Scrapie and Cellular Prion Proteins Differ in Their Kinetics of Synthesis and Topology in Cultured Cells

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Abstract. Both the cellular and scrapie isoforms of the prion protein (PrP) designated PrP^C and PrP^{Sc} are encoded by a single-copy chromosomal gene and appear to be translated from the same 2.1-kb mRNA. PrP^C can be distinguished from PrP^{Sc} by limited proteolysis under conditions where PrP^C is hydrolyzed and PrP^{Sc} is resistant. We report here that PrP^C can be released from the surface of both normal-control and scrapie-infected murine neuroblastoma (N$_2$a) cells by phosphatidylinositol-specific phospholipase C (PIPLC) digestion and it can be selectively labeled with sulfo-NHS-biotin, a membrane impermeant reagent. In contrast, PrP^{Sc} was neither released by PIPLC nor labeled with sulfo-NHS-biotin. Pulse-chase experiments showed that [^{35}S]methionine was incorporated almost immediately into PrP^C while incorporation into PrP^{Sc} molecules was observed only during the chase period. While PrP^C is synthesized and degraded relatively rapidly ($t_{1/2} \sim 5$ h), PrP^{Sc} is synthesized slowly ($t_{1/2} \sim 15$ h) and appears to accumulate. These results are consistent with several observations previously made on rodent brains where PrP mRNA and PrP^C levels did not change throughout the course of scrapie infection, yet PrP^{Sc} accumulated to levels exceeding that of PrP^C. Our kinetic studies demonstrate that PrP^{Sc} is derived from a protease-sensitive precursor and that the acquisition of proteinase K resistance results from a posttranslational event. Whether or not prolonged incubation periods, which are a cardinal feature of prion diseases, reflect the slow synthesis of PrP^{Sc} remains to be established.

O VER the last five years, there has been a remarkable convergence of information about the prion protein (PrP)[1] (26, 39). Biochemical studies have shown that scrapie prion infectivity and a protease-resistant core of scrapie prion protein (PrP^{Sc}) designated PrP 27-30 copurify (27, 28). Furthermore, prolonged exposure of purified prion fractions to proteases is accompanied by a concomitant degradation of PrP 27-30 and a loss of scrapie infectivity (21). Solubilization of scrapie prions into detergent-lipid-protein complexes (DLPCs) (13) made possible immunoaffinity chromatography with PrP monoclonal antibodies demonstrating the copurification of PrP^{Sc} and scrapie infectivity (14). Neutralization of scrapie infectivity in DLPCs with PrP antiserum has also been observed. Human and hamster prions purified by limited proteolysis, detergent extraction, differential centrifugation and sucrose gradient sedimentation were found to aggregate into insoluble, rod-shaped structures indistinguishable from many purified amyloids (6, 28). This observation was extended to the brains of humans and animals dying of prion diseases where amyloid plaques were found to stain intensely with PrP antibodies (17, 32).

The foregoing studies argue persuasively that PrP^{Sc} is a major and necessary component of the infectious prion. Molecular cloning studies established that PrP^{Sc} is encoded by a single-copy, chromosomal gene and not by a putative nucleic acid hiding within the prion (4, 10, 25). This is an important feature distinguishing prions from viruses. Although the PrP gene is highly regulated in the developing brain, it is constitutively expressed during the adult life of rodents (22, 24, 25). The product of the PrP gene in normal animals and humans is a protein designated PrP^C. Many lines of evidence suggest that PrP^C and PrP^{Sc} have the same amino acid sequence but differ in their properties because of an as yet unidentified posttranslational event (3, 4).

Until recently, cell culture systems have been disappointing with respect to extending our knowledge of prions since prion titers are low and there is no recognizable cytopathic effect associated with scrapie infection. The identification of PrP^C on the surface of cultured cells (37) and PrP^{Sc} in extracts of these cells (8) suggested that scrapie-infected cells in culture might be used to study PrP^{Sc} synthesis. We report here that PrP^C can be released from the surface of both normal-control and scrapie-infected murine neuroblastoma (N$_2$a) cells by phosphatidylinositol-specific phospholipase C (PIPLC) digestion and it can be selectively labeled with sulfo-NHS-biotin, a membrane impermeant reagent. In contrast, PrP^{Sc} was neither released by PIPLC nor labeled with

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1. *Abbreviations used in this paper:* DLPC, detergent-lipid-protein complex; N$_2$a, murine neuroblastoma; PIPLC, phosphatidylinositol-specific phospholipase C; PrP, prion protein; PrP^C, cellular isoform of the prion protein; PrP^{Sc}, scrapie isoform of the prion protein; Sarkosyl, sodium dodecyl sarcosinate; ScN$_2$a, scrapie-infected murine neuroblastoma.

sulfo-NHS biotin. Pulse-chase experiments showed that [^{35}S]methionine was incorporated almost immediately into PrP^C while incorporation into PrP^{Sc} molecules was delayed for several hours. While PrP^C is synthesized and degraded relatively rapidly, PrP^{Sc} is synthesized slowly and appears to accumulate. The kinetic findings reported here demonstrate that PrP^C and PrP^{Sc} have different rates of synthesis and degradation and that these differences arise from posttranslational events.

Materials and Methods

Reagents

PIPLC was a gift of Dr. Martin Low (Columbia University, NY). One unit of PIPLC will digest 1 μmol of phosphatidylinositol in 1 min (19). Proteinase K used in digestion of cellular extracts is a product of Beckman Instruments Inc. (Palo Alto, CA). Reagents for cell culture were obtained from the University of California San Francisco Cell Culture Facility except for Opti-MEM I which is a product of Gibco Laboratories (Grand Island, NY). Detergents for the extraction of cells and the solubilization of cellular proteins were obtained from Sigma Chemical Co. (St. Louis, MO) as was PMSF and aprotinin. PAGE reagents were obtained from Bio-Rad Laboratories (Richmond, CA).

Cell Lines

The uninfected N2a cell line used in this study was obtained from American Type Tissue Culture Collection. A clonal cell line of scrapie-infected N2a cells (ScN2a) was found to produce $\sim$10^5 ID$_{50}$ U of prions/10^7 cells, and protease-resistant PrP^{Sc} (8). The ScN2a subclone reported here was in continuous passage for $\sim$18 mo and continued to produce PrP^{Sc} at a constant level. Cell lines were maintained in DME with 10% FBS and penicillin/streptomycin in humidified incubators containing 5% CO$_2$. In general, scrapie-infected cultures were passaged every 7-10 d after a 1:5 or 1:10 split. Uninfected N2a cells were maintained in a 1:1 mixture of DME (+10% FBS) and Opti-MEM I (Gibco Laboratories) supplemented with penicillin and streptomycin, and passaged as required in 1:20 splits.

PrP Antibodies

All of the antisera used in this study to detect PrP^C and PrP^{Sc} molecules were prepared as previously described (2, 3, 5). Anti-PrP 27-30 rabbit sera R017 and anti-PrP peptide P1 rabbit antisera R013 have been described elsewhere and shown to react specifically with PrP^{Sc} and PrP^C molecules produced by ScN2a cells (2, 3, 8). Anti-PrP 27-30 rabbit sera R073 was recently produced (35). This antisera recognizes PrP^{Sc} or PrP^C molecules in extracts of N2a cells at a 1:5,000 dilution. R073 antiserum was used in Fig. 1 while immune sera R013 or R017 was employed in Figs. 2-9.

Extraction of Cellular Proteins for Immunoblot Analysis and Immunoprecipitation

Radiolabeled and nonradioactive cellular proteins were extracted from cells in detergent essentially as previously described (8, 34). Before extraction, cultures were washed 2-3 times in PBS, then harvested with a disposable scraper and pelleted by centrifugation. Cell pellets were then resuspended in 0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.005 M EDTA (TNE) and lysed on ice by adding Triton X-100 and deoxycholate to 0.5%. Insoluble material was then removed by centrifugation at 3,000 g before precipitation of protein extracts with at least 4 vol of methanol at -20°C for 30 min. After centrifugation at 5,000 g for 20 min, pellets were resuspended in TNE with 0.2% sodium dodecyl sarcosinate (Sarkosyl) and digested with 10-20 μg/ml proteinase K for 30-60 min at 37°C, where applicable. Proteinase K digestions were terminated by addition of PMSF to 2-5 mM and methanol precipitation (4 vol). We have recently modified this procedure and found that more efficient extraction was obtained when cells were treated directly on the culture vessel in 1-2 ml of ice-cold TNE, 0.5% Triton X-100, 0.5% deoxycholate. After removal of insoluble material by centrifugation, proteinase K digestion (10-20 μg/ml, 37°C, 60 min) can be carried out directly in the lysis buffer. All samples are then concentrated by methanol precipitation (4-10 vol) before immunoblot analysis or immunoaffinity purification. This

modified procedure was used for the experiments depicted in Figs. 5, 6 and 7. Each of these methods of extraction as well as proteinase K digestion yielded similar results.

Proteins released into the medium by PIPLC were concentrated by precipitation with 4 vol of methanol before further analysis. EDTA was added to a final concentration of 5 mM before methanol precipitation to reduce the formation of insoluble CaPO$_4$ precipitates.

Immunoblot Analysis

After PAGE separation of cellular proteins, PrP molecules were detected by electro-transfer to nitrocellulose membrane in 0.02 M Tris, 0.15 M glycine, 20% methanol, 0.02% SDS at 500 mA overnight at 4°C. After transfer, membranes were washed briefly in H$_2$O, then washed for 1 h in PBS with 5% nonfat dry milk (Carnation). After two additional washes in 0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl, 0.05% Tween 20 (TBST) (10 min each), membranes were further incubated in TBST with 10% horse serum for 45-60 min. After removal of the TBST serum, membranes were incubated overnight in TBST with anti-PrP sera as described in the figure legends. Complexes between antibodies and prion proteins were then revealed using Promega anti-rabbit Ig antibodies coupled to alkaline phosphatase as described by Promega Biotec (Madison, WI).

Immunoprecipitation

Methanol-precipitated cellular proteins were resuspended in DLPC buffer consisting of 0.5-2 ml of 0.05 M Tris-HCl pH 8.2, 0.15 M NaCl, 2% (wt/vol) Sarkosyl, 0.4% (wt/vol) phosphatidylcholine with 10^5 U/ml of aprotinin and sonicated for at least 3 min. This procedure forms DLPCs that enhance the solubility of PrP^{Sc} and allows immunoaffinity purification (14).

Anti-PrP serum was added at a 1:250 or 1:500 dilution, and after overnight incubation at 4°C, protein-antibody complexes were adsorbed to protein A-Sepharose (50 μl/500 μl of solubilized protein) for 30-60 min. Protein A-adsorbed material was then washed in DLPC buffer for 30 min at 4°C. This wash was followed by a second wash in 0.05 M Tris-HCl, pH 8.2, 0.5 M NaCl, 1% Sarkosyl (TN-1% Sarkosyl). After 30 min, protein A-Sepharose was transferred to a clean microfuge tube and further washed in TN-1% Sarkosyl 3 times for 5 min each. Protein A-Sepharose was then resuspended in 0.06275 M Tris-HCl pH 6.8, 3% SDS, 25% sucrose, 1% β-mercaptoethanol, 0.015% bromophenol blue, and boiled for 5 min before PAGE and autoradiography as described (34).

Metabolic Pulse-Chase Radiolabeling Experiments

Cell cultures were starved in methionine-free DME with 10% dialyzed FBS for 1 h prior to labeling. Labelings were generally for 2 h with 300 μCi/ml [^{35}S]methionine in methionine-free DME containing 2.5% dialyzed FBS. Chase incubations were performed in regular cell cutlure medium (DME with 10% FBS) or in Opti-MEM I (Gibco Laboratories), a defined serum-free medium. Cells were harvested by extraction as described above. In some experiments chase medium (Opti-MEM I) was also harvested by precipitation with 4 vol of methanol.

Densitometry of [^{35}S]-Met-Labeled PrP

Autoradiographic signals were quantitated by densitometric scanning using a model 620 instrument (Bio-Rad Laboratories). In each experiment, the relative amount of PrP present at any specific time during chase incubations is regarded as a percentage of the quantity of nascent PrP recovered immediately following the pulse-labeling period. The best fit to an exponential was calculated with a computer program that performs a nonlinear fit by minimizing the sums of the squares of the residuals between observed and calculated y-values.

Results

PIPLC Digestion Releases PrP^C but not PrP^{Sc}

The PIPLC-catalyzed release of PrP^C from the surface of ScN$_2$a cells is depicted in Fig. 1 A. Without PIPLC, little or no detectable PrP was found in the medium (lane 1) and most of the PrP immunoreactivity remained cell associated (lane 2). Proteinase K digestion of ScN$_2$a cell extracts revealed
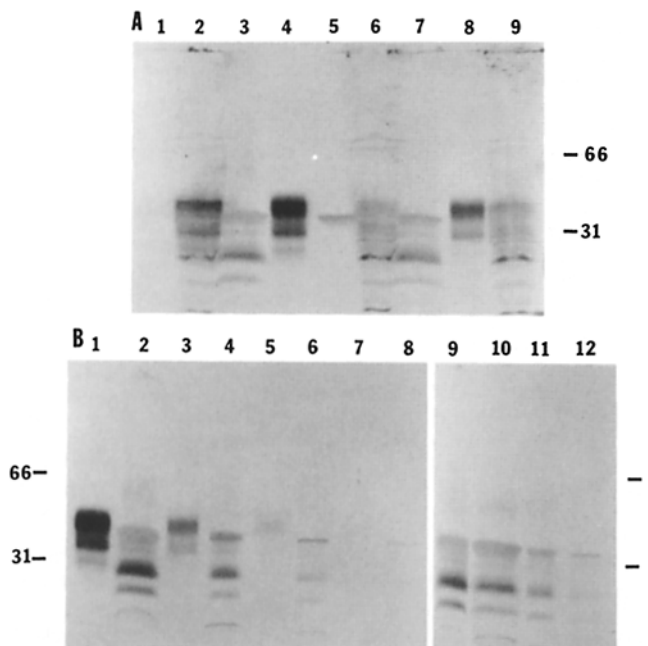
Figure 1. ScN₂a cells release PrP^C after exposure to PIPLC while PrP^Sc remains cell associated. This is an immunoblot that was developed with PrP antiserum R073. (A) Confluent 10-cm dishes of ScN₂a cells were exposed to PIPLC (0.2 U/ml) in PBS or PBS alone at room temperature for 3 h. Cells and medium were extracted or harvested, and digested with proteinase K as described in Materials and Methods. Lane *1* is culture medium without PIPLC. Lanes *2* and *3* contain extracts of untreated cells incubated without and with proteinase K, respectively. Lanes *4* and *5* are PIPLC-released proteins incubated without and with proteinase K, respectively. Lanes *6* and *7* are extracts of PIPLC-treated cells incubated without (lane *6*) or with proteinase K (lane *7*). Lane *8* contains the PIPLC-released PrP from one-half plate of ScN₂a cells while lane *9* contains a mixture of the other half of the sample in lane *8* and an extract of one-half a plate of ScN₂a cells. (B) Confluent cultures of ScN₂a cells were exposed to PIPLC (0.2 U/ml) in PBS. Cells were harvested, extracted in detergent, and proteinase K digested before comparative immunoblot analysis of PIPLC-released proteins and digested cell extracts. Lanes *1*, *3*, *5*, and *7* contain serial 1:3 dilutions of proteins released from one 10-cm dish of ScN₂a cells by PIPLC. Lanes *2*, *4*, *6*, and *8* contain serial 1:3 dilutions of proteins found in proteinase K-digested extracts of one 10-cm dish of ScN₂a cells. Lanes *9–12* are a control that shows that N₂a cells do not normally produce proteins which interfere with PrP^Sc immunoblot transfer and detection. Lane *9*, proteinase K-digested ScN₂a extract. Lane *10*, proteinase K-digested ScN₂a extract mixed with an equivalent amount of proteinase K-digested extract of uninfected N₂a cells. Lanes *11* and *12* are serial 1:3 dilutions of sample electrophoresed in lane *9*.

the characteristic pattern of PrP^Sc (lane *3*), which is found in prion-infected N₂a cells (8). PrP was found in the medium of ScN₂a cells digested with PIPLC (lane *4*); however, this protein was degraded by proteinase K (lane *5*) demonstrating that these molecules behave like PrP^C. The band seen in lane *5* is proteinase K, which reacts with some of our antisera raised against PrP 27-30 as previously described (5). In contrast, PrP^Sc was not released by PIPLC (lanes *6* and *7*). The immunostaining of PrP^C in cell extracts was problematic but was greatly enhanced after partial purification by PIPLC digestion, which released it into the medium (com-

pare lanes *2* with *4*). The poor immunostaining of PrP^C in cell extracts probably results from competition with cellular proteins for binding to the transfer membrane since mixing of a cell extract with PrP^C recovered after PIPLC (lane *8*) greatly reduced the immunostaining intensity (lane *9*).

To assess the relative amounts of PrP^C and PrP^Sc in ScN₂a cells, serial dilutions of the PrP^C released into the medium by PIPLC were compared to proteinase K-digested cell extracts (Fig. 1 *B*, lanes *1–8*). Since the immunostaining of the two samples exhibited a parallel decrease with the same dilutions, we conclude that ScN₂a cells contain roughly similar amounts of PrP^C and PrP^Sc. This estimate must be tempered because we are uncertain about the relative efficiencies of PrP^C and PrP^Sc transfer and binding to nitrocellulose as well as their immunoreactivity. PrP^Sc immunostaining was diminished by no more than a factor of 2 if mixed with an equivalent amount of proteinase K-digested extract from uninfected N₂a cells (lanes *9–12*).

All of the ScN₂a cells expressed PrP^C on their surface as assessed by indirect immunofluorescence (Fig. 2). The complete disappearance of immunostaining after PIPLC digestion of ScN₂a cells indicates that PrP^Sc cannot be visualized under the conditions used for these studies. Control N₂a cells before and after PIPLC digestion (in Fig. 2, *e* and *f*) are indistinguishable from the ScN₂a cells in PrP immunostaining.

PrP^Sc in N₂a Cells

The ScN₂a cell line used in this work is a clonal cell line originally isolated by Butler and colleagues (8). Other investigators have also reported long-term cultivation of ScN₂a cells (9, 30, 31). We have maintained this clonal line in continuous culture for over 18 mo. Occasionally cultures were found in which the PrP^Sc content was substantially diminished. However, we observed no decrease in the level of PrP^Sc production in most instances. 25 subclones were examined after ∼6 mo of cultivation. All were found to produce PrP^Sc molecules. 10 additional subclones were isolated after ∼12 mo of continuous cultivation. Again, all were found to produce PrP^Sc molecules although one subclone produced significantly less than the parental population.

During the course of this work, we have made several observations on the cultivation of ScN₂a cells that warrant comment. We found that passage of the cultures every 7–10 d split at a 1:5 or 1:10 ratio enhanced the reproducibility of experiments as well as the stability of the cell line. Cells that were stored in liquid nitrogen became unstable after thawing and subcultivation, and produced less PrP^Sc. In fact, less than one-half of new subclones isolated from cells that had been stored in liquid nitrogen for 6 mo produced PrP^Sc (data not shown).

Topology of PrP^C Differs from PrP^Sc

One explanation for the inability of PIPLC to release PrP^Sc from ScN₂a cells could be that PrP^Sc is not transported to the cell surface. To investigate the topology of PrP^Sc in ScN₂a cells, we employed a membrane impermeant reagent (sulfo-NHS-biotin) that covalently couples biotin to free amino groups of cell surface proteins (16). In control experiments, crude microsome preparations of scrapie-infected mouse brain were exposed to sulfo-NHS-biotin, digested
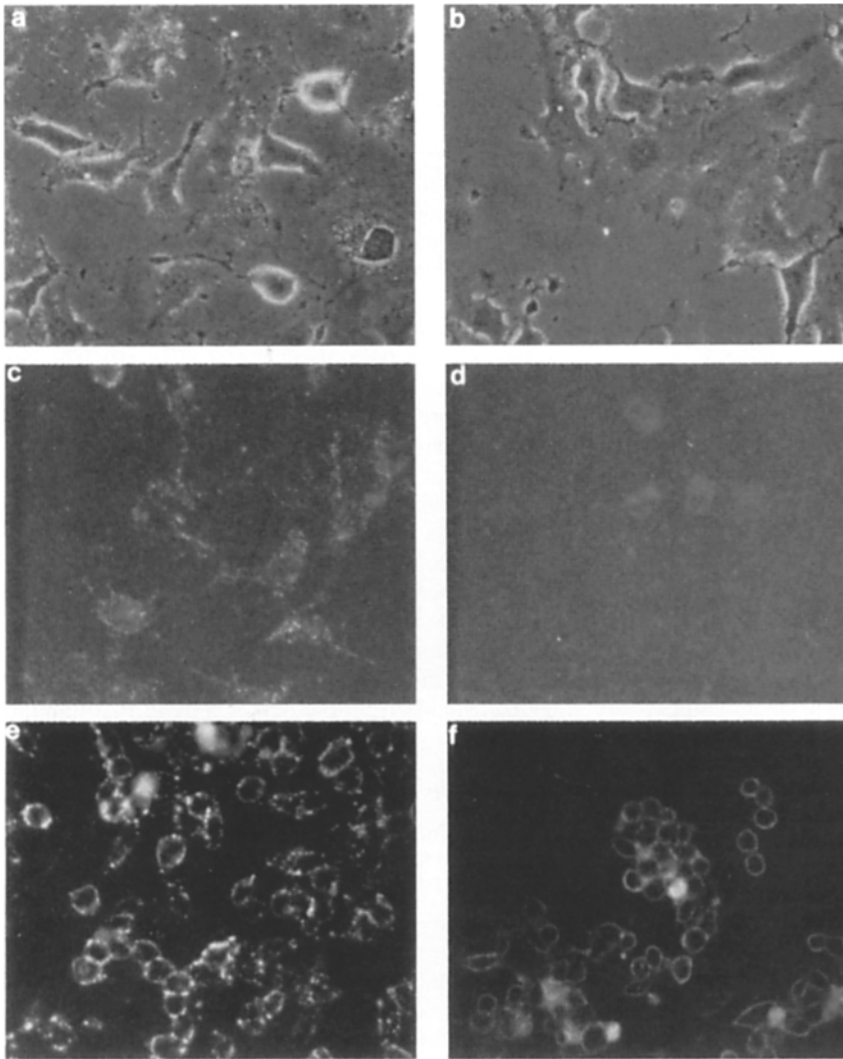
Figure 2. Indirect immunofluorescence of PrP on the surface of normal and infected N₂a cells. Control and ScN₂a cells were plated onto glass microslide culture vessels. Cells were then incubated without (*a, c,* and *e*) or with (*b, d,* and *f*) PIPLC (0.2 U/ml) in PBS for 3 h at room temperature, and then incubated with anti-PrP serum R017 and a fluorescein-conjugated goat anti-rabbit Ig serum (diluted 1:100) (Boehringer-Mannheim, Indianapolis, IN). *a* and *b* are ScN₂a cells; phase-contrast and immunofluorescent microscopy were used together to photograph the cells. *c* and *d* are an adjacent field of cells visualized by immunofluorescence only. *e* and *f* are control N₂a cells. Cells stained with preimmune serum R017 give an immunofluorescence signal similar to that seen for PIPLC-treated cells exposed to immune serum (*not shown*).

with proteinase K and analyzed by streptavidin shift PAGE/immunoblot analysis (Borchelt, D., A. Taraboulos, M. Scott, D. Bredesen, N. Stahl, and S. B. Prusiner, in preparation; N. Stahl, unpublished observations). PrP^{Sc} proteins extracted from nonbiotinylated microsomes did not bind streptavidin and their PAGE migration was unaffected (Fig. 3 *A*, lanes *1* and *2*). In contrast, PrP^{Sc} molecules extracted from biotinylated microsomes exhibited a marked shift in PAGE migration in the presence of streptavidin (Fig. 3 *A*, lanes *3* and *4*). In addition, large complexes of biotinylated PrP and streptavidin were found not to electrotransfer efficiently causing a reduction in immunostaining. These studies also showed that PrP^{Sc} retained its protease resistance after reacting with sulfo-NHS-biotin.

ScN₂a cells were exposed to sulfo-NHS-biotin followed by PIPLC digestion. Cellular proteins were extracted and an aliquot digested with proteinase K. PrP^C released from nonbiotinylated cultures did not bind streptavidin (Fig. 3 *B*, lanes *1* and *2*). In contrast, the PAGE migration of PrP^C released from cells exposed to sulfo-NHS-biotin was retarded in the presence of streptavidin (Fig. 3 *B*, lane *4*), but not by streptavidin preincubated with excess d-biotin (Fig. 3 *B*, lane *3*). Since PrP^C released from biotinylated cultures quan-

titatively bound streptavidin, the amount of sulfo-NHS-biotin used in these experiments (1 mg/ml) was clearly sufficient to react with cell-surface PrP. PrP^{Sc} extracted from biotinylated ScN₂a cultures did not quantitatively bind streptavidin (200 μg) (Fig. 3 *C*, lane *4*), while the PAGE migration of a biotinylated control PrP^{Sc} added to the extract (Fig. 3 *C*, lanes *1* and *2*) was completely retarded. The migration of neither PrP^C nor PrP^{Sc} was affected by streptavidin (200 μg) preincubated with d-biotin (100 μg) (Fig. 3 *C*, lane *3*). Similar analysis of extracts of biotinylated ScN₂a cells without the addition of control biotinylated PrP^C demonstrates that most PrP^{Sc} was not accessible to sulfo-NHS-biotin (Fig. 3 *C*, lanes *5* and *6*). The PAGE migration of PrP^{Sc} extracted from nonbiotinylated cultures appeared unaltered by streptavidin (Fig. 3 *C*, lane *7*). The inaccessibility of most PrP^{Sc} in ScN₂a cells to sulfo-NHS-biotin suggests that the majority of PrP^{Sc} does not accumulate on the cell surface.

Nascent PrP on the Surface of N₂a Cells

Uninfected N₂a (Fig. 4 *A*) and ScN₂a (Fig. 4 *B*) cells were incubated in medium with 300 μCi/ml [³⁵S]methionine for 2 h before exposure to PIPLC (Fig. 4, lanes *1* and *2*) or sulfo-NHS-biotin (Fig. 4, lanes *3* and *4*). The radiolabeled pro-
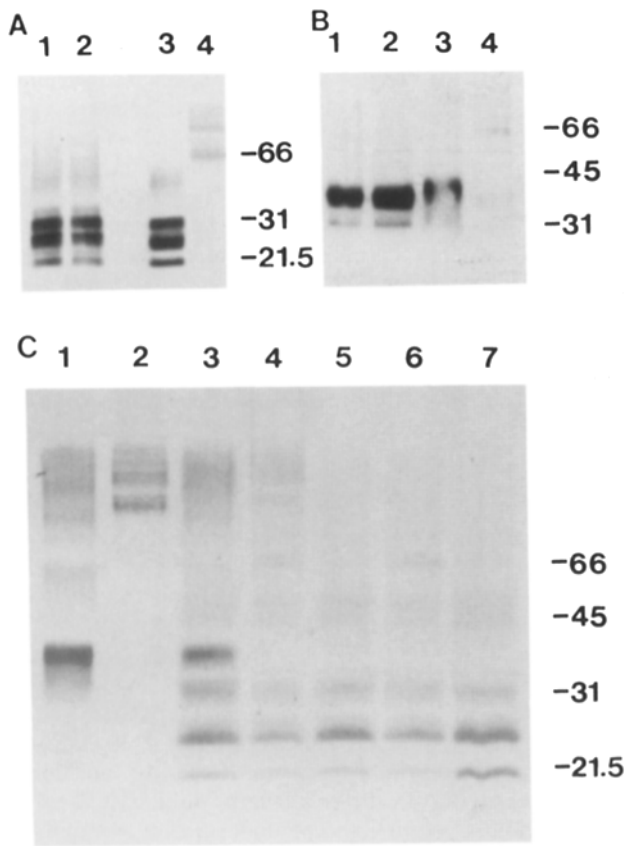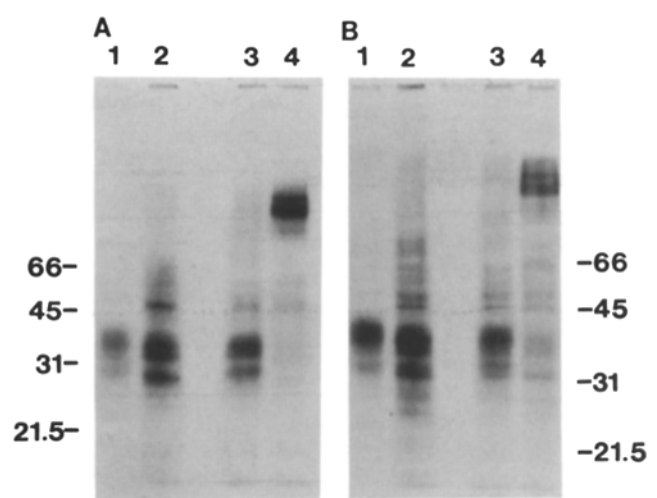
Figure 4. Nascent PrP^C in control and scrapie-infected N₂a cells is transported to the cell surface and partially released by PIPLC. Confluent flasks (25 cm²) of normal and scrapie N₂a cells were metabolically radiolabeled for 2 h with 300 μCi/ml [³⁵S]methionine in methionine-free DME with 2.5% dialyzed FBS. One set of flasks was then exposed to 0.2 U/ml PIPLC in PBS for 3 h while a second set was exposed to sulfo-NHS-biotin in PBS for 20 min. Cells and medium were extracted in detergent or harvested, and immunoprecipitated as described in Materials and Methods. (*A*) Normal N₂a cells. (*B*) ScN₂a cells. Lanes *1* and *2* show released and cell-associated proteins after exposure to PIPLC, respectively. Lanes *3* and *4* are proteins immunoprecipitated from biotinylated cultures electrophoresed in the presence of 50 μg streptavidin preincubated with 25 μg d-biotin (lane *3*) or 50 μg streptavidin (lane *4*).

Figure 3. PrP^C and PrP^Sc differ in their topology. (*A*) Initially, an examination of the ability of mouse PrP^Sc to react with sulfo-NHS-biotin and withstand proteinase K digestion was performed. Microsomal preparations (13) of scrapie-infected mouse brain were exposed to 1 mg/ml sulfo-NHS-biotin in H₂O after brief sonication. After 20 min, Tris-HCl (pH 7.5) was added to make a final concentration of 0.1 M before extraction in detergent, methanol precipitation, and digestion with proteinase K as described in Materials and Methods. Protein in each preparation was examined by streptavidin shift PAGE and immunoblot analysis with polyclonal anti-PrP 27-30 serum (R017). Biotinylated proteins bind streptavidin resulting in retarded migration on SDS-PAGE. Lanes *1* and *2* are nonbiotinylated microsomes without (lane *1*) or with (lane *2*) the addition of 50 μg of streptavidin. Lanes *3* and *4* are biotinylated microsomes without (lane *3*) and with (lane *4*) the addition of 50 μg of streptavidin. (*B* and *C*) Confluent cultures in 150 cm² flasks (1 per lane) were exposed to PBS containing 1 mg/ml sulfo-NHS-biotin, except where noted, for 20 min at room temperature. After two washes with 0.1 M Tris-HCl pH 7.5, 0.1 M NaCl, and 2 washes with PBS, the cells were exposed to PIPLC (0.2 U/ml) in PBS for 2 h at room temperature. The extent of PrP biotinylation in each fraction was then examined by streptavidin shift PAGE and immunoblot analysis for PrP proteins as in *A*. (*B*) Lanes *1* and *2* are PIPLC-released proteins from nonbiotinylated cultures electrophoresed in the presence of 20 μg of streptavidin preincubated with 20 μg d-biotin (lane *1*) or 20 μg of streptavidin (lane *2*). Lanes *3* and *4* are PIPLC-released proteins from biotinylated cultures electrophoresed with streptavidin/biotin or streptavidin as in lanes *1* and *2*, respectively. (*C*) Lanes *1* and *2* contain control biotinylated PrP^Sc isolated from scrapie-infected hamster brain (38) and electrophoresed with 20 μg of streptavidin preincubated with 20 μg of d-biotin or 20 μg of streptavidin only (lane *2*). Lanes *3* and *4* contain a mixture of added biotinylated hamster PrP^Sc and proteins extracted from biotinylated ScN₂a cultures after proteinase K digestion electrophoresed in the presence of 200 μg streptavidin preincubated with 100 μg d-biotin (lane *3*) or 200 μg streptavidin

teins were then immunoprecipitated as DLPCs before analysis by SDS-PAGE. Similar amounts of PrP^C were observed in the medium (Fig. 4, lane *1*), and the cell pellet (lane *2*) after exposure to either 0.2 or 2.0 U/ml PIPLC. In other experiments using immunofluorescence, as much as 90% of the PrP^C seemed to be released from the surface of N₂a cells upon PIPLC digestion (37). The majority of the newly synthesized PrP produced in both normal and scrapie N₂a cells during a 2-h pulse period was accessible to sulfo-NHS-biotin (Fig. 4, lanes *3* and *4*). After immunoprecipitation, the biotin-tagged samples were divided and one-half incubated with streptavidin in the presence of excess d-biotin (lane *3*) and the other aliquot incubated with streptavidin alone (lane *4*). Streptavidin binding to biotinylated PrP retarded its migration into SDS-PAGE (Fig. 4, lane *4*), indicating that the majority of newly synthesized PrP accumulates on the surface of both normal and scrapie N₂a cells. When the fourth lanes of Fig. 4, *A* and *B* are compared, slightly more newly synthesized PrP produced in ScN₂a cells was inaccessible to sulfo-NHS-biotin than in control, uninfected N₂a cells. Whether this difference is significant remains to be established. No difference between the uninfected N₂a and ScN₂a cells could be discerned with respect to the extent of nascent PrP release by PIPLC.

only (lane *4*). Lanes *5* and *6* are the same as lanes *3* and *4* without the added control biotinylated protein. Lane *7* is a proteinase K-digested extract of nonbiotinylated ScN₂a culture electrophoresed in the presence of 200 μg streptavidin.
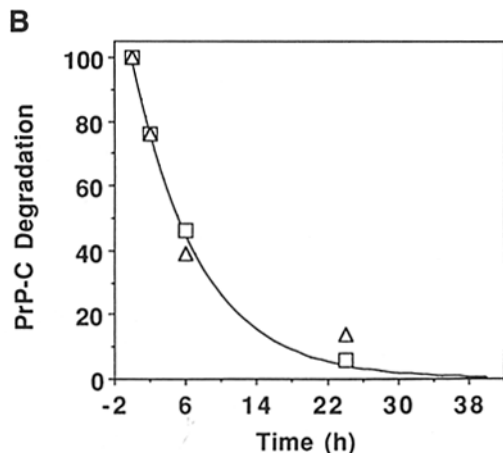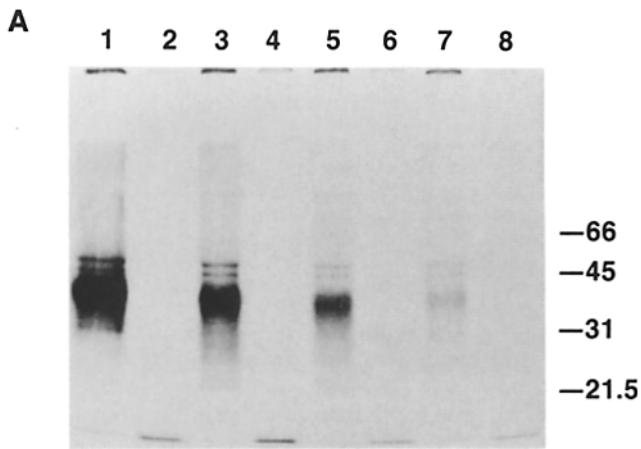
A

B

Figure 5. Turnover of PrP^C in normal N$_2$a cells. (A) Confluent N$_2$a cells in 25-cm^2 flasks were metabolically radiolabeled for 2 h with 300 μCi/ml [^{35}S]methionine in methionine-free DME with 2.5% dialyzed FBS after a 1-h preincubation in methionine-free medium. Cultures were then incubated in serum-free medium containing unlabeled methionine (Opti-MEM I) for increasing periods of time. Cell extracts were digested with proteinase K (even-numbered lanes only) followed by immunoprecipitation of DLPCs with immune R017 serum. Lanes *1* and *2*, no chase incubation. Lanes *3* and *4*, 2-h chase. Lanes *5* and *6*, 6-h chase. Lanes *7* and *8*, 24-h chase. (B) Degradation of PrP^C in uninfected N$_2$a cells after a 2-h pulse of [^{35}S]methionine. The radioautograph shown above (A) (−△−) and one from a duplicate experiment (−□−) were densitometrically scanned and the values plotted as a function of the chase period. Values for PrP^C are expressed as a percent of the signal detected at the end of the pulse period; i.e., no chase.

Synthesis and Degradation of PrP^C

Metabolic pulse-chase radiolabeling with [^{35}S]methionine was used to study PrP biosynthesis and degradation in normal and scrapie-infected N$_2$a cells. Radiolabeled PrP molecules were immunoprecipitated after increasing periods of chase and analyzed by SDS-PAGE. Prominent 33–35-kD PrP bands were observed for both uninfected N$_2$a (Fig. 5 A) and ScN$_2$a cells (Fig. 6 A) immediately following a 2-h pulse of radiolabeling (lane *1*). In uninfected N$_2$a cells, the PrP^C signal declined as a function of the chase period (Fig. 5 A; lanes *3*, *5*, and *7* correspond to 2 h, 6 h and 24 h of chase,

respectively). Digestion of N$_2$a cell extracts with proteinase K before immunoprecipitation resulted in no detectable radiolabeled products (Fig. 5 A, lanes *2*, *4*, *6*, and *8* correspond to 0 h, 2 h, 6 h, and 24 h of chase, respectively).

The autoradiograph shown in Fig. 5 A, as well as that from a similar experiment, was analyzed by densitometry and the peak areas are plotted in Fig. 5 B as the fraction of the signal observed in the absence of a chase. The two sets of data points show excellent reproducibility and can be fit by a line for an exponential decay with a half-time of 5.2 h. We must emphasize, however, that no effort was made to check the linearity of film response in these experiments, and the quantitation should be considered approximate. These results are in good agreement with those recently reported by other investigators (9).

Kinetics of PrP^{Sc} Synthesis

Metabolic radiolabeling studies gave a strikingly different result when ScN$_2$a cells were analyzed for proteinase K-resistant PrP molecules. Immediately following the 2-h pulse labeling period, no proteinase K-resistant PrP molecules were found (Fig. 6 A, lane *2*). With increasing intervals of chase, proteinase K-resistant PrP molecules became evident (Fig. 6 A, lanes *4*, *6*, *8*, *10*, *12*, and *14* correspond to 2 h, 4 h, 6 h, 10 h, 24 h, and 48 h of chase, respectively). This indicates that PrP^{Sc} is derived from a proteinase K-sensitive precursor. Lanes *15–17* are controls showing that the proteinase K-resistant PrP^{Sc} molecules are not immunopurified by preimmune monospecific antipeptide (P1) sera (3) (Fig. 6, lane *15*) while immune serum (Fig. 6, lane *16*) precipitates radiolabeled PrP^{Sc}. The immunoprecipitation of PrP^{Sc} with this antiserum was prevented by preincubation with the P1 synthetic peptide (Fig. 6, lane *17*), thereby confirming the identity of the putative PrP bands.

The total amount of PrP^C recovered in the nonproteinase K digested fractions of radiolabeled cell extracts declined with increasing chase time (Fig. 6 A, odd-numbered lanes). This suggests that a significant fraction of the newly synthesized PrP molecules produced in ScN$_2$a cells are destined for cellular degradation. Since we cannot distinguish the proteinase K digestable precursor of PrP^{Sc} from PrP^C, it is not possible, at present, to determine if the rate of PrP^C degradation in ScN$_2$a cells differs from that in uninfected N$_2$a cells.

Fig. 6 B shows a plot of sum of the densitometric values obtained for the three protease-resistant PrP bands in Fig. 6 A as a function of chase time, as well as that obtained from a similar experiment. The points are reasonably fit by a line describing an exponential appearance with a half-time of 15.2 h. Although this number should be considered only as a rough estimate of the rate constant for PrP^{Sc} synthesis, it is clear that it is much slower than that for PrP^C synthesis and significantly slower than the rate constant for PrP^C disappearance.

Release of PrP^C from ScN$_2$a Cells

To determine whether PrP^{Sc} is secreted from ScN$_2$a cells, aliquots of the culture medium were analyzed for PrP immunoreactivity. Figs. 7, A and B, show that similar amounts of PrP are released into the culture medium of uninfected N$_2$a and ScN$_2$a cells over a 16-h chase incubation (compare
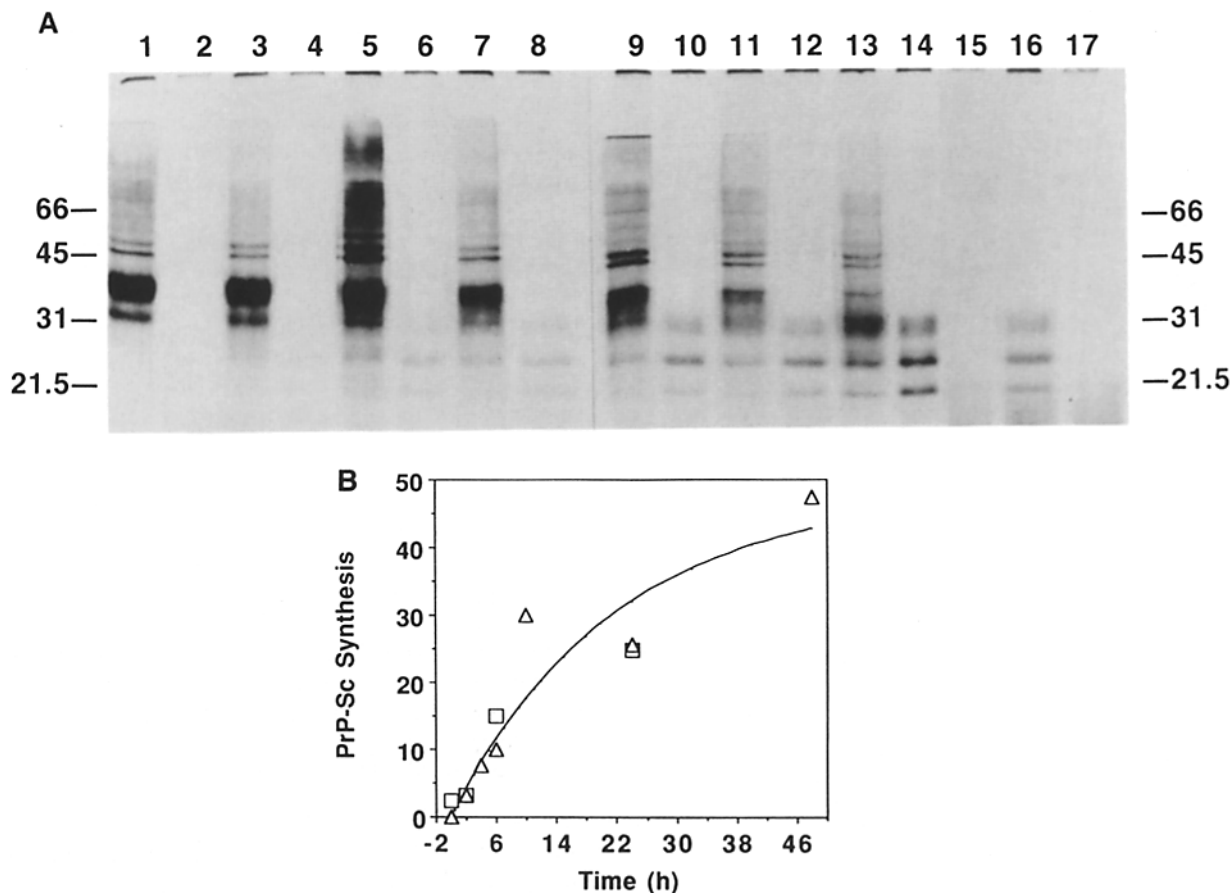
Figure 6. Kinetics of PrP^{Sc} biosynthesis. (*A*) Confluent T25 flasks of ScN₂a cultures were metabolically radiolabeled for 2 h as in Fig. 5. Cultures were then chase-incubated in Opti-MEM I before detergent extraction, proteinase K digestion, and immunoprecipitation in DLPCs as in Fig. 5. Lanes *1* and *2*, no chase without (−) and with exposure to proteinase K (+). Lanes *3* and *4*, 2-h chase (−) and proteinase K (+). Lanes *5* and *6*, 4-h chase (−) and proteinase K (+). Lanes *7* and *8*, 6-h chase (−) and proteinase K (+). Lanes *9* and *10*, 10-h chase (−) and proteinase K (+). Lanes *11* and *12*, 24-h chase (−) and (+) proteinase K. Lanes *13* and *14*, 48-h chase (−) and proteinase K (+). Lanes *15–17* are controls to demonstrate the specificity of antisera to the proteinase K-resistant PrP-reactive molecules found in ScN₂a cells. Lane *15*, preimmune antipeptide P1 sera R013 (Gly-Gln-Gly-Gly-Thr-His-Asn-Gln-Trp-Asn-Lys-Pro-Gly-Gly-Cys). Lane *16*, immune anti-P1 sera R013. Lane *17*, immune anti-P1 sera R013 preincubated with 25 μg P1 peptide. (*B*) Accumulation of PrP^{Sc} in ScN₂a cells after a 2-h pulse of [³⁵S]methionine. The radioautograph shown above (−△−) and one from a similar experiment (−□−) were densitometrically scanned. The sum of the values for the 3 PrP^{Sc} species (*a*, even-numbered lanes) were plotted as a function of the chase period. Values for PrP^{Sc} are expressed as a percent of the 33–35-kD PrP signal detected at the end of the pulse period (*a*, lane *1*).

lane *4* in Fig. 7, *A* and *B*, normal and ScN₂a, respectively). The PrP released from both cells into the media was found to be readily digested with proteinase K arguing that only PrP^C is released. This finding is consistent with studies by others (9) reporting that a portion of PrP^C is released from uninfected and scrapie-infected N₂a cells. The kinetics of PrP^C appearance in the culture medium from ScN₂a cells is depicted in Fig. 7 *C*. A plot of the densitometric values for the spontaneous release of PrP^C from ScN₂a cells is shown in Fig. 7 *D*. The best fit to a single exponential curve gives a half-time of 10.5 h. It is intriguing that the time course of PrP^C release parallels the synthesis of PrP^{Sc} as illustrated in Figs. 6 *B* and 7 *D*.

Discussion

Although a wealth of evidence argues persuasively that PrP^{Sc} is a major and necessary component of the infectious

prion particle, some investigators continue to challenge this premise (1, 11, 30, 36). In general, these investigators have encountered difficulty in detecting PrP^{Sc} in a specific extract or fraction and have concluded that its apparent absence is evidence for a dissociation between PrP^{Sc} and scrapie infectivity. In part, these problems are quantitative since the bioassay is extremely sensitive while PrP^{Sc} detection relies on immunodetection of samples subjected to limited proteolysis. The propensity of PrP^{Sc} to form aggregates and the difficulties attendant with its solubilization create an additional level of complexity.

Contrasting Properties of PrP^C and PrP^{Sc}

The studies described here on PrP^{Sc} biogenesis and topology are the first to report detection of radiolabeled PrP^{Sc}. These results complement those previously reported for the two PrP isoforms (Table I). Purified prions are composed largely, if not entirely, of PrP^{Sc} molecules. Limited proteol-
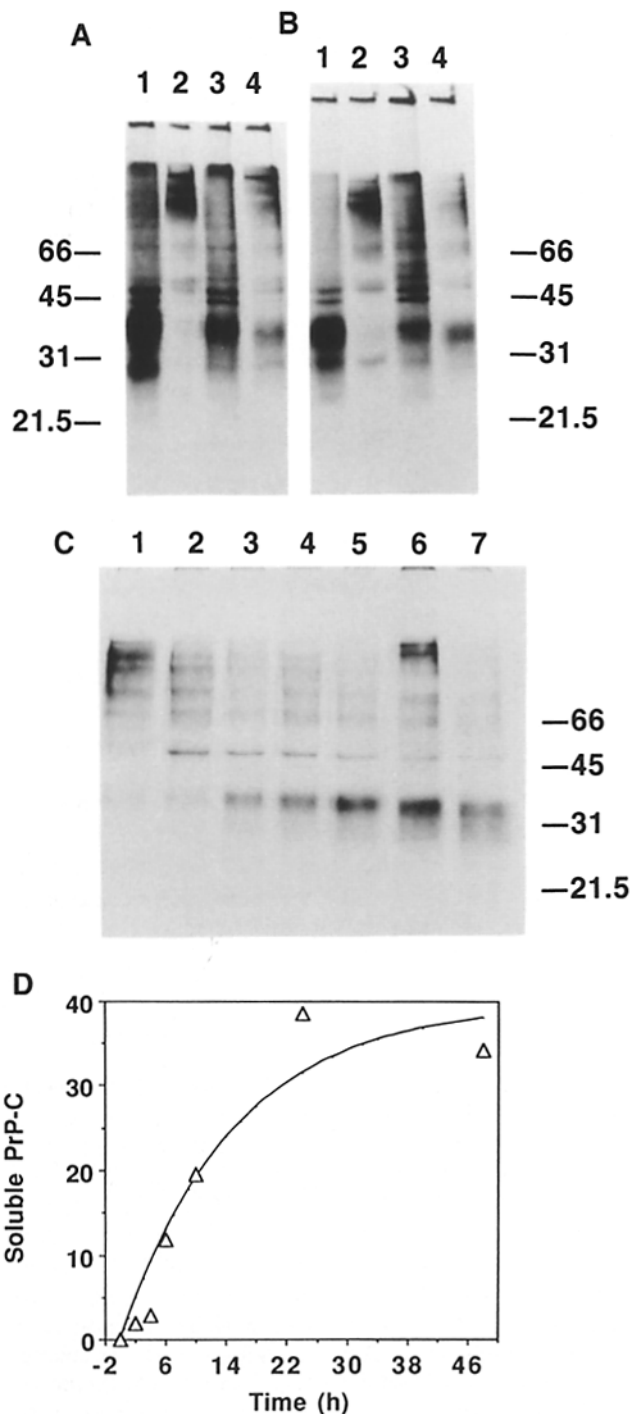
Figure 7. Release of PrP^C into cell culture medium from N$_2$a and ScN$_2$a cells. (*A*) N$_2$a and (*B*) ScN$_2$a cells were labeled with [^{35}S]methionine for 2 h followed by a chase period of 2 h (lanes *1* and *2*) or 16 h (lanes *3* and *4*). Cells (lanes *1* and *3*) and media (lanes *2* and *4*) were harvested and analyzed for PrP molecules. Immunoprecipitations were performed with R017 antisera. (*C*) Time course for release of PrP^C into culture medium from ScN$_2$a cells. The labeling and chase medium from cultures used in Fig. 6 *A* were harvested and examined. Lane *1*, labeling medium. Lane *2*, 2-h chase medium. Lane *3*, 4-h chase medium. Lane *4*, 6-h chase medium. Lane *5*, 10-h chase medium. Lane *6*, 24-h chase medium. Lane *7*, 48-h chase medium. (*D*) Radioautograph above in *C* was analyzed densitometrically and the values (−△−) plotted as a function of the chase time.

Table I. Properties of Cellular and Scrapie PrP Isoforms

	PrP^C	PrP^{Sc}
Normal cells	+	−
Scrapie-infected cells	+	+
Purified prions	−	+
Protease resistance	−	+ (PrP 27-30)
Amyloid rods	−	+ (PrP 27-30)
Subcellular localization	Cell surface	Primarily intracellular
PIPLC release	+	−
Synthesis	<2 h	~15 h
Turnover ($t_{1/2}$)	~5 h	>24 h

ysis degrades PrP^{Sc} by hydrolyzing only the NH$_2$ terminal 67 amino acids to produce PrP 27-30 (25, 29). Detergent extraction of membranes containing PrP 27-30 results in the polymerization of this protein in rod-shaped particles indistinguishable from amyloids (23, 28). Although both PrP isoforms possess glycosyl-phosphatidylinositol anchors, PrP^C is localized primarily on the cell surface while PrP^{Sc} is found mainly within the cell. PrP^C is released from the cell surface by PIPLC digestion but PrP^{Sc} is resistant to release. PrP^C synthesis and maturation are rapid as evidenced by the large amount of radiolabeled PrP^C found after a 2-h pulse. Other investigators suggest that the $t_{1/2}$ for PrP^C synthesis may be as small as 20 min (9). Our studies show that PrP^C turns over with a $t_{1/2}$ of 5.2 h. In contrast, PrP^{Sc} synthesis and maturation is slow with a $t_{1/2}$ of 15.2 h. We have not been able to demonstrate the degradation of PrP^{Sc} in cultured cells; presumably, this process is extremely slow with a $t_{1/2}$ > 24 h.

Subcellular Localization of PrP Isoforms

We found that ~50% of the PrP^C molecules produced in either uninfected, or scrapie-infected N$_2$a cells could be released by PIPLC digestion (see Fig. 4). In both uninfected N$_2$a cells and ScN$_2$a cells, most of the newly synthesized PrP^C molecules could be labeled with sulfo-NHS-biotin. This suggests that some cell surface PrP^C molecules may be resistant to PIPLC digestion. However, it appears that it is not uncommon for cells to produce subpopulations of GPI anchored proteins that cannot be released by PIPLC digestion (18, 19). Most PrP^{Sc} produced in ScN$_2$a cells appears to be inaccessible to labeling with sulfo-NHS-biotin (see Fig. 3). This result is consistent with recent immunofluorescence studies of ScN$_2$a cells showing that PrP^{Sc} accumulates within the interior of cells (Taraboulos, A., D. Serban, and S. B. Prusiner, submitted for publication).

Posttranslational Processing

Our experiments have demonstrated that a protein indistinguishable from PrP^C is present in ScN$_2$a cells. Since a high proportion of the cells also contain PrP^{Sc} and produce infectious prions, synthesis of PrP^C and PrP^{Sc} are not mutually exclusive. Whereas PrP^C may be observed in both normal and scrapie-infected N$_2$a cells following a brief (1–2 h) metabolic labeling period, detection of significant quantities of mature, protease-resistant PrP^{Sc} requires a prolonged chase period. Therefore, PrP^{Sc} is derived from a protease-sensitive precursor. Acquisition of protease resistance ensues slowly with an estimated half-time for synthesis of 15 h.

A significant fraction of PrP chains eventually acquire protease resistance. We estimate that ~30% of the PrP chains acquire protease resistance based on the data presented in Figs. 1 and 6. Our estimates suffer from uncertainties about the efficiencies of PrP^C and PrP^{Sc} transfer to nitrocellulose membranes as well as our assumption that virtually all PrP^C is released by PIPLC digestion. In addition, the relative efficiency of immunoprecipitation of radiolabeled PrP molecules in DLPCs is unknown. In spite of these uncertainties, immunoblot analysis and metabolic labeling studies give similar estimates.

Both normal and ScN_2a cells spontaneously release a protease-sensitive form of PrP. We note that the sum of the estimated rate constants for production of the extracellular form ($0.066h^{-1}$) and for formation of the protease-resistant form ($0.046h^{-1}$) approximates the rate constant for disappearance of PrP^C in normal cells ($0.13h^{-1}$). It follows that a large proportion of PrP molecules destined for intracellular degradation in uninfected cells might serve as substrates for conversion to PrP^{Sc} in infected cells.

The organization of the PrP gene argues that the cellular and scrapie isoforms do not arise from alternative splicing since the entire open reading frame is contained within a single exon (4). Furthermore, there is no evidence for DNA rearrangements of the PrP gene during scrapie infection. Based on these observations, we suggested that PrP^C and PrP^{Sc} differ because of some posttranslational event (4).

We are unable to determine from our present experiments whether the precursor of PrP^{Sc} is identical to or distinct from PrP^C. PrP^{Sc} might be derived directly from PrP^C via a subsequent posttranslational event, perhaps an abrogation of a normal cellular process. Alternatively, a distinct protease-sensitive precursor to PrP^{Sc} could be synthesized which would be committed toward formation of the protease-resistant form at an early stage of biosynthesis. In either event, it is clear from our present study that the biochemical property of protease-resistance is acquired posttranslationally.

Slow Accumulation of PrP^{Sc} and the Scrapie Incubation Period

Although scrapie has been successfully transmitted to cultured cells on many occasions (8, 20, 30, 31, 33), the low prion titer observed (usually 10^4–10^5 ID_{50}/ml of extract) contrasts markedly with those found in the brains of scrapie-infected hamsters exhibiting clinical signs of scrapie. In brain, titers in excess of 10^8 ID_{50}/ml of homogenate and correspondingly high concentrations of PrP^{Sc} are found. Our data suggest a simple explanation for this paradox. A continuous culture of scrapie-infected cells such as that described here may be unable to accumulate high titers of PrP^{Sc} (and by inference, infectious particles) because of constant dilution by ongoing cell division. In contrast, high titers in the brain may be achieved because prions replicate primarily in nondividing neurons. The extreme stability of PrP^{Sc} compared to PrP^C would allow accumulation to high concentrations during the extremely protracted incubation period that is characteristic of prion diseases.

New Approaches Arising from these Studies

The identification of conditions for the radiolabeling of PrP^{Sc} in scrapie-infected cultured cells and its detection af-

ter SDS-PAGE should open many new approaches to the study of prions. Learning which inhibitors of posttranslational processing and transport prevent PrP^{Sc} synthesis may elucidate the posttranslational event responsible for PrP^{Sc} formation. For example, numerous inhibitors have become available which block specific steps in the synthesis and processing of Asn-linked complex oligosaccharides. Both PrP^C and PrP^{Sc} are known to be modified by complex oligosaccharides (7, 12, 15). Elucidating the nature of these chemical reactions as well as identifying the rate limiting step will add significantly to our understanding of prion diseases. Thus, dynamic studies involving inhibitors of cellular functions and a more detailed exploration of precursor-product relationships in ScN_2a cells may elucidate the nature of the PrP^{Sc} precursor and its relationship to PrP^C.

References

1. Aiken, J. M., J. L. Williamson, and R. F. Marsh. 1989. Evidence of mitochondrial involvement in scrapie infection. J. Virol. 63:1686–1694.
2. Barry, R. A., M. P. McKinley, P. E. Bendheim, G. K. Lewis, S. J. DeArmond, and S. B. Prusiner. 1985. Antibodies to the scrapie protein decorate prion rods. J. Immunol. 135:603–613.
3. Barry, R. A., M. T. Vincent, S. B. H. Kent, L. E. Hood, and S. B. Prusiner. 1988. Characterization of prion proteins with monospecific antisera to synthetic peptides. J. Immunol. 140:1188–1193.
4. Basler, K., B. Oesch, M. Scott, D. Westaway, M. Wälchli, D. F. Groth, M. P. McKinley, S. B. Prusiner, and C. Weissmann. 1986. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell. 46:417–428.
5. Bendheim, P. E., R. A. Barry, S. J. DeArmond, D. P. Stites, and S. B. Prusiner. 1984. Antibodies to a scrapie prion protein. Nature (Lond.). 310:418–421.
6. Bockman, J. M., D. T. Kingsbury, M. P. McKinley, P. E. Bendheim, and S. B. Prusiner. 1985. Creutzfeldt-Jakob disease prion proteins in human brains. N. Engl. J. Med. 312:73–78.
7. Bolton, D. C., R. K. Meyer, and S. B. Prusiner. 1985. Scrapie PrP 27-30 is a sialoglycoprotein. J. Virol. 53:596–606.
8. Butler, D. A., M. R. D. Scott, J. M. Bockman, D. R. Borchelt, A. Taraboulos, K. K. Hsiao, D. T. Kingsbury, and S. B. Prusiner. 1988. Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. J. Virol. 62:1558–1564.
9. Caughey, B., R. E. Race, D. Ernst, M. J. Buchmeier, and B. Chesebro. 1989. Prion protein-biosynthesis in scrapie-infected and uninfected neuro-blastoma cells. J. Virol. 63:175–181.
10. Chesebro, B., R. Race, K. Wehrly, J. Nishio, M. Bloom, D. Lechner, S. Bergstrom, K. Robbins, L. Mayer, J. M. Keith, C. Garon, and A. Haase. 1985. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature (Lond.). 315:331–333.
11. Czub, M., H. R. Braig, and H. Diringer. 1988. Replication of the scrapie agent in hamsters infected intracerebrally confirms the pathogenesis of an amyloid-inducing virosis. J. Gen. Virol. 69:1753–1756.
12. Endo, T., D. Groth, S. B. Prusiner, and A. Kobata. 1989. Diversity of oligosaccharide structures linked to asparagines of the scrapie prion protein. Biochemistry. 28:8380–8388.
13. Gabizon, R., M. P. McKinley, and S. B. Prusiner. 1987. Purified prion proteins and scrapie infectivity copartition into liposomes. Proc. Natl. Acad. Sci. USA. 84:4017–4021.
14. Gabizon, R., M. P. McKinley, D. F. Groth, L. Kenaga, and S. B. Prusiner. 1988. Properties of scrapie prion liposomes. J. Biol. Chem. 263:4950–4955.
15. Haraguchi, T., S. Fisher, S. Olofsson, T. Endo, D. Groth, A. Tarantino, D. R. Borchelt, D. Teplow, L. Hood, A Burlingame, E. Lycke, A. Kobata, and S. B. Prusiner. 1989. Asparagine-linked glycosylation of the scrapie and cellular prion proteins. Arch. Biochem. Biophys. 274:1–13.
16. Ingalls, H. M., C. M. Goodloe-Holland, and E. J. Luna. 1986. Junctional plasma membrane domains isolated from aggregating Dictyostelium dis-

coideum membrane. *Proc. Natl. Acad. Sci. USA.* 83:4779–4783.

17. Kitamoto, T., K. Ogomori, J. Tateishi, and S. B. Prusiner. 1987. Formic acid pretreatment enhances immunostaining of amyloid deposits in cerebral and systemic amyloids. *Lab. Invest.* 57:230–236.

18. Low, M. G. 1987. Biochemistry of the glycosyl-phosphatidylinositol membrane protein anchors. *Biochem. J.* 244:1–13.

19. Low, M. G., J. Stiernberg, G. L. Waneck, R. A. Flavell, and P. W. Kincade. 1988. Cell-specific heterogeneity in sensitivity of phosphatidylinositol-anchored membrane antigens to release by phospholipase C. *J. Immunol. Methods.* 113:101–111.

20. Markovits, P., D. Dormont, B. Delpech, L. Court, and R. Latarjet. 1981. Essais de propagation in vitro de l'agent scrapie dans des cullules nerveuses de souris. *C. R. Acad. Sci. (Paris).* 293:413–417.

21. McKinley, M. P., D. C. Bolton, and S. B. Prusiner. 1983. A protease-resistant protein is a structural component of the scrapie prion. *Cell.* 35:57–62.

22. McKinley, M. P., B. Hay, V. R. Lingappa, I. Lieberburg, and S. B. Prusiner. 1987. Developmental expression of prion protein gene in brain. *Dev. Biol.* 121:105–110.

23. Meyer, R. K., M. P. McKinley, K. A. Bowman, M. B. Braunfeld, R. A. Barry, and S. B. Prusiner. 1986. Separation and properties of cellular and scrapie prion proteins. *Proc. Natl. Acad. Sci. USA.* 83:2310–2314.

24. Mobley, W. C., R. L. Neve, S. B. Prusiner, and M. P. McKinley. 1988. Nerve growth factor increases mRNA levels for the prion protein and the beta-amyloid protein precursor in developing hamster brain. *Proc. Natl. Acad. Sci. USA.* 85:9811–9815.

25. Oesch, B., D. Westaway, M. Wälchli, M. P. McKinley, S. B. H. Kent, R. Aebersold, R. A. Barry, P. Tempst, D. B. Teplow, L. E. Hood, S. B. Prusiner, and C. Weissmann. 1985. A cellular gene encodes scrapie PrP 27-30 protein. *Cell.* 40:735–746.

26. Prusiner, S. B. 1989. Scrapie prions. *Annu. Rev. Microbiol.* 43:345–374.

27. Prusiner, S. B., D. C. Bolton, D. F. Groth, K. A. Bowman, S. P. Cochran, and M. P. McKinley. 1982. Further purification and characterization of scrapie prions. *Biochemistry.* 21:6942–6950.

28. Prusiner, S. B., M. P. McKinley, K. A. Bowman, D. C. Bolton, P. E.

Bendheim, D. Groth, and G. G. Glenner. 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell.* 35:349–358.

29. Prusiner, S. B., D. F. Groth, D. C. Bolton, S. B. Kent, and L. E. Hood. 1984. Purification and structural studies of a major scrapie prion protein. *Cell.* 38:127–134.

30. Race, R. E., B. Caughey, K. Graham, D. Ernst, and B. Chesebro. 1988. Analyses of frequency of infection, specific infectivity, and prion protein biosynthesis in scrapie-infected neuroblastoma cell clones. *J. Virol.* 62:2845–2849.

31. Race, R. E., L. H. Fadness, and B. Chesebro. 1987. Characterization of scrapie infection in mouse neuroblastoma cells. *J. Gen. Virol.* 68:1391–1399.

32. Roberts, G. W., R. Lofthouse, R. Brown, T. J. Crow, R. A. Barry, and S. B. Prusiner. 1986. Prion protein immunoreactivity in human transmissible dementias. *N. Engl. J. Med.* 315:1231–1233.

33. Rubenstein, R., R. I. Carp, and S. H. Callahan. 1984. In vitro replication of scrapie agent in a neuronal model: infection of PC12 cells. *J. Gen. Virol.* 65:2191–2198.

34. Scott, M., D. Butler, D. Bredesen, M. Wälchli, K. Hsiao, and S. B. Prusiner. 1988. Prion protein gene expression in cultured cells. *Protein Eng.* 2:69–76.

35. Serban, D., A. Taraboulos, S. J. DeArmond, and S. B. Prusiner. 1989. Rapid detection of Creutzfeldt-Jakob disease and scrapie prion proteins enhances immunoreactivity. *Neurology.* In press.

36. Sklaviadis, T. K., L. Manuelidis, and E. E. Manuelidis. 1989. Physical properties of the Creutzfeldt-Jakob disease agent. *J. Virol.* 63:1212–1222.

37. Stahl, N., D. R. Borchelt, K. Hsiao, and S. B. Prusiner. 1987. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell.* 51:229–240.

38. Turk, E., D. B. Teplow, L. E. Hood, and S. B. Prusiner. 1988. Purification and properties of the cellular and scrapie hamster prion proteins. *Eur. J. Biochem.* 176:21–30.

39. Westaway, D., G. A. Carlson, and S. B. Prusiner. 1989. Unraveling prion diseases through molecular genetics. *Trends Neurosci.* 12:221–227.