Little is currently known about the biochemical mechanism by which induced prion protein (PrP) conformational change occurs during mammalian prion propagation. In this study, we describe the reconstitution of PrPres amplification in vitro using partially purified and synthetic components. Overnight incubation of purified PrP27–30 and PrPSc molecules at a molar ratio of 1:250 yielded ~2-fold baseline PrPres amplification. Addition of various polyanionic compounds increased the level of PrPres amplification to ~10-fold overall. Polyanionic compounds that stimulated purified PrPres amplification to varying degrees included synthetic, homopolymeric nucleic acids such as poly(A) and poly(dT), as well as non-nucleic acid polyanions, such as heparan sulfate proteoglycan. Size fractionation experiments showed that synthetic poly(A) polymers must be >0.2 kb in length to stimulate purified PrPres amplification. Thus, one possible set of minimal components for efficient conversion of PrP molecules in vitro may be surprisingly simple, consisting of PrP27–30, PrPSc, and a stimulatory polyanionic compound.

Transmissible spongiform encephalopathies such as Creutzfeldt-Jakob disease, bovine spongiform encephalopathy (BSE), chronic wasting disease, and scrapie are fatal infectious diseases of the central nervous system with an unusual etiology. Many biochemical and biophysical experiments have shown that the infectious agents of transmissible spongiform encephalopathies, termed prions, lack informational nucleic acids (1). Furthermore, the replication of infectious prions in vivo and in cultured cells is generally accompanied by the transformation of the normal cellular isoform of a neuronal membrane protein (PrPSc) into a protease-resistant state (PrPRes or PrPres) (2, 3), and PrPres co-purifies with prion infectivity (4). These observations can be explained by the protein-only hypothesis, which contends that infectious prions are exclusively composed of misfolded PrP molecules such as PrPres (5). In strong support of this hypothesis, Legname et al. (6) recently demonstrated the generation of infectious prions in vitro by refolding purified recombinant PrP.

Little is known about the molecular mechanism that mediates the self-propagating conversion of PrPSc to PrPres. Several investigators have used a biochemical approach to investigate this process in vitro. Caughey and colleagues (7–10) developed the first successful cell-free PrP conversion system by using a radiolabeled PrP substrate and showed that the efficiency of PrPSc to PrPres conversion in vitro was dependent upon PrP sequence and prion strain in a manner that precisely modeled the specificity of transmissible spongiform encephalopathy transmission in vivo. Later, Saborio and Soto (11) developed the protein-misfolding cyclic amplification technique, in which crude brain homogenates are intermittently sonicated to generate much more efficient conversion of PrPSc to PrPres than the radiolabel technique, resulting in high level amplification of PrPres levels. Even without sonication, a mixture of crude normal and diluted scrapie brain homogenates generates >6-fold amplification of PrPres after overnight incubation (11, 12). In contrast, a 50-fold stoichiometric excess of PrPres template is required to drive the conversion of radiolabeled PrPSc molecules in a cell-free system containing only purified prion proteins (9). This discrepancy in the efficiencies of crude versus purified systems suggests that factors other than PrP molecules are required for efficient PrP conversion in vitro, and that crude brain homogenates contain such factors. Furthermore, several genetic and biochemical experiments have provided evidence for the existence of PrP conversion cofactors (13, 14). Deleault et al. (15) demonstrated that treatment of crude brain homogenates with RNase abolished PrPres amplification in vitro and that efficient PrPres amplification could be reconstituted by addition of exogenous mammalian RNA. These results showed that RNA molecules within crude brain homogenates promote efficient PrPres amplification in non-purified systems such as protein-misfolding cyclic amplification. However, studies in crude homogenates cannot determine whether RNA molecules alone are sufficient to drive efficient PrPres amplification, because it is possible that other factors within the homogenates are also required for the amplification process. Furthermore, it is not known whether RNA molecules stimulate PrPres amplification directly by binding to prion proteins, or indirectly, for instance by sequestering a reaction inhibitor or activating a catalyst (16). To investigate the molecular requirements for PrPres amplification in vitro, we developed a series of protocols to isolate PrPSc and PrPRes molecules under
conditions that preserve their ability to reconstitute efficient PrPres amplification in vitro. Using these preparations, we have generated and characterized an efficient in vitro PrPres amplification system using only purified and synthetic components. Our results show that one possible set of minimal components for efficient amplification of PrPres in vitro may be surprisingly simple, consisting of PrPSc, PrPSc, and a polyanionic compound.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Total brain ganglioside extract, ammonium salt, was obtained from Avanti Polar Lipids (Alabaster, AL) and resuspended in 1% Triton X-100. Glycogen was obtained from In vitro. Sodium pentosan polysulfate was obtained from the TSE Resource Center (Compton, UK) and dissolved in RNase-free 1X TE (10 mM Tris, 1 mM EDTA, pH 8.0) (Ambion, Austin, TX). Heparan sulfate proteoglycan prepared from the basement membrane of Engelbreth-Holm-Swarm mouse sarcoma cells (molecular mass > 400 kDa, catalog number H4777) heparan sulfate, sodium salt from bovine kidney (molecular mass, ~12-14 kDa, catalog number H7640), and polyglutamate (molecular mass, ~50-100 kDa) were all obtained from Sigma.

All synthetic polynucleotides were purchased from Sigma. The size distributions of these various commercial preparations were determined by a combination of agarose gel electrophoresis and size exclusion high performance liquid chromatography techniques (data not shown). The preparations assayed were: poly(A) catalog number P9403 (0.2-6 kb by agarose gel electrophoresis), poly(C) catalog number P4960 (8-10 kb by high performance liquid chromatography), poly(G) catalog number P4404 (-0.2 kb by agarose gel electrophoresis), poly(U) catalog number P9528, (0.3-1 kb by size exclusion high performance liquid chromatography), poly(dA) catalog number P0087 (-1.5-4 kb by agarose gel electrophoresis), poly(dT) catalog number P6965 (-1.5-4 kb by agarose gel electrophoresis), and poly(dC) catalog number P5444 (0.39 kb, according to manufacturer). Stock solutions of synthetic polynucleotides were prepared in 1X TE pH 8.0, and concentrations were confirmed by A260 nm.

**Preparation of IgG Cross-linked Protein A-Agarose Beads**—All procedures were performed at room temperature. Four hundred microliters of ImmunoPure Immobilized Protein A Plus 50% slurry (Pierce) was mixed with 16 μg IgG per microliter of packed resin for 2 h. Following incubation, agarose beads were recovered by centrifugation at 1000 × g for 1 min and washed twice with 1 ml of 200 mM triethanolamine (pH 8.0) (Acros Organics, Geel, Belgium). Antibodies were cross-linked by incubation in 1 ml of 10 mM dimethyl pimelimidate hydrochloride (Pierce), 200 mM triethanolamine, pH 8.0, for 30 min. The reaction was quenched by the addition of 50 μl of 1 M Tris, pH 8.0, and beads were recovered by centrifugation at 1000 × g for 1 min. Cross-linked beads were then washed three times, once in phosphate-buffered saline without out calcium or magnesium (PBS), 0.25% sodium deoxycholate, 1% Triton X-100, and twice in PBS. Beads were resuspended in 200 μl of PBS and stored at 4 °C.

**Immunopurification of PrPSc from Hamster Brain**—All procedures were performed at 4 °C. Four brains, including cerebellum and brainstem, from 8- to 12-week-old specific-pathogen-free Golden Syrian hamsters of either sex were homogenized in 10 volumes (w/v) of ice-cold PBS plus Complete® protease inhibitors (Roche Applied Science) using a Potter homogenizer. The homogenate was centrifuged and the supernatant was discarded. The resin was washed twice in 20 ml of 200 mM triethanolamine, pH 8.0 (Acros Organics, Geel, Belgium). Antibodies were cross-linked by incubation in 1 ml of 10 mM dimethyl pimelimidate hydrochloride (Pierce), 200 mM triethanolamine, pH 8.0, for 30 min. The reaction was quenched by the addition of 50 μl of 1 M Tris, pH 8.0, and beads were recovered by centrifugation at 1000 × g for 1 min. Cross-linked beads were then washed three times, once in phosphate-buffered saline without out calcium or magnesium (PBS), 0.25% sodium deoxycholate, 1% Triton X-100, and twice in PBS. Beads were resuspended in 200 μl of PBS and stored at 4 °C.

**Quantification of PrPSc from PrP Sc, PrP Sc, and PrP Sc were quantified by comparing dilutions of these preparations against known amounts of recombinant PrPSc on Western blots (Prionics, Schlieren, Switzerland). Densitometric measurement of membrane marker film signals was performed through the analysis of multiple film exposures to ensure that comparisons were made within the linear range of the film. Signals within the linear range were quantified using the histogram functions in Adobe Photoshop and calibrated against the background signal. Serial dilutions of normal hamster brain were used to calibrate densitometric measurements.

**RNA Purification**—All total RNA preparations were isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Poly(A)+ RNA was isolated from hamster liver total RNA using the Poly(A) Purist Kit (Ambion), and the sample that did not bind to the oligo(dT) column was designated Poly(A)− RNA. All RNA preparations were resuspended in 1X TE, pH 8.0, and the concentration and quality of each preparation were determined by agarose gel electrophoresis and by measuring A260 nm/A280 nm.

**Size Fractionation of Poly(A)+**—Synthetic poly(A) (Sigma catalog number P5444, 0.39 kb, according to manufacturer). Stock solutions of synthetic poly(A) were prepared in 1X TE pH 8.0, and concentrations were confirmed by A260 nm.
number P9403) was resuspended in 1× TE, pH 8.0, and the concentration was confirmed by A 260 nm. A sample containing 200 µg of this preparation was electrophoresed on a 1% agarose gel. Unstained slices corresponding to different mobility ranges were excised and extracted using a gel-extraction kit (Qiagen, Valencia, CA). Poly(A) 45-, 25-, and 10-mer oligonucleotides were purchased from IDT (Coralville, IA) and resuspended in 1× TE, pH 8.0. Concentrations of each poly(A) fraction were determined by A 260 nm.

RESULTS

Because post-translational modifications of PrPc might affect its ability to convert efficiently to PrPres, we chose to purify mature, mammalian PrPc directly from normal brain tissue using detergent solubilization (19). To ensure that our results did not depend upon any peculiarities of one purification method, we developed two different protocols to generate PrPc molecules capable of undergoing efficient conversion to PrPres (see “Experimental Procedures”). In the first protocol, adapted from the method of Pan et al. (17, 18), PrPc molecules were purified from solubilized brain membranes by sequential adsorption to copper and lectin affinity columns. This procedure produced a preparation that contained conversion-competent PrPc molecules, but also contained many contaminating proteins, resulting in <10% purity (data not shown). In the second protocol, PrPc was immunopurified using immobilized anti-PrP antibodies. This procedure generates samples containing PrPc with ~50% purity; we identified one major contaminant as the immunoglobulin heavy chain (Fig. 1A). The three glycoforms of PrPc are identifiable as bands with molecular masses in the range from 30 to 33 kDa. As expected, these bands are more abundant in a sample purified from Tga20 transgenic PrP-overexpressing mice and absent from a sample prepared from Prnp0/0 mice (Fig. 1A).

A preparation of immunopurified hamster PrPc was incubated overnight with purified Syrian hamster Sc237 PrP27–30 template at a molar ratio of 250:1, and PrPres amplification was measured. Co-incubation of these two purified prion proteins alone yielded ~2-fold amplification of PrPres (Fig. 1B). Addition of Prnp0/0 mouse brain homogenate lacking PrPc to the mixture of purified proteins increased the PrPres amplification level to ~10-fold (Fig. 1B), similar to the level of PrPres amplification in crude brain homogenates (12). In control reactions, Prnp0/0 brain homogenate did not affect the protease resistance of either PrPc or PrP27–30 molecules in isolation (Fig. 1B). These results confirm that crude brain homogenates contain one or more cofactor(s) that promote the efficiency of PrPres amplification.

We previously found that RNA molecules are required for efficient PrPres amplification in crude brain homogenates (15). Therefore, we analyzed whether isolated RNA molecules might stimulate PrPres amplification from purified prion proteins. We found that addition of total hamster liver RNA to a mixture of PrP27–30 and immunopurified PrPc molecules yielded ~10-fold PrPres amplification (Fig. 1C). This result indicates that RNA molecules can act directly upon prion proteins without intermediary molecules and that purified PrPc, PrPsc, and RNA molecules are sufficient to reconstitute PrPres amplification to the same level as crude brain homogenates. The efficiency of purified PrPres amplification stimulated by RNA is further increased by protein-misfolding cyclic amplification (11), resulting in ~20-fold total PrPres amplification after 24 cycles, which again is similar to the level of PrPres amplification obtained with protein-misfolding cyclic amplification using reconstituted brain homogenate (Supplemental Fig. S1).

In crude brain homogenates, RNA concentrations between 100 and 500 µg/ml stimulate PrPres amplification in a species-specific manner (15). For example, addition of total RNA prepared from hamster or mouse tissues increases PrPres amplification in crude brain homogenates, but addition of the total RNA prepared from a variety of non-mammalian species, such as Caenorhabditis elegans and Escherichia coli, to crude homogenates does not affect amplification levels. To study the species specificity and potency of RNA stimulation in our purified system, we tested the ability of varying concentrations of total RNA prepared from a variety of species to stimulate purified PrPres amplification. Unexpectedly, we found that total RNA prepared from every species tested, including C. elegans and E. coli, potently stimulated PrPres amplification in our purified system (Fig. 2). For each preparation, the threshold RNA concentration for stimulation of purified PrPres amplification was ~1 µg/ml, and stimulation was optimal at an RNA concentration of ~10 µg/ml (Fig. 2). In contrast, the threshold concen-
Samples containing purified hamster PrPC, Sc237 PrP27–30, and varying concentrations of total RNA were incubated for 16 h at 37 °C and subjected to proteinase K digestion, except where indicated (-PK), as described under “Experimental Procedures.” Hamster and mouse RNA were prepared from livers, and worm and bacterial RNA were prepared from whole animals.

These results suggest that no specific RNA species is uniquely responsible for stimulating purified PrPres amplification. To confirm this hypothesis, we compared the potencies of poly(A) and poly(A) RNA for stimulation of purified PrPres amplification. The results indicate that, despite 100-fold enrichment of mRNA molecules in the poly(A) fraction compared with the poly(A) fraction, the two preparations stimulated purified PrPres amplification with equal potency (Supplemental Fig. S2). In addition, we found no difference in stimulation potency between brain and liver total RNA, indicating that stimulatory RNA molecules are not specifically enriched in brain tissue (data not shown).

Based on these results, we speculated that perhaps a broad range of polyanions might stimulate purified PrPres amplification, and therefore we tested a variety of pure compounds for their ability to stimulate PrPres amplification. We first assayed several commercially available preparations of synthetic homopolymeric nucleic acids with overlapping size distributions. Among the compounds tested, poly(A) and poly(dT) stimulated PrPres amplification at concentrations of 1 μg/ml; poly(dA) stimulated PrPres amplification at a concentration of 100 μg/ml; and poly(C) failed to stimulate PrPres amplification; a fraction containing monomeric nucleotides did not stimulate PrPres amplification; a fraction containing poly(U)-containing polymers stimulated PrPres amplification only moderately at a concentration of 1 ng/ml (Fig. 5). Other investigators have shown that copper affects the affinity of heparin binding to PrP (24), and therefore we also tested the compounds heparan sulfate and pentosan sulfate stimulate cell-free conversion of radiolabeled PrP. Therefore, we tested the compounds heparan sulfate (molecular mass, 12–14 kDa), pentosan sulfate, and HSPG (molecular mass, >400 kDa) for their ability to stimulate purified PrPres amplification. Pentosan sulfate and HSPG stimulated PrPres amplification only moderately at a concentration of 100 μg/ml, whereas heparan sulfate had no effect (Fig. 5). Other investigators have shown that copper affects the affinity of heparin binding to PrP (24), and therefore we also measured heparan sulfate stimulation of purified PrPres amplification in the presence of copper. We found no apparent stimulation of PrPres amplification by heparan sulfate in the presence of 1–100 μM CuCl2 (data not shown). The levels of stimulation induced by pentosan sulfate and HSPG were both <30% of the level of control stimulation by total hamster liver RNA (Fig. 5, compare lanes 3, 4, and 7). An artificial polyanionic compound, polyglutamate (molecular mass ~50–100 kDa), also stimulated purified PrPres amplification over a broad range of concentrations from 0.1 to 100 μg/ml (Fig. 5). However, the level of stimulation induced by polyglutamate was again less than the level of control stimulation induced by total hamster RNA, and some of the apparent increase in PrPres signal caused by polyglutamate may be attributable to a direct effect of this compound on the inherent protease resistance of PrP27–30 (Fig. 5, bottom panel, lane 6).

Some studies suggest a role for charged lipids in PrP struc-
DISCUSSION

In this report, we describe the first in vitro PrP conversion system using partially purified substrates capable of amplifying PrPres levels. The essential components for efficient conversion in this system include PrP\textsuperscript{C}, PrP\textsuperscript{Sc}, and a polyanionic scaffold. The molar ratio of PrP\textsuperscript{Sc}-to-PrP\textsuperscript{C} used in this purified PrPres amplification system is ~1:250, and ~10-fold amplification of PrPres is observed after a 16-h incubation period. In contrast, the radiolabel cell-free conversion assay requires a 50:1 molar ratio of PrP\textsuperscript{Sc}-to-PrP\textsuperscript{C} to trigger conversion (9). Reconstitution experiments show that the difference in PrP conversion efficiency between the two systems can be attributed mainly to the stimulatory effect of the polyanions (Fig. 1C). Another difference between the two systems is that the PrP\textsuperscript{C} substrate used in the cell-free conversion assay lacks a glycosphingolipid anchor (9); additional work will be required to determine whether presence of the glycosphingolipid anchor affects the efficiency of PrP conversion in vitro. Earlier experiments performed with the cell-free conversion assay as well as some of the experiments reported here show that the interaction of purified PrPC and PrPSc molecules is more potent at stimulating PrPres amplification in the purified system than the crude system. The precise nature of the interaction between polyanions and components of the crude homogenate that causes lower potency and higher specificity in stimulating PrPres amplification remains to be determined. Consistent with the low specificity of PrPres amplification by polyanions in our purified system, other investigators have reported that purified, recombinant PrP molecules bind to a number of different polyanionic molecules (24, 27–37). It is important to note that our results only identify one possible set of molecular components that is able to generate efficient PrPres amplification. It is possible that polyanions are not the only factor that affects the efficiency of PrPres amplification.

Brown, S. A., Main, K. A., and Prusiner, S. B. (2020) Prion Protein Conversion 26877

Fig. 6. Western blot of purified PrPres amplification assays testing stimulation by free nucleotides, brain gangliosides, and glycogen. Samples containing purified hamster PrP\textsuperscript{C}, Sc237 PrP27–30, and varying concentrations of each compound were incubated for 16 h at 37 °C and subjected to proteinase K digestion, except where indicated (-PK), as described under “Experimental Procedures.”

Fig. 5. Western blot of purified PrPres amplification assays testing stimulation by non-nucleic acid polyanions. Samples containing purified hamster PrP\textsuperscript{C}, Sc237 PrP27–30, and varying concentrations of polyanions were incubated for 16 h at 37 °C and subjected to proteinase K digestion, except where indicated (-PK), as described under “Experimental Procedures.”

Fig. 4. Stimulation of purified PrPres amplification assays by size-fractionated synthetic poly(A) RNA. Upper panel, Western blot of purified PrPres amplification testing stimulation by synthetic poly(A) size fractions. Samples containing purified hamster PrP\textsuperscript{C}, Sc237 PrP27–30, and 10 μg/ml size-fractionated poly(A) RNA were incubated for 16 h at 37 °C and subjected to proteinase K digestion, except where indicated (-PK), as described under “Experimental Procedures.” Lower panel, agarose gel electrophoresis of corresponding poly(A) size fractions. One microgram of each poly(A) size fraction was electrophoresed on a 1% agarose gel and visualized by staining with SybrGold (Molecular Probes, Eugene OR). Note that the poly(A) 10-mer was not visualized under these conditions, but its concentration was confirmed by A\textsubscript{260 nm}.
factors present in the brain homogenate that contribute to the efficiency of the reaction. Furthermore, because our PrP\textsuperscript{C} preparations are not 100% pure, it is possible that some of the additional polypeptides that co-purify with PrP\textsuperscript{C} may also be required for PrPres amplification. Nonetheless, our current results significantly extend our previous work in homogenates (15). In particular, the observation that polyanion stimulation of PrPres amplification is less selective in a purified system raises the possibility that polyanions other than RNA, or perhaps even multiple polyanions, can act as endogenous stimulators of PrPres formation.

Our observations are consistent with the explanation that endogenous polyanions may accelerate the rate of prion disease progression by acting as scaffolds or surfaces that facilitate interaction between PrP\textsuperscript{C} and PrP\textsuperscript{Sc} molecules. This explanation was originally proposed by Wong et al. (23) to explain the stimulation of PrPres formation by glycosaminoglycans. Candidate stimulatory endogenous polyanions include proteoglycans and host-encoded cellular nucleic acids, which could be released from dying cells into the extracellular space. It is also possible that the polyanionic compounds are able to stimulate PrPres amplification 	extit{in vitro} mimic negatively charged surfaces of specific accessory proteins and that such proteins facilitate prion propagation. Our pol\(\text{Y}\text{A})\text{ size fractionation experiments indicate that a minimum molecular size of \(\sim 300\) bases is required for full stimulation activity. There are several potential explanations for this observation as follows: 1) Optimal stimulation of PrP conversion may require the polyanion to adopt a particular surface or three-dimensional structure. This could explain why synthetic poly\(\text{C}\) failed to stimulate PrPres amplification (Fig. 3). It is known that, unlike other homopolymeric polynucleotides, poly\(\text{C}\) does not easily acquire secondary structure (38). 2) A scaffold length of at least 300 bases may be required to accommodate the minimum PrP\textsuperscript{C}/PrP\textsuperscript{Sc} “conversion unit,” the subunit composition of which remains unknown. 3) Large polyanions may disaggregate prion rods and thereby increase the number of infectious particles available to drive conversion. Further studies will be required to distinguish between these possibilities.

Our biochemical approach is inherently limited in its ability to model the process of prion propagation in vivo. However, the hypothesis that endogenous polyanions play a pathogenic role in prion disease is also supported by the observation that both small polyanionic compounds and polycationic dendraimers block prion propagation. Small polyanionic compounds may block prion propagation by competitively inhibiting endogenous stimulatory polyanions (20, 39, 40), and dendrimers may bind to and sequester endogenous polyanions, preventing their interaction with PrP molecules. Metabolic inhibitor and enzyme degradation studies in scrapie-infected neuroblastoma cells provide additional evidence that heparan sulfate molecules play a rate-limiting role in prion propagation (22). In our studies, HSPG molecules were significantly less potent than endogenous and synthetic nucleic acids in stimulating purified PrPres amplification. However, the HSPG preparation used in our studies was specifically prepared from the basement membrane of Engelbreth-Holm-Swarm mouse sarcoma cells, and it is possible that other proteoglycans would be more potent in stimulating PrPres amplification.

Our reconstitution of PrPres amplification using purified and synthetic components may also contribute to the development of sensitive prion detection assays such as protein-misfolding cyclic amplification (11, 41). Preparations of purified PrP\textsuperscript{C} and synthetic polyanions can be prepared more uniformly and quantitatively than crude homogenates, leading to more consistent amplification assays. Furthermore, defined components could be chemically manipulated to simplify assay formats. For instance, it may be possible to attach either PrP\textsuperscript{C} or a synthetic polyanion to a solid surface, where it could act as both an amplification substrate as well as a capture reagent for PrP\textsuperscript{Sc}.

The observation that certain polyanions directly stimulate induced PrP\textsuperscript{Sc} misfolding may also have relevance to other disease processes. It is interesting to speculate that polyanions such as nucleic acids and HSPG may play roles in the pathogenesis of other neurodegenerative diseases associated with protein misfolding. Notably, HSPG and specific neuronal RNA molecules accumulate in extracellular plaques associated with Alzheimer’s disease (42–44). The interaction between endogenous polyanions and misfolded neuronal polypeptides, such as PrP or A\textsubscript{\(\beta\)\textsubscript{42}}, may eventually prove to be a common therapeutic target for a broad range of neurodegenerative diseases.

Acknowledgments—We thank Lawrence Myers, Judy Rees, and William Wickner for their helpful suggestions, and Nicholas Orem for technical assistance.

REFERENCES

1. Prusiner, S. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13363–13383
2. Butler, D. A., Scott, M. R., Bockman, J. M., Borchelt, D. R., Taraboulos, A., Hsiao, K. R., Kingsbury, D. T., and Prusiner, S. B. (1988) J. Virol. 62, 1558–1564
3. Caughey, B., Neary, K., Buller, R., Ernst, D., Perry, L. L., Chesebro, B., and Raymond, G. J. (1993) J. Virol. 64, 1093–1102
4. Bolton, D. C., McKinley, M. P., and Prusiner, S. B. (1982) Science 218, 1309–1311
5. Reissner, S. B. (1982) Science 216, 136–144
6. Legname, G., Baskakov, I. V., Nguyen, H. O., Riesner, D., Cohen, F. E., DeArmond, S. J., and Prusiner, S. B. (2004) Science 305, 673–676
7. Bossers, A., Belt, P., Raymond, G. J., Caughey, B., de Vries, R., and Smits, M. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4931–4936
8. Kocisko, D. A., Caughey, B. J., Prilea, S. A., Chesebro, B., and Raymond, G. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1343–1352
9. Saborio, G. P., Permanne, B., and Soto, C. (2001) Nature 411, 810–813
10. Lucassen, R., Nishina, K., and Supattapone, S. (2003) Biochemistry 42, 4127–4135
11. Telling, G. C., Scott, M., Mastriani, J., Gabizon, R., Tarrhia, M., Cohen, F. E., DeArmond, S. J., and Prusiner, S. B. (1995) Cell 83, 79–90
12. Saborio, G. P., Soto, C., Kacsak, R. J., Levy, E., Kacsak, B., Harris, D. A., and Frangione, B. (1999) Biochem. Biophys. Res. Commun. 258, 470–475
13. Deleaut, N. R., Lucassen, R. W., and Supattapone, S. (2003) Nature 425, 717–720
14. Caughey, B. and Kocisko, D. A. (2003) Nature 425, 673–674
15. Peck, M. J., Baldwin, W., Nguyen, J., Gass, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E., and Prusiner, S. B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10962–10966
16. Pan, K. M., Stahl, N., and Caughey, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3923–3927
17. Nishina, K., Deleaut, N. R., Lucassen, R. W., and Supattapone, S. (2004) Biochemistry 43, 2613–2621
18. Caughey, B. and Raymond, G. J. (1993) J. Viral. 67, 443–450
19. Shahed, G. M., Meiner, Z., Avraham, I., Taraboulos, A., and Gabizon, R. (2001) J. Biol. Chem. 276, 14324–14328
20. Ben-Zaken, O., Tzaban, S., Tal, Y., Horonchik, L., Eoho, J. D., Vlodavsky, I., and Caughey, B. (2001) J. Biol. Chem. 276, 40041–40049
21. Wong, C., Xiong, L. W., Horiuchi, M., Raymond, L., Wehrly, K., Chesebro, B., and Raymond, G. J. (2002) Nature 417, 471–474
22. Kocisko, D. A., Prilea, S. A., Raymond, G. J., Chesebro, B., Lansbury, P. T., Jr., and Caughey, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3923–3927
23. Sanghera, N., and Pinheiro, T. J. (2002) Biotechnol. Appl. Mycol. 82, 377–386
Biochem. 37, 173–182
34. Nandi, P. K., Leclerc, E., Nicole, J. C., and Takahashi, M. (2002) J. Mol. Biol. 322, 153–161
35. Adler, V., Zeiler, B., Kryukov, V., Kascsak, R., Rubenstein, R., and Grossman, A. (2003) J. Mol. Biol. 332, 47–57
36. Nandi, P. K., and Nicole, J. C. (2004) J. Mol. Biol. 344, 827–837
37. Hundt, C., Peyrin, J. M., Haik, S., Gauczynski, S., Leuchtt, C., Rieger, R., Riley, M. L., Deslys, J. P., Dormont, D., Lasmezas, C. I., and Weiss, S. (2001) EMBO J. 20, 5876–5886
38. Ansevin, A. T., Macdonald, K. K., Smith, C. E., and Hnilica, L. S. (1975) J. Biol. Chem. 250, 281–289
39. Schonberger, O., Horonchik, L., Gabizon, R., Papy-Garcia, D., Barritault, D., and Taraboulos, A. (2003) Biochem. Biophys. Res. Commun. 312, 473–479
40. Adjou, K. T., Simoneau, S., Sales, N., Lamoury, F., Dormont, D., Papy-Garcia, D., Barritault, D., Deslys, J. P., and Lasmezas, C. I. (2003) J. Gen. Virol. 84, 2595–2603
41. Soto, C., Anderes, L., Suardi, S., Cardone, F., Castilla, J., Frussard, M. J., Peano, S., Saa, P., Limido, L., Carbonatto, M., Ironside, J., Torres, J. M., Pocchiari, M., and Tagliavini, F. (2005) FEBS Lett. 579, 638–642
42. Ginsberg, S. D., Crino, P. B., Hemby, S. E., Weingarten, J. A., Lee, V. M., Eberwine, J. H., and Trojanowski, J. Q. (1999) Ann. Neurol. 45, 174–181
43. Ginsberg, S. D., Crino, P. B., Lee, V. M., Eberwine, J. H., and Trojanowski, J. Q. (1997) Ann. Neurol. 41, 200–209
44. van Horsen, J., Wesseling, P., van den Heuvel, L. P., de Waal, R. M., and Verbeek, M. M. (2003) Lancet Neurol. 2, 482–492
45. Fischer, M., Ruliche, T., Raeber, A., Sailer, A., Moser, M., Oesch, B., Brandner, S., Aguzzi, A., and Weissmann, C. (1998) EMBO J. 17, 1255–1264
46. Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H. P., DeArmond, S. J., Prusiner, S. B., Aguet, M., and Weissmann, C. (1992) Nature 356, 577–582
47. Wessel, D., and Flugge, U. I. (1984) Anal. Biochem. 138, 141–143
Protease-resistant Prion Protein Amplification Reconstituted with Partially Purified Substrates and Synthetic Polyanions
Nathan R. Deleault, James C. Geoghegan, Koren Nishina, Richard Kascsak, R. Anthony Williamson and Surachai Supattapone

J. Biol. Chem. 2005, 280:26873-26879.
doi: 10.1074/jbc.M503973200 originally published online May 24, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503973200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/05/25/M503973200.DC1

This article cites 47 references, 18 of which can be accessed free at
http://www.jbc.org/content/280/29/26873.full.html#ref-list-1