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Inositol 1,4,5-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of *Saccharomyces cerevisiae*

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Inositol 1,4,5-trisphosphate (IP_3) induces a release of Ca^{2+} from vacuolar membrane vesicles of *Saccharomyces cerevisiae*. The amount released is dependent on IP_3 concentration (concentration for half maximal effect, $K_{m,\,apparent}$ = 0.4 μM). *Myo*-inositol, and inositol 1,4-bisphosphate up to 50 μM have no effect on Ca^{2+} levels in the vesicles. The IP_3-induced Ca^{2+} release is blocked by dantrolene and 8-(*N,N*-diethylamino)-octyl 3,4,5-trimethoxybenzoate-HCl (TMB-8), which are known to block Ca^{2+} release from Ca^{2+} stores in animal cells. IP_3-induced release of Ca^{2+} also occurs when Ca^{2+} is accumulated by means of an artificial pH gradient, indicating that the effect of IP_3 is not due to an effect on the vacuolar H^+-ATPase. The IP_3-induced Ca^{2+} release is not accompanied by a change in the pH gradient, which indicates that it is not due to a reversal of the Ca^{2+}/nH^+ antiport or to a decrease in ΔpH by IP_3. The present results suggest that IP_3 may act as a second messenger in the mobilization of Ca^{2+} in yeast cells As in plant cells, the vacuolar membrane of yeast seems to contain a Ca^{2+} channel, which can be opened by IP_3. In this respect the vacuole could function as an IP_3-regulated intracellular Ca^{2+} store, equivalent to the endoplasmic- and sarcoplasmic reticulum in animal cells, and play a role in Ca^{2+}-dependent signal transduction in yeast cells.

Inositol 1,4,5-trisphosphate-induced Ca^{2+} release; Vacuole; *Saccharomyces cerevisiae*

1. INTRODUCTION

The free cytosolic Ca^{2+} concentration is a major regulatory element in eukaryotic cells [1]. Functioning of Ca^{2+} in signal transduction pathways is dependent upon maintaining a low free cytosolic Ca^{2+} concentration, while at the same time permitting a rapid and transient increase in this concentration [2,3].

In yeast cells the role of Ca^{2+} in signal transduction has only begun to be elucidated. Cytosolic Ca^{2+} is found to play an essential role in the control of the cell cycle, in the budding process, and in the late reaction to the mating pheromone α [4–6]. Furthermore all the elements required for the functioning of a Ca^{2+} signal transduction pathway are present in yeast. Calmodulin, adenylate-cyclase, GTP-regulatory proteins and protein kinases in yeast are found to have similar functions as their mammalian counterparts [7–9]. Furthermore, like in mammalian cells, cytosolic free Ca^{2+} concentrations in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and the mycelial fungus *Neurospora crassa* are maintained at 0.1–0.3 μM [10–12].

Maintenance of a low cytosolic Ca^{2+} concentration in most eukaryotic cells is achieved by Ca^{2+} ATPases in addition to Na^+/Ca^{2+} exchangers [13–15] in the plasma membrane, and uptake into intracellular organelles like mitochondria and endoplasmic (ER) and sarcoplasmic reticulum (SR) in animal cells [2,3,16] and vacuoles in plant cells [17]. In fungi, however, Ca^{2+} extrusion by the cells has been ascribed to be mediated by a Ca^{2+}/nH^+ exchanger [18,19]. The recent identification of a yeast gene whose product shows strong homology to plasma membrane Ca^{2+}-ATPases of animal cells [20], indicates that Ca^{2+} extrusion by yeast cells is mediated by a Ca^{2+}-ATPase that exchanges Ca^{2+} for protons [12].

In yeast cells, like in plant cells, the vacuole is a major calcium store with a free calcium concentration of 1.3 mM in *S. cerevisiae* [10]. It has been suggested that in fungi vacuoles are involved in Ca^{2+} homeostasis [12,21]. Ohsumi and Anraku showed that Ca^{2+} transport into the vacuole is driven by a pH gradient which is generated by the vacuolar H^+ATPase [22]. It was found recently, however that disruption in *S. pombe* of *cta3*, a gene whose product shows similarity to the SR Ca^{2+}-ATPase in animal cells, affects Ca^{2+} homeostasis and transport, leading to the suggestion that other organelles besides the vacuole play a role in Ca^{2+} homeostasis as well [11]. As a matter of fact, recently non-vacuolar ATP-dependent intracellular Ca^{2+} transport mediated by a calcium pump encoded by the gene *cta3* has been described in the yeast *S. pombe* [23].

It is not known by which mechanisms the mobilization of intracellular Ca^{2+} from intracellular stores to the cytoplasm in yeast is achieved. In animal cells, inositol 1,4,5-trisphosphate (IP_3), which is formed by hydrolysis of the membrane lipid phosphatidylinositol 4,5-bisphosphate, can release Ca^{2+} from intracellular stores

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[16]. In these cells calcium is released mainly from the ER [16], but in plants also from the vacuole [24]. For fungi there is only one publication that reported on the ability of IP_3 to release Ca^{2+} from vacuoles in *N. crassa* [25]. Our present findings show that also in yeast IP_3 may cause mobilization of Ca^{2+} from intracellular stores, notably the vacuole.

2. MATERIALS AND METHODS

2.1. *Strain and culture conditions*

The diploid strain A294 (Whitbread & Co., UK) was used throughout. Cells were grown in YEPD medium (1% yeast extract, 2% bactopeptone and 2% glucose) in 250 ml batch cultures at 30°C on an orbital shaker at 120 r.p.m.

2.2. *Preparation of vacuolar membrane vesicles*

Preparation of vacuolar membrane vesicles was essentially according to Ohsumi and Anraku [26]. First vacuoles were isolated, which were almost free of any contamination as checked by phase contrast microscopy. The vacuoles were converted to right-side-out vesicles through mixing with a buffer of low osmotic strength, buffer A (10 mM MES-Tris pH 6.9, 5 mM $MgCl_2$ and 25 mM KCl). The vesicles were recovered by centrifugation and stored at −80°C. Purity of the prepared vesicles was checked by measuring the amount of ATPase activity which could be inhibited by vanadate (10 μM), which inhibits the plasma membrane H^+-ATPase, or azide (2 mM), which inhibits the mitochondrial ATPase [27].

2.3. *Generation and monitoring of pH gradient*

For an ATP-dependent pH gradient vesicles (5–10 μg protein/ml, final concentration) were diluted in buffer A containing 98 ng/ml of the pH-sensitive fluorescent dye 9-amino-6-chloro-2-methoxyacredine (ACMA) The reaction was started by the addition of 1.7 mM ATP The formation of the pH gradient was quantitatively assayed by quenching of ACMA fluorescence according to Okorokov et al. [28], in a quartz cuvet at 20°C, using an Aminco SPF 500 spectofluorimeter at excitation and emission wavelenghts of 410 and 482 nm, respectively. 60 nM valinomycin, 0.12 μM nigericin and 1.7 μg/ml CCCP (final concentrations) were added from stock solutions in ethanol, with a final concentration of less than 0.2% (v/v) ethanol.

A pH gradient could be generated artificially in the absence of ATP through the formation of a K^+ gradient, by diluting the vesicles containing 25 mM KCl in a K^+-free buffer, which was converted to a pH gradient using the K^+/H^+ ionophore nigericin [29]. The vesicles were preincubated during 30 s with 0.12 μM nigericin (final concentration). The formation of the artificially generated pH gradient was started by diluting vesicles (10 μg protein/ml in buffer A) 300-fold in buffer B (10 mM MES-Tris pH 6.9 and 40 mM choline chloride) containing 98 ng ACMA/ml

Divalent cations were added as dichloride salts from 1 or 0.1 M stock solutions.

2.4. *Ca^{2+} loading of the vesicles*

Ca^{2+} loading with an ATP-dependent pH gradient was performed in buffer A at 20°C. The vesicles (5–20 μg protein/ml) were preincubated with 2.2 mM ATP during 100 s, and the reaction was started by the addition of $^{45}CaCl_2$ (approximately 56 kBq/ml) together with 5 μM $CaCl_2$ (final concentration). Samples (100 μl) were diluted in 5 ml ice-cold stopping buffer (10 mM MES-Tris, pH 6.9, 40 mM choline-chloride and 250 μM $LaCl_3$) and the mix was filtered through Millipore filters (0.45 μm pore size). Radioactivity was determined by liquid scintillation counting.

For Ca^{2+} loading with an artificial pH gradient, vesicles (10–30 μg protein/ml) were diluted 100-fold in buffer B containing 0.3 μM nigericin and preincubated for 100 s. Further the assay was performed as above.

2.5. *Chemicals*

$^{45}CaCl_2$ was obtained from Amersham International (Amersham, UK). Yeast extract and Bactopeptone were from Difco (UK). Inositol 1,4,5-trisphosphate, inositol 1,4-bisphosphate, *myo*-inositol and Na_2ATP (vanadate-free) were from Sigma (USA). Zymolyase was from Seikagaku Kogyo Co (Japan). ACMA was a gift from Prof. Dr. R Kraayenhof at the Vrije Universiteit Amsterdam. All other chemicals were of analytical grade and obtained from commercial sources.

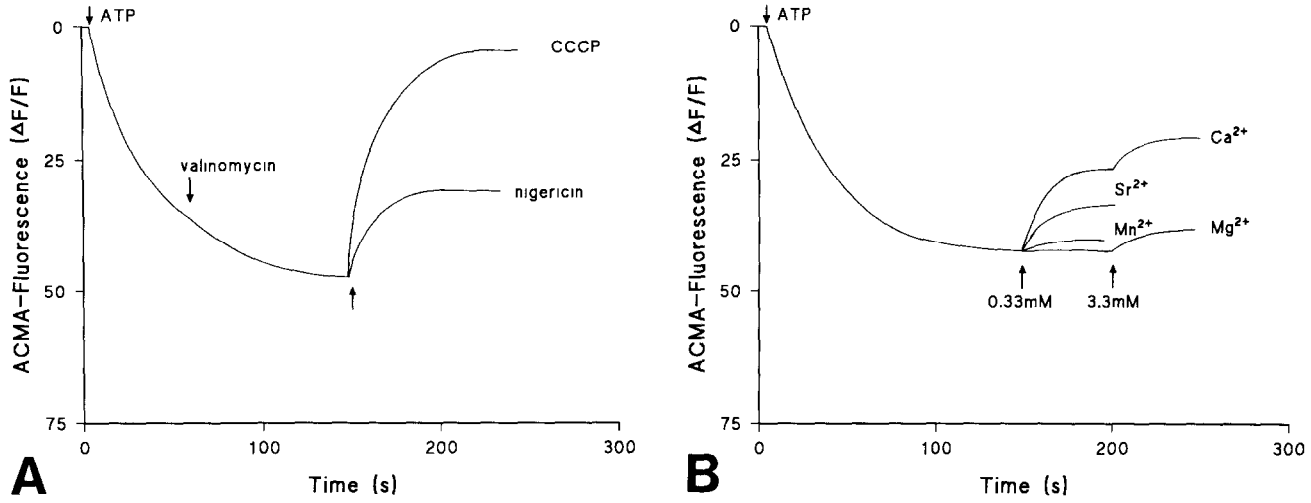

Fig. 1. Proton uptake driven by ATP and dissipation of the resulting pH gradient by nigericin, CCCP and divalent cations Formation of a ΔpH (acid inside) was followed by the quenching of ACMA fluoresence as described in section 2. Incubations were started by the addition of 1 7 mM ATP to the vesicles in buffer A also containing ACMA. (A) 60 nM valinomycin followed by either, 0.12 μM nigericin or 1.7 μg/ml CCCP were added at the times indicated by the arrows. (B) Dissipation of the ΔpH by addition at the time indicated by the first arrow of 0.33 mM $MgCl_2$, $MnCl_2$, $SrCl_2$ or $CaCl_2$, followed in the case of $MgCl_2$ and $CaCl_2$ by a further dissipation through the addition at the time indicated by the second arrow of 3.3 mM $MgCl_2$ or $CaCl_2$ The tracings are representative of three experiments done in duplicate.

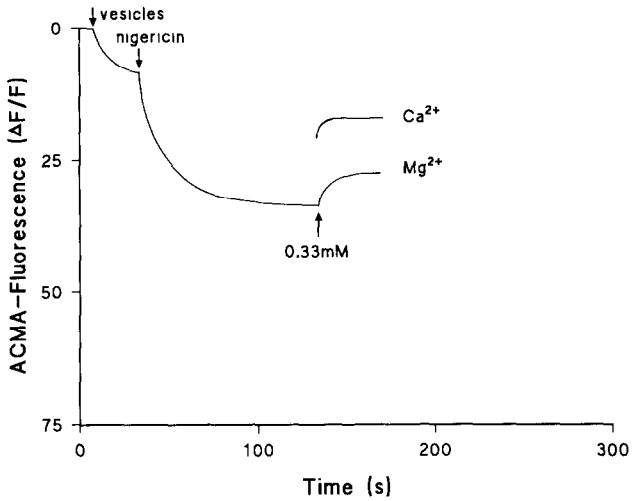

Fig. 2. Proton uptake driven by K^+ gradient and nigericin and dissipation of the resulting pH gradient by Ca^{2+} and Mg^{2+}. Formation of ΔpH (acid inside) was followed by measurement of the quenching of ACMA fluoresence as described in section 2. Incubation was started by diluting the vesicles 300-fold in buffer B also containing ACMA. Nigericin and $CaCl_2$ or $MgCl_2$ were added at the times indicated by the arrows. See also the legend to Fig. 1.

3. RESULTS

3.1. *ATP-dependent ΔpH formation*

As the vacuolar H^+-ATPase is not inhibited by vanadate or azide [27], these inhibitors of plasma membrane and mitochondrial ATPase activities, respectively [30], were used to determine the purity of our vacuolar membrane preparation. Inhibition of the ATPase activity of the vacuolar vesicles by vanadate and azide combined was less than 8%, showing that contamination by plasma membranes or mitochondria was low.

In the presence of ATP and Mg^{2+} a stable ΔpH (interior acid) was formed as measured by the quenching of ACMA fluorescence. Valinomycin gave a small increase in the ΔpH formed, while protonophore CCCP totally and H^+/K^+ exchanger nigericine partly dissipated the ΔpH (Fig. 1A). The ΔpH was partly dissipated by Ca^{2+} (depending upon the Ca^{2+} concentration added) until a new stable ΔpH plateau was reached. Sr^{2+} had a similar effect upon ΔpH though was less effective than Ca^{2+}. Mn^{2+} was still less effective in dissipating the pH gradient, while Mg^{2+} only slightly decreased ΔpH (Fig. 1B). Cd^{2+} also dissipated the ΔpH effectively, but after the initial fast decrease the pH gradient kept slowly dissipating (results not shown).

3.2. *Artificially generated ΔpH formation*

We also used an artificially generated pH gradient (interior acid) by loading vesicles with KCl, diluting in buffer without K^+ and Mg^{2+} and using nigericin to convert the K^+ gradient to a proton gradient. Already the addition of the vesicles to the buffer caused a small quenching of the ACMA fluorescence, indicating that a small pH gradient (interior acid) was formed. This quenching was reduced by addition of Mg^{2+} and was not found if the vesicles were added to buffer without K^+ but with 5 mM Mg^{2+}. Possibly the small ΔpH was formed as a consequence of diluting the Mg^{2+} containing vesicle suspension (5 mM $MgCl_2$) in Mg^{2+}-free buffer. After addition of nigericin a further ΔpH formation took place. The ΔpH formed was smaller than with ATP and also less stable (it was stable for about 10–20 min, whereas the ATP-generated pH gradient was stable for over an hour). Ca^{2+} partly dissipated the ΔpH as with the ATP-dependent ΔpH, but the plateau was reached very fast (Fig. 2). Mg^{2+} also dissipated the ΔpH though less effective and not as fast as Ca^{2+}.

3.3. *IP_3 stimulated Ca^{2+} release in Mg-ATP energized conditions*

Accumulation of $^{45}Ca^{2+}$ by an ATP-dependent pH gradient is shown in Fig. 3. It proceeded only in the presence of Mg^{2+} and ATP. The $^{45}Ca^{2+}$ accumulated was released completely after addition of 0.01% (w/v) Triton X-100 (not shown) or 0.5 μg/ml A23187, a Ca^{2+}-ionophore (Fig. 3), showing that the Ca^{2+} which was accumulated was not bound irreversibly inside the vesicles.

Addition of IP_3 to vesicles which had accumulated Ca^{2+} driven by the ATP-dependent ΔpH, resulted in a transient loss of up to 40% of the Ca^{2+} accumulated in the vesicles (Fig. 3). The maximum amount of Ca^{2+} release was reached within 45–60 s after addition of IP_3, thereafter part of the Ca^{2+} lost was taken up again. After about 200 s the Ca^{2+} concentration was recovered again partly. A second addition of IP_3 at this point

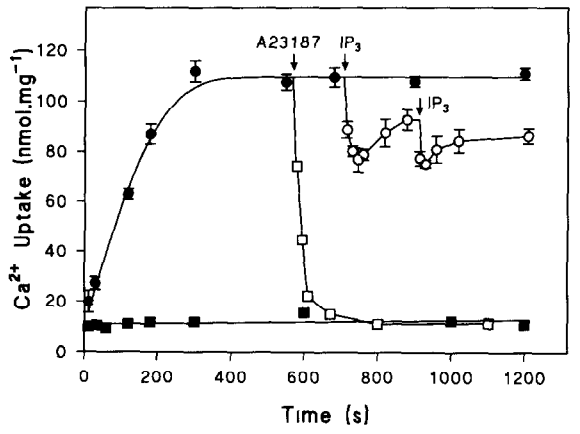

Fig. 3. Time course of Ca^{2+} uptake driven by an ATP-generated pH gradient and effect of IP_3 upon Ca^{2+} transport in tonoplast vesicles. Ca^{2+} uptake was assayed in the presence of 2.2 mM ATP and 5 μM Ca^{2+} in buffer A. Vesicles were preincubated 100 s with ATP before addition of Ca^{2+} (●). Control without ATP added (■). At the times indicated by the arrows IP_3 (○, 1 μM) or A23187 (□, 0.5 $\mu g \cdot ml^{-1}$) were added. The assay was performed as described in section 2. Means ± S.E.M. of triplicates are shown. Error bars were not drawn when smaller than the symbols.

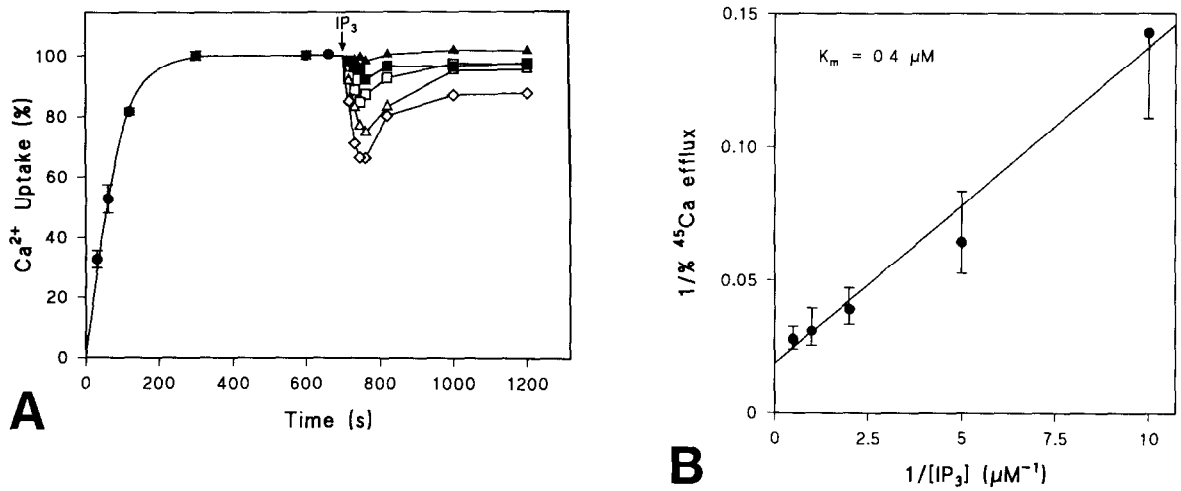

Fig. 4 Effect of IP_3, TMB-8 and dantrolene on Ca^{2+} release from tonoplast vesicles. (A) Ca^{2+} (5 μM) was accumulated into the vesicles driven by an ATP dependent ΔpH (●), and after equilibrium is reached IP_3 is added at the time indicated by the arrow. Final IP_3 concentration added was 0.1 μM (□), 0.5 μM (▲) or 2 μM (◇). 200 μM TMB8 or 20 μM dantrolene were added 100 s before the addition of 2 μM IP_3, (■) and (▲), respectively. The assay was performed as described in section 2. (B) The maximum amount of Ca^{2+} released (at 45–60 s) by different IP_3 concentrations is plotted in a Lineweaver–Burk plot. Means ± S.E.M. of 5 experiments are shown. The concentration of IP_3 needed for half maximal release (0.4 μM) is indicated by the (apparent) K_m.

again released Ca^{2+} followed by re-uptake of the Ca^{2+} (Fig. 3). The maximum amount of Ca^{2+} released was dependend upon the concentration of IP_3 used (Fig. 4A). Plotting the maximum amount of Ca^{2+} released against the IP_3 concentration in a Lineweaver–Burk plot gave a concentration for half-maximum effect of 0.4 μM IP_3 (Fig. 4B).

Specificity of the IP_3 induced Ca^{2+} release was tested with two other inositides; *myo*-inositol and inositol 1,4-bisphosphate. Both inositides released less than 5% of the Ca^{2+} accumulated up to a concentration of 50 μM, whereas 2 μM IP_3 was enough for near-maximum Ca^{2+} release (not shown). Also the IP_3 induced Ca^{2+} release could be inhibited for 90% with 20 μM dantrolene (Fig. 4A) which is known to inhibit IP_3 induced Ca^{2+} release from SR in animal cells [31,32]. Another inhibitor of IP_3 induced Ca^{2+} release is TMB-8 [33,34]. 200 μM TMB-8 inhibited Ca^{2+} release from these vesicles for more than 90% (Fig. 4A). TMB-8 nor dantrolene had an effect upon the equilibrium level of Ca^{2+} accumulation before addition of IP_3 (not shown).

3.4. *IP_3 stimulated Ca^{2+} release in artificially energized conditions*

The level of Ca^{2+} accumulation found with the artificial pH gradient was consistently lower than with the ATP-dependent pH gradient, being about 40 nmol·mg^{-1} and about 110 nmol·mg^{-1}, respectively (see Figs. 3 and 5). This may reflect the difference in the magnitude of the ΔpH formed, the artificially formed gradient being the smaller (see Figs. 1 and 2). The accumulation of $^{45}Ca^{2+}$ into the vacuolar vesicles driven by an artificial pH gradient is shown in Fig. 5. It only proceeded when nigericin was added. Again A23187 released all the Ca^{2+} which was accumulated in the vesicles (Fig. 5). Here IP_3 caused release of about 80% of the Ca^{2+} accumulated, and there was no re-uptake. The small amount of Ca^{2+} still present in the vesicles could be released by A23187.

Addition of IP_3 to Ca^{2+} loaded vesicles driven by both ATP-generated or K^+/nigericin generated pH gradients did not affect these pH gradients as deduced from ACMA fluorescence during the time measured (20–30 s) (not shown).

4. DISCUSSION

Ohsumi and Anraku provided indirect evidence that Ca^{2+} can be accumulated into vacuoles by means of the proton gradient generated by the vacuolar H^+-ATPase [22]. Okorokov et al. [35] showed that vacuolar Ca^{2+} uptake proceeds through an electroneutral $Ca^{2+}/2H^+$ antiport. Our work is in accordance with the notion that the pH gradient is the driving force for Ca^{2+} uptake. That ATP is only necessary for the generation of the proton gradient and not directly involved in Ca^{2+} transport is shown in a more direct way, through the use of an artificially generated pH gradient in the absence of ATP. The pH gradient generated through the use of a K^+ gradient and nigericin is sufficient to drive Ca^{2+} uptake into the vacuolar vesicles.

The dissipation of the pH gradient after the addition of Ca^{2+} is also found after the addition of Cd^{2+}, Sr^{2+}, Mn^{2+} and Mg^{2+}. Addition of Mg^{2+} has only a small effect upon the MgATP-generated ΔpH but a much larger effect upon the artificially generated ΔpH (compare Fig. 1B and Fig. 2). Furthermore, Ca^{2+} dissipates

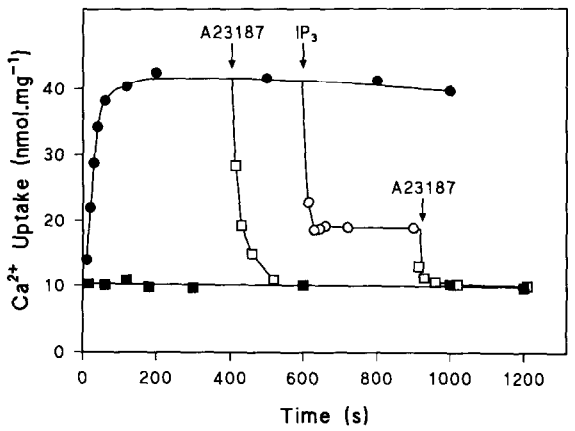

Fig. 5. Time course of Ca^{2+} uptake driven by an artificial pH gradient and effect of IP_3 upon Ca^{2+} transport in tonoplast vesicles. Ca^{2+} uptake was assayed in buffer B in the presence of 0.3 μM nigericin and 5 μM Ca^{2+}. Vesicles were preincubated for 100 s with nigericin before addition of Ca^{2+} (●). Control without nigericin added (■). At the times indicated by the arrows IP_3 (○, 1 μM) or A23187 (□, 0.5 $\mu g \cdot ml^{-1}$) were added. The assay was performed as described in section 2. The data are an average of 3 experiments.

the pH gradient much faster in case of the artificial ΔpH. These differences are probably due to the presence of 3.3 mM free Mg^{2+} in the buffer in the case of the ATP-dependent ΔpH, whereas no extra Mg^{2+} is added to the buffer applied in case of the artificial ΔpH. The small pH gradient formed after the addition of the vesicles to the buffer in case of the artificial ΔpH (see Fig. 2) can be explained by the movement of Mg^{2+} from the vesicles (obtained from a 5 mM Mg^{2+} containing buffer) to the Mg^{2+}-free buffer by means of the antiporter. Our results agree with previous findings of Okorokov et al. [35] that other divalent cations, including the physiologically important Mg^{2+}, are also transported by the antiporter. Ohsumi and Anraku also used excess $MgCl_2$ (about 3.7 mM free [22], which may explain why they did not find a decrease in the pH gradient on adding extra Mg^{2+}.

The values reported for yeast for the K_m of the exchanger are in the order of 60 to 100 μM [22,35]. These values are well above the value of 0.1–0.3 μM free calcium in the cytosol [10–12]. This led to the suggestion that the vacuolar $Ca^{2+}/2H^+$ exchanger functions as a low-affinity high capacity transporter under conditions of high extracellular Ca^{2+} concentrations or in Ca^{2+}-detoxification after membrane damage [23,36]. However, in the determination of the affinity of the antiport for Ca^{2+}, the fact that Mg^{2+} also seems to have affinity for the antiporter, and thus may act as a competitive cation, was not taken into account. Depending on how strong Mg^{2+} competes with Ca^{2+} for the transport-site and on the free Mg^{2+} concentration in the cytosol, the K_m of the antiport for Ca^{2+} in vivo may be much lower than the reported values. If this is the case, the vacuole could still play a physiological role in maintaining a low cytosolic Ca^{2+} concentration. Present investigations are aimed at this problem.

The recent identification of intracellular Ca^{2+}-ATPases in yeast [23], led to the suggestion that these Ca^{2+}-ATPases are the high affinity Ca^{2+}-transporters as they are in animal cells, and that the ER in yeast supplies the Ca^{2+} needed for the regulation of cell cycle processes [23]. But as argued above the vacuole could still play a role in high affinity Ca^{2+} uptake, possibly in parallel to non-vacuolar compartments in maintaining a low cytosolic Ca^{2+} concentration.

In this publication evidence for a possible role of the vacuole as an inositol 1,4,5-trisphosphate (IP_3) sensitive Ca^{2+} pool is presented. In the presence of ATP, IP_3 causes a transient release of Ca^{2+} from vacuolar vesicles in yeast. The effect of IP_3 upon the maximum amount of Ca^{2+} released is concentration dependent with a K_m of 0.4 μM. This value is close to the values of 0.2–0.6 μM found for plant vacuoles [24,37,38]. The only other report of an effect of IP_3 upon Ca^{2+} release in fungi describes an increase in Ca^{2+} efflux from whole vacuoles of *N. crassa* with a K_m for IP_3 of 5.28 μM [25], more than 10 times higher than the value reported here. This discrepancy could be due to a difference in membrane properties or to the different experimental approach.

A possible effect of IP_3 upon the vacuolar H^+-ATPase leading to a transient Ca^{2+} release is not very probable because IP_3 also induces a Ca^{2+} release from vesicles where Ca^{2+} accumulation is driven by an artificially generated ΔpH. Involvement of the $Ca^{2+}/2H^+$ antiport in the process of IP_3 induced Ca^{2+} release is improbable, because the Ca^{2+} release is not accompanied by a ΔpH formation, neither in the vesicles with an ATP-dependent ΔpH, nor in the vesicles with an artificially generated ΔpH. Thus IP_3 induced Ca^{2+} release from yeast vacuoles probably takes place through a separate IP_3 regulated Ca^{2+}-channel.

From the other inositolphosphates tested none caused a significant Ca^{2+} release, which is in agreement with the specificity of the IP_3-regulated Ca^{2+}-channel in animal microsomes and plant vacuoles [16,24,37,38]. The IP_3 receptor of the putative Ca^{2+} channel also shows other physiological similarities with the receptors from animal- and plant-cells in the effects of TMB8 and dantrolene, which both inhibit the IP_3-induced Ca^{2+} release. TMB8 and dantrolene are known to inhibit the IP_3-activated Ca^{2+} release from animal microsomes [31–34], and TMB8 also blocks Ca^{2+} release from plant cell vacuoles [24,37,38]. Dantrolene also inhibits the IP_3 induced Ca^{2+} leakage from vacuoles in *N. crassa* [25].

Although several processes in yeast are found to be regulated by the cytosolic Ca^{2+} concentration, it is not yet clear what the role of internal Ca^{2+} stores in the regulation of this concentration is. In animal cell signaling systems, IP_3 formed after hydrolysis of membrane phosphoinositide lipids mobilizes Ca^{2+} from intracellular stores [16]. In yeast cells the role of hydrolysis and

metabolism of phosphoinositide lipids in signal transduction is not yet clear. Indications for such a role are the enhancement of polyphospho-inositide synthesis by cAMP, which may lead to enhanced production of IP_3 [39], and the fact that the hydrolysis of phosphatidylinositol 4,5-bisphosphate is a requisite process for yeast cell growth [40]. Our results indicate that the vacuolar membrane contains a Ca^{2+} channel, which can be opened by IP_3. This suggests a possible role for IP_3 as a second messenger in mobilizing Ca^{2+} from the vacuole in yeast cells.

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