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Review

Evolution of acidocalcisomes and their role in polyphosphate storage and osmoregulation in eukaryotic microbes

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Acidocalcisomes are acidic electron-dense organelles, rich in polyphosphate (poly P) complexed with calcium and other cations. While its matrix contains enzymes related to poly P metabolism, the membrane of the acidocalcisomes has a number of pumps (Ca^{2+}-ATPase, V-H^+-ATPase, H^+-PPase), exchangers (Na^+/H^+, Ca^{2+}/H^+), and at least one channel (aquaporin). Acidocalcisomes are present in both prokaryotes and eukaryotes and are an important storage of cations and phosphorus. They also play an important role in osmoregulation and interact with the contractile vacuole complex in a number of eukaryotic microbes. Acidocalcisomes resemble lysosome-related organelles (LRO) from mammalian cells in many of their properties. They share similar morphological characteristics, acidic properties, phosphorus contents and a system for targeting of their membrane proteins through adaptor complex-3 (AP-3). Storage of phosphate and cations may represent the ancestral physiological function of acidocalcisomes, with cation and pH homeostasis and osmoregulatory functions derived following the divergence of prokaryotes and eukaryotes.

Keywords: acidocalcisome; calcium; polyphosphate; pyrophosphate; volutin granules; protists

1. INTRODUCTION

One of the first subcellular structures recognized in bacteria was the metachromatic (Babes 1895) or volutin (Meyer 1904) granule. The name *volutin granule* derives from their discovery in the bacterium *Spirillum volutans*, in which the granules stain red when treated with toluidine blue. The presence of these granules was used as a diagnostic feature of important bacteria such as *Corynebacterium diphtheriae* (Kornberg 1995). Over the years, volutin granules have also been described in lower eukaryotes such as algae, yeasts and protozoa.

Volutin granules were renamed polyphosphate (poly P) granules after Wiame (1947) found that the number of granules in yeast correlated with the amount of poly P. Poly P is a linear chain of a few to many hundreds of phosphate (Pi) residues linked by high-energy phosphoanhydride bonds (Kornberg *et al.* 1999). Their high electron density and a limiting membrane surrounding the granules in eukaryotes became evident with the advent of electron microscopy. Although early reports (Friedberg & Avigad 1968; Jensen 1968) suggested the presence of membranes surrounding the bacterial granules, this contradicted current thought that bacteria lack an endomembrane system, and for many years they were assumed to lack an internal structure or limiting membrane (Shively 1974; Shively *et al.* 1988).

Volutin or poly P granules were also found in a number of eukaryotic microbes using the 'Meyer test' based on their methachromasy, including coccidia (Kunze 1907), trypanosomes (Swellengrebel 1908) and Sarcosporidia (Erdnmann 1910). More recently, elemental analysis of these granules in different trypanosomatids revealed the presence of large amounts of phosphorus as well as calcium and other cations (Vickerman & Tetley 1977; Dvorak *et al.* 1988; LeFurgey *et al.* 1990). These granules were later identified (Scott *et al.* 1997) as the acidic, calcium-rich compartments of trypanosomes known as acidocalcisomes (Vercesi *et al.* 1994; Docampo *et al.* 1995).

Acidocalcisomes can thus be defined as electron-dense acidic organelles with a high concentration of phosphorus present as poly P complexed with calcium and other elements (Docampo *et al.* 2005). The identification of enzymes and transporters in the surrounding membranes of these granules in prokaryotes (Seufferheld *et al.* 2003, 2004) and eukaryotes (Docampo & Moreno 1999) established them as real organelles. The dense granules of human platelets possess similar characteristics to acidocalcisomes (Ruiz *et al.* 2004), suggesting that the organelles play important roles that may have been conserved following the divergence of prokaryotes and eukaryotes.

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One contribution of 12 to a Theme Issue 'Evolution of organellar metabolism in unicellular eukaryotes'.

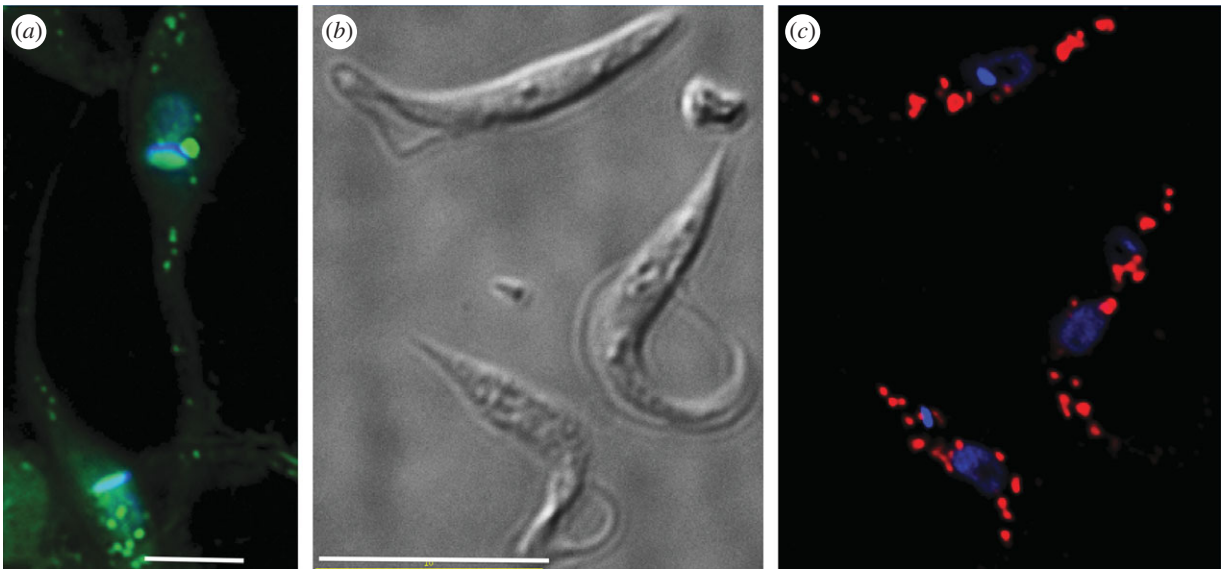

Figure 1. Acidocalcisomes in trypanosomatids. (*a*) DAPI staining of epimastigotes of *Trypanosoma cruzi*. Overlay of the *green* (poly P) and *blue* (DNA) channels. Acidocalcisomes correspond to the punctate labelling (*green*; scale bar, 5 μm). (*b*) Differential interference contrast (DIC) image of procyclic forms of *Trypanosoma brucei* (scale bar, 10 μm). (*c*) Immunofluorescence analysis of the same cells in (*b*), after reaction with polyclonal antibodies against TbVP1 (V-H^+-PPase, in *red*; scale bar, 10 μm). Figure 1*a* is adapted from Fang *et al.* (2007*b*).

Alternatively, the presence of acidocalcisomes in both prokaryotes and eukaryotes could be an example of convergent evolution.

Acidocalcisomal poly P stores are important for resistance to heavy metals in some eukaryotes and prokaryotes (Hashemi *et al.* 1994; Alvarez & Jerez 2004; Andrade *et al.* 2004; Remonsellez *et al.* 2006; Nagasaka & Yoshimura 2008). Thus, phosphate and heavy metal storage may represent an ancestral characteristic that was important for their evolutionary development. Phosphate storage is especially evident in marine plankton, in which nearly 10 per cent of the phosphorus pools are composed of poly P (Diaz *et al.* 2008), a reserve that some algae actively manage to overcome phosphate limitation in phosphate-poor water (Watanabe *et al.* 1988).

Acidocalcisomal poly P complexes protons in addition to sequestering heavy metals in eukaryotes, linking poly P inextricably to pH homeostasis. Poly P stores are inversely related to acidity in a wide variety of lower eukaryotes including *Candida humicola* (McGrath & Quinn 2000), *Dunaliella salina* (Pick & Weiss 1991) and trypanosomatids (Ruiz *et al.* 2001*a*; Lemercier *et al.* 2002; Rohloff & Docampo 2006). However, regulation of pH by acidocalcisomal poly P appears restricted to eukaryotes, suggesting that this characteristic was derived after divergence of the eukaryotic and prokaryotic lineages.

2. STRUCTURAL CHARACTERISTICS AND COMPOSITION OF ACIDOCALCISOMES

Acidocalcisomes of bacteria and eukaryotic microbes can be easily identified with dyes that accumulate into acidic compartments, such as acridine orange (Vercesi *et al.* 1994; Docampo *et al.* 1995; Miranda *et al.* 2008) and Lysosensor blue DND-167 (Seufferheld *et al.* 2003), and dyes that stain poly P, such as 4′-6′-diamino-2-phenylindole (DAPI; Scott & Docampo 2000; figure 1*a*). They typically are spherical with an average diameter of 0.2–0.5 μm although polymorphic morphologies occur in some cells (Docampo *et al.* 2005). Their position in the cells is random.

By transmission electron microscopy the organelle appears empty or with an inclusion of a thin layer of dense material that sticks to the inner face of the membrane (figure 2). While standard electron microscopy protocols extract electron dense material, acidocalcisomes can be directly observed with whole mounts of cells, bacteria or protists deposited onto carbon- and formvar-coated grids. They appear as electron-dense spheres (figure 3).

The elemental composition of the organelle has been analysed by electron microscopy techniques (X-ray microanalysis and elemental mapping) and has invariably revealed the presence of large deposits of phosphorus and calcium. Other cations (magnesium, sodium, potassium, zinc, iron) occur in lower amounts depending on the cell analysed (Docampo *et al.* 2005).

Acidocalcisomes from bacteria and eukaryote microbes are very rich in pyrophosphate (PPi) as well as in short-chain (fewer than 50 Pi units) and long-chain (50–800 Pi units) poly P. The concentration of poly P within these granules could reach molar levels (in terms of Pi units; Docampo *et al.* 2005), probably explaining their high electron density. In fact, solid-state condensed phosphates can be detected in isolated acidocalcisomes by magic-angle spinning NMR techniques (Moreno *et al.* 2002). Acidocalcisomes are enriched in basic amino acids that probably complex with the negatively charged poly P (Rohloff *et al.* 2003). Only a few soluble enzymes have been detected in the acidocalcisomal matrix, including an exopolyphosphatase (PPX) in *Leishmania*

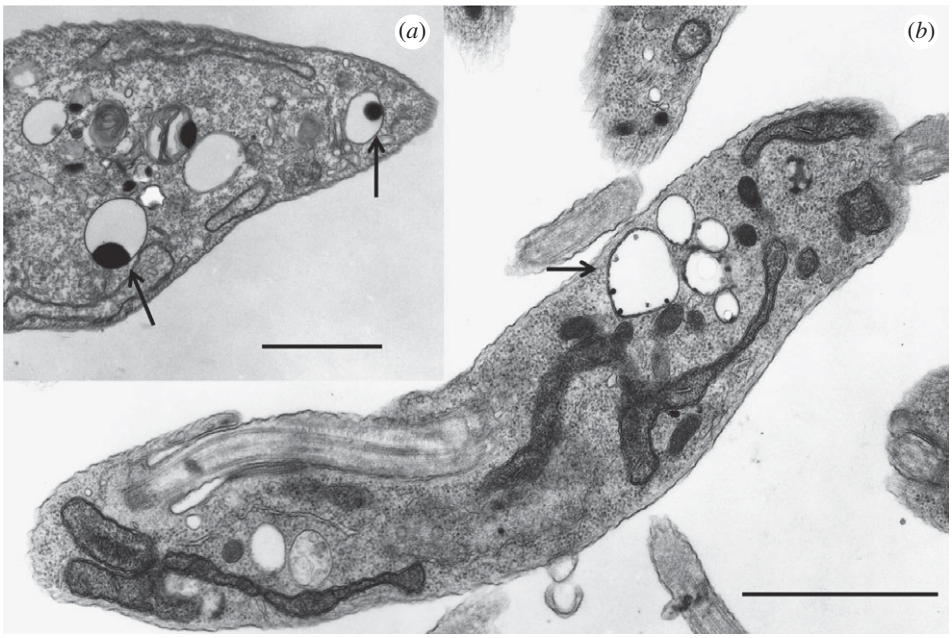

Figure 2. Thin section of procyclic forms of *Trypanosoma brucei* showing the morphology of acidocalcisomes. (*a*) acidocalcisomes appear as empty vacuoles or with an electron-dense inclusion (inclusion bodies, *arrows*). (*b*) A group of acidocalcisomes is observed at the anterior end of a cell with a thin layer of electron-dense material sticking to the inner phase of the membrane (*arrows*). Scale bar, 2 μm.

major (Rodrigues *et al.* 2002) and *Trypanosoma cruzi* (Fang *et al.* 2007*b*), a soluble inorganic pyrophosphatase in *Trypanosoma brucei* (Lemercier *et al.* 2004), and a metacaspase in *Leishmania donovani* (Lee *et al.* 2007). Acid phosphatase activity has also been detected using cytochemical methods (Gomes *et al.* 2006).

3. PUMPS, CHANNELS AND EXCHANGERS OF ACIDOCALCISOMES

Acidocalcisomes are similar to the plant vacuole in that they possess a number of pumps, channels and cation exchangers in their membranes. Both the plant vacuole and acidocalcisomes have two proton pumps: a vacuolar-type H^+-ATPase (V-H^+-ATPase; Lu *et al.* 1998) and a vacuolar proton translocating pyrophosphatase (V-H^+-PPase; Docampo *et al.* 2005; figure 1*c*), which is absent in animals and fungi. Coexistence of two different proton pumps in the membrane or tonoplast of the plant vacuole may play a role in energy conservation (Rocha-Façanha & de Meis 1998). The H^+ gradient generated across the plant vacuolar membrane by the hydrolysis of either PPi or ATP may drive both ATP and PPi synthesis by reversal of the tonoplast H^+-ATPase (Rocha-Façanha & de Meis 1998; Hirata *et al.* 2000) or the V-H^+-PPase (Rocha-Façanha & de Meis 1998), and a similar process could occur in acidocalcisomes. However, both pumps do not co-localize in all acidocalcisomes. Ruiz *et al.* (2001*b*) used antibodies against these two proteins in *Chlamydomonas reinhardtii* to demonstrate the presence of different populations of organelles, some containing both proteins and others containing only a single pump type. Similarly, co-localization studies of the V-H^+-PPase with a Ca^{2+}-ATPase in *T. cruzi* revealed two apparently different populations of acidocalcisomes (Lu *et al.* 1998). Physiological experiments in *L. donovani* also suggested the presence of the V-H^+-ATPase and the V-H^+-PPase in different compartments (Rodrigues *et al.* 1999*b*).

The V-H^+-PPase also exists elsewhere in some species. It was initially described in chromatophore membranes of *Rhodospirillum rubrum* (Baltscheffsky *et al.* 1966; Moyle *et al.* 1972) and in plant vacuoles (Rea & Poole 1986), but it has also been detected in the plasma membrane and the Golgi complex of some plants (Long *et al.* 1995; Robinson *et al.* 1996) and *T. cruzi* (Scott *et al.* 1998; Martinez *et al.* 2002). The V-H^+-PPase of *Toxoplasma gondii* has been found in a vacuolar compartment involved in microneme protein maturation (Harper *et al.* 2006), while the *Plasmodium spp.* enzyme has been found in the acidocalcisomes (Luo *et al.* 1999; Marchesini *et al.* 2000), digestive vacuole (Saliba *et al.* 2003) and plasma membrane (McIntosh *et al.* 2001).

There is also evidence for the presence of a Ca^{2+}-ATPase in acidocalcisomes from several eukaryotic microbes, such as *T. cruzi* (Scott & Docampo 2000), *T. brucei* (Rodrigues *et al.* 1999*a*; Luo *et al.* 2004), *Dictyostelium discoideum* (Marchesini *et al.* 2002) and *T. gondii* (*TgA1*; Luo *et al.* 2001; Luo *et al.* 2005).

Na^+/H^+ and Ca^{2+}/H^+ exchanger activities have been detected in acidocalcisomes of *T. brucei* procyclic forms (Vercesi & Docampo 1996; Vercesi *et al.* 1997) and in *L. donovani* promastigotes (Vercesi *et al.* 2000).

Finally, an aquaporin has also been identified in acidocalcisomes of *T. cruzi* (Montalvetti *et al.* 2004). The protein acts as a water channel and is unable to transport glycerol when expressed in *Xenopus* oocytes. This aquaporin is also localized to the contractile vacuole complex, suggesting a role in osmoregulation (Rohloff *et al.* 2004). Figure 4 shows a scheme of the

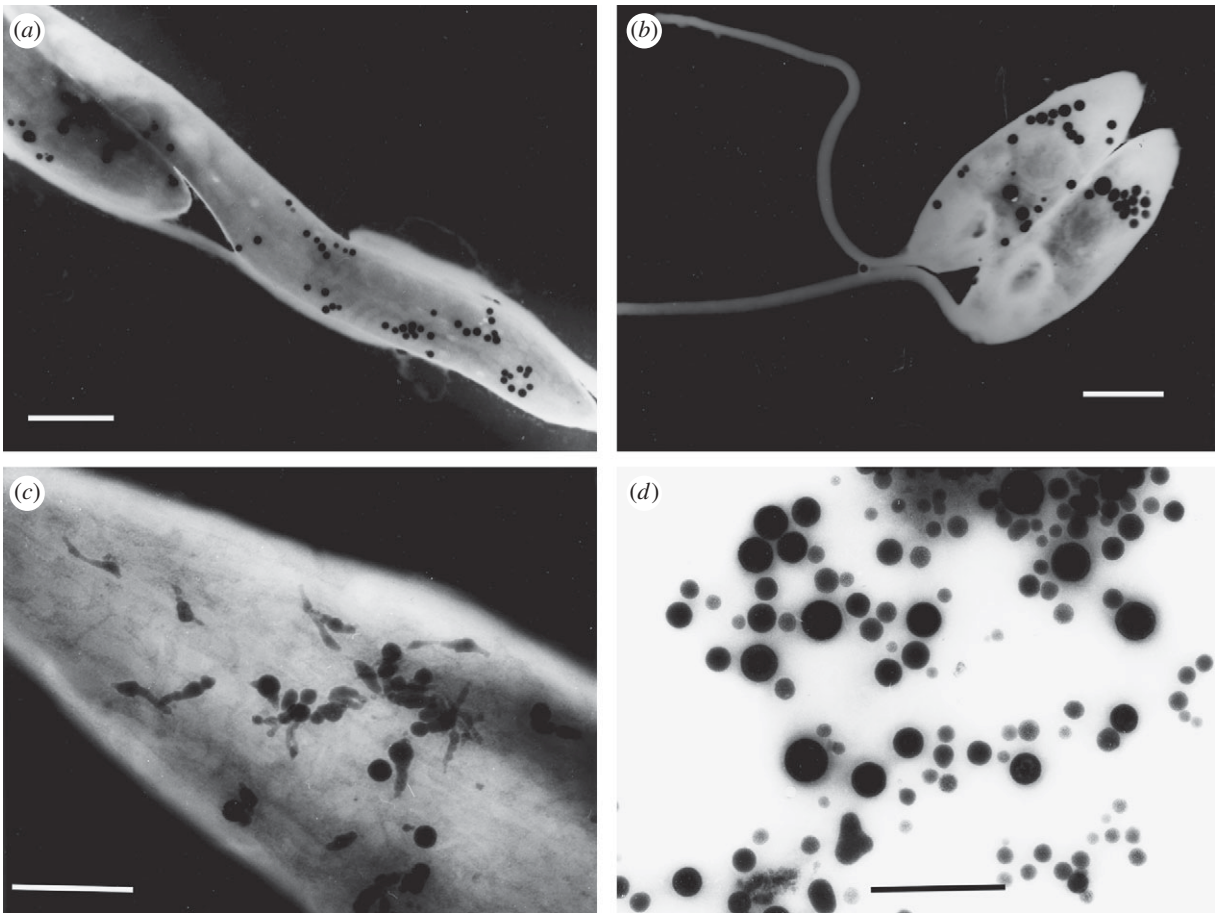

Figure 3. Morphology of trypanosomatid acidocalcisomes. Electron spectroscopic imaging (contrast tuning) of whole cells (*a–c*) or fractions (*d*) adhered to formvar-coated grids showing the shape, size and distribution of acidocalcisomes (black spots) in different species. (*a*) *Blastochritidia culicis*; scale bar, 2 μm. (*b*) *Herpetomonas anglusteri*; scale bar, 3 μm. (*c*) *Phytomonas serpens*; scale bar, 0.5 μm. (*d*) Isolated acidocalcisomes from *Trypanosoma cruzi*; scale bar, 0.5 μm. Note the polymorphic nature of acidocalcisomes in (*c*). *b*,*c* are adapted from Miranda *et al.* (2004, Copyright Elsevier).

known components of acidocalcisomes in early eukaryotes.

4. ACIDOCALCISOME ENZYMES INVOLVED IN POLYPHOSPHATE SYNTHESIS AND DEGRADATION

(a) *Poly P biosynthesis*

Biosynthetic enzymes for poly P were largely uncharacterized in eukaryotes until recently. A poly P kinase (PPK) homologous to prokaryotic PPK1 was reported in just a single eukaryote, *D. discoideum* (DdPPK1, Zhang *et al.* 2007), and proposed to be a product of horizontal gene transfer (Hooley *et al.* 2008). It is present in small vesicles, which may correspond to acidocalcisomes (Marchesini *et al.* 2002; Zhang *et al.* 2007), although no co-localization studies have been reported. DdPPK1 catalyses the following reaction:

$$\text{Poly P}_n + \text{ATP} \longrightarrow \text{Poly P}_{n+1} + \text{ADP}. \qquad (4.1)$$

A second PPK, termed DdPPK2, which catalyses the same reaction, was proposed to be present in acidocalcisomes of *D. discoideum* (Gomez-Garcia & Kornberg 2004). DdPPK2 shares characteristics and sequence identity with actin-related proteins, a group of proteins with homology to muscle actins. Actin inhibitors such as phalloidin and DNase I inhibited DdPPK2-mediated synthesis of poly P. This particular actin-related protein complex can polymerize into an actin-like filament concurrent with its synthesis of a poly P chain in a fully reversible reaction (Gomez-Garcia & Kornberg 2004). The presence of a DdPPK2-like activity in *C. reinhardtii* was also reported (Gomez-Garcia & Kornberg 2004), and an unidentified PPK activity was also detected in acidocalcisomes of *T. cruzi* (Ruiz *et al.* 2001*a*).

Using DNA microarray methodology, Ogawa *et al.* (2000) identified four *PHM* genes in *Saccharomyces cerevisiae* that encode proteins involved in poly P synthesis as shown by the lack of detectable poly P in *phm3*Δ and *phm4*Δ mutants or in *phm1*Δ–*phm2*Δ double mutants. These authors proposed that the protein products of these genes are poly P synthases (Ogawa *et al.* 2000). Since then protein sequence homologues from several organisms have been annotated in genome databases as poly P synthases. The *PHM* genes were independently identified by Cohen *et al.* (1999) and named vacuolar transporter chaperone (*VTC*) *1–4* (*VTC1*/*PHM4*; *VTC2*/*PHM1*, *VTC3*/*PHM2*, and *VTC4*/*PHM3*; Cohen *et al.* 1999; Nelson *et al.* 2000). A protein (TbVTC1) homologous to the yeast vacuolar transporter chaperone 1 (Vtc1p) was identified in *T. brucei* that was essential for poly P synthesis, acidocalcisome biogenesis and cytokinesis

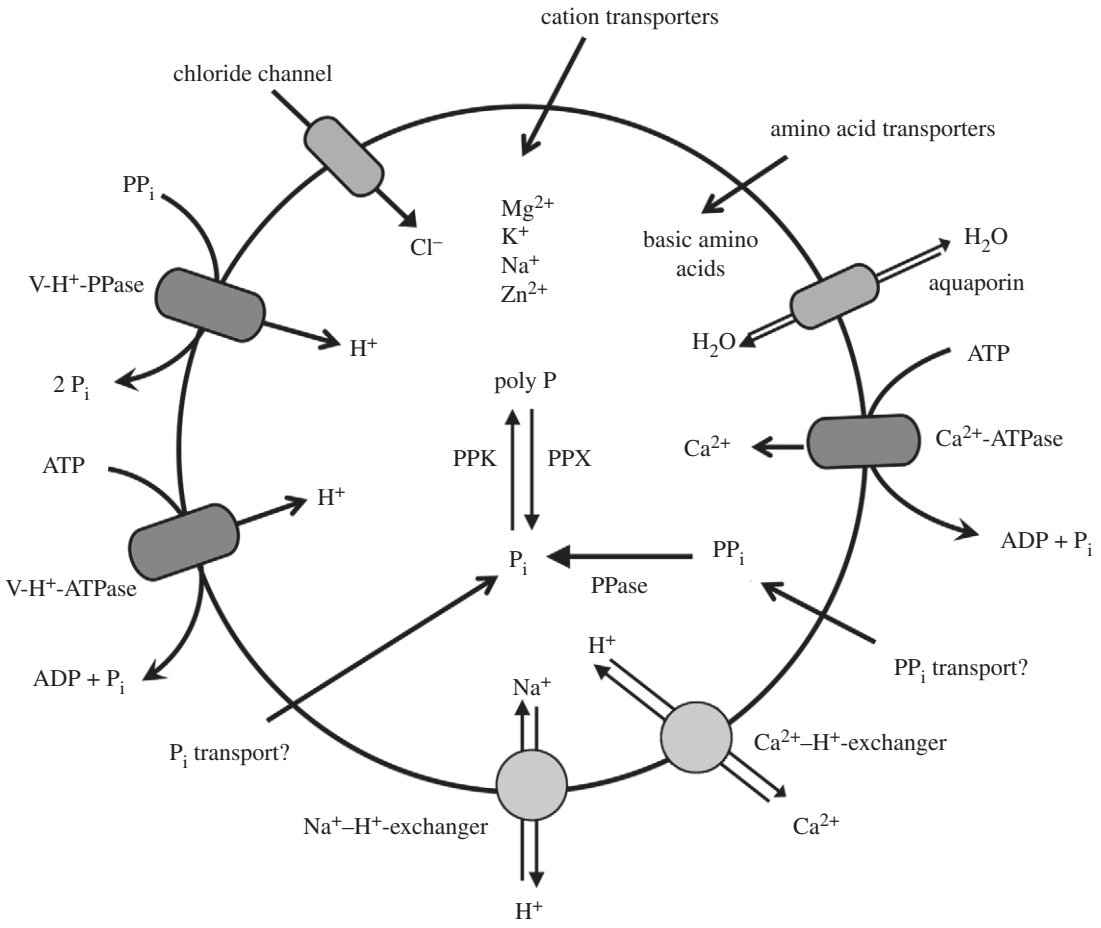

Figure 4. Schematic of an early eukaryote acidocalcisome. Ca^{2+} uptake occurs in exchange for H^+ by a reaction catalysed by a vacuolar Ca^{2+}-ATPase. A H^+ gradient is established by a vacuolar H^+-ATPase and a vacuolar H^+-pyrophosphatase (V-H^+-PPase). An aquaporin allows water transport. Ca^{2+} release occurs in exchange of H^+ and is favoured by sodium–proton exchange. Other transporters (for example, for Mg, Zn, inorganic phosphate (Pi), pyrophosphate (PPi), and basic amino acids) are probably present. The acidocalcisome is rich in pyrophosphate, short- and long-chain polyphosphate (poly P), magnesium, calcium, sodium and zinc. An exopolyphosphatase (PPX), a pyrophosphatase (PPase) and a polyphosphate kinase (PPK) may also be present. A question mark was added to indicate the lack of biochemical evidence for their presence.

(Fang *et al.* 2007*a*). TbVTC1 was shown to co-localize with the vacuolar V-H^+-PPase to the acidocalcisomes. RNA interference experiments altered acidocalcisomal morphology and significantly decreased the amount of poly P (Fang *et al.* 2007*a*).

Many apicomplexan and trypanosomatid parasite genomes include sequences with homology to Phm/Vtc proteins (Fang *et al.* 2007*a*). Analysis of these sequences revealed that there are both small and large proteins with Phm/Vtc homology. Large homologues (66.1–129.0 kDa) were detected in *Saccharomyces pombe*, *Candida albicans*, *Encephalitozoon cuniculi*, *T. gondii*, *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Plasmodium berghei*, *Plasmodium chabaudi*, *Plasmodium falciparum*, *L. major*, *T. brucei* and *T. cruzi*. Short homologues (13.4–19.9 kDa) to *S. cerevisiae* Phm4p/Vtc1 were detected in *S. pombe*, *T. cruzi*, *T. brucei*, and *L. major*. Regardless of size, all proteins examined shared a conserved motif, located centrally (*T. brucei*, *T. cruzi*, *L. major*) or near the N-terminus (*S. cerevisiae*, *S. pombe*) in the case of the short Phm4p/Vtc1 homologues and near the C-terminus in the case of the long homologues. Most of these sequences have not been experimentally examined (Fang *et al.* 2007*a*). The recent identification of the Phm/Vtc family as a poly P polymerase and translocase complex in *S. cerevisiae* (Hothorn *et al.* 2009) suggests that this complex is involved in the synthesis of acidocalcisomal poly P in most eukaryotic microbes. ScVtc4 was identified as the catalytic subunit of the complex (Hothorn *et al.* 2009), thus explaining the conservation of this subunit in other fungi as well as in protists, which usually have only two of the four subunits present in yeast. Vtc4 also possesses an SPX domain. SPX domains are usually at the N-termini of proteins and are though to have a regulatory function (Hürlimann *et al.* 2009). The VTC complex has been found only in marine organisms (diatoms), fungi and protists, but appears not to be conserved in animals or plants. It is not known whether all the organisms possessing the VTC complex possess acidocalcisomes, although structures closely resembling acidocalcisomes have also been found in fungi (Franzen *et al.* 2008).

(b) *Poly P hydrolysis*

Degradation of poly P in eukaryotic microbes is catalysed by PPXs and endopolyphosphatases (PPNs), but only a PPX (in *L. major*, LmPPX) has been

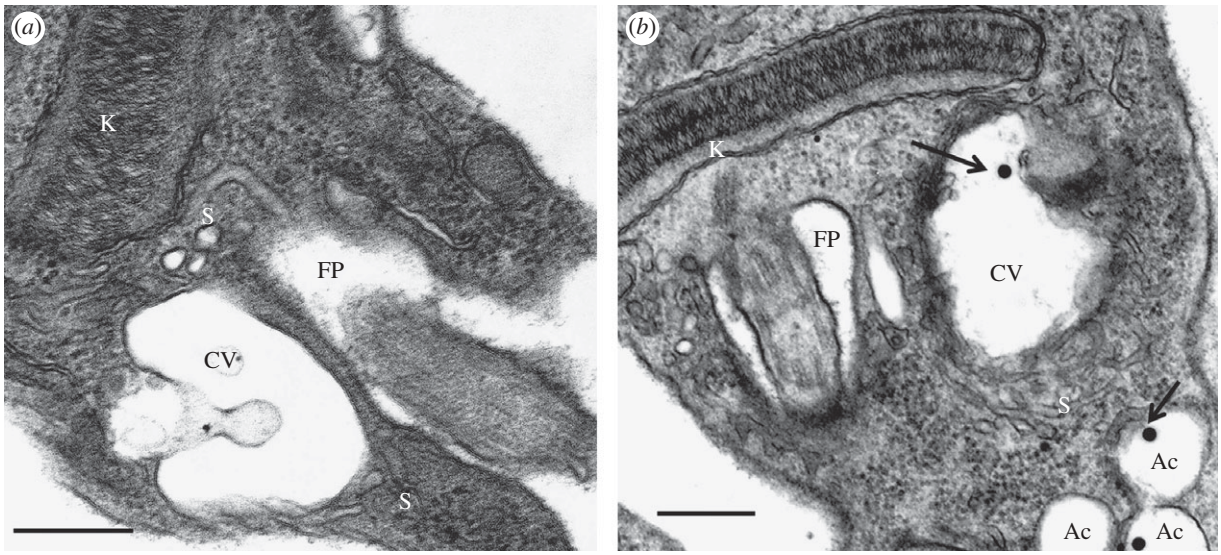

Figure 5. The contractile vacuole of *Trypanosoma cruzi*. Epimastigotes observed by transmission electron microscopy. Notations are flagellar pocket (FP), acidocalcisomes (Ac), kinetoplast (K), contractile vacuole (CV), tubules forming the spongiome (S). Note that similar electron-dense material (*arrows*) is observed in both the CV and the Ac. Scale bar, 0.25 μm. (*b*) is adapted from Montalvetti *et al.* (2004).

detected in acidocalcisomes (Rodrigues *et al.* 2002). The enzyme also localizes to the cytosol (Rodrigues *et al.* 2002) and catalyses the following reaction:

$$\mathrm{Poly}P_n + (n-2)H_2O \longrightarrow (n-2)\mathrm{Pi} + \mathrm{PPi}. \qquad (4.2)$$

PPX progressively hydrolyses poly P from the chain termini producing Pi until only PPi remains. Recombinant LmPPX is similar to yeast PPX (ScPPX, Wurst *et al.* 1995) with respect to its Mg^{2+} requirement, optimum pH, and sensitivity to cations, amino acids and heparin. In contrast to the yeast enzyme and other PPXs, LmPPX degrades short chain poly P with higher rates and affinity. The *T. cruzi* PPX is similar to the LmPPX although its localization has not been reported. Interestingly, overexpression of TcPPX led to a significant decrease in short-chain poly P and in the staining of acidocalcisomes with DAPI, suggesting that it is also localized to acidocalcisomes (Fang *et al.* 2007*b*).

Acidocalcisomal soluble vacuolar pyrophosphatases (VSP) have also been described in *T. brucei* (Lemercier *et al.* 2004) and *Leishmania amazonensis* (Espiau *et al.* 2006). VSP require the presence of transition metal ions such as Zn^{2+}, Mn^{2+} and Co^{2+} to hydrolyse poly P, a property that they share with the homologue *S. cerevisiae* pyrophosphatase (Oksanen *et al.* 2007). This could be physiologically important as acidocalcisomes of these parasites are rich in Zn^{2+}.

5. OSMOREGULATORY FUNCTIONS OF ACIDOCALCISOMES AND RELATION TO THE CONTRACTILE VACUOLE COMPLEX

In addition to their function as storage organelles for phosphorus and cations, acidocalcisomes appear to have an important role in osmoregulation.

The contractile vacuole is an organelle involved in osmoregulation in a number of free living and parasitic protists and has a bipartite structure consisting of a central vacuole, or bladder, and a surrounding network of microtubules and vesicles named the spongiome (Bowers & Korn 1968; figure 5). Early work demonstrated that acidocalcisomes are in close contact with the contractile vacuole of *D. discoideum* (Marchesini *et al.* 2002). Submission of *D. discoideum* amoebas to hyposmotic shock increased this association. In addition to poly P, both compartments possess a V-H^+-ATPase, a Ca^{2+}-ATPase and a H^+-PPase. Marchesini *et al.* (2002) proposed that poly P hydrolysis could lead to water uptake by the vacuole, thereby contributing to volume regulation under hyposmotic stress. Contractile vacuoles of *C. reinhardtii* are also rich in poly P, and also have a V-H^+-ATPase and a V-H^+-PPase (Ruiz *et al.* 2001*b*).

A more detailed study of the association of acidocalcisomes and the contractile vacuole complex was performed in trypanosomatids. When *T. cruzi* epimastigotes are exposed to hyposmotic or hyperosmotic stress conditions, there is a rapid hydrolysis or synthesis of acidocalcisomal poly P, respectively (Ruiz *et al.* 2001*a*), suggesting a link between acidocalcisomes and osmotic homeostasis. In addition, exposure of *L. major* promastigotes to hyposmotic stress alters sodium and chloride content of their acidocalcisomes, implicating their role in this response (LeFurgey *et al.* 2001). In *T. cruzi*, an aquaporin or water channel (TcAQP1) is located in both acidocalcisomes and the contractile vacuole complex (Montalvetti *et al.* 2004). Hyposmotic stress leads to an increase in cyclic adenosine monophosphate (AMP), which stimulates translocation of TcAQP1 from the acidocalcisome to the contractile vacuole. This translocation probably results in water movement leading to a decrease in cell volume (Rohloff *et al.* 2004). Additional evidence for a role of acidocalcisomes in osmoregulation resulted from studies on *T. brucei* (Lemercier *et al.* 2004; Fang *et al.* 2007*b*). The use of RNAi to reduce the expression of the acidocalcisomal soluble pyrophosphatase (TbVSP1) resulted in trypanosomes that were deficient in poly P

and in their response to hyposmotic stress (Lemercier *et al.* 2004). Ablation of a vacuolar transporter chaperone (VTC1) in *T. brucei* by RNAi resulted in abnormal morphology of acidocalcisomes, decrease in cellular poly P content, and a deficient response to hyposmotic stress (Fang *et al.* 2007*a*).

In addition to the cyclic AMP pathway, other signalling systems have been found to be involved in osmoregulation in *T. cruzi*. Overexpression of a phosphatidylinositol 3-kinase (PI3K, or Vps34) in *T. cruzi* resulted in morphological and functional alterations related to vesicular trafficking, and the cells were more resistant to hyposmotic stress (Schoijet *et al.* 2008). Interestingly, these cells had large contractile vacuole bladders (Schoijet *et al.* 2008).

6. RELATION OF ACIDOCALCISOMES TO LYSOSOME-RELATED ORGANELLES

Recent work has indicated that acidocalcisomes share characteristics with organelles known as lysosome-related organelles (LROs) and may be biogenically related. LROs comprise a heterogeneous set, many of which are secreted from the cell (Cutler 2002). Examples include melanosomes, lytic granules, major histocompatibility complex class II compartments, platelet dense granules, basophil granules and neutrophil azurophil granules (Dell'Angelica *et al.* 2000). Acidocalcisomes resemble LROs in many respects. For example, one type of LRO, platelet dense granules, have a similar size, high electron density, are acidic, and contain calcium and phosphorus in the form of poly P and PPi (Ruiz *et al.* 2004).

While endocytic tracers (transferrin, Scott *et al.* 1997; horseradish peroxidase, Coppens *et al.* 1993; FM4-64, Mullin *et al.* 2001) do not accumulate in acidocalcisomes, some accumulation of endocytic markers occur when *T. cruzi* is treated with an inhibitor of the sterol biosynthetic pathway (Vannier-Santos *et al.* 1999). In *L. major*, a mutant deficient in sphingolipid synthesis was found to be defective in biogenesis of both multivesicular bodies (or late endosomes) and acidocalcisomes, suggesting that these compartments have a common origin (Zhang *et al.* 2005). Besteiro *et al.* (2008) recently found that adaptor protein 3 (AP-3), a protein involved in transport of membrane proteins to lysosomes and LROs in other cells, has a similar function with respect to acidocalcisomes in *L. major*, providing support for a close similarity between acidocalcisomes and the endo/lysosomal system. Furthermore, mutants of *T. brucei* deficient in an orthologue of vacuolar sorting protein 41 (VSP41p), which interacts with the δ subunit of AP-3-coated carrier vesicles (Rehling *et al.* 1999) and is involved in the biogenesis of LROs (Dell'Angelica *et al.* 2000), had large numbers of small intracellular vesicles similar to acidocalcisomes (Lu *et al.* 2007). The finding that LROs and acidocalcisomes share the system for targeting of their membrane proteins reinforces the similarities between these organelles (Besteiro *et al.* 2008), supporting the hypothesis that LROs and acidocalcisomes are biogenically related.

7. CONCLUSION

Acidocalcisomes were known for many years as volutin or poly P granules and are present in both prokaryotes and eukaryotes. They are related to a group of eukaryotic organelles known as LROs. We know that acidocalcisomes are important storage compartments for phosphorus and cations as well as basic amino acids in some cells. Their acidity is maintained by proton pumps, one of which, a V-H^+-PPase, is only present in bacteria, plants, and early divergent eukaryotes. In addition to pumps, exchangers and aquaporin, acidocalcisomes possess several enzymes involved in PPi and poly P metabolism. Some of these enzymes, such as DdPPK2, have not yet been found in other organisms while the vacuolar transporter chaperone complex is only present in eukaryotic microbes. Many of the acidocalcisome enzymes are unique to different microbes and are therefore potential targets for new drugs, as we noted in a recent review (Docampo & Moreno 2008). Acidocalcisomes play an important role in response to osmotic stress, and their interactions with the contractile vacuole complex of free living and parasitic organisms are very relevant to this function. Many things are not yet known about acidocalcisomes, such as their biogenesis, the phylogenetic relationships of their various enzymes, the mechanism for accumulation and release of their phosphate and cationic components, and the functions of the components accumulated in their matrix. This is an exciting area of work and many novel functions of poly P and acidocalcisomes await discovery.

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