CURRENT MICROBIOLOGY Vol. 45 (2002), pp. 37–40
DOI: 10.1007/s00284-001-0046-0

Current Microbiology
An International Journal

MET3 Promoter: A Tightly Regulated Promoter and Its Application in Construction of Conditional Lethal Strain

Xicheng Mao, Yafang Hu, Chao Liang, Changde Lu

Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, The Chinese Academy of Sciences, Shanghai 200031, China

Received: 20 September 2001 / Accepted: 22 October 2001

Abstract. The β-galactosidase activity driven by MET3 promoter was assayed in the absence of methionine and in the presence of different concentration of methionine in medium. To compare its activity with GAL1 promoter and the data reported by Mumburg about MET25 promoter, the MET3 promoter was a weak but tightly controlled promoter. Its successful application in the construction of methionine-sensitive tri-mutant (*cln1*Δ*cln2*Δ*pho85*Δ) demonstrated that the MET3 promoter is a useful promoter in construction of conditional lethal strain and heterologous expression in *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae has become a popular model system for studying molecular biology of the eukaryotic cell and conditional lethal mutation of yeast has been a widely used tool to isolate functional homolog from mammalian cells by complementation. All these analyses need the ectopic and regulated expression of gene under the control of regulatable promoter. In budding yeast, several promoters of this kind have been applied in heterologous expression. They are GAL1-10 promoter, PHO5 promoter [1] and the regulatory promoter in methionine metabolism pathway, MET3 promoter, and MET25 promoter. Among these regulatable promoters, GAL1-10 promoter is the most widely used promoter for its complete repression by glucose in medium and massive induction when cell grown in medium containing galactose as the sole source of carbon [1].

The *Saccharomyces cerevisiae* MET3 gene encodes ATP sulphurylase, an enzyme in the methionine biosynthetic pathway. Like the MET25 gene encoding O-acethyl homoserine sulphydrylase, its physiological expression is repressed by the methionine in the medium [3]. There have been some reports about the use of the MET3 and MET25 promoters for regulatory expression [2, 5, 10]. Mumberg et al. have measured the MET25 promoter activity with the use of β-galactosidase as reporter gene and constructed series of vectors containing MET25 promoter for the regulated expression of heterologous protein at various levels [8], while there is still no data about the MET3 promoter activity and its kinetics of repression by methionine.

In this paper, we measured the activity of MET3 promoter with the β-galactosidase as reporter gene and compared it with GAL1 promoter at the same time and successfully used it in the construction of conditional lethal strain with deletion of genes CLN1, CLN2, and PHO85. The results present some special character of MET3 promoter and its promising application potential.

Material and Methods

Strain and medium. The *Saccharomyces cerevisiae* strain YPH499 used in this work was a gift from Prof. Shi-zhou Ao. YPH620 was derived from YPH499 with disruption of CLN1 and PHO85 gene, which was constructed in our previous work [6]. *Escherichia coli* strain XL1-Blue was used for plasmid construct and amplification.

The classical medium for yeast and *E. coli* were as described [1, 9].

PCR amplification of MET3 promoter. With the synthetic forward primer 5′TTGGTACCTTTAGTACTAACAGAGAC3′, the reward primer 5′TTGGATCCTGTTAATTATACTTTATTCTTG3′ and the YPH499 genomic DNA as template, the MET3 promoter sequence -1 to -490 was amplified [3] and cloned into T-EASY vector (Promega).

Plasmids construction. The MET3 promoter was inserted into pβ-gal basic plasmid to form a pMET3-LacZ expression cassette, which was cloned into yeast shuttle vector with 2μ replication sequence or ARS and CEN4 element respectively. The multi copy plasmid carrying

Correspondence to: Changde Lu; *email:* lucd@sunm.shcnc.ac.cn

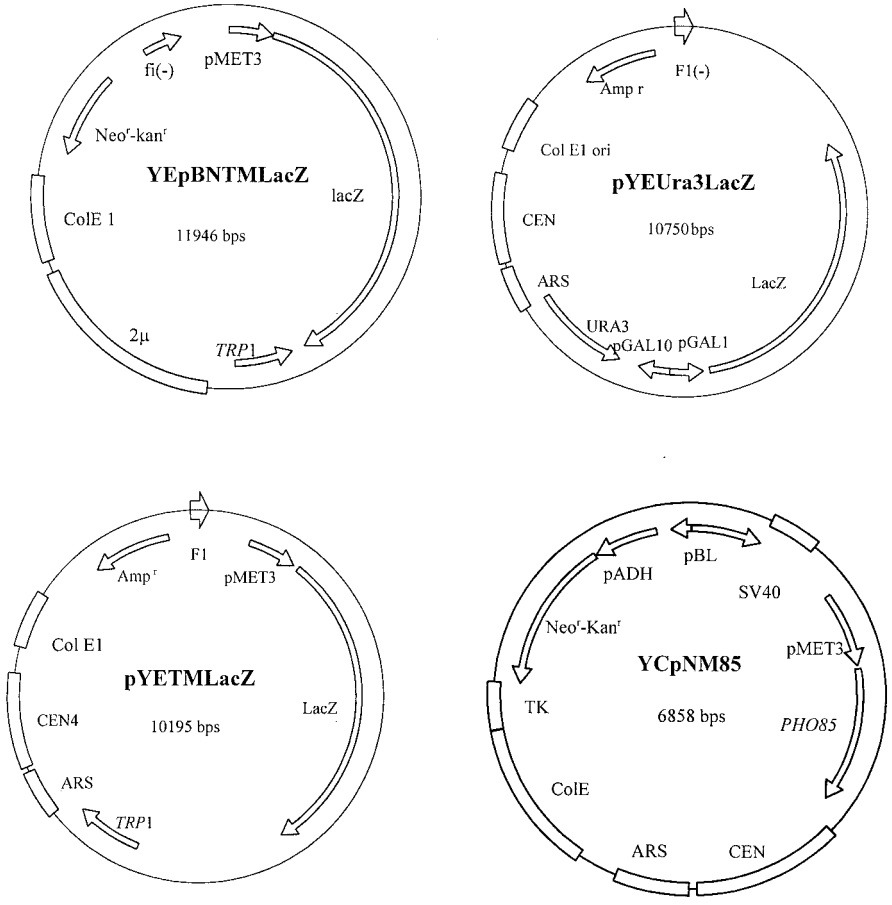

Fig. 1. Features of the plasmids.

pMET3-LacZ expression cassette was named YEpBNTMLacZ and the low copy plasmid carrying pMET3-LacZ expression cassette was pYETMLacZ. We also cloned the β-galactosidase gene into pYEUra3 (CLONTECH) to construct pGAL1-LacZ expression cassette. The plasmid was named pYEUra3LacZ. The construction YCpNM85 contained MET3 promoter driven PHO85 gene for rescue of the tri-mutant with disruption of CLN1, CLN2, and PHO85 gene (See Fig. 1 for the features of the plasmids).

Yeast transformation. The yeast was transformed by LioAc method described previously [9].

Assay of β-galactosidase in liquid culture [1]. Transformants, generally appearing 2–3 days after incubation (30°C), were inoculated in 5 ml of liquid SC media, grown to stationary phase and transfer the cell to another 5 ml SC medium in the presence of different concentration of methionine till the cell has grown to A600 0.5~0.8. The β-galactosidase activity was assayed as described [1]. Units of activity were calculated as Miller [7]: 1unit = 1000 × A420/time(min) × volume(ml) × A600. Each transformant was assayed in duplicate and reported β-galactosidase activity are the average of at least five separating randomly selected transformants.

Construction of the conditional lethal trimutant. Gene disruption and Southern blotting were as described in our previous work [6]. In this work, YPH620 which derived from YPH499 with the disruption of CLN1 and PHO85 gene by HIS3 and TRP1 selective marker was co-transformed with a linearized integrate plasmid to disrupt the *cln2* gene with nutrition marker LEU2 and a sustaining plasmid YcpNM85 to construct tri-mutant. The transformants were temporarily replicated to plate with or without methionine added. Several clones viable in the absence of methionine and inviable in the presence of methionine were obtained and their genomic DNA were extracted and digested with Hind III and Sph I respectively for Southern blotting with the probe upstream *Xho* I site of *cln2* gene.

Results

The MET3 promoter was repressed by methionine in medium. The β-galactosidase activity, driven by MET3 promoter in low copy and multicopy shuttle vector, was measured in the absence and presence of different concentration of methionine in medium. The addition of methionine to growth medium resulted in a dose-dependent decrease in β-galactosidase activity. In the absence of methionine supplementation, the β-galactosidase activity was 16.0 (low copy)~12.8 (multicopy) fold of that in the presence of methionine over 100 μM. (Figs. 2 and 3). These results coincide with the description about the regulatory character in a previous report [3].

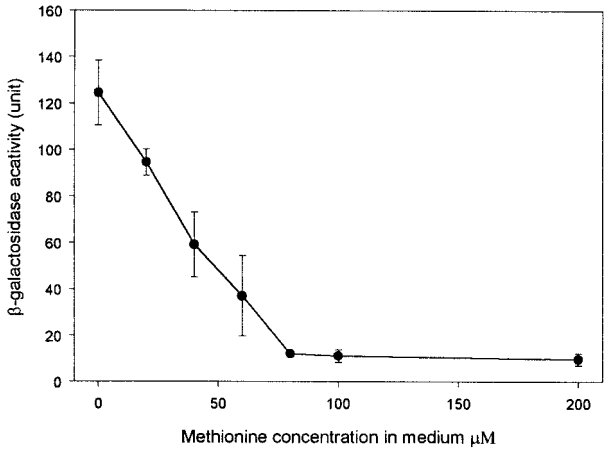

Fig. 2. The β-galactosidase activity driven by MET3 promoter in multicopy plasmid was measured in different concentration of methionine in medium.

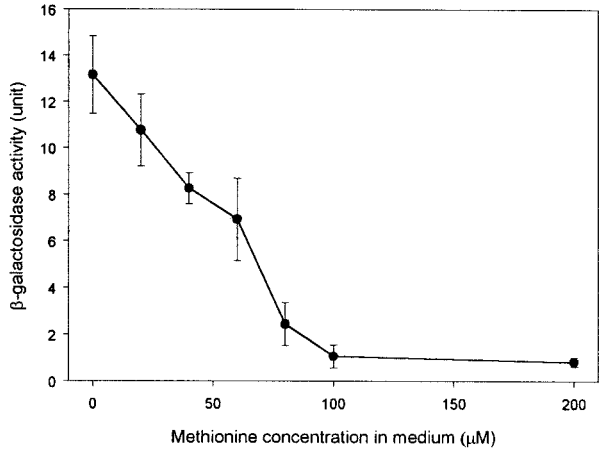

Fig. 3. The β-galactosidase activity driven by MET3 promoter in low copy plasmid was measured in different concentration of methionine in medium.

MET3 promoter is a weak but tightly controlled promoter. With the increase of the methionine concentration from 0 μM to 100 μM in growth medium, there is a dosage-dependent decrease of the β-galactosidase activity from maximum to minimum, regardless of the plasmid copy. As for as the data supplied by Mumberg about the transcriptional activity of MET25 promoter and GAL1 promoter was concerned [8], our data suggested that MET3 promoter is more sensitive to methionine in medium than MET25 promoter. The activity of the latter was repressed to minimum until the methionine amount to over 500 μM. The β-galactosidase activity without methionine in medium showed that MET3 promoter is a weaker but more tightly controlled promoter. Its transcriptional activity in low copy plasmid was only 13 units in the absence of methionine in medium and was

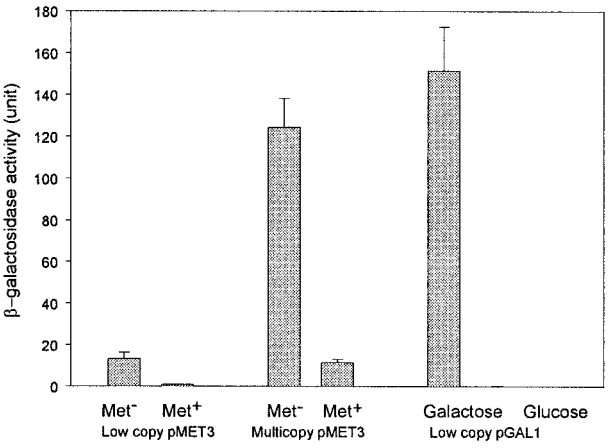

Fig. 4. Induction of expression level of MET3 and GAL1 promoter.

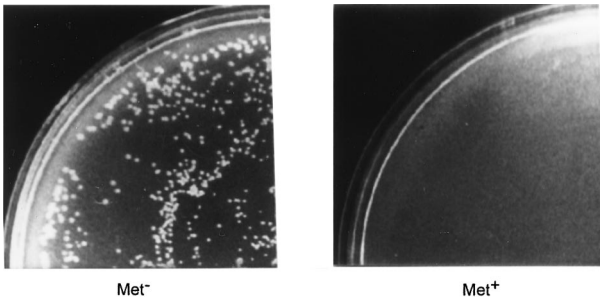

Fig. 5. The low copy sustaining plasmid carrying MET3 promoter controlled PHO85 gene render the tri-mutant (*cln1*Δ*cln2*Δ*pho85*Δ) growth sensitivity to the methionine in medium.

repressed to 0.8 unit in average in the presence of methionine over 100 μM (see Figs. 3 and 4).

MET3 promoter is feasible in the construction of conditional lethal tri-mutant. By disrupting the CLN2 gene with LEU2 nutrition marker and co-transforming with sustaining plasmid with PHO85 controlled by MET3 promoter, we got several clones viable in medium without methionine and inviable in medium with methionine over than 400 μM (as shown in Fig. 5). The Southern blotting demonstrated that the CLN2 gene was disrupted as expected (data not shown), since the G1 arrest of the *cln1*Δ*cln2*Δ*pho85*Δ tri-mutant has been demonstrated [4]. The result showed that the low copy plasmid carrying MET3 controlled PHO85 gene render the tri-mutant growth sensitive to methionine.

Discussion

Functional complementation is a classical and very useful method for isolation of function homologs from mammals. This requires the construction of conditional lethal strain. Besides the temperature-sensitive mutant,

the regulatable promoter is another tool to establish such a strain. As for the latter, the low residual activity of the promoter in repression condition is critical, especially for those low abundance regulatory factors. The β-galactosidase activity driven by MET3 promoter in low copy plasmid decreased to less than one unit, when the methionine concentration in medium was more than 100 μM. That means the promoter activity can be repressed to very low level. At the same time, we have successfully constructed conditional lethal strain *cln1*Δ*cln2*Δ*pho85*Δ by using the MET3 promoter. The low copy sustaining plasmid containing MET3 promoter controlled PHO85 gene renders the tri-mutant high sensitivity to methionine, i.e. growth repression by methionine. While the multicopy sustaining plasmid containing MET3 promoter controlled PHO85 gene was proved to be failure to give the tri-mutant complete growth repression by rather high concentration methionine (data not shown). All these results demonstrate the feasibility of the application of the MET3 promoter in the construction of conditional lethal strain.

To compare with the data about the MET25 promoter supplied by Mumberg et al. [8], MET3 promoter was a weaker but a strictly repressed promoter. In summary, MET3 promoter was a weaker promoter to compare with MET25 promoter, which shows more promising potential in the application of conditional lethal strain construction. The different character of the promoters gives the research more choice in the heterologous expression in *Saccharomyces cerevisiae*.

ACKNOWLEDGEMENTS

This work was supported by National Scientific Foundation of China Grant No. 39630160 (to Changde Lu).

Literature Cited

1. Ausubel FM, Brent R, Kingston RE (1995) Short protocol in molecular biology, 3rd ed. New York: John Wiley & Sons
2. Black S, Andrews PD, Sneddon AA (1995) A regulated MET3-GLC7 gene fusion provides evidence of a mitotic role for *Saccharomyces cerevisiae* protein phosphatase 1. Yeast 11:747–759
3. Cherest H, Nguyen NT, Surdin-Kerjan Y (1985) Transcriptional regulation of the *MET3* gene of *Saccharomyces cerevisiae*. Gene 34:269–281
4. Espinoza FH, Ogas J, Herskowitz I (1994) Cell cycle control by a complex of the cyclin HCS26 (PCL1) and the kinase PHO85. Science 266:1338–1391
5. Fuller KJ, Morse MA, White J (1998) Development of a yeast trihybrid screen using stable yeast strains and regulated protein expression. Biotechniques 25:85–88
6. Hu YF, Mao XH, Lu CD (1998) Role of PHO85 gene in the cell cycle control of budding yeast. Acta Biol Exp Sin 31:265–271
7. Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory
8. Mumberg D, Muller R, Funk M (1994) Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res 22:5767–5768
9. Sherman F (1991) Getting started with yeast. Methods Enzymol 194:3–21
10. Warit S, Zhang N, Short A (2000) Glycosylation deficiency phenotypes resulting from depletion of GDP-mannose pyrophosphorylase in two yeast species. Mol Microbiol 36:1156–1166