Amyloid formation is characterized by the conversion of soluble proteins into biochemically and structurally distinct fibers. Although amyloid formation is traditionally associated with diseases such as Alzheimer disease, a number of biologically functional amyloids have recently been described. Curli are amyloid fibers produced by Escherichia coli that contribute to biofilm formation and other important physiological processes. We characterized the polymerization properties of the major curli subunit protein CsgA. CsgA polymerizes into an amyloid fiber in a sigmoidal kinetic fashion with a distinct lag, growth, and stationary phase. Adding sonicated preformed CsgA fibers to the polymerization reaction can significantly shorten the duration of the lag phase. We also demonstrate that the conversion of soluble CsgA into an insoluble fiber involves the transient formation of an intermediate similar to that characterized for several disease-associated amyloids. The CsgA core amyloid domain can be divided into five repeating units that share sequence and structural hallmarks. We show that peptides representing three of these repeating units are amyloidogenic in vitro. Although the defining characteristics of CsgA polymerization appear conserved with disease-associated amyloids, these proteins evolved in diverse systems and for different purposes. Therefore, amyloidogenesis appears to be an innate protein folding pathway that can be capitalized on to fulfill normal physiological tasks.

Numerous studies have revealed that amyloidogenic proteins are mostly unstructured or contain mixtures of β-sheets and α-helices in their native state, but when polymerized into fibers, they invariably adopt a characteristic cross β-sheet structure (5–7). This cross β-sheet structure is common to all amyloids and is characterized by β-strands that orient perpendicular to the fiber axis. In vitro, disease-associated amyloids polymerize into fibers with nucleation-dependent kinetics with characteristic lag, growth, and stationary phase. The lag phase is proposed to contain folding intermediates that are key to the toxicity associated with certain amyloidogenic proteins (8, 9). During the lag phase, amyloidogenic proteins adopt a transient folding species that disrupts membrane integrity (3, 8, 10). Loss of membrane integrity is proposed to underlie the cell death and disease associated with many amyloids (3, 10). A conformational-specific antibody has been generated that recognizes a transient intermediate formed during amyloidogenesis of several disease-associated proteins (3).

A new class of amyloids has recently been found that plays important physiological roles for the cell. These so-called “functional” amyloids are found in bacteria (11–13), fungi (14, 15), and mammals (16). The first example of a functional amyloid in bacteria was curli (12). Curli compose part of the complex extracellular matrix that is required for biofilm formation (17–19), host cell adhesion (20), and invasion (21, 22), and they are proposed to be important stimulants of the host inflammatory response (23–25). An intriguing question is whether these functional amyloid proteins polymerize in a manner similar to disease-associated amyloids.

Curli formation is the result of an elegant biosynthetic pathway directed by the Csg proteins in Escherichia coli. The major curli subunit, CsgA, can be secreted to the cell surface as a soluble, unstructured protein (12, 26). CsgA is efficiently nucleated into an insoluble amyloid fiber in the presence of the outer membrane-associated protein, CsgB (27). After nucleation, the fibers are predicted to grow by subsequent CsgA addition to the tip of the amyloid fiber (26). Both CsgA and CsgB display a remarkable five-fold internal symmetry characterized by conserved polar residues. These five “repeating units” consist of 19–24 amino acids and align along serine, glutamine, and asparagine residues (26, 28). Each repeating unit is predicted to form a strand-loop-strand motif that closely resembles the cross β-spine structure described for many disease-associated amyloids (28–30).

Here we characterize the folding of purified CsgA and show that its polymerization is similar to that of disease-associated amyloids. CsgA polymerization involves a transient structurally
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conserved intermediate that implies a common polymerization pathway between functional and disease-associated amyloids. We found that the conserved folding intermediate for CsgA is a monomer or low molecular weight multimer. We demonstrate that at least three of five repeating units of CsgA are amyloidogenic. These results suggest that covalent linkage of multiple amyloidogenic units facilitates efficient fiber formation.

EXPERIMENTAL PROCEDURES

CsgA Purification—CsgG and CsgA-His were overexpressed in LSR12 (C600::Δcsg) as described previously (12). Following centrifugation for 15 min at 10,000 × g, the supernatant was clarified by filtration through a 0.22-μm polyethersulfone bottle-top filter (Corning, Acton, MA). Filtrates containing CsgA were passed over a HIS-Select™ HF nickel-nitrioltriacetic acid (Sigma) column, washed with 10 volumes of 10 mM potassium phosphate buffer (KPi),2 pH 7.2, and eluted with 10 mM KPi 100 mM imidazole, pH 7.2. CsgA-containing fractions were combined and passed through a 0.02-μm Anotop 10 filter (Whatman, Maidstone, UK). The N-terminal portion of purified CsgA-His was sequenced by mass spectrometry and found to be identical to the predicted CsgA amino acid sequence. A modified protocol using guanidine hydrochloride (GdnHCl) was employed to fully denature CsgA-His. Following the first wash, the column was equilibrated with 5 volumes of 10 mM KPi, 8 mM GdnHCl, pH 7.2, and eluted with 50 mM KPi, 8 mM GdnHCl, pH 2. Sephadex G25 was used for desalting/buffer exchange. To create CsgA-His seeds, 2-week-old fibers were sonicated using a Fisher Model 100 sonic dismembrator (Fisher) for three 15-s bursts on ice. Where indicated, CsgA samples were filtered through a prewashed Amicon Ultra-4 (Millipore, Bedford, MA) centrifugal filter device. Samples were centrifuged at 4,000 × g for 2 min, and the retentate and filtrate fractions were collected. A plasmid encoding CsgA-His allowed to dry for 5 min. The membrane was blocked in 5% milk in TBS-T (Tris-buffered saline with 0.01% Tween 20) for at least 1 h. The dot blots were developed using the SuperSignal® West Dura system (Pierce). Blots were stripped and reprobed with a 1:10,000 dilution of rabbit anti-CsgA antibody (31).

Electron Microscopy—Philips CM12 Scanning Transmission Electron Microscope was used to visualize the fiber aggregates. Samples (10 μl) were placed on Formvar-coated copper grids (Ernest F. Fullam, Inc., Latham, NY) for 2 min, washed with deionized water, and negatively stained with 2% uranyl acetate for 90 s.

Peptide Preparation—Peptides were chemically synthesized by Proteintech Group Inc., Chicago, IL. Purity was greater than 90% by high pressure liquid chromatography, and size was confirmed by mass spectroscopy. To equilibrate the pH of each sample and to remove any potential seed in the peptide preparations, the peptides were denatured using a modified protocol described previously (32). Briefly, peptides were dissolved to 0.5 mg/ml in trifluoroacetic acid/hexafluoroisopropanol (1:1 v/v) and sonicated for 10 min. The suspensions were incubated at room temperature until they visually cleared. The solvent was then removed by vacuum. Peptides were then dissolved in cold 2 mM HCl and centrifuged at 100,000 × g in a TLA-55 (Beckman Coulter) for 1 h at 4 °C. The supernatants were equilibrated to 50 mM KPi, pH 7.2, by 200 mM KPi, pH 7.2, on ice. When the samples were shifted to room temperature, the polymerization was measured by ThT.

RESULTS

CsgA Polymerization Kinetics—To determine the polymerization kinetics of CsgA, an in vitro polymerization assay was developed. The transition of freshly purified, soluble CsgA to amyloid fibers was monitored using ThT, an amyloid-specific dye commonly used to assay amyloid formation (33, 34). The ThT fluorescence of CsgA samples followed a sigmoidal curve (Fig. 1A). Two hours after purification, regular, unbranched amyloid fibers were readily observed (Fig. 1D). Dense fiber aggregates were also observed 7 days after purification (Fig. 1E).

2 The abbreviations used are: KPi, potassium phosphate buffer; ThT, thioflavin T; GdnHCl, guanidine hydrochloride; R, repeating unit; TEM, transmission electron microscopy; Aβ, amyloid β. 

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The A11 Antibody Recognizes a Transient CsgA Folding Species—The polymerization of eukaryotic amyloids involves the formation of an intermediate folding species proposed to cause amyloid-associated toxicity to host cells (2, 3). The A11 antibody recognizes an Aβ transient intermediate (3). Remarkably, this antibody also recognizes a transient intermediate formed by islet amyloid polypeptide, polyglutamine, prion peptide 106–126, and Sup35p, among others (3, 37). The A11 antibody recognizes only a transient intermediate species, not soluble monomers or mature amyloid fibers derived from these proteins.

The A11 antibody was used to determine whether CsgA shared a common polymerization intermediate with eukaryotic amyloids. We found that immediately after purification, CsgA was recognized by the A11 antibody (Fig. 2A). As fiber formation proceeded, evidenced by increased ThT fluorescence and the appearance of fiber aggregates by TEM, the A11 antibody lost its affinity for CsgA (Fig. 2A). A polyclonal antibody generated against CsgA recognized purified CsgA independent of its polymerization status (Fig. 2A).

The observation that the A11 antibody recognized CsgA suggested that CsgA polymerization intermediates might be structurally similar to those formed by disease-associated amyloid proteins. It also suggested that immediately after purification, CsgA had already begun its transition to an amyloid fiber. To prevent CsgA from folding during purification, the CsgA-containing fractions were amended with 8M GdnHCl. Under these strongly denaturing conditions, the A11 antibody did not recognize CsgA; however, denatured CsgA was strongly recognized by the CsgA antibody (Fig. 2B). Immediately after GdnHCl removal with a desalting column, CsgA was recognized purified CsgA independent of its polymerization status (Fig. 2A).

To determine the minimum size of the CsgA transient intermediate, freshly purified protein was passed through Amicon Ultra centrifugal membranes with different molecular weight cutoffs. The retentate and filtrate were probed with the A11 antibody (Fig. 2C). The A11 antibody recognized a species in the filtrate of the 30-kDa membrane, suggesting that
CsgA is composed of several amyloid-forming units—The observation that CsgA was recognized by the A11 antibody immediately after passing through a 30-kDa cutoff filter (Fig. 2C) was unexpected since the A11 antibody is thought to recognize an oligomeric form of amyloidogenic proteins (3, 9, 37). The number of molecules present in the oligomeric state recognized by A11 varies among amyloidogenic proteins, and Aβ oligomers have been estimated to be larger than tetramers (3, 9). However, CsgA is recognized by A11 as a monomer or at most a dimer as estimated by cutoff filtration. It is possible that a single CsgA molecule includes multiple amyloidogenic domains that collectively contribute to its interaction with the A11 antibody. The primary sequence of CsgA can be divided into three parts: the Sec-dependent signal sequence, the N-terminal 22 amino acids of the mature protein, and a repeat domain that contains five 19–22-amino-acid repeating units (Fig. 4A). The five repeating units form a protease-resistant structure that is proposed to be the amyloid core of CsgA (26, 28). Each repeat has four conserved polar amino acids: serine, glutamine, asparagine, and glutamine (Fig. 4A). The regular arrangement of glutamine and asparagine residues also occurs in CsgA homologs from different Enterobacteriaceae.3

We hypothesized that these repeating units might represent single amyloid-forming units. Peptides corresponding to each repeating unit were chemically synthesized and tested for their ability to form amyloid fibers. Two independently derived preparations of each peptide were assayed. Peptides corresponding to repeating unit 1, 3 or 5 (R1, R3, or R5) produced a ThT-positive signal and self-assembled into fibers as evidenced by TEM when incubated at 0.2 mg/ml (Fig. 4, B–E). Neither R2 nor R4 showed evidence of amyloidogenesis when resuspended at a concentration of 0.2 mg/ml, although fibers were observed by TEM when R2 or R4 was incubated at 2 mg/ml (Fig. 4B and data not shown). The morphology of R1 fibers was similar to that of those formed by purified CsgA, being generally longer than 1000 nm (compare Fig. 4C with Fig. 1, D and E). R3 fibers were consistently shorter (ranging from 200 to 1000 nm) than those formed by CsgA (compare Fig. 4D with Fig. 1, D and E). R5 fibers appeared more rigid and aggregated than CsgA fibers (Fig. 4E). The morphologies of the fibers did not appreciably change over the course of a 10-day incubation. This analysis suggests that CsgA contains at least three highly amyloidogenic domains, R1, R3, and R5, that likely drive fiber formation in vivo.

DISCUSSION

Amyloid formation is traditionally associated with uncontrolled protein misfolding and aggregation that results in many systemic and neurodegenerative disorders (1, 38). However, there are a growing number of functional amyloids that suggest amyloidogenesis is also a general tenet of normal cellular physiology. In fact, amyloid formation may be a common property of most proteins (39, 40).

The work presented here, as well as that published previously, demonstrates that both disease-associated and functional amyloids share a common amyloid formation pathway (41). CsgA polymerizes with nucleation-dependent kinetics, and fiber formation is ameliorated by the addition of preformed CsgA fibers. We also found that CsgA polymerization involves the formation of a transient species similar to that produced by other amyloidogenic proteins such as Aβ, synuclein, islet amyloid polypeptide, insulin, lysozyme, and polyglutamine (3).

The transient species that the A11 antibody recognizes during CsgA polymerization is a monomer or low molecular weight multimer (Fig. 2C). It was reported that the A11-recognized species of Aβ and Sup35p were probably large molecular

3 X. Wang and M. R. Chapman, unpublished observation.
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A

R1

R2

R3

R4

R5

C

N

alignment of internally conserved residues. The CsgA primary sequence shows the repeated consensus sequences. The identical amino acid residues of five repeats are in gray, and the conserved polar amino acid residues are enclosed in four boxes. N, N terminus; C, C terminus. B, oligopeptides of R1, R2, R3, R4, and R5 at 0.2 mg/ml in KPi were incubated at room temperature for 5 days before ThT fluorescence measurements were taken. The error bar indicates the S.E. for at least three separate experiments. CsgA fibers were diluted to 0.2 mg/ml and assayed for ThT fluorescence. C–E, TEM. 0.5 mg/ml R1, R3, and R5 in pH 7.2 KPi was incubated at room temperature for 5 days. Samples of different peptide solutions were directly applied on Formvar-coated grids and visualized with negative staining electron microscopy. The scale bar is equal to 500 nm.

weight oligomers (3, 37). Unlike Aβ and Sup35p (3, 37), CsgA was immediately recognized by the A11 antibody upon removal of strong denaturants such as GdnHCl or after its passage through a 30-kDa Amicon filter. We also found that freshly purified CsgA heated to 95 °C for 5 min was recognized by the A11 (data not shown). At least two hypotheses can be proposed to explain the ability of CsgA to be recognized by A11 immediately after denaturation or passage through a 30-kDa cutoff filter. First, CsgA may adopt an oligomeric conformation so quickly that our ability to measure this transition is lost in the time that it takes to immobilize CsgA on the blotting paper. Another possibility is that the CsgA species recognized by A11 is not an oligomer, but a monomer that contains multiple amyloidogenic units. In support of this hypothesis, we show that CsgA does indeed contain at least three amyloidogenic domains. Nevertheless, these two hypotheses are not mutually exclusive, and there may be other plausible interpretations.

Nonetheless, CsgA contains multiple amyloidogenic domains that may contribute to its ability to efficiently transition from a soluble protein to an amyloid fiber. Many studies have led to the proposal that amyloid fibers themselves are not toxic to cells; instead, toxicity is proposed to be caused by transient folding intermediates (2, 42, 43). Therefore, one mechanism that might be used by functional amyloids to prevent toxicity is to minimize the duration of toxic folding intermediates. This is apparently how Pmel17, an extremely rapidly forming functional amyloid found in mammalian cells, is able to assemble within the cell without eliciting a toxicity cascade (16).

CsgA has a striking primary sequence arrangement (Fig. 4A). The five repeats of CsgA are very similar and share greater than 30% sequence identity. We showed that each repeating unit is potentially a single amyloid domain and that R1, R3, and R5 are the highly amyloidogenic in vitro (Fig. 4, B–E). The covalent linkage of multiple amyloid domains may facilitate amyloid fiber formation by increasing the number of amyloidogenic building blocks. This also results in rapid formation of the intermediate recognized by the A11 antibody. Other amyloidogenic proteins contain repeat sequences that have been postulated to facilitate fiber formation (44, 45). For instance, the N-terminal prion-determining domain of Sup35p has five imperfect oligopeptide repeats, and certain deletions of the repeats are defective in propagation of Sup35p fibrils. Moreover, in vitro, repeat expansion peptides (with two extra repeats) were shown to be more amyloidogenic than wild-type peptides (46). Previous work suggested that the most amyloidogenic domains of CsgA were contained in the hexapeptide GHGGGN and QFGGGN, which are present in R2 and R4, respectively (47). However, our analysis suggests that R1, R3, and R5 contain the more highly amyloidogenic sequences. A thorough mutagenesis study is needed to define the residues that contribute to the highly amyloidogenic nature of CsgA.

The amyloidogenic peptides R1 and R5 contain sequences that contribute significantly to the ability of CsgA to bind human proteins such as fibronectin, plasminogen, tissue plasminogen activator, and β2-microglobulin (48). This correlation suggests that the amyloidogenicity of CsgA may be directly linked to these biological activities. In fact, work by Gebbink et al. (49) suggested that curli contribute to colonization in animal hosts by activating host proteases that are involved in hemostasis.

Curli can also enhance amyloid protein A amyloidosis in mice (50). It is proposed that cross-seeding may play a role in the development of amyloid diseases (50, 51). The in vitro system that we have established here provides an ideal vehicle to test the specificity of curli seeding with other amyloids. Understanding how functional amyloid proteins interact with other host proteins may lead to new ideas about cellular physiology and the processes that promote the toxicity associated with many amyloids.

Most amyloids are known to self-propagate in a process called seeding. In prion diseases such as bovine spongiform
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enveloped, seeding underlies protein infectivity (52). Amyloid self-propagation is also critical to disease development in the non-transmissible amyloid diseases (51, 53). Our demonstration of CsgA seeding suggests that functional amyloids also utilize a controlled self-propagation process to fulfill their biological function. In vivo CsgA polymerization is nucleated by the outer membrane-associated protein CsgB, which shares nearly 49% sequence similarity with CsgA (27). One proposed model of nucleation is that CsgB provides an amyloid-like template that initiates CsgA polymerization (26, 27). The growing fiber tip could then act as a template to direct subsequent CsgA polymerization.

Proteins that are not predicted to form stable globular folds may be prone to aggregation and amyloid formation, and indeed, most functional amyloid proteins have natively disordered segments (29, 54). Consistent with this, some proteins have been shown to form amyloid fibers only after the native, globular fold has been compromised by chemical denaturants or by mutations (39, 40, 55). The circular dichroism studies presented here suggest that CsgA is “natively unfolded” during the formation of a stably folded amyloid fiber. Importantly, in the case of functional amyloids, the amyloid fiber would not be the product of protein misfolding but that of protein folding. Certainly, the growing number of functional amyloids suggests that amyloid is an evolutionarily conserved structure. The selective processes that have been employed by functional amyloids to limit cellular toxicity provide a unique context from which to investigate disease-associated amyloidogenesis.

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REFERENCES

1. Chiti, F., and Dobson, C. M. (2006) Annu. Rev. Biochem. 75, 333–366
2. Hardy, J., and Selkoe, D. J. (2002) Science 297, 353–356
3. Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Kelly, J. W., and Eisenberg, D. (2005) J. Biol. Chem. 280, 26880–26885
4. Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S., and Hultgren, S. J. (2002) Science 295, 851–885
5. Elliott, M. A., Karoounthaisiri, N., Huang, J., Bibb, M. I., Cohen, S. N., Kao, C. M., and Buttner, M. J. (2003) Genes Dev. 17, 1727–1740
6. Coustou-Linares, V., Maddelein, M. L., Begueret, J., and Saupe, S. J. (2001) Mol. Microbiol. 42, 1325–1335
7. True, H. L., and Lindquist, S. L. (2000) Nature 407, 477–483
8. Lashuel, H. A., Hartley, D., Petre, B. M., Walz, T., and Lansbury, P. T., Jr. (2002) Nature 418, 291
9. Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M., and Ashe, K. H. (2006) Nature 440, 352–357
10. Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zordo, J., Taddei, N., Ramponi, G., Dobson, C. M., and Stefani, M. (2002) Nature 416, 507–511
11. Bieler, S., Estrada, L., Lagos, R., Baiza, M., Castilla, J., and Soto, C. (2005) J. Biol. Chem. 280, 26880–26885
12. Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S., and Hultgren, S. J. (2002) Science 295, 851–885
13. Elliott, M. A., Karoounthaisiri, N., Huang, J., Bibb, M. I., Cohen, S. N., Kao, C. M., and Buttner, M. J. (2003) Genes Dev. 17, 1727–1740
14. Coustou-Linares, V., Maddelein, M. L., Begueret, J., and Saupe, S. J. (2001) Mol. Microbiol. 42, 1325–1335
15. True, H. L., and Lindquist, S. L. (2000) Nature 407, 477–483
16. Dowler, D. M., Koulou, A. V., Alroy-Jost, C., Marks, M. S., Balch, W. E., and Kelly, J. W. (2005) PLoS Biol. 4, 66
17. Austin, J. W., Sanders, G., Kay, W. W., and Collinson, S. K. (1998) FEMS Microbiol. Lett. 162, 295–301
18. Zogaj, X., Bokranz, W., Nimtz, M., and Romling, U. (2003) Infect. Immun. 71, 4115–4118
19. Zogaj, X., Nimtz, M., Rohde, M., Bokranz, W., and Romling, U. (2001) Mol. Microbiol. 39, 1452–1463
20. Johansson, C., Nilsson, T., Olsen, A., and Wick, M. J. (2001) FEMS Immunol. Med. Microbiol. 30, 21–29
21. Gophna, U., Barlev, M., Seijffers, R., Oelschläger, T. A., Hacker, J., and Ron, E. Z. (2001) Infect. Immun. 69, 2659–2665
22. Gophna, U., Oelschläger, T. A., Hacker, J., and Ron, E. Z. (2002) FEMS Microbiol. Lett. 212, 55–58
23. Bian, Z., Brauner, A., Li, Y., and Normark, S. (2000) J. Infect. Dis. 181, 602–612
24. Bian, Z., Yan, Z. Q., Hansson, G. K., Thoren, P., and Normark, S. (2001) J. Infect. Dis. 183, 612–619
25. Tukel, C., Raffatellu, M., Humphries, A. D., Wilson, R. P., Andrews-Polymenis, H. L., Gull, T., Figueiredo, J. F., Wong, M. H., Michelsen, K. S., Akcelik, M., Adams, L. G., and Baumerl, A. J. (2005) Mol. Microbiol. 58, 289–304
26. Barnhart, M. M., and Chapman, M. R. (2006) Annu. Rev. Microbiol. 60, 131–147
27. Hammar, M., Bian, Z., and Normark, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6562–6566
28. Collinson, S. K., Parker, J. M., Hodges, R. S., and Kay, W. W. (1999) J. Mol. Biol. 290, 741–756
29. Nelson, R., and Eisenberg, D. (2006) Curr. Opin. Struct. Biol. 16, 260–265
30. Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. O., Riekel, C., Grothe, R., and Eisenberg, D. (2005) Nature 435, 773–778
31. Barnhart, M. M., Lynem, J., and Chapman, M. R. (2006) J. Bacteriol. 188, 5212–5219
32. Chen, S., and Wetzel, R. (2001) Protein Sci. 10, 887–891
33. LeVine, H., III (1993) Protein Sci. 2, 404–410
34. LeVine, H., III (1999) Methods Enzymol. 309, 274–284
35. Lomakin, A., Teplov, D. B., Kirschnitz, D. A., and Benedek, G. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7942–7947
36. Rosades, E., and Gafni, A. (2003) Biopolymers J. 84, 3480–3487
37. Shorter, J., and Lindquist, S. (2004) Science 304, 1793–1797
38. Shorter, J., and Lindquist, S. (2005) Nat. Rev. Genet. 6, 435–450
39. Fandrich, M., Fletcher, M. A., and Dobson, C. M. (2001) Nature 410, 165–166
40. Guijarro, J. I., Sunde, M., Jones, I. A., Campbell, I. D., and Dobson, C. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4224–4228
41. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J., and Lindquist, S. (1997) Cell 89, 811–819
42. Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I., and Glabe, C. G. (2005) J. Biol. Chem. 280, 17294–17300
43. Glabe, C. G., and Kayed, R. (2006) Neurology 66, 574–8
44. Ross, E. D., Minton, A., and Wickner, R. B. (2005) Nat. Cell Biol. 7, 1039–1044
45. Bieler, S., Estrada, L., Lagos, R., Baiza, M., Castilla, J., and Soto, C. (2005) J. Biol. Chem. 280, 26880–26885
45. Wright, C. F., Teichmann, S. A., Clarke, J., and Dobson, C. M. (2005) *Nature* **438**, 878–881
46. Liu, J. J., and Lindquist, S. (1999) *Nature* **400**, 573–576
47. Cherny, I., Rockah, L., Levy-Nissenbaum, O., Gophna, U., Ron, E. Z., and Gazit, E. (2005) *J. Mol. Biol.* **352**, 245–252
48. Olsen, A., Herwald, H., Wikstrom, M., Persson, K., Mattsson, E., and Bjorck, L. (2002) *J. Biol. Chem.* **277**, 34568–34572
49. Geppink, M. F., Claessen, D., Bouna, B., Dijkhuizen, L., and Wosten, H. A. (2005) *Nat. Rev. Microbiol.* **3**, 333–341
50. Lundmark, K., Westermark, G. T., Olsen, A., and Westermark, P. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6098–6102
51. Kisilevsky, R. (2000) *J. Struct. Biol.* **130**, 99–108
52. Prusiner, S. B. (1998) *Brain Pathol.* **8**, 499–513
53. Lansbury, P. T., Jr. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3342–3344
54. Uversky, V. N., Gillespie, J. R., and Fink, A. L. (2000) *Proteins* **41**, 415–427
55. Booth, D. R., Sunde, M., Bellotti, V., Robinson, C. V., Hutchinson, W. L., Fraser, P. E., Hawkins, P. N., Dobson, C. M., Radford, S. E., Blake, C. C., and Pepys, M. B. (1997) *Nature* **385**, 787–793
56. Galzitskaya, O. V., and Garbuzynskiy, S. O. (2006) *Proteins* **63**, 144–154