Supporting Information

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SI Text

Strain Construction. *Hho1*Δ carrying the *MET15* in the rDNA (AL001) was made by transformation of JS237 with a PCR fragment amplified with the PR and PF primers (Table S2) using DNA from strain Y02125 as a template, and selection on G418 containing plates. *Hho1* mutants a-e were created using the "delitto perfetto" technique (1). Briefly, JS237 was transformed with either a PCR fragment that directed a cassette carrying the *KanMX4* and counterselectable *KlURA3* genes that directed the cassette either into globular domain I or globular domain II of *Hho1*. Next, oligonucleotides were used to transform these strains on plates containing 1 mg/ml 5-fluoroorotic acid (5-FOA) to create the desired deletions. Transformants were plated on plates containing 20 μg/ml G418. Colonies growing on the 5-FOA plates, but unable to grow in the presence of G418 were selected for further study. To verify the mutant sequences, DNA isolated from these colonies was used as a template in PCR using PF and PR primers to amplify the mutant *Hho1* gene. The amplified DNA was then sequenced (Hy Laboratories, Rehovot, Israel). The oligonucleotides used for the cassette amplification (Metabion) and for the deletions are listed in Table S2.

Transcription Run-On Assay. The transcription run-on assay was performed essentially as described (2, 3). Early log phase cells (below 0.5 OD_{600}) grown in YPD were harvested and washed in 10 mM Tris-Cl pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, and then permeablized by resuspension in 0.5% sodium N-lauroyl sarcosine for 15 min. Next, transcription was allowed to proceed for 10 min following suspension of the cells in 50 mM Tris-Cl pH 7.9, 100 mM KCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 2 mM DTT, 0.5 mM ATP, 0.25 mM GTP, 0.25 mM CTP, 10 mM phosphocreatine, 12 μg/ml creatine phosphokinase and 1 mCi/ml α-^{32}P UTP at 3000 Ci/mmol. RNA was extracted by acid phenol. 10 μg plasmid DNA containing portions of the rDNA (gift of Susan Baserga, Yale University, New Haven, CT) were applied to Hybond-N (Amersham) following manufacturer's instructions. The RNA was denatured and hybridized overnight to the slot blot according to the Transcription Run-on Protocol by J. Michael Bishop at Bio.com (http://www.bio.com). Following washing, the radiolabel was visualized and quantitated using a phosphorimaging analyzer (Bio-Image Analyzer BAS-2500; Fujifilm).

RNA Extraction. Yeast cells were logarithmically grown in YPAD medium (1% yeast extract, 2% Bacto-Peptone, 2% dextrose, 100 mg/l adenine hemisulphate) at 25°C. Heat shocked cells were rapidly heated to 37°C (within 1 min) and allowed to grow for an additional 15 min. Total RNA was isolated using a hot acid-phenol procedure (4). RNA quality and integrity were determined using Eukaryote Total RNA Nano 6000 assay (Agilent RNA 6000 Nano LabChip Kit, part number 5065–4476) on the Agilent Technologies 2100 Bioanalyzer. RNA was quantified by measuring A_{260} on a Nanodrop spectrophotometer (ND-1000, NanoDrop Technologies).

Generation of Fluorescently Labeled cRNA Targets. Total RNA isolated from logarithmically growing wild type and *hho1*Δ strains was amplified and fluorescently labeled using the Agilent Technologies Low Input Linear Amplification Kit (part number 5184–3523) following the protocol described in the kit manual. The labeled cRNA was purified using Qiagen's RNeasy Kit (part number 74104). Mass yields and specific activities of the labeled cRNA targets were determined by measuring the absorbance spectra on a Nanodrop spectrophotometer.

Hybridization and Scanning of Microarrays. We used a reference design to hybridize three microarrays with the following cRNA samples labeled with Cy3 and Cy5, respectively: 1) wt25 and wt37, 2) mu37 and wt25, 3) mu25 and wt25. wt25, wt37, mu37 and mu25 represent cRNA made on RNA templates isolated from wild type cells grown at 25°C, wild type cells following heat shock at 37°C, *hho1*Δ cells following heat shock at 37°C and *hho1*Δ cells grown at 25°C, respectively.

For each hybridization, 750 ng of cyanine 3-labeled and 750 ng of cyanine 5-labeled cRNA were fragmented and hybridized to an Agilent Technologies Yeast (V2) Gene Expression Microarray (part number G4140B), using the Agilent Gene Expression hybridization kit (part number 5188–5242) as described in the manual. Following hybridization, all microarrays were washed and scanned using Agilent's dual laser DNA microarray scanner G2505B. The data were then extracted from images by using Feature Extraction 9.1 software (Agilent Technologies).

A summary of results is attached as a table showing fold change. Complete results are deposited with the Gene Expression Omnibus accession number GSE9095.

Data Analysis. The R 2.4 software and the LIMMA package (5) from the Bioconductor project (http://www.bioconductor.org) were used to normalize the microarray data using VSN normalization. Probes with average log intensities less than 5 or more than 15 were excluded from further analysis. All genes with fold change less than 1.3 were excluded from the list. The complete data set is available through the Gene Expression Omnibus (accession number GSE9095).

The above gene list was separated into two lists of up and down-regulated genes following *Hho1* disruption, and gene ontology analysis was done using the Gene Set Enrichment tool (6).

1. Storici F, Lewis LK, Resnick MA (2001) *In vivo* site-directed mutagenesis using oligonucleotides. *Nat Biotechnol* 19:773–776.
2. Elion EA, Warner JR (1986) An RNA polymerase I enhancer in *Saccharomyces cerevisiae*. *Mol Cell Biol* 6:2089–2097.
3. Gallagher JE, Dunbar DA, Granneman S, Mitchell BM, Osheim Y, Beyer AL, Baserga SJ (2004) RNA polymerase I transcription and pre-rRNA processing are linked by specific SSU processome components. *Genes Dev* 18:2506–2517.
4. Schmitt ME, Brown TA, Trumpower BL (1990) A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. *Nucleic Acids Res* 18:3091–3092.
5. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology* 3:Article 3.
6. Segal E, Friedman N, Koller D, Regev A (2004) A module map showing conditional activity of expression modules in cancer. *Nat Genet* 36:1090–1098.

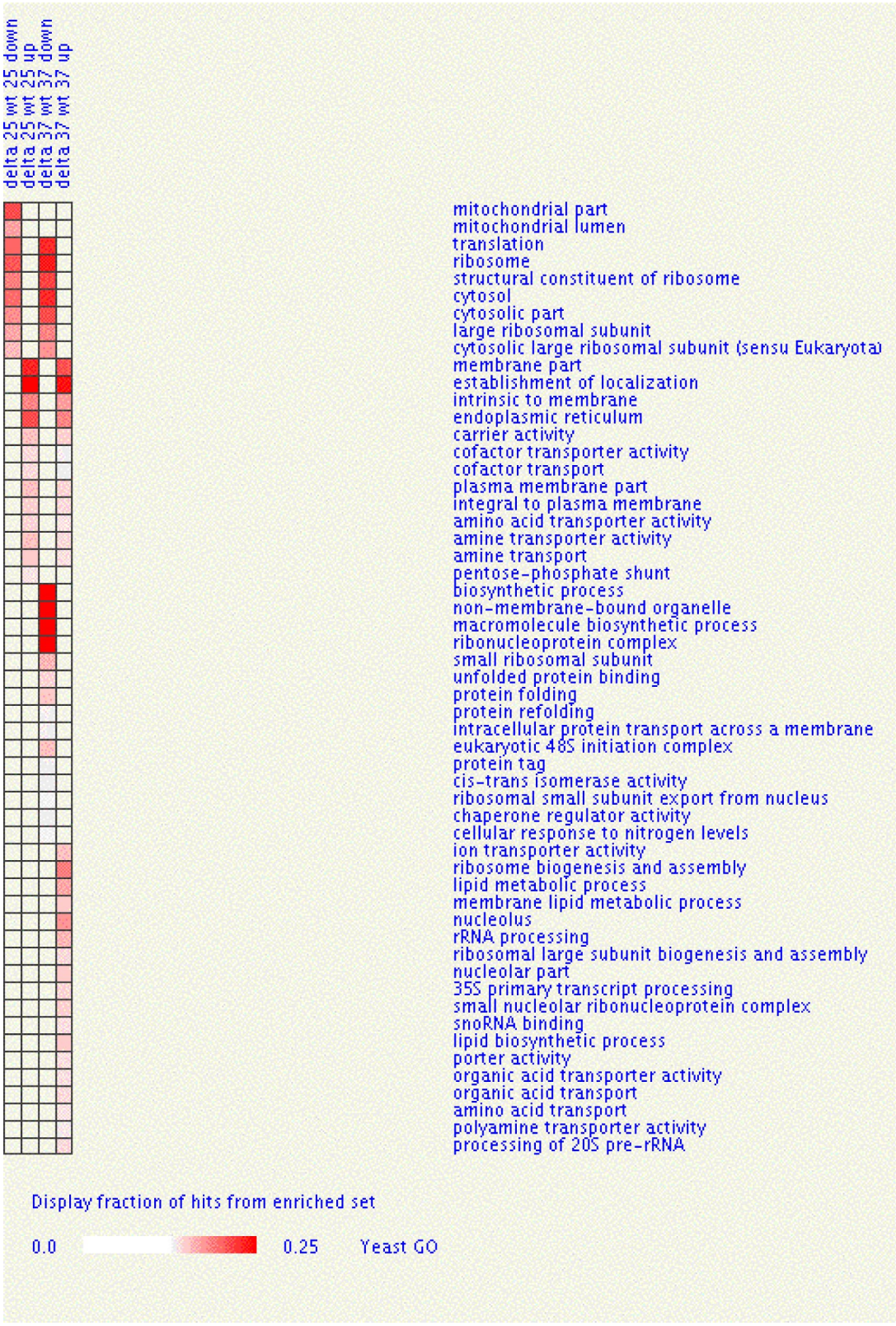
delta 25 wt 25 down
delta 25 wt 25 up
delta 37 wt 37 down
delta 37 wt 37 up
mitochondrial part
mitochondrial lumen
translation
ribosome
structural constituent of ribosome
cytosol
cytosolic part
large ribosomal subunit
cytosolic large ribosomal subunit (sensu Eukaryota)
membrane part
establishment of localization
intrinsic to membrane
endoplasmic reticulum
carrier activity
cofactor transporter activity
cofactor transport
plasma membrane part
integral to plasma membrane
amino acid transporter activity
amine transporter activity
amine transport
pentose-phosphate shunt
biosynthetic process
non-membrane-bound organelle
macromolecule biosynthetic process
ribonucleoprotein complex
small ribosomal subunit
unfolded protein binding
protein folding
protein refolding
intracellular protein transport across a membrane
eukaryotic 48S initiation complex
protein tag
cis-trans isomerase activity
ribosomal small subunit export from nucleus
chaperone regulator activity
cellular response to nitrogen levels
ion transporter activity
ribosome biogenesis and assembly
lipid metabolic process
membrane lipid metabolic process
nucleolus
rRNA processing
ribosomal large subunit biogenesis and assembly
nucleolar part
35S primary transcript processing
small nucleolar ribonucleoprotein complex
snoRNA binding
lipid biosynthetic process
porter activity
organic acid transporter activity
organic acid transport
amino acid transport
polyamine transporter activity
processing of 20S pre-rRNA
Display fraction of hits from enriched set
0.0
0.25
Yeast GO

Fig. S1.

Table S1. Quantitation of *Met15* RNA in Fig. 1 normalized to *Adh1* load control

Strain	Normalized Met15
Wild type	804
*hho1*Δ	1732
a	3000
b	958
c	4612
d	1030
e	6157
*sir2*Δ	8367

Table S2. Primers used for creation of *hho1* mutants

CGTCCGGCACTCAAGAAGTTTATCAAGGAAAACTACCCGATCGAGCTCGTTTTCGACACTGG	PCR primer for insertion of CORE into G1 domain of Hho1p
GGCATTGTTGAAGTACAAATCAAAGTTGCTTGCGGATCCGACTCCTTACCATTAAGTTGATC	PCR primer for insertion of CORE into G1 domain of Hho1p
CGTTTTAAAGAAGTATGTCAAGGACACTTTCTCCTCCAAGGAGCTCGTTTTCGACACTGG	PCR primer for insertion of CORE into G2 domain of Hho1p
GCGCTATTGAACAGATAGTCAAAATTTGAGCTTGTTTTCAATCCTTACCATTAAGTTGATC	PCR primer for insertion of CORE into G2 domain of Hho1p
GGAGAAATCAACTTCCAAGGCCGCTATCAAGAAAACCACGGCAGTCAGTCCAAAACCCA AGCAAGCCGCCACTTCTGTGAGTGC	Deletion a
GAAAGTAGTGAAAAAAAAATCGCCTACTGTTACCGCCAAGCTCTCCACGTAAAAAGTGA ATATCCAAACGAGAATGTCAATGG	Deletion b
GGCAGCGAGGGAAGCAATTATAATACAACTAAAGCAACATGAAGGCCTCTTCGCCTTCTT CATTGACCTACAAGGAAATGAT	Deletion c
GGAGAAATCAACTTCCAAGGCCGCTATCAAGAAAACCACGGCAAAGGCCTCTTCGCCTTC TTCATTGACCTACAAGGAAATGAT	Deletion d
GGCAGCGAGGGAAGCAATTATAATACAACTAAAGCAACATGGTCAGTCCAAAACCCAA GCAAGCCGCCACTTCTGTGAGTGC	Deletion e
CATTGACATTCTCGTTTGGA	PF
GCAGCGAGGGAAGCAATTATA	PR

For explanation of CORE, see ref. 1.

Table S3. Primers used to amplify control sequences in Fig. 2

AAGTAATGTCTGCTCCAGAA	RPS2 forward 5′
TTCAGTGTTTCTTGGTCCTC	RPS2 reverse 5′
AAACTTGTGGGCCGAACAA	RPS2 forward 3′
CAACAAGCTTAGAATCTCTTC	RPS2 reverse 3′
AACAATTCGTTGGCCTTTGG	ADH1 reverse 5′
TCCCAGAAACTCAAAAAGGTG	ADH1 forward 5′
AATCAAGGTTGTCGGCTTGT	ADH1 forward 3′
ATTCGCTTATTTAGAAGTGTC	ADH1 reverse 3′
TGCAAGAAAATGGTAGACGAA	RPN1 forward 5′
TCAGCCTTTCAACCAATAGC	RPN1 reverse 5′
ACGCAATCCACTCCTGTTTT	RPN1 forward 3′
TGCCCAAAAGCTATTCAGTTT	RPN1 reverse 3′
TCGAAAGATTAAAGCAAGTCA	PDC1 forward 5′
CAGCCCATCTCATACCTTCAA	PDC1 reverse 5′
TGATTGAAATCATGTTGCCAG	PDC1 forward 3′
TCGCTTATTGCTTAGCGTT	PDC1 reverse 3′

Other Supporting Information Files

Dataset S1 (PDF)
Dataset S2 (PDF)