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Calcium storage and function in apicomplexan parasites

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Abstract

Calcium is relevant for several vital functions in apicomplexan parasites including host cell invasion, parasite motility, and differentiation. The endoplasmic reticulum (ER) and calcium-rich acidocalcisomes have been identified as major calcium stores. Other potential calcium storage organelles include the Golgi, the mitochondrion, the apicoplast, and the recently described plant-like vacuole in *T. gondii*. Compared to most eukaryotic systems, apicomplexan parasites contain a reduced number of calcium-related genes, a vast majority of which remain uncharacterized. Several Ca^{2+}-ATPases have been described in apicomplexans, several of which are annotated in the different genomes. There is experimental evidence for an inositol 1,4,5-trisphosphate (IP_3)-dependent calcium response in *Plasmodium* spp. and *T. gondii* although no IP_3 or ryanodine receptors have been identified. Genes encoding potential calcium channels are present in *T. gondi* but not in *Plasmodium* spp. and *Cryptosporidium* spp. Effector calcium binding proteins including calmodulins and calcium-dependent protein kinase (CDPK) genes mainly found in plants have also been described. The characterized CDPKs were found to play important roles in protein secretion, host cell invasion and parasite differentiation. Together, the available information on calcium storage and function in apicomplexans, although fragmented, suggest the existence of unique calcium-mediated pathways in these parasites. An in-depth functional characterization of the apicomplexan calcium-related genes could lead to the identification of novel therapeutic targets, and will improve our understanding of the role of calcium in parasite development and virulence.

Introduction

Fluctuations of cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$) regulate a variety of cellular functions in all eukaryotes. The $[Ca^{2+}]_i$ is maintained at very low levels (of the order of 10^{-7} M) compared with that in the extracellular medium (about 10^{-3} M). A variety of mechanisms, enzyme transporters, channels and calcium binding proteins contribute to maintaining the $[Ca^{2+}]_i$ at 10^{-7} M. The total calcium inside the cell is much higher than 10^{-7} M, but the bulk of this calcium is either bound to proteins, polyphosphate, membranes and/or other cellular constituents, or is sequestered inside intracellular organelles such as mitochondria, endoplasmic reticulum (ER), Golgi apparatus, and nuclei [1]. A key event in calcium signaling is the influx of calcium across the plasma membrane. Ca^{2+} storage organelles capable of both high affinity uptake and rapid triggered release of Ca^{2+} are believed to be ubiquitous among eukaryotes [2].

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Ca^{2+} homeostasis and storage in apicomplexans

Apicomplexan parasites are a large group of protists, which include a number of pathogens of medical and agricultural relevance. These parasites are named for their peculiar apical end, which contains a number of unique organelles and structures.

Calcium homeostasis and storage has been studied mainly in two groups of apicomplexan parasites: *T. gondii* and *Plasmodium* spp. *T. gondii* is an important cause of congenital disease and infection in immunocompromised patients. *Plasmodium* spp. represent the causative agents of malaria, one of the most devastating human infectious diseases. Intracellular calcium measurements have been performed in apicomplexan parasites mainly using the fluorescent calcium indicator fura 2-AM (fura 2-acetoxymethylester). This reagent, crosses the plasma membrane and is converted inside the cell to fura 2 by endogenous cytosolic esterases. This de-esterified form of the reagent is unable to cross membranes and becomes trapped in the cytoplasm of the cell where it is able to monitor changes in the cytosolic calcium concentration. Using this methodology, the $[Ca^{2+}]_i$ in *T. gondii* tachyzoites was measured at 70 ± 6 nM in the absence of extracellular Ca^{2+} (with the Ca^{2+} chelator EGTA added to the medium) and 100 ± 9 nM in the presence of 1 mM extracellular Ca^{2+} [3]. In *Plasmodium chabaudi*, and *P. falciparum* the $[Ca^{2+}]_i$ was also measured using fura 2-loaded free parasites and the values obtained were also at nanomolar levels in single-cell imaging experiments [4] or in parasite suspensions [5]. When imaging *P. falciparum*-infected erythrocytes loaded with fura red, a ratiometric calcium indicator with a low sensitivity to pH, much higher levels of $[Ca^{2+}]_i$ (289–352 nM), were measured [6]. This high value may be the result of superposition of Ca^{2+} signals from the cytosol and the extensive ER compartment within these cells [6]. The $[Ca^{2+}]_i$ in the parasite is likely within the range of concentrations observed in other eukaryotic cells (i.e. 90–100 nM).

In addition to known eukaryotic calcium stores including acidocalcisomes, the ER, Golgi apparatus and mitochondria, apicomplexan parasites contain several unique organellar compartments that potentially could contribute to diverse calcium transients necessary for vital functions within the parasites. These include the apicoplast, a remnant plastid derived from a secondary endosymbiotic event, and various acidic organelles including a recently described plant-like vacuole.

The endoplasmic reticulum

The largest store of Ca^{2+} in cells is usually found in the ER (Fig. 1), with local concentration reaching millimolar levels. The ER possesses independent pathways for calcium influx and efflux. The influx is catalyzed by the very well known sarco-endoplasmic reticulum Ca^{2+}-ATPase referred to as SERCA-type Ca^{2+}-ATPase, that actively translocates 2 Ca^{2+} for the hydrolysis of 1 ATP molecule. SERCA-type Ca^{2+}-ATPases have been characterized in *T. gondii* (Fig. 1, *b*) [7] and in *Plasmodium falciparum* [8]. Thapsigargin, a specific inhibitor of SERCA-type Ca^{2+}-ATPases of other eukaryotes produces an increase in the cytosolic Ca^{2+} levels of *T. gondii* and, of *Plasmodium* spp. at high concentrations [4] [9]. Release of Ca^{2+} from the ER occurs through an inositol 1,4,5-trisphosphate (IP_3)-stimulated calcium channel. An IP_3/ryanodine-sensitive store has been postulated to be present in *T. gondii* on the basis of pharmacological studies (Fig. 1, *f*) [10] and the release of Ca^{2+} from intracellular stores of malaria parasites was also shown to respond to IP_3 [9, 11]. The enzyme that catalyzes the production of IP_3, the phosphoinositide phospholipase C (PI-PLC) has been characterized in *T. gondii* (TgPI-PLC) [12]. However, there is no genetic evidence to indicate the presence of an IP_3R in any of the apicomplexan parasites. This means that the parasite may use a different mechanism (probably responsive to IP_3) to release calcium from the ER.

Acidic organelles

Acidocalcisomes are organelles that contain large amounts of calcium in an acidic environment. This calcium is probably not free but bound to other molecules as short- and long-chain polyphosphate [13]. Acidocalcisomes were first studied in trypanosomes and apicomplexan parasites and later found to be similar to the previously described “volutin granules” in bacteria or polyphosphate bodies in algae [13]. These organelles were initially found because the stored Ca^{2+} could be released into the cell cytoplasm with nigericin (a K^+/H^+ exchanger) or the weak base NH_4Cl [3]. This compartment was further characterized and named acidocalcisomes (Fig. 1). More recent work in *Leishmania* suggests that acidocalcisomes are lysosome-related organelles [14]. Acidocalcisomes have also been found in Plasmodium spp. [13] and more recently in *Eimeria* parasites [15].

The calcium inside the acidocalcisome is probably pumped in by a Ca^{2+}-ATPase, which in *T. gondii* has been named as TgA1 (Fig. 1, *b* in acidocalcisome) [16]. Recent work with a purified acidocalcisome fraction from *T. gondii* tachyzoites shows that calcium uptake is sensitive to vanadate, (a Ca^{2+}-ATPase inhibitor) supporting the idea of calcium being stored in this organelle [17].

Two enzymes with proton pumping activity have been found to localize to acidocalcisomes. The vacuolar-H^+-pyrophosphatase is an enzyme that hydrolyses pyrophosphate and the energy released is used to pump protons toward the lumen of the organelle where it localizes. This enzyme was described and characterized in *Toxoplasma gondii* (TgVP1) (Fig. 1, *c*) and also in *Plasmodium falciparum* (PfVP1) [13]. The second enzyme is the vacuolar-H^+-ATPase (Fig. 1, *d*), which pumps protons and uses the energy from the hydrolysis of ATP. Acidification of the acidocalcisome by these pumps is important to maintain organellar calcium, as alkalinizing agents such as NH_4Cl, release calcium to the cytoplasm. This is postulated to occur through a calcium-proton exchanger (see the diagram of the acidocalcisome in Fig. 1, *e*).

The vacuolar-H^+-pyrophosphatase also localizes to other compartments, as for example the plant-like vacuole in *T. gondii*, the food vacuole in *Plasmodium* spp., the plasma membrane in both parasites and also to other endocytic compartments. This is unique because in mammalian cells, acidic compartments are maintained by the V-H^+-ATPase. One possible explanation for this difference is that the V-H^+-pyrophosphatase uses pyrophosphate as an energy source, which is quite abundant in these parasites. Some of the developmental stages of the parasites might run short in ATP supply because of their suboptimal mitochondrial function as for example in the blood stages of *Plasmodium* spp. and extracellular tachyzoites of *T. gondii* [18].

The plant-like vacuole (PLV), recently described in *T. gondii,* was also found to contain calcium. This was demonstrated by adding the compound glycyl-L-phenylalanine-naphthylamide (GPN) to intact parasites loaded with the Ca^{2+} indicator fura 2-AM. GPN is specifically hydrolyzed in the lysosome of a variety of different cell types by a cathepsin C protease, and the product of its hydrolysis has a swelling effect in the lysosome leading to calcium leaking out to the cytoplasm [19]. The presence of a cathepsin C (CPC) inside the PLV was demonstrated by proteomic data of enriched fractions and IFA analysis of cells expressing a C-terminal tagged CPC gene (Moreno and Carruthers, unpublished). This GPN-dependent calcium release was shown to be independent of other calcium stores such as the ER [19].

Further evidence supporting the presence of Ca^{2+} inside the PLV is the detection of a vacuolar PMCA-type calcium ATPase (TgA1). This was shown by proteomic analysis of subcellular fractions and by immunofluorescence assays. TgA1 has been characterized

previously and shown to have a role in intracellular Ca^{2+} homeostasis as well as in parasite virulence. The specific role of the PLV in Ca^{2+} homeostasis or Ca^{2+} related function is still not known.

Mitochondria

Mitochondria possess a high capacity to sequester Ca^{2+} although under physiologic conditions, total mitochondrial Ca^{2+} levels and free Ca^{2+} reflect and parallel cytosolic Ca^{2+}. The inner mitochondrial membrane possesses a uniport carrier for Ca^{2+}, which allows the electrogenic entry of the cation driven by the electrochemical gradient generated by respiration or ATP hydrolysis (Fig. 1, Ψ in the mitochondrion cartoon). Ca^{2+} efflux, on the other hand, takes place by a different pathway, which appears to catalyze the electroneutral exchange of internal Ca^{2+} by external sodium or protons.

Experiments performed with malaria parasites using digitonin to measure mitochondrial activity *in situ,* showed Ca^{2+} uptake from the incubation medium by a mechanism associated with depolarization of the membrane potential. These results support the presence of a Ca^{2+} uniport similar to that of mammalian mitochondria [20]. A Ca^{2+}/H^{+} antiporter was recently localized to *P. falciparum* mitochondria. Sensitivity to ruthenium red and ruthenium 360 suggested the presence of a Ca^{2+} uniport in these mitochondria [21]. Unlike the mammalian mitochondria, where intracellular Ca^{2+} regulates the activity of several dehydrogenases no such Ca^{2+}-regulated dehydrogenases have been reported in apicomplexan parasites.

Calcium signaling and functions in apicomplexan parasites

Ca^{2+} signaling involves the mobilization of Ca^{2+} from two sources: intracellular stores and the extracellular medium. Mechanisms to introduce Ca^{2+} into the cytoplasm are compensated by a coordinated set of removal mechanisms consisting of buffers, pumps and exchangers. Free Ca^{2+} binds a number of effectors, which are responsible for stimulating numerous Ca^{2+}-dependent processes. Each cell type expresses a unique set of mechanisms and effectors, which create a Ca^{2+}-signaling system with the appropriate spatial and temporal properties.

In excitable mammalian cells, such as in muscle cells, the primary signal (membrane depolarization) activates Ca^{2+} entry across the plasma membrane, and this Ca^{2+} signal is amplified and propagated by a mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum [22]. In non-excitable mammalian cells, such as endothelial cells, activation by a hormone or growth factor receptor coupled to a phospholipase C results in the hydrolysis of phosphatidylinositol 4,5-disphosphate (PIP_2) to generate inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 diffuses to the intracellular stores (ER) and causes the release of Ca^{2+} into the cytoplasm through IP_3 receptors [23]. Some mammalian cells, such as smooth muscle and neuroendocrine cells, can utilize both of these pathways.

Ca^{2+} release from the ER is followed by the entry of Ca^{2+} ions across the plasma membrane. This process is known as capacitative Ca^{2+} entry or store-operated Ca^{2+} entry (SOCE) [24]. The connection between the ER and the plasma membrane was discovered only in recent years and it is orchestrated mainly by two protein families: Stim (Stim 1 and 2), which appear to function as Ca^{2+} sensors within the ER, and Orai proteins (Orai 1, 2, and 3) which function as SOCE channels in the plasma membrane [25]. In addition, a number of newly discovered second messengers, such as cyclic adenosine diphosphate ribose (cADPr), sphingosine-1-phosphate, and nicotinic acid adenine dinucleotide phosphate (NAADP) have been observed to release or modulate the release of intracellular Ca^{2+} from different cells. The plasma membrane of eukaryotic cells contains a number of channels through which calcium gains access into the cytoplasm. Some of these channels respond to changes in the

membrane potential (voltage-gated Ca^{2+} channels), others are under the control of receptors (receptor-operated Ca^{2+} channels), and others are controlled by the content of intracellular stores (store-operated Ca^{2+} channels). The active export of calcium from eukaryotic cells is accomplished by the action of a Na^{+}/Ca^{2+} exchanger or the plasma membrane Ca^{2+}-ATPase (PMCA).

The information available on calcium signaling components in apicomplexan parasites is still fragmentary although important features of their life cycle such as motility, host cell invasion, and egress from infected cells, are linked to calcium. A sequence with general similarity to a voltage dependent Ca^{2+} channel has been found in the *T. gondii* genome (TGME49_005260). The demonstration that this gene product is functional as a Ca^{2+} channel awaits further work (Fig. 1, *a*). There is no direct evidence for receptor-operated Ca^{2+} influx and none of the genes that correspond to the SOCE machinery, Stim and Orai, [26] are present in any of the apicomplexan genomes. A Ca^{2+} channel has been postulated to be inserted in the plasma membrane of erythrocytes infected with *P. falciparum* [27]. There are several reports of PMCA-type-Ca^{2+}-ATPases in apicomplexan parasites [28]. Biochemical evidence for calmodulin (CaM) stimulation of this pump has been reported [29] although *Tg*A1 appears to lack a typical CaM-binding domain. This might suggest the presence of a different domain able to bind CaM. A gene encoding for a second putative PMCA has been found in the *T. gondii* genome (TGME29_033770) [28]. The deduced amino acid sequence (1200 aa) shows 45% identity with *Tg*A1 [16]. No homologues to this enzyme are found in the *Plasmodium* genomes.

Calcium binding proteins in apicomplexan parasites

Once inside the cell, Ca^{2+} can either interact with so-called soluble Ca^{2+}-binding proteins or become sequestered into intracellular organelles.

Calcium binding proteins (CBP) are characterized by the presence of a highly conserved helix-loop-helix structural motif known as an EF-hand. Typically, EF hand motifs occur in pairs (also called EF hand domain) and facilitate the cooperative binding of two Ca^{2+} ions per domain. However, CBPs with single or odd number of EF hand motifs have been reported in both bacteria and eukaryotes and are believed to function via dimerization mechanisms. At least 69 EF hand domain-containing proteins are encoded by the *P. falciparum* genome (PlasmoDB), 55 in *T. gondii* (ToxoDB), and 45 in *C. parvum* (CryptoDB). As with most apicomplexan protein families, a majority of these EF hand domain-containing proteins are hypothetical with no known functions.

CBPs are generally classified into three main families: the calmodulin (CaM) family, the calcineurin B-like (CBL) family, and the calcium-dependent protein kinase (CDPK) family. The CaM family includes classical calmodulins (sequence identity with human CaM > 75%), calmodulin-like (CML) proteins (sequence identity with human CaM < 75%), and all other CaM-related proteins (presence of at least one non-CaM domain structure). Structurally, calmodulins are acidic proteins comprised of two globular domains (each with a pair of EF hands) linked by a flexible helical region. Most apicomplexan genomes encode single prototype CaMs and a variable number of CMLs/CaM-related proteins. Of these proteins, only the *T. gondii* CaM has been cloned and shown to bind Ca^{2+} *in vitro* [30]. Additional evidence in support of a functional CaM in *T. gondii* has been provided by *in vitro* experiments using the CaM inhibitors calmidazolium and trifluoperazine [31]. These drugs significantly reduce host cell entry by *T. gondii* tachyzoites, suggesting a role of TgCaM in host cell invasion. CaM inhibitors are equally toxic to *P. falciparum*, affecting parasite development and erythrocyte invasion by merozoites [32, 33]. Unlike CaM proteins, CBLs have been identified only in higher plants and, to a limited extent, in some protist

genomes. Inspection of representative apicomplexan genomes reveals single gene sequences that potentially may encode functional CBLs in *T. gondii* and *P. falciparum*.

The third class of CBPs comprises several enzymes that are modulated through direct interactions with Ca^{2+}. These include a variety of kinases (e.g. CDPKs and CCaMKs), proteases, phosphatases, synthases, and nucleoside triphosphatases (NTPases). Most apicomplexan genomes encode several CDPKs, Ser/Thr protein phosphatases, tRNA synthases, ubiquitin C-terminal hydrolases and cathepsins.

The fourth group of CBPs encoded by the apicomplexan genomes includes several hypothetical proteins, heat-shock proteins, centrin/troponin C-like proteins, and variants of the *Plasmodium*-specific virulence factor PfEMP1. These proteins, which have barely been characterized in some apicomplexans, are likely to exhibit vital functions including a role in buffering and species-specific signaling processes.

Functional studies of calcium and CBPs in apicomplexan parasites

Microneme secretion, invasion, and egress

T. gondii and *Plasmodium* spp. contain micronemes (Fig. 1), specialized apical secretory organelles, which appear to play an important role in the early phase of the invasion process. These organelles contain protein complexes or adhesins, which are discharged and participate in the interaction with host cell surface. A large number of studies support the relevance of microneme secretion during invasion of the host cell.

The secretion of micronemes can be induced artificially by treating parasites with calcium ionophores [34]. This effect can be blocked with the intracellular Ca^{2+} chelator BAPTA-AM demonstrating that the secretion of micronemes is triggered by an increase in intracellular Ca^{2+}. BAPTA-AM was also used to demonstrate the role of calcium in conoid extrusion [35], gliding motility [36] and invasion [37]. The stimulation of microneme secretion by Ca^{2+} has also been demonstrated in *C. parvum* [38] and *P. berghei* [39].

The relevance of intracellular Ca^{2+} homeostasis was also highlighted by the phenotype of the TgA1 null mutant parasites. These cells have their intracellular calcium levels altered, are unable to maintain it at physiological level under the experimental conditions tested, and are deficient in microneme secretion. In addition, these cells have an invasion defect and reduced virulence in vivo [40].

T. gondii replicates inside its host cell but at some point needs to exit to be able to infect other cells and this egress process is still poorly understood. T. gondii egress is rapid and results in lysis of the infected host cell. It is also known that calcium ionophores like A23187 can stimulate this process [41]. Parasite mutants with a defect in egress (delayed egress) have been isolated and found to have their intracellular calcium levels elevated [42] and in addition have reduced pathogenicity [43].

Calcium and motility

Secretion of microneme proteins is also important for motility of *T. gondii*. Microneme secretion is triggered by an increase in intracellular calcium (see above) meaning calcium may have a role in motility. Several pieces of information available in the literature support this statement. *T. gondii* parasites were loaded with the Ca^{2+} indicator fluo 4 and analyzed by live imaging and it was observed that periodic oscillations in the intracellular Ca^{2+} levels were linked to gliding of the parasites [36].

The exit from the host cell is also a process dependent in motility of the parasite. The effect of Ca^{2+} ionophores on egress could be linked to the effect on the motility of the parasite. Changes in extracellular Ca^{2+} have not been evaluated as a possible trigger of microneme secretion and the natural agonist responsible for stimulating intracellular Ca^{2+} increase and subsequent microneme release is yet to be identified.

Role of CDPK

The Ca^{2+}-dependent protein kinase (CDPK) family constitutes a group of kinases that are only found in plants and protists. In plants, CDPKs are a required response mechanism that allows external Ca^{2+} signals to regulate a diverse number of pathways including cell cycle progression and stress responses. The typical CDPK is composed of an amino-terminal serine/threonine kinase domain, followed by a junction domain (also known as the autoinhibitory domain) that connects to the carboxy-terminal calmodulin-like domain. The calmodulin-like domain typically consists of multiple calcium binding domains (i.e., 4 EF hand domains).

Phylogenetic analyses of CDPKs in apicomplexans show that there is a large diversity of these kinases present in *Plasmodium*, *Toxoplasma*, and *Cryptosporidium* genera. In addition to the typical CDPKs described above and found in plants, apicomplexan parasites contain 4 additional structural variants. The primary source of this variation is the number of EF hand domains and the length of the amino-terminus preceding the kinase domain. *P. falciparum* possesses 7 annotated CDPKs while *T. gondii* possesses 12 CDPKs [44].

Ca^{2+} is known to have a key role in critical features of apicomplexan parasites as a second messenger system, and CDPKs have been implicated as a mechanistic link between Ca^{2+} signaling and differentiation, motility, invasion, and egress. The rodent malarial parasite, *P. berghei*, requires CDPKs for developmental differentiation. Genetic disruption of CDPK4 in *P. berghei* gametocytes (sexual stages located in mammalian blood stream) inhibits calcium dependent pathways that are required for microgamete differentiation [44]. Additionally, knockout of a different kinase, CDPK3, in *P. berghei* severely inhibits the ability of ookinetes to traverse the peritrophic membrane in the mosquito gut, thereby stopping oocysts production in the mosquito gut and short-circuiting the vector pathway of this parasite [45].

Essential attributes of Ca^{2+}-dependent protein kinases have also been observed in the related apicomplexan *T. gondii*. The protein kinase inhibitor, KT5926, which inhibits microneme secretion required for host cell attachment of the parasite, has been shown to target a CDPK in *T. gondii* [46]. More specifically, down regulation of *T. gondii* CDPK1, resulted in loss of parasite motility and host cell invasion and egress abilities, further demonstrating the essential nature of CDPKs in this important apicomplexan parasite [47].

The essential nature of CDPKs in regulating critical pathways of apicomplexan parasites has been clearly established. However, further efforts in determining the specific substrates of CDPKs will provide a more mechanistic understanding of their control of invasion and differentiation. Additionally, Ca^{2+}-dependent protein kinases exhibit a significant level of crosstalk with other protein kinases, most notably the cyclic nucleotide-dependent kinases. Taking this into account along with the large number and structural diversity of Ca^{2+}-dependent kinases, it is apparent that further research on the CDPKs in apicomplexans is likely to be very rewarding in terms of cell biology and development of potential drug targets for clinical use.

Conclusion

Ca^{2+} has important roles in secretion, motility, cell invasion and differentiation of apicomplexan parasites. Ca^{2+} regulation is controlled by a variety of systems for uptake and release that differ in several aspects from the processes that occur in other eukaryotic cells, providing excellent opportunities for targeting them for new therapies. Apicomplexan parasites contain several P-type Ca^{2+}-ATPases including a SERCA-type important for Ca^{2+} uptake in the endoplasmic reticulum, as well as Ca^{2+}/H^{+} antiporters. However, a PMCA-type Ca^{2+}-ATPase and voltage-dependent Ca^{2+} channels are only present in *T. gondii*. There is evidence for mechanisms of Ca^{2+} release stimulated by IP_3 and cADP ribose in some of these parasites but no receptors for these second messengers have been identified. A number of Ca^{2+}-binding proteins including calmodulins, calmodulin-like proteins, and an array of Ca^{2+}-dependent protein kinases are present in these parasites. Acidocalcisomes are present in *Plasmodium* spp., *T. gondii*, and *Eimeria* spp., but absent in *Cryptosporidium* spp. Other acidic organelles containing Ca^{2+} include the digestive vacuole of malaria parasites and the plant-like vacuole of *T. gondii*. Because of their situation at an early branch point in eukaryotic evolution studies of Ca^{2+} storage and signaling in these parasites could shed light about the origins of complex signaling networks in eukaryotes. Several apicomplexan genomes are completed, making it possible to look for conserved pathways through sequence-based phylogenetic comparisons. Additionally, the continuing availability and enhancement of experimental tools for genetic manipulation and molecular investigation of apicomplexan parasites (especially *T. gondii* and *Plasmodium* spp.) should allow for significant advances in deciphering calcium signaling pathways in these important eukaryotes.

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Summary

- Ca^{2+} is important for motility, secretion, invasion, egress, and differentiation of apicomplexan parasites
- There is insufficient evidence for the presence of some mechanisms for Ca^{2+} uptake and release, as for example IP_3 and cADP ribose receptors, PMCA-type Ca^{2+}-ATPases or voltage dependent Ca^{2+} channels in many of these organisms.
- Acidic calcium stores, such as the acidocalcisome, plant-like vacuole, and digestive vacuole appear to play important roles as Ca^{2+} stores.
- Apicomplexans contain a diversity of calcium-dependent kinases, which are commonly found in plants.

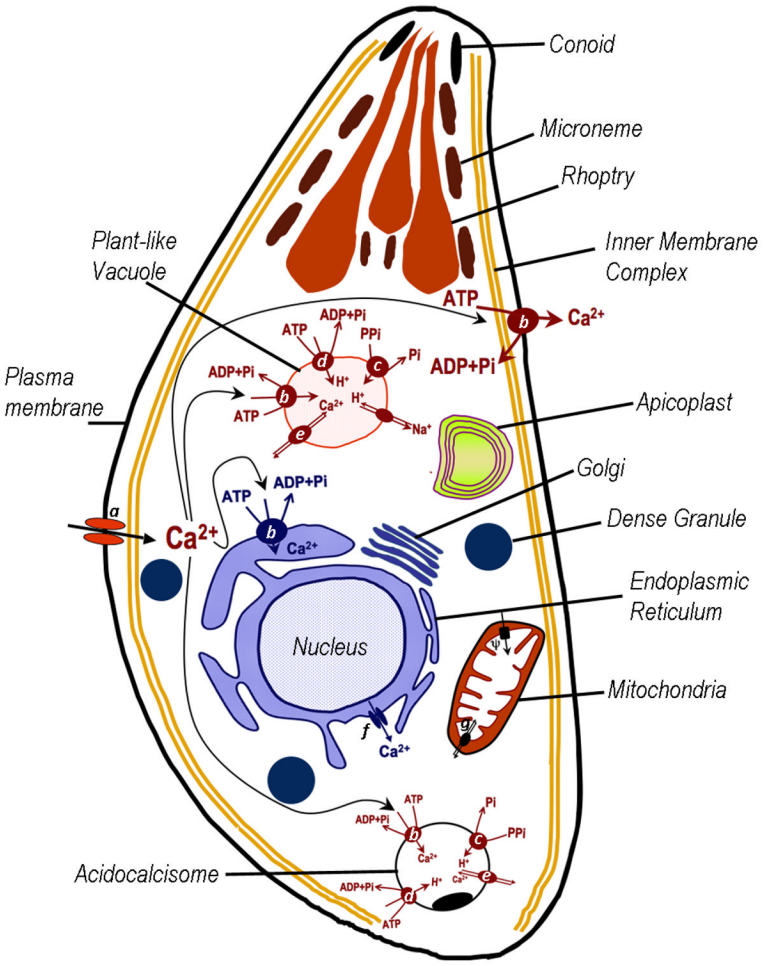

Figure 1.

Schematic representation of the distribution of Ca^{2+} in a *T. gondii* tachyzoite. Abbreviations: Ca^{2+} entry is probably through Ca^{2+} channels (a). Once inside the cells, Ca^{2+} can be translocated back to the extracellular environment, primarily by the action of the PMCA (b). In addition, Ca^{2+} will interact with Ca^{2+}-binding proteins or become sequestered by the ER by the action of the SERCA-Ca^{2+}-ATPase (b), sequestered by the mitochondrion through a postulated uniport driven by their membrane potential ($\Delta\Psi$), sequestered by the acidocalcisome or the plant-like vacuole by the action of a Ca^{2+}-ATPase (TgA1) (b). Ca^{2+} appears to diffuse freely into the nucleus. Calcium could also be released into the cytoplasm from the internal stores as the ER through an uncharacterized channel, which appears to respond to ryanodine and caffeine (f). It may also be released from the PLV and the acidocalcisome through a Ca^{2+}/H^{+} exchanger (e). Acidic compartments as the acidocalcisome and the PLV contain enzymes involved in their acidification as the H^{+}-ATPase (d) and the V-H^{+}-pyrophosphatase (c). Mitochondrial Ca^{2+} release is through a Ca^{2+}-H^{+} exchanger (g).