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Short communication

The *Trypanosoma brucei* CCCH zinc finger proteins ZC3H12 and ZC3H13

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ABSTRACT

CCCH-type zinc finger proteins have various roles in RNA metabolism. We here analysed the functional relevance of two such proteins from *Trypanosoma brucei*, *Tb*ZC3H12 and *Tb*ZC3H13. Each protein has a single CCCH motif very similar to those seen in metazoan proteins that regulate mRNA degradation. *Tb*ZC3H12 is expressed in bloodstream form parasites at low levels. It is phosphorylated, cytosolic and not required for normal growth of cultured bloodstream trypanosomes. RNA interference targeting *TbZC3H13*, on a *TbZC3H12* null background, also had no effect on bloodstream trypanosome growth, but over-expression of tagged *Tb*ZC3H13 inhibited procyclic trypanosome growth. Tandem affinity purification of both proteins revealed various interesting potential interactions; specificity was assessed against a list of proteins that were found in 24 other pull-down experiments, which is provided. The conservation of *Tb*ZC3H12 in all kinetoplastids, and *Tb*ZC3H13 in Salivaria, suggests that the two proteins may be required for optimal growth at some stage of the parasite life-cycle.

1. Introduction

Trypanosoma brucei multiplies in mammalian blood and tissue fluids as the bloodstream form (BS) and in the midgut of Tsetse flies as the procyclic form (PC). Since most trypanosome genes are constitutively transcribed, rates of mRNA turnover and translation are important in controlling trypanosome gene expression [1]. The control of mRNA turnover in eukaryotes is often mediated by proteins which bind the 3′-untranslated region of a target mRNA. One such protein class, the Tis11 family, contains two finger domains of the type C-x-C-x-C-x_3-H [2], separated by a short linker and immediately preceded by a consensus sequence, R/K-Y-K-T-E-L. Well-studied examples of mammalian Tis11-family proteins include tristetraprolin (TTP) and Butyrate Response Factors BRF1 and BRF2; each of these binds to AU-rich elements (AREs) in the 3′-UTRs of mRNAs and induces their decay [2].

Forty-nine genes encoding CCCH zinc finger proteins are found in the *T. brucei* genome. A few have known functions in splicing and mRNA export [3], and so far, four have been implicated in post transcriptional gene regulation [4]. *Tb*ZFP1, *Tb*ZFP2 and *Tb*ZFP3 are all required for normal differentiation [5–7]; *Tb*ZFP3 is required for normal patterns of translation of the major surface proteins of procyclic forms. Over-expression of *Tb*ZFP2 in the procyclic forms caused abnormal remodelling of the cytoskeleton [5]. Meanwhile *Tb*ZC3H20 is required for growth of procyclic forms, binding to and stabilizing at least two developmentally regulated mRNAs [8]. *Tb*ZC3H18 has been implicated in control of differentiation but the mechanism is unknown [9].

In this paper, we investigated the functions of two more *T. brucei* CCCH zinc finger proteins, *Tb*ZC3H12 (Tb927.5.1570) and *Tb*ZC3H13 (Tb927.5.1580) (henceforth written without the *Tb* prefix). Each has a single CCCH motif starting at residue 20 near the N-terminus. The proteins are 50% identical in the first 49 amino acid residues, and then diverge completely (Fig. 1A). ZC3H13 (60 kDa) is 400 residues longer than ZC3H12 (18.8 kDa). Within the core CCCH motif, the key aromatic residues that are involved in base stacking are conserved (Fig. 1A sequence, arrowheads). We were particularly interested in these two proteins because each has a sequence resembling the Tis11 consensus immediately preceding the CCCH motif: KYRTTL for ZC3H12 and KYKTSL for ZC3H13 (Fig. 1A, grey-shaded sequence).

The genes encoding ZC3H12 and ZC3H13 are located next to each other on chromosome 5 of *T. brucei* TREU927; the *ZC3H13* gene is the first ORF in a polycistronic transcription unit as indicated by both deep sequencing [10] and histone modification patterns [11] (Fig. 1B). *ZC3H12* is conserved in all Kinetoplastid parasite genomes sequenced to date, while *ZC3H13* is present in all salivarian trypanosomes (*T. brucei*, *T. congolense* and *T. vivax*). Messenger RNAs encoding ZC3H12 and ZC3H13 are present at similar levels in both BS and PC trypanosomes (Fig. 1B) [12]; the half-lives (12–18 min) and abundances (1–2 mRNAs per cell) are similar to those for most trypanosome open reading frames (ORFs) [13]. The

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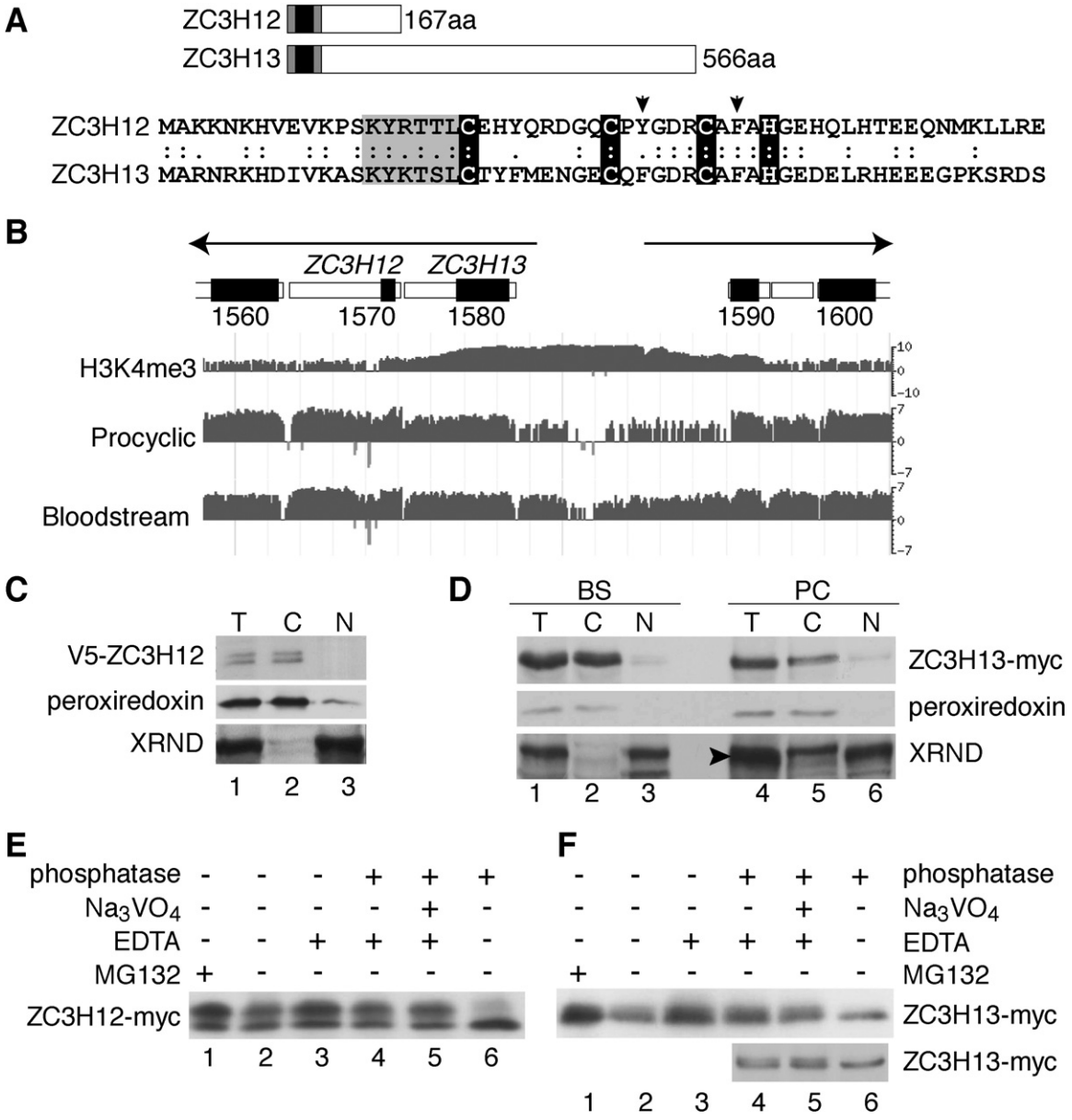

Fig. 1. ZC3H12 and ZC3H13 protein properties. (A) Above: Cartoon of *T. brucei* ZC3H12 and ZC3H13. The conserved motif with the CCCH domain is shown in black, and the remaining N-terminal conserved region in grey, with number of amino acid residues (aa) indicated. Below: The aligned N-terminal sequences, with double dots for identical residues and single dots for functional conservation: R/K (basic), F/Y (aromatic), S/T (aliphatic, hydroxyl group). The CCCH residues are white on black, and the conserved 6mer is underlain in grey. The arrows indicate the aromatic groups that are involved in base stacking in Tis11 proteins [22]. (B) Chromosomal context of ZC3H12 and ZC3H13. The gene map is shown at the top, with ORFs in black and the approximate extent of the mRNAs as open boxes. The arrows show the direction of transcription, and abbreviated gene numbers (*e.g.* Tb927.5.1570 is abbreviated to "1570"). Below are shown the map of histone H3 trimethylation at lysine 4, showing enrichment at the transcription start region [11], and the RNA sequencing read densities for PC and BS trypanosomes [12]. The figure is based on a screen shot from TritrypDB. (C) Localization of *in situ* V5-tagged *Tb*ZC3H12 in BS parasites. Nuclei and cytoplasm were separated by NP40 lysis and centrifugation, and the resulting fractions were analysed by Western blotting. Lysate equivalent to 2×10^7 cells was loaded for each fraction. T, total lysate; C, cytosol; N, Nuclei. The marker proteins are XRND (nucleus) and peroxiredoxin (cytosolic). All methods are described in the Supplementary Material. (D) Localization of inducibly expressed myc tagged *Tb*ZC3H13 in both BS and procyclic (PC) parasites. Experimental details as in (A). For each fraction, lysate equivalent to 5×10^6 cells was loaded on the gel. In PC cells, a cytoplasmic protein that migrates slightly slower than XRND cross-reacts with the anti-XRND antiserum. XRND is indicated by an arrowhead. (E) Phosphorylation of ZC3H12-myc. Lysates from myc- tagged *Tb*ZC3H12 bloodstream form cells were incubated at 30 °C for 20 min with the indicated chemicals and enzymes. The phosphatase was λ-phosphatase. MG132 is a proteasome inhibitor and was added 30 min prior to extract preparation, as well as to the extraction buffer. EDTA and Vanadate are λ-phosphatase inhibitors. *In situ* V5-tagged protein gave similar results but the bands were fainter (not shown). (F) Dephosphorylation of ZC3H13-myc. Experimental details as in (E); a second gel from another experiment, with slightly better resolution, is shown beneath the first.

ZC3H12 mRNA had the expected size of 1.5 kb (Supplementary Fig. S1B); the *ZC3H13* mRNA was usually not detectable (not shown).

To examine the properties of ZC3H12, we inserted a sequence encoding a V5 tag at the 5′-end of the endogenous ORF: this should result in expression of approximately normal levels of mRNA [14] (see Supplementary Material for methods). BS cells were obtained. Using 1×10^7 parasites, we detected the expected band migrating at 20 kDa, and an additional band migrating slightly slower (Fig. 1C and Supplementary Fig. S1B), but with smaller cell numbers, the protein could not be detected. In contrast, *in situ* V5-tagged UBP2 – estimated to be present at around 6×10^6 molecules per BS cell – was previously readily detected using 1×10^5 cells [15]. This comparison suggests that ZC3H12 is much less abundant than UBP2 in BS trypanosomes. Heat shock for 1 h at 41 °C, and cell densities