MOLECULAR AND CELLULAR BIOLOGY, Dec. 1995, p. 6526–6534 Vol. 15, No. 12
0270-7306/95/$04.00+0

Met30p, a Yeast Transcriptional Inhibitor That Responds to *S*-Adenosylmethionine, Is an Essential Protein with WD40 Repeats

DOMINIQUE THOMAS, LAURENT KURAS, RÉGINE BARBEY, HÉLÈNE CHEREST, PIERRE-LOUIS BLAISEAU, AND YOLANDE SURDIN-KERJAN*

Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France

Received 23 May 1995/Returned for modification 29 June 1995/Accepted 22 August 1995

A specific repression mechanism regulates the biosynthesis of sulfur amino acids in *Saccharomyces cerevisiae*. When the intracellular *S*-adenosylmethionine (AdoMet) concentration increases, transcription of the sulfur genes is repressed. Using a specific reporter system, we have isolated mutations impairing the AdoMet-mediated transcriptional regulation of the sulfur network. These mutations identified a new gene, *MET30*, and were shown to also affect the regulation of the methyl cycle. The *MET30* gene was isolated and sequenced. Sequence analysis reveals that Met30p contains five copies of the WD40 motif within its carboxy-terminal part, like the yeast transcriptional repressors Hir1p and Tup1p. We identified one target of Met30p as Met4p, a transcriptional activator regulating the sulfate assimilation pathway. By the two-hybrid method, we showed that Met30p interacts with Met4p and identified a region of Met4p involved in this interaction. Further analysis reveals that expression of Met30p is essential for cell viability.

Transcriptional regulation of gene expression can be achieved through either activation or repression mechanisms. While the processes by which transcription factors increase the rate of transcription have been extensively investigated, only a few systems of repression have been studied. The sulfur network of the yeast *Saccharomyces cerevisiae* constitutes one metabolic pathway that is negatively regulated at the level of transcription. This pathway involves a set of enzymes catalyzing the biosynthesis of cysteine, methionine, and *S*-adenosylmethionine (AdoMet). The sulfur network comprises more than 20 unlinked genes, the expression of which is regulated by AdoMet: in response to an increase in the intracellular AdoMet concentration, transcription of the sulfur genes is turned off (37, 39).

To date, two regulatory factors are known to be involved in the transcriptional control of this metabolic network. The first one, Met4p, belongs to the basic leucine zipper protein (bZIP) family and functions as a transcriptional activator (38). Its activity was shown to be sensitive to the level of intracellular AdoMet (27). The second one, Cbf1p (also called Cp1p and Cpf1p), belongs to the basic helix loop helix protein family, which recognizes the DNA motif RCACGTG (where R is a purine) (1, 7, 30). This motif is present in either one or two copies in the promoter region of almost all genes involved in sulfur metabolism (28). This motif also constitutes the centromere-determining element 1 (CDE1) of all the centromeres in *S. cerevisiae* (19). Accordingly, inactivation of the gene *CBF1* has two phenotypic consequences: methionine auxotrophy and a 9- to 25-fold increase in the rate of mitotic chromosome loss (1, 7). *trans* as well as *cis* experiments have shown that Cbf1p is indeed required for the expression of several genes of sulfur metabolism (28, 33, 38). However, DNA-bound Cbf1p does not appear to act as a transcriptional activator; it lacks transcription activation function but rather appears to aid local reconfiguration of the chromatin structure in the transcription initiation regions (33, 38).

* Corresponding author. Phone: 1-69-82-34-64. Fax: 1-69-82-31-29. Electronic mail address: kerjan@cgmvax.cgm.cnrs-gif.fr.

It is not yet known whether the transcriptional activation of sulfur genes involves additional factors. Furthermore, the molecular events responsible for the AdoMet-induced repression of the sulfur genes are not clearly established. Functional dissection of Met4p has shown that one part of the AdoMet-mediated repression occurs through the inhibition of the transcription activation function of Met4p (27). A second component of AdoMet-mediated repression involves a proximal sequence element that has been mapped on the *MET25* promoter (37).

To gain new insights into the mechanisms permitting the regulation of sulfur metabolism in *S. cerevisiae*, we have developed a genetic screen aimed at isolating mutations in genes encoding factors involved in transcriptional regulation of the sulfur genes. Our screen yielded mutations in two genes. In this report, we describe one of them, *MET30*, that encodes a protein containing five WD40 motifs. The Met30 protein (Met30p) was shown to function in the AdoMet-mediated inhibition of the transcription function of Met4p. Moreover, we have found that the role of Met30p extends to other parts of the cellular metabolism and that Met30p is essential for cell viability.

MATERIALS AND METHODS

Strains, media, and microbiological techniques. *Escherichia coli* JM103 was used as the host for plasmid maintenance. The yeast strains used in this work are listed in Table 1. Strain C161 was constructed by integrating the *MET25-xylE* gene fusion at the *LEU2* locus of strain W303-1B. Strain W303-1B was transformed by plasmid pYiX25 linearized at the unique *Kpn*I site located in the *LEU2* gene, and leucine prototophic transformants were selected. Correct integration was assessed in strain C161 by Southern blotting.

Standard yeast media were prepared as described by Cherest and Surdin-Kerjan (9). *S. cerevisiae* was transformed after lithium chloride treatment as described by Gietz et al. (16). Genetic crosses, sporulation, dissection, and scoring of markers were done as described by Sherman et al. (35).

Yeast strains expressing the *Pseudomonas putida* catechol oxidase were revealed by using a 0.25 M catechol aqueous solution sprayed on colonies grown for 48 h on plates. The yellow color develops within 10 min.

Mutagenesis and genetic analysis. Strain W303-1B was transformed with plasmid pYrX25 (bearing a *MET25-xylE* fusion gene [22]). Mutants were obtained by subjecting the transformed strain to UV mutagenesis. A thousand viable cells per petri dish were plated on minimal synthetic medium containing

TABLE 1. Yeast strains used

Strain	Relevant genotype	Reference or source
C119	*MAT***a** *his3 ura3 leu2*::*SAM2-lacZ*::*LEU2*	41
C121	*MAT***a** *his3 ura3 leu2*::*SAM1-lacZ*::*LEU2*	41
C161	*MAT***a** *his3 leu2 ade2 trp1 ura3 leu2*::*MET25-xylE*::LEU2	22
C170	*MAT***a** *his3 leu2 ade2 trp1 met4*::*TRP1 ura3*::*lexAop-lacZ*::*URA3*	27
C172	*MAT***a** *his3 leu2 ade2 trp1 met4*::*TRP1 MET30-1 ura3*::*lexAop-lacZ*::*URA3*	This study
CC572	*MAT***a**/*MAT*α *his3*/*his3 leu2*/*leu2 ura3*/*ura3 ade2*/*ade2 trp1*/*trp1*	22
CD126	*MAT***a**/*MAT*α *his3*/*his3 leu2*/*leu2 ura3*/*ura3 ade2*/*ade2 trp1*/*trp1 MET30*/*met30*::*URA3*	This study
CC682-24	*MAT***a** *his3 ura3 leu2*::*SAM1-lacZ*::*LEU2 MET30-1*	This study
CC687-1A	*MAT***a** *his3 ura3 leu2*::*SAM2-lacZ*::*LEU2 MET30-1*	This study
CI2-11D	*MAT*α *trp1 ura3 leu2*::*MET25-xylE*::*LEU2*	This study
CM100	*MAT*α/*MAT***a** *ade2*/*ADE2 his3*/*HIS3 trp1*/*trp1 ura3*/*ura3 leu2*::*MET25-xylE*::*LEU2*/*leu2*::*MET25-xylE*::*LEU2*	This study
CM100-1A	*MAT***a** *ade2 his3 MET30-1 trp1 ura3 leu2*::*MET25-xylE*::*LEU2*	This study
CM100-1B	*MAT*α *trp1 ura3 leu2*::*MET25-xylE*::*LEU2*	This study
CM100-1C	*MAT*α *his3 trp1 ura3 leu2*::*MET25-xylE*::*LEU2*	This study
CM100-1D	*MAT***a** *ade2 MET30-1 trp1 ura3 leu2*::*MET25-xylE*::*LEU2*	This study
X2180-1A	*MAT***a**	Yeast Genetic Stock Center
W303-1A	*MAT***a** *ade2 his3 leu2 trp1 ura3*	R. Rothstein

all auxotrophic requirements of the strain and 1 mM L-methionine. After 3 days at 28°C, the plates were sprayed with a catechol solution. Yellow colonies were picked and streaked for single colonies on selection medium. From 45,000 colonies, 14 mutants exhibiting yellow or faint yellow coloration in the catechol test were selected for further study. They were cured of the plasmid by growth for 30 generations on a nonselective medium. They were then retransformed with plasmid pYrX25 and tested once again by the catechol test. Of the primary isolates, three exhibited clearly a yellow phenotype in the catechol test after growth in repressive conditions (1 mM L-methionine). The *MET25-xylE* fusion gene was integrated at the *LEU2* locus of the three mutants, and the resulting strains were mated to strain CI2-11D. In the three cases, tetrad analyses of the progeny of heterozygotes displayed a 2+:2− segregation pattern of the yellow color. This indicates that a mutation affecting a single gene was causing the phenotype of each mutant. Diploids containing two different mutations were made, and tetrad analysis of the progeny showed that two of the three mutants studied belonged to the same complementation group. This group defined a new gene that was named *MET30.*

Recombinant DNA methods. Plasmids pEmblYe23, -25, and -31 (2) were used as shuttle vectors between *S. cerevisiae* and *E. coli*. The *S. cerevisiae* genomic library used for the cloning of the gene *MET30* was constructed by inserting the product of a partial *Sau*3A digest of chromosomal DNA from the wild-type strain X2180-1A in the *Bam*HI site of pEMBLYe23. Northern (RNA) blotting was performed as described by Thomas (42), with total cellular RNA extracted from yeast cells as described by Hoffman and Winston (21). Probes were made radioactive by the random priming method described by Hodgson and Fisk (20).

For the sequencing of *MET30*, systematic deletion subclones were generated by the method of Thomas and Surdin-Kerjan (40). Single-stranded phage DNA prepared from these deletions were sequenced with the Pharmacia T7 sequencing kit. Sequence analyses and comparisons were performed by using the Bisance service (12). To disrupt *MET30*, the *Bam*HI-*Bam*HI fragment of pM30-1 was first cloned into plasmid pUC19. The resulting plasmid was then digested with *Eco*RV, dephosphorylated, and ligated with a blunt-ended *Bgl*II-*Bgl*II fragment containing the *URA3* gene. The resulting plasmid was then digested with *Bam*HI, and the product of the digestion was used to transform a wild-type diploid strain.

The pGADM4Δi plasmids were constructed by transferring the *Eco*RI-*Bam*HI fragments from the corresponding plasmid pLexM4Δi (27) into plasmid pGAD424 (3). For the two-hybrid analysis, plasmids pLexM30-4 and pGadM4Δi were cotransformed into strain C170, which contains a *GAL1-lexAop-lacZ* gene fusion integrated at the *ura3* locus. In the *GAL1-lexAop-lacZ* fusion reporter, the *GAL* upstream activation sequence (UAS_{GAL}) has been deleted, and instead it contains four *lexA* operators at position −167 (18). We used oligonucleotides to amplify a fragment of pM30-1 that was cloned into plasmid pEG202 (17) digested by *Bam*HI, yielding pLexM30-4. Amplifications were performed with *Pfu* DNA polymerase (Stratagene, La Jolla, Calif.). The amplified fragment corresponds to the Met30p region 4 to 640. Plasmids JK1621 and CK30 were generously provided by A. D. Johnson.

Enzymatic assays. Yeast cells were grown to 10^7/ml in appropriate minimal synthetic medium. Acellular extracts were made as described by Thomas et al. (38) in the presence of 1 mM phenylmethylsulfonyl fluoride and 1 mM EDTA. After centrifugation for 15 min at 10,000 × *g*, the crude extract was used for enzymatic assays. ATP sulfurylase, 3′-phosphoadenosyl 5′-phosphosulfate (PAPS) reductase, and homocysteine synthase were assayed as described by Thomas et al. (38). β-Galactosidase activity was assayed as described by Kuras and Thomas (27). Protein concentrations were estimated by the method described by Lowry et al. (29).

Nucleotide sequence accession number. The *MET30* gene sequence has been assigned EMBL/GenBank accession number L26505.

RESULTS

As previously reported (22), we have developed a specific reporter system allowing the genetic analysis of the transcriptional mechanisms that regulate the sulfur network. In this system, heterologous expression of the catechol oxidase from *P. putida* (encoded by the *xylE* gene [47]) is provided under the control of the *MET25* promoter. Yeast colonies expressing the *P. putida* catechol oxidase turn yellow in a few minutes when sprayed with catechol. In growth conditions which lead to repression of the sulfur network, i.e., addition of 1 mM L-methionine to the medium, the colonies remain white after catechol spraying. A search for mutations that give rise to yellow colonies even in the presence of repressing amounts of methionine should thus identify genes involved in AdoMet-induced repression. Indeed, it was previously established that the repressive effect of added methionine is actually mediated through an increase in the intracellular AdoMet concentration as a result of the efficient transformation of methionine into AdoMet by the two AdoMet synthases.

Strain W303-1B was transformed with a plasmid bearing the *MET25-xylE* gene fusion and subjected to UV mutagenesis. Mutants that were yellow when sprayed with catechol after growth in presence of 1 mM L-methionine were isolated (see Materials and Methods). Here we report the analysis of one of these mutants, the yellow phenotype of which was shown to result from a mutation in a single gene that we called *MET30* (see Materials and Methods).

***MET30* mutations impair AdoMet-mediated repression of the sulfur network.** To confirm that a mutation in *MET30* impaired the repression of the sulfur network by AdoMet, we first performed enzymatic analyses with the four spores of a tetrad isolated after sporulation of diploid CM100 (*MET30-1*/*MET30*). As described below, *MET30-1* is a dominant allele, indicated by capital letters. In the two *MET30-1* spores, the activity of homocysteine synthase (encoded by *MET25* [24]), measured after growth in the presence of a repressing amount of methionine, was three- to fourfold higher than that measured in the two *MET30* spores grown in the same conditions (Table 2). The *MET30-1* mutation also affects the AdoMet-mediated repression of other genes from the sulfate assimilation pathway. As shown in Table 2, in *MET30-1* spores, both ATP sulfurylase and PAPS reductase activities were increased 5- to 10-fold after growth in the presence of a repressing amount of methionine compared with the activities measured in *MET30* spores.

We performed a Northern analysis to confirm that the differences in the level of homocysteine synthase activity are due to modifications in *MET25* gene transcription. The repression of *MET25* was monitored by shifting wild-type and *MET30-1* cells growing in a medium lacking methionine to one contain-

TABLE 2. Effect of the *MET30-1* mutation on the sulfate assimilation pathway[a]

Strain	Relevant genotype	Growth conditions	Sp act (nmol/min/mg of protein)		
			Homocysteine synthase	ATP sulfurylase	PAPS reductase
CM100-1A	*MET30-1*	NR	410	168	0.26
		R	208	40	0.10
CM100-1B	*MET30*	NR	254	150	0.18
		R	62	<5	<0.03
CM100-1C	*MET30*	NR	220	144	0.13
		R	68	<5	<0.03
CM100-1D	*MET30-1*	NR	340	170	0.21
		R	183	63	0.16

[a] The specific activities of enzymes involved in the sulfate assimilation pathway were assayed in wild-type and *MET30-1* spores grown in either nonrepressive (NR) or repressive (R, 1 mM L-methionine) growth conditions. Data are the averages of two independent experiments (deviation was less than 10%).

TABLE 3. Effect of the *MET30-2* mutation on the AdoMet-mediated regulation of *SAM1* and *SAM2*[a]

Strain	Relevant genotype	Growth conditions[b]	β-Galactosidase sp act (nmol/min/mg of protein)
C121	*SAM1-lacZ*	NR	685
		R	72
CC682-24	*SAM1-lacZ MET30-2*	NR	930
		R	550
C119	*SAM2-lacZ*	NR	2,620
		R	510
CC687-1A	*SAM2-lacZ MET30-2*	NR	2,120
		R	1,440

[a] β-Galactosidase activity was expressed from either a *SAM1-lacZ* or *SAM2-lacZ* fusion gene integrated in the chromosome of *MET30* and *MET30-2* mutant strains. Data are the averages of two independent experiments (deviation was less than 10%).
[b] NR, nonrepressive; R, repressive (1 mM L-methionine).

ing repressing amounts of methionine. Total RNA was then extracted at different times after the shift and analyzed with a *MET25*-specific probe. As shown in Fig. 1, in wild-type cells, addition of repressing amounts of methionine to the medium results in a rapid repression of transcription: *MET25* mRNAs are four times less abundant 10 min after the addition of methionine and are virtually undetectable 20 min after the shift. By contrast, in *MET30-1* cells, *MET25* mRNA levels decrease more slowly, and about 20% of *MET25* mRNAs remain 40 min after the addition of methionine to the growth medium. The *MET30-1* mutation thus prevents the complete repression of *MET25* transcription by AdoMet.

Next, genetic analyses showed that the *MET30-1* mutation was allelic to a mutation isolated in our laboratory several years ago and known to allow growth in presence of 0.1 mM *S*-adenosylethionine, a potent toxic analog of AdoMet (this allele is thereafter called *MET30-2*). This result led us to examine whether Met30p might control the regulation of the genes *SAM1* and *SAM2*, which encode the two AdoMet synthases in yeast cells. For this purpose, we measured the activity of two reporter genes, *SAM1-lacZ* and *SAM2-lacZ*, which were integrated in the chromosome of either a *MET30-1* mutant or a wild-type strain. The results of this experiment (Table 3) prove that Met30p indeed participates in the AdoMet-mediated regulation of these two genes. Accordingly, the resistance to *S*-adenosylethionine was shown to be achieved through a large accumulation of intracellular AdoMet (data not shown).

In addition, assays of homocysteine synthase activity in *MET30*/*MET30* and *MET30*/*MET30-1* diploid cells showed that the *MET30-1* allele is dominant (Table 4) (the same result was obtained for the *MET30-2* allele [not shown]).

Cloning of *MET30*. We first tried to clone the wild-type allele of the *MET30* gene by using the *MET25-xylE* gene reporter system. We transformed strain CM100-1A (*MET30-1 MET25-xylE*::*LEU2*) with a multicopy-plasmid-based genomic library and selected colonies that were white when sprayed with catechol on a medium containing 1 mM L-methionine. For unknown reasons, only false-positive clones were obtained. We thus determined whether the *MET30-1* mutation might result in a particular phenotype that could lead to a positive screening procedure. Selenate is a well-known toxic analog of sulfate that inhibits the growth of *S. cerevisiae* at concentrations higher than 1 mM (5). A wild-type yeast strain grown in the presence of high concentrations of organic sulfur compounds is resistant to selenate because of the repression of the sulfate transport system by AdoMet. A *MET30* mutant was expected to be sensitive to selenate even in the presence of organic sulfur compounds because, in these conditions, it expresses the sulfate transport system. Plate assays revealed that wild-type but not *MET30-1* strains were indeed capable of growth in the presence of both 5 mM selenate and 1 mM L-methionine.

Strain CM100-1A was transformed with a pEmblYe23-based genomic library, and about 30,000 Ura^+ transformants were selected on 25 plates. These transformants were recovered by

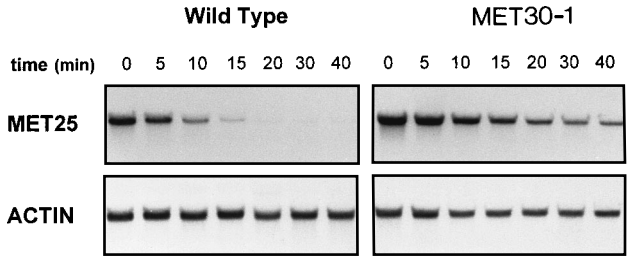

FIG. 1. Transcriptional repression of a sulfur gene in a *MET30-1* mutant. The repression kinetics of the transcription of gene *MET25* was monitored by shifting a wild-type and a *MET30-1* mutant strain from a medium without methionine to one containing a repressing amount of methionine. The strains used were W303-1A (*MET30*) and CM100-1A (*MET30-1*). Strains were grown in B medium (9) in the presence of 0.1 mM sulfate as the sulfur source. When the cell concentration reached about 10^7/ml, L-methionine was added to the medium at a final concentration of 1 mM. Samples were then withdrawn at different times, and total RNAs were extracted. For each time point, 10 μg of total RNA was electrophoresed on a 1% agarose gel and transferred to a nylon membrane. The transferred RNAs were hybridized to the radioactive *Xba*I-*Eco*RV fragment from the *MET25* gene (24). The half-life of *MET25* mRNA was previously shown to be about 8 min (24). The actin probe was used as a control for the amounts of RNA loaded.

TABLE 4. Dominance of the *MET30-1* mutation[a]

Strain	Relevant genotype	Growth conditions[b]	Homocysteine synthase sp act (nmol/min/mg of protein)
CC572	*MET30*/*MET30*	NR	345
		R	56
CM100	*MET30*/*MET30-1*	NR	370
		R	180

[a] Data are the averages of two independent experiments (deviation was less than 10%).
[b] NR, nonrepressive; R, repressive (1 mM L-methionine).

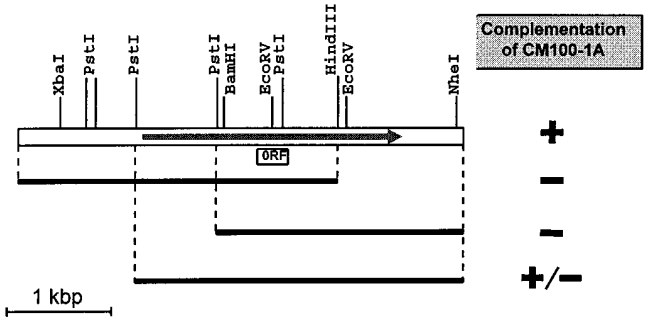

FIG. 2. Physical map of the *MET30* region. The fragments subcloned in plasmid pEmblYe23 and their ability to complement the mutation of strain CM100-1A (*ura3 MET30-1*) are shown. The ability of transformants plated on B medium containing 5 mM selenate plus 1 mM L-methionine to grow is indicated; ±, only small colonies that could not grow further appeared.

washing the plates, and the resulting cell suspensions were plated onto B medium containing 5 mM selenate and 1 mM L-methionine (B medium is a synthetic sulfur-free medium [9]). Cells that could grow in these conditions were derived from two plates of the first selection step. Plasmid DNA was isolated from eight colonies and used to retransform strain CM100-1A. All eight DNA preparations led to transformants that were resistant to selenate on B medium containing 1 mM L-methionine. These results were confirmed by using the catechol test. All transformants were white when sprayed with catechol after growth on a medium containing repressing amounts of methionine. The eight clones were found to harbor identical plasmids with a 2.9-kbp insert. Subcloning experiments showed that no smaller complementing fragment could be easily obtained (Fig. 2).

To determine whether the insert directed integration to the *MET30* region of the yeast genome, strain C161 (*ura3 MET30 MET25-xylE*::*LEU2*) was transformed with a plasmid lacking an autonomous yeast replication sequence but bearing the *URA3* gene and the complementing insert. Integration was directed by cleaving the plasmid within the insert with *Bam*HI. One Ura^+ transformant was crossed to strain CM100-1D (*ura3 MET30-1 MET25-xylE*::*LEU2*), the diploid was sporulated, and the tetrads were dissected. In all cases (20 tetrads), two spores were yellow and two spores were white when sprayed with catechol after growth in the presence of repressing amounts of methionine. Furthermore, all of the segregants that were white after catechol spraying were uracil prototrophs. These results indicate that the cloned insert directed integration to the *MET30* locus and therefore contained the *MET30* gene.

Five WD40 repeats in the C-terminal part of the *MET30*-encoded product. The complementing insert of pM30-1 was entirely sequenced on both strands, and the result is shown in Fig. 3. The DNA sequence contains a single long open reading frame with the potential to encode a protein of 640 amino acid residues. The predicted protein has a molecular mass of 72,800 and a calculated isoelectric point of 7.8. Inspection of the Met30p sequence does not reveal the presence of any canonical DNA-binding motif. However, the C-terminal part of Met30p contains five repeats of a structural motif of about 40 amino acid residues that resemble a peptide motif first identified in the β subunit of transducin, a G protein from photoreceptor rod cells, and called the β-transducin or WD40 motif (15). As shown in Fig. 4, the consensus sequence derived from the alignment of the repeats of Met30p is strongly similar to that derived for this repeating unit by Dalrymple et al. (10). The WD40 family is confined to eucaryotes and is composed of two classes of proteins. The first one includes the β subunits of the heterotrimeric G complexes; these subunits are made up almost entirely of WD40 repeats (31). The second class includes proteins which contain between four and eight repeats of the WD40 motif together with N-terminal or C-terminal extensions. These proteins function in numerous cellular processes, such as vesicular traffic, regulation of cytoskeletal assembly, RNA processing, and regulation of gene expression (reviewed by Neer et al. [32]). The function of WD40 repeats is not exactly known, although it has been suggested that they mediate protein-protein interactions. Interestingly, it seems that transcription factors bearing WD40 repeats, such as Tup1p, Cop1p, and Hir1p, are more specifically involved in repression mechanisms (11, 36, 45). One of the most studied among these factors is the protein Tup1 of *S. cerevisiae*. Tup1p forms a complex with Ssn6p, a protein that contains 10 repeats of the tetratricopeptide (TPR) motif (45, 46). The complex Ssn6-Tup1 is known to be a general repressor involved in the repression of several sets of genes, such as **a**-cell-specific genes, haploid genes, and glucose-repressible genes (14, 34, 43). Neither Tup1p nor Ssn6p possesses a DNA-binding motif.

DNA-bound Met30p does not repress transcription. Models explaining how a general repressor could recognize a wide variety of promoters invoke multiple protein interactions: the complex Ssn6-Tup1 locates the genes that it represses by recognizing specific DNA-bound proteins present at each promoter. Several lines of evidence support this model. Especially, it was shown that both the Tup1p and Ssn6p proteins can repress the transcription of a functional promoter when they are bound upstream through a LexA DNA-binding domain (23, 44). Since Met30p does not appear to be a DNA-binding protein, it seemed plausible that Met30p could function in a similar way in mediating the AdoMet-mediated repression of the sulfur network. We thus tested whether Met30p could act as a repressor when brought to DNA at a promoter. We constructed a fusion between LexA and the intact Met30 protein and tested it with two *CYC1-lacZ* gene fusions. One gene fusion (plasmid JK1621) contains four *lexA* operators upstream of the *CYC1* UAS; the other (plasmid CK30) contains one *lexA* operator positioned between the UAS and the TATA box of the *CYC1* promoter (23). β-Galactosidase assays of cotransformed cells (Table 5) grown in either nonrepressive or repressive conditions demonstrate that the LexA-Met30 fusion protein does not significantly affect the expression of the reporter genes. Additional results, using the interference assay developed by Brent and Ptashne (4), show that the LexA-Met30 fusion protein is indeed capable of binding efficiently to *lexA* operators (not shown). These results thus indicate that the Met30 protein functions in repression of transcription differently than Tup1p.

AdoMet-mediated inhibition of the transcription activation function of Met4p is Met30p dependent. Met4p, a member of the basic leucine zipper factor family, is a positive regulator of the sulfur network in *S. cerevisiae*. We have recently demonstrated that the transcription activation function of Met4p is specifically inhibited when the intracellular AdoMet level increases (27). Accordingly, when cells expressing a LexA-Met4 fusion protein and containing a *lacZ* gene placed downstream of *lexA* operators are grown in the presence of a repressing amount of methionine, a fourfold decrease in β-galactosidase activity compared with that under nonrepressive growth conditions is measured (27). The regulation of the transcriptional activation function of Met4p was shown to be mediated by two distinct domains within the protein, called the inhibitory region and the auxiliary domain (27). To determine whether the regulation of Met4p activity depends on the integrity of Met30p,

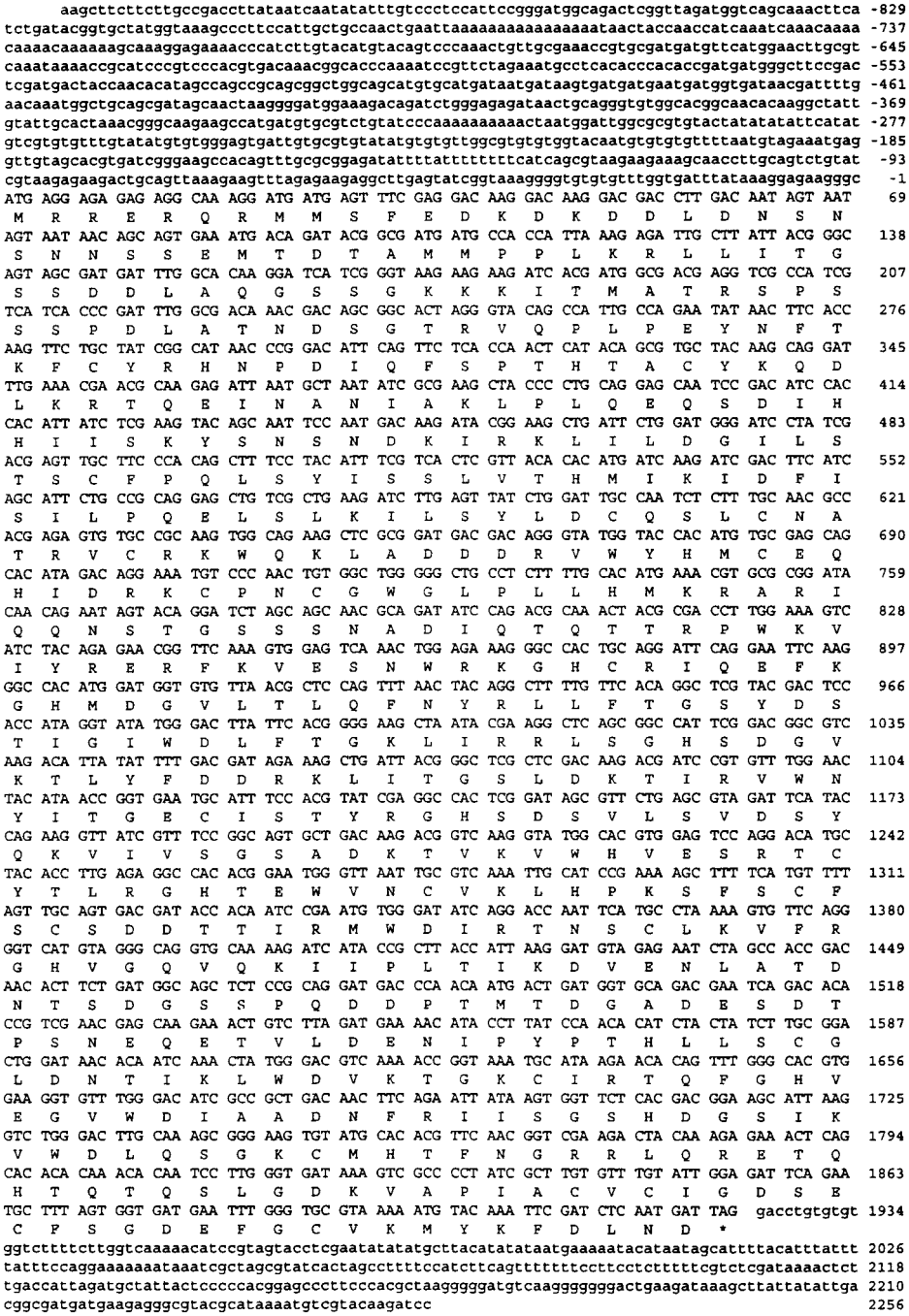

FIG. 3. Nucleotide and deduced amino acid sequences of the *MET30* region. The nucleotide sequence is numbered from nucleotide 1 of the presumed initiation codon of *MET30*.

various LexA-Met4p derivatives, each lacking one of the functional domains identified within Met4p, were expressed from plasmids introduced into *MET30-1* cells. These cells moreover contained a *lacZ* reporter gene placed downstream of six *lexA* operators in the chromosome. In contrast to what we measured in a wild-type strain, in *MET30-1* cells, all of the LexA-Met4p derivatives activate the reporter gene in repressive growth conditions as much as in nonrepressive growth conditions (Table 6). We therefore conclude that the AdoMet-mediated inhibition of the transcription function of Met4p requires the integrity of Met30p. However, it must be noted that the *MET30-1* mutation also lowered the activity of the fusion proteins in nonrepressive growth conditions.

In vivo interactions between Met4p and Met30p. The results presented above are consistent with the possibility that Met30p interacts directly with Met4p. To test this hypothesis, we carried out two-hybrid studies (13). We introduced various derivatives of Met4p fused to the activation domain of Gal4p (Gad) into strains expressing the LexA-Met30 fusion protein. The combination of LexA-Met30 fusion protein and the Gad-Met4


```
(300) GHMDGVLTLQFNYRLLFTGSYDSTIGIWD (328)
(340) GHSDGVKTLYFDDRKLITGSLDKTIRVWN (368)
(380) GHSDSVLSVDSYQKVIVSGSADKTVKVWH (408)
(419) GHTEWVNCVKLHPKSPSCPSCSDDTTIRMWD (449)
(525) ..............LLSCGLDNTIKLWD (538)
(550) GHVEGVWDIAADNFRIISGSHDGSIKVWD (578)

Met30p  GH.D.V..[I/L/V].....[I/L].SGS.D.TI[R/K]VWD
WD40    GH...I..L..D...I.SGS.D..I.IWD
```

FIG. 4. Met30p contains WD40 repeats. Amino acid residues are numbered on the left and on the right. Residues conserved within the Met30 protein are in boldface. Conservation of a given amino acid on at least three occasions yields the Met30p consensus sequence shown below the alignment. The reported consensus for the WD40 motif is from Dalrymple et al. (10). A schematic representation of the WD40 motifs (solid boxes) within Met30p is shown at the top.

TABLE 6. Dependence of AdoMet-mediated inhibition of the transcription function of Met4p on Met30p[a]

Expressed protein	Met4p domain deleted	Growth conditions[b]	β-Galactosidase sp act (nmol/min/mg of protein)			
			MET30	Ratio, NR/R	*MET30-1*	Ratio, NR/R
LexM4-1	None	NR	3,250		1,225	
		R	800	4.06	1,160	1.05
LexM4Δ14	Inhibitory	NR	3,150		1,200	
		R	2,850	1.14	1,170	1.02
LexM4Δ37	Auxiliary	NR	820		380	
		R	780	1.05	318	1.2

[a] Plasmids based on pLexM4Δi and expressing various LexA-Met4p derivatives were transformed into strain C170 (*met4*::*TRP1 MET30 ura3*::*lexAop-lacZ*::*URA3*) and C172 (*met4*::*TRP1 MET30-1 ura3*::*lexAop-lacZ*::*URA3*). Data are the averages of three assays performed with independent transformants (standard deviation was less than 10%).

[b] NR, nonrepressive (0.05 mM L-methionine), R, repressive (1 mM L-methionine).

derivative containing the entire length of Met4p confers 13-fold-higher expression of the reporter gene than either protein alone (Table 7). This result suggests that Met4p and Met30p indeed interact in vivo. Moreover, the use of several Gad-Met4 fusion proteins allowed us to identify the region of Met4p that associates with Met30p. Indeed, the two-hybrid interactions are significantly lowered by deletion of the inhibitory region of Met4p. Conversely, the Gad-Met4 derivatives lacking either the activation domain or the auxiliary domain of Met4p appear to interact with the LexA-Met30 fusion protein as well as the original Gad-Met4 hybrid (Table 7). These results suggest that the inhibitory region of Met4p is necessary for the formation of the Met4-Met30 complex. In addition, it must be noted that interactions between Met4p and Met30p cannot be easily detected by the two-hybrid method when the cells are grown in the presence of a repressing amount of methionine. Indeed, when fused to Met4p, the Gal4p activation domain is repressed by the Met4p regulatory regions when the intracellular AdoMet level is high (27) (not shown).

***MET30* essential for cell viability.** To determine whether the role of Met30p extends to other areas of metabolism, we constructed a strain that did not express a functional Met30 protein. The coding sequence of *MET30* from codon 264 to codon 448 was replaced with the selectable marker *URA3* (see Materials and Methods). This construction was used to transform the diploid strain CC572. Stable Ura^+ transformants were selected. DNA blot analysis of genomic DNA isolated from one of the transformed diploid strains (strain CD126) is shown in Fig. 5. A 1.8-kb wild-type *MET30* fragment is detected as well as the two smaller fragments that are expected from cleavage at the *Stu*I site within the *URA3* sequence. As shown in Fig. 5, sporulation of diploid CD126, heterozygous for *met30*::*URA3*, gave rise to tetrads containing no more than two viable spores that were invariably auxotrophs for uracil. The absence of uracil prototroph spores suggested that *MET30* inactivation is lethal. In order to confirm this result, we transformed diploid CD126 (*MET30*/*met30*::*URA3*) with a multicopy plasmid bearing the wild-type allele of *MET30* and the gene *LEU2* (pM30-5). The transformed diploid was sporulated. As expected, tetrads containing four, three, and two viable spores were obtained. Scoring of the nutritional requirements of these spores showed that all of the uracil prototroph spores obtained were also leucine prototrophs. These cells (*met30*::*URA3*/pM30-5) were shown to be incapable of losing plasmid pM30-5 under nonselective growth conditions: they were grown in complete medium for 50 generations and plated. The 150 resulting colonies were tested and shown to be, in all cases, both uracil and leucine prototrophs. In contrast, the same experiment performed with uracil auxotroph spores (*MET30*/pM30-5) leads to

TABLE 5. Function of LexA-Met30 fusion protein[a]

Expressed protein	Reporter plasmid	Growth conditions[b]	β-Galactosidase sp act (nmol/min/mg of protein)
None	JK1621	NR	1,930
		R	1,650
LexA-Met30p	JK1621	NR	2,300
		R	2,310
None	CK30	NR	1,595
		R	1,300
LexA-Met30p	CK30	NR	1,100
		R	950

[a] Wild-type strain W303-1A was transformed either with a plasmid (pLexM30-4) expressing the fusion protein LexA-Met30 or with vector plasmid pRS313, together with one plasmid containing the *lexAop-CYC1-lacZ* reporter gene. Plasmid JK1621 contains four *lexA* operators upstream of the *CYC1* UAS; plasmid CK30 contains one *lexA* operator between the UAS and the TATA box of *CYC1* (23). Data are the averages of two independent experiments performed with three independent transformants (standard deviation was less than 10%).

[b] NR, nonrepressive; R, repressive (1 mM L-methionine).

TABLE 7. Interactions of Met4p and Met30p in the two-hybrid system[a]

Plasmids	Met4p domain deleted	β-Galactosidase sp act (nmol/min/mg of protein)	Fold activation
pLexM30-4 + pRS315	None	1.5 ± 0.5	
pRS313 + pGadM4-1	None	1.3 ± 0.5	
pLexM30-4 + pGadM4-1	None	20.1 ± 1.0	13.4
pLexM30-4 + pGadM4Δ12	Activation	17.0 ± 1.0	11.3
pLexM30-4 + pGadM4Δ37	Inhibitory	5.5 ± 0.7	3.6
pLexM30-4 + pGadM4Δ30	Auxiliary	19.5 ± 2.1	13.0

[a] Strain C170 (*met4*::*TRP1 ura3*::*lexAop-lacZ*::*URA3*) was cotransformed with plasmid pLexM30-4 and different plasmids expressing various Gad-Met4p derivatives. The cells were grown in nonrepressive growth conditions (0.05 mM L-methionine). Data are the averages of four assays performed with independent transformants.

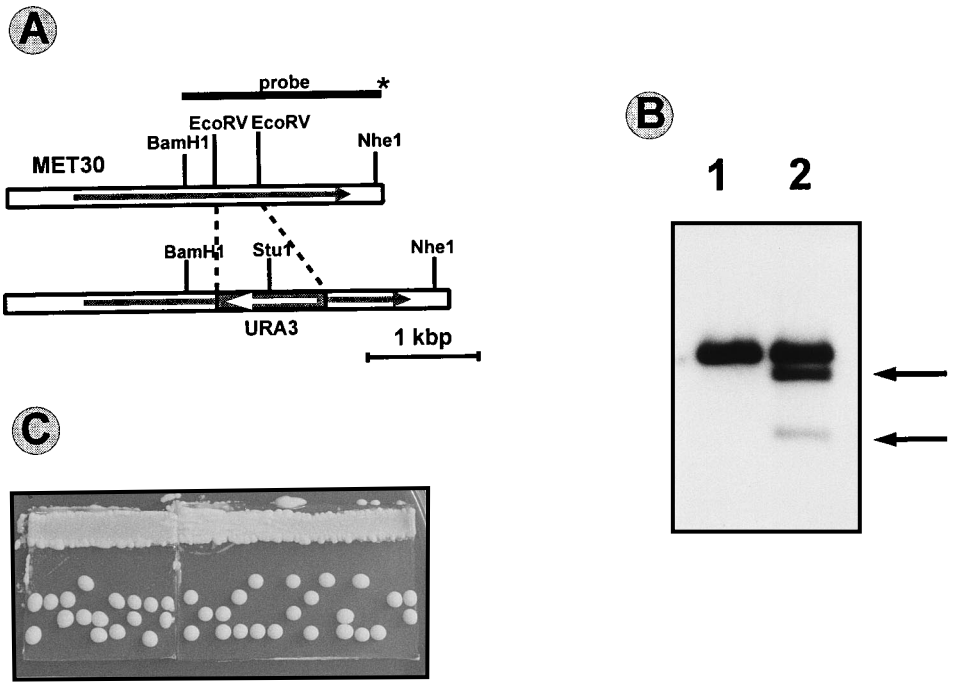

FIG. 5. *MET30* is essential for cell viability. (A) Physical map of the *met30*::*URA3* region. (B) Southern blot analysis of *MET30* gene disruption. Lane 1, *Bam*HI-*Stu*I-*Nhe*I digest of genomic DNA extracted from strain W303-1A; lane 2, *Bam*HI-*Stu*I-*Nhe*I digest of DNA extracted from strain CD126. The filter was probed with the *Bam*HI-*Nhe*I fragment of *MET30*. The arrows indicate the two fragments expected from *Stu*I cleavage within the *URA3* gene. (C) Tetrads obtained from the sporulation of diploid CD126 (*met30*::*URA3*/*MET30*).

85% leucine auxotrophs. Taken together, these results prove that *MET30* is indeed an essential gene.

Since the two *MET30* alleles isolated were shown to be dominant for the regulatory effect, it seemed important to determine whether the null *met30* allele, which is recessive for lethality, is also recessive for the regulatory effect. We thus assayed homocysteine synthase activity in the CD126 diploid strain (*MET30*/*met30*::*URA3*). The results show that, in strain CD126 as in the parental wild-type strain, homocysteine synthase activity is ninefold lower after growth in the presence of a repressing amount of methionine (Table 8). The *met30* null allele is thus recessive for the regulatory effect.

In addition, we transformed the CD126 diploid strain with plasmid pLexM30-4. A transformed diploid was sporulated. Tetrads containing four or three viable spores were obtained, and all the uracil prototroph spores were shown to be histidine prototrophs. This indicates that the pLexM30-4 plasmid is capable of complementing the *met30* null allele, demonstrating that the LexA-Met30 protein is active.

TABLE 8. Recessive nature of *met30*::*URA3* mutation[a]

Strain	Relevant genotype	Growth conditions[b]	Homocysteine synthase sp act (nmol/min/mg of protein)
CC572	*MET30*/*MET30*	NR	345
		R	56
CD126	*MET30*/*met30*::*URA3*	NR	350
		R	40

[a] Data are the averages of two independent experiments (deviation was less than 10%).

[b] NR, nonrepressive; R, repressive (1 mM L-methionine).

DISCUSSION

We report the identification of a new gene of *S. cerevisiae*, *MET30*, the product of which is involved in negative regulation of the sulfur network. This gene was isolated via a specific genetic screen that uses a fusion between the reporter gene *xylE* from *P. putida* and the promoter region of *MET25*, one gene governing the sulfate assimilation pathway. Enzymatic assays as well as Northern experiments confirm that mutations in *MET30* impair the transcriptional regulation of the sulfur network.

MET30 was cloned, and sequence analysis revealed that its encoded product belongs to an expanding family of proteins that all comprise several repeats of a 40-amino-acid segment, called the WD40 motif. The Met30 protein contains five well-conserved repeats as well as one degenerate repeat of the WD40 motif. The WD40 consensus sequence derived from the alignment of the Met30p repeats is extremely similar to that described by Dalrymple et al. (10). The WD40 proteins have been found in eucaryotes but not in procaryotes. They are involved in numerous cell processes, such as cell cycle regulation, transcription regulation, transmembrane signaling, and RNA splicing (32). All the members of the WD40 family seem to have a regulatory function, and none is known to be an enzyme. Within this family, Met30p shows the strongest similarity to the recently described protein Scon2 from *Neurospora crassa* (26). Scon2p actually appears to be the functional equivalent of Met30p in *N. crassa*, since this protein was identified as a negative regulator of the sulfur network in this fungus (6). However, nothing is known about how Scon2p functions (26).

The present identification of Met30p as a negative regulator of sulfur metabolism in *S. cerevisiae* reinforces the pattern suggesting that WD40 repeats may be found specifically in transcriptional factors that are involved in repression mecha-

nisms. For instance, before Met30p, the two yeast transcriptional factors known to include WD40 repeats were Hir1p, a negative regulator of histone gene expression, and Tup1p, which is involved in the repression of different sets of genes (36, 45). Like Met30p, Hir1p and Tup1p do not appear to be DNA-binding proteins. To explain how such molecules could repress transcription, it has been proposed that they are recruited to the target promoters by interacting with specific DNA-bound factors. Such a model was later confirmed by several studies on the function of Tup1p (23, 25, 44). In accord with such a model, Tup1p has the ability to repress transcription when brought to a promoter as a fusion with a DNA-binding domain (44). In contrast to Tup1p, we show here that Met30p did not repress transcription when it was tethered to a promoter through a fusion with the DNA-binding domain of LexA.

During this work, we have identified one target of Met30p as Met4p, a transcriptional activator regulating the sulfate assimilation pathway. Met4p was shown to respond to the level of intracellular AdoMet: an increase in the intracellular AdoMet level inhibits the transcription activation function of Met4p (27). A functional analysis of Met4p has demonstrated that this activator contains, in addition to its bZIP, three domains: one activation domain, located in the NH_2-terminal part of the protein; an inhibitory region, which mediates the AdoMet inhibition of the activation domain; and an auxiliary domain, which counteracts the function of the inhibitory region when the intracellular AdoMet level is low (27). Using LexA-Met4 fusion proteins, we show here that *MET30* mutations prevent the AdoMet-mediated inhibition of the Met4p activation function. Since a model explaining the intramolecular regulation of Met4p predicted the interaction of the Met4p inhibitory region with a regulatory protein (27), these results led us to look for physical interactions between Met4p and Met30p. Using various Gal4-Met4 derivatives, we demonstrate here by the two-hybrid method that Met4p and Met30p interact in vivo. Moreover, we show that the inhibitory region of Met4p is involved in this interaction. As the two-hybrid experiments were performed in nonrepressive growth conditions, the results suggest that Met4p and Met30p may form a complex even when the intracellular AdoMet level is low.

From these results, we propose that Met30p functions as a transcriptional inhibitor which interacts with the Met4p inhibitory region to repress the activation domain when the intracellular AdoMet level is high. Further studies are required to define more accurately how Met30p functions and to understand how the AdoMet signal is transduced to the Met4-Met30 couple. It would be also of importance to determine why, in contrast to the *met30* null mutation, the isolated alleles of *MET30* are dominant for the regulatory effect. In contrast to that of Met4p, the function of Met30p is not restricted to the sulfate assimilation pathway but also concerns the methyl cycle and the AdoMet-mediated regulation of *MET19*, which encodes the first enzyme of the pentose phosphate pathway (data not shown). This implies that Met30p has the ability to interact with transcriptional factors other than Met4p. Such multiple interactions would be possible because of the presence of WD40 repeats within Met30p. Indeed, each WD40 repeat is theoretically capable of interacting with at least one other protein, as demonstrated by the recent study of the interactions between Tup1p and homeodomain protein α2 (25). The lethality associated with *MET30* gene disruption is consistent with the involvement of Met30p in the regulation of numerous sets of genes. Perhaps this lethality originates from the central metabolic position filled by AdoMet, which is second only to ATP in the number of reactions in which it participates (8). It is thus conceivable that through Met30p, AdoMet controls the expression of a large number of genes, the global derepression of which is incompatible with cell viability.

In this regard, it is noteworthy that in yeast cells, the three transcriptional factors that belong to the WD40 protein family, Hir1p, Met30p, and Tup1p, are all negative regulators without a DNA-binding domain and that mutations in them were all found to produce pleiotropic effects on transcription (34, 36, 43; this study). This suggests that the ability to bind multiple targets may be the raison d'être of the WD40 repeats within transcriptional factors.

ACKNOWLEDGMENTS

We thank by R. Brent and A. D. Johnson for the gift of plasmids.

This work was supported by the Centre National de la Recherche Scientifique, the Association de la Recherche sur le Cancer, and the Institut National de la Santé et de la Recherche Médicale. L.K. is supported by a thesis fellowship from the Ministère de la Recherche et de l'Enseignement Supérieur.

REFERENCES

1. **Baker, R. E., and D. C. Masison.** 1990. Isolation of the gene encoding the *Saccharomyces cerevisiae* centromere binding protein CP1. Mol. Cell. Biol. **10:**2458–2467.
2. **Baldari, C., and G. Cesarini.** 1985. Plasmids pEMBLY: new single stranded shuttle vectors for the recovery and analysis of yeast DNA sequences. Gene **35:**27–32.
3. **Bartel, P. L., C. T. Chien, R. Sternglanz, and S. Fields.** 1993. Using the two-hybrid system to detect protein-protein interactions, p. 153–179. *In* D. A. Hartley (ed.), Cellular interactions in development: a practical approach. Oxford University Press, Oxford.
4. **Brent, R., and M. Ptashne.** 1984. A bacterial repressor or a yeast transcriptional terminator can block upstream activation of a yeast gene. Nature (London) **312:**612–615.
5. **Breton, A., and Y. Surdin-Kerjan.** 1977. Sulfate uptake in *Saccharomyces cerevisiae*: biochemical and genetic study. J. Bacteriol. **132:**224–232.
6. **Burton, E. G., and R. L. Metzenberg.** 1972. Novel mutation causing derepression of several enzymes of sulfur metabolism in *Neurospora crassa*. J. Bacteriol. **109:**140–151.
7. **Cai, M., and R. W. Davis.** 1990. Yeast centromere binding protein CBF1, of the helix-loop-helix protein family, is required for chromosome stability and methionine prototrophy. Cell **61:**437–446.
8. **Cantoni, G. L.** 1977. *S*-Adenosylmethionine: present status and future perspectives, p. 557–577. *In* F. Salvatore, E. Borek, V. Zappia, H. G. Williams-Ashman, and F. Schlenk (ed.), The biochemistry of *S*-adenosylmethionine. Columbia University Press, New York.
9. **Cherest, H., and Y. Surdin-Kerjan.** 1992. Genetic analysis of a new mutation conferring cysteine auxotrophy in *Saccharomyces cerevisiae*: updating of the sulfur metabolism pathway. Genetics **130:**51–58.
10. **Dalrymple, M. A., S. Petersen-Bjorn, J. D. Friesen, and J. D. Beggs.** 1989. The product of the *PRP4* gene of *S. cerevisiae* shows homology to β subunits of G proteins. Cell **58:**811–812.
11. **Deng, X. W., M. Matsui, N. Wei, D. Wagner, A. Chu, K. A. Feldmann, and P. H. Quail.** 1992. COP1, an arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a G_β homologous domain. Cell **71:**791–801.
12. **Dessen, P., C. Fondrat, C. Valencien, and C. Mugnier.** 1990. Bisance: a French service for access to biomolecular databases. Comput. Appl. Biosci. **6:**355–356.
13. **Fields, S., and O. K. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature (London) **340:**245–246.
14. **Flick, J. S., and M. Johnston.** 1990. Two systems of glucose repression of the *GAL1* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**4757–4769.
15. **Fong, H. K. W., J. B. Hurley, R. S. Hopkins, R. Miake-Lye, M. Johnson, R. Doolittle, and M. I. Simon.** 1986. Repetitive segmental structure of the transducin β subunit: homology with the *CDC4* gene and identification of related mRNAs. Proc. Natl. Acad. Sci. USA **83:**2162–2166.
16. **Gietz, D., A. St. Jean, R. A. Woods, and R. H. Schiestl.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:**1425–1426.
17. **Gyuris, J., E. Golemis, H. Chertkov, and R. Brent.** 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell **74:**205–214.
18. **Hanes, S. D., and R. Brent.** 1989. DNA specificity of the bicoid activator protein is determined by homeodomain recognition helix residue 9. Cell **57:**1275–1293.
19. **Hieter, P., D. Pridmore, J. H. Hegemann, H. Thomas, R. W. Davis, and P. Philippsen.** 1985. Functional selection and analysis of yeast centromeric DNA. Cell **42:**913–921.

20. **Hodgson, C. P., and R. Z. Fisk.** 1987. Hybridization probe size control: optimized "oligolabelling." Nucleic Acids Res. **15:**6295.
21. **Hoffman, C. S., and F. Winston.** 1987. A ten minute DNA preparation from yeast releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:**267–272.
22. **Jacquemin, I., D. Thomas, C. Cibert, J. Laporte, and Y. Surdin-Kerjan.** 1994. The vacuolar compartment is required for sulfur amino acid homeostasis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **244:**519–529.
23. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
24. **Kerjan, P., H. Cherest, and Y. Surdin-Kerjan.** 1986. Nucleotide sequence of the *Saccharomyces cerevisiae MET25* gene. Nucleic Acids Res. **14:**7861–7871.
25. **Komachi, K., M. J. Redd, and A. D. Johnson.** 1995. The WD repeats of Tup1 interact with the homeo domain protein α2. Genes Dev. **8:**2857–2867.
26. **Kumar, A., and J. V. Paietta.** 1995. The sulfur controller-2 negative regulatory gene of *Neurospora crassa* encodes a protein with β-transducin repeats. Proc. Natl. Acad. Sci. USA **92:**3343–3347.
27. **Kuras, L., and D. Thomas.** 1995. Functional analysis of Met4, a yeast transcriptional activator responsive to *S*-adenosylmethionine. Mol. Cell. Biol. **15:** 208–216.
28. **Kuras, L., and D. Thomas.** 1995. Identification of the yeast methionine biosynthetic genes that require the centromere binding factor 1 for their transcriptional activation. FEBS Lett. **367:**15–18.
29. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
30. **Mellor, J., W. Jiang, M. Funk, J. Rathjen, J. C. Barnes, T. Hinz, J. H. Hegemann, and P. Philippsen.** 1990. CPF1, a yeast protein which functions in centromeres and promoters. EMBO J. **9:**4017–4026.
31. **Neer, E. J., and D. E. Clapham.** 1988. Role of G protein subunits in transmembrane signalling. Nature (London) **333:**129–134.
32. **Neer, E. J., C. J. Schmidt, R. Nambudripad, and T. F. Smith.** 1994. The ancient regulatory-protein family of WD-repeat proteins. Nature (London) **371:**297–300.
33. **O'Connell, K., Y. Surdin-Kerjan, and R. Baker.** 1995. Role of the *Saccharomyces cerevisiae* general regulatory factor CP1 in methionine biosynthesis gene transcription. Mol. Cell. Biol. **15:**1879–1888.
34. **Schamhart, D. H. J., A. M. A. Ten Berge, and K. W. Van de Poll.** 1975. Isolation of a catabolite repression mutant of yeast as a revertant of a strain that is maltose negative in the respiratory-deficient state. J. Bacteriol. **121:** 747–752.
35. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1979. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Sherwood, P. W., S. V. M. Tsang, and M. A. Osley.** 1993. Characterization of *HIR1* and *HIR2*, two genes required for regulation of histone gene transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**28–38.
37. **Thomas, D., H. Cherest, and Y. Surdin-Kerjan.** 1989. Elements involved in *S*-adenosylmethionine-mediated regulation of the *Saccharomyces cerevisiae MET25* gene. Mol. Cell. Biol. **9:**3292–3298.
38. **Thomas, D., I. Jacquemin, and Y. Surdin-Kerjan.** 1992. MET4, a leucine zipper protein, and centromere binding factor I are both required for transcriptional activation of sulfur metabolism in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**1719–1727.
39. **Thomas, D., R. Rothstein, N. Rosenberg, and Y. Surdin-Kerjan.** 1988. *SAM2* encodes the second methionine *S*-adenosyl transferase in *Saccharomyces cerevisiae*: physiology and regulation of both enzymes. Mol. Cell. Biol. **8:** 5132–5139.
40. **Thomas, D., and Y. Surdin-Kerjan.** 1990. An improved strategy for generating a family of unidirectional deletions on large DNA fragments. Gene Anal. Tech. Appl. **7:**87–90.
41. **Thomas, D., and Y. Surdin-Kerjan.** 1991. The synthesis of the two *S*-adenosylmethionine synthetases is differently regulated in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **226:**224–232.
42. **Thomas, P. S.** 1980. Hybridization of denatured DNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77:**5201–5205.
43. **Trumbly, R. J.** 1986. Isolation of *Saccharomyces cerevisiae* mutants constitutive for invertase synthesis. J. Bacteriol. **166:**1123–1127.
44. **Tzamarias, D., and K. Struhl.** 1994. Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. Nature (London) **369:**758–761.
45. **Williams, F. E., and R. J. Trumbly.** 1990. Characterization of *TUP1*, a mediator of glucose repression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**6500–6511.
46. **Williams, F. E., U. Vanarasi, and R. J. Trumbly.** 1991. The CYC8 and TUP1 proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex. Mol. Cell. Biol. **11:**3307–3316.
47. **Worsay, M. J., and P. A. Williams.** 1975. Metabolism of toluene and xylene by *Pseudomonas putida* (arvilla) mt-2: evidence for a new function of the TOL plasmid. J. Bacteriol. **124:**7–13.