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FEMS Microbiology Letters 136 (1996) 269–273

FEMS MICROBIOLOGY LETTERS

Amplification of a gene for metallothionein by tandem repeat in a strain of cadmium-resistant yeast cells

Hiroshi Tohoyama *, Emi Shiraishi, Shinjiro Amano, Masahiro Inouhe, Masanori Joho, Tetsuo Murayama

Department of Biology, Faculty of Science, Ehime University, Bunkyo, Matsuyama 790-77, Japan

Received 11 November 1995; revised 22 December 1995; accepted 22 December 1995

Abstract

In a cadmium-resistant strain of *Saccharomyces cerevisiae*, cells are protected against cadmium toxicity by the production of large amounts of cadmium-binding metallothionein, as occurs similarly in a copper-resistant strain. The apoprotein of the metallothionein is encoded by the *CUP1* gene on chromosome VIII. The *CUP1* gene is present as 8–10 copies in the cadmium-resistant strain as a result of tandem repeat of a 2.0-kb fragment of DNA that includes *CUP1*, while the wild-type strain contains only a single copy of *CUP1*. In the cadmium-resistant strain, some evidence for elongation of chromosome VIII with variations in length (maximum to 200 kb) was obtained. However, the elongation was not due to the tandem repeats of the *CUP1*-containing region.

Keywords: *Saccharomyces cerevisiae*; Cadmium resistance; Metallothionein gene; *CUP1*; Gene amplification; Tandem repeat

1. Introduction

Heavy metals, such as copper, zinc, cadmium and mercury, impede the growth of yeast cells at concentrations that inhibit normal metabolism, and at higher concentrations the cells die. However, at such high concentrations, certain strains with effective metal-detoxification mechanisms survive [1]. The survival of cells depends on their ability to limit the intracellular concentration of the heavy metals. Detoxification can be achieved by regulating uptake and/or efflux, by intracellular compartmentalization, or by sequestration [2]. One of the major contributors to the intracellular sequestration of metals in yeast cells is metallothionein [3]. The metallothionein of *Saccharomyces cerevisiae* was first isolated from a copper-resistant strain as a copper-binding protein of 53 amino acid residues, which include 12 cysteine residues [4]. The metallothionein protects cells against the toxic effects of copper by chelating copper. It is encoded by the *CUP1* gene on chromosome VIII [5]. Resistance to copper is explained by the synthesis of large quantities of metallothionein in yeast cells [6]. More than ten copies of *CUP1* are generated by amplification of a fragment of DNA that contains *CUP1* on chromosome VIII in copper-resistant strains, while wild-type (copper-sensitive) strains have only a single copy of *CUP1* [7,8]. The tandemly repeated unit of the DNA is 1998 base pairs long and contains one copy of *CUP1* [9]. The

* Corresponding author. Tel.: +81 (899) 24 7111; Fax: +81 (899) 23 2545; E-mail: GCF00360@niftyserve.or.jp

PII S0378-1097(96)00015-8

number of copies of *CUP1* and the extent of resistance to external copper are directly correlated in copper-resistant yeasts [6,10,11].

We previously isolated a cadmium-resistant strain of *S. cerevisiae*, 301N, by repeated exposure of the wild-type strain to medium containing a defined concentration of cadmium ions [12]. 301N cells produce a cytoplasmic cadmium-binding protein for detoxification of cadmium in the cells [13]. This protein was characterized as a cadmium metallothionein with the same apoprotein as that of copper metallothionein from a copper-resistant strain [14]. As shown in a previous report [15], the structural gene for cadmium metallothionein in 301N is *CUP1*, as is the case in the copper-resistant strain, but amplification of the *CUP1* gene in 301N cells has not previously been investigated. The specificity for copper and cadmium might be due to some difference in transcription factors for *CUP1* in each resistant strain [16–19]. The production of metallothionein in *S. cerevisiae* has been detected only in copper- or cadmium-resistant strains [20]. In this study, we examined the amplification of a DNA fragment that contained *CUP1* and the size of chromosome VIII in cadmium-resistant strain 301N, comparing it with copper-resistant strains.

2. Materials and methods

2.1. Organisms and plasmids

Saccharomyces cerevisiae 301N (*MATa, ura1, CUP1*) is a cadmium-resistant strain that is able grow in medium that contains 0.5 mM cadmium sulfate [12]. Strain X2180-1B (*MATα, SUC2, mal, mel, gal2, CUP1*) and strain DTY22 (*MATα, his6, ura3-52, LEU::YipCL, CUP1*) are copper-resistant strains with about ten copies and three copies, respectively, of *CUP1* on chromosome VIII as a consequence of tandem repeat [6,9,21]. Strain IS318-4C (*MATa, his6, leu2-1*) is a wild-type strain with respect to resistance to cadmium and copper. *Escherichia coli* strain DH-1S was used for the propagation of plasmids. YEPD medium and LB medium were used for the cultivation of yeast cells at 30°C and *E. coli* at 37°C, respectively [22]. Plasmid YEp36 has an insert of 1.3 kb that includes *CUP1* at the *Bam*HI site of YEp13 [17]. Plasmid YIp5-GPA1 has an insert of 1.9 kb that includes *GPA1* (a gene for a G protein homologue) from chromosome VIII at the *Eco*RI site of YIp5 [23].

2.2. Gel electrophoresis and Southern hybridization

The preparation of yeast chromosomal DNA for digestion with restriction enzymes and for pulsed-field gel electrophoresis was carried out as described by Sherman et al. [24] and Spencer et al. [25], respectively. Standard techniques for manipulation of DNA, including preparation of plasmid DNA, restriction digestion and gel electrophoresis, were those described by Maniatis et al. [26]. For the separation of chromosomal DNA, pulsed-field gel electrophoresis (crossed-field electrophoresis system, AE-6800; Atto Co. Tokyo, Japan) was performed in 1% agarose gels for 40 h at 180 V with pulse intervals of 110 s in half-strength TBE buffer. The λ DNA ladder was used as the size makers for high-molecular-mass DNA [27]. The probes for hybridization were a 1.3-kb fragment that contained the *CUP1* gene obtained from plasmid YEp36 by digestion with *Bam*HI [17] and a 1.9-kb fragment that contained the *GPA1* gene obtained from plasmid YIp5-GPA1 by digestion with *Eco*RI [23]. A non-radioactive DNA Labelling and Detection Kit (Boehringer Mannheim, Mannheim, Germany) was used both to label the probe and for the detection of the hybridization signal, in accordance with specifications supplied with the kit.

3. Results and discussion

3.1. Amplification of CUP1 by tandem repeat of the DNA fragment that contains the CUP1 gene

The restriction enzyme *Eco*RI has recognition sites outside the 2.0-kb repeating unit on chromosome VIII and does not cleave within the unit, while there is single site for *Kpn*I in the basic repeated unit in copper-resistant strains [6–9]. In order to clarify the size of the repeating unit and the number of the *CUP1* gene in the cadmium-resistant strain 301N, we digested chromosomal DNA with each enzyme, fractionated the digests on an agarose gel by elec-

trophoresis and analyzed the fragments by Southern hybridization with a probe for *CUP1* (Fig. 1).

Digestion of DNA from 301N (lane 6) with *Eco*RI generated an intense broad band upon hybridization. The fragment was estimated to be 20–22 kb in length, as is the analogous fragment from the copper-resistant strain X2180-1B (lane 7). In contrast, DTY22, with three copies of *CUP1* (lane 8), and IS318-4C, with only one copy of *CUP1* (lane 9), gave hybridization signals that represented fragments of about 9 kb and 5 kb, respectively. *Eco*RI has a recognition site 0.8 kb away from the left end of the 2.0-kb basic repeating unit and another site 2.4 kb away from the right end of the unit in X2180-1B [6,7]. From our results, we conclude that the cadmium-resistant strain 301N has 8–10 repeats (a more accurate determination is difficult because of the broad band) of the 2.0-kb basic unit, as does the copper-resistant strain X2180-1B. The intensity of the hybridization signals reflected the number of tandem repeats.

Digestion with *Kpn*I generated a faint band of a 2.7-kb fragment that was common to all tested strains and a more intense band of a 2.0-kb fragment for 301N, X2180-1b and DTY22 (lane 10–12). The 2.0-kb fragment was identical in size to the basic repeating unit that includes *CUP1* in copper-resistant yeasts [6–9]. The absence of the 2.0-kb fragment from the digest of IS318-4C (lane 13) indicated that this metal-sensitive (wild-type) strain had only a single copy of the *CUP1* gene. The faint bands of 2.7-kb fragments generated from all DNAs by *Kpn*I correspond to the right-junction region of the repeating unit [6,7]. These results indicate that the genome of strain 301N contains 8–10 copies of *CUP1* as a consequence of the amplification of a 2.0-kb tandemly repeated unit, as is the case in copper-resistant strain.

3.2. Elongation of chromosome VIII

We can postulate that the DNA of chromosome VIII in copper- and cadmium-resistant strains should be longer than the DNA in the wild-type strain as a result of amplification of *CUP1* by tandem repeat. Chromosomal DNAs from 301N, X2180-1B, DTY22 and IS318-4C cells were separated by pulsed-field gel electrophoresis for a comparison of the length of chromosome VIII from each strain. The results of hybridization with the *CUP1* probe were very diffi-

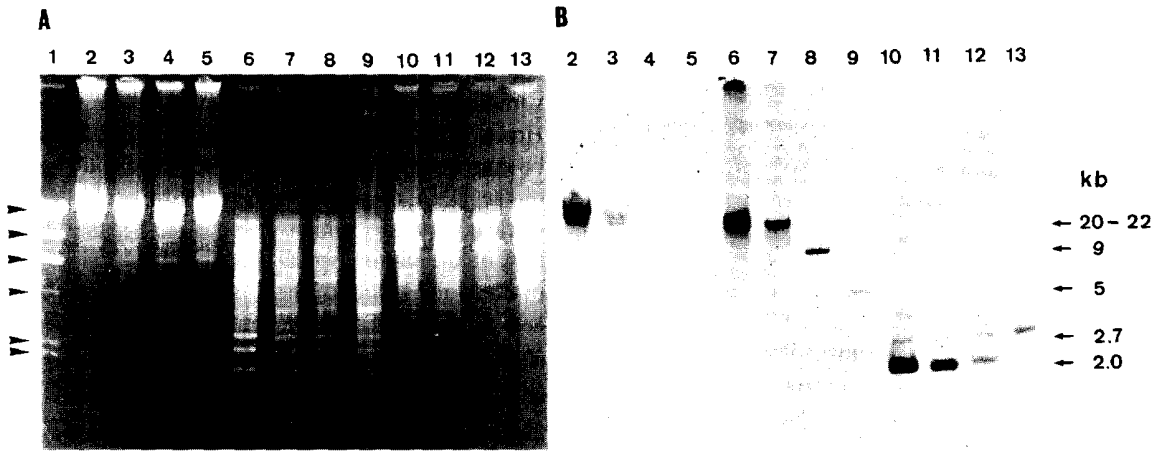

Fig. 1. Digestion of chromosomal DNAs by *Eco*RI and *Kpn*I, with subsequent Southern hybridization with a *CUP1* probe. (**A**) Electrophoresis of *Eco*RI- and *Kpn*I-digested chromosomal DNA from strains 301N, X2180-1B, DTY22 and IS318-4C. (**B**) Southern hybridization using a 1.3-kb DNA fragment that included *CUP1* as a probe. Lane 1 was loaded with λ DNA that had been digested with *Hin*dIII. DNA samples in lanes 2, 6 and 10 were extracted from 301N; in lanes 3, 7 and 11 from X2180-1B; in lanes 4, 8 and 12 from DTY22; and in lanes 5, 9 and 13 from IS318-4C. Lanes 2–5 were loaded with intact DNA, lanes 6–9 were loaded with DNA that had been digested with *Eco*RI and lanes 10–13 were loaded with DNA that had been digested with *Kpn*I. Arrows on the left side indicate the sizes of marker fragments (from the top): 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb, respectively. Arrows on the right side indicate the sizes of the fragments estimated by reference to the size markers.

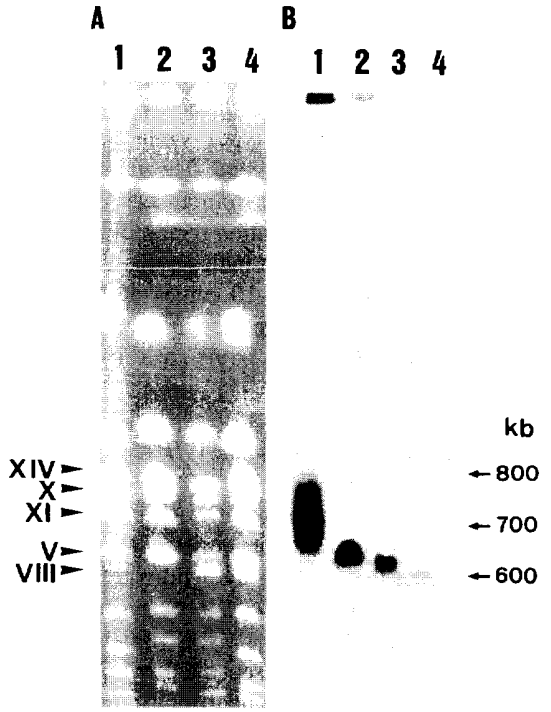

Fig. 2. Chromosomal DNAs separated by pulsed-field gel electrophoresis with subsequent Southern hybridization with the probe for *CUP1*. (**A**) Electrophoresis of chromosomal DNA from strains 301N, X2180-1B, DTY22 and IS318-4C. (**B**) Southern hybridization using the 1.3-kb DNA fragment that included *CUP1* as a probe. Chromosomal DNA in lane 1 was prepared from 301N, in lane 2 from X2180-1B, in lane 3 from DTY22, and in lane 4 from IS318-4C, respectively. Arrows (chromosome numbers) on the left side indicate the position of each chromosome, as anticipated from results from the standard strain [25]. Arrows on the right side indicate the positions of size markers (λ DNA ladder).

cult to interpret because it was hard to distinguish chromosome VIII from chromosome V on the gel under UV light (Fig. 2). The hybridization signal due to the *CUP1* probe was detected at the position expected for chromosome VIII (about 580–590 kb) in the strains X2180-1B, DTY22 and IS318-4C (lanes 2–4), although there were variations in the intensity of the signal that reflected differences in copy numbers of *CUP1* [22,25]. However, a broad hybridization signal (estimated to represented a region of about 600–800 kb) appeared in the analysis of strain 301N (lane 1). This result suggests the possibility that *CUP1* might also be present on chromosomes (for example, chromosomes V, XI, X and XIV) other than chromosome VIII, or that the migration of the DNA of chromosome VIII shifts to higher molecular positions with some variations in size [25]. In order to clarify this issue, a DNA fragment including the *GPA1* gene as stable marker of chromosome VIII was also used as a probe for Southern hybridization. The *GPA1* probe gave the same hybridization profile as the *CUP1* probe (data not shown). Therefore, we confirmed that the migration of the DNA of chromosome VIII from strain 301N shifted to positions of lower mobility with some variations. Two explanations can be considered for this shift in migration: the elongation of the DNA of chromosome VIII in strain 301N; and some artifact that affects the migration of the DNA of chromosome VIII without net elongation of the DNA. Although the maximum extent of the elongation of the DNA of chromosome VIII was estimated to be about 200 kb in strain 301N, elongation by more than 20–22 kb cannot be accounted for solely by the amplification of the *CUP1* gene by the tandem repeats discussed above. However, an artifact due to interactions between the DNA of chromosome VIII and the cadmium ions in the cells of strain 301N can be excluded because the cells for preparation of DNA were grown in medium without cadmium ions. From our results, we propose that strain 301N has an elongated chromosome VIII and the variation in the length of the DNA is due to variations in the cells in a population of strain 301N. However, the possibility of an artificial effect cannot be completely ignored. The reason for the elongation of the DNA of chromosome VIII in strain 301N is unclear. The mechanism responsible for the elongation of the chromosomal DNA and the relationship between the length of the DNA of chromosome VIII and cadmium resistance remain as problems to be solved in future studies.

Acknowledgements

The authors thank Dr. Dean Hamer of the National Institutes of Health (Bethesda, MD) for the generous gift of yeast strain DTY22, and Dr. Hiroaki Fujimura of Hoechst Japan Pharma Research for the generous gift of plasmid YIp5-GPA.

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