HOLI Mutations Confer Novel Ion Transport in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae histidine auxotrophs are unable to use L-histidinol as a source of histidine even when they have a functional histidinol dehydrogenase. Mutations in the *holl* gene permit growth of His⁻ cells on histidinol by enhancing the ability of cells to take up histidinol from the medium. Second-site mutations linked to *HOLI-1* further increase histidinol uptake. *HOLI* double mutants and, to a lesser extent, *HOLI-1* single mutants show hypersensitivity to specific cations added to the growth medium, including Na⁺, Li⁺, Cs⁺, Be²⁺, guanidinium ion, and histidinol, but not K⁺, Rb⁺, Ca²⁺, or Mg²⁺. The Na⁺-hypersensitive phenotype is correlated with increased uptake and accumulation of this ion. The *HOLI-1-101* gene was cloned and used to generate a viable haploid strain containing a *holl* deletion mutation (*holl*Δ). The uptake of cations, the dominance of the mutant alleles, and the relative inability of *holl*Δ cells to take up histidinol or Na⁺ suggest that *holl* encodes an ion transporter. The novel pattern of ion transport conferred by *HOLI-1* and *HOLI-1-101* mutants may be explained by reduced selectivity for the permeant ions.

Growth on biosynthetic pathway intermediates has been a useful tool for molecular biology. In bacteria, growth on the histidine intermediate histidinol (an amino alcohol) has permitted the development of selections for suppressors, deletions, and transposition (19, 20, 23). Since conversion of histidinol to histidine is the last step in the pathway of histidine biosynthesis, mutants with defects in earlier steps can grow on histidinol as the source of histidine. These histidine-requiring bacteria can transport sufficient histidinol to satisfy the internal histidine requirement. In *Saccharomyces cerevisiae*, by contrast, histidinol does not support the growth of a histidine auxotroph, even at extremely high concentrations. One possibility is that *S. cerevisiae* cannot transport histidinol.

The most likely systems to transport histidinol seem to be those normally used to transport histidine. In *S. cerevisiae*, histidine is transported via several different permeases, including the general amino acid permease (GAP) encoded by the *GAP* gene (10) and the histidine permease encoded by *HIP1* (7, 22). The GAP system transports many different amino acids (10), but *HIP1* permease transports only histidine (22). The arginine permease encoded by the *CAN1* gene takes up histidine with very low affinity. On media with proline as a nitrogen source, histidine enters through both the GAP and *HIP1* permeases, but even on this medium, histidine auxotrophs fail to grow on histidinol.

We have discovered mutations in a new gene, *holl*, which permit the growth of yeast histidine auxotrophs on histidinol. These mutations confer the ability to transport histidinol from the growth medium. Our evidence suggests that histidinol uptake in these mutants is not a consequence of mutation in one of the genes known to affect histidine transport; *holl* is genetically distinct from the histidine uptake systems (GAP, *HIP1*, and *CAN1*). Mutations in *holl* also confer significant uptake of Na⁺ and hypersensitivity to

a number of cations. The phenotypes of the *HOLI* mutations that permit growth on histidinol and the *holl* null mutation suggest that yeast cells acquire the ability to grow on histidinol by altering the specificity of an ion transporter.

MATERIALS AND METHODS

Media, strains, and plasmid constructions. YPD, YNB, and AA (YNB supplemented with all amino acids plus uracil and adenine) media and routine genetic techniques are described by Sherman et al. (21). AA-his is histidineless medium. AA-ura is medium with no uracil. Yeast transformation was performed by the cation method (12) using lithium acetate. The yeast strains used in this study are described in Table 1. The plasmids used in this study (see Fig. 8) were selected and propagated in *Escherichia coli* HB101 (5).

Isogenic *holl* (wild type) and *HOLI-1 HOLI-101* (*HOLI-1-101*) strains R757 and R855, respectively, were generated by the method of integration and excision (24). *S. cerevisiae* R853, containing the *holl URA3 HOLI-1-101* duplication, was constructed by transformation of strain R757 to a Ura⁺ phenotype with a plasmid (pRG131-1) carrying the *HOLI-1-101* gene. Recombinants of R853 without the plasmid sequences were obtained on 5-fluoro-orotic acid, which selects against Ura⁺ cells and permits the growth of Ura⁻ segregants (2).

S. cerevisiae R890 containing a deletion within the *HOLI-1-101* gene was generated by the method of integration and excision (24). *S. cerevisiae* R888 containing the *HOLI-1-101 URA3 holl*Δ1 duplication was constructed by transformation of strain R855 to a Ura⁺ phenotype with plasmid pRG166-1. Cells that lost the plasmid sequences but retained the *holl*Δ1 mutation were identified as mitotic Ura⁻ Hol⁻ segregants of strain R888 as described above.

Plasmid pRG126-2 was constructed by subcloning the 12-kilobase (kb) *Bam*HI-*Sal*I fragment from pRG120-1 into *Bam*HI-*Sal*I-digested YCp50 (17). Plasmid pRG127-1 was constructed by complete digestion of pRG126-2 with *Cla*I,

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TABLE 1. Strains used in this study

<i>S. cerevisiae</i> strain	Genotype	Source or reference
R650	a <i>his4-15^a holl</i>	This study
R651	α <i>his4-15 holl</i>	This study
R657	a <i>his4-15 ura3-52 HOL1-1</i>	This study
R658	α <i>his4-15 ura3-52 HOL1-1</i>	This study
R661	a/ α <i>his4-15/his4-15 HOL1-1/HOL101</i>	This study
R662	a/ α <i>his4-15/his4-15 HOL1-1/holl</i>	This study
R663	a/ α <i>his4-15/his4-15 holl/holl</i>	This study
R704	a <i>his4-15 ura3-52 HOL1-1-101</i>	This study
R757	α <i>his4-15 ura3-52 lys9 holl</i>	9
R806	α <i>his4-15 lys9 pet494-1</i>	This study
R832	a/ α <i>his4-15/his4-15 HOL1-1-101/holl</i>	This study
R833	a/ α <i>his4-15/his4-15 HOL1-1-101/HOL1-1</i>	This study
R834	α <i>his4-15 ura3-52 lys9 holl</i> pRG130-1	This study
R848	a <i>his4-401 ura3-52 HOL1-1-101</i>	This study
R850	a <i>his4-401 ura3-52 holl</i>	This study
R853	α <i>his4-15 ura3-52 lys9 holl</i> pRG131-1 <i>HOL1-1-101</i>	This study
R855	α <i>his4-15 ura3-52 lys9 HOL1-1-101</i>	This study
R878	a/ α <i>his4-15/his4-15 ura3-52/+ lys9/+</i> <i>HOL1-101/holl</i>	This study
R879	a/ α <i>his4-15/his4-15 ura3-52/+ lys9/+</i> <i>HOL1-101/HOL1-1</i>	This study
R890	α <i>his4-15 ura3-52 lys9 hollΔ1</i>	This study
R757/11-1	α <i>his4-15 ura3-52 lys9 HOL1-101</i>	This study
L1205	a <i>his4-401 ura3-52 holl</i>	T. Donahue

^a *his4-15* is a deletion of *HIS4A* and *HIS4B* with an intact *HIS4C* (histidinol dehydrogenase) region (8).

followed by self-ligation and recircularization. Plasmid pRG130-1 was constructed by subcloning the 4.3-kb *Bam*HI-*Xho*I fragment from pRG126-2 into *Bam*HI-*Sa*II-digested YIp5 (4). Plasmid pRG131-1 was constructed by subcloning the 6.3-kb *Xho*I fragment from pRG126-2 into *Sa*II-digested YIp5. Plasmids pRG147-1 and pRG148-1 were constructed by subcloning the 2.8-kb *Cla*I-*Hind*III fragment from pRG127-1 into *Cla*I-*Hind*III-digested YIp5 and YCp50, respectively. Plasmid pRG158 was constructed by complete digestion of pRG131-1 with *Cla*I, followed by ligation and recircularization. Plasmid pRG165-1, containing only part of the *HOL1-1-101* gene but containing the *HOL1-101* mutant site, was constructed by digesting pRG147-1 to completion with *Eco*RI, followed by ligation and recircularization. Plasmid pRG166-1, containing a 600-base-pair (bp) deletion within the *HOL1-1-101* gene, was constructed by complete digestion of pRG147-1 with *Kpn*I, followed by ligation and recircularization.

Genetic mapping. Standard linkage values were derived from tetrad data by using the equation x (in centimorgans) = $50[\text{tetatype asci} + 6(\text{nonparental ditype asci})]/\text{total asci}$ (15). Gene order in multipoint crosses was determined by analyzing recombinant asci containing crossovers in the regions of interest.

Isolation of *HOL1* mutants. Mutants able to grow on histidinol as the sole source of histidine (Hol^+) were isolated by plating Hol^- (R651 [*his4-15*]) cells onto AA-histidine supplemented with 2.5 mM histidinol. Spontaneous second-site mutants (Hol^{2+}) were isolated from strain R658 (*his4-14 HOL1-1*) by their ability to grow on AA-histidine supplemented with only 0.25 mM histidinol.

Physical mapping of *HOL1-1* and *HOL1-101* mutations. The presence of the *HOL1-1* mutation on integrative plasmid pRG166-1 was established by its ability to convert a recipient *holl* strain to the Hol^+ phenotype following recombination with the chromosome. The presence of the *HOL1-101* mu-

tation on plasmids pRG165-1 and pRG166-1 was similarly established by their ability to convert a recipient, a Hol^+ (*HOL1-1*) strain, to the Hol^{2+} phenotype following recombination with the chromosome.

DNA manipulations. Rapid plasmid DNA isolation was performed by the method of Holmes and Quigley (11); restriction analysis, gel electrophoresis, and Southern analysis were performed as described by Maniatis et al. (13). Hybridization probes were prepared as described by Maniatis et al. (13). A *holl*-specific probe was prepared by gel purification of the 600-bp *Kpn*I fragment of the *HOL1-1-101* gene that was subcloned from pRG148 into pUC18. Filters containing the hybridized probe were washed four times in $6\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 55°C before autoradiography.

Total yeast genomic DNA used in the construction of a cloning library was prepared as follows. One liter of stationary-phase cells was harvested and washed twice in double-distilled H_2O by centrifugation before suspension in SCE (1 M sorbitol, 0.1 M sodium citrate, 0.06 M EDTA, pH 7.0) at 1 g of cells per ml. Spheroplasts were made by treatment with 0.15 mg of zymolyase 100K (Miles Laboratories, Inc.) per ml of cell suspension. The spheroplasts were lysed by dilution of the suspension into 1.75 volumes of lysis buffer (3% *N*-lauroyl sarcosine, 0.5 M Tris [pH 9.0], 0.2 M EDTA) and immediate incubation at 65°C for 15 min. Cold (4°C) 5 M potassium acetate (0.16 volume) was added, and the mixture was kept on ice for at least 1 h. Cell debris was pelleted by centrifugation at $10,000 \times g$ for 40 min. Nucleic acids were precipitated from the supernatant with 2.5 volumes of ethanol and then solubilized in 16 ml of TE (10 mM Tris [pH 7.0], 0.1 mM EDTA). Insoluble material was removed from this suspension by centrifugation ($10,000 \times g$ for 10 min). The supernatant was treated with 0.5 ml of RNase A (5 mg/ml; Sigma Chemical Co.) at 37°C for 1 h. Isopropanol (0.6 volume) was added, and DNA was spooled onto a sealed capillary pipette. The DNA was further purified by density gradient centrifugation in 1.22 g of CsCl per ml.

Direct (yeast-to-yeast) cloning of the *HOL1-1-101* gene. A 100- μg sample of genomic DNA from a *HOL1-1-101* strain (R704) was prepared as described in the previous section and partially digested with *Sa*II (125 U at 37°C for 1 h). Following electroelution of fragments larger than 3 kb, the chromosomal DNA was ligated to 20 μg of YCp50 that had been treated with *Sa*II and calf intestinal phosphatase. The ligation mixture (600 μl) was ethanol precipitated and used to transform a *ura3-52 holl* strain (R757) to a Ura^+ phenotype. Yeast transformants that harbored plasmids carrying the dominant *HOL1-1-101* allele were identified by the ability to confer growth on AA-histidine supplemented with 2.5 mM histidinol. Following isolation of total DNA from the Ura^+ Hol^{2+} yeast transformants, plasmids carrying the *HOL1-1-101* gene were isolated by transformation of *E. coli* HB101 to ampicillin resistance.

[^{14}C]histidinol uptake assays. [^{14}C]histidinol was isolated from *Salmonella typhimurium* D1 (genotype *hisD1*) by the method of Ciesla et al. (6). Uptake of [^{14}C]histidinol was measured as follows. Mid-log-phase cells growing in AA were harvested and suspended at 10^8 cells per ml in 50 mM Tris succinate (pH 5.9)–50 mM KCl–4% glucose–[^{14}C]histidinol. Samples (100 μl) were taken at the times indicated, diluted into ice-cold wash solution (uptake medium without histidinol), and filtered with 45- μm -pore-size filters (Millipore Corp.), and the filters were washed twice with 5 ml of ice-cold wash solution. The radioactivity

retained within the cells was measured by suspending the cells in Aquasol and measuring radioactivity in a Beckman scintillation counter.

$^{22}\text{Na}^+$ transport assays. The growth conditions and methods for measuring $^{22}\text{Na}^+$ uptake were essentially the same as those used to measure [^{14}C]histidinol uptake. Na^+ efflux assays were performed in the following manner. Yeast cells at a concentration of $1 \times 10^8/\text{ml}$ were loaded with $^{22}\text{Na}^+$ by incubation in AA supplemented with $^{22}\text{Na}^+$ but lacking glucose. Under glucose starvation conditions, wild-type cells took up enough $^{22}\text{Na}^+$ to permit subsequent measurement of its efflux. Cells were harvested, washed twice in medium lacking $^{22}\text{Na}^+$, and suspended at a concentration of $1 \times 10^8/\text{ml}$. Upon addition of glucose to 4%, 100- μl samples of the cell suspension were taken, diluted into ice-cold medium lacking $^{22}\text{Na}^+$, and filtered through 45- μm -pore-size filters (Millipore). Radioactivity was measured in a Beckman scintillation counter.

Cation hypersensitivity assays. The sensitivities of wild-type and mutant yeast cells to different ions were measured by plating a lawn of approximately 2×10^5 cells onto AA solid medium and placing a solution containing the test compound onto a sterilized filter disk placed on the lawn. Zones of inhibition exhibited by mutant strains were compared with those of wild-type strains.

RESULTS

Isolation of *HOL1* mutations. Yeast histidine auxotrophs with a functional histidinol dehydrogenase are unable to grow on histidinol as a sole source of histidine (designated Hol^- ; Fig. 1). Mutations permitting growth on histidinol (Hol^+) were obtained by plating a Hol^- strain (R650; Table 1) onto AA-his agar medium containing 2.5 mM histidinol (AA-his-2.5 mM histidinol). Spontaneous Hol^+ mutants appeared on the 2.5 mM histidinol plates at approximately 10^{-8} per cell.

Each of the Hol^+ mutants was mated to a *his4-15 holl* mutant strain (R757; Table 1), and the diploid cells were tested for growth on AA-his-2.5 mM histidinol. All of the $\text{Hol}^+/\text{Hol}^-$ heterozygous diploids grew better than the homozygous wild-type *holl/holl* diploid on medium containing 2.5 mM histidinol (Fig. 1) but not as well as homozygous $\text{Hol}^+/\text{Hol}^+$ diploids, indicating that the ability to grow on histidinol is semidominant.

The genetic basis for the Hol^+ phenotype was examined by crossing 10 independent Hol^+ mutants with a Hol^- strain and analyzing the segregation pattern. In each case, a 2 Hol^+ :2 Hol^- pattern was observed among the meiotic tetrads, demonstrating that the Hol^+ phenotype was dependent on a single gene. Each of the 10 independent Hol^+ mutants was crossed with a strain containing the *HOL1-1* allele (R658; Table 1). Tetrad analysis of meiotic spores from these crosses showed a 4 Hol^+ :0 Hol^- segregation for growth on AA-his-2.5 mM histidinol. At least eight tetrads were analyzed from each of these crosses. No Hol^- recombinants were detected among the meiotic progeny. Although the number of tetrads analyzed from each cross was small, the absence of any Hol^- recombinants and the indistinguishable dominant Hol^+ phenotypes of these mutants suggest that all of the independent *HOL* mutations are alleles of *holl*.

Second-site mutations linked to *HOL1* confer growth on lower concentrations of histidinol. Mutants growing on a concentration of histidinol lower than 2.5 mM (0.25 mM) could not be isolated from *holl* strains ($<10^{-9}$), but they could be readily isolated from a strain which already had a

HOL1 mutation. Starting with the *HOL1-1* mutant, we obtained strains capable of growing on 0.25 mM histidinol (designated Hol^{2+}) at a frequency of 5×10^{-7} . Each of 16 independent Hol^{2+} mutants was crossed with a Hol^- strain (R757; Table 1) to examine the genetic basis for the Hol^{2+} phenotype. In every case, the ability to grow on 0.25 mM histidinol or 2.5 mM histidinol cosegregated 2 Hol^{2+} :2 Hol^- . No Hol^+ recombinants able to grow on 2.5 mM but not 0.25 mM histidinol were obtained. These data suggest that the mutation that confers the Hol^{2+} phenotype is tightly linked to the *HOL1-1* mutation. Further analysis of one cross yielded no Hol^+ recombinants in 43 tetrads. These results suggested that the Hol^{2+} phenotype is conferred by a combination of two mutations at the *holl* locus. Subsequent mitotic recombination and molecular cloning experiments (presented below) confirmed this interpretation. The new mutations are designated by the abbreviations *HOL1-1-101* through *HOL1-1-110*.

Dominance tests were performed by testing heterozygous *HOL1-1/holl* and *HOL1-1-101/holl* diploids (R662 and R832; Table 1) for growth on AA-his containing either 2.5 or 0.25 mM histidinol (Fig. 1). Growth was compared with that of homozygous *holl/holl*, *HOL1-1/HOL1-1*, and *HOL1-1-101/HOL1-1-101* diploids. The *HOL1-1-101* double mutant was completely dominant on medium containing 2.5 mM histidinol (Fig. 1) but was only semidominant on AA-his-0.25 mM histidinol. These phenotypes are consistent with the interpretation that gene dosage and the nature of the mutant alleles control the ability to grow on histidinol.

Genetic mapping of *HOL1-1*. The *holl* gene is on chromosome XIV. Data from a three-point cross including the markers *HOL1-1*, *pet494*, and *lys9* demonstrated that the probable gene order is *pet494 lys9 HOL1-1* (Fig. 2).

***HOL1* mutants take up histidinol.** Wild-type and *HOL1-1* and *HOL1-1-101* mutant strains were examined for the ability to take up [^{14}C]histidinol (Fig. 3). A striking difference in histidinol uptake was observed between *HOL1* and *holl* cells. *HOL1-1* cells showed significantly higher rates of histidinol uptake than wild-type *holl* cells (Fig. 3, inset). In addition, the *HOL1-101* second-site mutation further increased the rate of histidinol uptake by *HOL1-1* cells. Much of the radioactivity taken up by *holl* cells was due to contaminating material in the [^{14}C]histidinol preparation. When the [^{14}C]histidinol was preabsorbed with *holl* cells to remove the putative contaminant before the assay, *holl* cells showed essentially no uptake, whereas uptake by *HOL1* cells remained unaffected (data not shown).

Increased Na^+ uptake in *HOL1* mutants. *HOL1-1-101* cells exhibited sensitivity to YPD medium, although they grew well on synthetic medium (Fig. 4). YPD sensitivity was particularly striking when dilute inocula were applied. *HOL1-1-101* spores failed to germinate on YPD but germinated on synthetic medium. The basis of the YPD sensitivity phenotype of Hol^{2+} cells was investigated by testing the effects of various additions to YNB medium. *HOL1-1-101* cells (and, to a lesser degree, *HOL1-1* cells) were hypersensitive to some cations, including Na^+ , Li^+ , Cs^+ , Be^{2+} , and guanidinium ion $[(\text{NH}_2)_3\text{C}^+]$, but not others, including K^+ , Rb^+ , Tris hydrochloride, Ca^{2+} , and Mg^{2+} (Fig. 5). The hypersensitivity to a number of different cations suggests that *HOL1* cells are more efficient than *holl* cells in transporting these ions from the medium. To test this possibility, we assayed exponentially growing *holl* and *HOL1-1-101* cells for ^{22}Na uptake (Fig. 6). *HOL1-1-101* cells took up significantly more Na^+ than did wild-type *holl* cells. Thus, the hypersensitivity of *HOL1-1-101* cells to Na^+ can be

FIG. 1. Tests for histidinol-dependent growth of *hol1*, *HOL1-1*, and *HOL1-1-101* strains. Patches of homozygous *his4-15/his4-15* diploid strains containing all combinations of wild-type (*hol1*) and mutant (*HOL1-1* and *HOL1-1-101*) alleles were grown on YNB medium supplemented with all amino acids and replica plated to histidine-free medium supplemented with 0.25 or 2.5 mM histidinol or 2.5 mM histidine. Colonies were photographed after 48 h of incubation at 30°C. Heterozygous *HOL1-1/hol1* strains exhibited weak growth on 2.5 mM histidinol, indicating semidominance of the *HOL1-1* allele. The *HOL1-101* allele displayed clear dominance, but only on medium containing 2.5 mM histidinol and only when this mutation was in combination (*cis*) with the *HOL1-1* allele (see text for details). *his4-15 hol1*- and *his4-15 HOL1-101*-carrying cells failed to grow on medium supplemented with histidinol (see text for details). The strains used in the growth tests were R650/R651 (*hol1/hol1*), R657/R651 (*HOL1-1/hol1*), R704/R651 (*HOL1-1-101/hol1*), R657/R658 (*HOL1-1/HOL1-1*), R657/R855 (*HOL1-1/HOL1-1-101*), and R704/R855 (*HOL1-1-101/HOL1-1-101*). See Table 1 for complete genotypes.

attributed to increased uptake of this ion. In a separate experiment, *HOL1-1* cells exhibited rates of Na^+ uptake intermediate between those of *hol1* and *HOL1-1-101* cells (data not shown). Since the hypersensitivity of *HOL1* cells to Na^+ and the ability to grow on histidinol are correlated with increased uptake of these ions, it is likely that the hypersensitivity of these cells to Li^+ , Cs^+ , $[(\text{NH}_2)_3\text{C}^+]$, and Be^{2+} is due to increased uptake of these ions as well.

Wild-type and *HOL1-1-101* cells were preloaded with $^{22}\text{Na}^+$, and the relative rates of $^{22}\text{Na}^+$ efflux were measured

(Materials and Methods). Isogenic *hol1* and *HOL1-1-101* strains (R757 and R855) exhibited essentially indistinguishable rates of $^{22}\text{Na}^+$ efflux (Fig. 7), demonstrating that *HOL1-1-101* hypersensitivity to Na^+ was due entirely to increased uptake rather than decreased efflux of this ion. These results suggest that during growth, wild-type cells normally take up very little Na^+ . Indeed, yeast cells grow well in medium that is virtually sodium free (16).

Cloning of the *HOL1-1-101* gene. Attempts to clone mutant alleles of the *hol1* gene from yeast clone banks amplified in

FIG. 2. Genetic map of the right arm of chromosome XIV. The genetic distance between *HOL1-1* and linked markers on the right arm of chromosome XIV is indicated in centimorgans (cM). The genetic map positions are derived from tetrad analysis of meiotic progeny from a cross between R806 and R657 (Table 1). Among 131 tetrads, 10 were tetratype with respect to the *HOL1-1* and *lys9* markers. Nine *HOL1-1-lys9* tetratype asci were parental ditype, and one was tetratype for the *pet494-lys9* marker pair. Five tetrads were tetratype for the *pet494-lys9* marker pair. Four of the *pet494-lys9* tetratype asci were parental ditype, and one was tetratype for the *lys9-HOL1-1* marker pair. These results are consistent with the order *pet494-lys9-HOL1-1*. The genetic distance between *cenXIV* and the *pet494-lys9* marker pair and their order with respect to *cenXIV* are taken from Mortimer and Schild (14).

E. coli were unsuccessful. We considered the possibility that this was a consequence of toxic sequences within or adjacent to the *hol1* gene and used an experimental strategy designed to avoid the initial propagation and amplification of plasmid DNA in *E. coli*. We isolated the mutant *HOL1-1-101* gene from genomic yeast DNA prepared from a *Hol*²⁺ strain (R704; Table 1) by direct yeast-to-yeast cloning (Materials and Methods). This approach relied on expression of the dominant *HOL1-1-101* allele in a *ura3-52 hol1* recipient strain

(R757). Among approximately 5,000 *Ura*⁺ transformants screened, 3 were able to grow well on 2.5 mM histidinol and weakly on 0.25 mM histidinol. Each of the three *Hol*²⁺ transformants exhibited mitotic co-instability for the *Ura*⁺ and *Hol*²⁺ phenotypes, demonstrating the dependence of both phenotypes on the cloned plasmids. DNA was prepared from one of the *Ura*⁺ *Hol*²⁺ transformants, and the plasmid it contained was retrieved by transformation of *E. coli* HB101 to ampicillin resistance. *E. coli* transformants con-

FIG. 3. Uptake of [¹⁴C]histidinol by isogenic *hol1* and *HOL1-1-101* cells (see Materials and Methods for experimental details). The strains used were R848 (*HOL1-1-101*) and R850 (*hol1*). The inset shows the uptake of [¹⁴C]histidinol by nonisogenic *hol1*, *HOL1-1*, and *HOL1-1-101* strains. The strains used were L1205 (*hol1*), R657 (*HOL1-1*), and 4455-3B (*HOL1-1-101*). c.p.m., Counts per minute.

FIG. 4. Sensitivity of *HOL1-1-101* cells to rich medium (YPD). A series of 10-fold dilutions of isogenic R757 (*hol1*) and R855 (*HOL1-1-101*) cells were spotted onto rich (YPD) and synthetic (YNB-AA) media. Growth was photographed after 48 h of incubation at 30°C.

taining the putative *HOL1-1-101* clone (pRG120-7) grew extremely slowly. A 5.9-kb *ClaI-SalI* fragment subcloned into YCp50 (designated pRG127-1) did not confer the slow-growth phenotype in *E. coli* but retained the functional *HOL1-1-101* gene. These data suggest that sequences toxic to *E. coli* are near but not within the *HOL1* gene.

Two experiments demonstrated that the cloned sequences contain the *HOL1* gene with the *HOL1-1-101* mutations. (i) *Hol*⁻ recipients acquire a *Hol*²⁺ phenotype upon transformation with plasmid pRG127-1. (ii) Integrative transformation experiments showed that the cloned sequences integrate by homology to the *hol1* locus. Plasmid pRG130-1, an integrative plasmid incapable of replication in *S. cerevisiae*, contains DNA sequences from a region adjacent to but not including the *HOL1* gene (Fig. 8). pRG130-1 was used to transform a *Ura*⁻ *Hol*⁻ recipient (R834; Table 1) to a *Ura*⁺ *Hol*⁻ phenotype, and this transformant was crossed by a *Ura*⁻ *Hol*²⁺ strain (R704; Table 1) to detect linkage between the integrated plasmid and *hol1*. Among 19 four-spored tetrads obtained, all were parental ditype for the *Ura*⁺ *Hol*⁻ and *Ura*⁻ *Hol*²⁺ phenotypes, indicating complete genetic linkage between the integrated plasmid and the *hol1* gene. These results confirmed that the cloned fragment in plasmid pRG120-1 carries the *HOL1-1-101* gene. Subcloning experiments localized the *HOL1-1-101* gene to within a 2.8-kb DNA fragment (Fig. 8). Transformation of a *Ura*⁻ *Hol*⁻ recipient (R757) with pRG148, an autonomously replicating plasmid containing a 2.8-kb *HindIII-ClaI* fragment from pRG120-7, resulted in a *Ura*⁺ *Hol*²⁺ phenotype. A map of the relevant restriction endonuclease sites within the cloned insert from pRG120-7 is presented in Fig. 8.

***HOL1-1* and *HOL1-101* mutations are separable by recombination.** The *HOL1-101* mutation increased the ability of *HOL1-1* cells take up histidinol and sodium. We were interested in determining what phenotype, if any, the *HOL1-*

101 mutation produces without the *HOL1-1* mutation. The strategy we used to generate strains containing only the *HOL1-101* mutation was to integrate a plasmid carrying the *HOL1-1-101* double mutation at the wild-type *hol1* locus, resulting in a heterozygous duplication, and then isolate plasmid-free mitotic segregants that evicted the *HOL1-1* mutation but retained the *HOL1-101* mutation. Transformation of strain R757 (*Ura*⁻ *Hol*⁻) with integrative plasmid pRG131 carrying the *HOL1-1-101* gene resulted in several phenotypes. Among 35 *Ura*⁺ transformants, 15 were *Hol*⁻, 6 were *Hol*⁺, and 13 were *Hol*²⁺. One transformant exhibited stronger growth on 0.25 mM histidinol than did the parental *HOL1-1-101* mutant and was designated *Hol*³⁺. The occurrence of *Hol*⁺ transformants was consistent with the possibility that integration of pRG131 yielded chromosomal duplications in which the *HOL1-1* and *HOL1-101* mutant loci had been separated if the *HOL1-1-101* mutation by itself had no effect on histidinol uptake. Several possibilities could explain the phenotype of the single *Hol*³⁺ transformant (R757/131/11): homozygosity at either the *HOL1-1* site or the *HOL1-101* site, homozygosity at both sites, or integration of multiple copies of pRG131. Surprisingly, when plasmid-free segregants from the *Hol*³⁺ transformant were obtained by selecting for resistance to 5-fluoro-orotic acid (2), 2 of 10 segregants were *Ura*⁻ *Hol*⁻, indicating that the *Hol*³⁺ transformant was not homozygous for the *HOL1-1* mutation. Genetic recombination tests were performed with one of the *Hol*⁻ mitotic segregants of the *Hol*³⁺ transformant to determine whether it harbored the *HOL1-101* mutation. The *Hol*⁻ segregant (R757/11-1) was crossed with *HOL1-1* (R658) and *hol1* (R757), and the resulting diploids were tested for the ability to produce *Hol*²⁺ recombinants following UV irradiation. In these experiments, the putative *HOL1-101/HOL1-1* diploid cultures gave rise to *Hol*²⁺ colonies at high frequency (10^{-4} to 10^{-3}), whereas the putative *HOL1-101/hol1* diploids and control *HOL1-1/hol1* and *hol1/hol1* diploids failed to give rise to any *Hol*²⁺ colonies. These results indicate that *Hol*⁻ segregant R757/11-1 harbors only the *HOL1-101* mutation. Since the *HOL1-101* single mutant cannot grow on histidinol (2.5 mM) and is not hypersensitive to *Na*⁺ or *Li*⁺, we conclude that *HOL1-101* enhances the ability to take up these ions only in combination with *HOL1-1*. These results are also consistent with the interpretation that *HOL1-101* is a second-site mutation within *hol1*.

Deletion of the *hol1* gene. Deletion of a 600-bp *KpnI* fragment from within the functional *HOL1-1-101* gene was constructed in vitro (Materials and Methods), and the resulting plasmid (pRG166-1) was used to construct a *HOL1-1-101 URA3 hol1Δ* duplication by integrative transformation of a *HOL1-1-101* recipient yeast strain (R855). *Ura*⁻ segregants resulting from loss of the integrated plasmid (and one of the duplicated *HOL1* regions) were obtained by selection for resistance to 5-fluoro-orotic acid. The recovery of *Ura*⁻ *Hol*⁻ segregants signalled loss of the functional *HOL1-1-101* allele in these experiments. Southern analysis confirmed that the *Hol*⁻ segregants carry the deletion. The 600-bp *KpnI* fragment from the *hol1* gene was purified from plasmid pRG178-1 (Fig. 8) and used to probe Southern blots of digested genomic DNA from the *HOL1-1-101* (R855) parental strain and one of the *Ura*⁻ *Hol*⁻ segregants (R890). The autoradiogram in Fig. 9 demonstrates that *Ura*⁻ *Hol*⁻ segregant R890 contains the *hol1Δ1* mutation.

Isogenic *hol1*, *HOL1-1-101*, and *hol1Δ1* strains (R757, R855, and R890) were assayed and compared for the ability to transport ²²Na⁺ from the medium (Fig. 6). *HOL1-1-101* cells exhibited substantial uptake of ²²Na⁺, whereas *hol1* and

FIG. 5. Cation hypersensitivities of *HOL1-1*- and *HOL1-1-101*-carrying cells. Solutions containing chloride salts of cesium, guanidine, histidinol, lithium, potassium, rubidium, and sodium were placed onto filter disks on lawns of R650 (*hol1*), R657 (*HOL1-1*), and R855 (*HOL1-1-101*) cells. The medium was YNB-AA, and the plates were photographed following 48 h of incubation at 30°C. *HOL1-1*- and *HOL1-1-101*-carrying strains also showed hypersensitivity to beryllium (data not shown).

hol1Δ cells took up very little of the ion. $^{22}\text{Na}^+$ efflux from *hol1Δ* and *hol1* cells was indistinguishable (Fig. 7). Similar experiments that measured [^{14}C]histidinol uptake demonstrated that the *hol1Δ* and *hol1* strains exhibited indistinguishable phenotypes (data not shown). Since the *hol1Δ* mutation was generated in a *HOL1-1-101* cell, the resultant loss of histidinol and Na^+ uptake suggests that *hol1Δ* is a null allele. The viability of *hol1Δ* cells, therefore, indicates that *hol1* is likely to be a nonessential gene in *S. cerevisiae*.

Mapping of *HOL1-1* and *HOL1-101* mutant sites. To define more precisely the location of *HOL1-1* and *HOL1-101*, we performed recombination experiments using different DNA fragments derived from the cloned *HOL1-1-101* gene (see Materials and Methods). Transformation of a *hol1* strain

(R757) with *KpnI*-digested plasmid pRG165-1 gave rise to over 300 Ura^+ transformants, all of which retained the parental Hol^- phenotype. These results suggested that the 1.5-kb *EcoRI-HindIII* fragment carried on pRG165-1 does not carry the *HOL1-1* allele. Indeed, when plasmid pRG166-1, containing all of the *HOL1-1-101* gene except the 600-bp *KpnI* segment, was used to transform strain R757, Hol^- , Hol^+ , and Hol^{2+} transformants were obtained with roughly equal frequency. These results indicated that the *HOL1-1* mutation lies within the 1.5-kb *ClaI-KpnI* region carried by pRG166-1. These recombination experiments could not discern whether the *HOL1-101* mutation lies within the 800-bp *ClaI-KpnI* segment or the 1.5-kb *EcoRI-HindIII* segment of the gene. To determine the location of the *HOL1-101* muta-

FIG. 6. $^{22}\text{Na}^+$ uptake by *holl*, *HOL1-1-101*, and *hollΔ1* cells. See Materials and Methods for experimental details. The strains used were R757 (*holl*), R855 (*HOL1-1-101*), and R890 (*hollΔ1*). c.p.m., Counts per minute.

tion, we transformed *HOL1-1* recipient strain R657 with *KpnI*-digested pRG165-1 (Fig. 8). Of 61 transformants, 59 exhibited the Hol^{2+} phenotype, demonstrating the presence of the *HOL1-101* mutation on the 1.5-kb *EcoRI*-*HindIII* fragment carried by this plasmid. The results of these recombination experiments demonstrate that the *HOL1-1* and *HOL1-101* alleles lie on opposite sides of the *KpnI* segment. Preliminary DNA sequencing studies indicate that the *hollΔ1* mutation lies within the coding region of the gene between *HOL1-1* and *HOL1-101* (unpublished data).

DISCUSSION

Selection for mutants that can utilize histidinol as a source of histidine resulted in mutations that reside exclusively at the *holl* locus. Mutations at *holl* confer transport (uptake) of histidinol across the plasma membrane. Spontaneous mutations at *holl*, e.g., *HOL1-1*, that allow uptake of histidinol occur at a frequency of less than 10^{-8} . Second-site mutations linked to the *holl* locus, e.g., *HOL1-101*, that confer growth on lower concentrations of histidinol can occur only in the background of a *HOL1* mutation.

The simplest explanation for the acquisition of the ability to grow on histidinol is that our mutations in the *holl* gene alter the specificity of an ion transporter in such a way that it gains the ability to transport histidinol as well as several other cations. This explanation is consistent with both the genetic and physiological analyses of the Hol^+ mutants. Hol^+ cells actually take up more histidinol than do wild-type cells. Both the *HOL1-1* and *HOL1-1-101* mutations are dominant, suggesting the acquisition of a new function. Furthermore, Hol^+ cells are sensitive to a number of cations to which wild-type cells are relatively insensitive. Cells containing *HOL1-1* or *HOL1-1-101* show vastly increased

Na^+ uptake compared with wild-type cells. Presumably, the hypersensitivity to ions other than Na^+ is a reflection of their increased uptake as well. Moreover, strains carrying a null allele of *holl* (*hollΔ1*) are unable to grow on histidinol, a phenotype expected by our model and supporting the possibility that *holl* is the structural gene that encodes the ion transporter.

One possibility suggested by our results is that *holl* encodes an endogenous Na^+ transporter. The comparable rate of Na^+ efflux from *holl* and *HOL1-1-101* cells and the greatly increased rate of Na^+ influx in *HOL1-1-101* cells strongly suggest that transport of this ion by mutant cells is active and directional. Although Na^+ is not an essential ion for *S. cerevisiae*, the function of a Na^+ transporter has been clearly established: Na^+ can replace a significant amount of the cellular K^+ when yeast cells are grown in Na^+ -rich medium containing low concentrations of K^+ (1). In fact, when both ions are present, the sum of the intracellular K^+ and Na^+ concentrations is roughly constant (1, 18). In addition, several studies have quantitated the kinetic parameters of the Na^+ transport system(s) in *S. cerevisiae* (see reference 3 for a review). The finding that *holl* is genetically distinct from both *TRK1*, the gene that encodes the high-affinity K^+ transporter (9), and *KLA1*, the gene that encodes the low-affinity K^+ transporter (unpublished data), suggests that it does not encode either of the principal K^+ transporters in *S. cerevisiae*. Our results do not exclude the possibility that *holl* encodes a K^+ transporter whose function is simply masked by other transporters, such as *TRK1* and *KLA1*. The phenotypes of each of the *HOL1* mutants we examined are consistent with the interpretation that *HOL1-1* mutations either reduce the specificity of this hypothetical transporter or vastly increase its rate of transport. Our results suggest that both of these possibilities are correct. The positive effect of the *HOL1-101* mutation on the *HOL1-1*

FIG. 7. $^{22}\text{Na}^+$ efflux from preloaded *holl*-, *HOL1-1-101*-, and *hollΔ1*-carrying cells. Efflux is plotted on a log scale with initial counts per minute (c.p.m.) as indicated. See Materials and Methods for experimental details. The strains used were R757 (*holl*), R855 (*HOL1-1-101*), and R890 (*hollΔ1*).

FIG. 8. Restriction endonuclease sites of the cloned *S. cerevisiae* genomic fragment containing the *HOL1-1,101* gene and construction of the *hol1Δ* mutation within the *HOL1-1,101* gene. Genomic clone pRG120-7 and all subclones derived from it are described in Materials and Methods. The cloned DNA fragments containing the *HOL1-1* and *HOL1-101* mutations are indicated.

allele and the inability of the *HOL1-101* mutation by itself to confer sodium or histidinol uptake suggest that *HOL1-1* alters ion transport specificity and that *HOL1-101* increases the rate of transport.

Alternatively, *hol1* might encode a positive regulator of one or more ion transporters. If *hol1* is indeed the structural gene for an ion transporter, molecular analysis of *HOL1* alleles that alter transport specificity presents a unique opportunity to determine the relationship between its structure and function.

FIG. 9. Southern blot analysis of *hol1* and *hol1Δ* genomic DNAs. DNAs from strains R757 (*hol1*) and R890 (*hol1Δ*) were digested with *HindIII* and *ClaI* before electrophoresis and blotting to the filter. The 0.6-kb *KpnI* fragment from plasmid pRG178-1 used as a probe contains sequences entirely within the functional *HOL1-1,101* gene. The 6.7-kb band in the *hol1* lane was due to partial digestion of the DNA. Details of the experiments are provided in Materials and Methods.

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