BIOCHEMISTRY

What Makes a Prion Infectious?

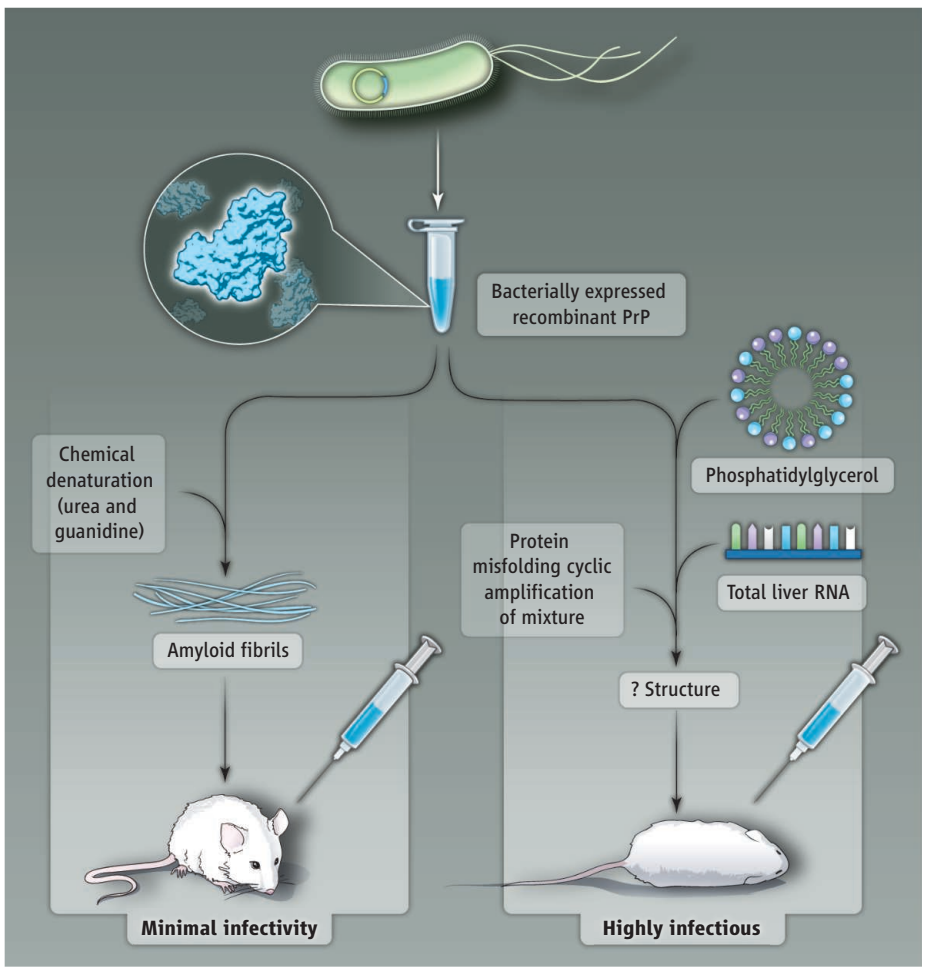
Nonproteinaceous cofactors may be essential to generate infectious prions.

Surachai Supattapone

Prions are unconventional infectious agents that cause fatal neurological illnesses such as Creutzfeldt-Jakob disease, bovine spongiform encephalopathy, and scrapie. Many hypotheses have been advanced to explain the chemical composition of infectious prions and the mechanism of their formation in the neurons of infected hosts, but none has yet been proven. Perhaps the most provocative proposal has been the "protein-only" hypothesis, which posits that the infectious agent is composed exclusively of a misfolded, host-encoded protein called the prion protein (PrP). However, three decades of investigation have yielded no direct experimental proof for this stringent hypothesis. Moreover, various biochemical studies have suggested that nonproteinaceous cofactors may be required to produce infectious prions, possibly by forming physical complexes with PrP (*1*–*4*). On page 1132 of this issue, Wang *et al*. demonstrate the importance of cofactors for producing recombinant infectious prions in vitro (*5*). Another study by Li *et al*. suggests that endogenous cofactors may also influence the strain properties of prions in cells (*6*).

Misfolded PrP that is associated with disease can convert normal PrP into an aberrant form. In a subset of cases, aggregates of misfolded PrP form amyloid fibers, which can accumulate and form plaques in the brain. A central prediction of the protein-only hypothesis is that it should be possible to generate prions with high specific infectivity by chemically refolding PrP in vitro in the absence of other cellular components. The specific infectivity of an infectious agent is measured by an end-point titration bioassay of a known quantity of the agent in susceptible, wild-type hosts. Previous studies have shown that using chemical denaturants to fold purified recombinant PrP (produced by genetically engineered bacteria) into amyloid fibrils yields products with minimal infectivity (*7*, *8*). Although end-point titration was not performed in these studies, extremely low specific infectivity may be inferred because wild-type rodents failed to develop clinical disease when inoculated with samples of highly concentrated protein.

Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA. E-mail: supattapone@dartmouth.edu



Extra ingredients. Two different biochemical protocols yield recombinant PrP with different infectivity. (**Left**) Minimally infectious amyloid fibrils are formed by incubating recombinant PrP with chemical denaturants. (**Right**) Mixing recombinant PrP with phospholipid and RNA produces highly infectious prions.

By contrast, a mixture of native PrP and lipid molecules purified from noninfected hamster brain, plus synthetic polyadenylic acid RNA molecules, resulted in the de novo formation of prions whose specific infectivity was comparable to that of naturally occurring prions (*3*). Although these results indicated that nonproteinaceous cofactors were necessary for generating native prions, it remained unknown whether the addition of such cofactors could facilitate the production of infectious prions from recombinant PrP (*9*).

In a major advance, Wang *et al*. report that mixing recombinant PrP with total liver RNA and synthetic 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) lipid molecules produces bona fide infectious prions (*5*). Building upon their earlier work showing that POPG promotes the conversion of PrP into an aberrant, protease-resistant conformation (*2*), Wang *et al*. used the protein misfolding cyclic amplification technique (*10*) to generate recombinant prions de novo. Remarkably, the resulting recombinant prions were infectious to wild-type mice and displayed unique strain characteristics (prion strains are self-propagating variants with distinct PrP conformations and infectious phenotypes). Although an end-point titration bioassay was not performed, a 100% fatality rate among inoculated animals and a short incubation period between inoculation and disease onset suggest high specific infectivity. The contrast between the bioassay results obtained with recombinant prions formed with lipid and polyanionic cofactors and those obtained using recombinant PrP

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amyloid fibrils (*7*, *8*) argues that cofactors likely facilitated the formation of prions with high specific infectivity from recombinant PrP (see the figure).

If endogenous cofactors participate in prion conversion, it is reasonable to anticipate that they might also constrain PrP structure and influence the properties of different prion strains in cells. Consistent with this possibility, Li *et al*. observed that infecting different cell types with prions can cause phenotypic "mutation" and selection of prion strains, as detected by a new rapid strain-typing assay. It may be that cell type–dependent differences in non-PrP factors could be responsible for the observed evolution of prion strains because all of the cell types examined expressed identical endogenous PrP molecules.

Wang *et al*. and Li *et al*. each have developed powerful methods that can be used to answer critical questions in future studies. It will be important to determine whether cofactors are essential components of an infectious complex, or simply catalyze the formation of prions exclusively composed of PrP. Identifying the endogenous cofactors that facilitate the formation of prion strains in different cell types is also of interest. And a biophysical comparison of the protein structures of the infectious recombinant prions produced by Wang *et al*. and the minimally infectious PrP amyloid (produced by chemically induced refolding of recombinant PrP) could reveal the structural features that encode prion infectivity. After decades of speculation, it may finally be possible to determine the molecular basis of prion infectivity experimentally.

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CLIMATE

Seawater Chemistry and Climate

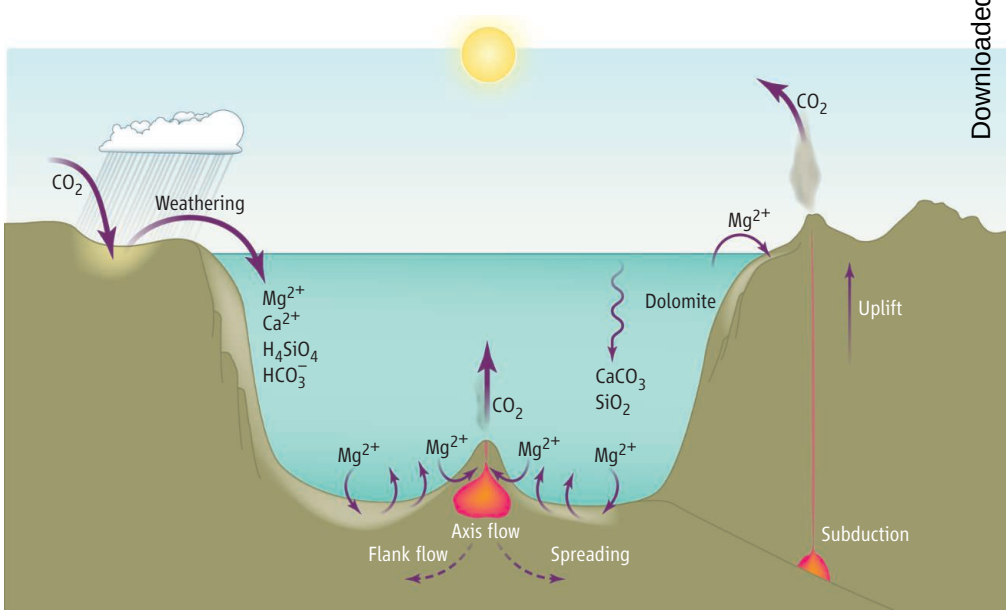
Reconstructions of past seawater chemistry provide insights into the driving forces behind long-term climate change.

Harry Elderfield

The chemical composition of the ocean is determined by rivers, submarine hot springs, and ocean sediments that add or remove elements to seawater. Throughout the oceans, the more abundant elements have near constant ratios to salinity (a measure of total dissolved salts). Thus, records of their past concentrations in seawater should tell us how active these sources and sinks were over long time scales. However, reliable archives of past seawater chemistry have been difficult to find (*1*). On page 1114 of this issue, Coggon *et al*. address this problem by measuring magnesium/calcium and strontium/calcium ratios in calcium carbonate (calcite) veins recovered from ocean crust buried under sediments (*2*). Their Mg/Ca record for the past 180 million years agrees with previous work (*1*), but the Sr/Ca record does not (*3*). The results have implications not only for seawater chemistry but also for climate change.

Earth's climate changes on several time scales. Over tens to hundreds of thousands of years, variations in Earth's orbit around the Sun alter the amount and distribution of external heating. The resulting changes in climate occur within the framework of tectonic processes that take place over millions of years. Driven by Earth's internal heat, tectonics shapes climate by recycling carbon between Earth's interior and surface. Tectonic forcing of climate is crucial for a habitable planet. Our closest planetary neighbors, Mars and Venus, have carbon dioxide in their atmospheres but were unable to escape runaway "icebox" (Mars) and "greenhouse" (Venus) conditions. For Earth to avoid such a fate, a negative feedback must keep runaway warming or cooling in check. Over million-year time scales, it is commonly thought that atmospheric CO_2 reflects a balance between input from volcanic activity and removal by silicate rock weathering feedback (see the figure). This balance, and how it may have changed, is reflected in the chemical composition of the oceans.

The calcite veins studied by Coggon *et al*. were formed by seawater flowing through the upper oceanic crust on the flanks of mid-ocean ridges. The authors dated the veins based on their $^{87}Sr/^{86}Sr$



Long-term climate change and ocean chemistry. On million-year time scales, climate is driven by the input of CO_2 to the atmosphere by plate tectonics. The atmospheric CO_2 reservoir is small and would raise global temperatures unchecked without a chemical weathering carbon feedback. Coggon *et al*. (*2*) have improved understanding of the global Mg cycle, which shares some similarities with the carbon cycle.

Earth Sciences Department, Cambridge University, Cambridge CB2 3EQ, UK. E-mail: he101@cam.ac.uk