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Acidocalcisomes

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Abstract

Acidocalcisomes are acidic organelles containing calcium and a high concentration of phosphorus in the form of pyrophosphate (PP_i) and polyphosphate (poly P). Organelles with these characteristics have been found from bacteria to human cells implying an early appearance and persistence over evolutionary time or their appearance by convergent evolution. Acidification of the organelles is driven by the presence of vacuolar proton pumps, one of which, the vacuolar proton pyrophosphatase, is absent in animals, where it is substituted by a vacuolar proton ATPase. A number of other pumps, antiporters, and channels have been described in acidocalcisomes of different species and are responsible for their internal content. Enzymes involved in the synthesis and degradation of PP_i and poly P are present within the organelle. Acidocalcisomes function as storage sites for cations and phosphorus, and participate in PP_i and poly P metabolism, calcium homeostasis, maintenance of intracellular pH, and osmoregulation. Experiments in which the acidocalcisome Ca²⁺-ATPase of different parasites were downregulated or eliminated, or acidocalcisome Ca²⁺ was depleted revealed the importance of this store in Ca²⁺ signaling needed for host invasion and virulence. Acidocalcisomes interact with other organelles in a number of organisms suggesting their association with the endosomal/lysosomal pathway, and are considered part of the lysosome-related group of organelles.

1. Introduction

Acidocalcisomes are acidic calcium-storage organelles present in a diverse range of organisms from bacteria to human cells. They were first recognized in bacteria [1, 2] and named the metachromatic or volutin granules. Their name derived from their property to stain red when treated with basic blue dyes and for their detection in the bacterium *Spirillum volutans*, respectively. In the early twentieth century, volutin granules were described in a number of protists, such as algae, yeasts, coccidian and trypanosomes [3]. After it was found that their numbers in yeasts increased as the amount of poly P increased in the cells [4] they became known as polyphosphate (poly P) granules. Poly P is a linear anionic polymer containing from a few to several hundred residues of orthophosphate linked by energy-rich phosphoanhydride bonds and was initially discovered in yeasts by Lieberman [5]. It was relatively recently [6–8] that the poly P granules were shown to have proton and calcium pumps, responsible for their acidity and calcium content, and this led to the current name of acidocalcisomes. The identification of organelles with similar composition and acidification

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mechanisms in bacteria [9, 10], and human cells [11] suggest that these organelles have been conserved over evolutionary time or have appeared more than one time by convergent evolution.

2. Acidocalcisomes in bacteria

For many years it was assumed that poly P granules in bacteria lacked a limiting membrane [12]. However, the presence of a membrane in acidocalcisomes of eukaryotes [7] suggested that this was probably not the case. Work in *Agrobacterium tumefaciens* and *Rhodospirillum rubrum* demonstrated that acidocalcisomes in bacteria are membrane-bound [9, 10]. Evidence for the presence of a limiting membrane included: (1) its detection by electron microscopy of intact bacteria and subcellular fractions; (2) the staining of the organelles by dyes that accumulate in acidic compartments, such as LysoSensor, and cycloprodigiosin; and (3) the detection in the acidocalcisome membranes of a vacuolar proton pyrophosphatase (V-H⁺-PPase), which contains several transmembrane domains, by immunofluorescence and immunoelectron microscopy (Fig. 1C and 1D) and by subcellular fractionation [9, 10].

Acidocalcisomes in bacteria are electron-dense, spherical, and from 15 to about 200 nm in diameter. Their number varies in different species although in most cases there are 1 to 3 per cell and they occupy less than 1% of the cell volume [13]. At the electron microscopy level they are recognizable as empty vacuoles or vacuoles containing a thin layer of dense material or an inclusion that sticks to the inner face of the membrane (Fig. 1A). By direct transmission electron microscopy they appear as electron-dense spheres. They strongly stain with 4'-6'-diamino-2-phenylindole (DAPI), which stains poly P (Fig. 1B) and their acidification, in at least *A. tumefaciens* and *R. rubrum*, is through the V-H⁺-PPase [13].

Most studies on the acidocalcisomes of bacteria have been on the role of poly P in phosphorus storage, detoxification of heavy metal cations, and removal of phosphorus from wastewaters, as well as on the enzymes involved in poly P synthesis and degradation [13, 14]. However, there have been no studies on how calcium and other cations are taken up by the organelles or on the role of this calcium store in bacteria.

3. Acidocalcisome in protists

Acidocalcisomes in protists have similar morphological characteristics to those in bacteria: they are electron-dense (Fig. 2A) and have an empty appearance (Fig. 2B) at the electron microscopy level, and they stain with DAPI and dyes that accumulate in acidic compartments (acridine orange (Fig. 2C), cycloprodigiosin, LysoSensor) when observed by light microscopy [6, 7, 15]. They are usually spherical and have an average diameter of 0.2-0.6 μ m but they could also have polymorphic appearance [3]. Acidocalcisomes are usually randomly distributed in the cells (Fig. 2A).

Acidocalcisomes of protists are rich in orthophosphate (Pi), PPi, and poly P complexed with cations (sodium, potassium, magnesium, calcium, zinc and iron) and basic amino acids [3]. Trypanosomatids are especially rich in short chain poly P such as poly P₃, poly P₄, and poly P₅ [16]. On the basis of its total concentration and the relative volume of the acidocalcisomes in some of these cells (about 1–2% of the total cell volume), its calculated concentration in the organelles would be in the molar range (3–5 M) [3]. This is congruent with the detection of solid-state condensed phosphates by magic-angle spinning NMR techniques, and with the very high electron density of acidocalcisomes [17]. Recent studies in *Trypanosoma cruzi* suggested that the presence of carbohydrate and lipids could be involved in maintaining these physical characteristics [18].

Several enzymes have been shown to be present in this organelle: a polyphosphate kinase [19], an exopolyphosphatase [20, 21], a soluble inorganic pyrophosphatase [22], and a metacaspase [23]. In addition, an acid phosphatase activity has also been detected using cytochemical methods [24]. Synthesis of poly P in the yeast vacuole and in acidocalcisomes is mediated by the “vacuolar transporter chaperone” (Vtc) complex, which comprises four proteins (Vtc 1– 4) anchored in the vacuole membrane of fungi [25] and probably two (Vtc1 and Vtc4) anchored in the acidocalcisomes membrane of trypanosomatids and Apicomplexan parasites [26]. Vtc4 is the catalytic subunit and has the function of polymerizing, and then threading the growing poly P chain through the vacuole membrane [25].

A scheme of all the enzymes and transporters identified in acidocalcisomes of protists is depicted in Fig. 3. Acidocalcisome membranes possess several pumps and antiporters, and at least one channel [3]. A Ca^{2+} -ATPase was found in acidocalcisomes of different protists [27–30]. Genes encoding for acidocalcisomal Ca^{2+} -ATPases were identified and the protein products were shown to be closely related to the family of plasma membrane calcium ATPases (PMCA) [31–33]. Knockdown of the Ca^{2+} -ATPase localized in the acidocalcisomes of *Trypanosoma brucei* results in reduced levels of mobilizable calcium from these stores and impaired growth [32]. Deletion of the acidocalcisome Ca^{2+} -ATPase in *Toxoplasma gondii* also affects growth, and has serious effects on invasion and virulence [34]. These results highlight the physiological importance of calcium uptake into acidocalcisomes for their normal functioning. Two proton pumps have been detected in acidocalcisomes of different protists. One is the multisubunit vacuolar-type H^{+} -ATPase and the other is the V- H^{+} -PPase, which uses PPI instead of ATP to transport protons [3]. In *T. cruzi* the V- H^{+} -ATPase was shown, by immunofluorescence and immunoelectron microscopy, to co-localize to acidocalcisomes with the vacuolar-type Ca^{2+} -ATPase [31]. The V- H^{+} -PPase was shown to localize to acidocalcisomes of different trypanosomatids, Apicomplexan parasites, *Dictyostelium discoideum*, the green algae *Chlamidomonas reinhardtii* [3], and the red algae *Cyanidioschyzon merolae* [35]. Only the gene for the *T. cruzi* V- H^{+} -PPase could be functionally expressed in yeast [36]. Interestingly, the N-terminal region of the *T. cruzi* V- H^{+} -PPase, when fused to other protist enzymes could enhance their functional expression in yeast [37]. The acidocalcisomal enzyme belongs to the K^{+} -stimulated group of V- H^{+} -PPases (type I), and has been successfully used as a marker for acidocalcisome purification. There is also evidence for the presence of $\text{Na}^{+}/\text{H}^{+}$ and $\text{Ca}^{2+}/\text{H}^{+}$ antiporters in acidocalcisomes of some trypanosomatids [38–40]. The $\text{Ca}^{2+}/\text{H}^{+}$ antiporter was proposed to be involved in Ca^{2+} release when Na^{+} was added to the organelles *in situ* or *in vitro* and this could be a mechanism of Ca^{2+} release from the organelles, since other second messengers, such as inositol trisphosphate (InsP_3), were unable to release Ca^{2+} from intracellular Ca^{2+} stores of trypanosomatids [41–43]. A water channel or aquaporin has also been identified in acidocalcisomes of *T. cruzi* [44]. The protein acts as a water channel and is unable to transport glycerol when expressed in *Xenopus* oocytes.

4. Acidocalcisomes in eggs

Eggs from insects [45], sea urchins [46] and chickens [47] have been shown to possess acidocalcisomes. In chicken eggs acidocalcisomes are inside larger acidic vacuoles, which were named compound organelles [47] (Fig. 4A-F). Interestingly, although a V- H^{+} -PPase activity was detected in acidocalcisomes of insect eggs [45, 48] there is no gene with homology to the V- H^{+} -PPases of plants and lower eukaryotes in their databases. In sea urchin and chicken eggs a V- H^{+} -ATPase is apparently responsible for their acidity [46, 47].

It is not known how calcium or other cations are transported into these acidocalcisomes, which have very similar morphology and composition to the acidocalcisomes of protists. They have preferentially short chain poly P (chains of less than 100 Pi units), PPi and Pi complexed with calcium, magnesium, and sodium (sea urchin eggs), calcium, magnesium, sodium, potassium, and zinc (chicken eggs), or calcium, magnesium, sodium, potassium, and iron (insect eggs). In sea urchin eggs it was found that neither NAADP nor InsP₃ could release Ca²⁺ from this compartment [46]. However, as occurs in trypanosomatids [38–40], Ca²⁺ could be released in exchange for protons coupled to Na⁺ entry through the combined operation of Ca²⁺/H⁺ and Na⁺/H⁺ antiporters [46]. Ca²⁺ release produced by alkalization of acidocalcisomes could contribute to the Ca²⁺ waves that occur upon fertilization of sea urchin oocytes [46]. Fig. 5 shows a model of the Ca²⁺ mobilization that could be involved in sea urchin eggs. NAADP would trigger Ca²⁺ release from yolk platelets that could then trigger endoplasmic reticulum Ca²⁺ mobilization through Ca²⁺-induced Ca²⁺ release with InsP₃ and ryanodine receptors. Alkalization of the egg cytoplasm by Na⁺ entry would release additional Ca²⁺ from acidocalcisomes [46].

5. Acidocalcisomes in mammalian cells

Acidocalcisomes resemble lysosome-related organelles, a heterogeneous group of organelles that share some physiological features with lysosomes [49, 50]. These organelles include melanosomes, lytic granules in lymphocytes, major histocompatibility complex class II compartments in antigen-presenting cells, platelet dense granules, basophilic granules, neutrophil azurophilic granules and others [50]. Lysosome-related organelles are acidic and most are rich in calcium. Platelet dense granules are the most similar to acidocalcisomes in that they contain PPi and poly P and are electron-dense when examined under direct transmission electron microscopy [11] (Fig. 6A, and 6B). They are also very rich in calcium [51]. The finding that poly P is released upon platelet activation [11] led to the discovery of the pro-coagulant [52] and pro inflammatory [53] activities of poly P. A V-H⁺-ATPase is apparently involved in acidification of dense granules but the mechanisms used for Ca²⁺ uptake and release are unknown. Platelet dense granules and other lysosome-related organelles share with acidocalcisome of protists the system for targeting of their membrane proteins through adaptor protein 3 (reviewed in [54]).

6. Functions of acidocalcisomes

Acidocalcisomes are a major storage compartment for phosphorus compounds (Pi, PPi and poly P) and cations (calcium, magnesium, sodium, potassium, zinc and iron) in different species. The storage of phosphorus as poly P reduces the osmotic effect of large pools of this compound as well as of cations, with which it is complexed. The ability of some bacteria to accumulate phosphorus in poly P granules has been used for the biological removal of phosphorus from wastewaters. Pi accumulates in wastewaters containing industrial discharges and run off of fertilizers resulting in algal blooms. Aerobically activated bacteria can take up the Pi in poly P granules, which can then be removed together with the bacteria as a sludge and prevent algal growth [55].

Poly P has several functions in bacteria in addition to its role as phosphorus and cation storage: as energy source to replace ATP; in cell membrane formation and function; in gene transcriptional control; in regulation of enzyme activities; response to stress and stationary-phase; and in the structure of channels and pumps [56]. By manipulating the expression of genes involved in poly P synthesis and degradation it was also demonstrated that poly P plays important roles in virulence of major bacterial pathogens [57].

Studies in protists revealed roles in development, sporulation and predation [58, 59], in stress adaptation [60–65] and in osmoregulation [19]. Several parasites are less virulent

when they contain lower amounts of poly P in their acidocalcisomes [22, 34]. The presence of poly P in many acidic calcium stores such as acidocalcisomes, yeast vacuoles, lysosomes and lysosome-related organelles highlight its role in calcium buffering [66]

In animals, poly P released from platelet dense granules (which are similar to acidocalcisomes) [11] is a potent modulator of blood coagulation affecting the intrinsic pathway, the fibrinolytic system, factor V activation, and fibrin structure [52, 67], increases vascular permeability [53], and induces apoptosis in plasma cells [68].

That acidocalcisomes contained very high concentrations of calcium was their first distinguished characteristic [6]. Depletion of acidocalcisomal calcium by pretreatment of invasive stages of *T. cruzi* with ionomycin plus nigericin or ionomycin plus NH_4Cl , inhibited invasion of host cells [69, 70]. Additional evidence that acidocalcisome calcium is important for invasion was observed in *T. gondii* tachyzoites knockouts of TgA1, the enzyme necessary for pumping calcium into the organelles. This resulted in de-regulation of cytosolic calcium, which then altered microneme secretion and decreased virulence [34]. These results highlight the importance of acidocalcisomes for Ca^{2+} signaling needed for invasion of intracellular parasites.

A role for acidocalcisomes in regulation of intracellular pH was shown in *T. brucei*. RNAi experiments to reduce acidocalcisome V- H^+ -PPase activity resulted in their inability to recover their pH when they were exposed to an external basic pH >7.4, and the same cells recovered from intracellular acidification at a slower rate and to a more acidic final intracellular pH [71].

Acidocalcisomes are also important for osmoregulation. Evidence supporting this role in trypanosomatids have been documented by the rapid hydrolysis or synthesis of acidocalcisome poly P during hypo- or hyperosmotic stress [19], and by the changes in sodium and chloride content in acidocalcisomes of *Leishmania major* in response to acute hyposmotic stress [72]. It has been found that cAMP levels increase when *T. cruzi* are subjected to hyposmotic stress and a cAMP phosphodiesterase (PDE) is important to terminate this activation [73]. Acidocalcisomes, which have an aquaporin (TcAQP), traffic towards the contractile vacuole after hyposmotic stress, as revealed by direct observation of cells expressing GFP-TcAQP. This traffic is stimulated by cAMP analogs or PDE inhibitors and is inhibited by adenylyl cyclase inhibitors. In agreement with these results cAMP levels significantly increase 1 min after hyposmotic stress. Fusion of acidocalcisomes to the contractile vacuole complex is suggested by electron microscopy observation of the presence of similar electron-dense material in both organelles, their apparent continuity in intact cells or subcellular fractions, and by the increase in brightness of the contractile vacuole complex of GFP-TcAQP labeled cells after hyposmotic stress. Furthermore, a cAMP analog stimulates the regulatory volume decrease (the mechanism by which cells recover their volume after swelling due to hyposmotic stress). A model for *T. cruzi* was proposed in which the stimulus of cell swelling causes a spike in intracellular cAMP through an as yet unidentified adenylyl cyclase, resulting in fusion of acidocalcisomes with the contractile vacuole and translocation of aquaporin [74]. This process helps the elimination of water by the contractile vacuole and is terminated by the action of a PDE [73]. Continuous cAMP oscillations, which are known to occur in other cells that have contractile vacuole mechanisms of water extrusion, such as *Dictyostelium discoideum* [75], could be responsible for the periodic contraction and water expulsion by the CVC that occurs under isosmotic conditions in *T. cruzi* (one contraction every min and a half [76]), and explain the absolute essentiality of this mechanism of water regulation in these organisms [73, 77]. The use of RNAi to reduce the expression of the acidocalcisomal soluble pyrophosphatase (TbVSP1) also resulted in trypanosomes that were deficient in poly P and in their response to

hyposmotic stress [22]. Ablation of a vacuolar transporter chaperone (VTC1) in *T. brucei* by RNAi resulted in abnormal morphology of acidocalcisomes, decrease in their poly P content, and deficient response to hyposmotic stress [26].

7. Acidocalcisomes and disease

In addition to the relevance of acidocalcisomes and poly P in the virulence of several pathogenic bacteria such as *Helicobacter pylori* [78], *Mycobacterium tuberculosis* [79], *Shigella flexneri* [80], *Salmonella enterica* [80] and others, as well as of parasites such as *Toxoplasma gondii* [34], *T. brucei* [26], and *Leishmania major* [81], a number of syndromes characterized by platelet dense granule (acidocalcisome) deficiency have been described. Both Hermansky-Pudlak (HPS) and Chediak-Higashi (CHS) syndromes have in common dense granule deficiency and bleeding tendency [82], which is congruent with the presence of poly P in dense granules [11] and its role in blood clotting [52]. Defects in dense granules also occur in δ -storage pool disease [82]. HPS has been associated with 8 genes in humans which encode proteins involved in trafficking proteins to the organelles such as adaptor complex 3 subunits, while CHS is linked to mutations in the lysosomal trafficking regulator (LYST) gene. The gene affected in δ -storage pool disease is not known [82].

8. Outlook

Based on their presence in bacteria, the acidocalcisome can be considered the earliest calcium-containing acidic compartment that appeared during evolutionary time. Calcium in the acidocalcisome is bound to a polyanionic matrix of poly P, and can be released after alkalization of the organelle. Ca^{2+} uptake into acidocalcisomes is driven by Ca^{2+} -ATPases in several protists and probably through $\text{Ca}^{2+}/\text{H}^{+}$ antiporter activities facilitated by the proton pumps in other cases. In contrast, calcium-permeable channels or second messengers that could stimulate their calcium release have yet to be identified in acidocalcisomes. Further studies are also necessary to understand the biogenesis and function of acidocalcisomes in different organisms and why this organelle has been conserved in so many species. Phylogenetic relationships of various acidocalcisomal enzymes need to be established, as sequence comparisons are important indicators of the evolution of these organelles. Intracellular PP_i , poly P, cations and basic amino acids are accumulated in large amounts in acidocalcisomes, but the mechanisms by which these compounds are transported into the organelle, except perhaps for poly P, are largely unknown. This is a promising area of research because of their potential for discovery of many novel functions in different species.

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References

1. Babes V. Beobachtungen über die methachromatischen Körperchen, Sporenbildung, Verzweigung, Kolben- und Kapselbildung Pathogener Bakterien. Z Hyg Infektr. 1985; 20:412–420.
2. Meyer A. Orientierende Untersuchungen über Verbreitung, Morphologie und chemie des volutins. Botanische Zeitung. 1904; VII:113–152.
3. Docampo R, de Souza W, Miranda K, Rohloff P, Moreno SN. Acidocalcisomes - conserved from bacteria to man. Nat Rev Microbiol. 2005; 3:251–261. [PubMed: 15738951]
4. Wiame JM. Etude d'une substance polyphosphoree, basophile et metachromatique chez les levures. Biochim Biophys Acta. 1947; 1:234–255.

5. Lieberman L. Über das Nuclein der Hefe und Kunstliche Darstellung eines Nucleus Eiweiss und Metaphosphatsäure. *Ber Chem-Ges.* 1888; 21:598–607.
6. Vercesi AE, Moreno SN, Docampo R. $\text{Ca}^{2+}/\text{H}^{+}$ exchange in acidic vacuoles of *Trypanosoma brucei*. *Biochem J.* 1994; 304:227–233. [PubMed: 7998937]
7. Docampo R, Scott DA, Vercesi AE, Moreno SN. Intracellular Ca^{2+} storage in acidocalcisomes of *Trypanosoma cruzi*. *Biochem J.* 1995; 310:1005–1012. [PubMed: 7575396]
8. Moreno SN, Zhong L. Acidocalcisomes in *Toxoplasma gondii* tachyzoites. *Biochem J.* 1996; 313:655–659. [PubMed: 8573106]
9. Seufferheld M, Lea CR, Vieira M, Oldfield E, Docampo R. The H^{+} -pyrophosphatase of *Rhodospirillum rubrum* is predominantly located in polyphosphate-rich acidocalcisomes. *J Biol Chem.* 2004; 279:51193–51202. [PubMed: 15371423]
10. Seufferheld M, Vieira MC, Ruiz FA, Rodrigues CO, Moreno SN, Docampo R. Identification of organelles in bacteria similar to acidocalcisomes of unicellular eukaryotes. *J Biol Chem.* 2003; 278:29971–29978. [PubMed: 12783865]
11. Ruiz FA, Lea CR, Oldfield E, Docampo R. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. *J Biol Chem.* 2004; 279:44250–44257. [PubMed: 15308650]
12. Shively JM, Bryant DA, Fuller RC, Konopka AE, Stevens SE Jr, Strohl WR. Functional inclusions in prokaryotic cells. *Int Rev Cytol.* 1988; 113:35–100. [PubMed: 3068183]
13. Docampo R. Acidocalcisomes and polyphosphate granules. *Microbiol Monogr.* 2006; 1:53–70.
14. Kornberg A. Inorganic polyphosphate: toward making a forgotten polymer unforgettable. *J Bacteriol.* 1995; 177:491–496. [PubMed: 7836277]
15. Miranda K, de Souza W, Plattner H, Hentschel J, Kawazoe U, Fang J, Moreno SN. Acidocalcisomes in Apicomplexan parasites. *Exp Parasitol.* 2008; 118:2–9. [PubMed: 17761167]
16. Moreno B, Urbina JA, Oldfield E, Bailey BN, Rodrigues CO, Docampo R. ^{31}P NMR spectroscopy of *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania major*. Evidence for high levels of condensed inorganic phosphates. *J Biol Chem.* 2000; 275:28356–28362. [PubMed: 10871617]
17. Moreno B, Rodrigues CO, Bailey BN, Urbina JA, Moreno SN, Docampo R, Oldfield E. Magic-angle spinning (^{31}P) NMR spectroscopy of condensed phosphates in parasitic protozoa: visualizing the invisible. *FEBS Lett.* 2002; 523:207–212. [PubMed: 12123833]
18. Salto ML, Kuhlenschmidt T, Kuhlenschmidt M, de Lederkremer RM, Docampo R. Phospholipid and glycolipid composition of acidocalcisomes of *Trypanosoma cruzi*. *Mol Biochem Parasitol.* 2008; 158:120–130. [PubMed: 18207579]
19. Ruiz FA, Rodrigues CO, Docampo R. Rapid changes in polyphosphate content within acidocalcisomes in response to cell growth, differentiation, and environmental stress in *Trypanosoma cruzi*. *J Biol Chem.* 2001; 276:26114–26121. [PubMed: 11371561]
20. Rodrigues CO, Ruiz FA, Vieira M, Hill JE, Docampo R. An acidocalcisomal exopolyphosphatase from *Leishmania major* with high affinity for short chain polyphosphate. *J Biol Chem.* 2002; 277:50899–50906. [PubMed: 12393865]
21. Fang J, Ruiz FA, Docampo M, Luo S, Rodrigues JC, Motta LS, Rohloff P, Docampo R. Overexpression of a Zn^{2+} -sensitive soluble exopolyphosphatase from *Trypanosoma cruzi* depletes polyphosphate and affects osmoregulation. *J Biol Chem.* 2007; 282:32501–32510. [PubMed: 17827150]
22. Lemercier G, Espiau B, Ruiz FA, Vieira M, Luo S, Baltz T, Docampo R, Bakalara N. A pyrophosphatase regulating polyphosphate metabolism in acidocalcisomes is essential for *Trypanosoma brucei* virulence in mice. *J Biol Chem.* 2004; 279:3420–3425. [PubMed: 14615483]
23. Lee N, Gannavaram S, Selvapandian A, Debrabant A. Characterization of metacaspases with trypsin-like activity and their putative role in programmed cell death in the protozoan parasite *Leishmania*. *Eukaryot Cell.* 2007; 6:1745–1757. [PubMed: 17715367]
24. Gomes SA, Fonseca de Souza AL, Silva BA, Kiffer-Moreira T, Santos-Mallet JR, Santos AL, Meyer-Fernandes JR. *Trypanosoma rangeli*: Differential expression of cell surface polypeptides and ecto-phosphatase activity in short and long epimastigote forms. *Exp Parasitol.* 2006; 112:253–262. [PubMed: 16442100]

25. Hothorn M, Neumann H, Lenherr ED, Wehner M, Rybin V, Hassa PO, Uttenweiler A, Reinhardt M, Schmidt A, Seiler J, Ladurner AG, Herrmann C, Scheffzek K, Mayer A. Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. *Science*. 2009; 324:513–516. [PubMed: 19390046]
26. Fang J, Rohloff P, Miranda K, Docampo R. Ablation of a small transmembrane protein of *Trypanosoma brucei* (TbVTC1) involved in the synthesis of polyphosphate alters acidocalcisome biogenesis and function, and leads to a cytokinesis defect. *Biochem J*. 2007; 407:161–170. [PubMed: 17635107]
27. Scott DA, Docampo R. Characterization of isolated acidocalcisomes of *Trypanosoma cruzi*. *J Biol Chem*. 2000; 275:24215–24221. [PubMed: 10816577]
28. Rodrigues CO, Scott DA, Docampo R. Characterization of a vacuolar pyrophosphatase in *Trypanosoma brucei* and its localization to acidocalcisomes. *Mol Cell Biol*. 1999; 19:7712–7723. [PubMed: 10523660]
29. Marchesini N, Ruiz FA, Vieira M, Docampo R. Acidocalcisomes are functionally linked to the contractile vacuole of *Dictyostelium discoideum*. *J Biol Chem*. 2002; 277:8146–8153. [PubMed: 11748243]
30. Ruiz FA, Marchesini N, Seufferheld M, Govindjee, Docampo R. The polyphosphate bodies of *Chlamydomonas reinhardtii* possess a proton-pumping pyrophosphatase and are similar to acidocalcisomes. *J Biol Chem*. 2001; 276:46196–46203. [PubMed: 11579086]
31. Lu HG, Zhong L, de Souza W, Benchimol M, Moreno S, Docampo R. Ca^{2+} content and expression of an acidocalcisomal calcium pump are elevated in intracellular forms of *Trypanosoma cruzi*. *Mol Cell Biol*. 1998; 18:2309–2323. [PubMed: 9528801]
32. Luo S, Rohloff P, Cox J, Uyemura SA, Docampo R. *Trypanosoma brucei* plasma membrane-type Ca^{2+} -ATPase 1 (TbPMC1) and 2 (TbPMC2) genes encode functional Ca^{2+} -ATPases localized to the acidocalcisomes and plasma membrane, and essential for Ca^{2+} homeostasis and growth. *J Biol Chem*. 2004; 279:14427–14439. [PubMed: 14724285]
33. Luo S, Vieira M, Graves J, Zhong L, Moreno SN. A plasma membrane-type Ca^{2+} -ATPase co-localizes with a vacuolar H^{+} -pyrophosphatase to acidocalcisomes of *Toxoplasma gondii*. *EMBO J*. 2001; 20:55–64. [PubMed: 11226155]
34. Luo S, Ruiz FA, Moreno SN. The acidocalcisome Ca^{2+} -ATPase (TgA1) of *Toxoplasma gondii* is required for polyphosphate storage, intracellular calcium homeostasis and virulence. *Mol Microbiol*. 2005; 55:1034–1045. [PubMed: 15686552]
35. Yagisawa F, Nishida K, Yoshida M, Ohnuma M, Shimada T, Fujiwara T, Yoshida Y, Misumi O, Kuroiwa H, Kuroiwa T. Identification of novel proteins in isolated polyphosphate vacuoles in the primitive red alga *Cyanidioschyzon merolae*. *Plant J*. 2009; 60:882–893. [PubMed: 19709388]
36. Hill JE, Scott DA, Luo S, Docampo R. Cloning and functional expression of a gene encoding a vacuolar-type proton-translocating pyrophosphatase from *Trypanosoma cruzi*. *Biochem J*. 2000; 351:281–288. [PubMed: 10998372]
37. Drake R, Serrano A, Perez-Castineira JR. N-terminal chimaeras with signal sequences enhance the functional expression and alter the subcellular localization of heterologous membrane-bound inorganic pyrophosphatases in yeast. *Biochem J*. 2010; 426:147–157. [PubMed: 20025609]
38. Vercesi AE, Docampo R. Sodium-proton exchange stimulates Ca^{2+} release from acidocalcisomes of *Trypanosoma brucei*. *Biochem J*. 1996; 315:265–270. [PubMed: 8670117]
39. Vercesi AE, Grijalba MT, Docampo R. Inhibition of Ca^{2+} release from *Trypanosoma brucei* acidocalcisomes by 3,5-dibutyl-4-hydroxytoluene: role of the $\text{Na}^{+}/\text{H}^{+}$ exchanger. *Biochem J*. 1997; 328:479–482. [PubMed: 9371704]
40. Vercesi AE, Rodrigues CO, Catisti R, Docampo R. Presence of a $\text{Na}^{+}/\text{H}^{+}$ exchanger in acidocalcisomes of *Leishmania donovani* and their alkalization by anti-leishmanial drugs. *FEBS Lett*. 2000; 473:203–206. [PubMed: 10812075]
41. Moreno SN, Vercesi AE, Pignataro OP, Docampo R. 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*. 1992; 52:251–261. [PubMed: 1620163]

42. Moreno SN, Docampo R, Vercesi AE. Calcium homeostasis in procyclic and bloodstream forms of *Trypanosoma brucei*. Lack of inositol 1,4,5-trisphosphate-sensitive Ca^{2+} release. *J Biol Chem*. 1992; 267:6020–6026. [PubMed: 1556113]
43. Docampo R, Moreno SN, Vercesi AE. Effect of thapsigargin on calcium homeostasis in *Trypanosoma cruzi* trypomastigotes and epimastigotes. *Mol Biochem Parasitol*. 1993; 59:305–313. [PubMed: 8341327]
44. Montalvetti A, Rohloff P, Docampo R. A functional aquaporin co-localizes with the vacuolar proton pyrophosphatase to acidocalcisomes and the contractile vacuole complex of *Trypanosoma cruzi*. *J Biol Chem*. 2004; 279:38673–38682. [PubMed: 15252016]
45. Motta LS, Ramos IB, Gomes FM, de Souza W, Champagne DE, Santiago MF, Docampo R, Miranda K, Machado EA. Proton-pyrophosphatase and polyphosphate in acidocalcisome-like vesicles from oocytes and eggs of *Periplaneta americana*. *Insect Biochem Mol Biol*. 2009; 39:198–206. [PubMed: 19111615]
46. Ramos IB, Miranda K, Pace DA, Verbist KC, Lin FY, Zhang Y, Oldfield E, Machado EA, De Souza W, Docampo R. Calcium- and polyphosphate-containing acidic granules of sea urchin eggs are similar to acidocalcisomes, but are not the targets for NAADP. *Biochem J*. 2010; 429:485–495. [PubMed: 20497125]
47. Ramos IB, Miranda K, Ulrich P, Ingram P, LeFurgey A, Machado EA, de Souza W, Docampo R. Calcium- and polyphosphate-containing acidocalcisomes in chicken egg yolk. *Biol Cell*. 2010; 102:421–434. [PubMed: 20196772]
48. Motta LS, da Silva WS, Oliveira DM, de Souza W, Machado EA. A new model for proton pumping in animal cells: the role of pyrophosphate. *Insect Biochem Mol Biol*. 2004; 34:19–27. [PubMed: 14723894]
49. Dell'Angelica EC, Mullins C, Caplan S, Bonifacino JS. Lysosome-related organelles. *FASEB J*. 2000; 14:1265–1278. [PubMed: 10877819]
50. Raposo G, Marks MS, Cutler DF. Lysosome-related organelles: driving post-Golgi compartments into specialisation. *Curr Opin Cell Biol*. 2007; 19:394–401. [PubMed: 17628466]
51. Holmsen H, Weiss HJ. Secretory storage pools in platelets. *Annu Rev Med*. 1979; 30:119–134. [PubMed: 233610]
52. Smith SA, Mutch NJ, Baskar D, Rohloff P, Docampo R, Morrissey JH. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci USA*. 2006; 103:903–908. [PubMed: 16410357]
53. Muller F, Mutch NJ, Schenk WA, Smith SA, Esterl L, Spronk HM, Schmidbauer S, Gahl WA, Morrissey JH, Renne T. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. *Cell*. 2009; 139:1143–1156. [PubMed: 20005807]
54. Moreno SN, Docampo R. The role of acidocalcisomes in parasitic protists. *J Eukaryot Microbiol*. 2009; 56:208–213. [PubMed: 19527347]
55. Hardoyo, Yamada K, Shinjo H, Kato J, Ohtake H. Production and release of polyphosphate by a genetically engineered strain of *Escherichia coli*. *Appl Environ Microbiol*. 1994; 60:3485–3490. [PubMed: 7986026]
56. Kornberg A, Rao NN, Ault-Riche D. Inorganic polyphosphate: a molecule of many functions. *Annu Rev Biochem*. 1999; 68:89–125. [PubMed: 10872445]
57. Rao NN, Gomez-Garcia MR, Kornberg A. Inorganic polyphosphate: essential for growth and survival. *Annu Rev Biochem*. 2009; 78:605–647. [PubMed: 19344251]
58. Zhang H, Rao NN, Shiba T, Kornberg A. Inorganic polyphosphate in the social life of *Myxococcus xanthus*: motility, development, and predation. *Proc Natl Acad Sci USA*. 2005; 102:13416–13420. [PubMed: 16174737]
59. Zhang H, Gomez-Garcia MR, Brown MR, Kornberg A. Inorganic polyphosphate in *Dictyostelium discoideum*: influence on development, sporulation, and predation. *Proc Natl Acad Sci USA*. 2005; 102:2731–2735. [PubMed: 15701689]
60. Castro CD, Koretsky AP, Domach MM. NMR-Observed phosphate trafficking and polyphosphate dynamics in wild-type and vph1-1 mutant *Saccharomyces cerevisiae* in response to stresses. *Biotechnol Prog*. 1999; 15:65–73. [PubMed: 9933515]

61. Castro CD, Meehan AJ, Koretsky AP, Domach MM. In situ ^{31}P nuclear magnetic resonance for observation of polyphosphate and catabolite responses of chemostat-cultivated *Saccharomyces cerevisiae* after alkalization. *Appl Environ Microbiol.* 1995; 61:4448–4453. [PubMed: 8534109]
62. Yang YC, Bastos M, Chen KY. Effects of osmotic stress and growth stage on cellular pH and polyphosphate metabolism in *Neurospora crassa* as studied by ^{31}P nuclear magnetic resonance spectroscopy. *Biochim Biophys Acta.* 1993; 1179:141–147. [PubMed: 8218356]
63. Pick U, Weiss M. Polyphosphate hydrolysis within acidic vacuoles in response to amine-Induced alkaline stress in the halotolerant alga *Dunaliella salina*. *Plant Physiol.* 1991; 97:1234–1240. [PubMed: 16668514]
64. Pick U, Zeelon O, Weiss M. Amine accumulation in acidic vacuoles protects the halotolerant alga *Dunaliella salina* against alkaline stress. *Plant Physiol.* 1991; 97:1226–1233. [PubMed: 16668513]
65. Weiss M, Bental M, Pick U. Hydrolysis of polyphosphates and permeability changes in response to osmotic shocks in cells of the halotolerant alga *Dunaliella*. *Plant Physiol.* 1991; 97:1241–1248. [PubMed: 16668515]
66. Patel S, Docampo R. Acidic calcium stores open for business: expanding the potential for intracellular Ca^{2+} signaling. *Trends Cell Biol.* 2010; 20:277–286. [PubMed: 20303271]
67. Smith SA, Morrissey JH. Polyphosphate as a general procoagulant agent. *J Thromb Haemost.* 2008; 6:1750–1756. [PubMed: 18665922]
68. Hernandez-Ruiz L, Gonzalez-Garcia I, Castro C, Brieva JA, Ruiz FA. Inorganic polyphosphate and specific induction of apoptosis in human plasma cells. *Haematologica.* 2006; 91:1180–1186. [PubMed: 16956816]
69. Neira I, Ferreira AT, Yoshida N. Activation of distinct signal transduction pathways in *Trypanosoma cruzi* isolates with differential capacity to invade host cells. *Int J Parasitol.* 2002; 32:405–414. [PubMed: 11849637]
70. Fernandes AB, Neira I, Ferreira AT, Mortara RA. Cell invasion by *Trypanosoma cruzi* amastigotes of distinct infectivities: studies on signaling pathways. *Parasitol Res.* 2006; 100:59–68. [PubMed: 16791632]
71. Lemercier G, Dutoya S, Luo S, Ruiz FA, Rodrigues CO, Baltz T, Docampo R, Bakalara N. A vacuolar-type H^{+} -pyrophosphatase governs maintenance of functional acidocalcisomes and growth of the insect and mammalian forms of *Trypanosoma brucei*. *J Biol Chem.* 2002; 277:37369–37376. [PubMed: 12121996]
72. LeFurgey A, Ingram P, Blum JJ. Compartmental responses to acute osmotic stress in *Leishmania major* result in rapid loss of Na^{+} and Cl. *Comp Biochem Physiol A Mol Integr Physiol.* 2001; 128:385–394. [PubMed: 11223400]
73. Rohloff P, Montalvetti A, Docampo R. Acidocalcisomes and the contractile vacuole complex are involved in osmoregulation in *Trypanosoma cruzi*. *J Biol Chem.* 2004; 279:52270–52281. [PubMed: 15466463]
74. Rohloff P, Docampo R. A contractile vacuole complex is involved in osmoregulation in *Trypanosoma cruzi*. *Exp Parasitol.* 2008; 118:17–24. [PubMed: 17574552]
75. Monk PB, Othmer HG. Cyclic AMP oscillations in suspensions of *Dictyostelium discoideum*. *Philos Trans R Soc Lond B Biol Sci.* 1989; 323:185–224. [PubMed: 2565584]
76. Clark TB. Comparative morphology of four genera of trypanosomatidae. *J Protozool.* 1959; 6
77. King-Keller S, Li M, Smith A, Zheng S, Kaur G, Yang X, Wang B, Docampo R. Chemical validation of phosphodiesterase C as a chemotherapeutic target in *Trypanosoma cruzi*, the etiological agent of Chagas' disease. *Antimicrob. Agents Chemother.* 2010; 54:3738–3745.
78. Ayraud S, Janvier B, Labigne A, Ecobichon C, Burucoa C, Fauchere JL. Polyphosphate kinase: a new colonization factor of *Helicobacter pylori*. *FEMS Microbiol Lett.* 2005; 243:45–50. [PubMed: 15667999]
79. Rifat D, Bishai WR, Karakousis PC. Phosphate depletion: a novel trigger for *Mycobacterium tuberculosis* persistence. *J Infect Dis.* 2009; 200:1126–1135. [PubMed: 19686042]
80. Kim KS, Rao NN, Fraley CD, Kornberg A. Inorganic polyphosphate is essential for long-term survival and virulence factors in *Shigella* and *Salmonella* spp. *Proc Natl Acad Sci USA.* 2002; 99:7675–7680. [PubMed: 12032342]

81. Zhang K, Hsu FF, Scott DA, Docampo R, Turk J, Beverley SM. *Leishmania* salvage and remodelling of host sphingolipids in amastigote survival and acidocalcisome biogenesis. *Mol Microbiol.* 2005; 55:1566–1578. [PubMed: 15720561]
82. Salles, Feys HB, Iserbyt BF, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Inherited traits affecting platelet function. *Blood Rev.* 2008; 22:155–172. [PubMed: 18180086]
82. Moreno, SN.; Miranda; de Souza, W. Calcium homeostasis and acidocalcisomes. In: Ajioka, JW.; Soldati, D., editors. *Toxoplasma Molecular and Cellular Biology*. Norfolk: Horizon Bioscience; 2007. p. 493-506.
83. Moreno, SN.; Miranda, K.; Fang, J.; Rohloff, P.; de Souza, W. Calcium storage and homeostasis in *Toxoplasma gondii*. In: Weiss, L.; Kim, K., editors. *Toxoplasma gondii, The Model Apicomplexan- Perspectives and Methods*. London: Elsevier Bioscience; 2007. p. 245-263.

Fig. 1.

Acidocalcisomes in *Agrobacterium tumefaciens*. (A) electron micrograph of intact bacteria. Arrow shows an electron-dense material in the periphery of an acidocalcisome. White arrowhead shows an electron-dense inclusion. (B) Staining of acidocalcisomes with DAPI (yellow). (C) (D) staining of acidocalcisomes with antibodies against a V-H⁺-PPase as detected by immunofluorescence (C) or immunoelectron microscopy (D). Arrowheads in (C) show the acidocalcisomes in green. Inset is at higher magnification. Arrowheads in (D) show the gold particles labeling the membrane of an acidocalcisome (vg, volutin granule). Inset in (D) shows an immunoblot analysis of a bacterial lysate. PPase, antibody against V-H⁺-PPase showing a band of 72 kDa. C, control treated with preimmune serum. Bars, A = 0.1 μ m; B, C = 0.5 μ m; D = 40 nm. A, C, and D are reproduced with permission from [10], © The American Society for Biochemistry and Molecular Biology.

Fig. 2. Morphology of acidocalcisomes in whole cells and sections. (A) Ultrathin sections of *Toxoplasma gondii* showing the acidocalcisomes as empty vesicles or containing electron dense material (arrows). (B) Acridine orange staining of acidocalcisomes (red vesicles) of *T. gondii* tachyzoites. (C) Visualization of acidocalcisomes in whole unfixed *Toxoplasma gondii* allowed to adhere to Formvar- and carbon-coated grids and then observed by direct transmission electron microscopy (using an energy filter). Acidocalcisomes (black granules) appear disperse in the cytoplasm. Bars, (A) = 500 nm; (B) = 3 μ m; (C) = 1 μ m. Reproduced with permission from [15], © Elsevier.

Fig. 3.

Schematic representation of an acidocalcisome. A H⁺ gradient is established by a vacuolar ATPase (V-H⁺-ATPase) and a vacuolar pyrophosphatase (V-H⁺-PPase). Ca²⁺ transport is driven by a Ca²⁺-ATPase. Other transporters include Na⁺/H⁺, and Ca²⁺/H⁺ exchangers, and a water channel or aquaporin. A vacuolar transporter chaperone (VTC) complex is involved in synthesis and translocation of poly P. Transporters for basic amino acids, P_i, PP_i, and cations are potentially present (in green). The matrix is rich in PP_i and polyphosphate (poly P) and enzymes involved in their metabolism (exopolyphosphatase (PPX), and pyrophosphatase (PPase)). Not all the enzymes and transporters are present in all acidocalcisomes.

Fig. 4. Acidocalcisomes in chicken egg yolk. (A) Compound organelle observed by energy-filtering transmission electron microscopy. The internal vesicles (acidocalcisomes) are electron-dense. (B) acridine orange staining of the compound organelle showing the more acidic internal vesicles (acidocalcisomes). (C-F) Elemental mapping of the compound organelle showed in (C) reveals the localization of phosphorus (P-Kα), calcium (Ca-Kα), and potassium (K-Kα). (G-H) freeze fracture analysis of acidocalcisome fractions showing the E (external face of the inner leaflet) (G) and P (internal face of the outer leaflet) (H) faces of fractured membranes. Intramembrane particles (IMPs) (arrows) are randomly distributed on the E and P of membranes. Scale bars, (A, B) = 5 μm; (C-F) = 10 μm; (G-H) = 2 μm. Reproduced with permission from [47], © Portland Press Ltd.

Fig. 5. Proposed Ca^{2+} mobilization pathways in sea urchin eggs

Endoplasmic reticulum (ER), acidocalcisomes, and yolk platelets are major Ca^{2+} storage organelles. IP_3 receptors (IP_3R) and ryanodine receptors (RyR) mediate Ca^{2+} release from the endoplasmic reticulum (ER) in response to increases in inositol 1,4,5-trisphosphate (IP_3) and cyclic ADP ribose (cADPR), respectively. Both receptors types are in addition, activated by Ca^{2+} via the so-called Ca^{2+} -induced Ca^{2+} release mechanism (CICR). NAADP triggers Ca^{2+} release from yolk platelets (gray) and this in turn triggers ER Ca^{2+} mobilization through Ca^{2+} -induced Ca^{2+} release via IP_3R and ryanodine (RyR) receptors. Na^+ entry after fertilization leads to alkalinization of the cytosol and stimulates Ca^{2+} release from acidocalcisomes by coupling the activity of Na^+/H^+ and $\text{Ca}^{2+}/\text{H}^+$ exchangers. GPN is hydrolyzed by a cathepsin C increasing the osmolarity of yolk platelets, attracting water, and leading to osmotic lysis and Ca^{2+} release. Both acidocalcisomes and yolk platelets possess bafilomycin A_1 -sensitive vacuolar type H^+ -ATPases to acidify the organelles. Poly P is hydrolyzed after alkalinization of acidocalcisomes. Reproduced with permission from [46], © Portland Press Ltd.

Fig. 6. Electron microscopy of whole platelets (A) or dense granule (acidocalcisome) fraction (B-C). (A) Transmission electron microscopy of an unfixed and unstained platelet preparation. Dense granules are identified by arrows. (C) Direct observation of unfixed and unstained dense granules air-dried directly onto microscope grids. The inset shows at higher magnification the sponge-like structure of the dense granules after exposure to the electron beam. (C) fixed and sectioned dense granule fraction. Bars, (A) = 1 μm ; (B-C) = 0.25 μm . Reproduced with permission from [11], © *The American Society for Biochemistry and Molecular Biology*.