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# Regulation of Cation-Coupled High-Affinity Phosphate Uptake in the Yeast *Saccharomyces cerevisiae*

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**Studies of the high-affinity phosphate transporters in the yeast *Saccharomyces cerevisiae* using mutant strains lacking either the Pho84 or the Pho89 permease revealed that the transporters are differentially regulated. Although both genes are induced by phosphate starvation, activation of the Pho89 transporter precedes that of the Pho84 transporter early in the growth phase in a way which may possibly reflect a fine tuning of the phosphate uptake process relative to the availability of external phosphate.**

*Saccharomyces cerevisiae* has over the years provided a model for studies of how a cell makes a coordinated response in adapting to environmental changes in phosphate levels (7, 11). This and other microorganisms have evolved complex mechanisms to efficiently take up this essential nutrient, which is often present in low amounts in the environment. When the cells meet a limitation in external phosphate, a high-affinity transport system with a $K_m$ for external phosphate of 0.5 to 10 μM is mobilized (3, 8). Of the proteins responsible for the high-affinity uptake, one is an $H^+$-coupled phosphate cotransporter encoded by the *PHO84* gene (3). The activity of the Pho84p transporter has been shown to be regulated by the external phosphate level through expression of the gene, sorting of the synthesized protein to the plasma membrane, and degradation by rerouting of the protein to the vacuole (9, 12). The other high-affinity phosphate transporter is encoded by the *PHO89* gene (8). The Pho89p transporter is largely inactive at the pH optimum for Pho84p-mediated transport, suggesting that this transporter has a complementary role in cellular phosphate acquisition. In this study, we have characterized the regulation and activity of the Pho89p transporter by use of mutants lacking either the Pho84p or the Pho89p transporter.

The *S. cerevisiae* strains used were MB191 (*MAT***a** *pho3-1 ade2 leu2-3,112 his3-532 trp1-289 ura3-1,2 can1*) (3), MB192 (*MAT***a** *pho3-1* Δ*pho84*::*HIS3 ade2 leu2-3,112 his3-532 trp1-289 ura3-1,2 can1*) (3), PAM1 (*MAT***a** *pho3-1* Δ*pho89*::*TRP1 ade2 leu2-3,112 his3-532 trp1-289 ura3-1,2 can1*) (8), and PAM2 (*MAT***a** *pho3-1* Δ*pho84*::*HIS3* Δ*pho89*::*TRP1 ade2 leu2-3,112 his3-532 trp1-289 ura3-1,2 can1*) (8). Cells were routinely grown in shaking Erlenmayer flasks at 30°C in low-phosphate ($LP_i$) medium (5), pH 4.5, to an optical density at 600 nm ($OD_{600}$) ranging from 0.1 to 4.5. Cells were harvested by centrifugation at 2,300 × *g* for 10 min and washed either once with 25 mM Tris-succinate (for $P_i$ uptake assays), at a different pH for each experiment, or twice with ice-cold bidistilled water (for $^{31}P$ nuclear magnetic resonance [NMR] measurements). The supernatants were subjected to phosphate concentration measurements spectrophotometrically essentially as described previously (10).

Phosphate uptake in Δ*pho84* cells was assayed by the addition of 1-μl volumes of [$^{32}P$]orthophosphate (0.18 Ci/μmol; 1 mCi = 37 MBq; Amersham-Pharmacia Biotech) to 30-μl aliquots containing (each) 3 mg (wet weight) of cells suspended in 25 mM Tris-succinate buffer, pH 8.5, supplemented with 3% glucose, to a final concentration of 50 μM $P_i$, in the presence of 25 mM NaCl. The suspension was immediately mixed and incubated at 25°C. $P_i$ transport was terminated at given time intervals, in the range of 0.5 to 15 min, by adding 1 ml of ice-cold Tris-succinate dilution buffer. The sample was filtered immediately, the filter (Whatman GF/F) was washed once with the same ice-cold buffer, and the radioactivity retained on the filters was determined by liquid scintillation spectrometry. Phosphate uptake in Δ*pho89* cells and in Δ*pho84* Δ*pho89* cells was assayed under the same condition used for the Δ*pho84* cells, with the exception that the pH and the $P_i$ concentration were 4.5 and 0.22 mM, respectively.

All NMR experiments were conducted on a Varian INOVA 500-MHz spectrometer. Wild-type, Δ*pho84*, and Δ*pho89* cells were harvested at $OD_{600}$ values of 0.5, 1.5, and 3.0. Samples analyzed consisted of 3.0-ml aliquots of cell suspensions (0.5 g [wet weight] of cells/ml) in 25 mM Tris-succinate buffer, pH 4.5. A broad-band probe designed for 10-mm-sample tubes was used. The spectral width was 7,267 Hz. Phosphoric acid (85%), 0 ppm, was used as an external reference. The pulse delay was 2 s, and 1,024 scans of 8,192 complex data points were collected during an experimental time range of approximately 40 min. The 90°C excitation pulse length was determined to be 22 μs. No deuterium frequency lock was used during the experiments. The relative contributions of different $^{31}P$-containing molecules were derived from the corresponding peak area intensities in the $^{31}P$ NMR spectra. The assignment of the $^{31}P$ NMR peaks of intra- and extracellular orthophosphate and nonterminal $P_i$ of polyphosphate were obtained from the literature (4).

Previous studies on $P_i$ transporter gene expression in *S. cerevisiae* have shown that the *PHO84* and the *PHO89* transcripts are induced under $P_i$-deficient conditions (3, 8). The induction of the *PHO84* transcript and synthesis of the transporter require that the concentration of external $P_i$ be lower than 100 μM (9, 12).

To further analyze the functional expression of the Pho84p and the Pho89p phosphate transporters in cells grown in $LP_i$ medium, we compared the phosphate transport properties of the three mutant strains (Δ*pho84*, Δ*pho89*, and Δ*pho84* Δ*pho89* mutants). In agreement with the behavior of wild-type cells (9), mutant cells lacking the Pho89p transporter revealed an activation of [$^{32}P$]phosphate uptake at pH 4.5 when measured at

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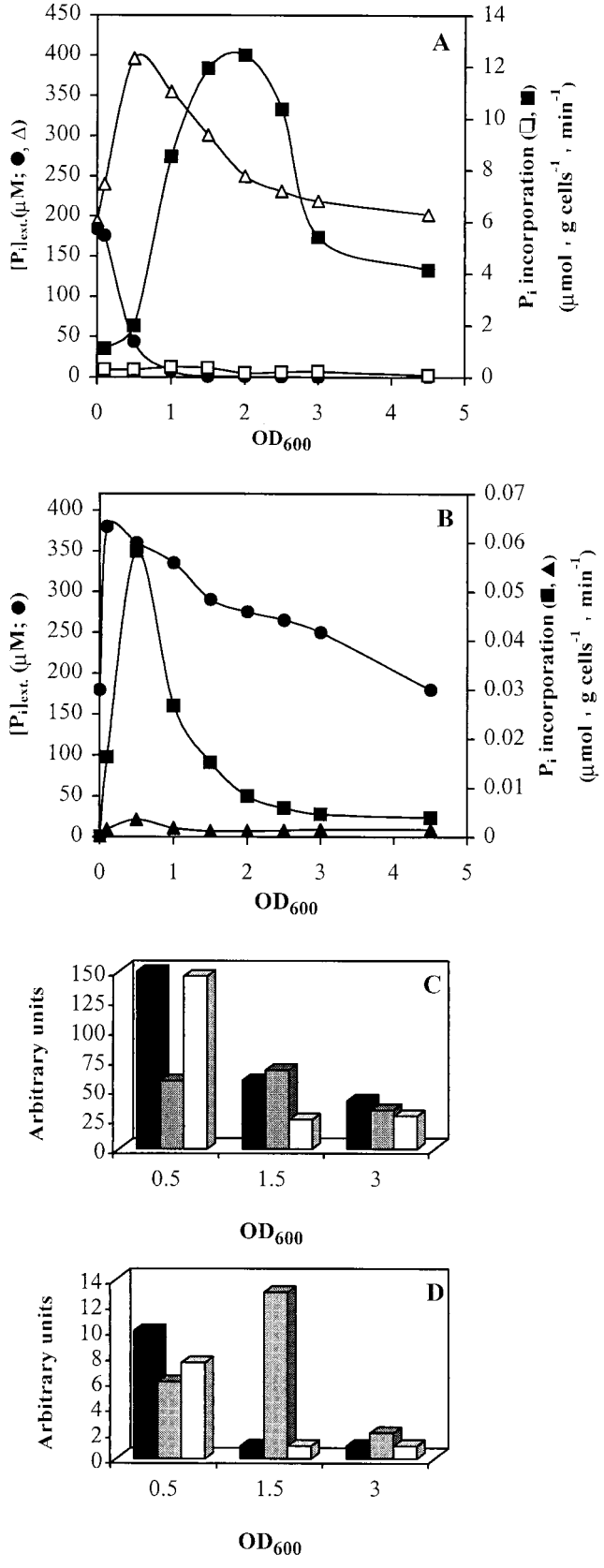


an $OD_{600}$ of 0.5, corresponding to a situation when the external $P_i$ concentration had decreased from the initial concentration of 180 μM to 40 μM. As in the case of wild-type cells (9), the mutant reached its maximum transport activity (12.5 μmol · g of cells$^{-1}$ · min$^{-1}$) at an $OD_{600}$ of close to 2 when the external $P_i$ was close to exhausted (Fig. 1A). Continued growth of the Δ*pho89* cells resulted in a rapid inactivation of the high-affinity [$^{32}$P]phosphate transport. In order to investigate the contribution of the low-affinity $P_i$ transport system in Δ*pho89* cells assayed at pH 4.5, the Δ*pho84* Δ*pho89* double-disruptant strain was used (Fig. 1A). The [$^{32}$P]phosphate transport catalyzed by these cells was at least 20-fold lower than that of the Δ*pho89* cells over the $OD_{600}$ range studied. In contrast to the Δ*pho89* cells, which at an $OD_{600}$ of 0.5 had consumed about 75% of the available $P_i$ in the growth medium, the double disruptant grown and assayed under identical conditions catalyzed a transient efflux of intracellular $P_i$, resulting in a twofold increase of the external $P_i$ concentration compared to that originally contained in the growth medium. The double-disruptant cells grown to $OD_{600}$ values exceeding 0.5, however, regained the ability to take up the excreted $P_i$ (Fig. 1A). Thus, it appears that the [$^{32}$P]phosphate transport activity and the rapid consumption of extracellular $P_i$ observed in Δ*pho89* cells is catalyzed by the high-affinity Pho84p transporter without a significant contribution by the low-affinity transport system.

In order to investigate whether the Pho89p transport activity is subjected to regulation by external $P_i$, $LP_i$-grown Δ*pho84* cells were assayed for [$^{32}$P]phosphate uptake at pH 8.5 (Fig. 1B). Although high-affinity $P_i$ transport in both Δ*pho89* and Δ*pho84* cells revealed a pronounced $OD_{600}$ dependence, activation of the Pho89p transporter occurred at an earlier stage of the growth phase, reaching its maximum (0.06 μmol · g cells$^{-1}$ · min$^{-1}$) at an $OD_{600}$ of 0.5, at which point the Δ*pho89* cells do not catalyze a significant [$^{32}$P]phosphate uptake (the level was 14-fold lower) (data not shown). In agreement with a previous proposal that the Pho89p catalyzes a cation-dependent transport (8), the activity of the Pho89p transporter expressed in Δ*pho84* cells in the absence of $Na^+$ was almost completely abolished (Fig. 1B). In contrast to the activation of the Pho84p transporter in Δ*pho89* cells at pH 4.5, which was paralleled by a lowered external $P_i$ concentration, Δ*pho84* cells catalyzed a rapid initial $P_i$ efflux at $OD_{600}$ values lower than 0.5, resulting in a twofold increase in external $P_i$, after which these cells, like the double-disruptant cells, regained the ability to take up external $P_i$.

Given the high degree of similarity in functional expression and external $P_i$ dependence of Pho84p in the wild-type and the Δ*pho89* cells and the difference observed in the case of Δ*pho84* cells, it was likely that activation of the two transporters would be reflected by an altered cellular level of $P_i$. $LP_i$-grown wild-type, Δ*pho84* and Δ*pho89* cells harvested at different $OD_{600}$ values were subjected to $^{31}$P NMR analysis of changes in intracellular $P_i$ (Fig. 1C) and polyphosphate (Fig. 1D) pools. In a composite of the results, it can be seen that the growth-dependent decrease in intracellular $P_i$ of the wild-type and

FIG. 1. (A) [$^{32}$P]orthophosphate uptake catalyzed by Δ*pho89* (■) and Δ*pho84* Δ*pho89* (□) cells at pH 4.5. Cells were grown in $LP_i$ medium and collected when the $OD_{600}$ reached the value indicated. The supernatants of Δ*pho89* cells (●) and of Δ*pho84* Δ*pho89* cells (△) were used for phosphate determination. (B) [$^{32}$P]orthophosphate uptake catalyzed by Δ*pho84* cells at pH 8.5 in the presence (■) or absence (▲) of $Na^+$. Cells were grown as described for panel A. The supernatant of the cells was used for phosphate determination (●). (C and D) Intracellular levels of inorganic phosphate and polyphosphate, respectively, in wild-type (black bars), Δ*pho84* (shaded bars), and Δ*pho89* (white bars) cells were measured by $^{31}$P NMR.

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Δ*pho89* cells was highly similar, while the cellular $P_i$ content of the Δ*pho84* cells was more than twofold lower at an $OD_{600}$ of 0.5. It is interesting that the twofold-lower content of intracellular free $P_i$ coincided with an approximately twofold increase in extracellular $P_i$ content. Moreover, the slight increase in intracellular free $P_i$ observable when these cells had reached an $OD_{600}$ of 1.5 was paralleled by a decrease in extracellular $P_i$ content (Fig. 1B). As in the case of the intracellular content of $P_i$, both wild-type and Δ*pho89* cells maintained polyphosphates at significant and comparable levels at an $OD_{600}$ of 0.5 while the polyphosphate content was close to exhausted at higher $OD_{600}$ values. In contrast, Δ*pho84* cells, which initially had a slightly lower polyphosphate content, had, at an $OD_{600}$ of 1.5, accumulated a high level of polyphosphates which, at an $OD_{600}$ of 3, had been reduced to a level comparable to that of wild-type and Δ*pho89* cells. The $P_i$ acquisition by Δ*pho84* cells following the initial rapid efflux was during prolonged growth ($OD_{600}$ of 1.5) paralleled by a pronounced synthesis of intracellular polyphosphate known to occur under conditions where phosphate and metabolic energy are available, especially when $P_i$ is added to cells previously starved for $P_i$, resulting in intracellular $P_i$ levels of up to 20 μmol/g (wet weight) of cells (2, 13). It has been suggested that when, with continued growth, the metabolic requirements of the cells exceed the extracellular supply of $P_i$ which can be taken up via the $P_i$ transporters, vacuolar polyphosphate is mobilized to replenish the cytosolic phosphate pool (1, 6).

In summary, the results presented in this work reveal that regulation of the Pho84p $P_i$ transport activity does not require the participation of the Pho89p, as the transport activity of the Pho84p in Δ*pho89* cells is regulated as in the wild-type cells. Interestingly, both Δ*pho84* cells and double-disruptant cells, devoid of a high-affinity transport system active at pH 4.5, catalyze an apparent rapid efflux of internal $P_i$. The obtained results suggest that the activation of the Pho84p transporter and that of the Pho89p transporter are independently regulated, with activation and inactivation of the Pho89p transport activity early in the growth phase and the Pho84p transport, in contrast, maximally active at mid-log phase.

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