Supplementary Materials

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Title: Heterogeneous interaction network of yeast prions and remodeling factors detected in live cells

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Running Title: Interaction network of yeast prions in live cells

Keywords: yeast prions, remodeling factors, fluorescence cross-correlation spectroscopy, live cell, protein interaction
Supplementary Materials and Methods

Plasmid Construction

Open reading frame (ORF) sequences of SUP35, RNQ1, URE2, and NEW1 genes were cloned directly from purified genomic DNA of S. cerevisiae BY4741 strain by a standard PCR method with appropriate pair of oligonucleotides. Each partial sequence corresponding to residues 1-253, 1-65 and 1-130 of Sup35, Ure2 and New1, respectively, was fused to the 5’-end of the sequence of TagRFP derived from pTagRFP-N (Evrogen) by an overlap-extension PCR method. Likewise, sequence corresponding to residues 134-405 of Rnq1 was fused to the 3’-end of the TagRFP-coding sequence. Finally, each chimeric DNA fragment was subcloned onto the pYES2 yeast expression plasmid vector (Invitrogen) to produce pYES-SUP35NM-TagRFP, pYES-URE2N-TagRFP, pYES-NEW1N-TagRFP and pYES-TagRFP-RNQ1C. As a negative control, a plasmid expressing thioredoxin-fused TagRFP (pYES-Trx-TagRFP) was also constructed by fusing the thioredoxin ORF originally coded on pThio-His B plasmid (Invitrogen) to the 5’-end of the TagRFP-coding sequence and subcloning onto pYES2 vector.

A plasmid that expresses Hsp104 fused with mCherry (pGAL1-HSP104-mCherry) was constructed as follows. The HSP104 containing SacI-BamHI DNA fragment was cloned into YCplac111GAL1p (1). The mCherry DNA fragment was amplified from a pmCherry-N1 vector (Clontech) and inserted into the BamHI-SalI site of the YCplac111GAL1pHSP104 plasmid. This includes GS linker between HSP104 and mCherry.

To visualize Sis1 in the yeast cell, pRS314-SIS1p-SIS1-mCherry was prepared. SIS1 gene including SIS1 own promoter was cloned from W303 PJ513a (2) and fused mCherry gene under SIS1 gene with Gly-Ser linker by PCR. SIS1p-SIS1-mCherry construct was cloned to pRS314 (3) and used for plasmid shuffling to obtain Sis1-mCherry expression strains.

[RNQ1p-RNQ1-GFP] plasmid, pRS413-RNQ1p-RNQ1-GFP, was from our previous work (2).
A plasmid (pRS413CYC1p-SUP35NM-GFP) encoding Sup35NM-GFP under the control of CYC1 promoter was constructed with SUP35NM gene cloned from W303 PJ513a and monomeric GFP gene (2).

**Yeast Strain**

In this study, *S. cerevisiae* G74-D694 strain, which we have established previously (4), was used as the parent strain. G74-D694 is a derivative of 74-D694 [MATa, ade1-14(UGA), his3, leu2, trpl, ura3] and carries a modified SUP35 gene (SUP35NGMC), in which a GFP gene was integrated between the N and M domains of the endogenous SUP35 gene. Either [psi⁻] or [PSI⁺] G74-D694, [gpsi⁻] or [GPSI⁺], respectively, was transformed with yeast expression plasmid described above by a standard lithium acetate method. Transformants were selected by synthetic medium lacking uracil (SC-Ura) or leucin (SC-Leu). To induce expression of each protein, 2% (w/v) galactose was added to synthetic medium containing 2% (w/v) raffinose instead of glucose and lacking uracil (SRaf (-ura)) for 4~ to 24h.

Details of W303 sis1-Δ::LEU2 [SIS1p-SIS1] [RNQ⁺] [psi⁻] or [rnq⁻] [PSI⁺] strains were described previously (2, 5). BY4741 MATa HSP104-GFP::HIS3MX6 was described in the previous study (6). W303 sis1-Δ::LEU2 [SIS1p-SIS1-mCherry] strains with [RNQ⁺] or [PSI⁺] were prepared by plasmid shuffling. Growth and prion maintenance of W303 sis1-Δ::LEU2 strain having expression of Sis1-mCherry was indistinguishable to W303 sis1-Δ::LEU2 strain with that of wild-type Sis1 from a [SIS1p-SIS1] plasmid. To measure FCS/FCCS, prion-GFP plasmid, pRS413-RNQ1p-RNQ1-GFP for [RNQ⁺] or [rnq⁻] or pRS413CYCp-SUP35NM-GFP for [PSI⁺] [psi⁻], was coexpressed in W303 sis1-Δ::LEU2 [SIS1p-SIS1-mCherry]. Semi-denaturing detergent agarose gel (5, 7) pattern of the prion-GFP coexpression strains showed identical pattern as of strains without coexpression. W303 [rnq⁻] and [psi⁻] strains were
obtained by 3 mM guanidine HCl hydrochloride (GdnHCl) treatment in the appropriate media for 2 days.

**Protein Expression**

Transformed yeast cells were cultured in SRaf (-ura). At mid-log phase, protein expression was induced by the addition of galactose at a final concentration of 0.2%. After a 12-h incubation at 30°C, cells were used for FCCS measurement. To estimate the expression level of each fusion protein conveniently, we utilized the fluorescence of TagRFP. Fluorescence images at green and red channel of a 100-µl droplet of each culture fluid were taken by LAS-4000 luminescence imager (Fuji Film, Japan) and the relative TagRFP fluorescence was analyzed by Multi Gauge (Fuji Film, Japan).

**Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS).** FCS and FCCS measurements were performed at 25°C on a confocal microscope system (LSM 510; Carl Zeiss) combined with a ConfoCor2 (Carl Zeiss). For confocal imaging followed by FCS or FCCS measurement, GFP and RFP were scanned independently in a multi-tracking mode. Details of the combined microscope system, analysis of fluorescence auto- function (FAF) obtained from FCS measurement, and analysis of two FAFs and one cross-correlation function (FCF) from FCCS measurement were described in previous studies (1, 6). Briefly, FAF and FCF, from which the absolute number and diffusion coefficient ($D$) of mobile fluorescent molecules, fractional ratio, and interaction amplitude represented by relative cross-correlation amplitude (RCA) are calculated, are obtained as follows:

The fluorescence auto-correlation functions of the red and green channels, $G_r(\tau)$ and $G_g(\tau)$, and the fluorescence cross-correlation function, $G_c(\tau)$, were calculated from
\[ G_x(\tau) = 1 + \frac{\langle \delta I_i(t) \cdot \delta I_j(t+\tau) \rangle}{\langle I_i(t) \rangle \langle I_j(t) \rangle} \]  

(1)

where \( \tau \) denotes the time delay, \( I_i \) is the fluorescence intensity of the red channel \( (i = r) \) or green channel \( (i = g) \), and \( G_r(\tau) \), \( G_g(\tau) \), and \( G_c(\tau) \) denote the auto correlation function of red \( (i = j = x = r) \), green \( (i = j = x = g) \), and cross \( (i = r, j = g, x = c) \), respectively. The acquired \( G_x(\tau) \)s were fitted using a one-component model for solution samples and a two-component model for live cells:

\[ G_x(\tau) = 1 + \frac{1}{N} \sum_i F_i \left( \frac{\tau}{\tau_i} \right)^{-1} \left( 1 + \frac{\tau}{s^2 \tau_i} \right)^{-\frac{1}{2}} \]  

(2)

where \( F_i \) and \( \tau_i \) are the fraction and diffusion time of component \( i \), respectively. \( N \) is the average number of fluorescent particles in the excitation-detection volume defined by radius \( w_0 \) and length \( 2z_0 \), and \( s \) is the structure parameter representing the ratio \( s = z_0/w_0 \). The structure parameter was calibrated using the known diffusion coefficient of Rhodamine-6G at room temperature \( (280 \mu m^2 s^{-1}) \). To estimate the diffusion coefficient and fractional ratio from FCS or FCCS measurements, FAF and FCFs in live cells were fitted by a two-component model \( (i = 2, D_{fast} \text{ and } D_{slow}) \) with a triplet term \( (6) \). Although the shape of FAFs originated from a one-component model \( (\text{i.e. single-species}) \) only depends on the diffusional mobility, it is emphasized that the shape of FAFs originated from a multi-component model \( (\text{multi-species}) \) depends not only on the diffusional mobility but also on fractional ratio of mobile species \( (1) \). For FCCS measurement, simultaneous excitation of GFP- and RFP-tagged proteins was carefully carried out at minimal and optimal excitation powers, chosen to obtain sufficiently high signal-to-noise ratios for the analysis of the diffusional coefficient and molecular interaction. Data containing severe photobleaching possibly resulting from a high proportion of immobilized fluorophores and non-stationary fluorescent signals resulting from
the drift of yeast cells were excluded from the analysis. For FCCS analysis, the amplitude of
the cross-correlation function was normalized by the amplitude of the autocorrelation
function of RFP to calculate the relative cross-correlation amplitude $((Gc(0)-1)/(Gr(0)-1);$
RCA value)(6).
**Supplementary Table S1. Theoretical molecular weights of prion proteins, Sis1, and Hsp104.**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Theoretical Mw (kD)</th>
<th>FP tagged proteins</th>
<th>Total theoretical Mw (kD)</th>
<th>Mw&lt;sub&gt;tot&lt;/sub&gt; &lt;sup&gt;c&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Sup35NM</td>
<td>49</td>
<td>Sup35NM-GFP</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>Sup35</td>
<td>79</td>
<td>NGMC</td>
<td>107</td>
<td>350</td>
</tr>
<tr>
<td>Rnq1</td>
<td>43</td>
<td>Rnq-GFP</td>
<td>71</td>
<td>76</td>
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<tr>
<td>Ure2</td>
<td>40</td>
<td>Ure2-mCherry</td>
<td>68</td>
<td>-</td>
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<tr>
<td>New1</td>
<td>134</td>
<td>New1-Ure2-mCherry</td>
<td>162</td>
<td>-</td>
</tr>
<tr>
<td>Sis1</td>
<td>40</td>
<td>Sis1-mCherry</td>
<td>68 (136 &lt;sup&gt;a&lt;/sup&gt;)</td>
<td>1720</td>
</tr>
<tr>
<td>Hsp104</td>
<td>104</td>
<td>Hsp104-GFP</td>
<td>132 (792 &lt;sup&gt;b&lt;/sup&gt;)</td>
<td>8600</td>
</tr>
</tbody>
</table>

<sup>a</sup> Molecular weight of Sis1-GFP as a homo-dimer.

<sup>b</sup> Molecular weight of Hsp104-GFP as a hexamer complex.

<sup>c</sup> Mw calculated from FCS analysis using lysis solution samples.
Supplementary Table S2. Molecular concentrations of Sup35NM-GFP and Sup35NGMC proteins in yeast cells

<table>
<thead>
<tr>
<th>Proteins and cell type</th>
<th>Concentration (nM)</th>
<th>SD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sup35NM-GFP in $[\psi^-]$</td>
<td>676</td>
<td>130</td>
</tr>
<tr>
<td>Sup35NGMC in $[g\psi^-]$</td>
<td>285</td>
<td>60</td>
</tr>
<tr>
<td>Sup35NM-GFP in $[P\Sigma^+]$</td>
<td>193</td>
<td>20</td>
</tr>
<tr>
<td>Sup35 NGMC in $[GPS^+]$</td>
<td>150</td>
<td>12</td>
</tr>
</tbody>
</table>
Supplementary Figure S4

A

Sis1-mCherry
Sup35NM-GFP ([PSI⁺])

Frequency

RCA

B

Sis1-mCherry
Rnq1-GFP ([RNQ⁺])

Frequency

RCA
Supplementary Figure Legends

Supplementary Figure S1. Diffusional properties of Rnq1-GFP in live cells. (A)
Fluorescence-confocal image of \([RNQ^+]\) prion cells expressing Rnq1-GFP are shown. Scale bar, 5\(\mu m\). Arrow and cross hair indicate a large immobile focus in a mother cell and the position of FCS measurement shown in (B), respectively. (B) Representative two FCS measurements carried on \([rnq^-]\) and \([RNQ^+]\) cells are respectively shown. (Upper) Time trace of average fluorescence intensity (counts per second; cps in kHz) of Rnq1-GFP observed in \([rnq^-]\) (black) and \([RNQ^+]\) (red) cells. (Bottom) The corresponding fluorescence auto-correlation functions (FAFs) calculated from the time trace are also shown. Fit curves (solid line) were obtained from two-component analysis. For comparison of mobility, the curves were normalized to the same amplitude, \(G(0) = 2\).

Supplementary Figure S2. Slow diffusional behavior of Hsp104-GFP in non-prion cells.
(A) Fluorescence-confocal image of yeast cells endogenously expressing Hsp104-GFP are shown. Scale bar, 5\(\mu m\). (B) Fluorescence-confocal image of \([psi^-]\) and \([PSI^+]\) cells expressing monomer GFP (mGFP) are respectively shown. Scale bar, 5\(\mu m\). (C) Representative normalized FAFs of Hsp104-GFP and mGFP are also shown. Fit curves (solid line) were obtained from two-component analysis. For comparison of mobility, the curves were normalized to the same amplitude, \(G(0) = 2\).

Supplementary Figure S3. FCCS measurements for detecting interactions among prion proteins in live cells. (A) ~ (D) Representative FCCS measurement carried on non-prion and prion cells are respectively shown (upper and bottom). (Inset) Measured cell type \(([gpsi^-]\) or \([GPSI^-]\)) and a pair of proteins tagged with Tag-RFP and GFP. (Upper) Time trace of average fluorescence intensity (counts per second; cps in kHz) of two prion proteins (red and blue).
(Bottom) Two corresponding fluorescence auto-correlation functions (FAFs) of mCherry signal (red) and GFP signal (blue), and one fluorescence cross-correlation function (FCF) are shown. (E) Representative FCCS measurement carried on a \([\psi^-]\) cell co-expressing Sis1-mCherry and Sup35NM-GFP are shown. (F) Representative FCCS measurement carried on a \([g\psi^-]\) cell co-expressing Hsp104-mCherry and Sup35NGMC are shown.

**Supplementary Figure S4. Histogram of RCA values for interaction between remodeling factor Sis1 and prion oligomers in yeast prion cells.** (A) Histogram of RCA values for interaction between Sis1-mCherry and Sup35NM-GFP oligomers. (B) Histogram for interaction between Sis1-mCherry and Rnq1-GFP oligomers.
References