Methods

Expression data
Individual experimental datasets (see list of published data in Supplementary material) were all put into a standardized *orf19* gene name format using conversion information provided by A. Nantel, C. D'Enfert and A. Tsong. Expression data were stored as log2 ratios. Dye swap data for the same experimental conditions were averaged when possible, resulting in a total of 198 conditions. Tab-delimited text files containing the log2 ratios are provided on our web page at http://www.weizmann.ac.il/home/barkai/Rewiring.

Definition of gene sets in *S. cerevisiae* and *C. albicans* (Figs. 1B and 2B)
S. cerevisiae gene sets were taken from a collection of transcription modules in *S. cerevisiae (1)*. The *C. albicans* orthologues of these genes were identified using the Inparanoid software available at http://inparanoid.cgb.ki.se (*2*). Only one-to-one orthologues were used in this analysis. To distinguish those orthologues that are also co-expressed in *C. albicans*, the 'raw' gene sets were further processed using the Signature algorithm, as described previously (*3, 4*). For consistency, we applied the same procedure also to the original *S. cerevisiae* gene sets (Supp. Fig.1), which yielded reduced gene sets for MRP, OXP and STR in *S. cerevisiae*. Full and refined sets of genes are listed in Supp. Tables 1 and 2, respectively. Figures 1B and 2B are based on the refined gene sets.

Gene sets in yeast phyla (Fig. 3)
Gene sets for the intermediate yeast species shown in Fig. 3 consist of the orthologues of the processed *S. cervevisiae* gene sets described above. Orthologues and upstream sequences for *S. paradoxus*, *S. bayanus* and *S. mikatae* were downloaded from http://www.broad.mit.edu/annotation/fungi/comp_yeasts (*5*). The proteins and genomic sequences of the nine additional yeast species shown in Fig. 3 were collected from the sources listed in Tab. 1:

Table 1. Sources for sequences

Species	Database	Web location
C. albicans	Stanford Genome Technology Center	ftp://cycle.stanford.edu/pub/projects/candida/
D. ansenii	Genolevures sequencing	Genbank entries CR382133 to CR382139
A. gossypiio	Ashbya Genome Database	http://agd.unibas.ch/Ashbya_gossypii/download_ashbya.fas
K. waltii	MIT Sequencing	http://www.broad.mit.edu/seq/YeastDuplication/
K. lactis	NCBI (Genolevures sequencing)	Genbank entries CR382121 to CR382126
S. catellii	SGD (WashU Sequencing)	ftp://genome-ftp.stanford.edu/pub/yeast/data_download/sequence/fungal_genomes/S_castellii/WashU/
S. glabratao	Genolevures sequencing	Genbank entries: CR380947 to CR380959
S. cerevisiae	SGD	ftp://genome-ftp.stanford.edu/pub/yeast/data_download/sequence/genomic_sequence/

Inparanoid (*2*) was used to identify the orthologues of each species with *S. cerevisiae* and the genomic sequences were used to retrieve the 600bp upstream of each gene. Only one-to-one orthologues were used in this analysis.

Data processing for Signature Algorithm
Expression data were normalized as described in reference (*4*). The element E_{gc} of the gene expression matrix contains the log-expression change of gene g in $G = \{1, ..., N_G\}$ at the experimental condition c in $C = \{1, ..., N_C\}$, where N_G and N_C denote the total number of genes and conditions, respectively. We introduce two normalized expression matrices E^G_{gc} and E^C_{gc}, which have zero mean and unit variance with respect to genes and conditions, respectively.

Frequency of RGE occurrence
Shown is the frequency of AATTTT (or reverse complement) sequence elements, observed in a window between x and x+50, where x is the position along the 600bp upstream sequence for the genes of each group. The observed number of occurrences for each gene set is divided by the number of genes assigned to the set. The red lines in Figs. 2B and 3 indicate the frequency when all genes of the genome are considered.

Western blot
All strains were derived from YJB6449 (ura3Δ::imm434/ura3Δ::imm434 his1::hist1G/his1::his1G arg4::hisG/arg4::hisG NOP1/NOP1::YFP-HIS1) (*6*) and were grown at 30C in SD medium containing 80 µg/ml uridine and 2% glucose. The promoter driving NOP1-YFP was replaced with 1000 bp of sequence 5' to the start codon of MRP7 (orf19.1906) using sequence information from the Stanford Genome Sequence Technology Center (http://www-sequence.stanford.edu/group/candida/). The MRP7 promoter was chosen because it is one of a few *C. albicans* mitochondrial ribosomal genes whose promoter included a single AATTTT sequence with minimal adjacent AT-rich sequence. The MRP7 promoter sequence was amplified using *C. albicans* genomic DNA as template. Primers for the wild-type, GC-rich (changing AATTTT to GGCGTT and AT-rich (changing AATTTT to ATATAT) versions of the promoter are listed in Table 1 below). The forward primer included an *Xho*1 site and the reverse primers included a *BamH*1 site. The PCR products were digested with Xho1 and BamH1 and were ligated into plasmid pURA3-pMET3-GFP (*7*), replacing the *MET3* promoter with the *MRP7* promoter sequence. The *MRP7* promoter sequence (wild-type, GC-rich or AT-rich) was then amplified from the appropriate plasmids using primers 1856 and 1857, which direct integration immediately upstream of the *NOP1* coding sequence in strain YJB6449(*7*). Correct integration into the NOP1-YFP tagged allele was verified by PCR and DNA sequencing.

A similar strategy was used for construction of MRPL37 and MRP10 promoters using the relevant oligonucleotides listed in Tab. 2.

Cells were grown to mid-log or stationary phase in rich medium (YPAD+uridine), harvested, lysed and analyzed on 7.5% SDS-polyacrylamide gels, transferred to PVDF (Millipore, Bedford, MA) membrane and probed with either anti-GFP antibody (Roche Applied Science, Indianapolis, IN) to detect Nop1-YFP or antibody raised against histone H4 (*8*) as a loading control. Amounts of Nop1-YFP protein were normalized to amounts of histone H4 protein in each lane.

Table 2. Oligonucleotides used for the construction of MRP7 promoter fusions with Nop1-YFP.

Name	Oligonucleotide sequence[1]	Oligonucleotide purpose
1856	CTTGTCAGACGCAAGATACGGTATTGGTCACGTGATAAC ATCCATGCACCAACGTTTGCCATCGCATCTCTCTAGAAG GACCACCTTTGATTG	Forward primer to insert promoters at NOP1 locus
1871	ccgctcgagGGTTCAGTCAATTATATGTTGATC	Forward primer to amplify all MRP7 promoters
1874	cgcggatccCGTTGTTAAATGACCAAAATAG	Reverse primer to amplify wt MRP7 promoter
1872	cgcggatccCGTTGTTAAATGACCAAAATAGTTGTGGGATTG GGTTGTTTCTCAAATAATAATATAAGTTAAA TGG**ggcgtt**TTTCTACACAAATGAAATGTTTCACC	Reverse primer to amplify MRP7 GC-rich mutant promoter
1873	cgcggatccCGTTGTTAAATGACCAAAATAGTTGTGGGATTG GGTTGTTTCTCAAATAATAATATAAGTTAAA TGG**atatat**TTTCTACACAAATGAAATGTTTCACC	Reverse primer to amplify MRP7 AT-rich mutant promoter
1839	ccgctcgagTTGGCAGTTAACAAAAACATTTGATTGATAATG	Forward primer to amplify all MRPL37 promoters
1840	cgcggatccTGTGAATGATATGTTTGTCTAACTTCTTGATGTA GATATCG	Reverse primer to amplify wt MRPL37 promoter
1838	cgcggatccTGTGAATGATATGTTTGTCTAACTTCTTGATGTA GATATCGTGAGCTAGATAAACTGTTAGAGTTAGGTATGG GTG**ggcgtt**TTCTTTCTCAGTTTCTGTTATTAAGTTCG	Reverse primer to amplify MRPL37 GC-rich mutant promoter
1854	cgcggatccTGTGAATGATATGTTTGTCTAACTTCTTGATGTA GATATCGTGAGCTAGATAAACTGTTAGAGTTAGGTATGG GTG**atatat**TTCTTTCTCAGTTTCTGTTATTAAGTTCG	Reverse primer to amplify MRPL37 AT-rich mutant promoter
1867	CcgctcgagCTTTGTATAATAGGAGATAAACCC	Forward primer to amplify all MRPL10 promoters
1870	CgcggatccTAGACAACAAGTTTATGTGGGATGTATAATC	Reverse primer to amplify wt MRPL10 promoter
1868	CgcggatccTAGACAACAAGTTTATGTGGGATGTATAATCGT TGTAAACCTTTGGTTATAATTTTGTTCAGAAAAG**ggcgtt**TT CACGCTTGATACTCAGTGCCAGAAAAAAAATATAC	Reverse primer to amplify MRPL10 GC-rich mutant promoter
1870	cgcggatccTAGACAACAAGTTTATGTGGGATGTATAATCGT TGTAAACCTTTGGTTATAATTTTGTTCAGAAAAG**atatat**TTC ACGCTTGAACTCAGTGCCAGAAAAAAAATATAC	Reverse primer to amplify MRPL10 AT-rich mutant promoter

All oligonucleotides were purchased from IDT Biosciences. Nucleotides in lowercase, bold font are those that replace the AATTTT sequence.

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