

Supplementary Methods

Yeast strain construction

The *DAL5* promoter region (568 bp) was amplified from genomic DNA (strain S288c) using primers OAB40 and OAB41 (Supplementary Table I) producing flanking sequences directing replacement of the most 3' 500 bp of *CAN1* promoter. This $P_{DAL5}:CAN1$ construct was transformed into strain 4132 which already contained the *URE2* expressing plasmid p644 (Masison & Wickner, 1995). Can^{res} clones were selected, producing strain BY103 (Figure 1A, Table I). BY103 was cured of plasmid p644 and $P_{DAL5}:CAN1$ *URE2* segregants were obtained from a cross with strain 4887-26C. In the resulting strain, *URE2* was deleted again by transformation with a *ure2Δ::kanMX* construct, obtained by amplification of *kanMX* from plasmid pFA6a-*kanMX* (Longtine et al, 1998) with primers OAB128 and OAB129 (Supplementary Table I) to generate strain BY178. The $P_{DAL5}:ADE2$ construct was introduced into BY178 by transformation of a 2598 bp $P_{DAL5}:ADE2$ fragment obtained by amplification from strain YMS23 genomic DNA (Schlumpberger et al, 2001) using primers OAB115 and OAB116 (Supplementary Table I), and selection of Ade^+ clones. The resulting strain BY179 was mated with the *URE2* strain BY228 to bring the two new reporters together with different auxotrophic markers into a *URE2*, as well as a *ure2* background. Strains BY241, BY245, BY248, BY250, BY251, BY252, BY254, and BY256 are progeny of this cross. In order to bring the reporters into different genetic backgrounds, strain BY241 was crossed with strain Σ 1278b and sporulated, resulting in strains BY315 and BY317, which were further crossed with strains 3742 and 3920, respectively, to yield segregants BY334, and BY327 and BY337, respectively. Cytoconduction of [URE3] was performed as previously described (Ridley et al, 1984).

Strain BY277 was derived from strain BY254 by transformation of plasmid pAB97 (linearized with *EcoRV* and *NheI*) containing 179 bp 5' of the *URE2* start site, the coding sequence for residues 90-354 of Ure2p, and 463 bp 3' of the *URE2* translation terminator. By homologous recombination, the *ure2Δ::kanMX* locus in BY254 was exchanged with a *URE2* locus in which the coding sequence for residues 3-89 of Ure2p is missing (residues 1 and 2 are MetMet). The resulting strain expresses Ure2p⁹⁰⁻³⁵⁴, could be selected due to its canavanine resistance, and is Ade^- and G418 sensitive. Strain BY277 was transformed with linearized plasmid pAB88 containing the same *URE2* promoter and terminator regions, but a frame-shift allele of *URE2*, in which the coding sequence for residues 1 and two is fused out of frame to the

coding sequence for residues 65-354. The resulting strain BY259 expresses the peptide MetMetHisArg, could be selected by its Ade⁺ phenotype, and is canavanine sensitive.

Preparation of yeast extracts

Yeast extracts were prepared in two different ways. For experiments in Figure 5 and Supplementary Figure 6, yeast strains were grown in 200 ml Synthetic Dextrose (SD) medium (Amberg et al, 2005) containing leucine, tryptophan, and uracil at concentrations of 20 mg/ml each at 30°C to OD₆₀₀ ≈ 1.5. Cultures were checked for frequency of [URE3] loss by streaking aliquots onto 1/2 YPD plates. [URE3] variant 1 was in all cases very stable, whereas in variants 2 and 3 roughly 50% of clones consistently reverted to [ure-o]. The cells were pelleted at 1500 x g for 5 min at room temperature, and washed twice with 40 ml ST buffer. Cells were resuspended in 25 ml ST buffer and spheroplasted with 1000 U lyticase for 1 hour at 30°C. Spheroplasts were pelleted at 250 x g for 5 min at room temperature, washed twice with 25 ml ST buffer, and resuspended in 1 ml H₂O to initiate osmotic lysis. Cell extracts were sonicated 4 times for 1 min each (Branson Sonifier 250; level 4, 20% duty cycle). The protein concentration was typically around 10 mg/ml. Cell survival was assessed by plating resulting whole cell extracts on YPAD plates, and was found to be between 0.01 and 0.1%. In order to make sure that these surviving cells did not show up as false positives in transformation experiments, we performed two control experiments. Cell extract incubated with pH125 using the standard transformation never yielded Leu⁺ transformants. And when we transformed an extract of a [URE3] ρ⁺ strain into BY241 made ρ⁻ (by growth on plates containing ethidium bromide), no [URE3] transformants were ρ⁺, indicating that they were neither surviving cells from the cell extract nor cell fusions with spheroplasts. These data were confirmed by the fact that when transformations were performed with cell extracts from strains with different auxotrophic markers, the transformants never showed these markers when tested for growth on respective selection plates.

For experiments in Figure 6, cells were grown overnight in 200 ml YPAD at 30°C to stationary phase, pelleted at 1500 x g for 5 min at room temperature, and washed twice with 40 ml H₂O. Cells were resuspended in 3 ml H₂O and a 1.5 ml aliquot was disrupted with 0.5 mm zirconia/silica glass beads 3 times for 1 min at 4°C using a Mini Beadbeater (Biospec Products). Protein concentration was typically around 50 mg/ml. Pelleted filaments that were treated in the same way did not exhibit an increase in infectivity (data not shown), indicating that the cell lysis

procedure did not disrupt filaments in the same fashion as did sonication. This suggests that Ure2p filaments in these extracts remained in their *in vivo* condition.