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Protein–protein interaction map of the *Trypanosoma cruzi* ribosomal P protein complex

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Abstract

The large subunit of the eukaryotic ribosome possesses a long and protruding stalk formed by the ribosomal P proteins. Four out of five ribosomal P proteins of *Trypanosoma cruzi*, TcP0, TcP1α, TcP2α, and TcP2β had been previously characterized. Data mining of the *T. cruzi* genome data base allowed the identification of the fifth member of this protein group, a novel P1 protein, named P1β. To gain insight into the assembly of the stalk, a yeast two-hybrid based protein interaction map was generated. A parasite specific profile of interactions amongst the ribosomal P proteins of *T. cruzi* was evident. The TcP0 protein was able to interact with all both P1 and both P2 proteins. Moreover, the interactions between P2β with P1α as well as with P2α were detected, as well as the ability of TcP2β to homodimerize. A quantitative evaluation of the interactions established that the strongest interacting pair was TcP0-TcP1β.

Keywords: *Trypanosoma cruzi*; Ribosome; Ribosomal P protein; Yeast two-hybrid; Protein–protein interaction

1. Introduction

The large subunit of ribosomes possesses a long and protruding stalk involved in the translocation step of protein synthesis. In eukaryotes, this structure is formed by the ribosomal P proteins (Liljas, 1991). These proteins include P0, a 34 kDa polypeptide, and two distinct, but closely related peptides of about 10 kDa, P1 and P2. All three share a conserved P protein motif at its C-terminal end. An additional P protein, named P3, has been described for plants (Bailey-Serres et al., 1997).

The number of ribosomal P proteins varies among species. In higher eukaryotes, the P1 and P2 families have only one member. However, in *Saccharomyces cerevisiae*, the families are made of two members, P1α/P1β and P2α/P2β (Planta and Mager, 1998). In the case of *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease, four components of the stalk have been identified: P0, of approximately 34 kDa, containing a C-terminal end that deviates from the eukaryotic P consensus and bares similarity to that of the L10 protein of Archaea, and three proteins of about 10 kDa, P1, P2α and P2β with the typical eukaryotic P consensus sequence at their C-terminal end (Levin et al., 1993).

These differences have consequences in the organization of the ribosomal stalk. In mammals, it is composed

Abbreviations: 2-D electrophoresis, two-dimension electrophoresis; cDNA, DNA complementary to RNA; Ig, immunoglobulin(s); kDa, kilodalton(s); mAb, monoclonal Ab; ONPG, o-nitrophenyl β-D-galactopyranoside; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside.

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by two copies of each P1 and P2 proteins, linked to P0 (Uchiumi and Kominami, 1997). However, the binding of the P2 protein to P0 could only be detected in the presence of P1, suggesting a pivotal role for the latter and P0 in the conformation of the stalk (Gonzalo et al., 2001). The stalk of *S. cerevisiae* ribosomes has a slightly different composition with one copy of each of the four 12-kDa acidic proteins forming preferential pairs; P1α/P2β and P1β/P2α. Again, P1 proteins seem to be necessary for the binding of the corresponding P2 partners to P0 (Guarinos et al., 2001). The use of a yeast two-hybrid approach confirmed the conclusions derived from genetic and complementation experiments in yeast, demonstrating the key role of the ribosomal P0, its interaction with the P1 proteins and provided evidence indicating that the P2α protein is also able to interact with P0 (Lalioti et al., 2002). Up to date, no information was available about the protein–protein interactions within the P protein complexes of protozoa. In *T. cruzi*, immunological evidences suggested that not all the P proteins had been identified and cloned (Schijman et al., 1992). Western blots of ribosomal proteins suggested the existence of a 19 kDa peptide containing the eukaryotic P consensus, represented by the epitope known as R13, a feature of the low molecular weight ribosomal P proteins of the parasite (Schijman et al., 1992). Phosphatase treatments of *T. cruzi* ribosomes did not change the electrophoresis migration pattern of these ribosomal proteins, suggesting that the 19-kDa peptide was not a phosphorylated form of the previously described 12-kDa P peptides (Gómez et al., 2001). Due to its antigenic properties, we hypothesized that the additional band could correspond to a novel, up to date non-characterized, member of the *T. cruzi* ribosomal P protein family. The aim of this work was to complete the characterization of the ribosomal P protein complex of *T. cruzi*, to determine the best conditions to study protein–protein interactions using a yeast two-hybrid approach, and to test it mapping the protein–protein interactions amongst the parasite P proteins. A novel ribosomal P1 protein of *T. cruzi*, named TcP1β, was identified, cloned and expressed, allowing the construction of a complete protein–protein interaction map that shows a *T. cruzi* specific profile of interactions for the ribosomal stalk of this microorganism.

2. Materials and methods

2.1. Data mining

The R13 amino acid sequence, EEEDDDMGFGLFD (Levin et al., 1993) was used to probe the *T. cruzi* genome database (www.tigr.org) using the tblastn program in search of additional ribosomal P proteins. In addition to previously reported P1, P2α and P2β sequences, a putative novel ribosomal P protein, named P1β, was found.

2.2. DNA recombinant techniques

The DNA encoding for *T. cruzi* P0 (GenBank Accession No X65066), TcP1α (X65025), TcP2α (X65065) and TcP2β (X75033) and the recently cloned TcP1β (AY618551) were obtained by PCR on cDNA from CL Brener strain. The DNA encoding P1/P2 proteins were subcloned into the BamHI and EcoRI restriction sites of the expression vector pRSET A (Invitrogen, Carlsbad, CA, USA) containing a sequence encoding for an N-terminal hexa-histidine tag that facilitates purification. TcP1α* was constructed essentially in the same way, but using a 3′ primer that modified the TAG stop codon to TAC (Tyr). As a consequence, a stop codon within pRSET A was used, yielding TcP1α* that contained a 27 amino acid long C-terminal extension. Proteins were expressed in *E. coli* BL21(DE3)pLysS strain (Invitrogen, Carlsbad, CA, USA) and purified under native conditions by affinity chromatography using Ni-NTA resin (Qiagen, Gmbh, Hilden, Germany) following the manufacturers instructions. For yeast two-hybrid experiments, all ORFs were cloned into the vectors from the ProQuest™ Yeast Two-Hybrid Gateway® System (Invitrogen, Carlsbad, CA, USA). In addition to the full length ORF encoding TcP0, a DNA fragment of 333 bp representing its C-terminal end was also cloned in this system. Identification was assessed by sequencing all the clones.

2.3. Yeast two-hybrid system

The ProQuest™ Yeast Two-Hybrid Gateway® System was used for the yeast two hybrid experiments, as indicated by the manufacturers (Invitrogen, Carlsbad, CA, USA). Once the ORFs were clonase transferred to the DNA Binding Domain (BD) and Activation Domain (AD) fusion vectors, their integrity was confirmed by sequencing. Mav203 yeast cells were first transformed with the BD fusion plasmids using the lithium acetate method (Sambrook and Russell, 2001). Transformants were selected on plates lacking Leu. Thereafter, cells carrying each BD plasmid were transformed with the corresponding AD plasmids. Double transformants were selected on plates lacking Leu and Trp. The identity and integrity of the BD and AD fusion products was confirmed by colony PCR on yeast colonies.

2.4. Detection of LacZ reporter gene

Positive transformed yeast colonies were plated onto selective solid medium, incubated overnight at 30 °C, and assayed for β-galactosidase activity. Briefly, a dry nitrocellulose filter was laid onto yeast colonies grown on selective medium. The filter was removed and placed 20 min at −70 °C. A Petri dish lid was prepared for the reaction by placing 2 ml buffer Z (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, pH 7) containing 70 μl of X-gal 2% and one Whatman filter

circle, followed by the nitrocellulose filter with the colonies facing up. The dish was incubated at 30 °C in humidified chamber.

2.5. Detection of His3 reporter gene

Activation of *His3* gene induced by interaction pairs was studied analyzing the ability of yeast expressing the corresponding GAL4 fusion proteins to grow on plates lacking histidine (SC Leu−Trp−His−), in the presence of up to 50 mM of 3-amino-1,2,3-trizole (3AT).

2.6. Detection of the Ura3 reporter gene

For the negative selection assay, selected colonies were plated separately in $Leu^{-}Trp^{-}$ plates in the presence of 5-fluororotic acid (5FOA 0.2%). Identical plates without 5FOA were used as control.

2.7. Quantitative β-Galactosidase assay in liquid medium

Yeast cells were grown in liquid medium overnight at 30 °C. 1 ml of cells was collected by centrifugation, resuspended in 0.3 ml of buffer Z, and stored at −70 °C 20 min. Thereafter, cells were thawed and 0.7 ml of buffer Z, supplemented with 2 μl of 2-mercaptoethanol, and 100 μl of ONPG 4 mg/ml was added. Cells were incubated 4 h and pelleted by centrifugation at 12000 *g* for 10 min. The supernatants were obtained and absorbance was measured at 420 nm. Measures of β-galactosidase activity were calculated as described (Platt et al., 1972).

2.8. SDS-PAGE and immunoblotting

SDS-PAGE under reducing conditions was performed using standard protocol (Laemmli, 1970). *T. cruzi* lysates were prepared resuspending a parasite pellet in SDS-PAGE sample buffer and boiling for 5 min. Purified ribosomes were prepared as described (Gómez et al., 2001). For immunoblotting, membranes were first incubated with mouse monoclonal antibodies 17.2 or 68.2 (Mahler et al., 2001) or monoclonal antibody against the BD domain of GAL4 (BD Biosciences Clontech, San Diego, CA, USA), followed by detection with anti-mouse IgG conjugated to peroxidase (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

2.9. Native and 2-D electrophoresis

Samples were prepared by mixing TcP1α* and TcP2β proteins in elution buffer (50 mM Na_2HPO_4 pH 8; 300 mM NaCl, 5 mM 2-mercaptoethanol, 100–200 mM imidazol) overnight at 4 °C before electrophoresis. When mixed, the molar ratio TcP2β:TcP1α* was 2:1. Native electrophoresis was performed using a 10% acrylamide gel essentially as described (Juri Ayub et al., 2001). For two-dimension electrophoresis, a lane of the native gel was loaded on the stacking layer of a denaturing SDS-PAGE 15% acrylamide gel and electrophoresed under standard conditions.

3. Results

3.1. Cloning of a novel P protein from T. cruzi

Western blots of *T. cruzi* lysates (not shown) and purified ribosomes (Fig. 1A) were probed with the monoclonal antibody 17.2 that recognizes the 13 amino acids C-terminal conserved eiptope (Mahler et al., 2001) of *T. cruzi* ribosomal P proteins, namely R13. This antibody reveals a triplet of bands migrating between 14 and 16 kDa and a fourth band of 19 kDa (p19 in Fig. 1 A), as well as TcP0. The central band of the triplet was characterized as the P2β protein due to its reaction with the monoclonal antibody 68.2, directed to an internal *T. cruzi* P2β-specific epitope (not shown). The identity of the fourth peptide migrating as a 19-kDa polypeptide (p19) was unknown (Fig. 1 A). In an attempt to identify the gene(s) that encodes the latter, we probed the database of the *T. cruzi* genome project (www.tigr.org) with the R13 peptide sequence. In addition to the already characterized P proteins, we found two genes ordered "in tandem" encoding for the same polypeptide. The deduced amino acid sequence contained the conserved R13 C-terminal peptide. This new protein, named TcP1β, was classified as belonging to the P1 type of the ribosomal P proteins, although it shows less homology to the yeast P1α (34.8%) and P1β (38.3%) proteins than the previously cloned TcP1 protein, now named TcP1α, 41.8% and 46.4% respectively (Table 1).

The TcP1β peptide sequence shared 42.6% identity with TcP1α, 40.2% with the TcP2α protein and 35.6% with TcP2β. In Fig. 1C, the amino acid sequence of the new protein is aligned along with the other *T. cruzi* P proteins. The pI and molecular weight of TcP1β deduced from its amino acid sequence was of 4.24 and 11.412 kDa, respectively, values that are similar to those of the other *T. cruzi* P1/P2 proteins. The complete cDNA encoding TcP1β was cloned and expressed as described in Materials and Methods. The migration pattern of the recombinant TcP1β protein on SDS-PAGE was compared to that of the other recombinant P proteins. Taking into account that the N-terminal hexa-histidine tag increased the molecular weight of each of the recombinants in about 4 kDa, the migration pattern of recombinant TcP1β (Fig. 1B) was slower than that of the other P1/P2 recombinant proteins. Indeed, the electrophoretic migration of recombinant TcP1β resembled the migration pattern of the ribosomal protein p19, strongly suggesting its identification as the ribosomal P1β protein.

Data mining revealed a similar set of five P protein components for *Trypanosoma brucei* and *Leishmania major*. The high homology among the members of each P

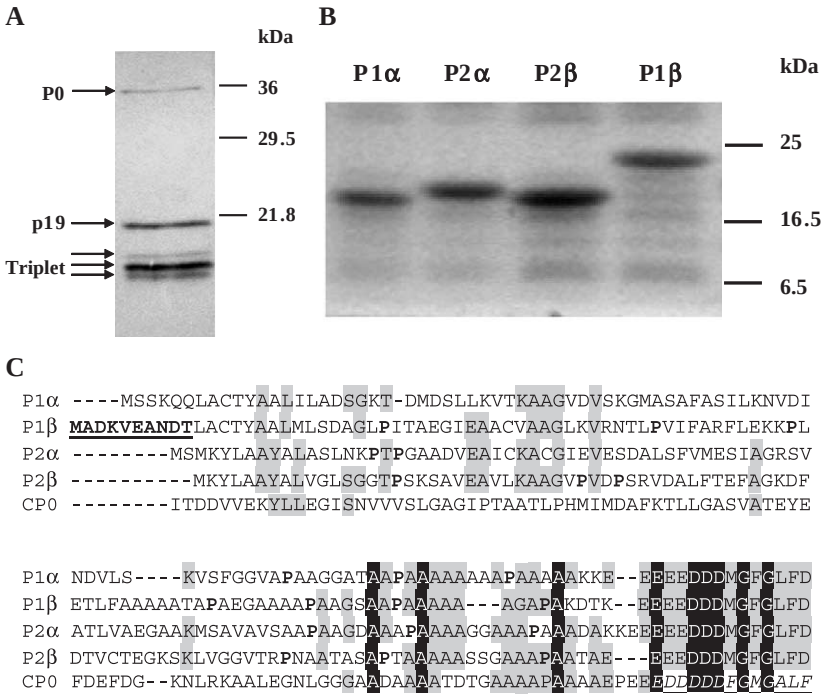

Fig. 1. A. Western blot of purified *T. cruzi* ribosomes revealed with monoclonal antibody 17.2. Fifteen micrograms of ribosomes were loaded. Molecular weights in kDa are shown on the right. The bands corresponding to P0 and p19 are indicated. The triplet corresponding to P1α, P2α and P2β is indicated with three arrows. B. One microgram of each recombinant P1/P2 protein from *T. cruzi* was analyzed by SDS-PAGE 15% and Coomasie blue staining. C. Sequence alignment of TcP1β with the other *T. cruzi* ribosomal P proteins. The 110 C-terminal amino acids of TcP0 are also shown (CP0). The acidic N terminal peptide of TcP1β is in bold and underlined. Proline residues in P1 and P2 proteins are in bold. The unusual C-terminal end of TcP0 is in italic and underlined.

protein family suggests that the composition of the different P protein families and their subtypes was a feature of the common ancestor to all three species. This is shown in Table 1 for the ribosomal P1 and P2 proteins.

3.2. Validation of the yeast two-hybrid system for T. cruzi ribosomal proteins

Conceptually, the biological information generated by two-hybrid analyses has often been questioned because of the inherent artificial nature of the assay. Therefore, it was necessary to assess if, in our hands, protein–protein interactions determined by classical biochemical protocols were correctly reproduced by the yeast two-hybrid approach.

Profiting from the availability of the monoclonal antibodies specific for R13 (mAb 17.2) and P2β (mAb 68.2) we studied the ability of previously cloned P1α and P2β proteins to interact in vitro. Gel mobility shift experiments were used to characterize their interaction. Due to the very similar electrophoretic migrating properties, we generated for this analysis a modified P1α protein with a C-terminal extension of 27 amino acids, named P1α*, that presented a migration pattern distinguishable from that of P2β. Details of this construction are given in Materials and Methods. P1α* and P2β were allowed to interact overnight at 4 °C. Since a two-fold excess of P2β was used, the detection of the interacting complex and free P2β was expected. In native PAGE, a band with an intermediate migration, representing the putative P1α*–P2β complex was evident (Fig. 2A, arrow). Western blots using 17.2 and 68.2 monoclonal antibodies support the hypothesis that the third band is composed by both P1α* and P2β polypeptides. The 17.2 antibody detected P1α*, P2β and the band with the interacting proteins (Fig. 2B, left). Free P1α* was not detected by mAb 17.2, whereas mAb 68.2 recognized the free P2β and a second band (Fig. 2B, right), demonstrating the composite nature of the latter.

Table 1
Percent of amino acid identity of *Trypanosoma cruzi* (Tc) P1/P2 proteins compared with proteins from *Saccharomyces cerevisiae* (Sc), *Trypanosoma brucei* (Tb) *and Leishmania major* (Lm)

	ScP1α	ScP1β	ScP2α	ScP2β	TbP1α	TbP1β	TbP2α	TbP2β	LmP1α	LmP1β	LmP2α	LmP2β
TcP1α	41.8	46.4	37.2	33.0	66.1	48.3	46.2	41.1	67.3	41.1	41.0	39.3
TcP1β	34.8	38.3	34.5	31.1	41.9	70.7	42.1	37.6	43.5	62.1	41.7	35.3
TcP2α	32.5	34.2	46.9	41.2	41.6	42.6	75.4	53.1	39.7	42.2	66.7	49.1
TcP2β	27.9	30.0	43.1	41.4	40.9	37.2	46.0	66.4	45.6	36.6	44.1	63.0

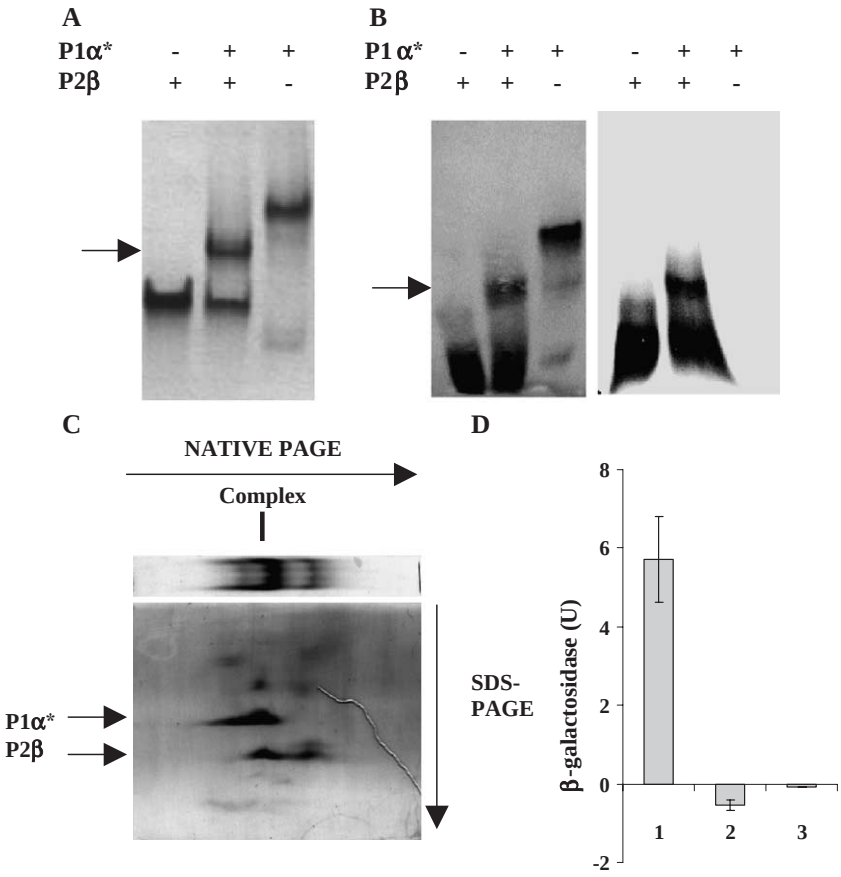

Fig. 2. In vitro and in vivo interaction between P1α and P2β. A. P1α* was preincubated with a two-fold excess of P2β and submitted to native PAGE. Controls in the absence of P1α* (left) or P2β (right) were included. Arrow indicates the P1α*–P2β complex band. B. Detection of P1α*–P2β complex with monoclonal antibodies against, in left panel: the C-terminal shared epitope (mAb 17.2) or right panel: the internal P2β epitope (mAb 68.2). C. Dissociation of the P1α*–P2β complex in the presence of SDS as evaluated by two-dimensional electrophoresis. D. Interaction between P1α and P2β evaluated by yeast two-hybrid. β-galactosidase activity of yeast extracts carrying BD-P1α and AD-P2β fusion proteins (1). Controls of self-activation in the absence of P2β (2) or P1α (3) are also showed.

To further demonstrate that P1α* and P2β were both present in the third band, the mixture was run in two-dimension gel (Fig. 2C). Under native conditions a pattern similar to the one shown in Fig. 2A, lane 2, was evident. Thereafter, a lane of this native gel (horizontal in Fig. 2C) was loaded over a denaturing gel and submitted to 15% SDS-PAGE (bottom part of Fig. 2C). As shown in Fig. 2C, P1α* and P2β proteins co-migrated under non-denaturing conditions, but were readily dissociated by SDS during the second run. P1α*/P2β interacting complex was also dissociated by 2–3 M urea, as evaluated by urea gradient electrophoresis (not shown).

These results were compared to those obtained using the yeast two-hybrid system, in which P1α and P2β were cloned as fusions to binding (BD) and activation (AD) domains of yeast GAL4 protein, respectively. Fig. 2D clearly showed that the interaction between P1α and P2β was also reproduced in yeast two-hybrid experiments, as determined, in this case, by β-galactosidase activity assays. This result prompted us to use the yeast two-hybrid approach to map the complete set of interactions of the components of the *T. cruzi* ribosomal stalk.

3.3. Interactions amongst T. cruzi P proteins

All the *T. cruzi* P proteins were subcloned into the corresponding vectors in order to obtain the DNA-binding domain (BD) and activation domain (AD) fusions. The first set of interactions tested were those involving the full length P0 protein, fused to both BD and AD domains, with all the low molecular weight P proteins. However, no interaction was detected with these P0 constructs. Taking into account results of two hybrid experiments reported by Lalioti et al. in which they demonstrate that the P1/P2 binding region of *S. cerevisiae* P0 was restricted to its C-terminal domain, we decided to represent TcP0 by constructions that carried only its 110 amino acid long C-terminal domain. The corresponding BD and AD fusions were named CP0. The full set of interactions was evaluated by means of two different reporter genes; *His3* and *LacZ* (Fig. 3). Filter β-galactosidase assays (Fig. 3, right panel, BD CP0) showed that CP0 interacted with P1α, P1β and P2α in the BD configuration. In the AD configuration the interactions of CP0 with P1α and P2α were confirmed, and the interaction with P2β was evidenced. Moreover, these β-galactosidase assays estab-

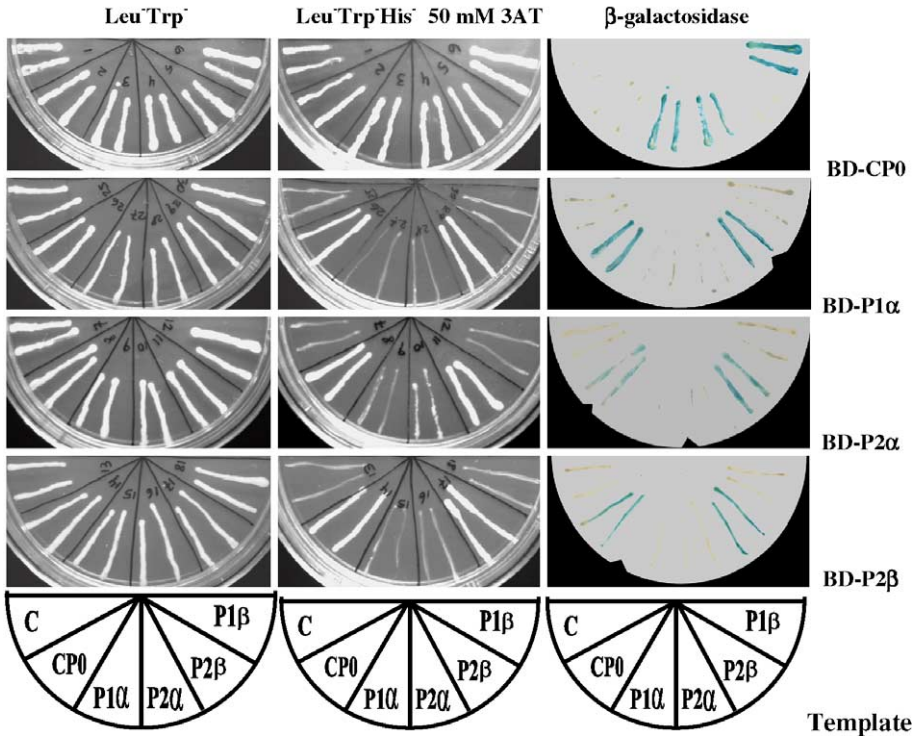

Fig. 3. Yeast-two-hybrid dependent induction of *His3* and *LacZ* reporter genes. Each BD fusion plasmid transformed cell (horizontal rows) was combined with the different AD fusions as indicated in the template. Cotransformats were grown in plates SC Leu−Trp− (control) and SC Leu−Trp−His− in the presence of 50 mM of 3AT, and assayed for β-galactosidase activity.

lished that P2β was also able to interact with P1α, P2α, and with itself. These results are summarized in Table 2. All the β-galactosidase positive interactions were confirmed with the *His 3* reporter gene (Fig. 3), excepting those with CP0 in the BD configuration, because the BD−CP0 fusion showed strong self-activation, even in the presence of 50 mM of 3AT (Fig. 3 upper row).

To corroborate the interactions involving BD−CP0 that were assessed by β-galactosidase assay (Fig. 3, right panel), the two-hybrid dependent induction of a third reporter gene, *Ura3*, was used. In the presence of 5-fluoroorotic acid (5FOA), the enzyme encoded by the *Ura3* gene generates 5-fluoroacil, which is toxic. All yeast cells positive for the β-galactosidase assay did not grow on 5FOA plates, confirming the interactions of BD−CP0 with P1α, P1β and P2α AD fusions (Fig. 4).

Altogether, results from Figs. 3 and 4 showed that CP0 was able to interact with each of the four *T. cruzi* P1 and P2 proteins, confirmed the P1α−P2β interaction and detected an unexpected P2α−P2β interaction (Table 2). The only P protein able to homodimerize was P2β.

It is noteworthy that attempts to transform yeast with the BD-P1β plasmid were repeatedly unsuccessful. When colonies were obtained, Westerns of yeast extracts with anti-GAL4BD monoclonal antibodies consistently failed to detect BD-P1β (not shown).

To evaluate quantitatively the mapped interactions the β-galactosidase liquid assay was employed. In Fig. 5 only the strongest interacting pair of both BD/AD configurations is shown. Remarkably, the highest signal was measured for the CP0-P1β interacting pair (Fig. 5, column 2). CP0 also interacted very strongly with the P1α and P2α proteins (Fig. 5, columns 1 and 3, respectively), and to a lesser extent, with P2β (Fig. 5, column 4) that showed a similar level of

Table 2
Summary of interactions amongst P proteins as detected by yeast two-hybrid assays

BD	AD				
	CP0	P1α	P1β	P2α	P2β
CP0	–	+	+	+	–
P1α	+	–	–	–	+
P2α	+	–	–	–	+
P2β	+	–	–	–	+

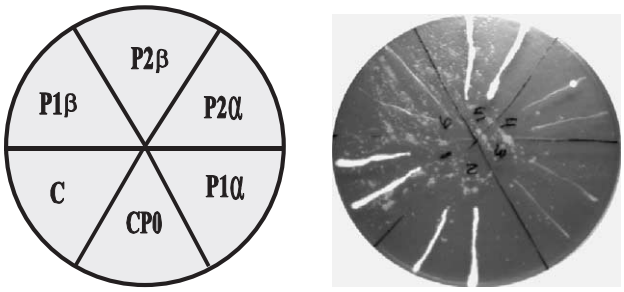

Fig. 4. Two-hybrid dependent induction of *Ura3* reporter gene. Yeast cells transformed with BD-CP0 and the indicated AD fusion plasmids (template) were grown in Leu−Trp− plates in the presence of 0.2% of 5FOA to detect interaction dependent growth inhibition.

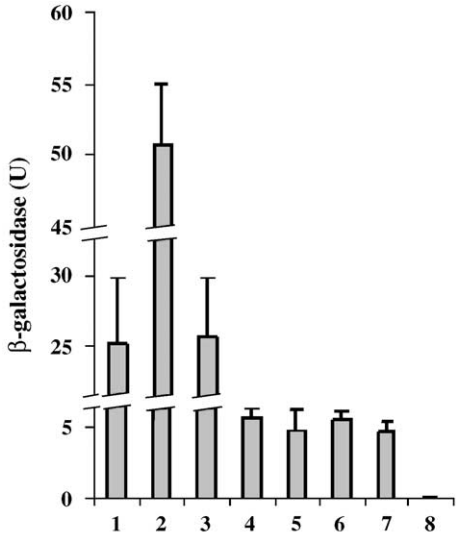

Fig. 5. Induction of β-galactosidase activity in yeast cells transformed with the following BD/AD interacting pairs: CP0/P1α (1), CP0/P1β (2), CP0/P2α (3), P2β/CP0 (4), P1α/P2β (5), P2α/P2β (6), P2β/P2β (7). The CP0/CP0 pair was included as negative control (8).

interaction with P1α, P2α and with itself (Fig. 5, columns 5,6,7, respectively). It is important to note that although the interactions involving P2β seemed weaker, they represent clear positive interactions as assessed in vitro for P1α–P2β, and by the fact that all four interactions involving P2β grow in plates lacking histidine, even in the presence of 50 mM of 3AT (Fig. 3).

4. Discussion

In 1992, Schijman et al. showed that the ribosomal P protein system of *Trypanosoma cruzi* was composed of five members. However, the identity of the ribosomal P protein of approximately 19 kDa remained unknown. Data mining allowed to identify a novel member of this protein group. Nevertheless, the molecular weight derived from nucleotide sequence was 11,400 far behind the almost 19 kDa revealed by Westerns. Its identity was confirmed only after the novel peptide was cloned and expressed. In SDS-PAGE, the recombinant protein showed a slow migration, similar to that of the native protein. Analysis of its sequence reveals two features that may be linked to its notable electrophoretic properties, the first one is the acidic nature of its N-terminal peptide MADKVEANDT (Alves et al., 2004), the second one is the distribution of Pro residues all along its sequence, particularly in the central portion of the molecule, and within the hinge region (Fig. 1C).

Sequence comparisons and phylogenetic analysis determined its classification as ribosomal protein P1, and was named P1β simply because it was the second P1 protein described for this parasite. The comparison of the parasite low molecular weight P proteins with those of yeast further suggests that the duplication of the P1 and P2 genes in each one of the species was an independent event.

The initial demonstration that in vitro detected interaction is correctly represented by a two-hybrid approach prompted us to analyze the interactions among these proteins using this method. The results indicate that P0 is able to interact with all the low molecular weight components of the system albeit differentially as indicated by the β-galactosidase quantification assays. Measures of this enzyme activity showed two types of interactions, those established by P2β, all of them around 4 β-Gal units, including its interaction with P0, and the interactions established between P0 and the other P1 and P2 peptides, ranging between 25 and 50 β-Gal units. It is tempting to speculate that this differential binding to P0 may be related to the different N-terminal sequences of the four P1 and P2 peptides (Tsurugi and Mitsui, 1991); if this is the case, the effect of the partial deletion and/or punctual mutations in this portion of these proteins could be easily measured. Experiments in this direction are currently underway in our laboratory.

In summary, the stalk of the large subunit of the ribosome of *T. cruzi* presents several parasite-specific features, such as the very unusual, archaea-like C-terminus of the P0 protein (Schijman and Levin, 1992), and the acidic N-terminus of P1β. It also presents a unique pattern of protein–protein

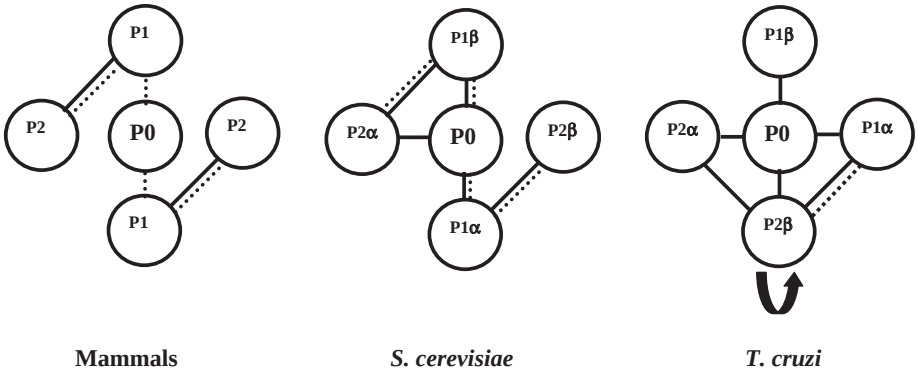

Fig. 6. Organization of the ribosomal stalk in different species. Detected protein–protein interactions amongst P proteins in different species are indicated as lines. Solid lines indicate interactions detected by two-hybrid technique. Dot lines indicate evidences from other in vivo and in vitro approaches. Arrow indicates the ability of TcP2β to interact with itself.

interactions, as P0 interacts with all four low molecular weight P proteins, particularly strongly with P1β, and P2β interacts with all P proteins, and with itself but not with P1β (Fig. 6).

The activity of several clinically powerful drugs against microbes is caused by their selective inhibition of protein synthesis (Fostel and Lartey, 2000), by analogy, compounds that may inhibit the parasite protein synthesis are likely to be useful anti-parasitic agents. In this regard, it is noteworthy that sordarin, an anti-fungal drug, seems to act stabilizing the binding of the elongation factor 2 (EF2) to the ribosome, an effect that seems to be enhanced by the presence of the fungal ribosomal P0 protein (Justice et al., 1999; Santos et al., 2004). Perhaps the very specific nature of the ribosomal P protein system of *T. cruzi* and the proteins that interact with them, L12 and EF2 (Uchiumi and Kominami, 1997; Bargis-Surgey et al., 1999; Lalioti et al., 2002) may enable the development of selective anti-parasite protein synthesis inhibitors.

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