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Materials and Methods

Yeast Strains and Growth Conditions. For microarray analysis and confirmation of microarray hits by Northern blot, wild-type (YPH499; ref. 1) and congenic *mot1-14* and *mot1-42* yeast strains were grown in rich medium (yeast extract/peptone/dextrose) at 30°C to an OD600 ~1.0, then shifted to 35°C for 45 min, and cells were harvested. To ensure a rapid and reproducible shift to 35°C, 30°C cell cultures were combined with an equal volume of culture prewarmed to 40°C. Cells were shifted to 35°C because this is the highest temperature that supports growth of our wild-type strain, YPH499. Descriptions of the *mot1* strains used in this study will be described in detail elsewhere, but briefly, the *mot1-14* allele encodes a premature stop codon in place of the codon for W496; cells with the *mot1-14* allele grow poorly at 30°C and virtually undetectably at 35°C. Slow growth at 30°C is attributable to a small degree of translational suppression of the premature stop codon, and cells expressing *mot1-14* contain no detectable immunoreactive Mot1 protein. Thus, the *mot1-14* strain mimics conditions of nearly complete loss of Mot1 protein. *mot1-42* encodes L383P; strains harboring this allele grow at near wild-type rates at 30°C but are growth-impaired at 35°C. For comparison, RNA was obtained from the following YPH499-derived strains: WCS132 (*tsm1*; ref. 2), JR374 (*taf145-869*; ref.3; Joe Reese, personal communication), and SHY258 (*toa2-3*; ref. 4). RNA was also prepared from JS306 (*BNA1+*) and JS663 (*bna1-delta*; ref.5) cells grown in the same way. The experiment in Fig. 3 was performed by using haploid yeast cells with a deletion of the chromosomal copy of *MOT1*, *mot1-42* carried on pRS313 (1) and *MOT1+*, *mot1-K1303A* (both pRS315-derived), or pRS315 (1) plasmid vector alone. Alternatively, RNA was analyzed from haploid strains containing plasmid-borne *mot1-42* and a second plasmid in which expression of *mot1-K1303A* was under control of the *GAL1* promoter. In this case, *mot1-K1303A* expression was induced by growth of cells in synthetic media (to select for plasmids) containing 2% galactose and 0.5% sucrose; cells were heat-shocked and harvested as above.

RNA Isolation and Northern Blots. Total yeast RNA was isolated by using a hot acid phenol extraction protocol (6. Poly(A)+ RNA was prepared from total RNA by using a Qiagen Oligotex Midi Kit according to the instructions supplied by the manufacturer. For Northern blots, 5-20 μg total RNA was separated by electrophoresis in formaldehyde agarose gels, transferred to nitrocellulose, and probed with random-primed DNA probes obtained from the cloned genes or by PCR amplification of portions of the ORFs for the genes of interest. The *ACT1* probe was derived from the ~1.1-kb *Xho*I–*Hin*dIII fragment containing the *ACT1* gene, the *HSP26* probe was derived from the ~3-kb *Pst*I–*Bam*HI fragment containing the *HSP26* gene, and the MET15 probe was obtained from the 715-bp *Eco*RI–*Hin*dIII fragment containing a portion of the *MET15* ORF. Probes for other genes were obtained by PCR of genomic DNA. The *THI5* probe probably crosshybridizes to messages derived from *THI11* and *THI13*, which are nearly identical. Blots were hybridized overnight at 42°C in 50% formamide and washed twice in 0.1× SSC, 0.1% SDS

at room temperature for 15 min each followed by washing twice in the same buffer at 50°C for 15 min each. Bands were detected by autoradiography and band intensities were quantitated by using a Phosphorimager.

Microarray Hybridization Experiments. cDNA microarray chips containing 6,024 yeast ORFs were prepared as described (7, 8). Briefly, primers specific for each ORF (Research Genetics, Birmingham AL) were used to amplify yeast ORFs from genomic DNA in a 100-μl PCR using Amplitaq or Pfu polymerases. The PCR products were run on 2% agarose gels to ensure quality of the reaction products and purified by ethanol precipitation. The purified cDNAs were resuspended in ArrayIt buffer (Telechem, San Jose, CA) and spotted onto poly-L-lysine-coated glass slides by using a modified, robotic DNA arrayer (Beecher Instruments, Bethesda). Poly(A)+ RNA (2–4 μg) was labeled with Cy3 and Cy5-conjugated dUTP (Amersham) by using a reverse transcription reaction and hybridized to the yeast cDNA microarray chip (7). cDNA chips were scanned by using an Axon Scanner (Axon Instruments, Foster City, CA), and images were analyzed by using the Microarray Suite Software (Scanalytics, Fairfax, VA). The relative fluorescence intensity was measured for each labeled RNA and a ratio of the values for the intensity of each fluor bound to each probe was calculated. The amount of autofluorescence generated in the Cy3 channel was measured and a minimum intensity cut-off was set just above this value. The distribution of the ratio of all of the genes was calculated and intensity ratio values that differed from the median with a confidence interval of greater than 95.0% (9) were scored as significant changes. The same RNA was labeled and hybridized in three independent reactions. The data for each array was normalized by using the mean of all of the targets on the array, and the coefficient of variance for each hybridization was less than 0.3. A database tool, MAPS (10) was used to compile the overall list of consistent, significantly changed genes across the multiple hybridizations. Analysis of the data at the 95% confidence level (not shown) indicated that *MOT1* controls the expression of 421 genes, 59 of which are activated by *MOT1* in wild-type cells. Thus, a less conservative statistical treatment suggests that the number of *MOT1*-activated genes in the 99% confidence data set may be an underestimate.

Analysis of Microarray Data. MOT1-controlled genes were functionally grouped manually by annotation of known features for each gene available from the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces/), the Munich Information Center for Protein Sequences (http://vms.mips.biochem.mpg.de), and the *Saccharomyces cerevisiae* Proteome Database (http://www.proteome.com). The alpha-factor time series (11) and diauxic shift expression pattern (7) were examined for each MOT1-regulated gene. The overlap between MOT1-repressed genes and genes induced during the diauxic shift was initially observed because a number of genes in the MOT1 data set displayed a log2 (ratio) 1 at 18.5 and/or 20.5 h in the diauxic shift time course. A number of MOT1-repressed genes were classified as alpha-factor-induced because they displayed a log2 (ratio) 1 at some point between 48 and 120 min after addition of alpha-factor.

To obtain quantitative estimates of the degree of overlap between the MOT1 microarray data and other data sets, the following analyses were performed. Microarray data for the diauxic shift and

TUP1 were taken from http://cmgm.stanford.edu/pbrown/explore/index.html. Ratios of induction or repression were taken directly from the data sets. Alignments of data sets were done in Microsoft Excel 5 and checked by using the comparative function [= if (*xn* = *yn*,0,100)], where *n* is the number of a row, and *x* and *y* are columns containing the ORF names that correspond to each data point (ORF names and microarray results from each data set are kept in adjoining columns). The function reports a value of zero when the ORF names are identical; when the summation of this function over all 6,000+ rows was zero, the alignment was complete. Entries in one data set that were not present in the other data set were deleted. In many cases the same ORF would appear two or three times in one data set; the second and third instances were discarded, except that for some of the diauxic shift data, multiple occurrences of a gene were averaged, using the functions [= exp((ln(*a*) + ln(*b*))/2)] or [= exp((ln(*a*) + ln(*b*) + ln(*c*))/3)], where *a*, *b*, and *c* are cells containing replicate results. For the purpose of counting genes significantly affected in a given microarray, another Microsoft Excel function was used: [= if (*x*2,1,0)] or [= if (*x*<0.5,1,0)], where *x* is a cell containing an induction/repression ratio. Genes affected significantly in two microarrays could be counted by using functions such as [= if (if (*xn*2,1,0) + if (*yn*2,1,0)1,1,0)], where *x* and *y* are columns containing induction or repression ratios, and *n* is a row. Summation of these functions over all rows gives the total number of genes in the intersection.

Microarray data for TAF145, TSM1, GCN5, and SPT3 was taken from the site http://web.wi.mit.edu/young/pub/expressionanalysis.html. These data were not reported in the form of ratios, but as intensities. To adjust for fluctuations in the readings, the operation [= if (*x*<10,10,0)] (where *x* is a cell) was used to set the baseline to 10, as recommended at the same web site. Repression ratios were then computed, and data sets were aligned as above. For many genes, the computed ratio is exactly equal to one; this was taken as an indicator that neither in the normal nor in the mutant strain did the intensity of the hybridization rise above the baseline, and these genes were removed from the alignment. The data for NC2 (BUR6) is available at the same web site; it had, however, already been edited so that the baseline was at 20. Genes for which neither reading was above baseline were again discarded.

Correlation coefficients were calculated by using the Excel CORREL function. Venn diagrams were constructed in Deneba Canvas 5 (Miami) as follows: first, a circle was drawn for one of the data sets; succeeding figures were made by scaling the first circle appropriately, then reshaped by eye to make the intersects the correct sizes. Areas of all shapes and intersections were checked in the Object Info window; errors were mostly in the range of 10%, but rising above 20% for some of the smaller objects and overlaps. The pie chart was made in Kaleidagraph (Synergy Software, Reading, PA).

Yeast stress response data used in making the expression tree were taken from http://genome-www.stanford.edu/yeast_stress/ (12). Cluster and Treeview programs are available at Michael Eisen's web site (http://rana.lbl.gov/EisenSoftware.htm).

The promoter sequences of the MOT1-activated genes were analyzed by using the Wconsensus

algorithm (http://ural.wustl.edu/-jhc1/project/Server/basic_wconsensus.cgi) with default parameters; no shared DNA sequence elements were identified with statistically significant expected frequencies (not shown).

Chromatin Immunoprecipitation. Chromatin immunoprecipitation was performed as described with minor modifications by using strains containing TAP-tagged (13) or untagged MOT1. Chromatin was prepared as described (14) with the following modifications. Cells were grown in 100 ml of yeast extract/peptone/dextrose to an OD_{600} of ~1.0 and were treated with 1% formaldehyde for 15 min at room temperature with occasional swirling. Cells were immediately harvested and washed twice with cold TBS (20 mM Tris-HCl, pH 7.5/150 mM NaCl) and once with FA-lysis buffer (50 mM Hepes-KOH, pH 7.5/140 mM NaCl/1 mM EDTA/0.1% sodium deoxycholate/1% Triton X-100/1 mM PMSF/1 μg/ml leupeptin/1 μg/ml pepstatin A). Cells were then resuspended in 800 ml FA lysis buffer and transferred to 2 ml screw-capped tubes. An equal volume of acid-washed glass beads (425–600 microns) was added and the cells were disrupted in FastPrepTM FP120 (Savant) at 4° C. The cell lysate was subsequently sonicated to yield an average DNA fragment size of 500 bp (range 100 to 700 bp). The cell debris was removed by centrifugation at 14,000 × *g* for 5 min. The lysate was transferred to another tube and further centrifuged at 14,000 × *g* for another 15 min to yield the chromatin solution ready for immunoprecipitation. Formaldehyde-crosslinked chromatin solution from the untagged and epitope-tagged MOT1 strain was incubated with 20 μl IgG sepharose beads (1:1 slurry equilibrated with FA-lysis buffer) for 2 h at 4°C on a rotator. The beads were then recovered by centrifugation at 14,000 × *g* for 15 s. The beads were washed twice for 5 min in 1.4 ml FA lysis buffer, twice in 1.4 ml FA-lysis buffer with 500 mM NaCl, and once in 10 mM Tris-HCl, pH 8.0/250 mM LiCl/0.5% NP-40/0.5% sodium deoxycholate/1 mM EDTA. The immunoprecipitated material was eluted with 190 ml of 2% SDS, 0.1 M $NaHCO_3$ at room temperature. This elution step was repeated once more and the eluates were combined.

To reverse crosslinks NaCl was added to 250 mM and the samples were incubated at 65°C for 5 h. About 10% of chromatin solutions with reversed crosslinks were reserved for later analysis as input controls. The protein and DNA was ethanol precipitated overnight at –20°C. After centrifugation at 14,000 × *g* at 4°C for 15 min, the pellet was washed with 70% ethanol and air dried and resuspended in 180 ml of TE (10 mM Tris-HCl, pH 8.0/1 mM EDTA). RNase was added to 20 μg/ml and the samples were incubated at 37°C for 30 min. After RNase digestion, 20 μl of 10× proteinase K digestion buffer (0.1 M Tris, pH 8.0/50 mM EDTA/5% SDS) and 1 μl of 20 μg/μl of proteinase K was added and the samples were incubated at 42°C for 2 h. After extraction with phenol-chloroform-isoamyl alcohol and chloroform, DNA was ethanol-precipitated overnight at –20°C in the presence of 20 μg glycogen and resuspended in TE buffer. Quantitative PCR was performed by using approximately 1/100 of the material recovered after the immunoprecipitation and 1/10,000 of the input DNA. Typically the PCRs were carried out in 50-μl reaction mixture (20 mM Tris-HCl, pH 8.4/1.5 mM $MgCl_2$/ 0.2 mM each dNTP/1 mM each primer/2.5 units Taq polymerase (GIBCO/BRL). The PCR was performed as follows: 2 min at 94°C, then 26 cycles of 30 s at 94°C, 1 min at 52°C and 1 min at 72°C; followed by a final

extension of 7 min at 72°C. All PCR products were separated on 15% polyacrylamide gels, which were stained with ethidium bromide and visualized with an AlphaImager digital camera (Alpha Innotech). AlphaEase program, version 4.0 was used to quantify the captured ethidium bromide-stained image. Quantification was performed by calculating the difference in band intensities between the tagged and untagged samples, normalized to the band intensities obtained by using the input samples. The normalized PCR signal obtained using primers for MOT1-controlled promoters was roughly 3-fold greater than the signal obtained using primers for other promoters or ORFs (except the ACT1 ORF), and the analysis was performed at least three times with two independently prepared batches of chromatin.

PCR was performed with the following pairs of primers (PCR product sizes in parentheses):

Northern probes: 5'-ACTACACCAATTAATATCGACAAATG-3' and 5'-ATTAGATTGAGGGCGTGCGTA-3' (*BNA1*, 522 bp); 5'-GACAGCCAGTTTAACTACCAAGTTCT-3' and 5'-TTCAACTTCCCACGGAACTGAT-3' (*URA1*, 933 bp); 5'-ATCAAAGCTACGGCGGTGTATT-3' and 5'-CCCTGTGTATTTGTTAAATTGTTCAC-3' (*SGA1*, 1,439 bp); 5'-TCTTTCGCTCATTTTACCTACCTG-3' and 5'-ACATTGCAAGCAACTGCCAT-3' (*AGA1*, 2,156 bp); 5'-CTCATCGTGGCATCTTTGTT-3' and 5'-TCAGGGGCAGTAGTTAGATCAT-3' (*TSL1*, 887 bp); 5'-GTCAAAGGCAGTAGGTGATTTAGG-3' and 5'-TAAGCTTGGTAGGTTGAGGAAGA-3' (*GND2*, 1,476 bp); 5'-CCGCTCGAGAAATGTTAGTTTTATCCTTGA-3' and 5'-CGGGATCCTTACAACAATCTCTCTTCGAAT-3' (*INO1*, ~1.67 kb); 5'-CCCAAAAAAAGTTTTACTCGCTC-3' and 5'-TAAAGCGTCGATGGATCTTACG-3' (*YDR533C*, 700 bp); 5'-GAGGAAGCTAAATCCAGCTTTAGA-3' and 5'-CCGTACCTTTTCCAATTTTCA-3' (*YDR539W*, 1,500 bp); 5'-TGTCAGAACCTTCAGAGAAAAAACA-3' and 5'-TCTTCAACCAGTTTGTACAGTGC-3' (*YGR043C*, 991 bp); 5'-TGTAACCAAATACTTTTACAAGGGTG-3' and 5'-AATTGTAGGCTTTGGTTCCG-3' (*YHR087W*, 326 bp); 5'-ACAAGATCACATTTTTGTTGAACTG-3' and 5'-CTGGAAGAGCCAATCTCTTGAA-3' (*THI5*, 1,008 bp).

Chromatin immunoprecipitation: 5'-TATTCTTTGATTGCGCTGCC-3' and 5'-CGATTTTTTTGGTAAATGTATGC-3' (*BNA1* promoter, 336 bp); 5'-GAAATGAAGATTCTTGTTCATGTG A-3' and 5'-TGTTGCTGAGATTTGTGACGGT-3' (*RPL5* promoter, 344 bp); 5'-CCTTTTGTTCTTCACGTCCTTTT-3' and 5'-CGACAACAGAACAAGCCAAA-3' (*INO1* promoter, 292bp); 5'-GGGAAAAAAGGAAAAGGAGCA-3' and 5'-GTTTGGTACGGAAGT TCAATTTT-3' (*URA1* promoter, 490 bp); 5'- AACTCCGTGTGTACCCCTAACT-3' and 5'-GTTTGTTTGTTTGCTTTTTTGG-3' (*HSP26* promoter, 479 bp); 5'-AACGTAAAATAAATAATACTGTTC-3' and 5'-AAGCTGAGGTTACAAGACTATGAG-3' (*SAN1* promoter, 326 bp); 5'-TCCTTATCGGATCCTCAAAACC-3' and 5'-CAGTAAATTTTCGATCTTGGGAAG (*ACT1* promoter, 479 bp); 5'-

TGTTCGTGCATTTTACACTCG-3' and 5'-AACTTCCCACGGAACTGATCTA-3' (*URA1* orf, 231 bp); 5'-ACATTCATTGCGGGAGACGA-3' and 5'-CCGACGGGCTTCATATATATTTGA-3' (*INO1* orf, 287 bp); 5'-CTGTGGGTATTGTTGTGGAACA-3' and 5'-ATTAGATTGAGGGCGTGCGTA-3' (*BNA1* orf, 218 bp); 5'-CTCACTGAACAACAACGCACTCTC-3' and 5'-CTCACTGAACAACAACGCACTCTC-3' and 5'-ATCTGTTCGCCAGAACTTGCATTT-3' (*SAN1* orf, 358 bp); 5'-TTAATAACATTCAGACATTATTGAAA-3' and 5'-CCTTCATATTAAGGAAACAACTCCTC-3' (*RAD16* orf, 187 bp); 5'-CTACCTCACGCCATTTTGAGAA-3' and 5'-AGTGATGACTTGACCATATGGAA-3' (*ACT1* orf, 237 bp).

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