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Review

Acidic calcium stores of *Saccharomyces cerevisiae*

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ABSTRACT

Fungi and animals constitute sister kingdoms in the eukaryotic domain of life. The major classes of transporters, channels, sensors, and effectors that move and respond to calcium ions were already highly networked in the common ancestor of fungi and animals. Since that time, some key components of the network have been moved, altered, relocalized, lost, or duplicated in the fungal and animal lineages and at the same time some of the regulatory circuitry has been dramatically rewired. Today the calcium transport and signaling networks in fungi provide a fresh perspective on the scene that has emerged from studies of the network in animal cells. This review provides an overview of calcium signaling networks in fungi, particularly the model yeast *Saccharomyces cerevisiae*, with special attention to the dominant roles of acidic calcium stores in fungal cell physiology.

1. Calcium signaling pathways in the fungal cytoplasm

Dozens of genome sequencing projects on phylogenetically diverse fungal species have revealed a basic toolkit of Ca^{2+}-binding proteins and Ca^{2+} pumps, exchangers, and channels and the remarkable conservation of the signaling network across the fungal kingdom [1]. The central Ca^{2+} sensor calmodulin can be easily spotted in the genomes of all sequenced fungi. Despite some lineage-specific drift in the amino acid sequence of yeast calmodulins, many targets of Ca^{2+}/calmodulin are also well preserved throughout the kingdom. Examples include two families of serine/threonine protein kinases and a family of serine/threonine protein phosphatases known as calcineurin. As in animal cells, these kinases and phosphatases in the bakers yeast *Saccharomyces cerevisiae* and in other fungi become activated upon binding of Ca^{2+}/calmodulin to conserved sequences within their autoregulatory tails and displacement of autoinhibitory motifs from their active sites (reviewed in [2,3]). Thus, the rise and fall of free Ca^{2+} concentrations in the cytoplasm can be directly sensed, decoded, and retransmitted to cellular targets through regulated protein phosphorylation and dephosphorylation. Myosins and other well-known targets of calmodulin have also been described in *S. cerevisiae* and many other fungi. Additionally, the genomes contain a spectrum of conserved proteins that bear EF-hand and C2 domains, which bind Ca^{2+} and may respond to fluctuation Ca^{2+} concentrations in their microenvironments. The emerging picture from these accounts is one where a multitude of Ca^{2+}-responsive regulatory pathways exist in fungal cells.

One of the best-studied Ca^{2+}-responsive signaling pathways in fungi (see Fig. 1) involves the calcineurin-dependent dephosphorylation of Crz1, a zinc-finger transcription factor first described in *S. cerevisiae* [4,5]. Crz1 is not related to the NFAT family of calcineurin-sensitive transcription factors that famously control many calcineurin-dependent processes in mammals. Similar to the NFAT story, activated calcineurin binds to canonical PxIxIT-like motifs and dephosphorylates several residues in Crz1, resulting in a conformational change that hides a nuclear export signal and exposes a nuclear localization signal (respectively recognized

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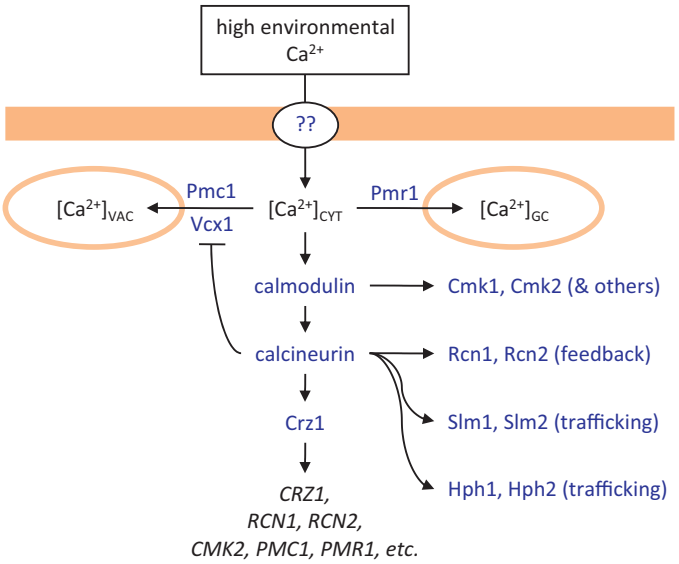

Fig. 1. Ca^{2+} transport and signaling in *S. cerevisiae* cells exposed to high environmental Ca^{2+}. Ca^{2+} enters the cell through unknown pathways and elevates $[Ca^{2+}]_{CYT}$, which results in activation of calmodulin, calcineurin, and a transcription factor (Crz1) that induces numerous genes. Activated calmodulin and calcineurin also regulate other cellular factors such as protein kinases (Cmk1, Cmk2), feedback regulators of calcineurin (Rcn1, Rcn2), membrane trafficking factors (Slm1, Slm2, Hph1, Hph2), and a vacuolar Ca^{2+}/H^+ exchanger (Vcx1). Ca^{2+} pumps in the vacuole (Pmc1) and the Golgi complex (Pmr1) become up-regulated and help to lower $[Ca^{2+}]_{CYT}$ to non-toxic levels.

by the β-importins Msn5 and Nmd5) [6–8]. After transport of dephosphorylated Crz1 into the nucleus, the Zn-finger domain binds specific DNA sequences (termed CDREs for Crz1-dependent response elements) present within the promoter regions of target genes and significantly increases expression above the resting basal level [6–12]. Inducible targets of Crz1 include *CRZ1* that encodes Crz1 [5], *RCN1* and *RCN2* that encode positive and negative regulators of calcineurin [13,14], *CMK2* that encodes a Ca^{2+}/calmodulin-dependent protein kinase [15], *PMC1*, *PMR1*, and *ENA1* that encode several P-type cation pumps responsible for efflux of Ca^{2+}, Mn^{2+}, Na^+, and Li^+ [5] and 60–100 other genes that control other processes [16,17]. Mutants of *S. cerevisiae* that lack Crz1 are hypersensitive to high environmental concentrations of these cations as a consequence of failed induction of the cation pumps and they exhibit other phenotypes that can be attributed to failed induction of other targets. Calcineurin-deficient mutants of *S. cerevisiae* exhibit an even larger set of phenotypes due to defects in the regulation of phosphoproteins other than Crz1. For example, calcineurin-dependent dephosphorylation of Hph1/Hph2 and Slm1/Slm2 protein pairs can alter sensitivity to high pH medium and alter trafficking of secretory and endocytic cargo proteins [18,19]. Calcineurin-dependent feedback regulation of Ca^{2+} channels and Ca^{2+} transporters will be discussed more fully later in this review. An overview of the known and suspected calcineurin targets in *S. cerevisiae* is given in Fig. 1.

In vegetatively growing *S. cerevisiae* cells, Crz1 is fully phosphorylated, localized to the cytoplasm, and transcriptionally inactive [16,20]. The simple inference from all these findings is that cytosolic free Ca^{2+} concentrations are maintained at low non-signaling levels in vegetatively growing *S. cerevisiae* cells and that $[Ca^{2+}]_{CYT}$ levels rise to levels capable of activating calcineurin and Crz1 in response to specific stimuli or stresses. Thus, cells of *S. cerevisiae* and its relatives probably behave very much like mammalian cells in their ability to dynamically control $[Ca^{2+}]_{CYT}$ and the downstream signaling pathways. A series of conserved Ca^{2+} pumps, exchangers, transporters, and channels accomplish that important task in fungal and animal cells. They also control intracellular pools of Ca^{2+} that have many important functions.

2. Ca^{2+} in secretory organelles and store-operated Ca^{2+} influx

The increasingly acidic lumens of the nuclear envelope, endoplasmic reticulum, Golgi complex, and trans-Golgi/endosomal network contain an array of conserved Ca^{2+} dependent enzymes that are involved in various aspects of protein secretion (reviewed in [21]). For instance, *S. cerevisiae* retains homologs of BiP, calnexin, UDP–glucose–glucosyltransferase, glucosidase II, and ERGIC-53 (termed Kar2, Cne1, Kre6, Rot2, and Emp46/47, respectively) in its nuclear envelope and endoplasmic reticulum [22–24]. In *S. cerevisiae*, many of these enzymes no longer retain an ability to bind Ca^{2+} and can function independently of Ca^{2+}. Such adaptations are probably related to the loss of SERCA-family Ca^{2+} pumps that normally supply the endoplasmic reticulum with sufficient Ca^{2+} for secretory functions. SERCA was probably present in the common ancestor of fungi and animals and subsequently lost several different times independently in the evolution of Ascomycetes (moulds, yeasts), Basidiomycetes (mushrooms, smuts, rusts), and other fungal phyla. A SERCA-family Ca^{2+} pump is expressed in the endoplasmic reticulum of the mould *Neurospora crassa* [25] but this enzyme has not yet been characterized biochemically or genetically. Though *S. cerevisiae* and other budding yeasts do not retain a SERCA-family Ca^{2+} pump and may have secretory machinery with reduced Ca^{2+} dependence, Ca^{2+} starvation of *S. cerevisiae* cells still causes activation of the so-called Unfolded Protein Response (UPR) signaling pathway that emanates from the endoplasmic reticulum upon its accumulation of misfolded or unassembled secretory proteins [26]. Inhibitors of SERCA elicit similar UPR responses in animal cells. Ca^{2+} starvation can also decrease the retention of foreign proteins expressed in yeasts [27] and thereby enhance the yield of recombinant protein preparations. Thus, luminal Ca^{2+} performs important secretory functions in the fungal endoplasmic reticulum, even in *S. cerevisiae* where both the supply and the demand seem greatly diminished.

The endoplasmic reticulum of *S. cerevisiae* concentrates Ca^{2+} approximately 100-fold relative to the cytoplasm largely through the Ca^{2+} transport activity of Pmr1 [28], the prototypical member of the SPCA-family of Ca^{2+}/Mn^{2+} pumps that are widely distributed among fungi, animals, and other eukaryotic kingdoms. Pmr1 localizes primarily to the Golgi complex of *S. cerevisiae* [27,29], like its homologs in mammals [30], and therefore supplies Ca^{2+} and Mn^{2+} to the endoplasmic reticulum during its early biogenesis or through vesicle-mediated trafficking in the retrograde direction from the Golgi complex. Mutants of *S. cerevisiae* that lack the Pmr1 exhibit a range of secretion defects that can be largely attributed to underperformance of late secretory pathway enzymes. A homolog of the Ca^{2+}-dependent pro-protein convertases or furins (termed Kex2) is highly dependent on Pmr1 function [29,31]. A Ca^{2+}-dependent lectin-like protein involved in sorting of specific cargo proteins to the lysosome-like vacuole (termed Vps10) also depends on Pmr1 for proper function [32]. The normal retention of foreign secretory proteins that are expressed heterologously in *S. cerevisiae* also depends on Pmr1 [27,33]. These defects of Pmr1-deficient mutants can be suppressed by elevating Ca^{2+} salts in the culture medium or by expressing SERCA in the endoplasmic reticulum, suggesting they are specifically a consequence of luminal Ca^{2+} insufficiency [32]. On the other hand, defects in N-glycosylation and O-glycosylation of secretory cargo are attributable to Mn^{2+} insufficiency in the Golgi complex [32]. All these findings are consistent with the hypothesis that the SPCA-family pump Pmr1 supplies the majority of the Ca^{2+} and Mn^{2+} that is crucial for normal processing functions in both the endoplasmic reticulum and the Golgi complex of *S. cerevisiae*.