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# Supplemental Data

# In Vivo Monitoring of the Prion Replication Cycle Reveals a Critical Role for Sis1 in Delivering Substrates to Hsp104

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## Supplemental Experimental Procedures

### Strains and Plasmids

| Sc Strain | Description | Source |
|---|---|---|
| [*PSI*$^+$]alpha | W303 *MATalpha leu2-3,112 his3-11,15 trp1-1 ade1-14 can1-100 ura3::nat* [*RNQ*$^+$] [*PSI*$^+$] | this study |
| [*PSI*$^+$]a | W303 *MATa leu2-3,112 his3-11,15 trp1-1 ade1-14 can1-100 ura3::kan* [*RNQ*$^+$] [*PSI*$^+$] | this study |
| Δ*sis1*-TET$^r$*SIS1* | W303 *MATalpha trp1-1 ura3-1 leu2-3,112 his3-11,15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2* Δ*sisl::LEU2*-[TET$^r$*SIS1*] [*RNQ*$^+$] [*psi*$^-$] | E. Craig |
| YKT12 | *hsp104::HIS3*/p316 *HSP104*pHSP104 [*PSI*$^+$]$^{Sc4}$ | this study |
| YKT24 | hsp104::HIS3/ p316 *HSP104*pHSP104 [*PSI*$^+$]$^{Sc37}$ | this study |
| YKT29 | hsp104::HIS3/ p316 *HSP104*pHSP104 [*PSI*$^+$] | this study |
| YKT101 | *hsp104::4BAP-TRP1*, Δ*sis1*-TET$^r$*SIS1*, [*PSI*$^+$]$^{Sc4}$ [*rnq*-] | |
| YKT44 | *hsp104::4BAP-TRP1*, Δ*sis1*-TET$^r$*SIS1*, [*PSI*$^+$]$^{Sc4}$ [*RNQ*+] | this study |
| YKT50 | *hsp104::444B-TRP1* [*PSI*$^+$] [*RNQ*$^+$] | this study |
| YKT52 | *hsp104::HIS3* | this study |
| YKT69 | *hsp104::4BAP-TRP1* [*psi*$^-$] | this study |
| YKT92 | *hsp104::4BAP-TRP1* [*PSI*$^+$]$^{Sc4}$ | this study |
| YKT96 | *hsp104::4BAP-TRP1* [*PSI*$^+$]$^{Sc37}$ | this study |
| YKV8 | *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$] | this study |
| YKT102 | *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$]$^{Sc37}$ [*rnq*$^-$] | this study |
| YKT111 | hsp104::4BAP-TRP1, *sis1*Δ-[TET$^r$*SIS1*], [*PSI*$^+$]$^{Sc4}$ [*rnq*$^-$] | this study |
| YKT99 | *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*psi*$^-$] [*rnq*$^-$] | this study |
| YKT141 | *hsp104::4BAP-TRP1*; *ura3::TDH3*p*ClpP*$^{Trap}$*FLAG*, [*psi*$^-$] [*rnq*$^-$] | this study |
| YKT143 | *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$]$^{Sc4}$ [*rnq*$^-$] | this study |
| YKT147 | *hsp104::4BAP-TRP1*; *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$]$^{Sc4}$ [*rnq*$^-$] | this study |
| YKT102 | *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$]$^{Sc37}$ [*rnq*$^-$] | this study |
| YKT149 | *hsp104::4BAP-TRP1*; *ura3::kanTDH3*p*ClpP*$^{Trap}$*FLAG*, [*PSI*$^+$]$^{Sc37}$ [*rnq*$^-$] | this study |

| E. coli Strain | Description | Source |
|---|---|---|
| MC4100 (CGSC #6152) | *[araD139], (*Δ*argF-lac)169, λ-; e14-, flhD5301,* Δ*(fruK-yeiR)725*(fruA25), *relA1, rpsL150*(strR)*, rbsR22,* Δ*(fimB-fimE)632*(::IS1)*, deoC1* | B. Bukau, *E.coli* GRC |
| Δ*clpB* | *[araD139], (*Δ*argF-lac)169, λ-; e14-, flhD5301,* Δ*(fruK-yeiR)725*(fruA25), *relA1, rpsL150*(strR)*, rbsR22,* Δ*(fimB-fimE)632*(::IS1)*, deoC1,* Δ*clpB::*Kan | B. Bukau, *E.coli* GRC |

| Plasmid | Description | Source |
|---|---|---|
| pKAT112 | pRS315 *HSP104*p*HSP104* (*HSP104*p = 500bp upstream of *HSP104*) | this study |
| pKAT116 | pRS315 *HSP104*p *ClpB* | this study |
| pKAT117 | pRS315*HSP104*p *444B* | this study |
| pKAT118 | pRS315*HSP104*p *4BAP* | this study |
| pKAT159 | pRS315*HSP104*p *4BBB* | this study |
| pKAT160 | pRS315*HSP104*p *B44B* | this study |
| pKAT162 | pRS315 *HSP104*p *BB4B* | this study |
| pKAT163 | pRS315*HSP104*p *4B4B* | this study |
| pKAT164 | pRS315 *HSP104*p *_44B* | this study |
| pKAT161 | pRS315_ *HSP104*p *BBB4* | this study |
| pKAT169 | pRS315_ *HSP104*p *4BB4* | this study |
| pKAT136 | pRS316 *HSP104*p *HSP104* | this study |
| pKAT172 | pSU18 *ClpB*pClpB (*ClpB*p = 500bp upstream of *ClpB*) | this study |
| pKAT173 | pSU18 *ClpB*p 444B | this study |
| pKAT174 | pSU18 *ClpB*p Hsp104 | this study |
| pKAT175 | pSU18 *ClpB*p BBB4 | this study |
| pKAT184 | p426 *GAL1*p*ClpP$^{trap}$FLAG* | this study |
| pGPDlux | p316 *GPD*p luciferase (firefly) | B. Bukau |
| pRnq-GFP | p316 *CUP1*p*RNQ1msGFP* | E. Craig |
| pKJV1 | *ura3::kan-TDH3*p*ClpP$^{trap}$ 3xFLAG-cyc1* | this study |

## Strains

All yeast strains are derived from W303 strains YJW1070 (MAT*a ura3::kan*) and YJW1068 (MAT*alpha ura3::nat*) [*RNQ*$^{+}$] [*PSI*$^{+}$] *leu2-3,112 his3-11,15 trp1-1 ade1-14 can1-100*. Defined [*PSI*$^{+}$]$^{SC4}$ and [*PSI*$^{+}$]$^{SC37}$ strains were constructed from [*psi*$^{-}$] [*rnq*$^{-}$] strains (created by curing on 3mM GdnHCl) by protein infection as described previously (Tanaka et al., 2004) or by mating into such strains. *HSP104* allele replacements were constructed by transforming wild-type or *hsp104::HIS3* yeast with DNA fragments containing the desired *Hsp100* allele marked by *TRP1* and selecting on Trp dropout media. Strains were confirmed by PCR and western blot. *ClpP$^{trap}$FLAG* (*Trap*) strains were constructed by replacing *ura3::nat* with *ura3::kanTDH3*p*Trap* in a similar fashion.
TET$^{r}$*SIS1* strains were constructed by mating into W303 *sis1*-Δ[TET$^{r}$*SIS1*], a gift of E. Craig and described previously (Aron et al., 2007).

## Plasmids

*Trap* knockin templates were constructed by amplifying *ClpP* without its 13-amino acid propeptide from DH5α and cloning into pFA6a-kanMX6. ClpP S97A (Trap) (Flynn et al., 2003) was constructed by QuikChange (Stratagene). Standard cloning techniques were used to insert the *TDH3* and *GAL1* promoters, the 3xFLAG epitope tag and to subclone the *GAL1*p*TrapFLAG* cassette into p426.
The *HSP104* coding region, promoter and 3'utr were amplified from *S. cerevisiae* genomic DNA, and the ClpB coding region, promoter and 3'utr were amplified from DH5α *E. coli*. A modular system of promoter-coding region-3'utr was created in p315 for yeast and pSU18 for bacteria for ease of gene replacement. All chimeras were constructed by fusion PCR using cloned *HSP104* and ClpB as templates, and all constructs were confirmed by sequencing.

**Thermotolerance Assays**

**Yeast**

YKT52 (*hsp104::CgHIS3*) transformed with LEU2 CEN-ARS plasmids carrying the indicated chaperone under the *HSP104* promoter were grown in synthetic media lacking leucine at 30°C to mid-log and shifted to 37°C for 30min. 1mL aliquots of the heat-treated cultures were added to prewarmed tubes in a 50°C water bath. Cells were taken at the indicated time points, serially diluted 5-fold each step in chilled microtiter plates, pinned on synthetic media lacking leucine and incubated 48-72 hours at 30°C.

**Bacteria**

MC4100 and Δ*clpB::kan* transformed with pSU18 carrying the indicated chaperone under the *ClpB* promoter were grown in LB + 34μg/mL chloramphenicol at 30°C to mid-log, shifted to 42°C for 15 minutes, and 1mL aliquots were added to prewarmed tubes in a 50°C water bath. Cells were removed at the indicated time points and serially diluted (10-fold each step) in chilled microtiter plates, pinned on LB+chloramphenicol media and incubated 24 hours at 30°C.

**Immunoprecipitations & Western Blots**

IP buffer: 50mM HEPES-KOH, pH 7.0, 150mM KOAc, 2mM MgOAc, 1mM $CaCl_2$, 0.1% Triton X-100) + Roche Complete Protease Inhibitor Cocktail, pepstatin A, 4mM EDTA and RNaseA.

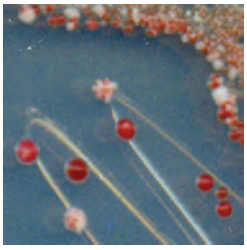
Western blots were performed using rabbit antisera specific for Sup35NM, for Hsp104 NTD (aa1-146), for full-length Hsp104 or ClpB (gifts of B. Bukau) or for Hsp104 C-terminal 15aa (Stressgen) and mouse monoclonal anti-FLAG (Sigma), mouse anti-Pgk1 (Molecular Probes), or mouse anti-RNAP beta (*E. coli*) (NeoClone) as primary antibodies and with AlexaFluor680- (Invitrogen) or IRDye800- (Rockland) conjugated goat anti-mouse and anti-rabbit antibodies as secondary antibodies. Blot signals were detected and quantitated using the LiCor Odyssey infrared imaging system and software.

**Figure S1. B44B can propagate an unstable [*PSI*$^+$]**
[*PSI*$^+$] was tested in pink or white colonies from otherwise [*psi*-] strains generated in Figure 1B experiments by passage on 3mM Gdn HCl and by allowing loss of the plasmid carrying the chaperone. Only *B44B* [*PSI*$^+$] candidates tested positive for the prion. Shown is a heavily sectoring *B44B* [*PSI*$^+$] isolate on low adenine media.

**Figure S2. Expression Levels of Hsp100s in yeast and bacteria**
Cultures of (A) yeast or (B) bacteria expressing the indicated Hsp100 were exposed to mild heat shock to induce Hsp100 expression and harvested. Cleared lysates of cells were subjected to SDS-PAGE, transferred to nitrocellulose and probed with a mixture of ClpB-reactive and Hsp104NTD-reactive antibodies plus antibodies reactive to abundant soluble proteins as internal loading controls (Pgk1 in yeast, RNA polymerase beta subunit (RNAPbeta) in bacteria). Fresh preparations of the same ratio of anti-ClpB/anti-Hsp104NTD were used for each blot to enable comparison of the ratio of Hsp100 expressed. Band intensities normalized to the internal control are shown with levels of Hsp104 set to 100.

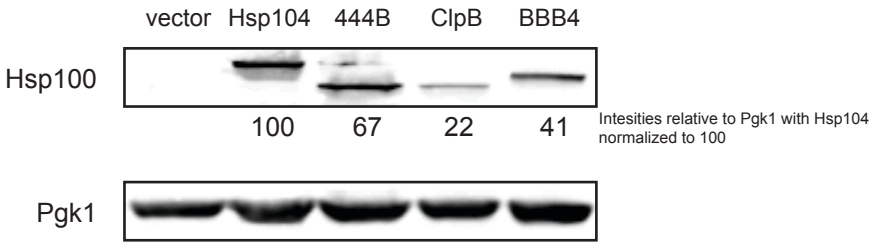
**Figure S3. The ClpP-coupling loop does not interfere with 4BAP function**
(A) Luciferase reactivation by 4BAP, performed as in Fig 1B. Mean values and stanard errors were calculated from at least three independent experiments.
(B) Induced thermotolerance function by 4BAP, performed as in Fig 1C.

**Figure S4. Sis1 inhibition reduces translocation of prion proteins from their aggregate substrates**
(A) Blot from Figure 4B, left, was probed for Sis1 to assess efficiency of doxycyline shutoff and for Pgk1 to assess the specificity of Sup35 immunoprecipitation. The abundant soluble protein Pgk1 failed to immunoprecipitate, confirming the specificity of the elution signals.
(B) Cultures at time of harvest for Sis1 shutoff experiment in Figure 4B, left. The doxycycline treatment, which resulted in a 12-fold decrease in Sup35 translocation, was insufficient to cure the cells of [*PSI*$^+$].
(C) Blot from Figure 4B, right, was probed for Sis1 to assess efficiency of doxycyline shutoff and for Pgk1 to assess the specificity of Rnq1 immunoprecipitation. The abundant soluble protein Pgk1 failed to immunoprecipitate, confirming the specificity of the elution signals. Faint bands in the IP lanes were from a previous probe.



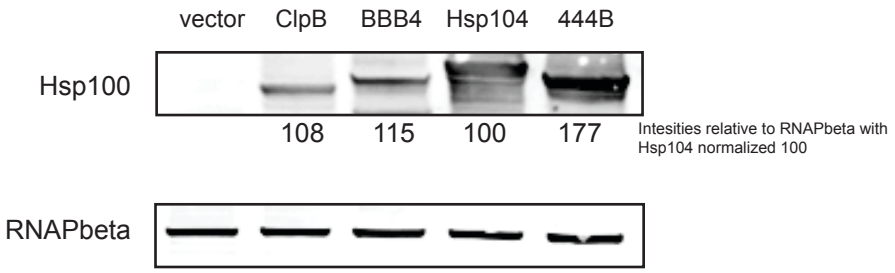
B44B

A



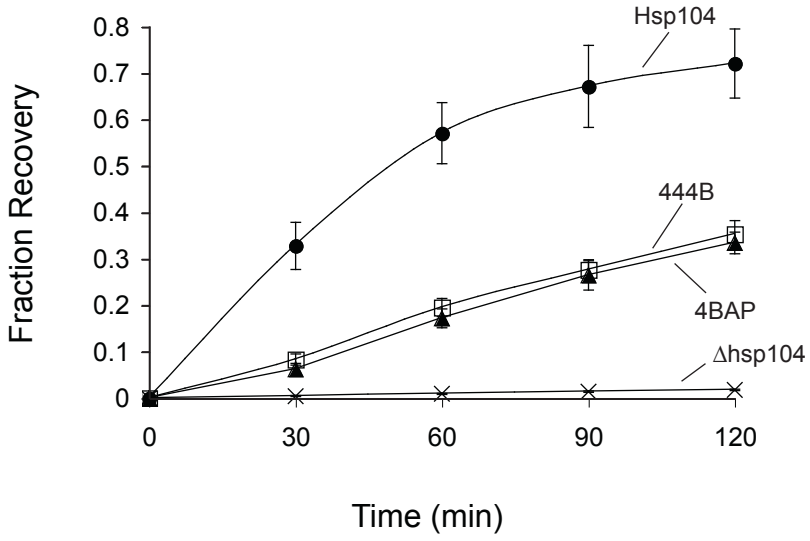

*S. cerevisiae*

B

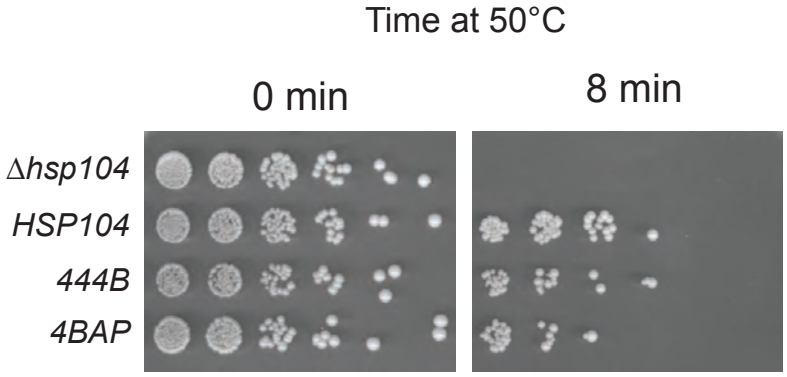



*E. coli*

A

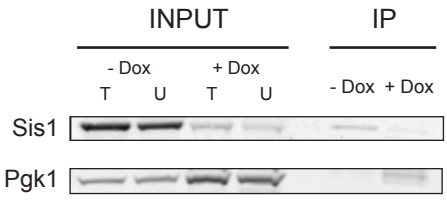

Hsp104
444B
4BAP
Δhsp104
Fraction Recovery
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
30
60
90
120
Time (min)

B

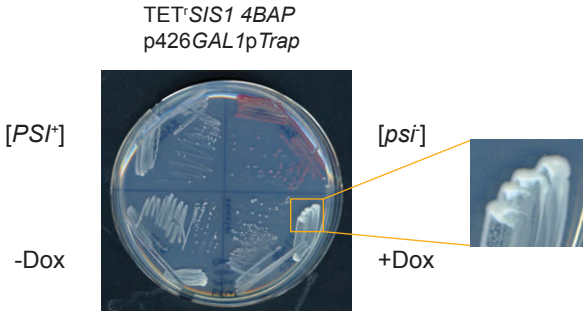

Time at 50°C
0 min
8 min
Δhsp104
HSP104
444B
4BAP

A

IP: α-FLAG (Trap)
IB: α-Sis1, α-Pgk1




B




C

IP: α-FLAG (Trap)
IB: α-Sis1, α-Pgk1