A General Model of Prion Strains and Their Pathogenicity

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Prions are lethal mammalian pathogens composed of aggregated conformational isomers of a host-encoded glycoprotein and which appear to lack nucleic acids. Their unique biology, allied with the public-health risks posed by prion zoonoses such as bovine spongiform encephalopathy, has focused much attention on the molecular basis of prion propagation and the "species barrier" that controls cross-species transmission. Both are intimately linked to understanding how multiple prion "strains" are encoded by a protein-only agent. The underlying mechanisms are clearly of much wider importance, and analogous protein-based inheritance mechanisms are recognized in yeast and fungi. Recent advances suggest that prions themselves are not directly neurotoxic, but rather their propagation involves production of toxic species, which may be uncoupled from infectivity.

According to the widely accepted "protein-only" hypothesis (1), an abnormal isoform of host-encoded cellular prion protein (PrP<sup>C</sup>) is the principal, and possibly the sole, constituent of the transmissible agent or prion (2). It is proposed that this isoform, PrP<sup>Sc</sup>, acts as a template that promotes the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> and that the difference between these isoforms lies purely in the monomer conformation and its state of aggregation.

The human prion diseases, such as Creutzfeldt-Jakob disease (CJD), can arise sporadically, be acquired by infection, or be inherited as autosomal dominant conditions caused by mutation and its state of aggregation. Some such biologically defined strains cannot be serially propagated in mice after passage in intermediate species with different PrP primary structures (Fig. 1B). Furthermore, strains can be re-isolated in mice after passage in inbred mice with identical PrP gene (3). These strains cannot be explained by differences in their nucleic acid coding sequence (Fig. 1B). Furthermore, strains can be re-isolated in mice after passage in intermediate species with different PrP primary structures (19) (Fig. 1C). Although distinct strains of conventional pathogens can be explained by differences in their nucleic acid genome, it has been less clear how a polypeptide chain could encode multiple disease phenotypes. Some such biologically defined prion strains show biochemical differences in the propagated PrP<sup>Sc</sup>. For two strains of TME prions, designated hyper (HY) and drowsy (DY) (22), limited proteolysis produced different PrP<sup>Sc</sup> fragment sizes, implying that the two strains have different conformations (23). Similarly, distinct human PrP<sup>Sc</sup> types have been identified by proteolytic fragment size and glycoform ratios following proteinase K digestion, and these are associated with different clinicopathological phenotypes of CJD (5, 24). To be plausible candidates for the molecular basis of strain diversity, such biochemical properties need to impose their

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characteristics on a recipient PrP whether they are of the same or a different species. In studies with human prion isolates, PrP\textsuperscript{Sc} fragment sizes following proteinase K digestion are maintained on passage in transgenic mice (5, 25) and, notably, ratios of the three principal PrP glycoforms are also maintained (5). Transmission of human and bovine prions to wild-type mice (expressing murine PrP\textsuperscript{C}) resulted in propagation of murine PrP\textsuperscript{Sc} with fragment sizes and glycoform ratios following protease digestion that correspond to the original inoculum (5), demonstrating imprinting of these biochemical characteristics onto PrP from another species. Indeed, a characteristic molecular signature of the BSE prion strain is maintained across several mammalian species, including humans (Fig. 1C) (5).

Further evidence that distinct prion strains are associated with different conformation states of PrP includes differential proteinase K digestion kinetics; thermal or chaotrope denaturation curves; conformation-dependent immunoassay; infrared spectroscopy and metal binding (26–29); and the use of methods to amplify PrP\textsuperscript{Sc} or prions in vitro that show faithful propagation of strain-associated biochemical characteristics (30–32).

Prion strains are associated with consistent ratios of the three principal PrP glycoforms, which can be maintained on serial passage in hosts with the same or different PrP sequences (5). How might ratios be maintained? One possibility is that this reflects different, strain-specific propagation and clearance kinetics of un-, mono- and diglycosylated forms. Alternatively, individual PrP\textsuperscript{Sc} fibrils could be composed of a regular repeating sequence of the different glycoforms, as in a linear crystal. Studies using monoclonal antibodies that precipitate native PrP\textsuperscript{Sc}, together with glycoform-specific antibodies, have shown that PrP glycoforms are physically associated in a strain-specific ratio in native PrP\textsuperscript{Sc} (33); such glycosylation ratios may be important in stabilizing particular protein conformations.

A critical test of the protein-only hypothesis, both with respect to infectivity and “strain-ness,” would be to produce discrete prion strains synthetically from defined components. Purified, bacterially expressed recombinant N-terminally truncated mouse PrP has been aggregated into fibrillar material and bioassayed in transgenic (Tg9949) mice expressing very high levels of the same truncated mouse PrP (14). After prolonged incubation periods, the Tg9949 mice develop a

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**Fig. 1.** Propagation of prion strains. (A) Prion propagation proceeds by recruitment of PrP monomers onto a preexisting PrP polymer template followed by fission to generate more templates in an autocatalytic manner. Distinct PrP polymer types can propagate, accounting for different strains. (B) Strains can be differentiated by characteristic incubation periods (length of arrow) and neuropathology (shaded brain area) when inoculated into defined inbred mice. Strain-specific PrP\textsuperscript{Sc} fragment patterns following proteolysis are illustrated in diagrammatic Western blots (vertical bars). Both biological and biochemical strain characteristics are closely maintained on serial passage in the same host expressing the same PrP\textsuperscript{C}. (C) Properties of a single strain may be retained after passage in a range of different species with distinct PrP\textsuperscript{C} sequences, when re-isolated in the original host.
prion disease transmissible to wild-type and Tg9949 mice. The transmission characteristics at both primary and second passage are consistent with production of a novel prion strain. Several “synthetic” prion strains have been described. Evaluating the strain properties of such synthetic prions on primary and second passage is complicated by the fact that primary material inoculated into mice lacks the posttranslational modifications PrP acquires in mammalian cells, notably N-linked glycosylation and C-terminal glycosylphosphatidylinositol anchor, while the putative prions then propagated in mice would be composed of processed mammalian PrP.

Though undoubtedly a major step forward, two key problems arise in the interpretation of these experiments. First, primary passage of infectivity requires transgenic mice with extremely high (~16-fold) overexpression of PrP<sup>Sc</sup>. Spontaneous development of prion disease–like pathology is seen in other transgenic lines with high levels of PrP<sup>Sc</sup> expression, although this does not appear to be transmissible (34, 35). Uninoculated or mock-inoculated Tg9949 mice do not appear to develop spontaneous pathology (14), although it has not been reported whether infectivity passageable in Tg9949 mice is present in the brains of aged un- or mock-inoculated Tg9949 mice. However, it is possible that these mice are “poised” to develop spontaneous disease and would do so if they lived long enough, and that onset of disease is simply accelerated or precipitated by inoculation of further PrP. Such a controversy is not new and continues with respect to interpretation of whether mice expressing high levels of PrP containing the murine equivalent (P101L) of the human pathogenic PrP mutation P102L (responsible for one of the inherited prion diseases) produce infectivity spontaneously. Putative prions produced de novo in such mice only infect mice expressing the same mutation at a lower level (some of which do develop spontaneous pathology at advanced age) (36). Prions may not then have been transmitted in this experiment; rather, this result may have represented acceleration of a spontaneous neurodegenerative disease (37).

The general question, then, is whether the putative synthetic prions are in fact prions, or simply agents that trigger prion production in hosts that have such high levels of PrP<sup>Sc</sup> expression that they are close to developing spontaneous prion disease. That is, are the prions generated in the cell-free system or only in the host? This is not a purely semantic issue. For example, one would probably not want to classify an entirely unrelated agent—conceivably an environmental stress factor, for example, that up-regulated PrP<sup>Sc</sup> expression and achieved the same apparent effect—as being a “prion.” The solution to this problem would be to show infection at first passage of wild-type animals with synthetic prions. This has not yet been reported. The second problem relates to infectious titer of the synthetic prions. The extremely prolonged incubation period at primary passage (516 ± 27 days) (14) could, as the authors conclude, reflect a prion strain effect. That is, the novel strain propagates only slowly in the host despite the high PrP<sup>Sc</sup> substrate concentration, but accepting the additional complication that the synthetic material lacks posttranslational modifications that may be important for pathogenicity.

However, there is another alternative explanation. As a prion isolate is serially diluted toward a single infectious unit per inoculum, incubation periods increase. Therefore, the long incubation periods seen with synthetic prions could simply reflect a low prion titer. With respect to the key issue of elucidating structural properties of prion strains, this is of fundamental importance. If the principal component of the synthetic material is the infectious prion, but they replicate only slowly in mice, biophysical studies can be performed to determine their structure and physical properties. Conversely, if the transmission characteristics reflect low prion titer, that would imply that the prions are only a minute fraction of the recombinant-derived protein, making physical and structural studies irrelevant (38). This question can be resolved, albeit laboriously, by serial titration of the synthetic prion inoculum in the transgenic mice. If the first interpretation is correct, the sample may be diluted many fold and would still produce the same incubation period. If the second is correct, even a 10-fold dilution is likely to result in nontransmission during the life span of these animals.

The recognition that certain heritable traits in yeast could be explained by conformational switching and aggregation in two yeast proteins, Ure2p and Sup35p, led to the emergence of the field of “yeast prions”; a fungal protein with prionlike properties, Het-s, has also been described (39). These proteins have no sequence similarity to PrP. While one can argue practical distinctions from mammalian prions, which are considered naturally infectious lethal pathogens, the study of these closely analogous phenomena has unquestionably led to rapid advances in investigating the processes of seeded fibril formation and the molecular basis of strain diversity and transmission barriers. Indeed, yeast prions are composed of protein fibrils, propagate by seeding, and possess strain diversity that is explained by distinct conformers (40–45).

### Unifying Strains and Transmission Barriers: Conformational and Kinetic Selection of Prions

Mammalian PrP genes are highly conserved, and the close similarity of PrP primary structures, and indeed folds (46), is presumably central to the ability of prions to cross-infect between mammalian species. Although a large number of PrP<sup>Sc</sup> types or “strains” are seen in the spectrum of mammalian prion diseases, presumably this number is limited by thermodynamic stability and the need to replicate at a rate above that of natural clearance in vivo. This number will constitute the theoretical portfolio of permissible and pathogenic mammalian prion strains and may be larger than the number of identified naturally occurring and experimentally derived strains. Some strains may be possible conformationally for a given PrP sequence and could be artificially synthesized, but they could not be indefinitely propagated in a normal host owing to efficient clearance. For the yeast prion [PS1<sup>+</sup>], for which the substrate protein is Sup35p, an analytical, steady-state model describing strains has been experimentally validated (47). This work supports a critical role of the fragmentation propensity of a prion strain in dictating its in vivo phenotype. To persist, yeast prions must propagate at a rate sufficient to compensate for the dilutional effect of cell division. Clearly, the in vivo situation in mammals is far more complex because predominantly postmitotic cells are infected and multiple cell types and tissues are involved. In the brain, clearance by cellular mechanisms to remove misfolded protein aggregates will be far more important than dilution by cell growth and division, although the situation may be different in the lymphoreticular system where prions also replicate. Mammalian prions also cause massive cell death, and cytotoxicity will be a major factor in any model of mammalian prion propagation. Also, although steady-state solutions are applicable in modeling yeast prion strains, a steady state is not reached in the mammalian central nervous system.

However, though a relatively large number of different PrP<sup>Sc</sup> types may be possible among the full range of mammalian PrP sequences, only a subset of these would be compatible with a given sequence (Fig. 2A). Substantial overlap between the permissible conformations for PrP<sup>Sc</sup> derived from species A and species B would thus result in relatively easy transmission of infection between these two species, whereas two species without any permissible PrP<sup>Sc</sup> conformations in common would have a large barrier to transmission. Any transmission of infectivity between such two species would require a change of strain type. According to the conformational selection model (8), host PrP<sup>Sc</sup> primary structure influences which of the portfolio of possible PrP<sup>Sc</sup> types are thermodynamically preferred with respect to conformation and kinetically selected during propagation. In this model, the transmission barrier is determined by the degree of overlap between the subset of PrP<sup>Sc</sup> types allowed or preferred by PrP<sup>Sc</sup> in the host and donor species. Strains and transmission barriers can therefore be considered opposite sides of the same coin.

Returning to the high cross-species pathogenicity of BSE, this strain may represent a thermodynamically highly favored PrP<sup>Sc</sup> conformation that is readily imprinted on PrP from a range of different species, accounting for the high promiscuity of this strain in mammals. Certainly, this strain was selected by an industrial cooking process (rendering of carcasses) involved in the recycling of infectivity that generated the UK BSE epidemic, and it is indeed known experimentally to be particularly thermostable (48).
Another illustration of the conformational selection model is provided by the common human PrP polymorphism (M129V), long known to be a key determinant of genetic susceptibility to prion disease. Notably, every patient with vCJD tested (~200) has been homozygous for the 129 methionine allele (a genotype seen in about a third of the normal population) because it appears that the valine 129 human PrP is not capable of adopting the PrPSc conformation associated with the BSE strain (49). Transmission across such a barrier thus results in strain switching with propagation of a distinct and sometimes novel strain type and a different disease phenotype (49).

**Prion Strain Stability and Mutation**

The phenomenon of strain mutation has been recognized for many years by biological strain typing methods (50). Classically, this occurs when a strain does not “breed true” on passage in a new host and a distinct strain is propagated. Mutation may (but does not necessarily) occur on crossing between species and also on intraspecies transmission where the PrP primary sequence of the host differs from that of the inoculum, for example, as a result of intraspecies PrP polymorphism at residue 129 in humans (5, 6, 49). In human PrP, residue 129 places constraints on which prion strains may propagate, but has no measurable effect on the folding, dynamics, and stability of PrPSc, suggesting that its effect is exerted through conformation of PrPSc, its precursors, or on the kinetics of their formation (51). Strain mutation may also occur on intraspecies transmission where host and prion donor have identical Prnp genes: This suggests an additional effect of background genes on strain selection (52, 53). Strain mutation can be accommodated within the conformational selection hypothesis by selection of a novel PrPSc conformation as a result of host PrPSc not being able to adopt the donor PrPSc conformation. However, the phenomenon poses an important question about strains. It has been long argued that strains can be biologically cloned (50). This is performed by serial passage at limiting dilution of an inoculum such that infection in the next host is initiated by a single prion. However, some strains are intrinsically unstable, readily reverting to another strain type, for example, the hamster DY strain (23). In addition, more than one strain has been isolated from some natural prion isolates, for example, sheep scrapie (54), and multiple PrPSc types are seen in a sizable fraction of CJD brains (55, 56). Certainly, a natural isolate shows considerable diversity in terms of N-terminal cleavage site, and its glycosylation is highly complex and diverse. Heat-inactivation studies also suggest heterogeneity of infectious species with thermostable subpopulations within a defined strain (57). Two (not mutually exclusive) possibilities can be envisaged (Fig. 2B): (i) A strain can exist as a molecular clone and strain mutation involves generation of a distinct PrPSc type; (ii) strains consist of an ensemble of molecular species (containing a dominant PrPSc type recognized on Western blot and preferentially propagated by its usual host) from which a less populous subspecies may be selected by an alternative host (whose propagation is most favored in that environment), resulting in a strain shift. Given the degree of molecular diversity observed in prion isolates, (ii) seems more plausible. The degree of diversity may also be strain dependent, with some strains approaching clonality in some hosts. Different cellular populations within a single host would also offer different environments for strain selection. It has long been argued that infection of a host with a “lymphotrophic strain,” which rapidly colonizes
lymphoid tissues with a long latency before neuroinvasion, is in part due to the need for selection of a “neuroinvasive strain” (38). Indeed, differences in PrPSc type in different peripheral tissues are well described in vCJD, in which prion colonization of lymphoid tissues long precedes neurological disease (59–61).

Essentially, a “prion” may be considered a rare subtype of PrP polymer that has the appropriate properties and replication kinetics to overcome and evade host defenses and propagate exponentially. Other, nascent prions are rapidly degraded in vivo and disappear. The crucial role of host selection on the ensemble that exists in a prion inoculum may be why it remains so difficult to demonstrate generation of synthetic prions. In vitro–generated “synthetic prions” may consist overwhelmingly of species that will be rapidly eliminated in a host. This would explain why highly purified PrP amyloid fibrils are apparently of low specific infectivity.

**Can Prion Infectivity and Toxicity Be Uncoupled?**

What is the cause of cell death in prion neurodegeneration? PrPSc loss of function is not a sufficient cause: PrP-null mice (Prnp0/0) are essentially normal (62). That adaptation during neurodevelopment in Prnp0/0 mice might compensate for loss of PrPSc function, while loss of PrPSc function in the developed brain by its sequestration to PrPSc might still be deleterious, is excluded by targeted PrPSc depletion in neurons of adult mice (63). However, conversion of PrPSc to PrPSc is clearly central to pathogenesis because Prnp0/0 mice are resistant to prion disease and do not propagate infectivity (64, 65). So, is PrPSc neurotoxic? In vitro neurotoxicity of PrPSc and short PrP peptides has been reported (66, 67), but several lines of in vivo evidence argue against direct toxicity: There are prion diseases in which PrPSc levels in the brain are very low (68–71); the distribution of PrPSc deposits does not necessarily mirror clinical signs, and PrPSc is not directly toxic to neurons that do not express PrPSc (64, 65, 72, 73). Furthermore, knockout of neuronal PrPSc expression during established brain infection completely protects mice from development of clinical disease, prevents neuronal loss, and reverses early spongiform neuropathology and behavioral abnormalities (74, 75). Notably, this recovery occurs despite continued PrPSc production and prion replication in glial cells, such that PrPSc and prion titers in brain reach levels seen in end-stage disease in conventional mice (74). So, do neurons need to express PrPSc and/or replicate prions themselves for toxicity to occur? Various mechanisms have been proposed, including aberrant signaling mediated by cross-linked cell surface PrPSc and altered PrPSc trafficking and topology (76–79). These alternatives are challenged, however, by the phenomenon of subclinical prion infection in wild-type animals. Such carrier states are sometimes established on

prion inoculation of a second species (80–82). Wild-type mice, with normal neuronal PrPSc expression and topology, inoculated with Sc237 hamster prions propagate mouse-adapted prions and yet live a normal life span without clinical disease. Prions propagate slowly but eventually reach titers (and levels of PrPSc) seen in end-stage conventional clinical disease (80). However, second passage of these prions in mice or hamsters results in conventional transmission with short incubation periods and 100% lethality (80).

**Linking Prion Propagation Kinetics to Neurotoxicity**

A possible explanation is that PrPSc is itself relatively inert, but toxicity resides in a smaller, labile, oligomeric PrP species (named PrPSc) for lethal, generated as an intermediate or side product during prion propagation (80, 82). Neurotoxicity may require a critical PrPSc concentration that is reached during conventional infections, but the slower kinetic of increase in infective titer in the subclinically infected mice may mean that toxic PrPSc levels are not reached (Fig. 3). This hypothesis can accommodate the

![Fig. 3. Toxicity and prion titer. According to the proposed models illustrated in Fig. 4, toxicity is due to the buildup of a templated intermediate or side-product, PrPSc, while the infectious agent itself is not directly toxic. This graph illustrates (in a highly schematic form) the separation of the two phenomena. Prion titer is shown in black and PrPSc concentration in red (66). Vertical dotted lines indicate time of onset of clinical signs and death. Green-shaded area denotes a level of PrPSc that can be tolerated without clinical symptoms. The upper, pink-shaded region represents a level of PrPSc that causes clinical illness; both titer and toxicity lines terminate at death of the animal. The incubation period is thus the time taken for PrPSc levels to cross the boundary. Four host/strain combinations are exemplified: tga20 mice express PrPSc at ~10-fold above levels seen in wild-type mice, whereas Prnp0/0 mice express at 50% of wild-type levels. The effect of PrPSc expression level on infection with mouse prions (RML strain) is demonstrated by the first three examples, whereas the fourth indicates wild-type mice infected with hamster (Sc237 strain) prions where prions propagate to high levels but without clinical onset during a normal life span (subclinical infection).](http://science.sciencemag.org/content/full/318/5845/934/F3)

The phenomena of prion propagation and toxicity can be considered from the standpoint of classical kinetic mechanisms. A formal model must accommodate experimental observations that demonstrate an apparent split between the identity of the propagating infectious agent and toxic species. It is increasingly proposed that the toxic species in amyloid diseases are intermediates on the pathway for formation of the amyloid structures (84). However, in this classical sense, such intermediates that occur before the favorable polymerization phase must reach a steady state, meaning that their concentration does not rise. That is, they are part of the priming process and not part of the autocatalytic phase.

Another, more promising mechanism can be proposed (Fig. 4) that accommodates both the autocatalytic nature of mammalian prion propagation and the lack of toxicity of PrPSc. In the protein-only hypothesis, it is axiomatic that PrPSc acts as a template for the PrPSc → PrPSc conversion. However, if PrPSc itself is the toxic agent, then the observed decoupling between the level of PrPSc and the extent of pathology is hard to explain. Without challenging the protein-
governed by the ratio of the initial rate of conversion ($k_1$) to the rate of maturation ($k_2$). Essentially, the proliferating, infectious PrP$^\text{Sc}$ acts as a catalytic surface upon which the intermediates form before they mature into the PrP$^\text{Sc}$ product. Consequently, their concentration will rise during the autocatalytic process because the population of the catalyst itself is rising exponentially. The difference between this model and the classical system is that formation of classical intermediates is not catalyzed by end products.

In the case of a subclinical infection, a relatively slow rate of initial conversion ($k_1$) would mean that the level of PrP$^\text{L}$ would be low, because its rate of loss through maturation ($k_2$) would be dominant. Thus, a large amount of PrP$^\text{Sc}$ or infectivity would build up, but with little toxicity (Fig. 3). By contrast, in a short-incubation disease such as RML prion infection in tga20 mice (with a high expression level of PrP$^\text{Sc}$), an increased rate of initial conversion leads to a rapid accumulation of PrP$^\text{L}$ and early death. In these circumstances, there is a higher probability of forming PrP$^\text{Sc}$:PrP$^\text{L}$ species through increased rates of encounter and, in turn, the level of PrP$^\text{Sc}$:PrP$^\text{L}$ is enhanced. Also, this model can explain transmission barrier (strain-adaptation) effects where primary passage is associated with very prolonged incubation periods (or indeed a persistent carrier state). Rapid propagation with an enhanced rate of initial conversion ($k_1$), which would then inevitably lead to a higher rate of formation of PrP$^\text{L}$ and a shorter incubation period. In this templated toxic intermediate model (Fig. 4A), the ability of a strain to propagate in a host depends on the rates of both initial conversion and of maturation; its ability to kill depends on the balance of these rates. An alternative, but related, model can be proposed in which PrP$^\text{Sc}$ is produced autocatalytically without toxic intermediates (Fig. 4B). However, PrP$^\text{Sc}$ then itself catalyzes the conversion of PrP$^\text{C}$ to PrP$^\text{L}$ as a toxic by-product. In this case, low-toxicity, high-titer infections would arise when the PrP$^\text{Sc}$-to-PrP$^\text{L}$ conversion rate is slow.

General Model

How may all these phenomena be brought together? The recent experimental evidence for these emerging concepts now allows a general model for mammalian prions to be proposed (table S1), which accommodates the known phenomena of exponential propagation of infectivity, strain diversity and mutation, transmission barriers, and the uncoupling of infectivity from neurotoxicity, while remaining within the constraint of requiring only a single polypeptide to constitute all strains of infective and toxic species.

Essentially, the phenomena of prion disease pathogenesis can be explained in terms of the kinetics of prion propagation, determined by interplay between prion strain type (dominant PrP$^\text{Sc}$ polymer and its ensemble) and tissue/host environment (PrP sequence and expression level, modifier genes, and clearance mechanisms); selection of preferred conformers determines transmission barriers. Neurotoxicity is mediated by a PrP species, PrP$^\text{L}$, distinct from PrP$^\text{Sc}$ but catalyzed by it, and occurs when PrP$^\text{L}$ concentration passes a local toxic threshold. Rapid propagation (with a host-adapted strain and normal or high levels of host PrP$^\text{Sc}$ expression) results in severe neurotoxicity and death at strain-specific incubation periods. Slow propagation (after infection across a transmission barrier or with low host PrP$^\text{L}$ expression) results in low neurotoxicity and prolonged and more variable incubation periods or a persistent carrier state.

The concept of prion strain originated from biological experiments, but at a molecular level there may be quite distinct infectious PrP polymers (PrP$^\text{Sc}$ types) that cannot be distinguished by transmission studies in inbred laboratory mouse lines. Although in practice, the converse situation of biologically distinct prion strains associated with PrP$^\text{Sc}$ that cannot be biochemically differentiated by current methods will also be observed, under this general protein-only model such biochemical or biophysical differences in PrP$^\text{Sc}$ must exist, and the model would predict that differences would be observed with more discriminating molecular methods, challenging the historical primacy of biological classification of prion strains.

Wider Implications

The impressive advances made in the field of yeast prions have clearly established a much wider biological importance of prion-like processes and have allowed direct experimental confirmation of molecular mechanisms proposed from work with mammalian prions. Understanding these phenomena will illuminate processes involving protein misfolding and aggregation, and protein-based inheritance, which clearly have far-reaching implications in pathobiology, aging, and the evolution of cellular processes. However, mammalian prions are distinctive. They are lethal pathogens par excellence—indeed, it is hard to think of other examples of infectious diseases with 100% mortality once the earliest clinical signs have developed.

Prions raise troubling questions in evolution. In particular, how did prions evolve as such
potent pathogens, complete with the ability to infect from the environment and travel to the brain? Conventional pathogens have evolved complex mechanisms to enable pathogenesis and evade destruction. If prions are composed purely of a polypeptide encoded by the host, how can they evolve? In essence, it seems that they had to arise de novo as an intact pathogenic system, and it is tempting to speculate that prions therefore represent malfunction of an evolved normal activity that has yet to be elucidated. How can they evolve? In essence, it seems that purely of a polypeptide encoded by the host, complex mechanisms to enable pathogenesis are required.

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