Supplemental Data

A non Q/N-rich prion domain of a foreign prion, [Het-s], can propagate as a prion in yeast

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Supplementary Experimental Procedures

Strains and Media

Yeast strains used were: L1749, a 74-D694 (*MATa ade1-14 leu2-3,112 his3-Δ200 trp1-289 ura3-52* [psi^{-}][PIN^{+}]) (Chernoff et al., 1995; Derkatch et al., 2000); L1751, a guanidine hydrochloride cured [pin^{-}] version of L1749 (Derkatch et al., 2001); L1802, an *HSP104* disruption of 74-D694 (Chernoff et al., 1995); L2598, a *kar*1 mutant, [rho^{-}] and guanidine hydrochloride cured [pin^{-}] version of L2176 (*MATα ade1-14 leu2-3,112 his3-Δ200 trp1-289 ura3-52 can*1^{R} *cyh2*^{R} [psi^{-}][PIN^{+}][RHO^{+}]) (Bradley et al., 2002). L2736, an *HSP104* disruption strain was created by transforming L2598 with a *Cla*I and *Hind*III fragment of plasmid p811 (also known as pYSU2) that has a *URA3* disruption of *HSP104* (Chernoff et al., 1995). The disruption was confirmed by PCR.

Standard media and growth conditions were used (Sherman, 1986). Unless otherwise specified, yeast were grown on organic complete medium, YPD. Transformants were grown on synthetic dextrose (SD) or raffinose media (SR) media lacking tryptophan (-Trp). To induce the expression of HET-s constructs, yeast were grown in SR–Trp containing 2% galactose (SR-Trp+2% gal) for high expression and SR–Trp containing 0.05% galactose (SR-Trp+0.05% gal) for low expression. The

cytoductants were selected on (-Trp) glycerol media containing 0.05% galactose and 3mg/l cyclohexamide (SG-Trp+0.05% gal+Cyh)

Plasmids

pHET-s(PrD)-GFP with the *TRP1* selectable marker is a centromeric plasmid encoding a fusion of GFP added to the C-terminus of the HET-s PrD (amino acids 218-289). The T266P mutant of HET-s (pT266P-GFP) was created by site-directed mutagenesis of pHET-s(PrD)-GFP using the QuikChange® Site-Directed Mutagenesis Kit from Stratagene and was confirmed by sequencing. The primers used for the mutagenesis were: 5' CTTCCCCACAACCGGCTCTACCGAGTTG 3' and 5' AACTCGGTAGAGCCGGTTGTGGGGAAGG 3'. A plasmid carrying a dominant negative allele of Hsp104 having Lys-to-Thr double substitutions at residues 218 and 620 (pKT218,620) was a gift from Susan Lindquist (Chernoff et al., 1995).

Preparation of Yeast Cell lysates for SDD-AGE:

Crude cell extracts were prepared using glass beads (Biospec, 0.5 mm) in 750 µl of lysis buffer containing 50mM Tris/HCl, pH7.5, 50 mM KCl, 10 mM $MgCl_2$ and 5% (w/v) glycerol supplemented with a protease inhibitor cocktail (P8215, Sigma) and 5 mM PMSF. Cells were lysed by vortexing (Vortex-Genie 2) at high speed, 15 times for 30 sec with cooling on ice for 30 sec between each vortexing. Crude lysates were precleared by centrifuging them two times at 600g for 2 min to remove unlysed cells. The supernatant (~30-80 µg of total protein) was treated with detergent (0.5 to 2% SDS or 2% and 3%

Sarkosyl) in sample buffer (25 mM Tris, 200 mM glycine, 5% glycerol and 0.025% bromophenol blue) for 10 min at room temperature unless otherwise mentioned.

Protein transfection of [Het-s*] mycelium with crude yeast extracts

Yeast crude extracts were prepared by disrupting a cell pellet of $5X10^8$ cells with glass beads (Sigma) in 500 µl in 10 mM Tris HCl pH 7.5 using a Fast Prep FP120 cell disruptor (run time 30 sec, speed 6 m/s). 20 µl of the crude yeast extract were added to three pieces of agar (about 3 mm^3) embedded with [Het-s*] mycelium in a 2 ml screw cap tube containing 500 µl of STC buffer (0.8M sorbitol 50mM $CaCl_2$ Tris HCl pH7.5 100mM). The mycelium were then fragmented in the disruptor (run time 30 sec, speed 6 m/s) and 80 µl aliquots of the suspension of mycelial fragments were plated onto 12 well plates containing SUHMEA regeneration medium (Bergès and Barreau, 1989) and incubated for 4-5 days at 26°C. Each regenerating mycelium was then assayed for the [Het-s] phenotype in barrage tests as previously described (Benkemoun et al., 2006).

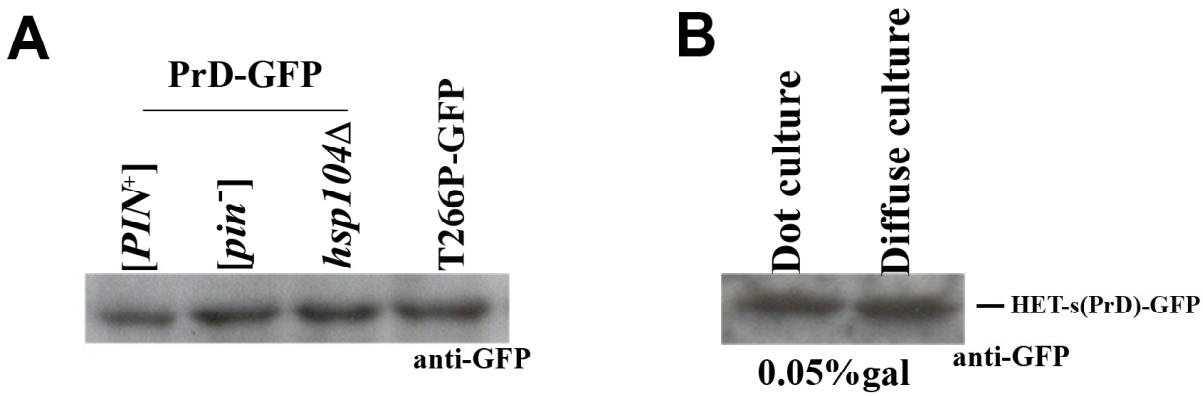

Figure S1. Expression levels of HET-s(PrD)-GFP fusion protein

A. Precleared yeast lysates from the indicated yeast strains expressing PrD-GFP and a [*PIN*$^+$] strain expressing T266P-GFP were normalized for total protein, treated with 2% SDS at 95°C for 10 min, resolved on a 10% polyacrylamide gel and probed with anti-GFP antibody.

B. Yeast lysates from $[Het\text{-}s]_y$ and $[het\text{-}s]_y$ cultures grown on 0.05% gal were normalized for total protein, treated with 2% SDS at 95°C for 10 min, resolved on a 10% polyacrylamide gel and probed with anti-GFP antibody. About 80% of the cells in the $[Het\text{-}s]_y$ culture (previously exposed to 2% gal) contained dots vs. the $[het\text{-}s]_y$ culture (grown only on 0.05% gal) which had essentially no cells with dots.

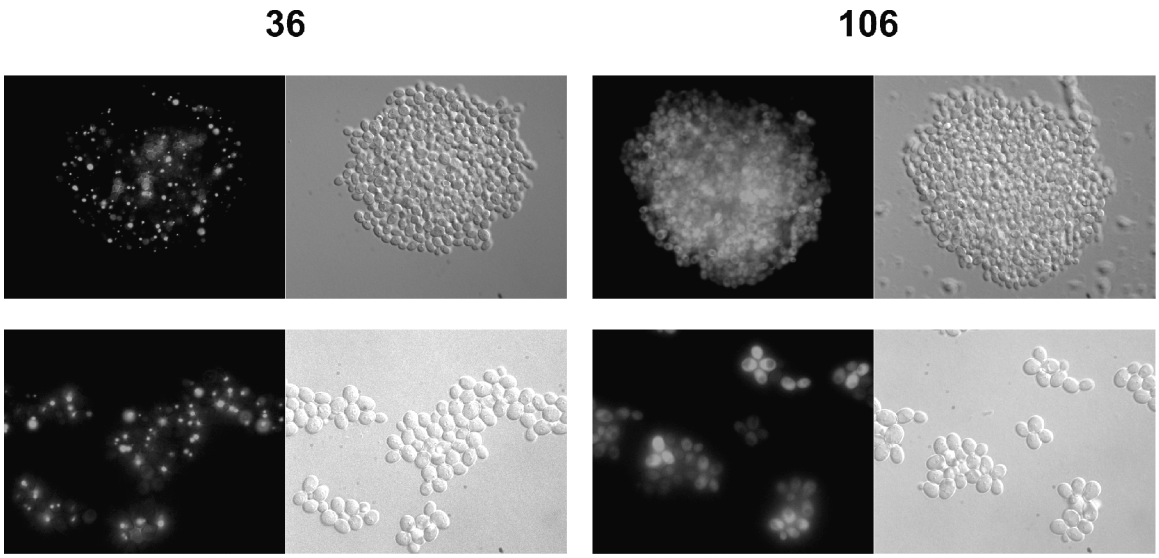

Figure S2. Isolation and propagation of colonies with dots

Cells of [*HET-s*$^{+}$]$_{y}$ clone 36 colonies containing dots and cells of [*het-s*$^{-}$]$_{y}$ clone 106 with diffuse fluorescence were plated on solid 0.05% gal medium. The [*HET-s*$^{+}$]$_{y}$ clone produced colonies composed of cells with dots, while the [*het-s*$^{-}$]$_{y}$ clone produced colonies composed of cells with diffuse fluorescence. The upper panel shows entire colonies, the lower panel partially dispersed cells.

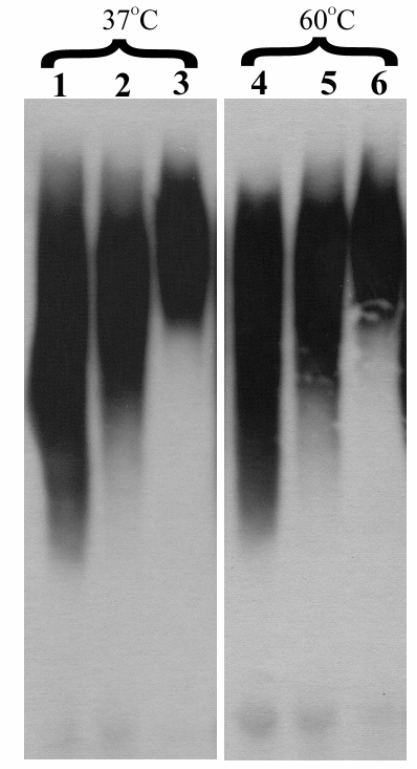

Figure S3. HET-s(PrD)-GFP subparticles are stable at higher temperatures

Precleared lysates of yeast cells with HET-s aggregates treated with 2% sarkosyl at the indicated temperatures for 10 min were resolved on a 1.5% agarose gel and probed with anti-GFP antibody. [*PIN*$^{+}$] (lanes 1 and 4), [*pin*$^{-}$] (lanes 2 and 5) and *hsp104*Δ (lanes 3 and 6).

Table S1. Stability of HET-s dot aggregates in mitotic growth

Culture duration in hr	Number of generations	Number of dot-colonies over total	Percentage of dot colonies over total	Cumulated loss rate per generation
0	-	499/505	98.81%	-
24	8	1218/1393	87.44%	0.03
48	16	557/844	65.99%	0.049
72	24	292/481	60.7%	**0.04**

A dot colony grown on solid 0.05% gal (t=0) was resuspended in 50 µl of water, dispersed by sonication for 10 sec and plated on solid 0.05% gal for single colonies. Also, these cells were simultaneously inoculated into a 0,05% gal liquid culture. After 24 hr, the numbers of colonies on the solid media with dot, vs. diffuse, fluorescence were counted under the fluorescence microscope to give the Culture duration = 0 hr data. The liquid culture was diluted regularly to keep the OD below 0.3. Under these conditions the doubling time was 3 hr. Every 24 hr, aliquots of the culture were sonicated for 10 sec and plated onto solid 0.05% gal at 10^4 cells per plate and after 24 hr, the numbers of colonies with dot vs. diffuse fluorescence was determined.

Loss rate per generation was calculated after Boe and Rasmussen (1996) using "model 1" that is based on the assumption that the growth rate is equivalent for prion containing and prion free cells.

θ, the loss rate per generation is obtained from:

$$\theta = 2(1-[\psi^+(g)/\ \psi^+(0)]^{1/g})$$

where $\psi^+(g)$ is the fraction of cells containing the element after g generations, $\psi^+(0)$ is the fraction of cells containing the element initially and g the number of generations.

Table S2. Stability of HET-s dot aggregates in mitotic growth

	Total cell count ($x10^6$)	Number of generations	Number of dot-colonies over total	Percentage of dot colonies over total	Loss rate per generation
Colony #2	5.62	~22	523/565	92.56%	0.007
Colony #6	5.84	~22	525/579	90.67%	0.0089
Colony #9	6.84	~22	541/583	92.79%	0.0068
Average	6.1	~22	-	92.01%	**0.0075**

A liquid culture with a majority of dot containing cells was sonicated for 10 sec and plated on 0.05% gal solid medium. Plates were incubated for 5-6 days at 30°C. Three colonies were picked (as entirely as possible) and resuspended in 500 µl of water. The total number of cells was counted yielding an estimate of 22 generations of growth in each colony. To determine how many of the cells in each of the three colonies retained HET-s dots that could be passed on to mitotic progeny, cells from each colony were plated on solid 0.05% gal medium to give individual colonies. After 24 hr the numbers of these colonies with dot and diffuse fluorescence were determined. The loss rate per generation during the 22 generations of growth in each of the original three colonies was then calculated as in Supplementary Table 1.

Supplementary References

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