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A non-universal transcription factor? The *Leishmania tarentolae* TATA box-binding protein *Lt*TBP associates with a subset of promoters ☆

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Abstract

In kinetoplastids a 39-nucleotide spliced leader RNA is trans-spliced to the 5′ end of nuclear mRNAs before they can be translated, thus the spliced leader is central to gene expression in kinetoplastid biology. The spliced leader RNA genes in *Leishmania tarentolae* contain promoters with important sites at approximately −60 and −30. A complex forms specifically on the −60 element as shown by electrophoretic mobility shift. The −60 shift complex has an estimated mass of 159 kDa. An *L. tarentolae* homologue of TATA-binding protein, *Lt*TBP, co-fractionates with the −60 shift complex. Inclusion of anti-*Lt*TBP antiserum in the shift assay disrupts the shift, indicating that *Lt*TBP is a component of the complex that interacts with the TATA-less −60 element of the spliced leader RNA gene promoter. Both *Lt*TBP and *Lt*SNAP$_{50}$ are found near the spliced leader RNA gene promoter and the promoters important for tRNA^{Ala} and/or U2 snRNA gene transcription, as demonstrated by chromatin immunoprecipitation. The *Lt*TBP appears to interact with a subset of promoters in kinetoplastids with an affinity for short transcription units.

Keywords: Kinetoplastid; Promoter; RNA polymerase II; RNA polymerase III; Small nuclear activating protein complex 50-kDa subunit; Transcription factor

1. Introduction

Kinetoplastid protozoa form a group of protists that include significant human parasites such as *Trypanosoma brucei* (African sleeping sickness), *Trypanosoma cruzi* (Chagas disease) and *Leishmania* species (leishmaniasis), as well as important plant and animal pathogens. Kinetoplastids diverged relatively early from the main eukaryotic tree and their biology is distinct in many ways from most model eukaryotes. One of the notable differences with regard to gene expression is that nuclear protein-coding genes are transcribed polycistronically (Johnson et al., 1987) by both RNA polymerase (pol) I and RNA pol II. As the polycistron is transcribed, a spliced leader (SL) is trans-spliced upstream of each coding region. Subsequent addition of a downstream poly (A) tail converts the polycistronic precursor into monocistronic mature mRNAs. The multicopy *SL RNA* are transcribed independently by RNA pol II (Gilinger and Bellofatto, 2001; Dossin and Schenkman, 2005) controlled by defined promoter elements (Campbell et al., 2000; Günzl, 2003; Palenchar and Bellofatto, 2006; Dossin and Schenkman, 2005) and a T-tract termination element (Sturm et al., 1999). Before participating in the trans-splicing reaction, the SL RNA primary transcript receives a m^{7}G cap and extensive 5′ methylations (Bangs et al., 1992) referred to as cap 4. In *Leishmania tarentolae*, undermethylation of cap 4 does not affect trans-splicing of the 96-nucleotide substrate SL RNA (Sturm et al.,

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1998), but has been correlated with a loss of mRNA loading onto polysomes (Zeiner et al., 2003).

RNA pol II presents two distinct modes of transcription in kinetoplastids: highly processive synthesis for protein coding genes with an apparently low rate of initiation (Martínez-Calvillo et al., 2003) and short punctuated synthesis with a high rate of initiation for SL RNAs (Kooter et al., 1984; Boothroyd et al., 1985). 'Switch regions', non-transcribed inter-cistronic areas likely containing sites for initiation of polycistronic transcription, have been characterised on chromosomes 1 and 3 in *Leishmania major* (Martínez-Calvillo et al., 2003, 2004). Identification of these regions could allow a precise definition of mRNA promoters in the near future. *SL RNA* promoters have been characterised in a number of kinetoplastids (Agami et al., 1994; Günzl et al., 1997; Nunes et al., 1997; Luo et al., 1999; Campbell et al., 2000), including single nucleotide resolution of the bipartite promoter of *L. tarentolae*, with −60 and −30 sequence elements (Yu et al., 1998). A pronounced difference between the activities of RNA pol II in kinetoplastids lies in the perception of transcription termination signals: a poly T tract leads to termination downstream of the *SL RNA* (Sturm et al., 1999), whereas each mRNA possesses a poly-pyrimidine tract upstream of each coding region as part of the trans-splicing signal (Curotto de Lafaille et al., 1992). Differential RNA pol II behaviour has been noted in the transcription of small nuclear (sn)RNA and mRNA genes in higher eukaryotes (Hernandez, 1992). Metazoan promoter and terminator elements for processive and punctuated transcription are not interchangeable (Hernandez and Weiner, 1986; Neuman de Vegvar et al., 1986; Dahlberg and Schenborn, 1988). It is reasonable to assume that distinct mechanisms of RNA pol II initiation will exist in kinetoplastids for the two modes.

Some of the cis-acting proteins involved in *SL RNA* transcription have been identified. The *L. tarentolae SL RNA* −60 promoter element (−60PE), which shows similarities to the proximal sequence element (PSE) of vertebrate snRNA gene promoters (Yu et al., 1998; Campbell et al., 2000), was bound specifically by a protein complex (Yu et al., 1998), as did a similar element from *Leptomonas seymouri*, from which the first *SL RNA* promoter binding proteins were reported: p36, p46, and p57 (Luo and Bellofatto, 1997). The p57 subunit is an orthologue of the human small nuclear activating protein complex (SNAPc) subunit $SNAP_{50}$ (Das and Bellofatto, 2003; Schimanski et al., 2005). In humans SNAPc directs transcription of *snRNA* by both RNA pol II and III (Hernandez, 2001). The kinetoplastid $SNAP_{50}$ protein has been associated with transcription by all three RNA pols, interacting with the *rRNA* (RNA pol I), *SL RNA* (RNA pol II) and *U2/U6 snRNA* (RNA pol III) gene promoters (Gilinger et al., 2004; Schimanski et al., 2004a); however the interaction with the *U2 snRNA* promoter was not detected in *T. brucei* (Schimanski et al., 2004b).

Searches of the three kinetoplastid genome databases (Berriman et al., 2005; El-Sayed et al., 2005; Ivens et al., 2005) have revealed few of the standard transcription factors common to many well-characterised eukaryotes, leading to the conclusion that the transcription machinery in kinetoplastids is reduced and/or divergent from that of higher eukaryotes. A *T. brucei* homologue of the TATA-binding protein (TBP), called TRF4, has been identified (Ruan et al., 2004). In eukaryotes and archaea, TBP is involved in most transcription initiation events (Thomm, 1996), and can direct transcription from TATA-less promoters (Pugh, 2000). Consistent with this specificity, *Tb*TBP is involved in transcription of multiple gene types including the *SL RNA* (Ruan et al., 2004).

In this paper, we present a molecular characterisation of a protein shift complex (designated −60SC) that forms on the *L. tarentolae* SL RNA −60PE. The genes for $SNAP_{50}$ and TBP homologues were cloned from *L. major* and *L. tarentolae*, respectively, and antibodies raised against recombinant proteins. The presence of *Lt*TBP in the −60SC was suggested in vitro by co-fractionation and confirmed by electrophoretic mobility shift assay (EMSA). Chromatin immunoprecipitation (ChIP) indicated that both *Lt*TBP and *Lt*$SNAP_{50}$ interact with the SL RNA gene promoter region in vivo. We discuss the implications of these results on formation of the transcription complex on the *SL RNA* promoter, as well as on RNA pol I and RNA pol III promoters in kinetoplastids.

2. Materials and methods

2.1. Nuclear extract preparation

Nuclear extracts were prepared as reported previously (Yu et al., 1998) with minor modifications. Mid-log UC(A) strain *L. tarentolae* cells (grown in brain–heart infusion supplemented with 100 μg ml^{-1} hemin) were centrifuged at 2000×*g* and 4 °C for 10 min, washed twice in cold PBS buffer. Each gram of pellet was resuspended with 5 ml of buffer A (0.3 M sucrose, 10 mM HEPES (pH 7.9), 10 mM K-Glu (potassium glutamate), 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.5% NP-40, 0.5 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulphonylfluoride (PMSF), 0.7 μg ml^{-1} pepstatin, 2 μg ml^{-1} leupeptin) by 20 strokes in a Dounce homogeniser. The lysate was centrifuged at 9800×*g* and 4 °C for 10 min. The resulting pellet was resuspended with 4 ml of buffer B (10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 1.5 mM EDTA, 5% glycerol and 100 mM K-Glu) for every gram of initial cell pellet. High salt extraction was performed by adding 3.0 M K-Glu dropwise to a final concentration of 400 mM with gentle stirring at 4 °C. The suspension was incubated for 30 min with continued gentle stirring and centrifuged in 1.5 ml microfuge tubes at 9800×*g* for 2 min at 4 °C. The supernatant was then dialysed overnight at 4 °C against buffer D (20 mM HEPES (pH 7.9), 100 mM K-Glu, 1.5 mM $MgCl_2$, 20% glycerol, 0.2 mM EDTA, 1.0 mM DTT and 0.5 mM PMSF).