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Lack of arginine- and polyphosphate-storage pools in a vacuole-deficient mutant (end1) of *Saccharomyces cerevisiae*

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Yeast cells accumulate large amounts of arginine and polyphosphate in their vacuoles and utilize these compounds as endogenous nitrogen or phosphate sources under conditions of starvation. We examined a vacuoleless mutant, end1, and found that it stored virtually no arginine or polyphosphate when grown on a medium with arginine as the sole nitrogen source. When starved of nitrogen or phosphate it stopped growing much faster than the wildtype. Unlike the wildtype, end1 showed no accumulation of polyphosphate and, concomitantly, of arginine after a period of phosphate starvation. The results support the concept that vacuoles contain the main reserves of nitrogen and phosphate in fungi.

Arginine; Polyphosphate; Starvation; Vacuole; (Yeast)

1. INTRODUCTION

Fungi store large pools of amino acids in the vacuoles [3,4,7,9,15,16,18]. Particularly basic amino acids rich in nitrogen, such as arginine, are accumulated in enormous amounts and play a central role as nitrogen reserves [3,6,7,9,17–19]. Moreover, fungi also store their main P reserve in the vacuoles in the form of polyphosphate (poly-P), a linear polymer of orthophosphate linked by energy rich phospho-anhydride bonds [3,11,14]. These prominent vacuolar reserves of opposite charge frequently occur in almost equal amounts suggesting a relationship of mutual charge compensation. However, since the two reserves can be mobilized independently by yeast and *Neurospora* under conditions of P or N starvation alternative counterions must be available for charge compensation under these starvation conditions [3,6,9].

Considering the important homeostatic function of vacuoles as storage organelles for N and P reserves as well as for the ionic and osmotic relationships in the cells [18,19], it was tempting so see how mutants lacking proper vacuoles [1,2,5,13] behaved under conditions of N or P starvation. We found that the vacuole-deficient mutant end1 (apparently an allelic mutation to the class *c* vpt11 mutation: [5,13]) contained virtually no arginine or poly-P. When starved for N or P it stopped growing much earlier than the wildtype strain.

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2. EXPERIMENTAL

2.1. *Organisms and cultivation*

Saccharomyces cerevisiae wildtype strain (X-2180-1A, a) and the end1 (110-1A, α) mutant in the same background, both obtained from H. Riezman, were grown on a defined liquid medium containing arginine (10 mM) as the sole nitrogen source and glucose (1%, w/v) as carbon source; mineral salts, vitamins and trace elements were as before [6]. Cultures were grown in conical flasks on a rotary shaker (145 rpm) at 27°C.

2.2. *Phosphate and nitrogen starvation*

Cultures in the exponential phase of growth ($\leq 7 \times 10^6$ cells/ml) were chilled on ice. Cells were collected rapidly by centrifugation (3000 rpm, 3 min), washed twice in cold distilled water (0°C) and resuspended in fresh, prewarmed (27°C) media lacking the P or N source. At different time intervals after initiating P starvation, 15 mM KH_2PO_4 was added again.

2.3. *Analytical methods*

For measuring, poly-P cells were harvested from 10 ml of

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culture by filtration (Whatman GF/C) and washed twice in 50 mM citric acid (NaOH) buffer, pH 5.1, at 0°C. Cells were resuspended in 1 ml of the same buffer at 0°C and boiled for 10 min. The cell suspension was used to determine poly-P as described [6]. For arginine measurements, cells were harvested and washed as described above, but resuspended in 1 ml of 80% ethanol. Arginine and other amino acids were extracted by incubating the suspension at 60°C for 15 min. The extract was centrifuged and the supernatant was brought to dryness under a stream of air at 50°C. Dry samples were dissolved in

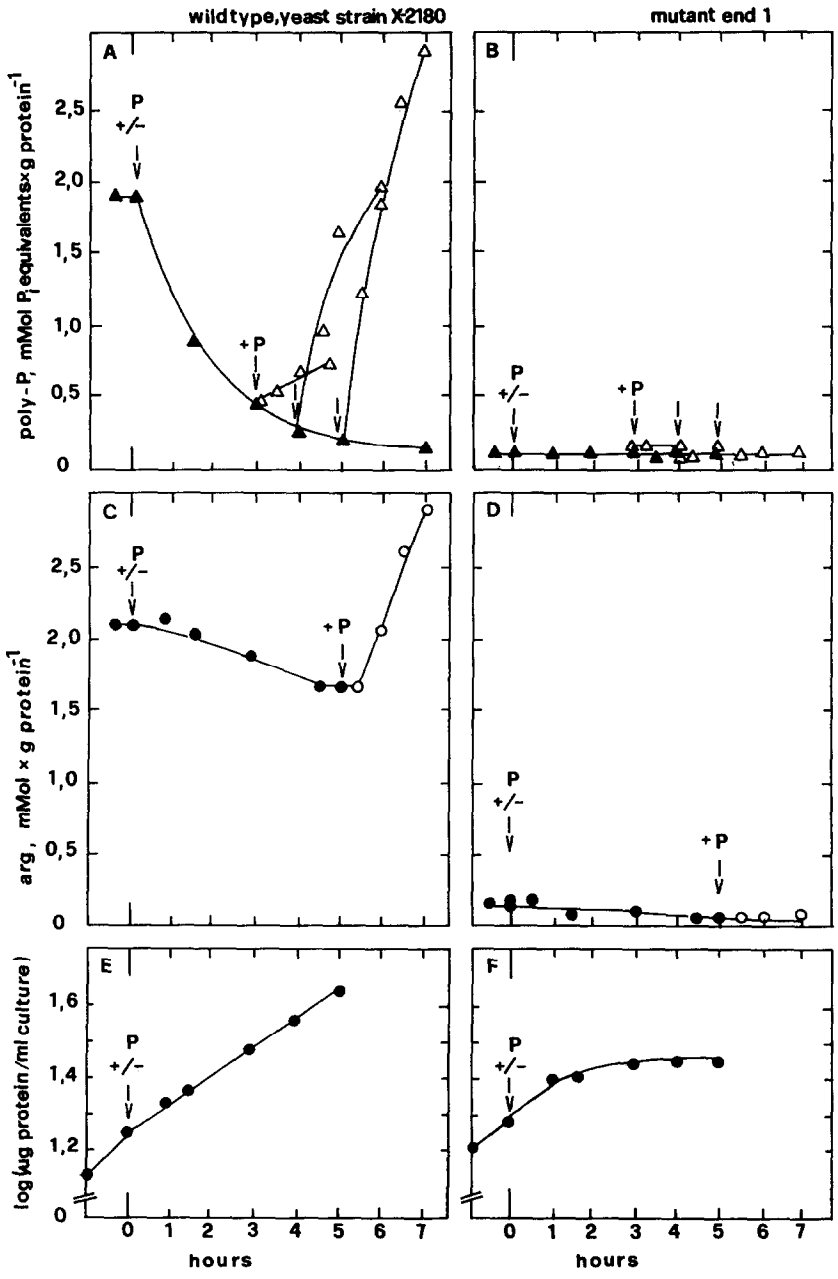

Fig.1. Transfer of *Saccharomyces cerevisiae* wildtype strains (A,C,E) and of the vacuoleless mutant end1 (B,D,F) from a complete medium (arginine as the nitrogen source) to a medium lacking phosphate (P + / −, at 0 h). After the time intervals indicated (+P) aliquots of the cultures were withdrawn and phosphate (15 mM KH_2PO_4) was added again. Contents of poly-P (A,B) and arginine (C,D) per protein. Growth (protein) of the cultures (E,F). Open symbols (○ △) give the parameters after addition of phosphate.

methanol/H_2O 1:1 before analysis. Arginine and other orthophthalaldehyde derivatized amino acids were determined fluorometrically by reverse-phase HPLC (C-18 column, Hypersil ODS, 5 μm, 250×4.6 mm) essentially according to Jones et al. [8]. Protein was measured according to Peterson [12] using BSA as a standard.

3. RESULTS AND DISCUSSION

Saccharomyces cerevisiae (X-2180) grown on arginine as the sole nitrogen source accumulated large quantities of poly-P (1.8 mmol P_i equivalents of poly-P/g protein) and arginine (2.1 mmol arginine/g protein). After transferring the culture to a medium lacking P, poly-P was mobilized (fig.1A) and growth continued for more than 5 h (fig.1E). The mean generation time (g) increased only slightly from 2.5 h before starvation to 3.7 h thereafter. The arginine pool was also reduced slightly (fig.2C). Hence anions other than poly-P had to compensate the charge of the arginine under these conditions.

Addition of phosphate after P starvation induced rapid accumulation of poly-P (fig.1A). The concentration finally reached, exceeded by far that present in the cells before P starvation. This is a well-known phenomenon called poly-P overcompensation or poly-P overplus occurring in many unicellular organisms storing poly-P [11]. The overcompensation only occurred if the period of P starvation was long enough to deplete the poly-P reserves in the cells (fig.1A). It is remarkable that the poly-P overcompensation was accompanied by an arginine accumulation in comparable amounts (fig.1B), which indicates that in this case storage of these two vacuolar reserves of opposite charge is related.

The vacuole-deficient mutant (end1) behaved quite differently under the same conditions. Grown

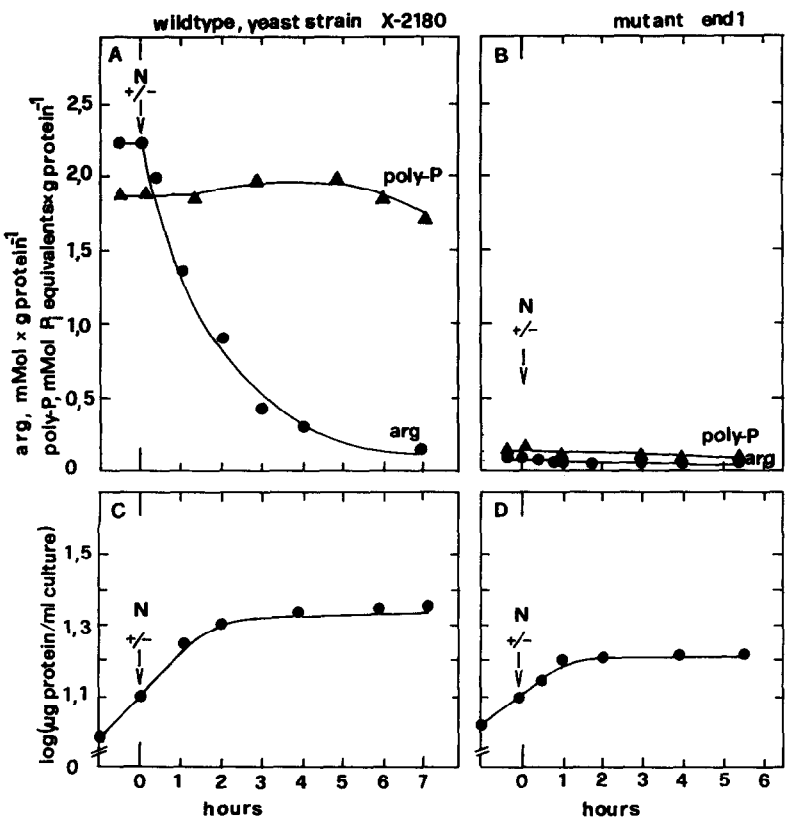

Fig.2. Transfer of *Saccharomyces cerevisiae* wildtype strain (A,C) and the vacuoleless mutant end1 (B,D) from a complete medium (arginine as the sole nitrogen source) to a medium lacking the nitrogen source (N+/− at 0 h). Amounts of arginine (●) and poly-P (▲) per protein (A,B). Growth (protein) of the cultures (C,D).

on arginine, it contained almost no poly-P or arginine (fig.1B and D). Considering the lack of the main P reserve, it was not surprising to find that the mutant stopped growing after 1 h of P starvation, allowing an increase of protein of about 30% only (fig.1F). Moreover the mutant did not show the phenomenon of poly-P and arginine overcompensation (fig.1B and D).

After being transferred to a medium lacking a N source, the wildtype strain continued to grow at a constant rate for nearly 2 h corresponding to about 1 generation (fig.2C). The stored arginine was mobilized as an endogenous N reserve whereas the poly-P content remained virtually constant (fig.2A). The charge of poly-P had to be balanced by cations other than arginine under these conditions. In contrast, the vacuoleless mutant subjected to N starvation stopped growing within 1 h corresponding to about 0.3 generations (fig.2B). The small arginine and poly-P pools present in the mutant changed only slightly (fig.2A). Microscopic observations showed that the mutant did not die after 6 h of P or N starvation. The results show that vacuole deficiency as induced by the end1 mutation and probably also by the allelic vpt11 mutation [2,5,13] causes a deficiency of the storage pools for both N and P. It is interesting that a lysine-sensitive mutant deficient in basic amino acid storage pools (slp1) also turned out to be lacking proper vacuoles [10]. In the natural habitats of many yeasts and molds, P and N are probably the nutrients whose supply is most often limited [20]. Therefore, it is certainly of great ecological importance for these fungi that they can increase phosphate and nitrogen stores rapidly in the vacuole when the nutrients are abundant in order to allow growth and metabolism during the frequently occurring periods of need.

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