A Presumptive Helicase (*MOT1* Gene Product) Affects Gene Expression and Is Required for Viability in the Yeast *Saccharomyces cerevisiae*

JENNIFER L. DAVIS,† RIYO KUNISAWA, AND JEREMY THORNER*

Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, Room 401, Barker Hall, University of California, Berkeley, California 94720

Exposure of a haploid yeast cell to mating pheromone induces transcription of a set of genes. Induction is mediated through a *cis*-acting DNA sequence found upstream of all pheromone-responsive genes. Although the *STE12* gene product binds specifically to this sequence element and is required for maximum levels of both basal and induced transcription, not all pheromone-responsive genes are regulated in an identical manner. To investigate whether additional factors may play a role in transcription of these genes, a genetic screen was used to identify mutants able to express pheromone-responsive genes constitutively in the absence of Ste12. In this way, we identified a recessive, single gene mutation (*mot1*, for modifier of transcription) which increases the basal level of expression of several, but not all, pheromone-responsive genes. The *mot1-1* allele also relaxes the requirement for at least one other class of upstream activating sequence and enhances the expression of another gene not previously thought to be involved in the mating pathway. Cells carrying *mot1-1* grow slowly at 30°C and are inviable at 38°C. The *MOT1* gene was cloned by complementation of this temperature-sensitive lethality. Construction of a null allele confirmed that *MOT1* is an essential gene. *MOT1* resides on chromosome XVI and encodes a large protein of 1,867 amino acids which contains all seven of the conserved domains found in known and putative helicases. The product of *MOT1* is strikingly homologous to the *Saccharomyces cerevisiae* SNF2/SWI2 and RAD54 gene products over the entire helicase region.

Coordinate transcriptional regulation of an appropriate set of genes is an essential feature of the response of cells to extracellular signals. In eukaryotes, such concurrent expression of a gene set often involves specific upstream DNA sequence elements and regulatory proteins that bind to these elements and interact in a complex manner with general transcription factors. One useful system for investigating such regulation is the mating response pathway of the unicellular eukaryote *Saccharomyces cerevisiae*. Haploid cells of this yeast exist as one of two cell types (*MATa* or *MATα*). Each cell type secretes a small oligopeptide pheromone (*MATa* cells secrete a-factor; *MATα* cells secrete α-factor) which acts on cells of the opposite mating type to induce a programmed set of physiological changes, including arrest of cell growth in the G_1 phase of the cell cycle, synthesis of cell surface agglutinins, and characteristic alterations in cell morphology. Collectively, these changes permit cell fusion and diploid formation (for a recent review, see reference 68).

Response to mating pheromone involves the transcriptional activation (and, in some cases, repression) of a set of genes, termed the pheromone-responsive genes, which initiate the mating program (16, 68, 69). The product of the *STE12* gene is a protein which binds specifically (17, 19) to an 8-bp upstream activating sequence (UAS) found in all pheromone-responsive promoters in one or more copies and in either orientation (44, 74). This UAS, called the pheromone response element (PRE), has the consensus sequence 5'-ATGAAACA-3' (44, 74).

Although the Ste12 protein is required for both basal and induced transcription from pheromone-responsive genes (23, 24), the requirement for Ste12 in basal transcription is not absolute. Fields et al. (23) demonstrated that several pheromone-responsive genes produced markedly lower, but readily detectable, levels of mRNA in a *ste12* null strain than in an isogenic *STE12*+ control, whereas in the same *ste12* strain, transcription from other pheromone-responsive genes was undetectable. In addition, Errede and Ammerer (19) observed that the apparent composition of Ste12-dependent DNA-binding complexes varied with the pheromone-responsive promoter examined. These findings suggest that not all pheromone-responsive genes are regulated in an identical manner and that additional factors may exist which differentially modulate transcription of various members of this set of genes.

To expand our understanding of the components that govern expression of pheromone-responsive genes, we devised a genetic screen to identify factors which operate downstream of, or independently of, Ste12. Here we describe the isolation of a mutation which increases basal-level expression of several pheromone-responsive genes in the absence of Ste12 and in the absence of pheromone. We have isolated the corresponding wild-type gene and determined that it encodes an unusually large, previously unidentified protein that is required for cell growth and viability.

MATERIALS AND METHODS

Strains, media and genetic methods. The yeast strains used in this study are listed in Table 1. Genetic manipulations and culture conditions were those described by Sherman et al. (63) except that twice the recommended levels of nutritional supplements were used in the synthetic media. Plates containing 0.02% 5-bromo-4-chloro-3-indolyl-β-D-galactoside

---

TABLE 1. Yeast strains used

Strain	Genotype	Source
EG123	*MATa leu2-3,112 ura3-52 trp1-1 his4-519 can1-101*	S. Fields
SF167-5a[a]	EG123 *ste12::LEU2*	S. Fields
1788	*MATa/α leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-1/trp1-1 his4-519/his4-519 can1-101/can1-101*	D. Levin
JD195[b]	*MATα* isogenic to EG123	This work
JD209[c]	JD195 *ste12::LEU2*	This work
JD100	SF167-5a *mot1-1*	This work
JD102C[d]	*MATα mot1-1 leu2-3,112 ura3-52 trp1-1 his4-519 can1-101*	This work
JD22/23[e]	*MATa/α mot1-1/mot1-1 leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-1/trp1-1 his4-519/his4-519 can1-101/can1-101*	This work
JD215A[f]	*MATα* isogenic to JD102C	This work
JD215B[f]	*MATa* isogenic to JD102C	This work
JD227A[f]	*MATa ste12::LEU2* isogenic to JD102C	This work
JD216D[f]	*MATα* isogenic to JD227A	This work
YPH499[g]	*MATa ura3-52 lys2-801^{am} ade2-101^{oc} trp1-Δ63 his3-Δ200 leu2-Δ1*	P. Hieter
YPH501[g]	*MATa/α ura3-52/ura3-52 lys2-801^{am}/lys2-801^{am} ade2-101^{oc}/ade2-101^{oc} trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*	P. Hieter

[a] Construction of this strain is described elsewhere (25).

[b] Constructed by DNA-mediated transformation of EG123 with a plasmid-borne copy of the *HO* gene (35, 61).

[c] Constructed by the one-step gene replacement method (60) using a *ste12::LEU2* plasmid (pLB1367), generously provided by G. Ammerer.

[d] A haploid segregant derived from the second backcross of strain JD100 carrying p*STE12(URA3)* to EG123 (see Materials and Methods).

[e] Two haploid segregants of opposite mating type derived as described in footnote *d* were mated.

[f] Haploid segregants derived from a backcross of JD102C to SF167-5a carrying p*STE12(URA3)* (see Materials and Methods). Prior to sporulation of the final resulting diploid, cells were grown nonselectively and plated on medium containing 5-fluoroorotic acid (4) to identify those cells that had lost p*STE12(URA3)*.

[g] Derivation of these strains is described in detail elsewhere (65).

TABLE 2. Gene probes used

Gene	Probe	Reference(s)
CMD1	0.8-kb *Eco*RI-*Sty*I fragment	13
DED1	1.8-kb *Bam*HI fragment	70
FUS1	0.5-kb *Nhe*I-*Sph*I fragment	72
MFa1	1.6-kb *Bam*HI fragment	6
MFa2	1.1-kb *Hpa*I fragment	6
PKC1	23-base specific oligonucleotide	48
STE2	14-base specific oligonucleotide	9, 52
STE6	0.8-kb *Spe*I fragment	45
SST2	1.3-kb *Cla*I-*Nhe*I fragment	12, 15

Plasmid constructions and gene probes. All recombinant DNA methods were performed by standard procedures (50). Plasmid pLGΔ178 (29) was digested within the *URA3* marker with *Apa*I, converted to flush ends by incubation with T4 DNA polymerase, and ligated to a *Bgl*II fragment containing the *TRP1* marker, excised from plasmid p330 (73), and converted to blunt ends by incubation with deoxyribonucleotides and the Klenow fragment of *E. coli* DNA polymerase I, to generate plasmid pLGΔ178T. Plasmid pLGΔ178T confers tryptophan, but not uracil, prototrophy to recipient *trp1 ura3* yeast cells. To construct pJD11, an *Xho*I cassette containing eight tandem consensus PRE boxes (provided by F. Gimble) was inserted into the *Xho*I site of pLGΔ178T. (The pJD11 plasmid contains two tandem head-to-tail inserts with the PRE consensus sequences on the noncoding strand.) Plasmid p*STE12(URA3)* contains a *Sac*I fragment bearing all but the most C-terminal 18 amino acids of the *STE12* coding sequence (19), isolated from plasmid pLB1212 (generously provided by G. Ammerer), inserted into pRS316 (65). Plasmid p*STE12(URA3)* fully restores mating competence to *ste12* strains (data not shown).

DNA probes used in this study are listed in Table 2. After cleavage with the indicated restriction enzymes, DNA fragments were purified by gel electrophoresis, recovered by electroelution, and labeled by the random-primer (hexanucleotide primers; Pharmacia) labeling method (22) and [α-^{32}P]dCTP (Amersham). Oligonucleotide probes were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (Amersham). Excess unincorporated label was removed from the probes by using NICK columns (Pharmacia). Restriction enzymes and DNA-modifying enzymes were purchased from Stratagene, Pharmacia, Boehringer-Mannheim, or New England Biolabs and used according to the manufacturers' recommendations.

Isolation of suppressors of *ste12*. Multiple cultures of strain SF167-5a carrying pJD11 were grown under selective conditions (no Trp) to ~2 × 10⁸ cells per ml and treated with various amounts of ethyl methanesulfonate (Kodak) according to standard protocols (63). Mutagenized cells were plated on synthetic complete medium lacking Trp at cell densities to yield isolated single colonies. Plates were incubated at 17°C (for 7 days), 30°C (for 3 days), or 37°C (for 2 days) and then replica plated onto X-Gal-containing plates. The indicator plates were incubated at 30°C for 1 to 2 days, and colonies were visually inspected for blue color. The *mot1-1* mutation described here was isolated from a culture of cells mutagenized to 50% survival and initially plated at 30°C. Plasmid p*STE12(URA3)* was introduced into the original *mot1-1* mutant strain (JD100), which was then backcrossed two times to EG123; each time, slowly growing, STE⁺ spores were selected and examined for temperature sensitivity and blue color on X-Gal medium. It was observed that

(X-Gal) (Bachem) were prepared by using synthetic medium buffered to pH 7.0 with 50 mM potassium phosphate. DNA-mediated transformation of yeast cells was performed by the lithium acetate procedure (39). Plasmid and genomic DNAs were isolated from yeast cells by the method of Hoffman and Winston (38), and plasmids were introduced into *Escherichia coli* by the method of Chung and Miller (10).

E. coli HB101 (5) was used for the propagation of most plasmids. Strains DH5αF' (33) and JM101 (51) were used for the propagation of certain plasmids and for the preparation of single-stranded and double-stranded DNAs for sequencing. Standard methods and culture conditions were used (50).

Chromosomal assignment was determined by hybridization of a *MOT1* probe to intact yeast chromosomes that were separated by pulsed-field gel electrophoresis (75) and transferred to nitrocellulose. Assignment to chromosome XVI was confirmed (i) by rehybridizing the same filter (after stripping and reexposing to film to ensure complete removal of the probe) with a gene probe (*PEP4*) known to reside on chromosome XVI (41) and (ii) by hybridizing the *MOT1* probe to an ordered collection of yeast genomic DNA fragments (in a bacteriophage λ vector) (55), generously provided by L. Riles and M. Olson (Washington University, St. Louis, Mo.).

TABLE 3. Effect of *mot1-1* on gene expression

Strain	Relevant genotype[a]	Plasmid (UAS)[b]	β-Galactosidase (U)[c]	
			−α-MF	+α-MF
SF167-5a	*MATa ste12::LEU2 MOT1*+	pJD11 (PRE)	Und	Und
JD227A	*MATa ste12::LEU2 mot1-1*	pJD11 (PRE)	1.1	0.5
EG123	*MATa STE12*+ *MOT1*+	pJD11 (PRE)	21.3	121.6
JD215B	*MATa STE12*+ *mot1-1*	pJD11 (PRE)	48.5	156.9
SF167-5a	*MATa ste12::LEU2 MOT1*+	pLGΔ178T (none)	Und	Und
JD227A	*MATa ste12::LEU2 mot1-1*	pLGΔ178T (none)	0.3	0.5
EG123	*MATa STE12*+ *MOT1*+	pLGΔ178T (none)	Und	Und
JD215B	*MATa STE12*+ *mot1-1*	pLGΔ178T (none)	0.6	0.5

[a] All strains are isogenic except at the *STE12* and *MOT1* loci.
[b] pLGΔ178T is a *cyc1* promoter-*lacZ* fusion lacking the normal UAS *cyc1*; in pJD11, synthetic PRE elements are inserted in place of the UAS *cyc1*.
[c] Values are averages from assays performed in duplicate (range, ≤30%). Und, undetectable (<0.1 U after a 6-h incubation); α-MF, α-factor mating pheromone.

the two growth phenotypes (slow growth at 30°C and lethality at 38°C) and the transcriptional phenotype always cosegregated. A final backcross with SF167-5a carrying p*STE12* (*URA3*) was performed to generate the strains used for the majority of the experiments presented here.

Procedures for phenotypic characterizations. For β-galactosidase assays, cultures were grown to early exponential phase in synthetic complete medium lacking Trp, buffered at pH 3.5, to select for maintenance of the *lacZ* reporter plasmid and were split into two portions. To one culture of each set, α-factor (Peninsula Laboratories) was added to a final concentration of 2.5 μM, and both the treated and untreated cultures were incubated at 30°C for 60 min. Cells were harvested and assayed for β-galactosidase as described elsewhere (31, 58). Independent assays were performed on duplicate transformants, and the data were averaged; the range observed was usually less than 10% and never greater than 30%.

Bioassays of a-factor production were performed as described by Kuchler et al. (45).

Quantitative mating assays were performed by mixing ~10^7 exponential-phase cells of the strain to be examined with ~10^8 exponential-phase cells of a tester strain of the opposite mating type, either DC17 (*MATα his1*) or DC14 (*MATa his1*), on a nitrocellulose filter. Filters were washed with YPD and then incubated on a YPD plate at 30°C for 5 to 6 h. Cells were then washed off the filters and resuspended in water. Dilutions of cells (in the case of *STE12*+ matings) or all cells (in the case of *ste12* matings) were plated on minimal plates selective for diploids. Mating frequency is defined as the total number of diploids produced divided by the total number of input haploids of the strain examined.

Isolation of poly(A)+ RNA and hybridization analysis. Cultures were grown to mid-exponential phase in YPD (pH 4.0) and split into two equal portions. One half of each culture was treated with α-factor (Peninsula Laboratories) at a final concentration of 2.5 μM at 30°C for 40 min; the other half was placed on ice during this period of time. Total RNA was prepared, and poly(A)+ RNA was isolated by using oligo(dT)-cellulose (Pharmacia), as described elsewhere (63). Poly(A)+ RNA (4 μg) was separated by electrophoresis through 1% agarose gels containing 2.2 M formaldehyde and transferred to Hybond-extraC membranes (Amersham) in 20× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaPO4, and 1 mM EDTA [pH 7.7]) (50). Molecular weight markers were from Bethesda Research Laboratories. Filters were hybridized to DNA probes at 42°C in a buffer containing 50% formamide, 6× SSPE, 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.4), 40 μg of single-

stranded herring sperm DNA per ml, 1× Denhardt's solution (50), 1% glycine, and 1% sodium dodecyl sulfate (SDS) and washed at 60°C in 0.1× SSPE–0.1% SDS. For hybridization with oligonucleotide probes, the buffer described above, but without formamide and HEPES, was used at 42°C; the blots were washed in 2× SSPE–0.1% SDS at 45°C (*STE2* probe) or 55°C (*PKC1* probe). The filters were used to expose Kodak XAR-5 film, using a DuPont Cronex Lightning-Plus intensifying screen. When it was necessary to remove a probe before rehybridizing the same filter, the filter was stripped (50) and exposed to film, using an intensifying screen for at least 20 h to ensure that removal of radioactivity was complete prior to reuse.

DNA sequence analysis. Restriction fragments from the region containing the *MOT1*-complementing activity (8-kb *BglII* segment) and adjacent fragments were subcloned into the polylinker regions of M13mp18 (54), M13mp19 (54), or pRS316 (65). Nested deletions of fragments in pRS316 were generated by using an exonuclease III-mung bean nuclease protocol supplied by the manufacturer of the enzymes (Stratagene). Both single-stranded and double-stranded templates were sequenced by using a Sequenase kit (United States Biochemical). Specific oligonucleotide primers were synthesized and used when necessary to close gaps in the sequence obtained from the deletion constructions and to confirm the sequence of junctions between restriction fragments. All nucleotide sequences were confirmed for each region of the DNA by sequencing both strands. [α-35S]dATP was from Amersham.

Nucleotide sequence accession number. The GenBank accession number for *MOT1* is M83224.

RESULTS

Isolation of the *mot1-1* mutation. A genetic screen was used to identify mutants which expressed pheromone-responsive genes in the absence of Ste12 and in the absence of pheromone. A reporter plasmid (pJD11) in which 16 PREs were inserted in place of the UAS in a *cyc1* promoter-*lacZ* gene fusion (see Materials and Methods) was introduced into a strain lacking a functional *STE12* gene (SF167-5a) (25). It has been amply documented that the level of β-galactosidase activity expressed from appropriate promoter-*lacZ* fusions directly reflects transcriptional activity of a gene (31, 58). In the absence of Ste12, expression of *lacZ* driven by PRE elements was extremely low both in the absence and in the presence of α-factor (Table 3). In agreement with this finding, SF167-5a[pJD11] was white on X-Gal-containing plates in both the absence and presence of pheromone (data

not shown). In contrast, when pJD11 was introduced into an isogenic STE12⁺ strain (EG123) (25), the level of β-galactosidase activity produced was 3 orders of magnitude higher than in the ste12 strain even in the absence of pheromone (Table 3). Furthermore, activity increased nearly sixfold when cells were exposed to α-factor (Table 3). Correspondingly, EG123[pJD11] was pale blue in the absence of pheromone and dark blue in the presence of α-factor on X-Gal-containing plates (data not shown). Thus, expression of pJD11 in both strains was regulated in a manner identical to that observed for natural pheromone-responsive genes in that maximum basal and induced levels of transcription required a functional STE12 gene (23, 24).

SF167-5a carrying pJD11 was mutagenized with ethyl methanesulfonate (see Materials and Methods), and 50,000 surviving colonies were screened for blue color on X-Gal-containing plates lacking α-factor. Candidate blue colonies (73 total) were assayed in vitro for β-galactosidase activity (see Materials and Methods) to identify mutants which specifically elevated expression of the PRE-driven lacZ gene and to eliminate those that displayed blue color due to increased permeability to X-Gal or other nonspecific effects. Of the 73 candidates tested, three displayed increased in vitro β-galactosidase activity. To rule out mutants in which expression of the indicator gene was elevated due to plasmid-linked alterations, expression of endogenous pheromone-responsive genes was examined in the remaining three candidates.

Both of the a-factor structural genes, MFa1 and MFa2 (6), and a gene required for a-factor secretion, STE6 (45), are induced by α-factor (6, 45) and require Ste12 for both basal and induced expression (23, 24). Mutations that increase expression of the reporter gene because they generally elevate expression from PRE-containing UAS elements should also increase expression of chromosomally located pheromone-responsive genes and thereby generate more a-factor than does the parental strain. Pheromone production can be judged by an a-factor bioassay that measures the amount of a-factor synthesized and secreted into the surrounding medium (45). Of the three remaining candidates, one isolate reproducibly displayed elevated a-factor production (data not shown) in addition to its elevated β-galactosidase activity. The level of production of a-factor was detected by the size of the zone of growth inhibition (halo) in a lawn of MATα cells onto which the MATa mutant cells were spotted. This mutant was called mot1 (for modifier of transcription), and the allele described here was designated mot1-1.

Phenotypic characterization of mot1-1. In a STE12⁺ background, basal expression of pJD11 is about twofold higher in cells carrying the mot1-1 allele (JD215B) than in wild-type cells (EG123) (Table 3). Following exposure to pheromone, the induced level in both strains was equivalent. As expected on the basis of the scheme for its isolation, in a ste12 background, a mot1-1 strain (JD227A) carrying pJD11 showed readily detectable expression of the PRE-driven lacZ gene, whereas its MOT1⁺ ste12 parent (SF167-5a) did not (Table 3). Expression was essentially unaffected by exposure to α-factor, indicating that mot1-1 does not restore response to pheromone action nor does it bypass the requirement for Ste12 for maximum levels of induction. This point was further supported by quantitative mating assays (Table 4). Regardless of mating type, no significant difference in mating efficiency was observed between MOT1⁺ and mot1-1 strains containing a functional STE12 gene; conversely, the

TABLE 4. Effect of mot1-1 on mating efficiency

Strain	Relevant genotype[a]	Mating frequency[b]
SF167-5a	MATa ste12::LEU2 MOT1⁺	<2.6 × 10⁻⁷
JD227A	MATa ste12::LEU2 mot1-1	<2.4 × 10⁻⁷
JD209	MATα ste12::LEU2 MOT1⁺	<1.7 × 10⁻⁷
JD216D	MATα ste12::LEU2 mot1-1	<2.0 × 10⁻⁷
EG123	MATa STE12⁺ MOT1⁺	0.59
JD215B	MATa STE12⁺ mot1-1	0.44
JD195	MATα STE12⁺ MOT1⁺	0.73
JD215A	MATα STE12⁺ mot1-1	0.40

[a] All strains are isogenic except at the MAT, STE12, and MOT1 loci.
[b] Total diploids per input number of haploid cells of strain examined. See Materials and Methods for experimental details.

mot1 mutation did not rescue the sterility of ste12 null mutants.

The effect of the mot1 mutation on expression of β-galactosidase from pLGΔ178T, which lacks a UAS (see Materials and Methods), was also examined (Table 3). Interestingly, the mot1-1 strains displayed a readily measurable level of expression, whereas the isogenic MOT1⁺ cells did not. This effect was independent of the presence of Ste12 and was not influenced by pheromone treatment. This relaxation of the requirement for a UAS for cyc1-lacZ expression suggested that mot1 might alter the expression of other genes in addition to pheromone-responsive genes (see below).

To rule out the possibility, however, that variations in plasmid copy number could explain the apparent effects observed on gene expression in the different strain backgrounds, plasmid DNA was recovered from each of the strains and examined by quantitative hybridization analysis (67). Comparison of the plasmid copy number across all of the various strains examined showed negligible differences (less than twofold) (data not shown), confirming that the different levels of β-galactosidase observed reflected changes in gene expression.

Effect of mot1 on gene transcription. To determine how general the effect of mot1 was on gene expression, hybridization analysis of poly(A)⁺ RNA from a variety of chromosomal genes was performed (Fig. 1). For pheromone-responsive genes whose expression is severely reduced, but not completely eliminated, by inactivation of STE12 (23), including MFa2 (6), STE2 (encoding the α-factor receptor) (9, 52), and STE6 (45), the mot1-1 allele caused significantly increased expression (at least threefold) in a ste12 background but only a modest increase in a STE12⁺ background (Fig. 1A and Table 3). In ste12 strains, no further α-factor induction was detectable in mot1 cells. In STE12⁺ strains, pheromone treatment elicited transcriptional induction in both mot1-1 and MOT1⁺ cells, although the levels in mot1 cells appeared to be slightly higher. The effect of mot1 on expression of these chromosomally located genes was qualitatively the same as its effect on the PRE-driven reporter plasmid (Table 3). To rule out the possibility that the transcriptional effects of mot1-1 were a-cell specific, the expression in MATα cells of STE3, an α-cell-specific gene that encodes the a-factor receptor (31, 52), was examined. STE3 expression is similar to that of MFa2, STE2, and STE6 in the sense that detectable basal transcription is observed in ste12 mutant cells (23). It was found that STE3 expression was increased about threefold in MATα mot1-1 cells compared with isogenic MATα MOT1⁺ cells (data not shown), indicating that the transcriptional phenotype of mot1-1 is not cell type specific.
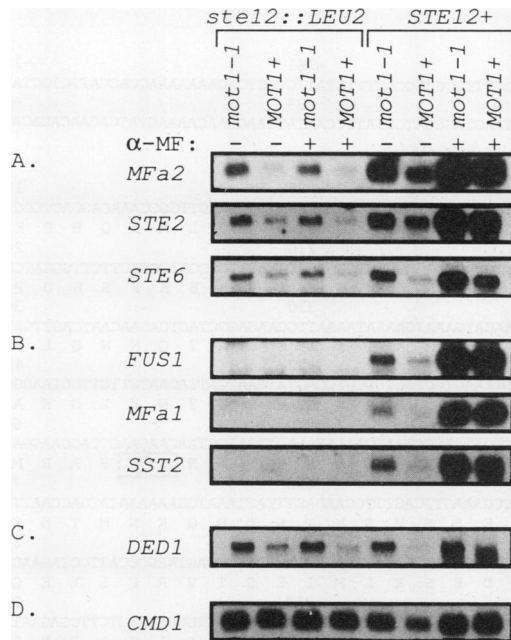
FIG. 1. Effect of *mot1* on gene transcription. Poly(A)$^+$ RNA (4 μg) from each strain was loaded into each sample well. The relevant genotype of each strain is indicated above each lane; strains are those listed in Table 3. Gene probes (see Table 2) are designated to the left of each strip. The same filter was used for all hybridizations (see Materials and Methods for conditions). α-MF, α-mating factor; −, not treated with α-factor; +, treated with α-factor. (A) Phero-mone-responsive genes that display Ste12-independent basal tran-scription; (B) pheromone-responsive genes that require Ste12 for basal transcription; (C) *DED1*, a gene whose product is thought not to be part of the mating pathway; (D) *CMD1*, used as a control for the uniformity of RNA loading.

For pheromone-responsive genes whose expression is reduced to undetectable levels by inactivation of *STE12* (23), including *FUS1* (encoding a protein required for cell fusion) (72), *MFa1* (6), and *SST2* (encoding a protein involved in recovery from α-factor-induced G$_1$ arrest) (12, 15), the *mot1-1* mutation did not lead to detectable transcription in the *ste12* background (Fig. 1B). These results may account for the fact that despite the elevated expression of certain mating genes, *mot1 ste12* strains are still mating deficient (Table 4). Remarkably, in the *STE12*$^+$ strains, the *mot1-1* mutation still had a detectable effect in enhancing basal transcription from these genes; however, α-factor induction of these genes was equivalent in *mot1* and *MOT1*$^+$ cells.

Expression of a gene thought to be unrelated to the mating pathway, *DED1* (for defines essential domain) (70), was also increased by the *mot1-1* mutation in both the *ste12* and *STE12*$^+$ backgrounds (Fig. 1C), in the same way as observed for the *MFa2*, *STE2*, *STE3*, and *STE6* genes. *DED1* tran-scription was not significantly affected by α-factor treatment in *STE12*$^+$ cells, in agreement with the fact that the 5′-flanking region of the *DED1* gene lacks canonical PRE sequences (70). The *DED1* gene product has not been implicated in the mating process previously. If it does not play a role in mating, these data further suggest that expres-sion of some genes outside of the pheromone response network is affected by the *mot1-1* mutation.

Two genes were identified whose expression was com-pletely unaltered by *mot1-1*, namely, *CMD1* (encoding yeast
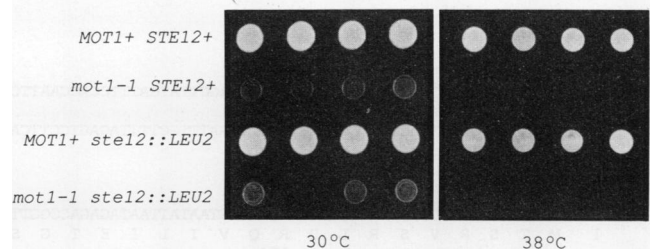
FIG. 2. Growth phenotypes of *mot1-1* cells. Four different spores of identical genotype, derived from a cross of JD102C (*MATα mot1-1*) with SF167-5a {*MATa ste12::LEU2* [p*STE12(URA3)*]} were streaked on a YPD plate and allowed to grow at 30°C for 3 days. Cells from these plates were resuspended in water at equal densities, placed into a multiwelled template dish, and spotted onto two fresh YPD plates by using a pronged inoculator. The plates were incu-bated at 30°C for 3 days or at 38°C for 3 days, as indicated.

calmodulin) (13) (Fig. 1D; see also Fig. 5B) and *PKC1* (encoding a yeast protein kinase C) (48) (see Fig. 5A). The level of the *CMD1* or the *PKC1* transcript was used to normalize for differences in RNA loading.

Effect of *mot1* mutation on growth. In addition to the transcriptional phenotypes described above, two growth phenotypes were observed in *mot1* mutants. Cells carrying the *mot1-1* allele grew slowly at 30°C, with a doubling time of about 3.5 h in rich medium (YPD), and were inviable at 38°C (Fig. 2). These growth phenotypes were manifest in either a *ste12* or a *STE12*$^+$ background. A temperature shift experi-ment was performed in which early-exponential-phase *mot1-1* cells were shifted from 30 to 38°C and examined at several time points after the shift. No uniform terminal cell morphology was observed (data not shown). Cells held at 38°C for up to 4 h retained nearly 100% viability when shifted back to 30°C. However, after longer periods at 38°C, viabil-ity dropped rapidly and progressively. For example, after 24 h at 38°C, viability was less than 0.1% of that of *MOT1*$^+$ cells subjected to the same treatment. After the *MOT1* gene was cloned (see below), expression of the *MOT1* transcript in *mot1-1* mutants was analyzed at 4 and 8 h after the shift to 38°C. Even after 8 h, the transcript was present at a level nearly equivalent to that observed initially (data not shown). Thus, the inviability at 38°C was likely due not to a lack of mRNA in the mutant cells but rather to a defect in the function of the *mot1-1* product at 38°C.

Isolation and sequence of the *MOT1* gene. When a *mot1-1* haploid was crossed against a normal haploid, the resulting heterozygous diploid cells grew normally at 30 and 38°C and did not display elevated expression of the pJD11 reporter plasmid; hence, the *mot1-1* allele was a recessive mutation.

FIG. 3. Restriction map of the *MOT1* gene. Rectangular regions represent areas that were subjected to DNA sequence analysis; dashed lines represent regions that were not sequenced. The open reading frame (ORF) is represented by the hatched rectangle; noncoding regions are represented by the open rectangles. All sites for each enzyme indicated are shown except for *ClaI* (a second *dam*-sensitive site exists at position 3435).

```
                    -221                        -191                        -161                        -130
      GTCCCACTCGCCGGCCGCTGCACTTGATATAAAAGCTTATGATTTCCACCAATTCCGTTCCCTTGGCGGTTTGCGCGCCATTTTCTATTGCGCTCAGAAAAAAACCACCATTCTGCTATT
                    -100                        -70                         -39                         -10
      ATAGCTCTGAACACACAGCTCGGCGGTTATTTTGATTTGCTTTTAGAGTCCTTCAGAACATAAAATATAAGTAGCGATCGTATTCCAATATTAACGAACAAAAGTATCACAACACACACA
                    -1
      GCACCTCAA
                         30                         60                         90                        120
      ATGACGTCACGAGTTTCGAGGCTGGACCGGCAGGTAATATTAATAGAGACCGGTTCCACCCAAGTAGTCCGGAACATGGCTGCCGATCAAATGGGAGACTTGGCCAAACAGCACCCCGAA
  1   M  T  S  R  V  S  R  L  D  R  Q  V  I  L  I  E  T  G  S  T  Q  V  V  R  N  M  A  A  D  Q  M  G  D  L  A  K  Q  H  P  E
                        150                        180                        210                        240
      GACATTCTTTCGTTGCTGTCCAGAGTTTACCCCTTTTTACTAGTGAAGAAATGGGAACGCGTGTTACTGCAGCAAGAGCTGTGGGTGGCATAGTTGCCCATGCTCCTTCTTGGGATCCG
 41   D  I  L  S  L  L  S  R  V  Y  P  F  L  L  V  K  K  W  E  T  R  V  T  A  A  R  A  V  G  G  I  V  A  H  A  P  S  W  D  P
                        270                        300                        330                        360
      AATGAATCTGACTTGGTAGGTGGTACAAATGAAGGTTCTCCATTGGATAATGCTCAGGTGAAGTTGGAACATGAAATGAAAATAAAAATTGGAAGAGGCTACTCAGAACAATCAGTTGAAT
 81   N  E  S  D  L  V  G  G  T  N  E  G  S  P  L  D  N  A  Q  V  K  L  E  H  E  M  K  I  K  E  E  A  T  Q  N  N  Q  L  N
                        390                        420                        450                        480
      CTCTTACAGGAGGACCATCACCTCAGTTCTCTGTCTGATTGGAAATTGAACGAAATTTTAAAGTCAGGTAAAGTGCTCCTGGCGTCGAGTATGAATGATTACAATGTTCTTGGTAAGGCT
121   L  L  Q  E  D  H  H  L  S  S  L  S  D  W  K  L  N  E  I  L  K  S  G  K  V  L  L  A  S  S  M  N  D  Y  N  V  L  G  K  A
                        510                        540                        570                        600
      GACGATAATATAAGAAAACAAGCTAAAACTGATGATATAAAACAGGAAACATCTATGCTAAATGCGTCTGATAAGGCGAATGAAAATAAAAGTAATGCTAACAAGAAGTCAGCAAGGATG
161   D  D  N  I  R  K  Q  A  K  T  D  D  I  K  Q  E  T  S  M  L  N  A  S  D  K  A  N  E  N  K  S  N  A  N [K  K] S  A  R  M
                        630                        660                        690                        700
      CTTGCTATGGCAAGGCGAAAAAAGAAAATGAGTGCTAAGAATACTCCCAAACATCCTGTAGATATAAACGGAAAGTTCAGTTTCCAAGACTTTATTAAATGGAAAAAATATGACCAATTCT
201   L  A  M  A [R  R  K  K  K] M  S  A  K  N  T  P  K  H  P  V  D  I  T  E  S  S  V  S  K  T  L  L  N  G  K  N  M  T  N  S
                        730                        760                        790                        820
      GCTGCATCGCTAGCCACTTCTCCCACCAGTAACCAGCTAAATCCCAAACTGGAGATTACTGAACAAGCCGATGAAAGTAAACTAATGATAGAATCCACAGTAAGGCCATTGCTAGAACAA
241   A  A  S  L  A  T  S  P  T  S  N  Q  L  N  P  K  L  E  I  T  E  Q  A  D  E  S  K  L  M  I  E  S  T  V  R  P  L  L  E  Q
                        850                        880                        910                        940
      CACGAAATTGTTGCTGGCCTAGTTTGGCAATTCCAAGGCATTTATGAACTACTTTTAGATAATCTAATGAGCGAAAATTGGGAAATAAGGCATGGAGCTGCGTTGGGTCTTCGAGAATTG
281   H  E  I  V  A  G  L  V  W  Q  F  Q  G  I  Y  E  L  L  L  D  N  L  M  S  E  N  W  E  I  R  H  G  A  A  L  G  L  R  E  L
                        970                       1000                       1030                       1060
      GTAAAAAAACATGCTTATGGAGTCAGCAGGGTTAAGGGGAATACCCGAGAGGAGAACAACCTGAGGAATTCTAGAAGCTTAGAAGACTTGGCGTCCCGTCTACTTACAGTATTTGCATTG
321   V  K  K  H  A  Y  G  V  S  R  V  K  G  N  T  R  E  E  N  N  L  R  N  S  R  S  L  E  D  L  A  S  R  L  L  T  V  F  A  L
                       1090                       1120                       1150                       1180
      GATAGAGATTTGGTGATTATGTTTATGATACAGTTGTGGCCCCTGTACGAGAGTCAGTTGCGCAAACTCTAGCTGCGTTATTAATTCACCTAGACAGTACCTTATCAATTAAAAATATTTAAT
361   D  R  F  G  D  Y  V  V  D  T  V  V  A  P  V  R  E  S  V  A  Q  T  L  A  A  L  L  I  H  L  D  S  T  L  S  I  K  I  F  N
                       1210                       1240                       1270                       1300
      TGTTTAGAACAACTGGTTCTACAAGATCCACTACAAACAGGTCTACCGAACAAAATATGGGAAGCTACCCATGGAGGGCTTTTAGGGATTCGTTACTTCGTCAGCATAAAGACAAATTC
401   C  L  E  Q  L  V  L  Q  D  P  L  Q  T  G  L  P  N  K  I  W  E  A  T  H  G  G  L  L  G  I  R  Y  F  V  S  I  K  T  N  F
                       1330                       1360                       1390                       1410
      CTATTTGCCCATGGCCTTTTAGAAAACGTTGTCCGTATAGTGCTATATGGTTTAAACCAAAGTGATGATGACGTTCAAAGCGTAGCAGCCTCAATTTTAACCCCAATAACCAGCGAATTT
441   L  F  A  H  G  L  L  E  N  V  V  R  I  V  L  Y  G  L  N  Q  S  D  D  D  V  Q  S  V  A  A  S  I  L  T  P  I  T  S  E  F
                       1440                       1470                       1500                       1530
      GTCAAATTAAACAACTCTACCATAGAAATTTTGGTGACCACGATATGGTCATTACTAGCTCGTTTAGATGATGATATATCTTCAAGCGTAGGTTCTATAATGGATCTATTGGCAAAGTTG
481   (V) K  L  N (N) S  T (I) E  I  L (V) T  T (I) W  S  L (A) R (L) D  D  D (I) S  S (S) V  G  S (I) M  D (L) L  A  K (L)
                       1560                       1590                       1620                       1650
      TGCGACCACCAAGAAGTACTAGATATTTTAAAGAACAAGGCTTTAGAGCATCCTTCAGAGTGGTCATTCAAGTCACTGGTACCGAAATTGTATCCCTTTTTACGCCATTCGATTTCTTCT
521   C  D  H  Q  E  V  L  D  I  L  K  N  K  A  L  E  H  P  S  E  W  S  F  K  S  L  V  P  K  L  Y  P  F  L  R  H  S  I  S  S
                       1680                       1710                       1740                       1770
      GTGAGGAGGGCTGTTTTGAATCTACTGATTGCATTCTTATCAATAAAGGATGATTCTACTAAAAACTGGTTAAACGGCAAGGTATTCCGTCTAGTTTTTCAGAATATTTTACTGGAGCAA
561   V  R  R  A  V  L  N  L  L  I  A  F  L  S  I  K  D  D  S  T  K  N  W  L  N  G  K  V  F  R  L  V  F  Q  N  I  L  L  E  Q
                       1800                       1830                       1860                       1890
      AACCCGGAACTATTGCAACTGTCTTTTGATGTTTATGTTGCGTTATTGGAGCATTATAAAGTTAAGCATACCGAAAAAAACTTTAGATCATGTTTTCAGTAAACATTTGCAGCCAATACTA
601   N  P  E  L  L  Q  L  S  F  D  V  Y  V  A  L  L  E  H  Y  K  V  K  H  T  E  K  T  L  D  H  V  F  S  K  H  L  Q  P  I  L
                       1920                       1950                       1980                       2010
      CATCTTCTAAATACGCCAGTAGGGGAAAAGGGAAAGAATTATGCCATGGAATCACAATATATTCTAAAGCCTTCCCAGCACTATCAATTACATCCAGAAAAAAAACGTTCTATATCTGAA
641   H  L  L  N  T  P  V  G  E  K  G  K  N  Y  A  M  E  S  Q  Y  I  L  K  P  S  Q  H  Y  Q  L  H  P  E  K  K  R  S  I  S  E
                       2040                       2070                       2100                       2130
      ACCACAACAGATTCTGACATTCCAATACCAAAAAATAATGAACATATCAATATCGATGCACCTATGATTGCTGGAGATATTACTTTGCTAGGATTGGACGTAATATTGAACACTAGAATA
681   T  T  T  D  S  D  I  P (I) P  K  N (N) E  H (I) N  I  D (A) P  M (I) A  G  D (I) T  L (L) G  L  D (V) I  L (N) T  R  I
                       2160                       2190                       2220                       2250
      ATGGGAGCAAAGGCATTTGCTTTGACATTGTCAATGTTTCAAGATTCCACACTTCAATCCTTCTTCACAAACGTTCTGGTTCGTTGTTTAGAGCTGCCATTCTCAACTCCACGTATGTTG
721   (M) G  A  K  A  F  A  L  T  L  S  M  F  Q  D  S  T  L  Q  S  F  F  T  N  V  L  V  R  C  L  E  L  P  F  S  T  P  R  M  L
                       2280                       2310                       2340                       2370
      GCAGGCATTATTGTTTCACAGTTTTGCTCCTCGTGGTTACAAAAGCATCCAGAAGGGGAAAAATTACCTTCTTTTGTATCAGAGATTTTCAGTCCTGTGATGAATAAACAGTTACTGAAT
761   A  G  I  I  V  S  Q  F  C  S  S  W  L  Q  K  H  P  E  G  E  K  L  P  S  F  V  S  E  I  F  S  P  V  M  N  K  Q  L  L  N
                       2400                       2430                       2460                       2490
      CGCGATGAATTTCCTGTATTTAGGGAGCTAGTACCTAGCTTAAAAGCTCTGAGGACTCAATGTCAAAGCCTACTGGCTACCTTTGTGGATGTAGGTATGTTACCTCAGTACAAACTACCC
801   R  D  E  F  P  V  F  R  E  L  V  P  S  L  K  A  L  R  T  Q  C  Q  S  L  L  A  T  F  V  D  V  G  M  L  P  Q  Y  K  L  P
                       2520                       2550                       2580                       2610
      AACGTAGCAATTGTGGTTCAAGGCGAAACAGAAGCCGGTCCACATGCGTTTGGTGTTGAAACAGCAGAAAAAGTGTACGGCGAATACTATGATAAAATGTTCAAATCCATGAACAACTCT
841   N  V  A  I  V  V  Q  G  E  T  E  A  G  P  H  A  F  G  V  E  T  A  E  K  V  Y  G  E  Y  Y  D  K  M  F  K  S  M  N  N  S
                       2640                       2670                       2700                       2730
      TACAAACTATTGGCCAAGAAGCCATTAGAAGATTCTAAGCATCGCGTGTTGATGGCAATCAATTCAGCCAAAGAATCCGCAAAACTTCGTACAGGCAGCATTCTCGCTAATTACGCATCA
881   Y  K  L  L  A  K  K  P  L  E  D  S  K  H  R  V  L  M  A  I  N  S  A  K  E  S  A  K  L  R  T  G  S  I  L  A  N  Y  A  S
```

FIG. 4. Nucleotide sequence and predicted amino acid sequence of *MOT1*. Nucleotide positions are shown above each line; amino acid positions are shown to the left of each line. Amino acid sequence is in the one-letter code below the nucleotide sequence. A potential nuclear targeting signal is boxed. The 568-residue domain (amino acids 1257 to 1825) of homology to helicases and to *SNF2* and *RAD54* is underlined. A number of segments in the amino-terminal domain, for example, residues 481 to 520 and 689 to 721 (circled), fit the 4-3 hydrophobic repeat that is diagnostic of amphipathic helices capable of forming coiled coils (11).

```
                    2760                    2790                    2820                    2850
     TCCATCTTGTTATTTGATGGTCTACCTCTTAAATTAAACCCCATTATCAGGTCGCTAATGGACAGTGTTAAAGAAGAAAGGAATGAAAAGTTACAAACGATGGCCGGAGAATCGGTCGTA
921  S  I  L  L  F  D  G  L  P  L  K  L  N  P  I  I  R  S  L  M  D  S  V  K  E  E  R  N  E  K  L  Q  T  M  A  G  E  S  V  V
                    2880                    2910                    2940                    2970
     CATTTGATTCAGCAACTGTTAGAAAATAATAAAGTTAATGTGTCTGGTAAGATAGTCAAAAATCTCTGCGGATTTTTATGCGTTGATACTTCAGAAGTTCCTGATTTCTCCGTAAATGCA
961  H  L  I  Q  Q  L  L  E  N  N  K  V  N  V  S  G  K  I  V  K  N  L  C  G  F  L  C  V  D  T  S  E  V  P  D  F  S  V  N  A
                    3000                    3030                    3060                    3090
     GAATATAAAGAAAAAATATTGACGTTAATAAAAGAATCTAATTCGATTGCCGCACAAGATGACATTAATCTCGCAAAGATGTCGGAAGAGGCTCAACTAAAAAGGAAGGGTGGGTTGATT
1001 E  Y  K  E  K  I  L  T  L  I  K  E  S  N  S  I  A  A  Q  D  D  I  N  L  A  K  M  S  E  E  A  Q  L  K  R  K  G  G  L  I
                    3120                    3150                    3180                    3210
     ACGTTAAAAATACTATTTGAAGTTCTTGGGCCTTCTATATTACAGAAACTACCTCAGCTTAGATCGATTTTATTTGATTCATTATCTGATCATGAAAATGAGGAAGCAAGTAAAGTTGAC
1041 T  L  K  I  L  F  E  V  L  G  P  S  I  L  Q  K  L  P  Q  L  R  S  I  L  F  D  S  L  S  D  H  E  N  E  E  A  S  K  V  D
                    3240                    3270                    3300                    3330
     AACGAGCAAGGTCAAAAAATAGTCGACTCATTTGGTGTACTAAGAGCTCTATTCCCATTTATGTCTGATTCGCTACGTTCTAGTGAGGTTTTTACCCGTTTCCCTGTTTTATTAACGTTC
1081 N  E  Q  G  Q  K  I  V  D  S  F  G  V  L  R  A  L  F  P  F  M  S  D  S  L  R  S  S  E  V  F  T  R  F  P  V  L  L  T  F
                    3360                    3390                    3420                    3450
     TTAAGATCAAACTTATCTGTTTTTCGTTATTCAGCTGCAAGAACTTTTGCTGATCTGGCTAAAATTTCCTCCGTGGAGGTAATGGCTTACACCATACGTGAGATTTTGCCTTTAATGAAT
1121 L  R  S  N  L  S  V  F  R  Y  S  A  A  R  T  F  A  D  L  A  K  I  S  S  V  E  V  M  A  Y  T  I  R  E  I  L  P  L  M  N
                    3480                    3510                    3540                    3570
     AGCGCTGGTTCTCTATCCGATCGTCAAGGGTCCACGGAGCTTATTTATCATTTATCACTTTCTATGGAAACCGATGTTCTACCTTACGTCATTTTCTTAATAGTTCCACTTTTGGGTCGC
1161 S  A  G  S  L  S  D  R  Q  G  S  T  E  L  I  Y  H  L  S  L  S  M  E  T  D  V  L  P  Y  V  I  F  L  I  V  P  L  L  G  R
                    3600                    3630                    3690                    3720
     ATGAGTGATTCGAACGAAGATGTGAGGAATTTAGCTACAACAACATTTGCTTCTATCATCAAATTAGTTCCTTTAGAAGCTGGTATCGCCGATCAAAAGGACTCCCAGAAGAACTAGTG
1201 M  S  D  S  N  E  D  V  R  N  L  A  T  T  T  F  A  S  I  I  K  L  V  P  L  E  A  G  I  A  D  P  K  G  L  P  E  E  L  V
                    3750                    3780                    3810                    3840
     GCAAGCAGAGAGAGGGAGCGTGATTTTATCCAACAAATGATGGATCCTTCTAAAGCTAAGCCATTTAAATTACCCATTGCCATCAAGGCGACTTTAAGAAAATATCAGCAAGATGGTGTT
1241 A  S  R  E  R  E  R  D  F  I  Q  Q  M  M  D  P  S̲ ̲K̲ ̲A̲ ̲K̲ ̲P̲ ̲F̲ ̲K̲ ̲L̲ ̲P̲ ̲I̲ ̲A̲ ̲I̲ ̲K̲ ̲A̲ ̲T̲ ̲L̲ ̲R̲ ̲K̲ ̲Y̲ ̲Q̲ ̲Q̲ ̲D̲ ̲G̲ ̲V
                    3870                    3900                    3930                    3960
     AACTGGTTGGCATTCCTGAACAAATATCATCTACATGGAATATTGTGTGACGATATGGGTTTGGGTAAAACATTACAAACCATCTGCATTATCGCAAGCGATCAATATTTGAGGAAGGAA
1281 N̲ ̲W̲ ̲L̲ ̲A̲ ̲F̲ ̲L̲ ̲N̲ ̲K̲ ̲Y̲ ̲H̲ ̲L̲ ̲H̲ ̲G̲ ̲I̲ ̲L̲ ̲C̲ ̲D̲ ̲D̲ ̲M̲ ̲G̲ ̲L̲ ̲G̲ ̲K̲ ̲T̲ ̲L̲ ̲Q̲ ̲T̲ ̲I̲ ̲C̲ ̲I̲ ̲I̲ ̲A̲ ̲S̲ ̲D̲ ̲Q̲ ̲Y̲ ̲L̲ ̲R̲ ̲K̲ ̲E
                    3990                    4020                    4050                    4080
     GATTATGAAAAAACGCGTTCTGTTGAAAGTAGAGCCTTACCATCTCTCATAATATGCCCCCCATCTTTAACTGGCCATTGGGAAAACGAGTTTGACCAGTATGCTCCCTTTTTAAAGGTT
1321 D̲ ̲Y̲ ̲E̲ ̲K̲ ̲T̲ ̲R̲ ̲S̲ ̲V̲ ̲E̲ ̲S̲ ̲R̲ ̲A̲ ̲L̲ ̲P̲ ̲S̲ ̲L̲ ̲I̲ ̲I̲ ̲C̲ ̲P̲ ̲P̲ ̲S̲ ̲L̲ ̲T̲ ̲G̲ ̲H̲ ̲W̲ ̲E̲ ̲N̲ ̲E̲ ̲F̲ ̲D̲ ̲Q̲ ̲Y̲ ̲A̲ ̲P̲ ̲F̲ ̲L̲ ̲K̲ ̲V
                    4110                    4140                    4170                    4200
     GTTGTGTATGCCGGTGGCCCAACTGTCAGATTAACTCTGAGACCACAGTTAAGTGATGCTGATATTATTGTAACATCATATGACGTAGCTAGAAATGATCTCGCAGTCCTAAACAAAACT
1361 V̲ ̲V̲ ̲Y̲ ̲A̲ ̲G̲ ̲G̲ ̲P̲ ̲T̲ ̲V̲ ̲R̲ ̲L̲ ̲T̲ ̲L̲ ̲R̲ ̲P̲ ̲Q̲ ̲L̲ ̲S̲ ̲D̲ ̲A̲ ̲D̲ ̲I̲ ̲I̲ ̲V̲ ̲T̲ ̲S̲ ̲Y̲ ̲D̲ ̲V̲ ̲A̲ ̲R̲ ̲N̲ ̲D̲ ̲L̲ ̲A̲ ̲V̲ ̲L̲ ̲N̲ ̲K̲ ̲T
                    4230                    4260                    4290                    4320
     GAATATAATTATTGTGTTTTGGATGAAGGACATATAATTAAAAACTCTCAATCAAAACTTGCTAAGGCTGTTAAAGAAATTACTGCAAACCATAGATTAATTTTAACCGGAACTCCCATC
1401 E  Y  N  Y  C  V  L  D  E  G  H  I  I  K  N  S  Q  S  K  L  A  K  A  V  K  E  I  T  A  N  H  R  L  I  L  T  G  T  P  I
                    4350                    4380                    4410                    4440
     CAAAACAACGTTTTAGAATTGTGGTCTCTGTTTGACTTCTTAATGCCGGGATTTTGGGAACGGAAAAGATGTTCCAGGAAAGGTTCGCAAAGCCAATCGCTGCGTCAAGAAATAGTAAA
1441 Q̲ ̲N̲ ̲N̲ ̲V̲ ̲L̲ ̲E̲ ̲L̲ ̲W̲ ̲S̲ ̲L̲ ̲F̲ ̲D̲ ̲F̲ ̲L̲ ̲M̲ ̲P̲ ̲G̲ ̲F̲ ̲L̲ ̲G̲ ̲T̲ ̲E̲ ̲K̲ ̲M̲ ̲F̲ ̲Q̲ ̲E̲ ̲R̲ ̲F̲ ̲A̲ ̲K̲ ̲P̲ ̲I̲ ̲A̲ ̲A̲ ̲S̲ ̲R̲ ̲N̲ ̲S̲ ̲K
                    4470                    4500                    4530                    4560
     ACATCATCTAAGGAGCAGGAAGCAGGTGTACTGGCTTTGGAGGCACTACATAAGCAAGTTCTACCTTTCATGCTGAGAAGATTGAAGGAAGACGTTCTATCTGATTTACCACCTAAAATT
1481 T̲ ̲S̲ ̲S̲ ̲K̲ ̲E̲ ̲Q̲ ̲E̲ ̲A̲ ̲G̲ ̲V̲ ̲L̲ ̲A̲ ̲L̲ ̲E̲ ̲A̲ ̲L̲ ̲H̲ ̲K̲ ̲Q̲ ̲V̲ ̲L̲ ̲P̲ ̲F̲ ̲M̲ ̲L̲ ̲R̲ ̲R̲ ̲L̲ ̲K̲ ̲E̲ ̲D̲ ̲V̲ ̲L̲ ̲S̲ ̲D̲ ̲L̲ ̲P̲ ̲P̲ ̲K̲ ̲I
                    4590                    4620                    4650                    4680
     ATTCAGGATTACTACTGTGAATTAGGTGATTTACAAAAACAACTATACATGGATTTTACTAAAAAACAAAAAAATGTAGTTGAGAAAGATATTGAAAATTCTGAGATTGCTGATGGCAAG
1521 I̲ ̲Q̲ ̲D̲ ̲Y̲ ̲Y̲ ̲C̲ ̲E̲ ̲L̲ ̲G̲ ̲D̲ ̲L̲ ̲Q̲ ̲K̲ ̲Q̲ ̲L̲ ̲Y̲ ̲M̲ ̲D̲ ̲F̲ ̲T̲ ̲K̲ ̲K̲ ̲Q̲ ̲K̲ ̲N̲ ̲V̲ ̲V̲ ̲E̲ ̲K̲ ̲D̲ ̲I̲ ̲E̲ ̲N̲ ̲S̲ ̲E̲ ̲I̲ ̲A̲ ̲D̲ ̲G̲ ̲K
                    4710                    4740                    4770                    4800
     CAACATATTTTCCAAGCTTTACAATACATGAGAAAATTGTGTAATCATCCAGCTTTGGTCCTTTCACCAAATCACCCGCAATTAGCGCAAGTACAAGACTATTTAAAGCAAACTGGTCTA
1561 Q̲ ̲H̲ ̲I̲ ̲F̲ ̲Q̲ ̲A̲ ̲L̲ ̲Q̲ ̲Y̲ ̲M̲ ̲R̲ ̲K̲ ̲L̲ ̲C̲ ̲N̲ ̲H̲ ̲P̲ ̲A̲ ̲L̲ ̲V̲ ̲L̲ ̲S̲ ̲P̲ ̲N̲ ̲H̲ ̲P̲ ̲Q̲ ̲L̲ ̲A̲ ̲Q̲ ̲V̲ ̲Q̲ ̲D̲ ̲Y̲ ̲L̲ ̲K̲ ̲Q̲ ̲T̲ ̲G̲ ̲L
                    4830                    4860                    4890                    4920
     GATTTACACGATATTATCAACGCTCCAAAACTGAGCGCATTGAGAACATTACTCTTTGAATGTGGTATAGGTGAAGAAGATATCGACAAAAAAGCAAGCCAAGATCAGAATTTTCCTATT
1601 D̲ ̲L̲ ̲H̲ ̲D̲ ̲I̲ ̲I̲ ̲N̲ ̲A̲ ̲P̲ ̲K̲ ̲L̲ ̲S̲ ̲A̲ ̲L̲ ̲R̲ ̲T̲ ̲L̲ ̲L̲ ̲F̲ ̲E̲ ̲C̲ ̲G̲ ̲I̲ ̲G̲ ̲E̲ ̲E̲ ̲D̲ ̲I̲ ̲D̲ ̲K̲ ̲K̲ ̲A̲ ̲S̲ ̲Q̲ ̲D̲ ̲Q̲ ̲N̲ ̲F̲ ̲P̲ ̲I
                    4950                    4980                    5010                    5040
     CAAAATGTCATATCACAACACGAGCCCTGATTTTCTGCCAACTAAAAGATATGTAGACATGGTTGAAAATGACTTGTTTAAAAAGTATATGCCCTCCGTAACCTATATGAGGCTAGAT
1641 Q̲ ̲N̲ ̲V̲ ̲I̲ ̲S̲ ̲Q̲ ̲H̲ ̲R̲ ̲A̲ ̲L̲ ̲I̲ ̲F̲ ̲C̲ ̲Q̲ ̲L̲ ̲K̲ ̲D̲ ̲M̲ ̲L̲ ̲D̲ ̲M̲ ̲V̲ ̲E̲ ̲N̲ ̲D̲ ̲L̲ ̲F̲ ̲K̲ ̲K̲ ̲Y̲ ̲M̲ ̲P̲ ̲S̲ ̲V̲ ̲T̲ ̲Y̲ ̲M̲ ̲R̲ ̲L̲ ̲D
                    5070                    5100                    5130                    5160
     GGAAGCATTGACCCAAGAGACAGACAAAAAGTTGTTCGGAAATTTAACGAAGATCCCTCTATTGATTGCCTACTGTTGACCACCAAGGTCGGAGGGCTGGGTCTGAATTTAACTGGTGCA
1681 G̲ ̲S̲ ̲I̲ ̲D̲ ̲P̲ ̲R̲ ̲D̲ ̲R̲ ̲Q̲ ̲K̲ ̲V̲ ̲V̲ ̲R̲ ̲K̲ ̲F̲ ̲N̲ ̲E̲ ̲D̲ ̲P̲ ̲S̲ ̲I̲ ̲D̲ ̲C̲ ̲L̲ ̲L̲ ̲L̲ ̲T̲ ̲T̲ ̲K̲ ̲V̲ ̲G̲ ̲G̲ ̲L̲ ̲G̲ ̲L̲ ̲N̲ ̲L̲ ̲T̲ ̲G̲ ̲A
                    5190                    5220                    5250                    5280
     GACACCGTCATTTTTGTAGAGCATGACTGGAATCCAATGAATGATCTACAGGCAATGGACAGAGCACATAGAATTGGTCAGAAAAAGGTTGTTAATGTTTACAGAATTATTACGAAAGGT
1721 D̲ ̲T̲ ̲V̲ ̲I̲ ̲F̲ ̲V̲ ̲E̲ ̲H̲ ̲D̲ ̲W̲ ̲N̲ ̲P̲ ̲M̲ ̲N̲ ̲D̲ ̲L̲ ̲Q̲ ̲A̲ ̲M̲ ̲D̲ ̲R̲ ̲A̲ ̲H̲ ̲R̲ ̲I̲ ̲G̲ ̲Q̲ ̲K̲ ̲K̲ ̲V̲ ̲V̲ ̲N̲ ̲V̲ ̲Y̲ ̲R̲ ̲I̲ ̲I̲ ̲T̲ ̲K̲ ̲G
                    5310                    5340                    5370                    5400
     ACGCTTGAAGAAAAAATCATGGGTTTGCAGAAATTCAAAATGAATATAGCTTCTACAGTTGTTAATCAGCAAAACAGTGGATTAGCATCCATGGATACACATCAGCTGCTTGATCTCTTC
1761 T̲ ̲L̲ ̲E̲ ̲E̲ ̲K̲ ̲I̲ ̲M̲ ̲G̲ ̲L̲ ̲Q̲ ̲K̲ ̲F̲ ̲K̲ ̲M̲ ̲N̲ ̲I̲ ̲A̲ ̲S̲ ̲T̲ ̲V̲ ̲V̲ ̲N̲ ̲Q̲ ̲Q̲ ̲N̲ ̲S̲ ̲G̲ ̲L̲ ̲A̲ ̲S̲ ̲M̲ ̲D̲ ̲T̲ ̲H̲ ̲Q̲ ̲L̲ ̲L̲ ̲D̲ ̲L̲ ̲F
                    5430                    5460                    5490                    5520
     GACCCCGATAACGTTACCTCACAGGACAATGAGGAAAAGAATAATGGCGATTCCCAAGCAGCCAAGGGCATGGAAGATATTGCCAACGAGACTGGTTTAACTGGCAAAGCCAAAGAGGCC
1801 D̲ ̲P̲ ̲D̲ ̲N̲ ̲V̲ ̲T̲ ̲S̲ ̲Q̲ ̲D̲ ̲N̲ ̲E̲ ̲E̲ ̲K̲ ̲N̲ ̲N̲ ̲G̲ ̲D̲ ̲S̲ ̲Q̲ ̲A̲ ̲A̲ ̲K̲ ̲G̲ ̲M̲ ̲E̲ D  I  A  N  E  T  G  L  T  G  K  A  K  E  A
                    5550                    5580
     CTCGGTGAATTGAAAGAGCTATGGGACCCATCTCAATACGAGGAGGAGTATAATTTAGCACCTTCATCAAAACTTTACGA
1841 L  G  E  L  K  E  L  W  D  P  S  Q  Y  E  E  E  Y  N  L  D  T  F  I  K  T  L  R
                                                                                                            5721
     TAAACAAATTCTTGCATTGGAATGACGCGTATACAAGGTCATTTTTGTTTTATTTAACGCATTTATGTATTATCAACTTGTAATATAATACGGTATATTAATGGAGTCTTATAGAATTTA
                                                                                                            5841
     CGGGTATATTCAAATCAGTATTGATAATGTTTTCATATTCTCTTGTTAAAATTCAGGTTATTAGCTGTCTATTGATTATGGCTTGGACTTTTTTTTTATTTTATTATTTTTCATAGTTAA
                                                                                                            5961
     GTTGCGCCAAAGGGAAACTAGAAACAGTTTAGTCGAACTCTGCAGAGCTTCATCTCAGTATGAAAAATTATTCATTTGAGCTATCGAAACGTTTGGATATAAACATACATAAAACCTCTG
                                                                                                            6081
     TTCTTCTGCCAATTTAAATGCAATTCGCTGGGAAGAAAACTGATCAAGTGACCACATCTTAATCCGGGCTTTGAAGAAGAAGAAGAAGAAGAAGAACTACAACAAGATTGGTCGCAA
                                                                                                            6201
     CTCGCATCGCTCGTTAGCAAAAATGCTGCTCTTTCACTTCCAAAGAGGGGTGAAAAAGACTATGAACCAGATGGGACTAATTTGCAAGATCTGCTTCTCTATAATGCAAGTAAAGCCATG
                                                                                                            6321
     TTTGATACCATATCAGACTCAATAAGAGGCACAACAGTAAAATCTGAAGTAAGAGGATACTATGTTCCCATAAACATCAAGCCGTTCTACTTAAACCCAAAGGCAGCTTTATGCAAACGA
                                                                                                            6441
     TGGGAAGGGCTGATAGCACAGGCGAACTCTGGCTTGACTTCCATGAGTTTGTTTATTTGGCAGAGAGAGGCACTATTCTTCCTTATTATAGATTAGAAGCAGGCTCTAACAAAAGCTCAA
                                                                                                            6561
     AGCACGAAACAGAGATTTTACTCAGTATGGAGGATTTGTATTCGCTGTTTTCATCGCAACAGGAAATGGACCAATATTTTGTGTTTGCTCATTTAAAAAAGGTTGGGTTTTATTCTAAAAC
     6596
     CCAGCAATCAAGAAGCAGCAGTCAAGACTTCTTTT
```

FIG. 4—Continued.

The growth phenotypes associated with the original motl-1 isolate segregated 2:2 and cosegregated with the transcriptional phenotypes associated with the original motl-1 mutation; therefore, the motl-1 allele represented an alteration of a single nuclear gene. The MOT1 gene was cloned from a YCp50 yeast genomic library (59) by complementation of the slow-growth/temperature-sensitive phenotypes. Eleven complementing plasmids were isolated which fell into three distinct but overlapping classes based on restriction mapping. The complementing region was localized to an approximately 8-kb BglII fragment (Fig. 3). To demonstrate that the cloned DNA corresponded to the MOT1 locus, the following experiment was performed. A SacI fragment (Fig. 3) containing part of the complementing region plus flanking sequences was subcloned into the integrating plasmid pRS306 (URA3) (65). This plasmid was linearized at either the unique HpaI site or the unique BglII site within the subcloned fragment (Fig. 3), to target integration to the homologous genomic locus, and introduced into a MATa strain (YPH499) (65) by DNA-mediated transformation. Ura$^+$ transformants were selected. These transformants exhibited no phenotype, and one representative was crossed to a MATα motl-1 strain (JD102C). Tetrad analysis of the resulting diploids showed that the site of integration was allelic to the MOT1 locus; in all 22 tetrads examined, there were no slowly growing, Ura$^+$ or normal-growing, Ura$^-$ recombinants. Hybridization of a BamHI fragment of the MOT1 coding region (Fig. 3) to a chromosome blot (see Fig. 5C), and to an ordered set of cloned yeast DNA fragments in a bacteriophage λ vector (see Materials and Methods), revealed that MOT1 resides on chromosome XVI. Two other genes that affect transcription, SIT3 (2) and SPT14 (21), also reside on chromosome XVI. The motl-1 mutation is not allelic to either of these two loci, as indicated by the following observations. First, a plasmid-borne copy of SIT3 (provided by K. Arndt) does not complement motl-1, nor do cloned SIT3 and MOT1 DNAs cross-hybridize (data not shown). Second, SPT14 encodes a small transcript and is tightly linked to MFα1 (21), whereas MOT1 encodes a large transcript of 6.2 kb (see Fig. 5A) and is unlinked to MFα1 (data not shown).

Nearly 7 kb from the complementing and surrounding regions was subjected to DNA sequence analysis. The nucleotide and predicted amino acid sequences are shown in Fig. 4. One large open reading frame of 5,601 nucleotides or 1,867 amino acids (predicted molecular weight of 209,989) was identified. There are no significant open reading frames on the opposite strand. An analysis of the deduced primary sequence is presented later.

MOT1 is expressed in all cell types. To examine expression of MOT1 in each cell type, an internal 3.5-kb BamHI fragment (Fig. 3) was used as a hybridization probe to a blot containing poly(A)$^+$ RNA from isogenic MATa, MATα, and MATa/α strains that were either MOT1$^+$ or motl-1. The probe detected a single transcript of about 6.2 kb that was present in all three cell types at about the same level (Fig. 5A). The size of this transcript is in good agreement with the size of the predicted open reading frame. The amount of MOT1 message in all of the motl-1 cells was higher than in the corresponding isogenic wild-type strains (Fig. 5A), suggesting that an autoregulatory mechanism may be involved in MOT1 expression.

Expression of the MOT1 transcript in response to α-factor was also examined. There was a decrease in MOT1 mRNA after α-factor treatment in both MOT1$^+$ cells and motl-1 cells (Fig. 5B). It is not clear from this experiment whether the decrease observed was a direct effect of α-factor action

or simply an indication that the MOT1 transcript is more unstable in G$_1$-arrested cells than in growing cultures.

MOT1 is essential for cell growth. A MOT1 gene disruption was constructed by the recently devised γ transformation procedure (65). The motlΔ1 allele was prepared by subcloning the 474-bp SacI-BamHI fragment (nucleotide positions 3539 to 4013) and the 1,058-bp HindIII-ClaI fragment (nucleotide positions 1286 to 2344) (Fig. 3 and 4) in tandem but reverse order into the integrating plasmid pRS306 (URA3) (65). This construct was linearized at a unique restriction site in between the two fragments (HindIII) and introduced into two unrelated diploid strains (YPH501 and 1788) by DNA-mediated transformation. The resulting disrupted gene is predicted to have the 1,194 bp (398 amino acids) between the ClaI and SacI sites (Fig. 3 and 4) deleted and replaced by plasmid sequences. A BamHI fragment which spans the site of the deletion/insertion mutation (Fig. 3) was used as a probe to detect both the wild-type and disrupted MOT1 alleles in Southern blot analysis (67) of genomic DNA isolated from the recipients and Ura$^+$ transformants that had been digested with BamHI (Fig. 6). The wild-type allele migrated at 3.5 kb and the larger deletion/insertion allele migrated at 6.5 kb, confirming that the two diploid strains were heterozygous for the motlΔ1 mutation. Both heterozygous diploids were subjected to tetrad analysis. For 11 of 12 and 10 of 11 tetrads from each strain, respectively, 2:2 segregation of live spores:dead spores was observed (Fig. 6). All of the live spores were Ura$^-$. The spores which did not form visible colonies were examined microscopically. In all cases, the spores had germinated and had yielded microcolonies consisting of 10 to 20 normal-looking cells. Thus, MOT1 is an essential gene required for cell growth. This result further supports the conclusion that Mot1 may affect the expression of genes outside of the mating response pathway because all of the pheromone-responsive genes described to date are nonessential. Of course, Mot1 may also be involved in additional cellular processes that are critical for cell viability.

Analysis of the MOT1 coding sequence. The putative protein sequence of Mot1 was compared with sequences in the GenBank data base, using the TFASTA algorithm (56). This search revealed (Fig. 7) that Mot1 is 40% identical to a stretch of 530 amino acids from the S. cerevisiae Snf2 protein (47), which is involved in the transcriptional regulation of the glucose-repressible invertase gene, SUC2. Cells carrying a snf2 mutation are unable to derepress SUC2 at the transcriptional level when grown on a carbon source such as sucrose or raffinose. In addition, like motl-1, snf2 mutations are pleiotropic, as indicated by defects in galactose and glycerol utilization and effects on expression of acid phosphatase, a non-glucose-repressible gene. Furthermore, the SNF2 message is present in all cells whether they are grown under repressing or nonrepressing conditions. However, unlike MOT1, a snf2 null mutation is not lethal but is identical in phenotype to the point mutations. Recent evidence suggests that Snf2 is a nuclear protein (47), which is consistent with its proposed role in transcription. The Mot1 protein has at least one sequence motif (residues 195 to 209) which could serve as a nuclear targeting signal (57).

Mot1 also is 33% identical to a 556-amino-acid domain from the S. cerevisiae RAD54 gene product (18), in exactly the same region in which Mot1 aligns with Snf2 (Fig. 7A).

Examination of the alignment shown in Fig. 7A revealed that the regions of amino acid identity were clustered, with significantly less identity in the intervening sequences. The first of these clusters matches exactly the A site of the
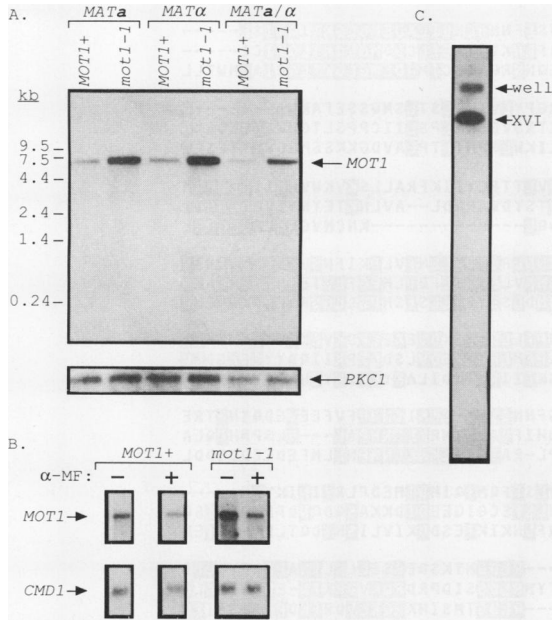
FIG. 5. Expression of *MOT1* mRNA. An internal 3.5-kb *Bam*HI fragment of *MOT1* (Fig. 3) was used as the probe. (A) Poly(A)+ RNA from *MAT*a, *MAT*α, and *MAT*a/α cells. The relevant genotype is indicated above each lane; strains are *STE12*+ and isogenic except at the *MAT* and *MOT1* loci. Migration of molecular weight markers is shown on the left. The *PKC1* probe (see Table 2) was used as a control for the uniformity of RNA loading. (B) Effect of pheromone treatment. Left two lanes, RNA from *MAT*a *MOT1*+ cells; right two lanes, RNA from *MAT*a *mot1-1* cells. The *CMD1* probe (see Table 2) was used as a control for RNA loading. (C) Chromosome blot showing hybridization of *MOT1* to chromosome XVI.

FIG. 6. Tetrad analysis of *MOT1/mot1Δ1* strains. (A) Hybridization analysis (left) and tetrad analysis (right) of a heterozygous *MOT1/mot1Δ1* derivative of strain YPH501. (B) Hybridization analysis (left) and tetrad analysis (right) of a heterozygous *MOT1/mot1Δ1* derivative of strain 1788. For Southern blots, genomic DNA was isolated from the wild-type (*MOT1/MOT1*) parent (lane 1) or from the heterozygous disruption strain (*MOT1/mot1Δ1*) (lane 2), digested with *Bam*HI, and probed with the *MOT1 Bam*HI fragment (Fig. 3). The wild-type allele migrates at 3.5 kb; the *mot1Δ1* allele migrates at 6.5 kb. Positions of migration of λ *Hin*dIII markers are shown to the left of each blot. For tetrad dissections, the four spores from each tetrad were placed in rows either to the left or to the right of the center streak of growth. One tetrad in each dissection yielded 1:3 segregation for live-to-dead spores, presumably as the result of a gene conversion event.

nucleotide-binding sequence found in many diverse enzymes that utilize ATP or GTP (76) (domain I; Fig. 7B). Furthermore, the B site of the nucleotide-binding domain (14) (domain II; Fig. 7B) is also present. Domain II is thought to be a Mg^{2+}-binding domain and to interact with Mg^{2+}-nucleoside triphosphate through the conserved Asp residue (14). Thus, it seems likely that Mot1, Snf2, and Rad54 are all nucleotide-binding proteins.

Mot1, Snf2, and Rad54 are putative helicases. Further examination of the Mot1, Snf2, and Rad54 alignments revealed that the nucleotide-binding site matched best a consensus for this kind of site found in helicases (27). In fact, the Mot1, Snf2, and Rad54 sequences possess all seven of the consecutively placed signature domains that are diagnostic of RNA and DNA helicases (27, 28, 37, 49). The primary sequence of these proteins always begins with the A and B sites of the nucleotide-binding domain, followed by five additional motifs. These seven helicase domains have been delineated by comparing the sequences of over 25 known and putative RNA and DNA helicases from a variety of different organisms, including *Escherichia coli*, various RNA and DNA viruses, *S. cerevisiae*, *Drosophila melanogaster*, and humans (27, 28, 37, 49). These helicases have been split into two superfamilies, SFI and SFII, based on the amino acid sequences in each of the seven domains (28). Proteins belonging to SFI include several viral proteins and the yeast mitochondrial protein Pif1 (37), which has been shown to have DNA helicase activity in vitro (46). Proteins belonging to SFII are exemplified by Rad3 (28), which has also been demonstrated to have DNA helicase activity in

vitro (71). Although the sequence signatures of the seven domains are distinct between SFI and SFII, they are clearly related (28). The composite consensus for each domain (28) is shown in Fig. 7B. Among the known and putative helicases described to date, domains I, II, and VI are the most highly conserved, while domains Ia, III, IV, and V are less well conserved. In some cases, only degenerate forms of these latter domains can be identified (28).

The homologies between the *MOT1*, *SNF2*, and *RAD54* coding sequences cluster in regions which are perfect, or near-perfect, matches to all seven of the diagnostic helicase domains, and they are in the correct order and have the appropriate spacing. In most cases (for example, domain III), the sequences of Mot1, Snf2, and Rad54 match the consensus even better than does the Rad3 sequence. Domain IV of Mot1, Snf2, and Rad54 is interesting from the standpoint that it seems to be intermediate between the two previously recognized superfamilies. The sequence of each of these proteins begins with the F residue that defines domain IV of SFII, but the rest of the sequence is more similar to proteins belonging to SFI. In particular, domain IV of Mot1 and Snf2 is quite similar to domain IV of the helicases from Semliki Forest virus, Sindbis virus, and brome mosaic virus (all members of SFI) in that all have the sequence R-R-X-X-X-D-V (28, 37).

To align domain V of Mot1, Snf2, and Rad54 with domain V of Rad3, two gaps need to be introduced (Fig. 7B). Gorbalenya et al. (28) chose to delete the corresponding residues of Rad3 as a means to maximize the alignment of Rad3 with other putative helicases. Apparently, the spacing between conserved residues in domain V can be somewhat variable.

Finally, for domain VI, although Mot1, Snf2, and Rad54 lack the G residue normally found in proteins belonging to

A

YSCSNF2	(751)	IKLDIKNQPSILVGGILKDYGIKGLDWMVSLFNNHLNGILADEMGLGKIIRIISL-----
YSCMOT1	(1257)	SNAKPFKLP-LAIKAILRKYGQDGVNDLAFLNKYHLHGILCDDMGLGKILQTICI-----
YSCRAD54	(294)	YLLAEAFMTSSEDPLKSDEKALTESDKTEQNRRGAYGCIMADEMGLGKILQCHALMWTLL
YSCNSF2	(806)	------------------LTHLYEMKNIRGPYLVIVPLSTLSNWSSEFAKW--------H
YSCMOT1	(1311)	------------------IASDQYLRKEDYEKTRSVESRALPSLIICPPSLTGHWEHEFDQYA
YSCRAD54	(354)	RQGPQGKRLIDKCIIVCPSSLVNNWANELIKWLGPNTLTPLAVDGKKSSMGGGNTTVSQA
YSCSNF2	(840)	PTLRTISFKGSPNERKAKQAKIRAGEFDWVLTTFEYIIKFRALLSRVKWVHNIIDEGHRM
YSCMOT1	(1356)	PFLKVVVYAGGPTVRLTLRPQLSDADIIVTSYDVARNDL--AVLNKTEYNYCVLDEGHII
YSCRAD54	(414)	IHAWAQAQGRNIVKPVLIISYETLRRNVDQL------------KNCNVGLMLADEGHRL
YSCSNF2	(900)	KNADSKLSLTLNTHYHADYRLILIGIPLQNNLPELWALLNIVLPKIFNSVKSIDEWINTP
YSCMOT1	(1414)	KNSQSKLAKAVK-EITANHRLILIGIPIQNNVLELWSLFDDLMPGFLGTEKMIQLRLAKP
YSCRAD54	(461)	QNGDSLTFTALDSISCPR-RVILSGIPIQNDLSEYFALLSFSNPGLLGSRAEFRKNIENP
YSCSNF2	(960)	FANTGGQDKIELSELETLLVIRRLHKVLRPFLLRRLKKDVEKELPDKVEKVVKCKMSALG
YSCMOT1	(1473)	LAASRNSKTSSKEQEAGVLALEALHKQVLPFMLRRLKEDVLSDLPPKIIQDYYCELGDLG
YSCRAD54	(520)	ILRGRDADATDKEITKGEAQLQKLSTIVSKFIIRRTNDILAKYLPCKYLHVIFVNLKPLQ
YSCSNF2	(1020)	QIMYQQHLNYRRLFIG-DQNNNKMVGLRGFNNQIMG-LKKICNHPFVFEEVEDQINPTRE
YSCMOT1	(1533)	KQLYMDFTRKQKNVVEKDIENSEIADGKQHIFDALDYMRKLCNHPALL---VLSPNHPQLA
YSCRAD54	(580)	NELYNKLIKSDEV---K-KVVKGVGGSQPL-RALGI-LKKLCNHPNLLNFEDEFDDEDDL
YSCSNF2	(1078)	TNDDIWRVAGKFELLDRILPKLLATGHRVLIHFQMIQIMDDIMEDFLRYINIKYL------
YSCMOT1	(1590)	QVQDYLKQTGLLHDIIN---APKLSALRTLLFECGIGEEDIDKKASQDQNDFPIQNVLSQ
YSCRAD54	(634)	ELPDDYNMPGSKARDVQTKYSAKFSILERFLHKIKTESDDKIVLISNYDQTLDLL---IEK
YSCSNF2	(1132)	------------------------------RLDGHTKSDERSELLRLENAPDSEYLCTI
YSCMOT1	(1647)	HRALIFCQLKDMLDMVENDLFKKYMPSVTYMRLDGSIDPRDRQKVVRKIER-EDPSIDCLL
YSCRAD54	(691)	MCRYKHYSAV-------------------RLDGTMSINKRQKLVDRFNDPEGQEFILL
YSCSNF2	(1161)	LSTRAGGLGLNLQTADIVILFDTDWNPHQDLQAQDRAHRIGQKNEVRILRLETTNSVEEV
YSCMOT1	(1706)	LTTNVGGLGLNLTGADIVIFVEHDWNPMNDLQAMDRAHRIGQKKVVNVYRILTKGTLEEK
YSCRAD54	(730)	LSSKAGGCGINLIGANRLILMDPDWNPAADQQALARVVRDGQKKDCFIYRFISTGIIEEK
YSCSNF2	(1221)	ILEWAYKRLDIDGKWIQAGKFDNKSTSEENEALLRSLLDAEEEERRKKRESGVEEEEELKD
YSCMOT1	(1766)	IMGLQKFKMNIASTVVNQQNSGLASMDTHQLLDLIDPDNVWSQDNELKNNGDSQAAKDME
YSCRAD54	(790)	IFQRQSMKMSLSSCVVDAKEDVERLFSSDNLRQLFQKNEMTICETHLTYHCKRCNAQGKQ

B

		I			Ia
YSCRAD3	(39)	ILEMPSGTGKIVSLL		(71)	YCSRTMSEIEKALV
YSCRAD54	(332)	IMADEMGLGKTLQCI		(374)	LVNNWANELIKWLG
YSCSNF2	(789)	ILADEMGLGKIIQII		(808)	YLYEMKNIRGPYLV
YSCMOT1	(1294)	ILCDDMGLGKILQTI		(1316)	YLRKEDYEKTRSVE
consensus		0 G GKT 0			00 Z 0 ZO
		S			T

		II			III
YSCRAD3	(226)	VSKDSIVIFDLAHNI		(457)	IITSGIISPLDMYPRMLNI
YSCRAD54	(446)	NCNVGLMLADEGHRL		(480)	VILSGIPIQNDLSEYFALL
YSCSNF2	(885)	KVKWVHNIIDEGHRM		(920)	LILIGIPLQNNLPELWALL
YSCMOT1	(1399)	KTEYNYCVLDEGHII		(1433)	LILIGIPIQNNVLELWSLF
consensus		Z 0000DE H			0 OTAT Z 0 Z
		D			SGS

		IV			V
YSCRAD3	(539)	VIFPSYLYMESIV		(590)	CSNGRGAILLSVARGKVSEGIDFDHDYGRIVL
YSCRAD54	(549)	KIIIRRTNDILAK		(721)	PEGQEFILLSSKAG--GCGINL--IGANRLI
YSCSNF2	(989)	PTLLRRLKKDVEK		(1152)	PDSEYLCIILSTRAG--GLGLNL--QTADIVI
YSCMOT1	(1502)	PFMLRRLKEDVLS		(1697)	EDPSIDCLLLTIKVG--GLGLNL--TGADIVI
consensus		F S Z Z			Z Z 000 SZO G ZO Z 00
		Y T			T S

		VI
YSCRAD3	(658)	AMRHAADCLGRVLEGKD
YSCRAD54	(755)	NPAADQQALARVVRDGQ
YSCSNF2	(1186)	NPHQDLQAQDRAHRIGQ
YSCMOT1	(1731)	NPMNDLQAMDRAHRIGQ
consensus		Z Z Q GR R
		H

FIG. 7. Homology of *MOT1* with *SNF2* and *RAD54*. (A) Alignment of the C terminus of Mot1 with those of Snf2 and Rad54. The alignment was generated by using the FASTA algorithm (56) and further optimized by visual inspection. Gene designation is on the far left, followed by the amino acid position in parentheses. Amino acids are depicted in the one-letter code; identities between two or more proteins are presented by white-on-black letters. (B) Comparison of the helicase domains of Rad3, Rad54, Snf2, and Mot1 with the seven consensus helicase domains. Gene designation is on the far left, with amino acid positions in parentheses. Amino acids are depicted in the one-letter code; identities between two or more proteins are given as white-on-black letters. The consensus sequence for each domain is as described by Gorbalenya et al. (28). O, hydrophobic residues (I, L, V, M, F, Y, and W); Z, charged/polar residues (S, T, D, E, N, Q, K, and R).

SFII, the rest of the domain is a perfect match to the consensus. Interestingly, all members of SFI also lack this G residue. Thus in Mot1, Snf2, and Rad54, domain VI, like domain IV, appears to be a hybrid of the SFI and SFII superfamilies.

DISCUSSION

The genetic screen described here was designed to identify factors which operate in the absence of the Ste12 protein, the DNA-binding protein which is required for transcriptional activity of pheromone-responsive genes (16, 17, 19, 23, 24). Such a screen might uncover mutations in specific factors directly downstream of Ste12, possibly belonging to the newly proposed class of coactivator proteins (3, 42), as well as factors operating independently of Ste12 such as those affecting the general transcriptional machinery, chromatin structure, or other nontranscriptional aspects of gene expression such as mRNA transport or stability. Cells carrying the *mot1-1* allele show increased expression of only those pheromone-responsive genes that exhibit low basal transcription in the absence of Ste12. Alteration of *MOT1* had no discernible effect on the expression of those pheromone-responsive genes which have a completely Ste12-dependent mode of transcription. Because *mot1-1* does not affect all pheromone-responsive genes equivalently, it seems probable that its effect on transcription is indirect rather than specific to pheromone-regulated genes.

Additional evidence presented here supports this view. First, we found that a *cyc1-lacZ* plasmid lacking a UAS exhibits a higher level of expression in *mot1-1* strains than in isogenic wild-type controls. Other mutations which appear to alter chromatin structure in vivo also have been shown to suppress a UAS-less promoter; for example, when cells are depleted for histone H2B or H4, elevated transcription from a *PHO5* promoter lacking its UAS is observed under repressing as well as derepressing conditions (32). Second, we have identified a gene (*DED1*) (70) not previously implicated in any way in the mating response whose expression is increased in the *mot1-1* background, raising the possibility that numerous cellular genes are also affected by alteration of *MOT1*. It is interesting to note that DNA sequence analysis of the *DED1* gene product reveals that it is also a presumptive helicase, like Mot1, and furthermore, it appears to have a role in mRNA splicing (40). Finally, we demonstrated that the *MOT1* gene is essential for cell growth and viability, consistent with the conclusion that the function of Mot1 includes vital cellular processes and is not exclusively confined to expression of genes in the mating pathway.

For those Ste12-dependent genes whose expression was elevated by the *mot1-1* mutation, the relative effect of the *mot1-1* allele was more pronounced in a *ste12* background than in a *STE12* background. In this sense, the presence of Ste12 appears to counteract somehow the influence of the *mot1-1* defect, even though the absolute level of expression of Ste12-dependent genes was, of course, higher in *STE12* cells. If Mot1 is a DNA helicase, one hypothesis that is

consistent with these data is that normal Mot1 operates in conjunction with the general transcription apparatus. Furthermore, because *mot1-1* is a recessive allele and is likely, therefore, to represent a loss-of-function mutation, the normal role of Mot1 may be to remove basal transcription factors from the DNA. The more persistent occupancy of the promoter region of a gene by these factors in a *mot1-1* cell could explain the enhancement of gene expression observed when Ste12 is absent. The higher level of expression of these genes supported by the presence of a specific transcriptional activator, like Ste12, could obscure the modest effect of the *mot1-1* mutation.

The *MOT1* gene contains an open reading frame that is capable of encoding a protein with a molecular weight of 210,000. The C-terminal 568 amino acids of Mot1 are homologous to the corresponding amino acids of the *S. cerevisiae* Snf2 and Rad54 proteins. All three of these proteins have the seven signature sequences of RNA, RNA/DNA, and DNA helicases (27, 28, 37, 49). On the basis of the following observations, we propose that Mot1, Snf2, and Rad54 define a new subfamily of these helicases. First, the degree of sequence identity in each of the helicase domains is greater among Mot1, Snf2, and Rad54 than between these proteins and any other previously described member of the helicase family. Second, these three proteins share two additional
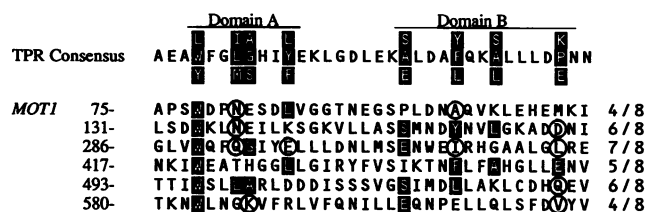

FIG. 8. Potential TPR motifs in the amino-terminal domain of the *MOT1* gene product. The consensus sequence derived from comparison of 47 34-amino-acid TPR repeats found in five different yeast nuclear proteins, as defined by Sikorski et al. (64) and Hirano et al. (36), is indicated on the top line. The amino acids indicated on this line by white-on-black letters are those found most frequently (in at least 40% of the repeats) at these positions; the amino acids indicated above this line are the next most frequently observed substitutions at these positions, and the amino acids indicated below the line are the third most frequently observed replacements at these positions. Six segments from the predicted primary sequence of the *MOT1* gene product, each beginning at the residue indicated by the number to the left, are shown. Amino acids indicated by white-on-black letters are perfect matches to the most highly conserved residues of the TPR consensus; amino acids that are circled match residues that are found at the identical position in at least one of the TPR repeats used to compile the consensus. The number of matches of the putative TPR repeats in Mot1 at the eight most highly conserved positions of the TPR consensus are given on the right. It should be noted that of the 47 TPR repeats used to derive the consensus sequence, the distribution, in terms of degree of match at the eight most conserved positions, was 1/8, 2; 2/8, 2; 3/8, 6; 4/8, 6; 5/8, 6; 6/8, 9; 7/8, 10; and 8/8, 6 (36, 64).

blocks of amino acid identity (1565 to 1579 and 1678 to 1696 in Mot1) that are not found in other helicases. Third, although most of the helicase domains in Mot1, Snf2, and Rad54 match those of the SFII subfamily, two of the domains, IV and VI, are somewhat unique in that they are a hybrid of the motifs present in the SFI and SFII subclasses. Based on sequence comparisons, two additional potential members of this newly recognized subfamily have been identified in S. cerevisiae, RAD16 (61a) and STH1, a homolog of SNF2 (47a). The fact that these proteins are so similar to each other in their respective helicase domains suggests that they may be involved in similar areas of nucleic acid metabolism, may interact with similar substrates or accessory molecules, and/or may be regulated in a similar manner.

Helicases have now been implicated in numerous cellular processes which require DNA or RNA unwinding, including DNA recombination and repair (43, 71). However, cells carrying the mot1-1 mutation are no more sensitive to UV or X-ray irradiation than isogenic normal cells (data not shown), making it unlikely that Mot1 is involved in a major DNA repair pathway. Other cellular processes in which helicases have been implicated are the initiation and progression of DNA replication (7), cell proliferation (26, 34), RNA splicing (30), ribosome assembly (53), initiation of translation (1), and transcription (66). Because a mutation in MOT1 has been shown to elevate the transcription of certain genes, it is tempting to speculate that it is a helicase involved in transcription. The mot1-1 allele is recessive and increases the basal level of expression of several classes of genes. Taken together, these observations suggest that the wild-type Mot1 protein functions, formally, as a negative regulator of transcription. Perhaps the function of Mot1 normally is to strip or expel resident DNA-binding proteins from their cognate sites by melting the duplex DNA in the regions where these transcriptional activators or initiation factors would bind. On the other hand, this putative helicase may be pivotally involved in a different nuclear process, and the transcriptional effects observed in mot1-1 cells may, therefore, be indirect. Such a situation might account for the relatively modest transcriptional effects (threefold) compared with the dramatic phenotypic effects of slow-growth and temperature-sensitive lethality conferred by the mot1-1 mutation. It will be important to determine whether the primary role of Mot1 is in transcription or whether it lies in some other aspect of cellular physiology.

There are several additional features of the MOT1 coding sequence which are worth noting. First, there are numerous potential phosphorylation sites for several different types of protein kinases, as revealed by a search of protein sequence motifs in the Keybank7 data base (8). Hence, Mot1 may be a phosphoprotein whose activity may be regulated via phosphorylation.

Second, the entire protein lacks any contiguous blocks of strikingly hydrophobic segments of any significant length, making it unlikely that Mot1 is a transmembrane protein.

Finally, within the N-terminal region that precedes the helicase domain, there are motifs that are similar to the so-called tetratricopeptide (TPR) units found in two proteins which modulate RNA synthesis, encoded by SSN6 and SKI3, as well as in several proteins important for mitosis, encoded by CDC23, nuc2$^+$, CDC16, and bimA (36, 64) (Fig. 8). In the case of the nuc2$^+$ gene product, a nuclear scaffold protein, and Ssn6, a nuclear phosphoprotein, these repeats have been shown to be essential for function (36, 62). In addition, there are several long segments in Mot1 that have

the 4-3 hydrophobic repeat characteristic of amphipathic α helices that have the potential to form either intramolecular or intermolecular coiled coils (11) (Fig. 4). Thus, it is tempting to speculate that Mot1 is a nucleoskeletal protein which is capable of interacting with DNA through its C-terminal helicase domain and of associating with or being a part of the nuclear matrix through its TPR units or through formation of helical bundles with other proteins. These latter structural features of the protein could serve to correctly position the C-terminal helicase domain so that it carries out its function properly.

ACKNOWLEDGMENTS

REFERENCES

1. Abramson, R. D., T. E. Dever, T. G. Lawson, B. K. Ray, R. E. Tach, and W. C. Merrick. 1987. The ATP-dependent interaction of eukaryotic initiation factors with mRNA. J. Biol. Chem. 262:3826–3832.
2. Arndt, K. T., C. A. Styles, and G. R. Fink. 1989. A suppressor of a HIS4 transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell 56:527–537.
3. Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente. 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61:1199–1208.
4. Boeke, J. D., F. LaCroute, and G. R. Fink. 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. 197:345–346.
5. Boyer, H., and D. Roilland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in Escherichia coli. J. Mol. Biol. 41:459–472.
6. Brake, A. J., C. Brenner, R. Najarian, P. Laybourn, and J. Merryweather. 1985. Structure of genes encoding precursors of the yeast peptide mating pheromone a-factor, p. 103–108. In M.-J. Gething (ed.), Protein transport and secretion. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. Brill, S. J., and B. Stillman. 1989. Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. Nature (London) 342:92–95.
8. Brutlag, D. L., J. P. Dautricourt, S. Maulik, and J. Relph. 1990. Improved sensitivity of biological sequence database searches. Comp. Appl. Biol. Sci. 6:237–245.
9. Burkholder, A. C., and L. H. Hartwell. 1985. The yeast α-factor receptor: structural properties deduced from the sequence of the STE2 gene. Nucleic Acids Res. 13:8463–8475.
10. Chung, C. T., and R. H. Miller. 1988. A rapid and convenient method for the preparation and storage of competent bacterial cells. Nucleic Acids Res. 16:3580.
11. Cohen, C., and D. A. D. Parry. 1986. α-Helical coiled coils—a widespread motif in proteins. Trends Biochem. Sci. 11:245–248.
12. Courschesne, W. E., and J. Thorner. 1986. Control of pheromone response in Saccharomyces cerevisiae: isolation of the SST2 gene. Yeast 2:S74.
13. Davis, T. N., M. S. Urdea, F. R. Masiarz, and J. Thorner. 1986. Isolation of the yeast calmodulin gene: calmodulin is an essential protein. Cell 47:423–431.

14. de Vos, A. M., L. M. Tong, M. V. Milburn, P. M. Natias, J. Jancarik, S. Noguchi, S. Nishimura, K. Miura, E. Ohtsuka, and S. H. Kim. 1988. Three dimensional structure of an oncogene protein: catalytic domain of human c-H-*ras* p21. Science **239**:888–893.

15. Dietzel, C., and J. Kurjan. 1987. Pheromonal regulation and sequence of the *Saccharomyces cerevisiae SST2* gene: a model for desensitization to pheromone. Mol. Cell. Biol. **7**:4169–4177.

16. Dolan, J. W., and S. Fields. 1990. Overproduction of the yeast Ste12 protein leads to constitutive transcriptional induction. Genes Dev. **4**:492–502.

17. Dolan, J. W., C. Kirkman, and S. Fields. 1989. The yeast Ste12 protein binds to the DNA sequence mediating pheromone induction. Proc. Natl. Acad. Sci. USA **86**:5703–5707.

18. Emery, H. S., D. Schild, D. E. Kellogg, and R. K. Mortimer. 1991. Sequence of *RAD54*, a *Saccharomyces cerevisiae* gene involved in recombination and repair. Gene **104**:103–106.

19. Errede, B., and G. Ammerer. 1989. STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein-DNA complexes. Genes Dev. **3**:1349–1361.

20. Estruch, F., and M. Carlson. 1990. *SNF6* encodes a nuclear protein that is required for expression of many genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:2544–2553.

21. Fassler, J. S., W. Gray, J. P. Lee, G. Yu, and G. Gingerich. 1991. The *Saccharomyces cerevisiae SPT14* gene is essential for normal expression of the yeast transposon, Ty, as well as for expression of the *HIS4* gene and several genes in the mating pathway. Mol. Gen. Genet. **230**:310–320.

22. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.

23. Fields, S., D. T. Chaleff, and G. F. Sprague, Jr. 1988. Yeast *STE7*, *STE11*, and *STE12* genes are required for expression of cell-type-specific genes. Mol. Cell. Biol. **8**:551–556.

24. Fields, S., and I. Herskowitz. 1985. The yeast *STE12* product is required for expression of two sets of cell-type-specific genes. Cell **42**:923–930.

25. Fields, S., and I. Herskowitz. 1987. Regulation by the yeast mating-type locus of *STE12*, a gene required for cell-type-specific expression. Mol. Cell. Biol. **7**:3818–3821.

26. Ford, M. J., I. A. Anton, and D. P. Lane. 1988. Nuclear protein with sequence homology to translation initiation factor eIF-4A. Nature (London) **332**:736–738.

27. Gorbalenya, E. G., E. V. Koonin, A. P. Donchenko, and V. M. Blinov. 1988. A conserved NTP-motif in putative helicases. Nature (London) **333**:22.

28. Gorbalenya, E. G., E. V. Koonin, A. P. Donchenko, and V. M. Blinov. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. Nucleic Acids Res. **17**:4713–4730.

29. Guarente, L., and M. Ptashne. 1981. Fusion of *Escherichia coli lacZ* to the cytochrome c gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **78**:2199–2203.

30. Guthrie, C. 1991. Messenger RNA splicing in yeast: clues to why the spliceosome is a ribonucleoprotein. Science **253**:157–163.

31. Hagan, D. C., and G. F. Sprague, Jr. 1984. Induction of the yeast α-specific *STE3* gene by the peptide pheromone a-factor. J. Mol. Biol. **178**:835–852.

32. Han, M., and M. Grunstein. 1988. Nucleosome loss activates yeast downstream promoter *in vivo*. Cell **55**:1137–1145.

33. Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.

34. Hay, B., L. Y. Jan, and Y. N. Yan. 1988. A protein component of *Drosophila* polar granules is encoded by *Vasa* and has extensive sequence similarity to ATP-dependent helicases. Cell **55**:577–587.

35. Herskowitz, I., and R. E. Jensen. 1991. Putting the HO gene to work: practical uses for mating-type switching, p. 132–145. *In* C. Guthrie and G. R. Fink (ed.), Guide to yeast genetics and molecular biology. Academic Press, Inc., San Diego, Calif.

36. Hirano, T., N. Kinoshita, K. Morikawa, and M. Yanagida. 1990. Snap helix with knob and hole: essential repeats in S. pombe

37. Hodgman, T. C. 1988. A new superfamily of replicative proteins. Nature (London) **333**:23. (Erratum, **333**:578.)

38. Hoffman, C. S., and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57**:267–272.

39. Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.

40. Jamieson, D. J., B. Rahe, J. Pringle, and J. D. Beggs. 1991. A suppressor of a yeast splicing mutation (*prp8-1*) encodes a putative ATP-dependent RNA helicase. Nature (London) **349**:715–717.

41. Jones, E. W. 1977. Proteinase mutants of *Saccharomyces cerevisiae*. Genetics **85**:23–33.

42. Kelleher, R. J., III, P. M. Flanagan, and R. D. Kornberg. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61**:1209–1215.

43. Kodahek, T., and B. Alberts. 1987. Stimulation of protein strand exchange by a DNA helicase. Nature (London) **326**:312–314.

44. Kronstad, J. W., J. A. Holly, and V. L. MacKay. 1987. A yeast operator overlaps an upstream activation site. Cell **50**:369–377.

45. Kuchler, K., R. E. Sterne, and J. Thorner. 1989. *Saccharomyces cerevisiae STE6* gene product: a novel pathway for protein export in eukaryotic cells. EMBO J. **8**:3973–3984.

46. Lahaye, A., H. Stahl, D. Thines-Sempoux, and F. Foury. 1991. PIF1: a DNA helicase in yeast mitochondria. EMBO J. **10**:997–1007.

47. Laurent, B. C., M. A. Treitel, and M. Carlson. 1991. Functional interdependence of the yeast *SNF2*, *SNF5* and *SNF6* proteins in transcriptional activation. Proc. Natl. Acad. Sci. USA **88**:2687–2691.

47a. Laurent, B. C., X. Yang, and M. Carlson. Personal communication.

48. Levin, D. E., F. O. Fields, R. Kunisawa, J. M. Bishop, and J. Thorner. 1990. A candidate protein kinase C gene, *PKC1*, is required for the *S. cerevisiae* cell cycle. Cell **62**:213–224.

49. Linder, P., P. F. Lasko, P. J. Nielsen, K. Nishi, and P. P. Slonimski. 1989. Birth of the D-E-A-D box. Nature (London) **337**:121–122.

50. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

51. Messing, J., R. Crea, and P. H. Seeburg. 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.

52. Nakayama, N. N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. **4**:2643–2648.

53. Nishi, K., F. Morel-Deville, J. W. B. Hershey, T. Leighton, and J. Schier. 1988. An eIF-4A-like protein is a suppressor of an *Escherichia coli* mutant defective in 50S ribosomal subunit assembly. Nature (London) **336**:496–499.

54. Norrander, J., T. Kempe, and J. Messing. 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.

55. Olson, M. V., J. E. Dutchik, M. Y. Graham, G. M. Brodeur, C. Helms, M. Frank, M. Maccollin, R. Scheinman, and T. Frank. 1986. Random-clone strategy for genomic restriction mapping in yeast. Proc. Natl. Acad. Sci. USA **83**:7826–7830.

56. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.

57. Robbins, J., S. M. Dilworth, R. A. Laskey, and C. Dingwall. 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64**:615–623.

58. Rose, M., M. J. Casadaban, and D. Botstein. 1981. Yeast genes fused to β-galactosidase in *Escherichia coli* can be expressed normally in yeast. Proc. Natl. Acad. Sci. USA **78**:2460–2464.

59. Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink. 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60**:237–243.

60. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.

61. **Russell, D. W., R. Jensen, M. J. Zoller, J. Burke, B. Errede, M. Smith, and I. Herskowitz.** 1986. Structure of the *Saccharomyces cerevisiae HO* gene and analysis of its upstream regulatory region. Mol. Cell. Biol. **6:**4281–4294.

61a.**Schild, D., B. Glassner, R. K. Mortimer, M. Carlson, and B. C. Laurent.** Personal communication.

62. **Schultz, J., L. Marshall-Carlson, and M. Carlson.** 1990. The N-terminal TPR region is the functional domain of SSN6, a nuclear phosphoprotein of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**4744–4756.

63. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

64. **Sikorski, R. S., M. S. Boguski, M. Goebl, and P. Hieter.** 1990. A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60:**307–317.

65. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.

66. **Sopta, M., Z. F. Burton, and J. Greenblatt.** 1989. Structure and associated DNA-helicase activity of a general transcription initiation factor that binds to RNA polymerase II. Nature (London) **341:**410–414.

67. **Southern, E. M.** 1975. Detecting specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.

68. **Sprague, G. F., Jr.** 1991. Signal transduction in yeast mating: receptors, transcription factors, and the kinase connection. Trends Genet. **7:**393–398.

69. **Stetler, G. L., and J. Thorner.** 1984. Molecular cloning of hormone responsive genes from the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **81:**1144–1148.

70. **Struhl, K.** 1985. Nucleotide sequence and transcriptional mapping of the yeast *pet56-his3-ded1* gene region. Nucleic Acids Res. **13:**8587–8601.

71. **Sung, P., L. Prakash, S. W. Matson, and S. Prakash.** 1987. RAD3 protein of *Saccharomyces cerevisiae* is a DNA helicase. Proc. Natl. Acad. Sci. USA **84:**8951–8955.

72. **Trueheart, J., J. D. Boeke, and G. R. Fink.** 1987. Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol. Cell. Biol. **7:**2316–2328.

73. **Tschumper, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *trp1* gene. Gene **10:**157–166.

74. **Van Arsdell, S. W., and J. Thorner.** 1987. Hormonal regulation of gene expression in yeast, p. 325–332. *In* D. Granner, M. G. Rosenfeld, and S. Chang (ed.), Transcriptional control mechanisms. Alan R. Liss, New York.

75. **Vollrath, D., R. W. Davis, C. Connelly, and P. Hieter.** 1988. Physical mapping of large DNA by chromosome fragmentation. Proc. Natl. Acad. Sci. USA **85:**6027–6031.

76. **Walker, J. E., M. Saraste, J. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes. EMBO J. **1:**945–951.