The calcium ion (Ca$^{2+}$) is used as a major signaling molecule in a diverse range of eukaryotic cells including several human parasitic protozoa, such as Trypanosoma cruzi, Trypanosoma brucei, Leishmania spp, Plasmodium spp, Toxoplasma gondii, Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis. Ca$^{2+}$ is critical for invasion of intracellular parasites, and its cytosolic concentration is regulated by the concerted operation of several transporters present in the plasma membrane, endoplasmic reticulum, mitochondria and acidocalcisomes. Recent findings have shed light on the function of these transporters, the roles that they play in cellular metabolism and their potential use for targeting them for new therapies.

**Introduction**

Intracellular calcium plays a crucial role as a second messenger for the control of a variety of cell functions in eukaryotes, including contraction, secretion, cell division and differentiation, and sodium and potassium permeability. The uptake and release of the calcium ion (Ca$^{2+}$) across the plasma membrane and intracellular organelles by the concerted operation of distinct calcium-transporting systems control the intracellular Ca$^{2+}$ concentration. Many aspects of calcium regulation are strikingly similar in diverse cell types. In this review, we focus on distinct peculiarities of the calcium homeostatic mechanisms of parasitic protozoa.

**Ca$^{2+}$ transport across the plasma membrane**

Ca$^{2+}$ gains access into eukaryotic cells across the plasma membrane through several channels (Figure 1), some of which are under the control of receptors (receptor-operated Ca$^{2+}$ channels), the potential across the plasma membrane (voltage-gated Ca$^{2+}$ channels) and the content of intracellular Ca$^{2+}$ stores (store-operated Ca$^{2+}$ channels), whereas others appear to be nonselective leak channels. Although voltage-dependent Ca$^{2+}$ channels have been detected in free-living protozoa, no such channels have yet been reported in any parasitic protozoa, including the malaria parasite, the genome of which has been completed [1**], suggesting that they might be different from their mammalian counterparts. There is also no evidence for a store-operated Ca$^{2+}$ channel and only one report suggests the activity of an L-glutamate receptor subtype specific for N-methyl-D-aspartate in Trypanosoma cruzi [2]. Receptors of this subtype control the voltage-dependent uptake of Ca$^{2+}$ in neural cells. Calcium entry in Trypanosoma brucei bloodstream trypomastigotes is apparently regulated via a novel signaling pathway involving phospholipase A2-mediated generation of arachidonic acid and stimulation of a plasma membrane-located calcium channel [3,4]. A Ca$^{2+}$ channel has been postulated to be inserted in the plasma membrane of erythrocytes infected with Plasmodium falciparum [5].

The active export of calcium from eukaryotic cells is accomplished by the action of a Na$^+$/Ca$^{2+}$ exchanger and a plasma membrane Ca$^{2+}$-ATPase (PMCA). Although there is no molecular evidence for the presence of a Na$^+$/Ca$^{2+}$ exchanger in any parasitic protozoa, there are several reports of PMCA-type Ca$^{2+}$-ATPases in different protozoan parasites (Table 1). Mammalian PMCA Ca$^{2+}$-ATPases are activated by the Ca$^{2+}$-binding protein calmodulin, and biochemical evidence for calmodulin stimulation has been reported for the enzymes from T. cruzi [6], Leishmania braziliensis [7] and Leishmania donovani [8]. The PMCA-type Ca$^{2+}$ ATPases described to date at the molecular level (Table 1) appear to lack a typical calmodulin-binding domain; this might suggest the presence of a different domain able to bind calmodulin, or a non-calmodulin-dependent regulation.

**Ca$^{2+}$-binding proteins**

Once inside the cell, Ca$^{2+}$ can either interact with so-called soluble Ca$^{2+}$-binding proteins or become sequestered into intracellular organelles. Some of these Ca$^{2+}$-binding proteins such as calmodulin act as Ca$^{2+}$ receptors. Other proteins appear to act as Ca$^{2+}$-storing devices (e.g. calsequestrin and calreticulin families). Calmodulin and other Ca$^{2+}$-binding proteins have been found in all parasitic protozoa that have been investigated (Table 2). Interestingly, the flagellar Ca$^{2+}$-binding protein (FCaBP) from T. cruzi is lipid modified. Although its function is
unknown, FCaBP has been shown to associate with the flagellar membrane in a calcium-dependent manner, reminiscent of the recoverin family of calcium-myristoyl switch proteins [9].

**Intracellular Ca²⁺ sequestration by organelles**

**Endoplasmic reticulum**

The largest store of Ca²⁺ in cells is usually found in the endoplasmic reticulum (ER), with local concentrations reaching millimolar levels. The ER also possesses two independent pathways for calcium influx and efflux. The influx is catalyzed by the well-studied sarco-endoplasmic reticulum Ca²⁺-ATPase (SERCA) that actively translocates 2Ca²⁺ for the hydrolysis of 1 ATP molecule. Several SERCA-type Ca²⁺-ATPases have been described in different parasitic protozoa (Table 1). A characteristic of some of these enzymes, especially in some trypanosomatids, is its insensitivity to thapsigargin, a specific inhibitor of SERCA-type Ca²⁺-ATPases of other eukaryotes [10]. The **Leishmania amazonensis** SERCA appears to be a virulence factor [11]. The ER has several distinct types of messenger-activated channels that are involved in the release of Ca²⁺. In parasitic protozoa, inositol 1,4,5-trisphosphate (InsP₃) releases Ca²⁺ from intracellular stores of malaria parasites [12] and *Entamoeba histolytica* [13] but was ineffective in trypanosomatids [14,15]. Inositol 1,3,4,5-tetraphosphate (InsP₄) was also shown to release Ca²⁺ from intracellular stores of *E. histolytica* [16]. InsP₃/ryanodine-sensitive stores have been postulated to be present in *Toxoplasma gondii* on the basis of pharmacological studies [17]. However, molecular evidence for the presence of an InsP₃ or ryanodine receptor homologous to those of mammalian cells in any parasitic protozoa, has not yet been provided. This probably means that these receptors might be different from their mammalian counterparts.

**Nucleus**

The transport of Ca²⁺ across the nuclear membrane has been the subject of much controversy. Large proteins permeate the nuclear membrane through the nuclear pores, although some authors have shown that the movement of Ca²⁺ might be restricted and require a SERCA-type pump. Studies in *T. brucei* using the calcium sensitive protein aequorin [18] have shown that calcium moves into and out of the nucleus in a manner that closely parallels changes in cytosolic Ca²⁺, therefore ruling out active accumulation of Ca²⁺ into the nucleus.

**Mitochondria**

Mitochondria possess a high capacity to sequester Ca²⁺, although under physiological conditions total mitochondrial Ca²⁺ levels and free Ca²⁺ reflect and parallel cytosolic Ca²⁺. This has also been demonstrated in *T. brucei* using recombinant aequorins [19]. The inner mitochondrial membrane possesses a uniport carrier for calcium, which allows the electrogenic entry of the cation driven by the electrochemical gradient generated by respiration or ATP hydrolysis. Calcium efflux, however, takes place by a different pathway, which appears to catalyze the electroneutral exchange of internal calcium by external sodium or protons. Biochemical evidence for Ca²⁺ uptake and for Ca²⁺-release channels is available in several parasitic protozoa including trypanosomatids [20–22] and apicomplexan parasites [23]. Other parasitic protozoa either do not have mitochondria (e.g. *Giardia lamblia*) or have homologous organelles such as the mitosome/cryp-tome of *E. histolytica* or the hydrogenosome of trichomonads [24]. The possible function in Ca²⁺ homeostasis of these novel organelles has not been studied until now.
Unlike mammalian mitochondria, where intracellular Ca\(^{2+}\) regulates the activity of several dehydrogenases, no such Ca\(^{2+}\)-regulated dehydrogenases have been reported in parasitic protozoa [20–22] and the elucidation of the role of intramitochondrial Ca\(^{2+}\) in these protozoa is a challenge that awaits further study.

**Acidocalcisomes**

The largest store of Ca\(^{2+}\) in many trypanosomatid and apicomplexan protozoa is found in the acidocalcisomes [25]. These are acidic calcium-storage organelles found in a diverse range of microorganisms, including algae [26] and slime molds [27], but absent in mammalian cells. They are characterized by their acidic nature, high density (both in weight and by electron microscopy) and high content of pyrophosphate, polyphosphate (polyP), calcium, magnesium and other elements [25,28,29]. Acidocalcisomes have been shown to possess a PMCA-type Ca\(^{2+}\)-ATPase, involved in Ca\(^{2+}\) influx, with similarity to vacuolar Ca\(^{2+}\)-ATPases of other unicellular eukaryotes, and a vacuolar H\(^{+}\)-pyrophosphatase, involved in their acidification [25]. In some protozoa, acidocalcisomes also possess a vacuolar H\(^{+}\)-ATPase and a Na\(^{+}\)/H\(^{+}\) exchanger [25]. A Ca\(^{2+}\)/H\(^{+}\) exchanger has been postulated to be...

**Table 1**

<table>
<thead>
<tr>
<th>Protozoa</th>
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<th>Accession number</th>
<th>Type</th>
<th>Expressed</th>
<th>Function confirmed*</th>
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<td>Yes</td>
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</table>

*Function confirmed by expression in the same parasite or in a heterologous system and correlation found between expression of the enzyme and Ca\(^{2+}\) transport.

Luo S., Uyemura SA., Moreno SNJ. and Docampo R., unpublished results.

Partial sequence in GenBank.

**Table 2**

<table>
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<th>Expected or demonstrated function</th>
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</thead>
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<td></td>
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<td>Leishmania donovani</td>
<td>Calreticulin</td>
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<td>Cell signaling</td>
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<tr>
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<td>Calflagin</td>
<td>U06644</td>
<td>Unknown</td>
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<tr>
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<td>Trichomonas vaginalis</td>
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</tbody>
</table>

*CaM, calmodulin.

Partial sequence.
involved in \( \text{Ca}^{2+} \) efflux on the basis of physiological studies [30]. In this regard, a gene encoding a \( \text{Ca}^{2+}/\text{H}^+ \) exchanger has been found in the genome of \textit{Plasmodium falciparum} [1**] but the localization of the protein has not been investigated. No second messenger has been demonstrated to be involved in \( \text{Ca}^{2+} \) release from acidocalcisomes [25*]. Although their \( \text{Ca}^{2+} \) content is very high (probably in the molar range), most of it is bound to polyP and can be released only upon alkalization [25*] or after polyP hydrolysis [28].

\textbf{Ca}^{2+} \text{ signaling in parasitic protozoa}

Changes in the intracellular \( \text{Ca}^{2+} \) concentration of \textit{T. cruzi} [31], \textit{L. amazonensis} [32] and \textit{T. gondii} [33] during their interaction with host cells have been demonstrated directly. When \( \text{Ca}^{2+} \) transients are prevented by intracellular \( \text{Ca}^{2+} \) chelators a decrease in host invasion by \textit{T. cruzi} trypomastigotes [31], \textit{L. amazonensis} amastigotes [32] or \textit{T. gondii} tachyzoites [33] is observed. Changes in intracellular \( \text{Ca}^{2+} \) have also been shown to be involved in \textit{T. gondii} egress from the host cells on the basis of the use of calcium ionophores [34]. This reveals the importance of intracellular \( \text{Ca}^{2+} \) in the process of parasite–host cell interaction. The stimulus and the sources for these \( \text{Ca}^{2+} \) changes are not known. In the case of \textit{T. gondii}, this increase in intracellular \( \text{Ca}^{2+} \) is required for microneme secretion [35] and conoid extrusion [36]. A role for \( \text{Ca}^{2+} \) signaling in differentiation has been postulated on the basis of changes in intracellular \( \text{Ca}^{2+} \) observed upon differentiation of \textit{T. cruzi} [37], \textit{T. brucei} [38] and \textit{L. donovani} [39]. \( \text{Ca}^{2+} \) signaling has also been postulated to be involved in differentiation of \textit{Plasmodium gallinaceum} zygotes to ookinetes [40], and in excystation of \textit{G. lamblia} on the basis of the use of inhibitors [41]. A \( \text{Ca}^{2+} \)-dependent cell-death pathway has also been described in \textit{T. brucei} [42]. \( \text{Ca}^{2+} \) was found to modulate promoter occupancy by the \textit{E. histolytica} \( \text{Ca}^{2+} \)-binding transcription factor URE3-BP suggesting a role of this ion in the control of gene expression [43]. A calcium-dependent modulation by melatonin of the circadian rhythm in malarial parasites has also been described [44].

\textbf{Conclusions}

\( \text{Ca}^{2+} \) regulation in parasitic protozoa differs in several aspects from the processes that occur in other eukaryotic cells, providing great opportunities for targeting them for new therapies. Acidocalcisomes are distinct calcium-storage organelles present in trypanosomatids and apicomplexan parasites and absent in mammalian cells. The calcium in these organelles is mostly bound to polyP, although no information is available on second messengers involved in \( \text{Ca}^{2+} \) release. Mitochondria are absent in several parasitic protozoa or have been replaced by homologous organelles, such as mitosomes and hydrogenosomes, with unknown roles in calcium homeostasis, but with metabolic pathways that are absent in mammalian cells. The role of intramitochondrial calcium is unknown in parasitic protozoa possessing mitochondria, as they apparently lack calcium-stimulated dehydrogenases. \( \text{Ca}^{2+} \)-ATPases are present but significantly different from their mammalian counterparts. SERCA-type \( \text{Ca}^{2+} \)-ATPases are in many cases insensitive to thapsigargin, a common inhibitor of the mammalian pumps, whereas PMCA-type \( \text{Ca}^{2+} \)-ATPases that also localize to acidocalcisomes, do not possess typical calmodulin-binding domains. The stimulation of microneme secretion by intracellular \( \text{Ca}^{2+} \) increase in \textit{T. gondii} is the only well-established function for calcium during invasion of mammalian cells by intracellular parasites. However, with the information provided by microbial genome sequencing and further work in the field, we might soon be able to predict many other functions and direct such information to effective therapeutic agents against specific pathways within protozoan parasites. Specially amenable to inhibition will be the mechanisms involved in \( \text{Ca}^{2+} \) storage in compartments like the acidocalcisomes and \( \text{Ca}^{2+} \) signaling pathways which are absent in mammalian cells.

\textbf{Update}

Recent work has revealed the presence of acidocalcisomes in the bacterium \textit{Agrobacterium tumefaciens} [45**]. Acidocalcisomes are therefore the first organelles described in prokaryotes that are also present in eukaryotes, and the results suggest that they arose before the prokaryotic and eukaryotic lineages diverged. \textit{Trypanosoma evansi}, an important animal parasite, was also shown to possess acidocalcisomes [46]. Using fluorescent dyes that compartmentalize in the food vacuole of the malaria parasite \textit{Plasmodium falciparum}, it was found that this organelle acts as a calcium store [47] similar to the previously described acidocalcisomes [25*] or acidic stores [48] of malaria parasites. Malaria parasites were described to be in contact with a high \( \text{Ca}^{2+} \) concentration in the parasitophorous vacuole (~40 \( \mu \text{M} \)) for at least one hour after invasion of erythrocytes, thus having access to an external source of \( \text{Ca}^{2+} \) for cell signaling [49]. A new calcium-binding protein (gGSP/AF293411), which localizes to the encystation-specific secretory granules was discovered in \textit{Giardia lamblia} [50].

\textbf{Acknowledgements}

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\textbf{References and recommended reading}

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

This paper reports the complete genome sequence of *Plasmodium falciparum*.


This review provides a general overview of the structure and function of acidocalcisomes in trypanosomatid and apicomplexan parasites.


45. Seufferheld M, Vieira MCF, Ruiz FA, Rodrigues CO, Moreno SNJ, Docampo R: Identification of organelles in bacteria similar to acidocalcisomes of unicellular eukaryotes. J Biol Chem, published online 3rd June 2003, DOI: 304548200. This is the first report of the presence of a discrete organelle in bacteria that has been conserved in eukaryotes.


