A SYNTHETIC OLIGONUCLEOTIDE PROBE THAT DISCRIMINATES BETWEEN THE SUBGENERA *SCHIZOTRYPANUM* AND *MEGATRYPANUM*

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Summary :

The article reports the use of synthetic oligonucleotides as an additional tool for discriminating between the subgenera *Schizotrypanum* and *Megatrypanum* of the genus *Trypanosoma*. Three oligonucleotides were synthesized based on conserved sequence blocks in the minirepeat of the kinetoplast DNA minicircles (kDNA) from *T. cruzi* and used as probes in dot-blot hybridization experiments against kDNA from trypanosomatids belonging to different genera. Under specific hybridization and washing conditions the oligoprobes were found to hybridize distinctly with protozoa from the subgenera *Schizotrypanum* and *Megatrypanum* and with two monogenetic insect trypanosomatids. One of the oligonucleotides showed to be specific for the subgenus *Schizotrypanum*. No cross-hybridization was detected with *T. rangeli*, which, in fact, belongs to another subgenus (Tejeraia, Añez, 1982), confirming the specificity of the probe. This promising result reinforces the use of oligoprobes for typing of trypanosomes.

KEY WORDS : oligonucleotides, kDNA, minicircles, hybridization, *Trypanosoma*.

Résumé : UNE SONDE OLIGONUCLÉOTIDIQUE SYNTHÉTIQUE CAPABLE DE FAIRE LA DISCRIMINATION ENTRE LES SOUS-GENRES *SCHIZOTRYPANUM* ET *MEGATRYPANUM*

L'article montre l'utilisation d'oligonucléotides synthétiques comme un outil additionnel capable de discriminer entre les sous-genres *Schizotrypanum* et *Megatrypanum* du genre *Trypanosoma*. Trois oligonucléotides ont été synthétisés selon les séquences conservées des régions des minicercles du DNA du kinétoplaste (kDNA) de *T. cruzi*. Ces oligonucléotides ont été utilisés comme sondes sur des hybridations en dot-blot contre des trypanosomatides appartenant à différents genres. Sous des conditions d'hybridation et lavage particulières les oligosondes ont hybridé spécifiquement avec des protozoaires des sous-genres *Schizotrypanum* et *Megatrypanum* et avec deux trypanosomatides monogénétiques d'insecte. Pourtant, un des oligonucléotides a révélé être spécifique pour le genre *Schizotrypanum*. Il n'y a pas eu d'hybridation croisée avec *T. rangeli*, qui appartient à un autre sous-genre (Tejeraia, Añez, 1982), ce qui a confirmé la spécificité de la sonde. Ce résultat encourageant suggère l'utilisation des oligosondes pour le typage des trypanosomes.

MOTS CLÉS : oligonucléotides, kDNA, minicercles, hybridation, *Trypanosoma*.

The genus *Trypanosoma* comprises parasites of a heteroxenous life cycle with great number of mammalian host and some species are pathogenic to man and domestic animals (Vickerman, 1976). The genus has been subdivided into subgenera according to Hoare (1964). In the subgenus *Schizotrypanum* the type-species *T. cruzi*, which is transmitted by triatomine bugs, is associated with Chagas' disease in Central and South America (Brener, 1977). Triatomine insects are also vectors of *T. rangeli* which belongs to a different subgenus, *Tejeraia* (Añez, 1982). The possibility of mixed infection in humans in endemic areas where both parasites are transmitted can be a factor confounding the diagnosis of Chagas disease. However, recently several molecular techniques have been reported which distinguish between these parasites (Houguin *et al.*, 1987; Murthy *et al.*, 1992; Souto & Zingales, 1993;

Steindel *et al.*, 1994). Besides these two subgenera triatomine bugs can be host to at least one species of the subgenus *Megatrypanum*, *T. conorhini* (Deane *et al.*, 1986). This latter subgenus has been reviewed by Wells (1976) and is considered of veterinary importance with some species being responsible for infections in Cervidae and Bovidae (Dirie *et al.*, 1990). Both *Schizotrypanum* and *Megatrypanum* are found in opossums which are the wild mammals most frequently examined in epidemiological investigations of Chagas' disease. Thus, the distinction between them is of importance in field studies.

In this short report we describe a simple method, using a synthetic oligonucleotide as a probe, to differentiate between the subgenera *Schizotrypanum* and *Megatrypanum* in dot-blot hybridization experiments. Different heteroxenous and monoxenous trypanosomatids of the genera *Trypanosoma*, *Leishmania*, *Endotrypanum*, *Phytomonas*, *Herpetomonas*, *Crithidia* and *Blastocrithidia* were tested in molecular hybridization experiments using synthetic oligoprobes. Three oligonucleotides were synthesized based on *T. cruzi* mini-

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Fig. 1. – Autoradiography showing dot-blot hybridizations with the synthetic oligoprobes TC4 (I), TC2 (II) and TC7 (III) against 20 ng of purified total kDNA from different trypanosomatids.

A1) *T.(S) cruzi* (Y strain), B1) *T.(S) cruzi* (CL strain), C1) *T.(S) cruzi* (opossum strain 234LI), D1) *T.(S) cruzi* (human isolate), E1) *T.(S) cruzi*-like (bat), F1) *T.(S) cruzi*-like (bat, Hastatus strain MJ7), G1) *T.(S) dionisii*, H1) *T.(T) rangeli* (San Agostin strain), A2) *T.(M) conorhini* (IOC strain), B2) *T.(M) freitasi* (IOC G219), C2) *Endotrypanum schaudinni* (IM217), D2) *Herpetomonas samuelpessoai* (ATCC 30252), E2) *Critibidia fasciculata* (ATCC 11745), F2) *C. deanei* (ATCC 30255), G2) *C.luciliae* (ATCC 14765), H2) *C. oncopelti* (ATCC 12982), A3) *Bastocrithidia culicis* (ATCC 30268), B3) *Phytomonas davidii*, C3) *Leishmania (L) enriettii* (LV90), D3) *Sauroleishmania tarentolae* (UCLA strain), E3) *L. (L) chagasi* (PP75), F3) *L. (V) guyanensis* (M4147), G3) *L.(L) amazonensis* (M2269), H3) *L. (L) major* (LRC L137).

circle repeat sequences (Degraeve *et al.*, 1988), encompassing the conserved blocks CSB1 (TC2 and TC4) and CSB2 (TC7) described by Ray (1989) with respective positions in the multiple alignment from Degraeve *et al.*, (1988): TC2 (26-mer, positions 31-56) TGGTTTTGGGAGGGG(C/G)(C/G)(T/G)TCAA(A/C)TTT; TC4 (22-mer, positions 31-52) TGGTTTTGGGAGGGGC-GTTCAA; and TC7 (26-mer, positions 69-94) TCATGCATCTCCCCGTACATTATTT. Probes were labelled with ³²P gamma ATP using polynucleotide kinase and hybridized in dot-blot experiments with purified total kDNA from different genera spotted onto nylon membranes. Techniques for kDNA extraction and dot-blot hybridization have been previously described (Pacheco *et al.*, 1986, 1990). Hybridizations were carried out at 42 °C overnight. The membranes were washed at the same temperature in 2X standard saline citrate (SSC)/0.5 % sodium dodecil sulphate (SDS) and exposed to X-ray films with intensifying screen at – 70 °C overnight.

The probes TC2 and TC4, the latter being 4 nucleotides shorter and lacking the redundancy in TC2, showed a very similar hybridization specificity and were found to hybridize with kDNA from parasites belonging to the subgenera *Schizotrypanum* and *Megatrypanum*. The probe TC2 was found to hybridize only with the genus *Trypanosoma* while TC4 showed an additional, albeit weak, signal with two monogenetic

insect trypanosomatids. However, the probe TC7 was found to be specific to the subgenus *Schizotrypanum*, showing no cross-hybridization with the kDNA from *T. rangeli* or any other tested genera (Fig. 1). This result is not unexpected, as the CSB2 motif (cCCCGTNC) is shorter than the CSB1 motif (AgGGGCGTTC) (Ray, 1989), and suggests the use of the TC7 oligoprobe as a tool for typing the subgenus *Schizotrypanum*. In addition, further applications for this probe can be found in preliminary screens of sylvatic animals captured in epidemiological surveys, in the quality control of strain banks and in the investigation of mixed infections.

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