

# Potassium and Rubidium Effluxes in *Saccharomyces cerevisiae*

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In growing cells,  $K^+$  and  $Rb^+$  effluxes followed first order kinetics, took place with slight differences between  $K^+$  and  $Rb^+$  and were stimulated at acidic pH values. In uncoupled cells and ATP-depleted cells,  $K^+$  efflux was higher than  $Rb^+$  efflux. In ATP-depleted cells, the effluxes were not stimulated at acidic pH values. Two different  $K^+$  effluxes are proposed: (i) the normal efflux, which may be a  $K^+/H^+$  antiport and does not function in ATP-depleted cells, and (ii) the backward reaction of the uptake system, which can be high in depolarized cells. The role of the normal efflux system in  $K^+$  content and pH regulation is discussed.

## Introduction

Transport of  $K^+$  in *Saccharomyces cerevisiae* is not exclusively unidirectional and some  $K^+/K^+$  exchange takes place during the process of uptake [1–3]. In  $Rb^+$ -loaded cells, an equivalent  $Rb^+/Rb^+$  exchange takes place [4]. The  $K^+/K^+$  exchange has been studied in connexion with the other two cation-exchange processes observed in yeast:  $K^+/H^+$  exchange during normal  $K^+$  uptake, and  $K^+/Na^+$  exchange in  $K^+$  uptake of  $Na^+$ -loaded cells. On the basis of the pH dependence of these processes, Ryan and Ryan (3) proposed that the  $K^+/K^+$  exchange and the  $K^+/Na^+$  exchange occur in the same system and that the system exchanging  $H^+$  and  $K^+$  is specific. However, according to Rothstein [5], the different pH dependence of the exchanges could also take place if there was only one transport system.

In analogy with  $Na^+$  transport in yeast [6] and  $K^+$  transport in bacteria [7], in yeast  $K^+$  influx and  $K^+$  efflux could be independent processes. If both processes take place simultaneously they would give rise to the observed  $K^+/K^+$  exchange and, in the steady-state, they would determine the actual level of the  $K^+$  content of the cell. In yeast, however, this hypothesis has not been demonstrated and the absence of a specific efflux system seems feasible. In fact, variation in influx can be so important [2, 8] that the existence of an efflux system has not to be postulated for the regulation of  $K^+$  content. In the absence of a

specific efflux system, the observed efflux would be the backward reaction of the influx process. In this case, efflux would be low when transport takes place far from the equilibrium of the system, in the accumulative direction, and high if the driving force is reversed. Consistent with this idea and with an electrogenic  $K^+$  transport [9, 10], high effluxes have been observed when the cells are depolarized. Depolarization occurs when the  $H^+$ -pump cannot cope with the leak (positive charge moving inward), as in ATP depletion [11], treatment with uncouplers [10], or by exposing the cells to  $H^+$ -cotransported substrates [11].

In the present communication we report that yeast has in fact a specific efflux system, whose activity can be distinguished from the backward reaction of influx by its different selectivity between  $K^+$  and  $Rb^+$ .

## Experimental

### Growth conditions

Strain X2180.1B was grown in arginine-phosphate medium [8]: 8 mM phosphoric acid, 10 mM L-arginine, 2 mM  $MgSO_4$ , 0.2 mM  $CaCl_2$ , and 2% glucose, brought to pH 6.5 with L-arginine, or pH 3.2 with tartaric acid, plus vitamins and trace elements. To this medium KCl and RbCl were added as required. Cells were grown overnight in a shaker up to a cell density of 0.5 mg (dry weight)  $ml^{-1}$ . To obtain low  $K^+$  and  $Rb^+$  cells, the cells were grown in 20  $\mu M$   $K^+$ , and 10  $\mu M$   $Rb^+$  and incubated until the external  $K^+$  dropped to 1  $\mu M$  or less [8].

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### $K^+$ and $Rb^+$ losses

The decrease in  $K^+$  and  $Rb^+$  contents was followed by atomic absorption analyses of the cells. Cells grown as described above were generally transferred to growth medium or buffers containing only  $K^+$  or  $Rb^+$ ; at times, samples were filtered and washed as previously described [8]. In long experiments in growth medium, the growth of the culture was followed spectrophotometrically at 550 nm and the cation content of each sample referred to the actual dry weight of the cells. In short experiments in buffers (20–30 min), the cation content was referred to the dry weight at time zero. In all cases, experiments were carried out at a cell density of 0.1–0.2 mg ml<sup>-1</sup>.

### $Rb^+$ fluxes

Under the conditions of each experiment,  $Rb^+$  influx was taken as the initial rate of  $^{86}Rb^+$  uptake. After  $^{86}Rb^+$  addition, samples of the cells were washed with the same medium without the isotope and then counted in a liquid scintillation counter. Influx was calculated from the count-accumulation rate and the counts per nmol in the medium.

For efflux determination, cells were labelled with  $^{86}Rb^+$  by adding the isotope to the cultures about 3–4 hours before harvesting. At the time of the experiment, the cells were transferred to the same medium without isotope, and efflux calculated from the rate of count loss and the  $Rb^+$  content of the cells. In some cases efflux was calculated in  $^{86}Rb^+$  uptake experiments. In these cases,  $^{86}Rb^+$  was added to a growing culture (exponential growth) in which  $Rb^+$  content was constant throughout the experiment and influx calculated as described before. Efflux was taken as the difference between influx and the instantaneous rate of  $Rb^+$  uptake. This last rate was calculated from the growth rate constant and the  $Rb^+$  content of the cells ( $0.0039 [Rb^+]_i$  nmol mg<sup>-1</sup> min<sup>-1</sup>).

## Results

### $K^+$ and $Rb^+$ effluxes

Cells grown in a medium containing  $K^+$  plus  $Rb^+$  lost both cations in similar ways when transferred to the same growth medium but containing only  $Rb^+$  or  $K^+$  (Fig. 1). The decreases in  $K^+$  and  $Rb^+$  contents followed first order kinetics with kinetic constants of 0.011 and 0.009 min<sup>-1</sup>. Considering that the growth rate constant of the yeast was 0.004 min<sup>-1</sup>, the actual

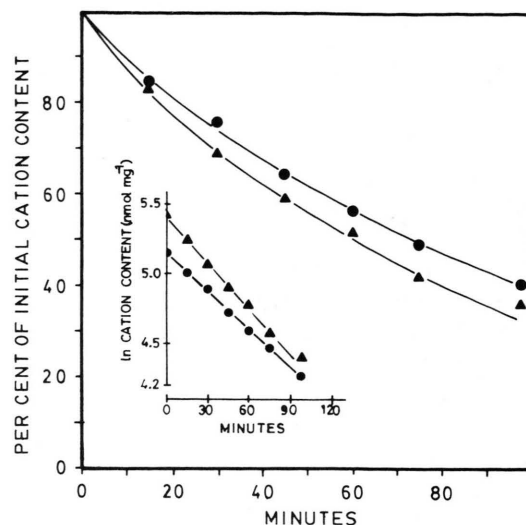


Fig. 1. Decrease in  $K^+$  or  $Rb^+$  contents of growing cells containing  $K^+$  and  $Rb^+$ . Cells grown at 1 mM  $K^+$  plus 3 mM  $Rb^+$ , which contained 250 nmol mg<sup>-1</sup> of  $K^+$  and 170 nmol mg<sup>-1</sup> of  $Rb^+$ , were transferred to fresh medium with either 5 mM  $Rb^+$  or 5 mM  $K^+$ . The  $K^+$  ( $\Delta$ ) or  $Rb^+$  ( $\bullet$ ) content of the cells were followed in the cells suspended in  $Rb^+$  or  $K^+$  respectively and plotted as per cent of the initial value. Inset, semilogarithmic plots of the actual cation content.

kinetic constants of the  $K^+$  and  $Rb^+$  effluxes were 0.007 and 0.005 min<sup>-1</sup> respectively.

The similarities between  $K^+$  and  $Rb^+$  losses observed in growth medium were also observed in buffers at different pH values (Fig. 2). Only at pH 3.0,

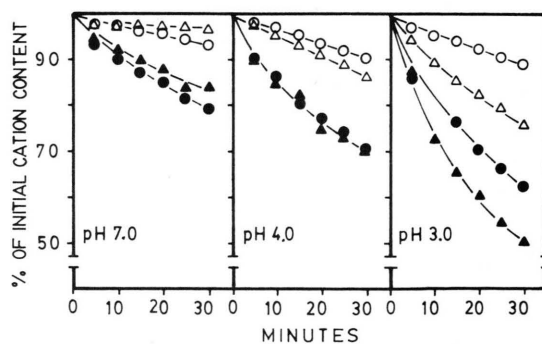


Fig. 2. Effect of pH on the loss of  $K^+$  or  $Rb^+$  in cell containing both cations. Cells grown at 1 mM  $K^+$  plus 3 mM  $Rb^+$  at pH 6.5 ( $\Delta$ ,  $\bullet$ ) and pH 3.2 ( $\triangle$ ,  $\circ$ ) were transferred to buffers containing either 5 mM  $Rb^+$  or 5 mM  $K^+$ . The  $K^+$  ( $\Delta$ ,  $\triangle$ ) or  $Rb^+$  ( $\circ$ ,  $\bullet$ ) content of the cells was followed in cells suspended in  $Rb^+$  or  $K^+$  respectively. Buffers (all 5 mM): 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid for pH 7.0 and L(+) tartaric acid for pH's 4.0 and 3.0. The acids were brought to pH with  $Ca(OH)_2$  and contained 0.1 mM  $MgCl_2$  and 1% glucose.

$K^+$  loss exceeded considerably  $Rb^+$  loss. Cells grown at pH 6.5 lost  $K^+$  or  $Rb^+$  very fast when transferred to acidic buffers (pH's 4.5 and 3.0) but the losses were not as fast in cells grown in an acid medium (pH 3.2) (Fig. 2).

The first order kinetic for  $Rb^+$  efflux was further supported by unidirectional fluxes obtained with  $^{86}Rb^+$  at different  $Rb^+$  concentrations at pH's 6.0 and 3.2 (Table I). The slight differences in the apparent kinetic constants in Table I and that calculated from data in Fig. 1 are probably not significant. The results at pH's 6.0 and 3.2 indicated that efflux in growing cells was independent of the external pH. The different  $Rb^+$  losses at different pH values in Fig. 2 were probably due to the sudden increase or decrease in the transmembrane  $\Delta$  pH, which do not reflect steady-state conditions.

In  $K^+$ - $Rb^+$ -starved cells, with a  $K^+$  plus  $Rb^+$  content about half of the normal content [8], the loss of  $K^+$  and  $Rb^+$  was much lower than in normal  $K^+$  cells. However, due to technical difficulties (these cells were in less than 1  $\mu M$  external  $K^+$  and 1–2  $\mu M$  external  $Rb^+$ ) we could not quantify efflux with precision.

#### $K^+$ and $Rb^+$ losses with uncouplers and in ATP-depleted cells

Cells treated with carbonylcyanide *m*-chlorophenylhydrazone, azide, dinitrophenol or with antimycin plus 2-deoxy-D-glucose, lost  $K^+$  and  $Rb^+$ , but, in contrast with untreated cells,  $K^+$  loss greatly exceeded  $Rb^+$  loss (Fig. 3). In these cases,  $K^+$  efflux was about three times higher than  $Rb^+$  efflux (zero-trans losses of  $K^+$  and  $Rb^+$ , referred to the  $K^+$  and  $Rb^+$  content), although the rates of the losses in the

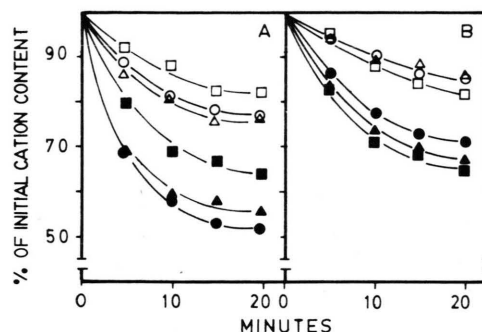


Fig. 3.  $K^+$  and  $Rb^+$  losses from cells containing  $K^+$  and  $Rb^+$  and treated with different uncouplers (A), and with antimycin (15  $\mu g\ ml^{-1}$ ) and 2-deoxy-D-glucose (5 mM) (B). Cells grown as described in the legend of Fig. 1 were transferred to buffers containing either 5 mM  $Rb^+$  or 5 mM  $K^+$ , and the  $K^+$  (full symbols) or  $Rb^+$  (open symbols) content followed in the cells suspended in  $Rb^+$  or  $K^+$  respectively. In (A), 150  $\mu M$  carbonylcyanide *m*-chlorophenylhydrazone pH 6.0 ( $\bullet$ ,  $\circ$ ); 150  $\mu M$  dinitrophenol pH 4.5 ( $\blacktriangle$ ,  $\triangle$ ); 1 mM azide pH 6.0 ( $\blacksquare$ ,  $\square$ ). In (B), pH 6.0 ( $\bullet$ ,  $\circ$ ); pH 4.5 ( $\blacktriangle$ ,  $\triangle$ ); pH 3.0 ( $\blacksquare$ ,  $\square$ ). Buffers (all 5 mM): 4-morpholineethanesulphonic acid for pH 6.0, and L(+) tartaric acid for pH's 3.0, 4.0 and 4.5. The acids were brought to pH with  $Ca(OH)_2$  and contained 0.1 mM  $MgCl_2$  and 1% glucose.

cells treated with the uncouplers were higher than in ATP-depleted cells. This difference between  $K^+$  and  $Rb^+$  losses contrasted with the similarity observed in non-treated cells, except at pH 3.0, and was considered the result of the depolarization of the cells. This depolarization was probably higher with uncouplers than in ATP-depleted cells, as observed in *Neurospora* [12, 13], explaining the higher losses with uncouplers. In ATP-depleted cells, the  $Rb^+$  and  $K^+$  losses were barely stimulated by decreasing the pH, in contrast with the results shown in Fig. 2 with un-

Table I. Unidirectional  $Rb^+$  fluxes in cells containing  $K^+$  and  $Rb^+$ .

$[K^+]_o$ [mM]	$[Rb^+U]_o$ [mM]	pH <sub>o</sub>	$[Rb^+]_i$ [nmol mg <sup>-1</sup> ]	Influx [nmol mg <sup>-1</sup> min <sup>-1</sup> ]	Efflux [nmol mg <sup>-1</sup> min <sup>-1</sup> ]	Efflux/ $[Rb^+]_i$ [min <sup>-1</sup> ]
1	3	6.0	170	1.32	—	0.004
1	2	6.0	120	0.90	—	0.004
1	1	6.0	65	0.50	—	0.004
1	0.5	6.0	35	0.30	—	0.005
1	3	6.0	170	—	0.84	0.005
1	3	3.2	180	—	0.80	0.004
1	3	3.2	180	1.43	—	0.004

Cells were grown as described in the text with the specified  $K^+$  and  $Rb^+$  and used in logarithmic growth, during which  $K^+$  and  $Rb^+$  contents were constant. Influx was calculated by adding  $^{86}Rb^+$  to the culture and analysing  $^{86}Rb^+$  uptake. To calculate efflux,  $^{86}Rb^+$  was added to the cultures, and after 3–4 h the cells were transferred to the same medium without the isotope. Each data is the mean of three or four experiments. SEM was in all cases lower than 10% of the mean. For the last column, in influx experiments, efflux was calculated subtracting net transport from influx (see text).

treated cells. These results suggest that the normal efflux system does not function in ATP-depleted cells.

*Effect of butyric acid and weak bases on  $K^+$  and  $Rb^+$  losses*

Cation fluxes in yeast depend on the cellular pH [3], and in bacteria [14] and *Chlorella* [15], it has been proposed that  $K^+$  efflux is involved in the control of the cellular pH. To test the effect of a lower cellular pH on efflux, we analysed the efflux in the presence of butyric acid, which is known to decrease the cellular pH [16]. Butyric acid (5 mM, pH 4.5) inhibited  $K^+$  and  $Rb^+$  losses without producing differences between them (conditions as in Fig. 2 using butyric and tartaric acids instead of 4-morpholineethanesulphonic acid). Similarly, in cells grown in 1 mM  $K^+$  plus 3 mM  $Rb^+$  labelled with  $^{86}Rb^+$  (as in Table I), efflux of  $Rb^+$  in  $Ca^{2+}$ -butyrate was less than half that in  $Ca^{2+}$ -tartrate (5 mM of the acid, 0.1 mM  $Mg^{2+}$ , 1 mM  $K^+$ , 3 mM  $Rb^+$ , 1% glucose, pH 4.5) amounting 0.41 nmol  $mg^{-1} min^{-1}$  and 0.93 nmol  $mg^{-1} min^{-1}$ , respectively.

When we tested the effect of the increase of cellular pH on  $K^+$  and  $Rb^+$  effluxes, it was found that the two bases used, ammonia and procaine at pH 8.0, produced different  $Rb^+$  and  $K^+$  losses (Fig. 4). Thus, the increase in  $K^+$  loss observed with these bases was probably due to a depolarizing effect rather than to an increase in the normal efflux.

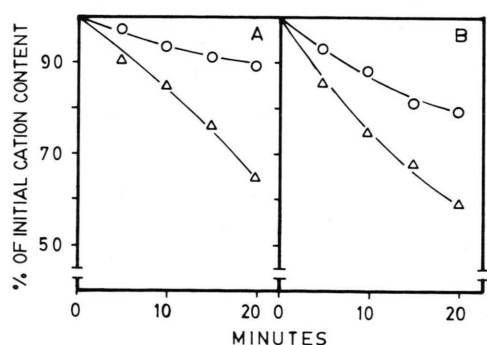


Fig. 4.  $K^+$  and  $Rb^+$  losses from cells containing  $K^+$  and  $Rb^+$  in presence of 5 mM procaine hydrochloride (A) and 20 mM  $NH_4Cl$  (B) at pH 8.0. Cells were prepared and assayed as described in the legend of Fig. 3 except that the buffers was N-2-[hydroxy-1,1bis(hydroxymethyl)ethyl]-glycine 5 mM, brought to pH 8.0 with  $Ca(OH)_2$  and containing 0.1 mM  $MgCl_2$  and 1% glucose. Symbols: ( $\Delta$ )  $K^+$ , ( $\circ$ )  $Rb^+$ .

## Discussion

The differences observed between  $K^+$  and  $Rb^+$  effluxes in control cells and those treated with uncouplers suggest two different  $K^+$  efflux processes in yeast. The discrimination between  $Rb^+$  and  $K^+$  observed in uncoupled cells is consistent with the idea that, in these cells, efflux is the backward reaction of influx (see ref. [11]), because this discrimination takes place during uptake. Thus, cells grown in 1 mM  $K^+$  plus 3 mM  $Rb^+$  contained 250 nmol  $K^+ mg^{-1}$  and 170 nmol  $Rb^+ mg^{-1}$ , proving that  $K^+$  is taken up more rapidly than  $Rb^+$ , because this difference is not due to a more active efflux of  $Rb^+$  (Fig. 1). Discrimination in the efflux between alkali metal cations has been observed previously with  $K^+$  and  $Li^+$  in dinitrophenol treated cells. In these conditions,  $K^+$  efflux greatly exceeds  $Li^+$  influx [17]. On the contrary, the efflux process in non-treated cells did not discriminate between  $K^+$  and  $Rb^+$ . The slightly higher loss of  $K^+$  in non-treated cells at pH 3.0 could be due to a depolarizing effect of low pH. In *Neurospora* [18], *Chlorella* [19] and yeast [20] the membrane potential decreases significantly at low pH values, suggesting that at this pH non-treated cells present a slight backward reaction through the influx system. An important observation about the normal efflux is that it is probably inhibited in ATP-depleted cells. This inhibition explains why non-treated cells at pH 3.0 lost much more  $Rb^+$  than ATP-depleted cells at the same pH. It is interesting that  $Li^+$  efflux in yeast has also been found to be inhibited in ATP-depleted cells [17].

The existence of a specific efflux system for  $K^+$  explains  $K^+/K^+$  exchange mainly as the result of the independent influx and efflux. The efflux system would cooperate to control the  $K^+$  content of the cell by keeping cells in a controlled steady-state. However, the most active control of  $K^+$  content of the cell is in the control of the influx, as deduced from comparing the fluxes in normal  $K^+$  cells with those in low- $K^+$  cells. Influx varies from about 4–5 nmol  $mg^{-1} min^{-1}$  in normal- $K^+$  cells, to 34 nmol  $mg^{-1} min^{-1}$  in low- $K^+$  cells [8], but efflux only from about 3 nmol  $mg^{-1} min^{-1}$  in normal cells (0.007  $min^{-1}$  rate constant and 450 nmol  $mg^{-1}$ ) to a minimum of zero in low- $K^+$  cells. Similarly, in the control of an acidic cellular pH, the inhibition of  $K^+$  efflux is probably insignificant in comparison to the response of other processes, as the increase in the rate of the pump and

leak induced by the low cellular pH [21]. In the control of an alkaline cellular pH, we did not find a significant increase in  $\text{Rb}^+$  efflux, which indicates that normal efflux did not accelerate very much. Previous reports referring to the increase of  $\text{K}^+$  efflux [14] may have resulted from a depolarizing effect, as we have found.

The existence of a specific system mediating efflux in yeast poses a question about the mechanism involved in the process. The high capacity of the cell for other exchanges (*e.g.*  $\text{K}^+$  uptake during  $\text{Rb}^+$  efflux, or  $\text{H}^+$  production) and the lack of inhibitors for

these processes made it impossible to obtain direct evidence of the mechanism of efflux. However, the great stimulation of an acidic pH shift suggests an involvement of the  $\text{H}^+$ . A  $\text{K}^+/\text{H}^+$  exchange as earlier suggested [22], is a possibility which could be handled as a working hypothesis.

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