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Regulation of cellular Mg^{2+} by *Saccharomyces cerevisiae*

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Abstract

Regulation of cellular Mg^{2+} by *S. cerevisiae* was investigated. The minimal concentration of Mg^{2+} that results in optimal growth of *S. cerevisiae* is about 30 μM and a half-maximum growth rate is attained at about 5 μM Mg^{2+}. Since the plasma membrane has an electrical potential greater than 100 mV, passive equilibration of Mg^{2+} across the plasma membrane would provide sufficient cytosolic Mg^{2+} (0.1–1 mM). The total cellular Mg^{2+} of cells grown in synthetic medium containing 1 mM Mg^{2+} is about 400 nmol/mg protein, most of which is bound to polyphosphate, nucleic acids, and ATP. Total cellular Mg^{2+} decreases to about 80 nmol/mg protein as the Mg^{2+} in synthetic growth medium is reduced to 0.02 mM, but remains relatively constant in growth medium containing 1 to 100 mM Mg^{2+}. Cells shifted into Mg^{2+}-free medium continue to grow by utilizing the vacuolar Mg^{2+} stores. Mg^{2+}-starved cells replenish vacuolar Mg^{2+} stores with a halftime of 30 min. following the addition of 1 mM Mg^{2+} to the growth medium. The data indicate that cytosolic Mg^{2+} is maintained by the regulation of Mg^{2+} fluxes across both the vacuolar and plasma membranes.

Keywords: Magnesium; Calcium; Polyphosphate; Vacuole; Dichlorophosphonazo III; (*S. cerevisiae*)

1. Introduction

Magnesium is an abundant physiological divalent cation of widespread importance in metabolic processes. Eukaryotic cells maintain the cytosolic Mg^{2+} concentration in the range of 0.1 to 1 mM by mechanisms yet to be identified [1]. The genetically tractable model eukaryote *S. cerevisiae* may be useful in identifying transport and regulatory proteins required for Mg^{2+} homeostasis. As a first step in establishing a screen to identify and characterize mutants with defects in Mg^{2+} homeostasis, an assay for cellular Mg^{2+} levels [2] was adapted to *S. cerevisiae* and the relationship between the Mg^{2+} concentration in the growth medium and the cellular Mg^{2+} content was investigated.

2. Materials and methods

2.1. Media and strains

CuH3 (*Mata* ura3-52 his4-619) and DBY 947 (*Matα ura3-52 ade2-101) S. cerevisiae* strains in the S288C background were used. Cells were grown in synthetic Wickerham's minimal media (SD) [3]; where indicated, Mg^{2+}, Ca^{2+} or phosphate was omitted or added to the stated concentration. For SD plates, electrophoresis grade agarose (1.0%) was substituted for Noble agar.

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2.2. Measurement of Mg^{2+}

Mg^{2+} was measured spectrophotometrically using dichlorophosphonazo III [2]. Cells were grown in a modified SD media without Ca^{2+}, harvested, washed by centrifugation at 4°C with 0.1 M KCl, 10 mM Pipes (pH 7.0), 1 mM EDTA to remove extracellular divalent cations and then with 0.1 M KCl, 10 mM Pipes (pH 7.0) to remove EDTA. All solutions used in the assay were passed through Chelex 100 columns to remove divalent cations. Cells were suspended to an A_{600} of 0.5 in 0.1 M KCl, 10 mM Pipes (pH 7.0), 10 μM TPEN (tetrakis(2-pyridylmethyl)ethylenediamine), and 20 μM dichlorophosphonazo III. TPEN was added to chelate Zn^{2+}, Cu^{2+} or Fe^{3+}[4]. The difference absorbance of the dichlorophosphonazo III-Mg^{2+} complex was measured at 672 nm using 600 nm as a reference wavelength using a SLM-Aminco DW2c dual wavelength spectrophotometer. After obtaining a baseline, digitonin (0.1 mg/ml) was added to permeabilize cellular membranes, and the released Mg^{2+} was determined by measuring the change in the difference spectrum. EDTA and Mg^{2+} were used to calibrate the response of the dye to Mg^{2+}. The change in the absorbance spectrum following digitonin addition was the same as that obtained by addition of Mg^{2+}, and different from that obtained by addition of Zn^{2+}, Cu^{2+} or Fe^{3+}. In parallel experiments using Arsenazo III to measure Ca^{2+} as previously described [5], there was no release of Ca^{2+} from the cell upon digitonin addition.

2.3. Measurement of Ca^{2+} accumulation by permeabilized cells

Vacuolar Ca^{2+} accumulation was measured using osmotically shocked partially regenerated spheroplasts as previously described [5]. Partially regenerated spheroplasts were resuspended in 0.1 M KCl, 10 mM Hepes/Pipes/Mes (pH 7.0), 0.6 M sorbitol to a protein concentration of 1.5 mg/ml. The cells were diluted 50-fold into 0.1 M KCl, 10 mM Hepes/Pipes/Mes (pH 7.0), 50 μM $CaSO_4$, 50 μM Arsenazo III, 1 mM Na ATP and the indicated Mg^{2+} concentration at 27°C. This osmotic shock resulted in permeabilization of the cell plasma membrane. Ca^{2+} uptake was monitored spectrophotometrically by measuring the difference absorbance of the Ca^{2+}-Arsenazo III complex at 660 nm using 685 as reference wavelength. The baseline absorbance was established by addition of 5 μM A23187, a Ca^{2+} ionophore that releases sequestered Ca^{2+}. The response of Arsenazo III to Ca^{2+} was calibrated by multiple additions of 2.5 μM Ca^{2+}.

2.4. Measurement of Ca^{2+} accumulation by S. cerevisiae cells

Ca^{2+} accumulation by whole cells was measured as previously described [5]. Cells were grown to an A_{600} of 1.0 in YPD medium and diluted into YPD medium + 50 mM $CaCl_2$ with varying concentrations of Mg^{2+}. After a 2-h incubation at 26°C, cells were washed by centrifugation (4°C) and resuspended to a density of 10^7 cells/ml in 0.1 M KCl, 10 mM Hepes/Pipes/Mes (pH 7.0), 50 μM $CaSO_4$, and 50 μM Arsenazo III. Ca^{2+} release from the cell was initiated by adding 1 mg/ml of the detergent digitonin which permeabilizes cell membranes. Ca^{2+} was measured spectrophotometrically by monitoring the difference absorbance of the Arsenazo III-Ca^{2+} complex at 660–685 nm.

3. Results

3.1. Mg^{2+}-requirement for growth

The effect of the Mg^{2+} concentration on the growth rate of *S. cerevisiae* cells was measured in Ca^{2+}-free synthetic dextrose (SD) media (Fig. 1A). The half-maximum growth rate is observed at about 5 μM Mg^{2+}. At 30 μM Mg^{2+}, the growth rate is about 90% that observed at 2000 μM. The cytoplasmic free Mg^{2+} concentration in eukaryotic cells is expected to be 0.1–1 mM [1]; therefore a 20–200-fold Mg^{2+} gradient across the plasma membrane is formed when the external Mg^{2+} is 5 μM. Proton transport by the plasma membrane H^+-ATPase is likely to polarize the membrane to greater than −100 mV [6] which has the potential to form a 10 000-fold Mg^{2+} gradient ($[Mg^{2+}]_{in}/[Mg^{2+}]_{out} = e^{-100\ mV\ nF/RT}$). Passive equilibration of Mg^{2+} across the polarized plasma membrane would be sufficient to provide the necessary cytosolic Mg^{2+} concentration.

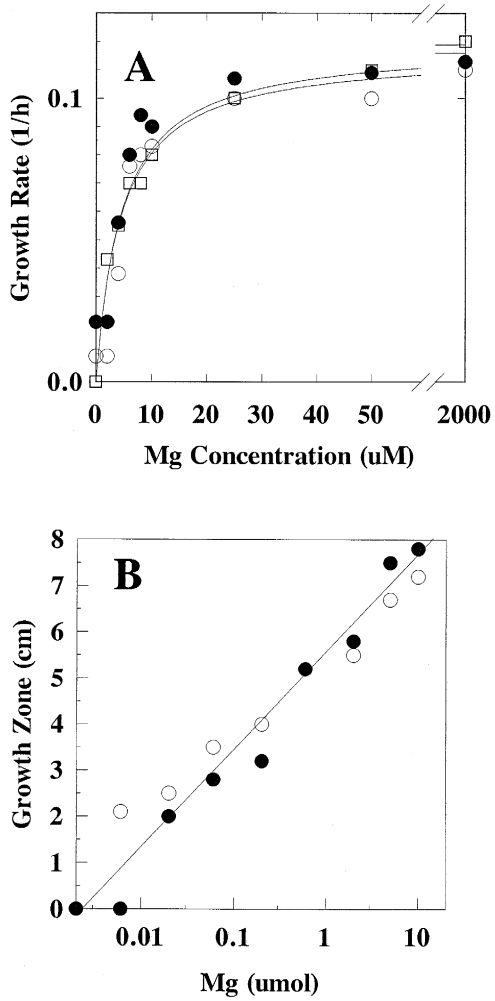

Fig. 1. Effect of the Mg^{2+} concentration on the growth rate of *S. cerevisiae* cells. (A) The effect of Mg^{2+} concentration on the growth rates of CuH3 (●) and 974 (○, □) cells was determined by monitoring the increase in the A_{600} over a 10-h period starting with an A_{600} of 0.1. A modified SD media was used; the $MgSO_4$ concentration was varied as indicated and the Ca^{2+} concentration was 15 μM (○, ●) or 20 mM (□). Cells were incubated in the media 16 h prior to measuring the growth rate. The growth rate was calculated from the slope of the line formed when the ln A_{600} was plotted against time. (B) The effect of Mg^{2+} on the growth of cells on SD agarose plates was determined. Cells were grown in YPD and washed with Mg^{2+}-free SD media. The cells were diluted to a concentration of 10^7 cells/ml and 0.3 ml were spread onto Mg^{2+}-free SD agarose plates containing 15 μM or 20 mM Ca^{2+}. Paper discs containing 20 μl of a solution containing the indicated amount of Mg^{2+} were placed on the plates. The plates were incubated for 3 days at 26°C. The diameter of the zone of growth surrounding the disc was measured (cm).

Addition of 20 mM Ca^{2+}, the second most abundant cellular divalent cation, does not significantly alter the dependency of growth on the Mg^{2+} concentration in liquid medium (Fig. 1A) or on agarose plates (Fig. 1B) indicating that the Mg^{2+} transport systems that mediate cellular Mg^{2+} accumulation are not significantly inhibited by Ca^{2+}.

3.2. Influence of extracellular [Mg^{2+}] on intracellular [Mg^{2+}]

The relationship between the growth medium Mg^{2+} concentration and the cellular Mg^{2+} content was determined (Fig. 2). Cells were grown in Ca^{2+}-free SD media containing varying amounts of Mg^{2+}, washed by centrifugation to remove extracellular Mg^{2+}, and placed in Chelex 100-treated KCl solution containing the Mg^{2+}-indicator dye, dichlorophosphonazo III. Mg^{2+} was released from the cells by permeabilization of the cellular membranes with the detergent digitonin and measured spectrophotometrically by monitoring the difference absorbance of the dichlorophosphonazo III-Mg^{2+} complex (Fig. 2A). Since Ca^{2+} also binds to dichlorophosphonazo III [2], Ca^{2+} was omitted from the growth medium. *S. cerevisiae* show normal growth in medium with only trace amounts of Ca^{2+}. Under these growth conditions, Ca^{2+} is not released from the cells upon permeabilization with digitonin [5]. The chelator tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) was included in the assay solution to prevent heavy metals from interfering with the Mg^{2+} determination [4]. Cells grown in media containing 0.3 to 100 mM Mg^{2+} accumulate about 400 nmol/mg Mg^{2+} (Fig. 2B). This value is similar to that reported by others using atomic absorption to determine cellular Mg^{2+} [7–9]. As the growth medium Mg^{2+} concentration is reduced from 0.3 mM to 0.03 mM, the cellular Mg^{2+} content decreases from about 400 nmol/mg protein to 90 nmol/mg although the growth rate does not change.

3.3. The role of the vacuole in Mg^{2+} storage

Yeast vacuoles are believed to be a major storage site for divalent cations in the cell [11]. The capacity of the vacuole to accumulate divalent cations is greatly increased by its high content of the polyanion

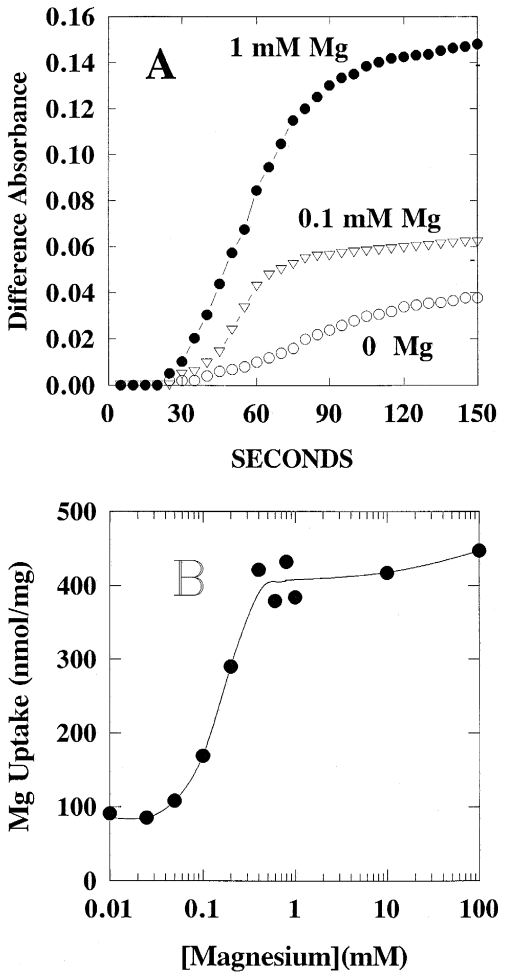

Fig. 2. Effect of the Mg^{2+} concentration on the level of cellular Mg^{2+}. (A) Kinetics of digitonin-induced release of cellular Mg^{2+}. Cells were grown in SD media and then transferred to Ca^{2+}-free SD medium containing the indicated Mg^{2+} concentration and incubated for 16 h at 26°C to give a final A_{600} of 0.2–1.0. The cells were washed by centrifugation three times with 0.1 M KCl, 10 mM Pipes (pH 7.0) and resuspended to A_{600} of 0.5 in KCl buffer containing 20 μM dichlorophosphonazo III and 10 μM TPEN. At t_o, 0.1 mg/ml digitonin was added to permeabilize the organellar and plasma membranes and the difference absorbance of the dichlorophosphonazo III-Mg^{2+} complex (672 nm/600 nm) was monitored to measure the release of cellular Mg^{2+}. (B) Cells were grown for 16 h to an A_{600} of 0.1 to 1.0 in Ca^{2+}-free SD media containing varying amounts of Mg^{2+}. The cells were then washed with cold KCl buffer, and resuspended in KCl buffer containing 20 μM dichlorophosphonazo III and 20 μM TPEN. Digitonin (0.1 mg/ml) was added to permeabilize the plasma and organellar membranes to release cellular Mg^{2+}. The difference absorbance of the Mg^{2+}-dichlorophosphonazo III complex (672 nm/600 nm) was measured.

polyphosphate (about 1 μmol phosphate residue/mg protein) [5]. Cellular Mg^{2+} can be reduced by 75% without affecting the growth rate suggesting that 75% of the cellular Mg^{2+} is storage Mg^{2+}. The vacuole is the likely site for Mg^{2+} storage. Mg^{2+} binding to synthetic polyphosphate was measured spectrophotometrically (Fig. 3A). Sodium polyphosphate (80 μg/ml, average chain length of 31 residues from Sigma) was titrated with Mg^{2+} in 0.1 M KCl, 10 mM Pipes buffer (pH 6.5), and 20 μM dichlorophosphonazo III. Polyphosphate binds 0.12 mol Mg^{2+}/mol polyphosphate residue with relatively high affinity (K_d about 4 μM) (Fig. 3B) and more than 0.2 mol Mg^{2+}/mol polyphosphate with lesser affinity. Since the polyphosphate content of vacuoles is about 0.1 mg polyphosphate/mg protein [5], 120 nmol Mg^{2+}/mg protein could be complexed with polyphosphate in the vacuole with relatively high affinity while more than 200 nmol Mg^{2+}/mg could be complexed at lower affinity. The free Mg^{2+} concentration in the vacuole is not known; however, the vacuolar membrane potential, generated by electrogenic H^+ transport by the vacuolar H^+-ATPase, is estimated to be 75 mV (inside-positive) [12]. Therefore, if Mg^{2+} is passively equilibrated across the vacuole membrane and the cytosolic Mg^{2+} concentration is 1 mM, the vacuolar Mg^{2+} concentration would be only 3 μM. At this free Mg^{2+}, about 50 nmol Mg^{2+}/mg cell protein would be bound. However if Mg^{2+} is transported into the vacuole in exchange for one proton, the lumenal Mg^{2+} concentration would be the same as the cytosolic Mg^{2+} concentration and the polyphosphate Mg^{2+} binding sites would be nearly saturated (300–400 nmol Mg^{2+}/mg protein).

3.4. The rate of Mg^{2+}-depletion of cells in Mg^{2+} free solution

When exponentially growing cells are shifted from Mg^{2+}-containing to Mg^{2+}-free medium, they continue to grow for about 9 h (Fig. 4A) and then cells begin to die. During this time the Mg^{2+} content of the cells decreases 4.8-fold from 390 nmol/mg to 80 nmol/mg (Fig. 4B) while the number of viable cells increases 3.5 fold indicating that stored Mg^{2+} can be utilized to support cell growth. However, growth ceases once cellular Mg^{2+} is lowered to about 80

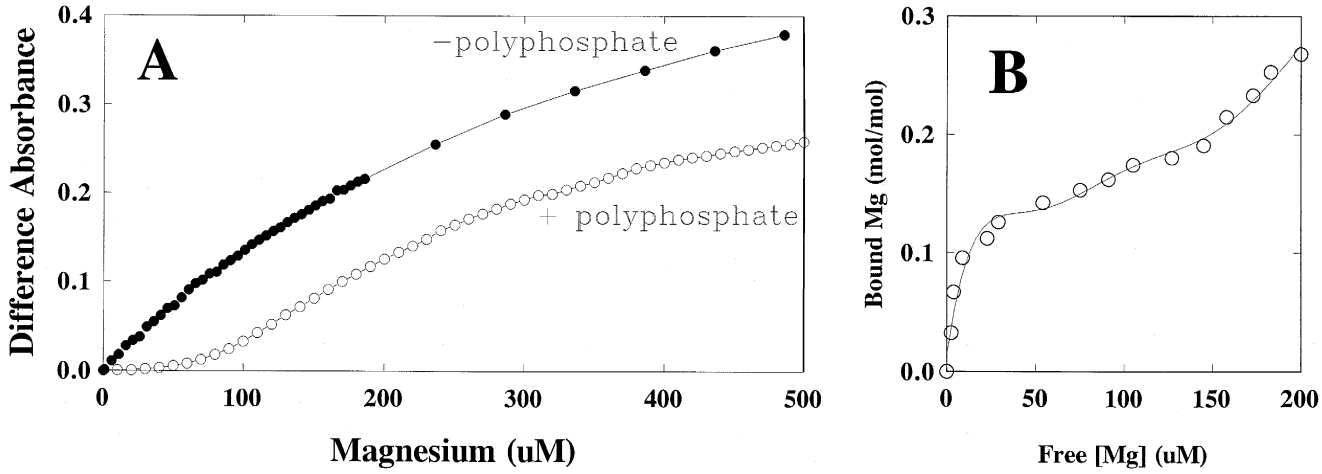

Fig. 3. Mg^{2+} binding to synthetic polyphosphate. (A) The Mg^{2+}-indicator dichlorophosphonazo III was titrated with Mg^{2+} in the presence (○) and absence (●) of 80 μg/ml polyphosphate. The assay solution contained 0.1 M KCl, 10 mM Mes (pH 6.5) and 20 μM dichlorophosphonazo III. The difference absorbance of the Mg^{2+}-dichlorophosphonazo III complex (672 nm/600 nm) was measured and plotted against the total Mg^{2+} concentration. (B) The polyphosphate-bound Mg^{2+} (Total Mg^{2+} minus dichlorophosphonazo III-bound and free Mg^{2+}) was plotted against free Mg^{2+}.

nmol Mg^{2+}/mg protein and between 9 and 50 h in Mg^{2+}-free YPD, the number of viable cells decreases 3.5-fold.

Cells grown in 20 μM Mg^{2+} have only 90 nmol Mg^{2+}/mg and stop growth immediately upon transfer to Mg^{2+}-free media (Fig. 5) indicating that the extra 300 nmol Mg^{2+}/mg protein in cells grown in 1 mM Mg^{2+} is utilized to permit continuous growth in low Mg^{2+} medium. Cells grown in media containing only 50 μM phosphate do not accumulate polyphosphate and also stop growth immediately upon transfer to Mg^{2+}-free, high-phosphate medium. Cells grown

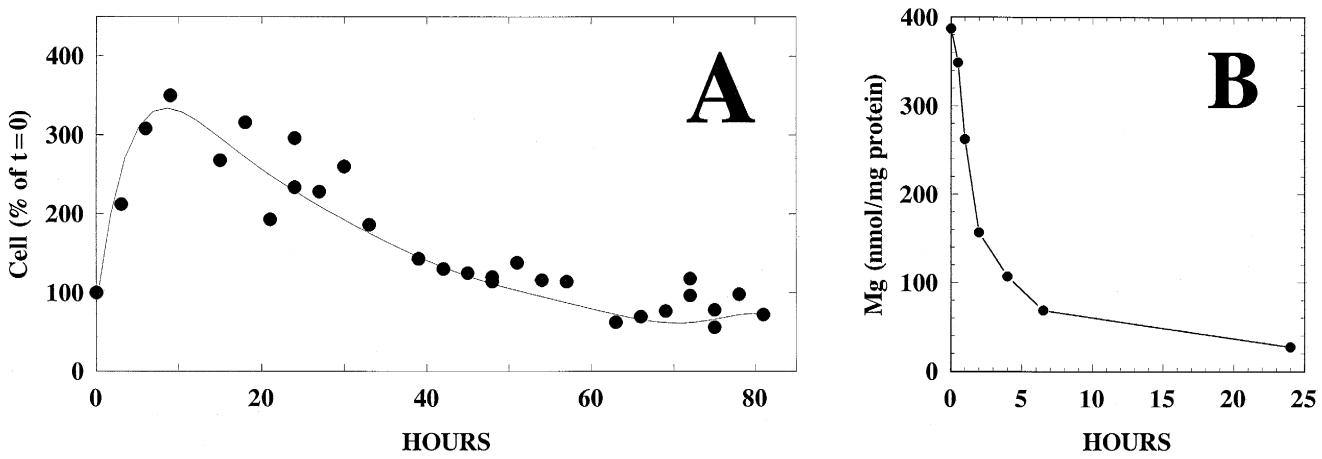

Fig. 4. Effect of Mg^{2+}-free medium on cell viability and cellular Mg^{2+}. (A) Cells grown in SD medium for several generations to an A_{600} of 0.1–1.0 were shifted to Mg^{2+}-free SD medium and at the indicated times aliquots of cells were plated onto YPD plates. After three days at 26°C, the number of viable cells (i.e., those that formed colonies) was determined. (B) The cellular Mg^{2+} levels were determined at the indicated times after shifting to Mg^{2+}-free SD media, using the dichlorophopho-nazo III indicator as described in the legend to Fig. 2.

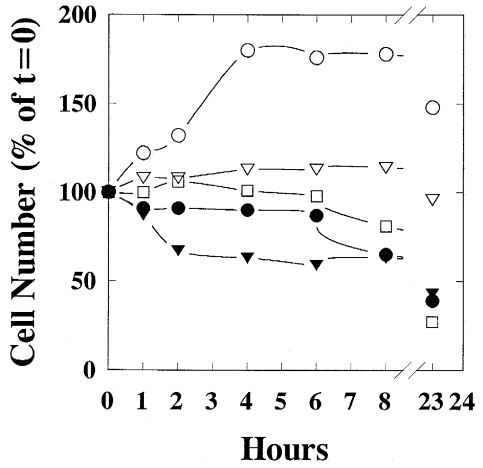

Fig. 5. Effect of preloading of cells with Ca^{2+}, Mg^{2+}, and polyphosphate on the ability of cells to grow in Mg^{2+}-free medium. Cells were grown in modified SD medium containing either 1 mM $MgSO_4$, 7 mM KH_2PO_4 (○); 20 μM $MgSO_4$, 7 mM KH_2PO_4 (low Mg^{2+}, ●); 1 mM $MgSO_4$, 50 μM KH_2PO_4 (low phosphate, □); 25 μM $MgSO_4$, 50 μM KH_2PO_4, (low Mg^{2+}, low phosphate, ▽); or 20 μM $MgSO_4$, 7 mM KH_2PO_4, 100 mM $CaCl_2$ (high Ca^{2+}, low Mg^{2+}, ▼). Cells were diluted into Mg^{2+}-free SD media and at the indicated times aliquots were removed and plated onto YPD plates to determine the number of viable cells.

in media containing 100 mM $CaCl_2$ accumulate 200–300 nmol Ca^{2+}/mg in their vacuole [5], presumably displacing vacuolar Mg^{2+}. Ca^{2+}-loaded cells not only stop growth immediately upon transfer to Mg^{2+}-free medium, but display a 40% drop in viability within 4 h. These data demonstrate that conditions that reduce Mg^{2+} storage capacity by the vacuole decrease the cell's ability to continue to grow in Mg^{2+}-free medium.

The rate at which the cellular Mg^{2+} level is restored to about 400 nmol/mg after it is reduced to 200 nmol/mg is shown in Fig. 6. Mg^{2+} accumulation follows first order kinetics with a rate constant of 1.4 h^{-1} (Fig. 6B).

Polyphosphate accumulation by cells is dependent on the Mg^{2+} concentration of the growth medium [13–15]. The effect of Mg^{2+} concentration on polyphosphate accumulation is especially striking when cells grown in low phosphate are shifted to high phosphate [16]. Under these conditions there is a burst of polyphosphate synthesis ('overcompensation'), the amount of which is dependent on the Mg^{2+} concentration in the growth media. 'Over-compensation' polyphosphate synthesis is accompanied by increased Mg^{2+} accumulation (Fig. 7), demonstrating a relationship between vacuolar Mg^{2+} levels and polyphosphate levels. Polyphosphate synthesis during 'overcompensation' might be limited by the availability of Mg^{2+} to form a complex with polyphosphate.

Effect of Mg^{2+} on vacuolar and cellular Ca^{2+} accumulation Cellular Ca^{2+} accumulation is dependent on the Mg^{2+} concentration of the growth medium [17]. Vacuolar Ca^{2+} accumulation is mediated by the $Ca^{2+}/2H^+$-exchanger [5]. The K_m (25 μM) of the vacuolar Ca^{2+} transporter is much higher than the cytosolic Ca^{2+} concentration and depends on

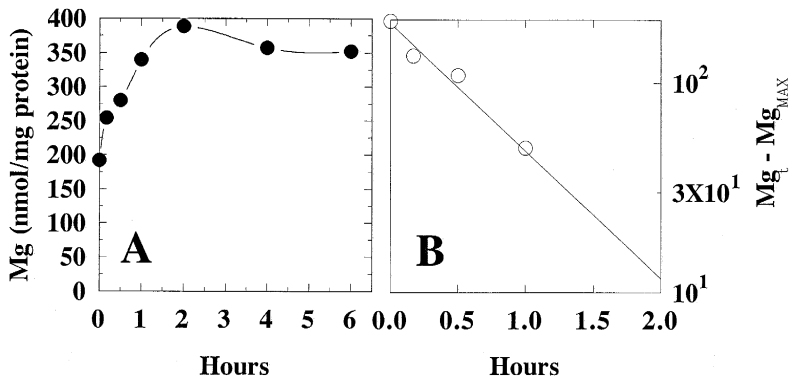

Fig. 6. Rate of Mg^{2+} accumulation by cells following a shift from Mg^{2+}-free medium to medium containing 1 mM Mg^{2+}. (A) Cells were grown in Mg^{2+}-free SD medium for several hours to reduce cellular Mg^{2+}, and then 1 mM $MgSO_4$ was added. At the indicated times after addition of Mg^{2+}, aliquots of the cells were assayed for accumulated Mg^{2+} using the dichlorophosphonazo III assay as described in the legend to Fig. 2. (B) The rate of Mg^{2+} loading of the Mg^{2+}-deficient cells is measured by plotting the logrithm of the difference between the maximum Mg^{2+}-loading level ($t = 2$ h) and the level at the indicated time vs. the time.

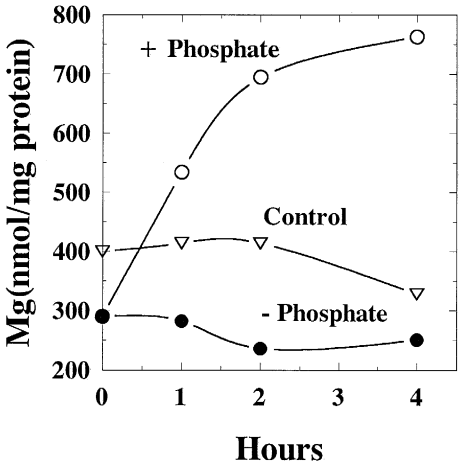

Fig. 7. Accumulation of Mg^{2+} by phosphate-starved cells following transfer into high phosphate medium. Cells were grown in SD medium +10 mM Mg^{2+}, and a portion of the cells was then shifted to SD-phosphate +10 mM Mg^{2+} for 4 h (○,●). The control cells were left in the SD +10 mM Mg^{2+} (▽). After the 4 h in phosphate-free medium, 7 mM phosphate was either added back (○) or the cells were maintained in the phosphate-free medium (●). The cellular Mg^{2+} levels were determined at the indicated times using the dichlorophosphonazo III assay as described in the legend to Fig. 2.

the Mg^{2+} concentration [5]; therefore, the rate of vacuolar Ca^{2+} uptake (Fig. 8A) depends on the Mg^{2+} concentration. As the free Mg^{2+} concentration increases from 0.2 mM to 9 mM the Ca^{2+} transport rate decreases at least 100-fold. The cellular Ca^{2+} loading level is also dependent on the Mg^{2+} concentration (Fig. 8B). When grown in YPD +50 mM Ca^{2+} for 2 h, cells accumulate about 90 nmol Ca^{2+}/mg protein. As the Mg^{2+} concentration is increased from 0.6 mM to 5 mM, the accumulated Ca^{2+} decreases to a level too low to be detected with Arsenazo III (less than 2 nmol/mg) (Fig. 8B).

4. Discussion

Based on the data reported here and from other laboratories, a model for the regulation of cytosolic Mg^{2+} in *S. cerevisiae* is proposed. Cytosolic Mg^{2+} is regulated by Mg^{2+} transport systems found in the vacuolar and plasma membranes. The free cytosolic Mg^{2+} concentration is unknown, but in general, eukaryotic cells are believed to have free cytosolic

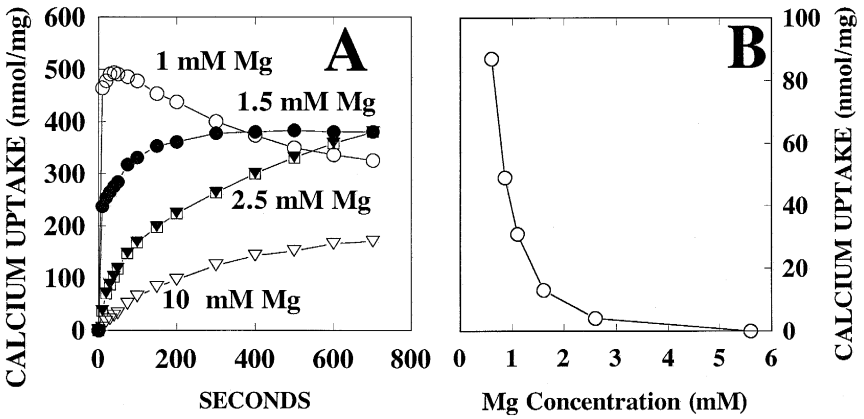

Fig. 8. Effect of Mg^{2+} concentration on the rate of vacuolar Ca^{2+} uptake and cellular Ca^{2+} accumulation. (A) Permeabilized partially regenerated spheroplasts were diluted 50-fold to a final protein concentration of 30 μg/ml into 0.1 M KCl, 10 mM Hepes/Pipes/Mes (pH 7.0), 50 μM $CaSO_4$, 50 μM Arsenazo III, 1 mM Na ATP and the indicated Mg^{2+} concentration at 27°C. Ca^{2+} uptake was monitored spectrophotometrically by measuring the decrease in the difference absorbance of the Ca^{2+}:Arsenazo III complex at 660 nm using 685 as reference wavelength. Baseline absorbance was established by addition of 5 μM A23187, a Ca^{2+} ionophore that releases accumulated Ca^{2+}. The response of Arsenazo III to Ca^{2+} was calibrated by multiple additions of 2.5 μM Ca^{2+}. (B) Cells were grown to an A_{600} of 1.0 and diluted into YPD medium +50 mM $CaCl_2$ with Mg^{2+} added to the indicated concentration (YPD has about 0.6 mM Mg^{2+}). After a 2-h incubation, cells were washed by centrifugation and resuspended to a density of 10^7 cells/ml in KCl solution containing 50 μM Arsenazo III. Accumulated Ca^{2+} was released by adding 1 mg/ml digitonin to permeabilize all membranes and measured spectrophotometrically by monitoring the difference absorbance of the Arsenazo III-Ca^{2+} complex at 660 nm/685 nm using an AMINCO-SLM dualbeam spectrophotometer.

Mg^{2+} concentrations in the range of 0.1 to 1 mM. Since the cell volume is about 12 μl/mg protein [10], the free Mg^{2+} would contribute 1.2–12 nmol Mg^{2+}/mg protein to the total cellular Mg^{2+} which, in cells grown in excess Mg^{2+}, is about 400 nmol/mg protein indicating only 0.3–3% of the cellular Mg^{2+} is free. In the cytosol, Mg^{2+} is bound mainly to ATP and to RNA. The MgATP concentation is about 1 mM [9] (12 nmol/mg protein). RNA binds about 0.2 Mg^{2+} per phosphate residue under physiological conditions [18,19] and cells contain about 590 nmol nucleotide residues per mg protein [10], so that about 117 nmol Mg^{2+}/mg protein would be expected to be bound to RNA. The free Mg^{2+} and that bound to ATP or RNA would account for about 140 nmol/mg protein Mg^{2+}. When cells are transferred into Mg^{2+}-free medium, cell growth continues until the cellular Mg^{2+} level is reduced to about 80 nmol/mg, while cells growing in 10–30 μM Mg^{2+} have normal growth rates and a cellular Mg^{2+} content of about 90 nmol/mg. Cells grown in media containing 0.5 mM to 100 mM Mg^{2+} contain about 400 nmol mg/ml. About 75% of this Mg^{2+} appears to be complexed to polyphosphate in the vacuole. Cells can be loaded with 750 nmol Mg^{2+}/mg by inducing polyphosphate synthesis by transfer of phosphate-starved cells into media containing 7 mM phosphate.

The growth of cells varies with the Mg^{2+} concentration in the range of 1 to 30 μM. Mg^{2+} transporters apparently can maintain 20- to 200-fold Mg^{2+} gradients across the plasma membrane. The polarization of the plasma membrane by the H^+-ATPase would provide a sufficient inside-negative membrane potential to form such gradients. Although the growth rate of cells does not significantly increase when the medium Mg^{2+} concentration increases from 0.1 to 1.0 mM, the cellular Mg^{2+} content increases 4-fold. The increased Mg^{2+} is likely due to increased polyphosphate-bound Mg^{2+} in the vacuole. The amount of vacuolar polyphosphate is about 0.1 mg/mg protein or about 1 μmol phosphate residue per mg protein [5]. Mg^{2+} bound to the polyphosphate provides a significant Mg^{2+} reservoir which can be mobilized when extracellular Mg^{2+} concentration is low. In Mg^{2+}-free medium, the cytosolic Mg^{2+} concentation is maintained by utilization of the vacuolar Mg^{2+}; thus the vacuole appears to be important in the regulation of cytosolic Mg^{2+} under conditions of low extracellular Mg^{2+}. Ca^{2+} and Mg^{2+} compete for the vacuolar storage sites but Mg^{2+} inhibits the $Ca^{2+}/2H^+$ exchanger. Therefore, when the Mg^{2+} concentration is relatively high, vacuolar Mg^{2+}-loading apparently has priority over Ca^{2+}-loading unless the cytosolic $[Ca^{2+}]$ is very high. At low Mg^{2+} concentrations, Ca^{2+} would displace Mg^{2+} thereby mobilizing the Mg^{2+} stores.

The plasma membrane Mg^{2+} transporter is regulated such that cells in Mg^{2+}-free medium do not leak Mg^{2+} out of the cell, and cells in media containing high Mg^{2+} concentrations do not over-accumulate Mg^{2+}.

Finally, it is interesting to note that when Mg^{2+} in the growth medium becomes limiting, the cells not only cease growing, but actually die. This is in contrast to starvation for most other nutrients where cells have mechanisms that allow them to enter G_o where they can survive for extended periods. Cells that enter G_o due to the lack of glucose remain viable in Mg^{2+}-free medium indicating that it is the dilution of cellular Mg^{2+} by growth that leads to cell death in Mg^{2+}-free medium.

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