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Identification and subcellular localization of TcHIP, a putative Golgi zDHHC palmitoyl transferase of *Trypanosoma cruzi*

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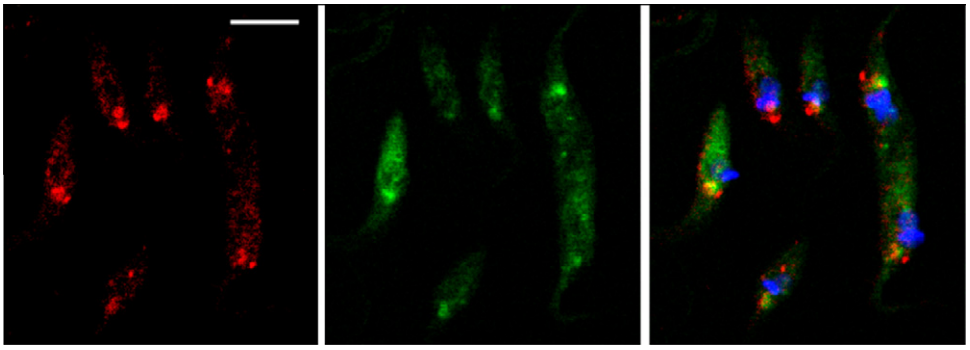
HIGHLIGHTS

- TcHIP is a putative zDHHC palmitoyl transferase of *T. cruzi*.
- TcHIP is expressed in all developmental forms of *Trypanosoma cruzi*.
- TcHIP colocalized with TcRab7, a Golgi marker of *T. cruzi*.

GRAPHICAL ABSTRACT

Confocal microscopy showing TcHIP immunolocalization at the Golgi complex of *T. cruzi* epimastigotes. Red: TcHIP; Green: TcRab7/GFP (Golgi marker); Merged image to the right: Blue–nucleus and kinetoplast; Yellow–TcHIP-TcRab7/GFP colocalization. Bar = 5 μm.



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ABSTRACT

Protein palmitoylation is a post-translational modification that contributes to determining protein localization and function. Palmitoylation has been described in trypanosomatid protozoa, but no zDHHC palmitoyl transferase has been identified in *Trypanosoma cruzi*, the etiological agent of Chagas disease in Latin America. In this study we identify and show the subcellular localization of TcHIP (Tc00.1047053508199.50), a putative *T. cruzi* zDHHC palmitoyl transferase. Analysis of the deduced protein sequence indicates that it contains ankyrin repeats (Ank and Ank2) and the zDHHC conserved domain, typical of zDHHC palmitoyl transferases. A TcHIP polyclonal antiserum obtained from mice immunized with the purified recombinant protein was used to study the presence and subcellular localization of the native enzyme. In western blots this antiserum recognized a protein of about 95 kDa, consistent with the predicted molecular mass of TcHIP (95.4 kDa), in whole extracts of *T. cruzi* epimastigotes, metacyclic trypomastigotes and intracellular amastigotes. Immunolocalization by confocal microscopy showed TcHIP labeling at the Golgi complex, co-localizing with the *T. cruzi* Golgi marker TcRab7-GFP. Transfectant *T. cruzi* epimastigotes containing a construct encoding TcHIP fused to proteins A and C (TcHIP/AC) were obtained. In western blotting experiments, the TcHIP polyclonal antiserum recognized both native and TcHIP/AC proteins in extracts of the transfectants. Confocal microscopy showed co-localization of native TcHIP with TcHIP/AC. These findings demonstrate the presence of a putative zDHHC

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palmitoyl transferase (TcHIP) containing ankyrin and zDHHC domains in different developmental forms of *T. cruzi*, and its association with the Golgi complex.

1. Introduction

Palmitoylation is a type of acylation that involves addition of palmitic acid to a protein. This modification can regulate the protein's localization, function, membrane anchoring, lipid raft segmentation and trafficking (Corvi et al., 2011). It is catalyzed by two different families of palmitoyl transferases. One group present a cysteine-rich domain, containing a zinc molecule and the "asp-his-his-cys" motif (zDHHC), which is responsible for their function (Putilina et al., 1999). The other enzyme type is the membrane-bound *O*-acyltransferase (MBOAT), which is involved in palmitoylation of various signaling proteins (Corvi et al., 2011).

The human Huntingtin Interacting Protein 14 (HIP-14) is a zDHHC palmitoyl transferase that is associated with the Golgi complex (Singaraja et al., 2002) and regulates the trafficking and function of the neuronal protein huntingtin (Yanai et al., 2006). The enzyme PfAnkDHHC palmitoyl transferase (containing ankyrin and zDHHC domains) is found in the protozoan *Plasmodium falciparum* and, like its human homologue HIP-14, is also found at the Golgi complex (Seydel et al., 2005). Although protein palmitoylation has been described in several apicomplexan protozoa, such as *Eimeria* spp. (Donald and Liberator, 2002), *Toxoplasma* spp. (Gilk et al., 2009) and *P. falciparum* (Rees Channer et al., 2006; Russo et al., 2009), little is known about the presence and function of palmitoyl transferases in trypanosomatid protozoa.

Myristoylation is an acyl modification that increases the total hydrophobicity of the protein. Some proteins also require palmitoylation at their N-terminus after myristoylation, for stable membrane attachment and/or protein translocation to rafts/caveolae or intracellular liquid-ordered domains (Levental et al., 2010; McCabe and Berthiaume, 1999; McCabe and Berthiaume, 2001; Shahinian and Silvius, 1995; Webb et al., 2000). No such dually acylated protein have been reported in the trypanosomatids *Trypanosoma brucei, Trypanosoma cruzi* or *Leishmania major* (Emmer et al., 2011; Godsel and Engman, 1999; Tull et al., 2004; Tyler et al., 2009). In *T. brucei*, the etiological agent of African trypanosomiasis, a palmitoyl transferase containing the zDHHC domain is required for sorting of calflagin (a Ca^{2+}-binding protein) to the flagellar membrane (Emmer et al., 2009). The localization of calflagins depends on their acylation status: calflagins that are only myristoylated are targeted to the cell body membrane; if after myristoylation they are palmitoylated at their N-terminus, they are directed to the flagellar membrane (Emmer et al., 2011). The flagellar calcium-binding protein (FCaBP) of *T. cruzi* is another dually acylated protein (Godsel and Engman, 1999), dual acylation being necessary for protein targeting to both flagellum and lipid rafts at the flagellar membrane of this parasite (Tyler et al., 2009). Dual acylation is also essential for the export of phosphatidylinositol-phospholipase C (PI-PLC) to the outer surface of *T. cruzi* intracellular amastigotes (Furuya et al., 2000; Martins et al., 2010). Furthermore, the small myristoylated and palmitoylated SMP-1 protein is targeted to the flagellar membrane in *Leishmania major* (Tull et al., 2004). These various data indicate that protein acylation plays an important role in targeting proteins to the flagellar and other cell membranes in trypanosomatids.

No zDHHC palmitoyl transferase has previously been described in *T. cruzi*, the etiological agent of Chagas disease in Latin America. The aim of this work was to identify a palmitoyl transferase in this parasite and assess its expression level and subcellular localization. We report the presence of a zDHHC palmitoyl transferase (TcHIP), containing ankyrin and zDHHC domains, in various developmental forms of *T. cruzi*, associated with the Golgi complex.

2. Material and methods

2.1. Reagents

Alkaline phosphatase-conjugated goat anti-mouse or goat anti-rabbit antibodies, mouse anti-histidine antibody, rabbit anti-protein A antibody, neomycin (G418), bromophenol blue, β-mercaptoethanol, bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), Dulbecco's Modified Eagle's Medium (DMEM) and Freund adjuvant were purchased from Sigma Co. (St. Louis, MO, USA). Hoechst 33342, goat anti-mouse antibodies coupled to AlexaFluor-488 or AlexaFluor-594, Bench Mark prestained Protein Ladder and Bench Mark Protein Ladder were purchased from Life Technologies-Invitrogen Co. (Carlsbad, CA, USA). Coomassie Blue R-250 was purchased from Merck Co. (Darmstadt, Germany). Alu-Gel-S adjuvant was purchased from Serva Electrophoresis GmbH Co. (Heidelberg, Germany). Fetal calf serum (FCS) was purchased from Cultilab Ltda. (Campinas, SP, Brazil). Isopropylthio-β-galactoside (IPTG) was purchased from Anresco Laboratories Inc. (San Francisco, CA, USA).

2.2. Parasites

Cultured epimastigotes of *T. cruzi* clone Dm28c (Contreras et al., 1988) were maintained by weekly passages at 28 °C in liver infusion tryptose (LIT) medium (Camargo, 1964) supplemented with 10% heat inactivated FCS. For TcHIP cloning, DNA was extracted from three-day-old cultured epimastigotes by a phenol–chloroform method (Sambrook et al., 1989).

In vitro-derived *T. cruzi* metacyclic trypomastigotes were obtained by incubating epimastigotes in TAU3AAG medium, according to a previously described metacyclogenesis (epimastigote-to-trypomastigote differentiation) protocol (Contreras et al., 1985). After 72 h of cultivation in this medium, about 80% of the cells in the supernatant were in the trypomastigote form.

To obtain *T. cruzi* intracellular amastigotes, 5×10^4 Vero cells (ATCC CCL-81) were seeded on circular glass coverslips and maintained at 37 °C in DMEM supplemented with 5% FCS in a humidified 5% CO_2 atmosphere. After 24 h, the cells were infected with *in vitro*-derived metacyclic trypomastigotes (ratio: 10 parasites per host cell). After 4 h of interaction, the cell monolayers were washed with PBS to remove non-adherent parasites and then further incubated in the same conditions. Intracellular amastigotes were obtained three days post-infection. Intracellular amastigotes were visualized by confocal microscopy of infected Vero cells.

2.3. In silico analysis

The TritrypDB database was searched for a *T. cruzi* gene encoding an aminoacid sequence of a putative palmitoyl transferase and a gene with the i.d. Tc00.1047053508199.50 (TcHIP) was identified. The deduced aminoacid sequence of TcHIP was aligned with the Protein Blast algorithm (Blastp-NCBI, Bethesda, MD, USA). For domain analysis pFAM software (Sanger Institute, Cambridge, UK) was used. The ClustalW algorithm (EMBL, Heildeberg, Germany) was used for multiple aminoacid sequence alignments by using the randomly selected sequences of putative zDHHC