The sequence of the yeast gene YDR205W places it within the family of cation diffusion facilitators: membrane proteins that transport transition metals. Deletion of YDR205W was reported to result in an increase in unequal sister chromatid recombination and was named meiotic sister chromatid recombination 2 (MSC2; Thompson, D. A., and Stahl, F. W. (1999) Genetics 153, 621–641). We report here that a msc2 strain shows a phenotype of decreased viability in glycerol-ethanol media at 37 °C. Associated with decreased growth is an abnormal morphology typified by an increase in size of both cells and vacuoles. Addition of extracellular Zn2+ completely suppresses the morphological changes and partially suppresses the growth defect. Regardless of the concentration of Zn2+ in the media, the msc2 strain had a higher Zn2+ content than wild type cells. Zinquin staining also revealed that msc2 had a marked increase in fluorescence compared with the wild type, again reflecting an increase in intracellular Zn2+. The deletion strain accumulated excess Zn2+ in nuclei-enriched membrane fractions, and when grown at 37 °C in glycerol-ethanol media, it showed a decreased expression of membrane fractions, and when grown at 37 °C in glycerol-ethanol; PCR, polymerase chain reaction.

The cation diffusion facilitator (CDF)1 family consists of genes that encode transition metal/H+ antiporters (1). Most CDF genes were discovered through overexpression experiments, which resulted in resistance to toxicity from transition metals (2, 3). More recently, additional CDF members have been identified through sequence homology. Members of the CDF family are found in all biological kingdoms, and in eukaryotes, different CDF proteins may be localized to different organelles. In many cases, the normal function of CDF proteins is unknown. In a few cases, gene deletion studies or studies of naturally occurring mutants have defined a physiological role for CDF proteins. Gene knockout studies in mice have demonstrated that ZNT3 is responsible for the accumulation of vesicular Zn2+ in nerve cells (4). A mutation in a homologous gene, ZNT4, was shown to be responsible for the murine toxic milk syndrome. ZNT4 proved to be required for secretion of Zn2+ into breast milk (5).

The sequence of at least five genes in the budding yeast Saccharomyces cerevisiae places these genes within the CDF family. Two family members, COT1 and ZRC1, are localized to the vacuole (6) and confer resistance to Co2+ (7) and Zn2+ (8), respectively. Other family members, MMT1 and MMT2, encode mitochondrial proteins that were identified through a suppressor screen. Overexpression of either MMT1 or MMT2 was shown to affect Fe2+ levels in the mitochondria and cytosol (9). However, deletion of these genes, either alone or in combination, did not affect essential Fe2+-dependent mitochondrial processes, leaving their precise role in mitochondrial Fe2+ metabolism unclarified.

The yeast gene YDR205W shows extensive homology to members of the CDF family, with some notable differences. Most members of the CDF family contain six transmembrane domains with an extensive histidine-rich cluster in a cytosolic loop. The protein sequence of YDR205W, however, reveals 12 transmembrane domains and two histidine-rich cytosolic clusters. A deletion in YDR205W was identified in a screen of mutants that affected the rate of meiotic sister chromatid exchange. The gene is referred to as MSC2 (10). The selection system was based on transposon insertion. When the gene was deleted, no increase in unequal sister chromatid exchange was detected in the deletion strain. Thus, the role of the gene in recombination events is unclear (10). We report here the characterization of a msc2 strain. The deletion strain shows an increased sensitivity to H2O2 and is not viable at 37 °C in glycerol-ethanol media. We further show that the deletion strain has an alteration in cellular Zn2+ content leading to an increase in nuclear Zn2+ content in cells grown on respiratory substrates.

**MATERIALS AND METHODS**

**Strains and Media**—The yeast strains used in this study were DY150 and DY1457, which were derived from a W303 background. The diploid DY1640 was derived from a cross between DY150 and an isogenic strain with the opposite mating type, DY151 (11). The media used included: 1% yeast extract, 2.0% peptone, and 2.0% glucose (YPD); 1.0% yeast extract, 2.0% peptone, 2.0% ethanol, and 2.0% glycerol (YPGE); growth media with glycerol-ethanol (1.0% yeast extract, 2.0% peptone, 2.0% ethanol, and 2.0% glycerol; CM, synthetic medium with glucose; CMGE, synthetic medium with glycerol-ethanol; PCR, polymerase chain reaction.

**Construction of the msc2 Deletion Strain**—A double fusion PCR technique was used to delete YDR205W, generating the yeast strain msc2 (12). The primers designed to amplify the 5′ end of YDR205W were 5′-GCAAGGCATTGTATACGTCG-3′ and 5′-GTCGTAGCTGGGAAAA-

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The yeast gene YDR205W shows extensive homology to members of the CDF family, with some notable differences. Most members of the CDF family contain six transmembrane domains with an extensive histidine-rich cluster in a cytosolic loop. The protein sequence of YDR205W, however, reveals 12 transmembrane domains and two histidine-rich cytosolic clusters. A deletion in YDR205W was identified in a screen of mutants that affected the rate of meiotic sister chromatid exchange. The gene is referred to as MSC2 (10). The selection system was based on transposon insertion. When the gene was deleted, no increase in unequal sister chromatid exchange was detected in the deletion strain. Thus, the role of the gene in recombination events is unclear (10). We report here the characterization of a msc2 strain. The deletion strain shows an increased sensitivity to H2O2 and is not viable at 37 °C in glycerol-ethanol media. We further show that the deletion strain has an alteration in cellular Zn2+ content leading to an increase in nuclear Zn2+ content in cells grown on respiratory substrates.
Cloning of YDR205W and an Epitope-tagged Construct—A probe to YDR205W open reading frame was generated by PCR. Southern blot colony analysis (13) was used to identify YDR205W in a yeast genomic library. The genomic library was obtained from the American Type Culture Collection (ATCC#37323) and transformed into DH5α Escherichia coli. A plasmid containing the YDR205W open reading frame and a 1000-base pair upstream region was subcloned into both low copy and high copy vectors. The primers used were 5′-CCCCCAGCGCTTATGAAAGCAGTAATGGA-3′ and 5′-GCTCTAGATTAAATTCTCAATGCCTCAACGGCATTTGGTCTCATATTGCTAGTGCTAGGG-3′. A FLAG epitope construct was also generated by PCR using the primers 5′-CCCCCAGCGCTTATGAAAGCAGTAATGGA-3′ and 5′-AGGGAAAAAGCGGC-GGCTATCACCTGTCATCTCCCTGAAATCGGTTATGCTGAGG-3′. The PCR products were ligated into both low copy and high copy vectors.

β-Galactosidase Assay—A Zn-responsive β-galactosidase reporter construct containing the upstream sequence of the ZRT1 gene (14) was a gift from Dr. David Eide (University of Missouri, Columbia, MO). A Cu-sensitive β-galactosidase reporter construct containing the upstream sequence of CTR1 (15) was a gift from Dr. Dennis Winge (University of Utah, Salt Lake City, UT). A Fe-sensitive β-galactosidase reporter construct was created from a PCR fragment of the FET3 promoter region. The PCR primers used to construct the fragment were 5′-CCCCCAGGGTGCTTGGCTTTGCGATTTTCTGTTTCTG-3′ and 5′-AAAACTGCGAGCCATCTAGTTTTGCGTCTTTC-3′. The PCR product was cloned into the SmaI-PstI sites of YEp354, a LacZ expression vector (16). A plasmid containing the Zap1′′ allele was a gift from Dr. Dennis Winge. All plasmids were transformed into either wild type or msc2 cells. Cells were grown in glucose media or glycerol-ethanol media with different concentrations of transition metals for 3 or 4 h before the assay. β-Galactosidase activity was assayed as described previously (17).

S1 Ribonuclease Protection Assay—S1 analysis was performed as described previously (18). Briefly, 50 ng of RNA was hybridized over-night at 55 °C with an end-labeled oligonucleotide probe for ZRT1 or CMD1, which was used as an internal control. The RNA was digested by the addition of 50 units of S1 ribonuclease. The samples were analyzed by polyacrylamide gel electrophoresis, and the developed gels were analyzed by PhosphorImager analysis.

Subcellular Fractionation and Zn²⁺ Measurement—Cells grown in YPD were transferred to YPGE and grown for 12 h at 37 °C. Subcellular fractionation and organelle isolation were performed as described previously (19), with some modifications. Briefly, spheroplasts were homogenized with a Dounce homogenizer ("B" pestle). The homogenate was centrifuged at 3,000 × g for 5 min. The 3,000 × g pellet was resuspended in mitochondria isolation buffer and centrifuged at 120 × g for 5 min. The supernatant was collected by centrifugation and found by microscopy to be enriched in nuclei. The 3,000 × g supernatant was centrifuged at 12,000 × g for 10 min and separated into a pellet and supernatant. The pellet was applied to a 15% Percoll gradient and centrifuged at 59,000 × g for 27 min, and the gradient was fractionated as described previously (20). The 12,000 × g supernatant was centrifuged at 100,000 × g for 30 min and separated into a pellet and supernatant. Each fraction was analyzed for protein concentration using the BCA reagent (Pierce) and for Zn²⁺ content using a PerkinElmer Life Sciences inductively coupled plasma atomic absorption spectrometer as described previously (6).

Zinquin Staining, Immunofluorescence, and Western Blot Analysis—Zinquin (CF-125) a gift from Dr. Thomas V. O’Halloran (Northwestern University, Evanston, IL) was used as described previously (3). Cells grown in YPD were washed and transferred to YPGE. At specified times, cells were incubated with 25 µm zinquin for 30 min at 37 °C. Cells were washed twice with phosphate-buffered saline, placed on concanavalin A (1 mg/ml)-coated slides, and examined by fluorescence microscopy.

For immunofluorescence analysis, cells transformed with the Myc-tagged construct of Msc2p were prepared as described previously (6).

**Fig. 1. Growth of wild type and msc2 cells in YPGE media and in YMGE supplemented with H₂O₂.** Cells were spotted on YPGE plates and incubated at 30 °C or 37 °C for 3 days. DY1640 is a wild type diploid, DY1640/msc2 is a heterozygote, and 1a, 1b, 1c, and 1d are haploid spores. 1a and 1c are the cells carrying the deletion of MSC2 as confirmed by genetic and PCR analysis (A). Similar dilutions of cells were plated on YPGE plates that contained different concentrations of H₂O₂ (B).

**Fig. 2. Morphology of wild type and msc2 cells grown in glycerol-ethanol medium.** Cells grown in YPD medium were transferred to YE medium and incubated at 37 °C for 12 h. Wild type cells (A) and msc2 cells (B) were examined by phase-contrast microscopy and photographed.
The cells were stained with a 1:100 dilution of a monoclonal anti-Myc antibody (Babco Inc.), followed by a 1:200 dilution of an Alexa Fluor 594-conjugated goat anti-mouse antibody (Molecular Probes). Cells treated with the primary and secondary antibody were incubated with 5 μg/ml 4′,6-diamidino-2-phenylindole (Molecular Probes) for 5 min.

Cytoplasm and membrane fractions were isolated from cells transformed with the FLAG-tagged Msc2p-containing plasmid. Samples were run on a 4–20% SDS-polyacrylamide gel electrophoresis gel (Bio-Rad) and transferred to nitrocellulose. The blot was probed with a 1:5000 dilution of a mouse anti-FLAG antibody (M2; Sigma) followed by a 1:10,000 dilution of peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). The blot was developed with chemiluminescence reagents (Renaissance) from PerkinElmer Life Sciences.

**RESULTS**

**Phenotypes of the Δmsc2 Strain—** A yeast strain with a deletion in YDR205W (Δmsc2) was generated as described under “Materials and Methods.” The deletion strain, msc2, grew well in both YPD and CM at 30 °C and 37 °C. However, the strain showed a temperature-sensitive growth defect in YPGE media at 37 °C (Fig. 1A). The deletion strain also showed an increased sensitivity to H₂O₂, which was exacerbated by growth on glycerol-ethanol medium (Fig. 1B). Both the growth defect and the increased sensitivity to H₂O₂ were recessive traits because they were not expressed in the heterozygotic diploid. Microscopic examination of msc2 cells grown in YPGE at 30 °C revealed that a small percentage of the population had an abnormal morphology, typified by the presence of large cells containing giant granules. The majority of Δmsc2 cells showed the abnormal phenotype upon a shift in temperature to 37 °C (Fig. 2). Transformation of msc2 cells with either high or low copy plasmids containing MSC2 restored both normal growth and morphology. Increasing the concentration of Zn²⁺ in the medium restored normal morphology to msc2 cells. Increased media Zn²⁺ attenuated the growth defect of msc2 cells (Fig. 3A, a) but did not restore a normal growth rate (Fig. 3B). Supplementation of YPEG with other transition metals, such as Fe²⁺ (Fig. 3A, b), Cu²⁺ (Fig. 3A, c), or Mn²⁺ (Fig. 3A, d) had no effect on the growth defect or morphological abnormality of msc2 cells at 37 °C (data not shown).
MSC2 Affects the Cellular Distribution of Zinc

Alterations in Zn\textsuperscript{2+} Levels and Distribution in Δmsc2—The deletion phenotype suggests that MSC2 affects intracellular Zn\textsuperscript{2+} homeostasis. To test this hypothesis, we measured the transition metal content of wild type and msc2 cells grown in both glucose-containing and glycerol-ethanol-containing media. Cells grown at 30 °C in glycerol-ethanol media had a higher content of Fe\textsuperscript{2+}, Cu\textsuperscript{2+}, and Zn\textsuperscript{2+} than did cells grown in glucose-containing media (Table I). Although the absolute content of metals differed in each experiment, the relative difference between metals was constant. Of the three transition metals assayed, growth in glycerol-ethanol had a greater affect on Zn\textsuperscript{2+} than it did on Fe\textsuperscript{2+} or Cu\textsuperscript{2+}. The msc2 strain grown in either glucose or glycerol-ethanol had similar levels of Fe\textsuperscript{2+} and Cu\textsuperscript{2+} when compared with the wild type parent. However, when grown in glycerol-ethanol, msc2 cells had a 50% increase in intracellular Zn\textsuperscript{2+} compared with wild type cells. Increases in the Zn\textsuperscript{2+} content of msc2 cells were seen at several different concentrations of media Zn\textsuperscript{2+} (data not shown). Zn\textsuperscript{2+} content was not uniformly increased in all msc2 organelles. A membrane fraction had a higher Zn\textsuperscript{2+} content than did the cytosol (Fig. 4). Of all membrane fractions examined, nuclei-enriched fractions had the highest Zn\textsuperscript{2+} content. A more extensive subcellular fractionation was precluded by the loss of Zn\textsuperscript{2+} from cellular organelles.

We examined intracellular Zn\textsuperscript{2+} levels in msc2 using zinquin. Zinquin is a fluoresceine derivative that fluoresces when bound to Zn\textsuperscript{2+} and can be visualized microscopically. The intensity of the zinquin fluorescence changes as the levels of Zn\textsuperscript{2+} change. Wild type cells grown in glycerol-ethanol and incubated in zinquin show a diffuse cytosolic fluorescence. Both the level and distribution of fluorescence change dramatically when intracellular Zn\textsuperscript{2+} levels are increased. The Zn\textsuperscript{2+}-regulated transcription factor ZAP1 induces the transcription of genes involved in Zn\textsuperscript{2+} transport and metabolism (22). In the absence of Zn\textsuperscript{2+}, Zap1p permits the transcription of Zn\textsuperscript{2+}-regulated genes through its binding to specific DNA sequences termed zinc-responsive elements. Zap1p regulates the transcription of ZRT1, which encodes a high affinity Zn\textsuperscript{2+} transporter. Transformation of wild type cells with an allele of the Zn transcriptional regulator ZAPI\textsuperscript{up} results in constitutive expression of Zn-regulated genes, leading to an increased concentration of cellular Zn\textsuperscript{2+} (22). Cells expressing ZAPI\textsuperscript{up} show increased zinquin fluorescence, with the fluorescence localized to punctate vesicles. Δmsc2 cells also show a robust zinquin fluorescence with the signal concentrated in punctate vesicles (Fig. 5). These data support the observation that cellular Zn\textsuperscript{2+} levels are altered in the Δmsc2 strain. As discussed below, we feel that the vesicular location of the zinquin fluorescence does not reflect the normal distribution of Zn\textsuperscript{2+}.

Zn\textsuperscript{2+}-regulated Transcription in the Δmsc2 Strain—We took advantage of the ability of Zap1p to sense intracellular Zn\textsuperscript{2+} levels to further demonstrate that msc2 cells show an alteration in Zn\textsuperscript{2+} homeostasis. Wild type and msc2 cells were transformed with a plasmid carrying a Zn\textsuperscript{2+}-regulated β-galactosidase reporter construct derived from the promoter elements of ZRT1. As expected, incubation of cells in high Zn\textsuperscript{2+} medium resulted in increased transcription of the Zn\textsuperscript{2+}-regulated β-galactosidase activity (Fig. 6). Conversely, incubation of wild type cells in low Zn\textsuperscript{2+} media resulted in increased expression of the Zn\textsuperscript{2+} regulon and increased levels of β-galactosidase. However, incubation of msc2 cells in low Zn\textsuperscript{2+} media did not result in increased β-galactosidase activity, suggesting that Zap1p may be occupied by Zn\textsuperscript{2+} and thus transcriptionally inactive. The effect of the MSC2 gene deletion on Zn\textsuperscript{2+}-dependent β-galactosidase activity was seen only when cells were grown in glycerol-ethanol medium. No effect was seen in cells grown in glucose- or galactose-containing media. Alteration in

|        | WT/CM | Δmsc2/CM | WT/CMGE | Δmsc2/CMGE |
|--------|-------|----------|---------|------------|
| Cu     | 0.44 ± 0.07 | 0.50 ± 0.07 | 0.60 ± 0.09 | 0.90 ± 0.17 |
| Fe     | 1.87 ± 0.18 | 1.78 ± 0.10 | 3.51 ± 0.06 | 3.14 ± 0.06 |
| Zn     | 3.29 ± 0.48 | 3.38 ± 0.38 | 10.32 ± 0.48 | 17.72 ± 1.62 |

Fig. 4. Distribution of Zn\textsuperscript{2+} in subcellular fractions obtained from wild type and msc2 cells. Wild type and msc2 cells grown in YPD medium were transferred to YPGE medium and incubated at 37 °C for 12 h. The cells were homogenized, and subcellular fractions were obtained as described under “Materials and Methods.” Each fraction was analyzed for protein and Zn\textsuperscript{2+} content.
intracellular metal homeostasis was selective for Zn\(^{2+}\) because reporter constructs designed to measure cytosolic levels of Cu\(^{2+}\) or Fe\(^{2+}\) showed no alteration in β-galactosidase activity in cells grown in either glucose or glycerol-ethanol. This result is consistent with the determination of metal concentrations in wild type and msc2 cells (Table I), which demonstrated that only Zn\(^{2+}\) was affected by deletion of MSC2.

Decreased expression of the Zn\(^{2+}\) regulon in glycerol-ethanol-grown msc2 cells was confirmed by S1 analysis using a probe designed to measure ZRT1 transcripts (Fig. 7). The amount of ZRT1 transcript was reduced in msc2 cells, but not to the same level as measured by the reporter construct. To test the possibility that deletion of MSC2 acted downstream of Zn\(^{2+}\)-regulated transcriptional control, both wild type and msc2 cells were transformed with a plasmid containing the ZAPI\(^{up}\) allele. Transformed wild type and msc2 cells had similar levels of ZRT1-β-galactosidase activity. Similar results were obtained measuring ZRT1 transcript levels. These results suggest that deletion of MSC2 alters the transcription of Zn\(^{2+}\)-responsive genes by affecting cellular Zn\(^{2+}\) levels.

Expression and Localization of Msc2p—We generated an epitope-tagged (FLAG) Msc2p to determine its location. MSC2 is predicted to encode a protein of 84 kDa. Western analysis showed the FLAG-tagged Msc2p migrating at a molecular mass of 75 kDa (Fig. 8A). Genetic studies confirmed that the epitope marked Msc2p expressed in both low or high copy plasmids complemented the deletion strain with regard to H\(_2\)O\(_2\) sensitivity and inability to grow on glycerol-ethanol at 37 °C (data not shown). Immunofluorescence studies using the high copy Myc-tagged plasmid indicated that the protein is localized to the endoplasmic reticulum/nucleus (Fig. 8B), as further demonstrated by the images of the Myc-tagged Msc2p surrounding the 4',6-diamidino-2-phenylindole-stained nucleus (Fig. 8C). The appearance of the Myc-tagged protein in the structure near the plasma membrane has been seen for other endoplasmic reticulum proteins (21).
DISCUSSION

The CDF transporters affect the concentration of transition metals within cells. Overexpression of CDF genes in bacteria results in increased transition metal resistance by elevating metal export. Increased expression of CDF genes in eukaryotes may also provide transition metal resistance, although eukaryotes sequester metals in intracellular compartments rather than exporting them. Sequestration of metals in vesicles has the effect of lowering their concentration in cytosol. As reported previously, some of yeast CDF proteins are in nonendocytic organelles, and the function of these proteins is less clear. The sequence of MSC2 clearly places it within the CDF family, although the deduced sequence is much larger than other members of the family. By direct sequencing of the isolated functional MSC2 gene, we have confirmed that the sequence reported in the data base is correct. The size of the epitope-tagged protein (75 kDa) is lower than predicted (84 kDa) but is still larger than the rest of the CDF members. One explanation for the difference between the observed and predicted size is that the protein undergoes posttranslational processing. Our localization data indicate that the protein is localized to an internal membrane system. Overexpressed protein is found in the endoplasmic reticulum/nucleus. We have not been able to detect the protein when expressed by a low copy vector. Thus, the localization of the protein to the nucleus or endoplasmic reticulum is tentative. We have identified a mammalian homologue of Msc2p, which is of a similar size and shows 63% homology to Msc2p, but expression of this homologue in yeast does not complement the msc2 phenotype.

The phenotype of the msc2 cells and suppression of the phenotype by increased media Zn\(^{2+}\) suggest that Msc2p is involved in Zn\(^{2+}\) homeostasis. Synthesis of either MSC2 mRNA or protein, however, is not regulated by Zn\(^{2+}\) (22).\(^2\) Deletion of MSC2 led to a dramatic morphological abnormality and a growth defect in glycerol-ethanol media at 37 °C. Increased media Zn\(^{2+}\) suppressed the morphological abnormality and reduced the growth deficit of msc2 cells. Surprisingly, when grown under identical conditions, msc2 cells had higher Zn\(^{2+}\) levels than control cells. Subcellular fractionation studies indicated that compared with wild type cells, msc2 cells show decreased cytosolic Zn\(^{2+}\) and increased membrane-associated Zn\(^{2+}\), specifically in fractions that were enriched in nuclei.

The increased fluorescence of zinquin observed in msc2 cells also suggests an increased Zn\(^{2+}\) content. We expected that the zinquin fluorescence would reflect not only the increased intracellular Zn\(^{2+}\) but also the localization of the Zn\(^{2+}\). The intensity of the zinquin fluorescence did increase in msc2, but the dye was localized in small punctate vesicles. Previously, we reported that overexpression of a vacuolar Zn\(^{2+}\) transporter,

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\(^2\) L. Li and J. Kaplan, unpublished results.
Zrc1p, protected cells against Zn$^{2+}$ toxicity (6). Cells expressing Zrc1p showed an increase in zinquin fluorescence. The fluorescence did not localize to the vacuole but was again found in small punctate vesicles, as we observed in this study (data not shown). Our interpretation of these results is that zinquin-bound Zn$^{2+}$ is accumulated in vesicles, regardless of the initial distribution of Zn$^{2+}$. Therefore, the vesicular localization of zinquin fluorescence may not represent the normal distribution of Zn$^{2+}$ and should be interpreted with caution. There is a precedent for this interpretation because in some mammalian cells, fluoresceine derivatives used for either Ca$^{2+}$ or pH measurements accumulate in small vesicles. This accumulation does not reflect the native distribution of either Ca$^{2+}$ or H$^+$ but is due to accumulation of the dye by a probenac-inhibitable organic anion transporter (24). We have not determined whether probenac will inhibit the vesicular accumulation of zinquin in yeast.

Measurement of the Zn$^{2+}$ content of subcellular organelles suggests that msc2 cells have a higher nuclear Zn$^{2+}$ content than wild type cells. This conclusion is supported by the differential response of Zn$^{2+}$-regulated genes in msc2 cells compared with wild type cells. msc2 cells grown in glycerol-ethanol show decreased expression of Zn$^{2+}$-regulated genes, which can be overcome by the constitutive allele of ZAP1 (ZAP1up). This result indicates that deletion of MSC2 does not prevent Zn$^{2+}$ from entering the nucleus but rather reduces the egress of Zn$^{2+}$. The function of MSC2 appears analogous to the CDF family member MMT1/MMT2 because these genes regulate the distribution of Fe$^{3+}$ between mitochondria and cytosol.

Why does the altered distribution of Zn$^{2+}$ result in a growth deficit seen only in glycerol-ethanol at the elevated temperature? Our data demonstrate that growth on glycerol-ethanol leads to an increase in transition metal content, particularly in Zn$^{2+}$ content. It is thought that increased transition metal content is a reflection of increased mitochondrial activity necessary for respiratory activity. A number of enzymes, even cytosolic enzymes, that are necessary for respiratory activity, such as ALD2 or ALD3, are Zn$^{2+}$-requiring enzymes. Transcripts for these enzymes are increased under stress or Zn$^{2+}$ deprivation (23). It might be expected that under the dual conditions of stress (due to increased temperature) and respiratory growth, alterations in cellular Zn$^{2+}$ metabolism would be exacerbated. Under these conditions, Δmsc2 cells show increased nuclear zinc, depressing the transcription of Zn$^{2+}$-regulated enzymes, and decreased cytosolic Zn$^{2+}$, decreasing the activity of Zn$^{2+}$-requiring enzymes. Supplementation of media Zn$^{2+}$ results in an increase in cellular zinc levels, decreasing transcription of the Zn$^{2+}$ regulon while, at the same time, increasing the absolute concentration of Zn$^{2+}$ in the cytosol. The increased cytosolic Zn$^{2+}$ may populate Zn$^{2+}$-requiring proteins. It is known that transition metals must gain access to the nucleus, both as structural components (Zn$^{2+}$ fingers) and as regulatory components (metal-sensing transcription factors). Zn$^{2+}$, as a small molecule, would be expected to diffuse freely between cytosol and nucleus without the need for a transporter to effect either entry or exit. Our data suggest that under the dual conditions of increased temperature and respiratory growth, movement between the nucleus and cytosol becomes rate-limiting, requiring the participation of a transporter. Based on sequence homology, we assume that Msc2p is a Zn$^{2+}$ transporter. The possibility exists that Msc2p may transport Zn$^{2+}$ chelates, which become rate-limiting under...
stress conditions. Whereas further experiments are required to determine the chemical nature of the transported molecule, our data do demonstrate that Msc2p is a zinc transporter that is localized to an internal membrane and can effect alterations in cellular Zn$^{2+}$ homeostasis.

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