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NOTES

Activation of the Potassium Uptake System during Fermentation in *Saccharomyces cerevisiae*

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Fermentable sugars activated the K^+ uptake system, increasing the V_{max}s of Rb^+, Na^+, and Li^+ influxes, but sugars did not affect the effluxes of these cations. This activation seems to be a direct effect of fermentation and not the consequence of the H^+ pump ATPase activation or internal pH decrease produced by fermentation.

K^+ uptake in *Saccharomyces cerevisiae* is a tightly regulated process whose kinetics depends on the K^+ content of the cells, internal pH, and activity of the H^+ pump ATPase (13, 14, 18). We now report that the activity of the K^+ uptake system is, in addition, regulated by fermentation.

The strains used in the present work are listed in Table 1, but unless otherwise indicated, experiments were carried out with strain XT300.3A. Normal-K^+ cells were harvested from mid-exponential-growth cultures in 0.7% yeast nitrogen base without amino acids (Difco) but with 2% glucose and then K^+ starved, when required, for 4 h in a K^+-free arginine-phosphate medium (18). Both media were supplemented to fulfill nutritional requirements. The initial rates of cation uptakes (influxes) (18), Na^+ and Li^+ effluxes from previously loaded cells (16, 17), K^+ efflux induced by uncouplers (13), and K^+ efflux in growing conditions (9) were determined by analysis of the cell cation contents. Samples of cells suspended in buffer were collected by filtration and washed with 20 mM $MgCl_2$ solution. Filters with cells were acid extracted and analyzed by atomic absorption spectrophotometry. K^+-starved cells expressing the high-affinity glucose transport system were obtained by 4 h of glucose starvation simultaneous with K^+ starvation. Unless otherwise indicated, the experiments were carried out with 10 mM 4-morpholineethanesulfonic acid brought to pH 6.0 with $Ca(OH)_2$ containing 0.1 mM $MgCl_2$ and the required amount of glucose (2% if nothing else is stated).

Rb^+ uptake activation. Both normal-K^+ cells and K^+-starved cells suspended in buffer or culture medium increased Rb^+ influx after the addition of glucose, completing the process of activation in less than 1 min. Kinetic analysis of Rb^+ influx showed that the presence of glucose induced a large increase in the V_{max} without affecting the K_m (Table 2). The results summarized in Table 3 indicate that the activation of Rb^+ uptake by glucose required the rapid transport of the sugar because the effective range of glucose concentration that produced the effect depended on the glucose transport capacity of the cells. Thus, in glucose-starved cells, activation by glucose occurred at lower glucose concentrations than in glucose-grown cells, in correlation with the lower K_m of glucose-starved cells (1 mM versus 27 mM glucose) (3, 15). In contrast, in a *snf3* mutant, lacking the high-affinity system for glucose transport (7, 8, 15), the glucose concentration dependence of activation was not affected by glucose starvation.

As expected from previous results (2), the ATP content of the cells in the conditions of the reported experiments was constant, except in 4-h-glucose-starved cells when assayed in the absence of glucose (ATP was determined as described in reference 19). This ruled out ATP depletion being the cause of the lower Rb^+ uptake in the absence of glucose. Besides, ethanol was respired by the cells but failed to activate Rb^+ uptake.

TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype	Source or reference
XT300.3A	*TRK1*	18
PC.1	*trk1-1*	12
MCY1093	*SNF3*	V. P. Cirillo (15)
MCY1408	*snf3-Δ4*	V. P. Cirillo (15)
RS-72	*PMA1*	R. Serrano (4)
RS-503	*pma1-Δ245*	F. Portillo (10)
ts321	*cdc25*	F. Portillo (11)

TABLE 2. Kinetic parameters for the initial rates of Rb^+ uptake in the presence and in the absence of glucose[a]

Conditions	Kinetic parameters for:			
	Normal-K^+ cells		K^+-starved cells	
	K_m (mM)	V_{max} (nmol · mg^{-1} · min^{-1})	K_m (mM)	V_{max} (nmol · mg^{-1} · min^{-1})
Glucose absent	16	1.5	0.12	7
Glucose present	16	10	0.12	30

[a] Cells (normal-K^+ cells and K^+-starved cells of strain XT300.3A) were harvested from the growth medium and suspended in buffer with or without glucose, and 2 min later Rb^+ was added from a concentrated solution. Initial rates were determined from the time courses of Rb^+ uptake by chemical analyses of the Rb^+ contents of the cells as described in the text. Data are means of the results from three independent experiments, which differed by less than 10%.

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TABLE 3. Initial rates of Rb^+ uptake at different glucose concentrations in glucose-starved cells and in cells grown in glucose[a]

Glucose concn in assay buffer (mM)	Rb^+ influx (nmol · mg^{-1} · min^{-1}) in:			
	MCY1093 cells		MCY1408 cells	
	Grown in glucose	Glucose starved	Grown in glucose	Glucose starved
0	2.0	2.0	2.3	2.2
1	5.5	10	5.8	6.0
2	6.7	16	7.5	7.9
20	18	26	18	17
100	28	30	30	30

[a] The uptake experiments were carried out at 50 mM Rb^+, as described in the text and Table 2, footnote *a*. All cells were K^+ starved. Data are means of the results from two or three experiments, which differed by less than 10%.

The lack of activation of Rb^+ uptake by ethanol, and the low activation produced by low concentrations of glucose (Table 3), which are weakly fermented (6), suggest that it was fermentation which triggered the activation of Rb^+ uptake. Consistent with this notion, in induced cells of a galactose-fermenting strain (RS-72), galactose activated Rb^+ uptake in the manner described for glucose (8- and 30-nmol · mg^{-1} · min^{-1} V_{max}s in the absence of substrate and with galactose, respectively).

The activation of Rb^+ uptake is not a consequence of the ATPase activation or internal pH decrease. The V_{max} of Rb^+ influx increases with the decrease of the internal pH of the cells and with the increase of the plasma membrane ATPase content (13). Consequently, activation of Rb^+ uptake upon the addition of glucose could be not only the result of the direct activation of the K^+ uptake system but also an indirect response of Rb^+ influx to either the decrease of the internal pH (5) or the ATPase activation produced by glucose (20, 21).

An indirect effect of the decrease of the internal pH was ruled out by the results obtained with a K^+ transport mutant (*trk1-1*). This mutant does not increase Rb^+ influx in response to an acid load (13) but was normal in the activation of Rb^+ uptake by glucose (Table 4). The second possibility, the activation of Rb^+ uptake being the consequence of ATPase activation, was addressed by the use of a *pma1* mutant and a *cdc25* mutant. In the first case we used a genetically engineered strain (RS-503) carrying in a plasmid a defective ATPase gene whose product is a permanently activated ATPase (10). In this strain, Rb^+ uptake was not permanently activated (Table 5), indicating that the activation of Rb^+ uptake by glucose was not the consequence of ATPase activation. This notion was also supported by the results obtained with the *cdc25* mutant. In this mutant, activation of the ATPase by glucose was very low at the restrictive temperature (11) but Rb^+ uptake was normally activated (at 37°C, K^+-starved cells at 50 mM Rb^+ presented an influx of 6.5 nmol of Rb^+ · mg^{-1} · min^{-1} in the absence of glucose and 20 nmol of Rb^+ · mg^{-1} · min^{-1} in its presence). This also indicates that the product of *CDC25* does not control the activation of Rb^+ uptake.

TABLE 4. Comparative effects of glucose and butyric acid on the initial rate of Rb^+ uptake in the wild type (strain XT300.3A) and in a *trk1-1* mutant (strain PC.1)[a]

Strain	Medium	Rb^+ influx (nmol · mg^{-1} · min^{-1})
XT300.3A	Buffer	7
	10 mM butyric acid	14
	100 mM glucose	30
PC.1	Buffer	0.7
	10 mM butyric acid	0.7
	100 mM glucose	8

[a] Experiments were carried out as described in the text and in Table 2, footnote *a*, with K^+-starved cells at 50 mM Rb^+. Data are means of the results from two or three experiments, which differed by less than 10%.

TABLE 5. Effect of glucose on ATPase activity and Rb^+ uptake in a strain with a permanently activated ATPase (RS-503) and in the control strain (RS-72)

Strain[a]	Glucose in buffer	ATPase activity (μmol · min^{-1} · mg of $protein^{-1}$)	Rb^+ influx (nmol · mg^{-1} · min^{-1})[b] in:	
			K^+-starved cells	Normal-K^+ cells
RS-72	No	0.16	10	1.2
	Yes	0.76	30	4.5
RS-503	No	0.63	16	1.7
	Yes	0.73	31	4.5

[a] Strain RS-72 (4) has a normal ATPase gene under the control of a promoter which is induced when cells are grown in a medium with galactose and repressed when cells are grown in a medium with glucose. RS-503 is a transformant of RS-72 with a plasmid containing the defective ATPase gene whose product is a permanently activated ATPase. For these experiments RS-72 was grown in galactose and RS-503 was grown in glucose.

[b] The assays were carried out at 50 mM Rb^+ as described in the text and Table 2, footnote *a*. Reported data are the means of the results from two or three experiments, which in all cases differed by less than 10%.

Activation of Na^+ and Li^+ uptakes. The K^+ uptake system of *S. cerevisiae* also mediates the uptakes of Na^+ and Li^+, although these cations show much higher K_ms than K^+ and Rb^+, especially in K^+-starved cells (1, 12). Experiments with Na^+ and Li^+ similar to those carried out with Rb^+ showed that their influxes were also stimulated by glucose (e.g., the V_{max}s of Li^+ influx in the absence and in the presence of glucose were 1 and 9 nmol · mg^{-1} · min^{-1}, respectively).

Effluxes are not activated by glucose. In addition to the K^+ uptake system, three other systems for alkali cation fluxes in *S. cerevisiae* have been identified: the sodium efflux system (17), the K^+ efflux system triggered by membrane depolarization (13), and the system mediating K^+ efflux in growing conditions (9). The functional relationships among all these systems are not known, and it was interesting to find out whether they responded to glucose. Flux experiments in the presence and in the absence of glucose showed that the described effluxes did not show any glucose effect.

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