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Short communication

Identification of core components of the exon junction complex in trypanosomes

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ABSTRACT

In animal cells, the exon junction complex (EJC) is deposited onto mRNAs during the second step of splicing, 20–24 nt upstream of the exon–exon junction. The EJC core contains four proteins: Mago, Y14, eIF4AIII and Btz. In trypanosomes, *cis*-splicing is very rare but all mRNAs are subject to 5′ *trans*-splicing of a 39-nt RNA sequence. Here we show that trypanosomes have a conserved Mago and a divergent Y14 protein, but we were unable to identify a Btz orthologue. We demonstrate that Mago and Y14 form a stable heterodimer using yeast two hybrid analyses. We also show that this complex co-purifies *in vivo* in trypanosomes with a protein containing an NTF2 domain, typically involved in mRNA transport.

1. Introduction, results and discussion

The expression of protein-coding genes in eukaryotes involves a series of interconnected steps: transcription initiation, mRNA processing, export from the nucleus, translation, and degradation. In opisthokont eukaryotes, the exon junction complex (EJC) is loaded onto mRNAs during the second step of splicing at a fixed distance upstream (20–24 nt) of exon–exon junctions. The EJC serves as a marker for completed splicing; other proteins that interact with it promote mRNA export. The EJC is eventually removed during the first "pioneering" round of translation in the cytoplasm [1,2]; failure to remove it, in mammals, results in degradation of the mRNA in a process known as Nonsense Mediated Decay (NMD).

The stable core of the EJC contains four proteins: Mago, Y14, eIF4AIII and Btz (MNLN51) [3,4]. eIF4AIII is a DExH/D-box helicase that binds to the RNA and interacts with the heterodimer Mago/Y14 and Btz (NLM51) [1,5]. Y14 is a small protein with an RNA Recognition Motif (RRM), but instead of binding RNA, its RRM domain is involved in a protein–protein interaction with Mago [6,7]. The EJC tetramer is stably engaged on mRNA because of inhibition of eIF4AIII ATPase activity by the C-terminal amino acids of Mago [8]. The dissociation of Mago/Y14 from eIF4AIII due to interaction with cytoplasmic factors such as PYM [9] liberates the ATPase function and induces the release of EJC from mRNA during the pioneer round of translation.

In trypanosomes, transcription by RNA polymerase II is polycistronic [10]. The 5′-ends of individual mRNAs are created by *trans*-splicing of a 39 nt spliced leader sequence (SL) [11]; this is followed by polyadenylation. So far there is no evidence for any coupling between RNA polymerase II transcription and splicing (M. Stewart, S. Haile and C. Clayton, in preparation) there is no published evidence for NMD and little is known about mRNA export. We were therefore interested to find out whether trypanosomes have an exon junction complex.

We first searched the *Trypanosoma brucei* and *Trypanosoma cruzi* genomes for EJC components. The predicted trypanosome Mago protein (accession numbers XP_818983 for *T. cruzi*, XP_845612 for *T. brucei*) has over 50% of identity with other eukaryotic Magos (Fig. 1A). In contrast, we were unable to find Y14 by direct BLAST analysis. We therefore restricted the searches to the complete set of trypanosome small proteins containing only one RRM domain [12], then manually searched for key Y14 amino acid residues present in the RNP-2 and RNP-1 signatures within the RRM. The resultant trypanosome Y14 candidate (accession numbers AAW82044 for *T. cruzi*, XP_845741 for *T. brucei*) has about 30% sequence identity with the human protein, mostly restricted to the RRM domain (Fig. 1B). No trypanosome homologue of Btz (NLM51) was found, but a nuclear eIF4AIII was previously suggested to be part of a putative EJC [13].

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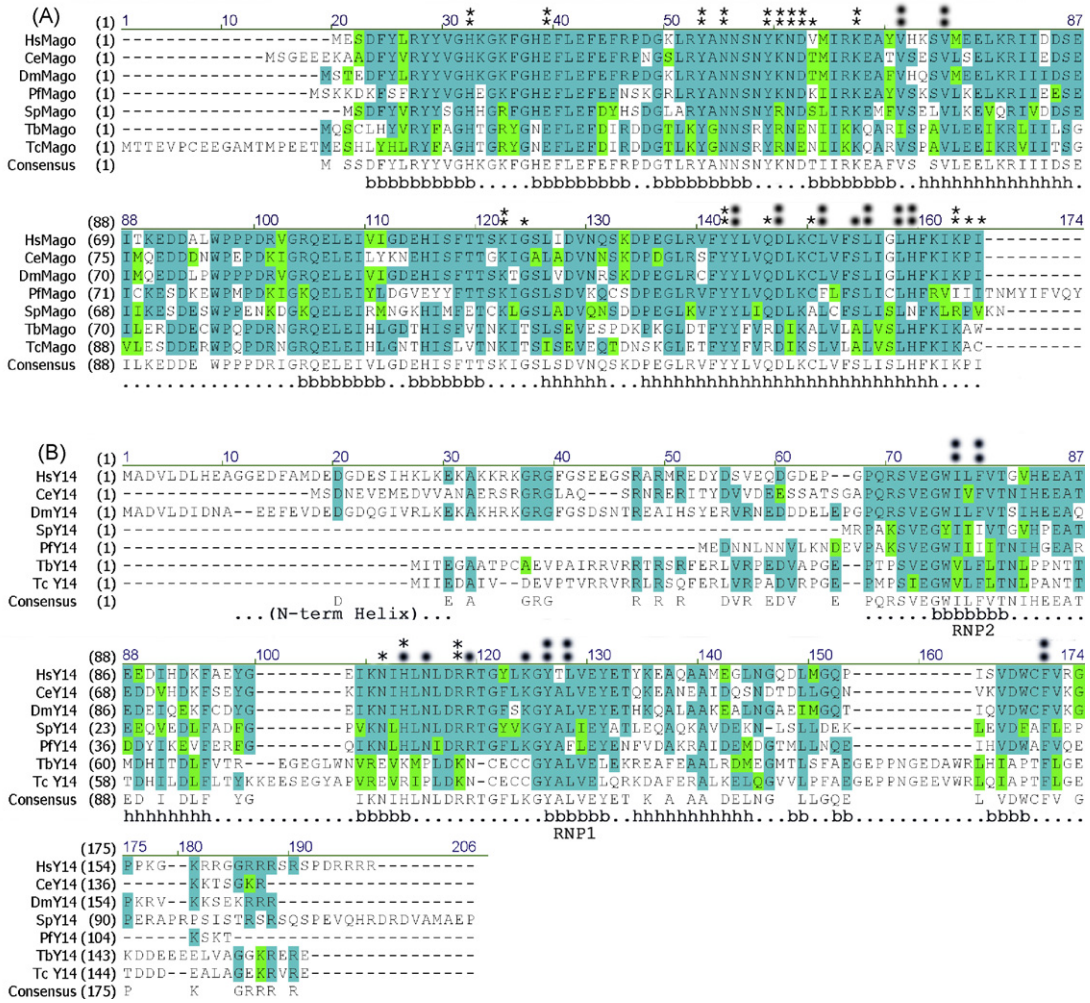

Fig. 1. (A) Mago sequence comparison. Residues that are involved in the Mago/Y14 interaction in the mammalian protein, and are conserved in the trypanosome protein, are shown by double dots; those required for interaction with eIF4AIII are shown by double stars. Mago/Y14-interacting residues that are not conserved in trypanosomes are indicated with single dots; altered residues for interaction with eIF4AIII are indicated by single stars. Structural predictions are below the sequence, b, beta-sheet; h, alpha helix; dot, loop. Hs, *Homo sapiens*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Pf, *Plasmodium falciparum*; Sp, *Schizosacharomyces pombe*; Tb, *Trypanosoma brucei*; Tc, *Trypanosoma cruzi*. (B) Y14 amino acids sequence comparison, symbols as in (A) except that double dots and single dots reference to the interaction with Mago.

Looking for EJC-type interactions, we cloned the human and trypanosome Mago and Y14 proteins in the Gateway compatible Proquest yeast-two hybrid system in both AD and DB configurations. Interactions were tested by activation of *HIS3* reporter gene in plates lacking histidine with 25 mM 3AT (not shown) and by activation of *LacZ* reporter gene in liquid culture assay (Fig. 2A). The results observed by activation of both reporter genes correlated perfectly. The interaction between *Tc*Y14 and *Tc*Mago appeared to be stronger than that of the human proteins (columns 2 and 5), and trypanosome Y14 also interacted with human Mago (columns 3 and 6). The Y14 RRM domain has two amino acids in the RNP-2 – Val_{46} and Phe_{48} – which are predicted to inhibit interactions with RNA [14] and might be involved in the interaction with Mago [6,7]. In agreement, mutation of these residues to Ala completely abolished interaction with *Tc*Mago further supporting the identity of the trypanosome Y14 (Fig. 2B).

To identify other components of the trypanosome EJC, we expressed a TAP-tagged version of Y14 in *T. brucei* procyclic cells, purified the complex, and identified the associated proteins by LC-MS/MS. Results are shown in Fig. 2C. In addition to *Tb*Mago, we found a novel protein of unknown function (encoded by locus Tb10.70.5500) with an NTF2 domain (Fig. 2D). NTF2 domains are typically found in nuclear transport factors [15–17]. Additionally, the protein has an Arg-rich region, separated from the NTF2 domain by a predicted coiled-coil (Fig. 2D). Surprisingly, we did not find the previously described [13] nuclear eIF4AIII protein. As expected, TAP-tagged *Tb*Y14 was mainly in the nucleus (Fig. 3A).

Finally, we depleted *Tb*Y14 and *Tb*Mago mRNAs by tetracycline inducible RNA interference. In one cell line, the knock down of *Tb*Y14 mRNA impaired trypanosome growth (Fig. 3B), suggesting that the trypanosome EJC is necessary for some aspect of mRNA metabolism. *Tb*Mago RNAi did not affect growth, but this result is uninformative since we do not know how much of the protein remains after RNAi induction.

Our results suggest that trypanosomes might contain a modified form of exon junction complex, containing Mago, a divergent Y14, and a novel protein with an NTF2 domain. The lack of any association with nuclear eIF4AIII, interestingly, correlates with the fact that two out of three residues required for Y14 to interact with eIF4AIII are mutated (Fig. 1B). Similarly, two key C-terminal residues necessary for Mago to inhibit ATPase function in eIF4AIII and lock the protein onto the mRNA are also lost (Fig. 1A). Nevertheless, we cannot rule out the possibility that this interaction indeed occurs *in vivo*, but was lost under the conditions used for the TAP purification. We do not yet know whether the trypanosome EJC is deposited on all mRNAs, or whether it is restricted to *cis* spliced mRNAs. We