ACIDOCALCISOMES — CONSERVED FROM BACTERIA TO MAN

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Abstract | Recent work has shown that acidocalcisomes, which are electron-dense acidic organelles rich in calcium and polyphosphate, are the only organelles that have been conserved during evolution from prokaryotes to eukaryotes. Acidocalcisomes were first described in trypanosomatids and have been characterized in most detail in these species. Acidocalcisomes have been linked with several functions, including storage of cations and phosphorus, polyphosphate metabolism, calcium homeostasis, maintenance of intracellular pH homeostasis and osmoregulation. Here, we review acidocalcisome ultrastructure, composition and function in different trypanosomatids and other organisms.

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Infection with trypanosomatid parasites, including African and American trypanosome species and parasites from the *Leishmania* and *Phytomonas* genera, are among the most widespread human, animal and plant parasitic diseases worldwide and are responsible for large socio-economic losses, especially in developing countries. *Trypanosoma cruzi* is the aetiological agent of Chagas disease or American trypanosomiasis[1]. At least 20 species of *Leishmania* are known to infect humans, causing cutaneous, mucocutaneous and visceral leishmaniasis[2]. African sleeping sickness is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*[3]. Most of these trypanosomatid species also infect animals, and in some areas, such as in sub-Saharan Africa, such infection has precluded using some domestic animals to provide food[3]. Plant trypanosomatids — known as *Phytomonas* spp. — can also result in devastating diseases, including phloem necrosis in many coffee plant species, heart rot in coconut plants and sudden wilt in oil palm[4].

Trypanosomatids belong to the family Trypanosomatidae and are of the order Kinetoplastida, and are characterized by their different cell morphologies during stages of the life cycle, the most important of which are shown in FIG. 1a. Trypanosomatids contain organelles that are typical of most eukaryotic organisms, including mitochondria, peroxisomes, lysosomes and the endoplasmic reticulum, and have well-developed endocytic and secretory pathways. Some of the trypanosomatid organelles have features that are unique to this group (FIG. 1b; BOX 1). Trypanosomatids were also the first cells in which acidocalcisomes were identified[5,6]. Acidocalcisomes are dense acidic organelles — both in terms of weight and as shown by electron microscopy — with a high concentration of phosphorus present as pyrophosphate and polyphosphate complexed with calcium and other elements[7]. Acidocalcisomes are related to organelles that were previously known as volutin or metachromatic granules[8] and polyphosphate vacuoles[9], and which were thought to contain nucleic acids and/or to function as storage granules[10] (TIMELINE). The discovery that trypanosomatid acidocalcisome membranes contain several pumps and exchangers suggested a metabolic function. After their identification in trypanosomatids, acidocalcisomes were found in several microorganisms such as *Toxoplasma gondii*[11], which is the aetiological agent of toxoplasmosis, *Plasmodium* spp.[12–14], which are the causative agents of malaria, the green alga *Chlamydomonas reinhardtii*[15] and the slime mould *Dictyostelium discoideum*[16]. The recent identification of acidocalcisomes in bacteria[17,18] and human platelets[19] indicates that these organelles have been conserved from bacteria to humans.

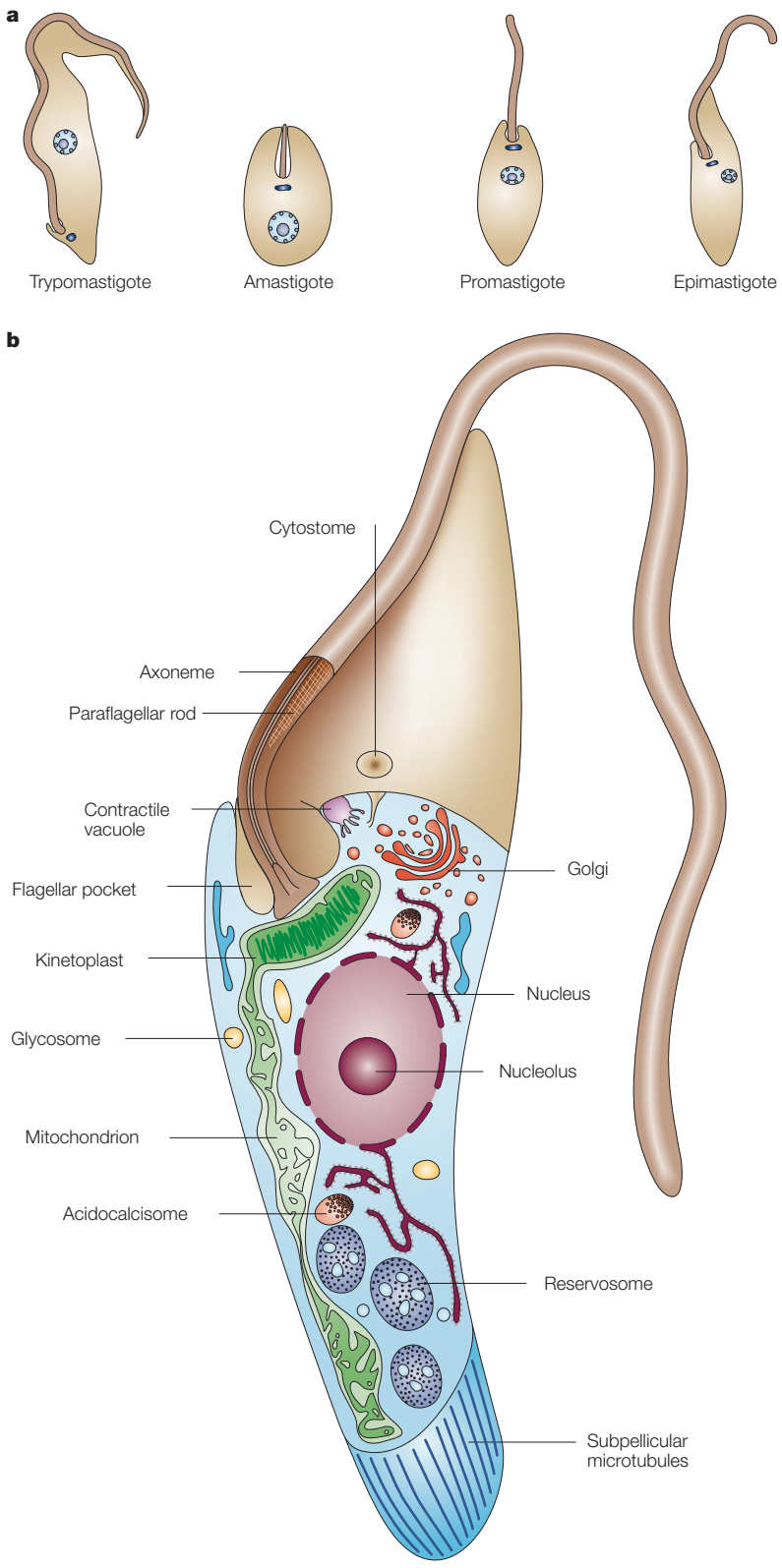


Figure 1 | **Schematic representation of trypanosomatids. a** | The main cellular forms of trypanosomatids as defined by cell shape, flagellum presence and attachment, and position of the basal body, kinetoplast and nucleus. In general, the epimastigote and promastigote forms of digenetic trypanosomatids are found in the vector, the trypomastigote form is found in the mammalian host and the amastigote form is intracellular. **b** | Schematic representation of longitudinal section of an epimastigote form of *T. cruzi*. Part **b** is modified from a drawing by Flavia Moreira-Leite, University of Oxford.

Acidocalcisome structure

Acidocalcisomes are rounded organelles with an 8-nm-thick membrane. They can be stained with dyes such as acridine orange[5,6] or cycloprodigiosin[20] that accumulate in acidic compartments and they can be observed as cytoplasmic granules in Giemsa-stained preparations[21].

The organelle contains an amorphous and electron-dense material (FIG. 2), but the amount seen is dependent on the method by which the sample is prepared for electron microscopy. Using standard staining methods for transmission electron microscopy, part of the dense material can be lost, leaving either an empty vacuole (FIG. 2e) or a thin layer of dense material that sticks to the inner face of the membrane. In some trypanosomatids[7,21] and bacteria[17], the dense material adheres to one side of the membrane as an inclusion (FIG. 2a). In some *Phytomonas* species, the electron-dense material seems to be arranged in a concentric pattern[22] (FIG. 2d). Electron-dense material is also seen to precipitate in cells that are fixed using potassium pyroantimoniate[23] or potassium fluoride (K.M. *et al.*, unpublished observations), both of which are known to precipitate calcium. Acidocalcisomes are best preserved in cells that are fixed using cryotechniques, such as physical fixation by high-pressure freezing followed by freeze substitution, where they seem completely filled with an electron-dense material[24] (FIG. 2b), or when frozen sections are directly observed at low temperature in the electron microscope[25] (FIG. 2c). Another useful method to observe acidocalcisomes is to allow whole cells to dry onto carbon- or formvar-coated grids in the transmission electron microscope[25], especially if it is equipped with an energy filter, so that electron spectroscopic images (contrast-tuned images) can be obtained[22,24,26,27] (FIG. 3a–d).

The general morphology of acidocalcisomes varies according to the species and the cultivation medium. Generally, the organelles are spherical structures with an average diameter of ~0.2 μm in *T. cruzi*[20,24] (FIG. 3c), *T. brucei*[28], *C. reinhardtii*[15], *D. discoideum*[16], bacteria[17,18] and human platelets[19], but they can be 0.6 μm in some *Leishmania* spp.[26] or 0.05 μm in merozoites of *Plasmodium falciparum*[29]. In some organisms, such as in some *Leishmania*[26] and *Phytomonas* isolates[22,27], acidocalcisomes are elongated and polymorphic. In trypanosomatids, the organelles are usually distributed throughout the cells, but seem to preferentially localize to the central portion of the cell body or in close proximity to the contractile vacuole[30]. They can also localize to the flagellum, only occasionally in epimastigotes of *T. cruzi*[24] but frequently in promastigotes of *Blastocrithidia culicis*[27] (FIG. 3a). In trypomastigotes of *T. cruzi,* they are preferentially localized to the anterior portion (the region of the parasite from which the flagellum emerges)[24]. In other cells[15–19], they are usually randomly distributed, although in some bacteria they can be close to one pole[17]. In addition, acidocalcisomes are sometimes aligned (FIG. 3c,d), which might indicate an interaction with cytoskeletal components. In some electron microscopy images apparent budding of new acidocalcisomes can be observed (FIG. 3c,d).

Box 1 | **Trypanosomatid model systems**

Although typanosomatids contain organelles and metabolic pathways that seem to be absent from prokaryotes and other eukaryotes, some of the organelles and metabolic routes first discovered in trypanosomatids were later found in other organisms. For this reason, and owing to the global burden of trypanosomatid diseases, these organisms are now used as model systems in cell biology.

GPI-linked surface molecules

The main cell surface molecules of trypanosomatids are rich in glycosylphosphatidylinositol (GPI)-anchored glycoproteins and GPI-related glycolipids. Early composition and structural studies on *Trypanosoma brucei* variant surface glycoprotein[93], which is responsible for antigenic variation in these parasites, were important for understanding this new type of membrane attachment molecule[94].

Cytoskeleton and cellular organization

The cytoskeleton of the uniflagellated trypanosomatids, which determines their cell shape, has a subpellicular array of microtubules that are crosslinked to each other and to the plasma membrane[95]. The organization of the helical pattern of the microtubules after cell division under the influence of a pre-existing cytoskeletal structure (by the flagellar connector) is one of the few examples of cytotaxis[96]. In addition to a conventional AXONEME, the flagellum of trypanosomatids has an associated structure known as the paraflagellar rod. It is formed by a complex array of filaments and is involved in flagellar motility[21].

Trypanosomatids are highly polarized cells. All their endocytic and exocytic functions occur through the flagellar pocket and, in some cases, the CYTOSTOME[21].

DNA and RNA biology

The single trypanosomatid mitochondrion contains a kinetoplast, which is a specific structure that is found adjacent to the basal body of the flagellum and contains approximately 5–20% of the total cellular DNA. The DNA-rich kinetoplast can be stained and viewed using light microscopy, and was the first extranuclear DNA to be discovered, long before mitochondria were shown to contain DNA[97]. Kinetoplast DNA is a large network of several thousand similar copies of minicircles and a few dozen copies of maxicircles[98]. The maxicircle DNA encodes ribosomal RNAs and a few mitochondrial proteins, in common with the mitochondrial DNA of other eukaryotes. Many maxicircle transcripts undergo RNA editing, a process first discovered in trypanosomatids[99], whereas the minicircles encode for small guide RNAs that control the specificity of editing[100].

Many trypanosome mRNAs are trans-spliced — the transfer of splice leader sequences or mini-exons to the polycystronic mRNAs[101]. This process, together with polyadenylation, functions to cleave polycistronic transcripts and attach a cap to mRNAs, and has subsequently been found in nematodes, euglenoids, trematodes and chordates[102].

Metabolic functions

In all trypanosomatids most glycolytic enzymes are found in specialized peroxisomes known as glycosomes[103], which contain typical peroxisomal enzymes and develop by a biogenesis pathway similar to peroxisomes. The compartmentalization of the glycolytic pathway in these organelles is important for glycolysis regulation and is unique to trypanosomatids[104].

One unique metabolic feature of trypanosomatids is their substitution of trypanothione (a glutathione–spermidine conjugate)[105] for glutathione in many reactions involved in protection against oxidative stress, such as trypanothione reductase and trypanothione-dependent peroxidase activities[106].

Several trypanosomatids contain a contractile vacuole that functions in water extrusion and osmoregulation[74,75]. Although the vacuole is important for free-living kinetoplastids like *Bodo* spp.[76], it is also relevant for parasites such as *Trypanosoma cruzi*[30,66], which are exposed to wide variations in osmolarity during their life cycle.

Close contact between the acidocalcisome and the nucleus, lipid inclusions, mitochondria and subpellicular microtubules has also been observed[24].

The number of acidocalcisomes varies from species to species and even among the developmental stages of the same species. For instance, in *T. cruzi*, amastigote forms (FIG. 3c) contain more acidocalcisomes (about 40 distributed throughout the cell) than epimastigotes and trypomastigotes[24]. A MORPHOMETRIC STUDY in different trypanosomatids showed that, although the numbers and sizes of acidocalcisomes vary, the volume of the cell that is occupied by acidocalcisomes remains ~2%. The size of the organelle seems to be inversely proportional to the number of organelles present — acidocalcisomes are often large when present in low numbers and small when there are many present[27].

AXONEME
A cytoskeletal structure of microtubules that forms flagella and cilia.

CYTOSTOME
An invagination of the plasma membrane that is used to incorporate external material.

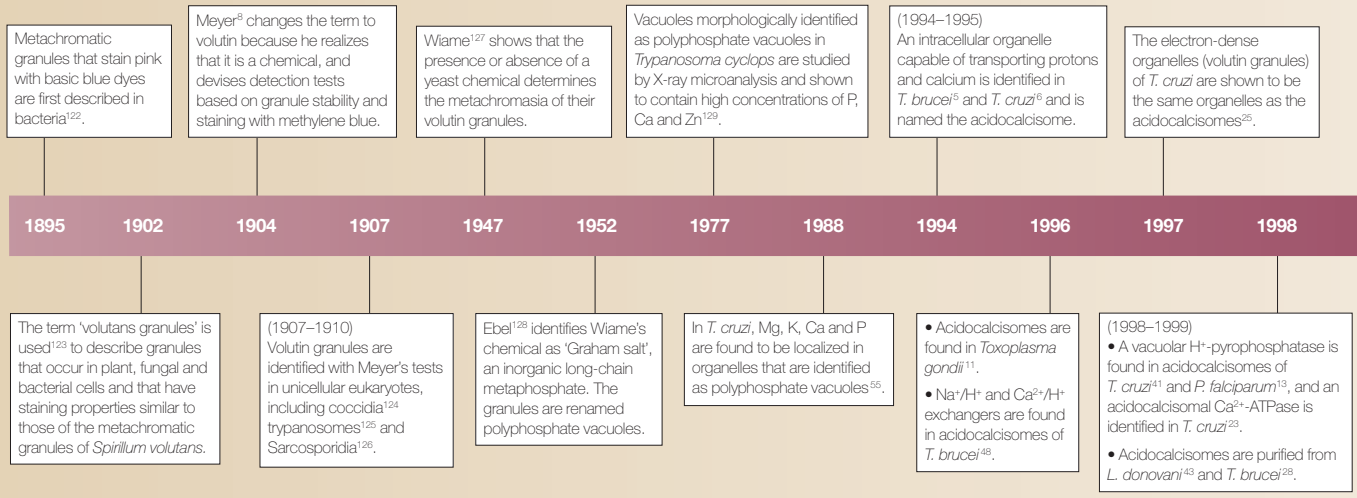
MORPHOMETRIC STUDY
The diameter of acidocalcisomes in electron microscopy sections is measured and their volume is calculated assuming that they are perfect spheres.

Acidocalcisome membrane

Several pumps and exchangers and at least one channel have been identified in the acidocalcisome membrane (FIG. 4).

Calcium pumps. A Ca^{2+}-ATPase that is sensitive to vanadate and present in an acidic compartment was first identified in experiments using permeabilized *T. brucei*[5] and *T. cruzi*[6] cells and later detected in isolated acidocalcisomes from both parasite species[20,28]. Genes encoding acidocalcisomal Ca^{2+}-ATPases were identified in *T. cruzi* (*tca1*)[23], *T. brucei* (*TbPMC1*)[31], *T. gondii*[32] and *D. discoideum*[16,33]. The *T. cruzi*, *T. brucei*, and *T. gondii* genes were able to complement yeast mutants that were deficient in the vacuolar Ca^{2+}-ATPase gene *PMC1*, providing evidence of their functionality.

Timeline | **Major developments in the history of acidocalcisome research**



These Ca^{2+}-ATPases are closely related to the family of plasma membrane calcium ATPases (PMCA) and localize to acidocalcisomes. Interestingly, *T. brucei* has a second PMCA-type Ca^{2+}-ATPase (TbPMC2) that localizes to the plasma membrane[31], whereas the *T. cruzi* PMCA-type Ca^{2+}-ATPase is present in both acidocalcisomes and plasma membranes[23]. The acidocalcisomal Ca^{2+}-ATPases of *T. cruzi*[23], *T. brucei*[31], *T. gondii*[32] and *D. discoideum*[16,33], and the vacuolar Ca^{2+}-ATPases of yeast[34] and *Entamoeba histolytica*[35] form a subcluster among the conserved core sequences of all PMCA-type Ca^{2+}-ATPases. A common feature of these pumps is the lack of a calmodulin-binding domain, which is found in other PMCA-type Ca^{2+}-ATPases.

Proton pumps. Two proton pumps — a vacuolar-type H^+-ATPase (V-H^+-ATPase) and a vacuolar-type H^+-pyrophosphatase (V-H^+-PPase) — have been found in acidocalcisomes from different microorganisms.

The V-H^+-ATPase was identified in permeabilized *T. brucei*[5] and *T. cruzi*[6] cells owing to its sensitivity to bafilomycin A_1, which is a specific inhibitor of this proton pump[36], and this finding was later confirmed in experiments using intact cells of *T. cruzi*[6], *T. brucei*[37], *Leishmania amazonensis*[38] and *T. evansi*[39]. Acidocalcisome V-H^+-ATPases were also found in *T. gondii*[40], *C. reinhardtii*[15], *D. discoideum*[16] and human platelets[19]. The V-H^+-ATPase co-localizes in acidocalcisomes with the vacuolar-type Ca^{2+}-ATPase in *T. cruzi*[23] but is absent from the endocytic pathway of these parasites[40], which is in contrast to its presence in this pathway in mammalian cells.

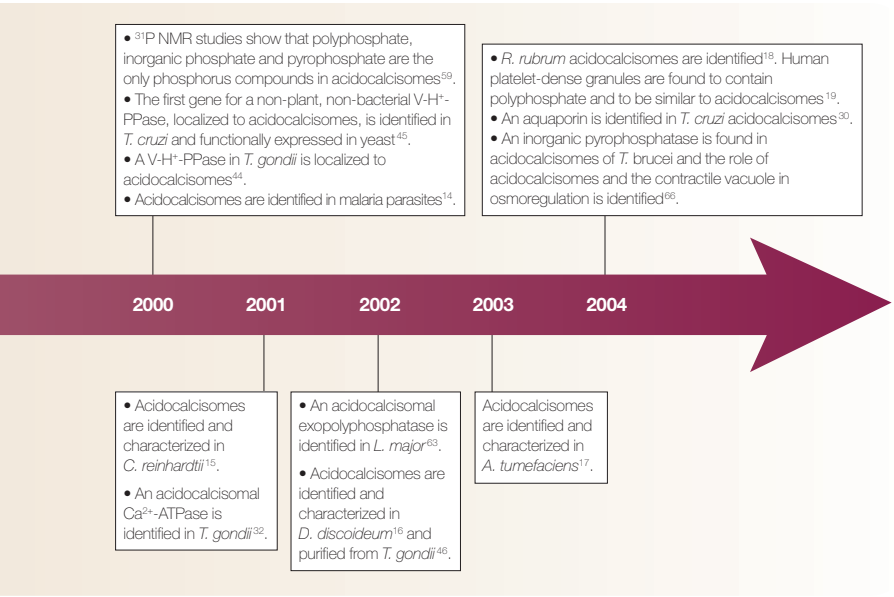
A V-H^+-PPase has been detected in *T. cruzi*[41], *T. brucei*[28,42], *Leishmania donovani*[43], *L. amazonensis*[26], *Phytomonas françai*[22], *T. gondii*[44], *P. falciparum*[13], *Plasmodium berghei*[14], *C. reinhardtii*[15] and *D. discoideum*[16]. This enzyme also localizes in acidocalcisomes in all these species (FIG. 2e). The V-H^+-PPase from *T. cruzi* functions in yeast[45]. The acidocalcisomal V-H^+-PPases are K^+-stimulated (type I) and can be used as markers for acidocalcisome purification[15,16,28,41–43,46]. Although they are not restricted to the acidocalcisome, they are concentrated in this organelle. The *T. cruzi* V-H^+-PPase is also found in the Golgi complex and in the plasma membrane[47].

Na^+/H^+ and Ca^{2+}/H^+ exchangers. A Na^+/H^+ exchanger has been found in *T. brucei* procyclic forms[48,49] and in *L. donovani* promastigotes[50]. This exchanger is sensitive to 3,5-dibutyl-4-hydroxy toluene (BHT) but tolerant to 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA), in contrast to other Na^+/H^+ exchangers. A Ca^{2+}/H^+ exchanger is thought to be involved in Ca^{2+} release when Na^+ is added to the organelles *in situ*[48–50] or *in vitro*[28], and it might function in the release of Ca^{2+} from the organelles because second messengers, such as inositol trisphosphate ($InsP_3$), did not release Ca^{2+} from intracellular Ca^{2+} stores of these organisms[51–53]. Although the isolated organelles from *T. brucei* procyclic trypomastigotes[28] and *L. donovani* promastigotes[43] have a Na^+/H^+ exchanger, this exchanger is absent from acidocalcisomes of *T. cruzi*[20]. They have different properties because ADP stimulates the *T. brucei*[49] exchanger but not the *L. donovani* exchanger[50].

Aquaporins. A water channel, or aquaporin, has been found in *T. cruzi* acidocalcisomes[30]. This protein can function as a water channel in *Xenopus* oocytes but is unable to transport glycerol. The *T. cruzi* aquaporin is localized to both the contractile vacuole complex and the acidocalcisome, which might indicate a role in osmoregulation[30].

Acidocalcisome matrix

The matrix of the acidocalcisome is electron-dense and, in common with volutin granules, this was thought to be due to the high concentrations of phosphorus compounds inside this compact structure. Incubating fixed



T. cruzi[54] or *T. evansi*[39] cells with a pyrophosphatase removed the electron-dense matrix, which indicates that inorganic pyrophosphate (PP_i) is an important component of the structure of this organelle. The matrix has been studied using electron microscopy, ^{31}P NMR and biochemical analyses.

The following elements are concentrated in the acidocalcisome: oxygen, magnesium, phosphorus and calcium[15–18,24,25,29,32,55–57]. Zinc has been found in acidocalcisomes from the trypanosomatids[24,25,55–57], *T. gondii*[32] and *C. reinhardtii*[15], whereas sodium and potassium are also frequently detected in trypanosomatids (TABLE 1). Iron has been found in acidocalcisomes of *T. cruzi* that were isolated from the bloodstream[57], and in *P. francai*[22], in *L. amazonensis*[26] and in several trypanosomatids that have been cultivated in complex medium[27].

The structure and composition of acidocalcisomes varies with cultivation conditions. For example, promastigotes of *L. amazonensis* have spherical iron-free acidocalcisomes if grown in a semi-defined medium but contain polymorphic and iron-rich organelles when grown using iron-rich complex medium[26]. However, when different trypanosomatids are cultivated under similar conditions, they can contain different elemental compositions in their acidocalcisomes[27], indicating that the elemental composition does not depend exclusively on the cultivation conditions but also on species-specific characteristics. Therefore, it seems that in some species the acidocalcisomes operate mostly as a storage compartment for other elements (for example, zinc) rather than calcium[27]. A remarkable property of the acidocalcisomes is the low variation in the concentration of the elements within organelles of the same cells, independent of their location.

All acidocalcisomes that have been described so far contain high concentrations of phosphorus in the form of inorganic pyrophosphate and polyphosphate (polyP) (TABLE 2). Trypanosomatids[54,59] and *T. gondii*[46] are especially rich in short-chain polyphosphates such as $polyP_3$, $polyP_4$ and $polyP_5$. ^{31}P NMR spectra of purified acidocalcisomes of *T. cruzi*, *T. brucei* and *Leishmania major* indicate that the polyphosphate has an average chain length of 3.2 phosphates[59]. Based on the total concentration of polyphosphates in different stages of *T. cruzi*[60] and the relative volumes of the acidocalcisomes at each stage of the life cycle (0.86%, 2.3% and 0.26% of the total cell volume of epimastigotes, amastigotes and trypomastigotes, respectively[24]), and assuming that these compounds are mainly concentrated in acidocalcisomes, the concentration in the organelles can be calculated as 3–8 M. This is consistent with the detection of solid-state condensed phosphates by magic-angle spinning NMR techniques and with the very high electron density of acidocalcisomes *in situ*[61]. Other components of these organelles, such as carbohydrates[25] or lipids, could be involved in maintaining this physical configuration.

Acidocalcisomes also contain high concentrations of free amino acids — 1,250 ± 297 nmol per mg protein were found in epimastigotes of *T. cruzi*. The basic amino acids arginine and lysine account for almost 90% of the amino acid pool of the acidocalcisomes, whereas whole-cell extracts contain high concentrations of neutral and acidic amino acids[62].

The low sulphur content detected by elemental analysis (TABLE 1) indicates that few proteins are present in acidocalcisomes, but a few acidocalcisome enzymes have been detected: polyphosphate kinase has been detected in *T. cruzi*[60], an acidocalcisome exopolyphosphatase has been detected in *L. major*[63] and a soluble inorganic pyrophosphatase has been identified in *T. brucei*[64].

Acidocalcisome functions

Storage function. Acidocalcisomes in several microorganisms are the main storage compartment for calcium, magnesium, sodium, potassium, zinc, iron, phosphorus compounds (inorganic pyrophosphate and polyphosphate) and basic amino acids. Most of these compounds are present at millimolar or molar concentrations. Ca^{2+} ions, and possibly other cations, are imported by the Ca^{2+}/H^+ countertransporting ATPase and can be released from acidocalcisomes when alkalinizing agents, such as the ionophores monensin and nigericin or NH_4Cl, are applied to intact cells[6,11,12,14,37,38,43,60,65] or isolated acidocalcisomes[20,28]. In *T. brucei*, monensin-induced Ca^{2+} that is released from acidocalcisomes is rapidly taken up by the mitochondria[65]. Hypoosmotic stress or alkalinizing agents are also able to produce hydrolysis of short- and long-chain polyphosphate[60], but the resulting inorganic phosphate (P_i) is not released to the cytosol or to the extracellular medium, which results in swelling of acidocalcisomes[66]. By removing water from the cytosol, this process helps the cells to recover their volume[66].

Although inorganic pyrophosphate is a byproduct of biosynthetic reactions (for example, synthesis of nucleic acids, coenzymes and proteins, activation of fatty acids and isoprenoid synthesis) in which hydrolysis by inorganic pyrophosphatases makes these reactions thermodynamically favourable, none of these pathways have been found in acidocalcisomes. Why is inorganic

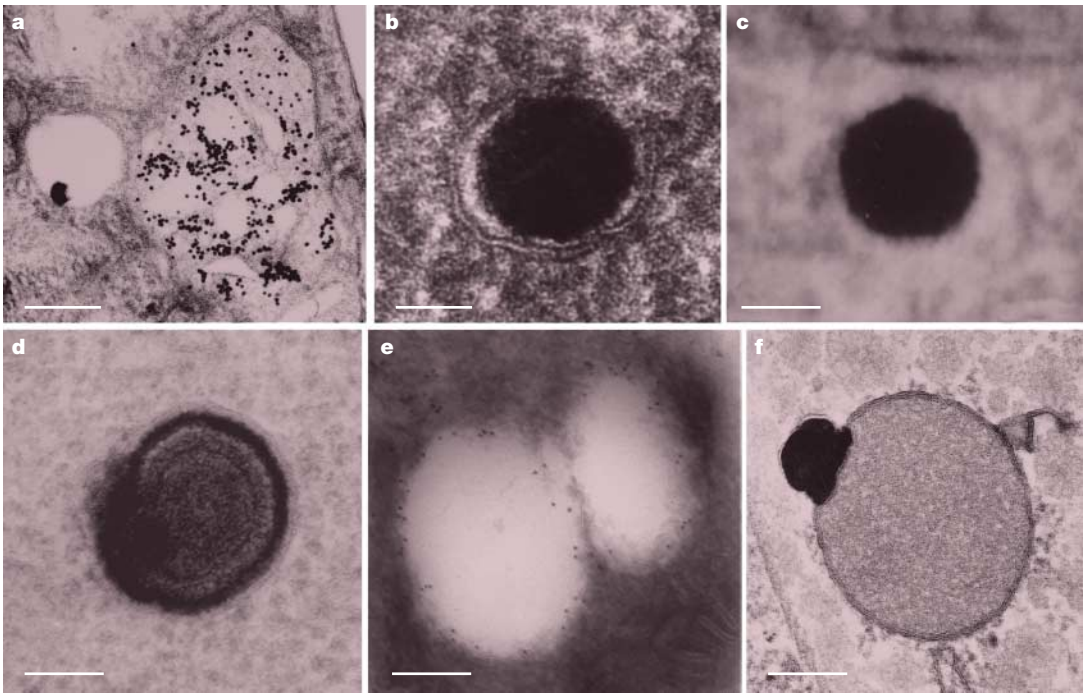


Figure 2 | **Thin sections of acidocalcisomes of trypanosomatid parasites prepared by different transmission electron microscopy methods and of hydrogenosomes of *Tritrichomonas foetus*.** Acidocalcisomes of epimastigote forms of *Trypanosoma cruzi* submitted to chemical fixation followed by conventional embedding in epoxide resin (**a**), cryofixation by high-pressure freezing followed by freeze substitution and epoxide embedding (**b**) and cryofixation by immersion in ethane, cryosection and observation at low temperature (**c**) (parts **a** and **b** are reproduced with permission from REF. 24 © (2000) Springer; part **c** is reproduced with permission from REF. 25 © (1997) American Society for Biochemistry and Molecular Biology). Note that the electron-dense material is better preserved with the use of cryomethods. Part **a** shows an acidocalcisome with an electron-dense inclusion and a vacuole of the endocytic pathway filled with endocytic tracers. Part **b** shows that a membrane surrounds the acidocalcisome. *Phytomonas françai* submitted to routine fixation and embedding in epoxide resin (**d**) (reproduced with permission from REF. 22 © (2004) Cambridge Univ. Press). Note the arrangement of the electron-dense material in concentric patterns in the acidocalcisome. **e** | Cryo-immunoelectron microscopy of *Leishmania amazonensis* using antibodies raised against the V-H$^+$-PPase (reproduced with permission from REF. 26 © (2004) Springer). **f** | *Tritrichomonas foetus* hydrogenosome. Cells were fixed according to a glutaraldehyde-osmium tetroxide-potassium ferrocyanide procedure with 5 mM $CaCl_2$ added to all solutions (reproduced with permission from REF. 130 © (1983) Society of Protozoologists). The electron-dense reaction product is visible in a vesicle-like structure (hydrogenosome vesicle) separated from the rest of the hydrogenosome, which has a double membrane. The scale bars represent 150 nm, 100 nm, 100 nm, 100 nm, 200 nm and 300 nm in parts **a**–**f**, respectively.

pyrophosphate stored in these organelles? Is it a byproduct of the polyphosphate hydrolysis or an intermediate for polyphosphate synthesis? Only three reactions are known to use inorganic pyrophosphate in trypanosomatids — one is catalysed by the pyruvate phosphate dikinase located in the glycosomes[67], another is catalysed by the V-H$^+$-PPase that is responsible for acidification of acidocalcisomes[41,42] and a third is catalysed by an inorganic pyrophosphatase located in the matrix of acidocalcisomes[64]. As inorganic pyrophosphate is charged and polar, it must presumably be transported through the acidocalcisomal membrane by a transporter, in common with the transmembrane transporters that shuttle inorganic pyrophosphate between intracellular and extracellular compartments in mammalian tissues[68]. A similar channel in the acidocalcisomal membrane could transport inorganic pyrophosphate into the acidocalcisome after synthesis in the cytosol or other compartments, or could transport inorganic pyrophosphate out into the cytosol, where it could be a substrate for the V-H$^+$-PPase.

Polyphosphate (BOX 2) accumulates to high concentrations in acidocalcisomes[60]. Storage of phosphate in the form of polyphosphate reduces the osmotic effect of large pools of this crucial nutrient element. A rapid increase in the concentrations of short- and long-chain polyphosphate was detected during *T. cruzi* trypomastigote to amastigote differentiation (within 2–4 hours) and during the lag phase of epimastigote growth (within 12–24 hours)[60]. Concentrations rapidly decreased after epimastigotes resumed growth[60]. The changes observed in the content of polyphosphate in *T. cruzi* epimastigotes when inorganic phosphate is present in the growth medium at high concentrations could indicate a requirement for these compounds as energy sources for resuming growth, whereas the changes observed during differentiation might indicate an adaptation to the intracellular life of amastigotes[60]. The concentrations of both short- and long-chain polyphosphate also rapidly decreased on exposure of epimastigotes to hypoosmotic stress, whereas concentrations increased after hyperosmotic stress[60]. This might indicate a role for storage of inorganic phosphate in the acidocalcisomes in the adaptation of the parasites to environmental stress.

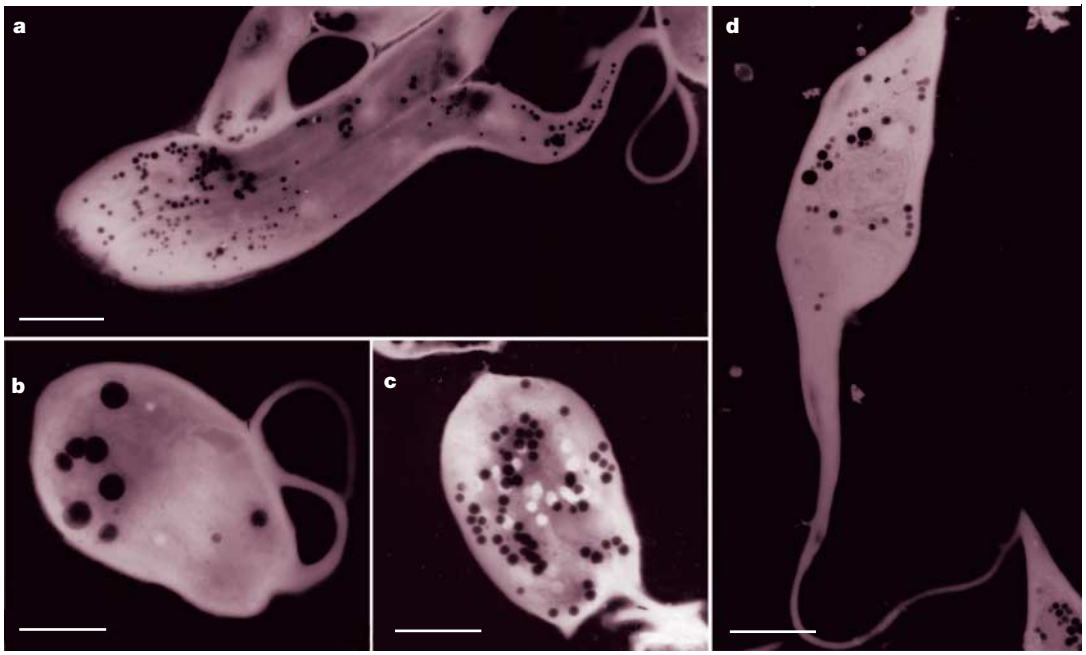


Figure 3 | **Morphology of acidocalcisomes in whole trypanosomatids.** Electron spectroscopic imaging (contrast tuning) of whole cells adhered to formvar-coated grids showing the shape, size and distribution of the acidocalcisomes (black spots) in different developmental forms of trypanosomatid species. **a** | Promastigote of *Blastocrithidia culicis* (scale bar 2 μm). Reproduced with permission from REF. 27 © (2000) Elsevier. **b** | Choanomastigote of *Crithidia deanei* (scale bar 1 μm). **c** | Amastigote of *Trypanosoma cruzi* (scale bar 1.5 μm). Reproduced with permission from REF. 24 © (2000) Springer. **d** | Epimastigote of *T. cruzi* (scale bar 2 μm). Reproduced with permission from REF. 131 © (2000) Academia Brasileira de Ciências.

pH homeostasis. It has been proposed that acidic intracellular compartments regulate intracellular pH[69] and are important under pathological conditions[70,71]. Polyphosphate could be involved in intracellular pH regulation because it has been shown that H^+ generation from polyphosphate hydrolysis can neutralize a pH change of up to 2.5 pH units in *S. cerevisiae*[72].

A role for acidocalcisomes in regulation of intracellular pH in *T. brucei* was shown by the phenotype changes that occurred in cells in which the acidocalcisome V-H^+-PPase activity was reduced by RNA interference[42]. pH homeostasis failed in these cells 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[42].

Osmoregulation. Osmoregulation is essential for DIGENETIC TRYPANOSOMATIDS as osmotic stress occurs in both the insect vector and the vertebrate host. The regulatory volume decrease mechanism, which involves the cellular release of ions and osmolytes, including amino acids, enables adaptation of trypanosomatids to hypoosmotic stress. However, a considerable amount of volume recovery could not be accounted for by release of amino acids and ions, and it was proposed that acidocalcisomes might be involved in this process[62,66].

Rapid hydrolysis or synthesis of acidocalcisomal polyphosphate occurs when epimastigotes of *T. cruzi* are exposed to hypoosmotic or hyperosmotic stress conditions, respectively[60], indicating a link between acidocalcisomes and osmotic homeostasis. A role for acidocalcisomes in the response of *L. major* promastigotes to osmotic stress was also shown by changes in the sodium and chlorine content of the acidocalcisomes after hypoosmotic stress[57]. A link between acidocalcisomes and the contractile vacuole complexes of *C. reinhardtii*[15] and *D. discoideum*[16], which are involved in water extrusion in hypoosmotic medium, was also demonstrated. The contractile vacuole complex is composed of two compartments — a collection of tubules and vesicles called the SPONGIOME, and a larger vacuole known as the bladder[73].

Early observations[74] of epimastigotes of *T. cruzi* by phase-contrast microscopy had detected the presence of a contractile vacuole complex as a group of small vacuoles that fuse after they enlarge. The PULSATION PERIOD was between 60 and 75 seconds[74]. Electron micrographs of the contractile vacuole and surrounding spongiome of other kinetoplastids have also been published[75,76]. Re-investigation of the presence of a contractile vacuole in trypanosomatids resulted in the identification of an aquaporin that is located in both the acidocalcisomes and the contractile vacuole complex of *T. cruzi*[30]. Microtubule- and cyclic AMP-mediated fusion of acidocalcisomes to the contractile vacuole complex results in translocation of aquaporin and the resulting water movement, which, in addition to swelling of acidocalcisomes, is responsible for the decrease in volume that is not accounted for by efflux of osmolytes[66]. Additional evidence for a role of acidocalcisomes in osmoregulation resulted from studies on *T. brucei*[64]. The use of RNAi to reduce the expression of the acidocalcisomal soluble pyrophosphatase (TbVSP1) resulted in trypanosomes that were deficient in polyphosphate and in their response to hypoosmotic stress[64].

DIGENETIC TRYPANOSOMATIDS
Trypanosomes that have two hosts, in contrast to monogenetic trypanosomatids which only have one host.

SPONGIOME
Tubules and vacuoles that are connected to the contractile vacuole.

PULSATION PERIOD
The period of time between contractions of the contractile vacuole.

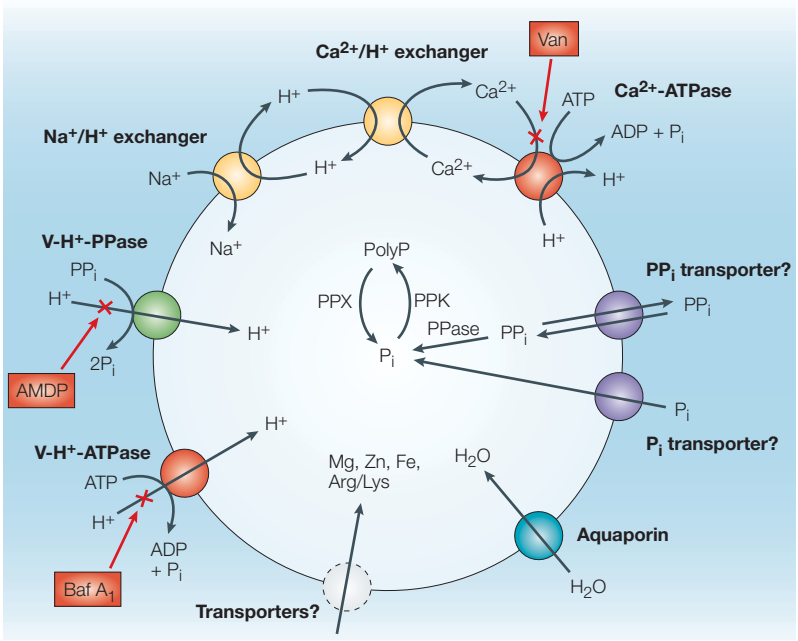


Figure 4 | **Schematic representation of a typical acidocalcisome.** Ca^{2+} uptake occurs in exchange for H^+ by a reaction catalysed by a vacuolar Ca^{2+}-ATPase that is inhibited by vanadate. A H^+ gradient is established by a bafilomycin A_1 (Baf A_1)-sensitive vacuolar H^+-ATPase and an amino-methylene-diphosphonate (AMDP)-sensitive vacuolar H^+-PPase (V-H^+-PPase). Ca^{2+} release occurs in exchange for H^+ and is favoured by sodium–proton exchange. An aquaporin allows water transport. Other transporters (for example, for Mg, Zn, Fe, inorganic phosphate (P_i) and pyrophosphate (PP_i), arginine and lysine) are probably present. The acidocalcisome is rich in pyrophosphate, short- and long-chain polyphosphate (polyP), magnesium, calcium, sodium, and zinc. An exopolyphophatase (PPX), a pyrophosphatase (PPase) and a polyphosphate kinase (PPK) are also present. Not all these enzymes are necessarily present in all acidocalcisomes described, and the internal concentration of elements may also vary.

Biogenesis of acidocalcisomes

Genetics and genomic sequencing have revealed that the regulation of vesicular transport in trypanosomatids is partially conserved in other eukaryotes, particularly the early steps in the secretory pathway (reviewed in REFS 77,78). In trypanosomatids, the endoplasmic reticulum is contiguous with the nuclear envelope, a Golgi apparatus consisting of a stack of 3–10 cisternae and a polymorphic trans-Golgi network[78]. Components of the vesicle budding, transport and fusion machinery, including *N*-ethylmaleimide-sensitive fusion protein (NSF), multiple Rab proteins and subunits of the coatamer and adaptor molecules, have been found in trypanosomatids[78]. However, little is known about trafficking of proteins to the acidocalcisome. Acidocalcisomes are not labelled with endocytic markers[79]. The presence of putative N-terminal leader sequences in the *T. cruzi* acidocalcisomal V-H^+-PPase (TcPPase) indicates that this protein is processed in the endoplasmic reticulum and trafficked to a location or locations within the secretory pathway, from where it is transported to the acidocalcisome[45]. N-terminal leader sequences have also been found in the acidocalcisomal V-H^+-PPase[80] and Ca^{2+}-ATPase[32] from *T. gondii*. Two recent studies have shown that defects in the biogenesis of acidocalcisomes result in distinct cellular phenotypes. RNAi-mediated downregulation of a kinesin-like protein from *T. brucei* (TbKIFC1) resulted in acidocalcisomes that were deficient in Ca^{2+} release[81], and it was suggested that this motor protein could be associated either with shuttle vesicles or macromolecular complexes moving to acidocalcisomes[81]. In a mutant form of *L. major* lacking the first enzyme in the sphingolipid biosynthesis pathway (serine palmitoyl transferase), the acidocalcisomes were shown to be 'empty' by electron microscopy and were devoid of long-chain polyphosphate, indicating that biogenesis of acidocalcisomes is linked to sphingolipid metabolism[82].

Table 1 | **Elemental analysis of acidocalcisomes**

Element	*Trypanosoma cruzi*	*Leishmania major*
Sodium	161 ± 18	148 ± 58
Magnesium	646 ± 19	515 ± 179
Phosphorus	1,390 ± 13	1,216 ± 316
Sulphur	10 ± 1	−3.1 ± 25.2
Chloride	2 ± 1	68 ± 34
Potassium	37 ± 2	237 ± 80
Calcium	171 ± 6	39 ± 18
Zinc	148 ± 6	74 ± 64

Concentrations of elements are expressed as nmol per mg dry weight. Elemental analysis is of acidocalcisomes from *T. cruzi* epimastigotes[25] and *L. major* promastigotes[56].

Acidocalcisomes and related organelles

Acidocalcisomes are now known to be similar to volutin granules, which were the first subcellular structures to be recognized in bacteria[8]. Volutin granules were later identified in algae and protists, and named polyphosphate vacuoles because this polymer is present at high concentrations in this organelle (TIMELINE). After their identification in trypanosomatids, the presence of acidocalcisomes in organisms previously known to contain volutin granules, such as the apicomplexan parasite *T. gondii*[11,32,46,80], the green alga *C. reinhardtii*[15] and the slime mould *D. discoideum*[16], was confirmed. Bacterial volutin granules were thought to lack an internal structure or limiting membranes[10]. Recently however, volutin granules that are surrounded by a membrane have been observed in *Agrobacterium tumefaciens*[17] and *Rhodospirillum rubrum*[18] using transmission electron microscopy. The membrane-bound volutin granules were stained with dyes that indicate the presence of acidic compartments and have also been shown to contain membrane-bound enzymes such as the V-H^+-PPase.

The digestive vacuole of *P. falciparum* trophozoites contains H^+ and Ca^{2+} pumps (V-H^+-ATPase, V-H^+-PPase and Ca^{2+}-ATPase)[83,84] that are similar to those of acidocalcisomes of other protozoa. Similarly, *D. discoideum*[16] and *T. cruzi*[30] contain acidocalcisomes and a contractile vacuole, both of which contain H^+ and Ca^{2+} pumps. The plant vacuole and the vacuole from yeast and other fungi have several similarities to the acidocalcisomes, such as an acidic nature, an abundance of polyphosphate and free basic amino acids, and the presence of proton and calcium pumps, Na^+/H^+ and Ca^{2+}/H^+ exchangers, and aquaporins[85].

Table 2 | **Polyphosphate (polyP) compounds in different life cycle stages of *T. cruzi* and *L. major***

Phosphorus compounds (mM)	*T. cruzi* epimastigotes	*T. cruzi* trypomastigotes	*T. cruzi* amastigotes	*L. major* promastigotes
Short-chain polyP	54.3 ± 0.3	3.1 ± 1.4	25.5 ± 5.1	21.4 ± 3.0
Long-chain polyP	2.89 ±0.29	0.82 ± 0.005	0.13 ± 0.01	55.9 ± 5.6

T. cruzi data are from REF. 60 and *L. major* data are from REF. 63.

Animal cells contain organelles that are similar to bacterial and unicellular eukaryotic acidocalcisomes, notably the dense granules in human platelets, which contain high concentrations of intracellular pryophosphate and polyphosphate and belong to the same class of organelles as acidocalcisomes[19]. This indicates that acidocalcisomes evolved before the prokaryotic and eukaryotic lineages diverged, and have been conserved during evolution in both bacteria and humans.

Two known organelles — the hydrogenosome and the protein storage vacuole — have internal compartments that are similar to acidocalcisomes. Hydrogenosomes are membrane-bound organelles that were first identified in the parabasalid flagellate *Tritrichomonas foetus*[86] and which are evolutionarily related to the mitochondria[87,88]. Hydrogenosomes of *T. foetus* contain a peripheral vesicle that, like the acidocalcisome, is electron-dense, contains large amounts of phosphorus, calcium, magnesium, iron and other elements[89], and is able to accumulate zinc when cells are cultivated in its presence[90] (FIG. 2f). The hydrogenosomal vesicles of the rumen anaerobic fungus *Neocallimastix frontalis*[91] are physiologically similar. Protein storage vacuoles store large concentrations of proteins during plant-seed development and maturation. They contain a membrane-bound acidic and electron-dense vacuole known as the globoid, which, like the acidocalcisome, is characterized by the presence of a V-H^+-PPase and an aquaporin (γ-TIP) and which is rich in the phosphorus compound phytic acid[92]. The evolutionary relationship of these 'organelles within organelles' to acidocalcisomes is intriguing and deserves further study.

Conclusions

Acidocalcisomes were found in bacteria more than one hundred years ago but their study, as well as the study of their main constituent, polyphosphate, has been neglected for many years. The conservation of this organelle from bacteria to man indicates that it has important functions that await discovery. Further studies are necessary to understand the biogenesis and function of acidocalcisomes in different organisms, why they have been conserved and how widely the organelle is distributed. Phylogenetic relationships of various acidocalcisomal enzymes need to be established as sequence comparisons are important indicators of the evolution of these organelles. We do not know how acidocalcisomes are distributed in daughter cells after cell division or why morphological changes occur in acidocalcisomes of some trypanosomatids. Intracellular pyrophosphate, polyphosphate, cations and basic amino acids are accumulated in large amounts in acidocalcisomes, but the mechanisms by which these compounds are transported into the organelle and the reasons for their accumulation are largely unknown. This is an exciting area of research, not least because these organelles have different characteristics in different organisms, which indicates that they could be targets for new drugs.

Box 2 | **Polyphosphate**

Polyphosphate is a linear chain of inorganic phosphate moieties (from a few to several hundred moieties) linked by high-energy phosphoanhydride bonds, and it is ubiquitous from bacteria to mammals[107,108].

Polyphosphate has several functions in bacteria — for example, it can be a phosphate store or an energy source to replace ATP, and can have roles in cation sequestration and storage, cell membrane formation and function, transcriptional control, regulation of enzyme activities, response to stress and stationary phase, and the structure of channels and pumps[107,108]. As the metabolic turnover of ATP is considerably higher than that of polyphosphate[109], it has been suggested[110] that polyphosphate is not an efficient supply of energy and that it has a regulatory role. Similar functions in adaptation to stress have been assigned to polyphosphate in eukaryotic cells such as yeast[72,111], fungi[112] and algae[113–115].

In many organisms, the mobilization of polyphosphate is mainly due to the action of enzymes that catalyse the synthesis and degradation of this polymer — the polyphosphate kinase and the endo- and exopolyphosphatases, respectively[107,108]. Until recently, the only genes to encode exopolyphosphatases[116] and endopolyphosphatases[117] in eukaryotes were from *Saccharomyces cerevisiae*, with the exception of a putative polyphosphate kinase gene (*DdPPK1*) in *Dictyostelium discoideum*[107,118] and a second polyphosphate kinase in *D. discoideum* (DdPPK2), which might be localized to the acidocalcisome[118,119]. DdPPK2 has a similar amino acid sequence to, and characteristics of, actin-related proteins, which in turn are similar to muscle actins. Actin inhibitors such as phalloidin and DNase I also inhibit DdPPK2-mediated synthesis of polyP. So, this particular actin-related protein complex is an enzyme that can polymerize into an actin-like filament concurrent with its synthesis of a polyphosphate chain in a fully reversible reaction[119]. It is interesting to note that actin-like proteins have been found in the electron-dense granules of *Entamoeba histolytica*, which are similar to acidocalcisomes in both morphology and composition[120], and that immunofluorescence with antibodies against actin revealed a granular pattern in different trypanosomatids[121].

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