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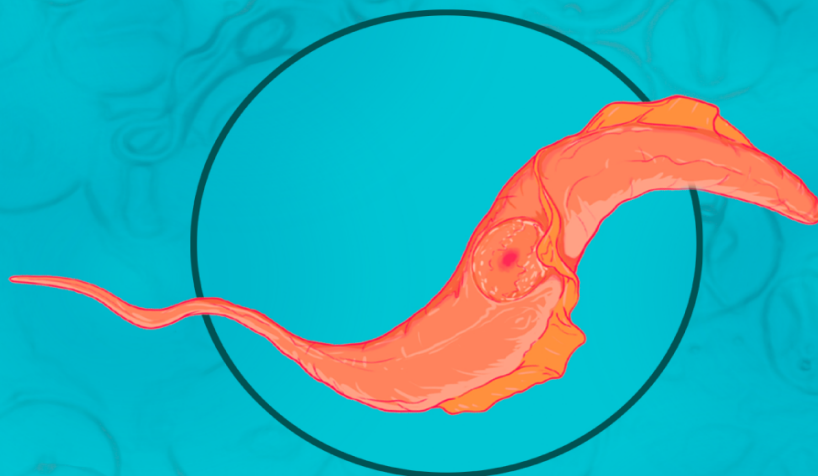
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Memorias



IX

Curso Internacional de
Tripanosomátidos

Simposio de Biología
Molecular de la
Enfermedad de Chagas



1803

**UNIVERSIDAD
DE ANTIOQUIA**

Facultad de Ciencias Exactas y Naturales

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Characterization of TcCAL1: a calcium binding protein and its role in the life cycle of *Trypanosoma cruzi*

Caracterización de TcCAL1: una proteína con dominios de unión a calcio y su función en el ciclo de vida de *Trypanosoma cruzi*

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In *Trypanosoma cruzi*, several studies have reported that intracellular calcium (iCa^{2+}) levels increase during metacyclogenesis and parasite adhesion to host cells. Also, channels that generate variations in iCa^{2+} concentration have been studied. However, most of the proteins that interact with this ion, possibly decoding its signals, have not been characterized. TcCAL1 is a 103 amino acid protein with EF-hand type domains for Ca^{2+} binding, with no known function and specific to kinetoplastids. In this work, we studied the role of TcCAL1 in some aspects of the parasite life cycle. By western blot, it was demonstrated that TcCAL1 is more abundantly expressed in the trypomastigote forms, compared to the amastigote and epimastigote stages. Through immunofluorescence microscopy, it was determined that TcCAL1 is localized throughout the cell body of the three stages mentioned. Also, cultures overexpressing TcCAL1 fused to a six-histidine tag (pTREX/TcCAL1x6His) and cultures containing the empty pTREX vector (controls) were obtained. In metacyclogenetic assays, overexpression of TcCAL1x6His significantly decreased the percentages of differentiation from epimastigote to metacyclic trypomastigote forms. When invasion processes were evaluated, overexpression of TcCAL1x6His caused an increase in the adhesion percentages of metacyclic trypomastigotes to the surface of Vero cells, as well as the number of parasites attached per cell. Similarly, the percentages of infected Vero cells and the number of intracellular amastigotes per cell increased in cultures overexpressing TcCAL1x6His. However, parasites overexpressing TcCAL1x6His showed similar epimastigote proliferation rates to controls, as well as differentiation of metacyclic trypomastigotes to axenic amastigotes. On the other hand, a yeast two-hybrid assay was performed expressing TcCAL1 as bait in conjunction with a *T. cruzi* cDNA library. As a result, two TcCAL1-interacting proteins were identified, with characteristic armadillo-like or prefoldin-like domains, respectively. Such interactions were studied by co-immunoprecipitation and mass spectrometry, where a protein with prefoldin domains was also identified. However, no proteins with armadillo domains were detected. Co-localization by immunofluorescence microscopy of epimastigotes expressing a fragment of the armadillo domain protein fused to an HA tag was evaluated using anti-HA and anti-TcCAL1 antibodies. As a result, signal intensity overlap was observed for both antibodies. These results allow us to hypothesize that TcCAL1x6His limits iCa^{2+} concentration in the parasite, negatively affecting metacyclogenesis. Also, we propose that overexpression of TcCAL1x6His promotes the invasiveness of *T. cruzi* to host cells by activating some Ca^{2+} -dependent function. Future studies aim to determine Ca^{2+} binding to TcCAL1 and to quantify iCa^{2+} levels in parasites overexpressing TcCAL1x6His against different components of the extracellular matrix. This study reaffirms the importance of studying uncharacterized proteins exclusive to the parasite to deepen our knowledge of *T. cruzi* biology.

Keywords: *Trypanosoma cruzi*, calcium binding protein, host-cell invasion

Palabras clave: *Trypanosoma cruzi*, proteína de unión a calcio, invasión en células hospedadoras