Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular & Biochemical Parasitology 149 (2006) 27–37
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Purification of an eight subunit RNA polymerase I complex in *Trypanosoma brucei*

Tu N. Nguyen [a], Bernd Schimanski [a,b], André Zahn [b], Birgit Klumpp [b], Arthur Günzl [a,b,*]

[a] *Department of Genetics and Developmental Biology and Department of Molecular, Microbial and Structural Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3301, USA*
[b] *Medizinisch-Naturwissenschaftliches Forschungszentrum der Universität Tübingen, Ob dem Himmelreich 7, 72074 Tübingen, Germany*

Received 13 December 2005; received in revised form 20 February 2006; accepted 28 February 2006
Available online 11 May 2006

Abstract

Trypanosoma brucei harbors a unique multifunctional RNA polymerase (pol) I which transcribes, in addition to ribosomal RNA genes, the gene units encoding the major cell surface antigens variant surface glycoprotein and procyclin. In consequence, this RNA pol I is recruited to three structurally different types of promoters and sequestered to two distinct nuclear locations, namely the nucleolus and the expression site body. This versatility may require parasite-specific protein–protein interactions, subunits or subunit domains. Thus far, data mining of trypanosomatid genomes have revealed 13 potential RNA pol I subunits which include two paralogous sets of RPB5, RPB6, and RPB10. Here, we analyzed a cDNA library prepared from procyclic insect form *T. brucei* and found that all 13 candidate subunits are co-expressed. Moreover, we PTP-tagged the largest subunit TbRPA1, tandem affinity-purified the enzyme complex to homogeneity, and determined its subunit composition. In addition to the already known subunits RPA1, RPA2, RPC40, 1RPB5, and RPA12, the complex contained RPC19, RPB8, and 1RPB10. Finally, to evaluate the absence of RPB6 in our purifications, we used a combination of epitope-tagging and reciprocal coimmunoprecipitation to demonstrate that 1RPB6 but not 2RPB6 binds to RNA pol I albeit in an unstable manner. Collectively, our data strongly suggest that *T. brucei* RNA pol I binds a distinct set of the RPB5, RPB6, and RPB10 paralogs.

Keywords: RNA polymerase I; *T. brucei*; RPB6; RPB8; RPB10; RPC19

1. Introduction

The unicellular eukaryote *Trypanosoma brucei*, causative agent of African sleeping sickness in humans and *Nagana* in animals, utilizes RNA polymerase (pol) I in a multifunctional way which is unique to this parasite. While typical eukaryotic RNA pol I serves exclusively for transcription of the large ribosomal RNA gene unit (*RRNA*), *T. brucei* RNA pol I additionally transcribes protein-coding gene units which encode the major cell surface antigens procyclin and variant surface glycoprotein (VSG; reference [1]). These gene units were denoted as *GPEET* and *EP1* procyclin loci and *VSG* expression sites, respectively. Characterization of the *RRNA* promoter [2,3], the *GPEET* and *EP1* promoters [4–6], and the *VSG* expression site promoters [7–9] as well as a study on the function of individual promoter elements in stable binding of *trans* activating factors [6] revealed that *T. brucei* class I promoters are structurally different (reviewed in reference [10]). Moreover, in bloodstream form trypanosomes, RNA pol I does not exclusively localize to the nucleolus but in addition is sequestered into a novel DNase I-resistant compartment inside the nucleus called the expression site body [11]. It is therefore possible that *T. brucei* RNA pol I utilizes specific subunits or subunit domains to accommodate its versatility.

In lower eukaryotes, RNA polymerases are best characterized in the yeast *Saccharomyces cerevisiae* (reviewed in reference [12]). Yeast RNA pol I consists of 14 subunits: the 3 core subunits RPA190, RPA135, and RPC40, the 5 specific subunits RPA49, RPA43, RPA34.5, RPA14, and RPA12, and the 6 common subunits RPB5, RPB6, RPB8, RPB10, RPB12, and RPC19. While five of the common subunits are shared by all three yeast RNA polymerases, RPC19, as well as the above cited RPC40, are shared between RNA pols I and III. Core and common subunits as well as the specific subunit RPA12 are highly conserved and

* Corresponding author. Tel.: +1 860 679 8878; fax: +1 860 679 8345.
E-mail address: gunzl@uchc.edu (A. Günzl).

0166-6851/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2006.02.023

orthologs can typically be found in other eukaryotes [12]. With the exception of RPB8 this is also true for archaebacteria [13]. In addition, the specific subunits RPA14 and RPA43 are structurally and functionally equivalent to the RPB4/RPB7 heterodimer of eukaryotic RNA pol II [14] and to the archaeal RNA pol subunits E and F [15] indicating that this protein pair represents a phylogenetically conserved feature of eukaryotic and archaeal RNA polymerases. Consequently, eukaryotic RNA pol I should consist of a minimum of 12 subunits.

Systematic data mining of trypanosomatid genomes revealed homologues to 10 of these subunits [16]. Moreover, these genomes contain two paralogs of RPB5, RPB6, and RPB10 (references [16,17] and this study), the significance of which is not understood. The largest subunit of *T. brucei* RNA pol I, TbRPA1, was initially cloned and characterized on the basis of sequence homology [18,19]. Subsequently, a first partial purification of this enzyme led to the identification of the second largest subunit TbRPA2 which was found to harbor an approximately 50 kDa-large parasite-specific N-terminal extension domain of yet unknown function [20]. Recently, an improved RNA pol I purification strategy facilitated the identification of TbRPC40, Tb1RPB5 and TbRPA12 as RNA pol I subunits in *T. brucei* [17].

In this study, we have tandem affinity-purified *T. brucei* RNA pol I to homogeneity by employing the recently established PTP tagging and purification protocol [21]. The purified complex consisted of eight subunits: the five previously characterized proteins as well as RPB8, RPC19, and 1RPB10. In addition, we evaluated binding of the two RPB6 paralogs to RNA pol I and found that 1RPB6 but not 2RPB6 binds RNA pol I. The association is unstable however, and the subunit was lost during purification. Taken together, our data suggest that trypanosome RNA pol I is associated with a specific set of RPB5, RPB6, and RPB10 paralogs and that the other set is part of one or both of the other two RNA polymerases.

2. Methods

2.1. Plasmid constructs

pTbRPA1-PTP-NEO is a derivative of the cassette-type construct pC-PTP-NEO ([22], accession no. DQ172900) and has 410 bp of the C-terminal *TbRPA1* coding sequence fused to the PTP tag sequence. pPURO-HA-1RPB6 and pPURO-HA-2RPB6 are derivatives of construct pN-PURO-PTP ([22], accession no. DQ172901); they contain the same puromycin resistance gene cassette followed by an HA cassette which was cloned in-between the pBlueskript SK(+) *Hin*dIII and *Kpn*I sites (Stratagene, La Jolla, CA). The HA cassettes of both constructs consist of 434 bp of the *TbRPA2* gene 5′ flank, a translation start codon, the HA tag sequence, an *Xho*I restriction site and either 418 bp of 1RPB6 or 400 bp of 2RPB6 N-terminal coding region.

2.2. Trypanosome culture and cell lines

Procyclic *T. brucei brucei* strain 427 cells were cultivated in SDM-79 medium at 27 °C and cells were stably transfected by electroporation of 10 μg of linearized plasmid as described previously [23,24]. Clonal cell lines were obtained by limiting dilution and selection with 40 μg/ml of G418, 20 μg/ml of hygromycin, and/or 4 μg/ml of puromycin (Sigma, St. Louis, MO). Using pI-81 cells, which have one *TbRPA1* allele replaced with a bleomycin resistance gene [1], as a parental line, cell line TbF10 was generated by targeted integration of *Sal*I-linearized pTbRPA1-PTP-NEO into the remaining *TbRPA1* allele. TbF10 cells were then further manipulated to N-terminally HA-tag 1RPB6 and 2RPB6 by targeted genome integration of *Bam*HI-linearized construct pPURO-HA-Tb1RPB6 (cell line TbE5) and *Apa*I-linearized construct pPURO-HA-Tb2RPB6 (cell line TbB5), respectively. Correct integration of constructs was verified by PCR analysis, and expression of epitope-tagged proteins was analyzed by immunoblotting with monoclonal anti-protein C and anti-HA antibodies (Roche, Indianapolis, IN).

2.3. cDNA analysis

The cDNA library was generated from procyclic *T. brucei brucei* strain 427 as detailed in a previous study [20]. In brief, poly(A)$^+$ RNA was isolated with the Dynabeads mRNA DIRECT kit (Dynal Biotech, Lake Success, NY) and cDNA was prepared using the Marathon cDNA Amplification Kit (Clontech, Mountain View, CA). Individual cDNAs were characterized by rapid amplification of cDNA ends (RACE) such that 5′ and 3′ RACE products overlapped by at least 50 bp. RACE products were cloned and sequenced on both strands, and complete cDNA sequences were deposited in the EMBL nucleotide sequence database (see Table 1 for accession numbers).

2.4. RNA pol I purification and analysis

Extract preparation and tandem affinity purification of PTP-tagged RNA pol I from TbF10 cells was carried out exactly as described in detail by Schimanski et al. [21]. For immunoblot analyses, PTP- or protein C-tagged proteins were separated on SDS-PAGE gels, electroblotted onto a polyvinylidene difluoride membrane, and detected by the anti-protein C antibody HPC4 in combination with the BM Chemiluminescence Blotting substrate according to the manufacturer's protocol (Roche). For protein identification, purified proteins were separated on 10–20% SDS/polyacrylamide gradient gels and stained with Pierce Gelcode coomassie stain (Pierce, Rockford, IL). Alternatively, purified proteins were separated by 2D gel electrophoresis. For this, purified proteins were resuspended in rehydration buffer (8 M urea, 3% CHAPS, 50 mM dithiothreitol, 0.2% Biolyte 3/10) and incubated with an 11-cm immobilized pH 3–10 gradient gel ReadyStrip (BioRad, Hercules, CA) overnight at room temperature. Isoelectric focusing was carried out in a PROTEAN IEF cell (BioRad) with conditioning at 250 V for 15 min, voltage ramping to 8000 V for about 2.5 h with a current limit of 50 μA, and the final focusing of 4.3 h for a total of 35,000 VH. For the second dimension, proteins were separated by denaturing PAGE and coomassie-stained as described above. Protein bands or spots were excised from the gel and identified by liquid chromatography–tandem mass spectrometry.