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Biochemical and genetic methods for characterization of [*PIN*$^+$] prions in yeast

Susan W. Liebman [a], Sviatoslav N. Bagriantsev [a], Irina L. Derkatch [b,*]

[a] *Department of Biological Sciences, Laboratory for Molecular Biology, University of Illinois, Chicago, IL 60607, USA*
[b] *Department of Microbiology, New York University School of Medicine, 550 First Avenue, MSB 217, New York, NY 10016, USA*

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Abstract

The glutamine- and asparagine-rich Rnq1p protein in *Saccharomyces cerevisiae* can exist in the cell as a soluble monomer or in one of several aggregated, infectious, prion forms called [*PIN*$^+$]. Interest in [*PIN*$^+$] is heightened by its ability to promote the conversion of other proteins into a prion or an aggregated amyloid state. However, little is known about the function of Rnq1p, which makes it difficult to assay the phenotypes associated with its normal vs. prion forms. In this chapter, we describe methods used to detect [*PIN*$^+$] and distinguish between different variations of the prion. Genetic methods are based on the ability of the [*PIN*$^+$] prion to facilitate the appearance of another yeast prion, [*PSI*$^+$], which has an easily detectable phenotype. Biochemical methods exploit the fact that the [*PIN*$^+$] prion exists in the yeast cytosol in the form of large aggregates, composed of SDS-stable subparticles. Sucrose gradient centrifugation, agarose SDS electrophoresis and GFP fusions are used to distinguish between aggregates and subparticles from different [*PIN*$^+$] variants.

Keywords: [*PSI*$^+$]; [*PIN*$^+$]; Prion; Yeast; Sup35p; Rnq1p; Amyloid; Cytoduction; Nonsense suppression

1. Introduction

Several yeast proteins have been shown to be able to form infectious aggregates, called prions (reviewed in [1]). These aggregates attract non-prion forms of the protein to join them. When the aggregates get large they are fragmented into smaller pieces, facilitating growth at their ends and transmission to daughter cells. This explains how prions propagate after the first prion aggregate or "seed" appears (reviewed in [2]). The discovery of the [*PIN*$^+$] prion shed some light on one of the mechanisms of the formation of that first "seed," and showed that different prion proteins present in the same cell can affect each other's appearance.

The story of [*PIN*$^+$] began in 1997 [3] when we cured cells of [*PSI*$^+$], the prion form of Sup35p, by growing them on medium containing guanidine hydrochloride (GuHCl)[1] and then attempted to re-induce [*PSI*$^+$] in several [*psi*$^-$] clones. We expected that [*PSI*$^+$] would re-appear in all of the clones. Indeed, in agreement with the predictions of the prion model, various [*psi*$^-$] strains, as well as [*PSI*$^+$] strains that were cured of [*PSI*$^+$] by a variety of agents, had previously been shown to become [*PSI*$^+$] at a low spontaneous rate [4,5]. Although the spontaneous reappearance of [*PSI*$^+$] was originally not detected in GuHCl-cured [*psi*$^-$] clones [4,6], [*PSI*$^+$] did reappear in such cells following the induction of [*PSI*$^+$] by overproducing the [*PSI*$^+$]-forming protein, Sup35p [7]. The overproduction of the complete Sup35p, or just the region of Sup35p necessary for it to form a prion (the prion domain) [7,8], was shown to signifi-

* Corresponding author. Fax: +1 212 263 8276.
E-mail address: irina.derkatch@med.nyu.edu (I.L. Derkatch).

[1] *Abbreviations used:* GuHCl, guanidine hydrochloride; GR, glucocorticoid receptor; HA, human influenza hemagglutinin; GFP, green fluorescent protein; RFP, red fluorescent protein; SDS, sodium dodecylsulphate; PMSF, phenylmethylsulphonyl fluoride; PVDF, polyvinylidenefluoride; RT, room temperature; Cyh, cycloheximide.

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cantly enhance the rate of appearance of [*PSI*$^+$], presumably because the high concentration of Sup35p protein molecules increases the chance of creating the first prion seed [9]. Thus, it was a great surprise to us to find that while overproduction of Sup35p could efficiently induce [*PSI*$^+$] in some of our GuHCl-cured [*psi*$^-$] derivatives, other derivatives could not be induced to become [*PSI*$^+$]. We called the derivatives that could or could not be induced to [*PSI*$^+$], respectively, Pin$^+$ and Pin$^-$, for [*PSI*$^+$]-inducible [3].

We investigated the difference between Pin$^+$ and Pin$^-$ cells by crossing them together and examining meiotic progeny. The diploids and meiotic progeny were all Pin$^+$. This type of non-Mendelian inheritance was reminiscent of the inheritance pattern of the [*PSI*$^+$] prion [10]. Also, like [*PSI*$^+$] [6,11], cells were cured of Pin$^+$ by growth in GuHCl or by deleting the gene encoding the Hsp104 chaperone [3]. Furthermore, this curing was reversible: Pin$^+$ reappeared at a low rate in cured cultures [12]. Reversible curing was a key characteristic proposed to distinguish non-Mendelian traits encoded by cytoplasmic organelles, plasmids and viruses, whose loss would not be reversible, from prions, which should reappear as long as the prion protein was still expressed in the cell [13]. Thus, we concluded that the Pin$^+$ phenotype we were studying was caused by a prion, and the prion nomenclature of [*PIN*$^+$] was adopted.

Using a candidate approach we showed that [*PIN*$^+$] was the prion form of Rnq1p [14]. Rnq1p stands for "rich in glutamine (Q) and asparagine (N)." This protein with unknown function was originally chosen as a prospective prion on the basis of its sequence similarity to the glutamine/asparagine-rich Sup35p prion domain, and had already been shown to be a prion [15]. Our work made it apparent that the [*PIN*$^+$] prion we were working with was identical to the [*RNQ1*$^+$] prion.

Interestingly, we found that there are different heritable variants of the [*PIN*$^+$] prion [16]. Similar prion variants have been described for PrP^{Sc}, [*PSI*$^+$] and [*URE3*] [7,17,18]. [*PIN*$^+$] variants differ in (i) the efficiency with which they promote the appearance of other prions ([*PSI*$^+$] and [*URE3*]); (ii) the level of growth inhibition caused by a high level of Sup35p overproduction; (iii) level of soluble vs. aggregated Rnq1p; (iv) the size and stability of the subparticles that the Rnq1p prion aggregates break into when solubilized with detergent [16,19].

The nomenclature of [*PIN*$^+$] variants is based on two characteristics. The first is the relative strength, with which the [*PIN*$^+$] variant promotes the de novo appearance of [*PSI*$^+$], upon overexpression of Sup35p (varies from a *low* to a very *high* frequency) [16]. The second is the fluorescence pattern of Rnq1-GFP in the cytosol: "single dot" (s.d.) or "multiple dot" (m.d.) [20]. The list of our [*PIN*$^+$] variants is given in Table 1. Different [*PIN*$^+$] variants do not co-exist in one cell [19,20]. Rather, when the cytosols of two cells bearing different [*PIN*$^+$] variants are mixed by mating, the progeny of the resulting zygote bears only one [*PIN*$^+$] variant, referred to as the "dominant" variant. "Dominance" of a [*PIN*$^+$] variant inversely correlates with the amount of non-prionized soluble Rnq1p found in the cell, i.e. in crosses between two [*PIN*$^+$] variants the one that has less soluble Rnq1p has always been found to be "dominant" [16]. A possible explanation of this phenomenon is that each [*PIN*$^+$] variant is characterized by a certain efficacy with which it recruits Rnq1p molecules into prion aggregates. When two [*PIN*$^+$] variants are present in the cytosol, the more efficient "recruiter" will take up most of the soluble Rnq1p available for aggregation, impeding the growth of the poorly recruiting variant and eventually leading to the dilution and disappearance of the less efficient variant as the cell divides.

Table 1
Nomenclature and "dominance" hierarchy of [*PIN*$^+$] variants

Rnq1-GFP fluorescence pattern	Relative [*PSI*$^+$] induction strength
m.d.	*High*
s.d.	*High*
s.d.	*Medium*
s.d.	*Low*
s.d.	*Very high*

A [*PIN*$^+$] variant is "dominant" over all variants listed below it in this table. m.d., multiple dot; s.d., single dot.

It is important to remember that theoretically the original phenotype of facilitating [*PSI*$^+$] induction may not be associated with all variants of the Rnq1p prion. Furthermore, other prions, e.g. [*URE3*], and overproduction of certain prion-like Q/N-rich proteins can facilitate the induction of [*PSI*$^+$] in the absence of [*pin*$^-$] [14,21]. These cells are said to have a Pin$^+$ phenotype, although they remain [*pin*$^-$] because the Rnq1p protein is not in the prion form. Methods described in Section 1 specifically score cells for the presence of the [*PIN*$^+$] prion, whereas methods described in Section 2 score cells for the Pin$^+$ phenotype generally associated with the [*PIN*$^+$] prion.

2. Methods

2.1. Scoring for [PIN$^+$] as a prion conformation of Rnq1p in any genetic background

A variety of methods have been used to distinguish between prionized and non-prionized forms of Rnq1p. The basic rationale for all of them is that prionized Rnq1p forms large aggregates, while its non-prionized counterpart is monomeric.

The methods described herein are aimed at distinguishing between [*pin*$^-$] and [*PIN*$^+$] cells as well as between different [*PIN*$^+$] variants. Indeed, all [*PIN*$^+$] variants that we tested exist in the cytosol in the form of large aggregates consisting of SDS-stable subparticles (probably, homo polymers of Rnq1p) and thus can be distinguished from the Rnq1p monomers in [*pin*$^-$] cells by centrifugation, electrophoresis, and fluorescent microscopy. On the other hand, the prion aggregates and subparticles of Rnq1p from different [*PIN*$^+$] variants have a variant-specific size distribution and thermal stability [19].