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ORIGINAL RESEARCH PAPER

Use of the cysteine-repressible *HpMET3* promoter as a novel tool to regulate gene expression in *Hansenula polymorpha*

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Abstract
Objectives The promoter of *HpMET3,* encoding an ATP sulfurylase, was evaluated for its potential as a repressible promoter to downregulate the expression of target genes in the thermotolerant, methylotrophic yeast *Hansenula polymorpha*.
Results The expression of *lacZ* under the control of the 0.6 kb *HpMET3* promoter was efficiently downregulated by cysteine, but not by methionine or sulfate. The *HpMET3* promoter was used to generate a conditional mutant of the *HpPMT2* gene encoding an *O*-mannosyltransferase, which is involved in post-translational protein modification. The addition of 0.5 mM cysteine adversely affected the growth of the conditional *HpMET3*(p)-*Hppmt2* mutant strain by downregulating transcription of *HpPMT2* to approx. 40 % of the normal levels, indicating that the *HpPMT2* gene is essential for cell viability. However, the *HpMET3* promoter was neither induced nor repressed in the heterologous host *Saccharomyces cerevisiae*.
Conclusion Our results reveal that the cysteine-repressible *HpMET3* promoter is a useful tool that downregulates the expression of various genes in *H. polymorpha.*

Keywords ATP sulfurylase · Cysteine · *Hansenula polymorpha* · *O*-Mannosyltransferase · *MET3* · Promoter · Regulation

Introduction

The transcription of a gene is initially controlled by its respective core promoter. Repressible promoters have been used to study the function of essential genes and to modulate the expression of target genes in biotechnological processes (Delic et al. 2013). In *Saccharomyces cerevisiae,* the promoters of *MET3*, *CUP1*, and *PHO5* have been used to downregulate various target genes. The *MET3* promoter is repressed by methionine (Mao et al. 2002), while the *CUP1* promoter is regulated by Cu^{2+} (Labbe and Thiele 1999), and the *PHO5* promoter is responsive to inorganic phosphate (Rudolph and Hinnen 1987). The *MET3* gene encodes ATP sulfurylase, the first enzyme involved in the sulfate assimilation and methionine biosynthetic pathway. It catalyzes the production of adenosine 5′-phosphosulfate (APS) from inorganic sulfate and ATP (Marzluf 1997; Thomas and Surdin-Kerjan 1997). APS is a high-energy molecule required for sulfate activation and reduction in the cell, and thus ATP sulfurylase plays a crucial role in the sulfur amino acid biosynthetic pathway when inorganic sulfate is used as a sole sulfur source (Ullrich et al.

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2001). In *S. cerevisiae*, *MET3* expression is repressed by exogenous methionine and *S*-adenosyl methionine (Cherest et al. 1985; Thomas et al. 1989). Due to its strictly controlled property, the *S. cerevisiae MET3* promoter has been widely used to generate conditional lethal strains for functional studies as well as to downregulate the expression of target genes for biotechnological processes (Mao et al. 2002; Asadollahi et al. 2008). The *MET3* promoters of other yeast species, such as *Ashbya gossypii, Candida albicans,* and *Pichia pastoris*, have been also evaluated as a useful molecular tool for regulated gene expression (Asadollahi et al. 2008; Care et al. 1999; Delic et al. 2013; Dunkler and Wendland 2007; Mao et al. 2002).

The thermotolerant, methylotrophic yeast *Hansenula polymorpha* is characterized by its high tolerance to various stressors, such as heavy metals, oxidants, xenobiotics, and environmental pollutants. It has attracted attention as an industrial yeast strain for various biotechnological applications, including heterologous protein production, bioconversion, environmental remediation, and glutathione production (Bartelsen et al. 2002). In addition, it has been extensively used as a model organism for investigating peroxisome biology and methanol metabolism (van Zutphen et al. 2010). The methanol-inducible promoters of the *MOX* and *FMD* genes, which encode alcohol oxidase and formate dehydrogenase, respectively, have been employed in the high-level expression of heterologous genes in *H. polymorpha* (Gellissen and Hollenberg 1997, 1999). The heat-inducible *TPS1* promoter (Amuel et al. 2000), the nitrogen-regulated promoters of nitrate and nitrite utilization genes (Brito et al. 1999), and the maltose- and sucrose-inducible, *MAL1* promoter that can also be repressed with glucose (Alamae et al. 2003), have all been used to express various genes in *H. polymorpha*. In the current study, we investigated the characteristics of the *HpMET3* promoter, and showed that it could be another tool that is suitable for modulating gene expression in *H. polymorpha*.

Materials and methods

Yeast strains, plasmids, primers, and culture media

The yeast strains used in this study are listed in Table 1. All plasmids and primers used in this study are listed in Supplementary Tables 1 and Table 2, respectively. Yeast cells were cultivated in YPD medium [1 % (w/v) yeast extract, 2 % (w/v) Bactopeptone, and 2 % (w/v) glucose], synthetic defined (SD) medium [0.67 % (w/v) yeast nitrogen base without amino acid and 2 % (w/v) glucose], or sulfur-free B-medium (synthetic medium with 2 % glucose without any sulfur source) (Cherest and Surdin-Kerjan 1992). Inorganic (sulfate; 40 mM final concentration) and organic (methionine, cysteine; 0.1, 0.5, or 1 mM) sulfur sources were added to SD or B-medium. Uracil (20 mg/ml), leucine (100 mg/ml), lysine (30 mg/ml), and histidine (20 mg/ml) were added to growth media according to the auxotrophic requirements of individual strains.

Construction of *MET3* promoter-based plasmids

To construct pDLM3P600-lacZ, the *H. polymorpha* expression vector for *lacZ* under the control of *HpMET3* promoter, a 0.6 kb DNA fragment corresponding to the promoter region of *HpMET3* was obtained by PCR amplification with primers MET3p600_3F and MET3p2B_RNC from the genomic DNA of the DL1-L strain and digested with *Cla*I and *Sal*I. The *HpMET3* promoter fragment was inserted into the *Cla*I/*Sal*I-digested pDLMOX-yEGFPm expression vector, which contains the selectable marker *HpLEU2*, to generate pDLM3P600. The *Eco*RI/*Nsi*I fragment of *lacZ*, encoding β-galactosidase, generated from pZAM522, was introduced into pDLM3P600, yielding pDLM3P600-lacZ. To construct the pDLMP600-PMT2 plasmid, the *H. polymorpha* expression vector for *PMT2* under the control of *HpMET3* promoter, the 0.7 kb *HpPMT2* fragment was amplified by PCR using primers Hp PMT2_11F_RI and HpPMT2_12B_StuI, and cloned between the *Eco*RI and *Stu*I sites of pDLM3P600.

To generate P_{ScMET3}-ScERG7(L), a 0.6 kb DNA fragment corresponding to the promoter of *ScMET3* and a 0.8 kb fragment of the *ScERG7* gene were amplified from the genomic DNA of the BY4742 strain. We used the primers SspI BglII pScMET3 1F and pScMET3 ATG EcoRI 2B to amplify the 0.6 kb *ScMET3* promoter, and primers EcoRI ScERG7 1F and ScERG7 SalI 2B to amplify the 0.8 kb *ScERG7* fragment. The amplified *ScMET3* promoter fragment was digested with *Bgl*II and *Eco*RI, while the *ScERG7* fragment was digested *Eco*RI and *Sal*I. The *ScMET3* promoter and *ScERG7* fragments were then inserted between the *Bam*HI and *Sal*I sites of

Table 1 Yeast strains used in this study

Strain name	Genotype	Reference
H. polymorpha		
DL1-L	*leu2*	(Kang et al. 2002)
DL1-L + L	*leu2*, pTHpLEU2-NS(c)	This study
HpMET3(p)	*leu2*Δ/pDLM3	This study
HpMET3(p)-*lacZ*	*leu2*Δ/pDLM3P600-lacZ	This study
ScMET3(p)-*lacZ*	*leu2*Δ/pSM3P600-lacZ	This study
HpMET3(p)-*Hppmt2*	*leu2* P_{PMT2}Δ*::LEU2*-P_{HpMET3}	This study
S. cerevisiae		
BY4742	*MATα his3* Δ*l eu2*Δ *ura3*Δ *lys2*Δ	Open Biosystems
ScMET3(p)	*MATα his3* Δ*l eu2*Δ *ura3*Δ *lys2*ΔYCpU-SM3P600	This study
ScMET3(p)-*lacZ*	*MATα his3* Δ*l eu2*Δ *ura3*Δ *lys2*ΔYCpU-SM3P600-lacZ	This study
ScMET3(p)-*ERG7*	BY4742, P_{ERG7}Δ::*LEU2*-P_{ScMET3}	This study
HpMET3(p)-*ERG7*	BY4742, P_{ERG7}Δ::*LEU2*-P_{HpMET3}	This study

pBluescript SK(+), to generate pBluSKP-P_{ScMET3}-ScERG7. The *Sma*I/*Hpa*I fragment containing the *ScLEU2* gene was generated from YEp351GAPII and ligated into *Nae*I-digested pBluSKP-P_{ScMET3}-ScERG7, yielding P_{ScMET3}-ScERG7(L). Using the same strategy, plasmid P_{HpMET3}-ScERG7(L) was constructed; however, the 0.6 kb *HpMET3* promoter obtained from pDLM3P600 via digestion with *Bam*HI and *Eco*RI, was used instead of the *ScMET3* promoter. To construct YCpU-SM3P600-lacZ, a *S. cerevisiae* expression vector for *lacZ* under the control of the *ScMET3* promoter, the *Bgl*II/*Eco*RI-digested 0.6 kb *ScMET3* promoter obtained from P_{ScMET3}-ScERG7(L) and the *Eco*RI/*Nsi*I-digested *lacZ* fragment from pDLM3P600-lacZ were ligated with the *Bam*HI/*Nsi*I-digested YCpU, a *S. cerevisiae* centromeric plasmid containing the *URA3* marker, yielding YCpU-SM3P600-lacZ.

Generation of *MET3* promoter-based conditional mutants

To generate *HpMET3*(p)-*Hppmt2* strains in which the native *PMT2* promoter was replaced with the *HpMET3* promoter, the *H. polymorpha* wild-type (WT) DL1-L strain was transformed with the *Cla*I-linearized pDLMP600-PMT2. Transformants were selected on synthetic complete medium lacking leucine (SC-LEU). Replacement of the *HpMET3* promoter with the native *HpPMT2* promoter was confirmed by PCR using primers MET3p600_3F and HpPMT2_2B_AscI_50. For the *HpMET3*(p)-*ERG7* and *ScMET3*(p)-*ERG7* strains, the *S. cerevisiae* BY4742 strain was transformed with an *Nsi*I-linearized P_{ScMET3}-ScERG7(L) and P_{HpMET3}-ScERG7(L) to replace the native *ERG7* promoter with the promoters of *ScMET3* or *HpMET3*, respectively. The correct replacement of the *ScMET3* promoter and the *HpMET3* promoter was confirmed by PCR using specific primer pairs (pSM3-SE7integ5F and pSM3-SE7integ4B, and MET3p600_3F and pSM3-SE7integ2B, respectively).

β-Galactosidase activity assay

Yeast cells were harvested and cell lysates were assayed for β-galactosidase activity. The assay was carried out as previously described (Rose and Botstein 1983) and the units of β-galactosidase activity were calculated according to Miller units (Miller 1972): 1 unit 1000 × A_{420}/time (min) × volume (ml) × A_{600}. The control is the transformants bearing the backbone vector without *lacZ* gene.

Quantitative PCR (qPCR) analysis

S. cerevisiae was grown at 30 °C and *H. polymorpha* at 37 °C in SD medium to the early growth phase (OD_{600} = 0.3). The medium was then changed to SD medium without any supplementation or SD medium supplemented with methionine or cysteine. After incubation for 2 or 3 h, cells were harvested and total RNA was isolated using the hot phenol extraction method (Chen et al. 2003). Total RNA was treated

with DNase I (Fermentas), and cDNA synthesized from 1 μg total RNA using RnaUsScript reverse transcriptase (LeGene Biosciences) and oligo(dT) primers. The resulting cDNA samples were used as templates in qPCR assay. Each amplification reaction contained 10 μl SYBR *Premix Ex Taq* (Takara) and 10 pmol of each forward and reverse oligonucleotide primer (Supplementary Table 2).

Results and discussion

Tight repression of the *HpMET3* promoter by cysteine

We previously observed that the expression of a set of *H. polymorpha* genes involved in sulfur metabolic pathway, including the *HpMET3* gene, was significantly repressed by exogenous cysteine, but only moderately repressed by methionine (Sohn et al. 2014). *In silico* analysis of the *HpMET3* promoter revealed that the structure of the *HpMET3* promoter is distinguishable from that of the *ScMET3* promoter, with respect to the number and the location of binding sites for transcription factors. Some of these transcription factors included Cbf1p, Met31p/Met32p, Gcn4p, Msn2p/Msn4p, and Yap1p (Fig. 1a). The *ScMET3* promoter contains three biding sites for Cbf1p, one for Met31p/Met32p, four for Gcn4p, one for Msn2p/Msn4p, and three for Yap1p. In contrast, the *HpMET3* promoter contains a single Cbf1 binding site and four Gcn4p binding sites. The organization of these two promoters differs with respect to the Cbf1p and Met31p/Met32p binding sites, which interact with the sole transcriptional activator of sulfur metabolism, Met4p, and recruit the Met4p transcriptional complex to target promoters (Thomas and Surdin-Kerjan 1997).

Serial deletion analysis of the *HpMET3* promoter fused to *lacZ* revealed that the 0.6 kb fragment of the *HpMET3* promoter, containing the Cbf1 and Gcn4p binding sites, retains its regulation pattern to be strongly repressed by cysteine (Supplementary Fig. 1A). The repression of the 0.6 kb *HpMET3* promoter by cysteine was shown to be concentration-dependent (Supplementary Fig. 1B). The *HpMET3* promoter exhibited a gradual repression by the increase of cysteine concentration. In particular, the promoter activity appeared to be completely repressed by 0.1 mM cysteine, indicating that 0.1 mM might be the minimal dose of cysteine required to repress efficiently the *HpMET3* promoter.

Transformants of *H. polymorpha* expressing β-galactosidase under the control of the 0.6 kb *HpMET3* promoter were cultivated in sulfur-free B medium without any supplementation and B medium supplemented with methionine, cysteine, or sulfate. The β-galactosidase activity of transformants cultured in the absence of a sulfur source was approx. 13-fold reduced by addition of 0.1 mM cysteine, but only threefold deceased by addition of 0.1 mM methionine (Fig. 1b). When we supplemented the culture medium with both methionine and cysteine, β-galactosidase activity decreased to levels similar to those observed when only cysteine was added to the medium. The addition of sulfate, an inorganic sulfur source, had little influence on the *HpMET3* promoter activity.

Our results show that the expression of β-galactosidase, which is driven by the *HpMET3* promoter, was almost completely repressed by low levels of cysteine, indicating that cysteine is central to downregulating the activity of the *HpMET3* promoter in *H. polymorpha*. This is a unique feature of *H. polymorpha MET3* promoter, considering that the *S. cerevisiae MET3* promoter is reported to be more efficiently repressed by methionine rather than by cysteine (Mao et al. 2002). We also analyzed *S. cerevisiae* transformants harboring *ScMET3*(p)-*lacZ* expression vector and confirmed the more tight repression of *S. cerevisiae MET3* promoter by methionine (Fig. 1c).

A conditional lethal mutant of *HpPMT2* with the *HpMET3* promoter

Given that the *HpMET3* promoter could be repressed with cysteine, we placed an essential gene under the control of this promoter to analyze its feasibility as a tool for generating a conditional lethal mutant. We selected the *HpPMT2* gene because it is thought to be essential for cell viability in *H. polymorpha* (Kim et al. 2013). We replaced the native promoter of *HpPMT2* with the *HpMET3* promoter and observed the growth of two independent conditional *Hppmt2* mutant strains under various conditions (Fig. 2a, b). The growth of the conditional *Hppmt2* mutant strains was severely retarded in the presence of 1 mM cysteine, indicating that *HpPMT2* is essential for cell

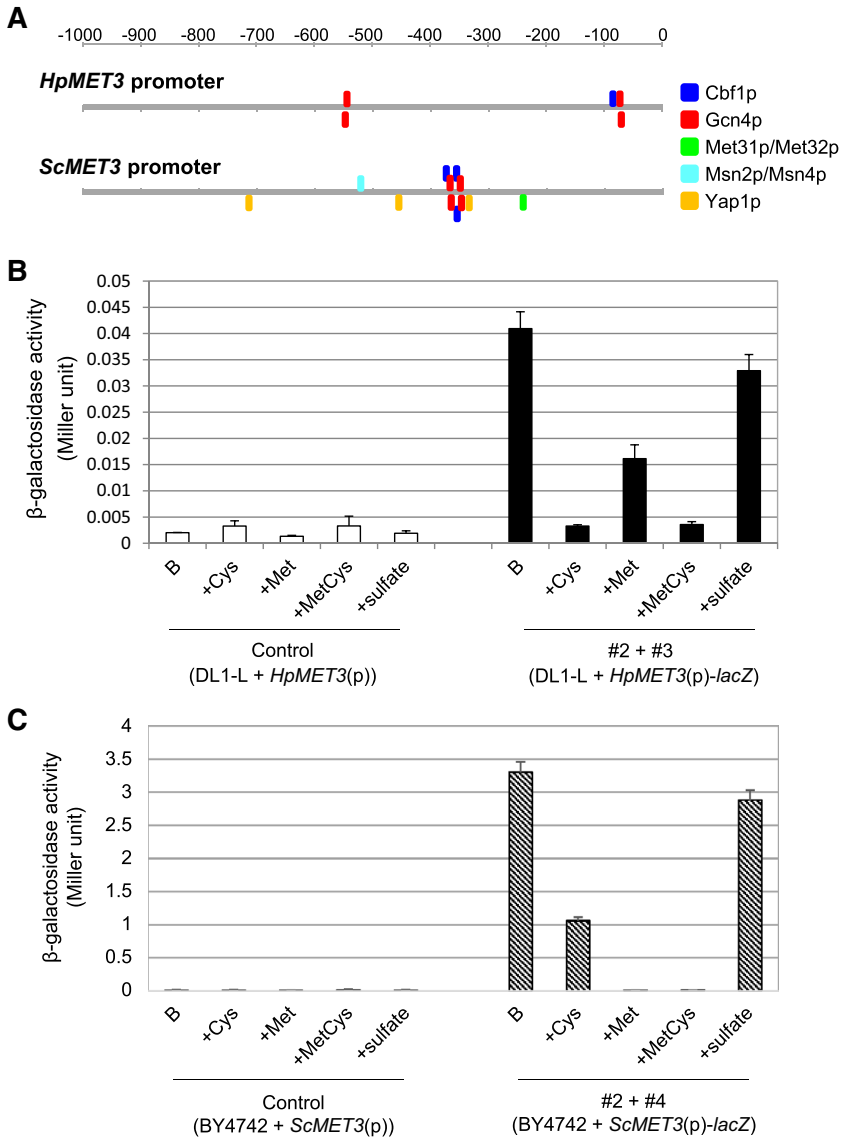

Fig. 1 ***HpMET3*** and *ScMET3* promoter analysis. **a** In silico analysis of the *HpMET3* and *ScMET3* promoters. Transcription factor binding sites were identified using RSAT (http://fungi.rsat.eu/) and Yeastract (http://www.yeastract.com/). **b** Activity analysis of the *H. polymorpha MET3* promoter fused to *lacZ* in the presence of different sulfur sources. Two *H. polymorpha* transformants (#2 and #3) containing the *HpMET3*(p)-*lacZ* expression vector were individually grown in sulfur-free B medium (B), with 0.1 mM cysteine (Cys), 0.1 mM methionine (Met), cysteine and methionine (MetCys) or 40 mM ammonium sulfate (sulfate). (C) Activity analysis of the *S. cerevisiae MET3* promoter fused to *lacZ* in *S. cerevisiae* in the presence of different sulfur sources. Two *S. cerevisiae* transformants (#2 and #4) containing the *ScMET3*(p)-*lacZ* expression vector were individually grown in sulfur-free B medium (B), with 0.1 mM cysteine (Cys), 0.1 mM methionine (Met), cysteine and methionine (MetCys) or 40 mM ammonium sulfate (sulfate), respectively. The β-galactosidase assay was carried out separately with each of transformants and the β-galactosidase activity presented is the average value from triplicate assays. The *error bars* represent the standard deviation from triplicate measurements

growth. We determined the transcript levels of *HpPMT2* in the conditional mutant strains that were cultured in the presence of cysteine, methionine, or cysteine and methionine. In the absence of sulfur compounds, expression levels of *HpPMT2* mRNAs driven by the *HpMET3* promoter were fivefold higher

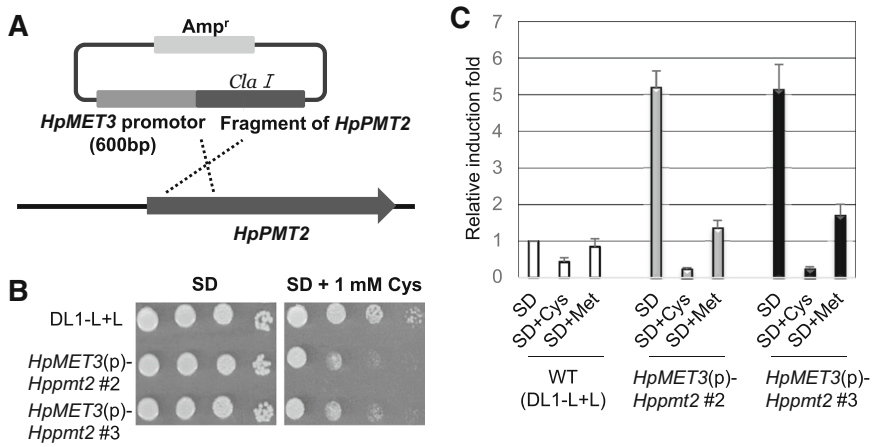

Fig. 2 Growth phenotype of the conditional *HpMET3*(p)-*HpPMT2* mutant strains. **a** Scheme of the conditional *HpPMT2* mutant strain under the control of the *HpMET3* promoter. **b** Ten-fold serial dilutions of wild-type (WT) *H. polymorpha* and conditional *HpPMT2* mutant strains were spotted onto SD medium with and without cysteine (1 mM Cys). **c** We used quantitative PCR assays to analyze the transcriptional regulation of *HpPMT2* driven by the *HpMET3* promoter. WT and conditional *HpPMT2* mutant cells were grown in SD medium with 0.5 mM cysteine (Cys), 1 mM methionine (Met), 0.5 mM cysteine and 1 mM methionine. Cells were harvested, and total RNA extracted and then subjected to qPCR analysis. Expression levels of *HpPMT2* transcripts were normalized to β-actin mRNA levels. Induced expression levels are presented as relative ratios to the expression levels of the WT cultivated in SD. The *error bars* represent the standard deviation from triplicate measurements

than those driven by the native *HpPMT2* promoter (Fig. 2c). In the presence of 0.5 mM cysteine, expression levels of *HpPMT2* mRNAs driven by the *HpMET3* promoter were decreased to around half of those driven by the native *HpPMT2* promoter. The addition of methionine had little effect on mRNA expression levels. Considering that only a 50 % decrease in *HpPMT2* expression was achieved by repressing the *HpMET3* promoter, compared with *HpPMT2* expression directed by its native promoter, the severe growth defects observed for the *HpMET3*(p)-*Hppmt2* mutant strains are strong indicators of the vital function of *HpPMT2* for cell viability.

We examined the cellular morphology of *HpMET3*(p)-*Hppmt2* mutants, to determine the physiological status of *H. polymorpha* when functional HpPmt2p was depleted. Confocal microscopy revealed that wild-type (WT) cells exhibited normal cell morphology with circular shape and smooth surface when cultured in the presence of 1 mM cysteine. In contrast, a high proportion of the *HpMET3*(p)-*Hppmt2* mutants showed rough and crumpled cell surface, with some cells having lysed (Fig. 3, right panel, indicated by arrows); thereby, reflecting defects in the maintenance of cell integrity. To examine the distribution of chitin in the cell wall, cells were stained with Calcofluor white. The cell wall along the rim was clearly seen for WT cells. However, *HpMET3*(p)-*Hppmt2* mutant cells were stained at the cell wall along the rim but also within enlarged vacuoles (Fig. 3, left panel).

These results indicate that loss of HpPmt2p function, which is involved in the *O*-mannosylation of proteins, resulted in major defects in cell wall integrity and the possible occurrence of adverse events during cell division. Taken together, these results strongly support the potential of the *HpMET3* promoter as a useful tool in generating conditional lethal mutant strains to obtain information regarding the physiological functions of an essential gene.

Incompatibility of the *HpMET3* promoter in *S. cerevisiae*

To determine whether the *HpMET3* promoter could be used as a repressible promoter in the heterologous host *S. cerevisiae*, we generated *S. cerevisiae* strains in which the *ERG7* gene encoding lanosterol synthase, an essential enzyme involved in ergosterol biosynthesis (Karst and Lacroute 1977; Shi et al. 1994), was placed under the control of the *HpMET3* promoter or *ScMET3* promoter. Transformants were cultured in SD medium in the presence or absence of cysteine or methionine.

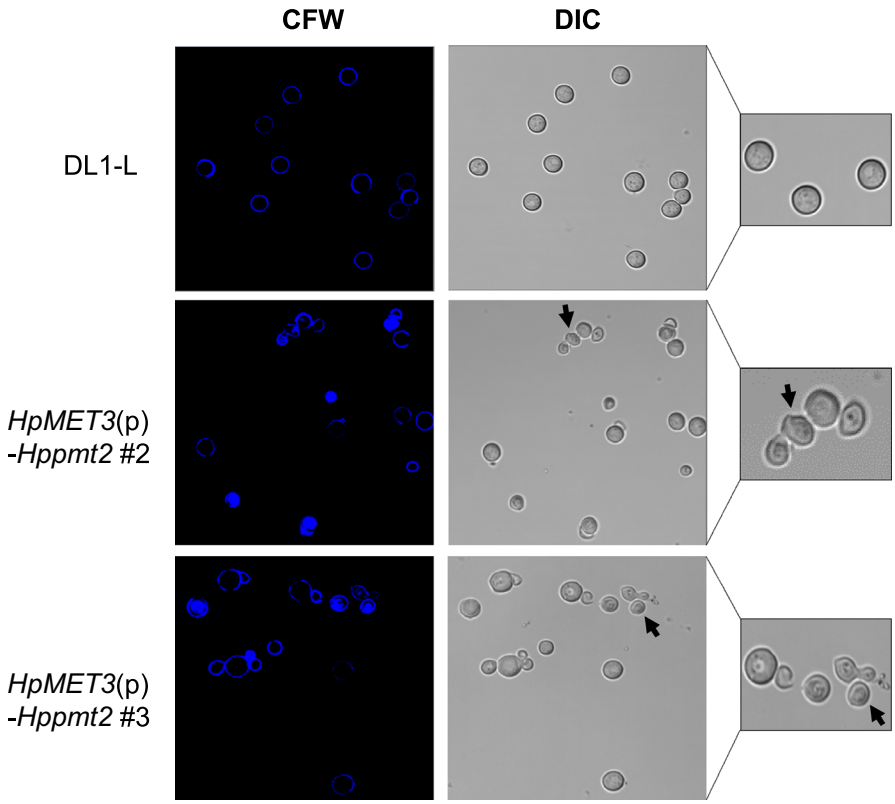

Fig. 3 Microscopy analysis of conditional *HpMET3*(p)-*HpPMT2* mutant strains Representative confocal microscopy images of the wild-type DL1-L strain of *H. polymorpha* and two conditional *HpMET3*(p)-*HpPMT2* mutants (#2 and #3). Cultures were grown in synthetic defined (SD) medium with 1 mM cysteine. Cells were stained with Calcofluor white (*blue*), adjusted to 2×10^8/ml and fixed with 3.7 % (v/v) formaldehyde in a rotator for 15 min at room temperature. After washing with phosphate-buffered saline (PBS) buffer (pH 7.4) three times, cells were stained with fluorescent brightener 28 (Calcofluor white, CFW; Sigma Aldrich) at 2 mg/ml in a rotator for 5 min and washed with PBS buffer (pH 7.4). *Inset* pictures on the right panel show yeast cells at a higher magnification. Phase differential interference contrast (DIC) and fluorescence microscopy pictures (CFW) were taken using a Zeiss LSM700 confocal equipped with Axio Observer. Images were processed by ZEN2011 software (Zeiss). Magnification = approx. x 1000

Transformants were also cultured in rich YPD medium to determine the basal transcript levels of *ERG7* driven by the two *MET3* promoters under sulfur-rich condition. In the absence of any sulfur-containing amino acids, *ERG7* expression levels driven by the *ScMET3* promoter were sixfold higher than those driven by the native promoter. Expression levels of *S. cerevisiae ERG7* under the control of the *ScMET3* promoter were downregulated to about 30 % of endogenous expression levels following the addition of methionine; *ERG7* expression levels were barely reduced when cysteine was added, as indicated in the expression of *lacZ* under the control of the *ScMET3* promoter (Fig. 1c). In contrast, the *HpMET3* promoter failed to drive induced expression of *ERG7*. The *HpMET3* promoter did not appear to downregulate expression of *ERG7* upon the addition of methionine or cysteine (Fig. 4).

The incompatibility of *HpMET3* promoter in *S. cerevisiae* could be attributed to the notable differences in promoter features between *S. cerevisiae* and *H. polymorpha* (Fig. 1a). Quite interestingly, we also observed no induced activity of the *ScMET3* promoter in *H. polymorpha* under sulfur-limited condition, supporting incompatibility of these two yeast *MET3* promoters (Supplementary Fig. 2). The *H. polymorpha MET3* promoter is unique, in that it is more efficiently regulated by cysteine than methionine.

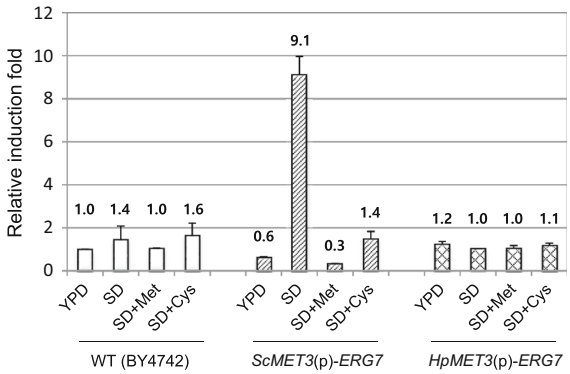

Fig. 4 *ScERG7* expression under the control of the *ScMET3* or *HpMET3* promoters in *S. cerevisiae* Wild-type *S. cerevisiae* BY4742, *ScMET3*(p)- and *HpMET3*(p)-*ScERG7* integrated strains were grown in YPD or synthetic defined (SD) medium supplemented with 1 mM cysteine (Cys) or 1 mM methionine (Met). Expression levels of *ScERG7* transcripts were determined by qPCR and normalized to β-actin mRNA levels. Induced expression levels are presented as relative ratios to the expression levels of *ScERG7* mRNAs driven by the native promoter on YPD medium. The error bars represent the standard deviation from triplicate measurements

Most studies regarding *MET3* promoter-based regulation in *S. cerevisiae*, *C. albicans* and *A. gossypii* have demonstrated the preferential use of methionine as an effective repressor (Thomas and Surdin-Kerjan 1997).

Conclusion

The cysteine-repressible *HpMET3* promoter can be used to downregulate expression of target genes in *H. polymorpha*. The *HpMET3* promoter can be efficiently repressed by low levels (0.1 mM) of cysteine, but is barely modulated by methionine in *H. polymorpha*. These findings are in contrast to those for the methionine-repressible *MET3* promoters in other yeast species. The *HpMET3* promoter can be exploited to generate conditional lethal mutants of an essential gene, allowing for the study of its physiological function. In addition, this promoter could also be used to investigate downregulation of target genes in metabolic pathways, thereby manipulating the metabolic flux in *H. polymorpha*.

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Supporting information Supplementary Table 1—Plasmids used in this study.

Supplementary Table 2—Oligonucleotide primers used in this study.

Supplementary Figure 1—Analysis of repressible activity of the HpMET3 promoter by cysteine.

Supplementary Figure 2—Activity analysis of the ScMET3 promoter in *H. polymorpha*.

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