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New Insights into Prion Structure and Toxicity

Minireview

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Prion diseases in humans and animals are due to conformational conversion of PrP^C, a cellular glycoprotein of unknown function, into PrP^{Sc}, an isoform that appears to be infectious in the absence of nucleic acids. Proteins that behave as prions are also found in yeast and filamentous fungi. Although there is now strong experimental support for the hypothesis that prions are infectious proteins, two subjects have remained poorly understood: the structure of prions, and the mechanisms by which they kill neurons. In this review, we will highlight recent studies that shed new light on these important issues.

Prion diseases, or transmissible spongiform encephalopathies, are fatal neurodegenerative disorders that have garnered enormous attention from both scientists and the public. These disorders include Creutzfeldt-Jakob disease and kuru in humans, as well as scrapie and bovine spongiform encephalopathy in animals. They are characterized clinically by dementia and motor dysfunction, and neuropathologically by spongiosis, amyloid deposition, and neuronal loss. Prion disorders have been of enormous concern from a public health standpoint because of the epidemic spread of bovine spongiform encephalopathy and its transmission to human beings as a variant form of Creutzfeldt-Jakob disease.

A prion is defined as an infectious protein. A wealth of evidence suggests that the central molecular event in prion diseases is the conformational conversion of PrP^C, a normal cell-surface glycoprotein, into PrP^{Sc}, an abnormal isoform that is infectious in the absence of nucleic acid (Prusiner, 1998). PrP^C is largely α-helical, monomeric, and protease sensitive, while PrP^{Sc} is rich in β sheets, aggregated, and protease resistant. Prion propagation is thought to involve a templating mechanism in which PrP^{Sc} seeds conversion of PrP^C substrate in an autocatalytic fashion. Prion diseases thus exemplify a novel mechanism of biological information transfer based on self-propagating changes in protein conformation, rather than inheritance of nucleic acid sequence.

Prions have also been described in yeast and other fungi, and studies of these more experimentally tractable organisms have provided powerful insights into prion biology (Shorter and Lindquist, 2005). [*PSI*$^+$] and [*URE3*], the two most well-studied prions in *Saccharomyces cerevisiae*, are composed, respectively, of Sup35p, a translation termination factor, and Ure2p, a transcriptional regulator involved in nitrogen metabolism. The prion found in the filamentous fungus *Podospora anserina*, designated [Het-s], is derived from the HET-s protein, which plays a role in heterokaryon incompatibility. Each of these proteins can exist in two alternate conformational states, with one state capable of imprinting its conformation on the other via cytoplasmic mixing or mating. Experiments on yeast and fungi have allowed more direct tests of hypotheses concerning protein-only transmissibility, species and strain barriers, and the role of molecular chaperones than has been possible in mammalian systems. In addition, these studies have led to the important conclusion that prions are not necessarily deleterious; indeed, they can be agents of phenotypic variation and evolutionary change (True and Lindquist, 2000), and it has been proposed that they play a role in memory storage (Shorter and Lindquist, 2005). It seems likely that additional prions will be discovered in mammals and other organisms and that the prion phenomenon will turn out to be quite general in nature.

When first proposed almost 25 years ago, the prion hypothesis was greeted with intense skepticism. Now, there is a large body of compelling evidence that this idea is correct. Perhaps the most definitive experiments involve generation of synthetic prions in the test tube. Purified, recombinant Sup35p, Ure2p, and HET-s can be polymerized in vitro and introduced into susceptible yeast or fungal cells to produce self-propagating phenotypic changes (Shorter and Lindquist, 2005). Production of infectious mammalian prions has also been reported recently, either by polymerization of recombinant PrP (Legname et al., 2004) or by PrP^{Sc}-catalyzed conversion of brain-derived PrP^C in a cyclic amplification process (Castilla et al., 2005). Although subject to some criticisms, these experiments have gone a long way toward demonstrating the protein-only mechanism of prion transmission.

Although we now know a great deal about prions and the mechanisms by which they propagate, two critical subjects have remained poorly understood: the physical structure of prions and the mechanism by which they cause pathology.

The Structure of Yeast and Other Fungal Prions

Although amyloid fibers are difficult to detect in yeast and *Podospora* cells, several lines of evidence support the notion that amyloid fibers polymerized in vitro from purified Sup35p, Ure2p, and HET-s are representative of prion structures found in vivo. Most notably, the fibers are able to "infect" yeast or *Podospora* and confer the prion state. Several recent studies of these amyloid forms have provided new insights into the structure of infectious prions, the process of self-assembly, and the origin of prion strains. The results have broad implications for prion biology, amyloid-related diseases, and principles of protein structure.

In the first study, Nelson et al. (2005) carried out X-ray crystallographic analysis of amyloid polymers of a peptide derived from the N-terminal, prion-forming domain of Sup35p. The deduced structure consists of a pair of β sheets running parallel to the fiber axis and bonded together by interdigitating side chains that form a dry, self-complementary interface referred to as a steric zipper. The β strands in each sheet run perpendicular to the fiber axis and are held together by hydrogen bonds

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between strands. It was proposed that the same basic structure is common to other kinds of amyloid fibrils, all of which display a cross-β diffraction pattern. Many proteins, regardless of whether they are associated with protein-misfolding diseases, have the capacity to form amyloid fibrils under suitable conditions, and it has been suggested that this is a generic property of all polypeptide chains (Dobson, 2003).

In a second study, Ritter et al. (2005) used NMR spectroscopy and fluorescent probe accessibility to determine the structure of amyloid fibrils formed from a fragment of the HET-s protein. The basic structural unit was remarkably similar to that determined for the fibrils of Sup35p peptide, consisting of two interlocking β sheets, although in this case each HET-s molecule contributed two adjacent β strands in each sheet. In a truly elegant series of experiments, these authors went on to show that proline substitutions that disrupted the amyloid core structure also abolished the infectivity of the HET-s fibrils when tested in biological assays in *Podospora*. In contrast, structurally nondisruptive substitutions did not affect infectivity. This satisfying correlation provides strong evidence that amyloid fibrils represent the infectious form of prions and that prion propagation is likely to involve seeded polymerization of these fibrils.

In a third study, Krishnan and Lindquist (2005) employed a variety of chemical techniques to analyze the structure of amyloid fibrils assembled from a fragment of Sup35p containing the N-terminal (N) and central (M) domains of the protein. They suggest a model in which the N domain consists of head, central core, and tail regions, with the amyloid fiber formed by head-to-head and tail-to-tail contacts between subunits. Their data also suggest that the critical nucleation step of polymer assembly is the result of an initial interaction between the head regions of individual subunits.

The study by Krishnan and Lindquist also illuminates another contentious subject in the prion field: prion strains. In mammals, prion strains are defined as infectious isolates that produce distinct incubation times and patterns of neuropathology when inoculated into a standard host (usually an inbred mouse) (Prusiner, 1998). For viruses or bacteria, strains are the result of variations in the nucleic acid genomes of the infectious agents. Since prions lack nucleic acid genomes, the best way to explain prion strains is to postulate that they result from variations in protein conformation. Consistent with this hypothesis, different strains of PrP^{Sc} are known to display distinctive biochemical signatures (e.g., glycosylation pattern, protease cleavage site). Strains of yeast prions can also be distinguished, based for example on strength of nonsense suppression (for Sup35p), mitotic stability, or aggregation state of the corresponding protein (Shorter and Lindquist, 2005). Until recently, however, there was no direct evidence that the three-dimensional structures of individual prion strains differed from each other.

To address this issue, Lindquist and Krishnan analyzed the structures of two strains of Sup35p NM amyloid, produced by assembly at different temperatures (4°C and 25°C), which create different [*PSI*$^{+}$] phenotypes when introduced into yeast. These two forms were found to differ in the length of the amyloid core region as well as in the nature of the interfaces between subunits. These results are remarkably similar to those obtained by Tanaka et al. (2005), who analyzed three different Sup35p strains using electron paramagnetic resonance. In a recent study that underscores the parallel between mammalian and yeast prions, it was reported that a truncated form of PrP (23-144) could be polymerized in vitro into two kinds of fibers with distinct morphologies and spectroscopic signatures; in this case, the nature of the seed used to initiate the polymerization reaction determined which of the two fiber variants was produced (Jones and Surewicz, 2005). Interestingly, self-propagating structural polymorphisms have also been described in fibrils assembled from Aβ, the protein subunit of amyloid plaques in Alzheimer's disease, raising the possibility that strain variation may be characteristic of non-prion amyloids as well (Petkova et al., 2005).

The Structure of Mammalian Prions

The three-dimensional structure of PrP^{C}, determined by NMR spectroscopy and X-ray crystallographic analysis of recombinant and brain-derived PrP, consists of a disordered N-terminal region (residues 23–124) and a C-terminal region (residues 125–228) composed of three α helices and two short β strands flanking the first α helix (Zahn et al., 2000). The structure of PrP^{Sc} has been much more difficult to determine. It is clear that the two isoforms are very different at the level of secondary structure, with PrP^{Sc} having a much higher proportion of β sheets than PrP^{C} (45% compared with 3%) (Prusiner, 1998). However, it has not been possible to resolve the tertiary structure of PrP^{Sc}, largely because of the tendency of the protein to form large, heterogeneous aggregates that are recalcitrant to analysis by high-resolution techniques. The aggregation tendency of PrP^{Sc} reflects the intrinsic nature of the PrP^{C}-PrP^{Sc} conversion process, which has properties of a seeded polymerization. Thus, a monomeric form of PrP^{Sc} is unlikely to exist. Electron microscopic analysis of two-dimensional crystals of PrP 27-30 (a proteolytic fragment of PrP^{Sc}) in conjunction with theoretical considerations has led to a model of PrP^{Sc} that is based on stacked, left-handed β helices (Govaerts et al., 2004). However, other models have also been proposed, based on computer modeling or on structural analysis of recombinant PrP or synthetic PrP peptides that have been induced to adopt β-rich conformations (see, for example, Lee and Eisenberg, 2003).

One structural issue that has received considerable attention concerns the oligomeric structure of infectious PrP^{Sc}. The main approach to this problem has been to dissociate highly infectious PrP^{Sc} using various detergents and chaotropic agents and then test the infectivity of the resulting PrP^{Sc} oligomers, either by animal bioassay or by an in vitro conversion assay. The primary conclusion from this work has consistently been that infectivity resides in oligomeric and not monomeric forms of PrP. In a particularly rigorous new study, Silveira et al. (2005) have characterized the biophysical properties of partially disaggregated PrP^{Sc} samples using chromatographic size fractionation. These authors conclude that the greatest infectivity (per unit mass of PrP) is associated with particles having an average size of 17–27 nm (300–600 kDa) and consisting of 14 to 28 PrP molecules. Much less specific infectivity was associated with monomeric PrP or large fibrils. A major implication of this

study is that small PrP^{Sc} oligomers are much more infectious than highly polymerized amyloid rods, and therefore attempts to limit prion propagation by breaking up PrP^{Sc} aggregates may actually have the opposite effect.

Considerable evidence indicates that prion propagation in both fungi and mammals has the characteristics of a nucleated polymerization reaction. For yeast and other fungal prions, the evidence is quite strong that polymerization results in an amyloid fibril structure that is correlated with infectivity (Ritter et al., 2005; Tanaka et al., 2005). For mammalian prions, the structural data are more equivocal. Certain forms of purified, recombinant PrP polymerize into amyloid fibers in vitro (Baskakov, 2004; Jones and Surewicz, 2005). However, in only one case has it been shown that these synthetic fibers are infectious in animal bioassays (Legname et al., 2004), and in that study the infective titer was low, implying that only a small subset of molecules possessed a conformation capable of self-propagation. It has also been surprisingly difficult to visualize PrP fibrils in brain tissue, and many cases of human and animal prion diseases lack cerebral plaques that stain with amyloid-specific dyes. Thus, there is continuing debate about whether infectivity is associated with amyloid forms of PrP^{Sc}, or with other kinds of β sheet-rich, oligomeric structures (Wille et al., 2000). In the study of Silveira et al. (2005), EM analysis of the most infectious PrP^{Sc} fractions revealed small amorphous aggregates lacking the features of amyloid fibrils and without clearly defined ends. Failure to demonstrate amyloid fibrils either in situ or in purified fractions may be due to the fact that these structures are below the resolution limit of the electron microscope or because they are disrupted by the methods used for tissue preservation or protein extraction. It is also possible that cellular factors such as membrane tethering or protein degradation restrict the size of fibrils or modify their morphology in an in vivo setting. Alternatively, there may be fundamental differences between fungal and mammalian prions in the structure of the replicating unit, with mammalian prions propagating via nonamyloid conformations.

A number of human diseases are characterized by the deposition of intracellular or extracellular protein aggregates in either the CNS or peripheral tissues. These include other neurodegenerative disorders like Alzheimer's, Parkinson's, and Huntington's diseases, as well as a number of systemic amyloidoses. In many of these cases, the protein component of the deposits polymerizes into fibrils that display the cross-β structure characteristic of amyloids (Dobson, 2003). Since all of these amyloidgenic proteins are capable of self-seeded assembly, at least in vitro, why is it that only prion diseases are transmissible in vivo? Possibly, localization of some of the proteins in intracellular compartments may render them inaccessible for transfer between cells. Cellular processes such as posttranslational modification or degradation might also reduce infectious transfer. In summary, then, not all amyloids are prions (i.e., are infectious), and not all infectious proteins (prions) are necessarily amyloids.

What Is the Neurotoxic Form of PrP?

Historically, a great deal of effort in the mammalian prion field has been devoted to defining the chemical nature of the infectious agent. In contrast, much less is known about the cellular mechanisms by which prions kill neurons and the toxic forms of PrP that are responsible. While it has commonly been assumed that PrP^{Sc} itself is the primary cause of neurodegeneration in prion diseases, this assumption is based primarily on the temporal and anatomical correlation between the accumulation of this form and the development of neuropathological changes. However, there are a number of situations where this correlation is weak or absent (Chiesa and Harris, 2001). In several kinds of transmission experiments, for example, significant pathology and/or clinical dysfunction develop with little accumulation of PrP^{Sc}. Conversely, there are subclinical infections in which there is abundant PrP^{Sc} but little symptomatology. Taken together, these results argue that PrP^{Sc}, the infectious form of PrP, may not be the proximate cause of neuronal dysfunction and degeneration in prion diseases.

What is the identity of PrP^{toxic}, the neurotoxic species? Several candidates have been proposed, each of which is thought to be pathogenic but not infectious. For example, transmembrane and cytosolic forms of PrP (^{Ctm}PrP and ^{Cyto}PrP, respectively), which differ from conventional PrP in their membrane topology, each cause a neurodegenerative illness without accumulation of PrP^{Sc} when expressed in transgenic mice (Ma et al., 2002; Stewart et al., 2005). PrP is normally tethered to the outside surface of cellular membranes via a glycosylphosphatidylinositol (GPI) anchor, which is a lipid- and glycan-containing structure that is added to the C terminus of the polypeptide chain during synthesis in the ER. In contrast, ^{Ctm}PrP spans the lipid bilayer once via a conserved hydrophobic segment, with the N terminus on the cytoplasmic side. ^{Cyto}PrP lies entirely in the cytoplasm and arises from abortive translocation or reverse translocation of the polypeptide chain at the ER. Both of these forms may be neurotoxic because they interact abnormally with membranes or with cytoplasmic proteins to which the PrP polypeptide chain is not normally exposed. Although ^{Ctm}PrP and ^{Cyto}PrP cause neurodegeneration when expressed at elevated levels in transgenic mice, it remains to be proven that these species accumulate naturally during the course of a prion infection.

Additional insights into the nature of PrP^{toxic} have derived from transgenic mice that express mutant forms of PrP associated with familial prion diseases. Mice expressing a mutant PrP molecule with octapeptide insertion found in some cases of familial Creutzfeldt-Jakob disease spontaneously develop a fatal neurodegenerative disorder accompanied by accumulation of a weakly protease-resistant, noninfectious form of the mutant protein (Chiesa et al., 2003). Biochemical characterization of this form demonstrates that it consists primarily of small oligomers of conformationally altered PrP, in contrast to the larger, more tightly packer polymers of PrP^{Sc}. Thus, the biophysical properties of infectious and toxic forms of PrP may differ, with the latter being less highly polymerized. Similar conclusions have been reached in a recent study of transgenic mice that express PrP carrying a point mutation linked to Gerstmann-Sträussler syndrome, an inherited prion disorder (Nazor et al., 2005).

A recent study by Chesebro et al. (2005) sheds new light on the molecular features that are required to

make PrP neurotoxic. These authors created transgenic mice that express a form of PrP that is lacking the GPI anchor. This was accomplished by deleting the C-terminal signal for anchor attachment. Amazingly, when these mice were inoculated with scrapie prions, they failed to develop clinical signs of scrapie, even though they could be shown to replicate prion infectivity (albeit at lower levels than wild-type mice) and produce protease-resistant PrP^{Sc}. Histological examination of the brains of inoculated animals demonstrated a striking accumulation of extracellular PrP deposits with the characteristics of amyloid.

How can these unusual findings be explained? One interpretation is that highly polymerized, amyloid forms of PrP are not intrinsically pathogenic, even though they are infectious. Thus, infectious and toxic forms of PrP may have distinct biophysical properties. This conclusion is consistent with the studies, discussed above, of transgenic mice expressing mutant forms of PrP that are pathogenic but not infectious (Chiesa et al., 2003; Nazor et al., 2005). It would be of interest to characterize the size distribution and biophysical properties of PrP^{Sc} found in the brains of the GPI^{-} mice, perhaps using the techniques described by Silveira et al. (2005), to determine whether large, amyloid polymers predominate at the expense of smaller, potentially neurotoxic oligomers.

A second possible implication of the study by Chesebro et al. is that GPI anchor of PrP somehow plays a role in the cellular response to prions. In this view, PrP^{C} would serve as a required transducer of a PrP^{Sc}-derived neurotoxic signal, and the absence of a membrane anchor on PrP^{C} would prevent generation of this signal. This hypothesis is consistent with the striking observation that neurons in the brains of PrP null mice are resistant to the toxic effects of PrP^{Sc} supplied from grafted brain tissue (Brandner et al., 1996) or from nearby astrocytes (Mallucci et al., 2003).

Cellular Mechanisms of Prion Toxicity: Loss, Gain, or Subversion of PrP^{C} Function?

If PrP^{C} transduces neurotoxic signals during prion infection, what is the normal, physiological function of this protein? Attempts to answer this question by analyzing the phenotypes of PrP null mice have been unrewarding, since lines of these mice in which the gene encoding Doppel (a PrP paralog) is not artifactually upregulated display no major anatomical or developmental deficits (Büeler et al., 1992). A variety of functions have been proposed for PrP^{C}, including roles in metal ion trafficking, cell adhesion, and signal transduction, but definitive evidence for any of these possibilities is lacking.

A particularly intriguing hypothesis, and one that may be relevant to the mechanism of prion toxicity, postulates that PrP^{C} functions as a cytoprotective molecule. Several experimental results support this idea (see Roucou and LeBlanc, 2005, and references therein). First, PrP overexpression rescues cultured neurons, some mammalian cell lines, and yeast (Li and Harris, 2005) from several kinds of death-inducing stimuli. Second, there is evidence that endogenous PrP protects cultured neurons against oxidative stress, and brain tissue against ischemia or trauma in vivo. In a recently published study, PrP was also found to be important for self-renewal of hematopoietic stem cells during serial transplantation, a phenomenon that may depend on an

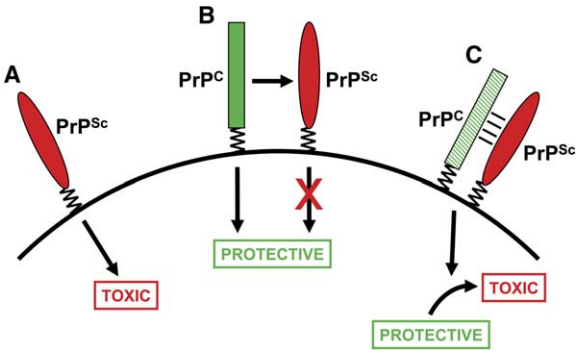

Figure 1. Models for the Cellular Toxicity of PrP^{Sc}

(A) Toxic gain-of-function mechanism. PrP^{Sc} (or PrP^{toxic}, a pathogenic intermediate) possesses a novel neurotoxic activity that is independent of the normal function of PrP^{C}. (B) Loss-of-function mechanism. PrP^{C} possesses a normal, physiological activity, in this case neuroprotection, that is lost upon conversion to PrP^{Sc}. (C) Subversion-of-function mechanism. The normal, neuroprotective activity of PrP^{C} is subverted by binding to PrP^{Sc} (or PrP^{toxic}). Cross-hatching of the rectangle representing PrP^{C} indicates a change in its signaling properties such that a neurotoxic rather than a neuroprotective signal is delivered. In the absence of the GPI anchor attaching PrP^{C} to the membrane, no signal would be delivered and disease would not occur, as was observed in the study of Chesebro et al. (2005).

antiapoptotic activity of the protein (Zhang et al., 2006). Finally, coexpression of even subphysiological levels of wild-type PrP completely abrogates the neurodegenerative phenotypes of mice expressing Doppel or N-terminally truncated forms of PrP (PrPΔ32-121 and Δ32-134) (Behrens and Aguzzi, 2002).

If the normal function of PrP^{C} is neuroprotection, then loss of this function by conversion to PrP^{Sc} might contribute to prion-induced neurodegeneration (Figure 1B). A loss-of-function mechanism appears to be incompatible with the observation that *Prn-p*$^{0/0}$ mice are relatively normal and do not display features of prion disease (Büeler et al., 1992). However, a biological activity of PrP^{C} that is dispensable under normal conditions may become essential in the disease state due to cellular or organismal stress.

Another possibility is that PrP^{Sc} subverts or modifies the normal function of PrP^{C}, rather than causing a complete loss of PrP^{C} function (Figure 1C). For example, the activity of a putative PrP^{C} signaling pathway might be altered by binding to PrP^{Sc} (or to another pathogenic intermediate), such that a neurotoxic rather than a neuroprotective stimulus is delivered. PrP^{Sc} might produce this effect by cross-linking of cell-surface PrP^{C}, which has been shown to induce apoptosis of CNS neurons in vivo (Solforosi et al., 2004), or by binding to and blocking specific functional domains of PrP^{C}. The neurodegenerative phenotype of transgenic mice expressing PrPΔ32-121/134 (Behrens and Aguzzi, 2002) suggests that specific domains of PrP are essential for its neuroprotective activity and that deletion of these domains unmasks a neurotoxic activity, perhaps by altering interaction with critical signaling proteins. Binding of PrP^{Sc} to PrP^{C} might produce a similar inversion of PrP^{C} signaling activity.

These two hypotheses stand in contrast to the toxic gain-of-function mechanism that is usually invoked to

explain dominantly inherited neurodegenerative disorders, including Alzheimer's, Huntington's, and Parkinson's diseases. In these cases, the protein aggregates that accumulate in the brain are presumed to possess a novel neurotoxic activity that is independent of the normal, physiological function of the parent protein (Figure 1A). For example, PrP^{Sc} aggregates may block axonal transport, interfere with synaptic transmission, or physically damage cellular membranes. Although plausible in many forms of prion disease, such toxic effects are difficult to invoke in certain familial cases. In these situations, the pathogenic mutation has no obvious effect on the biochemical properties or thermodynamic stability of PrP, and deposition of protein aggregates is minimal (Chiesa and Harris, 2001). These cases could result from a dominant-negative effect of the mutant protein on some aspect of PrP^{c} function. Thus, it is far from settled whether prion diseases are due to gain, loss, or subversion of PrP function, or perhaps to some combination of these mechanisms. It is also unclear whether the same mechanism is necessarily responsible for all categories of prion disease (transmissible, familial, and sporadic).

Conclusions

The prion field has advanced enormously during the past 25 years. The protein-only nature of prions has become much more firmly established, and it is now quite difficult to accommodate all the known experimental facts in a viral theory of transmission. The discovery of yeast and other fungal prions has demonstrated the generality of the prion phenomenon and made possible a number of powerful, new experimental approaches. The studies described in this review have begun to illuminate two previously mysterious subjects: the structure of prions and how they damage neurons. However, a number of outstanding issues remain to be resolved. These include the three-dimensional structure of PrP^{Sc}, the mechanism of the PrP^{C}-PrP^{Sc} conversion process, the identity and cellular action of neurotoxic PrP, the physiological function of PrP^{C}, and the role of prions in evolution and other biological processes. In addition, there is a pressing need for development of more sensitive diagnostic tests and more effective therapeutic strategies for prion diseases. With the current pace of research in the field, it seems certain that solutions to these problems will soon be forthcoming.

Selected Reading

Baskakov, I.V. (2004). J. Biol. Chem. *279*, 7671–7677.

Behrens, A., and Aguzzi, A. (2002). Trends Neurosci. *25*, 150–154.

Brandner, S., Isenmann, S., Raeber, A., Fischer, M., Sailer, A., Kobayashi, Y., Marino, S., Weissmann, C., and Aguzzi, A. (1996). Nature *379*, 339–343.

Büeler, H., Fischer, M., Lang, Y., Fluethmann, H., Lipp, H.-P., DeArmond, S.J., Prusiner, S.B., Aguet, M., and Weissmann, C. (1992). Nature *356*, 577–582.

Castilla, J., Saa, P., Hetz, C., and Soto, C. (2005). Cell *121*, 195–206.

Chesebro, B., Trifilo, M., Race, R., Meade-White, K., Teng, C., LaCasse, R., Raymond, L., Favara, C., Baron, G., Priola, S., et al. (2005). Science *308*, 1435–1439.

Chiesa, R., and Harris, D.A. (2001). Neurobiol. Dis. *8*, 743–763.

Chiesa, R., Piccardo, P., Quaglio, E., Drisaldi, B., Si-Hoe, S.L., Takao, M., Ghetti, B., and Harris, D.A. (2003). J. Virol. *77*, 7611–7622.

Dobson, C.M. (2003). Nature *426*, 884–890.

Govaerts, C., Wille, H., Prusiner, S.B., and Cohen, F.E. (2004). Proc. Natl. Acad. Sci. USA *101*, 8342–8347.

Jones, E.M., and Surewicz, W.K. (2005). Cell *121*, 63–72.

Krishnan, R., and Lindquist, S.L. (2005). Nature *435*, 765–772.

Lee, S., and Eisenberg, D. (2003). Nat. Struct. Biol. *10*, 725–730.

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.

Li, A., and Harris, D.A. (2005). J. Biol. Chem. *280*, 17430–17434.

Ma, J., Wollmann, R., and Lindquist, S. (2002). Science *298*, 1781–1785.

Mallucci, G., Dickinson, A., Linehan, J., Klohn, P.C., Brandner, S., and Collinge, J. (2003). Science *302*, 871–874.

Nazor, K.E., Kuhn, F., Seward, T., Green, M., Zwald, D., Purro, M., Schmid, J., Biffiger, K., Power, A.M., Oesch, B., et al. (2005). EMBO J. *24*, 2472–2480.

Nelson, R., Sawaya, M.R., Balbirnie, M., Madsen, A.O., Riekel, C., Grothe, R., and Eisenberg, D. (2005). Nature *435*, 773–778.

Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P., and Tycko, R. (2005). Science *307*, 262–265.

Prusiner, S.B. (1998). Proc. Natl. Acad. Sci. USA *95*, 13363–13383.

Ritter, C., Maddelein, M.L., Siemer, A.B., Luhrs, T., Ernst, M., Meier, B.H., Saupe, S.J., and Riek, R. (2005). Nature *435*, 844–848.

Roucou, X., and LeBlanc, A.C. (2005). J. Mol. Med. *83*, 3–11.

Shorter, J., and Lindquist, S. (2005). Nat. Rev. Genet. *6*, 435–450.

Silveira, J.R., Raymond, G.J., Hughson, A.G., Race, R.E., Sim, V.L., Hayes, S.F., and Caughey, B. (2005). Nature *437*, 257–261.

Solforosi, L., Criado, J.R., McGavern, D.B., Wirz, S., Sanchez-Alavez, M., Sugama, S., DeGiorgio, L.A., Volpe, B.T., Wiseman, E., Abalos, G., et al. (2004). Science *303*, 1514–1516.

Stewart, R.S., Piccardo, P., Ghetti, B., and Harris, D.A. (2005). J. Neurosci. *25*, 3469–3477.

Tanaka, M., Chien, P., Yonekura, K., and Weissman, J.S. (2005). Cell *121*, 49–62.

True, H.L., and Lindquist, S.L. (2000). Nature *407*, 477–483.

Wille, H., Prusiner, S.B., and Cohen, F.E. (2000). J. Struct. Biol. *130*, 323–338.

Zahn, R., Liu, A., Luhrs, T., Riek, R., von Schroetter, C., Lopez Garcia, F., Billeter, M., Calzolai, L., Wider, G., and Wüthrich, K. (2000). Proc. Natl. Acad. Sci. USA *97*, 145–150.

Zhang, C.C., Steele, A.D., Lindquist, S., and Lodish, H.F. (2006). Proc. Natl. Acad. Sci. USA *103*, 2184–2189.