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A novel cis-acting cysteine-responsive regulatory element of the gene for the high-affinity glutathione transporter of Saccharomyces cerevisiae

Received: 6 July 2001 / Accepted: 22 November 2001 / Published online: 12 January 2002
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Abstract We cloned a DNA fragment from Saccharomyces cerevisiae that complemented the deficiency in high-affinity glutathione transport activity conferred by a gsh11 mutation, and found that the ORF responsible was YJL212c, which had already been designated as OPT1 and HGT1 by others. Northern analysis clearly demonstrated that this ORF, now referred to as OPT1/HGT1/GSH11, was induced by sulfur starvation and repressed by adding cysteine to the growth medium. Reporter gene assays showed that a segment spanning the region between positions −371 and −355 was essential for the regulation of this gene. A sequence of 9 nt, CCGCCACAC (from −364 to −356), in this region was shown to be required for protein binding, using an electrophoretic mobility shift assay. Based on these results, we propose that CCGCCACAC comprises the core of a cis-acting element involved in cysteine-responsive gene regulation in S. cerevisiae.

Keywords Glutathione transporter · Oligo-peptide transporter · Regulatory motif · Sulfur metabolism · Saccharomyces cerevisiae

Introduction

Glutathione (γ-glutamylcysteinylglycine) is the most abundant sulfur-containing organic compound in the budding yeast Saccharomyces cerevisiae, as in many other organisms (Meister and Tate 1976; Penninckx and Jaspers 1982; Rennenberg 1982). Glutathione acts as a coenzyme for at least one enzyme involved in detoxification (Penninckx et al. 1983). Glutathione also acts as a mediator of oxidation/reduction reactions and as a radical scavenger (Taniguchi 1988), so that it is widely thought to play a central role in protecting cells against the effects of ionizing radiation (Deschavanne et al. 1981), and toxic chemicals such as heavy metals (Meister and Anderson 1983). Moreover, it is now well documented that glutathione plays an essential role in the response to oxidative stress (Izawa et al. 1995; Grant et al. 1996; Stephen and Jamieson 1996).

Besides these physiological functions, glutathione is thought to act as a metabolic reservoir of both cysteine and sulfur in cells (Elskens et al. 1991). This is because glutathione is synthesized by the consumption of cysteine and gives rise to cysteine by degradation. Glutathione synthesis is better understood (Ohtake and Yabuuchi 1991; Inoue et al. 1998) than is its transport or degradation. Because S. cerevisiae is a parasitic and/or saprophytic organism, it must utilize glutathione as an economical source of sulfur in the natural environment. It should be noted here that sulfate must be reduced to sulfide before it can be used for synthesis of sulfur-containing organic compounds, and this process requires large amounts of energy (Mountain et al. 1991; Thomas et al. 1992; Ono et al. 1996).

This is the context in which we initiated our work on utilization of glutathione as a sulfur source by S. cerevisiae. We found that this organism has at least two kinetically distinguishable glutathione transport systems: a high-affinity system (GSH-P1) that was induced by sulfur starvation, and a low-affinity system (GSH-P2) that was not (Miyake et al. 1998). We also obtained
mutants that were unable to utilize glutathione as a sulfur source and came to the conclusion that this phenotype resulted from simultaneous deficiency for the GSH-P1 activity (gsh11) and the sulfate assimilation activity (e.g., met17) (Miyake et al. 1999). By transforming a gsh11 met17 strain that did not grow on medium containing glutathione as a sole source of sulfur (GSH medium), we obtained a DNA fragment that restored the ability to grow on GSH medium. Here, we show that the ORF responsible for this phenotype codes for the high-affinity glutathione transporter. We therefore concluded that we had cloned GSH11 and that GSH11 is the structural gene for GSH-P1. We also describe attempts to characterize a regulatory element of GSH11 and discuss its significance in the regulation of the entire sulfur-metabolizing system in S. cerevisiae.

Materials and methods

Strains and plasmids

Strain SF1-1C (MATa leu2-1 trpl met17) does not grow on medium containing sulfate as a sole source of sulfur (SO₄²⁻ medium) because the strain carries a met17 mutation that results in deficiency for O-acetylserine and O-acetylmethionine sulphydrylase (Naiki and Iwata 1962; Yamagata et al. 1974, 1975; Masselon and Surdin-Kerjan 1977), but it does grow on medium containing an organic sulfur source such as methionine, cysteine or glutathione. Strain SF1-1CC8 (MATa leu2-1 trpl met17 gsh11), however, does not grow on SF medium supplemented with glutathione (GSH medium) because it carries a gsh11 mutation (deficiency of the activity of the high-affinity glutathione transport system, GSH-P1) in addition to met17 (Miyake et al. 1999). Strain SF1-1CC8-Aura3 (MATa leu2-1 trpl met17 gsh11 aura3::pBR322) is a ura3-disrupted derivative of SF1-1CC8 (described below).

The Escherichia coli strain JM109 (TaKaRa) was used to amplify plasmids. YCP50, a centromere-bearing vector (Parent et al. 1985), was used to construct the yeast genomic library. pBluecriptII SK⁺ (Toyobo) was used for DNA sequencing. YIp5 (Parent et al. 1985) and pBR322 (Sutcliffe 1979) were used for disruption of URA3. pMC1587, a plasmid containing the coding region of lacZ (Casadaban et al. 1983), was used for analysis of the promoter region of OPT1/HGT1/GSH11.

Growth media and growth conditions

Standard yeast growth media were used (Sherman et al. 1986). The YPD medium contained 1% yeast extract, 2% peptone and 2% glucose. The SD medium was the synthetic minimal medium described by Wickerham (1935). Sulfur-free (SF) medium was prepared by replacing all sulfate salts in the SD medium with the corresponding chloride salts (Ono et al. 1991). To provide for the nutritional requirements of the strains used in this study, L-leucine (30 µg/ml), L-tryptophan (20 µg/ml) and uracil (20 µg/ml) were added to the SD and SF media. Medium containing a defined sulfur source was obtained by adding cysteine (CYS), glutathione (GSH), methionine (MET) or sulfate (SO₄²⁻) to the SF medium at a concentration of 100 µM, unless specified otherwise. To select ura3 mutants, 5-fluoro-orotic acid (5-FOA) was added to the synthetic growth medium at a final concentration of 0.5 mg/ml (Rothstein et al. 1991). For solid media, 2% agar was added. The growth temperature was 30°C, and liquid cultures were incubated on a rotary shaker at 120 rpm.

LB medium (1% tryptone, 0.5% yeast extract and 0.5% NaCl; pH 7.2) was used for the cultivation of E. coli (Sambrook et al. 1989). To screen for Amp' clones, ampicillin was added to the LB medium at a concentration of 50 µg/ml. For solid media, 1.5% agar was added. The growth temperature was 37°C, and liquid cultures were incubated on a rotary shaker at 120 rpm.

DNA manipulations

Extraction of S. cerevisiae genomic DNA was carried out by the method of Philippsen et al. (1991). Transformation of S. cerevisiae was carried out by the method of Ito et al. (1983). Extraction of plasmids from S. cerevisiae followed the technique of Sherman et al. (1986). Other general DNA manipulation procedures were adopted from Sambrook et al. (1989).

Construction of a ura3 disruptant

Plasmid YIp5 containing the URA3 gene was digested with Sau3AI, and the resulting DNA fragments were inserted into the BamHI site of pBR322. Strain SF1-1CC8 was then treated with the ligated mixture, and spread on 5-FOA medium. After incubation for 7 days, the colonies that had developed were picked and tested for their dependence on added uracil. Of the five 5-FOA-resistant clones tested, all were uracil-dependent. One of them was further shown to lose the Ura' phenotype when transformed with plasmid YCP50 (URA3). When one of these Ura' transformants was grown in YPD medium and spread on the 5-FOA medium, the colonies obtained were all Ura−, indicating that the Ura' phenotype was conferred by YCP50. We concluded that this strain was a ura3 disruptant derived from strain SF1-1CC8. We therefore used this strain, designated SF1-1CC8-Aura3, for subsequent work.

Construction of a yeast genomic library using YCP50

Genomic DNA was extracted from strain SF1-1C (MATa leu2-1 trpl met17 GSH11) and partially digested with Sau3AI. The resulting DNA fragments were fractionated by means of sucrose-gradient centrifugation (Ausubel et al. 1987). DNA fragments of 8–15 kb were pooled and precipitated with ethanol. The precipitate was dissolved in water and ligated to BamHI-digested YCP50. E. coli JM109 was transformed with the ligation mixture, and about 7500 independent Amp' clones were picked. These were grown overnight in medium containing 50 µg/ml ampicillin. The cells were then harvested, and plasmids were extracted from them. The mixture of plasmids thus obtained served as the genomic library used in this study.

DNA sequencing and homology search

DNA fragments to be sequenced were digested with appropriate restriction enzymes, and the fragments were integrated into the corresponding cloning site in the multicloning region of pBluecriptII SK⁺. Then, a set of the plasmids obtained was subjected to DNA sequencing (carried out by Nissinbo, Tokyo, Japan). The homology search was done with the S. cerevisiae WU-BLAST2 Search program in the Saccharomyces Genome Database (http://genome-www.stanford.edu/).

Assay of glutathione transport activity

Cells were grown overnight in YPD medium. The cells were harvested and suspended in SF medium or CYS medium. After incubation for 12 h, the cells were transferred to fresh medium and incubated for an additional 4 h. A 5-ml aliquot of the culture was mixed with 55 µl of 10 mM [³⁵S]glutathione (18.6 MBq/nmol) and 445 µl of water. At intervals, 1-ml aliquots were removed and filtered through a membrane. The cells trapped on the membrane were washed with 1 mM glutathione and measured for radioactivity using a liquid scintillation counter as described by Miyake et al. (1998).