REVIEW ARTICLE

InsP3 Signaling in Apicomplexan Parasites

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Abstract: Background: Phosphoinositides (PIs) and their derivatives are essential cellular components that form the building blocks for cell membranes and regulate numerous cell functions. Specifically, the ability to generate myo-inositol 1,4,5-trisphosphate (InsP3) via phospholipase C (PLC) dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to InsP3 and diacylglycerol (DAG) initiates intracellular calcium signaling events representing a fundamental signaling mechanism dependent on PIs. InsP3 produced by PI turnover as a second messenger causes intracellular calcium release, especially from endoplasmic reticulum, by binding to the InsP3 receptor (InsP3R). Various PIs and the enzymes, such as phosphatidylinositol synthase and phosphatidylinositol 4-kinase, necessary for their turnover have been characterized in Apicomplexa, a large phylum of mostly commensal organisms that also includes several clinically relevant parasites. However, InsP3Rs have not been identified in genomes of apicomplexans, despite evidence that these parasites produce InsP3 that mediates intracellular Ca2+ signaling.

Conclusion: Evidence to supporting IP3-dependent signaling cascades in apicomplexans suggests that they may harbor a primitive or non-canonical InsP3,R. Understanding these pathways may be informative about early branching eukaryotes, where such signaling pathways also diverge from animal systems, thus identifying potential novel and essential targets for therapeutic intervention.

Keywords: Calcium signaling, InsP3 signaling, Apicomplexan parasites, Phosphoinositides, Plasmodium.

1. INTRODUCTION

Phosphoinositides (PIs) and their derivatives are important for controlling a variety of vital cell functions including intracellular signaling cascades, regulated secretion, and cytoskeleton integrity [1]. The inositol containing phospholipids are abundant constituents of cell membranes of Archaea and all eukaryotes [2]. The importance of inositol signaling has been known for many years. However, the seminal discovery that ligation of cell surface receptors and PLC activity coupled to PI turnover and calcium release [3,4] paved the way for the discovery of the secondary messengers InsP3 and diacylglycerol (DAG) and the downstream signaling cascades regulated by their generation and metabolism. InsP3 formation depends on phospholipase C (PLC) that is activated by a variety of specific cell surface receptors. PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP2), thus releasing soluble InsP3 [5,6]. Pharmacological evidence supports InsP3 signaling in unicellular eukaryotes such as members of the phylum Apicomplexa, a diverse group that includes Plasmodium, Toxoplasma and Cryptosporidium. The mobilization of intracellular Ca2+ level by InsP3 in single cell pathogens was described in Trypanosoma, a kinetoplastid member of the phylum Euglenozoa. Although the cell surface receptor mediated signaling cascades that lead to InsP3-dependent, and potentially DAG-dependent, signaling cascades have not been fully delineated, there is mounting evidence to suggest these pathways are operative in apicomplexans.

Interest in Ca2+ signaling in these parasites is motivated by a history of studies demonstrating the requirement of Ca2+ signaling for infection by apicomplexan parasites, as recently reviewed [7]. Elevation of intracellular calcium regulates secretion of adhesins from microneme organelles, as well as activating actin-myosin dependent motility. As described below, parasite pathways that recruit proteins sufficiently divergent from their mammalian hosts may be exploited for development of novel therapeutic interventions (Fig. 1).

Apicomplexans share many molecules pertinent to Ca2+ signaling with their closest relatives, the ciliates (phylum Ciliophora), despite these two groups being evolutionarily
Fig. (1). Schematic representation of the myo-inositol 1,4,5-triphosphate (InsP$_3$) turnover and its function in intracellular Ca$^{2+}$ release. Step 1: Inositol incorporation into CDP-DAG (CDP = cytidine diphosphate DAG = diacylglycerol) catalyzed by phosphatidylinositol synthase generating phosphatidylinositol (PI). Step 2: Phosphorylation of PI by phosphatidylinositol 4-kinase forming phosphatidylinositol 4-phosphate (PIP). Step 3: Phosphorylation of PIP by phosphatidylinositol 4-phosphate 5-kinase forming phosphatidyl 4,5-bisphosphate (PIP$_2$). Step 4: PIP$_2$ is cleaved by phospholipase C (PLC) generating a soluble molecule, inositol 1,4,5-trisphosphate (InsP$_3$), that is capable to bind to a InsP$_3$ receptor (InsP$_3$R) present in the membranes of intracellular compartments. InsP$_3$ binding temporarily opens the InsP$_3$R that acts as a Ca$^{2+}$-release channel, so that Ca$^{2+}$ can flow into the cytoplasm. The insoluble fraction formed by cleavage of PIP$_2$ is diacylglycerol (DAG). Step 5: InsP$_3$ is dephosphorylated by inositolpolyphosphate 5-phosphatase forming inositol 1,4-bisphosphate (InsP$_2$). Step 6: InsP$_2$ is desphosphorylated by inositol polyphosphate 1-phosphatase generating inositol 1-monophosphate (InsP). Step 7: Dephosphorylation of InsP to inositol by inositol monophosphatase. Step 8: phosphorylation of InsP$_3$ by inositol 1,4,5-trisphosphate 3-kinase generating inositol 1,3,4,5-tetrakisphosphate (InsP$_4$).

Fig. (2). Phylogenetic tree showing evolutionary relationships among organisms discussed in this review.

quite diverse [8]. Both groups are combined in the Alveolata, due to their endowment with subplasmalemmal flattened membranes, i.e. alveolar sacs in ciliates and inner membrane complex in apicomplexans. In ciliates, Ca$^{2+}$ signaling mechanisms are known in some detail, including the molecular identity of intracellular Ca$^{2+}$ channels involved in Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores [9,10]. In contrast, identifying Ca$^{2+}$-channels in apicomplexans such as Plasmodium and Toxoplasma has proved a major challenge. However, the recent development of transgenic parasites expressing genetically encoded sensors for detection of Ca$^{2+}$ in P. falcipa-

rum and T. gondii may reveal new aspects of Ca$^{2+}$ signaling [11,12] (Fig. 2).

2. EVIDENCE OF INS$_P$$_3$ TURNOVER AND INS$_P$$_3$-INDUCED CA$^{2+}$ RELEASE IN APICOMPLEXA

Inositol and inositol phospholipids have been described in most Archaea and eukaryotes indicating this system developed in a common ancestor of the Archaea kingdom approximately 2 billion years ago [2,13]. Inositol phospholipids (PI) are ubiquitous components of cell membranes, there is not a unique PI found only in apicomplexan. Table 1
Table 1. Occurrence of proteins important for the InsP$_3$ signaling pathway in some model organisms. The corresponding genes were searched for in the databases of Plasmodium, Toxoplasma, Homo sapiens, Trypanosoma and Paramaecium (PlasmoDB, ToxoDB, PDB/NCBI, TriTrypDB and ParameciumDB, respectively). Phospholipase C of Paramaecium tetraurelia is described in Klöppel et al., 2009 [27]. (1) Putative protein; (2) -like proteins; (3) encoded by pseudogene; (4) Leondaritis et al. (2013) [28], described 62 highly homologous phosphoinositide kinases genes; *genes that could not be found in databases.

| Species                  | Phosphatidylinositol 4-kinase | Phosphatidylinositol 4-phosphate 5-kinase | Phospholipase C | Inositol Polyphosphate 5-phosphatase | Inositol 1,4,5-Trisphosphate 3-kinase | Inositol Triphosphate Receptor |
|-------------------------|------------------------------|------------------------------------------|-----------------|-------------------------------------|--------------------------------------|---------------------------------|
| Plasmodium falciparum 3D7 | PF3D7_0509800; PF3D7_0419900(1) | PF3D7_1110600; PF3D7_1129600(1) | PF3D7_1013500 | PF3D7_1354200(1) | * | * |
| Toxoplasma gondii ME49  | TGME49_328200 | TGME49_245730 | TGME49_248830 | * | * | * |
| Homo sapiens            | AAA56839.1; BAA21661.1 | CAD99242.1 | AAA60112.1; BAA07668.1 | CAA74743.1; CAA67071.1 | AAH26331.1; CAC40650.1 | NP_002214.2 |
| Leishmania major Friedlin | LmjF.34.3590(2); LmjF.29.1450(3) | LmjF.36.0370(2); LmjF.34.3090(2); LmjF.35.0560(1) | LmjF.30.2950(5); LmjF.22.1680(5) | * | * | LmjF.16.0280(1) |
| Trypanosoma brucei gambiense | Tbg972.4.970(5) | Tbg972.10.4830(3) | Tbg972.11.6720(5) | * | * | Tbg972.8.2330(1) |
| Trypanosoma cruzi CL Brener | TcCLB.505987.70(1) | TcCLB.510289.30(1); TcCLB.511001.70(1) | TcCLB.508039.90(1) | * | * | BAM68694.1 |
| Paramaecium tetraurelia | XP_001346932.1 | XP_001347030.1; XP_001347013.1 | XP_001432600.1; XP_001426835.1 | * | (4) | PTMB.445c |

shows some important PI specific enzymes that have been reported in Apicomplexa and other unicellular parasites. The pathway by which PI is generated appears conserved amongst all eukaryotes [13]. The genes encoding the enzyme phosphatidylinositol synthase that catalyzes the formation of phosphatidylinositol (PI) are well characterized in P. falciparum and P. knowlesi [14], T. gondii [15] and T. brucei [16]. Phosphatidylinositol transfer protein encoding genes are also present, suggesting the existence of systems responsible for transporting PI from the ER to the plasma membrane [1]. In Plasmodium, Gardner et al., 2002 [17] reported a gene encoding the enzyme phosphatidylinositol 4-kinase (PI4K) that catalyses the formation of phosphatidylinositol 4-phosphate (PIP) from phosphatidylinositol (PI). Thus, apicomplexans have the machinery to generate the precursor of InsP$_3$. Table I summarizes known and putative proteins from the InsP$_3$ pathway in model organisms and parasites. In mammalian systems the ability of InsP$_3$ to act as a second messenger requires the enzymes to rapidly release InsP$_3$ from PIP$_2$, as well as the ability to reduce InsP$_3$ levels to basal. Importantly, genetic evidence for phospholipase C enzymes can be found in all apicomplexans, except Sarcocystis (presumably due to a lack of genetic data), and Kinetoplastids (See Table 1). The presence of the archetypal PLCδ isoform in all genomes indicates the ability to generate InsP$_3$ and DAG in unicellular parasites arising from a common ancestor. The enzymes involved in InsP$_3$ metabolism are less well conserved. Plasmodium falciparum have an annotated inositol polyphosphate 5-phosphatase, an indication that they too can metabolize InsP$_3$, to InsP$_2$, whilst a specific inositol 1,4,5 trisphosphate 3-kinase has not been annotated. However, inositol polyphosphate kinase genes can be found in both P. falciparum and T. gondii genomes and may metabolize InsP$_3$ to InsP$_4$.

3. PHARMACOLOGICAL EVIDENCE FOR INS$_{P3}$ SIGNALING IN APICOMPLEXA

Importantly, biochemical evidence to support PI turnover in apicomplexan parasites has also been reported in the literature. Despite the fact that mammalian RBCs have all the enzymes enabling the production of PIP$_2$ from PI [18], a non-infected RBC has limited biosynthesis of PIP$_2$ and other PIs [19]; in contrast, when a RBC is infected with Plasmodium knowlesi; P falciparum; and Babesia bovis [21] PI turnover increases due parasite metabolism. Incubating red blood cells (RBC) infected with P falciparum with radiolabeled myo-inositol [22] results in the biosynthesis of PI, PIP and PIP$_2$. Moreover, treatment with the Ca$^{2+}$ ionophore, ionomycin, caused an increase in inositol phosphate production with Ins1,4,5P$_3$ levels being the highest. The increase of intracellular Ca$^{2+}$ promoted by ionomycin presumably activates the Ca$^{2+}$ sensitive PLC [23] to cleave PIP$_2$, thus generating InsP$_3$. Martin et al. [24] demonstrated the formation of InsP$_3$ and diacylglycerol (DAG) during exflagellation of P. falciparum gamocytes, an important event in the sexual cycle that takes place in the Anopheles mosquitoes. Fang et al.,[25] identified a PI-PLCδ in T. gondii that cleaves PIP$_2$. Whilst recent bioinformatic approaches have failed to identify intracellular Ca$^{2+}$ release channels such as IP$_3$Rs and Ryanodine receptors (RyRs) within the genomes of apicomplexans [7, 26-28], a large body of pharmacological evidence suggests that a Ca$^{2+}$ release channel sensitive to InsP$_3$ does indeed
exist in these species. Prompted by evidence that malaria parasites maintain intracellular Ca\textsuperscript{2+} stores, Passos et al. [29] published the first work demonstrating InsP\textsubscript{3} mobilized Ca\textsuperscript{2+} from an intracellular store in *P. chabaudi* using a permeabilized cell system. The response was blocked by heparin (an inhibitor of InsP\textsubscript{3}Rs) providing pharmacological evidence for an InsP\textsubscript{3} dependent Ca\textsuperscript{2+} channel in *P. chabaudi*. Similarly, treatment of microscope preparations from *T. gondii* tachyzoites with InsP\textsubscript{3} initiated Ca\textsuperscript{2+} release which could be fully blocked by pharmacological inhibitors of InsP\textsubscript{3}Rs (Xestospongin C and heparin) [30].

Inhibitors of the InsP\textsubscript{3} signaling pathway, including agents that block InsP\textsubscript{3}R, such as heparin, xestospongic C and 2-aminoethoxydiphenyl borinate (2-APB), as well as inhibitors of PLC, such as U73122, have also been used to study Ca\textsuperscript{2+} signaling in Apicomplexa [31]. In *T. gondii*, the release of adhesins that mediate parasite attachment to host cells is an event regulated by Ca\textsuperscript{2+}-mediated microneme secretion [32,33]. Lovett et al. (2002) also reported that *T. gondii* mobilizes Ca\textsuperscript{2+} when exposed to xanomeline or caffeine, both RyRs [34]. Addition of xestospongic C inhibited the caffeine- or ethanol-induced increase of intracellular Ca\textsuperscript{2+}, thus preventing microneme secretion in *T. gondii* [35], and suggesting a role for InsP\textsubscript{3}R. In *Paramecium* this pathway controls stimulated trichocyst exocytosis, as found by energy-dispersive X-ray microanalysis and fluorochrome analysis [36], via activation of RyR-type Ca\textsuperscript{2+} release channels in alveolar sacs [10,37]. RyRs share common features and an evolutionary history with InsP\textsubscript{3}Rs [9,38,39], yet RyRs are activated by the intracellular messenger cADPR (cyclic ADP-ribose) [40]. In fact, cADPR has been shown to activate Ca\textsuperscript{2+} signaling pathways in *T. gondii* and *P. falciparum* [30,41].

In malaria melatonin dependent signaling via calcium is well established [29,42,43] enabling the investigation of a natural ligand to induce intracellular signaling. Hotta et al. [42] reported that intracellular Ca\textsuperscript{2+} mobilization induced by melatonin in *P. falciparum* and *P. chabaudi* is abolished by the PLC inhibitor U73122, but not by its inactive analogue, indicating that melatonin may activate signaling via PLC/InsP\textsubscript{3}. Enomoto et al. [44] blocked spontaneous Ca\textsuperscript{2+} mobilization in the ring and trophozoite forms of intraerythrocytic stage of *P. falciparum* using the InsP\textsubscript{3}R inhibitor, 2-APB [45]. The presence of 2-APB during intraerythrocytic development of *P. falciparum* compromised the asexual replication of this parasite suggesting that blocking the InsP\textsubscript{3}R can be a potential target for antimalarial treatment. Raabe et al. [46] investigated the role of PLP\textsubscript{2}/PLC/InsP\textsubscript{3} during *P. berghei* gametocyte exflagellation induced by xanthurenic acid (XA), a small metabolic intermediate found in Anopheles mosquito gut. Under XA stimulus, addition of the PLC inhibitor U73122 inhibits Ca\textsuperscript{2+} mobilization in this model. Together this work suggested a vital role of the InsP\textsubscript{3}/PLC signaling pathway at different points during the *Plasmodium* life cycle, and the important nature of this pathway suggests it may contain potential antimalarial targets expressed during all life cycle phases.

Evidence for a ryanoidine sensitive store in *T. gondii* [35] motivated Raabe et al. [46] to investigate the role of Ca\textsuperscript{2+} release via the RyR in *P. berghei* during gametocyte matura-

In the presence of RyR inhibitors, dantrolene and ruthenium red (RR), XA mediated Ca\textsuperscript{2+} increases were attenuated. Remarkably, RR treatment decreased InsP\textsubscript{3} levels and dantrolene inhibited gametocyte maturation during XA stimulation. These data also suggest the existence of ryanoidine sensitive channels in *P. berghei*. The development of cell permeant caged Ins\textsubscript{1,4,5}P\textsubscript{3} [47], enabled the investigation of InsP\textsubscript{3} dependent calcium signaling without compromising membrane integrity. With this tool, Alves et al. [43] reported that *P. falciparum* trophozoites within intact RBCs release Ca\textsuperscript{2+} from thapsigargin-sensitive stores due to liberation of caged Ins\textsubscript{1,4,5}P\textsubscript{3}. This observation was the first demonstration of a Ca\textsuperscript{2+}-increase induced by exogenous InsP\textsubscript{3} under physiological conditions in Apicomplexa. Furthermore, the authors reported an increase of InsP\textsubscript{3} in infected RBC treated with melatonin. These data further support the concept of PIP\textsubscript{2} hydrolysis to InsP\textsubscript{3} as a signaling pathway activated by melatonin in *P. falciparum*, as reported previously [42,48].

Mossaad et al. [49] first reported a complete reversal of chloroquine resistance in malaria parasites after treatment with 2-APB in vitro and in vivo, and this effect was justified by disturbance of Ca\textsuperscript{2+} homeostasis in the parasite cell. This result showed that 2-APB and other related compounds that block the InsP\textsubscript{3} pathway might be promising candidates in the search for new-resistance reversing agents that aid in treatment of the disease.

Beraldo et al. [50] showed that increase in cytosolic Ca\textsuperscript{2+} concentration in melatonin induced *P. falciparum* is abolished with the use of 2-APB, U73122 (PLC inhibitor) and luzindole (a melatonin antagonist). In the same work, capacitative calcium entry was also reported in malaria parasites.

Since then, other pharmacological agents have further been applied to investigate this pathway. It should be noted that most drugs used to interfere with signaling in protozoa are used solely on the basis of effects observed in mammalian cells [31], and all these drugs have potential off target effects. Despite this caveat, the combined evidence of a number of studies suggest the presence of an InsP\textsubscript{3}R and/or RyR calcium release channels in *Plasmodia* and *Toxoplasma*.

### 4. INS\textsubscript{P}\textsubscript{3}RS AND RYRS IN PROTOZOANS

The history of InsP\textsubscript{3}R discovery and the impact of this receptor on mammalian cell signaling has been previously reviewed [51]. Knowledge of receptor sequences and functional proprieties in mammals, together with increasing information and access to genome databases for different organisms, made bioinformatics tools such as BLAST searches a major strategy to identify orthologs to putative InsP\textsubscript{3}Rs and RyRs in metazoans.

Until recently no information about such channels has been available from protozoa. After successful identification of InsP\textsubscript{3}Rs and RyR-like proteins [9,37,52] in the ciliated protozoan, *Paramecium*, partial sequences suggested the occurrence of InsP\textsubscript{3}Rs in several protozoan phyla, including trypanosomatids [37,39]. Similarity with established InsP\textsubscript{3}Rs is often scattered throughout the protein, with highest similarity in the carboxy-terminal region containing the pore do-
main. Prole and Taylor [53] also used conserved regions of InsP₃R and RyR, specifically the amino-terminal RIH (RyR and InsP₃R homology) domain and the N-terminal InsP₃-binding domain, to successfully identify candidates for InsP₃R in *T. cruzi* and *T. brucei*. Such work supported the identification of an InsP₃R-type protein in *T. cruzi* [54,55] and *T. brucei* [56]. Hashimoto et al. [54] demonstrated that TcInsP₃R is essential for *T. cruzi* epimastigote survival, contributes to parasite invasiveness in mammalian cells, mediates Ca²⁺ release in trypomastigotes upon attachment to host cells, modulates parasite development and contributes to parasite virulence. These findings are consistent with previous evidence for an InsP₃/DAG pathway in *T. cruzi* (49) and *T. brucei* [47].

The identification of an InsP₃R candidate in apicomplexan parasites is a much more challenging task. When the same sequences of mammalian InsP₃Rs were used for BLAST searches, no InsP₃R orthologs were identified for *Plasmodium*, *Toxoplasma*, *Cryptosporidium* or *Babesia* species [53]. Previously, Naganume et al. [58], using a collection of Ca²⁺-associated protein orthologs to identify proteins participating in Ca²⁺ signaling, were also unable to find either any orthologs of InsP₃Rs, or of protein kinase C (PKC), a classic target for DAG activation [59]. Ladenburger et al. [47] were also unable to detect any InsP₃R or RyR orthologs in genomes of apicomplexans, in contrast to ciliates where they are readily found.

Recently, it was reported that, when TcInsP₃R expression is decreased by two thirds compared to wildtype, the invasion of trypomastigotes of *T. cruzi* is blocked, indicating TcInsP₃R as a potential therapeutic target [60]. After treatment of trypomastigotes with specific antisense oligonucleotides of TcInsP₃R, a reduction in infectivity, thus suggesting that the suppression of transcription of this gene led to reduced levels of TcInsP₃R protein. In Chagas disease, Ca²⁺ signaling mediated by InsP₃ plays a key role in multiple parasite differentiation steps [61]. A therapeutic approach using antisense transcripts is particularly feasible in the acute phase of infection in which trypomastigotes predominate in the bloodstream. It is possible that a treatment with antisense transcripts is effective in preventing the development of the disease by blocking the proliferation of trypomastigotes by inhibition of production of TcInsP₃R [62].

The presence of dense mitochondria in *T. cruzi* with non-functional InsP₃P,R provides new insights for the contribution of InsP₃Rs to organelle integrity and the fact that 2-APB does not completely inhibit the development of trypomastigotes [63] suggest structural difference to mammalian InsP₃Rs [64]. On the other hand, at high concentrations, 2-APB might influence functions of proteins other than InsP₃Rs.

5. LESSONS FROM OTHER SYSTEMS

Clues about the composition of Ca²⁺ signaling pathways in apicomplexans can be drawn from their closest ancestral group, free living ciliates. It is estimated that ciliates originated between ~800 and 850 million years [65,66]. Apicomplexa are assumed to have arisen ~550, and the genus *Plasmodium* ~400 million years ago [65]. These events occurred much before Apicomplexa became parasites of vertebrates and then of mammalian species, which occurred only ~13 million years ago [67]. *Paramecium tetraurelia* contains several genes encoding InsP₃Rs and RyR-like channels that have been identified at a genomic and proteomic level [9,37,52]. Considering the finding of InsP₃Rs and of RyR-like channels in *Paramecium* [10], the argument that the old age of some phyla may explain the failure to detect Ca²⁺ release channels in apicomplexans appears unlikely.

The lack of conservation at the gene level, suggests that InsP₃Rs in apicomplexans may diverge significantly from mammalian channels and those found in ciliates; one may ask whether they adopted a different, distinct protein or a complex of proteins for InsP₃R function. If this is the case, successful identification of InsP₃R in apicomplexans may require a different strategy focused on biochemical and physiological criteria. Specifically one should consider that any Ca²⁺ release channel requires a pore domain with six transmembrane stretches in InsP₃Rs and RyRs, from ciliates to humans, and a rather conserved, though slightly variable selectivity filter [10].

Currently the lack of identified Ca²⁺ release channels in Apicomplexa leaves a significant gap in our understanding of PI and calcium signaling mechanisms. There are clear physiological effects of stimulating production of InsP₃, and of inhibiting putative channels, and yet the molecular machinery in Apicomplexa is clearly diverse from other cells. Ultrastructurally the inner membrane complex of Apicomplexa looks very much like the alveolar sacs of ciliates; however, whereas the sacs of ciliates are well established Ca²⁺ stores [36], they appear largely independent of such function in Apicomplexa [8]. The third enigma is the presence of well defined InsP₃- and RyR-type Ca²⁺ release channels in ciliates [9,37,52], in contrast to their absence in their closest relatives, the Apicomplexa [68,69].

Similar to apicomplexans, plant genomes lack recognizable InsP₃Rs in despite of evidence for an InsP₃ signaling pathway [70]. Despite responding to InsP₃ to regulate Ca²⁺ increases, land plants typically lack an InsP₃R or RyR, leading to the suggestion that these channels were animal-specific, and perhaps evolved during the vertebrate lineage [70]. However, recent evidence from green algae *Chlamydomonas* indicates that chlorophyte plants contain InsP₃R, suggesting they were present in the ancestral eukaryotes prior to the plant-animal split [71]. Why InsP₃,R channels appear to have been lost in land plants remains a mystery, but it is intriguing to consider that like apicomplexans, they may have evolved different mechanism to release internal Ca²⁺ stores in response to InsP₃.

CONCLUSION

Differences in Ca²⁺ signaling between parasites and their hosts may present unique targets for developing interventions. For example, McNamara *et al.*, 2013 [72] reported PI4K as a target of imidazopyrazine, a class of new antimalarial drug that inhibits all stages of malaria parasite infection of the vertebrate host and also prevents transmission by mosquitoes to a murine malaria model. Additionally, as mentioned above, agents that disrupt InsP₃,R channels such as 2-ABP block malaria growth *in vitro* [44]. Given the apparent
divergent mechanism by which InsP₃ is sensed in parasites, identification of the molecular basis for this pathway might also identify novel targets for intervention.

The pharmacological literature and functional studies with exogenous InsP₃, discussed herein seem to support the divergent mechanism by which InsP₃ is sensed in parasites, InsP₃ Signaling in Apicomplexan Parasites Current Topics in Medicinal Chemistry,

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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