The intracellular dissipation of cytosolic calcium following glucose re-addition to carbohydrate depleted *Saccharomyces cerevisiae*

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Abstract Glucose re-addition to carbohydrate starved yeast cells leads to a transient elevation of cytosolic calcium (TECC). Concomitantly, a cytosolic proton extrusion occurs through the activation of the vacuolar H^+-ATPase and the plasma membrane H^+-ATPases. This study addressed the dissipation of the TECC through intracellular compartmentalization and the possible affects of the H^+-ATPases on this process. Both the vacuole and the Golgi-ER apparatus were found to play important roles in distributing calcium to internal stores. Additionally, the inhibition of cytosolic proton extrusion augmented cytosolic calcium responses. A model where pH dependent cytosolic calcium buffering plays an important role in the dissipation of the TECC in *Saccharomyces cerevisiae* is proposed.

Keywords: Yeast; Glucose; Calcium; VCX1; PMR1; pH

1. Introduction

Glucose re-addition to starved *Saccharomyces cerevisiae* has been extensively studied and shown to produce an array of signaling events. Among these are the cAMP mediated activation of protein kinase A [1], the deactivation of casein kinase [2], the activation of protein kinase C [3], the induction of calcium influx and efflux [1,4] and the induction of an extracellular calcium dependent cytosolic calcium signal [5,6]. Glucose re-addition mediated calcium uptake is eliminated by the addition of protonophores [7]. The glucose activated transient elevation of cytosolic calcium (TECC) is dependent on glucose uptake and phosphorylation [8]. Glucose, in the process of glucose re-addition, causes an initiation of cell proliferation in carbohydrate starved yeast similarly as growth factors in mammalian cells [1,9]. Accordingly, this experimental model is useful for gaining insights into cell cycle control.

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Abbreviations: TECC, transient elevation of cytoplasmic calcium; PMA, plasma membrane H^+-ATPases; V-ATPase, vacuolar H^+-ATPase; PKC, protein kinase C; PLC, phospholipase C

The addition of glucose to starved *Saccharomyces* causes a transient decrease in cytosolic pH due to glycolysis [9,10], which may lead to the induction of pH regulatory mechanisms [11]. These include the fast activation of the re-assembly of the V_0 and V_1 sub-units of the vacuolar H^+-ATPase (V-ATPase) [12,13] and the activation of the plasma membrane H^+-ATPases (PMA) via phosphorylation [14] by both Ca^{2+}/calmodulin dependent kinases and protein kinase C (PKC1) [15]. There are two plasma membrane H^+-ATPase isoforms in *S. cerevisiae*, PMA1 and PMA2, among which PMA1 is the major and accounts for practically the total plasma membrane H^+-ATPase activity [16]. The coordinative action of cytosolic hydrogen extrusion between the vacuolar- and plasma membrane ATPases has been shown in the pH regulation of *S. cerevisiae* and *Neurospora crassa* [10,17,18].

The consecutive activation of calcium signaling and pH regulation following glucose re-admission raises intriguing possibilities of the two influencing each other. The vacuolar Ca^{2+}/H^+ exchanger VCX1 has been shown to mediate short-term calcium sequestration in *S. cerevisiae* [19,20]. Additionally, the disruption of genes encoding for V-ATPase subunits (Vma^- mutants) leads to a Ca^{2+} sensitive phenotype in *S. cerevisiae* and *PMA1* mutants have also been shown to be calcium sensitive [21,22]. On the contrary, *vcx1*Δ mutants do not show a Ca^{2+} phenotype [20,23]. These observations describe an important relationship between pH regulation and Ca^{2+} homeostasis in yeast.

This study addressed the compartmentalization of Ca^{2+} following glucose re-addition and the possible effects of pH regulation on this process. Three major Ca^{2+} transporters were studied in *S. cerevisiae*. Namely, the roles of the vacuolar Ca^{2+}/H^+ exchanger VCX1 [23]; the vacuolar Ca^{2+} ATPase PMC1 [24]; and the Ca^{2+} ATPase of the Golgi-ER compartment PMR1 [25] were evaluated. Both the vacuole (through the activity of VCX1) and the Golgi-ER compartment (through the activity of PMR1) were found to compartmentalize Ca^{2+} following glucose re-administration in carbohydrate starved cells. Additionally, the effects of V-ATPase and PMA inhibition on the TECC response were tested. In *WT* and *vcx1*Δ mutants, cytosolic H^+ extrusion greatly influenced free cytosolic Ca^{2+} concentrations following glucose re-addition in yeast. In light of these findings a model is proposed, where pH dependent cytosolic Ca^{2+} buffering plays an important role in controlling free Ca^{2+} levels in *S. cerevisiae* following glucose starvation and re-addition.

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2. Materials and methods

2.1. Strains and media

Strains used in this study are enlisted in Table 1. For all measurements strains were grown in either liquid rich medium (YPD) or synthetic medium supplemented with 2% glucose (SMD) and amino acids as required [26].

2.2. Glucose starvation

Glucose starvation was carried out in the SEY6210 background according to Kaibuchi et al. [1]. Briefly, strains were grown to ~0.3 OD_{600} in SMD + amino acids at 30 °C, washed with double-distilled water and transferred to SM media + amino acids containing 0.02% glucose. The strains were incubated overnight at 30 °C in shaking water-bath. After this incubation, OD_{600} was regularly under 0.7–0.8. This treatment induces cell cycle arrest at the G_0/G_1 phase [1].

In the experiments with the YPH499 background strains, glucose starvation was carried out according to Tisi et al. [6] with minor modifications. Briefly, strains were grown to OD_{600} 0.7–0.9 in YPD at 30 °C, collected and washed twice in 40 mM Mes-Tris (pH 6.5) buffer followed by a 1.5 h incubation in the same buffer at 30 °C in shaking water-bath.

2.3. Calcium uptake

Ca^{2+} uptake experiments were done similarly as described before [27] with mild modifications. Glucose starved cells were collected, washed with double-distilled water and re-suspended (1 OD_{600}/ml) into 40 mM Mes-Tris, pH 6.5. The cells were incubated for 10 min at room temperature, and then glucose re-addition mediated calcium uptake was initiated by the addition of 25 mM glucose (from 50% stock) and 1 μCi/ml ^{45}Ca (from 10 μCi/μl stock). At indicated time-points, 1 ml of the culture (1 OD_{600}) was filtered through presoaked (ice-cold wash buffer/20 mM $MgCl_2$, 0.2 mM $LaCl_3$) 0.45 μm membrane filters (GelmanSciences) on a vacuum manifold. Membranes were washed immediately two times with 5 ml of ice-cold wash buffer, dried, and the cell associated radioactivity was measured by liquid scintillation.

2.4. Aequorin experiments

In the SEY6210 background, 10 OD_{600} units of cells were collected from glucose starved strains containing high copy aequorin expressing plasmids (pEVP11-Aeq [28] or pDB617 [29]). Cells were washed with SM (no glucose), buffered with 40 mM Mes-Tris (assay medium) and re-suspended in 200 μl of this medium. 20 μl of 590 μM coelenterazine (dissolved in methanol) was added and the cells were incubated for 20 min at room temperature. They were then centrifuged briefly in a micro-centrifuge and the supernatant containing excess coelenterazine was removed. The cells were washed in 0.5 ml of aequorin assay medium, re-suspended again in assay medium supplemented with 10 mM $CaCl_2$ [5] to 1 OD_{600}/100 μl and incubated at room temperature for 20 min before initiating the experiment. For experiments using BafilomycinA_1 or Staurosporine (Sigma): BafilomycinA_1 (5 μM) [19] from a 100 μM stock solution dissolved in DMSO, or/and Staurosporine (50 μM) [15] from a 1 mM stock, or DMSO (vehicle) alone was added to 100 μl aliquots of cells (1 OD) 10 min prior to the measurements. After detecting baseline light emission, 25 mM glucose from a 125 mM stock was administered into the chamber to generate a glucose re-addition mediated cytosolic Ca^{2+} increase [5]. A Berthold Lumat 9050 luminometer was used to collect aequorin light emission data at 0.5 s intervals (L). The data were downloaded directly to a computer using the MS Windows Terminal software and transferred to Microsoft Excel 5.0 for analysis. The values were plotted such that a 10 s baseline is shown on each figure before glucose administration (thus glucose administration always occurred at 10 s). After measuring maximal light emission from crude cell extracts upon Ca^{2+} addition (L_{max}), L/L_{max} values were plotted on a previously generated standard curve to estimate the free cytosolic calcium concentrations [30]. Under normal conditions (without drug treatment), L values did not exceed ~1% of the maximal light emission capacity. Figures are representative of a minimum of three experiments in each case.

In the YPH499 background, aequorin assay experiments were carried out according to Tisi et al. and Tokes-Fuzesi et al. [6,8]. YPH499 and JY314118A strains (generous gift from Martha Cyert) were transformed with the integrative expression vector pYX012-cytAEQ-G418 (generous gift from Enzo Martegani). 10 OD units of cells were collected from starved strains, re-suspended in 200 μl of 40 mM Mes-Tris (pH 6.5) and loaded with coelenterazine as described above. Following this treatment, the experiments were carried out as described with the SEY6210 background strains with the exception that the assay medium contained 40 mM Mes-Tris (pH 6.5) and 2 mM EGTA [8]; and 100 mM glucose was administered after detecting baseline luminescence from a 500 mM stock [6]. After each experiment, the expression level of aequorin was tested by lysing cells with 0.5% Triton X-100 [6].

3. Results

3.1. Increased cytosolic calcium levels in the pmr1Δ and the vcx1Δ mutant following glucose re-addition

To test how the major intracellular Ca^{2+} transporters mediate TECC, *pmc1*Δ, *pmr1*Δ and *vcx1*Δ mutants were challenged by starvation for glucose followed by glucose re-addition in an aequorin based experiment (see Section 2). In *WT* and mutant cells, cytosolic Ca^{2+} levels started to rise after 50–60 s following glucose administration, with maximal peaks around 80–90 s (Fig. 1). Interestingly, the *pmr1*Δ mutant possessed a 1.5–2 fold higher baseline of relative light units (RLU) emitted by aequorin (free cytosolic Ca^{2+} concentrations were calculated to be ~200 nM), while baseline levels in *pmc1*Δ and *vcx1*Δ mutants were not significantly different from *WT* (~140 nM). Additionally, *pmr1*Δ and *vcx1*Δ mutants exhibited around 2-fold higher peak cytosolic Ca^{2+} concentrations (~550 ± 50 nM free Ca^{2+}) than *WT* (250 ± 50 nM free cytosolic Ca^{2+}) cells. *Pmc1*Δ mutants always showed a milder peak response in consecutive experiments than *WT* cells, leading to a maximum of ~180 ± 20 nM free cytosolic Ca^{2+}. The recovery of cytosolic Ca^{2+} levels back to the original baseline was significantly slower in the *pmr1*Δ and the *vcx1*Δ mutant than in *WT* and *pmc1*Δ cells. While the *WT* and *pmc1*Δ mutant regained basal cytosolic Ca^{2+} levels in 4–5 min, the *pmr1*Δ and *vcx1*Δ mutants maintained ~450 ± 50 nM cytosolic Ca^{2+} concentrations even after 5 min following glucose administration.

It has been shown that upon extracellular Ca^{2+} challenge in logarithmically growing yeast, mainly VCX1 mediates cytosolic calcium compartmentalization [19,20]. In the same experiment, *pmr1*Δ mutants showed a similar response as *WT*

Table 1
Strains used in the study

Strain	Relevant genotype	Complete genotype	Source
SEY6210	Wild type	*MATa*, *ura3-52*, *leu2-3 112*, *his3*-Δ200, *trp1*-Δ901, *lys2-801*, *suc2*-Δ9	DM. Bedwell
YDB224	*Pmc1*Δ	*MATa*, *ura3-52*, *leu2-3 112*, *his3*-Δ200, *trp1*-Δ901, *lys2-801*, *suc2*-Δ9, *pmc1*Δ::*TRP1*	DM. Bedwell
YDB225	*Vcx1*Δ	*MATa*, *ura3-52*, *leu2-3 112*, *his3*-Δ200, *trp1*-Δ901, *lys2-801*, *suc2*-Δ9, *vcx1*Δ::*URA3*	DM. Bedwell
YDB279	*Pmr1*Δ	*MATa*, *ura3-52*, *leu2-3 112*, *his3*-Δ200, *trp1*-Δ901, *lys2-801*, *suc2*-Δ9?, *pmr1*Δ::*LEU2*	DM. Bedwell
YPH499	Wild type	*MAT***a** *ura3-52*, *lys2-801*, *ade2-101*, *trp1*-Δ63, *his3*-Δ200, *leu2-1*	MS. Cyert
JY34118A	*pma1-21*	*MAT***a** *ura3-52*, *lys2-801*, *ade2-101*, *trp1*-Δ63, *his3*-Δ200, *leu2*-Δ1, *pma1-21*	MS. Cyert

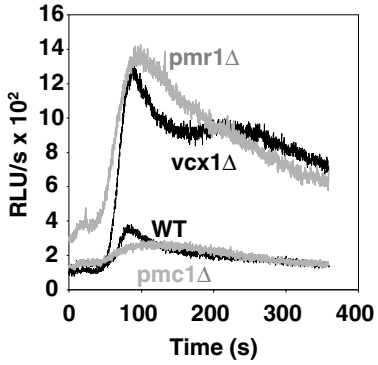

Fig. 1. Increased TECC response in the *pmr1Δ* and the *vcx1Δ* strain. Free cytosolic calcium dependent light emissions from aequorin expressing starved yeast cells following glucose re-admission were detected with a luminometer (Section 2). Glucose was administered at 10 s. RLU: relative light units.

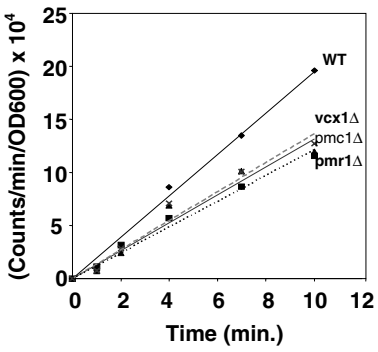

Fig. 2. Calcium uptake following glucose re-addition in *WT* and intracellular calcium transporter mutant yeast. ^{45}Ca uptake was induced by glucose administration to the carbohydrate starved strains and cell associated counts were evaluated at indicated time points.

cells did (unpublished results). Thus, it seems that VCX1 has a significant role in the fast distribution of Ca^{2+} from the cytosol both after acute changes in environmental calcium in logarithmically growing cells and following glucose re-addition to yeast arrested at the G_0/G_1 phase. Yet, PMR1 possesses an important role only in the later process.

3.2. Calcium uptake following glucose administration

The glucose re-administration mediated TECC response has been described as vastly extracellular Ca^{2+} dependent [6,8]. Consequently, increased cytosolic Ca^{2+} levels following glucose re-addition in mutant cells could be due to either increased Ca^{2+} uptake or decreased ability to distribute calcium to internal stores following the glucose exposure of starving cells. To address this question, a glucose mediated Ca^{2+} uptake experiment in starved *WT*, *pmc1*Δ, *pmr1*Δ and *vcx1*Δ mutants was conducted.

*Pmr1*Δ, *vcx1*Δ and *pmc1*Δ mutants all showed similar Ca^{2+} uptake abilities; while the Ca^{2+} uptake of *WT* cells was ~1.5-fold higher than of the mutant strains (Fig. 2). This finding eliminated the possibility of increased Ca^{2+} influx as a reason for the augmented glucose re-addition mediated TECC response in *vcx1*Δ and *pmr1*Δ mutants. Moreover, the observation suggested that it is the decreased ability to compartmentalize Ca^{2+} to vacuolar and Golgi-ER stores which causes the observed cytoplasmic free Ca^{2+} changes in the *vcx1*Δ and the *pmr1*Δ strain.

3.3. The effect of V-ATPase inhibition on the TECC response

The effect of acute V-ATPase inhibition on the TECC response of glucose starved *WT* and *vcx1*Δ mutants was tested. The selective V-ATPase-inhibitor BafilomycinA$_1$ was utilized. In earlier studies, BafilomycinA$_1$ rendered logarithmically growing *WT* cells to behave as the *vcx1*Δ mutant following a Ca^{2+} shock. The *vcx1*Δ mutant showed a further decrease in the ability to sequester calcium following the same treatment [19].

A short (10 min) pretreatment with BafilomycinA$_1$ had no significant effect on baseline Ca^{2+} levels in the *WT* or the *vcx1*Δ mutant, while peak cytosolic Ca^{2+} responses following glucose re-addition increased significantly compared to vehicle treated cells (Fig. 3). For *WT* cells, V-ATPase inhibition increased cytosolic Ca^{2+} peaks from ~200 to ~560 nM, while for *vcx1*Δ it increased peak levels from ~500 to ~630 nM. BafilomycinA$_1$ in *WT* cells regularly caused a 10–20% higher cytosolic Ca^{2+} level than the response of the *vcx1*Δ mutant with vehicle treatment, suggesting a mild, but additional role of vacuolar acidification in the glucose re-administration mediated Ca^{2+} response in the *WT* strain apart from mediating VCX1 activity. The results obtained from the *vcx1*Δ mutant showed that indeed, vacuolar acidification has a significant influence on free cytosolic calcium regulation following glucose addition to starved cells, which is independent of VCX1.

3.4. The effect of PMA inhibition on the TECC response

The observation that V-ATPase inhibition in the *vcx1*Δ mutant caused a significant increase in the cytosolic Ca^{2+} signal upon GRA raised the possibility of other putative mechanisms which utilize the proton gradient of the vacuole to compartmentalize Ca^{2+} into this organelle. Yet, it has been previously shown that vacuolar membranes isolated from *vcx1*Δ mutants are defective in BafilomycinA$_1$ sensitive, ATP dependent Ca^{2+} transport. This suggests that apart from VCX1 other direct Ca^{2+}/H^+ exchanging mechanisms do not exist in the vacuole of *S. cerevisiae* [23]. Consequently, we were interested if it might be the alteration of cytosolic H^+ extrusion

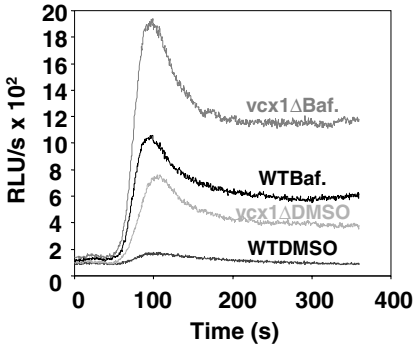

Fig. 3. The effect of BafilomycinA$_1$ on the glucose re-administration induced calcium response of the *WT* and the *vcx1*Δ mutant strain in an aequorin assay based experiment. The relative peak of light for the vehicle treated *WT* cells is low compared to the *vcx1*Δ mutant and the treated cells. Glucose was administered at 10 s. RLU: relative light units.

due to inhibited V-ATPase activity, which caused the rise in the free cytosolic calcium in our experiments. To test this hypothesis, the activation of the plasma membrane H^+ ATPases (PMA) was inhibited to see whether the hampering of the cytoplasmic H^+ extrusion by another mechanism would have the same effect on the TECC response as the inhibition of the V-ATPase.

Staurosporine has been shown to be a selective inhibitor of protein kinase C (PKC1) in *S. cerevisiae* [31] and to inhibit PMA mediated extracellular acidification following glucose re-addition [15,32]. Diacylglycerol (DAG) activated PKC plays a role in the activation of the plasma membrane H^+ ATPase following glucose re-addition to starved cells [15,33]. Thus, by inhibiting PKC activity, the extrusion of H^+ to the extracellular environment can be inhibited, leading to cytoplasmic H^+ stress. Consequently, we decided to test the effect of Staurosporine treatment on the TECC of our yeast strains.

Staurosporine treatment alone caused a significant increase in the glucose re-addition mediated calcium response both in the *WT* and in the *vcx1*Δ strains (Fig. 4). While baseline values were not affected, the peak free cytosolic calcium concentrations in *WT* cells rose from ~200 to ~350 nM, while in *vcx1*Δ mutants they rose from ~500 nM to ~1 μM following Staurosporine treatment. These observations supported our hypothesis that cytoplasmic H^+ stress due to inhibited vacuolar or plasma membrane H^+ ATPase activity may directly enhance free cytosolic calcium responses following glucose re-addition to starved yeast cells.

To further test the effects of the inhibition of H^+ extrusion on the TECC response, we decided to challenge the yeast cells by inhibiting both of the H^+ ATPase pathways at the same time. The double treatment of the cells with the inhibitors BafilomycinA$_1$ and Staurosporine caused an additive increase in free cytosolic calcium peaks and recovery values (Fig. 4). Namely, *WT* cells showed ~1 μM peak and ~650 nM recovery values after 5 min of glucose addition, while the *vcx1*Δ mutant showed ~1.2 μM peak and ~800 nM recovery free cytosolic Ca^{2+} concentrations following the simultaneous inhibition of V-ATPase and PMA activity.

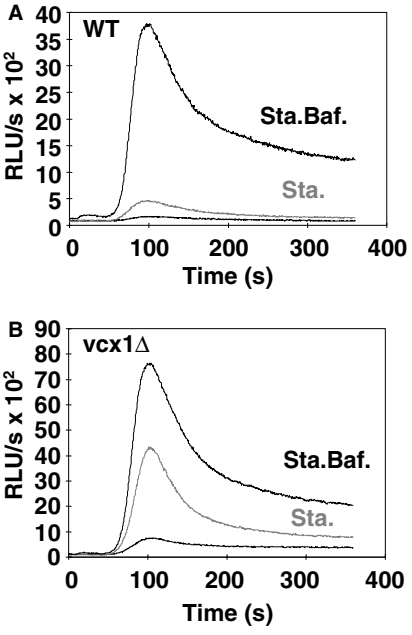

Fig. 4. Staurosporine induces augmented TECC. The effect of Staurosporine treatment alone (Sta.) or Staurosporine + BafilomycinA$_1$ (Sta. Baf.) treatment on the glucose re-addition mediated calcium response of the *WT* (A) and the *vcx1*Δ (B) mutant in an aequorin assay based experiment. The relative light activity of the vehicle treated cells was low compared to the treated cells. Glucose was administered at 10 s. RLU: relative light units.

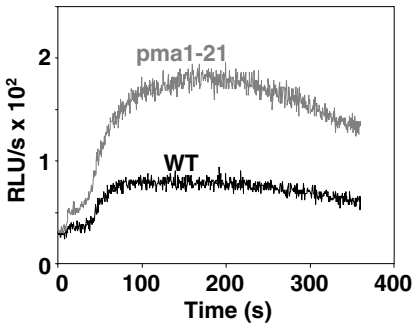

Fig. 5. Augmented TECC response in the *pma1-21* mutant. Aequorin related light emission of the strains carrying an aequorin expressing insertion vector was recorded following a short, 1.5 h carbohydrate starvation period. Glucose was administered at 10 s. RLU: relative light units.

In order to confirm the experiments with Staurosporine, the TECC response of a genetically altered *PMA1* mutant, *pma1-21*, was tested. This strain possesses decreased PMA1 activity and has been described to be sensitive to increased levels of extracellular Ca^{2+} while accumulating less cellular Ca^{2+} than *WT* yeast [21]. The *pma1-21* strain showed a significantly increased TECC response (Fig. 5). While baseline cytosolic Ca^{2+} levels were similar in the WT and the mutant strain (calculated to be approximately 130 nM), peak and recovery values of *pma1-21* cells were about 50% increased over the WT. These findings supported the results obtained with the pharmacologic inhibition of PMA activity.

4. Discussion

Both the vacuole and the Golgi-ER compartment were found to play a significant role in the distribution of the increased amounts of cytosolic Ca^{2+} following glucose re-addition in carbohydrate starved yeast cells. Namely, the vacuolar Ca^{2+}/H^+ antiporter VCX1 and the Golgi-ER Ca^{2+} ATPase PMR1 play important roles in the compartmentalization of the TECC response. Adequate levels of luminal calcium in the Golgi-ER compartment have been shown to be necessary for proper protein processing through the secretory pathway in yeast [25]. Thus, the involvement of the Golgi system in the elimination of the TECC signal following glucose re-addition could indicate a physiologic mechanism as to how calcium provides the capability of proper protein processing following a starvation period in *S. cerevisiae.*

In addition to evaluating the Ca^{2+} compartmentalization into sub-cellular organelles, the activity of the vacuolar H^+ ATPase was found to be an important factor in decreasing the Ca^{2+} signal after glucose re-addition. The importance of V-ATPase function in the Ca^{2+} homeostasis of logarithmically

growing *S. cerevisiae* has been already demonstrated [20]. This study revealed that following glucose addition to starving yeast, V-ATPase activity bears additional roles even in *WT* cells than just mediating Ca^{2+}/H^+ exchange through VCX1. The observations in the *vcx1*Δ mutant strain further supported the independent role of the V-ATPase in the Ca^{2+} homeostasis of starving yeast. Consecutively, it was observed that the pharmacologic inhibition of the plasma membrane H^+-ATPase had a similar effect on increasing the intensity of the cytoplasmic Ca^{2+} signal in both the *WT* and in the *vcx1*Δ mutant. The simultaneous blockage of the activation of both ATPases caused an even more drastic, additive increase in this response. Experiments with a *pma1-21* mutant supported the results obtained with Staurosporine treatment. Consequently, decreasing the ability of starving yeast cells to properly sequester H^+ from the cytosol following glucose administration induces augmented TECC. It should be emphasized that the inhibition of cytosolic H^+ extrusion following glucose re-addition only had an impact on the level of free cytosolic Ca^{2+} responses and not the pattern of it. This suggests that the concomitant pH regulatory changes following the provision of glucose to starved cells greatly modulate the TECC response in yeast. Such correlations have been indicated in earlier studies also [9].

Cytosolic calcium buffering has been described in various mammalian cells to bind 90–99.9% of the total cytosolic calcium, leaving only 0.1–10% free depending on the cell type [34]. The large amount of calcium buffering proteins not only protect cells from calcium toxicity, but may also be involved in setting parameters of intracellular Ca^{2+} signaling [35]. The importance of intracellular pH for calcium handling in the cytoplasm has been demonstrated [36]. Indeed, the consecutive decrease of cytosolic pH and increase in $[Ca^{2+}]_c$ has been reported in several mammalian studies [37,38] and the possibility of cytosolic buffers to bind Ca^{2+} and H^+ with the same affinity has been proposed [39]. Additionally, pH changes can modulate the calcium binding ability of proteins most likely through inducing conformational changes providing a greater calcium binding ability when pH increases [40]. A model based on the reports above, where H^+ and Ca^{2+} are competing for buffering sites in the cytoplasm of yeast cells, could explain our observations. Namely, inhibition of H+ extrusion would cause a significant rise in the cytosolic free calcium levels by competing with calcium for the negatively charged cytosolic protein residues. Consequently, free cytosolic calcium levels would increase when pH decreases. A pH dependent conformational change decreasing the calcium binding ability of the cytoplasmic proteins under these conditions would even augment the calcium signals. This would mean that the dissipation of the TECC not only occurs through sub-cellular compartmentalization into the vacuole and the Golgi-ER complex but through cytoplasmic protein buffering also. Hence, the cytoplasmic buffering proteins may participate in the calcium homeostasis of yeast as an additional, dynamic, sub-cellular compartment.

Phospholipase C (PLC1) activity has been shown to be necessary for the development of the TECC response following glucose re-addition in *S. cerevisiae* [6]. PLC1 appears to elucidate this function through inducing IP3 production in cooperation with the GPR1/GPA2 G-protein coupled receptor complex once glucose is phosphorylated [6,41,42]. A direct involvement of phospholipase C in the activation of protein kinase C has been described in mammalian cells [43]. Yet, in regards of mechanisms which induce calcium signaling – such as hypotonic shock – a direct interaction between PLC1 and PKC1 could not be shown in yeast [6]. On the contrary, several studies suggest a PLC1 mediated PKC1 activation to induce the activity of the plasma membrane H^+-ATPase following glucose re-addition in *S. cerevisiae* [3,15,33]. These studies and the observations of this report suggest that the direct activity of PLC1 in inducing the extracellular calcium dependent TECC signal is independent of PKC1, while the PLC1 activated PKC1-PMA pathway can indirectly modulate the TECC response once it occurs.

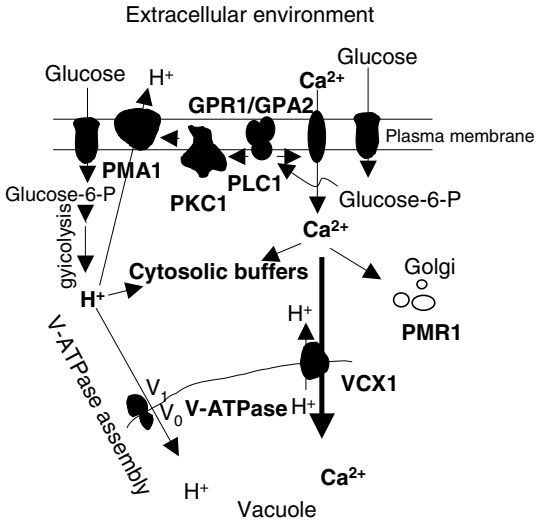

Fig. 6. Schematic model of the interaction between $[H^+]_c$ and $[Ca^{2+}]_c$ in starved *S. cerevisiae* following glucose re-addition. Cytosolic H^+ stress due to glycolysis is relieved by V-ATPase re-assembly, PMA1 activation (through phosphorylation by PKC1 and calmodulin dependent kinases) and cytosolic H^+ buffering. TECC (due to influx from the extracellular environment) is dissipated to the Golgi apparatus (through PMR1 activity) and to the vacuole (through VCX1 activity), while a proportion of it is bound to cytosolic buffering sites which are modulated by $[H^+]_c$ both by competing with calcium for negatively charged protein residues and also by inducing conformational change in the calcium binding proteins. Phosphorylated glucose activates PLC1 in complex with GPR1/GPA2. Activated PLC1 then induces the TECC response and activates PKC1 which phosphorylates PMA1.

This study proposes a delicate regulatory process involving a cooperative cytoplasmic H^+- and Ca^{2+} buffering protein network which acts in addition to the VCX1 mediated vacuolar- and the PMR1 mediated Golgi-ER compartmentalization of calcium following glucose admission to starved yeast. Additionally, a dual role of PLC1 is indicated in yeast in the development of the TECC signal. A direct one, independent of PKC1 and an indirect one, which manifests through the PKC1 mediated activation of the plasma membrane H^+-ATPases. In the latter process, PLC1 activity could augment cytoplasmic calcium buffering by inducing H^+ extrusion and intracellular pH elevation (Fig. 6). Such a system may promote the fine tuning of concomitant pH regulation and calcium signaling following glucose re-addition to starved yeast cells to optimize cell cycle initiation.

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