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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