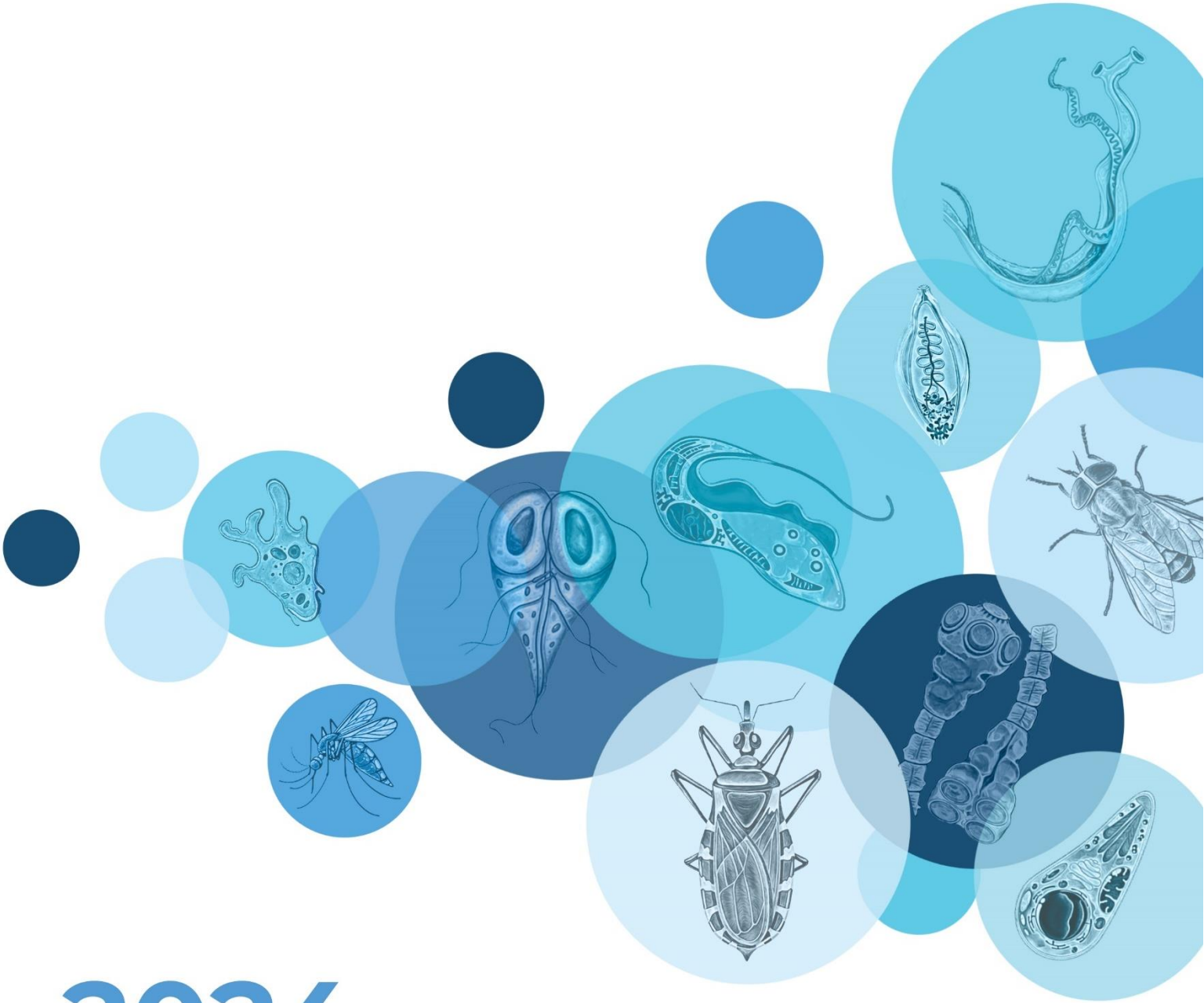
ISSN 2953-5751

Parasitus

Revista de la Sociedad Argentina de Protozoología



2024

PARASITUS - VOL.3

VOLUMEN 3 - 2024
ISSN 2953-5751

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Diseño de la Portada

"*Interdependencias parasitarias*"
Autor: Arturo Muñoz-Calderón

VOLUMEN 3 - 2024
ISSN 2953-5751

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La SUMOilación de proteínas es una modificación post traduccional que involucra la unión covalente de una proteína con homología estructural a ubiquitina llamada SUMO a residuos de lisina de distintas proteínas blanco generando cambios en la superficie de interacción de las mismas. La conjugación de SUMO a sus sustratos ocurre gracias a la acción secuencial de una enzima activadora (E1), una enzima conjugadora (E2) y en algunos casos una ligasa (E3). En nuestro laboratorio utilizamos como modelo de estudio *Trypanosoma brucei,* cuya superficie está cubierta por millones de copias de una única glicoproteína variable de superficie (VSG) inmunodominante. Este parásito es capaz de escapar del reconocimiento por parte del sistema inmune del hospedador mamífero mediante un proceso de variación antigénica mediante cambios de la expresión de un gen que codifica para una variante de VSG hacia otro distinto. Para que un gen de VSG sea transcripto debe localizarse en uno de los 20 sitios de expresión (ES) localizados en los telómeros. A su vez, la transcripción ocurre dentro de un compartimento extranucleolar específico llamado ESB ("expression site body") donde se encuentra la RNAPI, enzima responsable de la transcripción de VSG. Se ha visto que el ES activo está enriquecido en proteínas SUMOiladas, concentradas en un foco intenso denominado HSF (por "Highly SUMOylated Focus). Este HSF colocaliza tanto con la marca extranucleolar de la RNAPI como con el promotor del locus de VSG activo, y está ausente en los ES silenciados. En *T. brucei* se han identificado múltiples condensados nucleares asociados con el ESB, cada uno con funciones asociadas tales como splicing o la activación de la transcripción, y se ha observado que la SUMOilación de ciertos factores sería necesaria para la formación o la localización de ciertas proteínas en estos condensados. Para evaluar el rol de la SUMOilación en la formación de condensados que regulan la expresión de VSG utilizamos una herramienta basada en el sistema de operador:represor lac (LacO:LacI). Generamos una línea de parásitos sanguíneos que posee integradas repeticiones de lacO río arriba de un sitio de expresión de VSG. Además, estos parásitos expresan de manera inducible una fusión traduccional de la enzima E2 conjugadora de SUMO a LacI (LacI-E2) lo que permitirá que la enzima se dirija de manera específica al ES que posee integradas las repeticiones de lacO. De esta manera analizamos mediante inmunofluorescencia indirecta la localización de LacI-E2 luego de la inducción, así como también evaluamos si su reclutamiento a los operadores LacO da como resultado la formación de un nuevo HSF con consecuencias en la relocalización de la RNAPI y la expresión de una nueva variante de VSG.

BPA-39

Assessing the calcium binding properties of the kinetoplastid-specific protein TcCAL1

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Trypanosoma cruzi, the causative agent of Chagas disease, survives by invading different organisms and facing diverse environments. The parasite successfully completes its life cycle by differentiating into various developmental stages adapted to proliferate alternately in the extra- or intracellular space. Calcium (Ca^{2+}) plays important roles during mammalian host cell invasion and the differentiation from epimastigotes into infective metacyclic trypomastigotes, a process called metacyclogenesis. Although it is well established that the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ increases during these events, the mechanisms that generate and decode this signal are not yet fully elucidated. Recently, we identified a hypothetical protein with putative Ca^{2+}-binding domains named TcCAL1 to study its potential relevance in the parasite's biology. TcCAL1 lacks homologs in mammalian genomes, contains two predicted EF-hand domains for Ca^{2+} binding, and is expressed throughout the entire *T. cruzi* life cycle. We found that the overexpression of TcCAL1 facilitates both the adhesion and internalization of *T. cruzi* into *in vitro*-cultured mammalian cells, thereby increasing the parasite's virulence. We also demonstrated that

TcCAL1 overexpression impairs metacyclogenesis in a dose-dependent manner. In this study, we investigated whether this phenotype is mediated by the Ca^{2+}-binding properties of TcCAL1. To this end, we first employed different approaches to assay the association of Ca^{2+} using recombinant TcCAL1x6His protein produced in bacteria (rTcCAL1). We found that rTcCAL1 associates with Ca^{2+}, emitting a dose-dependent signal when incubated in the presence of the fluorescent probe Quin-2. By contrast, studies using circular dichroism and nuclear magnetic resonance could neither confirm nor refute that rTcCAL1 binds Ca^{2+}, suggesting that the wild-type version of the protein may be needed to demonstrate ion association by these methodologies[#]. We then measured the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in epimastigotes overexpressing TcCAL1 using the ratiometric indicator Fura-2AM and a FlexStation3 robotic multi-plate spectrofluorometer. These experiments showed that, in response to specific stimuli such as mild digitonin treatment, the increase in $[Ca^{2+}]_i$ is significantly higher in control parasites compared to those overexpressing TcCAL1. This suggests that transgenic cultures exhibit more efficient buffering capacity against a Ca^{2+} influx into the cytoplasm due to the overexpression of TcCAL1 and its ion-binding property. Overall, these results encourage us to move forward with more studies aimed at elucidating the role of this kinetoplastid-specific protein in the metacyclogenesis of *T. cruzi*, as well on the invasion into the mammalian hosts. [#]Work done at PLABEM-IBR-CONICET

BPA-40

Insights into the *Trypanosoma cruzi* deacetylase TcDAC1: Identification of protein interactors and impact on parasite metacyclogenesis and cell invasion

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Chagas disease or American trypanosomiasis, caused by a *Trypanosoma cruzi* infection, is an endemic illness in Latin America. The drugs presently used for Chagas disease treatment have shown limited efficacy due to the emergence of resistant parasites and severe side effects. Some of the most recent studies on anti-parasitic drugs have focused on protein acetylation, a regulatory pathway essential for parasite biology, which is modulated by acetyltransferases (KATs) and deacetylases (DACs). We and others have previously reported the anti-parasite effects of different drugs modulating DAC activity. This makes these enzymes attractive candidates as targets for the design of new drugs or for drug repurposing. Specifically, the zinc-dependent class of parasite DACs has significantly diverged from their human counterparts. In this context, the aim of the present study was to gain insights into the zinc-dependent function of TcDAC1 in parasite biology. We showed that the ectopic inducible expression of this enzyme has phenotypic effects on both the replicative and infective forms of the parasite. We found that TcDAC1 overexpression reduced the capacity of epimastigotes for metacyclogenesis and the infectivity of trypomastigotes. We also observed that TcDAC1 cellular localization is nuclear and cytoplasmic in all parasite forms, but in epimastigote cells the signal showed a granular display with partial co-localization with nucleus, mitochondria and the flagellar pocket. Finally, through bioinformatic sequence analysis and AlphaFold modeling, we identified candidate proteins that interact with this enzyme. We found six proteins with the highest numbers of contacts: TcCLB.503697.90 (Translator factor GUF1, 84 residues), TcCLB.503515.14 (Ubiquitin-conjugating enzyme E2, 51 residues), TcCLB.504431.64 (High mobility group protein TDP1, 53 residues), TcCLB.503473.20 (tRNA(guanine-N1-) methyltransferase activity MET10), TcCLB.504147.20 (60S ribosomal protein L18, RPL18) and TcCLB.511867.110 (flap endonuclease-1, FEN1), revealing a possible role in control of DNA replication and repair and in chromatin structural maintenance. In addition, we identified one protein, TcCLB.511585.190 (Eukaryotic initiation factor 4A-1), that has the putative acetylation site (TGKTG) in a