Biochemical and Biophysical Research Communications **282,** 79–83 (2001)
doi:10.1006/bbrc.2001.4522, available online at http://www.idealibrary.com on IDEAL®

The Zrc1 Is Involved in Zinc Transport System between Vacuole and Cytosol in *Saccharomyces cerevisiae*

Shinji Miyabe, Shingo Izawa, and Yoshiharu Inoue[1]
Research Institute for Food Science, Kyoto University, Uji, Kyoto 611-0011, Japan

Received February 13, 2001

The *ZRC1* gene encodes a multicopy suppressor of zinc toxicity in *Saccharomyces cerevisiae;* however, previously we found that the expression of *ZRC1* was induced when the intracellular zinc level was decreased. Zrc1 has six putative transmembrane domains and we determined that a Zrc1-GFP fusion protein was localized to the vacuolar membrane. The steady state level of intracellular zinc in a *zrc1*Δ mutant cultured in the zinc-abundant medium was lower than that in wild type. No distinct difference was observed in the basal activity of glyoxalase I, which is a cytosolic enzyme requiring zinc for catalytic function and is used here as a marker for cytosolic zinc-availability, between wild type and *zrc1*Δ mutant, although the activity was decreased much greater extent in the *zrc1*Δ mutant if the cells were exposed to the metal-limited medium. Similarly, the basal expression level of *ZRC1-lacZ* reporter gene in *zrc1*Δ mutant was the same as that in wild type; however, the fold of induction of *ZRC1-lacZ* expression in *zrc1*Δ mutant under the zinc-limited conditions was higher than that in the wild type. Based on these results, we present a tentative model for the function of Zrc1 as a mechanism to maintain the zinc homeostasis in yeast. © 2001 Academic Press

Key Words: **zinc homeostasis; *Saccharomyces cerevisiae; ZRC1; ZRT3;* vacuole; glyoxalase I.**

Zinc is an essential metal for all types of organisms and is involved in many biological activities in the cell. For example, zinc is required for the catalytic process of several metalloenzymes and stabilization of protein structure (1, 2). In particular, the studies on importance of Zn^{2+} in transcription factors have been progressed dramatically. Several conserved motifs, such as zinc finger, RING finger and zinc cluster, have been found in which zinc is required for stabilization of their structures. On the contrary, an excessive amount of zinc is toxic to the cell, therefore, intracellular zinc level must be controlled appropriately. Zinc homeostasis in *Saccharomyces cerevisiae* has being actively studied by Eide and his colleagues. For instance, Gitan *et al.* reported that Zrt1, a high-affinity zinc transporter protein on the cytoplasmic membrane (3), was ubiquitinated, taken into the cell by endocytosis and degraded in the vacuole when the cells were exposed to excess zinc (4, 5). This posttranslational regulation is one of the mechanisms which prevent the cell from overaccumulation of zinc. On the other hand, Kamizono *et al.* cloned the *ZRC1* gene as a multicopy suppressor of zinc toxicity from *S. cerevisiae* (6). The *ZRC1*-overexpressing strain could grow on an SD agar plate containing 40 mM Zn^{2+}, whereas wild type showed slow growth on the 5 mM Zn^{2+}-containing medium (6). They also showed that the *zrc1*Δ mutant exhibited slow growth on an SD agar plate containing 1 mM Zn^{2+}. However, the mechanisms explaining how Zrc1 confers resistance against excess amount of zinc have not yet been clarified in detail.

Because the overproduction of Zrc1 conferred resistance to excess zinc (6), the role of Zrc1 had been considered only in the environments where high concentrations of zinc are present. However, previously we showed that the growth of the *zrc1*Δ mutant was defective under zinc-limited conditions and it was restored only when the zinc is supplied (7). We also demonstrated that the expression of *ZRC1* was induced in response to a decrease in the intracellular zinc level (7). In this study, we present a tentative model for the function of Zrc1 in zinc homeostasis in yeast.

MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* YPH250 (*MAT***a** *trp1-*Δ*1 his3-*Δ*200 lys2-801 leu2-*Δ*1 ade2-101 ura3-52*) was obtained from the Yeast Genetic Stock Center, University of California, Berkeley. Construction of *zrc1::HIS3* mutant was described previously (7). The *ZRT3* gene was amplified by PCR using primers ZRT3-F2 (5′-GGTAGCAACGAAAGCTAGTCGACGAACGTT-3′) and ZRT3-R3H

Abbreviations used: GFP, green fluorescent protein; ZRE, zinc-responsive element; CDF, cation diffusion facilitator; ZIP, ZRT, IRT-like protein.

[1] To whom correspondence should be addressed. Fax: +81-774-33-3004. E-mail: inoue@food2.food.kyoto-u.ac.jp.

0006-291X/01 $35.00
Copyright © 2001 by Academic Press
All rights of reproduction in any form reserved.

(5′-TGACATCAAGCTTCCAATTCCCAGTGATGC-3′), where *Hin*dIII site for ZRT3-R3H is underlined. The amplified DNA fragment was digested with *Hin*dIII and the fragment was cloned into the *Hin*dIII site of pUC19 to form pUCZRT3. This plasmid was digested with *Nsp*V, followed by Klenow treatment, and a 784-bp fragment in the open reading frame of the *ZRT3* gene was replaced with the *LEU2* gene which was isolated from YEp13 by digesting with *Sal*I and *Xho*I followed by treatment with Klenow fragment. The resultant plasmid (pUzrt3ΔLEU2) was digested with *Hin*dIII, and the *zrt3*Δ::*LEU2* cassette was introduced into wild type and *zrc1*Δ mutant, respectively. Transformation of yeast was done by electroporation and disruption of the *ZRT3* gene was confirmed by PCR.

Media and growth conditions. Yeast cells were cultured at 30°C in SD minimal medium (2% glucose, 0.67% yeast nitrogen base, pH 5.5) supplemented with appropriate amino acids and bases. Metal-limited conditions were achieved by the addition of 1 mM EDTA in the SD minimal medium as described by Zhao and Eide (8).

Construction of Zrc1-GFP. The plasmid pKR1 contains the GFP (green fluorescent protein) gene fused with the cDNA of nucleoplasmin (9). The GFP gene with the stop codon was amplified by PCR using the pKR1 as a template. The primers used are GFP1 (5′-CCCAAGCTTATGAGTAAAGGAGAAGAACTT-3′) and GFP2 (5′-GTTGCGGAAGCGGCCGCGAATTTATTTGTA-3′), where *Hin*dIII site for GFP1 and *Not*I site for GFP2 are underlined, and the stop codon is shown by italic letters in GFP2. A 0.7-kb GFP fragment amplified by PCR was digested with *Hin*dIII and *Not*I, and inserted into the *Hin*dIII and *Not*I site of pKR1 to replace the GFP-nucleoplasmin fragment with the GFP gene, and the resultant plasmid was named pKR1-GFPw/STOP. To obtain the *ZRC1* lacking its original stop codon, the PCR primers were designed as follows: OSR1N (5′-AGAGTCATGATCACCGGTAAAGAATTGAGA-3′) and OSR1C (5′-CCAGGCAATTGGAAGTATTGCAGTTTACAG-3′). The initiation codon for the *ZRC1* in OSR1N is underlined. The 1.3-kb PCR fragment was treated with T4 polymerase to generate blunt ends, and then introduced to pKR1-GFPw/STOP, which was previously digested with *Hin*dIII and treated with Klenow fragment, to form pKR1-ZRC1-GFP. The recombinant plasmid was introduced to the *zrc1*::*HIS3* mutant, and the *ZRC1-GFP* was confirmed to complement the zinc sensitivity.

β-Galactosidase assay. *ZRC1-lacZ* fusion gene was constructed previously (7). Yeast cells cultured under various growth conditions were collected by centrifugation, washed twice with 0.85% NaCl solution, and suspended in 10 mM potassium phosphate buffer (pH 7.0). Cells were transferred to an Eppendorf tube containing an approximately equal volume of glass beads. Tubes were agitated with vortex mixer at maximum speed for 30 s and then placed on ice for 30 s. Agitating and cooling was repeated 6 times. Cell homogenates were centrifuged at 14000 rpm for 15 min at 4°C, and resultant supernatants were used as cell extracts. β-Galactosidase activity was determined as described by Miller (10). One unit of the activity was defined as $A_{420} \times 1000$ per minute normalized to protein used.

Glyoxalase I assay. After the cultivation of the cells in the medium with or without 1 mM EDTA in SD minimal medium, cells were collected by centrifugation, washed twice with 0.85% NaCl solution, and cell extracts were prepared as described above. Assay for glyoxalase I activity was done as described previously (11). One unit of the activity was defined as the amount of enzyme forming 1 μmol of *S*-D-lactoylglutathione per minute at 25°C (11). Protein concentration was determined by the method of Bradford (12).

Fluorescence microscopy. Yeast cells were cultured at 30°C in SD minimal medium to mid-log phase. Cells were collected, resuspended in SD medium at 40 OD_{610}unit/ml and 20 mM FM4-64 (Molecular Probes Inc., Eugene, OR) was added. After 30-min incubation at 30°C, cells were collected and resuspended in fresh SD medium without FM4-64 at 20 OD_{610}unit/ml. Cells were cultured for another 1 h as chase period prior to take a view of vacuolar membrane which is stained by FM4-64 (13).

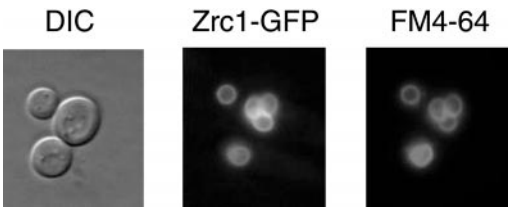

FIG. 1. Localization of Zrc1-GFP. The zrc1 mutant (*zrc1::HIS3*) expressing the *ZRC1-GFP* fusion gene under the control of *ADH1* promoter were cultured at 30°C in the SD medium until mid-log phase and analyzed by fluorescence microscopy. For visualization of the vacuolar membrane, cells were treated with FM4-64. DIC, difference interference contrast.

Atomic absorption analysis. After cultivation, cells were collected by centrifugation, washed twice with distilled water, and then twice with 1 mM EDTA. The cells were resuspended in zinc-free distilled water (atomic absorption analysis grade) (Nacalai tesque, Kyoto, Japan) at 5 OD_{610}unit/ml, and then applied to the atomic absorption analysis by using SPCA-6610 (Shimadzu, Kyoto, Japan). Zinc concentration was normalized to the OD_{610} unit.

RESULTS AND DISCUSSION

Localization of Zrc1

To explore the role of Zrc1 in zinc homeostasis in yeast, intracellular localization was investigated. Based on the amino acid sequence, Zrc1 is predicted to be an integral membrane protein with six putative transmembrane domains. Conklin *et al.* predicted that Zrc1 was localized to the mitochondrial membrane because of its sequence similarity to the Cot1, a cobalt-resistance protein, which was reported to be bound to the mitochondrial membrane (14). On the other hand, Li and Kaplan demonstrated that epitope-tagged Zrc1 was localized to the vacuole but they did not mention whether it binds to the vacuolar membrane or not (15). We then constructed the Zrc1-GFP fusion protein to determine the intracellular localization of Zrc1 more precisely. Since the *ZRC1-GFP* fusion gene expressed under the control of *ADH1* promoter could complement the zinc sensitivity of *zrc1*Δ mutant (data not shown), the fusion protein was confirmed to retain its original function. As shown in Fig. 1, when the exponentially growing cells were applied to fluorescence microscopy analysis, green fluorescence resulting from the Zrc1-GFP showed a ring-like pattern in the cells. We speculated that this pattern might reflect vacuolar membrane location. We then stained the vacuolar membrane with FM4-64 to identify the localization of this organelle. The staining pattern of Zrc1-GFP was coincident with the fluorescence of FM4-64, revealing that the Zrc1 was localized to the vacuolar membrane.

Zrc1 Modulates Intracellular Zinc Homeostasis

The expression of *ZRC1* was induced when the zinc level in the cells was lowered, although it was re-

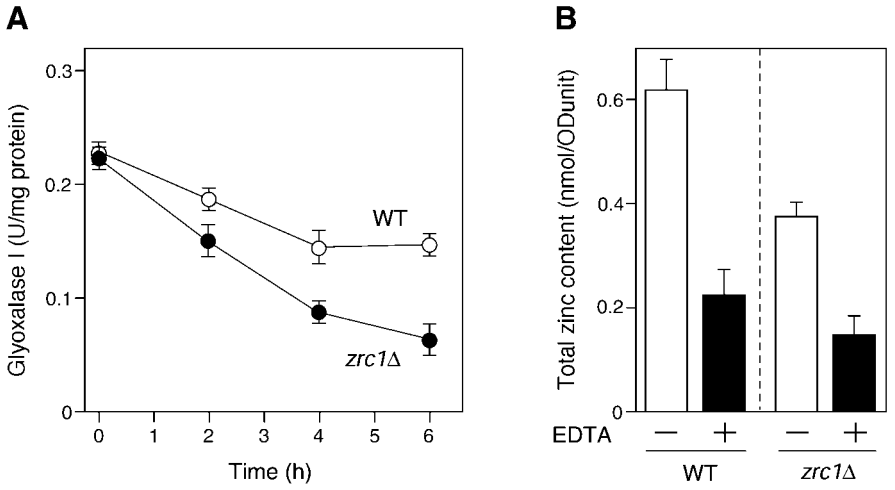

FIG. 2. Effect of disruption of *ZRC1* on glyoxalase I activity and zinc level under zinc-limited conditions. (A) Cells were cultured at 30°C in SD medium until $OD_{610} = 0.5$. After cultivation, the cells were collected, washed with 0.85% NaCl solution, and resuspended in SD medium with 1 mM EDTA. Cells were continued to culture at 30°C, collected periodically and glyoxalase I activity was measured. Symbols used: open circle, wild type; closed circle, *zrc1*Δ mutant. (B) Cells were cultured as described above. After shift to SD medium containing 1 mM EDTA, cells were cultured for 6 h and total zinc content was measured. Results indicate the average ± S.D. of three independent experiments.

pressed by the addition of zinc (7). These observations imply some roles of Zrc1 under zinc-limited conditions. On the other hand, the expression of *ZRC1* was constitutive and disruption of the gene enhanced the susceptibility to zinc, while the overexpression conferred resistance to zinc toxicity (6). These phenotypes can be explained by proposing that the Zrc1 expressed at normal levels transfers the zinc from cytosol to vacuole under the normal zinc-level conditions to form the zinc pool in vacuole. In addition, overexpressed Zrc1 transports elevated levels of zinc into the vacuole to reduce the toxicity of zinc in the cytosol. Since no one can measure zinc concentration in cytosol and vacuole separately (16), we measured glyoxalase I activity here to estimate zinc level in the cytosol. In *S. cerevisiae*, the glyoxalase I is present in cytosol and zinc directly participates in the catalytic function (11, 17, 18). As shown in Fig. 2A, no distinct difference was observed in the basal activity of glyoxalase I in the SD minimal medium without EDTA between wild type and *zrc1*Δ mutant. However, upon the imposition of the cells to zinc limitation in SD medium containing 1 mM EDTA, the glyoxalase I activity in the *zrc1*Δ mutant was lost much faster than that in wild type and 70% of the activity was lost within 6 h. In contrast, the wild type strain still maintained more than 50% activity after 6 h (Fig. 2A). We confirmed that expression of the structural gene for glyoxalase I (*GLO1*) was not affected by EDTA treatment (data not shown) by using the *GLO1-lacZ* reporter gene (19).

We also measured the steady state levels of zinc in wild type and an isogenic *zrc1*Δ mutant. As shown in Fig. 2B, the amount of zinc in the *zrc1*Δ mutant was approximately 40% lower than that in wild type. Furthermore, the zinc content in a *zrc1*Δ mutant strain decreased greater than in wild type if the cells were transferred to EDTA-containing medium. The fact that basal activity of glyoxalase I was the same in both wild type and *zrc1*Δ mutant (Fig. 2A) led us to suspect that the availability of zinc in cytosol was essentially equal between these two strains. Therefore, the difference in the steady state level of zinc in these strains was thought to reflect the difference in the zinc pool in the vacuole. Conklin *et al.* reported that a *zrc1*Δ mutant accumulated less amount of cobalt in the cells (20). However, as far as we tested, the growth defect of *zrc1*Δ mutant under metal-limited conditions was specifically restored by Zn^{2+}, while Co^{2+} did not rescue it (7). Furthermore, induced expression of *ZRC1* by metal-starvation stress was repressed by Zn^{2+} but not by Co^{2+}. The *ZRC1* promoter has ZRE (zinc-responsive element) and expression of *ZRC1* was regulated by Zap1-dependent fashion (7). Zap1 is a transcription factor which is critical for zinc-responsive genes (21–23). In addition, basal activity of cytosolic zinc-dependent enzyme, glyoxalase I, was normal in both wild type and *zrc1*Δ mutant (Fig. 2A), but steady state level of intracellular zinc in the *zrc1*Δ mutant was lower than that in wild type (Fig. 2B). This indicates that zinc levels in the vacuole, but not in the cytosol, are low. From these observations, we concluded that Zrc1 functions to maintain the vacuolar zinc pool.

Recently, MacDiarmid *et al.* (16) cloned the *ZRT3* gene which belongs to ZIP (ZRT, IRT-like protein) family (24–26). The proteins involved in this family have common structural similarity; i.e., they have eight transmembrane domains and have histidine-rich sequences. The expression of *ZRT3* was induced by zinc-starvation stress in a Zap1-dependent fashion. Zrt3 localizes to vacuolar membrane and its function is pre-

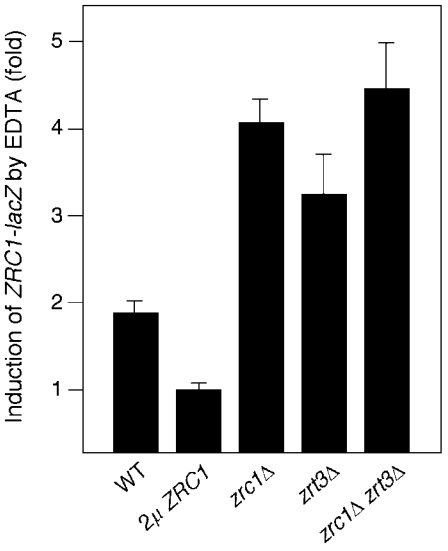

FIG. 3. Effect of disruption of *ZRC1* on expression of the *ZRC1-lacZ* reporter gene under zinc-limited conditions. Cells were cultured at 30°C in SD medium containing 1 mM $ZnCl_2$ until OD_{610} = 1.0. After cultivation, the cells were collected, washed with 0.85% NaCl solution, and resuspended in SD medium with 1 mM EDTA. Cells were continued to culture at 30°C for 16 h and β-galactosidase activity derived from *ZRC1-lacZ* (pZRC1F) (7) was assayed. Results indicate the average ± S.D. of three independent experiments.

dicted to mobilize zinc from vacuole if intracellular zinc level is lowered (16). On the other hand, Zrc1 structurally belongs to the CDF (cation diffusion facilitator) family and seems to function in zinc efflux. We have confirmed that Zrc1 was localized to the vacuolar membrane (Fig. 1) and it functioned to form zinc pool in vacuole. The notable feature of regulation of *ZRT3* and *ZRC1* is that expression of both genes is induced by Zap1 when intracellular zinc levels are decreased (7, 16). This indicates that both gene products function under zinc-limited conditions. If Zrc1 transports cytosolic zinc into vacuole and Zrt3 mobilizes it from this organelle, then expression of the ZRE-driven *ZRC1–lacZ* fusion gene would be elevated in the *zrc1*Δ mutant much higher extent compared with wild type under zinc-limited conditions. Because the zinc level in the vacuole in the *zrc1*Δ mutant background is thought to be lower than that in wild type (Fig. 2B) and only limited amount of zinc can be mobilized from zinc pool by Zrt3, concentration of zinc in cytoplasm in the *zrc1*Δ mutant may be much lower than that in wild type if the cells are exposed to zinc-limited conditions. As we expected, the β-galactosidase activity derived from *ZRC1-lacZ* was higher in the *zrc1*Δ mutant than in wild type under zinc-limited conditions (Fig. 3), although it was the same under zinc-abundant conditions between wild type and *zrc1*Δ mutant. Furthermore, if *ZRC1* gene was overexpressed (YEp13 + *ZRC1*, 2 μ *ZRC1*) under zinc-abundant conditions and then such cells were shifted to the zinc-limited medium, the expression of *ZRC1-lacZ* was not increased (Fig. 3). Presumably this may be due to that higher amount of zinc was pooled in the vacuole in the *ZRC1*-overexpressed cell. These results strongly suggest that the amount of zinc in vacuole in the *zrc1*Δ mutant is low. On the other hand, the expression of *ZRC1-lacZ* is expected to be increased much higher extent in the *zrt3*Δ mutant than that in wild type under zinc-starved conditions, because zinc can not be mobilized from vacuole if *ZRT3* gene is destroyed. To confirm this, we disrupted the *ZRT3* gene carrying the *ZRC1-lacZ* reporter gene. As shown in Fig. 3, induction rate of *ZRC1-lacZ* expression in the *zrt3*Δ mutant under zinc-limited conditions was higher than that in wild type. Similarly, simultaneous disruption of *ZRC1* and *ZRT3* genes enhanced induction of the ZRE-driven *ZRC1-lacZ* reporter gene under the same conditions.

Based on these observations, we propose a possible role of Zrc1 (Fig. 4). Both Zrt3 and Zrc1 are zinc transporter proteins on the vacuolar membrane. It has been well known that a clustered sequence of histidine and cysteine is involved in the binding site of Zn^{2+} (2). Zrc1 has three histidine repeat motifs (141-HSHSH-145; 163-HSHSH-167; and 216-HDHSH-220) between the 4th and 5th transmembrane domains and these motifs are predicted to be exposed to the cytoplasm (24). Zrc1 senses zinc availability in the cytosol, which might be performed through the histidine repeat motifs, and transports zinc from the cytosol to the vacuole if zinc in cytosol is abundant. This may be the reason why overproduction of Zrc1 confers resistance to zinc toxicity. On the other hand, if the cell is exposed to zinc-limited conditions, Zap1 is activated to induce expression of *ZRT1*, *ZRT2* and *ZRT3* to enhance the uptake of zinc from environment by Zrt1 and Zrt2 (3, 27) as well as

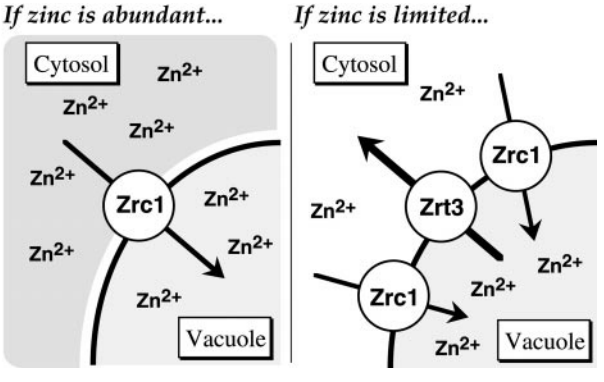

FIG. 4. A tentative model for function of Zrc1. If zinc is abundant in cytosol (left), Zrc1 transports Zn^{2+} into vacuole to form zinc pool. Zinc may be used by vacuolar enzymes. Once intracellular zinc level is lowered (right), Zap1 is activated to induce the expression of *ZRT1* and *ZRT2*. Both Zrt1 and Zrt2 take up Zn^{2+} from environment. In addition, expression of *ZRT3* is activated and Zrt3 mobilizes Zn^{2+} from vacuole. On the other hand, to maintain a certain level of zinc in vacuole, *ZRC1* expression is also enhanced in a Zap1-dependent manner and Zrc1 transports Zn^{2+} into the vacuole from cytosol.

the mobilization of zinc from vacuole by Zrt3 (16). However, if zinc level in the vacuole decreases too much extent, it may be harmful or inhibit normal metabolism in vacuole. Indeed, previously we showed that growth rate of *zrc1*Δ mutant was lowered under zinc-limited conditions (7). To warrant a certain level of zinc in vacuole, expression of the *ZRC1* gene is also induced by Zap1 under zinc-starved conditions (7, 25).

ACKNOWLEDGMENTS

We thank Dr. K. Kohno at Nara Institute of Science and Technology for pKR1. We also thank Dr. K. Fukuda for assistance in atomic absorption analysis and Y. Takatsume for technical assistance. We are grateful to Dr. W. S. Moye-Rowley for helpful discussion.

REFERENCES

1. Vallee, B. L., and Auld, D. S. (1990) Active-site zinc ligands and activated H_2O of zinc enzymes. *Proc. Natl. Acad. Sci. USA* **87,** 220–224.
2. Vallee, B. L., and Falchuk, K. H. (1993) The biochemical basis of zinc physiology. *Physiol. Rev.* **73,** 79–118.
3. 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.
4. Gitan, R. S., Luo, H., Rodgers, J., Broderius, M., and Eide, D. (1998) Zinc-induced inactivation of the yeast ZRT1 zinc transporter occurs through endocytosis and vacuolar degradation. *J. Biol. Chem.* **273,** 28617–28624.
5. Gitan, R. S., and Eide, D. J. (2000) Zinc-regulated ubiquitin conjugation signals endocytosis of the yeast ZRT1 zinc transporter. *Biochem. J.* **346,** 329–336.
6. Kamizono, A., Nishizawa, M., Teranishi, Y., Murata, K., and Kimura, A. (1989) Identification of a gene conferring resistance to zinc and cadmium ions in the yeast *Saccharomyces cerevisiae. Mol. Gen. Genet.* **219,** 161–167.
7. Miyabe, S., Izawa, S., and Inoue, Y. (2000) Expression of *ZRC1* coding for suppressor of zinc toxicity is induced by zinc-starvation stress in Zap1-dependent fashion in *Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun.* **276,** 879–884.
8. Zhao, H., and Eide, D. J. (1997) Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **17,** 5044–5052.
9. Lim, C. R., Kimata, Y., Oka, M., Nomaguchi, K., and Kohno, K. (1995) Thermosensitivity of green fluorescent protein fluorescence utilized to reveal novel nuclear-like compartments in a mutant nucleoporin NSP1. *J. Biochem.* (*Tokyo*) **118,** 13–17.
10. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
11. Inoue, Y., and Kimura, A. (1996) Identification of the structural gene for glyoxalase I from *Saccharomyces cerevisiae. J. Biol. Chem.* **271,** 25958–25965.
12. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72,** 248–254.
13. Vida, T. A., and Emr, S. D. (1995) A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. *J. Cell Biol.* **128,** 779–792.
14. 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.
15. Li, L., and Kaplan, J. (1998) Defects in the yeast high affinity iron transport system result in increased metal sensitivity because of the increased expression of transporters with a broad transition metal specificity. *J. Biol. Chem.* **273,** 22181–22187.
16. MacDiarmid, C. W., Gaither, L. A., and Eide, D. (2000) Zinc transporters that regulate vacuolar zinc storage in *Saccharomyces cerevisiae. EMBO J.* **19,** 2845–2855.
17. Marmstal, E., Aronsson, A. C., and Mannervik, B. (1979) Comparison of glyoxalase I purified from yeast (*Saccharomyces cerevisiae*) with the enzyme from mammalian sources. *Biochem. J.* **183,** 23–30.
18. Inoue, Y., and Kimura, A. (1999) Glycolytic-methylglyoxal pathway. Molecular evolution and stress response of glyoxalase I in *Saccharomyces cerevisiae. Proc. Jpn. Acad.* **75B,** 127–132.
19. Inoue, Y., Tsujimoto, T., and Kimura, A. (1998) Expression of the glyoxalase I gene of *Saccharomyces cerevisiae* is regulated by high osmolarity glycerol mitogen-activated protein kinase pathway in osmotic stress response. *J. Biol. Chem.* **273,** 2977–2983.
20. Conklin, D. S., Culbertson, M. R., and Kung, C. (1994) Interactions between gene products involved in divalent cation transport in *Saccharomyces cerevisiae. Mol. Gen. Genet.* **244,** 303–311.
21. Zhao, H., Butler, E., Rodgers, J., Spizzo, T., Duesterhoeft, S., and Eide, D. (1998) Regulation of zinc homeostasis in yeast by binding of the ZAP1 transcriptional activator to zinc-responsive promoter elements. *J. Biol. Chem.* **273,** 28713–28720.
22. Lyons, T. J., Gasch, A. P., Gaither, L. A., Botstein, D., Brown, P. O., and Eide, D. J. (2000) Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. *Proc. Natl. Acad. Sci. USA* **97,** 7957–7962.
23. Bird, A. J., Zhao, H., Luo, H., Jensen, L. T., Srinivasan, C., Evans-Galea, M., Winge, D. R., and Eide, D. J. (2000) A dual role for zinc fingers in both DNA binding and zinc sensing by the Zap1 transcriptional activator. *EMBO J.* **19,** 3704–3713.
24. Eide, D. J. (1998) The molecular biology of metal ion transport in *Saccharomyces cerevisiae. Annu. Rev. Nutr.* **18,** 441–469.
25. McMahon, R. J., and Cousins, R. J. (1998) Mammalian zinc transporters. *J. Nutr.* **128,** 667–670.
26. Paulsen, I. T., and Saier, M. J. (1997) A novel family of ubiquitous heavy metal ion transport proteins. *J. Membr. Biol.* **156,** 99–103.
27. Zhao, H., and Eide, D. (1996) The *ZRT2* gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae. J. Biol. Chem.* **271,** 23203–23210.