IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NUCLEOTIDE SUGAR TRANSPORTERS OF *TRYPANOSOMA CRUZI*

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Introduction: Glycoconjugates are essential for survival and infectivity of parasites. Their synthesis occurs in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus using nucleotide sugars as substrates. These molecules, however, are mostly synthesized in the cytosol and must therefore be transported across the ER and Golgi membranes. This intracellular transport of nucleotide sugars is essential for glycosylation and it is carried out by nucleotide-sugar transporters (NSTs). Objectives: In this study we have identified and partially characterized three transporters from *Trypanosoma cruzi*, the etiological agent of Chagas’ disease. TcNST1 transports UDP-N acetylglucosamine (UDP-GlcNAc), TcNST2 transports UDP-galactose (UDP-Gal) and TcNST3 transports GDP-mannose (GDP-Man). Materials and methods: Complementation assays in yeast (*Kluyveromices lactis*) and mammalian cells (CHO) were analyzed by flow cytometry. In *Saccharomyces cerevisiae*, complementation was based on a test growth in the presence of Congo red. Sub cellular localization studies using GFP fusion proteins were performed by fluorescence microscopy. Gene expression was analyzed by RT-PCR. Infection assays with *T. cruzi* mutants were performed in VERO mammalian cells. Results and Discussion: We have identified a family of 11 putative NSTs. Heterologous expression of these genes in a *K. lactis* mutant strain revealed only one UDP-GlcNAc transporter (TcNST1). TcNST2 and TcNST3 were identified by complementation of CHO and *S. cerevisiae* mutant cells, respectively. The three transporters are localized to the Golgi apparatus and seem to be expressed in all stages of the parasite life cycle. Knockout experiments indicate that TcNST1 is essential. Single knockout alleles are partially impaired in cellular differentiation and infectivity. Conclusions: We have identified three nucleotide sugar transporters from *T. cruzi*, which are localized to the Golgi apparatus. The fact that the TcNST1 gene is essential suggests that TcNST1 is the only transporter of UDP-GlcNAc in *T. cruzi* and shows the importance of NSTs in these parasites.

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