Supplementary Materials.

Yeast strains and plasmids.

Yeast strains were constructed using standard techniques of yeast transformation (Gietz et al. 1995), gene replacement (Rothstein 1983) and plasmid shuffling (Boeke et al. 1987). Preparation of media was done essentially as described (Sherman et al. 1974).

H2879 was derived from H1676 by integrating YIplac211-Δprt1 (pKHN44) linearized using BamH I. pKHN44 was made by digesting YIplac211 with Xma I and Xba I and ligating the isolated vector to the Xma I, Xba I fragment containing the 3'1629 bp of *PRT1* including a unique BamH I site. Transformants were selected on SC-ura and then counter selected on FOA plates to select for loss of the URA3 marker. Colonies not displaying a Ts^- phenotype were selected and further verified by PCR for having the wild type allele of *PRT1* instead of *prt1-1*.

H2880 was derived from H2879 by integrating a Bgl II-EcoR I fragment of B1756 containing a disruption cassette 5'trp1-hisG-URA3-hisG-3'trp1 to generate a *trp1*Δ by selecting for colonies on SC-ura and counter selecting on FOA to regain the URA3 marker. Cells that were screened for *trp1*Δ by replica on SC plates +/- trp

H2881 was derived from H2880 by integrating a Xba I-EcoR I fragment of B2806 containing a disruption cassette 5'gcn2-hisG-URA3-hisG-3'gcn2 to generate a *gcn2*Δ by selecting for colonies on SC-ura and counter selecting on FOA to regain the URA3 marker. Cells were screened for *gcn2*Δ by replica on SC plates +/- 40 mM 3-aminotriazole (3AT).

The construction of YAJ18-3 will be published elsewhere.

YKHN60 was made by tetrad analysis of a diploid H2881 that was generated by transformation of the haploid H2881 with B2086 encoding the endonuclease HO to switch mating types and obtain a diploid. This was followed by screening colonies by replica to SC plate +/- ura to obtain colonies that had lost the URA3 plasmid. The diploid had one allele of *PRT1* deleted, by integrating a Sal I-Xba 1 fragment of B3289 containing a disruption cassette 5'prt1-hisG-URA3-hisG-3'PRT1. After selection on SC-ura the URA3 marker was regained by selection on FOA plates. The resulting strain was transformed with pRS316-URA3-PRT1 and sporylated. Spores were analyzed by PCR to verify the *prt1*Δ deletion on the chromosome and transformed with either pRS315-LEU2-PRT1 or empty vector pRS315 and tested for FOA^S. To obtain YKHN60 the deletion strain containing pRS316-URA3-PRT1 was transformed with pRS315-LEU2-prt1-1 and colonies that had lost the URA3 plasmid was selected on FOA plates.

Other biochemical methods

The preparation of WCEs from non-cross-linked cells in the presence of heparin was conducted as described previously (Asano et al. 2000). β-galactosidase assays were conducted as described previously (Moehle and Hinnebusch 1991). Western analysis was carried out using 4%-20% gels from Biorad and the antibodies listed below. Northern analysis was conducted essentially as described (Intine et al. 2000) using the radioactive probes explained below.

Probes used in Northern analysis

Probe against *IMT4* tRNA was generated using end labeling of an oligo (5'ggtagcgccgctcggtttcgatccgaggtc 3') using T4 Polynucleotide Kinase (New England Biolabs) and radio labeled [γ-32P]ATP (Redivue 6000CI/mmol, Amersham) according to manufactures manual. The unincorporated radio labeled nucleotides were removed using MicroSpin™ G-25 spin columns (Amersham), according to manufactures manual. Probe against *RPL41A* mRNA was generated using Rediprime™ II Random Prime Labelling System (Amersham) and [α-32P]dCTP (Redivue 6000CI/mmol, Amersham), according to manufactures manual. The following primers, 5'TAAAAGAACCAGACCACATCGATTC3' and 5'GACTTGCCAAGCACAATTACAATG3' were used to obtain the template for the random labeling, by PCR on genomic DNA isolated from H1676 according to (Rose et al. 1989), 'Isolation of Genomic DNA for Southern Blot analysis. The unincorporated radio labeled nucleotides were removed using MicroSpin™ G-25 spin columns (Amersham), according to manufactures manual.

Antibodies

Antibodies against PRT1, NIP1, TIF34, eIF1, eIF5, GCD11, eIF4G (Phan et al. 1998), TIF32, TIF35 (Phan et al. 2001), SUI2 (Dever et al. 1992) have been described. Antibody against TIF11 was made in the lab against a GST-TIF11 fusion protein in rabbit (D.Olsen). HA antibody was obtained from 12CA5 ascites in the lab. HIS antibody (generated in rabbit) was obtained commercially from Santa Cruz. RPS22 antibody (generated in rabbit) was kindly provided by Jan van't Riet.

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