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The origin and evolution of the acidocalcisome and its interactions with other organelles

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Abstract

Acidocalcisomes are acidic calcium stores that have been found from bacteria to human cells. They are rich in phosphorus compounds in the form of orthophosphate (P_i), pyrophosphate (PP_i), and polyphosphate (polyP) and their acidity is maintained by proton pumps such as the vacuolar proton pyrophosphatase (V-H⁺-PPase, or VP1), the vacuolar proton ATPase (V-H⁺-ATPase), or both. Recent studies in trypanosomatids and in other species have revealed their role in phosphate metabolism, and cation and water homeostasis, as suggested by the presence of novel pumps, transporters, and channels. An important role in autophagy has also been described. The study of the biogenesis of acidocalcisomes as well as of the interactions of these lysosome-related organelles with other organelles have uncovered important roles in calcium signaling and osmoregulation. Significantly, despite conservation of acidocalcisomes across all of cellular life, there is evidence for intimate integration of these organelles with eukaryotic cellular functions, and which are directly relevant to parasites.

1. Introduction

We named these membrane-bounded organelles as *acidocalcisomes* when we first found them in trypanosomes [1, 2]. We later realized that, as early as in the nineteenth century [3], similar structures had been described in bacteria, and were named metachromatic [3] or volutin [4] granules. There was some initial discrepancy on the presence or absence of a surrounding membrane in these bacterial granules [5] until we described at least two species of bacteria in which a surrounding membrane is present [6, 7]. These volutin granules were also described early in the twentieth century in a number of unicellular protists, like trypanosomatids [8], coccidians [9], and Sarcosporidia [10] among others, although they were considered granules rather than organelles and were renamed as polyphosphate (polyP) bodies when it was found that this polymer was abundant within the acidocalcisome-like

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vacuole of yeast [11]. More recently polyP, which is a polymer from 3 to thousands of orthophosphate units bound by high-energy phosphoanhydride bonds, was found in membrane-bounded acid calcium stores [12] from mammalian cells such as human platelet dense granules [13] and mast cell and basophil granules [14], which were then considered as acidocalcisome-like organelles. Taken together, these studies revealed that acidocalcisomes are membrane-bounded organelles that, together with the lipid droplets [15], are the only organelles conserved from bacteria to human cells.

Early reviews [16–18] described the morphological and biochemical characteristics of acidocalcisomes of different species. In this review we will limit our discussion to the most recent work on the origin, biogenesis, and function of these organelles.

2. Origin of the acidocalcisome

Although most cell biology textbooks (see, for example [19]) still state that prokaryotes lack intracellular compartmentalization, many intracellular membrane-bounded organelles have been reported in several bacterial species (Table 1) [20]. Some of the membranes are lipid based as in, for example, acidocalcisomes [6, 7], magnetosomes [21], photosynthetic membranes (thylakoids, chlorosomes, and chromatophore membranes) [22], pyrellulosomes [23], anammoxosomes [24], lipid droplets [15], and polyhydroxyalkanoate granules [25], while others are protein based such as in carboxysomes, 1,2-propanediol use (Pdu) compartment, and Ethanol use (Etu) microcompartment [26]. The existence of such compartmentalization, which includes the discoveries that the Planctomycete *Gemmata obscuriglobus* has its chromosome surrounded by a double membrane with similarities to a nuclear membrane [27] and that it has an endocytosis-like protein uptake [28] support an autogenous origin of these organelles rather than an endosymbiotic one, although convergent evolution in their appearance cannot be ruled out [26, 29].

It has been claimed that acidocalcisomes could be present in all domains of life, including archaea, and may thus date back as far as to the last universal common ancestor [30]. On the other hand, acidocalcisomes of trypanosomes share characteristics with organelles known as lysosome-related organelles (LROs) such as human platelets dense granules and mast cell granules, which are also considered acidocalcisome-like organelles [18, 14]. For example, adaptor protein 3 (AP-3), a protein complex involved in transport of membrane proteins to LROs of mammalian cells [31] is involved in the biogenesis of acidocalcisomes of *Leishmania major* [32] and *Trypanosoma brucei* [33]. This does not necessarily suggest a different origin of acidocalcisomes in eukaryotes but a potential further adaptation in these cells. The point here is that similar membrane-bounded polyP-containing acidic calcium stores are present in both prokaryotes and eukaryotes and they could have appeared either autogenously or by convergent evolution.

Recent articles have reported the presence of acidocalcisomes and acidocalcisome-like organelles in a number of bacteria and eukaryotic species. The marine sulfide-oxidizing bacteria *Beggiatoa* were reported to accumulate polyP together with cations in well-delimited granules enclosed by a lipid layer with similarity to acidocalcisomes, although not particularly acidic [34]. Similar polyP and cation storage structures (small granules) were

found in the Gram-negative sporulating bacterium *Acetonebma longum* [35]. PolyP also accumulates in acidocalcisome-like vacuoles of the arbuscular mycorrhizal fungus *Rhizophagus* sp. [36]. Typical acidocalcisomes were recently found in the midgut of the caterpillar *Anticarsia gemmatilis*, although they were called spherites [37]. Acidocalcisomes have recently been described in other eukaryotes such as *Eimeria acervulina*, and *Eimeria tenella*, parasites that infect poultry flocks all over the world [38], and in the egg yolk of the insect *Rhodnius prolixus* [39], one of the insect vectors of *T. cruzi*. Mast cell granules and human basophil granules were also shown to contain polyP, calcium and an acidic pH and together with their elemental composition have the characteristics of typical acidocalcisomes [14]. PolyP released by mast cells and basophils could be important mediators of their pro-coagulant and pro-inflammatory activities [40].

3. New insights into the chemical and enzymatic composition

Recent biochemical and proteomic studies in trypanosomatids and in other species have identified new components of acidocalcisomes that clarify their role in phosphate and cation homeostasis, and in cell signaling.

Acidocalcisomes of *Chlamydomonas reinhardtii* [41] were found to contain copper, which becomes available for the synthesis of plastocyanin [42]. Copper trafficking to acidocalcisomes was proposed as a strategy for preventing mismetallation during zinc deficiency enabling efficient cuproprotein metallation or remetallation upon zinc resupply [42]. Copper accumulation was also detected in acidocalcisomes (spherites) of the midgut of the caterpillar *A. gemmatilis* [37] and in acidocalcisome-like vacuoles of *Euglena gracilis* [43].

PolyP is the polymer that defines acidocalcisomes and new information became available about its synthesis in eukaryotes. PolyP synthesis in eukaryotes was unclear until it was demonstrated that the *Sacharomyces cerevisiae* vacuolar transporter chaperone 4 (ScVtc4p) is a polyP polymerase that uses ATP to generate polyP [44] (Fig. 1). The Vtc complex in *S. cerevisiae* consists of four proteins (Vtc1–4) that form hetero-oligomeric complexes that couple synthesis and translocation of polyP to the acidocalcisome-like vacuole and prevents its toxicity when in the cytosol [45]. This polyP import requires an electrochemical gradient as a driving force [45]. A similar acidocalcisome localization of Vtc4 protein was also demonstrated in *T. brucei* [46] and *T. cruzi* [47], as detected by immunofluorescence and immunoelectron microscopy with antibodies against epitope-tagged proteins. The enzymes (TbVtc4 and TcVtc4) were characterized and shown to synthesize predominantly short chain polyP (~100–300 P_i residues). TbVtc4 is essential in both bloodstream and procyclic stages of *T. brucei*, as investigated using RNA interference (RNAi) and conditional knockout generation, respectively [46]. TbVtc1 is also localized to acidocalcisomes, essential and necessary for polyP synthesis, suggesting that trypanosomes possess at least two subunits of the complex (Vtc1 and Vtc4) [48]. Vtc2 and Vtc4 homologs were also identified in *Toxoplasma gondii*, and a plasmid insertion on the *TgVtc2* locus resulted in cells deficient in polyP synthesis and upregulation of some genes, among them a FIKK (phenylalanine, isoleucine, lysine, lysine motif)-containing kinase, which is only present in Apicomplexan [49]. HA-tagged TgVtc2 has a punctate localization although it is not clear whether it is in

acidocalcisomes since it does not co-localize with the V-H⁺-PPase (TgVP1) [49]. A Vtc1 homolog necessary for acidocalcisome formation and highly sensitive to sulfur-, phosphorus- or nitrogen-deficient conditions was also identified in *C. reinhardtii*, which also possesses a Vtc4 homolog [50]. Mutants deficient in CrVtc1 contain less polyP and acidocalcisomes [50]. Proteomic studies of acidocalcisomes of the red alga *Cyanidioschyzon merolae* (Vtc1) [51] and of *T. brucei* (Vtc1, Vtc4) [52] confirmed the presence of Vtc proteins in these organelles.

Definitive evidence of the co-localization in acidocalcisomes of *T. brucei* of two proton pumps, the V-H⁺-PPase (TbVP1) and the V-H⁺-ATPase, was obtained after proteomic identification of several V-H⁺-ATPase subunits in the acidocalcisome proteome and validation of their acidocalcisome localization by *in situ* epitope-tagging and western blot and immunofluorescence analyses using specific antibodies [52] (Fig. 1). ATP- and PP_i-driven proton uptake and ATP-dependent Ca²⁺ uptake was also described in isolated acidocalcisomes of *T. gondii*, where evidence of Ca²⁺/H⁺ and Na⁺/H⁺ exchangers was also obtained [53]. It is remarkable that proteomic analysis of acidocalcisomes of *C. merolae* also provided evidence of co-localization of V-H⁺-ATPase and V-H⁺-PPase [51]. Acidocalcisomes [54] are, together with the plant vacuole [55], the malaria vacuole [56], the *T. cruzi* [57], *C. reinhardtii* [41], and *Dictyostelium discoideum* [58] contractile vacuoles, and the *T. gondii* plant-like vacuole [59], organelles in which these two pumps co-localize. The V-H⁺-PPase of *T. gondii* (TgVP1), which also localizes to acidocalcisomes, was knocked out in the *ku80* strain, which favors homologous recombination [60]. The *null* mutants were deficient in microneme secretion, host cell invasion, and extracellular survival and were less virulent in mice [60].

Acidocalcisomes from trypanosomatids, *T. gondii*, and *D. discoideum* were known to possess a plasma membrane Ca²⁺ ATPase (PMCA)-type pump for Ca²⁺ uptake [17] but the mechanism involved in Ca²⁺ release was unknown until it was found that the inositol 1,4,5-trisphosphate receptor (IP₃R) localizes to acidocalcisomes of *T. brucei* [61] (Fig. 1). Immunofluorescence analysis with antibodies against the HA-tagged channel or with specific antibodies against the protein co-localized it with TbVP1, the acidocalcisome marker. IP₃ was able to release Ca²⁺ from a PP_i-acidified compartment in permeabilized procyclic trypomastigotes or from isolated acidocalcisomes [61]. IP₃ also releases Ca²⁺ from intracellular stores in live trypanosomes, as demonstrated using caged IP₃ [61]. The channel was expressed in DT40 cells in which all the vertebrate IP₃R genes were stably ablated and shown to be less sensitive to IP₃ than rat IP₃ receptor 1 (IP₃R1). RNAi of TbIP₃R in bloodstream forms revealed the essentiality of the receptor for infectivity [61]. In conclusion, the presence of Ca²⁺ uptake and Ca²⁺ release mechanisms suggest an active role of acidocalcisomes of *T. brucei* in Ca²⁺ signaling.

Proteomic and bioinformatic analyses of acidocalcisomes from *T. brucei* identified a number of proteins whose acidocalcisome localization was validated by *in situ* epitope-tagging and immunofluorescence analysis using antibodies [52] (Fig. 1). Among the newly identified transporters there is a zinc transporter (TbZnT) with similarity to an acidocalcisome zinc transporter of *T. cruzi* [62], and an ortholog (TbVIT1) to the vacuolar iron transporter (VIT) described in *Arabidopsis thaliana* and to the Ca²⁺-sensitive cross-complement 1 (CCC1) of

the yeast vacuole, which are involved in iron and manganese sequestration [52]. A phosphate transporter (TbPho91) with similarity to the *S. cerevisiae* Pho91 vacuolar P_i transporter was also identified [52]. This transporter would be involved in Na⁺-dependent export to P_i from acidocalcisomes. A putative polyamine transporter (TbPOL1), which also localizes to lysosomes was also identified [52], suggesting the accumulation of polyamines. Polyamines are known to accumulate in the acidocalcisome-like vacuole of yeast [63]. Interestingly, a soluble acid phosphatase (TbAP) was also localized to acidocalcisomes by *in situ* epitope-tagging [52], in agreement with previous reports of acid phosphatase activity detected by cytochemical methods in other trypanosomes [17]. Fig. 1 shows a schematic representation of the newly and previously identified pumps and transporters in acidocalcisomes from the procyclic stages of *T. brucei*.

4. Biogenesis

Previous work found that the AP-3 complex was involved in the biogenesis of acidocalcisomes of *L. major* [32] and *T. brucei* [33]. Two novel findings implicated other signaling pathways and organelles in the biogenesis of acidocalcisomes.

Target of rapamycin (TOR) kinases are key regulators of eukaryotic growth and trypanosomatids possess four TOR kinases. *L. major* promastigotes deficient in TOR kinase 3 exhibited slower growth without morphological alterations and were able to differentiate into metacyclic forms but were unable to survive or replicate in macrophages *in vitro*, or to establish infections in mice [64]. The loss of virulence was associated to a defect in acidocalcisome formation, less polyP accumulation and osmoregulatory defects, suggesting a role of TOR kinase 3 in acidocalcisome biogenesis [64]. Interestingly, although RNAi downregulation of TOR kinase 3 in *T. brucei* procyclic and bloodstream stages also affects growth, it results in increased PP_i and polyP levels in larger acidocalcisomes and in cells more sensitive to high osmolarity conditions [65]. The reason for these opposite results is unclear although they could be partially related to the way the results were quantified. Quantification of polyP and acidocalcisomes in *T. brucei* was based on biochemical measurements, and morphometric analysis of whole cells by transmission electron microscopy (TEM), respectively [65]. Quantification of polyP and acidocalcisomes in *L. major* was based on DAPI staining intensity, and immunofluorescence analysis with antibodies against VP1, or TEM of sections, respectively [64].

Work in *T. cruzi* found an important role of the contractile vacuole complex (CVC) in trafficking and exchange of proteins with acidocalcisomes [66]. Expression of dominant negative mutants of the CVC-located small GTPase Rab32 resulted in a lower number of less electron-dense acidocalcisomes, lower content of polyP, lower capacity for acidocalcisome acidification and Ca²⁺ uptake driven by the vacuolar proton pyrophosphatase (VP1) and the Ca²⁺-ATPase, respectively, and less infective parasites [66]. Fluorescence, and electron microscopy and electron tomography provided evidence of the active contact of acidocalcisomes with the CVC, suggesting an active exchange of proteins between the two organelles (Fig. 2). The acidocalcisome markers TcVP1 and TcVAMP7 were shown to traffic towards the CVC when the cells were submitted to osmotic stress [66]. The results complemented previous findings of translocation of an aquaporin (TcAQP1) from

acidocalcisomes to the CVC during osmotic stress [67]. In conclusion, there is extensive exchange of proteins between the CVC and the acidocalcisomes that can be enhanced under osmotic stress.

5. Novel functions

A role for acidocalcisomes in osmoregulation was proposed on the basis of the rapid hydrolysis or synthesis of acidocalcisome polyP and changes in cation levels when trypanosomatids are exposed to different osmotic stresses [16]. Under hyposmotic stress a microtubule- and cyclic AMP-mediated fusion of acidocalcisomes to the CVC of *T. cruzi* results in translocation of TcAQP1 to the CVC, and the resulting water elimination, which together with swelling of acidocalcisomes, is responsible for the decrease in volume during regulatory volume decrease [16]. TcAQP1 is also important for cell shrinkage of *T. cruzi* under hyperosmotic stress, which is accompanied by the increase in polyP synthesis, amino acid accumulation, and global gene expression changes [68, 69].

Experiments in *T. cruzi*, in which overexpression of a vacuolar soluble PPase (TcVSP, partially localized to acidocalcisomes and present in the cytosol) resulted in decreased levels of PP_i and polyP, defective growth of infective stages in host cells in vitro, less responsiveness to osmotic stress, and reduced persistence of the parasites in tissues of mice, suggested that PP_i and polyP are essential to resist the stressful conditions in the host and to maintain a persistent infection [70].

Another important function demonstrated recently was the involvement of acidocalcisomes in autophagy in *T. brucei* [71]. Treatment of procyclic stages with bafilomycin A₁ (a V-H⁺-ATPase inhibitor), monensin (a Na⁺/H⁺ ionophore), or the combination of NH₄Cl and ionomycin (alkalinizing agents) inhibited starvation-induced autophagosome formation, suggesting the need for functional acidic organelles for autophagy to occur. This effect was also observed by inhibiting acidocalcisome biogenesis by downregulating the expression of components of the AP-3 complex (adaptins β3 and δ). Surprisingly, downregulation of the expression of two subunits (A, and α) of the V-H⁺-ATPase by RNAi increases (instead of decreasing) starvation-induced autophagy while downregulation of TbVP1 or of both enzymes together does not affect it. Interestingly, staining of acidocalcisomes with BODIPY-chloroquine, acridine orange or LysoTracker Green, which usually are taken up by acidic compartments, increases upon starvation but downregulation of the expression of the V-H⁺-ATPase or TbVP1, or both, do not block LysoTracker Red accumulation, which the authors suggested is due to increased acidocalcisome acidification upon starvation. In summary, the experiments revealing inhibition of starvation-induced autophagy by downregulation of acidocalcisome biogenesis clearly indicate a role for acidocalcisomes in this process. What is not yet clear is the process involved in the increased dye uptake by acidocalcisomes under starvation conditions when proton pumps are less expressed.

6. Interactions with other organelles

Acidocalcisomes in trypanosomes are usually observed in close contact with other organelles and intracellular structures such as the mitochondria, nucleus, subpellicular

microtubules, and lipid inclusions [72]. The close contacts with mitochondria could be relevant for the regulation of the bioenergetics of the cells as occurs with the close contacts between the endoplasmic reticulum (ER) and mitochondria in vertebrate cells. The work by Rizzuto et al. [73] showed that IP₃R-induced Ca²⁺ release could be transmitted to the mitochondrial matrix because the organelles are very close and a local microdomain with a high Ca²⁺ concentration is created near the mitochondria. The presence of the IP₃R in acidocalcisomes [61] and the rapid transfer of Ca²⁺ from the acidocalcisomes to the mitochondria described by Xiong et al. [74] in *T. brucei* suggest that there must be a close proximity between acidocalcisomes and mitochondria (Fig. 3). The presence of a quasi-synaptic Ca²⁺ signal transmission between acidocalcisomes and mitochondria as the one present between the ER and mitochondria of mammalian cells is therefore possible. In mammalian mitochondria Ca²⁺ stimulates several dehydrogenases and carriers and similar functions in trypanosomes have been proposed [75]. Two potential targets are the proline dehydrogenase, which possesses EF-hand domains, and pyruvate dehydrogenase E1 α , which can potentially be activated by dephosphorylation catalyzed by a Ca²⁺-activated phosphatase [75].

A potential interaction of acidocalcisomes and mitochondria of *T. brucei* was suggested by a genome-scale RNAi library screening for isometamidium (ISM) resistance [76]. Genes encoding the fourteen V-ATPase subunits (which localize to acidocalcisomes, lysosomes and Golgi complex [52]), the four AP-3 subunits (which are involved in biogenesis of acidocalcisomes [33]) and six putative endoplasmic reticulum membrane complex (EMC) subunits (which in mammals play a role in transfer of phospholipids to mitochondria [77]), were implicated in drug resistance. Downregulation by RNAi of the expression of two V-ATPase subunits, one of the AP-3 subunits, or one of the EMC subunits or inhibition of the V-ATPase with bafilomycin A₁ also increased ISM resistance, validating the screening results. RNAi downregulation of these subunits also caused resistance to berenil, ethidium bromide, and to a lesser extent pentamidine. Resistance was apparently not due to less ISM accumulation, as detected by fluorescence changes after RNAi of the V-ATPase. Depletion of V-ATPase, AP-3 and EMC subunits by RNAi induced by tetracycline also increased resistance to acriflavin, which caused diskinetoplasty, but tetracycline removal caused death of the cells suggesting that V-ATPase, AP-3 or EMC depletion allows *T. brucei* to tolerate diskinetoplasty. Interestingly, V-ATPase depletion also increased resistance to growth inhibition by oligomycin, an inhibitor of the mitochondrial ATP synthase, and the authors suggested that the V-ATPase activity maintains ATPase synthase coupling in *T. brucei* [76]. However, this suggestion contradicts the authors' assertion that they did not detect changes in the mitochondrial membrane potential (Ψ_m) of the RNAi mutants, which in bloodstream forms is maintained by the ATP synthase working as an ATPase [78]. In any case, the results confirm the involvement of acidocalcisomes in maintaining the bioenergetics of *T. brucei* bloodstream forms [75]. When acidocalcisomes are deficient (by lower expression of the V-ATPase or by a biogenesis defect) there is less Ca²⁺ transfer through their IP₃ receptor to the mitochondria, which helps to maintain the cell bioenergetics through stimulation of mitochondrial dehydrogenases [75].

Acidocalcisomes also interact with the CVC upon osmotic stress and this interaction has been studied in more detail in *T. cruzi* (Fig. 2). Cyclic AMP (cAMP) levels increase when

epimastigotes are subjected to hyposmotic stress and compounds that modulate cyclic AMP levels and microtubule function affect translocation of TcAQP1 from the acidocalcisomes to the CVC [67]. It was proposed that stimulation of cell swelling causes a spike in intracellular cAMP, resulting in a microtubule-dependent fusion of acidocalcisomes with the CVC and transfer of TcAQP1 [68]. A rise in ammonia, and its accumulation in acidocalcisomes as NH_4^+ , would result in activation of an acidocalcisome exopolyphosphatase, cleaving polyP, and releasing P_i and polyP-chelated osmolytes, such as basic amino acids and cations. The resulting osmotic gradient would increase water uptake, through the aid of TcAQP1, leading to its release into the flagellar pocket. A cAMP phosphodiesterase (PDE) would terminate the signaling pathway by hydrolyzing cAMP to 5'-AMP [68]. Some recent evidence favors this model, such as the localization of a PDE C in the CVC (spongiome) [79], the inhibition of the regulatory volume decrease (RVD) by PDE inhibitors [80], and the finding that overexpression of a class III phosphatidylinositol 3-kinase (TcPI3K) affects RVD [81]. In addition, fusion of acidocalcisomes was detected by immunofluorescence and immune-electron microscopy analyses and by electron tomography [66] (Fig. 2). A sodium-phosphate transporter that could be involved in recycling of P_i produced by the hydrolysis of polyP during RVD was found in the bladder of the CVC [82]. In addition, proteins involved in organellar fusion were detected in the CVC (SNAREs, TcRab32), and in acidocalcisomes (TcVAMP7) [66, 57].

The involvement of acidocalcisomes and the CVC under hyperosmotic stress is quite different. When epimastigotes are subjected to hyperosmotic stress there is an initial great increase in the size of the CVC suggesting that water efflux is mediated through the CVC. Treatment of the epimastigotes with low concentrations of HgCl_2 , a known inhibitor of *T. cruzi* aquaporin 1 (TcAQP1), or knockdown of *TcAQP1* expression reduces the intensity of shrinking after hyperosmotic stress while overexpression of *TcAQP1* increased shrinking, suggesting that the CVC mediates water efflux during hyperosmotic challenge. Shrinking is also probably due to cation elimination through a cation channel (TcCAT) that is translocated to the plasma membrane of epimastigotes submitted to hyperosmotic stress. Inhibitors of TcCAT (BaCl_2 , 4-aminopyridine) inhibit shrinking of trypomastigotes under hyperosmotic stress. Early synthesis of polyP and sequestration of inorganic ions in acidocalcisomes of epimastigotes and the simultaneous increase in compatible osmolytes prevents the deleterious effects of a cellular increase in ionic strength. The response of epimastigotes to hyperosmotic stress is therefore different from that observed in mammalian cells or yeasts. An aquaporin and the contractile vacuole are involved in water efflux leading to cell shrinkage, and there is no early regulatory volume increase. The results suggest that the increase in ionic strength is counteracted by the early synthesis of polyP and sequestration of inorganic ions in acidocalcisomes. Amino acids are the compatible osmolytes that replace the inorganic ions sequestered in acidocalcisomes, and they initially accumulate by a reduction in their catabolism, and later on by protein degradation and by uptake through induced amino acid transporters.

7. Concluding remarks and areas for future studies

The acidocalcisome can be considered as the earliest acidic calcium store that appeared during evolutionary time [12]. Phylogenetic studies have led some authors to postulate that

acidocalcisomes were present in the last universal common ancestor [30]. Recent proteomic and bioinformatics studies of acidocalcisomes have revealed roles in phosphorus, cation, and water accumulation and release, in osmoregulation, in parasite persistence, and in Ca^{2+} signaling. Ca^{2+} and other cations (Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} , K^{+} , Na^{+} , which relative abundance depends on the species investigated) in the acidocalcisome are bound to a polyanionic matrix of polyP. The long sought mechanism of Ca^{2+} release, an inositol 1,4,5-trisphosphate receptor, was found in *T. brucei*, and the mechanism of synthesis and accumulation of polyP in the organelle was discovered in different species. Novel insights were gained on the biogenesis of this organelle and on its interactions with other organelles in the cell. Several novel transporters were identified and more similarities were found with the yeast and plant vacuoles, and with the lysosome-related organelles of mammalian cells. This continues to be a promising area of research for its potential for discovery of novel functions in a variety of species, especially in parasitic protists because of their essentiality and potential for targeting. Further studies in trypanosomes will be needed to confirm the role of putative enzymes and transporters identified in proteomic studies (52), to investigate the structural organization of phosphorus compounds and cations within the organelles, and the mechanisms of interaction between acidocalcisomes and other organelles, as for example the mitochondria and the contractile vacuole.

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Abbreviations

AP	Acid phosphatase
AP-3	adaptor protein 3
AQP	aquaporin
CVC	contractile vacuole complex
IP₃	inositol 1,4,5-trisphosphate
LRO	lysosome-related organelle
Pho91	phosphate transporter 91
PI3K	phosphatidyl inositol 3-kinase
PDE	phosphodiesterase
polyP	polyphosphate
PPi	pyrophosphate
RVD	regulatory volume decrease
TOR	target of rapamycin

VP1 of H⁺-PPase	vacuolar H ⁺ -pyrophosphatase
V-H⁺-ATPase	vacuolar H ⁺ -ATPase
VSP	vacuolar soluble pyrophosphatase
VTC	vacuolar transporter chaperone complex
VIT	vacuolar iron transporter

References

1. Docampo R, Scott DA, Vercesi AE, Moreno SN. Intracellular Ca²⁺ storage in acidocalcisomes of *Trypanosoma cruzi*. *Biochem J*. 1995; 310:1005–1012. [PubMed: 7575396]
2. Vercesi AE, Moreno SN, Docampo R. Ca²⁺/H⁺ exchange in acidic vacuoles of *Trypanosoma brucei*. *Biochemical J*. 1994; 304:227–233.
3. Babes V. Beobachtungen über die metachromatischen körperchen, sporenbindung, verzweigung, kolben- und kasselbildung pathogener bakterien. *Zentralbl Bakteriell Parasitenkd Infektionskr Hyg*. 1895; 20:412–420.
4. Meyer A. Orientierende untersuchungen über verbreitung. Morphologie, und chemie des volutins. *Bot Zeit*. 1904; 62:113–152.
5. Shively JM. Inclusion bodies of prokaryotes. *Annu Rev Microbiol*. 1974; 28(0):167–187. [PubMed: 4372937]
6. 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(49):51193–51202. [PubMed: 15371423]
7. Seufferheld M, Vieira MC, Ruiz FA, Rodrigues CO, Moreno SN, et al. Identification of organelles in bacteria similar to acidocalcisomes of unicellular eukaryotes. *J Biol Chem*. 2003; 278(32):29971–29978. [PubMed: 12783865]
8. Swellengrabel NH. La volutine chez les trypanosomes. *CR Soc Biol Paris*. 1908; 64:38–43.
9. Kunze W. Über Orcheobius herpobdellae schuberg et kunze. *Arch Protistenk*. 1907; 9:382–390.
10. Erdmann R. Kern und metachromatische körper bei sarkosporiden. *Arch Protistenk*. 1910; 20:239–243.
11. Wiame JM. Etude d'une substance polypshophoré, basophile et métachromatique chez les levures. *Biochim Biophys Acta*. 1947; 1:234–255.
12. Patel S, Docampo R. Acidic calcium stores open for business: expanding the potential for intracellular Ca²⁺ signaling. *Trends Cell Biol*. 2010; 20(5):277–286. [PubMed: 20303271]
13. 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(43):44250–44257. [PubMed: 15308650]
14. Moreno-Sanchez D, Hernandez-Ruiz L, Ruiz FA, Docampo R. Polyphosphate is a novel pro-inflammatory regulator of mast cells and is located in acidocalcisomes. *J Biol Chem*. 2012; 287(34):28435–28444. [PubMed: 22761438]
15. Yang L, Ding Y, Chen Y, Zhang S, Huo C, et al. The proteomics of lipid droplets: structure, dynamics, and functions of the organelle conserved from bacteria to humans. *J Lipid Res*. 2012; 53(7):1245–1253. [PubMed: 22534641]
16. Docampo R, De Souza W, Miranda K, Rohloff P, Moreno SN. Acidocalcisomes - conserved from bacteria to man. *Nat Rev Microbiol*. 2005; 3(3):251–261. [PubMed: 15738951]
17. Docampo R, Moreno SN. Acidocalcisomes. *Cell Calcium*. 2011; 50(2):113–119. [PubMed: 21752464]
18. Docampo R, Ulrich P, Moreno SN. Evolution of acidocalcisomes and their role in polyphosphate storage and osmoregulation in eukaryotic microbes. *Phil Trans Royal Soc London Series B, Biol Sci*. 2010; 365(1541):775–784.

19. Lingappa, VR.; And Lewin, B. What is a cell?. In: Plopper, G.; Sharp, D.; Sikorski, E., editors. *Lewin's Cells*. 3rd ed.. Burlington, MA: Jones & Bartlett Learning; 2015. p. 2-30.
20. Shively, Jm CS.; Heinhorst, S.; Fuerst, Ja; Bryant, Da; Dassarma, S.; Bazylnski, D.; Preiss, J.; Steinbüchel, a; Docampo, R.; Dahl, C. eLS. Chichester: John Wiley & Sons, Ltd; 2011. Bacterial and archaeal inclusions; p. 1-14.
21. Komeili A, Li Z, Newman DK, Jensen GJ. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science*. 2006; 311(5758):242–245. [PubMed: 16373532]
22. Murat D, Byrne M, Komeili A. Cell biology of prokaryotic organelles. *Cold Spring Harb Perspect Biol*. 2010; 2(10):a000422. [PubMed: 20739411]
23. Fuerst JA. Intracellular compartmentation in planctomycetes. *Annu Rev Microbiol*. 2005; 59:299–328. [PubMed: 15910279]
24. Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, et al. Missing lithotroph identified as new planctomycete. *Nature*. 1999; 400(6743):446–449. [PubMed: 10440372]
25. Lundgren DG, Pfister RM, Merrick JM. Structure of Poly-Beta-Hydroxybutyric Acid Granules. *J Gen Microbiol*. 1964; 34:441–446. [PubMed: 14135548]
26. Diekmann Y, Pereira-Leal JB. Evolution of intracellular compartmentalization. *Biochem J*. 2013; 449(2):319–331. [PubMed: 23240612]
27. Fuerst JA, Webb RI. Membrane-bounded nucleoid in the eubacterium *Gemmata obscuriglobus*. *Proc Natl Acad Sci U S A*. 1991; 88(18):8184–8188. [PubMed: 11607213]
28. Lonhienne TG, Sagulenko E, Webb RI, Lee KC, Franke J, et al. Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. *Proc Natl Acad Sci U S A*. 2010; 107(29):12883–12888. [PubMed: 20566852]
29. Forterre P, Gribaldo S. Bacteria with a eukaryotic touch: a glimpse of ancient evolution? *Proc Natl Acad Sci U S A*. 2010; 107(29):12739–12740. [PubMed: 20624972]
30. Seufferheld MJ, Kim KM, Whitfield J, Valerio A, Caetano-Anolles G. Evolution of vacuolar proton pyrophosphatase domains and volutin granules: clues into the early evolutionary origin of the acidocalcisome. *Biol Direct*. 2011; 6:50. [PubMed: 21974828]
31. Theos AC, Tenza D, Martina JA, Hurbain I, Peden AA, et al. Functions of adaptor protein (AP)-3 AP-1 in tyrosinase sorting from endosomes to melanosomes. *Mol Biol Cell*. 2005; 16(11):5356–5372. [PubMed: 16162817]
32. Besteiro S, Tonn D, Tetley L, Coombs GH, Mottram JC. The AP3 adaptor is involved in the transport of membrane proteins to acidocalcisomes of *Leishmania*. *J Cell Sci*. 2008; 121(Pt 5): 561–570. [PubMed: 18252798]
33. Huang G, Fang J, Sant'anna C, Li ZH, Wellems DL, et al. Adaptor protein-3 (AP-3) complex mediates the biogenesis of acidocalcisomes and is essential for growth and virulence of *Trypanosoma brucei*. *J Biol Chem*. 2011; 286(42):36619–36630. [PubMed: 21880705]
34. Brock J, Rhiel E, Beutler M, Salman V, Schulz-Vogt HN. Unusual polyphosphate inclusions observed in a marine *Beggiatoa* strain. *Antonie Van Leeuwenhoek*. 2012; 101(2):347–357. [PubMed: 21909788]
35. Tocheva EI, Dekas AE, McGlynn SE, Morris D, Orphan VJ, et al. Polyphosphate storage during sporulation in the gram-negative bacterium *Acetonebacterium longum*. *J Bacteriol*. 2013; 195(17):3940–3946. [PubMed: 23813732]
36. Kikuchi Y, Hijikata N, Yokoyama K, Ohtomo R, Handa Y, et al. Polyphosphate accumulation is driven by transcriptome alterations that lead to near-synchronous and near-equivalent uptake of inorganic cations in an arbuscular mycorrhizal fungus. *New Phytol*. 2014; 204(3):638–649. [PubMed: 25039900]
37. Gomes FM, Carvalho DB, Peron AC, Saito K, Miranda K, et al. Inorganic polyphosphates are stored in spherites within the midgut of *Anticarsia gemmatilis* and play a role in copper detoxification. *J Insect Physiol*. 2012; 58(2):211–219. [PubMed: 21946413]
38. Soares Medeiros LC, Gomes F, Maciel LR, Seabra SH, Docampo R, et al. Volutin granules of *Eimeria* parasites are acidic compartments and have physiological and structural characteristics similar to acidocalcisomes. *J Eukaryot Microbiol*. 2011; 58(5):416–423. [PubMed: 21699625]

39. Ramos I, Gomes F, Koeller CM, Saito K, Heise N, et al. Acidocalcisomes as calcium- and polyphosphate-storage compartments during embryogenesis of the insect *Rhodnius prolixus* Stahl. *PLoS One*. 2011; 6(11):e27276. [PubMed: 22096545]
40. Docampo R. Polyphosphate: a target for thrombosis attenuation. *Blood*. 2014; 124(22):3177–3178. [PubMed: 25414432]
41. 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(49):46196–46203. [PubMed: 11579086]
42. Hong-Hermesdorf A, Miethke M, Gallaher SD, Kropat J, Dodani SC, et al. Subcellular metal imaging identifies dynamic sites of Cu accumulation in *Chlamydomonas*. *Nat Chem Biol*. 2014; 10(12):1034–1042. [PubMed: 25344811]
43. Einicker-Lamas M, Mezian GA, Fernandes TB, Silva FL, Guerra F, et al. *Euglena gracilis* as a model for the study of Cu²⁺ and Zn²⁺ toxicity and accumulation in eukaryotic cells. *Environ Pollut*. 2002; 120(3):779–786. [PubMed: 12442801]
44. Hothorn M, Neumann H, Lenherr ED, Wehner M, Rybin V, et al. Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. *Science*. 2009; 324(5926):513–516. [PubMed: 19390046]
45. Gerasimaite R, Sharma S, Desfougeres Y, Schmidt A, Mayer A. Coupled synthesis and translocation restrains polyphosphate to acidocalcisome-like vacuoles and prevents its toxicity. *J Cell Sci*. 2014; 127(23):5093–5104. [PubMed: 25315834]
46. Lander N, Ulrich PN, Docampo R. *Trypanosoma brucei* vacuolar transporter chaperone 4 (TbVtc4) is an acidocalcisome polyphosphate kinase required for in vivo infection. *J Biol Chem*. 2013; 288(47):34205–34216. [PubMed: 24114837]
47. Ulrich PN, Lander N, Kurup SP, Reiss L, Brewer J, et al. The acidocalcisome vacuolar transporter chaperone 4 catalyzes the synthesis of polyphosphate in insect-stages of *Trypanosoma brucei* and *T. cruzi*. *J Eukaryot Microbiol*. 2014; 61(2):155–165. [PubMed: 24386955]
48. 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(2):161–170. [PubMed: 17635107]
49. Rooney PJ, Ayong L, Tobin CM, Moreno SN, Knoll LJ. TgVTC2 is involved in polyphosphate accumulation in *Toxoplasma gondii*. *Mol Biochem Parasitol*. 2011; 176(2):121–126. [PubMed: 21195114]
50. Aksoy M, Pootakham W, Grossman AR. Critical function of a *Chlamydomonas reinhardtii* putative polyphosphate polymerase subunit during nutrient deprivation. *Plant Cell*. 2014; 26(10):4214–4229. [PubMed: 25281687]
51. Yagisawa F, Nishida K, Yoshida M, Ohnuma M, Shimada T, et al. Identification of novel proteins in isolated polyphosphate vacuoles in the primitive red alga *Cyanidioschyzon merolae*. *Plant J*. 2009; 60(5):882–893. [PubMed: 19709388]
52. Huang G, Ulrich PN, Storey M, Johnson D, Tischer J, et al. Proteomic analysis of the acidocalcisome, an organelle conserved from bacteria to human cells. *PLoS Pathog*. 2014; 10(12):e1004555. [PubMed: 25503798]
53. Rohloff P, Miranda K, Rodrigues JC, Fang J, Galizzi M, et al. Calcium uptake and proton transport by acidocalcisomes of *Toxoplasma gondii*. *PLoS One*. 2011; 6(4):e18390. [PubMed: 21541023]
54. Scott DA, Docampo R. Characterization of isolated acidocalcisomes of *Trypanosoma cruzi*. *J Biol Chem*. 2000; 275(31):24215–24221. [PubMed: 10816577]
55. Maeshima M. Vacuolar H⁺-pyrophosphatase. *Biochim Biophys Acta*. 2000; 1465(1–2):37–51. [PubMed: 10748246]
56. Saliba KJ, Allen RJ, Zissis S, Bray PG, Ward SA, et al. Acidification of the malaria parasite's digestive vacuole by a H⁺-ATPase and a H⁺-pyrophosphatase. *J Biol Chem*. 2003; 278(8):5605–5612. [PubMed: 12427765]
57. Ulrich PN, Jimenez V, Park M, Martins VP, Atwood J 3rd, et al. Identification of contractile vacuole proteins in *Trypanosoma cruzi*. *PLoS One*. 2011; 6(3):e18013. [PubMed: 21437209]

58. Marchesini N, Ruiz FA, Vieira M, Docampo R. Acidocalcisomes are functionally linked to the contractile vacuole of *Dictyostelium discoideum*. *J Biol Chem*. 2002; 277(10):8146–8153. [PubMed: 11748243]
59. Miranda K, Pace DA, Cintron R, Rodrigues JC, Fang J, et al. Characterization of a novel organelle in *Toxoplasma gondii* with similar composition and function to the plant vacuole. *Mol Microbiol*. 2010; 76(6):1358–1375. [PubMed: 20398214]
60. Liu J, Pace D, Dou Z, King TP, Guidot D, et al. A vacuolar-H⁺-pyrophosphatase (TgVP1) is required for microneme secretion, host cell invasion, and extracellular survival of *Toxoplasma gondii*. *Mol Microbiol*. 2014; 93(4):698–712. [PubMed: 24975633]
61. Huang G, Bartlett PJ, Thomas AP, Moreno SN, Docampo R. Acidocalcisomes of *Trypanosoma brucei* have an inositol 1,4,5-trisphosphate receptor that is required for growth and infectivity. *Proc Natl Acad Sci U S A*. 2013; 110(5):1887–1892. [PubMed: 23319604]
62. Ferella M, Nilsson D, Darban H, Rodrigues C, Bontempi EJ, et al. Proteomics in *Trypanosoma cruzi*—localization of novel proteins to various organelles. *Proteomics*. 2008; 8(13):2735–2749. [PubMed: 18546153]
63. Tomitori H, Kashiwagi K, Asakawa T, Kakinuma Y, Michael AJ, et al. Multiple polyamine transport systems on the vacuolar membrane in yeast. *Biochem J*. 2001; 353(Pt 3):681–688. [PubMed: 11171066]
64. Madeira Da Silva L, Beverley SM. Expansion of the target of rapamycin (TOR) kinase family and function in *Leishmania* shows that TOR3 is required for acidocalcisome biogenesis and animal infectivity. *Proc Natl Acad Sci U S A*. 2010; 107(26):11965–11970. [PubMed: 20551225]
65. De Jesus TC, Tonelli RR, Nardelli SC, Da Silva Augusto L, Motta MC, et al. Target of rapamycin (TOR)-like 1 kinase is involved in the control of polyphosphate levels and acidocalcisome maintenance in *Trypanosoma brucei*. *J Biol Chem*. 2010; 285(31):24131–24140. [PubMed: 20495004]
66. Niyogi S, Jimenez V, Girard-Dias W, De Souza W, Miranda K, et al. Rab32 is essential for maintaining functional acidocalcisomes and for growth and infectivity of *Trypanosoma cruzi*. *J Cell Sci*. 2015; 128:2363–2373. [PubMed: 25964650]
67. 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(37):38673–38682. [PubMed: 15252016]
68. Docampo R, Jimenez V, Lander N, Li ZH, Niyogi S. New insights into roles of acidocalcisomes and contractile vacuole complex in osmoregulation in protists. *Int Rev Cell Mol Biol*. 2013; 305:69–113. [PubMed: 23890380]
69. Li ZH, Alvarez VE, De Gaudenzi JG, Sant'anna C, Frasch AC, et al. Hyperosmotic stress induces aquaporin-dependent cell shrinkage, polyphosphate synthesis, amino acid accumulation, and global gene expression changes in *Trypanosoma cruzi*. *J Biol Chem*. 2011; 286(51):43959–43971. [PubMed: 22039054]
70. Galizzi M, Bustamante JM, Fang J, Miranda K, Soares Medeiros LC, et al. Evidence for the role of vacuolar soluble pyrophosphatase and inorganic polyphosphate in *Trypanosoma cruzi* persistence. *Mol Microbiol*. 2013; 90(4):699–715. [PubMed: 24033456]
71. Li FJ, He CY. Acidocalcisome is required for autophagy in *Trypanosoma brucei*. *Autophagy*. 2014; 10(11):1978–1988. [PubMed: 25484093]
72. Miranda K, Benchimol M, Docampo R, De Souza W. The fine structure of acidocalcisomes in *Trypanosoma cruzi*. *Parasitol Res*. 2000; 86(5):373–384. [PubMed: 10836511]
73. Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca²⁺ close to IP₃-sensitive channels that are sensed by neighboring mitochondria. *Science*. 1993; 262(5134):744–747. [PubMed: 8235595]
74. Xiong ZH, Ridgley EL, Enis D, Olness F, Ruben L. Selective transfer of calcium from an acidic compartment to the mitochondrion of *Trypanosoma brucei*. Measurements with targeted aequorins. *J Biol Chem*. 1997; 272(49):31022–31028. [PubMed: 9388251]
75. Huang G, Vercesi AE, Docampo R. Essential regulation of cell bioenergetics in *Trypanosoma brucei* by the mitochondrial calcium uniporter. *Nat Commun*. 2013; 4:2865. [PubMed: 24305511]

76. Baker N, Hamilton G, Wilkes JM, Hutchinson S, Barrett MP, et al. Vacuolar ATPase depletion affects mitochondrial ATPase function, kinetoplast dependency, and drug sensitivity in trypanosomes. *Proc Natl Acad Sci U S A*. 2015; 112(29):9112–9117. [PubMed: 26150481]
77. Lahiri S, Chao JT, Tavassoli S, Wong AK, Choudhary V, et al. A conserved endoplasmic reticulum membrane protein complex (EMC) facilitates phospholipid transfer from the ER to mitochondria. *PLoS Biol*. 2014; 12(10):e1001969. [PubMed: 25313861]
78. Vercesi AE, Docampo R, Moreno SN. Energization-dependent Ca^{2+} accumulation in *Trypanosoma brucei* bloodstream and procyclic trypomastigotes mitochondria. *Mol Biochem Parasitol*. 1992; 56(2):251–257. [PubMed: 1484549]
79. Schoijet AC, Miranda K, Medeiros LC, De Souza W, Flawia MM, et al. Defining the role of a FYVE domain in the localization and activity of a cAMP phosphodiesterase implicated in osmoregulation in *Trypanosoma cruzi*. *Mol Microbiol*. 2011; 79(1):50–62. [PubMed: 21166893]
80. King-Keller S, Li M, Smith A, Zheng S, Kaur G, et al. Chemical validation of phosphodiesterase C as a chemotherapeutic target in *Trypanosoma cruzi*, the etiological agent of Chagas' disease. *Antimicrob Agents Chemother*. 2010; 54(9):3738–3745. [PubMed: 20625148]
81. Schoijet AC, Miranda K, Girard-Dias W, De Souza W, Flawia MM, et al. A *Trypanosoma cruzi* phosphatidylinositol 3-kinase (TcVps34) is involved in osmoregulation and receptormediated endocytosis. *J Biol Chem*. 2008; 283(46):31541–31550. [PubMed: 18801733]
82. Jimenez V, Docampo R. TcPho91 is a contractile vacuole phosphate sodium symporter that regulates phosphate and polyphosphate metabolism in *Trypanosoma cruzi*. *Mol Microbiol*. 2015

Highlights

- Acidocalcisomes are acidic calcium stores that have been found from bacteria to human cells
- They are rich in polyphosphate complexed with organic and inorganic cations
- Their membranes possess a number of transporters, pumps and channels that maintain their acidity and content
- Their components have roles in osmoregulation, parasites persistence, autophagy, blood coagulation and inflammation
- They interact with other organelles such as the contractile vacuole and mitochondria

Figure 1. Schematic representation of the acidocalcisome of procyclic stages of *T. brucei*
 Ca^{2+} is taken up by a H^+ -countertransporting Ca^{2+} -ATPase (TbPMC1) and released by the inositol 1,4,5,trisphosphate receptor (TbIP3R). H^+ are pumped in electrogenically by either the vacuolar H^+ -PPase (TbVP1) or the multisubunit vacuolar H^+ -ATPase (TbVATPase). A $\text{Na}^{2+}/\text{H}^+$ exchanger is used for Na^+ uptake in exchange for H^+ . A vacuolar iron transporter (TbVIT1) can be used for either Mn^{2+} or Fe^{2+} uptake and a Zn^{2+} transporter (TbZnT) for Zn^{2+} uptake. There is also a polyamine transporter (TbPOT1). A vacuolar transporter chaperone complex (VTC) with at least two subunits (TbVtc1 and TbVtc4) synthesizes polyP using ATP and translocates it into the organelle. A Na^+/P_i symporter (TbPho91) releases Na^+ and P_i from acidocalcisomes. Within acidocalcisomes there is a vacuolar soluble PPase (TbVSP1), an exopolyphosphatase (TbPPX) and an acid phosphatase (TbAP). Modified from reference 52, with permission.

Figure 2. Close apposition of an acidocalcisome (Ac) with the contractile vacuole complex (CVC), indicating a fusion event

(A–C) virtual section (1 nm thickness) sequence of a tomogram showing the anterior region of the parasite. The contractile vacuole complex is represented by the central vacuole or bladder (CV) and the spongiome (Sp). Acidocalcisomes (Ac) in the neighboring region are observed in close contact with the CVC. In the left lower corner, the section number is shown. In C it is possible to observe a close apposition between acidocalcisome and CVC membranes, which are suggestive of a fusion event (*arrow*) between the two organelles. (D–F) 3D models of the contractile vacuole complex (*blue*) and its close contact with an acidocalcisome (*orange*). (F) tilted view of the 3D model at 45° around the X axis. Spongiome (Sp) and flagellum (F) are shown. Scale bar = 200 nm. From reference 66, with permission.

Figure 3. Scheme of the potential contact between acidocalcisomes and mitochondrion in trypanosomes

The scheme depicts the molecules mediating Ca^{2+} influx (MCU), Ca^{2+} sensing at the intermembrane space (MICU1–MICU2) and Ca^{2+} efflux (Ca^{2+} /H⁺ exchanger, CAX) across the inner mitochondrial membrane (IMM) at an area of acidocalcisome (ACCSOME)-mitochondrial association. PMCA, plasma membrane-type Ca^{2+} -ATPase; OMM, outer mitochondrial membrane; VDAC, voltage-dependent anion-selective channel; IP₃R, inositol 1,4,5-trisphosphate receptor.

Table 1

Some of the organelles described in prokaryotes

Name	Membrane	Content	Present in	Evidence of membrane	Reference
Acidocalcisome	Lipid bilayer, proteins	PolyP, cations	<i>A. tumefaciens R. rubrum</i>	Biochemical, EM	[6, 7]
Magnetosome	Lipid bilayer, proteins	Magnetite (Fe ₃ O ₄) Greigite (Fe ₃ O ₄)	Magnetotactic bacteria (Gram-negative)	Biochemical, EM	[21]
Photosynthetic Membranes (thylakoids, chlorosomes, chromatophore membranes)	Lipid mono or bilayer, proteins	Photosynthetic reactions	Purple photosynthetic bacteria Green photosynthetic bacteria	Biochemical, EM	[22]
Anammoxosome	Ladderan lipids, proteins	Anaerobic ammonium oxidation pathway	Planctomycetes	Biochemical, EM	[24]
Polyhydroxyalkanoate granules	Lipid, proteins (phasins)	Hydroxyalkanoates, Mercaptoalkanoates,	Many bacteria	Biochemical, EM	[25]
Pyrellulosome	Lipid bilayer	DNA (nucleoid)	Planctomycetes	EM	[23]
Lipid droplet	Lipid monolayer	Lipids	Most bacteria	Biochemical, EM	[15]
Carboxysome	Protein	RuBisCO, CA	Actinobacteria, Cyanobacteria, Proteobacteria	Genetic, EM	[26]
Pdu compartment	Protein	Propanediol utilization	Actinobacteria, Firmicutes, Fusobacteria, Proteobacteria Spirochaetes, Synergistetes	Genetic, EM	[26]
Etu microcompartment	Protein	Ethanolamine utilization	Firmicutes	Genomic, EM	[26]