

N-Terminal Methionine Removal and Methionine Metabolism in *Saccharomyces cerevisiae*

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Abstract Methionine aminopeptidase (MetAP) catalyzes removal of the initiator methionine from nascent polypeptides. In eukaryotes, there are two forms of MetAP, type 1 and type 2, whose combined activities are essential, but whose relative intracellular roles are unclear. Methionine metabolism is an important aspect of cellular physiology, involved in oxidative stress, methylation, and cell cycle. Due to the potential of MetAP activity to provide a methionine salvage pathway, we evaluated the relationship between methionine metabolism and MetAP activity in *Saccharomyces cerevisiae*. We provide the first demonstration that yeast MetAP1 plays a significant role in methionine metabolism, namely, preventing premature activation of *MET* genes through MetAP function in methionine salvage. Interestingly, in cells lacking MetAP1, excess methionine dramatically inhibits cell growth. Growth inhibition is independent of the ability of methionine to repress *MET* genes and does not result from inhibition of synthesis of another metabolite, rather it results from product inhibition of MetAP2. Inhibition by methionine is selective for MetAP2 over MetAP1. These results provide an explanation for the previously observed dominance of MetAP1 in terms of N-terminal processing and cell growth in yeast. Additionally, differential regulation of the two isoforms may be indicative of different intracellular roles for the two enzymes. *J. Cell. Biochem.* 89: 964–974, 2003. © 2003 Wiley-Liss, Inc.

Key words: amino acid metabolism; product inhibition; protein processing; enzyme regulation; sulfur salvage

Protein synthesis initiates with methionine in the cytosol of eukaryotes and formyl-methionine in prokaryotes and eukaryotic organelles. When the penultimate residue is small and uncharged (Ala, Cys, Gly, Pro, Ser, Thr, or Val), the initiator methionine is removed co-translationally by methionine aminopeptidase (*MAP*, or *Map1p*, or *MetAP*) [Ben-Bassat et al., 1987; Huang et al., 1987; Miller et al., 1987; Moerschell et al., 1990]. Removal of methionine is an essential process; in certain cases, inhibition of MetAP inhibits cell growth [Chang et al., 1989, 1992; Li and Chang, 1995a; Griffith et al., 1997; Sin et al., 1997].

There are two forms of MetAP, designated as type 1 and type 2. Eukaryotes express both types of MetAP, while prokaryotes express only one. The significance of having two isoforms of MetAP is unclear, but the two isoforms have some known differences. Yeast MetAP1 and MetAP2 have slightly different substrate specificities in vivo [Chen et al., 2002]. The human isoforms have different substrate specificities in vitro [Turk et al., 1999]. Also, mammalian MetAP2 is a bi-functional protein, which, in addition to its MetAP activity, also has the ability to protect the alpha subunit of eukaryotic initiation factor 2 from phosphorylation, thus preventing inhibition of protein synthesis [Wu et al., 1993; Arfin et al., 1995; Li and Chang, 1995b, 1996]. In budding yeast, the only organism in which it has been properly tested, MetAP1 has been demonstrated to be the dominant isoform both in terms of in vivo protein processing and cell growth [Li and Chang, 1995a; Chen et al., 2002]. Thus, a deletion of the *MAP1* gene results in a significant slow growth phenotype, while loss of *MAP2* is largely innocuous.

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Human MetAP2 has been the subject of much investigation in recent years due to the ability of MetAP2 inhibitors to inhibit the growth of endothelial cells in culture, and inhibit angiogenesis both in vitro and in vivo [Kusaka et al., 1994; Yamamoto et al., 1994; Griffith et al., 1997; Sin et al., 1997]. Thus MetAP2 inhibitors are promising agents for the treatment of cancer. The extent to which the different isoforms of MetAP contribute to protein processing in mammalian cells has not been determined.

Methionine metabolism plays roles in many aspects of cellular physiology. The methionine metabolite *S*-adenosyl methionine (SAM) is the universal methyl donor in cells. Due to its capacity to be oxidized, it has been suggested that free methionine can act as a buffer against free radicals, and thus protect cells against oxidative stress [Moskovitz et al., 1997]. Metabolism of sulfur amino acids like methionine has been linked to cell cycle progression and cellular dysfunction [Outinen et al., 1999; Kaiser et al., 2000; Patton et al., 2000; Rouillon et al., 2000]. Additionally, methionine is a mediator of gene expression in budding yeast, specifically repressing genes involved in sulfur assimilation and methionine biosynthesis (*MET* genes) [Thomas and Surdin-Kerjan, 1997].

One of the sulfur salvage pathways in the cell is the recycling of methionine via its release from nascent polypeptides by the action of MetAP. The impact of this function on methionine metabolism has not been studied. We show that MetAP activity does play a measurable role in methionine metabolism, mainly by preventing the premature activation of genes involved in sulfur assimilation and methionine biosynthesis. Interestingly, *map1Δ* cells, which have the gene for MetAP1 deleted, are dramatically and specifically growth inhibited by methionine. This growth inhibition by methionine results from selective product inhibition of MetAP2. Selectivity for methionine inhibition of MetAP2

is conserved from yeast to humans and thus may play a role in the function of MetAP2. Additionally, the extent to which MetAP2 will be active in a particular cell type may be governed at least in part by the concentration of methionine.

MATERIALS AND METHODS

General Methods

Yeast transformation was performed by the lithium acetate procedure [Ito et al., 1983]. Protein assays were performed according to the method of Bradford [1976] using BSA as a standard. UV measurements were made on a DU-70 spectrophotometer.

Vectors and Strains

Yeast strains used in this study are listed in Table I. Strain C114 was a kind gift from Dr. Yolande Surdin-Kerjan. *E. coli* strain DH5 α (Stratagene, La Jolla, CA) was routinely used for plasmid propagation. Yeast strain W3031A, its derivative YHC001 (*map1::HIS3*), and plasmids pRS416-*yMAP1* (wild type yeast *MAP1* gene under the endogenous promoter), and pE1A (wild type yeast *MAP2* gene under the *ADH1* promoter) have been described previously [Li and Chang, 1995a; Klinkenberg et al., 1997; Vetro and Chang, 2002]. Plasmids pYCM4 and pYCM4-2, which contain the coding sequences for wild type Met4 and Met4-F156S mutant, respectively, were generous gifts from Dr. Fumihiko Omura. Plasmids pGAL-Met4-1 (wild type Met4 under *GAL1* promoter), pGAL-Met4 Δ ACT, pGAL-Met4 Δ bZIP, and pGAL-Met4 Δ INT were kind gifts from Dr. Dominique Thomas. Strain BD0010 (*map1::HIS3*) was created from strain C114 by single step transformation as described previously [Chang et al., 1992].

Strain BD1000, a *met4* knockout strain carrying plasmid pRS416-*yMAP1* was derived from strain YHC001 as follows. Two oligonucleotides

TABLE I. Yeast Strains

Strain	Genotype	Reference
W3031A	<i>MATa ade2-1 can1-100 ura3-1 leu2-3, 112 trp1-1 his3-11, 15</i>	Klinkenberg et al., 1997
YHC001	<i>MATa ade2-1 can1-100 ura3-1 leu2-3, 112 trp1-1 his3-11, 15 map1::HIS3</i>	Klinkenberg et al., 1997
C114	<i>MATa ura3 his3 leu2::MET25-lacZ::LEU2</i>	Thomas et al., 1989
BD0010	<i>MATa ura3 his3 leu2::MET25-lacZ::LEU2 map1::HIS3</i>	This study
BD1000	<i>MATa ade2-1 can1-100 ura3-1 leu2-3, 112 trp1-1 his3-11, 15 map1::HIS3 met4::LEU2 pRS416yMAP1</i>	This study
BD1001	<i>MATa ade2-1 can1-100 ura3-1 leu2-3, 112 trp1-1 his3-11, 15 map1::HIS3 met4::LEU2</i>	This study

(5'-GCGCTCTAGATCCTGATGCGGTATTTTCTCC-3' and 5'-GCGCTCTAGACGCGGTTTTCGGTGATGAC-3') were used to amplify the *LEU2* gene using pRS415 (Stratagene) as a template adding an *Xba*I site at each end. pYMC4 which contains the wild type *MET4* gene under the endogenous promoter was digested with *Xba*I to remove the *MET4* coding sequence while retaining some of the 5' and 3' gene flanking sequence. The *LEU2* fragment was then subcloned into *Xba*I-cut pYCM4, replacing the *MET4* ORF to generate pYMC4-*LEU2*. pYMC4-*LEU2* was then digested with *Bam*H1/*Sph*I to release the *LEU2* fragment containing endogenous *MET4* flanking sequences. This fragment was gel-purified and used to transform YHC001 pRS416-*yMAP1* cells. Potential *met4Δ* cells were selected first by growth on Leu⁻ plates. Positive clones were then tested for lack of growth on Met⁻ plates to verify the correct deletion. BD1001 cells lacking both the *MAP1* and *MET4* genes were created by selection of BD1000 cells on SD Leu⁻ + 5-FOA. Colonies were picked after several days and then streaked to new Leu⁻ 5-FOA plates. Afterwards cells were routinely grown in SD Leu⁻ media or YPD.

Yeast Growth

Single colonies were picked and grown in selective media to log phase. For generation of growth curves, cultures were diluted to an

OD_{600nm} of 0.1 and growth was continued aerobically at 30°C. At the indicated times, samples were withdrawn and OD_{600nm} was measured. Growth curves shown are representative. Experiments were performed at least three times and the deviation between results was never more than 10%. For plate assays, an equal number of cells were plated on the appropriate media. Plates were incubated at 30°C for 3–4 days and then photographed. To test compounds for growth inhibition or rescue of growth inhibition by methionine, cells were grown in SD minimal media containing candidate inhibitors (concentration was 5 mM unless otherwise indicated in Table II) with or without 5 mM methionine to late log phase. Cells were then diluted to an OD_{600nm} of 0.1 and growth was continued for 24–48 h. OD_{600nm} values were then compared to a reference culture with no inhibitor added to obtain a qualitative estimate of growth rate. For quantitation of methionine/cysteine growth inhibition, cells were grown in SD minimal media with or without inhibitor for at least 24 h, and then cultures were diluted to an OD_{600nm} of 0.025. Growth continued for 24 h (OD_{600nm} of 0.4 for cultures lacking inhibitor). OD_{600nm} was then determined for all cultures. Results are expressed as percentage of the OD_{600nm} of the reference cultures. Results are expressed as the mean ± standard error from three experiments.

TABLE II. Growth of *map1Δ* With Various Compounds

Nutrient added	Growth in minimal medium	Growth in medium +5 mM methionine
L-Arginine	+++	-
L-Asparagine	+++	-
L-Isoleucine	+++	+
L-Tryptophan	+++	-
L-Tyrosine (2.5 mM)	+++	-
L-Phenylalanine	+++	+
L-Lysine	+++	-
L-Histidine	+++	-
L-Serine	+++	-
L-Cysteine	-	-
L-Aspartate	+++	-
L-Glutamate	+++	-
L-Alanine	+++	-
L-Glutamine	+++	-
L-Glycine	+++	-
L-Proline	+++	-
L-Threonine	+++	-
DL-Homocysteine	++	-
S-adenosyl methionine	+++	-
Glutathione	+++	-
Guanine (0.1 mM)	+++	-
Adenine	+++	-
Uracil	+++	-

Semi-Quantitative RT-PCR

Cells were grown in minimal media to an OD_{600nm} of approximately 1.5. Aliquots were taken and then the cells were spun down, washed once with Met^- media, and then growth was continued in Met^- media for 60 min. Aliquots were withdrawn and total RNA was extracted from all samples using the Qiagen RNeasy kit. A portion of total RNA (3 μ g) was then digested with RQ1 DNase (Promega, Madison, WI), repurified, and resuspended at a final concentration of 0.1 mg/ml. From this reaction, 1 μ g of total RNA was then used for first strand cDNA synthesis with reverse transcriptase (RT) using an oligo dT₁₂₋₁₈ primer (Gibco/Invitrogen, Carlsbad, CA). A portion (10%) of the RT reactions was then used for PCR amplification with Taq polymerase using primers specific for *ACT1*, *MET16*, *MET25*, and *MET28*. Reactions were carried out under multiple cycling conditions to ensure that the product visualized was produced during the linear range of amplification. As a control, PCR reactions were carried out from RT reactions performed with the sense primer for *MET25*. No products were detected from these samples.

β -Galactosidase Assays

Strains C114 and BD0010, each containing an integrated *MET25-lacZ* allele, were grown in 5 ml cultures with varying concentrations of methionine to the indicated OD_{600nm} or to an OD_{600nm} of 1.0. For studies of *MET25* expression in different growth stages, media was prepared fresh and used as soon as possible thereafter. Cell extracts were prepared by vortexing the cells with glass beads in breaking buffer (20 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol) 5 times for 30 s each with 30 s intervals on ice. After vortexing, the supernatants were decanted and the beads were washed with breaking buffer. The combined supernatants were then microfuged for 5 min at 4°C. The extracts were diluted to 0.1 mg protein/ml and were then diluted 1:1 in 2 \times assay buffer (Promega) in microtiter plate wells. β -galactosidase activity was measured by kinetic analysis of ABS_{405nm} on a microplate reader (Molecular Devices, Sunnyvale, CA) at 30°C. Typically, the assay time was 30–60 min. The reaction rate was linear over the time course of the assay. Results are expressed as mean \pm standard error of three experiments.

MetAP Activity Assays

Recombinant human MetAP1 and two were expressed and purified in a baculovirus system as previously described [Turk et al., 1999]. Enzyme (1 μ M) was incubated in buffer H (10 mM Hepes pH 7.3, 0.1 mM NaCl, 0.1 mM Co^{++} , 10% glycerol) with the indicated concentration of methionine or vehicle, along with excess dipeptidyl peptidase IV (purified from bovine kidney) in microtiter plate wells for 5 min at room temperature. Reactions were initiated by the addition of 1 mM substrate (Met-Gly-Pro-pNA). Activity was then measured by monitoring Abs_{490nm} on a microplate reader continuously for 20 min at 37°C. The reaction rate was linear over the time course of the assay. Results are expressed as mean \pm standard error of three experiments.

Calculations for In Vivo Methionine Concentration

Calculations were based on cell volumes of 0.5 pl and 0.07 pl for endothelial and yeast cells, respectively [Hecker et al., 1990; Guthrie and Fink, 1991]. Values for the respective concentrations were either taken directly from the literature [Rincon and Benitez, 2001] or calculated from the given yield of methionine per cell number [Carmel and Jacobsen, 2001].

RESULTS

These experiments were initiated to test the hypothesis that the recycling of methionine by the activity of MetAP contributes significantly to methionine metabolism in *Saccharomyces cerevisiae*. Considering that yeast MetAP1 seems dominant both in terms of in vivo protein processing and cell growth, we examined the methionine biosynthetic gene expression in a *map1 Δ* strain and compared it to an isogenic wild type strain. A semi-quantitative RT-PCR assay indicated that the expression of *MET25*, *MET16*, and *MET28* was significantly increased in the *map1 Δ* strain when the cells were grown in minimal medium (Fig. 1). We tested whether this increased expression depended on the growth phase of the cells or the methionine concentration of the culture medium by measuring β -galactosidase activity from isogenic *map1 Δ* and wild type strains containing an integrated *MET25-lacZ* gene fusion. We observed a modest, but significant increase in β -galactosidase activity in the *map1 Δ* strain

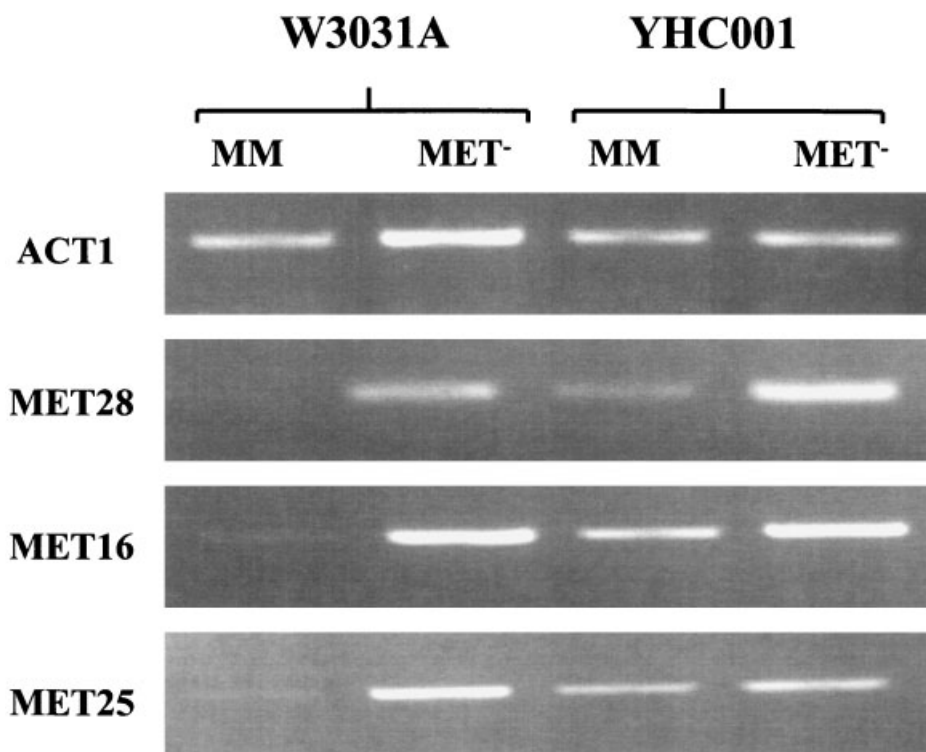


Fig. 1. Activation of *MET* genes in *map1Δ*. Semi-quantitative RT-PCR was performed to detect the expression of *MET16*, *MET25*, and *MET28*. Expression of *ACT1* serves as a positive control. Total RNA was used from wild type (W3031A) or *map1Δ* (YHC001) strains grown in either minimal media (MM) or media lacking methionine (MET^-).

under conditions where methionine was not limiting for wild type cells, namely at lower culture densities, or when methionine was available either at the concentration present in minimal media (0.1 mM) or in excess (5 mM) (Fig. 2A–C). When methionine was limiting, either at higher culture densities or during growth in the absence of methionine, β -galactosidase activity was substantially higher in both cell types. Importantly, elevated β -galactosidase activity in the *map1Δ* strain was rescued by overexpression of MetAP2 (Fig. 2D), which has been previously shown to largely compensate for loss of MetAP1 function [Chen et al., 2002]. This is the first demonstration that loss of MetAP activity has a significant measurable effect on methionine biosynthesis.

Considering that *map1Δ* cells do not seem to utilize methionine as efficiently as wild type cells, we evaluated whether the methionine levels in the media affected the growth of these cells. The absence of methionine did not affect the growth rate of *map1Δ* (data not shown). Interestingly, addition of excess methionine (5 mM) to the culture media caused a

specific, severe inhibition of *map1Δ* growth, such that the cells were barely viable (Fig. 3). These results were contrary to what we initially expected. In order to fully characterize the response of these cells to methionine we attempted to determine the mechanism of this growth inhibition.

When yeast are grown in the presence of a high concentration of methionine, the transcription of genes involved in sulfate assimilation and methionine biosynthesis are down-regulated [Thomas and Surdin-Kerjan, 1997]. One potential explanation for the observed growth inhibition by methionine was that one or more of the gene products involved in this pathway was required for normal growth in a *map1Δ* background. This hypothesis seemed reasonable, as some of these genes have been shown to be important for yeast survival under stress conditions [Jakubowski and Goldman, 1993].

To test whether methionine-mediated growth inhibition was caused by the down-regulation of *MET* genes, we evaluated whether there was a synthetic interaction between the *MAP1* and

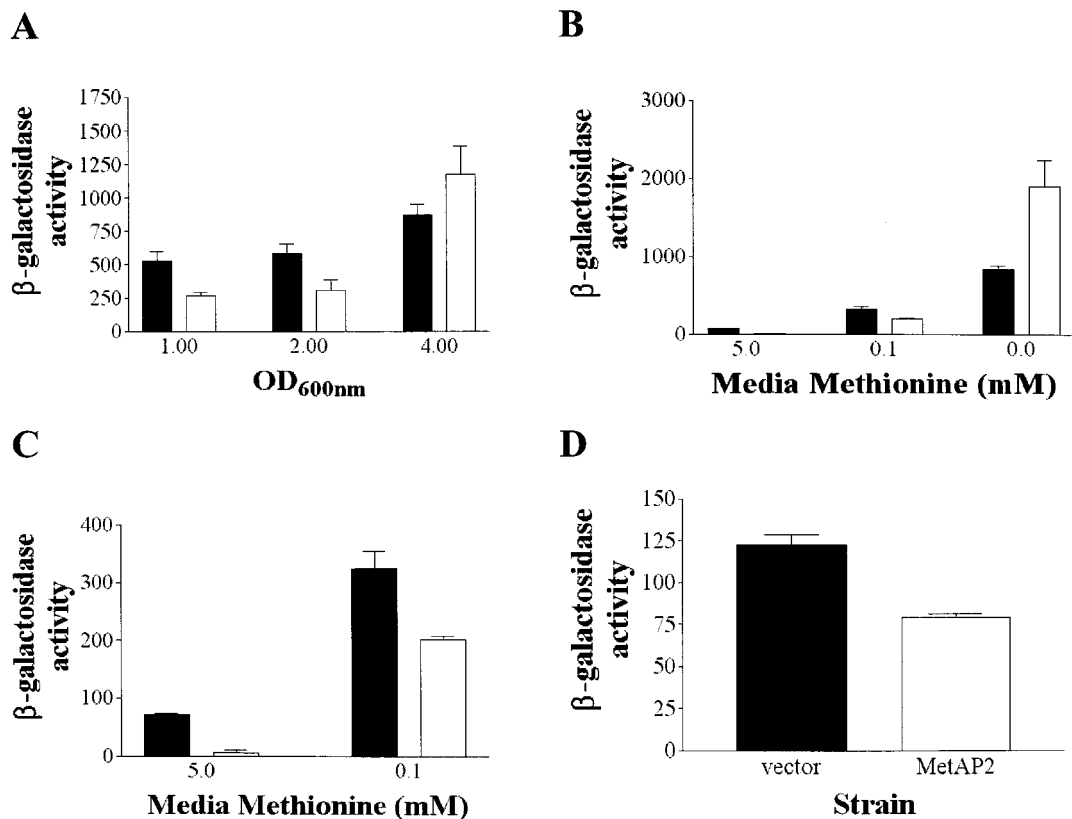


Fig. 2. Activation of *MET* genes in *map1Δ*. **A, B, C:** β -galactosidase activity was measured from *map1Δ* (filled bars) or wild type (open bars) cells expressing a *MET25-lacZ* gene fusion and grown under the indicated conditions. Figure 2C contains data from 2B shown at a different scale. **D:** β -galactosidase activity was measured from *map1Δ* strains overexpressing MetAP2 (open bar) or a vector control (filled bar). Activity is expressed as mOD/min/mg protein. Cells were grown in minimal media to an OD_{600nm} of 1.0.

MET4 genes. Met4 is the primary transcriptional activator of the genes involved in sulfur assimilation, and methionine-mediated repres-

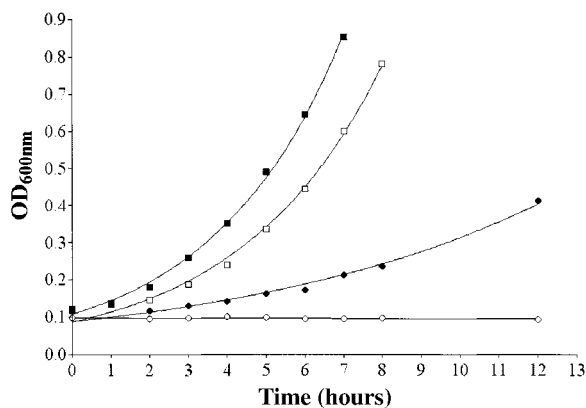


Fig. 3. Methionine-mediated growth inhibition of *map1Δ*. Shown is a representative growth curve. Symbol legends are as follows: (■) wild type, minimal media; (□) wild type, minimal media + 5 mM methionine; (●) *map1Δ*, minimal media; (○) *map1Δ*, minimal media + 5 mM methionine.

sion of these genes occurs principally through inactivation of Met4, most likely by proteolytic degradation [Kaiser et al., 2000; Rouillon et al., 2000; Kuras et al., 2002]. Thus, deletion of the *MET4* gene in a *map1Δ* background should mimic this physiological effect of methionine even under normal growth conditions.

We constructed isogenic strains containing single and double disruptions at the *MET4* and *MAP1* loci and compared the growth of the resultant strains to an isogenic wild type strain. As predicted, we observed a strong genetic interaction, resulting in an extreme slow-growth phenotype for the double knockout cells. This interaction was more pronounced in minimal media than in rich media (Fig. 4). However, several lines of evidence suggested that down-regulation of Met4 activity could not explain the methionine-mediated growth inhibition of *map1Δ*. First, expression of a dominant mutant of Met4, which is non-responsive to methionine inhibition [Omura et al., 1996],

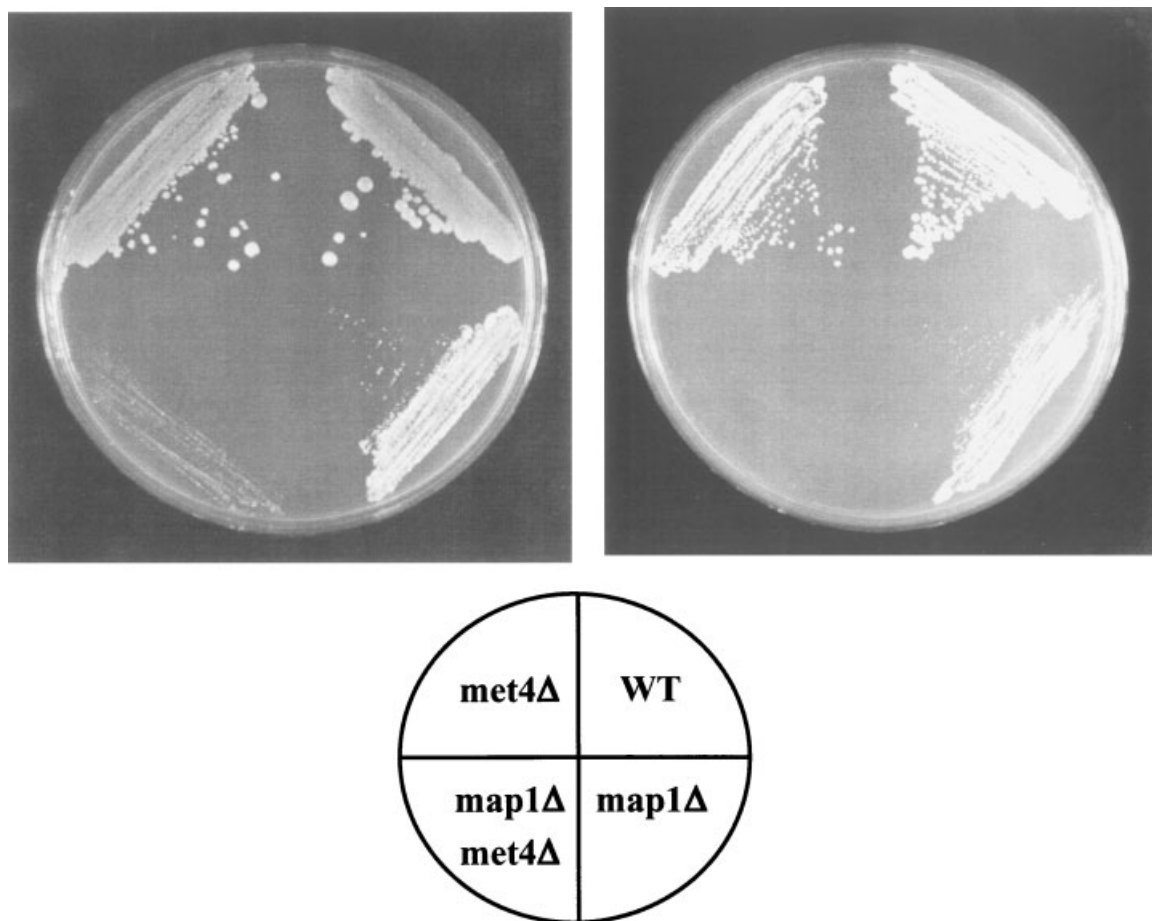


Fig. 4. Synthetic interaction between the *MET4* and *MAP1* genes. The indicated strains were plated on YPD (left) or minimal media (right).

was unable to overcome growth inhibition by methionine (data not shown). Secondly, addition of SAM, a methionine metabolite that is a more proximal mediator of *MET* gene repression than methionine [Thomas and Surdin-Kerjan, 1997], did not inhibit the growth of *map1Δ* (Table II). Considering these contrasting results, we directly evaluated whether the transcriptional activity of Met4 was important for the observed synthetic interaction with MetAP1. We tested this by attempting to rescue the slow growth phenotype of *map1Δ* with previously described Met4 mutants lacking the transcriptional activation domain of Met4, or lacking domains involved in interaction with cofactor DNA binding proteins [Patton et al., 2000]. Expression of any of these mutants complemented the slow growth of the double knockout strain as well as the wild type protein (Fig. 5). Thus, the *MAP1*–*MET4* interaction is indeed not dependent on transcriptional activa-

tion by Met4. Additionally, only very low levels of Met4 are required for complementation, as all of these Met4 variants expressed from the *GAL1* promoter could still complement when the cells were grown on glucose plates. Thus, methionine mediated growth inhibition of *map1Δ* does not depend on the negative regulation of *MET* genes by methionine.

Previously described cases of growth inhibition by methionine in fission and budding yeast often involved suppression of the synthesis of another metabolite, usually an amino acid or an adenine nucleotide [Meuris, 1969; Strauss, 1979; Hilti et al., 2000]. These defects could be overcome by adding the compound in question to the culture medium. In this case, growth inhibition could not be rescued by supplementation of the culture media with any of a number of compounds we tested, including the remaining amino acids, and several nucleotides; thus excess methionine does not interfere with the

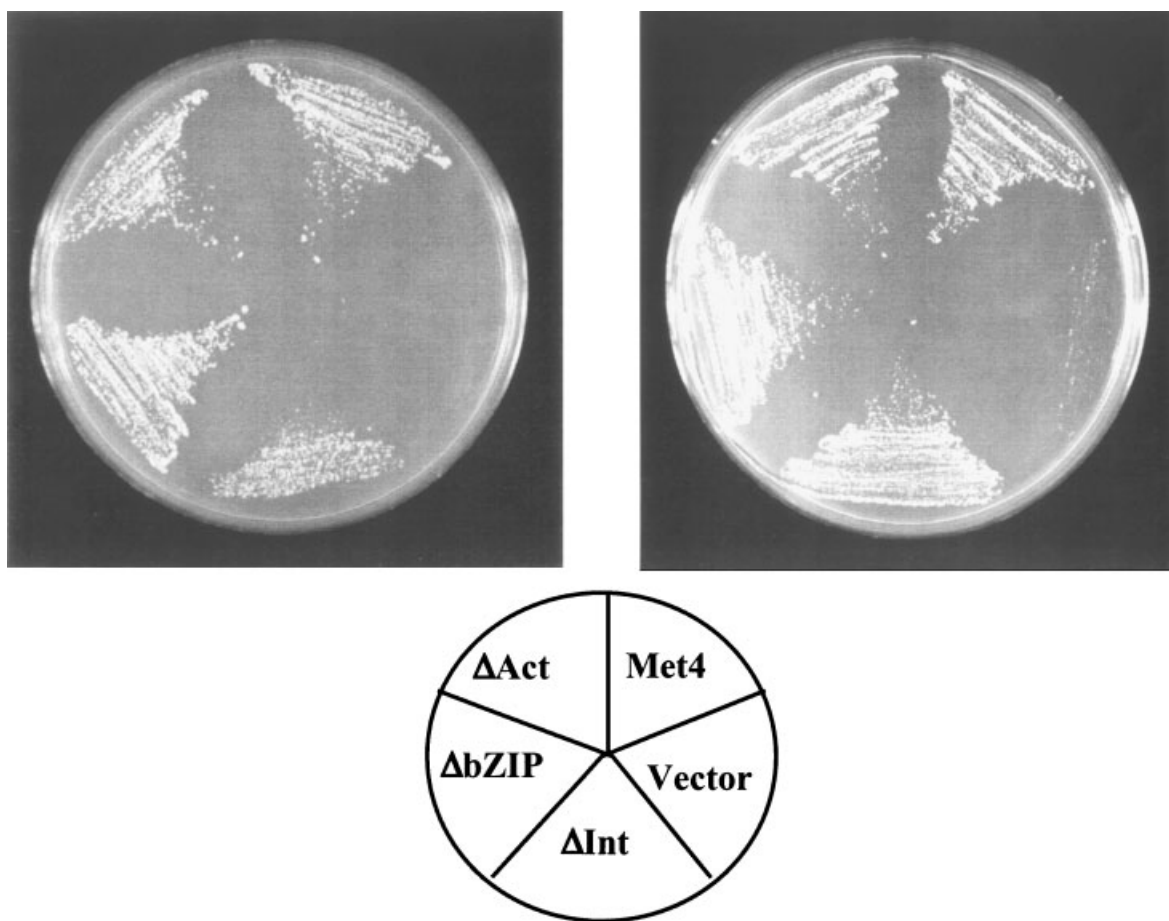


Fig. 5. Effect of Met4 transcriptional mutants on *MET4*–*MAP1* interaction. Double knockout yeast (*map1Δ*, *met4Δ*) were transformed with the indicated plasmids coding for either vector alone, wild type Met4 (Met4-1), Met4 lacking the activation domain (Δ Act), or Met4 lacking the domains involved in interaction with cofactor DNA binding proteins (Δ bZIP and Δ INT). Yeast were plated on galactose (**left**) or glucose (**right**).

synthesis of any of these compounds (Table II). We also tested glutathione in order to evaluate the growth inhibition potential of a compound that could be readily oxidized, and we observed no effect. Additionally, growth inhibition by methionine was specific. Of all the compounds we tested in addition to methionine, only cysteine and homocysteine could inhibit the growth of *map1Δ*.

Due to the fact that yeast, unlike human cells, can interconvert methionine, cysteine, and homocysteine [Thomas and Surdin-Kerjan, 1997], we thought that only one of these compounds was mediating the growth inhibition. Homocysteine had a significantly reduced potency, so we considered this an unlikely proximal mediator of growth inhibition. To determine whether growth inhibition was mediated by methionine or cysteine, we performed a dose

response analysis. Clearly, methionine was significantly more potent (Fig. 6). Thus, we concluded that methionine is the primary molecule that mediates this growth inhibition.

Since the growth inhibition was so specific for methionine, we next considered the possibility that methionine was inhibiting the activity of MetAP2. Methionine removal is essential, and thus the growth of *map1Δ* is dependent on MetAP2 function. However, for this explanation to be correct, the inhibition would have to be selective for MetAP2, as yeast cells clearly depend on MetAP1 for normal growth [Chang et al., 1992; Li and Chang, 1995a; Fig. 3], yet the growth of wild type yeast is only marginally affected by 5 mM methionine (Fig. 3).

We first tested the ability of methionine to inhibit the growth of *map1Δ* yeast, which were rescued by normal expression of MetAP1, as

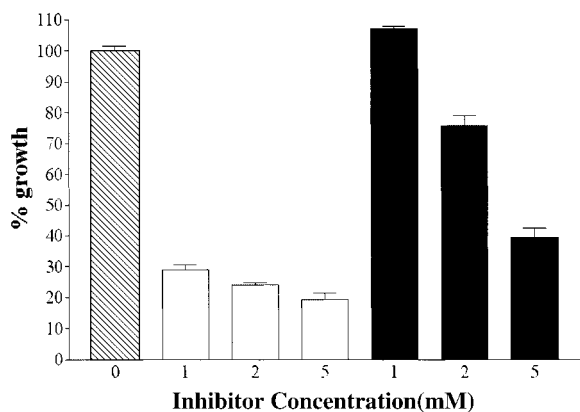


Fig. 6. Growth inhibition by methionine. Cells lacking MetAP1 were grown either in the absence of inhibitor (shaded bar), or in the indicated concentrations of methionine (open bars), or cysteine (black bars).

compared to overexpression of MetAP2. As expected, cells expressing MetAP1 were relatively insensitive to 5 mM methionine, while growth of the vector control was almost completely suppressed. Importantly, the growth of the strain rescued by overexpression of MetAP2 was still significantly impaired by 5 mM methionine (Fig. 7). Thus it seemed likely that methionine was inhibiting growth via product inhibition of MetAP2.

We tested this hypothesis directly using purified recombinant human MetAP1 and human MetAP2 in vitro. Methionine showed a fairly potent, selective inhibition of MetAP2 (Fig. 8). The IC_{50} for methionine inhibition of human MetAP2 was $\sim 150 \mu\text{M}$, while human

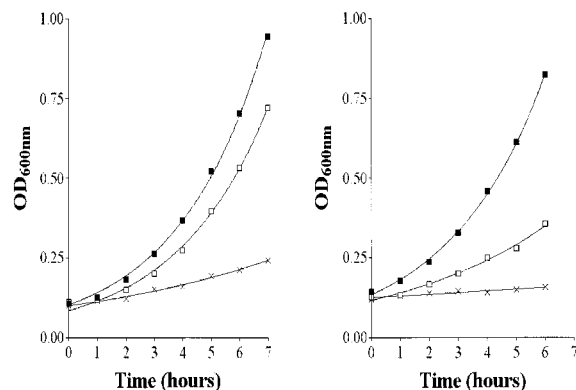


Fig. 7. Methionine sensitivity of strains rescued by overexpression of MetAP2. Shown are representative growth curves. Symbol legends are as follows: (■) *map1Δ*, pRS416yMAP1; (□) *map1Δ*, pE1A (*ADH1-MAP2*); (×) *map1Δ*, vector only. Cells were grown in SD media lacking uracil (left), or SD media lacking uracil + 5 mM methionine (right).

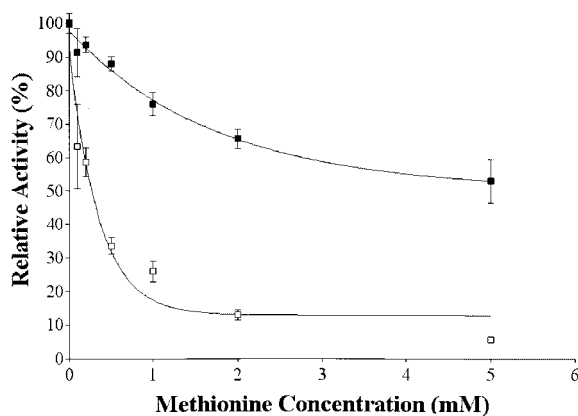


Fig. 8. Selective inhibition of human MetAP2 by methionine. The activity of purified human MetAP1 (■) and human MetAP2 (□) was assayed in vitro. The activity of each enzyme in the absence of inhibitor is set at 100%.

MetAP1 was still more than 50% active in the presence of 5 mM methionine. Thus, *map1Δ* growth inhibition by methionine results from selective product inhibition of MetAP2.

DISCUSSION

This report contains the first evidence that the recycling of methionine from nascent polypeptides catalyzed by MetAP plays a significant role in methionine metabolism, namely preventing the premature activation of *MET* genes. However, this role does not significantly affect the growth of the cells, as *map1Δ* cells grow as well in the absence of methionine as they do in minimal medium.

Interestingly, we observed that *map1Δ* cells are extremely sensitive to methionine, such that the addition of 5 mM methionine to the culture medium causes a drastic inhibition of cell growth. We determined that this effect results from selective product inhibition of MetAP2.

These observations raise the question of whether there is physiological significance to product inhibition of MetAP2. There is no obvious functional role for product inhibition of a MetAP by methionine. However, the observation that MetAP2 is specifically inhibited by methionine raises the question of whether MetAP2 and MetAP1, although sharing a common activity, have distinct functions, or function under different metabolic conditions. To this point, it is unclear why eukaryotic cells express both MetAP1 and MetAP2, whereas prokaryotes and eubacteria only express one or

the other. The fact that this selective inhibition is conserved from yeast to humans suggests that it may have functional importance. Whether this difference indeed points to a functional distinction between MetAP1 and MetAP2 remains to be determined.

Regardless of the functional implications of MetAP2 inhibition by methionine, this inhibition undoubtedly has physiological consequences. It has been established by our laboratory that MetAP1 is the dominant enzyme both in terms of in vivo protein processing and cell growth in yeast, and that MetAP2 seems to perform a largely redundant function [Chen et al., 2002]. Additionally, MetAP2 is somewhat compromised in its redundancy, as its ability to functionally substitute for MetAP1 requires overexpression [Li and Chang, 1995a; Chen et al., 2002; Fig. 7]. The concentration of free methionine in yeast cytosol seems to be on the order of 0.1–1 mM, depending on the growth conditions [Carmel and Jacobsen, 2001; Rincon and Benitez, 2001]. This concentration is near or above the IC₅₀ for human MetAP2 that we determined in vitro. Thus, these observations make sense, as we would expect that MetAP2 would be substantially inactivated under these conditions, and this inactivation may explain the reduced role for MetAP2 in yeast. Interestingly, in endothelial cells, the concentration of methionine has been measured in the range of 5–30 μM [Carmel and Jacobsen, 2001]. This reduced concentration of inhibitor may allow for an expanded role for MetAP2 in processing and cell growth in this cell type.

In the course of these experiments, we observed a synthetic interaction between the *MET4* and *MAP1* genes. Synthetic interactions can be viewed in two ways. One possibility is that a particular synthetic interaction is indicative of a functional relationship. Alternatively, a synthetic interaction may be the result of the additive effects of two milder phenotypes. We cannot yet ascribe a functional significance to the observed interaction between *MAP1* and *MET4* as this interaction does not seem to depend on the most well described function of Met4, namely the activation of *MET* genes. However, there are several reasons to believe that this interaction may have functional significance. Met4 is a large protein and is known to regulate genes other than *MET* genes [Kuras et al., 2002]. The mechanism(s) of Met4 regulation of alternative genes has not been explored

in detail, and thus we were unable to evaluate the impact of these functions on this genetic interaction. Additionally, deletion of Met4 alone has only a very modest effect on cell growth (Fig. 4), thus the defect of the double mutant is greater than what we would expect for simply the additive phenotypes of *map1Δ* and *met4Δ*. Whether this interaction points to a functional relationship between MetAP1 and Met4 has yet to be determined.

Clearly, MetAPs and methionine metabolism are linked in several ways, including recycling of methionine, product inhibition, and gene regulation. Understanding how these processes intertwine may lead to distinctions of function between MetAP1 and MetAP2 and clarify the roles for MetAPs in cellular growth and metabolism.

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