

The Yeast *MOT2* Gene Encodes a Putative Zinc Finger Protein That Serves as a Global Negative Regulator Affecting Expression of Several Categories of Genes, Including Mating-Pheromone-Responsive Genes

KENJI IRIE, KYOKO YAMAGUCHI, KAZUMITSU KAWASE, AND KUNIHIRO MATSUMOTO*

Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

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The *STE4* gene encodes the β subunit of a heterotrimeric G protein that is an essential component of the pheromone signal transduction pathway. To identify downstream component(s) of Ste4, we sought pseudo-revertants that restored mating competence to *ste4* mutants. The suppressor *mot2* was isolated as a recessive mutation that restored conjugational competence to a temperature-sensitive *ste4* mutant and simultaneously conferred a temperature-sensitive growth phenotype. The *MOT2* gene encodes a putative zinc finger protein, the deletion of which resulted in temperature-sensitive growth, increased expression of *FUS1* in the absence of pheromones, and suppression of a deletion of the α -factor receptor. On the other hand, sterility resulting from deletion of *STE4* was not suppressed by the *mot2* deletion. These phenotypes are similar to those associated with temperature-sensitive mutations in *CDC36* and *CDC39*, which are proposed to encode general negative regulators of transcription rather than factors involved in the pheromone response pathway. Deletion of *MOT2* also caused increased transcription of unrelated genes such as *GAL7* and *PHO84*. Overexpression of *MOT2* suppresses the growth defect of temperature-sensitive mutations in *CDC36* and *CDC39*. These observations suggest that Mot2 functions as a general negative regulator of transcription in the same processes as Cdc36 and Cdc39.

In the yeast *Saccharomyces cerevisiae*, conjugation of the two haploid cell types, a and α , yields an a/α diploid cell through a multistep mating process. The mating response is initiated by peptide pheromones secreted by each haploid cell type. α cells secrete α -factor pheromone that acts on a cells, and a cells secrete a -factor pheromone that acts on α cells. In response to mating pheromones, target cells undergo a number of major physiological changes. These include transcriptional activation of mating genes, morphological changes, and inhibition of cell cycle progression during G_1 phase (24).

Several genes involved in the signal transduction pathway have been identified by genetic and molecular analysis of the mating pheromone response. The *STE2* and *STE3* genes encode the α -factor receptor and a -factor receptor, respectively. The primary structures of these receptors are proposed to contain seven transmembrane domains and thus are similar to mammalian G protein-coupled receptors (7, 16, 28). *GPA1*, *STE4*, and *STE18*, respectively, encode the α , β , and γ subunits of a heterotrimeric G protein (4, 12, 20, 26, 42) that is thought to be functionally coupled to the mating pheromone receptors. Disruptions of *GPA1* cause constitutive activation of the pheromone response pathway (12, 20, 26), whereas disruption of either *STE4* or *STE18* leads to an inability to activate the response (4, 42). These results indicate that *STE4* and *STE18* play a positive role in the pheromone signaling pathway, whereas *GPA1* has a negative regulatory role.

Other components that act downstream of the receptor and G protein $\beta\gamma$ element include Ste5, a protein of unknown function, a group of protein kinases encoded by the *STE20*, *STE11*, *STE7*, *FUS3*, and *KSS1* genes (24), and the Ste12

transcription factor. These components are all required for the response to the pheromone, indicating that they define a set of positive elements in transmission of the signal.

Negative elements that inhibit the response pathway in the absence of pheromones have been identified. They include *CDC72*, *CDC73*, *SRM1*, *CDC36*, and *CDC39*. The first three of these genes have been defined by mutations that suppress the mating defect caused by deletion of pheromone receptor genes (9, 35). *CDC72* is allelic with *NMT1*, which encodes *N*-myristoyl transferase (13, 40). Gpa1 is myristoylated by Nmt1, and without this modification, Gpa1 is unable to inhibit pheromone signaling (40). *SRM1* is homologous to the mammalian *RCCI* gene that has been characterized as a regulator of the onset of mitosis (8, 32). The *SRM1* gene was also independently isolated in quite different screens. Mutations in the same gene, designated *PRP20*, were found in screens for mutants in pre-mRNA splicing and in 3'-end formations (1, 14). Another group identified this gene, designated *MTR1*, as a conditional mutation causing defective mRNA export (21). These findings suggest that *SRM1/PRP20/MTR1* plays some role in mediating processes as different as regulation of the mating pathway and processing and export of mRNAs. The *CDC36* and *CDC39* genes were originally identified in a screen for conditional cell division mutants that arrest cell division at the same step in the cycle as that controlled by pheromones (34). Mutations in *CDC36* and *CDC39* cause constitutive activation of the pheromone response pathway. Epistasis analysis with *ste2*, *ste4*, and *ste18* null mutations suggested that *CDC36* and *CDC39* gene products act at the level of the G protein (11, 29). However, Collart and Struhl isolated temperature-sensitive mutations in *CDC36* and *CDC39* by selecting for mutants that increase *HIS3* transcription by a Gcn4 derivative with a defective activator domain (10). Their results suggest that *CDC36* and *CDC39* encode general negative

* Corresponding author. Mailing address: Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan. Phone: (81)-52-789-3000. Fax: (81)-52-789-3001.

TABLE 1. Strains used in this study

| Strain | Genotype |
|----------|---|
| KA31 | <i>MATa/MATα ura3/ura3 his3/his3 leu2/leu2 trp1/trp1</i> |
| KMG4-7D | <i>MATα ura3 his3 trp1</i> |
| KMG61-1D | <i>MATa ste4-3 ura3 his3 leu2</i> |
| KMG62-1C | <i>MATα ste4-3 mot2-1 ura3 his3</i> |
| KYC36-2D | <i>MATa cdc36-16 ura3 leu2 ade1</i> |
| KYC39-3D | <i>MATα cdc39-1 ura3 leu2 trp1</i> |
| KY803 | <i>MATa gcn4-Δ1 ura3 leu2 trp1</i> |
| KY803-36 | <i>MATa cdc36 gcn4-Δ1 ura3 leu2 trp1</i> |
| KY803-39 | <i>MATa cdc39-2 gcn4-Δ1 ura3 leu2 trp1</i> |
| KY803-MO | <i>MATa mot2Δ::URA3 gcn4-Δ1 ura3 leu2 trp1</i> |
| 1411-1B | <i>MATα mot2Δ::HIS3 ste4Δ::LEU2 ura3 his3 leu2 trp1</i> |
| 1711-2D | <i>MATa mot2Δ::HIS3 ste2Δ::LEU2 ura3 his3 leu2 trp1</i> |
| 4611-2A | <i>MATa mot2Δ::HIS3 ura3 his3 leu2 trp1</i> |
| 4611-12C | <i>MATa ura3 his3 leu2 trp1</i> |

regulators of transcription rather than factors directly involved in the pheromone response pathway. Thus, the pheromone response phenotypes caused by mutations in these genes appear to arise indirectly.

In this study, we isolated a new mutation, *mot2*, that restored conjugational competence to a temperature-sensitive *ste4* mutant and simultaneously conferred a temperature-sensitive growth phenotype. *MOT2* encodes a putative zinc finger protein, and disruption of *MOT2* results in phenotypes similar to those of the *cdc36* and *cdc39* mutants. The molecular and genetic analyses presented here suggest that Mot2 functions as a general negative regulator of transcription in the same processes as Cdc36 and Cdc39.

MATERIALS AND METHODS

Microorganisms and plasmids. The *S. cerevisiae* strains used in this study are listed in Table 1. *Escherichia coli* DH5 α (18) was used for propagation of plasmid DNAs. Another *E. coli* strain, JM103, was used as the host for bacteriophages M13mp18 and M13mp19 (25), in the preparation of single-stranded DNAs, and for DNA sequencing. The plasmid vectors used were YCp50, YCplac33, YEp13, YEplac181, and YEplac195 (15, 33, 36). The genomic DNA library of *S. cerevisiae*, constructed by partial digestion of total genomic DNA with *Sau3A* and ligation at the *Bam*HI site of YCp50, was provided by Rose et al. (36). Plasmid YEpCDC36 is a YEp13 plasmid carrying the *CDC36* gene. Plasmid YEpCDC39 is a YEp13 plasmid carrying the *CDC39* gene. Plasmid pBG71 contains a *GAL7-lacZ* hybrid gene and *URA3* as a selection marker (31). Plasmid pHS6 contains a *PHO84-lacZ* hybrid gene and *URA3* as a selection marker (6). Plasmid pRS314-Sc4363 contains *gcn4-C163* and *TRP1* as a selection marker (10). Plasmid pKF1 is YCp50 carrying the 7.8-kb fragment which includes *MOT2*. Plasmid pRF11 is YCplac33 carrying the 2.4-kb *Sma*I-*Eco*RI fragment of the *MOT2* gene. Plasmid pKF3 is a YEplac181 plasmid carrying the 2.4-kb *Sma*I-*Eco*RI fragment of the *MOT2* gene. YEp vectors are high-copy-number plasmids, and YCp vectors are low-copy-number plasmids.

Media and genetic and biochemical methods. The compositions of the rich medium (YPD) and the synthetic medium (SD), supplemented with the appropriate nutrients for plasmid maintenance, have been described previously (26). Plates containing 5-fluoroorotic acid (5-FOA) were supplemented with 5-FOA at a concentration of 1 mg/ml (5). Standard genetic procedures for yeast manipulation were used (39).

Yeast transformations were carried out by the alkali cation method (19). *E. coli* was transformed as described by Morrison (27). Methods for preparation and manipulation of DNAs were described previously (26). The *MOT2* DNA sequence was determined for both strands by the dideoxy chain termination method (38) following subcloning of restriction endonuclease fragments into M13mp18 or M13mp19.

Construction of disruption strains of *MOT2*. Two disruption mutations of the *MOT2* gene were constructed by the one-step gene replacement method (see Fig. 2) (37). The 1.0-kb *Acc*III-*Pst*I fragment of *MOT2* was replaced with the 1.2-kb *Bam*HI-*Nsi*I fragment of *HIS3*, and the 1.1-kb *Acc*III-*Stu*I fragment of *MOT2* was replaced with the 2.1-kb *Bam*HI-*Sma*I fragment of *URA3*, after appropriate conversion of restriction sites. These constructions were made in the vector pBluescript SK+ carrying the 2.4-kb *Sma*I-*Eco*RI fragment of *MOT2*. The DNAs containing the entire *mot2::HIS3* or *mot2::URA3* constructions were used to transform a diploid strain, KA31, or a haploid strain, KY803, by selection for His⁺ or Ura⁺. Restriction mapping and Southern analysis of genomic DNAs from the resulting transformants were conducted to confirm that transplacement had occurred at the *MOT2* locus.

RNA analysis. Northern (RNA) blot analysis was performed as described previously (26). The *FUS1* hybridization probe was a 1.0-kb *Pst*I-*Bam*HI fragment of pSB231 (41). The hybridization signals corresponding to *FUS1* mRNA were quantified with a Fuji phosphoimager.

β -Galactosidase assays. β -Galactosidase assays were performed as described previously (26).

Flow cytometry. Flow cytometry assays were performed as described previously (30).

Mating assay. A patch mating assay was used for screening and as a qualitative measure of mating competence. Strains carrying plasmids were inoculated onto selective plates and allowed to grow to a heavy patch (2 days), replica plated onto a lawn of tester strain, allowed to mate overnight, and replica plated again on media selective for diploids.

Nucleotide sequence accession number. The GenBank/EMBL accession number for *MOT2* is L26309.

RESULTS

Isolation of the *mot2* mutation. To identify downstream components of the Ste4 G protein β subunit in the mating pheromone response pathway, we isolated mutants that had regained the ability to mate at the restrictive temperature in the background of the temperature-sensitive *ste4-3* cells. We reasoned that mutations which caused stimulation of the pheromone response pathway at a point downstream from Ste4 might restore conjugation in the *ste4-3* mutant cells at 35°C. Strain KMG61-1D (*MATa ste4-3*), which had been treated with the mutagen ethyl methanesulfonate, was challenged to mate with *MAT α* cells (strain KMG4-7D) at 35°C. The resulting diploid cells were induced to sporulate. Of the eight diploids analyzed, one (KMG62) produced tetrads that contained more than two spores that were fertile at 35°C. This segregation pattern suggested the presence of a suppressor mutation. The suppressor locus was designated *MOT2* (modulator of transcription; see the description below).

This *mot2-1* suppressor phenotype correlated with temperature-sensitive growth (37°C, restrictive temperature). The heterozygous diploid KMG62 could grow at 37°C and segregated two fully viable and two temperature-sensitive spores in 13 tetrads. All of the segregants that were unable to grow at 37°C could mate at 35°C. In addition, all of the segregants that were unable to mate at 35°C could grow at 37°C. Thus, the

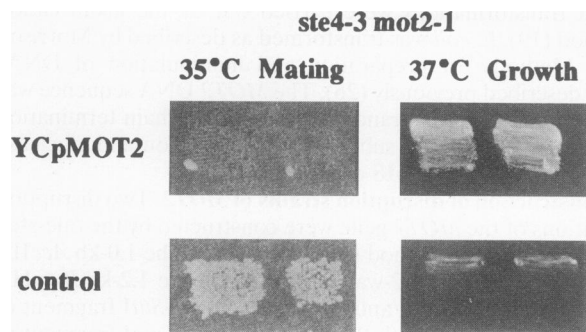


FIG. 1. Mating ability and temperature-sensitive growth of the *ste4-3 mot2-1* strain. To examine mating ability (left panel), each strain was grown as a patch on an SD plate lacking uracil at 25°C and replicated onto a lawn of strain AM242-1D (*MATa* wild type) on a YPD plate. After 24 h at 35°C, the plate was replicated onto an SD minimal plate to assess diploid formation. To examine temperature-sensitive growth (right panel), each strain was streaked onto a YPD plate and incubated for 2 days at 37°C. The strains used were KMG62-1C (*MATa ste4-3 mot2-1*) carrying pRF11 (YcMOT2) and KMG62-1C carrying Ycplac33 (control). Each patch represents an independent transformant.

temperature-sensitive growth phenotype and the ability to restore mating to *ste4-3* strains cosegregated in crosses. This cosegregation indicates that the *mot2-1* mutation is responsible for both phenotypes. To confirm this cosegregation, a cloned *MOT2* gene (see the description below) was shown to complement both the temperature-sensitive growth and mating phenotypes of the *ste4-3 mot2-1* mutant. The mutant harboring the *MOT2* gene on a YcP plasmid could grow at 37°C and could not mate at 35°C, whereas the mutant harboring the control plasmid, Ycplac33, could not grow at 37°C and could mate at 35°C (Fig. 1).

Cloning of the *MOT2* gene. The *MOT2* gene was cloned by

complementation of the temperature-sensitive phenotype of *mot2-1*, by using a library of yeast genomic fragments cloned into YcP50 (36). One plasmid (pKF1) identified in this manner carried an insert of 7.8 kb. The functional region of the 7.8-kb fragment was delimited by construction of various subclones in YcP50 or Ycplac33, followed by analysis of their ability to complement the *mot2-1* mutation. This analysis (Fig. 2) indicated that the *MOT2* gene was localized to a 2.4-kb *SmaI-EcoRI* fragment.

The nucleotide sequence of the 2.4-kb *SmaI-EcoRI* fragment was determined. DNA sequence analysis of the *MOT2* gene reveals a single large open reading frame with the potential to encode a protein of 587 amino acid residues (Fig. 3). The upstream region contains no obvious pheromone induction box (TGAAACA), as is found upstream of many pheromone-inducible genes such as *FUS1* (22). A search of the available data bases revealed that the putative protein shows no significant similarities to known proteins. However, the sequence near the N terminus (residues 33 to 77) contains a configuration of cysteine residues reminiscent of certain zinc finger nucleic acid-binding polypeptides. The pattern of cysteine residues is CX₂CX₁₃CXCX₄CX₂CX₁₃CX₂C (the sequence underlined in Fig. 3).

Genetic characterization of *MOT2*. To determine the phenotypic consequences of loss of *MOT2* function, a deletion mutant of *MOT2* was constructed as described in Materials and Methods. A 1.0-kb fragment of *MOT2* was replaced with the selectable marker *HIS3* (Fig. 2). This deletion allele (*mot2Δ::HIS3*) was transplanted into a diploid strain (KA31) by selecting for His⁺. Southern blot analysis of chromosomal DNA from transformants confirmed that the resulting diploid is heterozygous for the wild-type and disrupted *mot2* (*mot2Δ::HIS3*) alleles (data not shown). This diploid was induced to sporulate, and the tetrads were dissected. Four spores from 10 tetrads gave rise to colonies at 25°C, but two from each grew slowly. The slowly growing colonies carried the *mot2Δ::HIS3* allele, as judged by segregation of the *HIS3*

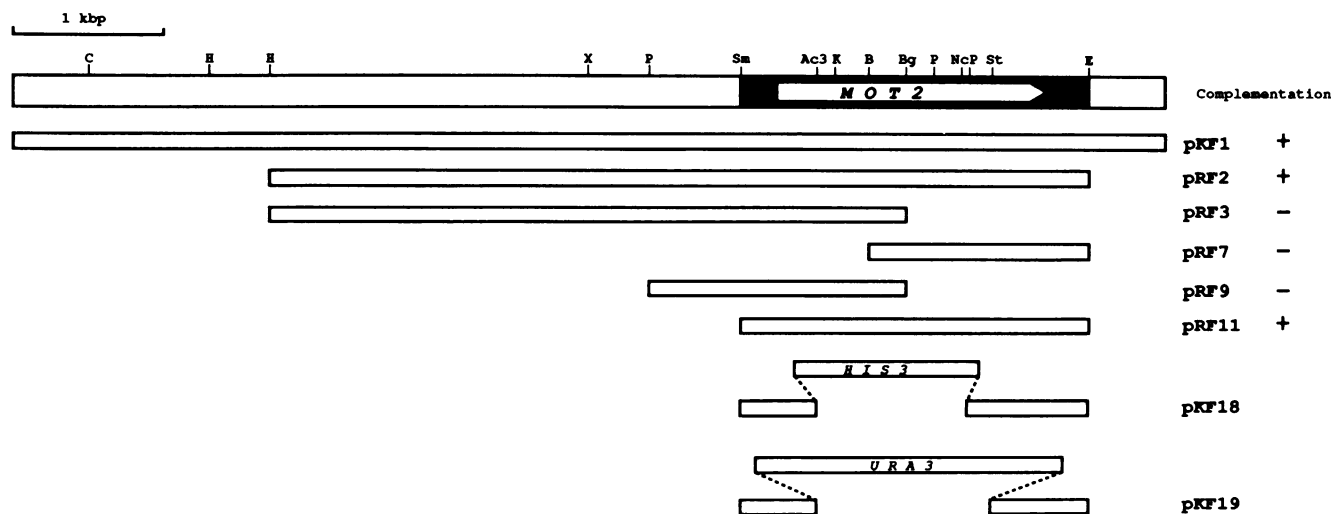


FIG. 2. Restriction map of the DNA fragment cloned on pKF1 and its derivatives. The nucleotide sequence of the region indicated (solid segment) was determined. The open arrow indicates the region and direction of the *MOT2* open reading frame. The open boxes indicate fragments subcloned into YcP50 or Ycplac33 for suppression tests. The ability of each plasmid to suppress temperature-sensitive growth of the *mot2* mutant is indicated on the right: +, suppression; -, no suppression. The two fragments at the bottom with the replaced open box labelled *HIS3* or *URA3* in pKF18 or pKF19 were used for construction of the deletion alleles. The restriction sites are *AccIII* (Ac3), *BamHI* (B), *BglII* (Bg), *EcoRI* (E), *HindIII* (H), *KpnI* (K), *NcoI* (Nc), *PstI* (P), *SmaI* (Sm), and *StuI* (St). The ligation site between the *BamHI* and *Sau3AI* cohesive ends is also indicated (B/Sau).

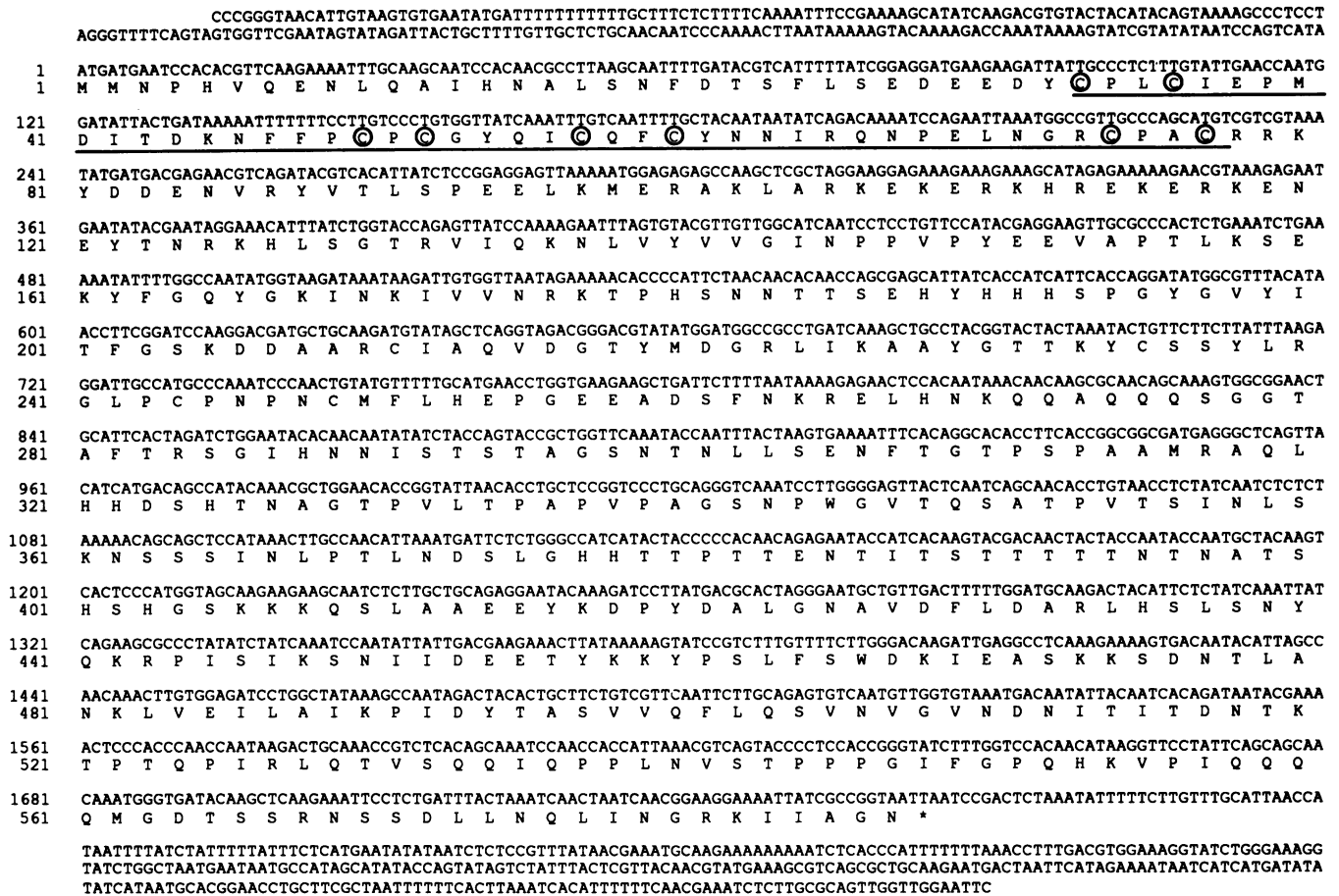


FIG. 3. Nucleotide sequence of the *MOT2* gene and its predicted amino acid sequence. The 2,355 nucleotides are shown, together with the predicted amino acid sequences. The zinc finger motif is underlined and the cysteine residues are circled. ★, stop codon.

marker. The *mot2Δ::HIS3* mutants failed to grow at 37°C. *MATa/MATα mot2Δ::HIS3/mot2Δ::HIS3* diploids were still temperature sensitive for growth. Moreover, the deletion and the original *mot2-1* mutation failed to complement each other for the growth defect at high temperatures, confirming that the cloned gene was indeed the same as that identified by the *mot2-1* mutation. Thus, the *MOT2* gene is necessary for cell growth at 37°C.

To determine the arrest phenotype of *mot2Δ::HIS3* mutants, cells were grown at the permissive temperature and shifted to the restrictive temperature of 37°C. Microscopic analysis revealed that *mot2* mutant cells were morphologically aberrant, showing a high percentage of large unbudded cells. Some of them formed projections at 37°C. The DNA content of the arrested cells was analyzed with a Flow cytometer (Fig. 4). After incubation at 37°C for 6 h, about 90% of the cells had a 1 N DNA content, indicating arrest in G₁ or early S phase.

The *mot2-1* mutation was originally identified on the basis of its suppression of the mating defect of temperature-sensitive *ste4-3* mutants. The *mot2Δ::HIS3* mutation also restored the mating ability of *ste4-3* cells at 35°C (data not shown). We examined whether the mating response pathway was activated constitutively in the *mot2* mutants. The pheromone response leads to the induction of transcription of mating-specific genes (24). One such gene, *FUS1*, is required for cell fusion during zygote formation (41). Since the transcript level of *FUS1* increases immediately after the addition of the pheromone, the

induction of this gene can serve to monitor the mating-pheromone response. In *mot2* mutant cells, *FUS1* transcription was higher than that in wild-type cells at the permissive temperature (25°C), indicating that the mating response pathway is activated constitutively in these cells in the absence of pheromone-receptor interaction (Fig. 5). Treatment of mutant cells with α-factor pheromone led to an increase in *FUS1* transcription. This level was slightly higher than that observed in stimulated wild-type cells. Thus, the constitutive pathway activity seen in *mot2* mutant cells is only partial and is still capable of further induction.

To determine whether the *mot2* mutation activates the mating response pathway in a pheromone-receptor-independent fashion, we examined the ability of *mot2* to suppress the sterility of cells caused by lack of the α-factor receptor. For this purpose, a *mot2Δ::HIS3 ste2Δ::URA3* mutant (strain 1711-12D) was constructed. To create isogenic *mot2* and *MOT2* strains, we transformed 1711-12D with YCplac33 or with a YCplac33 derivative carrying the *MOT2* gene (pRF11), and the mating ability was tested at both 25 and 35°C. As shown in Fig. 6, the *mot2* mutation suppressed the sterile phenotype conferred by *ste2Δ::URA3* at both temperatures. On the other hand, the *mot2* mutation was unable to suppress the mating defects caused by deletion of the *STE4* (Fig. 6), *STE5*, or *STE11* gene (data not shown).

Relationship of *MOT2* to *CDC36* and *CDC39*. The phenotype associated with the *mot2* mutation is reminiscent of *cdc36*,

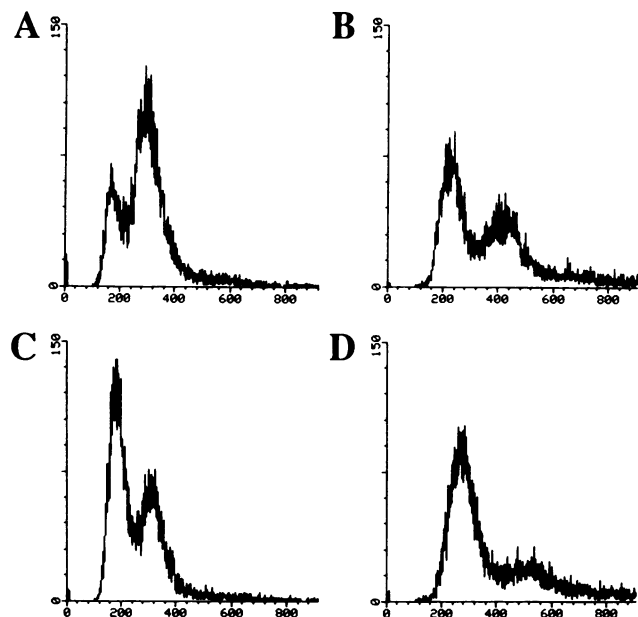


FIG. 4. Flow cytometric analysis of the *mot2Δ::HIS3* cells. Cells were cultured in YPD medium at 25°C (A and B) and shifted to 37°C for 6 h (C and D). Cultures were stained with propidium iodide and analyzed for nuclear DNA content by using a fluorescence-activated cell sorter analyzer. The left peak corresponds to cells in G₁, and the right peak corresponds to cells in G₂ and/or M phase. (A and C) 4611-12C (wild type); (B and D) 4611-2A (*mot2Δ::HIS3*).

cdc39, and *srm1* mutants, which express pheromone-inducible genes, conjugate in the absence of pheromone receptor, and arrest in G₁ when they are haploid (9, 11, 29). The *MOT2* gene is also similar to *CDC36*, *CDC39*, and *SRM1* in that they are required for growth in diploid cells, although diploid cells homozygous for the *cdc36*, *cdc39*, or *srm1* mutation do not exhibit the G₁ arrest (9, 11, 29). *CDC39* and *SRM1* are

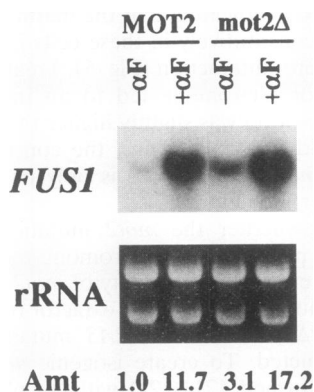


FIG. 5. Induction of *FUS1* transcription. Total RNA was harvested from cells growing at 25°C without (-) or with (+) induction by α-factor (αF) for 3 h. Blots of total RNA were hybridized to labelled *FUS1* probes. Ethidium bromide-stained rRNA was used as an internal control to quantitate expression. The ratio of hybridization signals for the *FUS1* mRNA was measured by phosphorimage analysis. The relative amounts (Amt) represent the *FUS1* mRNA levels relative to that of the wild-type sample in the absence of α-factor. Lanes 1 and 2, 4611-12C (*MOT2*, wild type); lanes 3 and 4, 4611-2A *mot2Δ::HIS3* (*mot2Δ*).

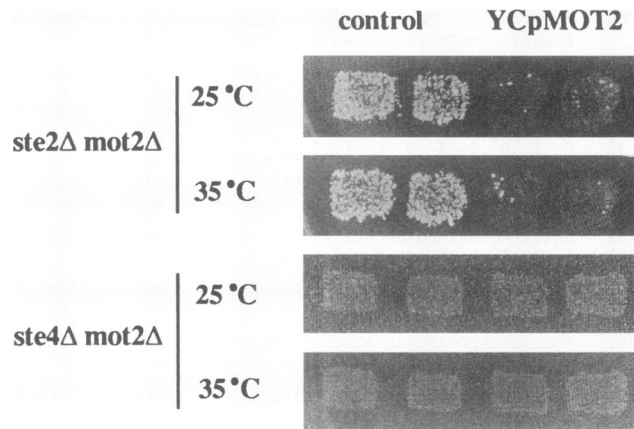


FIG. 6. Mating abilities of *ste2Δ::URA3 mot2Δ::HIS3* and *ste4Δ::LEU2 mot2Δ::HIS3* strains. The mating assay was performed at 25 or 35°C, as described in the legend to Fig. 1. Lines 1 and 2, 1711-12D (*MATa ste2Δ::URA3 mot2Δ::HIS3*) carrying YCplac33 (control) or pRF11 (YCpMOT2); lines 3 and 4, 1411-1B (*MATa ste4Δ::LEU2 mot2Δ::HIS3*) carrying YCplac33 or pRF11. Each patch represents an independent transformant.

essential for growth at any temperature (9, 10), while disruption of *CDC36* causes temperature-sensitive growth (23). As one means of probing the relationship between *MOT2* and these genes, we examined the effect of multiple copies of *MOT2* on temperature-sensitive *cdc36-16*, *cdc39-1*, and *srm1-1* mutations. Overexpression of *MOT2* did suppress *cdc36-16* and *cdc39-1* mutations (Fig. 7A and B) but not the *srm1* mutation (data not shown). Moreover, *MOT2* on a multicopy plasmid suppressed the growth defect at 37°C of a *cdc36* deletion mutation and another *cdc39* mutation, *cdc39-2* (data not shown), indicating that the effect of *MOT2* on *cdc36* and *cdc39* mutations is not allele specific. In contrast, overexpression of *CDC36* or *CDC39* was unable to suppress *mot2-1* temperature-sensitive (*mot2-1*) and deletion (*mot2Δ::HIS3*) mutations (data not shown). These results suggested a possible functional integration of *MOT2* with *CDC36* and *CDC39*.

To further test for genetic interaction between *MOT2*, *CDC36*, and *CDC39*, we constructed *cdc36-16 mot2Δ::HIS3* and *cdc39-1 mot2Δ::HIS3* double-mutant strains. A *mot2Δ::HIS3* strain was crossed with *cdc36-16* and *cdc39-1* strains, and the resulting diploids were sporulated and subjected to tetrad analysis at 25°C. An average of three viable spores to one nonviable spore was observed in at least 11 tetrads, suggesting that the two mutations segregated independently and that spores bearing both mutations were not viable. To confirm this possibility, plasmid pRF11 (YCplac33-*MOT2*) was transformed into the diploids of genotype *cdc36-16/CDC36 MOT2/mot2Δ::HIS3* and *cdc39-1/CDC39 MOT2/mot2Δ::HIS3*. Upon sporulation and tetrad dissection, the Ura⁺ transformants yielded a high proportion of tetrads with four viable spores, suggesting that the plasmid was capable of complementing the defect associated with *cdc36-16 mot2Δ::HIS3* and *cdc39-1 mot2Δ::HIS3*. We tested the ability of the segregants to survive the loss of plasmid pRF11 by using 5-FOA-containing medium which selects for cells that had lost the YCplac33 (*URA3*)-*MOT2* plasmid. As shown in Fig. 7C, *cdc36 mot2* and *cdc39 mot2* segregants harboring the *MOT2* gene on a plasmid could not grow on a 5-FOA plate even at 25°C, whereas *mot2*, *cdc36*, and *cdc39* single mutants harboring the plasmid could grow, indicating that the *mot2* mutation shows synthetic lethality with

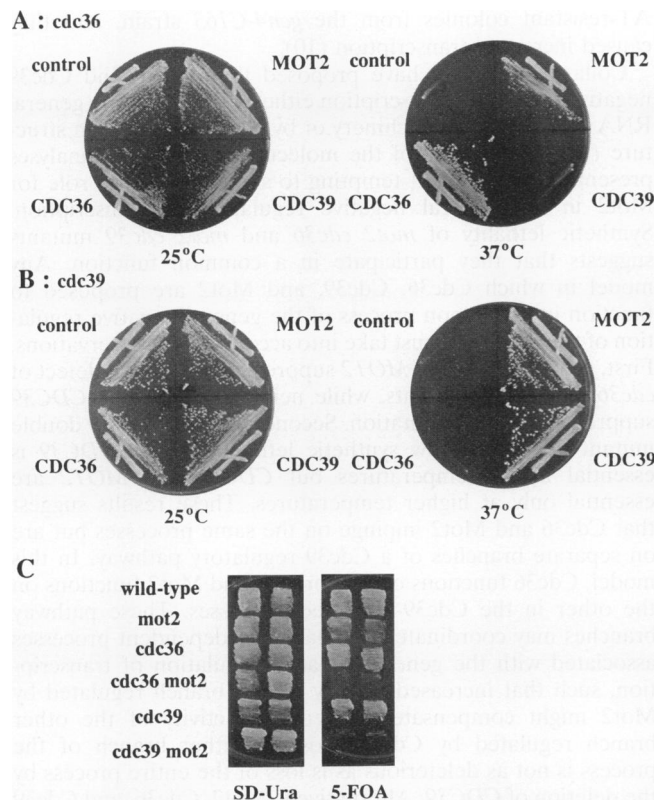


FIG. 7. Interaction of *MOT2* with *CDC36* and *CDC39*. (A) Effect of *MOT2* overexpression on *cdc36* mutation. Different plasmids were introduced in the *cdc36* mutant, KYC36-2D. Transformants were patched onto YPD medium and incubated for 2 days at 25°C (left plate) or 35°C (right plate). Plasmids are YEp13 (control), YEplac181 carrying *MOT2* (pKF3), YEp13 carrying *CDC36* (YEplac181), and YEp13 carrying *CDC39* (YEplac181). (B) Effect of *MOT2* overexpression on *cdc39* mutation. Different plasmids were introduced in the *cdc39* mutant, KYC39-6D. Transformants were patched onto YPD medium and incubated for 2 days at 25°C (left plate) or 35°C (right plate). Plasmids are the same as described in panel A. (C) Growth properties of *cdc36-16 mot2Δ::HIS3* and *cdc39-1 mot2Δ::HIS3* double mutants. Cells were transferred to SD-Ura or 5-FOA plates and incubated for 3 days at 25°C.

either the *cdc36* or *cdc39* mutation. This phenomenon often reflects the participation of the genes in a common process. Thus, these results suggest that Mot2, Cdc36, and Cdc39 affect a common, essential function.

Effect of the *mot2* mutation on transcription. Collart and Struhl isolated *cdc36* and *cdc39* mutants by selecting colonies that grew on 20 mM aminotriazole (AT), a competitive inhibitor of the *HIS3* gene product, from the strain (KY803)

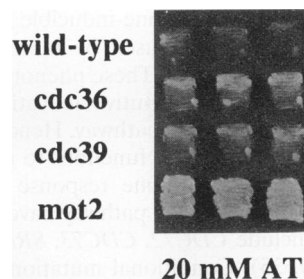


FIG. 8. Growth of the *mot2Δ::URA3* mutant expressing Gcn4-C163 on medium containing AT. Cells transformed with pRS314-Sc4363 were patched onto medium containing 20 mM AT: KY803 (wild type), KY803-36 (*cdc36*), KY803-39 (*cdc39-2*), and KY803-MO (*mot2Δ::URA3*). Each patch represents an independent transformant.

expressing a partially defective acidic activation domain of Gcn4, Gcn4-C163 (residues 119 to 281) (10). To examine whether the *mot2* mutation had the same effect as the *cdc36* and *cdc39* mutations, the *MOT2* gene was disrupted in KY803. The *mot2* mutant grew as well on 20 mM AT as *cdc36* and *cdc39*, suggesting that the *mot2* disruption suppresses the *gcn4-C163* activator mutation (Fig. 8).

If Mot2 works in the same manner as Cdc36 and Cdc39, the *mot2* mutation is predicted to result in increased levels of transcription of various genes. To test this possibility, *lacZ* reporter plasmids under the control of the *GAL7* or *PHO84* promoter region were introduced into *mot2Δ::HIS3* mutant and wild-type strains. The levels of *GAL7* and *PHO84* expression were analyzed by the β -galactosidase activity of the Gal7-LacZ and Pho84-LacZ fusion proteins (Table 2). Transcriptional induction of the *GAL7* promoter in galactose medium is mediated by the Gal4 activator. *PHO84* transcription in medium depleted of P_i is mediated by Pho2 and Pho4. The induced levels of *GAL7* transcription and derepressed levels of *PHO84* transcription were increased approximately four- and twofold, respectively, in the *mot2* mutant. The *mot2* mutant also appeared to have an effect on the basal levels of *GAL7* and *PHO84* transcription. Thus, the *mot2* mutation affects basal and activated transcription. Similarly, the *cdc36* and *cdc39* mutations cause increased transcriptional activation (10). Taken together, these results suggest that Mot2 is a transcriptional regulatory protein working at the same point as Cdc36 and Cdc39.

DISCUSSION

In this study, we identified a new gene, *MOT2*, as a mutational suppressor that restored mating competence to temperature-sensitive *ste4* mutant cells. *mot2* disruption causes the following phenotypes: temperature-sensitive growth, constitu-

TABLE 2. Effect of *mot2Δ::HIS3* on expression of *GAL7-lacZ* and *PHO84-lacZ*

| Strain | Genotype | β -Galactosidase activity (U) ^a | | | |
|----------|--------------------|--|-------------|--------------------------------|-----------------|
| | | <i>GAL7-lacZ</i> ^b | | <i>PHO84-lacZ</i> ^c | |
| | | Gly | Gly + Gal | +P _i | -P _i |
| 4611-12C | Wild type | 0.06 ± 0.01 | 730 ± 28 | 1.2 ± 0.1 | 250 ± 18 |
| 4611-2A | <i>mot2Δ::HIS3</i> | 0.27 ± 0.01 | 2,100 ± 160 | 2.5 ± 0.4 | 490 ± 67 |

^a β -Galactosidase activity was determined as described in Materials and Methods. The units shown are the averages of at least three independent transformants.

^b Cells were grown in 2% concentrations of the indicated carbon sources.

^c Cells were grown in the phosphate-depleted medium in the presence (+) or absence (-) of P_i.

tive expression of the pheromone-inducible gene *FUS1*, and suppression of the sterility caused by inactivation of *STE2* encoding the α -factor receptor. These phenotypes suggest that *mot2* disruption results in constitutive activation of the mating pheromone signal transduction pathway. Hence, in the absence of pheromones, wild-type Mot2 functions to prevent constitutive activation of the pheromone response pathway. Other negative control elements in the pathway have been previously described. They include *CDC72*, *CDC73*, *SRM1*, *CDC36*, and *CDC39* (9, 11, 29, 35). Conditional mutations in these genes result in G₁ arrest in haploids, constitutive expression of *FUS1*, and suppression of deletion of the pheromone receptors. This is similar to what was seen in the *mot2* mutant. These genes, as in the case of *MOT2*, are also required for growth in mating-type heterozygous diploid cells, indicating that they have a role in cellular responses other than the mating pheromone response.

Examination of epistasis relationships between *CDC72*, *SRM1*, *CDC36*, *CDC39*, and *MOT2* and *STE* genes suggested that all of them might act upstream from, or at the same level as, the transducing G protein, because neither could suppress the mating defect caused by the disruption of *STE4* (9, 11, 29, 35). Therefore, in the cases of *CDC72*, *SRM1*, *CDC36*, and *CDC39* it was hypothesized that they might regulate the activity of the G protein in the mating pheromone response pathway (9, 11, 29, 35). The action site of *CDC72* is consistent with this possibility. *CDC72* encodes *N*-myristoyl transferase, which affects the pheromone response pathway through the modification of the G protein α subunit (40). On the other hand, the effects of *Srm1*, *Cdc36*, and *Cdc39* on the pathway are not exerted at the level of the G protein that is localized in the cytoplasmic membrane. In fact, *Srm1* and *Cdc39* localize to the nucleus (10, 14). *Srm1* is involved in the processing and export of mRNA by acting as a GDP-GTP exchange factor for the small GTP-binding proteins *Gsp1* and *Gsp2* (2). *Cdc36* and *Cdc39* function as general negative regulators of transcription (10). Therefore, one mechanism whereby *srm1*, *cdc36*, and *cdc39* mutations could activate the mating pathway is simply by affecting the expression of *GPA1* and *STE4*. For example, these mutations could cause depletion of *Gpa1* or an increase in *Ste4*. However, this possibility was excluded by analyzing the levels of *GPA1* and *STE4* transcripts in *cdc36* and *cdc39* mutants (11). At the restrictive temperature, the level of *GPA1* mRNA was increased fivefold and that of *STE4* mRNA was increased slightly in the mutants. Another more likely explanation for the epistasis relationships is that suppression of the mating defects of receptor mutations by *srm1*, *cdc36*, *cdc39*, and *mot2* is dependent upon a basal level of signal intrinsic to the pheromone response pathway. The *ste2* disruption mutant has sufficient basal activity of the pathway, while the *ste4* disruption mutant does not (17).

Several lines of evidence presented here strongly argue that Mot2 functions in processes similar to *Cdc36* and *Cdc39*. First, the phenotype of the *mot2* disruption is very similar to those of the *cdc36* and *cdc39* mutants. Second, overexpression of *MOT2* suppresses the growth defects of *cdc36* and *cdc39* mutants. *MOT2* suppresses not only the *cdc36-16* point mutation but also the *cdc36* deletion, indicating that suppression by *MOT2* is not allele specific. Similarly, suppression of *cdc39* is not allele specific, because overexpression of *MOT2* suppresses both the *cdc39-1* and *cdc39-2* alleles. Third, the *mot2* disruption shows synthetic lethality with the *cdc36-16* and *cdc39-1* mutations. Fourth, the *mot2* mutation confers resistance to AT in the *gcn4-C163* strain and enhances the expression of genes such as *GAL7* and *PHO84*. *cdc36* and *cdc39* mutants were isolated as

AT-resistant colonies from the *gcn4-C163* strain, and they caused increased transcription (10).

Collart and Struhl have proposed that *Cdc36* and *Cdc39* negatively regulate transcription either by affecting the general RNA polymerase II machinery or by altering chromatin structure (10). The results of the molecular and genetic analyses presented here make it tempting to suggest a similar role for Mot2 in the general negative regulation of transcription. Synthetic lethality of *mot2 cdc36* and *mot2 cdc39* mutants suggests that they participate in a common function. Any model in which *Cdc36*, *Cdc39*, and *Mot2* are proposed to function in a common process of the general negative regulation of transcription must take into account three observations. First, overexpression of *MOT2* suppresses the growth defect of *cdc36* and *cdc39* mutants, while neither *CDC36* nor *CDC39* suppresses the *mot2* mutation. Second, the *cdc36 cdc39* double mutant does not show synthetic lethality. Third, *CDC39* is essential at all temperatures but *CDC36* and *MOT2* are essential only at higher temperatures. These results suggest that *Cdc36* and *Mot2* impinge on the same processes but are on separate branches of a *Cdc39* regulatory pathway. In this model, *Cdc36* functions on one branch and *Mot2* functions on the other in the *Cdc39*-mediated processes. These pathway branches may coordinately regulate interdependent processes associated with the general negative regulation of transcription, such that increased activity of one branch regulated by *Mot2* might compensate for reduced activity of the other branch regulated by *Cdc36*. Loss of either branch of the process is not as deleterious as is loss of the entire process by the deletion of *CDC39*. Alternatively, *Mot2*, *Cdc36*, and *Cdc39* may constitute a large multisubunit complex that functions as a negative regulator of transcription. In this case, *Mot2* can partially substitute or compensate for a loss of *Cdc36*. At the permissive temperature, a loss of *Mot2* or *Cdc36* can survive because of the compensation, while at a higher temperature this compensation is insufficient.

The *MOT2* coding sequence has a zinc finger-like motif in the N-terminal portion. The binding of zinc by such a domain may fold the protein in such a way as to make it suitable for interactions with other macromolecules. This motif is known to be responsible for binding proteins to DNA, or to a similar zinc finger-containing protein (3). Hence, *Mot2* may bind to DNA or interact with another protein carrying the zinc finger motif. Further biochemical analysis of *Mot2* will help in understanding the mechanism of the general negative regulation of transcription.

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