Prions of Yeast Are Genes Made of Protein: Amyloids and Enzymes

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In 1994 we described two infectious proteins (prions) of *Saccharomyces cerevisiae*, showing that the nonchromosomal genes [URE3] and [PSI] are inactive, self-propagating forms of Ure2p and Sup35p, respectively (Wickner 1994). Since then, [Het-s], a prion of *Podospora anserina* (Coustou et al. 1997), and [PIN⁺], another *S. cerevisiae* prion (Derkatch et al. 1997, 2001; Sondheimer and Lindquist 2000), have been found. All of the above prions are based on self-propagating amyloids. Recently, we described another prion, called [β], which is based on the *trans* self-activation by the yeast vacuolar protease B (Roberts and Wickner 2003). Evidence has appeared suggesting that C, the non-Mendelian gene of *Podospora anserina* determining Crippled Growth, is also a self-activating prion of a MAP kinase kinase kinase (Kicka and Silar 2004). Study of these yeast and fungal phenomena has established that proteins can be genes and infectious entities and has revealed many details of what makes a protein infectious.

“EPIGENETICS” AND “PRIONS”: WHAT DOES IT ALL MEAN?

“Epigenetic” has been defined as “. . . heritable changes in gene function that cannot be explained by changes in DNA sequence” (Riggs and Porter 1997). The prions (infectious proteins) alter the expression of genes without changes in DNA sequence, but do so by altering the gene product, without necessarily altering the function of the gene itself. However, “epigenetic” is more broadly used to mean any heritable change not due to a change in nucleic acid sequence.

The notion of infectious proteins originated in attempts to explain the relative radiation resistance of the scrapie agent (Alper et al. 1967; Griffith 1967). The term “prion,” which we now use to mean “infectious protein without a needed nucleic acid” (the protein-only model), was later coined as a synonym for the agent causing transmissible spongiform encephalopathies (TSEs), without requiring that this be its mechanism, but including this possibility (Prusiner 1982). In spite of two Nobel prizes, it seems that there is still doubt in some quarters about whether the TSEs are infectious proteins (Farquhar et al. 1998; Chesebro 2003; Priola et al. 2003). Our view is that there is considerable evidence pointing in this direction (see Wickner et al. 2004), but we remain agnostic about

whether this is enough. Two potential “final proofs” have famously not yet worked: (a) amyloid formed in vitro from recombinant PrP is not infectious for normal mice, and (b) brain extracts of transgenic mice dying with a scrapie-like syndrome as a result of carrying PrP genes with mutations that cause inherited Creutzfeldt–Jakob disease (CJD) in humans are again not infectious for normal mice.

FOUR TYPES OF PRION

There are four known types of prion, depending on whether the altered form of the protein is an amyloid or not, and whether the prion is toxic to the individual, is simply inactive for its normal function, or positively helps the cell (Fig. 1). In the TSEs, assuming they are infectious proteins, the amyloid form of PrP is cytotoxic. The [URE3] and [PSI⁺] prions of yeast are simply inac-

Four known prion types:

Figure 1. Four prion types. The known prions differ in whether they are based on amyloid or not, and what effect they have on the organism. The TSEs are lethal because of a novel harmful effect of the amyloid form of PrP. Cells with [URE3] and [PSI] partially lose the normal function of Ure2p and Sup35p, respectively. In [Het-s] strains, the HETs protein carries out a function for the cell only in the amyloid form (and is generally found in this form in wild isolates). The [β] prion is simply the active form of the vacuolar protease B, has no relation to amyloid, and functions for the cell in starvation and meiosis.

tive (or partially inactive) amyloid forms of yeast Ure2p and Sup35p, respectively. [Het-s] of *Podospira anserina* is an amyloid-based prion that performs a function for the cell, helping in the heterokaryon incompatibility that this organism uses to avoid harmful infectious agents (such as viruses and mitochondrial senility factors) that spread by fusion of cellular processes (hyphal anastomosis). The [β] prion of *S. cerevisiae* is not amyloid-based but is simply an enzyme whose active form is necessary *in trans* for activation of its own inactive precursor. [β] functions for the cell in promoting survival under starvation conditions and allowing meiosis and spore formation.

GENETIC CRITERIA FOR PRIONS

The first yeast prions were discovered based on their paradoxical genetic properties, properties that are unknown (and unexpected) for nucleic acid replicons, but are expected for prions in which the phenotype produced is due to simple inactivation of the prion protein's normal function (Fig. 2) (Wickner 1994). These properties were emphatically not the observed properties of the TSEs, both because they were designed for a prion of a microorganism and because TSEs cannot be a consequence of simple inactivation of PrP, since deletion of the *PRNP* gene produces essentially no phenotype (Bueler et al. 1992).

Reversible Curing

If a prion can be cured, then it should be possible for it to arise again in the cured strain, because the protein with the potential to become the prion is still present. Reversible curing is to be distinguished from reversible mutation. Curing is the efficient elimination of a nonchromosomal gene by some treatment or condition that, for a nucleic acid replicon, would mean elimination of the replicon—generally an irreversible event. Mutation is generally not so efficient and can revert.

Overproduction of the Protein Increases Frequency of Prion Generation

More of the protein with the potential to become a prion should result in a higher frequency of de novo prion generation, regardless of the mechanism.

Gene Required for Prion Propagation and Phenotype Relationship

The same gene whose overexpression increases prion generation should be essential for prion propagation. Furthermore, for prions of the “inactive amyloid” type (see Fig. 1), the phenotype of the presence of the prion should resemble the phenotype of a recessive mutation in the gene, since in each case there is a deficiency of the normal form.

All three genetic criteria apply strictly to the prions of the inactive amyloid type, indeed to any prion whose phenotype is due to self-propagating inactivation of the nor-

Diagnostic Features of Prions:

Figure 2. Genetic criteria for a prion. These three properties are expected of a nonchromosomal gene if it is a prion, but are not expected if it is a nucleic acid replicon (Wickner 1994).

mal form (whether by amyloid formation or not). The final genetic feature only half applies to other prions; the gene encoding the protein must be necessary for the propagation of the prion, but the phenotype relation will not be as described (Wickner 1994).

[URE3] AND [PSI⁺] ARE PRIONS OF Ure2p AND Sup35p, RESPECTIVELY

The non-Mendelian gene [URE3] and the chromosomal *ure2* mutant were discovered by Lacroute in the same genetic selection for strains that could take up ureidosuccinate to replace the absence of that intermediate in a *ura2* (aspartate transcarbamylase) mutant (USA⁺) (Lacroute 1971; Drillien and Lacroute 1972). The phenotypes of [URE3] and *ure2* strains are thus similar, and, remarkably, *ure2* mutants cannot propagate [URE3] (Drillien and Lacroute 1972; Aigle and Lacroute 1975; Wickner 1994). [URE3] can be cured by growth of cells in the presence of millimolar guanidine (Aigle and Lacroute 1975), but from the cured clones can again be isolated [URE3] subclones (Wickner 1994). Finally, overproduction of Ure2p (*URE2* mRNA does not suffice) induces a 100-fold increase in the frequency with which [URE3] arises de novo (Wickner 1994). This showed that [URE3] is a prion of Ure2p. When yeast is presented with a good nitrogen source, such as ammonia, glutamine, or glutamate, it represses the production of the enzymes and transporters needed to utilize poor nitrogen sources. Ure2p is a central player in this process, binding the Gln3p transcription factor and keeping it in the cytoplasm under repressing conditions (for review, see Cooper 2002).

[PSI⁺] is a nonchromosomal gene that increases the efficiency of weak non-sense suppressor tRNAs (Cox 1965; Cox et al. 1988). Results similar to those for [URE3] implied that [PSI⁺] is a prion of the Sup35 protein (Wickner 1994). [PSI⁺] is efficiently cured by growth in hyperosmotic media (Singh et al. 1979), but

[PSI⁺] derivatives can again be isolated from cured cells (Lund and Cox 1981). Overproduction of Sup35p increases the frequency with which [PSI⁺] arises (Chernoff et al. 1993; Derkatch et al. 1996). Finally, the phenotype of the presence of [PSI] resembles that of recessive *sup35* mutants, and [PSI] propagation depends on the *SUP35* gene (Doel et al. 1994; Ter-Avanesyan et al. 1994). Sup35p is a subunit of the translation termination factor (Frolova et al. 1994; Stansfield et al. 1995), explaining these effects on suppression of non-sense mutations.

These results showed that [URE3] and [PSI⁺] are prions of Ure2p and Sup35p (Wickner 1994), respectively; these conclusions have been amply confirmed by numerous later studies. For [URE3], it was shown that Ure2p becomes protease-resistant in [URE3] strains (Masison and Wickner 1995), and that this protease resistance was not a consequence of the altered nitrogen regulation (Masison et al. 1997). It is the Ure2 protein (not the mRNA or DNA gene) whose overproduction induces the de novo appearance of [URE3] (Masison and Wickner 1995; Masison et al. 1997), a result that may well be sufficient to prove a prion. Sup35p is aggregated in [PSI⁺] cells (Patino et al. 1996; Paushkin et al. 1996) and it is the protein, not the RNA or gene, that induces [PSI⁺] to arise de novo (Derkatch et al. 1996).

THE AMINO TERMINUS OF Ure2p CONSTITUTES A PRION DOMAIN

Deletion analysis of Ure2p showed that the amino-terminal 65 residues were sufficient to induce the appearance of [URE3] (Masison and Wickner 1995). This region was also sufficient to propagate [URE3] in the complete absence of the carboxy-terminal part of the molecule (Masison et al. 1997). In contrast, the carboxy-terminal residues 66–354 (Coschigano and Magasanik 1991; Masison and Wickner 1995) or even residues 90–354 (Madelain and Wickner 1999) are sufficient to carry out the nitrogen regulation function of Ure2p (Fig. 3). These results indicated that the interaction between Ure2p molecules that propagated the [URE3] prion were between one amino-terminal prion domain and another, and that the carboxy-terminal part of the molecule was only the indicator of the prion change, but not its mediator.

Figure 3. Prion domain of Ure2p. Deletion analysis showed that residues 1–65 are sufficient to induce [URE3] prion formation and necessary for Ure2p to participate in the prion change (Masison and Wickner 1995; Masison et al. 1997). This region can also propagate [URE3] in the absence of the carboxy-terminal domain. Residues 66–354 can carry out the nitrogen regulation function of Ure2p (Masison and Wickner 1995).

THE [URE3] PRION IS A SELF-PROPAGATING AMYLOID FORM OF Ure2p

The protease-resistance of Ure2p in [URE3] strains (Masison and Wickner 1995), and the appearance of Ure2-GFP fusion protein in [URE3] cells (Edskes et al. 1999a) suggested that the [URE3] change of Ure2p involved aggregation. Chemically synthesized Ure2p^{1–65}, the prion domain peptide, spontaneously forms amyloid in vitro, suggesting a role for this abnormal protein form in the [URE3] prion (Taylor et al. 1999). Just as overproduced Ure2p^{1–65} induces the full-length protein to undergo the prion change in vivo, the same prion domain peptide induces amyloid formation by the otherwise stably soluble full length Ure2p in vitro (Taylor et al. 1999). This amyloid formation could be further propagated by addition of more native Ure2p. The pattern of protease-resistant bands of Ure2p seen in extracts of [URE3] cells (Masison and Wickner 1995) is identical to that seen for amyloid of Ure2p formed in vitro (Taylor et al. 1999). In each case, the most protease-resistant part of the molecule is the prion domain itself (Masison and Wickner 1995; Taylor et al. 1999; Baxa et al. 2003). It is possible to detect networks of filaments specifically in [URE3]-carrying cells (Speransky et al. 2001). Using antibody to Ure2p linked to gold particles, it was shown that these filaments contained Ure2p.

ARCHITECTURE OF AMYLOID FILAMENT FORMED FROM Ure2p

The fact that the prion domain could propagate the [URE3] prion in the complete absence of the carboxy-terminal domain (Masison et al. 1997) implies that the primary interaction between molecules in the prion (amyloid) form of Ure2p is between one amino-terminal prion domain and another. This conclusion was confirmed by showing that protease digestion of filaments formed in vitro or Ure2p in extracts of [URE3] cells leaves a protease-resistant core consisting of the prion domain. The filaments become more narrow on digestion and are similar to those formed from the synthetic prion domain peptide (Taylor et al. 1999; Baxa et al. 2003).

The carboxy-terminal functional domain is only inactivated by prion formation if it is covalently attached to the prion domain (Masison et al. 1997). This could mean that the carboxy-terminal domain is conformationally changed by association with the amyloid-forming prion domain, or that amyloid formation makes the carboxy-terminal domain sterically unable to interact with Gln3p, its normal function. A series of fusion proteins were prepared consisting of the amino-terminal prion domain linked to enzymes whose structures are known and whose substrates are too small to be subject to steric hindrance by amyloid formation. Each of these fusion proteins readily formed amyloid filaments and in each case the enzyme was fully active in the amyloid filaments. This surprising result is particularly significant in the case of the Ure2p^{1–65}-glutathione-S-transferase fusion protein because the carboxy-terminal part of Ure2p is homologous to GSTs in both sequence and structure (Coschigano and Magasanik 1991; Bousset et al. 2001; Umland et al.

Ure2p Amyloid Architecture:

Figure 4. Architecture of amyloid filaments of Ure2p. Scanning transmission electron micrograph of a Ure2p amyloid filament showing the central core of amyloid, composed of the prion domain, and the appended carboxy-terminal domain (Baxa et al. 2003).

2001). This indicates that the carboxy-terminal domain of Ure2p is not conformationally changed in the prion (amyloid) form, but is unable to interact with Gln3p nonetheless. One intriguing possible explanation (Baxa et al. 2002) is that Gln3p association with Ure2p is a diffusion-limited reaction that is inefficient when Ure2p is restricted to a small part of the cytoplasm in the network of filaments (Speransky et al. 2001).

Scanning transmission electron microscopic images of Ure2p amyloid filaments show that each has a central core around which is arrayed larger masses attached rather loosely to the central core (Fig. 4) (Baxa et al. 2003). The central core of these filaments was shown by Western blot and mass spectrometry to be comprised of Ure2p residues 1–65 (with some fraying in some molecules) (Baxa et al. 2003). The masses arrayed around the core are the GST-like carboxy-terminal domains, consisting of residues

95–354. The carboxy-terminal domains are connected to the amyloid cores by a tether comprising roughly residues 70–95. This region remains highly protease-sensitive in the amyloid form, when both the prion domain and carboxy-terminal domain are structured (Baxa et al. 2003).

The structure of the amyloid core remains to be determined. The recent demonstration by Tycko and coworkers of a parallel β -sheet structure of the A β peptide (Petkova et al. 2002; Tycko 2003) suggests that a similar structure may be found for the Ure2p prion domain amyloid. All Ure2p amyloid filaments have one monomer per 0.45 nm, consistent with this type of structure (Baxa et al. 2003).

AMINO ACID COMPOSITION IS THE MAIN DETERMINANT OF Ure2p PRION FORMATION

The N/Q-rich nature of the Ure2p and Sup35p prion domains (Ter-Avanesyan et al. 1994; Masison and Wickner 1995) and the demonstrated importance of Q residues in Sup35p (DePace et al. 1998) and runs of N in Ure2p (Maddelein and Wickner 1999) implicate at least this feature as important in prion formation. However, there are over 100 yeast proteins with N/Q-rich regions, and, aside from Ure2p and Sup35p, only Rnq1p has been shown to be a prion (Derkatch et al. 1997, 2001; Sondheimer and Lindquist 2000). To determine whether there were sequence requirements for prion (or amyloid) formation by Ure2p, we constructed a series of derivatives in which the amino acids of the prion domain of Ure2p had been randomly shuffled without changing the overall composition or codon usage (Fig. 5). We found that all five such random sequences were capable of becoming prions *in vivo* and the purified proteins were able to form amyloid *in vitro* (Ross et al. 2004). This shows that, in the case of Ure2p, the major factor determining prion (and amyloid) formation is amino acid composition. There were differences in how robust were the prions formed by the various shuffled derivatives, showing that sequence is indeed a lesser factor but a factor nonetheless (Ross et al. 2004). Future studies will determine whether other prion domains can be shuffled without loss of activity, what aspects of composition (aside from N/Q richness) are important, and what are the sequence determinants of prion robustness.

CHAPERONES INVOLVED IN [URE3] PROPAGATION

That [PSI⁺] requires the disaggregating chaperone Hsp104 for its propagation and the curing of [PSI⁺] by overproduction of Hsp104 were crucial findings, opening up a vital new area in prion (and amyloid) studies (Chernoff et al. 1995). In addition, guanidine curing of yeast prions has been shown to occur by inhibition of Hsp104 (Ferreira et al. 2001; Jung and Masison 2001; Jung et al. 2002). Guanidine treatment was used as a tool to study the mechanism of Hsp104's contribution to [PSI⁺] propagation. It was found that guanidine treatment had no immediate effect on the incorporation of newly synthesized Sup35p into aggregates, but instead blocked the forma-

Shuffled prion domains:

	Prion	Amyloid
Wild-type Ure2:		
MMNNNGNQVSNLSNALRQVNIQNRNSNTTDDQSNINFEFTGVNNNNNN	+	+
SSNNNNNVQNNSSGNGSGQNDNENNIKNTLEQHRGQQQ		
Scrambled sequence #1:		
MVDGNQMNNKSRNNSQQRGNSNQVRVNNQENNFGLAQSSNNNSITTT	+	+
FTNNNQINSQNGINNHNVTQDQNVQNHGNSNENNSNL		
Scrambled sequence #2:		
MQSHQAESNSQNGDQNGTNNLQNRNSNGINNFQNNNRNNQNLQSRVNN	+	+
TINNKLNGFNGNEVNVNQSSDNTNNNNIVTTRNS		
Scrambled sequence #3:		
MNIRNQNSTAVLVNVQSSNGTSSNVNLFNNSGMQNHGRNFQSTRN	+	+
NNTEKGGNNILNSNDRINNQQQENNTVDNSQNNSS		
Scrambled sequence #4:		
MMQRNGQEGGTNNHNSINTQNRVFNNSANNRRNNEGLNNNSNFNNLV	+/-	+
SNQQVNVSSNINQDNNKSLISGTSNDTTENRGQQQ		
Scrambled sequence #5:		
MNTNSQGSFVDENQNRISVKSRTVNMSSQNNNTGNNNNAQLNNILNNTDS	+	+
GHVSNNEENRLGRQNDNFQNSQTTNNGNNQQSSNNNNNI		

Conclusion: For the Ure2 prion domain, prion-forming ability is determined by amino acid content, not by amino acid sequence.

Figure 5. Shuffled prion domains can be prions and form amyloid. Residues 1–89 of Ure2p were randomly ordered, and replaced the normal sequence at the normal *URE2* chromosomal locus. The amino acid content was unchanged, but the sequence was completely random. Ability of each of these five randomized proteins to form prions *in vivo* and amyloid *in vitro* was tested and each was capable of both (Ross et al. 2004).

tion of new prion seeds (Ness et al. 2002; Tuite and Cox 2003). Hsp70s have also been implicated in prion propagation (Chernoff et al. 1999; Newnam et al. 1999; Jung et al. 2000; Jones and Masison 2003). Likewise for the Hsp40 family and various cochaperones (Kushnirov et al. 2000; Jones et al. 2004).

We find that propagation of [URE3] requires Hsp104, but that, unlike [PSI⁺], overproduction of Hsp104 does not cure [URE3] (Moriyama et al. 2000). However, overproduction of Ydj1p, an Hsp40 family member, does cure [URE3] (Moriyama et al. 2000). Mutation of *SSA2*, encoding the most abundant of the cytoplasmic Hsp70s, also results in loss of [URE3] (Roberts et al. 2004). Interestingly, overexpression of *Ssa1p* eliminates [URE3], although identical overexpression of *Ssa2p* does not (Schwimmer and Masison 2002).

GENETIC CONTROL OF [URE3] GENERATION: Mks1p AND [PIN⁺]

Our finding that overproduction of Mks1p negatively affects the ability of Ure2p to regulate nitrogen catabolism (Edskes et al. 1999b) suggested the possibility that Mks1p might likewise affect prion generation or propagation. We found that *mks1Δ* strains were nearly unable to generate [URE3] derivatives, even when Ure2p or one of its highly prion-inducing derivatives was overexpressed (Edskes and Wickner 2000). Modest overproduction of Mks1p resulted in a substantial increase in [URE3] prion generation. The same *mks1Δ* strains were fully competent to propagate [URE3] and to show the USA⁺ phenotype typical of [URE3] strains (Edskes and Wickner 2000). This shows that the effect of *mks1Δ* on prion formation was an effect on prion generation, not an effect on propagation or detection of the [URE3] prion.

MKS1 was first defined as a gene whose overexpression slows growth, an effect antagonized by the *RAS*-cAMP pathway (Matsuura and Anraku 1993). We found that expression of the constitutive ("oncogenic") Ras^{val19} mutant dramatically inhibited [URE3] generation, as one would expect if it inhibited Mks1p action (Edskes and Wickner 2000).

The [PIN⁺] nonchromosomal genetic element was discovered as a factor necessary for [PSI⁺] inducibility on overproduction of Sup35p (Derkatch et al. 1997). Subsequent genetic analysis showed that [PIN⁺] is a prion of the Rnq1 protein, a nonessential protein rich in asparagine and glutamine residues throughout most of its length (Derkatch et al. 2000, 2001; Sondheimer and Lindquist 2000). The [PIN⁺] effect may be a result of cross-seeding between Q/N-rich amyloid formed by Rnq1 and the prion domain of Sup35p. A similar, but less dramatic, effect is seen with [URE3]. [PIN⁺] strains are 10- to 100-fold more frequently induced to form [URE3] than are isogenic [pin⁻] strains (Bradley et al. 2002).

Ure2 HOMOLOGS IN PATHOGENIC YEASTS

Although Ure2p is weakly homologous to many GSTs, and it has not been found to have GST activity in vitro, it is reported to confer a low level of metal resistance (Rai et

al. 2003). However, close relatives of Ure2p are found in a series of yeasts, including other species of *Saccharomyces*, *Schizosaccharomyces pombe*, *Candida albicans*, *Candida lypolytica*, *Candida maltosa*, *Ashbya gossypii*, and others (Edskes and Wickner 2002). Most of these foreign *URE2* genes were able to complement an *S. cerevisiae ure2Δ* mutant, reflecting the 80–100% identity in their carboxy-terminal domains (Edskes and Wickner 2002). Their amino-terminal domains show far lower similarity, and, in the case of *Saccharomyces pombe*, no amino-terminal domain at all. Those amino-terminal extensions are all Q/N-rich, suggesting some utility for the prion domain. Indeed, expression of only the carboxy-terminal domain gives a leaky phenotype, with some expression of normally nitrogen-repressed genes (Masison and Wickner 1995). Since [URE3] and *ure2* mutant cells grow slowly on most media, it is likely that the evolutionarily selected function of the Ure2p prion domain is not for the rare prion formation but rather for the promotion of the normal function of Ure2p. However, there is at least one case of a prion that is functional for its host, the [Het-s] prion of *Podospora*.

[Het-s], AN ADAPTIVE AMYLOID PRION OF *PODOSPORA ANSERINA*

When two colonies of a filamentous fungus meet, they fuse their cellular processes (hyphae) perhaps to share nutrients, but only if they are genetically identical. Genetic identity is tested by checking identity at around a dozen polymorphic loci scattered around the genome. These are called *het* loci in *Podospora* and include *het-s*, whose alleles are *het-s* and *het-S*. When colonies meet, a few hyphae fuse in a sort of trial. If they differ in alleles at even a single *het* locus, these fused hyphae degenerate and die and set up a barrier to further fusions. This reaction is called "heterokaryon incompatibility." The *het-s* locus encodes a 289-amino-acid protein differing at 13 residues between the *het-s* and *het-S* alleles.

Studies by Saupe and colleagues at Bordeaux have shown that only if the *het-s* protein is in a prion form is the proper incompatibility reaction observed (Coustou et al. 1997; Saupe et al. 2000; Maddelein et al. 2002). This prion is called [Het-s] and is based on a self-propagating amyloid form of the protein encoded by the *het-s* allele. Since heterokaryon incompatibility is a normal fungal function, possibly designed to prevent the spread of debilitating fungal viruses and plasmids between unrelated strains, it is reasonable to assume that this prion is carrying out a function for the cell. This hypothesis is supported by the fact that essentially all isolates of *Podospora* with the *het-s* allele carry the prion.

All yeast and fungal prions are nonchromosomal genes that spread via cell–cell fusion such as occurs in sexual matings or in nonsexual matings such as the hyphal anastomosis discussed above for *Podospora*. This infectious mode of spread means that these elements should gradually invade the population unless they are a disadvantage to their hosts. Thus, finding a prion in the wild does not imply it is an advantage, but failure to find it in the wild is a strong indication that the prion is a disadvantage to

Figure 6. A new type of prion: an enzyme needed for its own activation. Vacuolar protease B of *S. cerevisiae* can be a prion in the absence of protease A, which normally catalyzes its activation (Roberts and Wickner 2003). The properties of the “C” nonchromosomal gene of the filamentous fungus *Podospora anserina* suggest that it too may be a prion of this type, based on self-activation of a MAP kinase kinase kinase (Kicka and Silar 2004). It is possible that the MAP KKK activation is done by one of the kinases further down in the cascade, MAP KK or MAP K.

the host. For example, two efforts to find the [PSI⁺] prion in wild strains have been unsuccessful (Chernoff et al. 2000; Resende et al. 2002), indicating that this prion is, on the net, a disadvantage to the host.

[β], A DIFFERENT KIND OF PRION

The word prion means “infectious protein,” and, as first discussed by Griffith (Griffith 1967), need not involve amyloid. If the active (or, more generally, modified) form of an enzyme is necessary for its own activation (or modification), then a cell that starts out without the active (modified) form will never develop it unless it comes from outside the cell. A cell with the active (modified) form will continue to activate (modify) newly synthesized protein molecules and should pass the trait “has active enzyme” on to its offspring. Transmission of the active protein to a cell lacking it should likewise transmit the self-propagating trait as well (Roberts and Wickner 2003).

Based on extensive work by Beth Jones (Carnegie Mellon University; Zubenko and Jones 1982) and Deiter Wolf (University of Stuttgart; Teichert et al. 1989), we have identified such a prion—namely, the active form of the vacuolar protease B of *S. cerevisiae* encoded by the *PRB1* gene (Fig. 6) (Roberts and Wickner 2003). Protease B, encoded by *PRB1*, is made as an inactive precursor, whose activation by deletion of amino-terminal and carboxy-terminal extensions is carried out in normal cells by combined action of itself and protease A (for re-

view, see Jones 1991). However, in mutants deleted for the protease A structural gene (*pep4 Δ*), protease B self-activation can continue indefinitely as cells grow (Roberts and Wickner 2003). The active state can be transmitted by cytoplasmic mixing from a cell with active protease B to one lacking it, showing that this trait is a nonchromosomal gene. The state in which cells have protease B activity is called [β] and its absence [β -o].

[β] has the characteristics expected of a prion for which the prion form is the active form of the protein. Cells that start out with protease B activity give rise to offspring, nearly all of which have active enzyme. Such a strain is efficiently cured of protease B activity by growth on rich dextrose media, a condition that partially represses *PRB1* transcription, leading to the self-activation cycle rapidly dying out. But of these cured cells, about 1 in 10⁵ descendants has spontaneously developed protease B activity (reversible curing). Overexpression of *PRB1* increases this frequency to several percent (genetic criterion 2 for a prion) (Roberts and Wickner 2003). [β] depends on *PRB1* for its propagation, but, of course, the phenotype of [β] is not the same as a *prb1* mutant because the prion form is the active form of the protein.

[β] is advantageous to the cell, since [β] cells can undergo meiosis, while [β -o] cells cannot, and cells with [β] survive starvation better than do those without (Roberts and Wickner 2003).

The importance of the [β] prion lies not in its biochemical mechanism, which has been recognized for decades, but in the facts that (1) a protease can be a gene and (2) there are many potentially self-modifying enzymes (protein kinases, protein acetylases, protein methylases, etc.) that, under some circumstances, might become prions. Indeed, recent work on the Crippled Growth phenomenon of *Podospora anserina* appears to be just such a new prion (Kicka and Silar 2004).

CRIPPLED GROWTH AS A NEW PRION OF *PODOSPORA ANSERINA*

Crippled growth (CG) is a condition that can develop in *Podospora* which is characterized by increased pigmentation and slowed growth (Silar et al. 1999). CG is the result of carrying the nonchromosomal gene “C.” If a piece of a colony with CG is placed on a non-CG colony, heterokaryon formation leads to cytoplasmic mixing and the non-CG colony acquires the CG trait and the C genetic element.

Recent work has shown that the propagation of C (not just the phenotype, but the cytoplasmic gene) requires a MAP kinase kinase kinase similar to that encoded by *BCK1* of *S. cerevisiae*, and called PaASK1 because of its homology to human ASK1 (Kicka and Silar 2004). Overexpression of PaASK1 results in generation of the C element under conditions where its formation would be rare. C can be cured, but will arise anew in the cured strains. The phenotype of C is different from that of mutants in PaASK1, indicating that it is not inactivity of PaASK1 that produces C (Kicka and Silar 2004). While evidence of altered activity of the kinase cascade in C-carrying strains will be needed, it seems likely that C is a new case of a prion of the same type as [β] (Fig. 6). As previously sug-

gested by Silar (Silar and Daboussi 1999), filamentous fungi may prove to be a rich source of prions. Their syn-cytial structure allows newly arisen prions to spread throughout the colony, and heterokaryon formation provides a rapid route for infection of neighbors.

CONCLUSIONS

The discovery of yeast prions has ended the debate about whether there can be such a thing as a prion, although it has not ended the debate about whether TSEs are such entities. The ease of genetic manipulation of yeast has led to identification of a number of cellular components affecting the generation and propagation of prions, and produced a great deal of insight into prion phenomena, long pioneered in the more difficult animal systems. The new class of nonamyloid prions (now that there are apparently two of them, we are justified in calling it a "class") expands the possibilities for "infectious proteins" to a host of new mechanisms. It is expected that yeast and other fungi will continue to be useful in revealing the range of prion phenomena and their mechanisms.

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