Phosphoglucomutase (PGM) is a key enzyme in glucose metabolism, where it catalyzes the interconversion of glucose 1-phosphate (Glc-1-P) and glucose 6-phosphate (Glc-6-P). In this study, we make the novel observation that PGM is also involved in the regulation of cellular Ca\(^{2+}\) homeostasis in *Saccharomyces cerevisiae*. When a strain lacking the major isoform of PGM (pgm2Δ) was grown on media containing galactose as sole carbon source, its rate of Ca\(^{2+}\) uptake was 5-fold higher than an isogenic wild-type strain. This increased rate of Ca\(^{2+}\) uptake resulted in a 9-fold increase in the steady-state total cellular Ca\(^{2+}\) level. The fraction of cellular Ca\(^{2+}\) located in the exchangeable pool in the pgm2Δ strain was found to be as large as the exchangeable fraction observed in wild-type cells, suggesting that the depletion of Golgi Ca\(^{2+}\) stores is not responsible for the increased rate of Ca\(^{2+}\) uptake. We also found that growth of the pgm2Δ strain on galactose media is inhibited by 10 \(\mu\)M cyclosporin A, suggesting that activation of the calmodulin/calcineurin signaling pathway is required to activate the Ca\(^{2+}\) transporters that sequester the increased cytosolic Ca\(^{2+}\) load caused by this high rate of Ca\(^{2+}\) uptake. We propose that these Ca\(^{2+}\)-related alterations are attributable to a reduced metabolic flux between Glc-1-P and Glc-6-P due to a limitation of PGM enzymatic activity in the pgm2Δ strain. Consistent with this hypothesis, we found that this “metabolic bottleneck” resulted in an 8-fold increase in the Glc-1-P level compared with the wild-type strain, while the Glc-6-P and ATP levels were normal. These results suggest that Glc-1-P (or a related metabolite) may participate in the control of Ca\(^{2+}\) uptake from the environment.

Saccharomyces cerevisiae shares many features of Ca\(^{2+}\) homeostasis and signaling pathways with mammals (1). Like other eukaryotes, yeast maintain a low resting free Ca\(^{2+}\) concentration in their cytosol in the range of 50–200 nM (2–5). Considering that the total cellular Ca\(^{2+}\) content is in the millimolar range, more than 99% of the total cell-associated Ca\(^{2+}\) is either bound to proteins within the cytosol or compartmentalized within intracellular organelles. This remarkably tight control of free cytosolic Ca\(^{2+}\) is a universal phenomenon among living organisms that apparently relates to the low solubility of Ca\(^{2+}\) complexes of many phosphorylated metabolites (6). This tight control of free cytosolic Ca\(^{2+}\) is also an essential feature of Ca\(^{2+}\) signaling, where Ca\(^{2+}\)-sensing proteins like calmodulin utilize a transient increase in the steady-state cytosolic Ca\(^{2+}\) concentration to activate various signal transduction pathways (7).

The great majority of cellular Ca\(^{2+}\) is stored within intracellular organelles in *S. cerevisiae* (8). The vacuole is believed to contain the largest (~90%) reservoir of Ca\(^{2+}\), where it is thought to reside primarily in a complex with polyphosphate (9, 10). In contrast, the endoplasmic reticulum (ER) and the Golgi apparatus hold Ca\(^{2+}\) in a more readily mobilized form, which, together with cytosolic Ca\(^{2+}\), is often referred to as the exchangeable Ca\(^{2+}\) pool (11). Cytosolic Ca\(^{2+}\) can be transported into the Golgi apparatus by the Ca\(^{2+}\)-ATPase Pmr1p (12–15) and possibly by another putative Ca\(^{2+}\) and Mn\(^{2+}\) transporter, Ccc1p (16–18). Consistent with its capacity to store Ca\(^{2+}\), a recent study demonstrated that the Golgi apparatus can play a significant role in maintaining cellular Ca\(^{2+}\) homeostasis under conditions where vacuolar Ca\(^{2+}\) storage is compromised (4). Cytosolic Ca\(^{2+}\) can also be sequestered into the vacuole through the action of the Ca\(^{2+}\)-ATPase Pmc1p and the vacuolar H\(^+\)/Ca\(^{2+}\) exchanger Vcx1p/Hum1p (17, 19, 20). Of these two transporters, Vcx1p has been shown to play a predominant role in pumping cytosolic Ca\(^{2+}\) into the vacuole and restoring basal cytosolic Ca\(^{2+}\) conditions following an increase in cytosolic Ca\(^{2+}\) (5, 17, 20). The expression and function of each of these Ca\(^{2+}\) transporters is regulated by calcineurin, a highly conserved protein phosphatase that is activated by Ca\(^{2+}\)/calmodulin (4, 17, 20). While mitochondria have been shown to play an important role in Ca\(^{2+}\) signaling in mammalian cells, there is currently little or no evidence that mitochondria play any role in Ca\(^{2+}\) sequestration in *S. cerevisiae* (21, 22).

A number of stimuli can trigger a transient increase in the cytosolic Ca\(^{2+}\) concentration in yeast. These stimuli include various nutrients, mating pheromones, or a hypotonic shock (3, 23). While glucose is frequently considered to be primarily a carbon and energy source in yeast, it can also activate several signaling pathways in yeast, including the RAS-cAMP pathway, phosphatidylinositol turnover, Ca\(^{2+}\) influx and efflux, and the glucose repression/de-repression pathway (24). It is unclear whether these distinct pathways are regulated by glucose, a glucose derivative, a glycosylated protein, or by some other mechanism. Eilam and co-workers (25, 26) have shown that glucose-stimulated Ca\(^{2+}\) uptake is not controlled by changes in

---

Loss of the Major Isoform of Phosphoglucomutase Results in Altered Calcium Homeostasis in *Saccharomyces cerevisiae* *

(Rceived for publication, September 22, 1999, and in revised form, November 2, 1999)

Lianwu Fü‡, Attila Miseta‡§, Dacia Hunton¶, Richard B. Marchase¶, and David M. Bedwell¶§

From the Departments of *¶*Microbiology and *§*Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

---

*This work was supported by Grant JDF 99502 from the Juvenile Diabetes Foundation International. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Dept. of Clinical Chemistry, University Medical School, 7624 Pecs, Hungary.

‡ To whom correspondence should be addressed: Dept. of Microbiology, Bevill Biomedical Research Bldg., Rm. 432, 845 19th St. S., University of Alabama at Birmingham, Birmingham, AL 35294-2170. Tel.: 205-934-6593; Fax: 205-975-5482; E-mail: dbedwell@uab.edu.

---

This paper is available online at http://www.jbc.org

Printed in U.S.A.
either the ATP level or by a cAMP-dependent pathway. Interestingly, Glc-6-P has been shown to stimulate the ATP-dependent transport of Ca²⁺ into mammalian microsomes (27, 28), indicating that glucose metabolites may directly influence Ca²⁺ transport mechanisms in higher eukaryotes.

The biochemical pathways required to utilize glucose as a carbon and energy source are highly conserved from bacteria to humans. A key enzyme involved in this process is phosphoglucomutase (PGM; EC 2.7.5.1), which catalyzes the interconversion of Glc-1-P and Glc-6-P. PGM acts at a key metabolic trafficking point that controls the synthesis and degradation of carbohydrates. The direction of metabolic flow through PGM depends on the available carbon source. Both the degradation of glycogen and the metabolism of galactose as carbon source require PGM activity to convert Glc-1-P to Glc-6-P. The Glc-6-P produced can then be utilized in glycolysis and the pentose-derivative (or a derivative) may regulate Ca²⁺ uptake from the environment.

**MATERIALS AND METHODS**

**Strains Used**—Yeast strains used in this study are listed in Table I. Sc252 and Sc451 were kindly provided by J. Hopper (30). W303–1C and YRP032 were gifts from H. K. Rudolph (33). Yeast strains YDB0171, YDB0200, YDB0202, and YDB0201 were generated using a one-step gene replacement method (34). Briefly, the PGM2 gene in YDB0171, YDB0200, and YDB0201 was disrupted by the insertion of the LEU2 gene. A 1.5-kilobase pair HpaI/HindIII DNA fragment containing most of the structural gene of PGM2 in pDB0197 was removed by the insertion of a BamHI/HindIII fragment containing the LEU2 gene from pJJ282 (35). The PGM1 genes in YDB0200 and YDB0201 were disrupted by the insertion of the URA3 gene. A 2-kilobase pair fragment of the PGM1 gene was generated by polymerase chain reaction using wild-type genomic DNA as template. The forward primer used was DB225 (5'-CAA GAC TCG AGA AGG GCG CAT CAC) and DB226 (5'-GAT CTT GAA TTC CTG TAC GGC TCT GC). These primers contain XhoI and EcoRI restriction endonuclease sites, respectively (underlined). The polymerase chain reaction product was digested with EcoRI and XhoI and cloned into a pBlueScript KS II (pJJ244) (35). The EcoRI/XhoI fragment containing the disrupted PGM1::URA3 was then used to transform yeast. In all cases, the correct gene replacements were confirmed by Southern blot analysis (data not shown). YDB0310 and YDB0312 were generated by backcrossing the Sc451 strain with YR122 (36) by standard methods (37).

**Culture Medium**—Bacteria strains were grown on standard media (38). Media for yeast growth were prepared as described (37). YP media and synthetic media (SM) was supplemented with 2% glucose (YPD or YPMD) or 2% galactose (YPGal or YPGal) All media containing Ca²⁺ or Mg²⁺ were buffered with 40 mM MES-Tris, pH 5.5 or 6.5 as specified. In all experiments, cultures were grown for a minimum of 5–6 generations to an A₆₀₀ of ≤1.0.

**Measurement of Total Cellular Ca²⁺ Levels**, **Cytosolic Free Ca²⁺ Concentration**, **Rate of Ca²⁺ Uptake**, and **Efflux of Ca²⁺**—Total cell calcium levels were measured as described earlier (4). Briefly, 50–100 OD₆₀₀ units of yeast cells grown in YPD or YPGal were harvested and washed. The cell pellets were dried in a Savant SpeedVac system and resuspended in 1 M HCl. The total calcium levels were then measured by using a flame photometry method and expressed in moles per kilogram of dry mass.

The cytosolic free Ca²⁺ was measured by using an aequorin-based method as described (4, 33). Briefly, the cells of the wild-type and pgrpΔ strains containing the apoaequorin-expressing plasmid were grown in YPGal media supplemented with the required amino acids.
Alteration of Calcium Homeostasis in a Phosphoglucomutase Mutant

The cells were then harvested and loaded with coelenterazine. For glucose- or galactose-stimulated Ca2+ uptake, the cells were loaded with coelenterazine, then incubated without a carbon source for 2 h in SM medium supplemented with 2 mM EGTA. To confirm that the glucose- or galactose-stimulated increase in cytosolic Ca2+ was dependent upon extracellular Ca2+, cells were incubated in the presence or absence of 5 mM CaCl2 for 10 min prior to the addition of glucose or galactose. As previously reported (3), the increase in cytosolic Ca2+ was strictly dependent upon extracellular Ca2+. In these experiments, aequorin luminescence was measured using a Berthold Lumat 9050 luminometer. The data were recorded and transferred to a personal computer for analysis. The cytosolic free Ca2+ was calculated using a standard curve (4).

The rate of Ca2+ uptake was measured as described before (14). The cells were harvested from an exponential phase culture, washed three times with distilled water, and resuspended in a buffer containing 40 mM MES-Tris, pH 5.5, and 20 mM n-glucose. The Ca2+ uptake experiment was then initiated by the addition of [45Ca2+] to a concentration of 1 μCi/ml. At the times indicated, aliquots were filtered through a 0.45-μm Gelman filter pre-washed with buffer containing 20 mM MgCl2 and 0.2 mM LaCl3. The cells were then rapidly washed three times with the same washing solution, and cell-associated [45Ca2+] was determined by scintillation counting. For the measurement of [45Ca2+] uptake in glucose-starved cells, strains were grown in SMGal supplemented with required amino acids and then starved for carbon source for 2 h. The cells were then harvested, washed three times with water, and resuspended in 40 mM MES-Tris buffer, pH 5.5. Ca2+ uptake was initiated by the addition of 1% glucose and 1 μCi of [45Ca2+] to. At the indicated times, cells were filtered and washed, and the cell-associated radioactivity was determined by scintillation counting. Nonspecific binding of [45Ca2+] to the cells at the zero time point was subtracted. The Ca2+ exchange experiments were done as described previously (4, 19). Wild-type and pgm2Δ strains were grown in YPGal for 5–6 generations in the presence of 10 μCi/ml of [45Ca2+] before harvest at logarithmic phase. The cells were harvested by centrifugation at room temperature, washed with YP medium, and then resuspended in YPGal plus 20 mM CaCl2. After incubating at 30 °C for the time indicated, aliquots of cells were harvested by rapid filtration, washed, and processed for scintillation counting as described above.

Phosphoglucomutase Assay—To assay for PGM activity, frozen cell pellets were resuspended to 50 OD600 units/ml in assay buffer (50 mM Bis-Tris, pH 6.0, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride). Cells were disrupted by mechanical agitation with glass beads in a mini-bead beater (Biospec Products). Assays were carried out on total cellular homogenates at 22 °C using a coupled enzyme assay as described (29). Specific activity is expressed as micromoles of product formed/min/mg of protein. Protein concentrations were determined by the method of Bradford (39), with bovine serum albumin used to generate the standard curve.

Measure of the Metabolite Levels—Extracts were made using a method published previously (40). An aliquot of exponentially growing yeast cells was filtered under vacuum through a 0.45-μm Gelman filter. The wet weight of cells was rapidly determined, and the cells were then frozen in liquid nitrogen. 3 ml of 3 M HClO4 was added to the frozen cells, and they were then ground in the presence of glass beads in a mortar cooled with liquid nitrogen. The extract was centrifuged at 4 °C for 10 min at 10,000 × g, and the supernatant was neutralized by the addition of 5 M KOH. Glc-6-P levels in the extract were measured using an assay coupled with Glc-6-P dehydrogenase (41). Glc-1-P levels were measured similarly, with the addition of PGM. For the calculation of concentrations of each metabolite, it was assumed that 1.67 g of wet yeast contain 1 ml of cell volume (40).

The levels of ATP, ADP, and AMP were measured by high performance liquid chromatography analysis. Briefly, yeast extracts were resolved using a Keystone Partisil-10 SAX column using a linear salt and pH gradient from 5 to 750 mM (NH4)2HPO4, and from pH 2.8 to 3.7, respectively, at a flow rate of 2 ml/min over 40 min. External standards of known concentration were used to identify and quantitate specific peaks that corresponded to each metabolite. The energy charge of each strain was calculated as the sum of the ATP concentration plus one-half the ADP concentration divided by the sum of the concentrations of ATP, ADP, and AMP.

Analysis of Invertase Glycosylation and CyP1+ Degradation—Invertase glycosylation was determined from cells labeled with [35S]methionine/cysteine (Expre585; NEN Life Science Products). Immunoprecipitation was carried out using an invertase antibody (a gift from Scott Emr) as described previously (42). To amplify the invertase signal, strains containing pRB58 (43), a high copy invertase-expressing plasmid, were grown in SMGal medium supplemented with the required amino acids. 10 A600 units of cells were used for the initial immunoprecipitation, followed by a second immunoprecipitation using the same antibody. CyP1+ degradation was assayed by a pulse-chase experiment as described previously (33), except the cells were grown in SMGal medium.

RESULTS

Growth Phenotype of a pgm2Δ Yeast Strain—PGM is a key enzyme in glucose and galactose metabolism. In S. cerevisiae there are two isoforms of PGM that are subject to distinct patterns of regulation (29, 30). To better understand the physiological roles of PGM, we constructed strains containing single pgm1Δ or pgm2Δ knockouts, as well as a strain containing knockouts of both genes (pgm1Δ/pgm2Δ). Initially, we compared the growth of these strains on YPD and YPGal media. Consistent with the results reported by Boles et al. (31), the pgm1Δ, pgm2Δ, and pgm1Δ/pgm2Δ strains could all grow as well as the WT strain in YPD medium (data not shown). On YPGal medium, the pgm1Δ strain grew as well as the WT strain, while the growth of the pgm2Δ mutant was somewhat reduced. In contrast, the pgm1Δ/pgm2Δ strain could not grow on YPGal media, confirming that PGM activity is essential for growth when galactose is the sole carbon source (31).

The pgm2Δ Strain Accumulates Glc-1-P When Grown on Galactose Media—PGM catalyzes the interconversion of Glc-6-P and Glc-1-P. To determine how the pgm1Δ and pgm2Δ mutations affect the levels of sugar metabolites and energy metabolism in cells grown on galactose media, we measured the specific activity of PGM and the concentrations of Glc-1-P and Glc-6-P in strains containing deletions of each PGM gene. As shown in Fig. 1A, inactivation of the PGM2 gene greatly reduced the enzymatic activity of PGM in galactose-grown cells. The specific activity of PGM in the WT strain was about 0.9 μmol/min/mg of protein, while the pgm2Δ mutant was about 0.03 μmol/min/mg of protein. Thus, the pgm2Δ mutant has 30-fold less PGM activity than the WT strain.

We next measured the levels of Glc-6-P and Glc-1-P in these strains. We found that the steady-state levels of Glc-6-P and Glc-1-P in the WT strain were 0.23 and 0.11 mM, respectively, when the cells were grown in galactose-containing media. While the pgm2Δ strain had a level of Glc-6-P that was not significantly different from the WT strain (0.20 mM), the Glc-1-P level in this strain was increased to 0.75 mM (Fig. 1B). Hence, the intracellular Glc-1-P concentration was 7-fold higher in the pgm2Δ mutant than in the WT strain. These results suggest that the limitation of PGM enzymatic activity caused by the pgm2Δ mutation results in a metabolic bottleneck that restricts the conversion of Glc-1-P to Glc-6-P. Like the Glc-6-P level, the cellular energy charge in these strains was also found to be similar (Fig. 1C), indicating that the pgm2Δ mutation did not cause a reduction in the ATP level when galactose was utilized as the carbon source. Since the pgm2Δ strain exhibits a moderately slower growth rate than the WT strain, these results suggest that cellular growth may be limited by the ability to generate a normal level of Glc-6-P (and ultimately, ATP). In contrast, no significant differences in the levels of Glc-1-P, Glc-6-P, or the energy charge were measured in these strains when they were grown in YPD media (data not shown).

The pgm2Δ Strain Contains an Elevated Level of Total Cellular Ca2+—Previous studies have shown that glucose can transiently stimulate Ca2+ uptake in glucose-deprived yeast cells (3, 26). In addition, glucose can induce phosphatidylinositol turnover and activation of the plasma membrane H+-ATPase in glucose-deprived cells (24). While the mechanism by which glucose activates these (and other) signaling pathways is not well understood, it is thought that glucose or phosphoryl-
ated glucose derivatives may be responsible. This led us to ask whether an alteration in the concentration of glucose metabolites such as those observed in the pgm2Δ strain when grown on YPGal could lead to an imbalance in Ca²⁺ homeostasis. We initially measured the total cellular Ca²⁺ level in the WT and pgm2Δ strains by flame photometry (4). We found that the total cellular Ca²⁺ level in the WT strain was 2.85 mmol/kg dry weight (Fig. 2A). In contrast, the pgm2Δ mutant contained 25.83 mmol of Ca²⁺/kg dry weight. Thus, the amount of total cellular Ca²⁺ in the pgm2Δ strain was 9-fold higher than the wild-type strain in YPGal medium. We confirmed that the pgm2Δ strain had a much higher level of total cellular Ca²⁺ by measuring the accumulation of [⁴⁵Ca²⁺] (data not shown).

When these strains were grown on YPD medium, we did not observe a significant difference in the total cellular Ca²⁺ levels between the WT and pgm2Δ strains, which contained 2.40 and 2.56 mmol of Ca²⁺/kg of dry weight, respectively (Fig. 2B). These results indicate that the increased accumulation of cellular Ca²⁺ in the pgm2Δ mutant is dependent upon utilization of galactose as the carbon source.

The yeast PMR1 gene encodes a P-type Ca²⁺-ATPase that localizes to the Golgi membrane (13). Previous studies have documented the role of Pmr1p in Ca²⁺ transport into the Golgi apparatus (12, 14) and in the maintenance of cellular Ca²⁺ homeostasis in yeast (4). It has been reported that the total cellular Ca²⁺ level in the pmr1Δ strain is increased 5–10-fold compared with the WT strain when grown on YPD medium (14). Consistent with these reports, we found that the pmr1Δ strain grown in YPD medium contained 20.91 mmol of Ca²⁺/kg of dry weight, a level that was 8.7-fold higher than the WT strain (Fig. 2B). When the pmr1Δ strain was grown in YPGal medium, we found that the total cellular Ca²⁺ level was 7.85 mmol/kg dry mass, a level that was 2.7-fold lower than was observed in cells grown in YPD (Fig. 2A). This observation suggests that the carbon source may also have a moderate effect on the absolute level of Ca²⁺ accumulation in the pmr1Δ strain. However, only the pgm2Δ mutant showed a conditional
defect in Ca\(^{2+}\) accumulation that was evident only when a specific carbon source was utilized.

\textbf{Ca}^{2+} \textbf{Uptake Is Increased in the pgm2\Delta Strain—}The massive Ca\(^{2+}\) accumulation observed when the \textit{pgm2}\Delta mutant was grown in YPGal suggested that the rate of Ca\(^{2+}\) uptake might also be increased. According to Eilam and co-workers (44), the time course of Ca\(^{2+}\) influx into yeast is composed of two components. The initial component represents Ca\(^{2+}\) transported across the plasma membrane into the cytosol. The second component represents the subsequent distribution of the cytosolic Ca\(^{2+}\) into intracellular organelles. These two components of Ca\(^{2+}\) uptake can be determined by measuring \([^{45}\text{Ca}^{2+}]\) uptake during short and long time intervals, respectively. Yeast cells were grown in YPGal and \([^{45}\text{Ca}^{2+}]\) uptake experiments were done as described (14, 44). We found that the initial \([^{45}\text{Ca}^{2+}]\) uptake rate in the \textit{pgm2}\Delta strain was 5 times faster than was observed in the WT strain, indicating that the elevated Ca\(^{2+}\) accumulation in the \textit{pgm2}\Delta strain correlates well with an increase in the rate of Ca\(^{2+}\) uptake across the cytoplasmic membrane (Fig. 2C). By comparison, under the same conditions, we found that Ca\(^{2+}\) uptake by the \textit{pmr1}\Delta strain was only 2-fold greater than the WT strain. These values correlate well with the total cellular Ca\(^{2+}\) levels measured in each of these mutant strains.

\textbf{The Fraction of Total Cellular Ca}^{2+} \textbf{in the Exchangeable Pool Is Not Reduced in the pgm2\Delta Strain—}Intracellular Ca\(^{2+}\) in yeast exists in two kinetically distinguishable pools, the exchangeable pool (representing Ca\(^{2+}\) in the cytosol, ER, and Golgi apparatus) and the non-exchangeable pool (representing Ca\(^{2+}\) in a more stable form within the vacuole) (19, 45). To determine whether the partitioning of cellular Ca\(^{2+}\) between the exchangeable and non-exchangeable pools is altered in the \textit{pgm2}\Delta strain, cells growing in YPGal medium were loaded for several generations with \([^{45}\text{Ca}^{2+}]\). They were then harvested, resuspended in YPGal containing 20 mM CaCl\(_2\), and the radioactivity that remained associated with cells was determined as a function of time (Fig. 3). Under these conditions, –13% of the total cellular \([^{45}\text{Ca}^{2+}]\) was measured in the exchangeable pool in WT cells after 40 min, while the remaining 87% of cellular \([^{45}\text{Ca}^{2+}]\) was located within the non-exchangeable pool (Fig. 3C).

Since the \textit{pmr1}\Delta strain contains a much larger absolute amount of cellular Ca\(^{2+}\) than the WT strain, it also contains more Ca\(^{2+}\) in both the non-exchangeable and exchangeable pools (Fig. 3, A and B, respectively). However, a much smaller fraction of the total cellular Ca\(^{2+}\) (–7%) was found in the exchangeable pool in the \textit{pmr1}\Delta mutant, consistent with its reduced ability to transport Ca\(^{2+}\) into the ER and Golgi (Fig. 3C) (12, 13, 33). Like the \textit{pmr1}\Delta strain, the \textit{pgm2}\Delta strain also contains more total cellular Ca\(^{2+}\) than the WT strain. Consequently, the amount of Ca\(^{2+}\) present in both the non-exchangeable and exchangeable pools in this strain was also larger than was observed in the WT strain (Fig. 3, A and B). However, the absolute amount of Ca\(^{2+}\) present in the exchangeable pool of the \textit{pgm2}\Delta strain was >4-fold larger than was observed in the \textit{pmr1}\Delta strain. Accordingly, the percentage of exchangeable Ca\(^{2+}\) in the \textit{pgm2}\Delta mutant (–20%) was also much higher than the \textit{pmr1}\Delta strain (and actually somewhat higher than the WT strain). These results indicate that the \textit{pgm2}\Delta strain does not have a diminished capacity to maintain cellular Ca\(^{2+}\) in the exchangeable pool (which includes the ER and/or Golgi), as was previously shown for the \textit{pmr1}\Delta strain.

\textbf{Galactose Metabolism Blocks Growth of a pgm2\Delta/pmrm1\Delta Double Mutant—}To gain a better understanding of the relationship between Pgm2p and Pmr1p in the maintenance of Ca\(^{2+}\) homeostasis, we generated a \textit{pgm2}\Delta/pmrm1\Delta double mutant. While strains carrying the \textit{pgm2}\Delta or \textit{pmr1}\Delta mutations alone were able to grow on YPGal plates, the \textit{pgm2}\Delta/pmrm1\Delta double mutant was unable to grow on galactose-containing media (Fig. 4A). Interestingly, the growth inhibition of the \textit{pgm2}\Delta/pmrm1\Delta double mutant was relieved by the addition of 100 mM CaCl\(_2\) to the medium (Fig. 4B). This conditional synthetic lethality associated with combining the \textit{pmr1}\Delta and \textit{pgm2}\Delta mutations suggests that the defects associated with these mutations are additive in nature.
Altered Calcium Homeostasis in a Phosphoglucomutase Mutant

Fig. 4. The pgm2Δ/pmrlΔ strain is unable to grow in galactose-containing medium. The strains indicated were plated on YPGal (A), YPGal plus 100 mM CaCl2 (B), YPLactate (C), and YPGal plus lactate (D). The plates were incubated at 30 °C for 5–7 days.

possibilities, the cells were inoculated onto YP plates containing either lactate or lactate and galactose as carbon source(s). We found that each of the mutant strains (pgm2Δ, pmr1Δ, and pgm2Δ/pmrlΔ) and the WT strain were able to grow when lactate alone was provided as sole carbon source (Fig. 4C). When both galactose and lactate were present, the WT and each of the single mutants were again able to grow. In contrast, the pgm2Δ/pmrlΔ double mutant was unable to grow under these conditions (Fig. 4D), indicating that the metabolism of galactose disrupted cellular physiology (presumably Ca2+ homeostasis) to the extent that growth could not occur even through the utilization of lactate as carbon source.

The pgm2Δ Strain Is Sensitive to Cyclosporin A When Grown in Galactose-containing Media—Under normal conditions, the cytosolic Ca2+ concentration in yeast is maintained within the 50–200 nM range (2, 3). Since we observed an elevated level of total Ca2+ and exchangeable Ca2+ in the pgm2Δ strain when grown in galactose-containing media, we next asked whether the signaling pathway mediated by calcineurin and calmodulin was activated. To test this possibility, we asked whether each of these strains could grow on YPGal medium in the presence of 10 μg/ml cyclosporin A (CsA), an inhibitor of calcineurin. We found that growth of the WT and pmr1Δ strains was unaffected by CsA, while growth of the pgm2Δ strain was completely inhibited (Fig. 5). These results suggest that the pgm2Δ mutation may cause an imbalance in cellular Ca2+ homeostasis to the extent that activation of the calcineurin pathway is required to maintain growth of the pgm2Δ strain in galactose-containing media.

The pgm2Δ Mutation Leads to Increased Sequestration of Cytosolic Ca2+ into Intracellular Compartments—In a previous study, it was shown that the re-addition of glucose to glucose-starved cells led to a transient increase in cytosolic Ca2+ that was dependent upon the presence of extracellular Ca2+ (3). Since our CsA experiment indicated that the pgm2Δ strain required constitutive calcineurin activity to grow in galactose media, we next asked whether this state of calcineurin activation could reduce or abolish the increase in cytosolic Ca2+ levels previously shown to accompany the addition of glucose to glucose-starved cells. To make these measurements, we utilized an aequorin reporter system (23, 46) that recently proved useful in measuring transient changes in the cytosolic Ca2+ concentration of yeast (4, 5). An aequorin expression plasmid was transformed into the WT and pgm2Δ strains, and cultures of each strain were grown in SMGal medium to mid-log phase. The cells were then harvested and resuspended in SM medium lacking a carbon source for 2 h. We found that the resting concentration of cytosolic Ca2+ in the WT strain was ~100 nM. When glucose was injected into the chamber to a final concentration of 1%, we found that the cytosolic Ca2+ began to rise after a lag of 40–60 s and reached a peak of ~340 nM within 2–3 min (Fig. 6A). The Ca2+ level then dropped over the next 5 min until it reached a basal cytosolic Ca2+ concentration similar to the level measured before glucose addition.

When we repeated this protocol with the pgm2Δ strain, we found that the unstimulated cytosolic Ca2+ concentration after carbon source depletion was ~90 nM, slightly lower than the WT strain. However, when glucose was added to the carbon source-starved pgm2Δ strain, no increase in cytosolic Ca2+ was observed. To confirm that this absence of glucose-stimulated Ca2+ uptake was not due to the fact that the strains were grown in SMGal medium, we repeated the experiment and examined the effect of galactose addition following the starvation period. With the WT strain, we measured a basal cytosolic Ca2+ concentration of ~60 nM. When galactose was added, we again observed a 40–60 s delay, followed by a rapid increase in cytosolic Ca2+ that reached a peak of ~160 nM. With the pgm2Δ strain, we observed a resting cytosolic Ca2+ concentration of ~50 nM (again, slightly lower than the WT strain). When galactose was added to the starved pgm2Δ cells, we observed a
very slight increase in cytosolic Ca$^{2+}$ to ~60 nM, but the response was still much smaller than was observed in the WT strain (Fig. 6B).

The results of the above experiments indicate that the pgm2Δ strain is either unable to carry out Ca$^{2+}$ uptake from the extracellular environment under these conditions, or it removes Ca$^{2+}$ from its cytosol so rapidly that the cytosolic Ca$^{2+}$ concentration cannot increase to the level observed in the WT strain. To distinguish between these possibilities, we used a similar experimental protocol to determine whether $[^{45}\text{Ca}^{2+}]$ uptake was altered in the pgm2Δ strain. Cells were grown in SMGal medium, harvested, and resuspended in SM lacking a carbon source for 2 h. The rate of $[^{45}\text{Ca}^{2+}]$ uptake was then measured upon the re-addition of glucose to the medium. We found that both strains had similar rates of $[^{45}\text{Ca}^{2+}]$ uptake (Fig. 6C), indicating that the pgm2Δ strain was not significantly different from the WT strain in its ability to take up Ca$^{2+}$ following carbon source starvation. We conclude that the transient rise in cytosolic Ca$^{2+}$ was not observed in the pgm2Δ strain upon glucose addition because the entering Ca$^{2+}$ was rapidly sequestered into intracellular compartments. This increased rate of Ca$^{2+}$ sequestration is presumably mediated by Ca$^{2+}$ transporters activated by calcineurin in response to the elevated influx of Ca$^{2+}$ in the pgm2Δ strain.

**The pgm2Δ Mutation Does Not Affect Ca$^{2+}$-dependent ER and Golgi Functions**—The PMR1 gene encodes a Ca$^{2+}$-ATPase localized to the Golgi membrane (12). Although a direct measurement of the Golgi Ca$^{2+}$ level has not been made in the pmr1Δ mutant, the size of its exchangeable Ca$^{2+}$ pool is reduced in a manner that is consistent with reduced Golgi Ca$^{2+}$ levels. Furthermore, the pmr1Δ mutation results in incomplete outer chain glycosylation of invertase, a process that is carried out by enzymes in the Golgi compartment (12, 13). Recently, it was shown that deletion of the PMR1 gene also results in a delayed degradation of CpY*, a mutant form of the vacuolar protein carboxypeptidase Y (CpY) that is unable to acquire a properly folded conformation upon translocation across the ER membrane (33). As a consequence, it is recognized by the quality control apparatus within the ER and rapidly transported back out of the ER to proteosomes for degradation (47, 48). The pgm2Δ mutant shares some alterations in Ca$^{2+}$ homeostasis with the pmr1Δ strain, such as an elevated total cellular Ca$^{2+}$ level and increased Ca$^{2+}$ uptake. On the other hand, the pgm2Δ mutant (unlike the pmr1Δ mutant) retains a normal distribution of Ca$^{2+}$ between the exchangeable and non-exchangeable pools.

To determine whether the pgm2Δ mutation also affects the retrograde transport of misfolded proteins from the ER like the pmr1Δ mutation, we determined the rate of CpY* degradation by enzymes in the Golgi compartment (12, 13). Recently, it was shown that deletion of the PMR1 gene also results in a delayed degradation of CpY*, a mutant form of the vacuolar protein carboxypeptidase Y (CpY) that is unable to acquire a properly folded conformation upon translocation across the ER membrane (33). As a consequence, it is recognized by the quality control apparatus within the ER and rapidly transported back out of the ER to proteosomes for degradation (47, 48). The pgm2Δ mutant shares some alterations in Ca$^{2+}$ homeostasis with the pmr1Δ strain, such as an elevated total cellular Ca$^{2+}$ level and increased Ca$^{2+}$ uptake. On the other hand, the pgm2Δ mutant (unlike the pmr1Δ mutant) retains a normal distribution of Ca$^{2+}$ between the exchangeable and non-exchangeable pools.

To determine whether the pgm2Δ mutation also affects the retrograde transport of misfolded proteins from the ER like the pmr1Δ mutation, we determined the rate of CpY* degradation

---

**Fig. 5. Growth of the pgm2Δ strain is inhibited by CsA.** Strains were streaked onto YPGal (A) and YPGal supplemented with 10 μg/ml CsA (B).

**Fig. 6. The pgm2Δ strain is defective in glucose-induced Ca$^{2+}$ signaling.** The carbon source re-added was 1% glucose (A) or 1% galactose (B). Yeast cells of the wild-type or the pgm2Δ strain, which were transformed previously with apoaequorin-containing plasmid, were grown in SMGal, loaded with coelenterazine, and starved for carbon source for 2 h. The indicated carbon source was then added (as indicated by the arrow). The rate of $[^{45}\text{Ca}^{2+}]$ uptake following the addition of 1% glucose is shown in C.
the pgm2Δ mutant was glycosylated in a manner similar to the WT strain, indicating that glycosylation within the Golgi is not significantly altered by the pgm2Δ mutation. We conclude that the pgm2Δ mutation does not alter the Ca²⁺ concentration within the ER and Golgi compartments to the extent that these enzymatic processes are compromised.

**DISCUSSION**

The results of this study indicate that the loss of the major isoform of PGM causes large alterations in cellular Ca²⁺ homeostasis and signaling in galactose-grown cells. Under these conditions, the pgm2Δ strain exhibited a much higher rate of Ca²⁺ uptake and more total cellular Ca²⁺ than the WT strain. The pgm2Δ strain was unable to grow on YPGal plates supplemented with CsA, suggesting that the activation of Ca²⁺ transporters by the calcineurin pathway may be required to adequately sequester the large quantity of Ca²⁺ that enters the cytosol of these cells. Such an increased capacity to rapidly remove cytosolic Ca²⁺ could also explain why the pgm2Δ strain is unable to properly mediate the transient increase in cytosolic Ca²⁺ that accompanies the addition of glucose to glucose-starved cells as described in previous studies (3, 26). In contrast, we did not observe any of these Ca²⁺-related alterations when the pgm2Δ strain was grown in either glucose or lactate media. The pgm2Δ strain thus exhibits a carbon source-dependent defect in both cellular Ca²⁺ homeostasis and signaling. Since the normal enzymatic function of PGM is to interconvert Glc-1-P and Glc-6-P, the loss of the majority of cellular PGM activity could be predicted to limit the conversion of Glc-1-P to Glc-6-P in cells grown on galactose, resulting in a "metabolic bottleneck." Consistent with this prediction, we found that the pgm2Δ strain accumulates a large amount of Glc-1-P when grown on galactose media. The simplest interpretation of these results is that the intracellular level of Glc-1-P (or a related glucose metabolite) stimulates the accumulation of Ca²⁺ in the pgm2Δ strain under these conditions. Interestingly, we found that the total cellular Ca²⁺ level in the pmr1Δ strain was 2.5-fold higher when grown in YPD medium than in YPGal, providing further evidence that glucose metabolites may play a more general role in controlling cellular Ca²⁺ levels than was previously appreciated.

The PMR1 gene encodes a Ca²⁺-ATPase involved in the transport of Ca²⁺ into the Golgi apparatus. Several of the Ca²⁺-related defects of the pgm2Δ strain grown in galactose-containing media are similar to those observed with the pmr1Δ strain, including elevated Ca²⁺ uptake and accumulation (12–14). Durr and co-workers (33) proposed that Pmr1p also mediates the uptake of Ca²⁺ into the ER, since the pmr1Δ strain also exhibits a defect in retrograde protein transport out of the ER. In certain mammalian cell types, the depletion of Ca²⁺ stores within the ER has been proposed to induce the synthesis of a diffusible molecule termed calcium influx factor (CIF). This molecule is thought to stimulate the uptake of Ca²⁺ across the plasma membrane to amplify and prolong the propagation of the cytosolic Ca²⁺ signal. Interestingly, the yeast pmr1Δ strain is missing the Ca²⁺-ATPase responsible for transporting Ca²⁺ into the ER and the Golgi apparatus, and it also exhibits an increased rate of Ca²⁺ uptake and accumulation. This phenotype would be predicted if the depletion of Ca²⁺ from the compartments of the secretory pathway resulted in the production of a CIF-like molecule. Consistent with this reasoning, a recent study found that this strain produces a high level of a CIF-like activity (49). Several groups have reported that CIF is a soluble molecule with a molecular mass of ~700 Da (49–51). Although the structure of CIF has not been determined, it was suggested to contain hydroxyl groups on adjacent carbons and a phosphate moiety (51, 52).
structural characteristics are consistent with the features expected if CIF were related to (or derived from) a sugar phosphate or sugar nucleotide (53). This raises the possibility that Glc-1-P may be a metabolic precursor in the CIF biosynthetic pathway. While the increased rate of Ca²⁺ uptake and total cellular Ca²⁺ levels in the pgm2Δ strain could be attributable to a store depletion mechanism that involves a CIF-like signaling molecule, other aspects of the phenotype of the pgm2Δ strain are inconsistent with such a model. First, our data indicate that the pgm2Δ strain, unlike the pmr1Δ strain, contains a fraction of total cellular Ca²⁺ in the exchangeable pool that is at least as large (if not larger) than the exchangeable pool in the WT strain. This suggests that the level of Ca²⁺ contained in the ER and the Golgi apparatus is not significantly reduced. Consistent with this, we did not observe any evidence of reduced Ca²⁺ levels within the ER or Golgi apparatus of the pgm2Δ strain in functional assays that measured retrograde transport of CpY² from the ER, or invertase glycosylation in the Golgi apparatus. These results indicate that the elevated rate of Ca²⁺ uptake in the pgm2Δ strain is not coupled to a defect in filling the Ca²⁺ stores within the ER and/or the Golgi apparatus. Instead, it appears that the pmr1Δ and pgm2Δ mutations induce the uptake of Ca²⁺ in mechanistically distinct ways. Since the pgm2Δ strain accumulates a high level of Glc-1-P, we hypothesize that Glc-1-P accumulation may be coupled to elevated Ca²⁺ uptake by some mechanism. It is possible that the accumulation of Glc-1-P may bypass the need to deplete ER or Golgi Ca²⁺ stores prior to increased Ca²⁺ uptake. This could occur if an increased concentration of Glc-1-P stimulates the synthesis of CIF in the absence of store depletion, or if Glc-1-P directly stimulates the rate of Ca²⁺ accumulation into one or more intracellular compartments (such as the ER, Golgi apparatus, or vacuole). This latter possibility would not be unprecedented, since it has been shown that Glc-6-P can stimulate ATP-dependent Ca²⁺ uptake into isolated mammalian microsomes (27, 28). The existence of a Glc-6-P transporter in mammalian ER membranes is well established. While Glc-6-P transporter activity in the ER is coupled to Glc-6-P phosphatase activity in the ER lumen of gluconeogenic tissues such as liver, the much broader tissue distribution of the Glc-6-P transporter suggests that it may also participate in another general cellular process such as Ca²⁺ homeostasis (54). Consistent with this, it has been shown that neutrophils and monocytes from patients with Glc-6-P transporter deficiency are unable to efficiently sequester Ca²⁺ (55). As would be predicted from our model that Glc-1-P can also function in this way, it was recently found that Glc-1-P can also stimulate the ATP-dependent uptake of Ca²⁺ into isolated cardiac microsomes.²

To better understand how Pgm2p and Pmr1p each influence cellular Ca²⁺ homeostasis, we constructed a pgm2Δ/pmrlΔ strain. To our surprise, the pgm2Δ/pmrlΔ double mutant was unable to grow on YPGal medium, even when an alternate carbon source (lactate) was present. This suggests that the metabolism of galactose in the pgm2Δ/pmrlΔ double mutant mouse may cause such a severe imbalance in ion homeostasis that cell growth is inhibited. This finding again suggests that the pmr1Δ and pgm2Δ mutations may increase Ca²⁺ uptake in distinct ways, since the phenotypic consequences of combining these two mutations were more severe than the phenotype of strains carrying either mutation alone. Although our results indicate that the free Ca²⁺ level within the ER and the Golgi apparatus is normal in the pgm2Δ strain, we cannot exclude the possibility that the elevated Ca²⁺ uptake associated with the pgm2Δ mutation is caused by the inability to fill another intracellular compartment (or subcompartment) with Ca²⁺. If this were the case, the combination of the pgm2Δ and pmr1Δ mutations could further compromise the ability to adequately fill the effector compartments when grown in standard YPGal medium (which contains only 0.3 mM Ca²⁺). Our finding that the addition of 100 mM CaCl₂ can restore the growth of the pgm2Δ/pmrlΔ strain on this growth medium is consistent with such a model. Clearly, further studies are required to determine the mechanism that couples glucose metabolism to Ca²⁺ homeostasis. The elucidation of this process may provide important new insights into the control of Ca²⁺ homeostasis in yeast. Ultimately, it may also lead to a better understanding of human diseases such as diabetes that are also caused by defects in glucose metabolism.

Acknowledgments—We thank Dr. James Hopper, Dr. Hans K. Rudolph, and Dr. Todd R. Graham for providing strains and plasmids; Dr. Scott Emr for antiserum; and Dr. Richard Kellermayer for helpful discussions and for critically reading the manuscript.

REFERENCES

1. Cunningham, K. W., and Fink, G. R. (1994) J. Exp. Biol. 196, 157–166
2. Iida, H., Yagawa, Y., and Anraku, Y. (1990) J. Biol. Chem. 265, 13391–13399
3. Nakajima-Shimada, J., Iida, H., Tsuji, F. I., and Anraku, Y. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 6878–6882
4. Miseta, A., Fu, L., Kellermayer, R., Buckley, J., and Bedwell, D. M. (1999) J. Biol. Chem. 274, 5904–5907
5. Miseta, A., Kellermayer, R., Acierno, D. P., Fu, L., and Bedwell, D. M. (1999) FEBS Lett. 451, 132–136
6. Williams, R. J. (1990) Adv. Exp. Med. Biol. 269, 7–16
7. Putney, J. W., Jr., and Bird, G. S. J. (1993) Cell 75, 199–201
8. Halachmi, D., and Eilam, Y. (1989) FEBS Lett. 255, 56–61
9. Eilam, Y., Lavi, H., and Grosswohl, N. (1985) J. Gen. Microbiol. 131, 623–629
10. Miseta, A., and Fink, G. R. (1992) Mol. Biol. Cell 3, 633–654
11. Rudolph, H. K., Antebi, A., Fink, G. R., Buckley, C. M., Dorman, T. E., LeVitre, J., Davidow, L. S., Mao, J. I., and Marc, D. T. (1989) Cell 58, 133–145
12. Halachmi, D., and Eilam, Y. (1996) FEBS Lett. 392, 194–200
13. Soria, A., Rosas, G., and Rao, R. (1997) J. Biol. Chem. 272, 9695–9901
14. Fu, D., Beeler, T., and Dunn, T. (1994) Yeast 10, 515–521
15. Pozo, T. C., Sekler, I., and Cyert, M. S. (1996) Mol. Cell. Biol. 16, 3730–3741
16. Lapinskas, P. J., Lin, S. J., and Culotta, V. C. (1996) Mol. Microbiol. 21, 55–61
17. Cunningham, K. W., and Fink, G. R. (1994) J. Cell Biol. 124, 351–363
18. Cunningham, K. W., and Fink, G. R. (1996) Mol. Cell. Biol. 16, 2226–2237
19. Pinto, F., Brn, M., Bastianutto, C., Tuft, R. A., Pozzan, T., and Rizzuto, R. (1998) Biochim. Biophys. Acta 1361, 391–397
20. Pino, L., Dráppel, A., and Sležkovič, D. (1994) Yeast 10, 515–521
21. Chan, P. Y., Coutera, P., Veyna-Burke, N., and Marchase, R. B. (1998) Diabetes 47, 874–881
22. Fu, L., Bouniani, P., Dey, N., Browne, B. L., Marchase, R. B., and Bedwell, D. M. (1995) J. Bacteriol. 177, 2563–2567
23. Oh, D., and Hopper, J. E. (1996) Mol. Cell. Biol. 16, 3373–3377
24. Boles, E., Liebtrau, W., Hofmann, M., and Zimmermann, F. K. (1994) Eur. J. Biochem. 220, 83–96
25. Hofmann, M., Boles, E., and Zimmermann, F. K. (1994) Eur. J. Biochem. 221, 741–747
26. Durr, G., Straley, J., Piemper, R., Elbs, S., Klees, S. K., Catty, P., Wolf, D. H., and Rudolph, H. K. (1998) Mol. Biol. Cell 9, 3623–3625
27. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–209
28. Jones, J. S., and Prakash, L. (1990) Yeast 6, 363–366
29. Harmens, M. M., Langedijk, A. C., van Tuienen, E., Geese, R. H., Raue, H. A., and Maat, J. (1993) Gene (Amst.) 135, 115–123
30. Rose, M. D., Kellems, R. E., and Fink, G. R. (1996) Methods in Yeast Genetics, 3rd Ed., Vol. 6, pp. 163–199, Verlag Chemie, Weinheim, Germany
31. Bedwell, D. M., Klionsky, D. J., and Emr, S. D. (1987) Mol. Cell. Biol. 7, 4038–4047
32. Carlson, M., and Botstein, D. (1982) Cell 28, 145–154
33. Eilam, Y., and Chernichovsky, D. (1987) J. Microbiol. 133, 1614–1649

² R. Marchase, unpublished results.
45. Dunn, T., Gable, K., and Beeler, T. (1994) J. Biol. Chem. 269, 7273–7278
46. Allen, D. G., Blinks, J. R., and Prendergast, F. G. (1977) Science 195, 996–998
47. Finger, A., Knop, M., and Wolf, D. H. (1993) Eur. J. Biochem. 218, 565–574
48. Hiller, M. M., Finger, A., Schweiger, M., and Wolf, D. H. (1996) Science 273, 1725–1728
49. Csutora, P., Su, Z., Kim, H. Y., Bugrim, A., Cunningham, K. W., Nuccitelli, R., Keizer, J. E., Hanley, M. R., Blalock, J. E., and Marchase, R. B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 121–126
50. Thomas, D., and Hanley, M. R. (1995) J. Biol. Chem. 270, 6429–6432
51. Randriamampita, C., and Tsien, R. Y. (1993) Nature 364, 809–814
52. Randriamampita, C., and Tsien, R. Y. (1995) J. Biol. Chem. 270, 29–32
53. Kim, H. Y., Thomas, D., and Hanley, M. R. (1995) J. Biol. Chem. 270, 9706–9708
54. Hiraiwa, H., Pan, C. J., Lin, B., Moses, S. W., and Chou, J. Y. (1999) J. Biol. Chem. 274, 5523–5536
55. Kilpatrick, L., Garty, B. Z., Lundquist, K. F., Hunter, K., Stanley, C. A., Baker, L., Douglas, S. D., and Korchak, H. M. (1990) J. Clin. Invest. 86, 196–202
