Abnormal Properties of Prion Protein with Insertional Mutations in Different Cell Types*

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Inherited forms of the human transmissible spongiform encephalopathy Creutzfeldt-Jakob disease (CJD) have been associated with mutations in the normal soluble, protease-sensitive form of the host prion protein (PrP-sen). Normal PrP protein contains five copies of a repeating eight-amino acid region, and PrP molecules with six or more copies of this region are associated with disease in familial CJD. It has been hypothesized that these mutations might facilitate spontaneous formation of the abnormal, aggregated protease-resistant PrP isoform, PrP-res, associated with clinical CJD and other transmissible spongiform encephalopathies (TSE). In the present experiments, hamster PrP molecules with 5 (wild-type), 7, 9, or 11 copies of this repeat region were generated and expressed in mouse fibroblast cells or mouse neuroblastoma cells. In mouse fibroblast cells, mutant hamster PrP molecules expressing 7, 9, and 11 copies of the octapeptide repeat sequence showed altered cell surface expression, but both mutant and wild-type hamster PrP-sen molecules demonstrated abnormal properties of aggregation and increased protease resistance. By contrast in mouse neuroblastoma cells, hamster PrP-sen with 5, 9, and 11 octapeptide repeats were expressed normally on the cell surface, but only PrP-sen molecules with 9 or 11 copies of the repeat motif had abnormal properties of aggregation and increased protease resistance. Overall, regardless of cell type, hamster PrP molecules with greater than 7 octapeptide repeats were more aggregated and more protease-resistant than molecules with 7 repeats or less. However, these abnormal molecules were at least 1000-fold less protease-resistant than bona fide PrP-res derived from TSE-infected brain tissue, and they showed no increased ability to form PrP-res in a cell-free system.

A key event in the pathogenesis of the TSE is the conversion of a normal, proteinase K-sensitive host protein, PrP-sen, into an abnormal partially proteinase K-resistant form, PrP-res. Direct interactions between PrP-sen and PrP-res are necessary for this conversion to occur (9–11). PrP-sen and PrP-res are encoded by the same host gene, and there are no known differences in post-translational modifications between the two molecules which distinguishes them (12, 13). PrP-res has been detected in most familial CJD patients with octapeptide repeat insertions in the PrP gene (6, 7). However, it is unclear whether the PrP-res is derived from the mutant PrP allele, the normal PrP allele, or both.

One possible explanation for the occurrence of familial CJD is that the mutant PrP protein can spontaneously form PrP-res which slowly accumulates over time, eventually leading to disease. In partial support of this hypothesis, a mutant mouse PrP protein containing 11 copies of the amino acid repeat was shown to have a slightly increased resistance to low concentrations of proteinase K (PK) and was not expressed normally on the surface of Chinese hamster ovary (CHO) cells (14–16). In vivo, PrP molecules with six or more copies of the repeat region are all associated with disease, but the effect of increasing numbers of the repeat region on aggregation and protease resistance is unknown.

To more closely examine the relationship between the number of amino acid repeats and increased aggregation and protease resistance, we engineered hamster PrP (HaPrP) molecules which contained 5 (wild-type), 7, 9, and 11 copies of this region and assayed their characteristics in two different cell types. In mouse fibroblast cells, PrP molecules with greater than 5 copies of the octapeptide repeat were expressed abnormally on the cell surface, but all PrP molecules tested showed a significant increase in aggregation and a slight increase in protease resistance. However, in mouse neuroblastoma cells, all PrP molecules tested were expressed normally on the cell surface.

The transmissible spongiform encephalopathies (TSE) are a group of rare, neurodegenerative diseases which include scrapie in sheep, bovine spongiform encephalopathy in cattle and kuru, and Gerstmann-Sträussler-Scheinker syndrome and Creutzfeldt-Jakob disease (CJD) in humans. In humans, TSE disease can occur in iatrogenic, sporadic, and familial forms. Iatrogenic CJD is associated with exposure to CJD contaminated materials such as dura mater, corneal transplants, or cadaver-derived human growth hormone. Sporadic CJD occurs with a worldwide incidence of 1 in 1–2 million. In sporadic CJD there is no known exposure to the infectious CJD agent and the exact cause of disease is unclear (1).

Familial TSE are forms of CJD or Gerstmann-Sträussler-Scheinker syndrome which vary in disease incubation times and clinical presentation and are associated with both point and insertion mutations in the PrP gene (see Pocchiari (1) for review). The insertion mutations occur in a portion of the PrP gene that has five copies of a repeating eight amino acid region and involve extra copies of this motif (2–7). In families with insertion mutations onset of disease is usually early in life and the disease course is long, sometimes lasting several years. Generally, the greater the number of insertions the longer the disease and the earlier the age of onset (6, 8).

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† The abbreviations used are: TSE, transmissible spongiform encephalopathy; CJD, Creutzfeldt-Jakob disease; PK, proteinase K; PrP, prion protein; PrP-sen, protease-sensitive PrP; PrP-res, protease-resistant PrP; HaPrP, hamster PrP; PIPLC, phosphotidylinositol-specific phospholipase C; MNB, mouse neuroblastoma; CHO, Chinese hamster ovary.
surface and only PrP molecules with 9 or 11 copies of the octapeptide motif demonstrated an increase in aggregation and protease resistance. Our studies suggest that in vivo the cell type in which PrP is expressed may be an important determinant of whether or not mutant PrP-sen acquires abnormal properties.

**EXPERIMENTAL PROCEDURES**

**Clones**—The wild-type HaPrP clone has been described previously (17). The clones made are summarized in Fig. 1A (see “Results”). To insert additional copies of the octapeptide motif into HaPrP, a BamHI site was engineered into wild-type HaPrP cloned into the vector Bluescript-SK (Stratagene). The primers used for amplification were the M13 primer and the HaPrP primer 5′-TACCCACCTCGGTTGGCGG-GGCGATCTGGGGGAG-3′ which introduces a BamHI site at codons 55 and 56 of HaPrP. The mutated bases are underlined. Although this introduces a Thr to Ser change at codon 56 in HaPrP, the sequence of the octapeptide repeat is the same as repeat motifs in other PrP molecules (1). The polymerase chain reaction product was confirmed by sequencing and the HaPrP with the BamHI site was subcloned into the Bluescript-SK vector or the retroviral vector pSFF (17) from which the mouse PrP proteins. Individual cell clones were not derived as >95% of the cells were positive for the expressed construct by immunofluorescence analysis and expression levels were stable over multiple cell passages (data not shown). The mouse neuroblastoma (MNB) cell line N2a and the MNB cell line that expresses the wild-type HaPrP gene have been described previously (18). All of the MNB cell lines expressing exogenous HaPrP constructs with 9 or 11 copies of the octapeptide repeat region were produced by transducing MNB cells with retroviral vectors expressing the various PrP constructs. Single-cell clones expressing high levels of HaPrP protein recognized by the hamster-specific monoclonal antibody 3F4 were derived as described previously (18). At least two independent cell lines were assayed for each HaPrP. All MNB cell lines were maintained in Dulbecco’s modified essential medium supplemented with 10% fetal bovine serum and 300 units/ml penicillin plus 10% bovine serum. The 9/10 was treated with 80 μg/ml PK for 1 h at 37 °C, and no difference was observed in the kinetics of biosynthesis or the turnover rate of the mutant HaPrP proteins when compared with wild-type HaPrP (data not shown).

**Expression of Mutant HaPrP—**Hamster PrP contains a repeating region of amino acids consisting of one nine-amino acid motif and four tandem copies of an eight-amino acid region. Hamster PrP with 5 (HaPrP-5, wild-type), 7 (HaPrP-7), 9 (HaPrP-9), or 11 (HaPrP-11) copies of the octapeptide repeat regions (Fig. 1A) were derived and expressed in the retroviral packaging cell line mix 2/PA317. These cell lines were derived from NIH 3T3 mouse fibroblasts. Cell-free conversion of the three mutant HaPrP proteins by treating the 2/PA317 cells with PIPLC which specifically cleaves the phosphotidylinositol anchor attaching PrP to the cell surface. As the number of repeats increased, the amount of monomeric HaPrP released by PIPLC decreased (Fig. 2A). Consistent with a previous study (18), the HaPrP dimer was not releasable by PIPLC treatment. These results demonstrated that in 2/PA317 cells aberrant cell surface expression of HaPrP correlated with increasing numbers of octapeptide repeats.

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buffer cushion for 45 min at 353,000 × g as described previously (18). Detection of HaPrP was by SDS-polyacrylamide gel electrophoresis and immunoblotting using the HaPrP-specific monoclonal antibody 3F4 as described previously (18).

**Cell-free Conversion**—The in *vitro* conversion of HaPrP-sen to HaPrP-res was as described previously (11). Briefly, 1 μg of HaPrP-sen was incubated with 10–20,000 cpm of radiolabeled HaPrP-sen at 37 °C for 2 days. After incubation, 1/10 of the reaction was precipitated in methanol. The remaining 9/10 was treated with 80 μg/ml PK for 1 h at 37 °C, PK was inactivated with protease inhibitors, and the protein was methyl-lysine-precipitated. Radiolabeled protease-resistant products were analyzed by SDS-polyacrylamide gel electrophoresis. The amount of protease-resistant (17–32 kDa) and protease-sensitive (25–70 kDa) radiolabeled protein was determined using the Molecular Dynamics Storm PhosphorImager system. Bands were quantified in terms of volume, and the percent conversion was calculated using the formula

\[
\text{Percent conversion} = \left( \frac{\text{vol of protease-resistant}}{\text{vol of protease-sensitive}} \right) \times 100
\]

**RESULTS**

**Expression of Mutant HaPrP—**Hamster PrP contains a repeating region of amino acids consisting of one nine-amino acid motif and four tandem copies of an eight-amino acid region. Hamster PrP with 5 (HaPrP-5, wild-type), 7 (HaPrP-7), 9 (HaPrP-9), or 11 (HaPrP-11) copies of the octapeptide repeat regions (Fig. 1A) were derived and expressed in the retroviral packaging cell line mix 2/PA317. These cell lines were derived from NIH 3T3 mouse fibroblasts (17). All cells expressed similar levels of the different glycosylated forms of monomeric HaPrP as well as the dimeric form of HaPrP we have described previously for HaPrP-5 (18). The expected increase in molecular mass was observed with the successive addition of the eight amino acid repeat region (Fig. 1B). No difference was observed in the kinetics of biosynthesis or the turnover rate of the mutant HaPrP proteins when compared with wild-type HaPrP (data not shown).

**Cell Surface Expression of Mutant HaPrP—**PrP is normally attached to the cell surface via a phosphotidylinositol anchor (19), but mutant PrP molecules can be aberrantly expressed on the cell surface (14, 15, 20). We tested the cell-surface expression of the three mutant HaPrP proteins by treating the 2/PA317 cells with PIPLC which specifically cleaves the phosphotidylinositol anchor attaching PrP to the cell surface. As the number of repeats increased, the amount of monomeric HaPrP released by PIPLC decreased (Fig. 2A). Consistent with a previous study (18), the HaPrP dimer was not releasable by PIPLC treatment. These results demonstrated that in 2/PA317 cells aberrant cell surface expression of HaPrP correlated with increasing numbers of octapeptide repeats.

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*Phosphotidylinositol-specific Phospholipase C (PIPLC)* Treatment of Radiolabeled Cells—Treatment of radiolabeled cell monolayers with PIPLC was done as described previously (18). Briefly, a confluent 25-cm² flask of cells was incubated in the presence of Tran35S-labeled methionine/cysteine (ICN) for 90 min at 37 °C and then incubated for 30 min in 10% fetal bovine serum, Dulbecco’s modified essential medium. Cells were next incubated in the presence of 0.2 unit/ml PIPLC at 37 °C for 30 min. The medium was collected and assays for the presence of radiolabeled PrP. The cell monolayer was lysed and the lysate was also assayed for radiolabeled PrP.
but could also be dependent upon the cell type in which the mutant was expressed. Consequently, other characteristics of normal and mutant HaPrP were assayed in both \( \Psi/2PA317 \) fibroblast cells and in mouse neuroblastoma cells.

**Aggregation of Mutant HaPrP Proteins**—One of the distinguishing characteristics of PrP-res is its ability to form large aggregates (23–25). To test the aggregation of mutant HaPrP proteins expressed in \( \Psi/2PA317 \) cells and MNB cells, cell lysates were centrifuged under conditions which pelleted particles \( \geq 20 \) S in size (18). In \( \Psi/2PA317 \) cells, monomeric HaPrP proteins with 5, 7, 9, and 11 copies of the octapeptide repeat sequence aggregated to a greater extent than the same constructs in MNB cells, while the dimeric forms of these constructs aggregated less in \( \Psi/2PA317 \) cells when compared with MNB cells (Fig. 3, A and B). The differences in aggregation of HaPrP-5 and HaPrP-11 in \( \Psi/2PA317 \) cells and MNB cells were significant (\( p < 0.0006 \)). In general, as the number of repeats increased so too did the tendency of the PrP protein to aggregate. HaPrP proteins with greater than 7 copies of the octapeptide repeat region were almost always more aggregated than wild-type HaPrP-5 (Fig. 3). These differences suggested that the extent of aggregation was not only a consequence of the protein sequence but also a consequence of the cell-type in which the protein was expressed.

**PK Resistance of Mutant HaPrP Protein**—The mutant HaPrP proteins with extra copies of the octapeptide repeat region resembled PrP-res in that they showed a greater tendency to form aggregates than did wild-type HaPrP protein. Next, we tested whether or not the mutant HaPrP proteins had an increased resistance to PK, another characteristic of PrP-res. Cell lysates were digested with increasing concentrations of PK and the amount of HaPrP remaining was assayed. The monomeric and dimeric forms of the protein were similarly resistant to PK and the combined data for both PrP forms is shown in Fig. 4. In both cell types, constructs with greater than 7 copies of the octapeptide repeat regions showed an increase in PK resistance with HaPrP-11 > HaPrP-9 > HaPrP-7 or -5. All of the constructs in \( \Psi/2PA317 \) cells were more resistant to PK treatment (Fig. 4A) than the same constructs expressed in MNB cells (Fig. 4B). These data demonstrated that an increase in the number of octapeptide repeats in HaPrP led to an increase in the resistance of HaPrP to digestion by PK. However, this PK-resistance is 1000-fold less than that of PrP-res derived from scrapie-infected hamster brain which is greater than 95% resistant to digestion with up to 500 \( \mu \)g/ml PK for 30 min at 37 °C (26).

**Cell-free Formation of HaPrP-res from Wild-type and Mutant HaPrP-sen Protein**—PrP-sen mutants containing extra copies of the octapeptide motif may induce familial TSE by forming PrP-res more easily than wild-type PrP-sen. To determine if the mutant HaPrP proteins formed PrP-res more efficiently than wild-type HaPrP, mutant and wild-type HaPrP-sen proteins were assayed for their ability to form protease-resistant products in a cell-free conversion assay (11). In the absence of HaPrP-res, none of the HaPrP proteins tested was able to spontaneously convert to HaPrP-res under the conditions used (Fig. 5, A and B). In the presence of HaPrP-res, wild-type and mutant HaPrP-sen proteins from either \( \Psi/2PA317 \) or MNB cells were induced to convert to PrP-res with similar yields (Fig. 5, A and B). The results demonstrated that the insertion of extra octapeptide repeats did not result in a mutant PrP-sen molecule which formed PrP-res more efficiently than wild-type PrP-sen in this cell-free system.

**DISCUSSION**

In this study we examined the relationship between an increase in the number of octapeptide repeats and the acquisition of abnormal properties in PrP. As the number of repeats increased so did the aggregation and PK resistance of the mutant PrP protein. Interestingly, our data correlate with \textit{in vivo} observations that increasing numbers of the repeat motif are associated with an earlier onset of familial CJD (6, 8). The increased aggregation and/or protease-resistance of the mutant PrPs could be responsible for the onset of disease associated with these mutations.

The type of familial TSE associated with extra copies of the PrP octapeptide repeat region is reminiscent of Huntington’s disease. Onset of Huntington’s disease is associated with the expansion of a polyglutamine repeat sequence in the huntingtin gene from the normal 10–34 copies to the disease-associated 37–100 copies (27). As in familial CJD, the greater the number of repeats, the earlier the disease onset; expansions of 70 and above are always associated with the juvenile form of Huntington’s disease (28). Recent studies with huntingtin protein have shown that as the number of glutamine repeats increases, so does the tendency of the huntingtin protein to aggregate and form amyloid fibrils similar to those observed in the TSE and Alzheimer’s disease (28–30). Our data are very similar in that increasing numbers of the octapeptide repeat
region in PrP also appeared to increase the extent to which PrP aggregated. It is unknown, however, whether or not the mutant PrP-sen, in the absence of PrP-res, can form fibrils or intranuclear inclusions similar to those formed by the mutant huntingtin protein (28, 29).

The mechanism by which extra copies of the octapeptide repeat region influence aggregation of PrP is unclear. Other glycine-proline rich repeat regions are present in proteins such as wheat high molecular weight glutenin (31), keratin, and type I collagen (32, 33), which aggregate or form filaments. The PrP octapeptide repeats might also be involved in PrP polymerization. One possibility is that the repeat region may act as a

FIG. 2. Expression of HaPrP molecules on the cell surface. %2/PA317 fibroblast cells (A) or mouse neuroblastoma cell clones (B) expressing normal and mutant HaPrP proteins were radiolabeled with [%35S]methionine/cysteine and treated with PIPLC as described under “Experimental Procedures.” After treatment, HaPrP was immunoprecipitated from either the cell lysate (C) or the cell culture medium (M). The number of repeats in each clone are indicated at the top. The 60-kDa dimeric form of HaPrP and the fully glycosylated (32–40 kDa), partially glycosylated (30 kDa) and unglycosylated (25 kDa) monomeric forms of HaPrP are indicated on the left. Molecular mass markers in kilodaltons are shown on the right.

FIG. 3. Aggregation of HaPrP expressed in %2/PA317 or MNB cells increases with increasing numbers of the amino acid repeat regions. Cell lysates were centrifuged at 353,000 × g for 45 min, and the amount of PrP present in the pellet was assayed as described under “Experimental Procedures.” Under these conditions, particles greater than 20 S in size will pellet (18). Results for both the monomeric (dark bar) and dimeric forms (open bar) of HaPrP constructs expressed in either %2/PA317 fibroblast cells (A) or mouse neuroblastoma cells (B) are shown. The results are the average ± standard error of the mean for at least three experiments.

FIG. 4. Proteinase K resistance of HaPrP expressed in %2/PA317 cells or MNB cells increases with increasing numbers of the amino acid repeat region. Individual aliquots of the cell lysates from either %2/PA317 fibroblast cells or mouse neuroblastoma cells expressing the different HaPrP constructs were treated with increasing concentrations of proteinase K and assayed by Western blot as described under “Experimental Procedures.” The amount of HaPrP remaining was quantitated either by densitometry or PhosphorImager analysis. There was no difference in the PK resistance of the monomeric or dimeric forms within each sample, and the results have been combined. The results are the average ± standard error of the mean for multiple experiments. A, proteinase K resistance of HaPrP constructs expressed in %2/PA317 fibroblast cells. The number of repeats in each HaPrP clone is indicated above the line. B, proteinase K resistance of HaPrP constructs expressed in MNB cells. The number of repeats in each HaPrP clone are indicated above the line.
site which promotes either PrP self-aggregation or aggregation between PrP and other cellular factors (34). This is consistent with the idea that PrP mutants with extra copies of the octapeptide repeat region could more efficiently form PrP-res spontaneously in vivo.

The differences in aggregation of different PrP-sen molecules in our cells might also be influenced by overexpression of the protein. However, all of the fibroblast cells expressed similar levels of the constructs (Fig. 1B), so overexpression cannot explain the increased aggregation of the mutant HaPrP-7, HaPrP-9, and HaPrP-11 proteins versus the normal HaPrP-5 protein within fibroblast cells. The same is true for the MNB cells. However, expression level could at least in part explain the higher levels of aggregation and PK resistance in fibroblast cells compared with MNB cells, since, in general, the fibroblast cells used here expressed higher levels of recombinant PrP protein (data not shown).

In our studies in mouse fibroblast cells, there was a correlation between an increase in the number of octapeptide repeats, a decrease in expression of PrP-sen on the cell surface in a form accessible to PIPLC, and an increase in aggregation and protease resistance. In other studies in CHO cells a mutant PrP molecule which was inaccessible to PIPLC demonstrated increased aggregation and protease resistance (14–16, 35). In contrast, in our studies in MNB cells at least two different mutants (HaPrP-9 and HaPrP-11) were susceptible to PIPLC while still showing increased aggregation and PK-resistance. These findings suggest that, whereas increased aggregation and increased PK resistance correlate with increasing numbers of octapeptide repeats, sensitivity to PIPLC may be a consequence of cell type.

The altered behavior of mutant PrP following PIPLC treatment in CHO cells suggests that mutant PrP molecules begin to acquire abnormal properties as early as the translocation of PrP into the endoplasmic reticulum (35). While our data in mouse fibroblast cells is in agreement with this hypothesis, our data in mouse neuroblastoma cells is not. In neuroblastoma cells, mutant and wild-type PrP are similarly sensitive to PIPLC added to the extracellular medium (Fig. 2), but only mutant PrP molecules aggregate and are mildly PK-resistant. Therefore, in neuroblastoma cells, aggregation and PK-resistance may be acquired after translocation through the endoplasmic reticulum at a later step during biosynthesis than in CHO or fibroblast cells. This is in keeping with previous results in scrapie-infected mouse neuroblastoma cells, which demonstrated that protease-resistant PrP is made from mature PrP after it reaches the cell surface (9, 10).

The inability of our mutants to spontaneously convert to PrP-res in a cell-free assay argues that the increase in aggregation and protease-resistance of PrP-sen detected in our analyses may not be sufficient to promote the spontaneous formation of PrP-res in familial TSE. Even when HaPrP-res was added to the cell-free assay system, mutant HaPrP molecules were not induced to convert to PrP-res any more efficiently than normal HaPrP (Fig. 5). The situation could be very different in vivo, however, where over the course of time the mutant PrP proteins may eventually potentiate the spontaneous formation of PrP-res. Another possibility is that abnormal PrP proteins could facilitate an early step in pathogenesis of familial TSE. For example, mutations in PrP could act as susceptibility factors which might eventually initiate disease in vivo when combined with other events such as aging, interactions with other molecules such as chaperones (36, 37) or copper (38–40), or even exposure to an infectious agent.

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19. Stahl, N., Borchelt, D. R., Hsiao, K., and Prusiner, S. B. (1987) Cell 51, 229–240
20. Petersen, R. B., Parchi, P., Richardson, S. L., Urig, C. B., and Gambetti, P. (1996) J. Biol. Chem. 271, 12661–12668
21. Race, R. E., Fadness, L. H., and Chesebro, B. (1987) J. Gen. Virol. 68, 1391–1399
22. Race, R. E., Caughey, B., Graham, K., Ernst, D., and Chesebro, B. (1988) J. Virol. 62, 2845–2849
23. Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bendheim, P. E., Bolton, D. C., Grath, D. F., and Glenner, G. G. (1983) Cell 35, 349–358
24. Bolton, D. C., McKinley, M. P., and Prusiner, S. B. (1984) Biochemistry 23, 5898–5906
25. Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A., and Prusiner, S. B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2310–2314
26. McKinley, M. P., Bolton, D. C., and Prusiner, S. B. (1983) Cell 35, 57–62
27. Huntington’s Disease Collaborative Research Group (1993) Cell 72, 971–983
28. Davies, S. W., Turmaine, M., Corense, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., and Bates, G. P. (1997) Cell 90, 537–548
29. DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., and Aronin, N. (1997) Science 277, 1990–1993
30. Scherzinger, E., Lurz, R., Turmaine, M., Mangiarini, L., Hollenbach, B., Hasenbank, R., Bates, G. P., Davies, S. W., Lehrch, H., and Wanker, E. E. (1997) Cell 90, 549–558
31. Sugiyama, T., Raffalski, A., Peterson, D., and Soll, D. (1985) Nucleic Acids Res. 13, 8729–8737
32. Marchuk, D., McCrohon, S., and Fuchs, E. (1984) Cell 39, 491–498
33. Piez, K. A. (1984) in The Protein Folding Problem (Wetlaufer, D. B. ed) pp. 47–63, Westview, Boulder, CO
34. Jarrett, J. T., and Lansbury, P. T., Jr. (1993) Cell 73, 1055–1058
35. Daude, N., Lehmann, S., and Harris, D. A. (1997) J. Biol. Chem. 272, 11604–11612
36. Deblburman, S. K., Raymond, G. J., Caughey, B., and Lindquist, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13938–13943
37. Schirmer, E. C., and Lindquist, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13932–13937
38. Hornshaw, M. P., McDermott, J. R., and Candy, J. M. (1995) BBRC 207, 621–629
39. Hornshaw, M. P., McDermott, J. R., Candy, J. M., and Lakey, J. H. (1995) BBRC 214, 993–999
40. Brown, D. R., Qin, K., Herms, J. W., Madlung, A., Manson, J., Stromme, R., Fraser, P. E., Kruck, T., von Bohlen, A., Schule-Schaeffer, W., Giese, A., Westaway, D., and Kretzschmar, H. (1997) Nature 390, 684–687
