

***Trypanosoma rangeli* protein tyrosine phosphatase is associated with the parasite's flagellum**

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Protein tyrosine phosphatases (PTPs) play an essential role in the regulation of cell differentiation in pathogenic trypanosomatids. In this study, we describe a PTP expressed by the non-pathogenic protozoan Trypanosoma rangeli (TrPTP2). The gene for this PTP is orthologous to the T. brucei TbPTP1 and Trypanosoma cruzi (TcPTP2) genes. Cloning and expression of the TrPTP2 and TcPTP2 proteins allowed anti-PTP2 monoclonal antibodies to be generated in BALB/c mice. When expressed by T. rangeli epimastigotes and trypomastigotes, native TrPTP2 is detected as a ~65 kDa protein associated with the parasite's flagellum. Given that the flagellum is an important structure for cell differentiation in trypanosomatids, the presence of a protein responsible for tyrosine dephosphorylation in the T. rangeli flagellum could represent an interesting mechanism of regulation in this structure.

Key words: *Trypanosoma rangeli* - protein tyrosine phosphatase - flagellum

Tyrosine phosphorylation is a key mechanism involved in cell regulation and differentiation. In kinetoplastid protozoans, such as trypanosomes, there is evidence that several proteins are phosphorylated on tyrosine residues (Andreeva & Kutuzov 2008). Determination of the TriTryp phosphatome (Brenchley et al. 2007) revealed that the *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* protein tyrosine phosphatases (PTPs) comprise 2.3%, 2.6% and 3.4% of the phosphatase complements of these organisms, respectively, in contrast to the 16% observed in humans. In addition, the significant difference between kinetoplastid PTPs and their human homolog (33% amino acid identity) suggests a promising role for these enzymes as targets for therapeutic drugs (Brenchley et al. 2007).

Several phosphatases have been described in protozoans, suggesting their role in regulating the development of these organisms. *T. brucei* PTP (*TbPTP1*) is associated with the cytoskeleton and has been reported to be intrinsically involved in this parasite's life cycle (Szoor et al. 2006), as its inactivation through chemical and genetic methods triggered the differentiation of morphologies ranging from infective stumpy forms to non-infective procyclic forms. The downstream step in this developmental signalling pathway was determined to involve a DxDxT phosphatase found in *T. brucei* glycosomes (Szoor et al. 2010). Additionally, a *T. cruzi* PTP was recently purified and biochemically characterized

(Gallo et al. 2011). Inhibition of this PTP using BZ3, a human PTP1b inhibitor, reduced the time required for the differentiation of epimastigotes to metacyclic forms and decreased the infectivity of trypomastigotes in L6 rat skeletal muscle cells by 50% (Gallo et al. 2011).

Considering the importance of PTPs in pathogenic trypanosomes, the present study focuses on the description of a PTP in *T. rangeli* (*TrPTP2*), a non-pathogenic Latin American protozoan closely related to *T. cruzi*. *T. rangeli* strains are divided into two major lineages that differ in their ability to infect certain *Rhodnius* species (Vallejo et al. 2002). These lineages are designated KPI(+) and KPI(-) based on the presence/absence of distinct types of kinetoplast DNA (kDNA) minicircles.

The life cycle of *T. rangeli* requires complex machinery to regulate cell differentiation, in which PTPs must play an important role. The first study on PTPs in *T. rangeli* showed that there are differences in tyrosine phosphatase activity between the long and short epimastigote forms (Gomes et al. 2006). The importance of this group of proteins for the *T. rangeli* life cycle was reaffirmed by the finding that inhibition of surface ecto-phosphatases impaired parasite proliferation (Fonseca-de-Souza et al. 2009). Additionally, ecto-phosphatase activity appears to be modulated by hydrogen peroxide (Cosentino-Gomes et al. 2009), which may be produced by the parasite, as well as by the concentration of inorganic phosphates in the medium (Dick et al. 2010). Although these studies have evaluated total ecto-phosphatase activity, the inhibitors used suggest the presence of PTPs among the surface phosphatases.

The aim of this study was to characterize the *T. rangeli* PTP gene by assessing both its intra and inter-specific variability and to comparatively evaluate the levels and sites of *TrPTP2* protein expression in different forms and strains of this parasite.

Financial support: CAPES, CNPq, FINEP
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Received 13 February 2012
Accepted 27 May 2012

MATERIALS AND METHODS

Parasites - The *T. cruzi* Y strain and 17 *T. rangeli* strains from different hosts and geographical origins were used in this study (Table). The epimastigote forms were cultivated by weekly passages at 27°C in liver infusion tryptose medium supplemented with 10% foetal bovine serum after a cyclic mouse-triatomine-mouse passage. *T. rangeli* trypomastigotes were obtained through in vitro differentiation (Koerich et al. 2002) and *T. cruzi* trypomastigotes were obtained from the supernatant of infected Vero cells (Eger-Mangrich et al. 2001).

Nucleic acid extraction and gene amplification - Total DNA and RNA were extracted (Sambrook & Russell 2001) from the *T. cruzi* Y strain and 17 *T. rangeli* strains isolated from different hosts and geographical origins (Table). Different primer sets based on previously obtained *T. rangeli* cDNA sequences (Grisard et al. 2010) were designed for amplification and sequencing of the complete *TrPTP2* coding region, as well as the 3' and 5' UTRs (Supplementary data).

Sequence assembly and phylogenetic analyses - The obtained amplification products were sequenced using the Megabace 1000[®] DNA Analysis System with the DYEnamic ET terminator kit (GE Healthcare, Uppsala, Sweden) according to the manufacturer's instructions. Clusters were assembled with the Phred/Phrap/Consed[®] package and aligned using CLUSTALW software. Sequences of *T. cruzi* (GenBank XM_816368), *T. brucei* (GenBank XM_817818.1), *Trypanosoma vivax* (TriTrypDB TvY486_1006690) and *Trypanosoma congolense* (TriTrypDB TcIL3000.10.5750) PTP genes were

retrieved from the GenBank or TriTrypDB database for comparative analysis. To construct a phylogenetic tree, the sequences were analysed with bootstrapped maximum parsimony and neighbour-joining methods using complete deletion and the Kimura-2 parameters in MEGA software version 4.0.2. The predicted *TrPTP2* protein sequence was analysed using InterPro.

Southern and northern blotting - Southern and northern blot analyses were performed according to standard protocols (Sambrook & Russell 2001). A peroxidase-labelled probe was prepared using an 821 bp polymerase chain reaction fragment and the ECL Direct Nucleic Acid Labelling and Detection System (GE Healthcare).

Recombinant proteins and mass spectrometry (MS/MS) analysis - Recombinant His-tagged *TrPTP2* and *Trypanosoma cruzi* *TcPTP2* were produced in *Escherichia coli* BL21(DE3) cells transformed with the pET-14b vector (Novagen) containing the coding region from either *TrPTP2* from the *T. rangeli* Choachí strain or *TcPTP2* from the *T. cruzi* Y strain. Expression was induced by treatment with 1 mM IPTG (isopropyl-β-d-thiogalactopyranoside) at 37°C for 3 h and the recombinant proteins were purified through electroelution (4 h at 70 V) of the gel-excised proteins following dialysis in a buffer containing 25 mM Tris, 250 mM glycine and 0.1% sodium dodecyl sulfate (SDS) and assessment *via* SDS-polyacrylamide gel electrophoresis (PAGE). Mass spectrometry analysis (On-line Nano-LC MS-MS/MS) of gel-excised recombinant *TrPTP2* was carried out with the NanoAcquity System (Waters Corporation) coupled to the LTQ Orbitrap Velos nanospray (Thermo-Scientific) using an established protocol (Williamson et al. 2010)

TABLE

Trypanosoma rangeli strains used in this study, their original geographical region and hosts, kDNA group classification according to presence (+) or absence (-) of KP1 minicircle and their respective GenBank accessions for *TrPTP2* gene sequences

| Strain | Original host | Geographical origin | KP1 grouping | GenBank |
|-------------|-------------------------------|---------------------|--------------|----------|
| SC-58 | <i>Echimys dasythrix</i> | Brazil | - | EU325665 |
| SC-61 | <i>E. dasythrix</i> | Brazil | - | EU325657 |
| SC-76 | <i>Panstrongylus megistus</i> | Brazil | - | EU325660 |
| PIT10 | <i>P. megistus</i> | Brazil | - | EU325656 |
| C23 | <i>Aotus</i> sp. | Colombia | - | EU325667 |
| 5048 | <i>Homo sapiens</i> | Colombia | - | EU325666 |
| TRE | Not determined | Colombia | - | EU325668 |
| B450 | <i>Rhodnius</i> sp. | Brazil | + | EU325654 |
| R1625 | <i>Homo sapiens</i> | El Salvador | + | EU325664 |
| H9 | <i>H. sapiens</i> | Honduras | + | EU325662 |
| H14 | <i>H. sapiens</i> | Honduras | + | EU325658 |
| Macias | <i>H. sapiens</i> | Venezuela | + | EU325659 |
| Palma-2 | <i>Rhodnius prolixus</i> | Venezuela | + | EU325663 |
| Choachí | <i>R. prolixus</i> | Colombia | + | EU325653 |
| D3493 | <i>R. prolixus</i> | Colombia | + | EU325655 |
| San Agustín | <i>H. sapiens</i> | Colombia | + | EU325661 |
| 1545 | <i>R. prolixus</i> | Colombia | + | EU325652 |

with slight modifications. The m/z spectrum was processed using Mascot Distiller and Mascot Software (Matrix Science) was employed to perform database searches among *T. rangeli* expressed sequence tags (Grisard et al. 2010) and predicted open reading frames (ORFs).

Monoclonal antibody (mAb) production - mAbs were obtained through immunization of BALB/c mice with *Tr*PTP2 (50 µg) according to an established protocol (Mazuratto et al. 2009). Clones were selected based on exclusive recognition of recombinant and/or native *Tr*PTP2.

Western blotting - Parasite protein extracts were obtained by lysis in an appropriate buffer (50 mM NaCl, 200 mM Tris-HCl pH 8.0, 1% Triton X-100) at 80°C. NP-40 fractionation was carried out according to a previously described protocol (Woods et al. 1989), with ethylene glycolbis(beta-aminoethyl ether)-N,N,N',N'-tetra acetic acid substituted for ethylene-diamine tetraacetic acid to enable concentration of proteins from the cytoskeletal fraction. After resolution in SDS-PAGE gels, the proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad) in an appropriate buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3) using a blotting transfer unit (Bio-Rad). After blocking with non-fat milk, the membranes were incubated for 90 min with anti-*Tr*PTP2 or anti-*Tc*PTP2 polyclonal antisera (1:250), anti-PTP2 mAbs (1:2), an anti-His-Tag mAb (Sigma-Aldrich, 1:12,000) or an anti- α -tubulin mAb (1:10,000), the last of which was used as a loading control.

Immunofluorescence assays - Indirect immunofluorescence assays were carried out as described previously (Stoco et al. 2012) in *T. rangeli* epimastigotes and trypomastigotes. Anti-PTP2 mAbs (no dilution) and a mAb directed against the flagellar calcium-binding protein (FCaBP) (1:50) (Schenkman et al. 1991) were used as primary antibodies, with anti-mouse IgG antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 594 (Molecular Probes; diluted 1:1,000) being used as secondary antibodies and 4',6-diamidino-2-phenylindole was added to a final concentration of 1 µg/mL. The slides were examined using a Leica TCS SP5 confocal microscope (Leica Microsystems).

Ethics - All procedures involving animals were previously approved by the Ethical Committee on Animal Use of Federal University of Santa Catarina (reference 23080.025618/2009-81).

RESULTS

The *Tr*PTP2 gene has a 987 bp ORF, with a predicted polypeptide product of ~36 kDa, which is approximately the same as the predicted size of *Tc*PTP2. The *Tr*PTP2 sequences from all strains were of high quality (Phred \geq 60) and are available in the GenBank database under the accessions listed in Table.

The same tree topology was obtained from the phylogenetic analyses using nucleotide sequences regardless of the methodology employed, illustrating that *Tr*PTP2 is useful as a marker for interspecific characterization, but lacks the appropriate bootstrap support for intraspecific characterization (Fig. 1). Interestingly, the KP1(-) strains from Brazil were separated from the Colombian

KP1(-) strains, which were grouped with KP1(+), indicating a reduced, but clear intraspecific variability between the KP1(-) lineages.

The complete *Tr*PTP2 gene sequence was obtained for the 17 *T. rangeli* strains and nucleotide alignment revealed several substitutions within the coding region, as well as in the 5' and 3' UTRs (data not shown). A comparison of the PTP nucleotide sequences from *T. rangeli* KP1(+) with KP1(-) strains showed 23 substitutions. The Colombian KP1(-) strains differed from the KP1(+) strains and from the Brazilian KP1(-) strains in three exclusive nucleotide substitutions. However, the identity between the nucleotide sequences from all strains was still close to 99%.

The predicted *Tr*PTP2 protein sequence showed a single PTP domain between amino acids 238 and 247, similar to what is observed for the TriTryps PTPs. Comparisons with orthologous sequences from other trypanosomatid species showed high intra and interspecific conservation (Supplementary data). The amino acid sequence identities between *Tr*PTP2 and its orthologs are as follows: *Tc*PTP2, 73%, *T. brucei* *Tb*PTP1, 59%, *T. congolense*, 58%, *T. vivax*, 57%, and *L. major*, 37%.

Southern blot analysis revealed that *Tr*PTP2 is present as a single copy gene in the genome of the *T. rangeli* Choachi strain (Fig. 2A, B), which is in agreement with the number of copies of orthologous PTP genes observed in other kinetoplastid species. Northern blot assays showed that *Tr*PTP2 encodes a ~1.5 kb mRNA transcript in both the epimastigote and trypomastigote forms of the *T. rangeli* Choachi strain (Fig. 2C).

Heterologous expression of both *Tr*PTP2 and *Tc*PTP2 using the pET-14b vector produced a protein of approximately 36 kDa in the insoluble fraction of the lysate, corresponding to the theoretically expected size for these

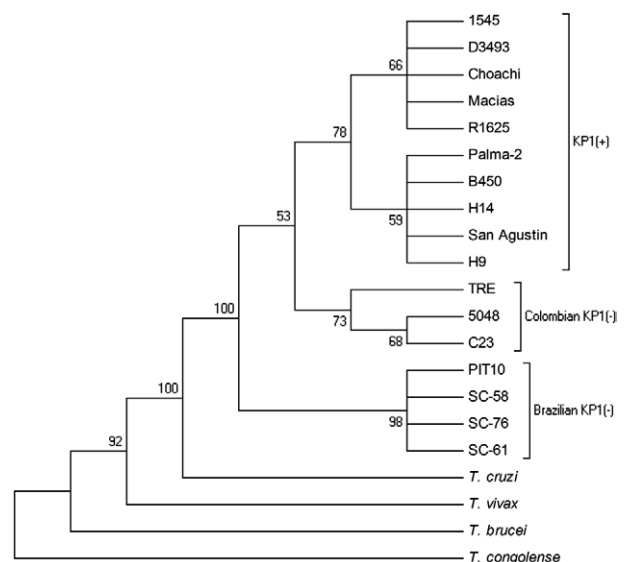


Fig. 1: dendrogram analysis based on the *Trypanosoma rangeli* protein tyrosine phosphatase (*Tr*PTP2) gene. A neighbour-joining (1,000 replicates) based-dendrogram derived from the analysis of the *Tr*PTP2 gene and its orthologous sequences from distinct trypanosome species. Branches with bootstrap values < 50 are not shown.

proteins (data not shown). Mass spectrometry analysis of the recombinant *Tr*PTP2 covered more than 50% of its peptides and confirmed its identity (data not shown).

Immunization of BALB/c mice with the purified recombinant proteins resulted in the production of anti-*Tr*PTP2 and anti-*Tc*PTP2 polyclonal antisera. The anti-*Tr*PTP2 antiserum recognized a ~65 kDa protein in *T. rangeli* extracts, but not in *T. cruzi* extracts, whereas the anti-*Tc*PTP2 antiserum recognized both *Tc*PTP2 in *T. cruzi* extracts and *Tr*PTP2 in the *T. rangeli* extracts (data not shown). Based on the cross-reactivity obtained using the polyclonal antisera, as well as the identification of a protein that was larger (~65 kDa) than predicted, anti-PTP2 mAbs were produced and selected based on the recognition of both recombinant and native *Tr*PTP2 in *T. rangeli* extracts.

The anti-PTP2 mAbs showed a strong signal when tested against the recombinant *Tr*PTP2 and specifically recognized the ~65 kDa protein in both epimastigotes

and trypomastigotes from the *T. rangeli* Choachí strain (Fig. 3A). Similar to *Tb*PTP1 in *T. brucei*, *Tr*PTP2 is detected in the cytoskeletal fraction in *T. rangeli* (Fig. 3B). Remarkably, mAbs preincubated twice with recombinant *Tr*PTP2 embedded in PVDF membranes could no longer recognize the ~65 kDa protein in *T. rangeli* protein extracts (data not shown). Although *Tc*PTP2 is predicted to be 73% identical to *Tr*PTP2, native *Tc*PTP2 was not detected in *T. cruzi* by the mAbs generated in this study. This absence of cross-reactivity could be due to the few differences in the amino acid compositions of *Tr*PTP2 and *Tc*PTP2, as shown in the supplementary data.

The observed ~65 kDa protein is almost 30 kDa heavier than the predicted mass of 36 kDa for both *Tr*PTP2 and *Tc*PTP2. This unexpected increase in mass was investigated in several different ways. Although glycosylation sites have been predicted within the amino acid sequence of *Tr*PTP2, analyses using staining with Schiff's reagent did not detect aldehydes in the region corresponding to the ~65 kDa protein in the *T. rangeli* extracts (data not shown), suggesting a lack of significant glycosylation sites within this region. Because dimer formation could also explain the ~30 kDa increase in mass, *T. rangeli* extracts were resolved *via* PAGE using 8 M urea as a denaturing agent. This process produced the same ~65 kDa protein (Fig. 3C). Thus, if *Tr*PTP2 forms dimers, they are very stable and resistant to strong denaturing conditions.

Staining with anti-PTP2 mAbs revealed a strong signal in the *T. rangeli* flagellum in both epimastigotes and trypomastigotes that overlaps with the signal from anti-FCaBP mAb, suggesting that *Tr*PTP2 is concentrated in

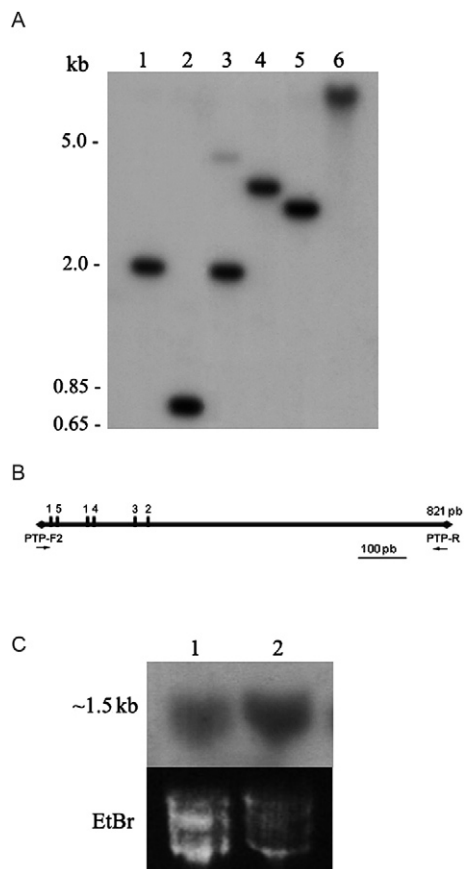


Fig. 2. *Trypanosoma rangeli* protein tyrosine phosphatase (*Tr*PTP2) is present as a single copy gene and is expressed as a ~1.5 kb transcript. *Tr*PTP2 gene is present as a single copy in the *T. rangeli* genome as revealed by southern blot analysis (A) using total DNA digested with *Pst*I (1), *Hinf*I (2), *Bgl*I (3), *Bam*HI (4), *Hind*III (5) and non-digested DNA as control (6). B: restriction map of the *Tr*PTP2 gene fragment used as a probe [*Pst*I (1), *Hinf*I (2), *Bgl*I (3), *Bam*HI (4), *Hind*III (5)]; C: *Tr*PTP2 is expressed as a ~1.5 Kb transcript as revealed by northern blot analysis using total RNA from epimastigote (1) and trypomastigote (2) forms of Choachí strain.

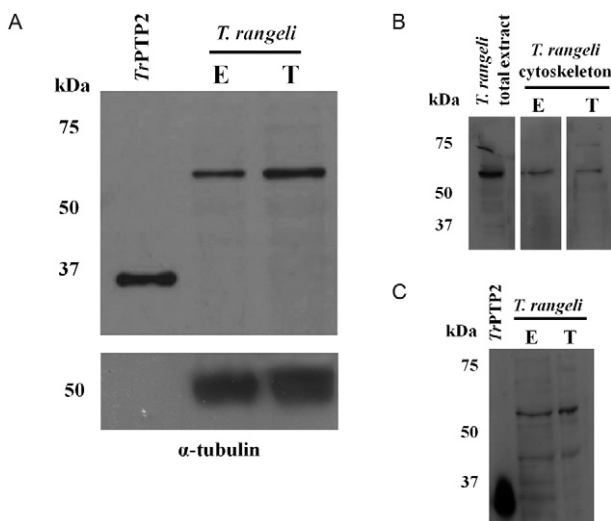


Fig. 3. *Trypanosoma rangeli* protein tyrosine phosphatase (*Tr*PTP2) expression in *T. rangeli* epimastigotes and trypomastigotes. A: *Tr*PTP2 expression is slightly higher in *T. rangeli* trypomastigotes than epimastigotes as revealed by the anti-PTP2 monoclonal antibodies. Recombinant *Tr*PTP2 and α -tubulin were used as positive and loading controls, respectively; B: *Tr*PTP2 is detected in the cytoskeletal protein fraction from *T. rangeli* epimastigotes and trypomastigotes; C: when *T. rangeli* protein extracts are resolved in a gel containing 8 M urea as denaturant *Tr*PTP2 is still detected as a ~65 kDa protein.

this structure and co-localizes with a flagellar protein (Fig. 4). In epimastigotes, there was also a weak signal detected in the cell body that was not observed in trypomastigotes. In *T. cruzi*, the staining with the mAbs was observed to be subtle and diffuse.

DISCUSSION

Given that PTPs from pathogenic trypanosomatids, such as *T. brucei* and *T. cruzi*, have been reported to play a role in the parasites' in vitro differentiation into infective forms (Szoor et al. 2006) and mammalian host cell infectivity (Gallo et al. 2011), finding a highly similar PTP in a non-virulent species like *T. rangeli* is intriguing.

The *TrPTP2* gene is present in a single copy in this parasite's genome and encodes a ~1.5 kb transcript, which is in accordance with a former transcriptome analysis (Grisard et al. 2010). The *TrPTP2* gene proved to be a good phylogenetic marker to differentiate *T. rangeli* from other trypanosomes, but did not exhibit high intraspecificity, despite the variability between the KPI(+) and KPI(-) strains, as well as among the KPI(-) strains.

The *TrPTP2* transcript is close in size to the *T. brucei* transcript (~1.8 kb) (Szoor et al. 2006), predicting a polypeptide product with a size of approximately 36 kDa. This predicted molecular mass is almost identical for both *TrPTP2* and *TcPTP2* and is close to the mass reported for the homologous *TbPTP1* protein (~34 kDa) (Szoor et al. 2006). In contrast to the recombinant forms of *TrPTP2* and *TcPTP2* produced in this study, which were detected in the insoluble fraction of the lysate, Gallo et al. (2011) expressed a recombinant, active 36 kDa *T. cruzi* PTP in the soluble fraction, which is consistent with the similar sizes of *T. rangeli* and *T. cruzi* PTP2 obtained herein.

While the molecular mass of ~65 kDa detected for PTP2 in *T. rangeli* is similar to a 55-60 kDa *L. major* PTP (Aguirre-Garcia et al. 2006), the increase in mass compared to the predicted 36 kDa protein was unexpected and was investigated in several different ways. Post-translational modifications, such as glycosylations, have been predicted for *TrPTP2* and may dramatically increase protein mass (Sakurai et al. 2008). Formation of dimers could also account for the detection of a protein that was heavier than expected; however, no specific antibody signal near 36 kDa (the expected mass for *TrPTP2*) was detectable under strong denaturing conditions. We cannot rule out the possibility of covalent oligomerization within the *TrPTP2* product itself or with another protein, but the results obtained from urea denaturation suggest that if *TrPTP2* forms dimers, they are very stable. A previous study in *T. cruzi* (D'Orso & Frasch 2001) detected a difference of 20 kDa between the deduced and obtained mass of the *TcUBP-1* protein and parasite transfection was employed to confirm that this increase in mass occurred naturally, possibly due to oligomerization or post-translational modifications. A similar situation might involve *TrPTP2*, which could be post-translationally modified through phosphorylation or methylation, both of which are predicted to occur within the *TrPTP2* sequence. Additionally, the anomalous electrophoretic migration observed might be due to the estimated high pI of *TrPTP2* (8.95) (Chiou & Wu 1999).

The detection of *TrPTP2* in the *T. rangeli* flagellum was a surprising and interesting result obtained in this study. Given that the flagellum in eukaryotic cells is constantly modulated by phosphorylation, it is very likely that dephosphorylation and thus phosphatases play an

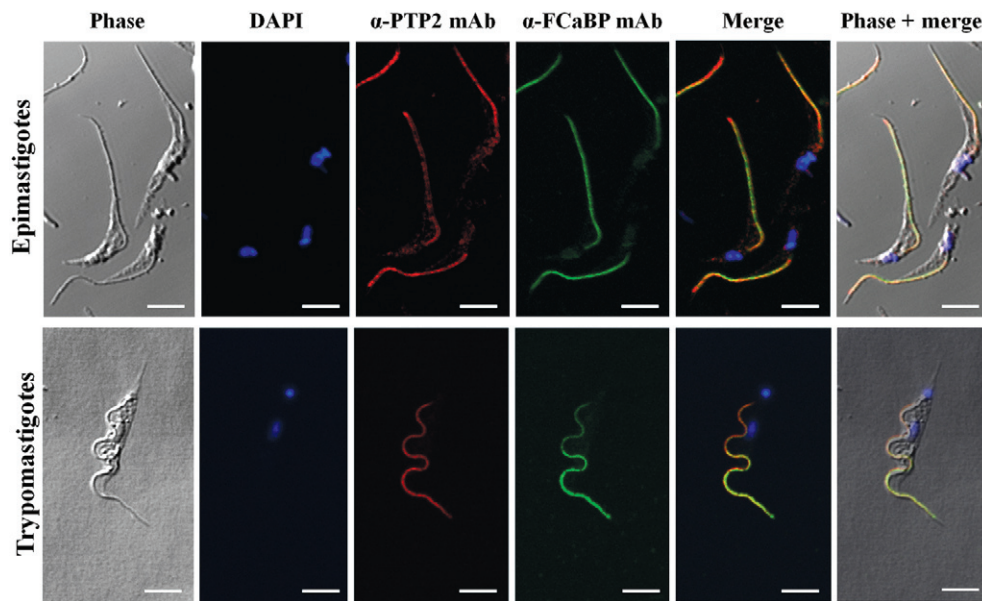


Fig. 4: *Trypanosoma rangeli* protein tyrosine phosphatase (*TrPTP2*) is associated with the *T. rangeli* flagellum. *TrPTP2* concentrates in the flagellum and co-localizes with the flagellum calcium-binding protein (FCaBP) in *T. rangeli* epimastigotes and trypomastigotes as revealed by immunofluorescence assays using anti-PTP2 monoclonal antibodies and anti-FCaBP monoclonal antibodies. Parasites were counter-stained with 4',6-diamidino-2-phenylindole (DAPI). White bars represent 5 μ m. mAb: monoclonal antibody.

important role in this structure. It has been shown that phosphorylation of axonemal proteins is crucial for regulating flagellar motility, independent of cyclic AMP, in response to an increase in pH (Nakajima et al. 2005). Interestingly, the *in vitro* differentiation of *T. rangeli* is successful when the culture pH is 8.0, but is drastically impaired when it is 6.0 or 7.0 (Koerich et al. 2002).

Although *Tb*PTP1 is mainly found in the *T. brucei* cytoskeletal fraction, a specific association with the flagellum was not detected (Szoor et al. 2006). However, a mAb directed against phosphotyrosine allowed the identification and cytolocalization of tyrosine-phosphorylated proteins in *T. brucei* (Nett et al. 2009), which were found to be concentrated in the nucleolus region, the flagellum basal body and the flagellum itself. The recognition signal detected throughout the parasite's flagellum disappears in the distal extremity, suggesting that tyrosine-phosphorylated proteins could be directly associated with the axoneme, either adjacent to it or connected to membranous structures near the flagellum (Nett et al. 2009). With the exception of the basal body, the recognition signal for *Tr*PTP2 observed in *T. rangeli* epimastigotes and trypomastigotes precisely follows the same distribution pattern throughout the flagellum.

Given that the *T. brucei* flagellum contains proteins modulated by specific phosphorylation on tyrosine residues, it is likely that these proteins may also be found in phylogenetically related organisms, such as *T. rangeli*, *T. cruzi* and *Leishmania* species. Therefore, the presence of *Tr*PTP2 in the flagellum is quite intriguing, as concentration of proteins regulated by tyrosine phosphorylation most likely requires tyrosine kinases as well as tyrosine phosphatase enzyme activity.

The mechanisms involved in adhesion to a substrate in *T. brucei* (Ersfeld & Gull 2001) *T. cruzi* (Ferreira et al. 2008) and *T. rangeli* (Meirelles et al. 2005) require the crucial and active participation of the flagella of these parasites. During metacyclogenesis, the flagellar membrane remains connected to the epithelial surface (Ersfeld & Gull 2001) representing an important interface between the parasite and its host that is essential for the fulfilment of its biological cycle. The presence of proteins such as PTPs in the flagellum reinforces the role of this structure in both cell differentiation and cell cycle regulation.

ACKNOWLEDGEMENTS

To Dr. Sergio Schenkman, for the anti-FCaBP antibody, to Dr Claudia Nunes Duarte dos Santos, for providing facilities for mAbs production, and to Dr Hercules Moura, for the MS/MS analysis.

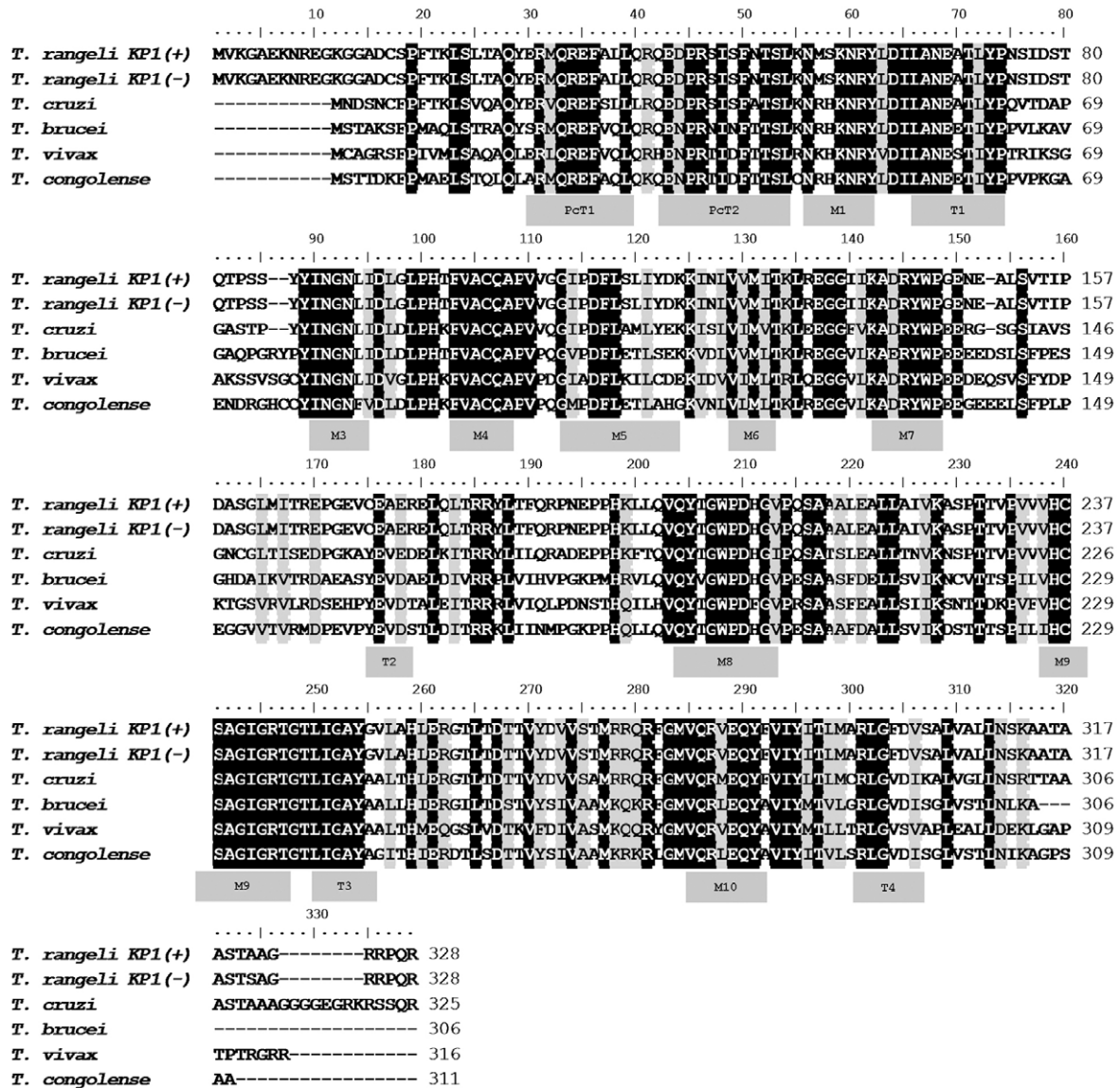
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| Forward primers (5' to 3') | | Reverse primers (5' to 3') | |
|----------------------------|--|----------------------------|---|
| PTP-F1 | CGC TAC CTG ACT TTT CAG CG | OligodT-anchor | GAC CAC GCG TAT CGA TGT CGA C (T) ₁₆ V |
| | | Anchor | GAC CAC GCG TAT CGA TGT CGA CT |
| PTP-F2 | GGG AAG GAA AAG GTG GGG C | PTP-R | CAC CAT TCC AAA (A/G)CG CTG TC |
| TrPTP2 | CAT ATG GAC ACG CCC AGC ATG GTG AAG | TrPTP2 | CTC GAG TTA TCT CTG TGG CCT CCT TCC |
| TcPTP2 | CAT ATG ATG AAT GAT TCG AAC TGC TTC | TcPTP2 | CTC GAG TTA TCT CTG TGA ACT TCT TTT TCT |

List of primers used for amplification and sequencing of *Trypanosoma rangeli* protein tyrosine phosphatase (TrPTP2) and *Trypanosoma cruzi* PTP2 (TcPTP2) genes. Sites for NdeI and XhoI restriction enzymes are underlined on forward and reverse primers, respectively.



Deduced amino acid sequence alignment of the *Trypanosoma rangeli* protein tyrosine phosphatase (TrPTP2) gene with orthologous sequences from different trypanosome species. Motifs related to catalytic activity (M1: binding motive of phospho-Tyr; M8: WPD loop; M9: P-loop catalytic motive; M10: Q-loop), structural motifs inside the catalytic domain (M3-M7), trypanosome-specific motifs inside the catalytic region (T1-T4) and pre-catalytic motifs (PcT1, PcT2) are indicated. Dark background indicates identity and gray background indicates conservation of amino acids.