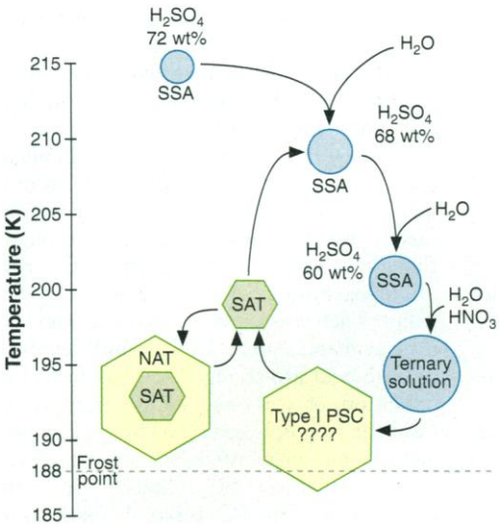


**Fig. 2.** Life cycle of a polar stratospheric cloud. In one scenario, falling temperatures cause sulfate aerosols to take up water and nitric acid to form droplets of a ternary solution of $H_2SO_4$, $HNO_3$, and $H_2O$. Nitric acid trihydrate might then nucleate out of solution, forming a type I PSC. As the temperature rises, the PSC evaporates, leaving behind a frozen SAT aerosol. This frozen aerosol may then serve as an effective nuclei for further PSC condensation at low temperature or may melt to reform a liquid aerosol if the temperature rises above 210 K.

The main controversy centers on what happens next. As the temperature continues to drop, it had generally been believed that the supercooled sulfuric acid aerosols would eventually freeze as sulfuric acid tetrahydrate (SAT). However, laboratory and theoretical studies have shown that sulfuric acid droplets supercool quite readily, and that the freezing point for these aerosols is difficult to predict because of the stochastic nature of freezing. Once frozen, the aerosols were thought to serve as good nuclei for the formation of PSCs. In 1991, Dye and co-workers (*8*) used this theory to explain why PSCs were often not observed in the Arctic until well below the NAT threshold temperature. They proposed that under slow cooling conditions, only a fraction of the sulfate aerosols would freeze by 192 K. Although this fraction could then serve as effective NAT nuclei, the remaining liquid aerosols would not develop into PSCs. Our group has shown that the SAT aerosols, once frozen, are likely to remain frozen until a temperature of at least 210 K (*14*). Thus, after the PSCs evaporate, it is thought that a frozen sulfate seed will be available for further nucleation of PSCs.

An alternative theory for PSC nucleation was proposed recently by Molina and co-workers (*15*). Through laboratory experiments at low temperatures, these authors showed that dilute sulfuric acid will take up significant amounts of $HNO_3$, and they proposed that the SSAs could be ternary solutions of $H_2SO_4$, $HNO_3$, and $H_2O$ with up to 10% $HNO_3$ by weight under polar conditions. The Molina group found evidence that the increased $HNO_3$ in solution caused NAT to crystallize out of the ternary mixture. They proposed that compositional changes in the droplet resulting from NAT crystallization would cause the entire droplet to freeze. The NAT would then grow by additional condensation of $HNO_3$ and $H_2O$ onto the seed crystal.

A third theory for PSC formation was presented at the Boulder workshop by T. Peter (Max-Planck-Institut für Chemie). He and his co-workers have proposed that it may not be necessary for the sulfuric acid aerosols to freeze at all for PSC nucleation. In their model, the supercooled sulfuric acid droplets remain liquid as they cool and continue to absorb $HNO_3$ and $H_2O$. As the temperature drops to 190 K, the droplets contain 40% $HNO_3$ by weight, and have sequestered much of the atmospheric $HNO_3$. These authors propose that the droplets will remain liquid until the ice frost point is reached. In this model, the type I PSCs at 190 K are thus a supercooled solution of 4% $H_2SO_4$, 40% $HNO_3$, and 56% $H_2O$.

Future laboratory and field experiments may yield insight into which, if any, of the above mechanisms for PSC formation dominate in the stratosphere. Researchers are currently performing laboratory experiments with actual aerosol samples to measure parameters such as the freezing nucleation rate for sulfuric acid, the uptake of $HNO_3$ by liquid sulfuric acid, and the growth of nitric acid hydrates on liquid and frozen sulfuric acid. The final answer about the PSC composition and nucleation may, however, have to await field observations. There are several projects in progress to aid in the identification of actual stratospheric aerosols. Infrared extinction data from satellites and aircraft are already being used to identify the average properties of collections of particles. In situ methods are also under development for the characterization of single aerosol particles. As we learn more about the chemical composition of the stratospheric clouds, we may be able to improve our predictions of when and where they might form. This, in turn, will help us understand when heterogeneous chemistry will be important in controlling the abundance of stratospheric ozone.

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# The Prion Connection: Now in Yeast?


Charles Weissmann


Phenomena discovered in higher eukaryotes acquire additional respectability if they are also found in yeast. This is now true for the prion, the agent responsible for a group of transmissible diseases of the central nervous system—Creutzfeldt-Jakob disease in humans, scrapie in sheep, and "mad cow" disease. The propagation of prions has been attributed to a conformational rearrangement of a normal host protein into a pathological form, a rearrangement that is catalyzed by the pathological form itself (see accompanying Perspective). In a report in this issue of *Science*, R. Wickner suggests that the pathogenesis of an obscure mutational phenotype in yeast is based on the same principle.

Prion diseases are unusual because they can arise spontaneously in the population at large without any apparent cause—so-called sporadic forms—or they can be familial and tightly linked to certain mutations of the *Prn-p* (prion protein) gene; in either case, the disease can usually be transmitted by inoculation. The peculiar properties of the transmissible agent, the prion, has given rise to speculations that it might be devoid of nucleic acid and consist of protein only (*1*, *2*). Currently, the most


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widely accepted proposal is the "protein only" hypothesis, first outlined in general terms by Griffith (*1*) and enunciated in its updated and detailed form by Prusiner and his colleagues (*3*, *4*). It states that the prion is identical with $PrP^{Sc}$, a modified form of $PrP^{C}$ (*3*). $PrP^{C}$ is a normal host protein (*5*, *6*) found predominantly on the outer surface of neurons. $PrP^{Sc}$ is defined as a form of $PrP^{C}$ that readily forms protease-resistant aggregates after treatment with detergents (*5*, *7*). $PrP^{Sc}$, when introduced into a normal cell, causes the conversion of $PrP^{C}$ into $PrP^{Sc}$, likely because of a conformational modification. Familial forms of prion disease are linked to mutations in $PrP^{C}$ that are thought to facilitate spontaneous conversion of $PrP^{C}$ into $PrP^{Sc}$. Sporadic cases of prion disease are attributed to a very rare spontaneous conversion of normal $PrP^{C}$ or to a somatic mutation in the *Prn-p* gene.

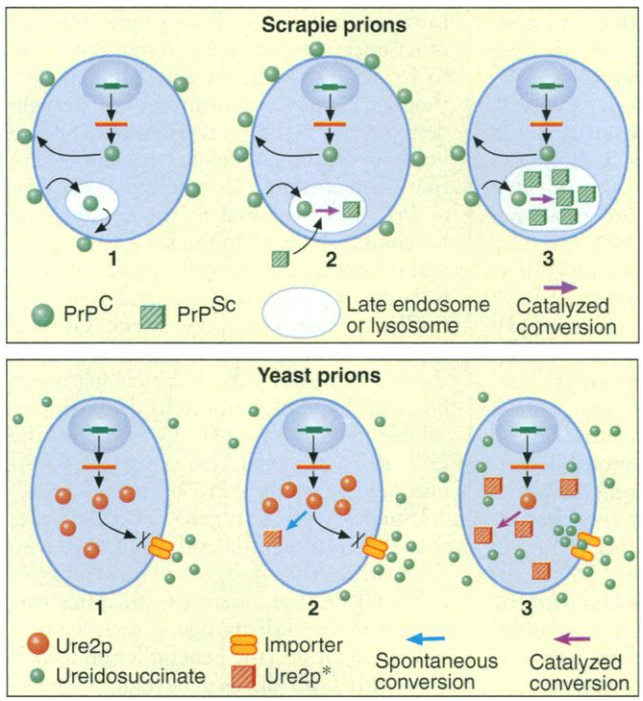
The most important biochemical evidence in favor of the protein only hypothesis is that highly purified infectious prion preparations appear to contain no nucleic acids (*8*) and comprise $PrP^{Sc}$ as the major if not only protein. The hypothesis is strongly supported by several lines of genetic evidence provided by Prusiner and his colleagues (*4*). The essential role of $PrP^{C}$ in scrapie was demonstrated by Büeler *et al.* (*9*), who generated mice devoid of $PrP^{C}$ and showed that they were resistant to scrapie disease and unable to propagate scrapie prions.

What in yeast is now thought to be analogous to prion disease? Wickner proposes that a yeast protein, called Ure2p, which is involved in the metabolism of nitrogenous compounds (*10*), can on rare occasions spontaneously convert into an inactive form, Ure2p* (my terminology), which then promotes the conversion of all functional Ure2p in the cell into the inactive form. The resulting phenotype is called "URE" and the underlying "mutation" [URE3]. [URE3] would be dominant either because Ure2p converts all Ure2p into Ure2p* or because it interferes with the function of whatever Ure2p is left. Why is [URE3] not just an ordinary genetic mutation resulting in a modified amino acid sequence?

The protein Ure2p is encoded by a chromosomal gene, *URE2*, which has been cloned and sequenced; it shows homology to a glutathione S-transferase (*10*). Mutations inactivating or deleting *URE2* (designated ure2 or ure2Δ, respectively) give rise to the same physiological phenotype as [URE3]. However, ure2 mutations are recessive, as shown by the fact that introduction of a plasmid encoding the wild-type gene restores function.

Lacroute and his colleagues (*11*, *12*) showed more than 20 years ago that, although ure2 mutations behaved exactly the way chromosomal mutations should, [URE3] behaved as though it were associated with a nonmitochondrial cytoplasmic element (the brackets indicate the non-chromosomal nature and the capitals the dominance of the trait). [URE3] mutants arise spontaneously with a frequency of about $10^{-5}$ and can be easily selected for (because the mutation allows the utilization of ureidosuccinate in the presence of $NH_4^+$ and thus enables yeast mutants incapable of synthesizing the uracil precursor to grow in the absence of uracil). The Lacroute group (*11*, *12*) made the remarkable discovery that the two mutations, ure2 and [URE3], cannot coexist in the same yeast cell. This observation, now confirmed by Wickner with modern techniques, had never been satisfactorily accounted for by classical genetics, but it is easily understood within the framework of Wickner's "yeast prion" hypothesis: If the [URE3] mutation represents the stable conformational change of normal Ure2p into the inactive form Ure2p*, and if the ure2 mutation modifies or abolishes the protein, then [URE3] cannot be maintained in the yeast strain because there is no longer any Ure2p propagate to the conformational change.

How was it possible to tell that ure2 and [URE3] cannot coexist if both mutations give the same phenotype? Wickner introduced a multicopy episomal plasmid expressing Ure2p into yeast carrying a ure2Δ mutation, thereby restoring the wild phenotype, and from this strain he selected a spontaneous [URE3] mutant as described above. From this mutant strain Wickner then isolated a substrain that had lost the episomal plasmid; this substrain still retained the mutant URE phenotype, which could be attributed to the presence of ure2Δ, [URE3], or both. However, when the multicopy plasmid expressing Ure2p was reintroduced, the yeast showed the wild phenotype. Because the [URE3] mutation is dominant, this means that the [URE3] mutation had been lost during the period in which the ure2Δ strain was unable to express normal Ure2p. Wickner also noted that the frequency with which the [URE3] mutation arose in cells overexpressing Ure2p was increased about 50 to 100 times; within the framework of the prion hypothesis this can be ascribed to the larger supply of Ure2p that can undergo a spontaneous conversion.




**Scrapie prions.** In the normal neuronal cell, $PrP^{C}$ is transported to the cell surface from where it is endocytosed and probably recycled (**1**). On infection with $PrP^{Sc}$ (the prion), $PrP^{C}$ is converted to $PrP^{Sc}$, probably at the surface or in the endosomal-lysosomal compartment. The familial prion disease, $PrP^{C}$ may convert spontaneously (**2**). $PrP^{Sc}$ is very stable, accumulates in the cell, and damages it (**3**). **Conjectured "yeast prions."** In normal yeast the protein Ure2p inhibits (among other processes) import of ureidosuccinate in the presence of $NH_4^+$ (**1**). Occasionally Ure2p converts spontaneously to Ure2p*, which is functionally inactive (**2**). Eventually most or all Ure2p has been converted, and the yeast cell can take up ureidosuccinate (**3**).

Finally, yeast carrying the [URE3] trait can be "cured" by growing them in the presence of 5 mM guanidinium chloride. Because the growth rate and plating efficiency of the mutant is not significantly affected, the "cure" cannot be due to selection and so is ascribed to inhibition of the postulated conformational conversion of Ure2p that ultimately leads to dilution of Ure2p*. From such cured strains, spontaneous [URE3] mutants can again be isolated, suggesting that curing is not due to loss of an autonomous replicon.

Can the data be interpreted otherwise? One could, for example, envisage that there exists an episome whose sequence is also encoded on chromosomal DNA and which depends on Ure2p for its cytoplasmic propagation. [URE3] would be a mu-

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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