Identification of Intracellular and Plasma Membrane Calcium Channel Homologues in Pathogenic Parasites

David L. Prole*, Colin W. Taylor
Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom

Abstract
Ca\(^{2+}\) channels regulate many crucial processes within cells and their abnormal activity can be damaging to cell survival, suggesting that they might represent attractive therapeutic targets in pathogenic organisms. Parasitic diseases such as malaria, leishmaniasis, trypanosomiasis and schistosomiasis are responsible for millions of deaths each year worldwide. The genomes of many pathogenic parasites have recently been sequenced, opening the way for rational design of targeted therapies. We analyzed genomes of pathogenic protozoan parasites as well as the genome of *Schistosoma mansoni*, and show the existence within them of genes encoding homologues of mammalian intracellular Ca\(^{2+}\) release channels: inositol 1,4,5-trisphosphate receptors (IP\(_3\)Rs), ryanodine receptors (RyRs), two-pore Ca\(^{2+}\) channels (TPCs) and intracellular transient receptor potential (Trp) channels. The genomes of *Trypanosoma, Leishmania* and *S. mansoni* parasites encode IP\(_3\)-R/RyR and Trp channel homologues, and that of *S. mansoni* additionally encodes a TPC homologue. In contrast, apicomplexan parasites lack genes encoding IP\(_3\)-R/RyR homologues and possess only genes encoding TPC and Trp channel homologues (*Toxoplasma gondii*) or Trp channel homologues alone. The genomes of parasites also encode homologues of mammalian Ca\(^{2+}\) homeostasis and some are known modulators of mammalian Ca\(^{2+}\) channels, suggesting that parasite Ca\(^{2+}\) channel homologues might be the targets of some current anti-parasitic drugs. Differences between human and parasite Ca\(^{2+}\) channels suggest that pathogen-specific targeting of these channels may be an attractive therapeutic prospect.

Introduction
Parasitic diseases collectively affect billions of people and cause millions of deaths worldwide each year (Table 1). Many parasitic diseases are caused by single-celled protozoa such as various species of the apicomplexan parasites *Plasmodium*, *Toxoplasma*, *Cryptosporidium* and *Babesia*, as well as several species of the kinetoplastid *Trypanosoma* and *Leishmania* parasites. Other protozoan parasites that are major contributors to worldwide disease include *Trichomonas vaginalis*, *Entamoeba histolytica* and *Giardia intestinalis*. Another major cause of human death and mortality, second only to malaria among parasitic diseases, is the *Schistosoma* family of parasitic blood flukes. Many of these parasites maintain stringent control over their intracellular Ca\(^{2+}\) concentration [1,2] and have been shown to exhibit Ca\(^{2+}\) signals in response to physiological stimuli. These Ca\(^{2+}\) dynamics are critical for parasite function, egress, invasion, virulence and survival [2–4]. However, the molecular basis for these parasite Ca\(^{2+}\) responses is largely unknown.

Ca\(^{2+}\) release via intracellular Ca\(^{2+}\) channels controls numerous cellular processes, ranging from receptor signalling to growth and apoptosis [5]. Four main families of these channels have been identified in mammals: inositol 1,4,5-trisphosphate receptors (IP\(_3\)Rs), ryanodine receptors (RyRs), two-pore channels (TPCs), and some transient receptor potential (Trp) channels. Mammalian IP\(_3\)Rs and RyRs are responsible for release of Ca\(^{2+}\) from endoplasmic reticulum (ER) in response to the intracellular messengers IP\(_3\) and Ca\(^{2+}\) (IP\(_3\)Rs), or cyclic ADP ribose (cADPR) and Ca\(^{2+}\) (RyRs), produced via a wide variety of cellular signalling pathways [6,7]. The recently described TPCs are nicotinic acid adenine dinucleotide phosphate (NAADP)-activated channels responsible for Ca\(^{2+}\) release from acidic organelles, including lysosomes [8,9]. Mammalian intracellular Trp channels such as TrpM, TrpML and TrpP2 (polycystin-2) have also been shown to play a role in release of Ca\(^{2+}\) from intracellular stores [10,11]. Many parasites possess intracellular Ca\(^{2+}\) stores within their ER, mitochondria, glycosomes and acidocalcinsomes [2,3,12,13], but whether channels homologous to mammalian intracellular Ca\(^{2+}\) channels are present in these parasite organelles and whether they mediate Ca\(^{2+}\) release are unknown.

Ca\(^{2+}\) entry in mammalian cells is mediated by plasma membrane Ca\(^{2+}\) channels such as voltage-gated Ca\(^{2+}\) (Ca\(_{v}\)) channels [14], Trp channels [11] and store-operated Orai channels [15]. These channels are essential components in a multitude of signalling pathways and are also required for refilling of intracellular Ca\(^{2+}\) stores following intracellular Ca\(^{2+}\) release. Mechanisms of Ca\(^{2+}\) influx and demand for it are likely to differ between life cycle stages of many parasites, due to the differential ionic conditions of their respective environments. The intracellular...
S. mansoni may be essential for parasite survival. In the extracellular and plasma membrane Ca\(^{2+}\) parasites. Some currently used anti-parasitic agents alter Ca\(^{2+}\) cloning of three for muscle contraction and viability [17]. However, apart from the parasite channels may therefore represent attractive novel targets that are important for channel activation, ion conduction or drug binding may result in distinct pharmacological profiles. These parasite channels from their mammalian counterparts in regions of pathogenic parasitic protozoa, and that of S. mansoni (Table 1). In this study we examined the genomes of pathogenic parasitic protozoa, and that of S. mansoni, for the presence of genes that might encode Ca\(^{2+}\) channels in pathogenic parasites remain largely unknown.

Pharmacological or genetic modulation of Ca\(^{2+}\) channel activity in the plasma membrane or intracellular organelles has profound effects on cell function and survival in many organisms. This suggests that the channels underlying Ca\(^{2+}\) signals in parasites might represent novel drug targets. Recent advances in genomics have led to whole-genome sequencing of several parasites that are pathogenic to whole-genome sequencing of several parasites that are pathogenic to their large size (2700 residues for RyRs and 2700 residues for RyRs). These homologues were found in the vertebrates [7,20], each species of Trypanosoma and Leishmania parasite possessed only a single homologue. In contrast, S. mansoni possessed two homologues: one similar to mammalian IP3Rs and RyR against the whole-genome sequences of pathogenic parasites identified several predicted protein products displaying significant similarity in this pore region (Figure 1 and Table 2). These homologues were found in the kinetoplastid Leishmania and Trypanosoma parasites, as well as in S. mansoni. In contrast to the multiple isoforms of IP3R and RyR found in vertebrates [7,20], each species of Trypanosoma and Leishmania parasite possessed only a single homologue. In contrast, S. mansoni possessed two homologues: one similar to mammalian IP3Rs (XP_002576843/42 in Figure 1), which showed 34% identity with human IP3R3 (hIP3R3) and 31% identity with human RyR1 (hRyR1) in the full-length proteins; and one more similar to mammalian RyRs (XP_002578360), which showed 45% identity with hRyR2 and 32% identity with hIP3R1 (Figure 1). No IP3R/RyR homologues of either pore or full-length sequences) were found in the genomes of any of the apicomplexan parasites, or in the protozoan parasites E. histolytica, G. intestinalis and T. vaginalis.

### Table 1. Pathogenic parasites with completed whole-genome sequences, their associated diseases and worldwide disease burden.

| Parasite                  | Disease                              | Estimated worldwide cases of infection | Genome References |
|---------------------------|--------------------------------------|----------------------------------------|-------------------|
| Plasmodium falciparum     | malaria                              | 3 billion [103,126]                    | [127–130]         |
| Plasmodium knowlesi        | malaria                              |                                        |                   |
| Plasmodium vivax           | malaria                              |                                        |                   |
| Toxoplasma gondii          | toxoplasmosis                        | 6–75% of population [131]              | [132]             |
| Cryptosporidium hominis    | cryptosporidiosis, diarrhoea          | 1–20% of population [131,133]          | [134–136]         |
| Cryptosporidium muris      |                                      |                                        |                   |
| Cryptosporidium parvum     |                                      |                                        |                   |
| Babesia bovis              | babesiosis                           | humans: >500 cattle: 400 million       | [137]             |
| Leishmania major           | leishmaniasis                        | 12 million [131]                      | [138–140]         |
| Leishmania infantum        | leishmaniasis                        |                                        |                   |
| Leishmania braziliensis    |                                        |                                        |                   |
| Trypanosoma brucei         | african trypanosomiasis (sleeping sickness) | 500,000                              | [141]             |
| Trypanosoma cruzi          | Chagas’ disease                      | 10 million [142]                      |                   |
| Entamoeba histolytica      | amoebiasis, dysentry                 | 50 million [131]                      | [143]             |
| Giardia intestinalis       | giardiasis                           | 280 million [144]                     | [145–148]         |
| Trichomonas vaginalis      | trichomoniassis                      | 174 million [149]                     | [150]             |
| Schistosoma mansoni        | schistosomiasis (bilharzia)          | 200 million [121]                     |                   |

World Health Organization (http://www.who.int/en) and Center for Disease Control (http://www.cdc.gov) figures, in addition to the references cited, were used as sources of worldwide epidemiology, doi:10.1371/journal.pone.0026218.t001

Results

Parasite homologues of IP3R and RyR pores

Mammalian IP3Rs and RyRs show a high degree of similarity, particularly in the pore region responsible for ion conduction. BLAST analysis of the conserved pore-region sequences of mammalian IP3Rs and RyRs against the whole-genome sequences of pathogenic parasites identified several predicted protein products displaying significant similarity in this pore region (Figure 1 and Table 2). These homologues were found in the kinetoplastid Leishmania and Trypanosoma parasites, as well as in S. mansoni. In contrast to the multiple isoforms of IP3R and RyR found in vertebrates [7,20], each species of Trypanosoma and Leishmania parasite possessed only a single homologue. In contrast, S. mansoni possessed two homologues: one similar to mammalian IP3Rs (XP_002576843/42 in Figure 1), which showed 34% identity with human IP3R3 (hIP3R3) and 31% identity with human RyR1 (hRyR1) in the full-length proteins; and one more similar to mammalian RyRs (XP_002578360), which showed 45% identity with hRyR2 and 32% identity with hIP3R1 (Figure 1). No IP3R/RyR homologues (of either pore or full-length sequences) were found in the genomes of any of the apicomplexan parasites, or in the protozoan parasites E. histolytica, G. intestinalis and T. vaginalis.

Structures of parasite IP3R and RyR homologues

Although the Leishmania, Trypanosoma and S. mansoni parasite proteins show a high degree of sequence similarity to IP3Rs/RyRs in the pore region, it was necessary to examine similarity in other regions before establishing them as potential functional correlates. Defining characteristics of vertebrate IP3Rs and RyRs include their large size (~5000 residues for RyRs and ~2700 residues for IP3Rs) and the presence of: multiple transmembrane domains near their C-terminal ends, conserved N-terminal MIR (mannosyltransferase, IP3R and RyR) domains, conserved R1H (internal RyR and IP3R homology) sequences with homology to the IP3-binding core of IP3Rs [21], conserved “R1H-associated domains” preceding their C-terminal transmembrane regions, and conserved N-terminal domains with homology to the suppressor domain of IP3Rs [22] (Figure 2A). Mammalian RyRs are also distinguished by the presence of multiple copies of a repeat termed an SPRY (SPFa and RyR) domain and multiple copies of a repeat termed a RyR domain, both of which have unknown functions.
The parasite IP$_3$/RyR pore homologues are large proteins (2216–4998 residues) [Figure 1]. The *S. mansoni* IP$_3$/R-like and RyR-like homologues are 2216 and 4998 residues in length, and phylogenetic analyses are consistent with those proteins representing IP$_3$R and RyR homologues respectively [Figures 2A and 2B]. Significantly, most homologues have predicted N-terminal RIH domains [Figure 2A], and searches of parasite genomes using the N-terminal IP$_3$-binding domain sequence of mammalian IP$_3$Rs (residues 224–604 of rat IP$_3$R1) resulted in alignment with the same *Leishmania*, *Trypanosoma* and *S. mansoni* proteins found in the pore homology search. However, alignments suggested that like RyRs, the parasite homologues lack many of the ten basic residues known to be important for high-affinity binding of IP$_3$ to mouse IP$_3$R1 [23] (data not shown). The *S. mansoni* protein (XP_002576843/42), which has the greatest sequence similarity to full-length human IP$_3$Rs, also has the highest conservation of these basic residues (5 out of 10) (data not shown). All parasite homologues are predicted to contain multiple putative transmembrane domains near their C-termini, and to contain N-terminal domains with similarity to the suppressor domain of mammalian IP$_3$Rs and the analogous A-domain of RyRs [22] [Figure 2A]. In addition, most homologues have N-terminal RIH-associated sequences [Figure 2A]. Both *S. mansoni* homologues have N-terminal MIR domains, while the *S. mansoni* RyR homologue, like mammalian RyRs, has four copies of a RyR motif and multiple copies of an SPRY domain [Figure 2A]. Several consensus Ca$^{2+}$-binding EF-hands are also present in the C-terminus of the *S. mansoni* RyR homologue [Figure 2A], suggesting that this channel, like mammalian IP$_3$Rs and RyRs, might be regulated by cytosolic Ca$^{2+}$. Taken together, these sequence similarities suggest that these parasite channels are structural and functional analogues of mammalian IP$_3$Rs and RyRs.

The close similarity of the pore regions of parasite channels to those of mammalian IP$_3$Rs/RyRs, including the conserved GGGXGD selectivity filter motif [Figure 1] suggests that these parasite channels are likely to form cation channels with high single-channel conductance and permeability to Ca$^{2+}$. Despite this significant similarity, parasite homologues show some potentially important sequence divergence from human IP$_3$Rs/RyRs in the pore region [Figure 1]. For example, both *Leishmania* homologues and the *S. mansoni* IP$_3$R-like homologues differ from human IP$_3$Rs/RyRs near the selectivity filter region, at positions analogous to hRyR1 residues G4909 and R4993 that are known to affect ryanodine binding [24]. Also, all parasite homologues (except the *S. mansoni* RyR homologue) differ from human RyRs in the pore-lining transmembrane domain, at the position analogous to Q4934 of hRyR1, which is a determinant of ryanodine binding [25]. Some pathogenic parasites therefore possess homologues of both IP$_3$Rs and RyRs in which key functional domains are well enough conserved to suggest that these proteins might function as intracellular Ca$^{2+}$ channels. However, the sensitivity of these parasite channels to drugs may differ sufficiently from those of their host to perhaps allow their selective targeting by drugs.

**TPC homologues**

We searched next for parasite homologues of mammalian TPCs, which mediate release of Ca$^{2+}$ from acidic organelles in mammalian cells. A defining characteristic of both mammalian and plant TPCs is the presence of two independent pore-forming domains in series, each consisting of multiple transmembrane domains [8, 9, 26, 27]. We therefore searched parasite genomes, using the full-length sequences of human TPC1 and TPC2, for homologues that contained at least four transmembrane domains in two distinct regions. Reciprocal BLAST searches were then carried out with sequences of the identified parasite proteins, to confirm specific homology with mammalian TPCs. This approach identified TPC homologues in only *S. mansoni* and *T. gondii* (Figures 3A and 3B). These parasite homologues show substantial similarity to mammalian TPCs in the pore regions responsible for ion conduction (Figure 3A) suggesting that they, like their mammalian counterparts, may act as Ca$^{2+}$-permeable channels. The *S. mansoni* homologue shows most similarity to human TPC2, while the *T. gondii* homologue is more distantly related to mammalian TPCs (Figure 3C). Interestingly, the *S. mansoni* TPC homologue has only two predicted transmembrane segments in each of its pore domains, compared to the six found in each domain of mammalian TPCs [8, 9, 26, 27].
Intracellular Trp channel homologues

In addition to IP₃Rs, RyRs and TPCs, several subtypes of mammalian Trp channel are located within the membranes of intracellular organelles and mediate Ca²⁺ release in mammalian cells. These include TrpM, TrpML, and TrpP2 channels [10,11,28]. We therefore searched parasite genomes for homologues of these Trp channels, using the full-length and N-terminally truncated (to remove common ankyrin domains) sequences of human isoforms, followed by further searches using the sequences of identified homologues from T. gondii. We identified many parasite homologues of these Trp channels (Table 2). Putative Trp channel homologues in *Plasmodium spp* were only identified by homology with a *T. gondii* Trp channel homologue, and show only weak similarity to mammalian Trp channel homologues identified here.
channels. Overall, these results are consistent with a recent report of Trp channels in various parasites [19], although we describe here additional putative homologues in *Plasmodium* spp., *T. gondii*, *T. vaginalis*, *Cryptosporidium* spp., *Trypanosoma* spp., and *S. mansoni* (Table 2). Definitive categorization of homologues as subtypes of Trp channel was difficult, as multiple homologies existed between these proteins. Homologues were therefore categorized by reference to the human Trp channels to which they showed the greatest sequence similarity (Table 2). TrpML and TrpP homologues were found in most parasites examined, while TrpM channel homologues were found only in *S. mansoni* (Table 2). We were most interested in the TrpML/TrpP homologues found in kinetoplastid parasites, which show considerable similarity to human TrpML and TrpP2 channel subunits in the putative pore region (Figure 4A). They also have conserved predicted polycystin (PKD) domains and long TMD1-TMD2 linkers that are characteristic of TrpML and TrpP channel subunits (Figure 4B). In addition, several of these parasite proteins have a cluster of basic residues preceding their TMD1 regions, similar to the cluster responsible for binding phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2) in mouse TrpML1 [29] (Figure 4B). The Trp channel homologue Yvc1p (TrpY1) is responsible for mediating Ca2+ release from vacuolar stores of *Saccharomyces cerevisiae* [30,31]. We therefore searched parasite genomes for homologues of full-length Yvc1p, but none were found.

### Plasma membrane Trp channel homologues

Ca2+-permeable Trp channels located in the plasma membrane of mammalian cells include TrpC, TrpA, TrpV and TrpP subtypes [11,28]. TrpC channels may contribute to store-operated Ca2+ entry in many cells [32], TrpA and TrpV channels respond to external chemical and thermal stimuli [11], and TrpP2 channels at the plasma membrane respond to stimuli including mechanical stress and receptor activation [33]. We searched parasite genomes for homologues of these Trp channels, using the full-length and N-terminally truncated (again, to remove ankyrin domain hits) sequences of human isoforms, and identified several parasite homologues (Table 2). The protozoan parasite genomes examined were found to lack homologues of TrpA and TrpC channels, and only *T. vaginalis* had a homologue of TrpV channels.
In contrast, *S. mansoni* was found to contain TrpA and TrpC channel homologues (Table 2). The presence of TrpP homologues was discussed earlier, in the context of intracellular Trp channels. The Trp channel homologues identified are consistent with a recent report of Trp channels in parasites [19], with additional homologues found to exist in *S. mansoni* (XP_002576118) and *T. vaginalis* (XP_001296819) in the current study (Table 2).

**Orai and STIM homologues.** Store-operated Ca\(^{2+}\) entry (SOCE) is an almost ubiquitous feature of mammalian cells, where plasma membrane channels composed of Orai subunits mediate Ca\(^{2+}\) influx in response to emptying of intracellular Ca\(^{2+}\) stores [34-37]. STIM subunits act as the sensors of ER Ca\(^{2+}\) depletion and are necessary for activation of Orai channels [38–40]. However, apart from a single study suggesting the presence of SOCE in *Plasmodium falciparum* [41], the existence of SOCE in parasites is largely unexplored, and whether Orai and STIM homologues exist in parasites is not known.

Parasite genomes were searched for homologues of these proteins, using full-length sequences of human Orai1 and STIM1 proteins. All pathogenic apicomplexan parasites examined were found to lack both Orai and STIM homologues. In contrast, the *S. mansoni* genome contains a gene encoding an Orai1 homologue (XP_002578837), with an alternatively spliced variant (XP_002578838) (Figures 5A and 5B), and a homologue of STIM1 (XP_002581211). The Orai homologue shows pronounced identity with human isoforms in the pore region (Figure 5A), including residues R91 and E106 of human Orai1, which are critical for pore function [34,42,43]. The occurrence of both Orai and STIM homologues in *S. mansoni* strongly suggests that a SOCE process similar to that found in mammalian cells exists in this parasite.

**Ca\(_{\text{v}}\) channels**

Many subtypes of plasma membrane Ca\(_{\text{v}}\) channel exist that are crucial for voltage-dependent Ca\(^{2+}\) influx in a wide variety of
mammalian cells [14]. Human Ca\textsubscript{v} channel sequences and the sequence of the Cch1 Ca\textsubscript{v} channel homologue from S. cerevisiae [44,45] were used to search for homologues in parasites. S. mansoni was found to possess genes encoding several Ca\textsubscript{v} channel homologues. In addition to the previously described and cloned Cav1, Cav2A and Cav2B channels [18], we identified two novel putative Ca\textsubscript{2+} channels (XP_002578175 and XP_002575006) (Table 2). A partial sequence of another potentially novel Cav homologue was also identified (XP_002571932), which shows pronounced (but not perfect) similarity with Cav2B, but insufficient sequence was available to conclusively categorize this protein. Cav channel homologues were also found in all other parasites examined except Plasmodium spp., Cryptosporidium spp., T. vaginalis, G. intestinalis and E. histolytica (Table 2 and Figure 6). We also searched parasite genomes for homologues of the CatSper channels (relatives of Cav, TPC and Trp channels) that are responsible for Ca\textsubscript{2+} entry into mammalian spermatozoa [46]. All CatSper homologues found were identical to the Ca\textsubscript{v} homologues identified above. Some of the Ca\textsubscript{v} channel homologues identified in parasites are predicted to possess 18–24 transmembrane domains (Table 2), consistent with a four-domain structure formed from a single subunit, similar to the organization of mammalian Cav channels (Figure 6A) [14]. In contrast, some of the parasite Cav channel homologues, such as those found in T. gondii and B. bovis, possess only 2–6 predicted transmembrane domains (Table 2), suggesting that these may be a novel class of Cav channel homologue, formed by tetramerization of four individual subunits. Like mammalian Cav channels and Cch1, most parasite Cav channel homologues have acidic residues within their putative pore loops (Figure 6B). In mammalian Ca\textsubscript{v} channels these residues form a negatively charged ring in the intact tetrameric channel, which facilitates selectivity for Ca\textsubscript{2+} [14]. Fewer acidic residues are present at the analogous positions in some homologues from Leishmania spp. and Trypanosoma spp.
Figure 5. Comparison of human and S. mansoni Orai homologues. (A) Alignments of human Orai subunits with predicted homologues from S. mansoni are shown (XP_002578837 is a shorter alternatively spliced variant of XP_002578837). Residues within the putative pore that are crucial for hOrai1 function (R91 and E106) are highlighted by grey bars. Predicted transmembrane regions of hOrai1 are indicated by red bars above the alignment, and asterisks below the alignment denote sequence identity amongst all isoforms. (B) Phylogram showing the relationship between human and S. mansoni Orai homologues as well as those of the model invertebrates C. elegans, D. melanogaster and C. intestinalis (see Methods: based on 141 high confidence positions from a multiple sequence alignment; gamma shape parameter 1.541; proportion of invariant sites 0.156). Branch length scale bar and branch support values are shown. doi:10.1371/journal.pone.0026218.g005
although several acidic residues are present at other positions within the putative pore loops of these proteins (Figure 6B). Voltage-gated sodium (Nav) channels are thought to have evolved from Cav channels and share a high degree of sequence similarity with them [47]. It is possible therefore that some of the Cav homologues identified here may be selective for Na\(^+\) rather than Ca\(^{2+}\) (or they may be non-selective), although experimental testing will be required to determine this. However, all parasite homologues identified lacked the characteristic DEKA selectivity filter motif of mammalian Nav channels, which is necessary for Na\(^+\) selectivity [48] (Figure 6B). Many of the parasite Cav channel homologues possess several regularly spaced basic residues in the TMD4 region of each domain (Figure 7A and 7B), suggesting functional equivalence of these regions to the voltage sensors of mammalian Cav channels [14].

Other Ca\(^{2+}\)-permeable channels

Given the relative sparsity of Ca\(^{2+}\) channel homologues in the protozoan parasites studied, we also searched their genomes for genes encoding homologues of other mammalian Ca\(^{2+}\)-permeable channels. Cyclic nucleotide-gated (CNG) cation channels can mediate Ca\(^{2+}\) influx in mammalian cells [49]. We searched parasite genomes for CNG homologues, using the full-length and transmembrane region-only (to remove common cyclic nucleotide

Figure 6. Parasite Ca\(^{2+}\) channel homologues show similarity to human Ca\(^{2+}\) channels in the pore region. (A) Schematic showing the four-domain structure of human Ca\(^{2+}\) channels, with the pore loop of each domain shown in red. Cylinders indicate TMDs, and plus signs indicate the charged voltage sensor regions. (B) Multiple sequence alignments of the pore domains of human Cav channels with parasite Cav channel homologues. Only those parasite homologues containing four putative domains are shown. Sequences of human Ca\(_{1.2}\) (L-type), Ca\(_{2.1}\) (P/Q-type), Ca\(_{2.2}\) (N-type) and Ca\(_{3.2}\) (T-type) channels, as well as the S. cerevisiae Cch1 Ca\(^{2+}\) channel (ScCch1), are shown. L. braz denotes L. braziliensis. Selected human Nav isoforms are included, to allow comparison with the related Cav channels. The locations of acidic residues (underlined) forming an acidic ring motif in human Cav\(_{1.2}\) channels are indicated by asterisks. The overall motif formed by all four domains at this locus is indicated to the right of the Domain IV alignment for each channel homologue.

doi:10.1371/journal.pone.0026218.g006
binding domains) sequences of human CNGA1 and CNGA2. Only hits containing multiple transmembrane domains and which showed similarity in the pore and ligand-binding regions were acknowledged. No homologues were found in any of the protozoan parasites examined, except for several homologues in T. vaginalis which all contain the pore motif GYGD reminiscent of potassium channels [50]. This suggests that they may be K⁺-selective channels rather than Ca²⁺ channels, although this requires experimental testing.

We also searched protozoan genomes for genes encoding homologues of potentially Ca²⁺-permeable mammalian NMDA receptors, kainate receptors, AMPA receptors, pannexins, P2X4 purinergic receptors and nicotinic acetylcholine receptors. No convincing homologues (i.e., showing multiple transmembrane domains, pore homology, and reciprocal BLAST output) of these channels were found in any of the protozoan parasite genomes examined. In contrast to protozoan parasites, S. mansoni is known to have homologues of some of these receptors, such as nicotinic acetylcholine receptors [51,52] and P2X receptors [53], but analysis of these likely non-selective cation channel homologues in this organism was not extended further.

**Discussion**

Ca²⁺ channels are critical for many of the most fundamental cellular processes and pharmacological modulation of these channels can lead to marked changes in cell growth and viability. These channels therefore represent attractive drug targets for treatment of infectious disease. Complex Ca²⁺ signalling processes exist in parasites and Ca²⁺-handling machinery including Ca²⁺-
ATPases is present in these organisms [2,3]. We have shown that genes encoding proteins homologous to mammalian intracellular Ca\(^{2+}\) channels and Ca\(^{2+}\) influx channels are present in many of the most clinically relevant pathogenic parasites (Table 2). Many of these putative channels are not yet annotated in available pathogen databases, such as ToxoDB and TriTrypDB (http://eupathdb.org/eupathdb) [54]. The presence of these Ca\(^{2+}\) channel homologues, along with the occurrence of physiological Ca\(^{2+}\) signals in parasites, suggests that they participate in Ca\(^{2+}\) signalling within these cells. Our analysis has attempted to distinguish intracellular from plasma membrane Ca\(^{2+}\) channels because the distribution of Ca\(^{2+}\) channels profoundly affects their regulation, the amount of Ca\(^{2+}\) to which they have access, and the subcellular organization of the Ca\(^{2+}\) signals they evoke [55]. It is however increasingly clear that many Ca\(^{2+}\) channels can function effectively in both intracellular organelles and the plasma membrane [55]. Some parasites, whose extracellular surface may, at different stages of their lifecycle, be exposed to typical extracellular Ca\(^{2+}\) concentrations or to the 10,000-fold lower Ca\(^{2+}\) concentration of their host cell’s cytosol, may perhaps exploit this plasticity in targeting of Ca\(^{2+}\) channels even more extensively than mammalian cells. Despite the difficulty of unambiguously assigning Ca\(^{2+}\) channel homologues to specific membrane compartments on the basis of their primary sequence alone, subsequent sections consider the parasite homologues by reference to the distribution of their mammalian counterparts.

Parasite intracellular Ca\(^{2+}\) channels

Pathogenic parasites contain a variety of intracellular Ca\(^{2+}\) stores including ER, mitochondria, glycosomes and acidocalcisomes, and biochemical signalling pathways analogous to those of mammalian cells have been shown to exist in parasites. Evidence exists for the presence of phospholipase C signalling pathways in parasites [56–58], which may sustain IP\(_3\)-based effects on IP\(_3\)R homologues. The intracellular messenger cADPR that activates mammalian RyRs has also been shown to be involved in parasite signalling pathways [59–60]. Which of these pathways might regulate the IP\(_3\)-R/RyR homologues in kinetoplastid parasites containing a cluster of basic residues required for high-affinity binding of IP\(_3\) in mouse IP\(_3\)R1 [23]. These observations suggest that the IP\(_3\)-R/RyR homologues in these organelles might respond to a different intracellular messenger (such as cADPR), or that they might require specific conditions for activity in response to IP\(_3\).

Apicomplexan parasite genomes appeared to lack IP\(_3\)-R/RyR homologues, as reported by others [61]. Despite the absence of IP\(_3\)-R/RyR homologues in these parasites, IP\(_3\) has been reported to elicit Ca\(^{2+}\) release from intracellular stores of Plasmodium chabaudi [64] and E. histolytica [65], and Ca\(^{2+}\) release has been attributed to IP\(_3\)-R/RyR-like channels in T. gondii [66] and Plasmodium berghei [67]. These observations suggest that another type of IP\(_3\)-sensitive intracellular Ca\(^{2+}\) channel exists in apicomplexan parasites. A similar situation is seen in S. cerevisiae, which lacks IP\(_3\)-R/RyR homologues, but has been reported to show IP\(_3\)-induced Ca\(^{2+}\) release from vacuolar vesicles [68]. The Yvc1p (TrpY1) channel is responsible for mediating Ca\(^{2+}\) release from vacuolar stores of S. cerevisiae [30–31] and this channel is sensitive to Pl(3,5)P\(_2\) [29], although whether this channel is also sensitive to IP\(_3\) is not yet known. Our searches found no homologues of Yvc1p among the genomes of pathogenic apicomplexa. However, we did identify apicomplexan homologues of the Pl(3,5)P\(_2\)-sensitive mammalian endoplasmic reticulum TrpML1 channel [29]. Whether mammalian TrpML channels are sensitive to IP\(_3\) has not been tested, so whether these homologues might mediate the IP\(_3\)-sensitive Ca\(^{2+}\) release in apicomplexan parasites remains uncertain. The molecular determinants of IP\(_3\)-evoked Ca\(^{2+}\) release in apicomplexan parasites therefore remain to be determined. The Trp, TPC, Cch1 and Ca, channel homologues identified in this study (see below and Table 2) are also potential candidates for the role of novel IP\(_3\)-sensitive intracellular Ca\(^{2+}\) channels in the pathogenic parasites that lack IP\(_3\)/R/RyR homologues.

Trp channels in mammalian cells can mediate Ca\(^{2+}\) release from intracellular stores in response to a wide variety of stimuli [10,11]. In addition, the Trp channel homologue Yvc1p is the dominant Ca\(^{2+}\) release channel in S. cerevisiae [30,31]. Trp channel homologues are therefore likely candidates for mediating intracellular Ca\(^{2+}\) release in parasites, consistent with previous identification of TrpML genes in L. major [69] and a recent report published during the course of this study which noted the presence of Trp channel homologues in a variety of parasites [19]. We identified a striking abundance of Trp channel homologues in parasites (Table 2), including many TrpML homologues, which in mammalian cells have been shown to play roles in signal transduction, ion homeostasis and membrane trafficking [29,70]. TrpP homologues are also apparent in many parasites (Table 2). In mammalian cells these channels can mediate release of Ca\(^{2+}\) from ER stores and can be modulated by cytosolic Ca\(^{2+}\) via an EF-hand domain [10,71,72]. Whether these parasite Trp channels play similarly diverse roles in Ca\(^{2+}\) release, signal transduction and membrane trafficking remains an exciting avenue for future research.

The parasite acidocalcisome is similar in several ways to mammalian lysosomes from which TPCs [8,9], TrpM [73] and TrpML [29,70] channels mediate Ca\(^{2+}\) release in mammalian cells. Both organelles have a low luminal pH, high Ca\(^{2+}\) content and enzymatic activity [74]. TPCs in mammals and sea-urchins are activated by intracellular NAADP [8,9], plant TPCs may be regulated by Ca\(^{2+}\) [75], TrpML channels are modulated by Pl(3,5)P\(_2\) [29] and TrpM2 channels are modulated primarily by adenosine diphosphoribose (ADPR), as well as cADPR and Ca\(^{2+}\) [73]. However, no second messenger has yet been shown to cause release of Ca\(^{2+}\) from acidocalcisomes [74]; neither NAADP nor IP\(_3\) releases Ca\(^{2+}\) from acidocalcisomes of sea urchin eggs [76]; and the effect of NAADP and ADPR on Ca\(^{2+}\) dynamics in parasites has not been studied. Interestingly, three of the TrpM homologues in S. mansoni (XP_002579069, XP_002571459 and XP_002578454) contain Nudix hydrolase domains, suggesting that they might be involved in signalling pathways utilizing nucleoside diphosphate derivatives such as ADPR or cADPR, as is the case with mammalian TPC channels [77,78]. Some TrpML homologues in kinetoplastid parasites contain a cluster of basic residues before their TMD1 regions. A similar cluster allows binding of Pl(3,5)P\(_2\) to mammalian TrpML channels and subsequent channel activation [29], suggesting that the parasite homologues may also form phosphoinositide-sensitive channels. Whether the parasite homologues of mammalian TPCs or other intracellular Ca\(^{2+}\) channels identified in this study release Ca\(^{2+}\) from acidocalcisomes or other organelles in response to NAADP, ADPR, cADPR, Pl(3,5)P\(_2\), IP\(_3\), Ca\(^{2+}\), or other intracellular messengers therefore remains to be determined.

Parasite Ca\(^{2+}\) influx channels

Ca\(^{2+}\) influx mediates physiological signalling pathways in parasites [79–84] and allows refilling of intracellular Ca\(^{2+}\) stores following intracellular Ca\(^{2+}\) release. The importance of Ca\(^{2+}\) influx
pathways for cell survival in general has been demonstrated in S. cerevisiae, where lack of the plasma membrane Cch1 Ca^{2+} channel impairs high-affinity Ca^{2+} uptake, and leads to cell death in conditions of low Ca^{2+} concentration or when Ca^{2+} influx is required [44,45]. Recently it has likewise been shown that in Leishmania parasites, Ca^{2+} influx is necessary for thermotolerance [94]. Many protozoan parasites exist within mammalian cells for part of their life cycle, where Ca^{2+} levels are maintained at low levels (typically <100 nM). These parasites therefore seem likely to require unique strategies in order to enhance Ca^{2+} uptake.

Residence of some parasites within parasitophorous vacuoles or other membraneous compartments [16,85] helps to circumvent this problem by surrounding the parasite with elevated Ca^{2+}, but parasite plasma membrane Ca^{2+} channels or transporters with novel properties may also be essential, especially for those parasites directly exposed to the cytosol, such as T. cruzi [16,86]. The Ca^{2+} channel homologues described in this study may differentially contribute to Ca^{2+} influx during different phases of parasite life cycles and may have novel properties that allow their function in ionic conditions that differ substantially from those experienced by their mammalian counterparts.

Ca^{2+} channel homologues were found in many of the pathogenic parasites examined, suggesting that these proteins have a widespread function in parasites. The presence in these homologues of charged regions analogous to the voltage sensors of Ca^{2+} channels suggests that they might be gated by transmembrane voltage, although this remains to be tested experimentally. Homologues of mammalian Ca^{2+}-permeable TrpA, TrpC and TrpV subtypes capable of mediating plasma membrane Ca^{2+} influx were absent from most protozoan parasites. In contrast, TrpA and TrpC channel homologues were found in S. mansoni, although their exact subtype categorization was unclear. Since in addition to mediating intracellular Ca^{2+} release, TrpP channels are also capable of mediating Ca^{2+} influx in mammalian cells when located in the plasma membrane [33,87], the “intracellular” Trp channel homologues identified in this study may also contribute to Ca^{2+} influx in parasites. Mammalian Trp channels are modulated by diverse stimuli [10,11,32] making them interesting candidates for the transduction of environmental stimuli into physiological responses in S. mansoni.

Homologues of the Orai channels which mediate SOCE in mammalian cells [34–37], were found to be absent from all pathogenic protozoa examined. In addition, the ER Ca^{2+}-sensing STIM proteins responsible for activation of Orai proteins [38–40] were also absent from these parasites. This suggests that either these parasites lack SOCE, or that other proteins might fulfil the roles of both the pore-forming and Ca^{2+}-sensing subunits of SOCE channels in these organisms. In mammalian cells, TrpC channels have also been shown to contribute to SOCE in a variety of cells [88], suggesting the possibility that Trp channel homologues (Table 2) might fulfil roles as SOCE channels in some of these protozoa. Interestingly, in contrast to the absence of Orai/STIM proteins in the protozoan parasites tested, S. mansoni has an alternatively spliced Orai homologue and a STIM1 homologue, suggesting that Orai/STIM-mediated SOCE exists in this parasite.

The expression and function of the putative Ca^{2+} channel homologues identified in this study will in future need to be measured experimentally in order to confirm their status as Ca^{2+} channels. Homologues of other mammalian non-selective cation channels may also be found in parasites and may contribute to Ca^{2+} signalling pathways in these organisms. In addition, some of the Ca^{2+} channel homologues identified in this study may play roles in flux of other ions such as Na^{+} or K^{+}, perhaps in a lifecycle-dependent manner, given the substantial changes in ionic composition of the extracellular environment during different stages of the lifecycle in many parasites.

Ca^{2+} channel homologues in parasites and their free-living relatives

Comparison of the genomes of the parasitic Apicomplexa examined here (Plasmodium spp., T. gondii and Cryptosporidium spp.) with their free-living ciliate relative Paramecium reveals differences in the complement of genes encoding Ca^{2+} channels. IP_{3}/R/RyR homologues are absent in the Apicomplexa examined, whereas they are present in Paramecium [89]. Likewise, Ca^{2+} channels with a four-domain architecture similar to mammalian Ca^{2+} channels are absent in the Apicomplexa (T. gondii has several more distantly related homologues, but these do not have a four-domain structure), whereas they are present in Paramecium (data not shown). Whether the apparent absence of IP_{3}/R/RyR and Ca^{2+} channels in Apicomplexa occurred as a result of the acquisition of a parasitic existence is unclear. In contrast, both the Apicomplexa examined here and Paramecium (data not shown) appear to have Trp channel homologues. Paramecium [90] and the apicomplexan parasite P. falciparum [41] have been reported to display SOCE, although the molecular basis is unknown. As with the Apicomplexa, our searches did not reveal any genes encoding convincing homologues of Orai1 in Paramecium (data not shown), suggesting that lack of Orai homologues in Apicomplexa may not be a result of transition to parasitism. The channel-forming proteins underlying SOCE in these organisms remain to be identified.

A comparison of the flagellate parasites examined here (Trypanosoma spp. and Leishmania spp.) with their free-living chonogloablative relative Monosiga breviscillis also reveals differences. Both M. breviscilllis [91] and the kinetoplastid parasites examined here have homologues of IP_{3}, Ca^{2+}, and Trp channels, although M. breviscilllis has a greater diversity of these channels [91]. However, while Orai and STIM homologues are present in M. breviscilllis [91], they appear to be absent in the kinetoplastid parasites. Again, whether these differences arose due to the transition from a free-living to a parasitic existence is unclear.

Relevance of parasite Ca^{2+} channels to therapy

A role for Ca^{2+} in the action of some anti-parasitic drugs has long been appreciated [92] and many anti-parasitic drugs that are known to affect mammalian Ca^{2+} channels have effects on Ca^{2+} signalling in parasites. For example, amiodarone [93], nimodipine [94] and several other 1,4-dihydropyridines [95], as well as a range of Ca^{2+} channel and calmodulin antagonists [96] may exert anti-parasitic effects via disruption of Ca^{2+} homeostasis in parasites. Anti-parasitic actions of other agents such as antimicrobial peptides [97], parasite-specific antibodies [98] and curcumin [99] also affect Ca^{2+} homeostasis in parasites. In addition, the widely used antimalarial artemisinin may affect Ca^{2+} homeostasis via inhibition of parasite sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) [100], although this has recently been contested [101,102] and other mechanisms for its action have been hypothesized [103]. Praziquantel [104,105], chloroquine [106,107], dantrolene [59,108], and suramin [109,110] are also currently used anti-parasitic drugs with complex and unclear mechanisms of action, that may involve modulation of Ca^{2+} signalling. We speculate that in addition to affecting their currently known targets, such as the plasmoidal surface anion channel in the case of dantrolene [111] and Ca,β subunits in the case of praziquantel [112], these drugs may also alter the activity of the parasitic Ca^{2+} channel homologues described in this study and hence perturb Ca^{2+} signalling pathways involved in parasite
survival. In particular, we suggest that suramin, a known RyR agonist [113,114] and dantrolene, a known RyR antagonist [115,116], may affect the RyR/IP3R homologues shown in this study to exist in Trypanosoma, Leishmania and S. mansoni parasites. This represents a potentially novel mechanism of action for these clinically useful drugs.

The apparent scarcity of Ca\textsuperscript{2+} channels in parasites relative to the wide array of isoforms in mammalian cells suggests that Ca\textsuperscript{2+} signalling in parasites may rely on a less redundant repertoire of Ca\textsuperscript{2+} channels. This characteristic may increase the susceptibility of parasites to drugs that target these channels.

Although parasite Ca\textsuperscript{2+} channel homologues show significant similarity to their mammalian counterparts, sequence divergence suggests that there is no homologue of any Ca\textsuperscript{2+} channels examined in any pathogenic parasite. This represents a potentially novel mechanism of action for these clinically useful drugs.

Materials and Methods

Genomes analyzed

The genomes of the following pathogenic parasites were examined (annotation release date in parentheses): Plasmodium falciparum 3D7 (May 2007), Plasmodium vivax Sal I (Jan 2008), Plasmodium knowlesi strain H (Apr 2008), Toxoplasma gondii ME49 (May 2008), Babesia bovis T2Bo (Mar 2008), Cryptosporidium hominis T0592 (Mar 2008), Cryptosporidium muris RN66 (Oct 2008), Cryptosporidium parvum Isocla H (Nov 2006), Leishmania braziliensis MHOM/BR/75/M2994 (Oct 2007), Leishmania infantum JPCM5 (May 2007), Leishmania major Friedlin (Mar 2008), Trypanosoma brucei TRE5/927 (Dec 2006), Trypanosoma cruzi CL Brener (Dec 2007), Entamoeba histolytica HM-1:IMSS (Jun 2010), Giardia intestinalis (Mar 2008), Trichomonas vaginalis G3 (Mar 2008), and Schistosoma mansoni (Jul 2011). No homologues of any Ca\textsuperscript{2+} channels examined were identified in the genomes of E. histolytica or G. intestinalis. Other genomes analyzed included those of: Homo sapiens (Dec 2010), Caenorhabditis elegans (Apr 2011), Drosophila melanogaster (May 2011), Paramecium tetraurelia (Mar 2008) and Ciona intestinalis (Sept 2008).

BLAST searches, alignments and topology analysis

BLASTP analysis was carried out in all cases, using the following human sequences (GenBank accession number in parentheses): full-length or pore sequences of IP3R1 (Q14643.2; pore region amino acids 2356-2605) or RyR1 (P21017.3; pore region residues 4677-4945), and full-length human sequences of TriP1 (NP_015328; N-truncated sequence residues 763-end), TriP2 (NP_061197; N-truncated sequence residues 439-end), TriPC1 (P40995; N-truncated sequence residues 350-end), CNSG1 (AAW93904; transmembrane sequence residues 200-420), CNSG1 (NP_001288), NMBA receptor NR1 (O05506), NMBA receptor N2 (Q12397), AMPA receptor GRIK4 (P24261.2), kainate receptor GRIK1 (P39096), nAChR-alpha1 (ABR09427), purinergic receptor P2X4 (NP_002512.1), panxinin-1 (AAH16951), Orai1 (NP_116179.2), STIM1 (AAH12300), TPC1 (NP_001137291.1), TPC2 (NP_620714.2), TriP1 (NP_00109944), TriP2 (NP_000228), TriPM1 (NP_002411), TriPM1 (NP_063394), CatSper1 (Q0NE5C.3), mitochondrial unipporter (NP_612366.1) and Ca_1.2 (NP_955630.2). Sequences of the S. cerevisiae Ca\textsuperscript{2+} channel subunit Cch1 (CAA97244) and Arabidopsis thaliana TCP1 (AAK95545) were also used to search for parasite homologues.

BLASTP searches of the genomes of protozoan parasites, H. sapiens, C. elegans, D. melanogaster and C. intestinalis were carried out against the National Center for Biotechnology (NCBI) genomic protein databases. Searches of the P. tetraurelia genome were carried out using the BLASTP facility of ParameciumDB (v.1.59) [115,116] to search for parasite homologues. Experimental observations also suggest the possibility of developing drugs with specificity for parasite homologues. Experimental observations also suggest the possibility of developing drugs with specificity for parasite homologues.
References

1. Philosoph H, Zilberstein D (1989) Regulation of intracellular calcium in promastigotes of the human protozoan parasite Leishmania donovani. J Biol Chem 264: 10420–4.

2. Moreno SN, Docamo R (2003) Calcium regulation in protozoan parasites. Curr Opin Microbiol 6: 359–64.

3. Nagamune K, Moreno SN, Chini EN, Sibley LD (2008) Calcium regulation and signaling in apicomplexan parasites. Subcell Biochem 47: 70–81.

4. Billker O, Lourido S, Sibley LD (2009) Calcium-dependent signaling and kinases in apicomplexan parasites. Cell Host Microbe 5: 612–22.

5. Marks AR (1997) Intracellular calcium-release channels: regulators of cell life and death. Am J Physiol 272: H567–605.

6. Berридge MJ (2009) Inositol trisphosphate and calcium signalling mechanisms. Biochim Biophys Acta 1793: 933–40.

7. Fill M, Copello JA (2002) Ryanodine receptor calcium release channels. Physiol Rev 82: 893–922.

8. Calafat PJ, Ruas M, Pan Z, Cheng X, Arre夺aoua A, et al. (2009) NAADP mobilizes calcium from acid organelles through two-pore channels. Nature 459: 596–600.

9. Braibon E, Charamnani D, Cai X, Schlau MG, Braibon GC, et al. (2009) Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling. J Cell Biol 186: 201–9.

10. Dong XP, Wang X, Xu H (2010) TRP channels of intracellular membranes. J Neurosci 19: 25–28.

11. Gees M, Colsoul B, Nilius B (2010) The role of transient receptor potential cation channels in Ca2+ signaling. Cold Spring Harb Perspect Biol 2: a003962.

12. Docamo R, Moreno SN (2001) The aciddoicicosome. Mol Biochem Parasitol 114: 151–9.

13. Gupta S, Raychaudhury B, Banerjee S, Das B, Datta SC (2006) An entry. Biochim Biophys Acta 1793: 223–30.

14. Catterall WA (2000) Structure and regulation of voltage-gated Ca2+ channels. Annu Rev Cell Dev Biol 16: 521–55.

15. Smyth JT, Dehaven WI, Jones BF, Sibelle SH, Sibley LD (2002) Role of the proposed pore-forming domain of the cardiac ryanodine receptor channel alters both the kinetics of ryanoid-induced Ca2+ release and the resting Ca2+ concentration. Am J Physiol 282: C877–82.

16. Ishibashi K, Suzuki M, Imai M (2000) Molecular cloning of a novel form (two-pore domain) of Ca2+ release channels. J Biol Chem 275: 27510–5.

17. Furuichi T, Cunningham KW, Muto S (2001) A putative two-pore channel associated with the Ca2+ release channel in the yeast vacuolar membrane. Proc Natl Acad Sci U S A 98: 4596–901.

18. Sibley LD (2011) Invasion and intracellular survival by protozoan parasites. Immun Rev 240: 72–91.

19. Green RM, Leulier F, Temperley M, Anderson PA, Sibley LD (2003). Structure of three high voltage-activated calcium channel alpha1 subunits from Schistosoma mansoni. Parasitology 125: 489–97.

20. Wohlenhohme AJ, Williamson SM, Reaves BJ (2011) TRP channels in parasites. Exp Biol Med (Maywood) 236: 359–71.

21. Foskett JK, White C, Cheng KH, Mak DO (2007) Inositol 1,4,5-trisphosphate receptor Ca2+ release channels. Physiol Rev 87: 593–638.

22. Ponting CP (2000) Novel repeats in ryanodine and IP3 receptors and protein O-mannosyltransferases. Trends Biochem Sci 25: 49–50.

23. Bosanac I, Yamazaki H, Matsu-Ura T, Michikawa T, Mikoshiba K, et al. (2005) Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor. Mol Cell 17: 193–203.

24. Yoshikawa F, Morita M, Monkawa T, Michikawa T, Furuichi T, et al. (1996) AtTPC1 mediates Ca2+ uptake and exocytosis in Orai1 mutated yeast vacuolar membrane. Proc Natl Acad Sci U S A 103: 4096–4091.

25. Chini EN, Nagamune K, Wetzel DM, Sibley LD (2005) Evidence that the CRAC channel by altered ion selectivity in a mutant of Schistosoma mansoni promotes cytokine production. J Pineal Res 43: 360–4.

26. Ronen A, Navarro-Borelly L, McNally B, Prakriya M (2007) Orai1 is a Ca2+-dependent sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. J Cell Biol 166: 1255–64.

27. Yamashita M, Navarro-Borelly L, McNally B, Prakriya M (2007) Orai1 mutation alters ion permeation and Ca2+-dependent fast inactivation of CRAC channels: evidence for coupling of permeation and gating. J Gen Physiol 130: 525–40.

28. Zhou Y, Ramachandran S, Oh-Hora M, Rao A, Hogan PG (2010) Pore architecture of the ORAI1 stored-operated calcium channel. Proc Natl Acad Sci U S A 107: 4986–901.

29. Paidhungat M, Garrett S (1997) A homolog of mammalian, voltage-gated calcium channels mediates yeast phenotype-stimulated Ca2+ uptake and exocytosis of the cdc15(1-ts) growth defect. Mol Cell Biol 17: 6339–47.

30. Fischer M, Schnell N, Chattawon J, Davies P, Dixon G, et al. (1997) The Saccharomyces cerevisiae CCH1 gene is involved in calcium influx and mating. FEBS Lett 419: 259–62.

31. Ronen A, Navarro B, Perez G, Jackson AC, Hsu S, et al. (2001) A sperm ion channel required for sperm motility and male fertility. Nature 413: 603–9.

32. Yu FH, Catterall WA (2004) The VGL-chanome: a protein superfamily specialized for electrical signaling and ion homeostasis. Cell 125: 1783–4. re3.

33. Paidhungat M, Garrett S (1997) A homolog of mammalian, voltage-gated calcium channels mediates yeast phenotype-stimulated Ca2+ uptake and exocytosis of the cdc15(1-ts) growth defect. Mol Cell Biol 17: 6339–47.

34. Iwahashi L, Zong X, Hofmann F (1996) Cyclic nucleotide-gated cation channels: evidence for coupling of permeation and gating. J Gen Physiol 107: 4896–901.

35. Iwahashi L, Zong X, Hofmann F (1996) Cyclic nucleotide-gated cation channels: evidence for coupling of permeation and gating. J Gen Physiol 107: 4896–901.

36. Yeromin AV, Jiang W, Yu Y, Safrina O, et al. (2006) Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Schistosoma mansoni. Parasitology 133: 161–7.

37. Biel M, Zong X, Hofmann F (1996) Cyclic nucleotide-gated cation channels: evidence for coupling of permeation and gating. J Gen Physiol 107: 4896–901.

38. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, et al. (2005) STIM1, an essential and conserved component of store-operated Ca2+ channel complex. Proc Natl Acad Sci U S A 102: 15892–8.

39. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, et al. (2005) STIM is a Ca2+-sensing protein that is lipid modified and localized to the ER membrane. J Biol Chem 280: 27539–47.

40. Zhang SL, Yu Y, Roos J, Kosak JA, Deerinck TJ, et al. (2005) STIM1 is a Ca2+-sensing protein that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. J Cell Biol 166: 1255–64.

41. Beraldo FH, Mikoshiba K, Garcia CR (2007) Human malarial parasite, Plasmodium falciparum, displays capacitative calcium entry: 2-aminoethyl diphenylborate blocks the signal transduction pathway of melanotin action on the P.falciparum cell cycle. J Pineal Res 43: 360–4.

42. Paidhungat M, Garrett S (1997) A homolog of mammalian, voltage-gated calcium channels mediates yeast phenotype-stimulated Ca2+ uptake and exocytosis of the cdc15(1-ts) growth defect. Mol Cell Biol 17: 6339–47.

43. Paidhungat M, Garrett S (1997) A homolog of mammalian, voltage-gated calcium channels mediates yeast phenotype-stimulated Ca2+ uptake and exocytosis of the cdc15(1-ts) growth defect. Mol Cell Biol 17: 6339–47.

44. Biel M, Zong X, Hofmann F (1996) Cyclic nucleotide-gated cation channels: evidence for coupling of permeation and gating. J Gen Physiol 107: 4896–901.

45. Iwahashi L, Zong X, Hofmann F (1996) Cyclic nucleotide-gated cation channels: evidence for coupling of permeation and gating. J Gen Physiol 107: 4896–901.
61. Moreno SN, Vrecesi AE, Pignataro OP, Docampo R (1992) Calcium homeostasis in Trypanosoma cruzi amastigotes: presence of inositol phosphates and lack of an inositol 1,4,5-trisphosphate-sensitive calcium pool. Mol Biochem Parasitol 52: 251–61.

62. Docampo R, Moreno SN, Vrecesi AE (1993) Effect of thapsigargin on calcium homeostasis in Trypanosoma cruzi trypomastigotes and epimastigotes. Mol Biochem Parasitol 59: 305–13.

63. Nagamune K, Moreno SN, Chini EN, Sibley LD (2008) Calcium regulationCharacterization of two different mucolipin-like genes from Schizosaccharomyces cerevisiae. J Eukaryot Microbiol 56: 208–13.

64. Peiter E, Maathuis FJ, Mills LN, Knight H, Pelloux J, et al. (2005) The calcium channel blocker norepinephrine stimulates the Tol2 transposon in malaria parasites. Mol Biochem Parasitol 141: 120–7.

65. Lange I, Yamamoto S, Partida-Sanchez S, Mori Y, Fleig A, et al. (2009) Structure of the EF-hand domain of polycystin-2 suggests a mechanism for Ca2+-dependent regulation of polycystin-2 channel activity. Proc Natl Acad Sci U S A 107: 9176–61.

66. Lovett JL, Marchesini N, Moreno SN, Sibley LD (2002) Calcium-mediated calcium release from internal stores of Entamoeba histolytica. Mol Biochem Parasitol 126: 219–30.

67. Chenik M, Douagi F, Ben Achour Y, Khalef NB, Ouakad M, et al. (2005) Calcium Channel Homologues in Pathogenic Parasites. Biochem Soc Trans 33: 823–31.

68. Belde PJ, Vossen JH, Borst-Pauwels GW, Theuvenet AP (1993) Inositol 1,4,5-trisphosphate (IP3)/ryanodine-sensitive stores. J Biol Chem 268: 25870–6.

69. Raha S, Dalal B, Biswas S, Biswas BB (1994) Myo-inositol triphosphate–mediated calcium release from internal stores of Entamoeba histolytica. Mol Biochem Parasitol 65: 63–71.

70. Kolisek M, Beck A, Fleig A, Penner R (2005) Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TrpM2 channels. Mol Pharmacol 68: 1303–15.

71. Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, et al. (2002) Polycystin-2 is an intracellular recycling of calcium from internal stores of Entamoeba histolytica. Mol Biochem Parasitol 65: 63–71.

72. Petri ET, Celic A, Kennedy SD, Ehrlich BE, Boggon TJ, et al. (2010) Structure of the cytoplasmic free Ca2+ system. J Cell Sci 119: 3705–17.

73. Serral-Romero X, Garcia-Marchan Y, Fernandez A, Rodrigo R, Rojas H, et al. (2009) Amiodarone destabilizes intracellular Ca2+ homeostasis and biosynthesis of sterols in Leishmania mexicana. Antimicrob Agents Chemother 53: 1405–10.

74. Clarkston AB, Jr., Amole BO (1982) Role of calcium in trypanocidal drug action. Science 216: 1232–3.

75. Schiefler IW, Colombani PM, Hess AD, Aikawa M, Atkinson CT, et al. (1997) Calcium and calmodulin antagonists inhibit human malaria parasites (Plasmodium falciparum): implications for drug design. Proc Natl Acad Sci U S A 94: 7310–4.

76. Kulkarni MM, Mcmaster WR, Kamysz W, McQuive BS (2009) Antimicrobial peptide-induced apoptosis of Leishmania results from calcium-dependent, caspase-independent mitochondrial toxicity. J Biol Chem 284: 15496–504.

77. Mendoza M, Uzcanga GL, Pacheco R, Rojas H, Carraquiel LM, et al. (2008) Anti-VSG antibodies induce an increase in Trypanosoma cruzi intracellular Ca2+ and regulate Ca2+ uptake. J Innate Immun 10: 36–41.

78. Cheng X, Shen D, Samie M, Xu H (2010) Mucolipins: Intracellular TRPML1- and polyphosphate-containing acidic granules of sea urchin eggs are similar to the cytoplasmic free Ca2+ system. J Cell Biol 161: 103–10.

79. Sarkar D, Bhaduri A (1995) Temperature-induced rapid increase in intracellular calcium from chloroquine-sensitive and -insensitive intracellular stores in the intracellular calcium release channel. Mol Biochem Parasitol 59: 305–13.

80. Valderramos SG, Scanfeld D, Voigt G, Krishna S (2010) Investigations into the role of the Plasmodium falciparum SERCA (PfATP6) in transporting intracellular calcium in response to ATPase inhibition. Mol Biochem Parasitol 169: 87–92.

81. McGeary RP, Bennett AJ, Tran QB, Cosgrove KL, Ross BP (2008) Suramin: mode of action and potential drug target. Biochem Soc Trans 36: 821–3.

82. Kang M, Lisk G, Hollingworth S, Baylor SM, Desai SA (2005) Antileishmanial activity and ultrastructural alterations of Leishmania (L.) chagasi treated with the calcium channel blocker nimodipine. Parasitol Res 98: 5–13.

83. Fruen BR, Mickelson JR, Louis CF (1997) Dantrolene inhibition of sarcoplasmic reticulum Ca2+ release. J Biol Chem 272: 15951–6.

84. Hohenegger M, Matyash M, Poussu K, Herrmann-Frank A, Sarkozi S, et al. (2010) Structure-activity relationship study. Bioorg Med Chem 18: 8044–53.

85. Kosten GC, Annes DL, De Jongh S, De Meyer E, De Smedt R, et al. (2010) Identification of a dantrolene-binding sequence on the skeletal muscle ryanodine receptor. J Biol Chem 285: 36873–6.

86. McMillan KR, Piletz DJ, Marchant JS (2009) A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel beta subunits: subversion of flavonoid regenerative polarity. Plos Negl Trop Dis 3: e464.

87. Misra UK, Gawdi G, Pizzo SV (1997) Chloroquine, quinine and quinidine lower the intracellular calcium of flatworm regenerative polarity. PLoS Negl Trop Dis 3: e464.

88. Kohn AB, Anderson PA, Roberts-Minterly JM, Greenberg RM (2001) Activation of the skeletal muscle ryanodine receptor by suramin and effects of ryanodine receptors by suramin. J Membr Biol 153: 93–103.

89. Kang M, Lisk G, Hollingworth S, Baylor SM, Desai SA (2005) Malaria parasites are rapidly killed by dantrolene derivatives specific for the plasmodial surface anion channel. Mol Pharmacol 68: 34–40.

90. Docampo R, Moreno SN (2003) Current chemotherapy of human African trypanosomiasis. Parasitol Res 99(Suppl 1): S10–9.

91. Meyer RP, Bennett AJ, Tran QB, Cosgrove KL, Ross BP (2008) Suramin: clinical uses and structure-activity relationships. Mini Rev Med Chem 8: 1304–14.

92. Kang M, Lisk G, Cohn JY, Desai SA (2006) Specific inhibition of the plasmodial surface anion channel by dantrolene. Eur J Cell Biol 85: 1082–93.

93. Kohn AB, Anderson PA, Roberts-Misterly JM, Greenberg RM (2001) Schistosoma calcium channel beta subunits. Unusual modulatory effects and potential role in the action of the antischistosomal drug praziquantel. J Biol Chem 276: 36873–6.

94. Nogueira JR, Zhang D, Chan JD, Marchant JS (2009) A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel beta subunits: subversion of flavonoid regenerative polarity. PLoS Negl Trop Dis 3: e464.

95. Misra UK, Gawdi G, Pizzo SV (1997) Chloroquine, quinine and quinidine lower the intracellular calcium of flatworm regenerative polarity. PLoS Negl Trop Dis 3: e464.

96. McMillan KR, Piletz DJ, Marchant JS (2009) A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel beta subunits: subversion of flavonoid regenerative polarity. PLoS Negl Trop Dis 3: e464.

97. Misra UK, Gawdi G, Pizzo SV (1997) Chloroquine, quinine and quinidine lower the intracellular calcium of flatworm regenerative polarity. PLoS Negl Trop Dis 3: e464.

98. Nogueira JR, Zhang D, Chan JD, Marchant JS (2009) A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel beta subunits: subversion of flavonoid regenerative polarity. PLoS Negl Trop Dis 3: e464.
