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Short communication

Trypanosoma rangeli expresses a gene of the group II *trans*-sialidase superfamily☆

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Trypanosoma cruzi, the etiological agent of Chagas disease, and *Trypanosoma rangeli*, a nonpathogenic protozoa for mammals, present surface glycoproteins of the *trans*-sialidase superfamily (TSASF). According to sequence identity, molecular weight, and function [1–3], members of TSASF are classified into four groups. The first group includes *T. cruzi trans*-sialidase (TcTS) and *T. rangeli* sialidase (TrSial). TrSial expressed in *T. rangeli* epimastigotes forms is a strict hydrolytic enzyme that releases sialic acid residues from the host cell surface glycoconjugates [4–6]. In contrast, *T. cruzi trans*-sialidase transfers sialic residues from the host surface onto mucin molecules on the parasite's surface [3]. Although TrSial has been well characterized [4–9], its biological role remains unknown.

Members of group II *TSASF*, collectively known as gp85 (or *gp85*/*trans*-sialidase), are expressed in *T. cruzi* infective trypomastigotes forms, and intracellular amastigotes stages [1–3]. This group, which has only been described in *T. cruzi*, includes a set of heterogeneous GPI-anchored surface glycoproteins with similar molecular masses but different electrical charges. *gp85*/*trans*-sialidase proteins have been implicated in adhesion and/or internalization of the parasite to host cells [10,11], but none of its members have sialidase or *trans*-sialidase activity.

In a previous work, we cloned telomeric sequences from a *T. rangeli* [12]. One of the recombinants obtained, namely TrTel 4 (3376 bp), had an ORF with high percent identity with all members of the gp85/*trans*-sialidase family at 1 kb from the telomeric end, and with the transcription sense oriented from the centromere towards the telomere.

The putative 1953-bp long gene (*TrGP*) (Fig. 1A) encoded for 651 aminoacids (aa) putative protein with estimated mass of 71 kDa. At the nucleotide level, *TrGP* sequence displayed 62–67% identity (83–96% in some blocks) with *T. cruzi gp85*/*trans*-sialidase genes. In addition, the translated sequence of *TrGP* exhibited 45–50% identity (reaching 60% considering conservative amino acid substitutions) with proteins encoded by group II *TSASF* genes [10,13–17], and to a lesser degree (25–30% identity) with group I members of this superfamily, including *T. rangeli* sialidases (GenBank U83180, L14943). Blocks of sequence identity between TrGP and gp85 proteins are shown in Fig. 1B.

TrGP shares with all members of the TSASF [3] the following features:

(i) Two conserved copies of the bacterial neuraminidase motif SxDxGxTW (Asp Box) (Fig. 1A and B).
(ii) A partially complete copy of the subterminal element VTVxNVfLYNR (Fig. 1A and B). This motif, known

Abbreviations: aa, aminoacids; bp, base pair; CHEF, clamped homogeneous gel electrophoresis; gp85, surface glycoprotein of 85 kDa; GPI, glycosylphosphatidylinositol; kb, kilobase; kDa, kilodaltons; TBS, Tris-buffered saline; TcTS, active *trans*-sialidase of *T. cruzi*; TrSial, sialidase of *T. rangeli*; TSA, *trans*-sialidase

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Fig. 1. (A) Schematic representation of *T. rangeli* Trtel 4 clone. Blocks are to indicate major sequence features. Arrows indicate the sense of the coding sequence. Diagonal hatched bar represents TrGP-Nterm probe used in hybridization experiments. SubTR represents subterminal conserved region characteristics of *T. rangeli* telomere [12]. Relative positions of Asp-boxes and VTVxNVfLYNR motifs are shown (thick vertical lines). (B) Clustal W multiple alignment of deduced amino acid sequences from TrGP and four *T. cruzi* surface proteins of group II of *TSA* gene superfamily. Sequences are as follows: TrGP (AF426022), TcASP-2* (U77951), Tcsp-2* (AY186573), TcTSA-E2 (U02613) and Tc85KD (M64836). Conserved residues are in black (100% conservation), dark gray (75% conservation), and light gray (50% conservation). Overlining indicates the following motif of *gp85*/*trans*-sialidase in the TrGP: a predicted N-terminal signal peptide, two Asp boxes, VTVxNVfLYNR motif (⋆), and the partially conserved fRiP sialidase motif (✱). Sequence enclosed in pointed line is $TrGP^{Nterm}$ peptide. In order to improve the alignment, we did not consider a section of 38 amino acids 5′ the N-terminal signal peptide in TcASP-2 and Tcsp-2 sequences.

as peptide J, has been found in gp85 from *T. cruzi* bloodstream trypomastigotes, and it has been implicated in the binding to the mammalian host laminin [10].

(iii) At its N-terminus, it has a signal peptide to direct the protein to the endoplasmatic reticulum that shared a higher percent of identity with *T. cruzi* gp85/*trans*-sialidase members (77%) than with *T. rangeli* sialidase (45%) (Fig. 1B).

However, TrGP is devoid of a recognition site for the GPI anchor, a characteristic of many members of TSASF (Fig. 1B). Many residues regarded as important for TrSial

catalytic activity are absent in TrGP, for instance, the Arg residue in the conserved fRiP element is substituted by an uncharged aa (like *T. cruzi gp85*/*trans*-sialidase proteins) (Fig. 1B) [5,7,8]. Missing as well, is the N-terminal aa sequence LAPGSS, a characteristic of mature TrSial and TcTS [5].

To study the genomic organization of *TrGP* genes, we carried out Southern blot experiments using a 460-bp probe based on the N-terminal region of the ORF (TrGP-Nterm). This region was selected for its low nucleotide identity with other *TSA* superfamily genes. TrGP-Nterm was PCR amplified with the following primers: forward (5′-GTGTTGATGGCCTTTGGC-3′) and reverse (5′-GTATTTGTGATGCAGGCC-3′) (Fig. 1A). Fig. 2A shows the results of hybridizing TrGP-Nterm with *T. rangeli* (DOG-82 strain) genomic DNA digested with several restriction enzymes, the probe recognized many genomic fragments, indicating that *TrGP* related sequences are present in many copies in *T. rangeli* genome. A similar sample of *T. cruzi* YBM strain was negative (Fig. 2A, lane 4). Fig. 2B shows a hybridization experiment using TrGP-Nterm probe against *T. rangeli* chromosomal bands separated by pulsed field gel electrophoresis. Confirming the widespread presence of *TrGP* in *T. rangeli* genome, the probe recognized most chromosomal bands.

To check for TrGP transcription, we performed Northern blot assays using total RNA isolated from *T. rangeli* epimastigote stage. The result of this experiment revealed that *TrGP* is transcribed in mRNA species of about 4.7 kb (Fig. 2C). No hybridization was observed with an mRNA fraction isolated from *T. cruzi* YBM strain epimastigotes (not shown). A positive control consisting of a probe derived from *T. cruzi* ubiquitin gene, detected mRNA bands in both parasites (not shown).

To check for TrGP translation, we first generated an anti-TrGP rabbit antisera using as antigen a recombinant peptide derived from its N-terminal region ($TrGP^{Nterm}$). The 65 aa peptide had low identity with other members of TSA superfamily, including TrSial [4–6]. Briefly, recombinant pET-$TrGP^{Nterm}$ was constructed as follows: $TrGP^{Nterm}$ coding sequence was amplified from TrTel 4 recombinant using primers FwNterm (5′-CTTTTAGTGCATATGGCCTTT-3′) and RvNterm (5′-TGTTTAAAGCATATGTTACCCT-3′). For the insertion in the expression vector pET-28a (Novagen), *Nde*I sites were incorporated into both primers. The PCR product was digested with *Nde*I, and then inserted in-frame in pET-28a plasmid downstream from the region encoding for the initiation codon ATG and 6× His tag. Recombinant plasmids were transferred to *E. coli* BL21 cells, grown to mid-log phase, and finally induced with IPTG. After incubation for 3 h, protein expression was checked in cell extracts using SDS-PAGE. In the gels, a peptide with an apparent molecular mass of 6.9 kDa was observed; this peptide was purified with the B-PER® purification kit (PIERCE), and then used to immunize two rabbits as previously described [18]. Rabbit antiserum reactivity and specificity was tested in Western blotting experiments using epimastigotes cell lysates from

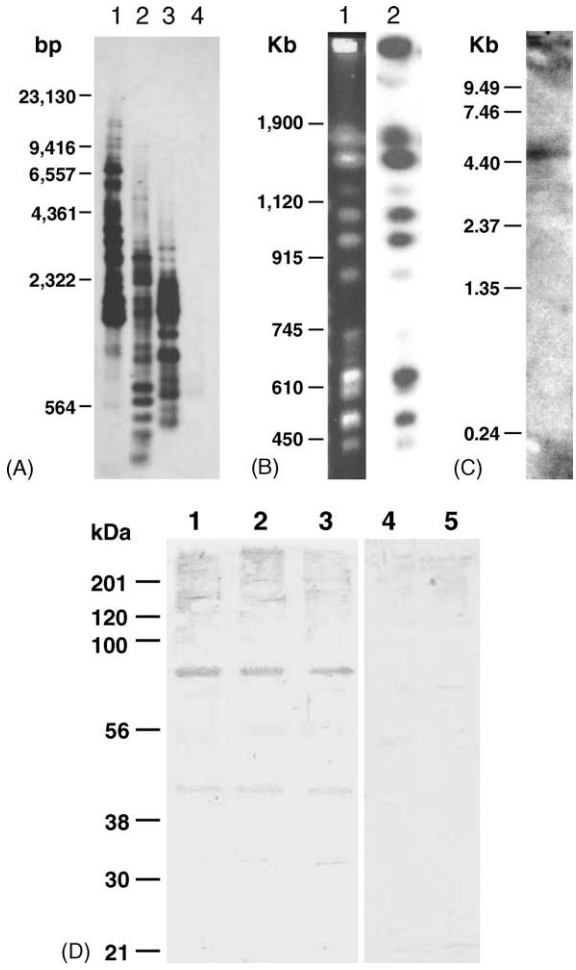

Fig. 2. Genomic organization and expression of *TrGP*. All the hybridization analyses were carried out with the ^{32}P-labelled TrGP-Nterm probe at high stringency conditions as previously described [19]. (A) Identification of *TrGP* sequences in *T. rangeli* genomic DNA by Southern blot. Lanes 1–3, *T. rangeli* DNA digested with *Pst*I, *Rsa*I and *Sau*3AI, respectively; lane 4, *T. cruzi* DNA digested with *Sau*3AI. Molecular sizes in kilobases (kb). (B) Chromosomal mapping of *TrGP* genes. Chromosomal bands of *T. rangeli* (DOG-82) were separated by CHEF as described in [12]. Lanes: 1, ethidium bromide stained gel; 2, blot after hybridization. Molecular sizes in kilobases (kb). (C) Northern blot analysis of the *TrGP* with total RNA from *T. rangeli* epimastigotes. Molecular sizes in kilobases (kb). (D) Identification of *T. rangeli* TrGP native protein by western blot. Proteins from lysates of parasites were separated by SDS-PAGE and transferred to Immobilon-P (Millipore) membranes. Filters were blocked with a solution of 5% non-fat dry milk in TBS (50 mM Tris, pH 8.0, 150 mM NaCl), incubated with $TrGP^{Nterm}$ antiserum, and the binding of antisera was revealed with alkaline phosphatase-conjugated goat anti-rabbit antibodies and the chromogen 3,3′-diaminobenzidine. Extracts proteins are from parasite isolates and stages: *T. rangeli* epimastigotes: M/HOM/VE/03/CARMEN HERNANDEZ (lane 1) and M/HOM/VE/98/ALBA (lane 2), *T. cruzi* M/HOM/VE/92/YBM metacyclic trypomastigotes (lane 3). Molecular sizes in kilodaltons (kDa).

three *T. rangeli* isolates, and epimastigotes and trypomastigotes extracts from one *T. cruzi* strain. The result of these experiments revealed that the antiserum recognized two protein bands with molecular masses of 77 and 45 kDa in the three *T. rangeli* samples (Fig. 2D shows two of them, lanes 1 and 2), but failed to react with the *T. cruzi* one (Fig. 2D, lane

3). Pre-immune rabbit serum did not react with any *T. rangeli* proteins (not shown). The 77 kDa band was slightly higher than the expected size, opening the possibility that the telomeric *TrGP* is in fact a truncated form of a gp85-like protein, and/or that the native protein undergoes post-translational modifications that alter its molecular mass. It is possible that the smaller 45 kDa band is a degradation product.

In summary, in this work we characterized an ORF encoding for a protein with high identity with members of *T. cruzi gp85*/*trans*-sialidase family. Although the gene is expressed in *T. rangeli* epimastigotes cells, at present we do not know its role in the parasite life cycle. Presence of *gp85*/*trans*-sialidase in *T. rangeli* suggests that these genes were present in a common ancestor with *T. cruzi*. However, in *T. cruzi*, *gp85* genes suffered an expansion and adopted important roles in invasiveness and infectivity and also became a structural part of its telomeres [3,19,20]. The high epitopic variation of *T. cruzi* gp85 proteins has been pointed out as the cause of an anergic $CD4^+$ T cell response that hampers an effective host attack against the parasite [3]. In contrast, since *T. rangeli* does not enter mammalian cells and its presence is considered harmless to the vertebrate host [21], gp85-like proteins may play a different role. The fact that the *TrGP* gene is located next to the telomeres makes us wonder whether the presence of surface proteins genes at this position is a universal feature in all trypanosomes. Efforts are under way to determine the function of TrGP in the biology of *T. rangeli*.

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