Short communication

Minicircle classes heterogeneity within the TcIII and TcIV discrete typing units of Trypanosoma cruzi

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Abstract

The taxon Trypanosoma cruzi, causative agent of Chagas disease, is composed of several discrete typing units (DTUs) named TcI-TcVI, and Tcbat. The history of the taxon T. cruzi is known, even though several controversial aspects remain as the relationships between TcIII and TcIV. We analyzed cloned T. cruzi stocks pertaining to the seven DTUs by filter hybridization tests of PCR amplicons from minicircle variable regions and kinetoplast DNA probes. Minicircle DNA blots from the cloned stocks and filter hybridization with one TcI, one TcII, one TcV, one TcVI, three TcIII, one TcIV from North America and one TcIV kinetoplast DNA probes from South America revealed minicircle variable region cross-reaction in some T. cruzi DTUs probed. TcIII was heterogeneous in minicircle class composition, even though two TcIII probes revealed that a small fraction of minicircles cross-hybridized with the minicircles from the TcIII, TcV and TcVI DTUs. The minicircles of TcIV from North America cross-reacted only with TcIV from North America but not with TcIV stocks from Brazil and Bolivia. The results on minicircle cross-hybridizations are discussed in the context of RNA editing, mitochondrial function in T. cruzi DTUs.

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1. Introduction

Kinetoplast DNA (kDNA) is a unique structurally complex mitochondrial DNA in nature with maxicircles and minicircles present in Trypanosoma cruzi (order Kinetoplastida). Minicircles are present in several thousand copies per network and usually nearly identical in size (0.9–2.5 kb) (Schnaufer et al., 2002). They are frequently heterogeneous in sequence and encoding gRNAs. These gRNAs are coded in the variable region of the minicircles which show absence of cross-hybridization with cloned minicircle from heterologous DTUs (Sanchez et al., 1984; Macina et al., 1985; Britto et al., 1995). TcI and TcII stocks are ancient and heterogeneous, while TcV and TcVI stocks are recent and display low genetic diversity (Lewis et al., 2011; Flores-López and Machado, 2011). The relationships between T. cruzi DTUs TcIII and TcIV are not sufficiently demonstrated. A three ancestor model (TcI, TcII, and TcIII) suggests two independent hybridization events between TcII and TcIII to generate TcV and TcVI. However no information on the origin of TcIV was presented in that study (de Freitas et al., 2006). Three significant clusters were found with mitochondrial genes (mtTcI, mtTcII, mtTcIII) corresponding to TcI, TcII and TcIII-TcIV-TcV-TcVI (Barnabé et al., 2016). The aim of this study is to analyze the minicircles classes present in T. cruzi DTUs TcII and TcIV cloned stocks and to address the following questions: Do TcII and TcIV share minicircle classes with each other DTUs? Do TcIII and TcIV share minicircle classes with the other DTUs?

2. Material and methods

The panel of 21 T. cruzi stocks belonging to the six T. cruzi DTUs and Tcbat were examined by hybridization assays. We used the same DNA purified of these stocks for probes preparation and for minicircle variable region PCR assay (Table 1).

The amplification reactions were performed with oligonucleotides 121 and 122 (Wincker et al., 1994). PCR samples were transferred onto ten identical Hybond N⁺ nylon membranes (Amer sham, Little Chalfont, United Kingdom). The minimum amount of amplified DNA to perform filter hybridization tests is 30 ng, and under high stringency conditions any probe used should cross-hybridize and determine identity with the immobilized DNA in the membranes, unless they are heterologous DNAs. (See Fig. 1.)

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Ten kDNA minicircles probes (Table 1) were obtained as described in Véas et al. (1991). Finally, the probes were labeled using the random primer method with [α-32P] dATP. The electrophoresis signal and the hybridization profiles were analyzed by densitometry (NIH Image J software). The intensity of the radioactive signal with the corresponding hybridization probe was quantified as percentage of the positive control. Signals similar to background in areas without DNA weren't considered (as percentage of the positive control). Signals similar to background in areas without DNA weren't considered (as percentage of the positive control). Signals similar to background in areas without DNA weren't considered (as percentage of the positive control).

### 3. Results and discussion

The hybridization results with the same *T. cruzi* stock are maximum (100%) (Fig.1). However when a kDNA probe from TcII or TcIV stock hybridizes with another *T. cruzi* stock belonging to a similar DTU the hybridization signal is low or absent, indicating that each *T. cruzi* within the same DTU contains very different minicircle classes or the proportions of these are quite different. That means TcII and TcIV stocks are heterogeneous in their minicircle classes composition. Some minicircle classes of TcII (X109 and J2A) are found in other DTUs in very low amount suggesting they were conserved in the similar DTU TcII, TcV and TcVI as well. Some minicircle classes of TcV (StC10R) are found in other DTUs from North America and TcIV but not in TcII stocks from South America. Minicircle classes from TcII and TcIV DTUs, do not hybridize with minicircles belonging to the TcII, Tcbat, and the TcII stocks used in this study. This result also confirms that cross hybridizations are due to remnants or the constant regions present in the kDNA probes.

Each minicircle is replicated once/generation when released from the concatenated network, generating nicked minicircles, to avoid progeny code for the different gRNA present in a *T. cruzi* clone. Similar minicircle classes are detected between TcII of X109 with other TcII, and TcVI. The same occur with TcII of J2A with other TcII; TcIV from North and South America, TcV and TcVI. The high similarity in minicircle classes between the J2A clone with TcIV stocks could indicate the identity of the parental TcII which fused with the parental TcII. This result suggests that some minor minicircle classes are conserved after the fusion of TcII with TcIII and later recombination independent events to generate TcV and TcVI. Between TcII most of the *T. cruzi* clones tested contain very different minicircle classes to code for gRNAs. Restoration of the disrupted reading frames of mitochondri al transcript seems to be accomplished by strain specific RNA editing (Westenberger et al., 2006). The cross-hybridization signals obtained with one TcIV probe (Stc10R) with two TcIV samples from North America, but not with other two TcIV samples from South America suggests that minicircle class composition is quite different between North and South American Tc IV stocks. The same differentiation was shown by phylogenetic analysis with other genetic markers (Tomasini and Diosque, 2015). This TcIV probe also revealed that some minor minicircle classes are conserved within TcIV and TcVI but not in any other DTU. The heterogeneity of minicircle classes observed within the TcII and TcIV stocks represent different sets of gRNAs.

### 4. Conclusions

We report minicircle classes' heterogeneity in some Trypanosoma cruzi TcII and TcIV DTUs which represent different sets of guide RNAs within those DTUs. In some TcII stocks a fraction of minicircles classes are conserved in other TcII, TcIV, TcV and TcVI cloned stocks but in someone is strain specific as most of the cases. Future studies should be necessary using different methodologies to validate these conclusions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2017.03.017.

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References


Fig. 1. Hybridization patterns of different *Trypanosoma cruzi* stocks belonging to different DTUs. Different *T. cruzi* DTUs probes (TcI-sp 104 cl 1; TcII-CBB cl 3; TcIII-X109 cl 1; M5631 cl 5; Arma 1Bcl 3; JA 2 cl 2; TcIV-CAN III cl 1; STC10R cl 1; TcV-VCF cl 1; TcVI-V195 cl 1). Minicircle PCR amplicons stained with ethidium bromide Lane M, 100-base pair (bp) DNA ladder. A. Hybridization with probe TcI; B. Hybridization with probe TcII; C, D, E, F. Hybridizations with probes TcIII; G, H. Hybridizations with probes TcIV; I. Hybridization with probe TcV; J. Hybridization with probe TcVI.


