

## Evidence for a Secretory Form of the Cellular Prion Protein<sup>†</sup>

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**ABSTRACT:** The biogenesis of hamster brain prion protein (PrP) has been studied by expression of RNA transcribed from a full-length PrP cDNA in *Xenopus* oocytes and cell-free systems. Earlier studies in the wheat germ cell-free system showed that one form of PrP is a transmembrane protein that spans the bilayer at least twice [Hay, B., Barry, R. A., Lieberburg, I., Prusiner, S. B., & Lingappa, V. R. (1987) *Mol. Cell. Biol.* 7, 914-920]. We now report that PrP can also exist as a secreted protein. SP6 PrP RNA microinjected into *Xenopus* oocytes produced two forms of PrP: one that remained in the cell and another that was secreted into the medium. Cell-free translation studies in rabbit reticulocyte lysates supplemented with microsomal membranes gave similar results: while one form of PrP was found as an integral membrane protein spanning the membrane at least twice, another form of PrP was found to be completely translocated to the microsomal membrane vesicle lumen. Both the membrane and secretory forms of PrP appear to be generated from the same pool of nascent chains. The mechanism governing the alternative fates of nascent PrP remains to be elucidated but may have significance for understanding the pathogenesis of scrapie and other prion diseases.

**S**crapie and several other transmissible, degenerative diseases of the central nervous system are caused by novel infectious pathogens or prions (Gajdusek, 1977; Prusiner, 1987). Considerable evidence indicates that prions contain a protein (PrP<sup>Sc</sup>)<sup>1</sup> that is an abnormal isoform of a normal cellular protein (PrP<sup>C</sup>). Despite numerous attempts to identify a nucleic acid genome within the scrapie prion, none has been found to date. Although the chromosomal gene encoding PrP has been sequenced and cloned (Basler et al., 1986), the mechanism whereby PrP<sup>C</sup> is converted to PrP<sup>Sc</sup> is unknown.

Recent studies show that PrP<sup>Sc</sup> is specific for prion diseases and that this protein has molecular properties which distinguish it from its cellular isoform (PrP<sup>C</sup>) (Oesch et al., 1985; Meyer et al., 1986). Both PrP<sup>Sc</sup> and PrP<sup>C</sup> purify with membrane fractions, but upon detergent extraction, PrP<sup>C</sup> is solubilized while PrP<sup>Sc</sup> polymerizes into rod-shaped particles. Digestion of PrP<sup>C</sup> with proteinase K completely degrades the protein; however, digestion of PrP<sup>Sc</sup> produces a smaller protein designated PrP 27-30, which still is associated with scrapie prion infectivity.

Learning about the biogenesis of PrP and its conversion to an abnormal isoform (PrP<sup>Sc</sup>) is not only important in understanding the pathogenesis of scrapie but is also significant for understanding the mechanism of three human brain disorders: kuru, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler syndrome (Prusiner, 1987). Molecular cloning of a PrP cDNA that contains the entire protein coding region (Basler et al., 1986) provides an opportunity to study the biosynthesis of PrP

by transcription-linked translation in *Xenopus* oocytes (XO) as well as cell-free systems.

In earlier studies, we reported the cell-free synthesis of a transmembrane form of PrP that resembles PrP<sup>C</sup> in its sensitivity to protease digestion (Hay et al., 1987). We report here on the results of further studies which show that this PrP molecule may exist both as an integral membrane protein and as a soluble secretory protein. Although we have not succeeded in synthesizing a molecule with the properties of PrP<sup>Sc</sup>, we believe that the results of our studies have important implications for understanding how scrapie prions may spread during infection and how prion amyloid plaques arise.

### MATERIALS AND METHODS

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

**Antisera.** Rabbit antisera to the PrP synthetic peptide GQGGGTHNQWNKP from the amino terminus were prepared as previously described (Barry et al., 1986).

**Construction of SP6 Expression Plasmids.** An *EcoRI* restriction fragment from pHaPrPcDNA-S11 was isolated (Basler et al., 1986). The fragment, which included the entire hamster PrP-coding region, was engineered into the *EcoRI* site of pSP64.

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<sup>1</sup> Abbreviations: PrP, prion protein; PrP<sup>Sc</sup>, scrapie isoform of the prion protein; PrP<sup>C</sup>, cellular isoform of the prion protein; XO, *Xenopus* oocytes; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RRL, rabbit reticulocyte lysate; *s*, sedimentation coefficient; Tris, tris-(hydroxymethyl)aminomethane; kDa, kilodaltons.

Plasmid pSPGM2C contains the  $\beta$ -lactamase signal sequence engineered behind the SP6 promoter. This plasmid contained sites downstream from the signal-coding region that were convenient for construction of expression clones of PrP, without its own amino-terminal signal sequence. Plasmid pSPGM2C was linearized with *Bam*HI and ligated by T4 DNA ligase with a *Bam*HI fragment containing the PrP cDNA insert, which had been purified by gel electrophoresis from the remainder of the plasmid pHaPrP cDNA-1 (Oesch et al., 1985). After transformation of *E. coli*, plasmid DNA was prepared from individual ampicillin-resistant colonies and screened for correct orientation by restriction enzyme mapping with *Eco*RI and *Apa*I. Plasmid in the correct orientation was designated pSPGM2D. In order to remove GC tailing sequences upstream of the *Bam*HI insert and to generate the proper reading frame for the fusion protein, pSPGM2D was linearized with *Xba*I and subjected to *Bal*31 nuclease digestion. The linearized vector was then cut with either *Bgl*III, which places the PrP cDNA behind the SP6 promoter (pSPGM2H), or with *Eco*RI, which places it behind the  $\beta$ -lactamase signal sequence (pSPGM2G), with a poly(G) region (which codes for glycines) linking the two coding regions. In both cases, ragged ends were filled in with *E. coli* DNA polymerase I Klenow fragment in the presence of all four dNTPs and ligated with T4 DNA ligase. Plasmid DNA was prepared from individual ampicillin-resistant colonies after transformation of *E. coli* and screened for size and immunoreactivity of encoded products by transcription-linked translation in a wheat germ system.

**Cell-Free Transcription-Linked Translation.** SP6 plasmids were transcribed in vitro (Krieg & Melton, 1984) at a concentration of 0.2 mg/mL in a reaction mixture containing 40 mM Tris-HCl (pH 7.5), 6 mM magnesium chloride, 2 mM spermidine, 10 mM dithiothreitol, 25  $\mu$ g of calf liver tRNA/mL, 0.5 mM each ATP, CTP, and UTP, 0.1 mM GTP, 0.5 mM diguanosine triphosphate, 0.9 unit of RNase inhibitor/ $\mu$ L, and 0.4 unit of SP6 RNA polymerase/ $\mu$ L. Reactions were performed at 40 °C for 1 h, and aliquots were used directly in transcription-linked translations in the rabbit reticulocyte lysate (RRL) cell-free system at a concentration of 20%. Translation reactions were performed in 20–200- $\mu$ L volumes that contained 45% RRL in a final volume of 100  $\mu$ L and contained 20  $\mu$ Ci of [<sup>35</sup>S]methionine, 1.0 *A*<sub>260</sub> unit of RNA, 20 mM *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (Hepes) (pH 7.5), 140 mM potassium acetate, 3 mM dithiothreitol, 2.2 mM magnesium acetate, 10 mM Tris-HCl (pH 7.5), 0.4 mM spermidine, 1 mM each ATP and GTP, 10 mM creatine phosphate, 40  $\mu$ M each of 19 L amino acids minus methionine, 0.1 mg of calf liver tRNA/mL, 20  $\mu$ g of creatine phosphokinase/mL, and 1 unit of ribonuclease inhibitor/mL. Dog pancreas microsomes were prepared from rough microsomes (Walter & Blobel, 1983) and added at a concentration of 2.5 *A*<sub>280</sub> units/mL. Reaction mixtures were incubated at 25 °C for 60 min.

**Posttranslational Assays.** Including proteolysis with proteinase K, endoglycosidase H digestion, and immunoprecipitation, posttranslational assays were performed essentially as previously described (Perara & Lingappa, 1985) except that proteolysis proceeded at 24 °C rather than at 0 °C. Products were visualized by fluorography after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bonner & Laskey, 1974).

**Protein Synthesis in *Xenopus* Oocytes.** Stage VI *Xenopus laevis* oocytes were dissected and injected with 50 nL each of PrP transcript prepared from linearized plasmid DNA in the

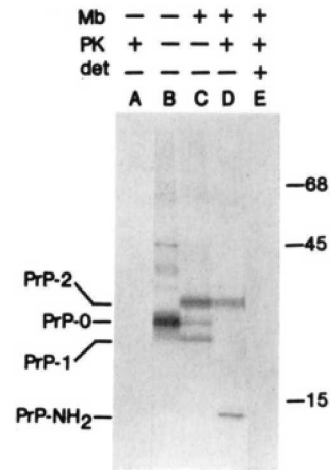


FIGURE 1: Characterization of transmembrane orientation of PrP synthesized in the RRL cell-free system. Cell-free transcription-linked translation using full-length PrP cDNA cloned behind the SP6 promoter was carried out in RRL as previously described (Perara & Lingappa, 1985). Translation reactions were carried out in the absence (lanes A and B) or presence (lanes C–E) of dog pancreas microsomal membranes (Mb). Samples were subjected to immunoprecipitation with peptide antisera directed to the amino terminus either directly (lanes B and C) or after proteolysis (PK) with proteinase K (lanes A, D, and E). Triton X-100 was added to a final concentration of 1% during proteinase K digestion in lane E, as indicated by det. Proteolysis and immunoprecipitation were carried out as previously described (Perara & Lingappa, 1985). Molecular weights  $\times 10^3$  are indicated to the right and the positions of PrP<sub>2</sub>, PrP<sub>1</sub>, PrP<sub>0</sub>, and PrP-NH<sub>2</sub> to the left.

presence of diguanosine triphosphate and all four ribonucleoside triphosphates. Oocytes were incubated in modified Barth's saline solution containing 10% fetal calf serum. Newly synthesized proteins were detected by inclusion of 100  $\mu$ Ci/mL [<sup>35</sup>S]methionine in the incubation medium for periods up to 4 h, followed by incubation in fresh medium containing 1 mM cold methionine.

## RESULTS

Translation of plasmid pSPPPrP RNA in the rabbit reticulocyte lysate (RRL) generated the full-length nonglycosylated precursor, termed PrP<sub>0</sub> (Figure 1). In the absence of membranes or with membranes added after completion of translation (data not shown), PrP<sub>0</sub> was completely digestible by proteinase K (Figure 1, lanes A and B). When membranes were present during translation, products corresponding to the forms seen in wheat germ translations (Hay et al., 1987), namely, glycosylated and signal peptide cleaved (PrP<sub>2</sub>) and nonglycosylated but signal peptide cleaved (PrP<sub>1</sub>), were observed (Figure 1, lane C). When newly synthesized PrP in RRL was subjected to proteolysis, the characteristic immunoreactive cleavage fragments of the transmembrane form described by Hay et al. (1987) were generated: PrP-NH<sub>2</sub> (Figure 1, lane D) and PrP-COOH (data not shown). However, approximately two-thirds of the newly synthesized PrP<sub>2</sub> was found to be resistant to proteinase K digestion. Addition of the nondenaturing detergent Triton X-100 abolished the resistance to proteinase K digestion (Figure 1, lane E) and resulted in degradation of both PrP<sub>2</sub> as well as PrP-NH<sub>2</sub>. These results were consistent either with a protease-resistant variant of a membrane-bound PrP or with a completely translocated species.

To explore the topology of this protected form of PrP<sub>2</sub>, we turned to XO. By expressing PrP RNA in XO, we were able to demonstrate that a proportion of PrP is secreted from these cells. The expression of PrP transcripts in XO labeled with [<sup>35</sup>S]methionine is demonstrated in Figure 2. A product of

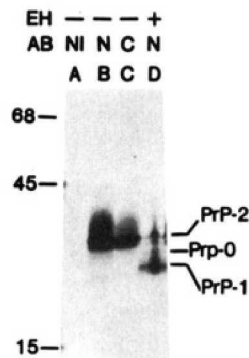


FIGURE 2: Expression of PrP in *Xenopus laevis* oocytes. Approximately 2–4 h after injection of transcript, oocytes were reinjected with [<sup>35</sup>S]methionine and incubated in Barth's saline solution. After 2 h of incubation, batches of oocytes were homogenized in 1% Triton X-100, 100 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 100 mM Tris-HCl (pH 8), and 1 mM phenylmethanesulfonyl fluoride and cleared of insoluble debris by centrifugation at 15000g for 5 min, and the supernatants were prepared for immunoprecipitation with PrP antisera (N, C) or nonimmune serum (NI) and endoglycosidase H (EH) digestion as previously described (Perara & Lingappa, 1985). Total translation products in the presence of dog pancreas microsomal membranes as described in Figure 1 were used for alignment of oocyte PrP immunoreactive products with PrP<sub>2</sub>, PrP<sub>1</sub>, and PrP<sub>0</sub>, as indicated to the right.

similar mobility to PrP<sub>2</sub> is observed upon immunoprecipitation with either amino- or carboxy-terminal peptide-specific sera but not with nonimmune serum (Figure 2, lanes A–C), followed by SDS-PAGE. Digestion of PrP immunoprecipitates with endoglycosidase H generated a product comigrating with PrP<sub>1</sub> (Figure 2, lane D). Thus, PrP expressed in XO after pulse labeling appears to be comparable to the PrP products of cell-free synthesis with respect to size, immunoreactivity, and glycosylation.

When XO injected with PrP transcripts (either alone or mixed with transcripts for globin, a cytoplasmic protein) were pulsed with methionine and then subjected to a cold chase for periods of 12, 24, 48, and 96 h, secretion of PrP into the medium was observed (Figure 3). PrP secreted into the medium was completely proteinase K digestible in the absence of detergent (data not shown). Terminal processing of carbohydrates of intracellular PrP, presumably in transit through the Golgi apparatus, results in heterogeneity (PrP in Figure 2 compared to Figure 3) as well as a shift in mobility by SDS-PAGE of PrP in homogenates as compared to medium (Figure 3, lanes A, C, E, and G compared to lanes B, D, F, and J). Thus, PrP appears to traverse the secretory pathway in XO. Coinjected transcripts encoding the cytosolic polypeptide globin demonstrated that subcellular integrity of XO was maintained throughout the course of the experiment. Even 5 days after expression of both globin and PrP, globin immunoreactive chains were detectable exclusively intracellularly (Figure 3, lane I, upward-pointing arrowhead) while approximately 60% of PrP chains was chased into the medium (Figure 3, lane G vs J, upward-pointing arrowhead). The globin controls establish that the appearance of PrP in the media is not the result of nonspecific protein leakage from the XO.

Sucrose gradient sedimentation was used to characterize the apparent *s* value of the secreted PrP product in the XO medium (Figure 4), compared to cytochrome *c* and bovine serum albumin, globular proteins of defined sedimentation coefficients and molecular weights. These experiments demonstrated that

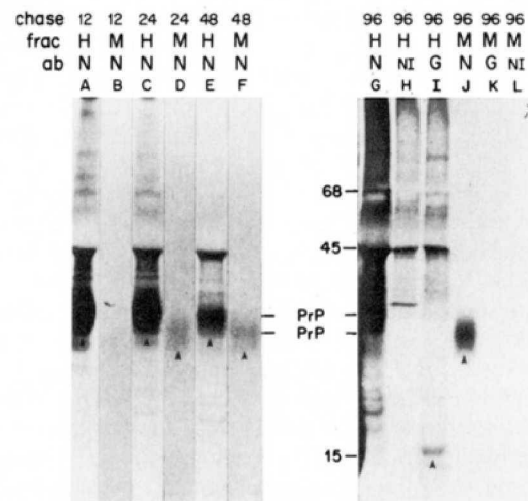


FIGURE 3: Time course of PrP secretion from *Xenopus* oocytes. Stage VI *Xenopus laevis* oocytes were dissected and injected with 50 nL each of PrP transcript alone (12-, 24-, 48-h time points) or mixed with chimpanzee globin transcript (96-h points). Microinjected oocytes were incubated in Barth's saline solution containing 5 mCi/mL [<sup>35</sup>S]methionine for 5 h before transfer to Barth's saline solution containing 20 mM unlabeled methionine,  $5 \times 10^{-4}$  M cycloheximide, 5% fetal calf serum, and penicillin, streptomycin, and gentamycin for the indicated chase time periods. Media were collected, oocytes washed, and all samples subjected to immunoprecipitation as described elsewhere (Simon et al., 1987). Fraction or frac refers to either oocyte (H) or medium (M) immunoprecipitated with either anti-PrP amino-terminal peptide serum (N), nonimmune serum (NI), or rabbit anti-human globin serum (G). The upward-pointing band refers to PrP in oocytes (lanes A, C, E, and G) or medium (lanes B, D, F, and J) or to globin in oocytes (lane I). Molecular weights  $\times 10^3$  are indicated as are the positions of PrP from homogenates and medium. Each sample represents products from 10 oocytes incubated in 10  $\mu$ L of medium per oocyte.

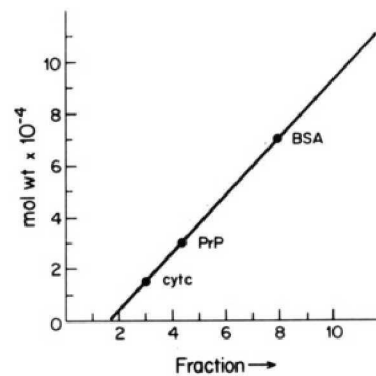


FIGURE 4: Characterization of PrP secreted from *Xenopus* oocytes by sucrose gradient ultracentrifugation. Samples of [<sup>35</sup>S]-methionine-labeled oocyte medium (50  $\mu$ L) were mixed with 10–20  $\mu$ g of horse heart cytochrome *c* and bovine serum albumin and applied to 5-mL 5–20% sucrose gradients containing Barth's saline solution. Samples were centrifuged in an SW50 rotor for 20 h at 45000 rpm and fractionated into 25 200- $\mu$ L aliquots, which were divided into samples applied directly to SDS-PAGE and others subjected first to immunoprecipitation with PrP antisera. Peaks of cytochrome *c* and bovine serum albumin were determined from the total gradient sample gel, while the PrP peak was determined from a fluorogram of immunoprecipitates and plotted as a function of known molecular weights of marker proteins.

PrP was secreted as a soluble monomeric protein and not as an oligomer. The observed sedimentation coefficient of secreted PrP was  $\sim 4.5$  S, a value intermediate between those of the marker proteins and consistent with a monomeric globular protein of  $\sim 30$  kDa. While it is possible that secreted PrP actually exists as a dimer of unusual shape giving a falsely low *s* value, these results rule out a possible role for vesicle budding or particulate protein micelle formation as mecha-

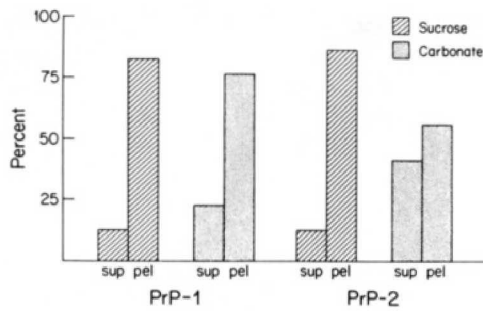


FIGURE 5: Carbonate extraction of PrP translation products. Cell-free translation of PrP was carried out in the presence of dog pancreas microsomal membranes as previously described (Hay et al., 1987). Ten-microliter aliquots of products were added to 1 mL of either 0.25 M sucrose, 10 mM Tris-HCl (pH 7.5) (sucrose), or 0.1 M sodium carbonate (pH 11.5) (carbonate) at 4 °C and incubated for 30 min. Samples were then subjected to ultracentrifugation at 50 000 rpm in a Beckman 50.2 Ti rotor for 1 h. Supernatants were removed carefully and pellets rinsed once with ice-cold distilled water. Supernatants were then adjusted to pH 7.5 with glacial acetic acid and precipitated with 2 volumes of 15% trichloroacetic acid, washed once with ethanol-ether (1:1), and prepared for SDS-PAGE. Pellets were resuspended in 100  $\mu$ L of 1% SDS and 0.0 M Tris-HCl (pH 8.9) and treated similarly. PrP<sub>1</sub> and PrP<sub>2</sub> bands were identified from fluorograms and ratios in supernatant and pellet fractions as determined by quantitative densitometry with an LKB 2222 Ultrascan XL laser densitometer.

nisms of PrP secretion. It seemed likely that secreted PrP from XO corresponds to the protease-protected fraction of PrP<sub>2</sub> found in RRL.

Since the extraction of membrane vesicles with alkaline sodium carbonate (pH 11.5) has proved useful in distinguishing integral membrane proteins from those that are either loosely bound or contained within vesicles, we utilized this procedure to study PrP<sub>1</sub> and PrP<sub>2</sub> synthesized in RRL. PrP translation products were extracted with either sucrose buffer (pH 7.5) or sodium carbonate (pH 11.5). Whereas the former treatment leaves the vesicles intact, the latter treatment strips off peripheral proteins from vesicles and releases secreted proteins from the interior of vesicles while integral membrane proteins remain attached to the residual membrane lipid bilayer (Fujiki et al., 1982). More than 80% of both PrP<sub>1</sub> and PrP<sub>2</sub> were sedimented with the vesicles through sucrose buffer (Figure 5). Carbonate extraction of PrP<sub>1</sub> gave results similar to those obtained with sucrose, suggesting that most of the PrP<sub>1</sub> is tightly bound to the membrane. In contrast, almost half of PrP<sub>2</sub> was released upon carbonate extraction (Figure 5). These results are consistent with the hypothesis that PrP<sub>2</sub> exists in two different forms: the carbonate-extractable form being equivalent to the secretory form observed with XO and the carbonate-resistant form tightly bound or integrated into membranes.

Thus, while PrP<sub>1</sub> is composed almost entirely of transmembrane polypeptides, PrP<sub>2</sub> appears to consist of both transmembrane and secreted molecules. The existence of membrane-bound PrP is not surprising in view of the extensive literature demonstrating an association of both PrP and scrapie infection with membranes (Millson et al., 1971; Semancik et al., 1976; Prusiner et al., 1978; Meyer et al., 1986; Gabizon et al., 1987). However, the existence of a secreted form of the same polypeptide that spans the bilayer twice in its integral membrane configuration was unexpected.

To explore further the biogenesis of secreted PrP, we asked if secreted PrP was derived from membrane PrP or vice versa. To investigate the possibility of a precursor product relationship between the two forms of PrP, aliquots of membrane-supplemented translation reaction were removed at the earliest

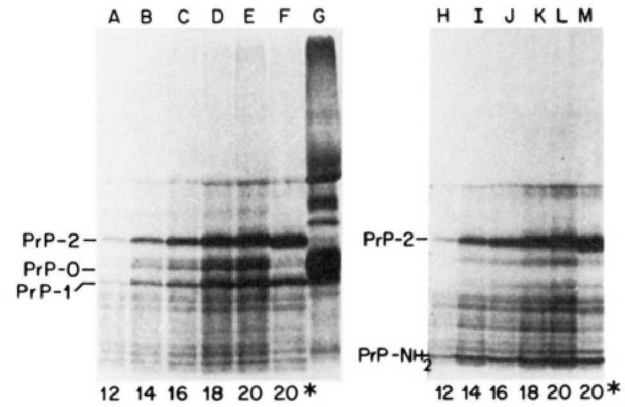


FIGURE 6: Kinetics of secretory and membrane PrP synthesis. Cell-free transcription-linked translation was carried out in RRL as described in Figure 1, except that aliquots were taken at various times of incubation (12, 14, 16, 18, and 20 min) and protein synthesis was terminated by addition of cycloheximide, and samples were transferred to ice. In some cases (20\*) incubation was continued after addition of cycloheximide for an additional 60 min at 24 °C before transferring to ice. Half of each sample was subjected to proteolysis as in Figure 1, and total products before (lanes A-F) and after (lanes H-M) proteolysis were analyzed by SDS-PAGE. Time points are indicated below each lane; positions of PrP<sub>2</sub>, PrP<sub>0</sub>, PrP<sub>1</sub>, and PrP-NH<sub>2</sub> are indicated. Lane G contains translation products in the absence of membranes.

time point when completed chains of PrP were found. The immunoprecipitated PrP was analyzed by SDS-PAGE either directly or after proteinase K digestion (Figure 6, lanes A-E and H-L) to determine the relative amounts of membrane and secretory forms of PrP. An additional aliquot was removed at 20 min and treated with inhibitors of protein synthesis, and incubation was allowed to continue for an additional 40 min in the absence of further protein synthesis (lane F). After completion of incubation, this aliquot was digested with proteinase K at 4 °C (lane M). Both the membrane and secretory forms of PrP were demonstrable from the earliest time point at which completed chains of PrP were detected. Furthermore, the ratio of the two PrP forms remained relatively constant throughout the time course of the experiment (Figure 6). Densitometry of lanes E and F, compared to lanes L and M, revealed that further incubation in the absence of protein synthesis did not alter the ratio of the two PrP forms. Hence, it appears the nascent chains of PrP are committed to either integral transmembrane or secretory fates either during or immediately after their synthesis. Once committed to one fate or the other, no detectable precursor product relationship could be demonstrated between the two completed forms.

To explore the role of the N-terminal signal peptide of PrP, mutants were constructed in which the amino-terminal signal sequence codons of PrP were either deleted or replaced in frame with a sequence including the signal sequence of *E. coli* lactamase. Previously, one of us (V.R.L.) has shown that this signal sequence is competent to direct translocation of heterologous passenger sequences even when those passengers are normally cytoplasmic proteins (Lingappa et al., 1984).

We found that, in the absence of its amino-terminal 14 codons (pSPGM2H), PrP was expressed exclusively as a cytoplasmic protein completely digested by proteinase K, with no evidence for glycosylated, integral membrane, or secretory species (Figure 7, lanes A-D). When PrP was provided with the lactamase signal sequence (pSPGM2G), it was expressed as both integral membrane and secretory forms in cell-free systems (Figure 7, lanes E-I). While an amino-terminal signal sequence (native or heterologous) is required to generate both the membrane and secretory forms of PrP, some element other

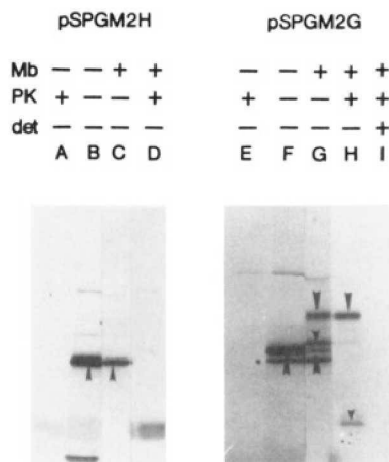


FIGURE 7: PrP topology is directed by signal sequences. Expression plasmids behind an SP6 promoter were prepared for a partial cDNA of PrP lacking the amino-terminal 14 codons compared to the full-length PrP cDNA (pSPGM2H, lanes A–D) and with the lactamase signal sequence engineered in its place (pSPGM2G, lanes E–I). Plasmids were expressed by transcription-linked translation in rabbit reticulocyte lysates in the absence (lanes A, B, E, and F) or presence (lanes C, D, G–I) of dog pancreas microsomal membranes as described previously (Perara & Lingappa, 1985). In some cases, products were digested with proteinase K in the absence (lanes A, D, E, and H) or presence (lane I) of 0.5% Triton X-100. All samples were subjected to immunoprecipitation with PrP-anti-NH<sub>2</sub> peptide antisera and analyzed by SDS-PAGE autoradiography as described (Perara & Lingappa, 1985). The upward-pointing arrow refers to the full-length product of pSPGM2H (lanes B and C) and pSPGM2G (lanes F and G). The downward-pointing arrow (lanes G and H) and arrowhead (lane G) refer to glycosylated forms of product encoded by pSPGM2G expressed in the presence of dog pancreas microsomal membranes, as determined by endoglycosidase H digestion (data not shown). The downward-pointing arrowhead in lane H refers to the nonglycosylated amino-terminal protected fragment of pSPGM2G corresponding to PrP-NH<sub>2</sub> in Figure 1. The band marked with an asterisk (\*) in lane F is presumed to be an internal initiation product.

than the signal peptide must control the fate of PrP as it is synthesized.

## DISCUSSION

The finding that PrP RNA can be translated in both cell-free systems and XO to produce both membrane and secretory forms of PrP is intriguing. Whether these results accurately reflect the topology of PrP in brain and other organs remains to be established. Previous work has suggested that these systems proceed with high fidelity.

From the studies reported here and those previously (Hay et al., 1987), we conclude that both the membrane and secretory forms of PrP synthesized in cell-free systems or XO resemble PrP<sup>C</sup> and are distinct from PrP<sup>Sc</sup>. Other investigations have suggested that PrP<sup>Sc</sup> and PrP<sup>C</sup> probably have the same amino acid sequence but differ in their posttranslational modifications (Basler et al., 1986). Thus, we conclude that, under the conditions of our experiments, the posttranslational modifications necessary to create PrP<sup>Sc</sup> are not occurring.

Earlier studies on the biogenesis of PrP demonstrated a transmembrane orientation for this protein in cell-free systems; moreover, the membrane form of PrP was shown to span the bilayer twice (Hay et al., 1987). The deduced amino acid sequence of PrP reveals a transmembrane region between amino acid residues 90 and 114 that contains a segment of 24 hydrophobic and uncharged amino acids (Oesch et al., 1985; Bazan et al., 1987; Hay et al., 1987). The second transmembrane region from approximately residue 135 to residue 154 is less typical for a transmembrane segment, containing at least three charged amino acids and a poor

overall hydrophobicity index (Bazan et al., 1987; Hay et al., 1987). Just why these two regions span the bilayer and whether PrP biogenesis would proceed in this unusual fashion if they were replaced by other considerably more or less hydrophobic transmembrane sequences remain to be determined.

Our studies show that the same molecule shown to span the membrane twice is also found secreted, not only in cell-free systems but also from XO. Topogenic sequences appear to participate in biogenesis of both of these forms since deletion of the amino-terminal signal abolishes both forms which can be regenerated by complementation with a heterologous signal sequence. Whether the dual secretory nature and transmembrane nature of this polypeptide are the result of heretofore undescribed topogenic sequences (e.g., a stop-transfer override sequence) or novel forms of signal and stop-transfer sequences themselves or simply of properties specific to domains of PrP, which modify the function of otherwise prototypic signal and stop-transfer sequences, remains to be established. It has recently been demonstrated (Mize et al., 1986) that stop-transfer sequences recognize a subset of receptors in common with signal sequences and can, in certain contexts, initiate translocation. If the first transmembrane region of PrP is a stop-transfer sequence, perhaps under certain physiologic conditions its translocation termination function is subsumed to its potential for initiation of translocation.

In nature, several strategies have been discovered whereby the subcellular disposition of a protein is converted from integral transmembrane to secretory. One strategy typified by immunoglobulin M involves RNA splicing to generate two distinct mRNAs encoding secretory and transmembrane species of a given polypeptide as a consequence of insertion or deletion of a stop-transfer coding region (Early et al., 1980). A second strategy is selective proteolytic release of an extra-cytoplasmic domain of an integral membrane polypeptide as is the case in conversion of the IgA receptor into secretory component (Mostov et al., 1980). A third recognized pathway is conversion of an intracellular integral membrane polypeptide into a secretory particle by a process of aggregation in the plane of the endoplasmic reticulum membrane, lipid exclusion and extrusion of a particulate protein micelle into the vesicle lumen. Such a process has been proposed for hepatitis B virus surface antigen (Eble et al., 1986). Still another mechanism involves the addition of a glycolipid linkage to anchor an otherwise secreted polypeptide to membranes (Bangs et al., 1986). All of these mechanisms involve posttranscriptional or posttranslational processes. The features described for integral transmembrane and secreted PrP as described here are unprecedented in that they appear to involve control at the level of endoplasmic reticulum membrane, a biosynthetic pathway heretofore considered to be constitutive and not subject to regulation. Further work should reveal whether or not this novel form of control of protein phenotype is a general mechanism of regulation of gene expression. Recent studies have demonstrated that both PrP<sup>Sc</sup> and PrP<sup>C</sup> possess covalently attached glycolipids, making them possibly the first example of a transmembrane protein that can also undergo glycolipid addition (Stahl et al., 1987). Alternatively, glycolipid addition may occur only when PrP is synthesized or translocated in a nontransmembrane form. More studies are needed to clarify the membrane associations of the cellular and scrapie PrP isoforms before the relationship between the transmembrane, secretory, and glycolipid-anchored forms of these molecules can be accurately assessed.

The results presented in this paper raise many questions about the role of PrP in cellular metabolism as well as in the

pathogenesis of scrapie. The function of PrP<sup>C</sup> remains undefined; whether PrP<sup>C</sup> is primarily a membrane protein or secretory molecule remains to be established. While considerable evidence implicates PrP<sup>Sc</sup> in the pathogenesis of scrapie, little is known about the mechanism of neuronal degeneration caused by the disease. Our finding of a secreted form of PrP suggests that the spread of scrapie prions from cell to cell might occur through a mechanism involving PrP<sup>Sc</sup> secretion. Evidence for an extracellular form of PrP in the disease is well documented since PrP<sup>Sc</sup> molecules have been shown to polymerize into amyloids and the amyloid plaques in scrapie-infected brains contain PrP immunoreactive amyloid filaments (Prusiner et al., 1983; Bendheim et al., 1984; DeArmond et al., 1985). As we learn more about the chemical structure of the PrP isoforms, this knowledge should help in elucidating the mechanisms responsible for producing both the transmembrane and secretory forms of PrP.

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