dog pancreas microsomal membranes. Translation products were either first immunoprecipitated (lanes A to C and F to H) or prepared directly (lanes D and E) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12 to 17% gradient gels. Immunoprecipitation was carried out with either anti-$NH_2$ or anti-COOH peptide rabbit serum or preimmune serum as indicated. Molecular weight markers are indicated on the left, and designations of PrP related products are indicated on the right.

encoded by native brain nor the cell-free transcribed mRNA displayed the proteinase K resistance of $PrP^{Sc}$. Our results are consistent with the possibility that the differences in properties between $PrP^{C}$ and $PrP^{Sc}$ may arise from post-translational modifications or from association with other molecules which modify the properties of the PrP in the normal brain (3).

## MATERIALS AND METHODS

**Materials.** All chemicals were of the highest commercial grade available. All restriction endonucleases, SP6 RNA polymerase, T4 DNA ligase, and Klenow fragment of *Escherichia coli* DNA polymerase I were from Boehringer Mannheim Biochemicals, Indianapolis, Ind., or from New England BioLabs, Inc., Beverly, Mass. RNase inhibitor was from Promega Biotec, Madison, Wis.; staphylococcal protein A-Sepharose was from Pharmacia, Inc., Piscataway N.J.; proteinase K was obtained from E. Merck AG, Darmstadt, Federal Republic of Germany; endoglycosidase H and [$^{35}$S]methionine (translation grade, >800 Ci/mmol) were from New England Nuclear Corp., Boston, Mass.

**Source and propagation of scrapie prions.** A hamster-adapted scrapie prion isolate (29) was passaged as previously described and was used to inoculate hamsters intracerebrally (38).

**Antisera.** Rabbit antisera to purified scrapie PrP 27-30 (4) and to synthetic peptides GQGGGTHNQWNKP from the amino terminus and KESQAYYDGRSSA from the carboxy terminus were prepared as previously described (2).

**mRNA isolation.** Syrian Golden hamsters (LVG/LAK) purchased from Charles River Breeding Laboratories Lakeview, N.J., were used for the preparation of normal and infected-brain RNA. Freshly dissected hamster brains were isolated, and total cellular RNA was prepared by the method of Chirgwin et al. (13).

**Cell-free translation of brain RNA.** Cell-free translation experiments using brain RNA were performed in a final volume of 100 μl and contained 20 μCi of [$^{35}$S]methionine, 1.0 $A_{260}$ U of RNA, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5), 140 mM potassium acetate, 3 mM dithiothreitol, 2.2 mM magnesium acetate, 10 mM Tris hydrochloride (pH 7.5), 0.4 mM spermidine, 1 mM each ATP and GTP, 10 mM creatine phosphate, 40 μM each of 19 L-amino acids minus methionine, 0.1 mg of calf liver tRNA per ml, 20 μg of creatine phosphokinase per ml, 1 U of ribonuclease inhibitor per ml, and 20% (by volume) wheat germ extract prepared by the method of Erickson and Blobel (15) at a concentration of 2.5 $A_{280}$ U/ml. Incubations were at 25°C for 60 min. Dog pancreas microsomes were prepared from rough microsomes (47) and added at a concentration of 2.5 $A_{280}$ U/ml.

**Construction of SP6 expression plasmid.** An *Eco*RI restriction fragment from pHaPrPcDNA-S11 was isolated (3). The fragment, which included the entire hamster PrP-coding region, was engineered into the *Eco*RI site of pSP64.

**Cell-free transcription-linked translation.** SP6 plasmids were transcribed in vitro (25) at a concentration of 0.2 mg/ml in a reaction mixture containing 40 mM Tris hydrochloride (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, 25 μg of calf liver tRNA per ml, 0.5 mM each ATP, CTP, and UTP, 0.1 mM GTP, 0.5 mM diguanosine triphosphate, 0.9 U of RNase inhibitor per μl, and 0.4 U of SP6 RNA polymerase per μl. Reactions were performed at 40°C for 1 h, and aliquots were used directly in transcription-linked translations in the wheat germ cell-free system at a concentration of 20%. Translation reactions were performed in 20- to 200-μl volumes that contained 20% wheat germ extract essentially as previously described (15). Reaction mixtures were incubated at 24°C for 60 min.

**Posttranslational assays.** Including proteolysis with proteinase K, endoglycosidase H digestion, and immunoprecipitation, posttranslational assays were performed essentially as previously described (35) except that proteolysis proceeded at 24°C rather than at 0°C. Products were visualized by fluorography after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9).

**Hydrophobicity index calculations.** Hydrophobicity index calculations were made by using the values for individual amino acid residue hydrophobicities of Kyte and Doolittle (26). The value for a given residue was multiplied by the number of times that residue appeared in the peptide sequence of interest and was added to the values derived for all other amino acid residues of that sequence.

## RESULTS

A full-length cDNA encoding PrP from scrapie-infected hamster brain (3) was engineered behind the SP6 promoter, and RNA was transcribed in a cell-free system by using SP6 polymerase (25). The transcription products were translated in a wheat germ cell-free protein synthesizing system, including [$^{35}$S]methionine to label newly synthesized chains (15). The translation products were characterized by determining their reactivities with either preimmune or immune rabbit serum directed against synthetic peptides corresponding to unique sequences of the amino- or carboxy-terminal region of PrP and then subjecting the total or immunoprecipitated products to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1, lanes A to D). The major translation product ($M_r$, 28,000) was immunoprecipitated by either amino- or carboxy-terminal PrP-

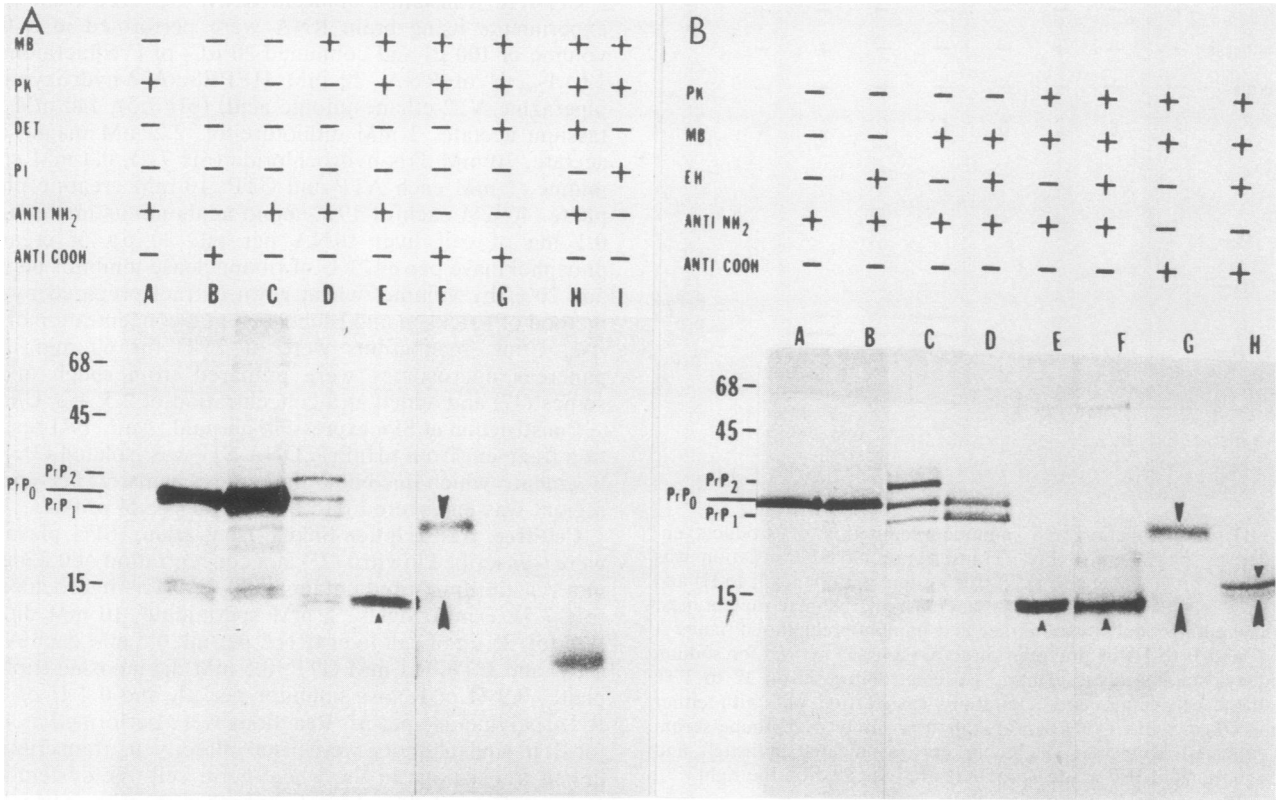


FIG. 2. (A) Translation, proteolysis, digestion, and immunoprecipitation of products encoded by plasmid pSP PrP. Translations were performed as described in the legend to Fig. 1. Some reactions were performed in the absence (lanes A to C) or presence (lanes D to I) of dog pancreas microsomal membranes. In some cases (lanes A and B), membranes were added after the completion of translation and incubation was continued for an additional 45 min. Some aliquots (lanes A, E, F, G, H, and I) were subjected to proteolysis either with (lanes G and H) or without (lanes A, E, F, and I) Nikkol (Nikko Chemical Co., Tokyo, Japan) added to 0.5%. Immunoprecipitation with various sera was as described in the legend to Fig. 1 and is indicated by the heading. PrP-related product markers are indicated on the left. △, PrP-$NH_2$ proteolytic fragment; ▽, PrP-COOH proteolytic fragment. (B) Aliquots of PrP translation products in the absence (lanes A and B) and presence (lanes C to H) of membranes and after proteolytic digestion (lanes E to H) were subjected to incubation with (lanes B, D, F, and H) and without (lanes A, C, E, and G) endoglycosidase H. Markers and arrowheads are as described in the legend to Fig. 1 except for downward- and upward-pointing long arrowheads indicating PrP-COOH and PrP-COOH′ fragments containing and lacking carbohydrate, respectively. The lane H band indicated by the short arrowhead has been shifted by removal of carbohydrate after endoglycosidase H digestion. Note the anomalous migration of PrP-COOH before but not after endoglycosidase H digestion.

specific antiserum (lanes B and C) but not by preimmune serum (lane A). This putative precursor form of PrP was termed $PrP_0$. When microsomal membranes were added cotranslationally, two new products termed $PrP_1$ and $PrP_2$ of $M_r$s 25,000 and 33,000, respectively, were identified as having identical immunoreactivities to that of $PrP_0$. By analogy to previous results with biogenesis of glycoproteins, we suspected that these forms represented glycosylated ($PrP_2$) and unglycosylated ($PrP_1$) species lacking a cleaved amino-terminal signal sequence. In the case of $PrP_1$, removal of the signal sequence without glycosylation of the authentic PrP chain resulted in a shorter polypeptide, whereas for $PrP_2$, the presence of the added carbohydrate moiety more than offset the increased mobility caused by removal of a signal sequence. This interpretation was confirmed by endoglycosidase H digestion, which abolished $PrP_2$ and increased the intensity of products migrating close to $PrP_1$ (Fig. 2B, lane D).

It is well established that endoglycosidase H removes the N-linked oligosaccharides but leaves the most proximal sugar which is attached to an Asn residue; the enzyme does not remove O-linked sugars (46). The shift in molecular weight after endoglycosidase digestion is an important observation with respect to the transmembrane orientation of PrP, since there are only 2 potential N-glycosylation sites (3, 33) and these are located close together near the COOH-terminal domain of the molecule.

Because glycosylation and signal peptide cleavage are cotranslational modifications occurring exclusively in the lumen of the endoplasmic reticulum (22, 28), it seemed likely that $PrP_1$ and $PrP_2$ represented forms integrated into or translocated across the microsomal membranes. To characterize their orientation with respect to the membrane, PrP was synthesized either in the presence of microsomal membranes or with the membranes added posttranslationally. These products were subjected to proteolysis with proteinase K, either under conditions which maintained the microsomal membrane bilayer intact or in the presence of nonionic detergents which solubilized the bilayer. When membranes were added after the completion of synthesis (Fig. 2A, lane B), only $PrP_0$ was observed and it was completely degraded by added protease (lane A). When the products synthesized in the presence of microsomal membranes (lane D) were subjected to proteinase K digestion,

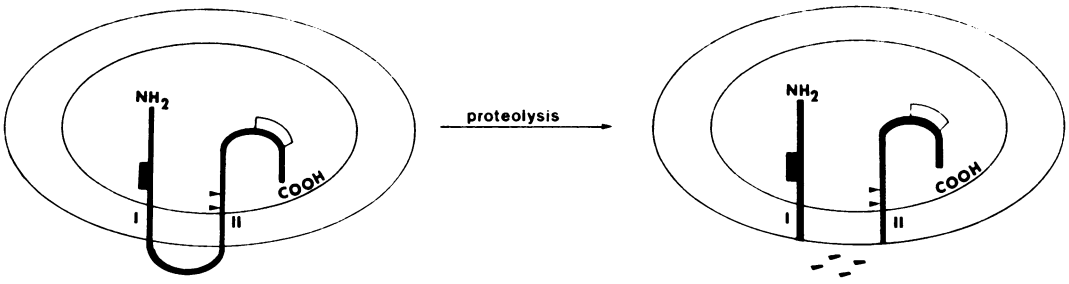


FIG. 3. Model for transmembrane orientation of integral-membrane form of PrP. Microsomal vesicles depicting transmembrane orientation of PrP before (left) and after (right) proteolysis. $NH_2$ and COOH refer to amino and carboxy termini; solid and open bars refer to peptide sequences against which $NH_2$- and COOH-specific antisera, respectively, were raised. Arrowheads refer to N-linked glycosylation sites. I and II refer to transmembrane domains.

three new species were detected, termed PrP-$NH_2$ (lane E). PrP-COOH, and PrP-COOH′ (lane F). These PrP polypeptides reacted with either monospecific amino-terminal antiserum (anti-PrP-$NH_2$) or monospecific carboxy-terminal antiserum (anti-PrP-COOH) but not with both (lanes E and F). Immunoreactivity was abolished by proteolysis in the presence of detergents (lanes G and H). Thus, at least two domains corresponding to the amino and carboxy terminus of PrP were localized to the vesicle lumen and hence protected from protease in the absence of detergents. Since both putative glycosylation sites were located in the carboxy-terminal region of the molecule, this orientation was confirmed for $PrP_1$ and $PrP_2$ by the observation that PrP-COOH but not PrP-$NH_2$ was shifted by digestion with endo H (Fig. 2B, lanes E to H) to a position just above PrP-COOH′ (lanes E to H). These observations indicate that both the processed glycosylated ($PrP_2$) and the processed nonglycosylated ($PrP_1$) species had the same transmembrane orientations, with luminal amino- and carboxy-terminal domains. In some but not all experiments, various amounts of another form of $PrP_2$ were detected (data not shown). This species, which was degraded by proteinase K in the presence of detergent, behaved either as a proteinase K-resistant transmembrane species or an unusual secretory form of PrP (B. Hay, S. B. Prusiner, and V. R. Lingappa, manuscript in preparation).

It is important that the proteinase K resistance of the PrP cell-free translation products described here was abolished by nondenaturing detergents. This indicates that these proteins or portions of them were protected by the microsome membranes. This resistance of the PrP translation products contrasts with that of $PrP^{Sc}$, in which proteinase K in the presence or absence of nondenaturing detergent generates PrP 27-30 by removal of the amino-terminal 67 amino acids as well as removal of up to 7 amino acids from the carboxy terminus (3).

The data presented here demonstrate that the major species of PrP generated in the wheat germ cell-free protein synthesizing system was a polytopic integral transmembrane protein which must span the bilayer at least twice, with amino- and carboxy-terminal regions in the extracytoplasmic space (Fig. 3). Similar analysis of translation products of native mRNA from either normal or scrapie-infected hamster brain revealed a single PrP immunoreactive species comigrating with $PrP_0$ (Fig. 4). The PrP immunoreactive translation products of native mRNA from both normal and scrapie-infected hamster brain represent a small fraction (less than 0.01%) of total-brain mRNA (Fig. 4). When translated in the presence of microsomal membranes and subjected to proteolysis with proteinase K, cleavage products characteristic of the integral membrane species $PrP_1$ and $PrP_2$ were obtained (data not shown), indicating that the topogenesis of the cloned gene expression product was faithfully reproduced by that of native-brain mRNA translation products.

It is of interest that the PrP translation products produced from both normal and scrapie-infected mRNA were present in equal concentrations. This observation is in agreement with Northern blot analyses showing that the level of poly(A)$^+$ PrP mRNA does not change during the course of scrapie infection (12, 33). In contrast to the unchanged PrP mRNA level, $PrP^{Sc}$ accumulates during infection to a level ~10-fold greater than that of $PrP^C$, which remains constant (2, 31). Recent studies suggest that $PrP^{Sc}$ and $PrP^C$ are translated from the same mRNA but that their different properties arise most probably from posttranslational modifications (3).

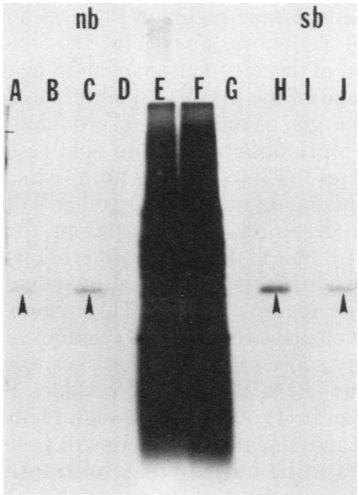


FIG. 4. Immunoprecipitation of brain translation products with PrP 27-30 and PrP-$NH_2$ antisera. Translations of 100 μl containing 1 $A_{260}$ U of total cellular RNA from normal (nb) and scrapie-infected (sb) hamster brains were performed as described in Materials and Methods. Immunoprecipitation was with anti-PrP 27-30 serum (lanes A and J), preimmune serum (lanes B and I), anti-PrP $NH_2$ serum (lanes C and H), and anti PrP-$NH_2$ serum plus 500 ng of synthetic peptide (lanes D and G). Lanes E and F contain 1 μl of total-brain translation products.

## DISCUSSION

We studied the topogenesis of the cell-free translation product encoded by the hamster brain scrapie PrP mRNA as expressed from both a cloned full-length cDNA and from native-brain mRNA. Our data suggest (to the limits of resolution of one-dimensional gel electrophoresis) that there is a single species of PrP mRNA common to normal and infected brain and that its translation product can be expressed as an integral membrane protein spanning the bilayer at least twice, with defined extracytoplasmic domains at both the amino and carboxy termini (Fig. 3). The size of the primary translation product from PrP mRNA was as predicted from the known sequence for the PrP chromosomal gene (13). The size of $PrP_2$ is similar to that of the highest-molecular-weight forms observed in vivo (31).

Current views on the biogenesis of complex integral membrane proteins suggest that multiple and internal signal and stop transfer sequences are engaged in generating transmembrane loops (5, 17, 27, 45, 48; B. Eble, D. McRae, V. R. Lingappa, and D. Ganem, submitted for publication). The transmembrane orientation demonstrated here for PrP suggests the action of two signal sequences and at least one stop transfer sequence. Construction of chimeric proteins to identify the putative two signal and one stop transfer sequences of PrP is in progress and should provide a further test of the proposed orientation and elucidate the role of topogenic sequences in PrP biogenesis (35, 49).

Analysis of the amino acid sequence deduced from the PrP cDNA (33) together with a consideration of the sizes of PrP-$NH_2$ and PrP-COOH proteinase K digestion fragments after endoglycosidase H treatment suggests that the first transmembrane region is located at approximately amino acid residues 90 to 113 (Fig. 5). This region comprised some 24 contiguous uncharged amino acids, with a calculated hydrophobicity index of +28.6 (26). By comparison, the best hydrophobicity score for 24 contiguous amino acid residues of the transmembrane domain of membrane-bound immunoglobulin M heavy chain was +36.8. An unusual feature of this first transmembrane region of PrP was a preponderance of glycine and alanine residues (Fig. 5).

The location of the second transmembrane region was less certain. The end of the first transmembrane domain and the position of the glycosylation sites provided endpoints between which were about 45 amino acid residues (approximately residues 115 to 160) within which the second transmembrane region must lie. Together with the results of proteinase K digestion and an attempt to maximize the hydrophobicity index, these considerations suggest that amino acid residues 135 to 158 are included in the second transmembrane region (Fig. 5). This region included two prolines as well as two charged residues and had a low hydrophobicity score of −29.1. However, the hydrophobicity score of the alternative region (residues 115 to 135) was even lower, i.e., −41.1. Although such features are clearly not typical of transmembrane regions in general, they have been proposed to be characteristic of transmembrane segments of polypeptides which compose channel subunits (10, 16). For example, a similarly calculated hydrophobicity index of the fifth transmembrane region of acetylcholine receptor, based on the model of Finer-Moore and Stroud (16), is −19.4. It is tempting, therefore, to consider the possibility that at least one function of cellular PrP may be that of an ion-channel component. In such a view, aggregation of PrP monomers in the plane of the bilayer at some point after synthesis, perhaps by self-association of the second transmembrane region, would form a functional complex, stable in the lipid bilayer. Whether the involvement of PrP in either normal brain or in scrapie pathology is related to such putative functions remains to be determined. Expression of this polypeptide in xenopus oocytes should permit an initial test of possible ion-channel functions of PrP (14, 43).

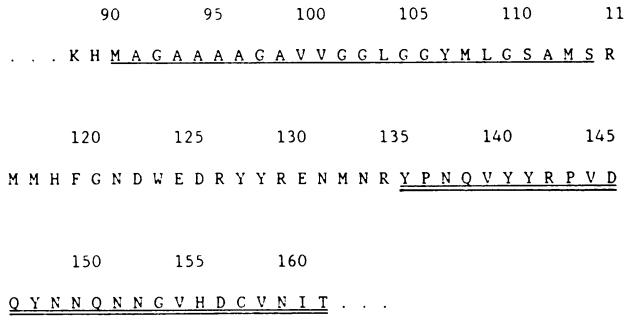


FIG. 5. Assignment of transmembrane regions in PrP. PrP-coding region from amino acid 88 to 161 is indicated, including the sequence of amino acid residues in the predicted transmembrane domains illustrating a typical hydrophobic domain for transmembrane region I (underlined once) and the proposed nonhydrophobic transmembrane domain II (underlined twice).

Although a single product was observed upon translation of both brain and cloned gene transcripts in the absence of membranes, multiple forms were generated by the cotranslational presence of dog pancreas membranes. The major species, namely $PrP_1$ and $PrP_2$, represent nonglycosylated and glycosylated variants, respectively, reflecting the relative inefficiency of glycosylation compared with translocation and signal peptide cleavage in cell-free systems. Although all of $PrP_1$ and most of $PrP_2$ appeared to display the transmembrane orientation described here, various amounts of a minor component of $PrP_2$ were noted to be resistant to proteinase K in the absence but not in the presence of detergents (i.e., behaving as a polypeptide completely translocated into the microsomal vesicle lumen). Elsewhere, we will describe the properties of this species of $PrP_2$ in greater detail (Hay, Prusiner, and Lingappa, in preparation).

Studies to demonstrate the orientation directly in the brain have been hampered by the intrinsic protease resistance of $PrP^{Sc}$ and the low levels of $PrP^{C}$ expressed (D. Andrews and V. R. Lingappa, unpublished observation). We cannot, therefore, rule out that the orientation achieved in the wheat germ cell-free system is different from that achieved in the brain. However, previous studies of membrane protein biogenesis in cell-free systems have demonstrated fidelity with the early events in living cells (1, 21, 23), and other studies of the scrapie agent are suggestive of an integral membrane protein (31, 32, 44). Thus, it seems likely that the initial stages of PrP biogenesis in the brain would include molecules in this orientation.

From our data, PrP translation products appear to lack the biochemical hallmark of PrP from infectious preparations, namely proteinase K resistance, even in the presence of nondenaturing detergents. Our data as well as recent studies on the organization of the PrP gene (3) suggest that $PrP^{Sc}$ in hamsters is generated by either a posttranslational modification or by the association of a PrP precursor molecule with other components. Our inability to produce PrP molecules in cell-free translation systems that are proteinase K resistant

in the presence of detergent suggests that the necessary posttranslational modification or association with other molecules (1) was not achieved.

## ACKNOWLEDGMENTS

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# ERRATA

## Biogenesis and Transmembrane Orientation of the Cellular Isoform of the Scrapie Prion Protein

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Volume 7, no. 2, p. 914, column 1, line 20: "(*prnP*)" should read "(*Prn-p*)".

Page 914, column 1, line 25: "(*prnI*)" should read "(*Prn-i*)."

Page 914, column 1, line 28: "Prp" should read "PrP."

Page 914, column 1, line 29: "($Prp^{Sc}$)" should read "($PrP^{Sc}$)."

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## The *SPT6* Gene Is Essential for Growth and Is Required for δ-Mediated Transcription in *Saccharomyces cerevisiae*

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Volume 7, no. 2, p. 684, column 2: The last five lines should be deleted.

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## Isolation and Characterization of *MOD5*, a Gene Required for Isopentenylation of Cytoplasmic and Mitochondrial tRNAs of *Saccharomyces cerevisiae*

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Volume 7, no. 1, p. 183, column 2, reference 9a: "Ellis, S. R., M. J. Morales, J.-M. Li, and A. K. Hopper" should read "Ellis, S. R., M. J. Morales, J.-M. Li, A. K. Hopper, and N. C. Martin."