Ultrastructural Characterization of the Adhesion Plaque in Trypanosoma cruzi

AUGUSTO, I.[1], Girard Dias, W.[2] and Miranda, K.[3]

[1] Federal University of Rio de Janeiro, Brazil, [2] Oswaldo Cruz Foundation, Brazil, [3] Federal University of Rio de Janeiro, Brazil

Chagas disease, a neglected disease endemic in Latin America and spreading around the globe due to human migration, affects 7 million people worldwide and cause about 7.000 deaths per year (1). The current treatment is only efficient for its acute phase and limited by severe side effects. In this regard, the research aiming new therapeutic targets is extremely important for the development of more efficient drugs. This disease is caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi),* which faces several environmental challenges during its biological cycle between the invertebrate and mammalian hosts (2). One of them is the osmotic stress due to different environments inhabited by the parasite. *T. cruzi* possess a precise osmoregulatory system that involves acidic organelles rich in calcium, polyphosphates and other cations, called acidocalcisomes and the contractile vacuole complex (CVC), an organized structure formed by a bladder vacuole surrounded by a set of vesicles and tubules called spongiome, responsible for accumulating and release water during an osmotic shock (3). The process of efflux of the accumulated water occurs probably through flagellar pocket by unknown mechanisms. Evidences indicate the presence of an adhesion plaque (AP) between CVC and flagellar pocket that would act as a water exit site during osmoregulation, but its structure and composition was never described (4). Thus, the aim of this work was to study the ultrastructure of adhesion plaque in *T. cruzi*, using as tool mutant epimastigotes (TcVps34) that present super-efficient responses to hyposmotic shock. The samples were processed for freeze-fracturing and high-pressure freezing, followed by freezing substitution and visualized by advanced three-dimensional electron microscopy methods, such as electron tomography and dual beam electron microscopy. The results showed no significant differences between adhesion plaque's structure in wild type and TcVps34 parasites, despite dramatic differences on both CVC systems. We observed the AP with irregular shape and with variations in the volume along its extension (Fig. 2), making the membranes of the bladder vacuole and flagellar pocket parallel and rectilinear (Fig 1 and 2A). Several filaments measuring 6-8 nm thick were also observed connecting both flagellar pocket and bladder, forming a dense filamentous network (Fig. 2F-G and Fig. 3). Some cells presented two APs, suggesting their possible importance for CVC division (data not shown). No pores or disrupted membranes were observed on the plaque region. These data suggest the adhesion plaque as a microdomain of the CVC and flagellar pocket, attaching CVC on flagellar pocket and participating in the response to cell volume changes.

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References

1. World Health Organization. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015;(6):33 - 44.

2. Jimenez V. Dealing with environmental challenges: mechanisms of adaptation in Trypanosoma cruzi. Res Microbiol. 2014;165(3):155 - 65.

3. Rohloff P, Montalvetti A, Docampo R. Acidocalcisomes and the contractile vacuole complex are involved in osmoregulation in Trypanosoma cruzi. J Biol Chem. 2004;279(50):52270 - 81.

4. Girard-Dias W, Alcantara CL, Cunha-e-Silva N, De Souza W, Miranda K. On the ultrastructural organization of trypanosoma cruzi using cryopreparation methods and electron tomography. Histochem Cell Biol. 2012;138(6).

Figure's caption

Figure 1. Freeze fracture imaging of wild-type (WT) and mutant TcVps34 cells. (A) Arrows show a set of IMP's in adhesion plaque. (B) asterisk points to parallelism of flagellar pocket and CVC membrane. (C) Row organization of IMPs in plaque. (D) Bladder in lamellar shape connect to flagellar pocket by adhesion plaque. B - bladder; Sp - Spongiome; FP - flagellar pocket; F - flagellum; K - kinetoplast; N-nucleus.

Figure 2. Electon tomography and 3D model of TcVps34 cell. (A) Eletrondense region between arrows suggests presence of adhesion plaque. (B) 3D model of CVC linked to flagellar pocket by AP. (C) front view of 3D model of AP. (D) Different thicknesses along the plaque are observed. (E) AP located close to flagellar pocket base, pointed by arrow. (F) and (G) 3D model and virtual section in YZ plane of tomogram, it's possible to observe the presence of filaments. B - bladder; Sp - spongiome; FP - flagellar pocket; AP - adhesion plaque. Scale bar=200 nm.

Figure 3. Closer view of adhesion plaque filaments. (A) AP is circled and arrowheads are pointing to filaments orientation; (B) 3D model associated with virtual section. (C) filamentous network in 3D model.

Figure 1

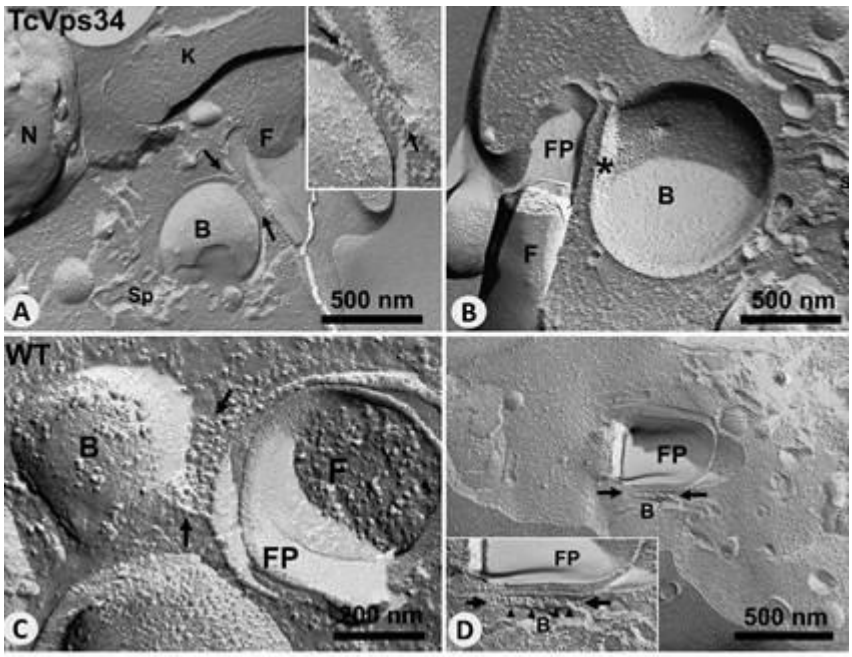

Figure 2

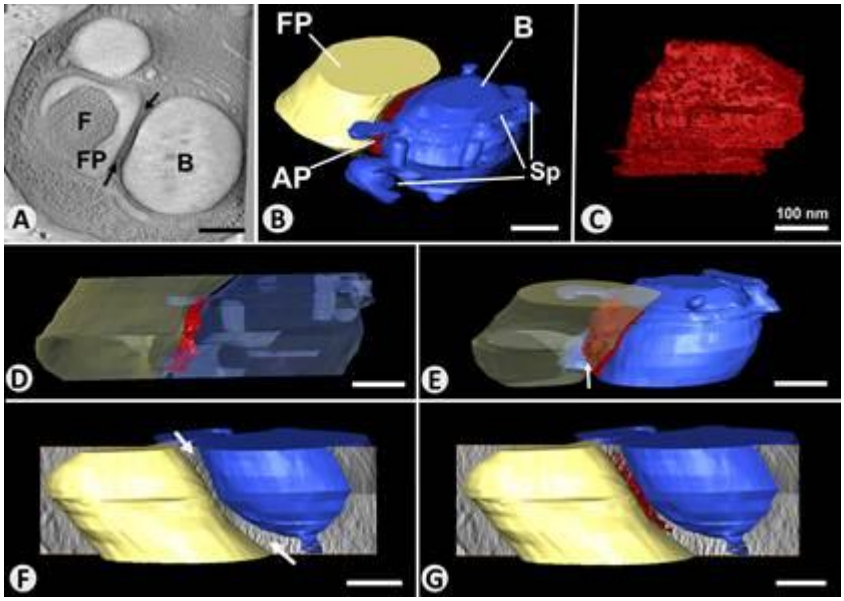

Figure 3