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The [*PSI*$^+$] prion of yeast: A problem of inheritance

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Abstract

The [*PSI*$^+$] prion of the yeast *Saccharomyces cerevisiae* was first identified by Brian Cox some 40 years ago as a non-Mendelian genetic element that modulated the efficiency of nonsense suppression. Following the suggestion by Reed Wickner in 1994 that such elements might be accounted for by invoking a prion-based model, it was subsequently established that the [*PSI*$^+$] determinant was the prion form of the Sup35p protein. In this article, we review how a combination of classical genetic approaches and modern molecular and biochemical methods has provided conclusive evidence of the prion basis of the [*PSI*$^+$] determinant. In so doing we have tried to provide a historical context, but also describe the results of more recent experiments aimed at elucidating the mechanism by which the [*PSI*$^+$] (and other yeast prions) are efficiently propagated in dividing cells. While understanding of the [*PSI*$^+$] prion and its mode of propagation has, and will continue to have, an impact on mammalian prion biology nevertheless the very existence of a protein-based mechanism that can have a beneficial impact on a cell's fitness provides equally sound justification to fully explore yeast prions.

Keywords: Yeast; Prion; [*PSI*$^+$]; Chaperone

1. Introduction

That fungi, and in particular the yeast *Saccharomyces cerevisiae*, harbour protein-only infectious agents ('prions') was first established by Reed Wickner in 1994 [1]. In the decade preceding this paradigm shifting report, prions had been the subject of considerable debate and controversy not least because their very existence challenged one of the fundamental dogmas of genetics, namely that all inheritance was nucleic acid based. The possibility of a self-replicating protein-based infectious agent was first discussed by Griffiths in 1967 [2] in connection with transmission of scrapie, a fatal neurodegenerative disease of sheep. The subsequent development of the 'protein-only' hypothesis of scrapie infection by Stanley Prusiner [3,4] led to the discovery that PrP^{Sc}, a protein-based infectious agent, was associated with several related fatal neurodegenerative diseases of both man and other animals known as the transmissible spongiform encephalopathies (TSE). PrP^{Sc} is a self-propagating alternative conformer of the cellular protein PrP that can capture and convert the α-helical rich cellular form (PrP^{c}) to the β-sheet rich PrP^{Sc} conformer associated with the TSEs [4, review]. Remarkably, the acquisition of the infectious property associated with PrP^{Sc} is not due to any inherited change in the amino acid sequence of PrP, but rather is a gross conformational change to PrP^{Sc} which in time leads to the formation of the diagnostic high molecular weight, amyloid-like deposits of PrP found in post-mortem samples of brain tissue taken from sufferers.

There is now overwhelming evidence that *S. cerevisiae* encodes at least three different proteins—Sup35p, Ure2p, and Rnq1p—that show similar behaviour to PrP^{Sc} i.e., are able to form transmissible (infectious) and self-perpetuating alternative conformations that cause distinct extrachromosomally inherited phenotypes [5,6; reviews]. Each of these proteins can exist in a stable prion form and cells containing the prion form (i.e., [*PRION*$^+$] cells) have one (or more) phenotypes that distinguish them from the prion-free (i.e., [*prion*$^-$]) cell. In contrast to PrP^{Sc} infection

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however, the phenotype associated with the inheritance of a yeast prion is not necessarily a negative one; indeed in some cases it may be of benefit to the [*PRION*$^+$] cell when exposed to certain environmental conditions [7,8]. As for PrP^{Sc}, the presence of any of the three different yeast prions is not associated with any underlying change in the host cell's DNA sequence and therefore represent bona fide protein-based epigenetic determinants.

The study of prions in yeast and in the unrelated filamentous fungus *Podospora anserina* [9,10] has greatly contributed to the wide acceptance within the scientific community that prions exist and are replicated by a protein-only mechanism. With so many fundamental questions remaining about prions and how they are replicated, the attractions of working with yeast prions as 'models' are several-fold: experiments can be carried out without the need for high biological containment facilities, prion formation in the cell can occur, and be monitored, within a few hours [11], and an overwhelming battery of genetic, biochemical, and cell biological tools can be applied to the study of native prion behaviour in the cell. Perhaps early on the rationale for studying yeast prions was driven by a desire to further our understanding of the propagation and transmission of PrP^{Sc}. While this justification still remains valid, and important findings continue to emerge that have had an impact on mammalian prion biology [see 12], nevertheless the very existence of a protein-based mechanism that can have a beneficial impact on a cell's fitness and perhaps also contribute to its evolution, provides sufficient justification to fully explore fungal prions.

The three prions so far described in *S. cerevisiae* have their impacts on the cell through their modulation of different cellular processes: Sup35p (which gives rise to the [*PSI*$^+$] prion) is an essential component of the translation termination machinery and Ure2p (which gives rise to the [*URE3*] prion) regulates nitrogen catabolic gene expression at the level of transcription. The cellular function of Rnq1p (which gives rise to the [*PIN*$^+$] prion) remains to be established but it may play a role in ascus formation [13]. However, the [*PIN*$^+$] prion is clearly crucial for the de novo formation of the [*PSI*$^+$] and [*URE3*] prions in yeast [14, review]. Yet in spite of their different cellular roles and their minimal amino acid sequence similarity, the three yeast prions nevertheless share a number of properties in common including a non-Mendelian pattern of inheritance indicative of a genetic determinant that is 'extranuclear'.

In this article, we will only focus on one of the yeast prions, [*PSI*$^+$] to illustrate the impact genetic, biochemical, and cell biological methodologies have had in furthering our understanding of a unique epigenetic phenomenon.

2. The discovery of [*PSI*$^+$] as a non-Mendelian genetic element

Brian Cox, then at the University of Oxford, stumbled across the first [*PSI*$^+$] strain in 1964 while studying the genetics of yeast nonsense suppressor mutants [15]. In this study, he was using a strain carrying a suppressible *ade2-1* allele which when expressed gave rise to red Ade$^-$ colonies but when suppressed by a nonsense suppressor mutation, gave rise to white Ade$^+$ colonies. In studies on one particular nonsense suppressor, *SUQ5*, the emergence of a high frequency of red sectors in one cross from spore-derived colonies led to the remarkable discovery that the underlying 'mutation' giving rise to the red sectors, failed to segregate in further crosses. Cox went on to show that the red sectors still contained the *SUQ5* mutation but that such strains had lost a cytoplasmic determinant which he designated Ψ [15] (now more commonly written as [*PSI*]). This led to his proposal that the *SUQ5* mutation required an extranuclear factor in order for it to suppress the *ade2-1* allele sufficiently to generate a prototrophic Ade$^+$ phenotype i.e.,

SUQ5 ade2-1 [*PSI*$^+$] →→ White Ade$^+$ colonies

SUQ5 ade2-1 [*psi*$^-$] →→ Red Ade$^-$ colonies

The *SUQ5* gene (also designated *SUP16)* encodes a mutant form of a UCA-decoding tRNA^{Ser} that has a single base change in the anticodon allowing it to translate the premature UAA codon in the *ade2-1* allele [16]. The presence of [*PSI*$^+$] is not necessary for all known tRNA suppressors to act efficiently; for example mutation in the anticodon of various tRNA^{Tyr} genes (e.g., *SUP4*) generates 'strong' suppressor tRNAs that can suppress the *ade2-1* allele in a [*psi*$^-$] strain; in fact such suppressors are often lethal in combination with [*PSI*$^+$] [17].

The ability of the [*PSI*$^+$] determinant to allow *SUQ5*/*SUP16* to suppress a nonsense mutation is not restricted to the *ade2-1* allele although it is restricted to ochre mutations as nonsense suppressor tRNAs in yeast are codon specific. For example the *his5-2*, *can1-100*, and *lys1-1* ochre alleles are all only suppressed by *SUQ5*/*SUP16* in a [*PSI*$^+$] background. However, subsequent studies have shown that [*PSI*$^+$] can also suppress certain nonsense mutations in the absence of a known suppressor tRNA. Liebman and Sherman [18] originally described [*PSI*$^+$]-mediated suppression of *cyc1-72* an ochre mutant in the iso-1-cytochrome C gene. Subsequently a number of nonsense alleles (including UAG, amber and UGA, opal mutations) have been reported as suppressible by [*PSI*$^+$]. This has led to the widespread use of one particular nonsense allele for studies involving [*PSI*$^+$], namely the opal mutant allele *ade1-14* which contains a premature UGA codon in the *ADE1* gene in place of a UGG codon at position 244 (Fig. 1) [19].

In the 30 years that followed Cox's original description of the [*PSI*$^+$] factor there was considerable debate about the nature of the underlying genetic determinant [20]. All known cytoplasmic nucleic acid species were ruled out as potential determinants i.e., mitochondrial DNA, dsRNA virus-like genomes, and 2 μm plasmid DNA [21] which left little option other than to invoke rather radical models to account for its genetic properties. One of these models, put forward in 1988, was that [*PSI*$^+$] might represent an