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Regulation of potassium fluxes in *Saccharomyces cerevisiae*

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To investigate the regulation of K^+ fluxes in *Saccharomyces cerevisiae* the dependence of K^+ efflux and Rb^+ influx on $[K^+]_i$, pH_i, $[Na^+]_i$, membrane potential, cell volume, and turgor pressure were studied in cells with different K^+ contents. By decreasing the cell volume with osmotic shocks and the cellular pH with butyric acid the following was found. (1) The K^+ efflux induced by uncouplers decreases simultaneously with the decrease of the K^+ content of the cell, but the process was insensitive to $[K^+]_i$, pH_i, cell volume and turgor pressure. The internal presence of Na^+ inhibited this K^+ efflux. (2) The increase of the V_{max} of Rb^+ influx observed in low-K^+ cells is due to the decrease of the pH_i and probably mediated by the increase of the activity of the plasma membrane ATPase. The V_{max} is independent of $[K^+]_i$, $[Na^+]_i$, cell volume and turgor pressure. (3) The decrease in the K_m of Rb^+ influx observed in low-K^+ cells does not depend directly on $[K^+]_i$, pH_i, cell volume or turgor pressure. If Na^+ is present, $[Na^+]_i$ might be directly involved in the regulation of the K_m.

Introduction

In all living cells K^+ neutralizes an important part of the negative charges of cellular anions, and internal K^+ is kept normally at much higher concentrations than that in the external environment. Except in Na^+-loaded cells, fungi take up K^+ in exchange for H^+ [1–3]. This K^+/H^+ exchange balances the acidifying processes and controls the cellular pH [4,5] and, as a consequence, H^+-symported anions or organic acids produced by the metabolism are converted into K^+ salts. Obviously, an excessive or insufficient exchange would lead to an exceedingly high or low internal pH, but the kinetics of the H^+ pump and the K^+ uptake oppose these deviations. Thus, in *Neurospora crassa*, if pH_i became too acidic due to an acid load, the pump increases its activity in response to the increase of its substrate, delivering more current and hyperpolarizing the membrane [6]. Hyperpolarization enhances K^+ influx [7], and, as a consequence of the increase in the K^+/H^+ exchange, $[K^+]_i$ increases and $[H^+]$ decreases. In the case of alkalinization, the decrease in the activity of the pump decreases K^+ uptake.

It is well established that, both in *Saccharomyces cerevisiae* [8,9] and in *N. crassa* [10], any decrease in the cell K^+ content enhances K^+ uptake. Very small decreases, hardly detected by cell analysis, significantly decrease the K_m of K^+ (or Rb^+) influx without affecting the V_{max}. A more extensive K^+ loss further decreases the K_m and increases the V_{max}. Since cells with a lower K^+ content have a lower pH_i [11,12], a simple mechanism to explain this response is by the effect of pH_i either directly [13] or activating the pump, as described above. However, this model has not been tested by manipulating $[K^+]_i$ without affecting $[H^+]_i$, a condition required for a direct confirmation of the model.

The K^+ content of the cells of *S. cerevisiae* and *N. crassa* also regulates the K^+ efflux induced by uncouplers, which is inhibited by the decrease of the K^+ content of the cell [9,12]. Conceivably this behaviour has a teleological significance, because K^+ efflux may be the form to use the energy stored as a K^+ gradient, as proposed in bacteria [14], maintaining the membrane potential during the rapid uptake of positive charges [15]. However, this loss would turn out to be a prejudice if it became too extensive, suggesting that efflux should be tightly regulated.

In addition to the regulations described, K^+ could be also affected by changes in the cellular volume or in the

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Mes, 4-morpholineethanesulfonic acid; Hepes, 4-(2-hydroxyl)-1-piperazineethanosulphonic acid.

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turgor pressure. In fact, K^+ is one of the most abundant osmotic components in the cell, and it may be involved in osmoregulation, as it occurs in bacteria [16].

The experiments reported in this paper were undertaken to investigate whether the variations in $[K^+]_i$, pH_i, volume, and turgor pressure are responsible for changes in the kinetics of K^+ uptake and K^+ efflux in yeast.

Materials and Methods

Strains and preparation of cells

The wild strain XT300.3A (*MATα, ade2-1*) has been used throughout the work, unless otherwise stated, but some experiments were also performed with the K^+-transport mutant PC-1 (*MATα, ade2-1, trk1-1*) [17]. Rb^+ uptake in cells with a low plasma membrane H^+-ATPase was studied in the strain RS-72, in which the promoter of the ATPase is replaced by a galactose-dependent promoter (control strain RS-74) [18], and in strains RS-515 and RS-516, in which the promoter contains insertions and deletions (control strain RS-514) [19]. Although strains RS-515 and RS-516 were maintained in galactose, all the experiments were performed with glucose grown cells (see Ref. 19).

Normal-K^+ cells were grown in the arginine/phosphate synthetic medium previously described [8] with 2 mM K^+, and harvested in the middle of the exponential growth phase. K^+-starved cells were grown in the same medium with 20 μM K^+ and harvested when external K^+ dropped to 2 μM [8,9]. Azide-treated cells, with approximately half the normal K^+ content, were prepared by exposing normal-K^+ cells to 10 mM sodium azide for 7 min as described in Ref. 9. Cells with an intermediate K^+ content, between normal-K^+ cells and K^+-starved cells, were prepared inoculating 20 μM K^+ medium and harvesting the cells when the external K^+ dropped to 5 μM.

Cytoplasmic and vacuolar K^+ contents

Differential extraction of K^+ from the cytoplasm and vacuole was essentially achieved as previously described [20], using cytochrome *c* to permeabilize the membranes, 0.7 M mannitol to stabilize the vacuoles, and 10 mM Hepes as buffer instead of acetic acid. The cells were treated with cytochrome *c* in 0.7 M mannitol and washed three times with 0.7 M mannitol, then transferred to the buffer without mannitol and washed three times with this buffer. In K^+-starved cells, the buffer was supplemented with 5 mM deoxyglucose and 18 μg ml^{-1} antimycin A to prevent non permeabilized cells from taking up the K^+ released by the permeabilized cells. The K^+ remaining in non permeabilized cells and in unburst vacuoles was extracted with 0.2 M HCl.

Cellular volumes and water contents

Cells were harvested from the growth medium and washed with buffer. Aliquots with 10 mg of cells (dry weight) were suspended in approx. 60 μl of the standard buffer without glucose, and the whole volume transferred to two or three microhematocrit calibrated capillary tubes. After centrifugation the volumes of the packed cells and supernatants were calculated from their respective lengths in the capillary tubes.

To calculate the cellular water, ^3H_2O (200 Bq · $mmol^{-1}$) was used to label the total water, and D-[U-^{14}C]sorbitol or L-[1-^{14}C]glucose at 0.5 mM (185 MBq · $mmol^{-1}$) to label the extracellular water. The isotopes, each one in independent experiments, were added to the thick suspensions of cells, mixed and allowed to equilibrate for 3 min. Then the suspension was transferred to the capillary tubes, as in unlabeled experiments. After centrifugation, the volumes of pellets and supernatants were measured, and the capillary tubes were cut just on the limits of the pellets. Pellets and supernatants were counted in a liquid scintillation counter and the data were corrected for quenching. Isotopic activities in the supernatants were used to calculate the total water (^{3}H counts) or the extracellular water (^{14}C counts) of the pellets.

Cation contents and fluxes

Samples of cells were filtered through Millipore membrane filters, 0.8 μm pore size, and the collected cells were rapidly washed with 20 mM $MgCl_2$ solution. If any of the cations to be analyzed exceeded 100 μM in the medium, the collected cells on the filter were washed out onto a new filter, and washed again in the same manner. Filters with cells were acid extracted and analyzed by atomic absorption spectrophotometry.

The initial rates of Rb^+ uptake (Rb^+ influx) were determined by analysis of the cells as described previously [8,9]. When the cells had been osmotic shocked with sorbitol, the same concentration of sorbitol was kept during the washes.

In K^+-loss experiments, 150 μM CCCP was added to the cell suspension at time zero.

Flux experiments were carried out at a cell density of 0.2 to 0.5 mg · ml^{-1} (dry weight).

Internal pH

The internal pH was determined by the propionic acid distribution method [21]. The experiments were performed at 120 μM [1-^{14}C]propionic acid/propionate (60 MBq · $mmol^{-1}$) (pH 5.8) at a cell density of 5 mg · ml^{-1}, allowing 12 min for equilibration.

ATP content

The ATP content of the cells was determined by using the luciferine-luciferase method as described in Ref. 22.

Buffers

Unless otherwise stated the experiments were carried out in the standard buffer, 10 mM Mes brought to pH 6.0 with $Ca(OH)_2$, 0.1 mM $MgCl_2$ and 2% glucose. For other pH values: tartaric acid, Hepes or 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulphonic acid substituted for Mes, as required. In buffers with 10 mM butyric acid, this acid was brought to pH 5.0 with $Ca(OH)_2$ and the buffer was completed as before.

Results

K^+ loss in uncoupler-treated cells

In the presence of a sufficient concentration of uncouplers, yeast cells lose K^+, but this loss is limited to about half the normal K^+ content [9]. The time-course of the loss showed that the rate decayed with time during the treatment, concomitantly with the decrease of the K^+ content of the cell (Fig. 1). K^+-starved cells with different K^+ contents behaved as cells that had lost K^+ due to the uncoupler treatment, and the time-courses of the K^+ losses in these cells superposed to segments of that of the normal-K^+ cells, when compared in the same range of cellular K^+ contents. In all cases the K^+ loss was insensitive to the external pH (from pH 3.0 to pH 8.0, the time-courses of K^+ losses were similar to those shown in Fig. 1). It is worth mentioning that the CCCP concentrations used in the described experiments (150 μM) inhibited completely Rb^+ influx. Therefore, the net losses recorded in Fig. 1 are virtually K^+ effluxes.

To investigate the regulation of the K^+ efflux induced by uncouplers, it has to be considered that both uncoupler treatment [23] and K^+ starvation decrease simultaneously the K^+ content and the internal pH of the cells (Table I). Therefore, both K^+ and H^+ were candidates as regulators of K^+ efflux. By treating the cells with 10 mM butyric acid the internal pH was reduced 0.4 pH units in normal-K^+ cells (from 7.1 to 6.7), but this treatment did not affect the time-course of the K^+ loss (the presence of 10 mM butyric acid at pH 5.0 in experiments similar to those shown in Fig. 1 did not affect the time-course). Therefore, the low pH_i was not the cause of the low rate of K^+ loss in low-K^+ cells.

TABLE I

Internal pH values of different types of cell as measured by [^{14}C]propionic acid distribution

Type of cell	pH_i
Normal-K^+	7.10 ± 0.03
Intermediate-K^+	6.90 ± 0.02
Azide-treated	6.64 ± 0.04
K^+-starved	6.56 ± 0.03

K^+ loss in a trk1 mutant

In the search for identifying the pathway of K^+ efflux, a K^+ transport mutant strain (PC-1) was used. This mutant does not decrease significantly the K_m when the K^+ content decreases and the V_{max} is completely independent of the K^+ content [17]. The original strain and the mutant were grown in the same medium at a K^+ concentration that did not limit the growth of the mutant, and both strains were treated with CCCP. Fig. 2 shows that the losses in both strains were identical. This indicated that K^+ efflux is perfectly well regulated in the mutant and that the system affected by the mutation does not mediate the K^+ efflux induced by uncouplers.

Rb^+ uptake and K^+ efflux in osmotic shocked cells

K^+-starved cells, and cells depleted of K^+ by uncoupler treatment take up K^+ and Rb^+ at much higher rates than K^+-repleted cells [8,9], and lose K^+ at lower

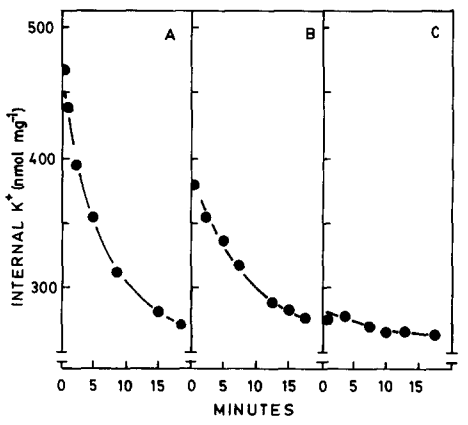

Fig. 1. K^+ loss in cells treated with 150 μM CCCP, in standard buffer: (A) normal-K^+ cells; (B) intermediate-K^+ cells; and (C) K^+-starved cells. Cells were prepared and assayed as described in text.

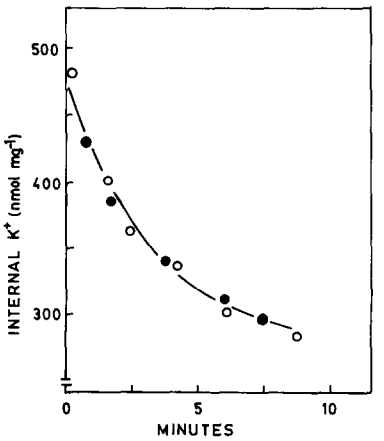

Fig. 2. K^+ loss induced by 150 μM CCCP in the wild-type strain XT300.3A (○) and in the PC-1 mutant (●). Wild-type and mutant were grown in the arginine/phosphate medium with 3 mM K^+. Assays in buffer as described in text.

TABLE II

Cell water content ($\mu l \cdot mg^{-1}$) of normal-K^+ cells and K^+-starved cells in buffer and after an osmotic shock with sorbitol

Sorbitol (molal)	Cell water content ($\mu l \cdot mg^{-1}$)	
	normal-K^+ cells	K^+-starved cells
0	2.06 ± 0.02	1.46 ± 0.03
0.3	1.38 ± 0.05	1.13 ± 0.04
0.5	1.10 ± 0.05	1.02 ± 0.05

rates (Fig. 1). These correlations between K^+ fluxes (influx and efflux) and K^+ contents indicate that fluxes are not independent of their effects on the concentration of cellular K^+, but they do not prove that fluxes respond directly to the internal K^+ concentration. It is worth observing that during K^+ starvation or K^+ loss the cells contract (Table II), and that the actual concentration of K^+ did not change as much as the K^+ content when referred to dry weight. Furthermore, since both K^+ starvation and uncoupler treatment decrease the K^+ content and the internal pH (Table I) simultaneously, nothing is known about the effect of the K^+ concentration if the internal pH is constant. Therefore, it was decided to investigate K^+ fluxes in osmotic shocked cells. Cells shocked with sorbitol decreased their volume significantly (Table II), but they did not lose K^+ or change their internal pH during the time required for the experiments (15 min).

Although osmotic shocked cells did not lose K^+, it was necessary to find out whether the shock induced cytoplasmic–vacuolar K^+ exchanges. To investigate this point normal-K^+ cells and K^+-starved cells were shocked with 0.7 M sorbitol (in the conditions used for the analysis of cytoplasmic and vacuolar contents) and, at intervals between zero and 15 min, cytoplasmic and vacuolar K^+ were analyzed. The analyses showed that cytoplasmic and vacuolar K^+ were constant during this

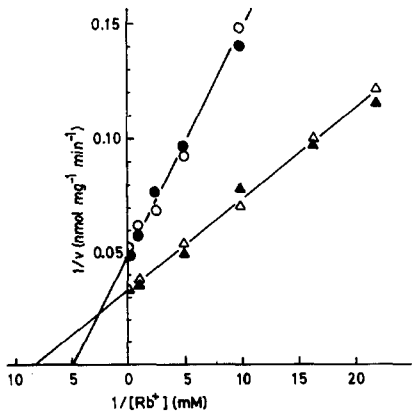

Fig. 3. Double-reciprocal plots of the initial rates of Rb^+ uptake in K^+ starved cells (△, ▲) and intermediate-K^+ cells (○, ●) in buffer (○, △) and in buffer containing 0.3 molal sorbitol (●, ▲). Intermediate-K^+ cells contained 300 nmol $K^+ \cdot mg^{-1}$.

time (respectively, 260 nmol · mg^{-1} vs. 245 nmol · mg^{-1} in normal-K^+ cells, and 105 nmol · mg vs. 205 nmol · mg^{-1} in K^+-starved cells).

The increase in the internal K^+ concentration brought about by the loss of water consequent to an osmotic shock affected very slightly, if at all, the kinetics of Rb^+ uptake in all kind of cells tested: normal-K^+ cells (not shown), K^+-starved cells or intermediate-K^+ cells (Fig. 3). Similarly, the K^+ efflux induced by CCCP was not affected by the osmotic shocks (the time-courses of K^+ loss in the presence of 0.3 and 0.5 molal sorbitol were identical to those shown in Fig. 1). These results ruled out the possibility that $[K^+]_i$ regulates K^+ fluxes directly, since the osmotic shocks increased $[K^+]_i$ very significantly.

Rb^+ uptake and K^+ efflux in Na^+ cells

Another possibility to decrease the K^+ content of yeast cells is to substitute Na^+ for the cellular K^+ by growing the cells in low-K^+, high-Na^+ media [11] (normal-K^+ cells contain approx. 500 nmol $K^+ \cdot mg^{-1}$ and do not contain Na^+). Yeast cells in which Na^+ substitutes half of the K^+ content (250 nmol of K^+ and 250 nmol of Na^+ per mg) had a normal pH_i (7.02) and a normal water content (2.07 $\mu l \cdot mg^{-1}$). In these cells, the kinetics of Rb^+ uptake showed the V_{max} characteristic of normal-K^+ cells (10 nmol · mg^{-1} min^{-1}), but the K_m corresponded to that of K^+-starved cells (0.2 mM Rb^+, see Ref. 9). Remarkably, the K^+ loss induced by uncouplers in these cells was completely inhibited (conditions as those in Fig. 1, but with Na^+ cells). We repeated the efflux experiments in cells containing a lower amount of Na^+ (380 nmol K^+ and 120 nmol Na^+ per mg), obtaining again a complete inhibition of K^+ efflux.

Rb^+ uptake in cells with a low membrane potential

One of the most important findings for the understanding of the regulation of K^+ uptake in *N. crassa* was the voltage dependence of the process [7]. In *S. cerevisiae* electrophysiological methods are not available; therefore, the voltage dependence of K^+ uptake was examined by using uncouplers, and cells with a low ATPase content. In the first case the membrane potential should be decreased because the pump has to cope with a higher leak (see below that in the conditions of the assay K^+ efflux was not triggered). In the second case the lower membrane potential should be the result of the lower activity of the pump [19].

Consistent with the results in *N. crassa*, 10 μM and 25 μM CCCP inhibited Rb^+ uptake, although at 10 μM CCCP the growth rate was very slightly affected, and at 25 μM CCCP the cells still grew (at 25 μM CCCP the doubling time increased from 2.5 h to 7.0 h). Interestingly, the effect of these concentrations of CCCP was only on the V_{max} of Rb^+ influx, leaving the K_m com-

TABLE III

Kinetic parameters for the initial rates of Rb^+ uptake in presence of CCCP

Cells were prepared as described in text, and Rb^+ was added immediately after CCCP.

CCCP (μM)	Normal-K^+ cells [a]		K^+-starved cells	
	K_m (mM)	V_{max} (nmol·mg^{-1}·min^{-1})	K_m (mM)	V_{max} (nmol·mg^{-1}·min^{-1}
Control	16	10	0.12	30
10	–	–	0.12	19
25	16	3.3	0.12	14.5

[a] In the presence of 0.5 mM K^+, see Ref 7.

pletely unchanged both in K^+-starved cells and in normal-K^+ cells (Table III).

It is worth mentioning that 10 μM and 25 μM CCCP did not produce K^+ efflux. In K^+-starved cells it could not occur due to the low K^+ content of the cells (Fig. 1), and in normal-K^+ cells the low amount of the uncoupler probably did not depolarize the membrane sufficiently to trigger the efflux. Furthermore, the internal pH was insignificantly affected by 10 μM CCCP, and very slightly decreased by the treatment with 25 μM CCCP (0.1 pH units in K^+-starved cells and 0.2 pH units in normal-K^+ cells).

To measure Rb^+ uptake in cells with a low plasma membrane ATPase activity we used the strain RS-72, in which the constitutive promoter of the ATPase gene has been replaced by a galactose-dependent promoter [18], and the strains RS-515 and RS-516, which are mutants containing insertions and deletions in the promoter [19]. The strain RS-72 was incubated in glucose up to 24 h to decrease the ATPase content [18], and the kinetics of Rb^+ uptake was determined at different intervals during this time. In all the experiments performed during glucose incubation, the cells were treated with azide to standardize the cells at the same K^+ content and at the same internal pH, thus ruling out the possibility that differences in Rb^+ influxes could be due to differences in K^+ contents or internal pH values. Azide-treated cells of the strain RS-72 decreased continuously the V_{max} of Rb^+ influx along with the incubation in glucose. In 24 h the V_{max} decreased to less than a half of the normal value (Table IV). In the control strain (RS-74) Rb^+ influx was not significantly affected by the incubation in glucose.

TABLE IV

Kinetic constants of Rb^+ influx in cells with a low plasma membrane ATPase activity

Strain	Conditions	K_m (mM)	V_{max} (nmol·mg^{-1}·min^{-1})
RS-72	0 h in glucose azide-treated cells	0.30	31
RS-72	24 h in glucose azide-treated cells	0.37	13
RS-74	0 h in glucose azide-treated cells	0.29	26
RS-74	24 h in glucose azide-treated cells	0.36	24
RS-515	intermediate-K^+ cells tartaric acid (pH 5.0)	0.66	12
RS-516	intermediate-K^+ cells tartaric acid (pH 5.0)	0.66	11
RS-514	intermediate-K^+ cells tartaric acid (pH 5.0)	0.66	17
RS-515	intermediate-K^+ cells 10 mM butyric acid (pH 5.0)	0.66	19
RS-516	Intermediate-K^+ cells 10 mM butyric acid (pH 5.0)	0.66	21
RS-514	Intermediate-K^+ cells 10 mM butyric acid (pH 5.0)	0.66	26

Since the growth rate of strain RS-72 is affected by the incubation in glucose, it was decided to use the low ATPase content strains RS-515 and RS-516 which grew normally in our medium (the growth of these strains is normal at pH values above 5.5 [19]). Again, K^+-starved cells, azide-treated cells, and intermediate-K^+ cells of these strains presented lower V_{max} values but the same K_m levels of Rb^+ influx compared with the control strain RS-514 (Table IV presents the results with intermediate-K^+ cells).

Acidification of the cytoplasm stimulates Rb^+ influx [8,13], a result that may be explained by the stimulation of the pump and the consequent response of the K^+ uptake system to the membrane potential [6,7]. Consistent with this hypothesis, cells with a low ATPase activity compensated apparently their defective Rb^+ influx in the presence of permeant acids (Table IV summarizes the results with intermediate-K^+ cells of the mutants RS-515 and RS-516, and with the control strain RS-514, at 10 mM butyric acid). By using butyric acid instead of propionic acid it was found that only the V_{max} was increased, leaving the K_m unaffected. This modifies a previous observation of increase of the K_m with propionic acid [8], and is consistent with the result of other authors [.13]. It is also consistent with the fact that the uncoupler treatment and the decrease of the ATPase activity affected only the V_{max}.

Rb^+ uptake in a trk1 mutant

A *trk1-1* mutant (strain PC-1) has been described which is unable to increase the V_{max} of Rb^+ influx in response to the decrease of K^+ [17]. Therefore, if the increase in the V_{max} of the low-K^+ cells in the wild type is caused by a decrease in their pH_i, the mutant should not increase the V_{max} when treated with permeant acids. Consistent with this notion, it was found that the V_{max} and the K_m of Rb^+ influx in K^+-starved cells and

intermediate-K^+ cells of the strain PC-1 were completely insensitive to the presence of 10 mM butyric acid in the buffer (compare with the results in Table IV).

Discussion

K^+ efflux

Present results on the K^+ efflux induced by uncouplers indicate that the decrease in the efflux correlates with the decrease in the K^+ content of the cell, but that neither $[K^+]_i$ nor pH_i is a direct regulator of the process. When Na^+ is present inside the cell, Na^+ may be directly the inhibitor of the efflux, because in its presence K^+ efflux stops before it would do in response to the decrease of $[K^+]_i$ (in Na^+ cells with 380 nmol $K^+ \cdot mg^{-1}$, efflux is completely inhibited, but not in cells without Na^+ and the same K^+ content). In the absence of Na^+ a signal released by the depletion of K^+ has to be postulated.

In previous reports, both in *S. cerevisiae* and *N. crassa*, the number of the K^+ transport systems has been discussed. Normal efflux occurring in growing yeast can be distinguished from the K^+ efflux induced by uncouplers or by ATP depletion on the basis of their different discrimination between K^+ and Rb^+ [24], but no way has been found to decide whether the efflux induced by uncouplers in both fungi is mediated by the same or by a different system than that of the influx [12,24]. The results obtained with the *trk1-1* mutant of *S. cerevisiae* (Fig. 2) indicate that the efflux induced by uncouplers occurs through a system different from that of influx.

A likely possibility is that the K^+ efflux induced by uncouplers could be mediated by a voltage-gated K^+ channel [25]. The normal function of this channel would be to restore the membrane potential when the cell is depolarized by the high influx of either a charged substrate or a H^+-cotransported neutral substrate [15]. Addition of an uncoupler, above a certain concentration, would produce the same depolarizing effect and the same response. So far, we have not been able to inhibit K^+ efflux with tetraethylamonium, Ba^+, or quinine, but there is a dramatic inhibitory effect of internal Na^+, and in *Chara* Na^+ inhibits K^+ channels [26].

In ATP-depleted cells exposed to low external K^+, the plasma membrane ATPase does not pump H^+ but K^+ efflux can neutralize the uptake of cations. Thus, the addition of many compounds which are cotransported with H^+ to ATP-depleted cells increases K^+ efflux, and the uptake of these compounds occurs coupled to K^+ efflux [27]. Obviously, electrophoretic uptake in ATP-depleted cells requires K^+ efflux, and it cannot take place if K^+ efflux is inhibited. The here reported inhibition of K^+ efflux by internal Na^+ and by the decrease of $[K^+]_i$ explains the inhibition of amino acid uptake in ATP-depleted cells of *S. cerevisiae* when they contain Na^+ [28] or when they have lost K^+ [29,30].

The presence of voltage-gated K^+-channels, independent of the K^+ uptake system, is consistent with the $I-V$ curves of K^+ uptake in *N. crassa*. Subtracting the currents in presence and absence of K^+, the differential curves show increasing negative inward currents at membrane potentials positive to -100 mV [7], which can be explained by the decrease in K^+ efflux through the channel in the presence of external K^+. Interestingly, these differential currents are inhibited by the iontophoretic injection of vanadate (see Fig. 8 in Ref. 7), which does not affect the differential currents at membrane potentials negative to -100 mV, suggesting that inward and outward currents are mediated by different systems.

K^+ influx

The model proposed for the K^+–H^+ symport in *N. crassa* [7] predicts that K^+ uptake has to be rather insensitive to changes in the intracellular K^+ concentration. Present results with *S. cerevisiae* confirm that prediction. The insensitivity of Rb^+ influx to the osmotic shocks with sorbitol leaves it clear that changes in $[K^+]_i$ have no significant effects on the kinetics of Rb^+ influx. It is remarkable that this kinetics in K^+-starved cells was not affected by the osmotic shock with sorbitol, although the shrinkage of the cells increased $[K^+]_i$ almost up to the level of normal-K^+ cells. More remarkable are the results of a previous report [31] which show that after increasing $[K^+]_i$ more than 2-fold by a 2.9 osmolal shock, the K_m for Rb^+ uptake was not affected. In that report the V_{max} of the net K^+ uptake was decreased by the osmotic shock, suggesting that either the K^+ uptake system or the pump (see below) is partially inactivated by such a large osmotic shock.

A common observation in *S. cerevisiae* [8,9,32] and *N. crassa* [10] is the correlation between the rates of K^+ and Rb^+ uptakes with the K^+ content (referred to dry weight). The low internal pH of low-K^+ cells, and the increase of the V_{max} of Rb^+ influx in response to the acidification of the cell ([8,13] and present results) strongly suggest that the increase of the V_{max} in low-K^+ cells is due to the low internal pH. This idea is also supported by the results obtained with low-K^+, high-Na^+ cells, which have a normal pH_i, and the V_{max} of normal-K^+ cells. The increase of the internal concentration of H has not reason to accelerate directly Rb^+ influx. On the contrary, it should inhibit the Rb^+-H^+ symport. Therefore, the most likely explanation is that the stimulation of Rb^+ influx is in response to the acceleration of the pump [6]. The response of the V_{max} of Rb^+ influx to uncouplers and to the decrease of the plasma membrane ATPase content is consistent with

the idea of the electrical link between the V_{max} of Rb^+ influx and the activity of the pump.

Contrarily to the clear framework for V_{max} responses, variations in the K_m of K^+ (Rb^+) influx in response to K^+ depletion are more difficult to explain with an unifying hypothesis. The K_m of Rb^+ influx decreases in cells that have lost K^+ in exchange for H^+ and also in cells that have exchanged K^+ for Na^+. This decrease in the K_m requires that a signal (released as a function of the K^+ content) interacts with the K^+ uptake system. This signal is neither turgor pressure nor cell volume, because the osmotic shock with sorbitol did not affect the kinetics of Rb^+ influx. The possibility that the internal pH is involved in the regulation of the K_m is also ruled out because the K_m does not decrease when pH_i is decreased without affecting the K^+ content, and because Na^+-cells exhibit a very low K_m but a normal pH_i. Internal Na^+ may regulate the K_m, but this need not be postulated because Na^+ cells are already low-K^+ cells, as are K^+-starved cells, and the signal may be released in function of something related to the K^+ status. Finally, the fact that propionic acid increases the K_m [8] but not butyric acid (this work), and the fact that low concentrations of dinitrophenol, inactive on the V_{max}, also increases the K_m [33] suggest that the K_m is sensitive to some metabolic changes not easy to understand at this moment.

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