RESEARCH COMMUNICATION

Heritable activity: a prion that propagates by covalent autoactivation

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Keywords

Known prions (infectious proteins) are self-propagating amyloids or conformationally altered proteins, but in theory an enzyme necessary for its own activation could also be a prion (or a gene composed of protein). We show that yeast protease B is such a prion, called [β]. [β] is infectious, reversibly curable, and its de novo generation is induced by overexpression of the pro-protease. Present in normal cells but masked by the functionally redundant protease A, [β] is advantageous during starvation and necessary for sporulation. We propose that other enzymes whose active, modified, form is necessary for their maturation might also be prions.

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The word “prion” [Prusiner 1982] has come to mean “infectious protein.” It is believed that the transmissible spongiform encephalopathies of mammals are caused by infectious protein. “It is believed that the transmissible word “prion” [Prusiner 1982] has come to mean “infectious protein.” It is believed that the transmissible spongiform encephalopathies of mammals are caused by infectious protein. “It is believed that the transmissible word “prion” [Prusiner 1982] has come to mean “infectious protein.” It is believed that the transmissible spongiform encephalopathies of mammals are caused by infectious protein. “It is believed that the transmissible

Figure 1. Activation diagram of three vacuolar hydrolases. Arrows point from an activating enzyme toward the enzyme[s] it activates. Orange arrows indicate autoactivation in cis (intramolecular) or in trans (mature form activating a separate molecule of the pro-form) as indicated.

Results and Discussion

Because phenotypic lag is observed during growth on dextrose medium (YPAD), which represses transcription of PRB1 [Moehle and Jones 1990], we tested whether the lag might be prolonged on glycerol medium (YPG). We sporulated a diploid heterozygous for a pep4 deletion (pep4Δ/PEP4), and germinated the meiotic products on YPG. We found that CpY, frequently used to follow pep4 mutants in crosses [Jones 2002], remained active indefinitely. CpY activity disappeared rapidly when pep4Δ cells were transferred to YPAD and was not restored by a return to YPG [Fig. 2A–C]. Perhaps the phenotypic lag we observe is shorter than that reported for primary pep4Δ segregants because in our experiments PrA need not be diluted out before PrB dilution begins. As expected [Jones 2002], CpY activity is a reflection of PrB activity in pep4 strains [Fig. 2D]. CpY activity was fully dependent on PRB1, because 12 of 12 pep4Δ prb1Δ meiotic segregants of a diploid heterozygous for both genes were CpY− even on YPG. These data suggest that continuous expression of PrB suppresses the loss of down-

like yeast vacuole, catalyzed by Protease A (PrA) and PrB itself in that order [Nebes and Jones 1991]. The PrB-mediated step would seem to depend on the PrA-catalyzed cleavage, because deletion of PEP4 (encoding pro-PrA) leads to accumulation of the immature form and loss of PrB activity [Jones et al. 1982]. This disappearance of mature PrB is delayed after loss of PEP4: The activity of PrB, and other vacuolar hydrolases activated by either PrA or PrB such as carboxypeptidase Y (CpY), may be detectable for 20 generations or more [a cytoplasmic dilution of 106], an effect referred to as “phenotypic lag” (Zubenko et al. 1982). Phenotypic lag is thought to reflect sequential dilution, during growth, of PEP4 mRNA, PrA, and PrB, combined with an imperfect capacity of PrB to bypass PrA in processing pro-PrB [Jones 1991]. The final steps in the activation of these proteases are summarized in Figure 1.

[Keywords: Prion; protease; vacuole; epigenetics; yeast]

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stream hydrolase activities usually observed in pep4 mutants.

Phenotypic lag has also been observed in pep4 mutants following transfer of cytoplasm from wild-type [PEP4] cells via cytoplasmic mixing (cytoduction), similar to the mode of yeast virus and prion transmission [Zubenko et al. 1982]. Using this method, we showed that our stable PrB+ state can be “infectious” when both donor and recipient are pep4Δ mutants. Many cytoductants were CpY+ [Table 1], indicating that the PrB+ state is inherited as a cytoplasmic factor we call [β] [cells without it are called [β-0]] rather than as a nuclear gene. Extensive studies have documented the mechanisms by which vacuolar contents and membrane structures are transferred from mother to daughter cells [Wickner and Haas 2000]. Our results suggest that horizontal transmission of vacuolar material [via cytoduction] is less efficient than vertical [mitotic] transmission.

Table 1. Cytoplasmic transfer experiments

| Experiment A | Experiment B | Experiment C |
|--------------|--------------|--------------|
| PrB− → PrB+ | PrB+ → PrB− | PrB+ → PrB+ |
| PrB− | 0 | 4 | 10 |
| Total cytoductants | 20 | 17 | 18 |

Cytoduction was carried out for 8 h on raffinose. Experiment A is the negative control; B and C are repetitions of the same experiment with different recipient strains. The donors for A, B, and C are 4868-5A PrB−, 4868-5A PrB+, and 4868-10C PrB+, respectively. The recipients were 4870-3C PrB− pβ for A and B, and 4876-7B PrB− pβ for C. Donors and recipients are all pep4Δ. The PrB phenotype is inferred from the CpY phenotype.

To be certain that “curing” of [β] by growth on YPAD and its stability on YPG were caused by the effects on transcription of PRB1 rather than some other metabolic effect, we placed PRB1 on a plasmid under control of the strong, constitutive ADH1 promoter into the pep4Δ prb1Δ mutant strain 4868-2C. The transformants, which lacked CpY activity, became CpY+ following cytoduction from [β] cells, and remained so even when grown on dextrose [data not shown]. Cells that lost the plasmid during a period of nonselective growth also lost CpY activity. Cells harboring a control plasmid that expresses prb1-519, a protease-inactive missense mutant [Nebes and Jones 1991], were unable to propagate [β]. These data establish that it is PrB activity, rather than culture conditions or the PRB1 transcript, that propagates [β].

Known yeast prions can, rarely, arise spontaneously from a strain previously cured of the prion. This frequency is increased by overproduction of the normal cellular protein [Wickner 1994], pep4Δ strain 4868-10C, cured of [β] by extended growth on YPAD, was plated for single colonies on YPG, and ~1 colony in 105 was found to be CpY+. Furthermore, although 4868-2C transformed with the ADH1-promoter-driven PRB1 is initially CpY+, CpY-isolates appear during culture at a frequency >1 in 102. Thus, PrA function is dispensable for the initial formation of [β], as well as for its propagation, and overexpression of PRB1 increases the frequency of [β] appearance.

When dextrose is nearly exhausted, transcription of PRB1 rises dramatically, and, after a delay, so does PrB activity [Naik et al. 1997]. This rise in protease activity is evidently important because pep4 and prb1 mutants rapidly lose viability during starvation [Teichert et al. 1989]. However, PrB and other vacuolar hydrolases were presumably inactive in pep4Δ mutants, leaving open the question of whether PrA is directly required in starvation, or important only for activating the other hydrolases. We compared the ability to survive starvation by pep4Δ prb1Δ [β] and [β-0] cells harboring the PRB1 overexpression plasmid with pep4Δ prb1Δ cells overexpressing the protease-inactive prb1-519 mutant protein and with wild-type cells carrying a control vector [Fig. 3]. Each strain was grown on SC-uracil to select for the plasmid. Cells containing active PrB were far more resistant to starvation than those in other cultures, indicating that [β] confers a survival advantage on pep4Δ cells. That pep4 [β] cells do not survive as well as wild-type suggests that PrA does have a role in survival of starvation unrelated to its activation of other vacuolar hydrolases. However, it may be that PrB activity remains lower in the [β] cells than in wild-type cells, particularly because transcription from PADH1 diminishes significantly during diauxic growth [Denis et al. 1983].

Homozygous pep4 diploids are unable to sporulate [Zubenko and Jones 1981]. We found, however, that pep4Δ [β] diploids sporulated as well as wild-type cells. We were unable to detect tetrads in [β-0] or the prb1-
Prion propagation by autoactivation

519-expressing pep4Δ homozygotes (Fig. 4). Asci formed in the [β] culture appeared normal, and the germination efficiency was high (67 of 68 dissected spores). None of the germinated spores had active Cpy. This is surprising because the phenotypic lag observed in pep4Δ spores of heterozygotes suggests transmission of vacuolar material from the parental diploid (Zubenko et al. 1982). However, other groups find that vacuoles appear to be excluded during spore formation (Roeder and Shaw 1996). Our findings suggest that spores create new vacuoles de novo, using PEP4 mRNA, pro-PrA, or PrA present prior to the completion of the prospore wall. Alternatively, spores may form with protovacuoles lacking sufficient PrB activity to perpetuate [β] without PrA. However, given that 25% of protein in normal cells is degraded by PrB in meiosis (Zubenko and Jones 1981), it is unlikely that the effect is a consequence of a deficiency of PrB. Previous studies showed that cells carrying certain missense alleles of pep4 lacking measurable PrA activity not only display increased phenotypic lag, but also do not exhibit other phenotypes associated with pep4Δ [Rupp and Wolf 1995], provided the cells remain PrB+. However, in those studies survival of starvation and ability to sporulate still required the presence of a PEP4-derived gene product.

[β] satisfies two of the three criteria proposed to distinguish yeast prions from other infectious particles (Wickner 1994); (1) [β] is reversibly curable—cells that become [β-0] through growth on media that repress PRB1 transcription may rarely redevelop [β] during subsequent culture on YPG; (2) overexpression of PRB1 increases the frequency with which [β] emerges. The third criterion, that the phenotype of the prion should be similar to the phenotype of a loss-of-function mutation in the gene, applies only to prions that are self-propagating inactive forms of a protein, such as [URE3] and [PSI]; it does not apply to [β] because the prion form of PrB is itself the active product of PRB1. Notably, the [Het-s], [PIN], and PrPSc (Bueler et al. 1992; Coustou et al. 1997; Derkatch et al. 2001) phenotypes are also not similar to those of mutations in their respective genes. Taken with the fact that [β] depends on the continuous supply of normal pro-PrB, these results constitute a proof of principle that prion propagation can be mediated by the autocatalytic covalent modification of an enzyme.

Our results are somewhat analogous to the “cortical inheritance” phenomenon described by Beisson and Sonneborn (1965). The pattern of cilia on the surface of Paramecium altered by accident or surgical manipulation is propagated to mitotic offspring (although it is not infectious). However, instead of a simple protein bearing the information, cortical inheritance involves a complex self-propagating structure including membrane and protein components.

Theoretically a kinase, phosphatase, acetylase, methylase, or isoprenylase could be a prion, provided that its activity depends on self-modification in trans and that there is a mechanism by which it can be transmitted from individual to individual. It is also not inconceivable that an enzyme activated in one germ layer of an embryo could influence the development of neighboring tissues later in development by transmitting the mature protein to them. In any case, maturation events need not be activating per se; they might increase substrate specificity for the unmodified enzyme, reducing capacity to modify other substrates. A kinase has been reported to have the

**Figure 3.** Survival of strains during culture on dextrose minimal plates, (w.t.) PEP4& PRB1+ [4771-1A] with pH972 [PRB1 vector]; [β] pep4Δ prb1Δ [4868-2C] with P_{ADH1} & PRB1 [β-0]; pep4Δ prb1Δ [4868-2C] with P_{ADH1} & PRB1 [β-0]; pep4Δ prb1Δ [4868-2C] with P_{ADH1} & PRB1 [β-0]. Error bars encompass quartiles above and below the median.

**Figure 4.** Diploids of the indicated genotypes were grown on sporulation media for 5 d, digested with glusulase, and visualized by differential interference contrast microscopy; (wild type) PEP4& PRB1+; (pep4Δ/pep4Δ P_{ADH1} & PRB1 [β]) TRY256; (pep4Δ/pep4Δ P_{ADH1} & PRB1 [β-0]) TRY257; (pep4Δ/pep4Δ P_{ADH1} & prb1-519) TRY258. Arrows indicate tetrads.
capacity to autoactivate in trans [Wang et al. 1997], rais-
ing the possibility that it could behave as a prion. We note that certain viral transcription factors stimulate ex-
pression from their own promoters (Flemington and Speck 1990, Deng et al. 2000; Ragozy and Miller 2001; 
Wang and Gao 2003), a phenomenon that has also been suggested to allow protein infectivity [Griffith 1967].

Materials and methods

Strains and plasmids

The yeast strains used in this study are: 4771-1A MATa met15Δ his3- 
Δ1 ura3-Δ0 leu2-Δ0, 4868-2C MATα met15Δ his3 ura3 prb1Δ; KanMX 
pep4::His3 K+; 5′x47 MATaα his1::trpl1 ura3 K+; 4870-5A MATα 
his3 α ura3 pep4::His3 K+ [pBl]; 4870-3C MATα kar1-1 met15Δ leu2 his3 
pep4::His3 α K− [pBl]; 4868-10C MATa his3 leu2 ura3 pep4::His3 K+ 
[pBl]; 4876-7B MATα kar1-1 met15Δ leu2 his3 pep4::His3 K− [pBl]; 
TRY250 MATα kar1-1 ade5 met15Δ leu2 his3 pep4::His3 K− [pBl]; 
TRY251 same as TRY250 except [pBl]; TRY256 MATα [α met15Δ his3 leu3 
ura3 ura3Δ leu2Δ prb1::KanMX::pep4::His3 pep4::his3 K+ pCR1 [pBl]; 
TRY257 same as TRY256 except [pBl], TRY258 same as TRY257 except 
pCR1, TRY260 MATα kar1-1 met15Δ leu2 his3 pep4::His3 K− [pBl]; TRY261 same as TRY260 except [pBl].

Plasmid pCR1 was made by amplifying wild-type PRB1 using primers TATTCTAC GTA CACCAAGGACACAACTCACAGGCTG 
ATTCAGAATGGAAAAATCCTATTACAC and ACCCTTCG 
CGGAGAAGCCGTTGCTGCGGAGCAGATGTTCTT 
AAATGAAAGGAATTATAATTTG. The PCR product was cotrans- 
fomed into strain 4868-2C along with HIS3 PRB1 (Saccharomyces cerevisiae Ura3 α, Prb1−) (caughey 2000; F. Begueret, J. V. 2000. Transmissible spongiform encephalopathies, amyloi- 
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Prion propagation by autoactivation

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Heritable activity: a prion that propagates by covalent autoactivation

B. Tibor Roberts and Reed B. Wickner

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