Activation of Ca^{2+} influx by metabolic substrates in *Saccharomyces cerevisiae*: role of membrane potential and cellular ATP levels

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Influx of Ca^{2+} into cells of *Saccharomyces cerevisiae* was measured under non-steady-state conditions, which enable measurements of the initial rate of transport across plasma membranes without interference by the vacuolar Ca^{2+} transport system. Removal of glucose from the incubation medium led to inactivation of Ca^{2+} influx within 5 min. Readdition of glucose led to a transient increase in the rate of Ca^{2+} transport, reaching a peak after 3–5 min. A second increase was observed 60–80 min later. To examine whether the first transient activation of Ca^{2+} influx by glucose was mediated by membrane hyperpolarization, influx of $^{45}\text{Ca}^{2+}$ was measured in the presence and absence of metabolic substrates (glucose, glycerol, and glucose plus antimycin A) in cells hyperpolarized to different values of membrane potential ($\Delta\psi$). Logarithms of the rate of Ca^{2+} influx were plotted against values of $\Delta\psi$. Two different slopes were obtained, depending upon whether the metabolic substrate was present or absent. Ca^{2+} influx in the presence of the metabolic substrates was always higher than expected by their effect on $\Delta\psi$. Glycerol plus antimycin A did not affect Ca^{2+} influx. It was concluded that metabolized substrates activate Ca^{2+} influx not only by effects on $\Delta\psi$ but also by additional mechanism(s). Since no simple correlation between Ca^{2+} influx and intracellular ATP levels was observed, it was concluded that ATP levels do not affect the initial rates of Ca^{2+} transport across the plasma membrane of *S. cerevisiae*.

Introduction

Influx of Ca^{2+} into yeast cells proceeds only in the presence of a metabolic substrate (Boutry *et al.*, 1977), but the mechanism of this activation is not yet understood (Borst-Pauwels, 1981). Since in yeast cells most of the cellular Ca^{2+} is sequestered within the vacuole (Eilam *et al.*, 1985a) it is necessary to distinguish between Ca^{2+} transport across the plasma membrane and the subsequent accumulation of Ca^{2+} in the vacuole by the vacuolar $\text{nH}^+/\text{Ca}^{2+}$ antiporter (Ohsumi & Anraku, 1983), which drives active transport of Ca^{2+} into the vacuole.

A method for measuring the initial rate of Ca^{2+} influx across the cell membrane without interference by the vacuolar Ca^{2+} transport system has been developed (Eilam & Chernichovsky, 1987). Influx of Ca^{2+} occurred in the absence of metabolic substrates in energy-depleted cells which were hyperpolarized by preincubation with low concentrations of trifluoperazine (TFP) (Eilam *et al.*, 1985b). Below a threshold value of membrane potential ($\Delta\psi$) a linear relationship was observed between the

logarithms of the initial rates of Ca^{2+} transport and the values of $\Delta\psi$. These results led to the suggestion that Ca^{2+} influx in yeast cells is mediated by gated Ca^{2+} channels, which open below the threshold $\Delta\psi$ value (Eilam & Chernichovsky, 1987).

In the present study, we investigated the role of metabolic substrates in activating Ca^{2+} influx. Two hypotheses are examined. (a) Addition of a metabolic substrate causes hyperpolarization of the cell membrane (van de Mortel *et al.*, 1988; Borst-Pauwels *et al.*, 1988). Since Ca^{2+} influx is strongly dependent on $\Delta\psi$ (Eilam & Chernichovsky, 1987) the activation of Ca^{2+} influx by glucose may be mediated by the glucose-induced hyperpolarization which may reduce the negative $\Delta\psi$ below the threshold value. (b) The effect of metabolic substrate on Ca^{2+} influx may be mediated via glucose-induced changes in cellular ATP concentration. A preliminary report of these results has been presented (Eilam & Othman, 1988).

Methods

Organism and culture conditions. *Saccharomyces cerevisiae* strain N123 (genotype *MATa/α his1*) was maintained at 4 °C on YPD agar slopes and grown at 30 °C in YPD broth (Bacto yeast extract 10 g l⁻¹, Bacto

Abbreviations: TPP⁺, tetraphenylphosphonium ion; TFP, trifluoperazine; $\Delta\psi$, membrane potential.

peptone 20 g l⁻¹, glucose 20 g l⁻¹). Cells were collected from an overnight culture by centrifugation, washed three times by resuspension in distilled water and finally resuspended in the indicated medium. To obtain non-steady-state conditions for measurement of ⁴⁵Ca²⁺ influx, cells were preincubated for 90 min at 30 °C with shaking in medium containing MES/Tris (10 mM, pH 6.0) and glucose (100 mM) (buffer-glucose medium) at a density of 10⁸ cells ml⁻¹.

Preparation of cells with different $\Delta\psi$ values. Cells were preincubated for 90 min in buffer-glucose medium to obtain non-steady-state conditions, and then preincubated for an additional 30 min in buffer-glucose medium containing 0–30 μ M-TFP, in order to obtain cells with different $\Delta\psi$ values. Cells were then collected by centrifugation and washed once with MES/Tris buffer (10 mM, pH 6.0) (buffer-medium).

Effect of metabolic substrates on rates of Ca²⁺ influx. After preincubation with TFP and washing in buffer (see above) cells were preincubated for 5 min in buffer-medium at a concentration of 10⁸ cells ml⁻¹. Medium (0.5 ml) containing MES/Tris (10 mM, pH 6.0) alone or with a metabolic substrate (20 mM or as indicated) was added to 0.5 ml of the cell suspension. After 3 min at 30 °C, transport was initiated by the addition of ⁴⁵CaCl₂ to yield a final concentration of 1 μ M-Ca²⁺, 1 μ Ci (37 kBq) ml⁻¹, and the cell suspension was immediately mixed. Transport was terminated after 20 s by the addition of 1 ml washing solution containing MgCl₂ (20 mM) and LaCl₃ (0.1 mM); the cells were then filtered immediately on membrane filters (0.45 μ m pore size), and washed five times with 2 ml portions of the washing solution. The filters were dried and the radioactivity was determined, after the addition of toluene-based scintillation fluid, using a liquid scintillation counter. Ca²⁺ binding was determined by zero-time measurements, which were obtained by the addition of 0.5 ml cell suspension to 0.5 ml buffer-medium containing ⁴⁵CaCl₂ (2 μ M, 1 μ Ci per sample), and 1 ml washing solution (see Eilam & Chernichovsky, 1987). The suspensions were immediately filtered and washed as described above. In preliminary experiments, it was determined that the time course of Ca²⁺ uptake was linear for 20 s under all conditions tested in the present work.

$\Delta\psi$ measurements. These were based on the steady-state distribution of [³H]TPP⁺ between cells and medium. $\Delta\psi$ was determined in every experiment in parallel with Ca²⁺ transport measurements. Samples of the cell suspension underwent the same procedure of preincubation and washing as described above but [³H]TPP⁺ (1 μ M; 0.05 μ Ci ml⁻¹) was included in all preincubation and incubation media, for a total of 128 min. These samples were filtered at the time of Ca²⁺ influx measurements, after incubation for 3 min in the presence or absence of a metabolic substrate and with [³H]TPP, as described above, but glass-fibre filters (Whatman GF/C) were used instead of membrane filters. All filters were prewashed with washing solution. The results were corrected for [³H]TPP⁺ binding. Cell volume was determined and $\Delta\psi$ values were calculated as described by Eilam *et al.* (1985b). Preliminary experiments using a double labelling technique (Eilam & Chernichovsky, 1987) indicated no change in [³H]TPP distribution during 20 s of ⁴⁵Ca²⁺ influx.

Measurements of intracellular ATP content. Cells were preincubated as described above for Ca²⁺ influx measurements. Samples (0.1 ml) were removed, diluted in 2 ml boiling distilled water and maintained at 100 °C for 5 min. After cooling, 0.2 ml volumes of these solutions were added to 2.8 ml of the reaction mixture in scintillation vials. The reaction mixture contained 1 ml solution A, 1 ml solution B and 0.8 ml H₂O. Solution A contained sodium arsenate (0.1 M) and MgSO₄ (40 mM), and was brought to pH 7.4 with H₂SO₄; solution B contained potassium phosphate buffer pH 7.4 (0.4 M) and MgSO₄ (4 mM). The reaction was initiated by the addition of 75 μ l of a solution of luciferin/luciferase (4 mg ml⁻¹; Sigma), followed by immediate mixing and the placing of the vial into the well of a liquid scintillation counter

that had been set for maximum sensitivity with the coincidence circuit off. Each sample was immediately counted for 10 s. Calibration curves were prepared in every experiment by adding different volumes of solution B containing 0.5 μ M-ATP to the reaction mixture, in which 0.2 ml water replaced the sample volume; the volume of solution B was adjusted according to the volume of the ATP solution. TFP (up to 100 μ M), glucose (10 mM) or antimycin A (15 μ M), when added to cell extracts, did not affect the ATP assay.

Results

We have developed a technique which enables the initial rate of Ca²⁺ transport across the plasma membrane to be measured without interference by the vacuolar Ca²⁺ transport system (Eilam & Chernichovsky, 1987). All experiments reported here were done by this technique, which involved preincubation of the cells in medium containing glucose and buffer, without Ca²⁺, and measuring the initial rate of ⁴⁵Ca²⁺ influx (non-steady-state Ca²⁺ influx).

In the first group of experiments the kinetics of activation/inactivation of ⁴⁵Ca²⁺ influx by glucose were investigated (Fig. 1). Preincubating the cells for 90 min in buffer-glucose medium led to a high rate of Ca²⁺ transport. Removal of glucose caused a rapid decrease in the rate of Ca²⁺ influx within 5 min. Readdition of glucose (10 mM) caused a transient increase in the rate of Ca²⁺ influx, which reached a maximum after 3–5 min and then decreased. A second increase was observed after 60–80 min incubation in the presence of glucose.

To examine the hypothesis that the effect of glucose on Ca²⁺ influx may be mediated by glucose-induced hyperpolarization, the following experiments were done. After preincubation in buffer-glucose medium (to obtain non-steady-state conditions), and with low concentrations of TFP (to obtain cells with different values of $\Delta\psi$), cells were washed and preincubated in buffer-medium (without glucose) for 5 min, to inactivate Ca²⁺ influx. Each sample of cells was then split into two parts, to measure the initial Ca²⁺ influx in the absence or presence of a metabolic substrate. The latter was added 3 min before the measurement of Ca²⁺ influx and the determination of $\Delta\psi$. Values of log (initial rate of Ca²⁺ influx) were plotted against the values of $\Delta\psi$. The aim of the experiments was to distinguish between the two models (see Introduction). (a) Effects of the metabolic substrate may be mediated only via an effect on $\Delta\psi$. In this case, a single slope would represent the relation between log (rate of Ca²⁺ influx) and $\Delta\psi$. This slope should fit data obtained in either the presence or absence of the metabolic substrate. (b) The effect of metabolic substrates may be mediated by additional mechanisms(s), in which case two different slopes would be observed. The results in Fig. 2(a–c) and Table 1 show that with glucose, glycerol, or glucose plus antimycin A as metabolic substrate, two different slopes were obtained, one in the

Fig. 1. Effect of removal and addition of glucose on Ca^{2+} influx. Cells were preincubated for 90 min at 30°C in buffer-glucose medium (10 mM-MES/Tris, pH 6.0, and 100 mM-glucose). Rates of Ca^{2+} influx were then determined, following removal of glucose by centrifugation and resuspension in buffer-medium (10 mM-MES/Tris, pH 6.0) ($\circ$), and after re-addition of glucose (10 mM), as indicated ($\bullet$). Rates of Ca^{2+} influx were calculated from the difference between the 20 s and zero-time uptake data. The results are means $\pm$ SD of three determinations.

absence and one in the presence of the substrate. Linear relations ($r > 0.9$) were observed between $\log$ (rate of Ca^{2+} influx) and $\Delta\psi$ in all experiments. Cells with similar values of $\Delta\psi$ displayed higher rates of Ca^{2+} influx in the presence of a metabolic substrate than in its absence. The differences between the slopes at the same $\Delta\psi$ values represent the part of the influx independent of $\Delta\psi$. This effect was most pronounced at low values of $\Delta\psi$. At the higher values of $\Delta\psi$, the predominant effect on influx was exerted by $\Delta\psi$, independent of experimental variables. Thus, the metabolic substrates induced a greater effect on Ca^{2+} influx than expected from their effect on $\Delta\psi$.

The metabolic substrates which were selected are metabolized via different pathways: via glycolysis and the tricarboxylic acid (TCA) cycle (glucose), only by glycolysis (glucose + antimycin A), or only via the TCA cycle (glycerol). It is clear (Fig. 2*a-c*; Table 1) that the strongest effect on Ca^{2+} influx was exerted by glucose. Glycerol in the presence of antimycin A is not metabolized. The results in Fig. 2(*d*) show that in these

Fig. 2. Effect of metabolic substrates on the initial Ca^{2+} influx in cells with different values of $\Delta\psi$. Cells were preincubated as described in Methods. Initial rates of $^{45}\text{Ca}^{2+}$ influx were measured in the absence of added substrate ($\circ$) or 3 min after the addition of the indicated substrate ($\triangle$). Substrates were: (a) 10 mM-glucose; (b) 10 mM-glycerol; (c) 10 mM-glucose plus $15\ \mu\text{M}$ -antimycin A; (d) 10 mM-glycerol plus $15\ \mu\text{M}$ -antimycin A. Rates of Ca^{2+} influx, v ($10^{-15} \times \text{mol Ca}^{2+} \text{ s}^{-1} \text{ per } 10^8 \text{ cells}$) were calculated from the difference between 20 s and zero-time uptake data. $\Delta\psi$ (mV) was calculated from $[^3\text{H}]\text{TTP}^+$ accumulation. Lines were drawn by linear regression; r values are shown in the respective figure. Data of a representative experiment are shown. Mean values of the slopes of three different experiments are given in Table 1.

Table 1. Effects of various substrates on the slopes of the lines representing the relation between $\log$ (rate of Ca^{2+} influx) and $\Delta\psi$

The values represent means $\pm$ SD of three different experiments which were done as shown in Fig. 2. Due to some variability between the values obtained in different experiments, in every experiment the measurements were done in the absence and presence of the substrate. Each experiment was done in triplicate.

Substrate	Slope [$\log(10^{-15} \times \text{mol Ca}^{2+} \text{ s}^{-1} \text{ per } 10^8 \text{ cells}) \times \text{mV}^{-1}$]	
	Minus substrate	Plus substrate
Glucose	$(4.1 \pm 0.2) \times 10^{-2}$	$(2.6 \pm 0.2) \times 10^{-2}$
Glycerol	$(4.0 \pm 0.3) \times 10^{-2}$	$(3.0 \pm 0.1) \times 10^{-2}$
Glucose + antimycin A	$(4.1 \pm 0.2) \times 10^{-2}$	$(2.7 \pm 0.3) \times 10^{-2}$
Glycerol + antimycin A	$(3.9 \pm 0.4) \times 10^{-2}$	$(3.9 \pm 0.4) \times 10^{-2}$

Table 2. Effect of various substrates on intracellular ATP concentration

Cells were preincubated as described in Methods and suspended in buffer-medium (10 mM-MES/Tris, pH 6.0). After 5 min incubation, the indicated substrates were added. Concentration of ATP was determined 3 min after addition of each substrate. The results are means $\pm$ SD of three determinations.

Substrate added	$10^{15} \times \text{ATP content (mol per cell)}$	
	Preincubation in buffer-glucose (120 min)	Preincubation in buffer-glucose (90 min), then TFP (15 μM) added for 30 min
None	12.6 ± 0.6	9.2 ± 0.5
Glucose (10 mM)	14.0 ± 0.6	10.6 ± 0.4
Glycerol (10 mM)	13.5 ± 0.6	10.3 ± 0.6
Glucose 10 mM + antimycin A (15 μM)	10.3 ± 0.5	6.7 ± 0.4
Glycerol (10 mM) + antimycin A (15 μM)	9.2 ± 0.4	6.3 ± 0.4
Antimycin A (15 μM)	8.6 ± 0.4	5.7 ± 0.4

conditions glycerol did not activate Ca^{2+} influx since the slope of the relation between $\log$ (rate of Ca^{2+} influx) and $\Delta\psi$ was similar in the presence or absence of glycerol + antimycin A (see also Table 1). We conclude that the selected metabolic substrates activate Ca^{2+} influx not only by the hyperpolarizing effect but also by additional mechanism(s).

The requirement for a metabolic substrate for the activation of Ca^{2+} influx suggested that the latter process may be related to the intracellular level of ATP. Intracellular concentrations of ATP were therefore

measured at the time of $^{45}\text{Ca}^{2+}$ influx measurement. TFP caused a marked decrease in intracellular ATP levels, as reported previously (Eilam *et al.*, 1985b). Incubation with glucose or glycerol for 3 min resulted in a consistent small increase in intracellular ATP level as compared with cells incubated in buffer alone (Table 2). However, incubation with glucose and antimycin A for 3 min caused a small decrease in ATP level. A greater decrease was observed when cells were incubated for 3 min with antimycin A alone (Table 2). This decrease was probably associated with the inhibitory effect of antimycin A on mitochondrial metabolism. The results in Table 2 are incompatible with the model suggesting effects of intracellular ATP levels on the stimulation of $^{45}\text{Ca}^{2+}$ influx across the plasma membrane, since addition of glucose and antimycin A stimulated $^{45}\text{Ca}^{2+}$ influx but the intracellular levels of ATP decreased.

Discussion

Previous results, which showed that in energy-depleted cells the Ca^{2+} influx system is activated by hyperpolarization (Eilam & Chernichovsky, 1987), led to the suggestion that Ca^{2+} influx is mediated via gated channels in the cell membrane, which open at $\Delta\psi$ values below a threshold value (approx. -70 mV). In the absence of metabolic substrate, the value of the negative $\Delta\psi$ may be above the threshold value; most of Ca^{2+} channels would therefore be closed and Ca^{2+} influx inhibited.

In the present study, the effect of glucose on Ca^{2+} influx was found to be very rapid. Inactivation was observed 5 min after the removal of glucose. A transient increase in the rate of Ca^{2+} influx was observed 3–5 min after addition of glucose, and a second increase 60–80 min later. Glucose-dependent fluctuations in $\Delta\psi$ were reported by Kuschmitz & Hess (1987) using rhodamine 6G. Van de Mortel *et al.* (1988) found that addition of glucose to starved yeast cells caused a transient increase in $\Delta\psi$, reaching a peak 3 min after the addition. A second increase was observed 60–80 min later. Thus, the kinetics of changes in $\Delta\psi$ were similar to the changes we observed in the rates of $^{45}\text{Ca}^{2+}$ influx. The time scale of Ca^{2+} -influx activation/inactivation is also similar to that of activation/inactivation of plasma membrane H^+ATPase following addition or removal of glucose (Serrano, 1983). These results may indicate that the hyperpolarizing effect of glucose is the mechanism which activates $^{45}\text{Ca}^{2+}$ influx. Alternatively one may consider that additional mechanisms may be involved.

To test the 'hyperpolarization model', cells were prepared with different $\Delta\psi$ values, and their Ca^{2+} influx and $\Delta\psi$ were measured simultaneously, in the presence or

absence of metabolic substrates. Only the first transient peak of activation (3 min after addition of a metabolic substrate) was examined. Plots of $\log$ (rate of Ca^{2+} influx) versus $\Delta\psi$, gave different slopes in the presence and absence of each substrate, indicating that hyperpolarization is not the only mechanism involved in the activation of Ca^{2+} influx.

Preincubation with TFP was used to hyperpolarize the cells to different values of $\Delta\psi$ (Eilam, 1984). Preincubation with low concentrations of TFP ($< 35 \mu\text{M}$) for short periods (30 min) does not damage cell membranes, as indicated by the complete inhibition of Ca^{2+} influx by the competitive ion La^{3+} , after preincubation with TFP ($35 \mu\text{M}$) (Eilam *et al.*, 1985b). These results indicate that the enhanced Ca^{2+} influx, stimulated by low concentrations of TFP, is mediated via channels or carriers, and not by simple permeation through disrupted cell membranes. Since experiments were done after the complete removal of TFP, as determined previously (Eilam & Chernichovsky, 1987), the effects on transport were not exerted by TFP itself but by the changes in $\Delta\psi$.

The use of TPP^+ for measuring $\Delta\psi$ has been previously discussed (Eilam, 1984). The possibility should be considered that in the present work, TPP^+ may have accumulated in the mitochondria and may have led to an overestimation of $\Delta\psi$, which would be more pronounced in the presence of a metabolic substrate; however, since such an overestimation would tend to decrease the difference between the two slopes, it would not invalidate the conclusion.

We considered the possibility that glucose-induced activation of Ca^{2+} influx may be mediated by an effect of glucose on intracellular ATP levels. ATP may affect transport by its energetic contribution, or by ATP binding (see, for example, Smith *et al.*, 1986). Intracellular ATP levels were therefore measured at the time of $^{45}\text{Ca}^{2+}$ influx measurements. Since no simple correlation between Ca^{2+} influx and ATP levels was observed, we conclude that intracellular ATP levels do not affect initial rates of Ca^{2+} influx across the cell membrane.

The transient increase in the rate of Ca^{2+} influx across the plasma membrane 3–5 min after the addition of glucose has not been described before. This increase is not dependent on glucose as an energy source, but glucose seems to act as a signal which induces transient hyperpolarization, and a transient increase in Ca^{2+} influx via hyperpolarization and additional mechanism(s). Addition of glucose to glucose-starved yeast cells is known to cause transition from the G0 to the G1 stage of the cell cycle and initiation of proliferation (Kaibuchi *et al.*, 1986). Thus glucose acts in yeast like growth factors in mammalian cells. The role of changes in intracellular Ca^{2+} concentrations as a signal for cell proliferation in various mammalian cells has been established (Moolen-

aar *et al.*, 1984; Truneh *et al.*, 1985; Alkon & Rasmussen, 1988). In addition, increased Ca^{2+} cycling across cell membranes has been suggested to play a regulatory role in sustained cellular responses to various stimuli (Alkon & Rasmussen, 1988). The transient rise in Ca^{2+} influx induced by glucose in yeast may suggest a role of Ca^{2+} in the G0/G1 transition caused by addition of glucose.

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References

- ALKON, D. L. & RASMUSSEN, H. (1988). A spatial-temporal model of cell activation. *Science* **239**, 998–1004.
- BORST-PAUWELS, G. W. F. H. (1981). Ion transport in yeast. *Biochimica et Biophysica Acta* **650**, 88–127.
- BORST-PAUWELS, G. W. F. H., VAN DE MORTEL, J. B. J., MULDER, D., KORTHOOT, H. & THEUENET, A. P. R. (1988). Indications that the transient hyperpolarization of yeast by glucose at pH 7 is caused by opening of specific K^+ channels. In *Proceedings of Contributions to the 6th Small Meeting on Yeast-Transport and Energetics, Dortmund, FRG*, pp. 15–16. Edited by D. Kuschmitz. Dortmund: Max Planck Institut.
- BOUTRY, M., FOURY, F. & GOFFEAU, A. (1977). Energy dependent uptake of calcium by the yeast *Schizosaccharomyces pombe*. *Biochimica et Biophysica Acta* **464**, 602–612.
- EILAM, Y. (1984). Effects of phenothiazine on inhibition of plasma membrane ATPase and hyperpolarization of cell membranes in the yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta* **769**, 601–610.
- EILAM, Y. & CHERNICHOVSKY, D. (1987). Uptake of Ca^{2+} driven by membrane potential in energy depleted yeast cells. *Journal of General Microbiology* **133**, 1641–1649.
- EILAM, Y. & OTHMAN, M. (1988). Mechanism of activation of Ca^{2+} influx by metabolic substrate in *Saccharomyces cerevisiae*. In *Proceedings of Contributions to the 6th Small Meeting on Yeast Transport and Energetics, Dortmund, FRG*, pp. 17–18. Edited by D. Kuschmitz. Dortmund: Max Planck Institut.
- EILAM, Y., LAVI, H. & GROSSOWICZ, N. (1985a). Cytoplasmic Ca^{2+} homeostasis maintained by a vacuolar Ca^{2+} transport system in the yeast *Saccharomyces cerevisiae*. *Journal of General Microbiology* **131**, 623–629.
- EILAM, Y., LAVI, H. & GROSSOWICZ, N. (1985b). Active extrusion of potassium in the yeast *Saccharomyces cerevisiae* induced by low concentrations of trifluoperazine. *Journal of General Microbiology* **131**, 2555–2564.
- KAIBUCHI, K., MIYAJIMA, A., ARAI, K. I. & MATSUMOTO, K. (1986). Possible involvement of RAS-encoded proteins in glucose-induced inositol phospholipid turnover in *Saccharomyces cerevisiae*. *Journal of Cell Biology* **83**, 8172–8176.
- KUSCHMITZ, D. & HESS, B. (1987). Linear and non linear phenomena in the interaction between glycolysis and plasma membrane in yeast. In *Proceedings of Contributions to the 5th Small Meeting on Yeast Transport and Energetics, Louvain La Neuve, Belgium*, pp. 44–45. Edited by A. Goffeau. Louvain La Neuve: Université Catholique de Louvain.
- MOOLENAAR, W. H., TERTOOLEN, L. G. J. & DELAAT, S. W. (1984). Growth factors immediately raise cytoplasmic free Ca^{2+} in human fibroblasts. *Journal of Biological Chemistry* **259**, 8066–8069.
- OHSUMI, Y. & ANRAKU, Y. (1983). Calcium transport driven by a proton motive force in vacuolar membrane vesicles of *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **258**, 5614–5617.
- ROOMANS, G. M., THEUENET, A. P. R., VAN DEN BERG, TH. P. R. & BORST-PAUWELS, C. W. F. H. (1979). Kinetics of Ca^{2+} and Sr^{2+} uptake by yeast. Effects of pH, cations and phosphate. *Biochimica et Biophysica Acta* **551**, 187–196.

- SERRANO, R. (1983). In vivo glucose activation of the yeast plasma membrane ATPase. *FEBS Letters* **156**, 11–14.
- SMITH, J. S., CORONADO, R. & MEISSNER, G. (1986). Single channel measurements of calcium release channel from skeletal muscle sarcoplasmic reticulum. Activation by Ca^{2+} and ATP and modulation by Mg^{2+} . *Journal of General Physiology* **88**, 573–588.
- TRUNEH, A., ALBERT, F., GOLSTEIN, P. & SCHMITT-VERHULST, A. M. (1985). Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Nature, London* **313**, 318–320.
- VAN DE MORTEL, J. B. J., MULDER, D., KORTHOUT, H., THEUVENET, A. P. R. & BORST-PAUWELS, G. W. F. H. (1988). Transient hyperpolarization of yeast by glucose and ethanol. *Biochimica et Biophysica Acta* **936**, 421–428.