tant form of this episome that inhibits the function of Ure2p directly or indirectly, thereby generating the URE phenotype. Overproduction of Ure2p would promote overproduction of the episome and increase the probability of its mutation. In the absence of Ure2p the mutated episome could not be propagated and would be lost. Upon introduction of URE2 into ure2Δ, the normal episome could be regenerated from the genome, propagated in the cytoplasm, and again undergo mutations.

One of the striking properties of prion diseases in mammals is their transmissibility by crude or purified cell-free preparations. Transmission of [URE3] has been achieved in vivo by cytoduction, that is, by transfer of cytoplasm from a [URE3] donor to a recipient yeast cell by means of an abortive mating event; however, transmission by cell-free extracts has not been achieved. The latter is clearly an important goal and should reveal whether one is dealing with modified Ure2p or an episome.

In Prusiner's model of conformational conversion, a molecule of PrP^{Sc} interacts with PrP^{C} and thereby imposes its conformation on it. The β-sheet content of PrP^{Sc} is high, whereas that of PrP^{C} is low (*13*). PrP^{C} and PrP^{Sc} are both very stable, suggesting that a high activation energy barrier between the two states must almost completely prevent spontaneous conversion. Such an energy barrier probably cannot be provided by minor conformational changes. The conversion may therefore require extensive unfolding of PrP^{C} and refolding under the direction of PrP^{Sc}, perhaps mediated by a chaperone and with the use of an energy source. Such a mechanism would explain why it has not yet been possible to achieve conversion in vitro in mixing experiments (*14*). Maybe the proposed conformational changes could be studied more readily in yeast if and when cell-free transmission of [URE3] is achieved.

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Structural Clues to Prion Replication

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Prions cause a variety of degenerative neurologic diseases that can be infectious, inherited, or sporadic in origin. These diseases include scrapie in sheep, bovine spongiform encephalopathy ("mad cow" disease) in cattle, and Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease, kuru, and fatal familial insomnia in humans. Prions differ from all other infectious pathogens in that they seem to be devoid of nucleic acid genomes (*1*); their only known constituent is a protein denoted PrP^{Sc}. This protein is an altered version of a normal cellular protein (PrP^{C}) that is encoded by a chromosomal gene (*2*).

PrP^{C} is synthesized in the endoplasmic reticulum, modified in the Golgi apparatus, and transported to the cell surface, where it is bound by a glycophosphatidyl inositol (GPI) anchor (*3*). Like other GPI-anchored proteins, PrP^{C} appears to reenter the cell through a subcellular compartment bounded by cholesterol-rich, detergent-insoluble membranes (*4*). Deletion of the GPI addition signal results in greatly diminished synthesis of PrP^{Sc} (*5*).

Studies with transgenic mice indicate that PrP^{C} is most efficiently converted into PrP^{Sc} when the amino acid sequences of PrP^{C} and PrP^{Sc} are identical (*6*). Mice carrying a mouse PrP transgene with a Pro^{102} to Leu mutation (the genetic lesion associated with GSS disease) develop a neurodegenerative disorder that is indistinguishable from experimental scrapie, and they produce prions de novo (*7*). Overexpression of the mouse wild-type PrP transgene in older mice produces disease characterized by spongiform degeneration of the central nervous system, a necrotizing myopathy involving virtually all skeletal muscle, and a demyelinating peripheral neuropathy (*8*). Mice in which the wild-type PrP gene has been ablated are resistant to scrapie and do not produce prions, confirming that a source of PrP^{C} is necessary for transmission and pathogenesis of disease (*9*).

How is PrP^{Sc} formed? The possibility that it is generated by alternative splicing is unlikely, as the PrP^{C} open reading frame resides within a single exon in all species examined (*10, 11*). PrP^{Sc} might also arise by posttranslational chemical modification of PrP^{C}, but thus far no such modifications have been detected (*12*). Recent structural studies demonstrate that PrP^{C} and PrP^{Sc} differ in conformation. Both isoforms have been purified under nondenaturing conditions, and their secondary structures determined by Fourier transform infrared and circular dichroism spectroscopy. PrP^{C} was found to have a high content of α helix (42%) and essentially no β sheet (3%), whereas PrP^{Sc} had a β-sheet content of 43% and an α-helix content of 30% (*13*). Neither PrP^{C} nor PrP^{Sc} formed aggregates detectable by electron microscopy.

Although it has not yet been possible to obtain crystals of PrP^{C} or PrP^{Sc} for x-ray structure determinations, a model for the tertiary structure of PrP^{C} has been deduced by computational methods (*14*). PrP^{C} sequences were analyzed by secondary structure prediction algorithms that had been optimized for the study of globular proteins with a unique folded state. Four putative regions of secondary structure were identified, but these algorithms did not allow unambiguous assignment of the regions as α helices or β sheets (*13*). Synthetic peptides corresponding to the four regions had been produced in earlier work, and three of the four were found to adopt a β-sheet conformation in water and polymerize into rods with the tinctorial properties of amyloid (*15*). This incongruency may simply reflect the intrinsic limitations of current algorithms for predicting secondary structure, or it may indicate that PrP^{C} contains domains that can assume more than one conformation. A plausible model for the tertiary structure of PrP^{C} was generated by examining all possible ways that the four putative α helices could be arranged into a compact globular structure (*14*).

These structural findings suggest a conformational model for prion replication that, in principle, can explain the pathogenesis of infectious, inherited, and sporadic forms of prion disease (Fig. 1). According to this model, stochastic fluctuations in the structure of PrP^{C} would generate a partially unfolded monomer (PrP*) that is an intermediate in the formation of PrP^{Sc}. PrP* can revert to PrP^{C}, be degraded, or form PrP^{Sc}. Normally, the concentration of PrP* would be low, and PrP^{Sc} would be formed in

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insignificant amounts. Whether PrP^{Sc} formation involves oligomerization remains uncertain, because the insolubility of PrP^{Sc} has precluded analysis of its physical state.

In the case of infectious prion diseases, exogenous prions containing PrP^{Sc} would act as templates to promote the conversion of PrP* into PrP^{Sc} (Fig. 1A). The insolubility of PrP^{Sc} would make this process irreversible and drive the formation of PrP* (and PrP^{Sc}) by mass action. In the case of inherited prion diseases, PrP mutations (Δ) would destabilize ΔPrP^{C} and promote its conversion into ΔPrP*, which would increase the likelihood of ΔPrP^{Sc} formation (Fig. 1B). Current information on the location of PrP mutations (*11*, *16*) is consistent with this hypothesis. Ten of eleven point mutations that segregate with inherited prion diseases fall within or near the four putative α helices, and five mutations cluster surround a central cavity that might be essential for the structural stability of PrP^{C} (*14*).

Finally, the sporadic prion diseases may result from the rare occasions in which there is sufficient accumulation of PrP* to produce PrP^{Sc} (Fig. 1A). In support of this proposal is the observation that transgenic mice overexpressing the wild-type PrP gene develop spongiform degeneration and produce prions (*8*). Alternatively, sporadic prion diseases may arise because of somatic mutations that destabilize PrP^{C} and promote its conversion into PrP^{Sc} through PrP*. Of note is the common Met/Val polymorphism at amino acid 129 in human PrP; this amino acid lies at the amino-terminal border of the second putative α helix in PrP^{C}, but may be at the end of a β strand in PrP^{Sc}. Homozygosity at codon 129 appears to predispose to sporadic CJD (*17*), an interesting observation given that this residue may lie at a multimeric interface joining two β sheets. Studies of other proteins suggest that Met-Met and Val-Val are common neighbors in β sheets, whereas juxtaposition of Met and Val is unusual (*18*). Patients with the Asp^{178} to Asn mutation present with insomnia if codon 129 specifies Met in the mutant allele, or dementia if codon 129 specifies Val (*19*).

The transmission of prions from one species to another is accompanied by a prolonged incubation time on the initial passage to the host, with subsequent passages being considerably shorter. As noted above, this "species barrier" has been shown to be due to differences in PrP sequence (6). Differences in incubation time have also been observed between different strains of mice; the PrP genes of mice with short and long incubation times differ at two nucleotide positions. When prions are passaged into mice with a nonmatching PrP sequence, the incubation time is longer than that seen in mice with a matching PrP sequence (*20*). These allotypic interactions between variant PrP^{C} and PrP^{Sc} molecules appear to have a profound influence on incubation times with different "strains" of prions (*21*). Whether prion strains represent different conformers of PrP^{Sc} remains to be established (*1*, *22*).

The unprecedented nature of prions raises questions about how cells monitor protein conformation—in particular, how they dispose of misfolded proteins when these conformers are potentially pathogenic. Diseases caused by prions will undoubtedly require novel therapeutic approaches. The model discussed here suggests that these diseases might be preventable or treatable by interventions that stabilize the putative α helices of PrP^{C} and thereby inhibit their conversion into β sheets.

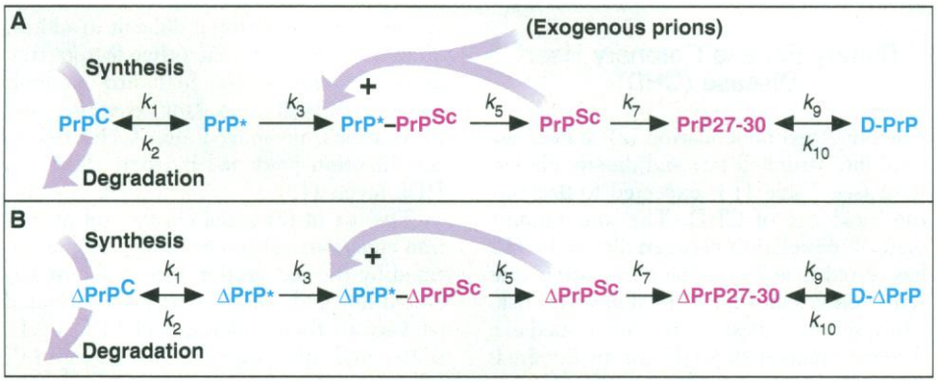

Fig. 1. A conformational model for prion replication. Infectious forms of PrP are shown in red and noninfectious forms in blue. (**A**) Postulated events in infectious and sporadic prion diseases. Wild-type PrP^{C} is synthesized and degraded as part of normal cellular metabolism. Stochastic fluctuations in the structure of PrP^{C} can create (k_1) a rare, partially unfolded monomer (PrP*) that is an intermediate in the formation of PrP^{Sc}. PrP* can revert (k_2) to PrP^{C}, be degraded, or form a complex (k_3) with PrP^{Sc}. Normally, the concentration of PrP* is low and PrP^{Sc} formation is insignificant. In infectious prion diseases, exogenous prions enter the cell and stimulate conversion (k_5) of PrP* into PrP^{Sc}, which is likely to be an irreversible process. In sporadic prion diseases, where there are no exogenous prions, the concentration of PrP^{Sc} may eventually reach a threshold level upon which a positive feedback loop would stimulate the formation of PrP^{Sc}. Limited proteolysis of the amino terminus of PrP^{Sc} produces (k_7) PrP27-30, a truncated form of PrP^{Sc} that polymerizes into amyloid and has a high content of β sheet (*23*). Denaturation (k_9) of PrP^{Sc} or PrP27-30 into D-PrP renders these molecules protease-sensitive and abolishes scrapie infectivity; attempts to renature (k_{10}) D-PrP have been largely unsuccessful (*24*, *25*). (**B**) Postulated events in inherited prion diseases. Mutant (Δ) PrP^{C} is synthesized and degraded as part of normal cellular metabolism. Stochastic fluctuations in the structure of ΔPrP^{C} are greater than those in wild-type PrP^{C}; these fluctuations create (k_1) significant amounts of a partially unfolded monomer (ΔPrP*) that is an intermediate in the formation of ΔPrP^{Sc}. ΔPrP* can revert (k_2) to ΔPrP^{C}, be degraded, or be converted (k_5) into ΔPrP^{Sc}. Limited proteolysis of the amino terminus of ΔPrP^{Sc} produces (k_7) ΔPrP27-30, which in some cases may be less protease resistant than wild-type PrP27-30.

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