Evaluating the Role of MCU and MCUb Components of the Mitochondrial Calcium Uniporter in *Trypanosoma cruzi* using the CRISPR/Cas9 System.

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INTRODUCTION: Calcium (Ca^{2+}) is a key-signaling ion for a variety of cell processes in trypanosomatids, whose mitochondria possess a channel (mitochondrial calcium uniporter or MCU) to take up Ca^{2+} that is predicted to be structurally simpler than those found in vertebrates. MCU protein is the pore-forming subunit, whereas its paralogue MCUb is considered a negative regulator of the uniporter complex. **OBJECTIVE:** To study the physiological role of mitochondrial Ca^{2+} uptake in *Trypanosoma cruzi* (causative agent of Chagas disease) by phenotypic characterization of mutant cell lines where genes encoding for TcMCU and TcMCUb have been knocked out by CRISPR/Cas9 genome editing or overexpressed. **MATERIALS AND METHODS:** We performed the ablation or disruption of both genes by co-transfecting *T. cruzi* epimastigotes with the Cas9/pTREX-n vector - including a specific sgRNA- and a DNA donor cassette containing a blasticidin resistance marker to induce DNA double-strand break repair by homologous recombination. Additionally, we cloned each gene in pTREX-n vector to overexpress both proteins. **RESULTS AND DISCUSSION:** *TcMCU*-knockout (KO) cells displayed a complete absence of mitochondrial Ca^{2+} uptake without affecting the membrane potential of digitonin-permeabilized *T. cruzi* epimastigotes. Additionally, the overexpression of *TcMCU* caused a significant increase in the ability of mitochondria to accumulate Ca^{2+} and generated an increase in reactive oxygen species production. Moreover, *TcMCU*-KO epimastigotes exhibit a higher growth rate at late exponential phase, and a long-lived phenotype in low-glucose LIT medium, while *TcMCUb*-KO cells have an important growth defect, suggesting that MCUb is essential for parasite survival. Increased expression of autophagy markers in *TcMCU*-KO cells suggests that the decreased mitochondria ability to take up Ca^{2+} promotes pro-survival mechanisms. Evaluation of other phenotypic features is currently in progress. **CONCLUSIONS:** *T. cruzi* mitochondrial Ca^{2+} uptake is solely performed by MCU. TcMUb is essential for *T. cruzi* epimastigotes growth *in vitro.*

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