Single point mutations in Met4p impair the transcriptional repression of *MET* genes in *Saccharomyces cerevisiae*

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Abstract Transcription of *MET* genes in *Saccharomyces cerevisiae* depends on a transcriptional activator, the *MET4* gene product (Met4p). Using in vitro mutagenesis, we isolated two mutant *MET4* alleles encoding [Pro^{215}]Met4p and [Ser^{156}]-Met4p. These mutations impeded Met4p's responsiveness to methionine in the media, and yeast cells carrying mutant alleles exhibited enhanced transcription of *MET* genes under repressing conditions. The enhanced transcription was dependent on the *CBF1* gene, but did not compete with an excess of wild-type Met4p, suggesting that some changes in the affinity of Met4p to other factors might be involved in *S*-adenosylmethionine-mediated transcriptional regulation

Key words: Sulfur metabolism; *MET4*; Transcriptional regulation; Random mutagenesis; *CBF1*; *S*-Adenosylmethionine

1. Introduction

Reductive assimilation of sulfate and methionine biosynthesis constitute a metabolic network in *Saccharomyces cerevisiae*. This network consists of more than 20 unlinked genes, the expression of which is under the control of a negative regulation system: the transcription level of sulfur genes is lowered in response to an increase in *S*-adenosylmethionine (AdoMet), which is enzymatically converted from methionine [1].

The corresponding *cis*-acting sequences have been found in the promoter region of almost all sulfur genes, and were identified as upstream activating sequences (UAS_{Met}) [2,3]. In contrast, in the 5′ upstream region of the *MET25* gene, upstream repressing sequences (URS_{Met}) have been found to play an important role in the negative regulation of gene expression [3].

In the case of *trans*-acting elements, there are a few regulatory factors involved in the transcription of sulfur genes. A unique protein, called Cbf1p (also referred to as Cp1p and Cpf1p), recognizes the DNA motif RCACGTG (where R is a purine). This motif is called centromere-determining element 1 (CDE 1) [4,5], and is identical to the UAS_{Met}. Cbf1p is required for methionine prototrophy in some cases, but does not appear to act by itself as a transcriptional activator [6–8]. Met4p belongs to the basic region-leucine zipper (bZIP) protein, and mediates transcriptional activation of sulfur genes [9,10]. Functional dissection of Met4p has identified domains required for transcriptional activation and susceptibility to negative regulation via AdoMet [11]. A recent study has revealed that another participant in the transcriptional control, Met30p, associates with Met4p and acts as a negative regulator [12].

In spite of the increasing number of regulatory elements essential for proper transcriptional regulation of sulfur genes, it remains obscure how AdoMet molecules exert the switching of Met4p. To address this issue, we have isolated mutant Met4 proteins, which are defective in normal response to the elevation of extracellular methionine. The mutant proteins with enhanced activities have amino acid substitutions within or in the vicinity of the inhibitory region of Met4p [11]. Moreover, we have gained some insights into the mechanism of the negative regulation of *MET* genes from the results of competitive experiments using mutant and wild-type *MET4* genes.

2. Materials and methods

2.1. Yeast strains and media

Rich medium (YPD) and synthetic media (SD) supplemented with auxotropic components to maintain plasmids were prepared essentially as described elsewhere [13]. The media were supplemented with methionine (5 mM) for repression of *MET* genes. The strains used in this study are listed in Table 1. Yeast strains were constructed by transformation using the lithium acetate method or by standard genetic crosses [13].

2.2. Recombinant DNA

The promoter region of the *MET25* gene (nucleotide positions −675 to −19) [14] was amplified by polymerase chain reaction (PCR) from *S. cerevisiae* (strain X2180-1A) genomic DNA, using oligonucleotides 5′-ctcaagcttATCCTTGCGTTTCAGCTTCCAC-3′ (where the nucleotides written in lower-case letters were added to generate a restriction site) and 5′-ctcggatccTCTATGTATCTATGTTAAGA-3′. The 6.2-kb *Hin*dIII-*Sph*I fragment from YIp33 [15] was ligated with the 2.2-kb *Hin*dIII-*Sph*I fragment from p714 [16] to give p813. The PCR product for *MET25* promoter (*MET25p*) was doubly digested with *Hin*dIII and *Bam*HI, and then inserted into the *Hin*dIII-*Bam*HI gap of pSH39 [17] and p813 to construct p886 and p953, respectively. The plasmids p886 and p953 were used for the integration of the *MET25p-PHO5* reporter gene at *ura3* and *leu2* loci, respectively. An *Eco*RI-*Bam*HI DNA fragment including the *MET4* locus (nucleotide positions 43–2411) [9] was amplified from X2180-1A DNA by PCR with the primers 5′-TACAGCACGGAATTCATAAATCTCT-3′ and 5′-gaggatccATTTCGAGCGGCTTGCA-3′. After digestion with *Eco*RI and *Bam*HI, the fragment was inserted into pUC118 [18] at the *Eco*RI and *Bam*HI sites to construct pUC-MET4. The promoter region of the *MET4* gene (positions −693 to 48) was amplified with 5′-CACATACATGCATGCCACATACATGC-3′ and 5′-AGAGATTTATGAATTCCGTGCTGTAT-3′, and inserted into the *Sph*I and *Eco*RI sites of pUC18 to obtain pMET4SpE. To obtain plasmid pYCM4, the 2.4-kb *Eco*RI-*Bam*HI

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Abbreviations: Met4p, a *MET4* gene product; Cbf1p, a *CBF1* gene product; *MET25p*, *MET25* promoter; rAPase, repressible acid phosphatase

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fragment from pUC-MET4 and the 737-bp *Sph*I-*Eco*RI fragment from pMET4SpE were ligated together with the 7.8-kb *Sph*I-*Bam*HI fragment from YCp50 [13], in which the original *Eco*RI site at position 1 had been eliminated. The *Sph*I site in pYCM4 was converted to an *Xho*I site, and the 3.1-kb *Xho*I-*Bam*HI fragment was excised and cloned into the *Sal*I-*Bam*HI gap of YEp24 [13] to give pYEM4U. The 3.5-kb *Nru*I-*Bam*HI fragment was cut out from pYCM4, and inserted into the *Pvu*I-*Bam*HI gap of YEp13 [13] to obtain pYEM4L. The *TRP1* gene used in this study was derived from YRp7 [13], and subcloned into the *Pst*I site of pUC18 with *Pst*I linkers to give pUC-TRP1. Plasmid pTRPdMET4 bearing a disrupted allele of *MET4* (i.e. *met4::TRP1*) was constructed by insertion of a 1.1-kb *Sph*I-*Sal*I fragment of the *TRP*1 gene from pUC-TRP1 into the *Nco*I and *Sal*I sites of the *MET4* gene in pUC-MET4 after blunt-ending the *Sph*I and *Nco*I ends. Plasmid pTRPdCBF1 carrying a disrupted *CBF1* allele (*cbf1::TRP1*) was constructed by insertion of a 1.1-kb *Sph*I-*Hin*cII fragment of *TRP1* gene from pUC-TRP1 into the *Sph*I and *Bal*I sites of the CBF1 gene fragment (nucleotide positions 49–974) cloned in pUC119 using *Bam*HI and *Eco*RI linkers. Plasmids pTRPdMET4 and pTRPdCBF1 were used for the establishment of disruptant strains, FDM441 and FDC101, respectively.

2.3. Oligonucleotide-directed random mutagenesis

To obtain a set of random mutated *MET4* genes, Kunkel's modification [18] of oligonucleotide-directed mutagenesis was carried out using the single-stranded DNA prepared from pUC-MET4 as a template. The 6-mers of synthetic oligonucleotides with random sequences were used as mutagenic primers, and hybridized to the template DNA during the mutagenesis procedure to generate random mutations in the *MET4* open reading frame (ORF). The double-stranded DNA of pUC-MET4 was then prepared and used as a pool of mutant *MET4* genes. The 2.4-kb *Eco*RI-*Bam*HI fragment was excised from the pool, and inserted into the *Eco*RI-*Bam*HI gap of pYCM4. *Escherichia coli* strain DH5α was transformed with the ligation mixture, and the resulting colonies were collected and used as the source of DNA library for mutant *MET4* genes.

The 2.8-kb *Sal*I fragments from *MET4-1* and *MET4-2* alleles cloned in pYCM4 were inserted into the *Sal*I site of pUC-TRP1 to give pTRP-MET4-1 and pTRP-MET4-2, respectively. These constructs were digested with *Nco*I and used for the integration of mutant alleles into the chromosomal *MET4* locus to obtain FMC22 (*met4::TRP1::MET4-1*) and FMC99 (*met4::TRP1::MET4-2*) strains.

2.4. Measurement of repressible acid phosphatase (rAPase) activity

The methods employed to detect the rAPase activity of colonies [19] and that in cell suspensions [20] of *S. cerevisiae* have been described previously. For plate assay, the media were supplemented with 5 or 0 mM methionine to to create repressing or nonrepressing conditions, respectively.

2.5. Northern analysis

The total RNA sample was prepared according to a standard method [13] from yeast cells cultivated under the conditions indicated. Agarose gel electrophoresis was carried out with 40 μg RNA per lane, and subsequent Northern analysis was performed with ^{32}P-labeled DNA fragments exiced from the ORF of *MET4*, *MET25*, and *ACT1* genes [18]. The relative amount of each mRNA was measured based on the corresponding radioactive signal of the membrane filter using an imaging plate (Fujix BAS 2000, Fuji Photo Co., Ltd., Japan).

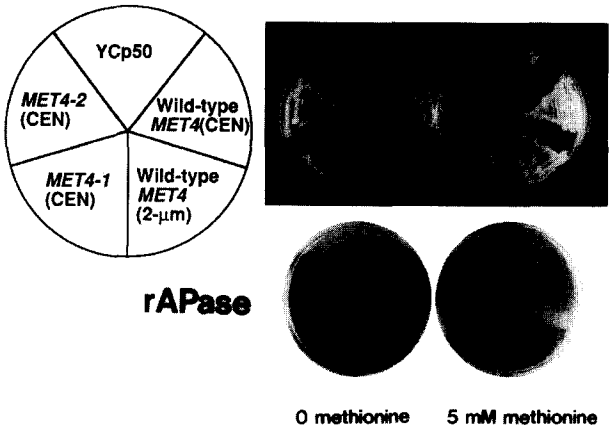

Fig. 1. The rAPase activities with mutant *MET4* alleles. Strain FDM441 (*leu2::MET25p-PHO5::LEU2 Δmet4*) was transformed with a wild-type *MET4* gene on a centromeric vector (CEN), pYCM4, or on pYEM4U (2 μm vector). YCp50 was used as a control. The resulting transformants and two other clones carrying *MET4-1* and *MET4-2* alleles on a centromeric vector were examined for cell growth and rAPase activity in the absence or presence of 5 mM methionine in the media after 2 days incubation at 30°C.

3. Results

3.1. Isolation of mutant MET4 alleles that are not responsive to extracellular methionine

To investigate the structure-function relationship of Met4p, we attempted to isolate mutant *MET4* proteins that are not under the control of AdoMet, which is derived from methionine. For this purpose, we took advantage of the detection system for rAPase activity with a specific staining method on plates [19]. The reporter gene *MET25p-PHO5* inserted at the *leu2* locus of strain FDM441 (*leu2::MET25p-PHO5::LEU2 Δmet4*), was expected to be subject to the transcriptional control for sulfur genes. The pool of mutant *MET4* genes was screened on the basis of the methionine-nonresponsive expression of the reporter gene. The 20 000 transformants carrying mutant *MET4* genes were checked by the rAPase assay for their ability to confer nonresponsive expression of the reporter

Table 1
List of yeast strains

Strain	Relevant genotype	Source
SH2553	*MATα ura3 trp1 his3 pho3 pho5*	S.Harashima
SH2676	*MATa ura3 leu2 trp1 his1 pho3 pho5*	S.Harashima
SH3685	*MATa leu2 trp1 his1 pho3 pho5 ura3::MET25p-PHO5::URA3*	This study
SH4378	*MATa ura3 trp1 his1 pho3 pho5 leu2::MET25p-PHO5::LEU2*	This study
FDM441	*MATa ura3 trp1 his1 pho3 pho5 leu2::MET25p-PHO5::LEU2 met4::TRP1*	This study
FDC101	*MATa ura3 trp1 his1 pho3 pho5 leu2::MET25p-PHO5::LEU2 cbf1::TRP1*	This study
FMC22	*MATa leu2 trp1 his1 pho3 pho5 ura3::MET25p-PHO5::URA3 met4::MET4-1::TRP1*	This study
FMC99	*MATa leu2 trp1 his1 pho3 pho5 ura3::MET25p-PHO5::URA3 met4::MET4-2::TRP1*	This study
FMC221	*MATa/MATα LEU2/leu2 trp1/trp1 HIS1/his1 HIS3/his3 pho3/pho3 pho5/pho5 ura3/ura3::MET25p-PHO5::URA3 MET4/met4::MET4-1::TRP1*	This study
FMC991	*MATa/MATα LEU2/leu2 trp1/trp1 HIS1/his1 HIS3/his3 pho3/pho3 pho5/pho5 ura3/ura3::MET25p-PHO5::URA3 MET4/met4::MET4-2::TRP1*	This study
FM001	*MATa/MATα LEU2/leu2 trp1/trp1 HIS1/his1 HIS3/his3 pho3/pho3 pho5/pho5 ura3/ura3::MET25p-PHO5::URA3*	This study
X2180-1A	*MATa*	Yeast Genetic Stock Center

gene to the 5 mM methionine in the medium. The 30 positive candidates were chosen and the plasmid DNA was recovered from each clone. The whole DNA sequence throughout the ORF of mutated *MET4* genes was determined by the dideoxy sequencing method [18], revealing that two distinct mutant alleles were included in the positive clones. The *MET4-1* mutation was caused by a T→C change at nucleotide position 643 [9] replacing the corresponding serine residue with proline ([Pro^{215}]Met4p). The *MET4-2* included a T→C change at position 467 replacing the corresponding phenylalanine residue with serine ([Ser^{156}]Met4p). Fig. 1 shows the nonrepressive expression of the *MET25p-PHO5* reporter gene due to the *MET4-1* and *MET4-2* genes. The yeast strains carrying one of those mutant alleles exhibited considerable levels of rAPase activity even in the presence of methionine in the medium, while wild-type MET4 gene resulted in methionine-responsive expression of rAPase regardless of the copy number of introduced *MET4* gene.

To examine the dominancy of the mutant alleles isolated in this study, *MET4-1* and *MET4-2* alleles were integrated into the *MET4* locus of the SH3685 (*MET4*) strain. The resulting strains FMC22 (*met4::TRP1::MET4-1*) and FMC99 (*met4::TRP1::MET4-2*) were crossed with SH2553 (*MET4*) to obtain diploid strains FMC221 (*MET4/MET4-1*) and FMC991 (*MET4/MET4-2*), respectively. These diploid strains were tested for the methionine independence of rAPase activity. As shown in Fig. 2, *MET4-2* allele exhibited a dominant phenotype, whereas the dominancy of *MET4-1* was rather ambiguous. The genetic background of constructed diploid strains seemed to reduce the level of rAPase activity somewhat. However, as shown below in Fig. 5, the *MET4-1* allele showed a clear dominancy under the different genotypic conditions. Therefore, we designate the mutant alleles as *MET4-1* and *MET4-2*, respectively.

We next attempted to make it clear that the *MET4-1* and *MET4-2* alleles influenced the transcription level of the intrinsic *MET25* gene as well as the reporter gene tested. A set of transformants used in Fig. 1 were analyzed with respect to mRNA expression from the chromosomal *MET25* gene (Fig. 3). When transferred into the strain FDM441 (Δ*met4*), the wild-type *MET4* gene functioned in *MET25* expression in a methionine-responsive manner regardless of the copy number of the *MET4* gene. However, when *MET4-1* and *MET4-2* mutant genes were introduced, the chromosomal *MET25* gene was expressed in a semi-constitutive manner. The expression levels of *MET25* mRNA in the presence of methionine with *MET4-1* and *MET4-2* were 67 and 81% of the level without

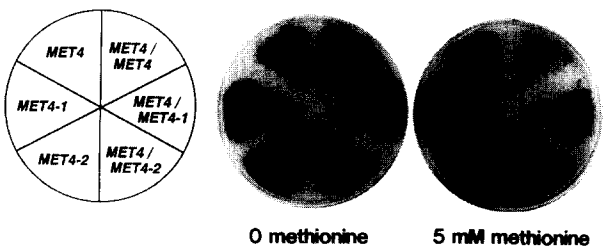

Fig. 2. Analysis of diploid cells with rAPase assay. Yeast cells were spread onto the plates, incubated at 30°C for 2 days with or without 5 mM methionine, and subjected to the rAPase assay as described above. Strains clockwise from the top were FM001 (*MET4/MET4*), FMC221 (*MET4/MET4-1*), FMC991 (*MET4/MET4-2*), FMC99 (*MET4-2*), FMC22 (*MET4-1*), SH3685 (*MET4*).

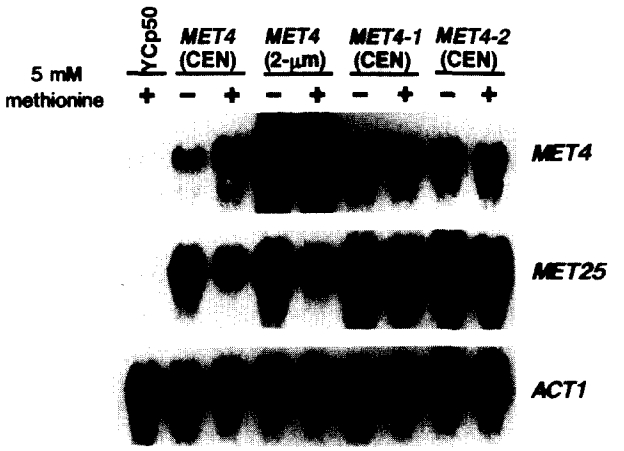

Fig. 3. Mutant *MET4* alleles influence the mRNA of chromosomal *MET25* gene. Total RNA was prepared from a set of transformants shown in Fig. 1 after 12 h cultivation in SD media with or without 5 mM methionine. The samples were then processed for Northern analysis using ^{32}P-labeled *MET4*, *MET25*, and *ACT1* gene fragments as probes. The *MET4* genes were introduced into the transformants with a centromeric (CEN) or a 2 μm plasmid.

methionine, respectively, whereas that with the wild-type *MET4* gene was repressed to 44% of the level for the methionine-free conditions. We also investigated the expression levels of four other *MET* genes, the expression of which was under the control of AdoMet. In the FDM441 strains carrying mutant *MET4* genes, the *MET2* and *MET3* genes were found to show enhanced expression under repressing conditions (data not shown). In the case of *MET14* and *MET16*, however, not only under repressing conditions, but also under nonrepressing conditions, a 2–3-fold increase in expression level was observed as compared to the strain with the wild-type *MET4* gene (data not shown).

3.2. *Cbf1p is requisite for enhanced expression via the MET25 promoter with mutant MET4 genes*

Cbf1p is known to promote the expression of several *MET* genes. Therefore, we assessed the possibility of mutant *MET4* alleles to bypass the function of Cbf1p in the course of *MET* gene expression. We assayed the dependency on methionine

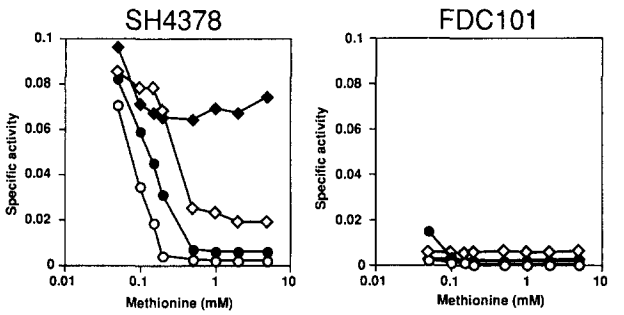

Fig. 4. Mutant *MET4* alleles do not bypass Cbf1p's function. Strains SH4378(*CBF1*) and FDC101(Δ*cbf1*) were transformed with YCp50 (○) and pYCM4 (●), and with *MET4-1* (◇) and *MET4-2* (◆) genes (mutated derivatives of pYCM4). The transformants were cultivated in SD medium supplemented with methionine (0.05–5 mM) for 12 h, and subjected to the rAPase analysis using cell suspensions in accordance with the method reported previously [20]. Specific activity was expressed as units per unit of optical density at 660 nm.

concentration of the function of wild-type and mutant Met4 proteins in SH4378 (*MET4 CBF1*) strain using the rAPase assay with cell suspensions [20]. As shown in Fig. 4, wild-type Met4p resulted in methionine-responsive expression of the *MET25p-PHO5* gene whereas additional *MET4-1* and *MET4-2* gene products conferred semi-constitutive and constitutive expression, respectively, regardless of the methionine concentration. However, when those extragenous mutant *MET4* genes were expressed in the FDC101 strain (*MET4 Δcbf1*), almost no rAPase activity was detected suggesting the necessity of Cbf1p for the emergence of the *MET4-1* and *MET4-2* phenotypes.

3.3. *Excess wild-type Met4p does not interfere with the function of mutant Met4p in the presence of methionine*

To obtain a clue to understanding how the transcriptional activity of Met4p is implemented and regulated by an effector molecule such as AdoMet, we investigated the capability of inactivated wild-type Met4p to compete with the function of *MET4-1* gene product ([Pro^{215}]Met4p) under repressing conditions. The wild-type *MET4* gene was introduced into the strain FMC22 (*met4::TRP1::MET4-1*) *via* 2 μm-based plasmid (pYEM4L) that was expected to be largely amplified. The resulting transformant expectedly showed a pronounced increase in *MET4* mRNA, however, no changes were observed in the level of *MET25* mRNA when compared to the parental FMC22 (Fig. 5A). This result was confirmed in another experiment using the rAPase assay (Fig. 5B). The semi-constitutive expression pattern of rAPase by [Pro^{215}]Met4p was not affected by an excess of wild-type Met4p.

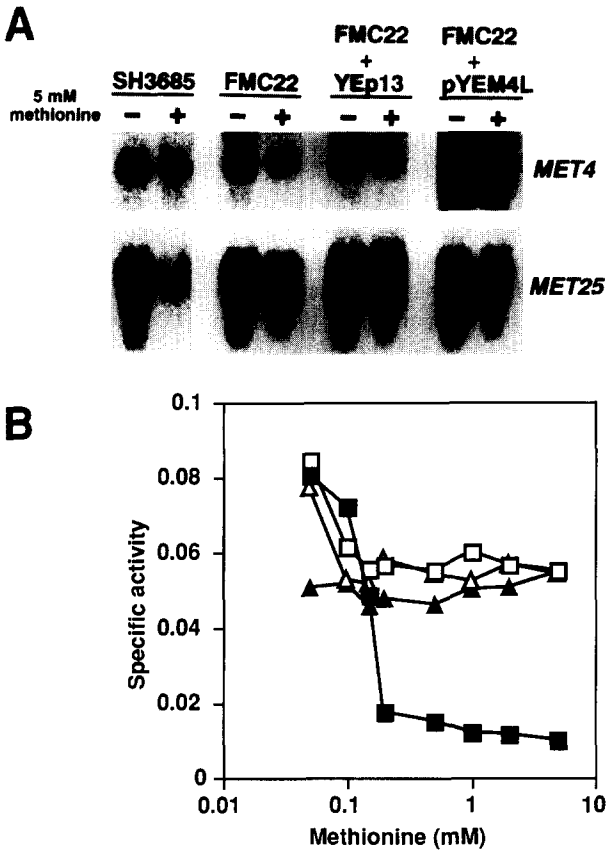

Fig. 5. Wild-type Met4p does not compete with the function of mutant Met4p. (A) Northern analysis was adapted to the total RNA prepared from strains SH3685 (*MET4*) and FMC22 (*met4::TRP1::MET4-1*), and FMC22 transformed with YEp13 and pYEM4L (YEp13+wild-type *MET4*) using ^{32}P-labeled *MET4* and MET25 gene fragments as probes. Cells were cultivated in the presence or absence of 5 mM methionine. (B) Yeast cells were cultivated in SD media supplemented with various concentrations of methionine for 12 h, and processed for the rAPase assay system with cell suspensions. Specific activity was expressed as units per unit of optical density at 660 nm. (■) SH3685; (□) FMC22; (△) FMC22+YEp13; (▲) FMC22+pYEM4L.

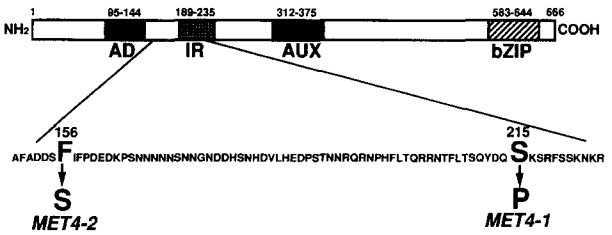

Fig. 6. Schematic representation of Met4p and amino acid substitutions in mutant proteins. Part of the amino acid sequence surrounding the mutated sites caused by *MET4-1* and *MET4-2* mutations is indicated using the standard single-letter code. Numbers indicate the positions of amino acid residues numbered from the initiation ATG. This protein model is based on [11]. AD, activation domain; IR, inhibitory region; AUX, auxiliary domain; bZIP, basic region-leucine zipper motif.

4. Discussion

Molecular dissection of Met4p has been carried out by Kuras and Thomas [11] using a LexA-Met4 fusion protein along with a *LexAop-lacZ* reporter gene, identifying three functional domains of Met4p (i.e. the activation domain, inhibitory region, and auxiliary domain) in addition to the bZIP region (Fig. 6). The LexA-Met4 protein is designed to bind to DNA molecules at its NH_2-terminal part, which is distal from the bZIP motif. In contrast, our mutational approach was based on the topologically native form of Met4p. The combination of random mutagenesis and rAPase assay facilitated the isolation of two methionine-nonresponsive mutants *MET4-1* ([Pro^{215}]Met4p) and *MET4-2* ([Ser^{156}]Met4p). The mutation sites were in good agreement with the results of functional analysis on the LexA-Met4p [11] (Fig. 6). The Ser → Pro change at residue 215 (numbered from the initiation ATG defined [9]) is exactly in the inhibitory region required for AdoMet responsiveness. Another substitution at residue 156 (Phe → Ser) was found between the activation domain and inhibitory region, however, a LexA-Met4 protein with deletion of residues 115–184 seems to have a similar phenotype to our mutants [11]. In our experiments, [Pro^{215}]Met4p always exhibited lower transcriptional activation than [Ser^{156}]Met4p in the presence of methionine. It is unclear whether this is due to the incomplete relief of the protein by the Ser^{215} → Pro mutation from inactivation via AdoMet, or to instability of this mutant protein under repressing conditions. Further site-directed mutagenesis revealed that the substitution of residue 156 with Pro, Gly, Leu, and Glu resulted in the same phenotype as [Ser^{156}]Met4p, whilst [Asp^{215}]Met4p showed the same moderate phenotype as [Pro^{215}]Met4p (data not shown). The requirement for the properties of amino acid residues at those positions to acquire nonresponsiveness to AdoMet is unclear.

It is known that the transcription of the *MET4* gene itself is not repressed by the presence of methionine, but is regulated by the general amino acid control mechanism, and that weak

stimulation of the *MET4* transcript level is observed when 2–20 mM methionine is added to the media [10]. Therefore, the elevation in the wild-type *MET4* mRNA level in the FDM441 strain in the presence of 5 mM methionine (Fig. 3) is probably attributable to the general amino acid control [1,10]. It is noteworthy that, in the yeast strains carrying mutant *MET4* genes, the level of *MET4* transcripts is higher under nonrepressing conditions without methionine, as compared to the strains carrying a wild-type gene (Figs. 3 and 5). This observation may be explained by a possible increase in intracellular methionine (i.e. an effector molecule for the general amino acid control system) resulting from the activation of some *MET* genes (e.g. *MET14* and *MET16*), the expression of which is enhanced by the mutant *MET4* genes under nonrepressing as well as repressing conditions. Moreover, the parental strain SH3685 used in Fig. 5 was found to exhibit tighter repression of the *MET25* gene with 5 mM methionine, as compared to the FDM441 strain carrying a wild-type *MET4* gene (Fig. 3). This is possibly due to the difference in the amino acids supplemented to complement their auxotrophy, which may influence the transcription of the *MET4* gene itself via the general amino acid control.

We investigated whether the *MET4-1* and *MET4-2* mutations could bypass an activation step performed by Cbf1p during or prior to the Met4p-dependent transcription. An experiment using a Δ*cbf1* strain clearly showed that the function of Cbf1p was also required for methionine-nonresponsive activation with [Pro^{215}]Met4p and [Ser^{156}]Met4p. This suggests that mutant the Met4 proteins isolated in this report are affected only in respect of the responsiveness to AdoMet, and that they still require other transcriptional regulatory factors as does wild-type Met4p.

Functional analysis of the LexA-Met4p derivative has demonstrated that the negative regulation of transcriptional activity is independent of the DNA-binding capacity of the fusion protein [11]. The function of LexA-Met4p has also been shown to be regulated by the interaction of a regulatory protein, Met30p, with the Met4p inhibitory region [12]. Met30p seems to be associated with Met4p even when the intracellular AdoMet level is low. However, these results do not exclude the possibility that the changes in affinity of a native Met4p to the *cis*-acting elements on DNA or other regulatory proteins might be involved in the functional regulation that is responsive to the AdoMet level. In our experiments, the methionine nonresponsiveness of [Pro^{215}]Met4p was not affected by a large amount of wild-type Met4p, suggesting that wild-type Met4p differs in its conformation under repressing and nonrepressing conditions. Therefore, it did not compete with target molecules (e.g. *cis*-acting DNA sequences or other proteins) required for the transcription activity governed by [Pro^{215}]Met4p. In conjunction with genetical and immunological approaches, the AdoMet-nonresponsive Met4 proteins might be useful to isolate factors involved in the active complex for the transcription of *MET* genes, since those mutant proteins probably tend to be fixed stably in an active state.

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