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Molecules and Cells

The Putative Transcriptional Activator *MSN1* Promotes Chromium Accumulation in *Saccharomyces cerevisiae*

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Yeast is a good system for studying molecular mechanisms of metal tolerance. Using a mini-Tn mutagenized yeast pool, we isolated a chromate-tolerant mutant, CrT9, that displayed metal-specific tolerance since it was only tolerant to Cr(VI), not to Cr(III), Cd, As, or Fe. The Cr-tolerance of CrT9 appeared to be due to reduced Cr accumulation as it accumulated only 56% as much as WT (Y800). Using IPCR (inverse PCR), we found that the mini-Tn had been inserted at nt 741 of the transcriptional activator, *MSN1*. MSN1 is a multifunctional protein involved in invertase activity, iron uptake, starch degradation, pseudohyphal growth, and osmotic gene expression. We found that there was only one mini-Tn insertion in CrT9 since *MSN1* and mini-Tn probes hybridized to the same DNA fragment, and the *MSN1* probe detected an enlarged *MSN1* mRNA. When we over-expressed *MSN1* in CrT9 and WT, both accumulated larger amounts of Cr. We conclude that Cr accumulation in *S. cerevisiae* is promoted by the transcriptional activator *MSN1*.

Keywords: Accumulation; Chromium; IPCR; Mini-Tn; *MSN1*; Mutant; *S. cerevisiae*; Transcriptional Activator.

Introduction

Chromium is a major heavy metal that threatens humans, animals, and plants by contaminating air, water and soil. Chromium is released into the environment by industrial processes such as leather tanning, electroplating, and paint manufacture. Major forms of chromium in nature are Cr(VI) (chromate) and Cr(III) (chromite). Since chromate is absorbed more easily by cells and chromite is impermeable due to its low water solubility, chromate is more toxic than chromite (Cary, 1982).

It is believed that chromate enters *S. cerevisiae* via its sulfate transport systems since sulfate uptake mutants are resistant to chromate (Smith *et al.*, 1995). The observation of reduced oxygen uptake by *S. cerevisiae* exposed to high concentrations of chromate indicates that the mitochondria are targets of chromate (Henderson, 1989). Chromate also has a genotoxic effect in *S. cerevisiae*, inducing chromosome aberrations and mutation, as in animals, plants and bacteria (Galli *et al.*, 1985). Since chromate-sensitive mutant of *S. cerevisiae* accumulate higher amount of Cr than WT, it has been suggested that tolerance is due to reduced uptake (Gharieb and Gadd, 1998).

MSN1 is a multi-functional protein known by a variety of names such as: MSN1, involved in invertase activation (Estruch and Carlson, 1990) and the expression of osmotic stress-induced genes (Rep *et al.*, 1999) and SWI6-dependent genes (Sidorova and Breeden, 1999); FUP1, whose over-expression increases iron-uptake in iron-limited cells (Eide and Guarente, 1992); PHD2, identified as inducing pseudohyphal growth of diploid cells on nitrogen-limiting medium (Gimeno and Fink, 1994); and MSS10, that acts as an activator of STA2 in starch degradation (Lambrechts *et al.*, 1996). Since MSN1 is located in the nucleus of *S. cerevisiae* and has weak, non-specific DNA-binding activity, it was suggested to be a transcriptional activator (Estruch and Carlson, 1990).

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To understand the molecular mechanism of Cr-tolerance in *S. cerevisiae* we isolated a chromate-tolerant mutant, CrT9. The mutant accumulates less Cr than WT and its chromate-tolerance is the result of disruption of *MSN1* by a mini-Tn insertion.

Materials and Methods

Strains, cultures, and mutant isolation We used a *Saccharomyces cerevisiae* mutant pool supplied by Dr. Eu-Yol Choi (Hallym Univ., Korea) to isolate the chromate-tolerant mutant. The pool was generated by inserting a 6654 nt mini-transposon (a synthetic *E. coli* Tn3-derived transposon containing *leu2*, the complete β-lactamase coding sequence and a β-galactosidase partial sequence) (Hong *et al.*, 1999) into wild type Y800 (MATa/ MATα leu2/leu2 ade2/ade2 HIS3/his3 ura3/ura3 can1/CAN1 lys2/lys2 CYH2/cyh2 trp1/TRP1) (Ross-Macdonald *et al.*, 1997). Both Y800 and the mutant were grown on YPD or CM (complete minimal) media (Ausubel *et al.*, 1999). To isolate the Cr-tolerant mutant, the mutant pool was plated on CM agar containing 300 μM potassium chromate without leucine, and colonies that appeared were retested for chromate tolerance on chromate medium. *Escherichia coli* strain DH5α was used for routine subcloning.

Isolation of yeast DNA and RNA Genomic DNA and RNA were isolated from Y800 and CrT9 cells by the glass bead method as described by Ausubel *et al*. (1999).

Inverse PCR CrT9 genomic DNA was digested with *Eco*RI, and self-ligated to form circular DNA fragments. These were amplified by PCR using Ex *Taq* polymerase (TaKaRa) and a set of primers corresponding to the termini of the *lacZ* sequence in the mini-transposon (GenBank accession number U351112). The 5′ primer was 5′-CGTTGTAAAACGACGGGATCCCCCT-3′, and the 3′ primer 5′-CTGCACGCGGAAGAAGGCACAT GGC-3′. Temperature cycles were as follows: 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, 60°C for 3 min, and 72°C for 1 min. A 1.2 kb PCR product containing parts of the mini-Tn and *MSN1* sequences, was sub-cloned into pGEM-T easy vector (Promega), and sequenced by the dideoxy-chain termination method using Sequenase 2.0 (U.S. Biochemical).

Manipulation of *MSN1* and yeast transformation Full length *MSN1* DNA was generated by PCR from the genomic DNA of Y800 using *pfu* polymerase (Genenmed) and primers corresponding to the termini of the *MSN1* ORF. The sense and antisense primers were 5′-CCCCTTGCTTATAAGAAAAGAAA CC-3′ and 5′-GCTAATTTGGGATAAAGGTGAATAC-3′, respectively and PCR was performed as described above. The resulting 1.2 kb DNA was cloned into pGEM-T easy vector (Promega) and the product was digested with *Not*I and subcloned into *Not*I-digested pFL61 vector (Minet *et al.*, 1992), which contains the PGK (phosphoglycerate kinase) promoter (Mellor *et al.*, 1985) and URA3 marker for selection. The resulting pFL61 vector containing *MSN1* was transformed into CrT9 by the LiAc/PEG method (Ausubel *et al.*, 1999), and transformants were selected on CM agar medium without uracil. To test the chromium tolerance of the transformants, they were grown in CM-Ura liquid medium overnight, spotted on CM-Ura agar plates supplemented with 300 μM K_2CrO_4, and their growth monitored after 4 days.

Northern and Southern blot analysis For Northern blot analysis, total RNA was isolated using the glass bead method as described by Ausubel *et al.* (1999) and Northern blot analysis was carried out as described by Hwang and Herrin (1994). A full-length *MSN1* DNA probe was prepared by random primer labeling with [α-^{32}P]dCTP. For Southern blot analysis, 10 μg of the genomic DNA of CrT9 was digested with *Eco*RI and *Sac*I, separated on a 0.7% agarose gel, transferred to a positively charged nylon membrane (Q-BIOgene), and fixed for 2 h at 80°C. The 3′ terminus of the 0.8 kbp *MSN1* probe was prepared from *MSN1* DNA by digesting with *Sac*I. The 0.7 kbp mini-Tn probe, a fragment of *lacZ*, was prepared from mini-Tn by digesting with *Pvu*I. Hybridization was performed as described by Hwang and Herrin (1994).

Measurement of chromium *S. cerevisiae* cells were cultured in CM medium for 24 h in the presence of 50 μM potassium chromate, harvested, washed with CM buffer, and dried at 60°C. 1.0 g of dried cells was used to determine Cr content by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy, Perkin Elmer ELAN3000) at a wavelength of 267.7 nm at the Korean Basic Science Institute (KBSI).

Preparation of fragments of metal transporter genes DNA fragments for use as hybridization probes were generated by PCR. Primers for *Zrt1* (YGL255W; accession no. Z72777) PCR product (620 bp) were 5′-GACACTGTTGTGAGAAAC ACTGCA-3′ and 5′-TACCGATCAAAGCCATGATACC-3′. The *Cot1* (YOR316C; M88252) PCR product (607 bp) was generated using primers 5′-AGCGCAAGAGATCTTTAAATATGC-3′ and 5′-AAGCAAT-CAGCTGTGTTGCAGT-3′. PCR primers for the *Ctr1* (YPR124W; U02511) fragment (606 bp) were 5′-GACGAAGGAAAACATT-ATGACACA-3′ and 5′-TTTTTCGG CCGG-AAGTAAGT-3′. The PCR product (684 bp) of *FIT1* (YDR534C) was produced using primers 5′-AGACTTCCTCTG CTGTTGAGACTT-3′ and 5′-CAAAAAGATAACGGCAGCGA-3′. PCR primers for *SUL1* (YBR294W; Z36163) were 5′-CTCCTCCACCAGGTGTCATA-3′ and 5′-TCCCATTTAGAAA AATCGGG-3′. Primers 5′-CCACCAGACGGTGTTCTTG-3′ and 5′-CCTGAAGCGGTAT-AAACGC-3′ were used for generating the *SUL2* (YLR092W) gene fragment.

Results and Discussion

Isolation of the chromate tolerant mutant, CrT9 To

isolate *S. cerevisiae* mutants with increased tolerance to chromate, the most toxic form of chromium, a mini-Tn mutant pool (see **Materials and Methods**) was plated on CM agar containing 300 μM potassium chromate (K_2CrO_4) and lacking leucine. This concentration of potassium chromate was chosen as the minimal concentration inhibiting growth of the parental strain, Y800 (data not shown). Colonies formed from about 10^6 plated cells were retested for chromate tolerance on chromate-containing agar. CrT9 exhibited the highest tolerance of the 11 mutants tested; it was able to grow on 300 μM of Cr(VI), whereas the other mutants grew on up to 250–280 μM of Cr(VI). Therefore CrT9 was characterized further. To see whether it was tolerant to other heavy metals, its growth was monitored on agar media containing Cr(VI) (potassium chromate, 300 μM), Cr(III) (chromium chloride, 3 mM), Cd (cadmium sulfate, 230 μM), Fe (iron sulfate, 35 mM) and As (sodium arsenite, 700 μM). As shown in Fig. 1A, it was only tolerant to Cr(VI). This specific tolerance is a unique phenotype as other chromate-tolerant strains of yeasts are cross-resistant to a variety of metals (Czakó-Vér *et al.*, 1999). In addition, WT and CrT9 showed a similar tolerance to chromite.

We compared Cr accumulation in WT (Y800) and CrT9 cells after growth in liquid medium containing 50 μM of chromate for 24 h. Interestingly, the mutant accumulated less (56%) Cr (12.0 ppm), than WT (21.5 ppm) (Fig. 1B). Evidently it has a mutation that reduces Cr accumulation; if instead it had an increased ability to detoxify Cr it might be expected to accumulate more Cr than WT. Thus chromate-sensitive mutant of *Schizosaccaromyces pombe* accumulate more Cr, whereas chromate-resistant mutant accumulate less (Czakó-Vér, 1999). Chromate-sensitive mutant of *S. cerevisiae*, which lack vacuoles or have defective vacuoles, also accumulate more Cr in their cytosol (Gharieb and Gadd, 1998).

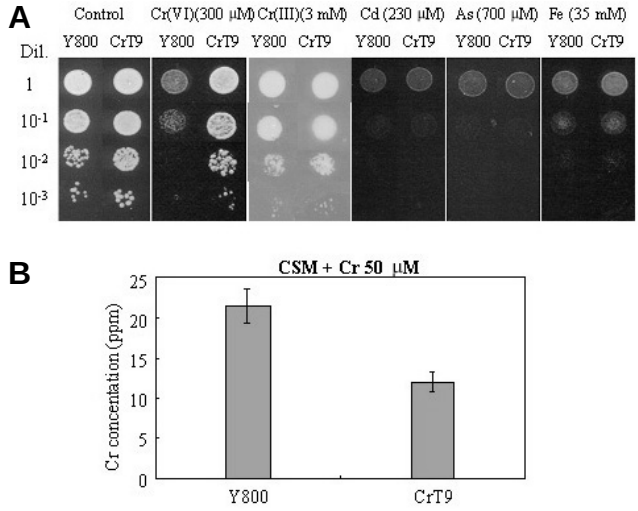

Fig. 1. Chromate tolerant CrT9 mutant accumulates less Cr. **A.** Chromate tolerance of CrT9. WT (Y800) and CrT9 cells were grown to OD_{600} 1.2 in CM liquid medium, diluted and cultured for 4 days on CM agar containing potassium chromate (300 μM), chromium chloride (3 mM), cadmium chloride (230 μM), sodium arsenite (700 μM) or iron sulfate (35 mM). **B.** Lower accumulation of Cr in CrT9. Y800 and CrT9 cells were cultured for 24 h in CM liquid medium containing 50 μM potassium chromate, harvested, dried, and Cr content measured. All values are averages of 3 measurements, and error bars represent SE.

***MSN1* is disrupted by mini-Tn in CrT9** To clone the gene mutated in CrT9, we performed IPCR (inverse PCR) (see **Materials and Methods**). A 1.2 kb IPCR product, consisting of 1,110 nt of genomic DNA and 90 nt of mini-Tn sequence (data not shown) was generated and sequenced, and the disrupted gene was identified as *MSN1* by searching the *Saccharomyces* genome database (SGD). By comparing the sequence of the IPCR product with the complete sequence of *MSN1*, we found that mini-Tn was inserted at position nt 741 of the 1,148 bp ORF (Fig. 2A).

We analyzed *MSN1* mRNA in CrT9 by Northern hybridization to confirm that *MSN1* was non-functional. As shown in Fig. 2B, the *MSN1* mRNA of CrT9 detected with an *MSN1* DNA probe migrated more slowly than that of WT, no doubt due to the insertion of mini-Tn. Hence it is clear that *MSN1* had been disrupted by the insertion of mini-Tn.

In order to rule out the possibility that the increased tolerance of CrT9 was attributable to insertion into some other gene than *MSN1*, we performed a genomic DNA Southern blot of CrT9 to see if mini-Tn had been inserted into any other genes. Because *MSN1* has single *Eco*RI and *Sac*I restriction sites, genomic DNA digested with *Eco*RI (E) or with *Sac*I (S) is cut into two fragments. As a result the *MSN1* probe hybridized with two genomic DNA fragments (Fig. 2C, left panel). When a mini-Tn probe was used to probe *Eco*RI (E) or *Sac*I (S) digested genomic DNA, it detected a single band of about 4.0 or 2.0 kbp, respectively (Fig. 2C, right panel) indicating that mini-Tn had inserted in just one site. The *MSN1* probe detected the same bands. Evidently the phenotype of increased Cr tolerance and lower Cr accumulation, is the result of a single insertion of mini-Tn into the *MSN1* gene.

***MSN1* is involved in chromate accumulation** To confirm that the reduced chromium accumulation in CrT9 is due to disruption of *MSN1*, wild type *MSN1* was overexpressed in CrT9 and its parent, under the control of the constitutive phosphoglycerate kinase (PGK) promoter (Mellor *et al.*, 1985). In brief, *MSN1* was introduced into vector pFL61 (Minet *et al.*, 1992) with URA3 as selective marker, and this was transformed into CrT9 and WT by the LiAc/PEG method (Ausubel *et al.*, 1999), and transformants were selected on CM agar medium without uracil. 20 μg of total RNA was extracted from three inde

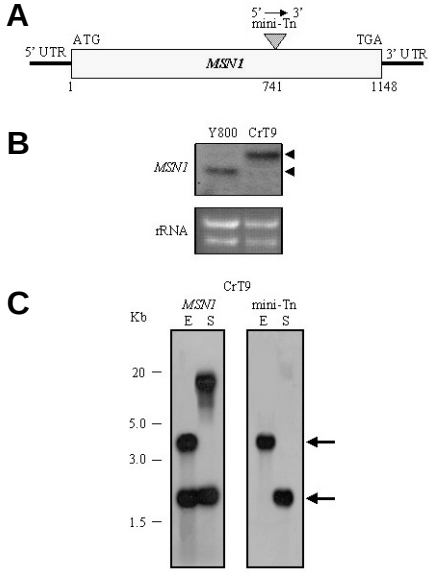

Fig. 2. *MSN1* is disrupted by a single insertion of mini-Tn in CrT9. **A.** Diagram of the disrupted *MSN1* gene as identified by IPCR. The position of mini-Tn insertion is indicated by a triangle. **B.** Larger *MSN1* mRNA in CrT9. Total RNA (20 μg) was electrophoresed, blotted and hybridized with an *MSN1* DNA probe. Equal loading of mRNA was confirmed by the amount of rRNA. **C.** Detection of the same CrT9 genomic DNA fragments by *MSN1* and mini-Tn probes. Genomic DNA of CrT9 was digested with *Eco*RI (E) and *Sac*I (S) respectively, blotted and hybridized with *MSN1* or mini-Tn probes. Since *MSN1* has a single internal site for *Eco*RI and *Sac*I, the *MSN1* probe hybridized to two bands. The mini-Tn probe also hybridized with one of the two bands, as indicated by the arrowheads.

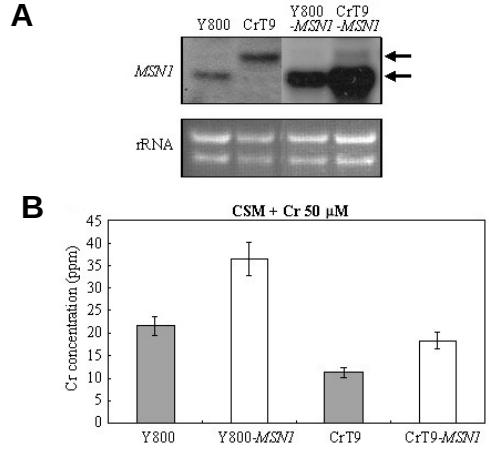

Fig. 3. Increased Cr accumulation due to over-expression of *MSN1* in WT (Y800) and the CrT9 mutant. **A.** Increased *MSN1* mRNA levels in Y800 and CrT9 *MSN1* transformants. 20 μg of total RNA of Y800, CrT9, *MSN1*-expressing Y800 and *MSN1*-expressing CrT9 cells were electrophoresed, blotted and hybridized with an *MSN1* DNA probe. The fast migrating band is *MSN1* mRNA, and the slowly migrating one is *MSN1* mRNA with a mini-Tn insert found in CrT9. **B.** Increased Cr accumulation in Y800 and CrT9 *MSN1* transformants. Cells were cultured for 24 h in CM liquid medium with 50 μM potassium chromate, harvested, dried, and their Cr content determined by ICP-AES. All values are averages of 3 experiments, and error bars indicate SE.

pendent transformants and Northern blot analysis performed. Over 90% of the WT and CrT9 transformants showed increased *MSN1* mRNA levels (data not shown). As there was no difference in *MSN1* mRNA expression between the three selected transformants examined that had higher levels of *MSN1*mRNA, we show the *MSN1* mRNA from only one of them (Fig. 3A). The larger-sized *MSN1* mRNA was also present. The WT (Y800) transformants also had higher levels of *MSN1* mRNA.

To test whether increased *MSN1* expression augments Cr accumulation, Cr levels were measured in the transformed yeast cells (Fig. 3B): the Cr level of CrT9 expressing *MSN1* (18.2 ppm) was higher than that of CrT9 (12.0 ppm) but lower than that of Y800 (WT) (21.5 ppm). Over-expression of *MSN1* also increased Cr accumulation in Y800 expressing *MSN1* (36.5 ppm). Cr tolerance was not altered in Y800 or CrT9 over-expressing *MSN1* (data not shown). These findings indicate that *MSN1* is involved in prmoting Cr accumulation in *S. cerevisiae*.

However, *MSN1* mRNA levels did not exactly correlate with Cr levels: *MSN1*-expressing CrT9 cells had the highest level of *MSN1* mRNA, but their Cr accumulation was less than WT (Y800) or WT expressing *MSN1*. We speculate that the protein level of *MSN1* may not be increased to the level of WT in the *MSN1*-expressing CrT9 transformant, even though its mRNA level is increased. We were able to rule out the possibility that *MSN1* was not expressed in the *MSN1* transformants of CrT9, since Cr accumulation was increased in all them and we did not detect increased Cr levels in those CrT9 transformants in which no wild type *MSN1* mRNA was detected (data not shown). It is possible that the level of MSN1 protein does rise in *MSN1*-expressing CrT9, but downstream genes involved in chromate accumulation are not activated to the same extent by MSN1. Finally, it is conceivable that a mutant form of MSN1 derived from the disrupted *MSN1* gene may act as dominant negative mutant interfering with expression of the wild type MSN1 protein.

How does MSN1 increase Cr accumulation? The most obvious possibility is that MSN1 increases Cr uptake by inducing or activating a transporter involved in chromate uptake. Chromate seems to enter yeast cells via a sulfate transporter (Cherest *et al.*, 1997; Smith *et al.*, 1995). Therefore, to examine if MSN1 affects the expression of sulfate transporter genes in yeasts, we compared the levels of mRNA for the high affinity sulfate transporters *SUL1* (Smith *et al.*, 1995) and *SUL2* (Cherest *et al.*, 1997) in WT(Y800) and CrT9. As shown in Fig. 4 (right panel), transcripts of *Sul1* and *Sul2* were lower in CrT9, implying

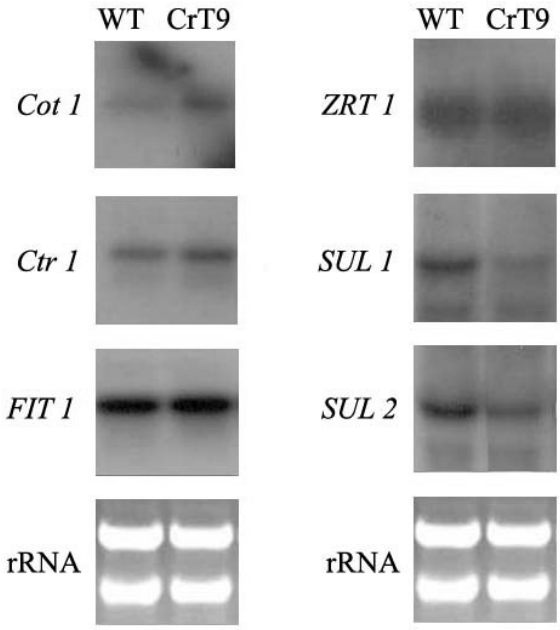

Fig. 4. Decreased levels of *SUL1* and *SUL2* mRNA in CrT9. 20 μg of total RNA of Y800 and CrT9 was electrophoresed, blotted and hybridized with DNA probes for *Cot1*, *Ctr1*, *FIT1*, *ZRT1*, *SUL1 and SUL2* prepared as described in **Materials and Methods**. The *rRNA* indicates equal loading.

that *MSN1* plays a role in the expression of *Sul1* and *Sul2*. Levels of messages for transporter *Cot1* for cobalt (Conklin *et al.*, 1992), *Ctr1* for copper (Dancis *et al.*, 1994), *Fit1* for iron (Protchenko *et al*., 2001), and *Zrt1* for zinc (Zhao and Eide, 1996) were unaltered. We infer therefore that *MSN1* increases Cr accumulation by activating high affinity sulfate transporters.

Involvement of *MSN1* in accumulating heavy metals was reported by Eide and Guarante (1992). When they expressed MSN1 in *S. cerevisiae* using a high copy number plasmid, growth in iron-limiting medium was promoted. However, when they disrupted *MSN1* by homologous recombination, the rate of iron uptake did not change in glucose medium, but it did decline two-fold in raffinose medium. In contrast, we found that *MSN1* expression elevated Cr accumulation, and *MSN1* disruption reduced it, regardless of the amount of Cr, or the presence of raffinose, in the medium (data not shown). We conclude that even though the transporters for Fe and Cr may be different, the accumulation of both metals is influenced by *MSN1*.

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References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1999) *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., Unit13.

Cary, E. E. (1982) Chromium in air, soil and natural waters; in *Biological and Environmental Aspects of Chromium*, Langard, S. (ed..), pp. 48-64, Elsevier, Amsterdam.

Cherest, H., Davidian, J. C., Thomas, D., Benes, V., Ansorge, W., and Surdin-Kerjan, Y. (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* **145**, 627–635.

Conklin, D. S., McMaster, J. A., Culbertson, M. R., and Kung, C. (1992) *Cot1*, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 3678–3688.

Czakó-Vér, K., Batiè, M., Raspor, P., Sipiczki, M., and Pesti, M. (1999) Hexavalent chromium uptake by sensitive and tolerant mutants of *Schizosaccharomyces pombe*. *FEMS Microbiol. Lett.* **178**, 109–115.

Dancis, A., Yuan, D. S., Haile, D., Askwith, C., Eide, D., Moehle, C., Kaplan, J., and Klausner, R. D. (1994) Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. *Cell* **76**, 393–402.

Eide, D. and Guarente, L. (1992) Increased dosage of a transcriptional activator gene enhances iron-limited growth of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **138**, 347–354.

Estruch, F. and Carlson, M. (1990) Increased dosage of the *MSN1* gene restores invertase expression in yeast mutants defective in the SNF1 protein kinase. *Nucleic Acids Res.* **18**, 6959–6964.

Galli, A., Boccardo, P., Del Carratore, R., Cundari, E., and Bronzetti, G. (1985) Conditions that influence the genetic activity of potassium dichromate and chromium chloride in *Saccharomyces cerevisiae*. *Mutat. Res.* **144**, 165–169.

Gharieb, M. M. and Gadd, G. M. (1998) Evidence for the involvement of vacuolar activity in metal(loid) tolerance: vacuolar-lacking and -defective mutants of *Saccharomyces cerevisiae* display higher sensitivity to chromate, tellurite and selenite. *BioMetals* **11**, 101–106.

Gimeno, C. J. and Fink, G. R. (1994) Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol. Cell. Biol.* **14**, 2100–2112.

Henderson, G. (1989) A comparison of the effects of chromate, molybdate and cadmium oxide on respiration in the yeast *Saccharomyces cerevisiae*. *Biol. Met.* **2**, 83–88.

Hong, S. K., Han, S. B., Snyder, M., and Choi, E. Y. (1999) SHC1, a high pH inducible gene required for growth at alkaline pH in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* **255**, 116–122.

Hwang, S. and Herrin, D. L. (1994) Control of *lhc* gene tran-

scription by the circadian clock in *Chlamydomonas reinhardtii*. *Plant Mol. Biol.* **26**, 557–569.

Lambrechts, M. G., Sollitti, P., Marmur, J., and Pretorius, I. S. (1996) A multicopy suppressor gene, *MSS10*, restores *STA2* expression in *Saccharomyces cerevisiae* strains containing the *STA10* repressor gene. *Curr. Genet.* **29**, 523–529.

Mellor, J., Dobson, M. J., Roberts, N. A., Kingsman, A. J., and Kingsman, S. M. (1985) Factors affecting heterologous gene expression in *Saccharomyces cerevisiae*. *Gene* **33**, 215–226.

Minet, M., Dufour, M. E., and Lacroute, F. (1992) Complementation of *Saccharomyces cerevisiae* auxotrophic mutants by *Arabidopsis thaliana* cDNAs. *Plant J.* **2**, 417–422.

Protchenko, O., Ferea, T., Rashford, J., Tiedeman, J., Brown, P. O., Botstein, D., and Philpott, C. C. (2001) Three cell wall mannoproteins facilitate the uptake of iron in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**, 49244–49250.

Rep, M., Reiser, V., Gartner, U., Thevelein, J. M., Hohmann, S., Ammerer, G., and Ruis, H. (1999) Osmotic stess-induced gene expression in *Saccharomyces cerevisiae* requires Msn1p and the novel nuclear factor Hot1p. *Mol. Cell. Biol.* **19**, 5474–5485.

Ross-Macdonald, P., Sheehan, A., Roeder, G. S., and Snyder, M. (1997) A multipurpose transposon system for analyzing protein production, localization, and function in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**, 190–195.

Sidorova, J. and Breeden, L. (1999) The *MSN1* and *NHP6A* genes suppress *SWI6* defects in *Saccharomyces cerevisiae*. *Genetics* **151**, 45–55.

Smith, F. W., Hawkesford, M. J., Prosser, I. M., and Clarkson, D. T. (1995) Isolation of a cDNA from *Saccharomyces cerevisiae* that encodes a high affinity sulfate transporter at the plasma membrane. *Mol. Gen. Genet.* **247**, 709–715.

Zhao, H. and Eide D. (1996) The yeast *ZRT1* gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proc. Natl. Acad. Sci. USA* **93**, 2454–2458.