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Role of Vacuolar Ion Pool in *Saccharomyces carlsbergensis*: Potassium Efflux from Vacuoles Is Coupled with Manganese or Magnesium Influx

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Saccharomyces carlsbergensis cells accumulated Mn^{2+} (or Mg^{2+}) ions in the presence of glucose, fructose, or mannose, but not of deoxyglucose, 3-*O*-methylglucose, and sorbose. Accumulation of one equivalent of Mn^{2+} was coupled with the efflux of two equivalents of K^+ from the cells. Mg^{2+} did not exit during Mn^{2+} uptake. Preliminary treatment of cells with various proton conductors or glucose led to the loss of K^+ and to the proportional inhibition of Mn^{2+} uptake. Polyene antibiotic candicidin together with glucose elicited rapid efflux of K^+ and completely inhibited Mn^{2+} accumulation. Exogenous K^+ (more than 1 mM), 100 μM *N,N'*-dicyclohexylcarbodiimide, and 30 mM sodium arsenate inhibited both K^+ efflux and Mn^{2+} influx. K^+ efflux from *S. carlsbergensis* cells affected the vacuolar pool of K^+ both during the accumulation of Mn^{2+} or Mg^{2+} and during glucose uptake.

According to the information presently available, the transport of amino acids (22), sugars (3), and purines (18) in yeast cells is coupled with the proton influx and K^+ efflux. It has been reported (7, 13) that Co^{2+} accumulation by yeasts also stimulates the loss of K^+ by cells. As we recently found, K^+ is unequally distributed in *Saccharomyces carlsbergensis* cells, its concentration being 60 mM in cytoplasm and 470 mM in vacuoles (15). In the present work, the K^+ content in various pools of *S. carlsbergensis* during the accumulation of Mn^{2+} or Mg^{2+} in the vacuoles has been studied in vivo. The decisive role of the vacuolar pool of K^+ in the uptake of Mn^{2+} and Mg^{2+} has been demonstrated.

MATERIALS AND METHODS

S. carlsbergensis, strain IBPhM-366, was grown on synthetic medium supplemented with yeast extract (15). Cells were harvested by centrifugation in the mid-exponential growth stage (5 h) and, after being washed with twice distilled water, were used for the study of Mn^{2+} or Mg^{2+} uptake.

Usually the yeast was incubated for 60 min at 30°C in the medium with 100 mM glucose and 3 mM Mn^{2+} under constant shaking (1 g of wet yeast per 20 ml of medium). In some experiments the cells were preincubated in the medium with 33 mM potassium phosphate and 500 mM glucose at 37°C and pH 6 under constant shaking for 60 min. After being washed with water, the cells were placed into the incubation medium. After incubation the cells were separated from the medium by centrifugation and then washed three times with water.

In the experiments with inhibitors, the yeast cells were incubated with carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) or 2,4-dinitrophenol (DNP) for 15 min and then washed with water and placed into the incubation medium with glucose and Mn^{2+}. With NaN_3 the inhibitor was present during both the preincubation (15 min) and the further incubation with Mn^{2+} and glucose (60 min).

In the experiments with candicidin, the yeast cells were preincubated for 10 min in 50 mM imidazole buffer, pH 6.5, with 20 mM glucose (1 g of yeast in 100 ml). Candicidin (500 μg/g of cells) dissolved in dimethylsulfoxide (DMS) was then added to the incubation mixture. The final DMS concentration in the incubation medium was not more than 1%. After 3 min of incubation with candicidin, the yeast cells were rapidly (3 min) centrifuged, washed with water, and placed into incubation medium with Mn^{2+} and glucose.

The conditions of incubation with *N,N'*-dicyclohexylcarbodiimide (DCCD) and Na_3AsO_4 are described below.

Differential extraction of ions and the determination of intracellular ion content (expressed as the difference between ion content in whole cells and cell walls) have been described in the accompanying paper (15). K^+, Mg^{2+}, and Mn^{2+} were determined by atomic absorbtion spectrophotometry (Hitachi model 207).

Chemicals. D-Fructose, DNP, sodium azide, DMS, imidazole, and cytochrome *c* were purchased from Serva; 2-deoxy-D-glucose was from Fluka; 3-*O*-methylglucose was from Koch-Light; L-sorbose was from Merck; D-mannose was from Schuchardt; DCCD was from Ferack; and candicidin and CCCP were from Calbiochem. All other chemicals were pure.

RESULTS

K^+ efflux during Mn^{2+} accumulation. In the presence of glucose, *S. carlsbergensis* cells lose K^+ and accumulate Mn^{2+}, both of these

processes depending on glucose concentration (Fig. 1). Deoxyglucose did not stimulate either K^+ efflux or Mn^{2+} uptake (Table 1). In the presence of this sugar, the intracellular K^+ content even increased, probably due to the uptake of cell wall K^+. 3-*O*-Methylglucose and L-sorbose behaved as deoxyglucose, and fructose and mannose behaved like glucose (data not shown). Glucose also caused the efflux of intracellular K^+ in the medium deprived of Mn^{2+} or Mg^{2+} (Table 1). Thus, in the presence of Mn^{2+}, and glucose, K^+ efflux is the complex response of the cells to glucose and Mn^{2+} uptake. During the first 30 min of Mn^{2+} uptake, the ratio of movements of K^+/Mn^{2+} equaled approximately 2 (Fig. 2).

It is accepted now that K^+ accumulation in yeast and fungal cells occurs due to K^+-H^+ exchange energized by the plasma membrane

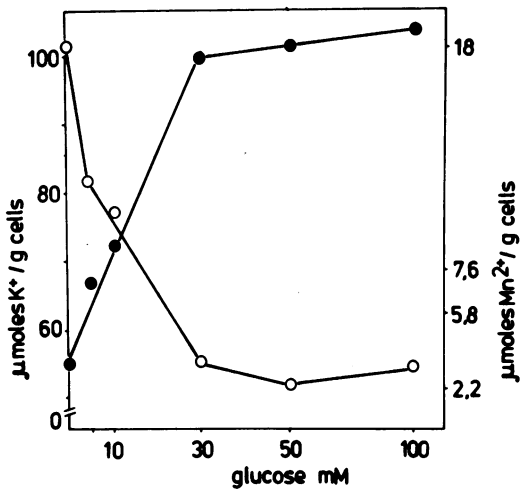

FIG. 1. *Effect of glucose concentration on the intracellular content of K^+* (○) *and Mn^{2+}* (●). *Incubation time: 60 min. Mn^{2+} was added to 3 mM.*

TABLE 1. *Influence of sugar on intracellular K^+ and Mn^{2+} content*[a]

Sugar (100 mM)	Intracellular K^+		Intracellular Mn^{2+} (incubated with 3 mM Mn^{2+})
	Incubated without Mn^{2+}	Incubated with 3 mM Mn^{2+}	
Initial cells	100	100	<0.5
2-Deoxyglucose	105	114	7
Glucose	83	57	100

[a] Intracellular K^+ expressed as the percentage of the intracellular K^+ of initial cells (111 µmol/g of wet cells). Intracellular Mn^{2+} expressed as the percentage of the intracellular Mn^{2+} content of cells after incubation with 3 mM Mn^{2+} and 100 mM glucose (18 µmol/g of wet cells).

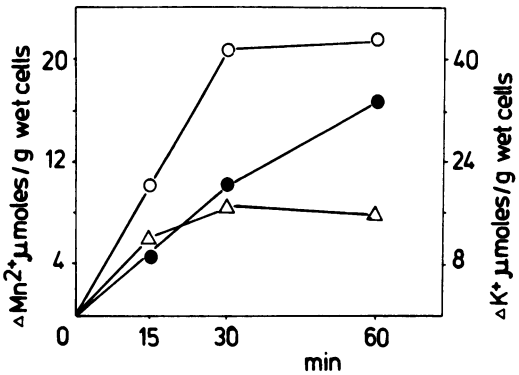

FIG. 2. *Mn^{2+} influx into S. carlsbergensis cells is coupled with K^+ efflux. Mn^{2+} influx* (●); *total K^+ efflux* (○) *stimulated by glucose and Mn^{2+}; net K^+ efflux stimulated by Mn^{2+} only* (△). (*K^+ efflux stimulated by glucose was substracted from total K^+ efflux*).

Mg^{2+}-dependent ATPase (10). Treatment of *S. cerevisiae* cells with proton conductors resulted in the inhibition of K^+ accumulation and even its efflux from the cells at higher concentrations of uncouplers (16). We have observed the identical effect of proton conductors with *S. carlsbergensis* cells: they lose K^+ in the presence of various proton conductors (Table 2). Simultaneously, they become unable to accumulate Mn^{2+}. Partial inhibition of Mn^{2+} accumulation (30%) may be reached by a 60-min preincubation of the yeast with glucose: K^+ loss is responsible for Mn^{2+} transport inhibition (data not shown).

The treatment of *S. carlsbergensis* with the polyene antibiotic candicidin in the presence of glucose (Table 2) resulted in complete and very rapid loss of intracellular K^+ and the complete inhibition of Mn^{2+} accumulation. Under such conditions [^{14}C]glucose uptake was not inhibited for 10 min, and by 60 min, 40 to 45% inhibition was observed (data not shown). It is important that the rapid lowering of the concentration gradient of K^+ on both plasma and vacuolar membranes is not coupled with the lowering of the concentration gradient of Mg^{2+} (data not shown). Our findings, unlike those of Liras and Lampen (11), show that only K^+, not Mg^{2+}, leaves the yeast cells upon candicidin treatment.

Thus, in yeast cells with K^+ and Mg^{2+} gradients across the tonoplast and plasmalemma (15), Mn^{2+} influx was coupled with the efflux of K^+ but not of Mg^{2+}. Even when the K^+ gradient was dissipated, Mg^{2+} did not leave the cells. In this way yeast cells differ from bacterial ones which lose Mg^{2+} during Mn^{2+} or Co^{2+} accumulation (23).

DCCD inhibits various ATPases, including the ATPase of the plasma membrane of the

fungi (2, 4, 21). DCCD inhibited both Mn^{2+} transport and K^+ efflux (Table 3), whereas glucose accumulation was inhibited only slightly (15%) (data not shown). Arsenate inhibited the accumulation of Mn^{2+} and Mg^{2+} to 45 and 25%, respectively (taking into account the influence of Na^+). The total inhibition of Mn^{2+} and Mg^{2+} accumulation by arsenate and Na^+ equaled 56 and 45%, respectively, (Table 3). In this case K^+ efflux was also inhibited (76% during Mn^{2+} accumulation).

Influence of exogenous K^+ and Na^+ ions. If the influx of Mn^{2+} (or Mg^{2+}) ions is relatively tightly coupled with K^+ efflux in *S. carlsbergensis*, inhibition of bivalent ion accumulation with increasing concentrations of exogenous K^+ should be expected. Indeed, even 1 mM KCl slightly inhibited Mn^{2+} accumulation (Fig. 3). K^+ at 60 mM, i.e., a concentration similar to that in cytosol (15), fully inhibited Mn^{2+} accumulation stimulated by glucose. Mn^{2+} transport inhibition even at relatively low concentrations of exogenous K^+ is in good agreement with the data on Mg^{2+} inhibition by exogenous K^+ (12). Apparently, another transport system works at lower (0.75 mM) concentrations of Mg^{2+}: in this case Mg^{2+} accumulation is even stimulated at exogenous K^+ concentrations up to 20 mM (20).

Na^+ at 30 mM stimulated K^+ efflux from yeast cells due to K^+-Na^+ exchange, thus inhibiting Mn^{2+} accumulation, although to a lesser extent than with K^+ (Table 3).

Role of the vacuolar pool of K^+. The analysis of different *S. carlsbergensis* ion pools before and after Mn^{2+} accumulation demonstrated that the effluxing K^+ was represented mainly by vacuolar ions (Table 4). Correspondingly, Mn^{2+} accumulation was observed mainly in vacuoles (Tables 4 and 5). The physicochemical state of K^+ and Mn^{2+} depended on physiological conditions. In vacuoles, probably, the redistribution of osmotically free and bound ions may occur. The bulk of the vacuolar K^+ is osmotically free if the cells are analyzed after the growth without preincubation with potassium phosphate and glucose (Table 4). A considerable part of Mn^{2+} accumulated in such cells is also osmotically free and vacuolar. When the cells were analyzed after preincubation with potassium phosphate and glucose, the bulk of vacuolar K^+ was bound. Mn^{2+} accumulation by cells after preliminary incubation with glucose and phosphate was coupled with the decrease of the content of this form of K^+ (Table 5). Correspondingly, the bulk of Mn^{2+} accumulated by these cells was represented by bound ions. During the accumulation of Mg^{2+} in the vacuoles of *S. carlsbergensis* cells preincubated with glucose and potassium phosphate, K^+ efflux from the vacuoles occurred at the expense of the bound potassium (Table 5).

TABLE 2. *Influence of proton conductors and candicidin upon intracellular Mn^{2+} and K^+ content*

Incubation conditions	ΔK^+ [a] (μmol/g of cells)	ΔMn^{2+} [a] (μmol/g of cells)
3 mM Mn^{2+} (60 min)	16.1	2.6
Water (15 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−45.0	22.2
0.1 mM CCCP (15 min)	−18.2	<0.1
0.1 mM CCCP (15 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−59.3	6.9
0.5 mM DNP (15 min)	−5.1	<0.1
1.0 mM DNP (15 min)	−9.5	<0.1
2.0 mM DNP (15 min)	−18.7	<0.1
0.5 mM DNP (15 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−41.2	19.2
1.0 mM DNP (15 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−46.8	14.7
2.0 mM DNP (15 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−50.5	5.6
10 mM NaN_3 (15 min), then 3 mM Mn^{2+}, 100 mM glucose and 10 mM NaN_3 (60 min)	−45.5	3.4
0.5 mg of candicidin per g of cells and 20 mM glucose (3 min)	−169.8	<0.1
0.5 mg of candicidin per g of cells and 20 mM glucose (3 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	−169.8	2.5

[a] ΔK^+ and ΔMn^{2+} denote the change in intracellular content of ions after corresponding incubations of initial cells. Intracellular K^+ of initial cells = 170 μmol/g of cells; Mn^{2+} < 0.1 μmol/g of cells.

It is noteworthy that K^+ efflux from yeast cells affected the vacuolar pool of K^+ during glucose uptake, too (data not shown).

DISCUSSION

Recent studies of transport systems of bivalent metal cations suggest that microbial cells have both the general transport system for bivalent cations with limited specificity and the highly specific transport systems for each cation (23). The total transport system of divalent cations operates at comparatively high ion concentrations (higher than 1 mM) and, as a rule, transports Mg^{2+} (in vivo) (7, 9, 12, 23). The limited specificity of this system may lead to the accumulation of toxic metals, for instance, Co^{2+} accumulation by *Escherichia coli* cells (23) or Ca^{2+} by yeast cells (8, 13).

Alongside this system, bacteria have highly specific transport systems of Mg^{2+}, Ca^{2+}, and

TABLE 3. *Influence of arsenate and DCCD upon Mn^{2+} or Mg^{2+} accumulation*

Incubation conditions	Mn^{2+} accumulation (μmol/g of cells, 60 min)		Mg^{2+} accumulation (μmol/g of cells, 60 min) (ΔMg^{2+})
	ΔMn^{2+}	ΔK^+	
Water, pH 6.0 (15 min), then 100 mM glucose and 3 mM Mn^{2+} or Mg^{2+}	18.4	−37.9	13.4
30 mM NaCl, pH 6.0 (15 min), then 100 mM glucose and 3 mM Mn^{2+} or Mg^{2+}	14.4	−40.4	9.9
30 mM Na_3AsO_4, pH 6.0 (15 min), then 100 mM glucose and 3 mM Mn^{2+} or Mg^{2+}	8.0	−8.9	7.4[a]
Water (60 min), then 3 mM Mn^{2+} and 100 mM glucose (60 min)	22.1	−44.0	
100 μM DCCD (60 min), then 3 mM Mn^{2+} and 100 mM glucose	10.2	−31.2	

[a] Na_3AsO_4 was present during the 60-min accumulation of Mg^{2+}.

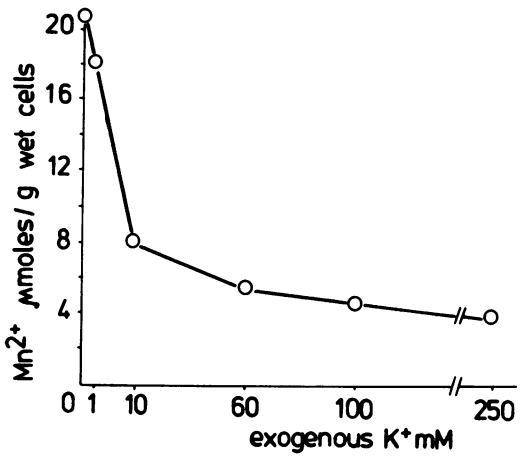

FIG. 3. *Influence of exogenous K^+ on Mn^{2+} uptake by S. carlsbergensis. Mn^{2+} uptake lasted for 60 min from 3 mM Mn^{2+} with 100 mM glucose.*

Mn^{2+} ions and trace elements. These systems function at low ion concentrations and transport only one element (23). The discovery of the specific transport system of Zn^{2+} (5) in yeast cells suggests the existence of the analogous systems both for Mg^{2+} and Mn^{2+} ions.

The present work deals with the bivalent cation transport system which is responsible for accumulation of Mg^{2+} and Mn^{2+} (7, 12, 14).

TABLE 4. *Compartmentation[a] of K^+ and Mn^{2+} in S. carlsbergensis*

Ion compartment	K^+ (μmol/g of cells)			
	Before Mn^{2+} accumulation	After Mn^{2+} accumulation	ΔK^+	Mn^{2+}
Intracellular cations (sum)	177.0	126.7	−50.3	21.1
Cytosol	37.7	28.0	−9.7	1.1
Vacuoles:				
Osmotically free ions	94.9	50.8	−44.1	7.5
Bound ions	31.1	35.9	+4.8	11.5
Other compartments	13.3	12.0	−1.3	1.0

[a] Yeast cells were analyzed after the growth without preincubation with glucose and KH_2PO_4.

TABLE 5. *Influence of Mn^{2+} or Mg^{2+} accumulation on K^+ content[a]*

Compartment	Mn^{2+} accumulation (μmol/g of cells)		Mg^{2+} accumulation (μmol/g of cells)	
	ΔK^+	ΔMn^{2+}	ΔK^+	ΔMg^{2+}
Intracellular	−62.1	13.5	−54.5	22.3
Cytosol	−1.0	1.2	2.3	0.4
Vacuoles				
Osmotically free ions	4.5	2.8	8.1	9.4
Bound ions	−51.1	7.1	−55.9	5.4
Other compartments	−14.5	2.4	−9.0	7.1

[a] Yeast cells were preincubated with 500 mM glucose and 33 mM KH_2PO_4 for 60 min before incubation with 3 mM Mn^{2+} or Mg^{2+} and 100 mM glucose for 60 min. Control was yeast cells after preincubation.

The Mn^{2+} uptake in the yeast was stimulated severalfold by glucose (Fig. 1, reference 14). Fructose and mannose showed the same effect, but sorbose, 2-deoxyglucose, and 3-*O*-methylglucose did not. It is generally accepted (but not strictly proven) that ATPase of fungal plasmalemma is a H^+ pump (2, 4, 6, 10, 21). Therefore, the ATPase activation can stimulate Mn^{2+}/H^+ exchange and Mn^{2+} uptake. This occurs probably after 30 min of Mn^{2+} uptake (the interval of 30 to 60 min, Fig. 2). Norris and Kelly found similar kinetics of Co^{2+} uptake (13). They supposed that Co^{2+} influx can take place without K^+ efflux. It was shown in the case of plasma membrane vesicles of *S. cerevisiae* that Mn^{2+}/H^+ exchange resulted in the Mn^{2+} transport (L. Okorokov and F. Fuhrmann, manuscript in preparation). Mn^{2+} transport in the plasma membrane vesicles was inhibited by the exogenous K^+ as a result of K^+/H^+ exchange (which is

probably more rapid). The inhibition of Mn^{2+} uptake by exogenous K^+ (Fig. 3) in the case of whole cells was probably a consequence of K^+/H^+ exchange also, in spite of the glycolysis activation (19) and stimulation of the glucose transport in *S. carlsbergensis* (data not shown). The leveling up of K^+ gradient on the plasma membrane is an alternative interpretation of the inhibition of Mn^{2+} uptake by the exogenous K^+. The same is probably true for the inhibition of Mn^{2+} uptake by protonophores (Table 2). Arsenate inhibiting the glycolysis can decrease the Mn^{2+} uptake (Table 3). DCCD can inhibit both ATPase and other components of transport system; this will decrease Mn^{2+} uptake also (Table 3). However the decrease in the K^+ gradient after their action on yeast cells also takes place, but not so dramatically as after candicidin (Table 2).

The stoichiometry Mn^{2+}/K^+ fluxes was equal to 1:2 (Fig. 2, or reference 13 in the case of Co^{2+} uptake). The H^+ pump was probably inoperable in these conditions, and the outward K^+ gradient might provide the driving force for Mn^{2+} uptake. Why was this gradient not utilized in the absence of the effective sugars? One can suppose that only sugars, which are able to symport with H^+ (3), can facilitate K^+ efflux in result of the membrane depolarization.

The data from different laboratories confirm that the K^+ gradient might provide the driving force for the transport of divalent cations and amino acids. K^+ efflux stimulated by candicidin was shown to increase the leucine transport and even the ATP synthesis (11). The Ca^{2+} influx was increased if the antibiotic Dio-9 or CCCP (low concentrations) was the effector of K^+ efflux (1). DNP (low concentrations also) stimulated Mg^{2+} influx (12). Addition of several cationic dyes resulted both in K^+ efflux and strong increase in Ca^{2+}, Mn^{2+}, or glycine uptake (17). The effects of candicidin and Dio-9 were found only in the presence of glucose, and they were stimulated by glucose in case of cationic dyes. Therefore, K^+ gradient might drive the transport of ions and metabolites. Since the K^+ gradient is decreased, its potential for transport energization will fall also, but the potential of the H^+ pump (ATPase) will increase. This will result in a decrease in K^+/Mn^{2+} stoichiometry. For example, Mn^{2+} uptake occurred between 30 and 60 min entirely without net K^+ efflux (Fig. 2; reference 13 for Co^{2+} uptake). The efficiency of the K^+ gradient depends on the capacity of the K^+ vacuolar pool because the vacuolar K^+ first of all is involved (through the cytoplasm K^+) in the K^+ efflux and the energization of Mn^{2+}, Mg^{2+} (Tables 4 and 5), or glucose uptake (data not shown). The study of the participation of K^+ gradient in the energization of transport systems in *S. carlsbergensis* is in progress in this laboratory.

LITERATURE CITED

1. **Boutry, M., F. Foury, and A. Goffeau.** 1977. Energy dependent uptake of calcium by the yeast *Schizosaccharomyces pombe*. Biochim. Biophys. Acta **464**:602–612.
2. **Bowman, B. J., and C. W. Slayman.** 1977. Characterization of plasma membrane adenosine triphosphatase of *Neurospora crassa*. J. Biol. Chem. **252**:3357–3363.
3. **Brocklehurst, R., D. Gardner, and A. A. Eddy.** 1977. The absorption of protons with α-methyl glucoside and α-thioethyl glucoside by the yeast N.C.Y.C. 240. Evidence against the phosphorylation hypothesis. Biochem. J. **162**:591–599.
4. **Delhez, J., J. P. Dufour, D. Thines, and A. Goffeau.** 1977. Comparison of the properties of plasma membrane-bound and mitochondria-bound ATPase in the yeast *Schizosaccharomyces pombe*. Eur. J. Biochem. **79**:319–328.
5. **Failla, M. L., and E. D. Weinberg.** 1977. Cyclic accumulation of zinc by *Candida utilis* during growth in batch culture. J. Gen. Microbiol. **99**:85–97.
6. **Foury, F., M. Boutry, and A. Goffeau.** 1977. Efflux of potassium induced by Dio-9, a plasma membrane ATPase inhibitor in the yeast *Schizosaccharomyces pombe*. J. Biol. Chem. **252**:4577–4583.
7. **Fuhrmann, G. F., and A. Rothstein.** 1968. The transport of Zn^{2+}, Co^{2+} and Ni^{2+} into yeast cells. Biochim. Biophys. Acta **163**:325–330.
8. **Heldwein, R., H. W. Tromballa, and E. Broda.** 1977. Aufnahme von Cobalt, Blei und Cadmium durch Bäckerhefe. Z. Allgem. Mikrobiol. **17**:299–308.
9. **Jasper, P., and S. Silver.** 1977. Magnesium transport in microorganisms, p. 7–47. *In* E. D. Weinberg (ed.), Microorganisms and minerals. Marcel Dekker, New York.
10. **Jennings, D. H.** 1976. Transport in fungi, p. 189–228. *In* V. Lüttge and M. G. Pitman (ed.), Transport in plants, vol II. Part A. Cells. Springer-Verlag, Berlin.
11. **Liras, P., and J. O. Lampen.** 1974. Sequence of candicidin action on yeast cells. Biochim. Biophys. Acta **372**: 141–153.
12. **Netter, H., and L. Sachs.** 1961. Stoffwechselabhangige Magnesiumaufnahme bei Backerhefe. Biochem. Z. **334**: 18–36.
13. **Norris, P. R., and D. P. Kelly.** 1977. Accumulation of cadmium and cobalt by *Saccharomyces cerevisiae*. J. Gen. Microbiol **99**:317–324.
14. **Okorokov, L. A., L. P. Lichko, V. M. Kadomtseva, L. P. Kholodenko, V. Titovsky, and I. S. Kulaev.** 1977. Energy-dependent transport of manganese into yeast cells and distribution of accumulated ions. Eur. J. Biochem. **75**:373–377.
15. **Okorokov, L. A., L. P. Lichko, and I. S. Kulaev.** 1980. Vacuoles: main compartments of potassium, magnesium, and phosphate ions in *Saccharomyces carlsbergensis* cells. J. Bacteriol. **144**: 661–665.
16. **Penã, A.** 1975. Studies on the mechanism of K^+ transport in yeast. Arch. Biochem. Biophys. **167**:397–409.
17. **Penã, A.** 1978. Effect of ethidium bromide on Ca^{2+} uptake by yeast. J. Membr. Biol. **42**:199–213.
18. **Reichert, M., R. Schmidt, and M. Foret.** 1975. A possible mechanism of energy coupling in purine transport of *Saccharomyces cerevisiae*. FEBS Lett. **52**:100–102.
19. **Rothstein, A., and C. Demis.** 1953. The relationship of the cell surface to metabolism. The stimulation of fermentation by extracellular potassium. Arch. Biochem.

Biophys. 44:18–29.
20. **Rothstein, A., A. Hayes, D. Jehnings, and D. Hooper.** 1958. The active transport of Mg^{2+} and Mn^{2+} into the yeast cell. J. Gen. Physiol. 41:585–594.
21. **Scarborough, G. A.** 1977. Properties of the *Neurospora crassa* plasma membrane ATPase. Arch. Biochem. Biophys. 180:384–393.
22. **Seaston, A., C. Inkson, and A. A. Eddy.** 1973. The absorption of protons with specific amino acids and carbohydrates by yeast. Biochem. J. 134:1031–1043.
23. **Silver, S.** 1978. Transport of cations and anions, p. 221–324. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, New York.