Supplementary information for:

Dom34-Hbs1 mediated dissociation of inactive 80S ribosomes promotes restart of translation after stress.

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Running title: Dom34-Hbs1 modulates ribosomal subunit availability after stress

Supplementary Material and Methods

In vitro translation

Translational extracts (Tuite & Plesset, 1986) were prepared from a *dom34*Δ*hbs1*Δ strain (BSY2550) as follows. 4 L of culture at OD_{600} = 1 was washed in 200 ml cold water, incubated in 100 ml β-mercaptoethanol 10mM; EDTA 2 mM for 30 minutes at room temperature, washed in 100 ml cold sorbitol 1M and resuspended in 1M sorbitol at room temperature at a concentration of 10 ml/g cells, spinning at 2000 x g for 5 min at 4°C in between. Zymolyase was added at 4 µg/ml final concentration and the reaction was stopped by pelleting the resulting spheroplast at 1000 x g for 10 min at room temperature when 75% of cells had converted to spheroplasts. Spheroplasts were washed in 200 ml sorbitol 1.2 M and incubated in 500 ml YPDA-sorbitol 1M at 25°C 40 rpm. Spheroplasts were harvested at 1000 x g for 10 min at 4°C and lysed using glass beads (0.5 ml/g cells) in lysis buffer (20 mM Hepes-KOH pH7.4; 100 mM KOAc; 2 mM $Mg(OAC)_2$; 2 mM DTT; 0.5 mM PMSF; protease

inhibitor cocktail) (1 ml/g cells) in 5 cycles of shaking vigorously at 2 Hz for 20 seconds with 1 minute intervals on ice. Lysates were cleared spinning 30 000 x g for 15 min at 4°C, then 100 000 x g for 30 min at 4°C. Glycerol was added at 10% final concentration for storage at -80°C.

In vitro translation was performed basically as described in (Tarun & Sachs, 1995). Translational extracts were incubated with 150 U/ml micrococcal nuclease (New England Biolabs) in presence of 480 µM $CaCl_2$ for 5 minutes at 26°C, before adding 2 mM EGTA on ice. 7.5 µl extract was added to 7.5 µl translation mix containing 0,1 µl RNasin (Promega), 500 ng firefly luciferase-A(50) mRNA (Gallie et al, 1991) and 4 µg creatine phosphokinase (Roche) in 22 mM Hepes-KOH pH 7.4; 120 mM KOAc; 2 mM MgOAc; 750 µM ATP; 100 µM GTP; 25 mM creatine phosphate; 40 µM amino acid mixture (Promega); 1.7 mM DTT. After 1 h incubation at 26°C luciferase activity was measured in 10 s measurements using a Lumat LB 9507 luminometer (Berthold technologies) adding 1 µl translation reaction to 50 µl luciferine mix (470 µM luciferine; 530 µM ATP; 270 µM coenzyme A; 20 mM Tris-phosphate pH 7.8; 1.07 mM $MgCl_2$; 2.7 mM $MgSO_4$; 100 µM EDTA; 33.3 mM DTT).

Dom34 added to the translation reaction was purified as described in (Shoemaker et al, 2010; Shoemaker & Green, 2011). Hbs1 was purified from BL21 codon+ cells expressing C-terminally 6xHis-tagged Hbs1 grown in Autoinduction media Terrific Broth Base including Trace elements (Formedium) over Ni-NTA agarose (Qiagen) after cell lysis in a Cell Disruptor (Constant Systems) at 1.55 kbar, using lysis buffer (75 mM Hepes pH 7.9, 300 mM NaCl, 5 mM β-mercaptoethanol, 1% Tween, 20 mM imidazole, 10% glycerol, 2 mM $MgCl_2$), wash buffer (50 mM Hepes pH 7.9, 500 mM NaCl, 5 mM β-mercaptoethanol, 20 mM imidazole, 10% glycerol, 2 mM $MgCl_2$) and elution buffer (50 mM Hepes pH 7.9, 500 mM NaCl, 5 mM β-mercaptoethanol, 300 mM imidazole, 10% glycerol, 2 mM $MgCl_2$). Both Hbs1 and the Dom34-Hbs1 complex were further purified over a Superdex 75 10/300 GL column (GE Healthcare) in 20mM Tris-Cl pH 7.5, 200mM NaCl, 5mM β-mercaptoethanol, 5% glycerol.

Yeast growth curves

Yeast was grown to OD_{600} 0.6 in YPDA, shifted to the indicated temperature and grown for another 2 hours. The rest of the experiment was done at the indicated temperatures. Cultures were split into 2 x 10 ml and resuspended in 10 ml YPA or YPDA for 10 minutes. Then 3 ml of each cultures was pelleted and resuspended in 15 ml YPDA (to an OD_{600} of ~0.2). OD_{600}

was measured at the indicated time points. OD_{600} series corresponding to the same cultures were normalized for the OD_{600} at time = 0 minutes to be 0.2.

Supplementary table 1: Strains and plasmids

Strain or plasmid name	Description (genotype for strains; vector/insert/marker for plasmids)	Reference
BMA64	*MAT α, ura31, trp1Δ, ade21, leu23,112, his311,15*	(Baudin-Baillieu et al, 1997)
BSY1970	*MAT α, ura31, trp1Δ, ade21, leu23,112, his311,15, dom34Δ::HIS3*	(van den Elzen et al, 2010)
BSY2145	*MAT a, ura31, trp1Δ, ade 21, leu23,112, his311,15, hbs1Δ::KanR*	(van den Elzen et al, 2010)
BSY2550	*MAT a, ade 2-1 his3-11,15, leu2-3,112, trp1delta ura3-1, dom34Δ::HIS3, hbs1Δ::KanR*	This study
N20T20	*MAT a, ura3-1, trp1Δ, ade2-1, leu2-3,112, his3-11,15, stm1Δ::TRP1*	Gift from F. Lacroute and F. Wyers
BSY2626	*MAT a, ura3-1, trp1Δ, ade2-1, leu2-3,112, his3-11,15, stm1Δ::TRP1, dom34Δ::HIS3*	This study
pBS3614	pRS415/ *HBS1-PROTEIN A* + promoter/*LEU2*	(van den Elzen et al, 2010)
pBS3675	pRS415/ *hbs1 V176G-PROTEIN A* + promoter/*LEU2*	(van den Elzen et al, 2010)
pBS3685	pRS415/ *DOM34-3HA* + promoter/*LEU2*	(van den Elzen et al, 2010)
pBS3701	pRS415/ *dom34 E361R-3HA* + promoter/*LEU2*	(van den Elzen et al, 2010)
pBS4415	pRS415/ *hbs1ΔN-ter (2-149)-PROTEIN A* + promoter/*LEU2*	This study