Involvement of histidine permease (Hip1p) in manganese transport in *Saccharomyces cerevisiae*

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Abstract In a search for components involved in Mn\(^{2+}\) homeostasis in the budding yeast *Saccharomyces cerevisiae*, we isolated a mutant with modifications in Mn\(^{2+}\) transport. The mutation was found to be located in *HIP1*, a gene known to encode a high-affinity permease for histidine. The mutation, designated hip1–272, caused a frameshift that resulted in a stop codon at position 816 of the 1812-bp ORF. This mutation led to Mn\(^{2+}\) resistance, whereas the corresponding null mutation did not. Both hip1–272 cells and the null mutant exhibited low tolerance to divalent cations such as Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), and Cu\(^{2+}\). The Mn\(^{2+}\) phenotype was not influenced by supplementary histidine in either mutant, whereas the sensitivity to other divalent cations was alleviated by the addition of histidine. The cellular Mn\(^{2+}\) content of the hip1–272 mutant was lower than that of wild type or null mutant, due to increased rates of Mn\(^{2+}\) efflux. We propose that Hip1p is involved in Mn\(^{2+}\) transport, carrying out a function related to Mn\(^{2+}\) export.

Key words Manganese · Divalent cations · Transport · *HIP1* · *Saccharomyces cerevisiae*

Introduction

Trace metals (Mn, Co, Cu, Ni, Zn) play an important role in cell metabolism, especially as coenzymes and cofactors in various cellular processes. Usually, the environmental concentration of such ions is low and cells must develop active transport processes to accumulate them. On the other hand, when the internal concentrations of these ions rise above physiological levels, they become toxic, mainly as a result of non-specific binding to proteins or interference with the metabolism of other metals; under such circumstances, cells must find a means of detoxification, either by exporting the ions back into the environment or by sequestering them in a non-toxic form.

Mn\(^{2+}\) is one of the essential trace elements and competes with Mg\(^{2+}\) for binding to biological molecules such as ATP, Mg\(^{2+}\)-requiring enzymes, and nucleic acids. In higher than normal concentrations, it exhibits growth inhibitory and mutagenic properties in yeast (Putrament et al. 1977). Several studies on its transport have been performed, and systems for both high affinity (Gadd and Laurence 1996; Supek et al. 1996) and low-affinity transport (Gadd and Laurence 1996) have been described in *S. cerevisiae*. Under high Mn\(^{2+}\) conditions, mechanisms involved in blocking entry of the ions into the cell (Farcasanu et al. 1995), or in the export of Mn\(^{2+}\) (Miyahara et al. 1996) have been described. Mn\(^{2+}\) is thought to be stored mainly in the vacuole in the polyphosphate form (Okorokov et al. 1977, Kihn et al. 1988), but evidence suggests that a Mn\(^{2+}\)-sequestering protein might also exist in the Golgi-like organelle (Lapinskas et al. 1996).

In a search for other components involved in Mn\(^{2+}\) homeostasis, we isolated mutants that exhibited defects in Mn\(^{2+}\) transport in the *S. cerevisiae* cells. One of these mutants (initially designated as *mrl1–1*) displayed Mn\(^{2+}\) resistance caused by altered transport. The mutation was found to be located in the *HIP1* gene, previously described as a high-affinity permease for histidine (Tanaka and Fink 1985). In this work we demonstrate that Hip1p is also involved in Mn\(^{2+}\) export and in the nonspecific uptake of divalent cations.

Materials and methods

Strains, media and growth conditions

*S. cerevisiae* strains used are presented in Table 1. YPD (yeast extract-polypeptone-dextrose) and SD (synthetic medium contain-
ing dextrose) were prepared as described (Sherman et al. 1986). For solid media, 2% agar was added. All cations, as well as histidine, were added from sterile stocks to autoclaved media cooled to 65 °C. Tests for the effects of various cations on growth were conducted in both liquid and on solid media. To determine cation toxicity in liquid media, yeast cells were inoculated from a preculture in media containing various concentrations of cations. Toxicity was then assessed by measuring the total growth (as OD600) after incubation for various periods of time at 28°C.

Table 1 Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303 1 A</td>
<td>MATa ade2–1 his3–11, 15 leu2–3, 112 trp1–1 ura3–1 can1–100</td>
<td>Rothstein (1983)</td>
</tr>
<tr>
<td>W303</td>
<td>MATa/MATa ade2–1 his3–11, 15/ his3–11, 15 leu2–3, 112/ leu2–3, 112 trp1–1/ trp1–1 ura3–1/ ura3–1 can1–100/ can1–100</td>
<td>Rothstein (1983)</td>
</tr>
<tr>
<td>IFH 12</td>
<td>Same as W303–1A, except hipl::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>NMY400</td>
<td>Same as W303–1A, except hipl–272</td>
<td>This study</td>
</tr>
<tr>
<td>IFH 272</td>
<td>Same as W303–1A, except hipl–272-Ylp5 hipl::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>IFH 283</td>
<td>Same as W303–1A, except hipl–283-Ylp5 hipl::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>IFH181</td>
<td>Same as W303–1A, except hipl–181-Ylp5 hipl::LEU2</td>
<td>This study</td>
</tr>
</tbody>
</table>

DNA manipulation, gene cloning, sequencing techniques

Cloning techniques and DNA manipulation were as described by Sambrook et al. (1989). A YCP50-based wild-type genomic library was used to clone the wild-type gene by complementation. Sequencing of double-stranded DNA was carried out by the cycle sequencing method (Kretz et al. 1995), using an ALF sequencer (Pharmacia Biotech). For PCR amplification of the genomic DNA, the Thermo Sequenase fluorescence-labeled primer sequencing method (Kretz et al. 1995), using an ALF Sequencer (Pharmacia Biotech) was employed. The cellular distribution of Mn2+ was determined by differential extraction of soluble pools from the cytosol and from vacuoles (Huber-Wächli and Wiemken 1979). Cellular cation levels were measured by particle-induced X-ray emission (PIXE) and were expressed as nmoles/mg total cellular protein; cellular protein content was measured using the method described by Bradford (1976).

Cation transport assay

Cation uptake and efflux were monitored as described by Farcassanu et al. (1995). The cellular distribution of Mn2+ was determined by differential extraction of soluble pools from the cytosol and from vacuoles (Huber-Wächli and Wiemken 1979). Cellular cation levels were measured by particle-induced X-ray emission (PIXE) and were expressed as nmoles/mg total cellular protein; cellular protein content was measured using the method described by Bradford (1976).

Plasmid and gene disruption

Construct p85 (Fig. 1A) contains a 7.5-kb genomic DNA fragment in YCp50. p85ΔXbaI and p85ΔSalI were obtained by deleting the internal 1.4-kb XbaI and 3.5-kb SalI (one site in YCp50) fragments of p85, respectively. The 3.4-kb XhoI-SalI fragment of p85 was subcloned in the SalI sites of YCp50, YEp24, and YIp5, to give pHPl, pHIP24, and pHIP5, respectively. Plasmid pHPl9 was constructed by introducing the ClaI-XbaI fragment of pHPl into the AccI/XbaI sites of pUC19. The 1.24-kb HindIII fragment of pHPl9 was replaced by a fragment carrying the LEU2 gene, yielding plasmid pHPl1. Plasmid pHPl400 was constructed by introducing the HindIII-XbaI fragment of hipl–272 gene, isolated by PCR, into the HindIII-XbaI sites of pUC19. Plasmid pHM1272 was constructed by introducing the HindIII-EcoRI fragment of pHPl400 into the corresponding sites of YIp5. Plasmids containing the truncated HIP1 gene were pHThD2 (HindIII-DraI fragment of pHPl9 in YIp5), and pHIS2 (HindIII-Styl1 fragment of pHPl9 in YIp5). A strain bearing the null mutation hipl::LEU2 was constructed by one-step gene disruption (Rothstein 1983): plasmid pHPl1 was cut with SphI + XbaI and used to transform diploid W303 cells. Correct disruption was verified by Southern hybridization.

Results

Isolation of a mutant with altered Mn2+ transport

To identify components involved in Mn2+ homeostasis, we searched for yeast mutants that showed changes in Mn2+ transport. Some 50 000 EMS-mutagenized cells were screened for growth properties in media containing Mn2+. Sixty-eight mutants were obtained, which were either resistant or sensitive to the cation, when compared to the wild type. One Mn2+-resistant mutant (strain NMY400, Table 1) showed modifications in Mn2+ transport (low Mn2+ uptake) and was chosen for further study. By crossing the original mutant to wild-type cells, it was confirmed that the mutation affected one chromosomal gene. The mutation was designated mrl1–1 (for manganese resistance due to low uptake). The mrl1–1 cells could grow on YPD agar in the presence of MnCl2 at concentrations of up to 12 mM, whereas the wild-type cells could grow on MnCl2 up to only 8 mM.

In addition to the selection phenotype, the mutant exhibited slow growth on YPD, sensitivity to H2O2 and lack of growth on SD medium. Mn2+ resistance, H2O2 sensitivity, and poor growth on YPD were (semi)dominant features. In contrast, the inability to grow on synthetic medium was a recessive phenotype.