Supplementary Information

Strain conformation, primary structure and the propagation of the yeast prion [*PSI*$^{+}$]

Katherine J. Verges*[1], Melanie H. Smith*[1,2], Brandon H. Toyama[1,3], Jonathan S. Weissman[1]

[1]Department of Cellular and Molecular Pharmacology, and Howard Hughes Medical Institute, University of California, San Francisco, 94158, California, USA.

[2] Graduate Group in Biophysics, University of California, San Francisco, 94158, California, USA.

[3]Present address: The Salk Institute for Biological Studies, La Jolla, 92037, California, USA.

*These authors contributed equally to this work.

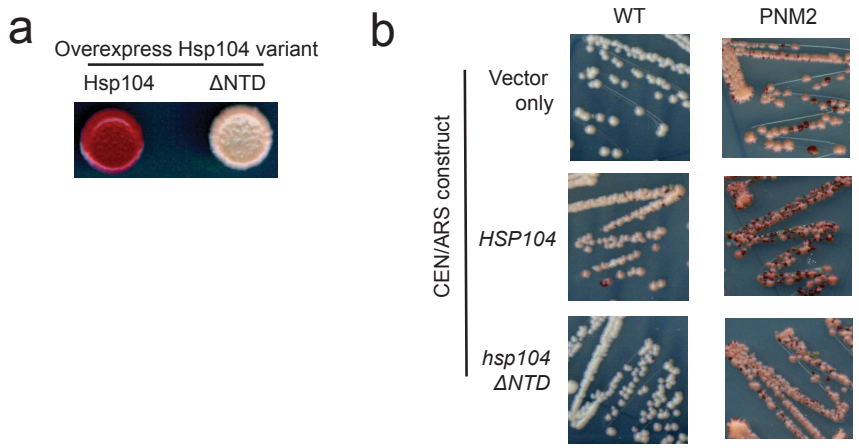
Correspondence:

Jonathan S. Weissman

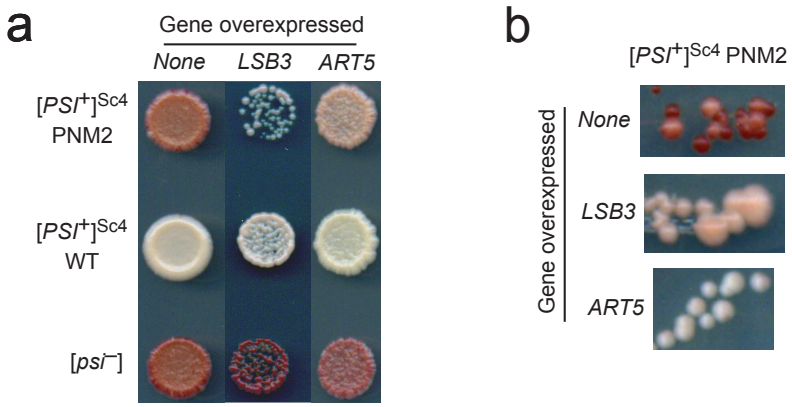
415-502-7642 (phone)

415-514-0273 (fax)

weissman@cmp.ucsf.edu



Supplementary Figure 1. The Hsp104ΔNTD variant is deficient in curing but propagates [*PSI*$^{+}$]^{Sc4} and maintains the PNM2 phenotype. **(a)** *HSP104* or *hsp104ΔNTD* was expressed from a high copy (2μ) plasmid in [*PSI*$^{+}$]^{Sc4} yeast spotted onto low adenine media. **(b)** Streak out of yeast from Figure 5 on low adenine media for better visualization of phenotype. WT or PNM2 [*PSI*$^{+}$]^{Sc4} yeast that express: an empty CEN/ARS plasmid ("vector only"), a CEN/ARS plasmid containing *HSP104* or a CEN/ARS plasmid containing *hsp104ΔNTD*.



Supplementary Figure 2. Overexpression of either *LSB3* or *ART5* suppresses the PNM2 phenotype. **(a)** [*PSI*$^{+}$]^{Sc4} yeast with PNM2 (top row) or WT (middle row) Sup35p or WT cured to [*psi*$^{-}$] (bottom row) transformed with an overexpression (2μ) plasmid that contains: no gene (left column), *LSB3* (middle column) or *ART5* (right column). All yeast are spotted onto low adenine media. **(b)** Single colony photographs of PNM2 [*PSI*$^{+}$]^{Sc4} yeast with an overexpression (2μ) plasmid that contains: no gene (top), *LSB3* (middle) or *ART5* (bottom).

Supplementary Table 1: Strains

Sc Strain	Description	Genotype	Source
YJW1109	74D-694 [*psi*$^{-}$]	74D-694 *ade1-14 his3Δ200 leu2-3,112 trp1-289 ura3-52 sup35::TRP1*/pRS316 [*psi*$^{-}$]	this study
YJW1110	74D-694 [*PSI*$^{+}$]^{Sc4}	74D-694 *ade1-14 his3Δ200 leu2-3,112 trp1-289 ura3-52 sup35::TRP1*/pRS316 [*PSI*$^{+}$]^{Sc4}	this study
YJW1111	74D-694 [*PSI*$^{+}$]^{Sc37}	74D-694 *ade1-14 his3Δ200 leu2-3,112 trp1-289 ura3-52 sup35::TRP1*/pRS316 [*PSI*$^{+}$]^{Sc37}	this study
YJW1112	W303 [*psi*$^{-}$] HAP	W303 *ade1-14 leu2-3,112 his3-11,15 trp1-1 can1-100 ura3::nat hsp104::TRP1*/phs313HAP [*psi*$^{-}$]	this study
YJW1113	W303 [*PSI*$^{+}$]^{Sc4} HAP	W303 *ade1-14 leu2-3,112 his3-11,15 trp1-1 can1-100 ura3::nat hsp104::TRP1*/phs313HAP [*PSI*$^{+}$]^{Sc4}	this study
YJW1114	W303 [*PSI*$^{+}$]^{Sc4} Hsp104	W303 *ade1-14 leu2-3,112 his3-11,15 trp1-1 can1-100 ura3::nat hsp104::TRP1*/phs313*Hsp104* [*PSI*$^{+}$]^{Sc4}	this study
YJW1115	W303 [*PSI*$^{+}$]^{Sc4} HAP	W303 *ade1-14 leu2-3,112 his3-11,15 trp1-1 can1-100 ura3::kan hsp104::*HAP-*TRP1 Sup35::nat*/pRS316 WTSup35 [*PSI*$^{+}$]^{Sc4}	this study
YJW1667	74D-694 [*PSI*$^{+}$]^{Sc4} Hsp104ΔNTD	74D-694 *ade1-14 his3Δ200 leu2-3,112 trp1-289 ura3-52 sup35::TRP1*/pRS316 *hsp104*::*nat-hsp104ΔNTD (resi 1-146 deleted)* [*PSI*$^{+}$]^{Sc4}	this study; Hung and Masison[35]

Supplementary Table 2: Plasmids

Plasmid	Description	Source
pRS316 WT Sup35p	pRS316; *SUP35*	this study
pRS315 WT Sup35p	pRS315; *SUP35*	this study
pRS315 PNM2 Sup35p	pRS315; *SUP35 G58D*	this study
phs313 Hsp104p	phs313; *HSP104*	Tessarz et al.[34]
phs313 HAP	phs313; HAP	Tessarz et al.[34]
pmCUP425-ClpP^{trap}	pmCUP425; ClpP^{trap} (ClpPΔ1–13, S111A; SBP-tagged)	Tessarz et al.[34]
pRS313 WT Sup35p	pRS313; *SUP35*	this study
pRS313 PNM2 Sup35p	pRS313; *SUP35 G58D*	this study
pRS313 WT Sup35HA	pRS313; *SUP35* with 3HA inserted after amino acid 216	this study
pRS313 PNM2 Sup35HA	pRS313 *SUP35 G58D* with 3HA inserted after amino acid 216	this study
pRS399 WT Hsp104p	pRS399 (pRS315 backbone with *Leu*::*KanR*); *HSP104*	this study
pRS399 Hsp104ΔNTDp	pRS399 (pRS315 backbone with *Leu*::*KanR*); *hsp104ΔNTD*	this study; Hung and Masison[35]
pGP564	pGP564; empty vector	Jones et al.[55]
pGP564 Lsb3p	pGP564; *LSB3*	this study
pGP564 Art5p	pGP564; *ART5*	this study

Supplementary Methods

High copy PNM2 suppression screen. Systematic overexpression of the full complement of yeast proteins was carried out using the Thermo Scientific Open Biosystems Yeast Genomic Tiling Collection[55]. The entire yeast genome was overexpressed in segments, each containing 4–5 genes, from 1588 clones using a pGP564 (2μ) vector in PNM2 [*PSI*$^{+}$]^{Sc4} yeast. Colonies were visually screened for reduced sectoring and five were identified as meeting this criteria. Individual clones were recovered from these colonies and sequenced to determine the identity of the clone. Clone YGPM21g14 was recovered 2 times and clone YGPM34g01 was recovered 3 times. Individual genes from each clone were overexpressed using the pGP546 vector to determine the causative genes. Both *LSB3* (YFR024C) and *ART5* (YGR068C) were identified as high copy suppressors of the PNM2 [*PSI*$^{+}$]^{Sc4} phenotype.

Supplementary References

34 Tessarz, P., Mogk, A. & Bukau, B. Substrate threading through the central pore of the Hsp104 chaperone as a common mechanism for protein disaggregation and prion propagation. *Mol Microbiol* **68**, 87-97, (2008).

35 Hung, G.-C. & Masison, D.C. N-terminal domain of yeast Hsp104 chaperone is dispebensable for thermotolerance and prion propagation but necessary for curing prions by Hsp104 overexpression. *Genetics* **173**, 611-620, (2006).

55 Jones, G. M., et al. A systematic library for comprehensive overexpression screens in Saccharomyces cerevisiae. *Nat Methods* **5**, 239-41 (2008).