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Minireview

A novel alkali-tolerant *Yarrowia lipolytica* strain for dissecting Na^+-coupled phosphate transport systems in yeasts

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Abstract

The newly isolated osmo-, salt- and alkali-tolerant *Yarrowia lipolytica* yeast strain is remarkable by its capacity to grow at alkaline pH values (pH 9.7), which makes it an excellent model system for studying Na^+-coupled phosphate transport systems in yeast cells grown at alkaline conditions. In cells *Y. lipolytica* grown at pH 9.7, phosphate uptake was mediated by several kinetically discrete Na^+-dependent systems that are specifically activated by Na^+ ions. One of these, a low-affinity transporter, operated at high-phosphate concentrations. The other two, derepressible, high-affinity, high-capacity systems, functioned during phosphate starvation. Both H^+- and Na^+-coupled high-affinity phosphate transport systems of *Y. lipolytica* cells were under the dual control of the prevailing extracellular phosphate concentrations and pH values. The contribution of the Na^+/P_i-cotransport systems into the total cellular phosphate uptake activity was progressively increased with increasing pH, reaching its maximum at pH $\geq$ 9.

Keywords: *Yarrowia lipolytica*; Yeast; Plasma membrane; Phosphate transport; Na^+/P_i-cotransport systems; Regulation

1. Introduction

Phosphorus is one of the major, essential macronutrient acquired by yeasts. It is a structural element of diverse cellular components, including nucleic acids, proteins, lipids, and sugars, a constituent of energy transfer reactions, and a regulator in signal transduction cascades. However, despite its widespread occurrence, inorganic phosphate (P_i) is often present in low amounts in many ecosystems (Harold, 1966). Therefore, yeasts, like other organisms, have to evolve complex mechanisms for sensing P_i availability and adjusting coordinated gene expression and metabolic activities in response to varying P_i levels. In *Saccharomyces cerevisiae*, the PHO regulatory pathway regulates expression of the "PHO" genes involved in the scavenging, specific uptake, integrating and storing of P_i (Lenburg and O'Shea, 1996; Oshima, 1997; Persson et al., 1999; Lagerstedt et al., 2000; Persson et al., 2003). When extracellular P_i concentrations are low, many genes encoding P_i transporters, phosphatases, phosphodiesterase(s), RNases, polyphosphate kinase(s), and endopolyphosphatase(s) are trancriptionally induced (see Persson et al., 2003).

Abbreviations: CAPS, 3-[cyclohexylamino]-1-propanesulfonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanine iodide.

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2. Inorganic phosphate uptake

A primary step in the utilization of P_i is its uptake by plasma membrane transporters, concentrating P_i against its thermodynamic gradient by a cotransport with H^+ or Na^+ (for a recent review, see Persson et al., 2003). Among all eukaryotic non-animal cells, most of the available information on the P_i transport systems has been confined to the *S. cerevisiae* yeast. In this fungus, three different systems have been proposed to be involved in uptake of P_i from the cultivation medium (see Persson et al., 2003; Lagerstedt et al., 2004). The so-called low-affinity system, with an apparent K_m for extracellular P_i of approximately 1 mM at its pH optimum of 4.5, has been suggested to be a constitutively expressed H^+/P_i cotransporter (Borst-Pauwels, 1981; Nieuwenhuis and Borst-Pauwels, 1984; Tamai et al., 1985). Recently, two new PHO genes, *PHO90* and *PHO91*, have been identified as possibly constituting the expression of the low-affinity transporter (Wykoff and O'Shea, 2001).

Besides this low-affinity system, a growing family of potentially derepressible P_i transporters is now believed to exist, including Pho84p, Pho88p, Pho89p (see Giots et al., 2003; Persson et al., 2003). The Pho84p of *S. cerevisiae*, a 65-kDa hydrophobic membrane protein, the product of the *PHO84* gene (Oshima, 1991; Bun-Ya et al., 1991), is responsible for the major cellular phosphate uptake under P_i limiting conditions. It specifies a high-affinity H^+/P_i cotransporter, being maximally active at pH 4.5 and having an apparent K_m value for phosphate of 1–15 μM (Cockburn et al., 1975; Roomans and Borst-Pauwels, 1979; Borst-Pauwels, 1993; Behre et al., 1995). Pho84p belongs to the class of 12-transmembrane helix transporters, displaying a high degree of similarity to members of the yeast hexose transporter family (Bisson et al., 1993; Henderson, 1993) and to both Snf3p and Rgt2p involved in sensing of external glucose concentrations (Özcan et al., 1996). Although Pho84p has been shown to be solely responsible for H^+-coupled P_i uptake in the model systems (plasma membrane vesicles enriched in the Pho84p or proteoliposomes loaded with Pho84p) (Behre et al., 1995; Fristedt et al., 1996, 1999a,b), several other auxiliary proteins, including Pho87p (Bun-Ya et al., 1996; Yompakdee et al., 1996a,b), Pho88p (Yompakdee et al., 1996a,b) and Gtr1 (Bun-Ya et al., 1992) are proposed to be associated with the Pho84p-mediated transport system, possibly serving as receptors for P_i signaling or altering the intrinsic stability of Pho84p. Under P_i-starvation, the Pho84p is transcriptionally up-regulated and sorted to the plasma membrane. The correct sorting of Pho84p depends on Pho86p, an endoplasmic reticulum resident protein, possibly required for packaging of Pho84p into COPII vesicles (Lau et al., 2000). The rate of P_i uptake by Pho84p increases during the exponential growth in low-P_i medium, reaching its maximum at the late-exponential growth phase, and then rapidly declined. The onset of the decline in P_i transport activity coincides with decreasing of the extracellular P_i concentration up to 10 μM, which is close to the reported K_m value for the transporter (Martinez et al., 1998), thus supporting the idea that both the derepression and inactivation of the Pho84p is under the control of extracellular phosphate level (Bun-Ya et al., 1991; Martinez et al., 1998). As external phosphate is totally exhausted, the Pho84p is inactivated and routed to the vacuole to be degraded (Petersson et al., 1999; Lagerstedt et al., 2000).

The other high-affinity transporter, corresponding to the *PHO89* gene product (Martinez and Persson, 1998), is a Na^+-coupled P_i uptake system, active predominantly at pH 9.5, with a K_m for P_i of 1 μM at pH 7.2 (Roomans and Borst-Pauwels, 1979; Martinez and Persson, 1998). Like Pho84p, the pho89p is organized into 12 discrete hydrophobic domains (see Persson et al., 2003) and is a homoloque of the mammalian type III Na^+-P_i transporters. However, the activity of the Na^+-coupled transporter in *S. cerevisiae* is very low, casting some doubt on the physiological significance of this P_i transporter. Obviously, the *S. cerevisiae* yeast, thriving under acidic conditions and only barely growing at pH $\geq$ 8.0, is not the best model organism for studying Na^+-coupled transporters, active predominantly at alkaline conditions. Clearly, more appropriate yeast species are needed to gain precise resolution of P_i transport mechanisms in yeast cells grown at alkaline conditions. For this purpose, in our studies we used the recently isolated novel osmo-, salt- and alkali-tolerant strain of *Yarrowia lipolytica* (Zvyagilskaya et al., 2001a). The strain was isolated from salt-excreting leaves of desert plants, containing a fauna that copes in different ways with fluctuating and often extreme temperature, pH, salinity, and water activity (Simon et al., 1994). Furthermore, the salt-excreting leaves of arid plants were commonly colonized by the novel *Y. lipolytica* strain, thus indicating its perfect adaptation to extreme growth conditions. The isolated strain shares all advantages of the *Y. lipolytica* yeast, being non-toxic, growing to very high densities, having a haploid genome and sexual life cycle, and therefore being amenable to both classical and molecular genetic techniques. The new strain, however, differs from other typical *Y. lipolytica* strains and, more generally, from yeast species by its inherent ability to grow over a wide range of pH values, from 3.0 to 10.0 (Zvyagilskaya et al., 2001a). It is worthwhile to note that pH 10.0 is the upper pH limit for yeast growth; the overwhelming majority of yeast species thrive at pH 5.5–6.5 and can only barely grow at pH $\geq$ 8. The capacity to grow vigorously at alkaline pH values makes the new *Y. lipolytica* strain a promising model in clarifying general principles of adaptation to extreme environmental factors and an exceptionally