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Yeast Prions

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Introduction

The term “PRION” stands for “protein only” and means “infectious protein.” In mammals, prions cause lethal neurodegenerative diseases (transmissible spongiform encephalopathies, TSEs) such as Scrapie in sheep, bovine spongiform encephalopathies (BSE; mad cow disease) of cattle and Creutzfeldt-Jakob disease (CJD) in humans. The first prion disease Scrapie, was first described in the early 18th century, its human variety in the 1920s. The nature of the TSE disease-causing agent remained a mystery until the suggestion by Prusiner (45) of the involvement of novel "proteinaceous particles", which he termed prions. It should be noted that the initial idea that protein may be the causative scrapie agent was made in 1967 (2, 20).

Wickner (56) proposed that the genetic behavior of the *Saccharomyces cerevisiae* non-mendelian genetic elements [PSI$^+$] (10) and [URE3] (1) could be explained if they were prions of the Sup35 and Ure2 proteins respectively. Since then much evidence has accumulated to support this proposal. There are currently four confirmed prions in yeast, [PSI$^+$], [URE3], [RNQ$^+$] (12) and [NU$^+$] (42) and genetic evidence suggests there may be many more (12). The fact that prions exist in yeast provides an ideal environment for detailed genetic analysis of factors affecting prion propagation and maintenance.

What is a prion?

When proteins are synthesized on the ribosome, they usually adopt only one final 3-dimensional conformation. Prion proteins somehow adopt an altered conformation from its normal form (by a mechanism that is not understood, but is thought to involve some kind of nucleation event, 46) a state referred to as the prion conformation. In the case of yeast prions, once present the prion form of the protein is capable of recruiting newly synthesized protein into the prion form and thus deplete the cell of functional protein (Fig. 1). We should emphasize here that there is no difference in the genome between cells carrying or lacking a prion, the only difference is the conformational state of the particular protein in question. It is therefore reasonable to state that proteins can indeed behave as genes, in that they can contain inheritable information.

The prion conversion does not occur in the whole protein but appears to occur in a so-called prion domain. Typical prion domains consist of 60 ~ 150 amino acids and the conformational change within the prion domain takes the credit for prion conversion (27, 34-36). The prion conformation is rich in β-sheets and is prone to aggregation in which amyloid fibers are formed (17, 18, 28, 52). Amyloid is a type of protein aggregate, which forms self-sustaining orderly fibers. Amyloid fibers are found associated with many chronic diseases (amyloidosis) such as prion diseases, Alzheimer disease, polyglutamine expansion diseases and type II diabetes (4). The difference between prion proteins and other amyloids is that prions are transmissible and infectious. Thus, yeast prions have been used as a genetic model to study aspects of amyloidosis and prion propagation in mammals.

Prions are not always disease associated

In mammals, prions cause incurable brain diseases. However, the prion killer image doesn’t apply in yeast. Although, [URE3] causes slower growth of yeast, there appears to be no general toxicity of prion aggregates in yeast. To the contrary it has been suggested that the [PSI$^+$] prion may be beneficial to yeast under certain circumstances. The presence of [PSI$^+$] can provide protection to some yeast strains against various stresses such as heat shock (14). It has also been suggested that the possible production of C-terminally extended proteins due to nonsense suppression by [PSI$^+$] could act as an aid to evolution (53). Moreover the [Het-s] prion of the filamentous fungus *Podospora anserina*

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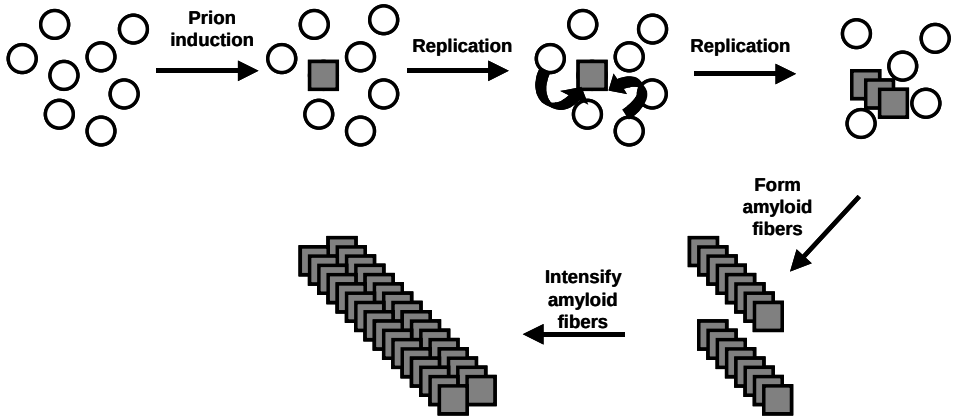

Figure 1. Illustration of the prion propagation.

is clearly carrying out a normal cellular function (9). The possible existence of many eukaryotic prions has been postulated by computer aided methods (37) and in genetic screens (12). It therefore seems highly likely that many different prions will exist naturally and will probably carry out normal cellular functions.

Confirming the protein-only hypothesis?

Yeast prion research has addressed a key feature of the prion theory, that is, prion phenomena occur not by classical genetic elements (virus, plasmid or other nucleic acid replicon) but by infectious proteins. The genetic criteria applied by Wickner (56) to suggest that [PSI^+] and [URE3] are prions is in itself compelling evidence for protein-only inheritance. Firstly, he showed [URE3] appears spontaneously in certain ratio. This experiment was possible because yeast prions are not lethal in yeast and could be cured simply by treating them in extremely low concentrations (1 ~ 5 mM conc.) of guanidine hydrochloride (Gdn-HCl). He found that [URE3] appears spontaneously at a low frequency ($1/10^6$) cells, and when cured could spontaneously appear at a similar frequency [URE3] (Fig. 2). This was termed “reversible curability.” Such behavior would not be expected for a nucleic acid replicon. Secondly, when he overproduced the Ure2 protein the ratio of prion appearance was increased ($1/10^4$). The idea of this experiment is that the frequency with which a prion arises should increase if the cellular content of the normal form is increased, regardless of the mechanism of prion generation. However, a nucleic acid replicon generally does not arise *de novo* regardless of what proteins in the cell overproduced.

Yeast and filamentous fungi have provided the only environments were the actually infective nature of the prion form of a protein have been directly assessed. Infectious behavior has been demonstrated to varying degrees for [PSI^+] (51) and [HET-s] (33).

Yeast prion dependence of the Hsp104 protein and the effects of protein chaperones on prion maintenance also add credibility to the protein-only nature of these non-mendelian genetic elements.

The role of chaperones in prion propagation

A major contribution of yeast prion research has been to identify essential roles for protein chaperones in prion appearance and propagation. Chaperones are proteins that aid and regulate the correct folding of other proteins. Many types of chaperones have been described but only a subset exert effects upon yeast prions. The finding that chaperones can cure or enhance prion propagation may provide a new approach to the treatment of prion/amyloid diseases. Chaperones are usually induced by cellular insults such as heat shock. Indeed, many protein chaperones have been identified in screens to identify heat-shock proteins and thus are termed Hsp's. At least 6 groups of Hsp's have been identified and are grouped together by molecular weight, (Hsp100, 90, 70, 60, 40 and 20). Some Hsp groups have sub-family members, for instance, in yeast the Hsp70 family consists of 14 different proteins classed together by amino acid homology. These Hsp70's function in various compartments of the cell and carry out functions such as preventing aggregation of denatured or incorrectly folded proteins, and aiding protein transport across cell membranes.

The first reports describing the effects of a protein chaperone on prion propagation identified a critical role for the Hsp104 protein (6, 8). Hsp104 is a member of the ClpB family of Hsp100's and is a "dis-aggregase". It cannot prevent unfolded proteins aggregating but it can resolubilize an aggregate once it has formed (19, 24, 25, 43). An interme-

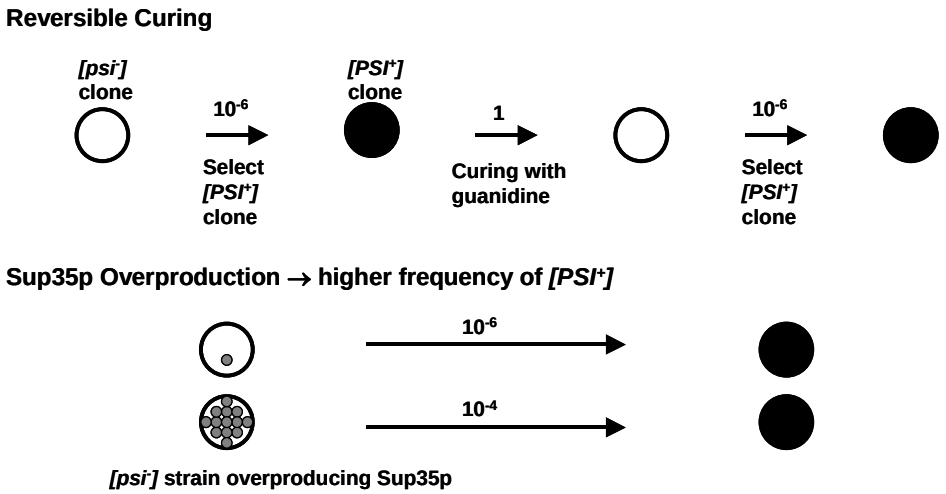

Figure 2. Genetic criteria for a prion.

diate level of Hsp104 is required for efficient maintenance of [PSI$^+$]. Depletion of Hsp104 activity in the cell by either gene deletion or protein inhibition causes the curing of all known yeast prions (12, 13, 38, 50). It is postulated that Hsp104's ability to break up prion aggregates into smaller particles or "seeds" is responsible for efficient transfer of the prion from cell to cell (55). Over-expression of Hsp104 can cure [PSI$^+$] but has no effect on [URE3] or [RNQ$^+$] (12, 13, 38). This finding suggests structural differences exist between these prions. A single amino acid substitution in Hsp104 protein causes dramatic weakening of two prions, [PSI$^+$] and [URE3] (24). Also, alterations in the AAA protease motif of Hsp104 can affect [PSI$^+$] stability (21).

Cytosolic Hsp70's have also been implicated in maintenance of [PSI$^+$]. Yeast has two sub-classes of cytosolic Hsp70's, the Ssa family comprising the Ssa1-4 proteins and also the Ssb family comprising the Ssb1 and 2 proteins (11). The Ssa1 protein has been implicated in [PSI$^+$] propagation both as an antagonist of prion curing by over-expression of Hsp104 (41) and in general prion stability (22, 26). Although the Ssa1 and Ssa2 proteins are 97% identical they can have different effects upon yeast prions. Over-expression of Ssa1 was capable of efficiently curing [URE3] whereas over-expression of Ssa2 was not (49). Deletion of the SSB genes caused an increase in spontaneous appearance of [PSI$^+$] (7) which can be explained by these ribosome-associated protein chaperones having a role in folding of newly synthesized polypeptides (39, 44). Over-expression of Ssb1 can cure certain "strains" of [PSI$^+$] (5).

The functions of Hsp70's in protein folding are usually carried out in conjunction with an Hsp40 co-chaperone partner. This also seems to be the case for Hsp70 effects on prions. Over-expression of the Ydj1 protein can cure weak forms of [PSI$^+$] (30) and can also cure [URE3] (38). The Hsp40 protein Sis1 has been shown to be important for maintenance of [RNQ$^+$] prion (32, 50), and the uncharacterized Apj1 protein, when over-expressed can cure some "strains" of [PSI$^+$] (29). The importance of a direct interaction between Hsp70 and Hsp40 in [PSI$^+$] propagation is demonstrated by suppression of the prion destabilizing SSA1-21 mutation by a second site suppressor that disrupts interaction with Hsp40's (24).

Recently co-chaperones of the tetratricopeptide repeat (TPR) family have been implicated in yeast prion propagation. Over-expression of the Sti1 protein can cure "strains" of [PSI$^+$] (29) while mutations in the C-terminal Hsp70 motif EEVD, required for interaction with TPR proteins are capable of suppressing the SSA1-21 mutation (22).

Amyloid- a "special" type of chaperone substrate?

Because amyloid is formed into a regular ordered structure, this raises the possibility that recognition of amyloid and prion aggregates by protein chaperones may be different from recognition of heat-denatured amorphous aggregates. Genetic evidence for this proposal has arisen from the array of Hsp104 and Hsp70 mutants available in yeast that affect prion propagation. Many mutations exist in Hsp70 (22, 26) and Hsp104 (24) that affect prion propagation but have no effect on cell growth or cellular thermotolerance. This suggests that either amyloid-aggregates are more sensitive to the effects of these chaperones or that they are recognized differently than denatured amorphous aggregates.

Prions can affect the appearance of other prions

The increased appearance of [PSI$^+$] due to overexpression of the Sup35 protein was shown to be dependent on the presence of another suspected prion called [PIN$^+$] (13). A genetic screen revealed the identity of [PIN$^{+]}$ as the [RNQ$^+$] prion and also identified an array of putative yeast prions that could also behave in a [PIN$^+$]-like manner (12). Similar results were observed for strains carrying the less well-characterized [NU$^+$] prion (42).

An antagonistic effect on prion strength and induction has also been reported for the [PSI$^+$] and [URE3] prions (49). The mechanism for how prions can affect each others induction and stability is unknown. Possible models include established prion aggregates providing a template for new prion aggregates to initiate or due to the sequestration of protein chaperones providing an environment favorable for new prion formation.

Mechanism of prion curing by guanidine hydrochloride

Tuite et al (54) showed that an array of chemicals or stress treatments were capable of curing [PSI$^+$] to some degree. One potent and widely used prion-curing agent is guanidine hydrochloride (Gdn-HCl). Medium containing 1-5mM Gdn-HCl efficiently cures yeast of all confirmed prions by a process that inhibits replication of the prion (15). Yeast cells grown in the presence of 1mM Gdn-HCl in liquid culture have been estimated to have intra-cellular Gdn-HCl concentrations around 20mM (23). The mechanism of curing by Gdn-HCl has recently been shown to involve the inhibition of Hsp104, effectively creating conditions mimicking a deletion of Hsp104 (16, 24, 25, 40). The most likely nature of this inhibition is to disrupt the ATPase activity of the Hsp104 protein. Although it is beyond contention that Gdn-HCl is curing prions predominantly through an Hsp104 dependent mechanism, there is some evidence that other proteins or pathways capable of exerting effects on [PSI$^+$] may also be affected by Gdn-HCl (31, 55). The elucidation of this curing mechanism further emphasizes the importance of Hsp104 in prion propagation.

Fungal and plant homologues of Hsp104 have been identified, but as yet no mammalian counterpart has been isolated. It seems reasonable to assume that such a mechanism as protein dis-aggregation will have been conserved through evolution and a mammalian protein analogous to Hsp104 will eventually be identified. Inhibition of such a protein may be a potential treatment for prion and amyloid diseases.

Conclusion and future prospects

In almost a decade since Wickner (56) proposed that prions exist in yeast, researches in this area have gone a long was in proving the protein-only hypothesis. Many cellular factors (mostly protein chaperones) have been identified that affect yeast prion propagation, most of these factors are conserved in mammals. It is through the genetic and biochemical analysis of the existing chaperone mutants and other cellular factors, in relation to their known cellular functions and prion phenotypes, that will allow us to unravel the complex nature of prion propagation in yeast. Due to the high degree of conservation in protein chaperones, findings are likely to be directly relevant to amyloid and prion formation in higher eukaryotes.

Researchers are also exploring some novel uses of prions. Prion domains appear modular and transferable. Proteins that are not natural prions can be made to behave like prions by the addition of the Sup35 prion domain (47). This raises the possibility of protein engineering using prion domains. Most recently prion fibres have been coated with metal particles and been shown to conduct electricity (48). Others have found forming prion aggregates does not cause a total loss of enzymatic function (3). It may therefore be possible to develop immobilized functional proteins using prion fibres that will have industrial applications.

The relatively youthful area of yeast prion research has already produced many significant findings and will continue to do so for many years to come. The relevance of prions in biology is not yet realized and it is through research on yeast prions were most of our understanding of this relevance has been and will continue to be based upon.

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