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Research brief

Trypanosoma cruzi: Molecular characterization of TcPUF6, a Pumilio protein

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Abstract

In trypanosomes regulation of gene expression occurs mainly at the post-transcriptional level. Pumilio proteins are RNA-binding proteins that modulate gene expression in lower and higher eukaryotes. Here we present the characterization of TcPUF6, a member of the Pumilio family in *Trypanosoma cruzi*. TcPUF6 is expressed in the different life cycle forms of the parasite showing no clear stage specific regulation and it is localized to multiple discrete foci in the cytoplasm of epimastigotes. The recombinant TcPUF6 fusion protein specifically binds to the *Drosophila hunchback* NRE (*nanos* response element). TcPUF6 conserves functional properties that characterize the Pumilio family throughout evolution.

Index Descriptors and Abbreviations: GST, Glutathione-*S*-transferase; NRE, nanos response element; SL, spliced leader; UTR, untranslated region; WGS, whole genome shotgun
keywords: Kinetoplastida; *Trypanosoma cruzi*; Pumilio family; RNA-binding proteins; Post-transcriptional regulation

The kinetoplastid protozoan *Trypanosoma cruzi* is the causative agent of American trypanosomiasis or Chagas' disease (Chagas, 1909), which afflicts millions of people in Central and South America. During its life cycle, the parasite invades two different hosts (a reduviid insect and a mammal) displaying at least four distinct developmental stages: trypomastigotes, epimastigotes, metacyclic trypomastigotes, and intracellular amastigotes (Tyler and Engman, 2001). Gene expression in trypanosomes involves peculiar mechanisms such as polycistronic transcription, addition of a mini exon sequence by trans-splicing, and editing of mitochondrial transcripts. Unlike higher eukaryotic cells, regulation of gene expression is considered to occur mainly at post-transcriptional stages since individual genes belonging to a common polycistronic unit may show differential expression (Teixeira, 1998). Mechanisms regulating gene expression could involve mRNA modulation by differential processing of long polycistronic transcripts by trans-splicing and poly(A) tail addition, changes in mRNA stability (Clayton, 2002), and mRNA mobilization to polysomes (Avila et al., 2001). Sequence or structural elements present in the 3′UTR of some mRNAs have been shown to confer stage specificity (Nozaki and Cross, 1995) through the binding of protein factors (Coughlin et al., 2000; D'Orso et al., 2003).

Regulation at the level of mRNA translation is a major mechanism in the control of gene expression. Particularly, the products of *nanos* and *pumilio* genes in *Drosophila* are key components of translation repression of target mRNAs (Parisi and Lin, 2000). Pumilio was first described as a protein that represses translation of the *hunchback* transcript in a ternary complex with Nanos, contributing to the posterior patterning of the *Drosophila* embryo (Wharton and Struhl, 1991). In *Caenorhabditis elegans* the fem-3-binding factors (FBF) 1 and 2 regulate the sperm/oocyte switch and germ line stem cell maintenance (Zhang et al., 1997). Based on structural similarities, FBF and Pumilio were proposed as members of a family of sequence-specific RNA-binding proteins named Puf (for Pumilio and FBF) (Zamore et al., 1997). In *Drosophila*, Pumilio has an RNA-binding domain with eight imperfect repeats of ~36 amino acids (Edwards et al., 2001) that recognizes a pair of highly conserved 32 bp elements (5′-AUUAUUUUGUUGU CGAAAAUGUUACAUAAGCC-3′) known as the *nanos* response element (NRE) of the *hunchback* mRNA (Murata and Wharton,

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1995; Wang et al., 2002). The Pumilio proteins modulate mRNA expression by enhancing turnover or repressing translation via interactions with other regulator proteins such as Nanos and Brain Tumor in *Drosophila* (Sonoda and Wharton, 1999), Nos3 in *C. elegans* (Kraemer et al., 1999) or DAZ-like proteins in human cells (Moore et al., 2003). The mechanism of action of these ternary complexes is not yet known.

In kinetoplastids a member of the Puf family, TbPUF1, has been described in *Trypanosoma brucei* being essential for cell viability. In addition, transfection assays suggest that its overexpression affects parasite virulence. TbPUF1 interacts with the ESAG8 putative regulatory protein and may control mRNA stability (Hoek et al., 2002).

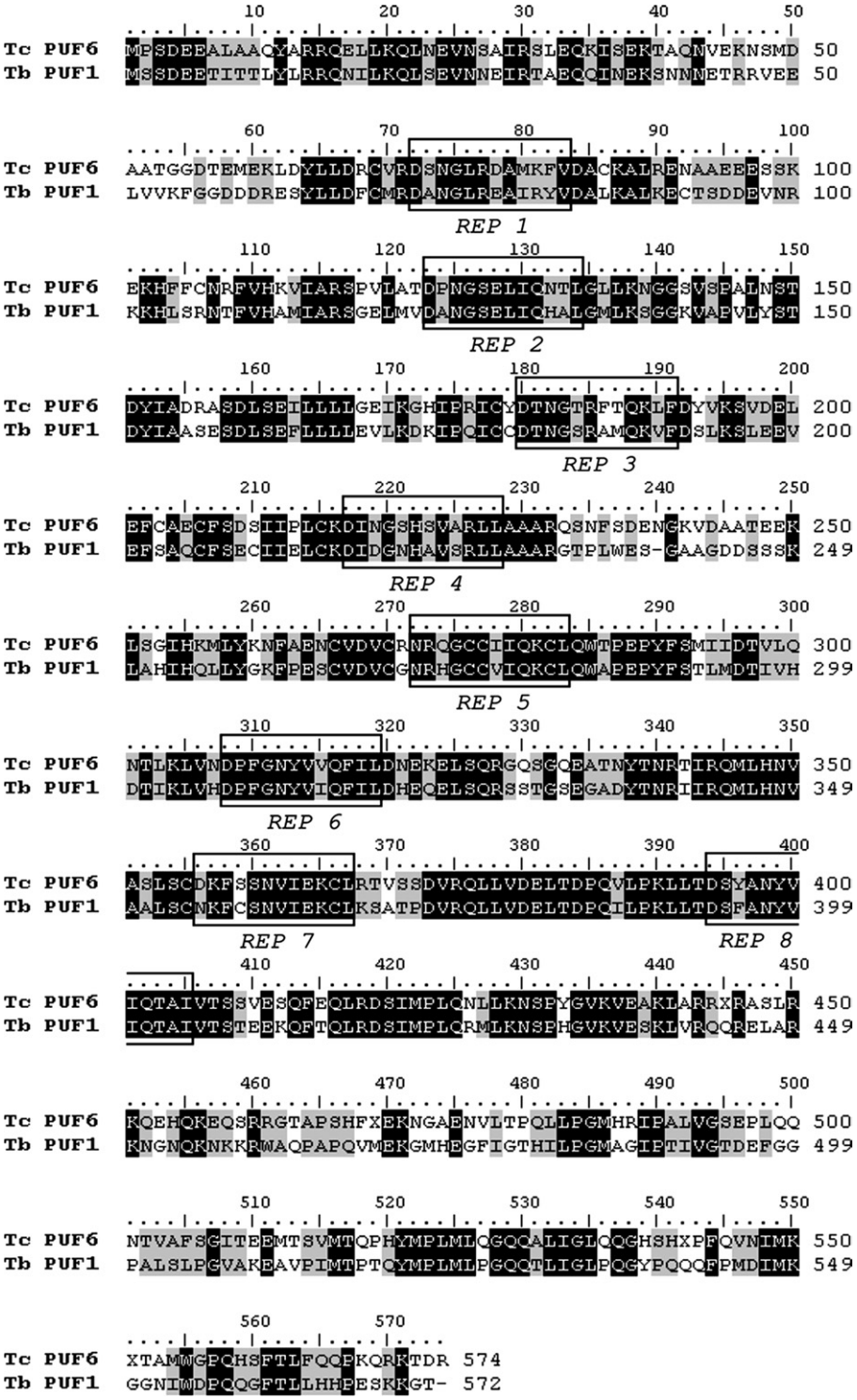

Fig. 1. Alignment of the Pumilio proteins from *T. cruzi* (TcPUF6) and the *T. brucei* orthologue (TbPUF1). Similarities (PAM250) are gray highlighted and identities are black shaded. Pumilio repeats are boxed.