Short communication

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

S. Ortiz a, G. Osorio b, A. Solari a,⁎

a Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile
b Programa de Microbiología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 14 March 2017
Accepted 15 March 2017
Available online 16 March 2017

Keywords:
Trypanosoma cruzi
TcIII
TcIV
kDNA minicircles

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 TcVI 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 TcIII and TcIV cloned stocks and to address the following questions: Do TcIII and TcIV share minicircles classes within each DTUs? Do TcIII and TcIV share minicircles 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 (Amersham, 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.)
Table 1

Set of stocks and reference strains representing the known T. cruzi DTUs used in the study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>DTU</th>
<th>Origin</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tcbat</td>
<td>TcII</td>
<td>Brazil Sao Paulo</td>
<td>Myotis levis</td>
</tr>
<tr>
<td>Tcbat S1</td>
<td>TcII</td>
<td>Brazil Sao Paulo</td>
<td>Myotis albuscens</td>
</tr>
<tr>
<td>Cuica c1</td>
<td>TcI</td>
<td>Brazil Sao Paulo</td>
<td>Philander opossum</td>
</tr>
<tr>
<td>Sp104 c1a</td>
<td>TcI</td>
<td>Chile IV Region</td>
<td>Mepraia spinolai</td>
</tr>
<tr>
<td>CB4 c3*</td>
<td>TcII</td>
<td>Chile Talahuen</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>Es2 c1</td>
<td>TcI</td>
<td>Brazil Sao Felipe</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>X109 c2*</td>
<td>TcII</td>
<td>Paraguay Makhilwalaia</td>
<td>Canis familiaris</td>
</tr>
<tr>
<td>M6241 c1g</td>
<td>TcII</td>
<td>Brazil Belen</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>M5631 c1a</td>
<td>TcII</td>
<td>Brazil Marajo</td>
<td>Dasyus novenscintus</td>
</tr>
<tr>
<td>ARMA18 c3j</td>
<td>TcII</td>
<td>Paraguay Campo Loro</td>
<td>Dasyus novenscintus</td>
</tr>
<tr>
<td>ARMA13 c7f</td>
<td>TcII</td>
<td>Paraguay Campo Loro</td>
<td>Dasyus novenscintus</td>
</tr>
<tr>
<td>JAC c3a</td>
<td>TcII</td>
<td>Brazil Amazonas</td>
<td>Monodelphis sp.</td>
</tr>
<tr>
<td>Canll c1+</td>
<td>TcIV</td>
<td>Brazil Belen</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>DogTheis c1</td>
<td>TcIV</td>
<td>USA Oklahoma</td>
<td>Canis familiaris</td>
</tr>
<tr>
<td>9212102R c1</td>
<td>TcIV</td>
<td>USA Georgia</td>
<td>Procyon lotor</td>
</tr>
<tr>
<td>10 K26 c1</td>
<td>TcIV</td>
<td>Bolivia Santa Cruz</td>
<td>Astus sp.</td>
</tr>
<tr>
<td>St108 c1c</td>
<td>TcIV</td>
<td>USA Georgia</td>
<td>Procyon lotor</td>
</tr>
<tr>
<td>S2.80 c12</td>
<td>TcV</td>
<td>Bolivia Santa Cruz</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>VCR c1b</td>
<td>TcV</td>
<td>Chile San Felix</td>
<td>Triatoma infestans</td>
</tr>
<tr>
<td>V195 c1+</td>
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<td>Triatoma infestans</td>
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<tr>
<td>CH2 c1</td>
<td>TcVI</td>
<td>Chile San Pedro de Atacama</td>
<td>Triatoma infestans</td>
</tr>
</tbody>
</table>

The T. cruzi clones used in this study are described in Lewis et al., 2011.

* KDNA minicircles used as probe (see Materials and methods).

Ten KDNA minicircles probes (Table 1) were obtained as described in Veas et al. (1991). Finally, the probes were labeled using the random primer method with [$^\alpha$-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 considered as 100% (positive control). The identity of any heterologous sample tested against each probe was quantified as percentage of the positive control. Signals similar to background measured in areas without DNA weren't considered (≤5%). The hybridizations were repeated twice (see Supplementary material).

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 JAC) are found in other DTUs in very low amount suggesting they were conserved in the similar DTU TcV and TcVI as well. Some minicircle classes of TcV (St10R) are found in other similar DTUs from North America and TcII but not in TcIV stocks from South America. Minicircle classes from TcII and TcIV DTUs do not hybridize with minicircles belonging to the Tc, Tcbat, and the TcII stocks used in this study. This result also confirms that cross hybridizations are not 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 another replication event/generation (Kitchin et al., 1985). Available minicircle sequences show great heterogeneity in the whole T. cruzi taxon and within a parasite clone (Frasch et al., 1984; Macina et al., 1986; González, 1986; Degrave et al., 1988; Velazquez et al., 2008). The analysis of the variable regions from 170 minicircle sequences of four T. cruzi DTUs showed that only 56% exhibited significant homology and the other 44% corresponded to unique sequences (Telleria et al., 2006). These sequence studies also revealed that the most frequent mutations observed in those minicircle variable regions were mainly substitutions, transversions and also short insertions/deletions without large sequence rearrangements, suggesting they are the result of replication errors of mitochondrial DNA by DNA polymerase β, which generates a higher mutation rate in kDNA than in nuclear DNA (Schambacher-Reis et al., 2012; Maldonado et al., 2015). An unequal segregation of minicircles after the number of proliferation cycles could also contribute to their heterogeneous generation. The heterogeneity of different minicircle classes in T. cruzi Mexican TcI stocks was observed by KDNA hybridization tests as described, or a great heterogeneity of hybridization patterns, with T. cruzi populations in Brazil (Britto et al., 1995; Bosso et al., 2000). These results and other studies with highly characterized T. cruzi stocks from several countries of South America demonstrate that KDNA probes of T. cruzi DTUs TcI, TcII, TcV and TcVI are specific in hybridization tests under high stringency conditions and hybridize only with identical minicircle sequences (Brenière et al., 1998; Arenas et al., 2012; Rumi et al., 2013; Egaña et al., 2016; Bontempi et al., 2016). The results of positive cross-hybridization in this study, using the TcII and TcIV KDNA probes suggest that the total composition of minicircle classes varies between each TcII and TcIV stock as reported previously in TcI and TcII stocks (Arenas et al., 2012). This observation suggests that minicircle classes heterogeneity 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 JAC with other TcII; TcIV from North and South America, TcV and TcVI. The high similarity in minicircle classes between the JA2 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 mitochondrial 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 (Tomassini 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 some case 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.

Acknowledgements

We are grateful to Dr. Patrick Hamilton for send us Tcbat DNA samples, and to Dr. M. A. Miles for epimastigote T. cruzi clones DNA samples (Epi Net project. Contract N° 223034). We are grateful to Constanza Blanco for make the densitometry measurements. This work was supported by FONDECYT-Chile 1120122 to A. Solari.
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, TcII-X109 cl 1, M5631 cl 5, Arma 1Bcl 3, JA 2 cl 2; TcV-CAN III cl 1, STC10R cl 1; TcV-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.


