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Yeast Prions and Their Prion-Forming Domain

Minireview

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Over a period of some 25 years the genetic analysis of two completely unrelated mutants of the yeast *Saccharomyces cerevisiae* posed a considerable dilemma to yeast researchers. The two mutants in question, the [*PSI*$^+$] mutant (which modifies the efficiency of nonsense suppression) and the [*URE3*] mutant (which shows an alteration in nitrogen metabolism), failed to behave according to Mendel's laws. Non-Mendelian inheritance in yeast of itself was not a surprising discovery. What was surprising was that no underlying cytoplasmically located nucleic acid determinant could be assigned to either mutant, prompting a wide-ranging debate about the nature of the underlying genetic determinant(s) (reviewed in Cox et al., 1988).

The realization in 1994 that the unusual genetic behavior of both the [*PSI*$^+$] and [*URE3*] mutants could be explained by the prion-like behavior of two previously identified cellular proteins (Wickner, 1994) provided a satisfying solution to the conundrum of how stable phenotypic traits in yeast can be inherited in a non-Mendelian fashion yet have no apparent nucleic acid determinant. By direct analogy with the well-studied behavior of the mammalian prion protein PrP (Prusiner, 1999), the proposed protein-based non-Mendelian inheritance of [*PSI*$^+$] and [*URE3*] would arise as a consequence of a self-perpetuating change, most likely in conformation, of a cellular protein. This in turn would result in the partial (or complete) functional inactivation of the protein as a consequence of its ultimate aggregation leading to an apparent loss-of-function phenotype. The self-propagating prion "seeds," which may not necessarily be the aggregates leading to the [*PSI*$^+$] or [*URE3*] phenotypes, would be inherited both mitotically and meiotically through the cytoplasm. In the presence of these "seeds" the newly synthesized normal cellular form of the protein then undergoes prion conversion leading to aggregate formation and the apparent dominant genetic trait.

The identity and the prion-like behavior of the prion determinants in question have been rigorously established; [*PSI*$^+$] is associated with Sup35p (otherwise known as eRF3), an essential component of the translation termination machinery, while [*URE3*] is associated with Ure2p, a protein that acts as a negative regulator of nitrogen metabolism. That neither protein shows amino acid identity between one another or with mammalian PrP immediately raises the question: What sequence and/or structural features of a prion protein make it behave as such? Recent studies on the two yeast prions have begun to provide some answers.

A detailed overview of the evidence supporting Wickner's proposal that Sup35p and Ure2p are prions can be found in a review by Wickner and Chernoff (1999). The focus of research on yeast prions is now moving to exploit this established and highly tractable model system to elucidate the molecular basis of prion conversion and to extrapolate these findings to mammalian prions. This of course raises the question of whether such extrapolation is valid.

Yeast and Mammalian Prion Conversion: A Common Mechanism?

There is no direct evidence that the mechanism of establishment and transmission of the prion state of Sup35p and Ure2p parallels the mechanism of mammalian PrP infectivity. Unlike Sup35p and Ure2p, PrP is not a cytoplasmic protein, but rather is a GPI-anchored glycoprotein that transits through the secretory pathway ultimately emerging on the cell surface where it becomes attached to the plasma membrane via the GPI anchor (Prusiner, 1999). Formation of the infectious prion form of PrP (PrP^{Sc}) occurs after it subsequently transits through the plasma membrane into a subcellular compartment; the formation of PrP^{Sc} therefore occurs in a very different molecular and ionic environment to that in which the yeast prions reside. This raises the question of whether there can be any commonality of "cofactor" requirements in yeast and mammalian prion conversion.

Although the cytoplasmically located molecular chaperone Hsp104 is essential for the formation and maintenance of the prion form of Sup35p in yeast (Chernoff et al., 1995), Hsp104 is not implicated in PrP^{Sc} formation. No mammalian Hsp104 homolog has been reported nor would it be expected to be found in the subcellular compartment to which PrP is delivered. In fact, no requirement for a protein cofactor in PrP^{Sc} formation has been directly demonstrated. The recent demonstration by Ma and Lindquist (1999) that mouse PrP expressed in the yeast cytoplasm acquires the biochemical characteristics of PrP^{Sc}—increased resistance to proteinase K digestion and detergent insolubility—implies that the glycosylation status of PrP and oxidative-reductive balances may be two important parameters in defining the conformation of PrP. Neither parameter is likely to be important in yeast prion formation in the cytoplasm. Ma and Lindquist (1999) did not report whether mouse PrP^{Sc} formation in yeast was Hsp104-dependent so it remains to be established whether PrP^{Sc} formation does require one or more "molecular chaperones."

Caution must therefore be taken in directly extrapolating from findings with yeast prions. Nevertheless, a series of new papers on yeast prions has now provided important clues to two of the major problems of prion biology: what features of a prion protein make it behave as such, and what prevents efficient interspecies transfer of these novel cytoplasmically located "infectious agents"?

Sup35p and Ure2p Have Prion-Forming Domains that Are Both Modular and Transferable

The replication of yeast prions occurs by a protein-only mechanism. Studies in vivo and in vitro show that the N-terminal regions of both Sup35p and Ure2p are necessary for the formation of their respective prion states, although sequences in the C terminus of Ure2p may also

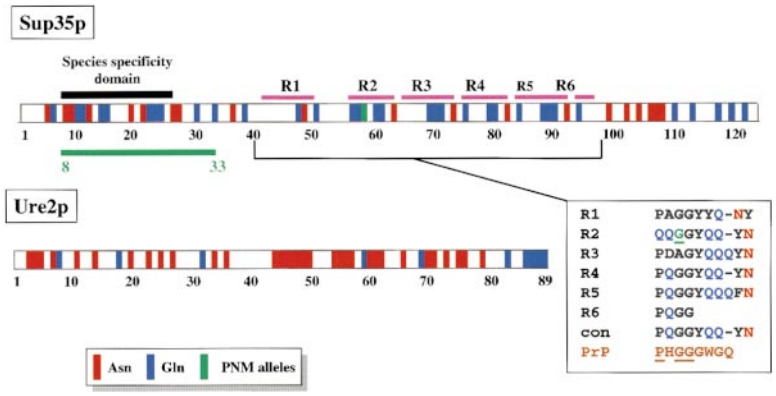

Figure 1. The N-terminally Located Prion-Forming Domains (PrD) of Sup35p and Ure2p of *Saccharomyces cerevisiae* Contain a Number of Important Sequence Features

contribute to the prion behavior of Ure2p (Maddelein and Wickner, 1999). In both proteins the N-terminally located prion-forming domain (PrD) is not essential for protein function per se, nor do the two PrDs share significant amino acid sequence identity. What the two PrDs do share, however, is an atypically high proportion of Gln and Asn residues (>40%, Figure 1 and Table 1), two polar amino acids with uncharged R groups, and an overall low charge content.

The Sup35p- and Ure2p-PrDs are both modular and transferable as fusion of either with the green fluorescent protein (GFP) leads to the formation of discrete GFP foci in cells carrying the prion form of the corresponding protein or PrD (Wickner and Chernoff, 1999). A striking demonstration of the transferability of the Sup35p-PrD has come from the work of Li and Lindquist (2000) who constructed a recombinant version of the rat glucocorticoid receptor fused to the Sup35p-PrD. The resulting fusion protein, when expressed in yeast, was found to exist in distinct stable and heritable functional states, maintaining the epigenetic characteristics of Sup35p. The resulting phenotypic changes (in transcription of a glucocorticoid-regulated promoter driving expression of the *E.coli lacZ* gene) reflected changes in glucocorticoid receptor function, but not Sup35p function. It remains to be established whether yeast prion PrDs can be used to engineer such a novel means of regulating "loss of function" to all types of proteins. The modular and transferable properties of yeast PrDs also has important evolutionary implications.

The Role of the Yeast PrD in Prion Conversion

One of the most tantalizing sequence features of the Sup35p-PrD is the presence of five complete (and one partial) copies of an oligopeptide repeat that bears striking resemblance to the octapeptide repeat (the "octarepeat") of mammalian PrP (Figure 1). Amino acid substitution within one of the Sup35p-PrD repeats, or changes in the numbers of copies of these repeats, can have dramatic effects on both the [*PSI*$^+$] phenotype in vivo and on the seeded aggregation of Sup35p in vitro. For example, a Gly58 to Asp58 substitution within repeat number 2 (R2) results in a dominant "Psi-No-More" (PNM) phenotype (Doel et al., 1994) while deletions encompassing repeats R1–R3 (Ter-Avanesyan et al., 1994) or R2–R5 (Liu and Lindquist, 1999) give a similar PNM phenotype.

An indication that the R2 repeat may play a key role in prion conversion has come from studying a constructed mutant of Sup35p that contains two additional copies of the R2 repeat (R2E2). Liu and Lindquist (1999) found that a [*psi*$^-$] R2E2 mutant spontaneously reverted to [*PSI*$^+$] at a frequency some 5000-fold higher than the wild-type [*psi*$^-$] strain (i.e., a Sup35p-PrD containing seven rather than five oligopeptide repeats has a [*PSI*$^+$] "mutator" phenotype). These authors further showed that the number of copies of the repeat within the Sup35p-PrD also influenced the kinetics of Sup35p fiber formation in vitro in a manner that parallels its in vivo behavior. Thus, both the number of oligopeptide repeats and the amino acid composition of at least one of these repeats in the Sup35p-PrD are important for the self-perpetuating change associated with the prion-like inheritance of the [*PSI*$^+$]determinant.

Mammalian PrP contains five complete copies of the octarepeat PHGGGWGQ at its N terminus, and expansion in the number of octarepeats has been associated

Table 1. Yeast Proteins Showing Prion-like Behavior

			PrD			
Gene	ORF	PrD	%Q+N	GFP Foci	Hsp104	Ag^n
SUP35	YDR172W	aa 1–123	43	Yes	Yes	Yes
URE2	YNL229C	aa 1–89	47	Yes	ND	Yes
RNQ1	YCL028W	aa 153–405	43	Yes	Yes	Yes
NEW1	YPL226W	aa 1–153	26	ND	ND	Yes

The data for *RNQ1* are from Sondheimer and Lindquist (2000) and for *NEW1* from Santoso et al. (2000). Two other proteins, YBR016W (a hypothetical ORF) and Hrp1p (YOL123W) generate GFP foci when fused to GFP (Sondheimer and Lindquist, 2000). A yeast protein has, on average, 9% Gln+Asn residues (Santoso et al., 2000). ND, not determined; PrD, prion-forming domain; Ag^n, ability to form high mol. wt. aggregates in vivo; Hsp104, maintenance of the aggregated state is dependent upon Hsp104.

with certain mammalian prion diseases. Charles Weissmann and his colleagues (Weissmann et al., 1999) have, however, reported that transgenic mice carrying a *PrP* allele lacking the N-terminal region encompassing the octarepeats are still susceptible to scrapie infection, although the resulting neurodegenerative disease requires a longer than usual incubation period and has a much lower level of infectivity. The nonessentiality of the mammalian PrP octarepeats in prion conversion suggests that other more general features of a prion protein may be equally important. This must also be true for prion formation in yeast not least because the Ure2p-PrD has no oligopeptide repeats yet is able to efficiently direct prion formation in vivo and to form amyloid-like fibers in vitro (Taylor et al., 1999; Thual et al., 1999).

The oligopeptide repeats in the Sup35p-PrD are not the sole determinants that confer prion-like behavior to Sup35p. De Pace et al. (1998) have identified *PNM* alleles of Sup35p that contain single amino acid substitutions in a region particularly rich in Gln and Asn residues (amino acids 8–33, Figure 1) which lies outside the region carrying the oligopeptide repeats. Their findings suggest that the high density of these two residues in the PrD is a major contributor to the prion-like behavior of Sup35p. This is further supported by Maddelein and Wickner (1999), who showed that deletion of poly-Asn tracts in the Ure2p-PrD impair [*URE3*] formation. Such localized high densities of Gln residues also increase the efficiency of amyloid formation both in vivo and in vitro. The poly-Gln expansions in the huntingtin protein, associated with Huntington's Disease, is a particularly striking example, although there is no evidence that the huntingtin amyloid is infectious while the infectious PrP^{Sc} is rich in neither Gln or Asn.

Are All Gln+Asn-Rich Yeast Proteins Prions?

Sup35p and Ure2p are not the only Gln+Asn-rich proteins in the yeast proteome. Surveys by Sondheimer and Lindquist (2000) and Santoso et al. (2000) have revealed several proteins with Gln-Asn-rich domains that behave like the Sup35p- and Ure2p-PrDs in vivo (Table 1). The most thorough analysis was undertaken on Rnq1p, which satisfied all of the in vivo criteria used to diagnose the Sup35p-PrD: modular, transferable to GFP, Hsp104-dependent aggregation, and inherited in a non-Mendelian fashion (Sondheimer and Lindquist, 2000). The striking difference between the Sup35p- and Rnq1p-PrDs is that the Rnq1p-PrD is C-terminally located: the region aa 153–405 is able to substitute for the Sup35p-PrD fully recapitulating the phenotypic and epigenetic behavior of [*PSI*$^+$]. It is therefore not essential that a yeast PrD is N-terminally located.

Another striking feature of the Rnq1p "prion" is that it can be present in yeast laboratory strains in one of at least three distinct states of aggregation as defined by ultracentrifugation (Sondheimer and Lindquist, 2000). It remains to be seen whether Rnq1p shows seeded in vitro polymerization into amyloid-like fibers, but it can be considered a third yeast prion in spite of the fact that there appears to be no phenotypic consequence to the loss-of-function prion switch.

Yeast PrDs and the Species Barrier

One aspect of prion biology that is receiving considerable attention is the so-called "species barrier," i.e., the inability of a prion from one species to infect a different species. This cannot be an absolute barrier as the appearance of BSE-derived new variant CJD in humans stands testimony to. Nonetheless, numerous transgenic studies in mice have shown that the *PrP* gene itself governs the species barrier, although the requirement for a homologous PrP:cofactor interaction has not been ruled out as a contributing factor. Recent efforts have therefore been made to probe the molecular basis of the species barrier using yeast prions.

While it is highly improbable that *S. cerevisiae* is ever "infected" by a prion from another yeast species, it is relatively straightforward to experimentally introduce a heterologous yeast prion gene into *S. cerevisiae* to study the consequences. This is exactly what three different groups have now done (Chernoff et al., 2000; Kushnirov et al., 2000; Santoso et al., 2000) and all have come to the same conclusion, namely that *S. cerevisiae* does show a species barrier to Sup35p prion replication. As with the mammalian prion species barrier, continued propagation of the heterologous prion requires the presence of the corresponding heterologous prion gene. In these studies, the PrD domain of Sup35p from a range of evolutionarily diverged yeast species including *Candida albicans* (CaSup35p), *Pichia methanolica* (PmSup35p), and *Kuyveromyces lactis* (KlSup35p) was fused to either the functional C domain of the *S. cerevisiae* Sup35p (ScSup35p) or to GFP. The data obtained unequivocally demonstrate that it is the PrD that confers the high degree of specificity to prion polymerization both in vivo and in vitro.

Particularly enlightening have been the studies with the CaSup35p-PrD fusions (Santoso et al., 2000). The CaSup35p-PrD-ScSup35p fusion, when expressed at low levels under the control of the *ScSUP35* gene promoter, remained soluble in a [*PSI*$^+$] strain demonstrating that the heterologous CaSup35p-PrD could not become incorporated into the endogenous ScSup35p prion aggregate when expressed at low levels. However, when a CaSup35p-PrD-GFP fusion was expressed at high levels, prion-like foci formed in both [*PSI*$^+$] and [*psi*$^-$] cells with similar kinetics. Thus, the heterologous CaSup35p-PrD can direct prion formation when overexpressed, but its polymerization is not influenced by the endogenous prion. Neither are the heterologous CaSup35p-PrD-containing aggregates able to seed the polymerization of soluble ScSup35p in the [*psi*$^-$] strain, i.e., the two prions replicate independently in the same cell. The homologous Rnq1p prion also appears to replicate independently of Sup35p (Sondheimer and Lindquist, 2000).

Using a clever two-plasmid-based assay, Santoso et al. (2000) were also able to demonstrate that the heterologous CaSup35p-PrD can support prion-based inheritance and that a barrier prevents cross-seeding between PrDs from different yeast species. However, Chernoff et al. (2000) have reported that over expression of full-length PmSup35p can induce the prion form of ScSup35p, albeit inefficiently, suggesting that the species barrier can be by-passed with high-level overexpression of the heterologous PrD.

The specificity imparted by the PrD appears to be controlled by the Gln$^+$Asn-rich region at the N terminus of ScSup35p. By replacing the corresponding sequence in the CaSup35p-PrD with amino acids 8–26 from the ScSup35p (see Figure 1), Santoso et al. (2000) showed

that this chimeric PrD now behaves as the wild-type ScSup35p. This result implies that this 19–amino acid sequence is sufficient to confer species specificity to Sup35p polymerization and thus defines this region, which lies outside the oligopeptide repeat–containing region, as a major determinant of the "species barrier" in yeast.

What now remains to be established is the mechanism underlying the species barrier to yeast prion propagation. It might simply be at the level of PrD:PrD interactions during polymerization. We know from studies with the *PNM* alleles of Sup35p that single amino acid changes in the PrD can inhibit prion replication in yeast. These findings might also be explained if this region of the PrD were the binding site for a "helper" molecule. The obvious candidate would be Hsp104, although there is no evidence that a stable Hsp104:Sup35p interaction occurs in vivo. An important *transient* interaction can not be ruled out (Wickner and Chernoff, 1999). Alternatively, it may be part of the binding site for some other cellular protein involved in yeast prion propagation; for example, the cytoskeletal assembly protein Sla1p stably interacts with the ScSup35p-PrD and influences [PSI^+] maintenance (Ballieul et al., 1999).

Conclusion

We have learned much about prion biology through the study of yeast prions and their associated PrDs, but caution must be exercised in extrapolating these findings directly to mammalian prion behavior. It must not be forgotten that mammalian PrP^{Sc} is an infectious agent that can spread from cell to cell while there is no evidence of cell-to-cell transmission of yeast prions other than through cytoplasmic mixing. This apparent lack of infectivity of yeast prions may simply reflect their cytoplasmic location. Nevertheless, the recently published studies have added significantly to our understanding of the molecular basis of yeast prion conversion and maintenance. We now know the key sequence features of a yeast PrD that drive prion conversion, and the new data are consistent with a highly specific PrD–PrD interaction mediating polymerization both in vivo and in vitro. For both Sup35p and Ure2p the in vitro–formed polymers are clearly amyloid-like, but we must now determine whether Sup35p, Ure2p, and other yeast prions are present in cells in the same form. This, together with an understanding of the underlying protein structural framework, will allow us to build mechanistic models for prion conversion in yeast.

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