International Journal for Parasitology 48 (2018) 179–189

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Tail characteristics of *Trypanosoma brucei* mitochondrial transcripts are developmentally altered in a transcript-specific manner

Vahid H. Gazestani [a,1], Marshall Hampton [b], Aubie K. Shaw [c], Reza Salavati [a,*], Sara L. Zimmer [c,*]

[a] Institute of Parasitology, McGill University, Montreal, QC H9X 3V9, Canada
[b] Department of Mathematics and Statistics, University of Minnesota Duluth, Duluth, MN 55812, USA
[c] Department of Biomedical Sciences, University of Minnesota Medical School, Duluth, MN 55812, USA

ARTICLE INFO

Article history:
Received 16 June 2017
Received in revised form 22 August 2017
Accepted 26 August 2017
Available online 1 November 2017

Keywords:
RNA tail
Mitochondrion
Trypanosoma
Uridylation
Polyadenylation
Hidden Markov modelling

ABSTRACT

The intricate life cycle of *Trypanosoma brucei* requires extensive regulation of gene expression levels of the mtRNAs for adaptation. Post-transcriptional gene regulatory programs, including unencoded mtRNA 3′ tail additions, potentially play major roles in this adaptation process. Intriguingly, *T. brucei* mitochondrial transcripts possess two distinct unencoded 3′ tails, each with a differing functional role; i.e., while one type is implicated in RNA stability (in-tails), the other type appears associated with translation (ex-tails). We examined the degree to which tail characteristics differ among cytochrome c oxidase subunits I and III (CO1 and CO3), and NADH dehydrogenase subunit 1 (ND1) transcripts, and to what extent these characteristics differ developmentally. We found that CO1, CO3 and ND1 transcripts possess longer in-tails in the mammalian life stage. By mathematically modelling states of in-tail and ex-tail addition, we determined that the typical length at which an in-tail is extended to become an ex-tail differs by transcript and, in the case of ND1, by life stage. To the best of our knowledge, we provide the first evidence that developmental differences exist in tail length distributions of mtRNAs, underscoring the potential involvement of in-tail and ex-tail populations in mitochondrial post-transcriptional regulation mechanisms.

1. Introduction

Vector-borne *Trypanosoma brucei* parasites cause sleeping sickness in humans and major production losses (e.g., meat, milk and fertility) in livestock. These pathogens shuttle between mammalian hosts (bloodstage form, BSF) and the tsetse fly vector (procyclic form, PCF), encountering extremely different environmental conditions, particularly in available energy sources. To cope with such extreme changes, *T. brucei* possesses a complicated, multilayer mitochondrial gene regulatory pathway that is essential for its survival in both life stages (Aphasizhev and Aphasizheva, 2011). Such dependence on unique mitochondrial gene regulatory pathways presents an attractive target for drug development. Accordingly, several compounds with trypanosomacidal activity, including pentamidine and ethidium bromide, target the mitochondrial genome or its fitness (Roy Chowdhury et al., 2010; Fidalgo and Gille, 2011). However, many aspects of *T. brucei* mitochondrial gene regulation are still poorly understood.

Trypanosoma brucei mitochondrial gene expression is significantly divergent from that of yeast or mammals (Verner et al., 2015). Illustrated in Fig. 1A, gene expression starts with the transcription of two rRNAs and 18 protein-encoding transcripts (mtRNAs) from mitochondrial DNA "maxicircles" in a polycistronic manner. Post-transcriptional events are the main mechanism of mtRNA expression regulation, mediating cleavage/trimming of RNA precursors into monocistrons, and mtRNA decay and translation rates (Aphasizhev and Aphasizheva, 2011; Carnes et al., 2015; Suematsu et al., 2016; Zhang et al., 2017). Twelve of the 18 mtRNAs undergo RNA editing in which uridine(s) are inserted in and/or deleted from specific positions of mtRNAs to form correct translatable transcripts. This is a transcript-specific process that is directed by a set of template-containing guide RNAs (gRNAs) (Hashimi et al., 2013; Aphasizheva et al., 2014; Aphasizheva and Aphasizhev, 2016; Read et al., 2016). While the role of RNA editing in mtRNA post-transcriptional regulation has been studied extensively, the importance of mtRNA stability and translation regulation, and how those relate to RNA editing, has been analysed less thoroughly.

* Corresponding authors.
E-mail addresses: reza.salavati@mcgill.ca (R. Salavati), szimmer3@d.umn.edu (S.L. Zimmer).
[1] Present address: Department of Pediatrics, University of California, San Diego School of Medicine, La Jolla, CA 92093, USA.

https://doi.org/10.1016/j.ijpara.2017.08.012
0020-7519/

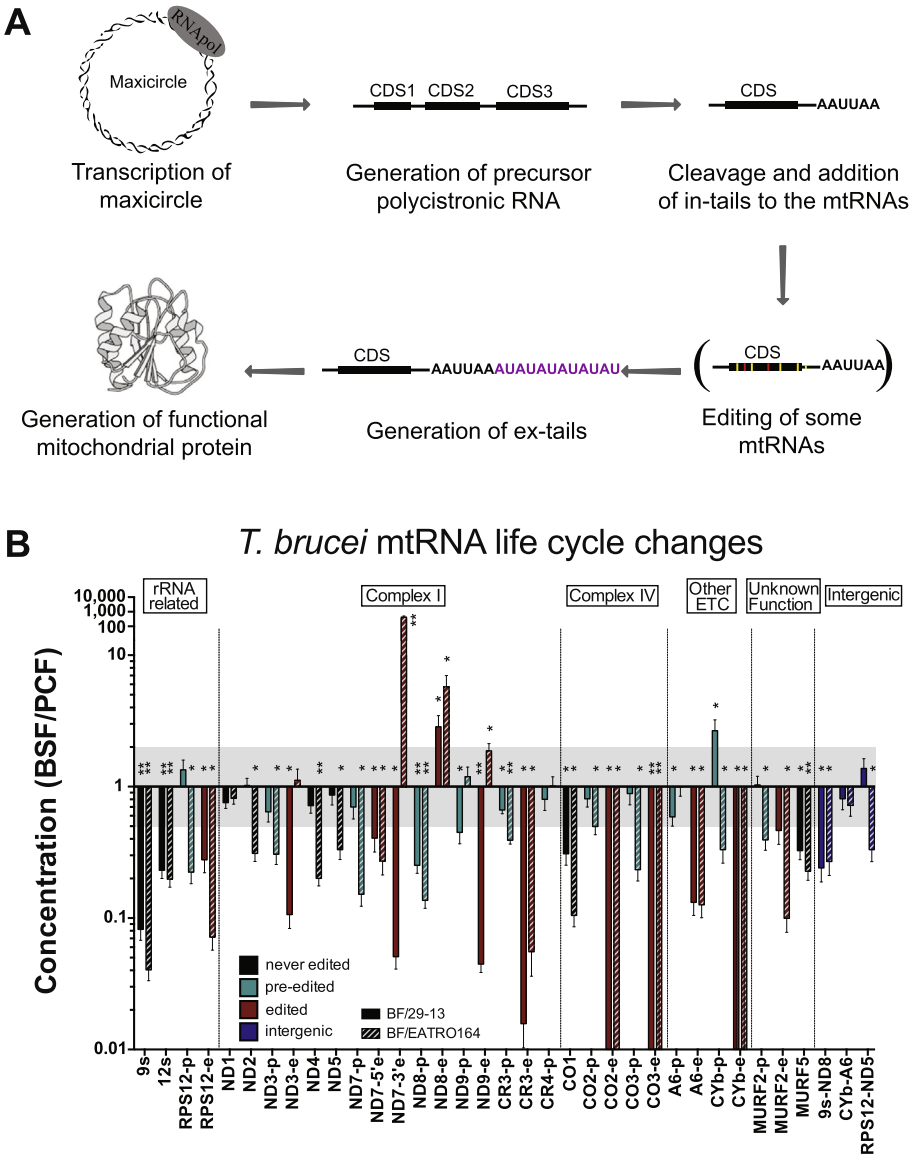

Fig. 1. *Trypanosoma brucei* maxicircle gene expression. (A) Pathway of mtRNA processing and expression. CDS, coding sequence. (B) Log-scale comparison of relative abundances of maxicircle-derived RNAs (arranged on the *X* axis) from cultured *T. brucei* in the bloodstage form compared with the procyclic form life stage. RNAs are arranged by gene product function. Never edited RNAs are shown in black, pre-edited (-p) RNAs in green, and edited (-e) RNAs in brown. ND7 contains a "constitutively edited" 5′ region and a 3′ region believed to be edited in the bloodstage form stage only; hence, each site was analysed separately. Three primer pairs spanning from one gene product to the next capture abundances of polycistronic RNAs containing those two sequences prior to cleavage into monocistrons (shown in blue). The relative expression of RNAs from the 427-derived BSF SM strain with both 427-derived PCF 29-13 strain (solid) and 927-derived PCF EATRO164 Istar1 strain (hatched) are shown. Error bars represent S.E.M. $^{**}P < 0.001$, $^{*}P < 0.05$. Where a ratio is displayed as reaching 0.01 (CO2-p, CO3-p and CYb-e), the transcript was undetectable in the bloodstage form cells.

In vitro and in vivo experiments have revealed distinguishable tailing processes that impact mtRNA stability and translation. mtRNA tails can be composed of adenine (A), uridine (U), or combinations thereof (Verner et al., 2015). mtRNA tails further fall into distinct categories of in-tails (initially added tails) and ex-tails (extended tails) with differences in their length and biological functions (Fig. 1A). In-tails are fairly ubiquitous oligomer tails added to mtRNAs. The As in in-tails are added by the poly(A) polymerase KPAP1 (Etheridge et al., 2008) and Us by the terminal uridyltransferase RET1 (Aphasizhev et al., 2002, 2003) in a manner influenced by the pentatricopeptide protein KPAF3 (Zhang et al., 2017). In-tails are recognised as stability elements that are ubiquitously added to mtRNAs upon maturation of their 3′ ends, but other potential roles for them have not been explored (Ryan et al., 2003; Kao and Read, 2005; Etheridge et al., 2008; Aphasizheva and Aphasizhev, 2010; Zhang et al., 2017). Transcript-specific variation in sequence composition and length of in-tails have been suggested by studies that either had low sequencing depth or were limited to one life stage of parasite (Decker and Sollner-Webb, 1990; Souza et al., 1992; Kao and Read, 2007; Zimmer et al., 2012; Gazestani et al., 2016, Zhang et al., 2017). However, systematic studies of adequate depth to explore the relationship between transcript-specific in-tail variation and mtRNA regulation are lacking.

Ex-tails are longer, with potential roles in translational regulation. Ex-tails are generated by extensions appended to a subset of in-tails of translatable mtRNAs. The nucleotide extensions appear to be fairly homogenous in A/U composition with KPAP1/RET1 addition of A and U (a 7:3 A/U ratio (Etheridge et al., 2008)) controlled by the pentatricopeptide protein KPAF1 and possibly KPAF2 (Aphasizheva et al., 2011). Extensions exhibit a fairly consistent frequency in switching of addition from A to U and back.