PERSPECTIVES: CELL BIOLOGY

Sowing the Protein Seeds of Prion Propagation

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According to the "protein-only" hypothesis, an "infectious protein" (prion) adopts an altered conformation, forming a seed that induces normal cellular versions of that protein to adopt the aberrant form. In this way the infectious protein can be propagated and transmitted to other cells in the absence of nucleic acid. Prions have been invoked to explain the transmission of a group of neurodegenerative diseases that include Creutzfeldt-Jakob disease in humans and scrapie in sheep. In these diseases, cells appear to have both a normal cellular version of the prion protein (PrP), which can be readily digested by proteolytic enzymes, and an infectious form (PrP^{Sc}), which has a β sheet–rich conformation and is protease-resistant. Although it has been possible to generate a variety of mammalian protease-resistant forms of PrP^{Sc} in the test tube (*1*), none of them are infectious even though they have all of the physico-chemical properties of prions isolated from infected animal brain tissue (*2*). To prove the protein-only hypothesis, a purified soluble form of the prion protein must be shown to form an infectious agent spontaneously in vitro. Although mammalian prions have not yielded the necessary proof, Sparrer *et al.* (*3*) report on page 595 of this issue that yeast prions have been a lot more cooperative. They show that the prion form of the normal yeast protein, Sup35p, effectively seeds a self-propagating conformational change in normal Sup35p of living yeast cells. Their demonstration provides the finishing touches to an extensive body of data that supports the protein-only hypothesis.

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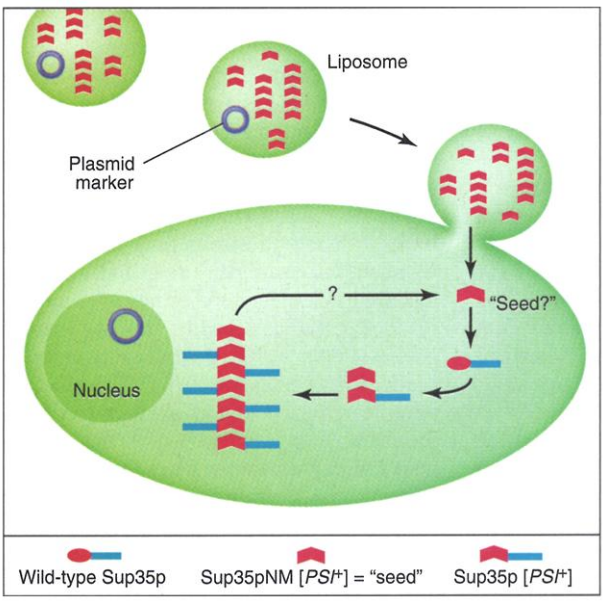
Normal Sup35p (encoded by the *SUP35* gene) is one of at least two, and possibly up to 20, proteins in yeast that can acquire the self-propagating properties associated with mammalian prions (*4*). The normal form of Sup35p is essential for decoding mRNA, ensuring that ribosomes terminate correctly at the end of the coding region. The functionally inactive prion form of Sup35p confers the so-called [*PSI*$^+$] phenotype on yeast cells. In such strains, Sup35p is found almost exclusively as a protease-resistant, high molecular weight aggregate. The other well-characterized yeast prion is the nitrogen regulatory protein Ure2p. Strains with the prion form of Ure2p have the [*URE3*$^+$] phenotype, characterized by an inherited alteration in nitrogen metabolism. Until now the only way to transmit the [*PSI*$^+$] and [*URE3*$^+$] phenotypes between yeast cells has been through fusion of the cytoplasm of two cells (cytoduction) either with or without concomitant nuclear fusion. Spontaneous appearance of the aggregated prion form of these two proteins does occur (albeit at a rate of $<10^{-6}$ in most strains) as long as the corresponding nuclear genes—*URE2* and *SUP35*—are present (*5*).



A soupçon of prion seeds. Liposome delivery of the Sup35p prion seed. A fragment of Sup35p (Sup35pNM) in the prion form can "seed" the adoption of the prion conformation by normal Sup35p. New seeds are then generated from the accumulating aggregates of prion Sup35p and are transmitted to daughter cells, thus propagating the [*PSI*$^+$] phenotype.

In 1994, Wickner first proposed that the non-Mendelian inheritance of the [*URE3*$^+$] and [*PSI*$^+$] genetic determinants could be explained by the prionlike behavior of the Ure2p and Sup35p proteins (*6*). So far, the most convincing evidence in support of the protein-only hypothesis has come from induction experiments in which parts of the prion forms of either of these proteins are transiently overexpressed in yeast cells. In some cases, such overexpression resulted in a 1000-fold increase in the rate of appearance of the prion form. The normal soluble form of Ure2p or Sup35p can be "seeded" with a small amount of the corresponding prion (*7, 8*). Microscopic analysis of the resulting aggregates reveals that they are ultrastructurally very similar to the fibrillar polymers formed by mammalian prions and other amyloid-like proteins. The relevance of this in vitro conversion to the prionlike behavior of Sup35p in vivo is highlighted by studies with mutant forms of Sup35p (*9*). Several mutants that are unable to propagate the [*PSI*$^+$] phenotype in vivo are also less efficient at seeding the conversion of the normal protein to the prion form in vitro. The Sparrer study now unequivocally demonstrates that the establishment of the [*PSI*$^+$] phenotype in yeast in vivo is a direct consequence of seeding by an altered conformation of Sup35p.

These investigators selected an amino-terminal fragment of Sup35p (Sup35pNM) for their experiments because this region is essential for [*PSI*$^+$] propagation (*10*). They encapsulated into liposomes a recombinant form of the Sup35pNM fragment that aggregates in vitro. They then fused the liposomes with yeast cells that had the normal (but not the prion) form of Sup35p (see the figure). To demonstrate successful delivery of Sup35pNM to yeast cells, the authors included a plasmid marker. Less than 1 in 10^5 of the cells that were mixed with the loaded liposomes acquired the plasmid marker; but, of these, approximately 2% became [*PSI*$^+$]. The rate of acquisition of the [*PSI*$^+$] phenotype was about 10-fold higher than that for yeast cells fused with control liposomes that did not carry Sup35pNM. The increased appearance of [*PSI*$^+$] cells did not seem to be a consequence of a dramatic increase in the amount of Sup35pNM in the yeast cells after fusion. Sparrer *et al.* calculated that the amount of Sup35pNM delivered by each liposome represented less than 10% of the endogenous amount of Sup35p in the yeast cells after fusion. Clearly, some of the lipo-

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somes delivered an infectious agent that provided a seed, enabling endogenous Sup35p to convert to the self-propagating prion form. This form was subsequently transmitted from the original yeast cells to daughter cells through cell division, and was then propagated over a number of generations of growth. Two variants of the Sup35pNM fragment known to be defective in seeding conversion of normal Sup35p to the prion form (*9*) were unable to seed this conversion when delivered by liposomes to yeast cells.

These experiments prompt the question, What is the nature of the Sup35p seed that catalyzes Sup35p conversion? Sparrer and co-workers show that only a small percentage of the Sup35pNM-loaded liposomes contain, or at least are able to deliver, seeding activity. This hints that it is not simply aggregated Sup35p that acts as the seed. Fibrils of Sup35p formed by conversion of the normal protein to the prion form exist for the most part as amyloid-like fibrils (*7*), although there has been no direct demonstration that these fibrils are the seeds that initiate the normal-to-prion conversion. Tellingly, agitation (and the presumed consequent fragmentation of Sup35p fibrils) greatly increases their seeding activity in vitro (*7, 9*). In vivo, the picture is even less clear. Sedimentation analysis of Sup35p has confirmed that it is found primarily as part of an aggregate (that can be pelleted by centrifugation) in [*PSI*$^+$] cells. But there is no evidence that these aggregates consist of fibrillar polymers of Sup35p even though they can seed prion conversion in vitro.

Fibrils of Sup35p formed in vitro and the high molecular weight Sup35p aggregates found in vivo may simply be "dead-end" products of the polymerization process. The prion-forming seeds may be lower molecular weight, possibly monomeric, forms of Sup35p that have acquired a new and inheritable conformation. We know from studies of chemically induced [*PSI*$^+$] loss in yeast that there are at least 60 seeds per haploid cell (*11*). Until the identity of these seeds has been established, we can only speculate about the mechanism of protein-mediated inheritance in yeast and the transmission of prions in mammals.

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PERSPECTIVES: APPLIED PHYSICS

How to Be Truly Photonic

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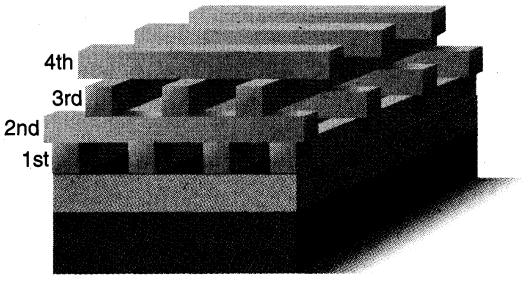
Photonic crystals are three-dimensional (3D) dielectric structures with a forbidden gap for electromagnetic waves, analogous (*1*) to the electronic band gap in semiconductors that lies at the heart of silicon technology. A photonic band gap allows light to be trapped in the tiniest volumes and optoelectronic devices, which interface optical and electronic components, to reach their ultimate limit of miniaturization.

The dream of photonic integrated circuits—microchips for light—remains yet to be fulfilled, and many believe that tiny photonic crystal devices will hold the key. The Internet is demanding more and more communications capacity that will require vast numbers of such optical components. As reported on page 604, Noda *et al.* (*2*) now bring these devices one step closer to reality by creating a photonic crystal with unprecedented performance.

The first photonic crystals were reported about 10 years ago (*3*, *4*), but they were hardly photonic at all. They were large structures and had gaps at microwave frequencies and centimeter wavelengths. Since then, the race has been on to shrink the structures down to optical wavelengths. This has not been an easy task, not least because photonic crystals are intricate 3D objects that must be created with nanometer precision.

Among the variety of optical materials, only those with a refractive index greater than roughly 2.0 are capable of supporting a photonic band gap. For fully functional optoelectronics, the classic III-V semiconductors, such as GaAs, will ultimately be preferred, because they combine both optical and electronic function. Although there has been considerable progress with other substances, such as TiO_2 and silicon, the III-V semiconductors remain the preeminent materials of choice.



Stacking assembly of photonic crystals. Just eight layers make a respectable photonic crystal. With the strategy of Noda *et al.* (*2*), this requires only three stacking and alignment steps.

The ideal structure for photonic band gaps must recreate, at the optical wavelength scale, the beautiful valence bond structure of diamond crystals at the atomic scale. Diamondlike connectivity or geometry in photonic crystals has provided the widest photonic band gaps observed to date even for relatively low refractive index contrast. Other crystal structures are easier to make. For example, face-centered cubic (fcc) crystals will self-assemble from microspheres in many types of colloidal solutions. But these fcc crystals have a relatively feeble gap that requires a refractive index greater than 2.8. These materials are likely to have other optical applications but are unlikely to provide full optoelectronic function, wherein electricity directly creates light.

Strategies for meeting the exacting set of requirements for a 3D, diamondlike nanostructure, with a III-V material base, for photonic crystals have long been sought. There have been some mildly successful efforts in the past (*5*, *6*). A figure of merit has emerged to measure their success, with the rejection of optical intensity within the forbidden band gap becoming the accepted gauge. For a photonic band gap to be interesting, a rejection factor of 10 is deemed rather inadequate. Optical rejection should be much higher, at least 100 and up to 10,000 or more according to need.

The excellent report by Noda *et al.* (*2*) represents a watershed in photonic crystal research. The authors demonstrate unprecedented optical rejection >10,000 in a GaAs photonic band gap structure. All of the key requirements for photonic crystal–based optoelectronics are demonstrated in this work.

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