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 ER (endoplasmic reticulum) 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 *Toxoplasma gondii*. Compared with 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 IP$_3$ (inositol 1,4,5-trisphosphate)-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. gondii*, but not in *Plasmodium* spp. and *Cryptosporidium* spp. Effector calcium-binding proteins including calmodulins and CDPK (calcium-dependent protein kinase) 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. Taken together, the available information on calcium storage and function in apicomplexans, although fragmented, suggest the

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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 (approximately 10$^{-3}$ M). A variety of mechanisms, enzyme transporters, channels and CBPs (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, ER (endoplasmic reticulum), Golgi apparatus and nuclei [1]. A key event in calcium signalling 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].

**Ca$^{2+}$ homoeostasis and storage in apicoplexans**

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 homoeostasis and storage has been studied mainly in two groups of apicomplexan parasites: *Toxoplasma 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 acetoxymethyl ester). This reagent crosses the plasma membrane and is converted inside the cell into 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 *Plasmodium 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 probably 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 the recently described PLV (plant-like vacuole).

**ER**
The largest store of $Ca^{2+}$ in cells is usually found in the ER (Figure 1), with the local concentration reaching millimolar levels. The ER possesses independent pathways for calcium influx and efflux. The influx is catalysed by the very well known SERCA (sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase), which actively translocates two $Ca^{2+}$ molecules for the hydrolysis of one ATP molecule. SERCA-type $Ca^{2+}$-ATPases have been characterized in *T. gondii* (Figure 1, b) [7] and in *P. 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 IP$_3$ (inositol 1,4,5-trisphosphate)-stimulated calcium channel. An IP$_3$/ryanodine-sensitive store has been postulated to be present in *T. gondii* on the basis of pharmacological studies (Figure 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 catalyses the production of IP$_3$, PI-PLC [phosphoinositide PLC (phospholipase C)], has been characterized in *T. gondii* (TgPI-PLC) [12]. However, there is no genetic evidence to indicate the presence of an IP$_3$ receptor 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 NH₄Cl [3]. This compartment was characterized further and named the acidocalcisome (Figure 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 (Figure 1, b in the 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 towards the lumen of the organelle where it localizes. This enzyme was described and characterized in *T. gondii* (TgVP1) (Figure 1, c) and also in *P. falciparum* (PfVP1) [13]. The second enzyme is the vacuolar-H\(^{+}\)-ATPase (Figure 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\(_4\)Cl release calcium into the cytoplasm. This is postulated to occur through a Ca\(^{2+}\)/H\(^{+}\) exchanger (Figure 1, e in the acidocalcisome).

The vacuolar-H\(^{+}\)-pyrophosphatase also localizes to other compartments, for example the PLV 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 vacuolar-H\(^{+}\)-ATPase. One possible explanation for this difference is that the vacuolar-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 of an 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 PLV, recently described in *T. gondii*, was also found to contain calcium. This was demonstrated by adding the compound GPN (glycyl-L-phenylalanine-naphthylamide) to intact parasites loaded with the Ca\(^{2+}\) indicator fura 2/AM. GPN is specifically hydrolysed in the lysosome of a variety of different cell types by a CPC (cathepsin C) protease, which results in swelling and loss of membrane integrity of the lysosome, leading to calcium leaking out to the cytoplasm [19]. The presence of a CPC inside the PLV was demonstrated by proteomic data of enriched fractions and IFA (immunofluorescence assay) analysis of cells expressing a C-terminal tagged CPC gene (S.N.J. Moreno and V.B. Carruthers, unpublished work). 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 (plasma membrane Ca\(^{2+}\)-ATPase)-type calcium ATPase (TgA1). This was shown by proteomic analysis of subcellular fractions and by IFAs. 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 physiological 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 (Figure 1, $\Psi$ in the mitochondrion). Ca\(^{2+}\) efflux, on the other hand, takes place by a different pathway, which appears to catalyse 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 [21]. 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 signalling and functions in apicomplexan parasites
Ca\(^{2+}\) signalling 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 co-ordinated 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\(^{2+}\) signalling 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 CICR (Ca\(^{2+}\)-induced Ca\(^{2+}\) release) 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 PLC results in the hydrolysis of PIP\(_2\) (phosphatidylinositol 4,5-bisphosphate) to generate 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\(^{2+}\) across the plasma membrane. This process is known as capacitative Ca\(^{2+}\) entry or SOCE (store-operated Ca\(^{2+}\) entry) [24]. The connection between the ER and the plasma membrane was only discovered in recent years and is orchestrated
mainly by two protein families: Stim (stromal interaction molecule; Stim1 and 2), which appear to function as Ca\(^{2+}\) sensors within the ER, and Orai proteins (Orai1, 2 and 3), which function as SOCE channels in the plasma membrane [25]. In addition, a number of newly discovered second messengers, such as cADPr (cyclic adenosine diphosphate ribose), sphingosine 1-phosphate and NAADP (nicotinic acid–adenine dinucleotide phosphate) 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 the rest 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 an Na\(^{+}\)/Ca\(^{2+}\) exchanger or the PMCA.

The information available on calcium signalling 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 \textit{T. gondii} genome (TDR Targets database number TGME49_005260). The demonstration that this gene product is functional as a Ca\(^{2+}\) channel awaits further work (Figure 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 \textit{P. falciparum} [27]. There are several reports of PMCA-type-Ca\(^{2+}\)-ATPases in apicomplexan parasites [28]. Biochemical evidence for CaM (calmodulin) stimulation of this pump has been reported [29], although TgA1 appears to lack a typical CaM-binding domain. This might suggest the presence of a different domain able to bind CaM. A gene encoding a second putative PMCA has been found in the \textit{T. gondii} genome (TDR Targets database number TGME49_033770) [28]. The deduced amino acid sequence (1200 amino acids) shows 45% identity with TgA1 [16]. No homologues to this enzyme are found in the \textit{Plasmodium} genomes.

**CBPs in apicomplexan parasites**

Once inside the cell, Ca\(^{2+}\) can either interact with so-called soluble CBPs or become sequestered into intracellular organelles. CBPs 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 the EF-hand domain) and facilitate the co-operative binding of two Ca\(^{2+}\) ions per domain. However, CBPs with a 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 *Cryptosporidium 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 CaM family, the CBL (calcineurin B-like) family, and the CDPK (calcium-dependent protein kinase) family. The CaM family includes classical CaMs (sequence identity with human CaM >75%), CML (CaM-like) proteins (sequence identity with human CaM <75%) and all other CaM-related proteins (presence of at least one non-CaM domain structure). Structurally, CaMs are acidic proteins composed 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 for TgCaM (*T. gondii* CaM) 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 (calcium/CaM-dependent protein kinases)], proteases, phosphatases, synthases and NTPases. Most apicomplexan genomes encode several CDPKs and serine/threonine protein phosphatases.

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 signalling processes.

**Functional studies of calcium and CBPs in apicomplexan parasites**

**Microneme secretion, invasion and egress**

*T. gondii* and *Plasmodium* spp. contain micronemes (Figure 1), specialized apical secretory organelles that 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 the 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 \( \text{Ca}^{2+} \) chelator BAPTA/AM [1,2-bis-(o-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid tetrakis(acetoxyethyl ester)], demonstrating that the secretion of micronemes is triggered by an increase in intracellular \( \text{Ca}^{2+} \). 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 \( \text{Ca}^{2+} \) has also been demonstrated in \textit{C. parvum} [38] and \textit{Plasmodium berghei} [39].

The relevance of intracellular \( \text{Ca}^{2+} \) homoeostasis 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 a physiological level under the experimental conditions tested and are deficient in microneme secretion. In addition, these cells have an invasion defect and reduced virulence \textit{in vivo} [40].

\textit{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. \textit{T. gondii} egress is rapid and results in lysis of the infected host cell. It is also known that calcium ionophores such as 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 \textit{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. \textit{T. gondii} parasites were loaded with the \( \text{Ca}^{2+} \) indicator Fluo-4 and analysed by live imaging, and it was observed that periodic oscillations in the intracellular \( \text{Ca}^{2+} \) levels were linked to gliding of the parasites [36]. The exit from the host cell is also a process dependent on motility of the parasite.

The effect of \( \text{Ca}^{2+} \) ionophores on egress could be linked to the effect on the motility of the parasite. Changes in extracellular \( \text{Ca}^{2+} \) have not been evaluated as a possible trigger of microneme secretion, and the natural agonist responsible for stimulating intracellular \( \text{Ca}^{2+} \) increase and subsequent microneme release is yet to be identified.

**Role of CDPK**

The 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 \( \text{Ca}^{2+} \) signals to regulate a diverse number of pathways including cell-cycle progression and stress responses. The typical CDPK is
composed of an N-terminal serine/threonine kinase domain, followed by a
junction domain (also known as the auto-inhibitory domain) that connects to
the C-terminal CML domain. The CML domain typically consists of multiple
calcium-binding domains (i.e. four EF-hand domains).

Phylogenetic analyses of CDPKs in apicomplexans show that there is a
large diversity of these kinases present in the *Plasmodium*, *Toxoplasma* and
*Cryptosporidium* genera. In addition to the typical CDPKs described above and
found in plants, apicomplexan parasites contain four additional structural vari-
nants. The primary source of this variation is the number of EF-hand domains
and the length of the N-terminus preceding the kinase domain. *P. falciparum*
possesses seven annotated CDPKs, whereas *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\(^{2+}\) signalling 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 the mammalian bloodstream) 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 ookinete to traverse the peritrophic membrane
in the mosquito gut, thereby stopping oocyst production in the mosquito gut
and short-circuiting the vector pathway of this parasite [45].

Essential attributes of CDPKs have also been observed in the relat-
ed apicomplexan *T. gondii*. The protein kinase inhibitor KT5926, which
inhibits microneme secretion required for host cell attachment of the para-
site, 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 api-
complexan 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 cross-talk 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.

**Conclusions**

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 that is important for Ca$^{2+}$ uptake in the ER, 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 cADPr in some of these parasites, but no receptors for these second messengers have been identified. A number of CBPs, including CaMs, CML proteins and an array of CDPKs, are present in these parasites. Acidocalcisomes are present in *Plasmodium* spp., *T. gondii*, and *Eimeria* spp., but are absent in *Cryptosporidium* spp. Other acidic organelles containing Ca$^{2+}$ include the digestive vacuole of malaria parasites and the PLV of *T. gondii*. Because of their situation at an early branch point in eukaryotic evolution, studies of Ca$^{2+}$ storage and signalling in these parasites could shed light about the origins of complex signalling networks in eukaryotes. Several apicomplexan genomes have been 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 signalling pathways in these important eukaryotes.

**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, for example IP$_3$ and cADPr receptors, PMCA-type Ca$^{2+}$-ATPases or voltage-dependent Ca$^{2+}$ channels, in many of these organisms.
- Acidic calcium stores, such as the acidocalcisome, PLV 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.

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