HHS Public Access

Author manuscript

Biochem Soc Trans. Author manuscript; available in PMC 2017 February 15.

Published in final edited form as:

Biochem Soc Trans. 2016 February 15; 44(1): 1–6. doi:10.1042/BST20150193.

Polyphosphate and Acidocalcisomes

Noelia Lander[1,2], **Ciro Cordeiro**[1], **Guozhong Huang**[1], and **Roberto Docampo**[1,2,*]

[1]Center for Tropical and Global Emerging Diseases and Department of Cellular Biology, University of Georgia, Athens, 30602, USA

[2]Departamento de Patología Clínica, Universidade Estadual de Campinas, São Paulo, 13083-877, Brazil

Abstract

Inorganic polyphosphate (polyP) accumulates in acidocalcisomes, acidic calcium stores that have been found from bacteria to human cells. Proton pumps, such as the vacuolar proton pyrophosphatase (V-H^+-PPase, or VP1), the vacuolar proton ATPase (V-H^+-ATPase), or both, maintain their acidity. A vacuolar transporter chaperone complex (VTC) is involved in the synthesis and translocation of polyP to these organelles in several eukaryotes, such as yeast, trypanosomatids, Apicomplexan, and algae. Studies in trypanosomatids have revealed the role of polyP and acidocalcisomes in osmoregulation and calcium signaling.

Introduction

The organelles known as acidocalcisomes were first identified in trypanosomatids [1, 2], although they were known before with other names such as metachromatic [3] or volutin [4] granules. However, these granules, first described in bacteria, were though to lack a delimiting membrane [5] until two species of bacteria were found in which a surrounding membrane is present [6, 7]. When Wiame reported the presence of polyphosphate (polyP) in the yeast vacuole [8] they were also named as polyP granules or bodies. Recent work is mammalian cells have discovered the presence of polyP in organelles which were then recognized as acidocalcisomes, such as the human platelet dense granules [9], and mast cell and basophil granules [10]. These studies established that acidocalcisomes are membrane-bounded organelles that have been conserved from bacteria to human cells, and are defined by their common properties: the abundant presence of polyP, calcium and other cations, and their acidity.

It has been proposed that acidocalcisomes occur in all domains of life, including archaea, and may date back as far as to the last universal common ancestor or unancestor [11]. Some acidocalcisomes, like those of trypanosomes, share their biogenesis mechanism with organelles known as lysosome-related organelles (LROs) [10, 12]. Adaptor protein 3 (AP-3), a protein complex involved in transport of membrane proteins to LROs of mammalian cells

*Corresponding author: Center for Tropical and Emerging Global Diseases, and Department of Cellular Biology, University of Georgia, 350A Paul D. Coverdell Center, 500 D.W. Brooks Dr., Athens, GA 30602, USA, Tel: 706-542-8104; Fax: 706-542-9493; ; Email: rdocampo@cb.uga.edu

[13] is involved in the biogenesis of acidocalcisomes of *Leishmania major* [14] and *Trypanosoma brucei* [15]. These results suggest that similar membrane-bounded polyP-containing acidic calcium stores could have appeared either autogenously or by convergent evolution.

Acidocalcisomes have been well studied in bacteria [6, 7], *Dictyostelium discoideum* [16], *Chlamydomonas reinhardtii* [17, 18], trypanosomatids [1, 2], Apicomplexan parasites [19, 20], human cells [9, 10] and eggs of different origin [21, 22]. PolyP was found to accumulate in other acidic organelles that could also be considered acidocalcisomes, such as the small granules found in the Gram-negative sporulating bacterium *Acetonema longum* [23], the acidocalcisome-like vacuoles of the arbuscular mycorrhizal fungus *Rhizophagus* sp. [24], or the spherites found in the midgut of the caterpillar *Anticarsia gemmatalis* [25].

Transporters and channels in acidocalcisomes

The acidity of acidocalcisomes is maintained by two proton pumps, a vacuolar H^+-pyrophosphatase (V-H^+-PPase), and a vacuolar H^+-ATPase (V-H^+-ATPase), or by both [26]. Few organelles are known to have these two proton pumps together in addition to acidocalcisomes [26–28], such as the plant vacuole [29], the malaria vacuole [30], the *T. cruzi* [31], *C. reinhardtii* [17], and *Dictyostelium discoideum* [16] contractile vacuoles, and the *T. gondii* plant-like vacuole [32]. A Ca^{2+}-ATPase involved in Ca^{2+} uptake was characterized in acidocalcisomes of different species [33] and a recent proteomic analysis of acidocalcisome of *Trypanosoma brucei* revealed the presence of other transporters involved in transport of P_i, Zn^{2+}, Fe^{2+}, and polyamines [26], in addition to Ca^{2+}/H^+ and Na^+/H^+ exchangers [20, 34]. At least two channels have been described in acidocalcisomes, an aquaporin or water channel in *T. cruzi* [35], and an inositol 1,4,5-trisphosphate receptor or calcium channel in *T. brucei* [36]. The presence of mechanisms for uptake and efflux of Ca^{2+} suggests an important role for acidocalcisomes in Ca^{2+} signaling. Fig. 1 shows a scheme of the pumps, channels, and exchangers present in procyclic stages of *T. brucei*.

Polyphosphate synthesis and degradation, and acidocalcisomes

Early work in *T. cruzi* demonstrated the presence of large amounts of polyP (Fig. 2) and polyP kinase and exopolyphosphatase activities in isolated acidocalcisomes [37]. Work in *D. discoideum* localized a polyP kinase 1 (DdPPK1) to small vesicles that could correspond to acidocalcisomes [38]. A second PPK, named DdPPK2, which share characteristics and sequence identity with actin-related proteins, was also proposed to be in acidocalcisomes [39]. More recent work in *Sacharomyces cerevisiae* found that a vacuolar transporter chaperone 4 (ScVtc4p) is a polyP polymerase that uses ATP to generate polyP [40]. A previous report already revealed that *null* mutants of several Vtc proteins resulted in less polyP synthesis [41]. Vtc4p is the catalytic subunit of a complex of four proteins (Vtc1-4p) that form heterotrimeric complexes that couple synthesis and translocation of polyP to the acidocalcisome-like vacuole preventing its toxicity when in the cytosol and using an electrochemical gradient as a driving force [42]. A similar role was found for the products of the *VTC4* genes present in acidocalcisomes of *T. brucei* [43] and *T. cruzi* [44]. These enzymes (TbVtc4 and TcVtc4) synthesize predominantly short chain polyP (~100–300 P_i

residues). TbVtc4 [43] and TbVtc1 [45] are essential in *T. brucei*. Vtc2 and Vtc4 homologs were also found in *Toxoplasma gondii*, where they are also involved in polyP synthesis although it is not clear whether they are in acidocalcisomes because Vtc2 does not co-localize with the V-H^+-PPase [46]. A Vtc1 homolog necessary for acidocalcisome formation was also found in *C. reinhardtii*, which also possesses a Vtc4 homolog [47]. Mutants deficient in CrVtc1 contain less polyP and acidocalcisomes [47]. Proteomic studies of acidocalcisomes of the red alga *Cyanidioschyzon merolae* (Vtc1) [28] and of *T. brucei* (Vtc1, Vtc4) [26] revealed the presence of Vtc proteins in these organelles.

A vacuolar soluble pyrophosphatase (VSP) localizes to acidocalcisomes and the cytosol of different trypanosomatids [48–50]. This enzyme has two distinct domains, an N-terminal EF-hand-like domain and a C-terminal catalytic domain and can hydrolize either PP_i or polyP. Its overexpression in *T. cruzi* results in significant decrease in cytosolic PP_i, and short and long chain polyP levels, accompanied by a growth defect, less responsiveness to hyperosmotic stress and reduced persistence of the parasite in tissues of mice, suggesting a role of PP_i and polyP under stressful conditions in the host and in maintaining a persistent infection [50]. Table 1 summarizes the acidocalcisome enzymes involved in polyP metabolism that have been identified in eukaryotes

Function of acidocalcisome polyphosphate in osmoregulation

The role of acidocalcisome polyphosphate in osmoregulation was better studied in *T. cruzi*. When epimastigote stages are exposed to hyposmotic stress there is a microtubule- and cyclic AMP-mediated fusion of acidocalcisomes to the contractile vacuole that results in translocation of a water channel or aquaporin (TcAQP1) to this organelle [35]. A model has been proposed according to which this fusion of acidocalcisomes with the contractile vacuole together with a rise in ammonia and its accumulation in acidocalcisomes as NH_4^+ would result in activation of acidocalcisome polyP hydrolysis and the resulting transfer of osmolytes (amino acids, cations and P_i) and water (through the AQP) to the contractile vacuole facilitating its swelling. After water release to the extracellular medium amino acids, cations and P_i would return to the cytosol helping the regulatory volume decrease (RVD). Evidence in favor of this model is the presence of a phosphodiesterase C (PDEC), which would terminate cyclic AMP stimulation, in the spongiome of the contractile vacuole [51], the inhibition of RVD by PDEC inhibitors [52], microscopic evidence of fusion of these organelles [53], and the presence in the contractile vacuole of a sodium-phosphate symporter that could be involved in recycling of P_i produced by the hydrolysis of polyP during RVD [54]. In addition, proteins involved in organellar fusion were detected in the contractile vacuole (SNAREs, TcRab32), and in acidocalcisomes (TcVAMP7) [31, 53].

In contrast to what occurs under hyposmotic stress, hyperosmotic stress results in increased synthesis of acidocalcisome polyP, which by complexing cations results in decreased cytosolic ions that, together with water release by the contractile vacuole through TcAQP1, results in shrinking of the cells. In support of this model treatment of epimastigotes with $HgCl_2$, 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 contractile vacuole mediates water

efflux during hyperosmotic challenge. Shrinking is also favored by cation elimination through a cation channel (TcCAT) that is translocated to the plasma membrane of epimastigotes submitted to hyperosmotic stress [55]. Inhibitors of TcCAT ($BaCl_2$, 4-aminopyridine) inhibit shrinking of trypomastigotes under hyperosmotic stress [55]. Synthesis of polyP and inorganic ions sequestration in acidocalcisomes prevents the deleterious effects of a cellular increase in ionic strength. 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 [56, 57].

Osmoregulation defects are observed when expression of acidocalcisome enzymes involved in polyP synthesis [43] or degradation [50] are altered, evidencing the involvement of acidocalcisomes in this process.

Acknowledgments

Funding

This work was supported in part by the United States National Institutes of Health [grant numbers AI-077538 and AI-108222] and from the São Paulo Research Foundation (FAPESP), Brazil [grant number 2013/50624-0]. N.L. is a postdoctoral fellow of FAPESP [grant number 2014/08995-4].

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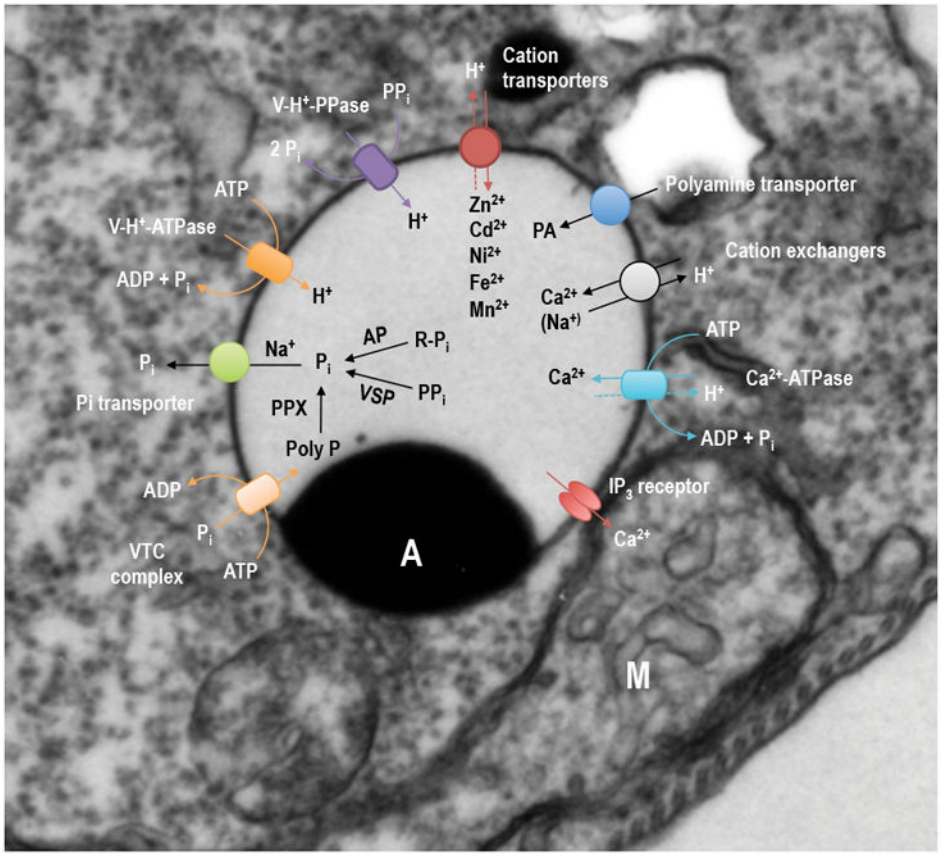

Figure 1. Pumps, channels and exchangers in acidocalcisomes of procyclic stages of *T. brucei*

Electron micrograph of a procyclic trypomastigote of *T. brucei* showing an acidocalcisome (A, recognized by the presence of an electron-dense inclusion of electron dense material) in close contact with the mitochondrion (M). Ca^{2+} is taken up by a H^+-countertransporting Ca^{2+}-ATPase and released by the inositol 1,4,5, trisphosphate receptor (IP_3 receptor). H^+ are pumped in electrogenically by either the vacuolar H^+-PPase (V-H^+-PPase) or the multisubunit vacuolar H^+-ATPase (V-H^+-ATPase). Na^+/H^+ or Ca^{2+}/H^+ exchangers are used for Na^+ uptake in exchange for H^+ or Ca^{2+} release in exchange of H^+. Cations transporters are used for either Zn^{2+}, Cd^{2+}, Ni^{2+}, Fe^2 or Mn^{2+} uptake. There is also a polyamine (PA) transporter. A vacuolar transporter chaperone complex (VTC) with at least two subunits (Vtc1 and Vtc4) synthesizes polyP using ATP and translocates it into the organelle. A Na^+/P_i symporter releases Na^+ and Pi form acidocalcisomes. Within acidocalcisomes there is a vacuolar soluble PPase (VSP), an exopolyphosphatase (PPX) and an acid phosphatase (AP).

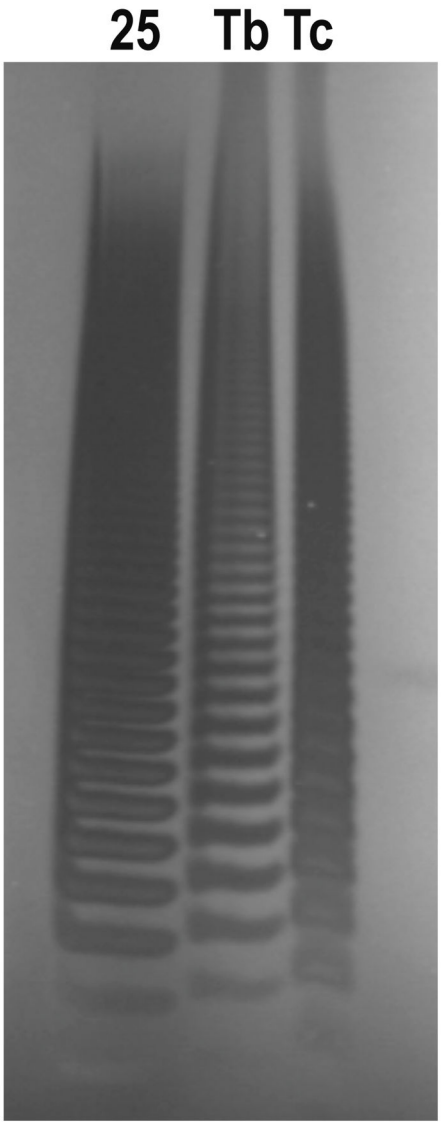

Figure 2. Short chain polyphosphate from *T. brucei* (Tb) and *T. cruzi* (Tc)

PolyP was extracted with titanium dioxide beads (62) and resolved by 35% polyacrylamide gel electrophoresis. 25 corresponds to short chain polyphosphate of maximum length of 25 P_i units. Gels were stained with 4′,6-diamidino-2-phenylindole (DAPI).

Table 1

Enzymes involved in polyP metabolism studied in eukaryotes.

Enzyme abbreviation	Enzyme name	Protein Function	Organism	NCBI Reference number	Ref.
DdPPK1	PolyP kinase 1	PolyP synthesis	*D. discoideum*	AAD53165.1	38
DdPPK2	PolyP kinase 2	Reversible polyP synthesis	*D. discoideum*	XP_636500.1, XP_645275.1, XP_636191.1	39
Vtc1p	Vacuolar Transporter Chaperone 1	PolyP synthesis (subunit of VTC complex)	*S. cerevisiae*	NP_010995.1	41, 42
Vtc2p	Vacuolar Transporter Chaperone 2	PolyP synthesis (subunit of VTC complex)	*S. cerevisiae*	NP_116651.1	41, 42
Vtc3p	Vacuolar Transporter Chaperone 3	PolyP synthesis (subunit of VTC complex)	*S. cerevisiae*	NP_015306.1	41, 42
Vtc4p	Vacuolar Transporter Chaperone 4	PolyP synthesis (catalytic subunit of VTC complex)	*S. cerevisiae*	NP_012522.2	40–42
CrVtc1	Vacuolar Transporter Chaperone 1	PolyP synthesis (subunit of VTC complex)	*C. reinhardtii*	XP_001690865.1	47
CmVtc1	Vacuolar Transporter Chaperone 1	PolyP synthesis (subunit of VTC complex)	*C. merolae*	XP_005537755.1	28
TbVtc1	Vacuolar Transporter Chaperone 1	PolyP synthesis (subunit of VTC complex)	*T. brucei*	XP_846013.1	26, 45
TbVtc4	Vacuolar Transporter Chaperone 4	PolyP synthesis (catalytic subunit of VTC complex)	*T. brucei*	XP_829284.1	26, 43, 44
TcVtc4	Vacuolar Transporter Chaperone 4	PolyP synthesis (catalytic subunit of VTC complex)	*T. cruzi*	XP_821342.1	44
TgVtc2	Vacuolar Transporter Chaperone 2	PolyP synthesis (subunit of VTC complex)	*T. gondii*	XP_002372055.1	46
ScPPX1	Exopoly phosphatase 1	PolyP hydrolysis	*S. cerevisiae*	AAA65933.1	58
ScPPN1	Endopoly phosphatase 1	PolyP hydrolysis	*S. cerevisiae*	NP_010740.3	59
TcPPX	Exopoly phosphatase	PolyP hydrolysis	*T. cruzi*	AAQ11880.1	60
LmPPX	Exopoly phosphatase	PolyP hydrolysis	*L. major*	CBZ11834.1	61
TbPPX	Exopoly phosphatase	PolyP hydrolysis	*T. brucei*	AAP74699.1	62
TbVSP	Vacuolar Soluble Pyrophosphate	PolyP and PPi hydrolysis	*T. brucei*	AAP74702.1	48
LaVSP	Vacuolar Soluble Pyrophosphate	PolyP and PPi hydrolysis	*L. amazonensis*	AAP74700.1	49
TcVSP	Vacuolar Soluble Pyrophosphate	PolyP and PPi hydrolysis	*T. cruzi*	XP_807207.1	50