Molecular & Biochemical Parasitology 205 (2016) 1–5

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Short communication

Polycistronic trypanosome mRNAs are a target for the exosome

Susanne Kramer[a,b,*], Sophie Piper[b], Antonio Estevez[c], Mark Carrington[b]

[a] Biozentrum, Julius-Maximilians Universität Würzburg, Am Hubland, 97074 Würzburg, Germany
[b] Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK
[c] Instituto de Parasitología y Biomedicina "López-Neyra", IPBL N-CSIC, Parque Tecnológico de Ciencias de la Salud, Avda. del Conocimiento, s/n.18100 Armilla, Granada, Spain

ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 26 February 2016
Accepted 29 February 2016
Available online 3 March 2016

Keywords:
Trypanosoma brucei
Exosome
NMD
Polycistronic mRNA
trans-splicing
Trypanosomes

ABSTRACT

Eukaryotic cells have several mRNA quality control checkpoints to avoid the production of aberrant proteins. Intron-containing mRNAs are actively degraded by the nuclear exosome, prevented from nuclear exit and, if these systems fail, degraded by the cytoplasmic NMD machinery. Trypanosomes have only two introns. However, they process mRNAs from long polycistronic precursors by *trans*-splicing and polycistronic mRNA molecules frequently arise from any missed splice site. Here, we show that RNAi depletion of the trypanosome exosome, but not of the cytoplasmic 5′-3′ exoribonuclease XRNA or the NMD helicase UPF1, causes accumulation of oligocistronic mRNAs. We have also revisited the localization of the trypanosome exosome by expressing eYFP-fusion proteins of the exosome subunits RRP44 and RRP6. Both proteins are significantly enriched in the nucleus. Together with published data, our data suggest a major nuclear function of the trypanosome exosome in rRNA, snoRNA and mRNA quality control.

1. Introduction, results and discussion

Splicing of pre-mRNAs is not 100% efficient. The translation of intron-containing mRNAs would be harmful to the cell and eukaryotic cells have developed several control systems that act in parallel to avoid the production of aberrant proteins. One major system is the active retention of unspliced mRNAs in the nucleus with several components of the nuclear pores being involved [1,2]. If this system fails, intron-containing mRNAs are recognized and degraded by the cytoplasmic nonsense mediated decay (NMD) system. In yeast for example, many mRNA precursors accumulate in strains carrying mutations of the two essential NMD proteins Upf1p or Xrn1p [3]. A third system is the active degradation of intron-containing mRNAs by the nuclear exosome/TRAMP (Trf–Air–Mtr4 polyadenylation) complex, with the processive 3′-5′ *exo*- and *endo*ribonuclease Dis3p/Rrp44 being the responsible catalytic component [1]. In yeast, both the spliceosome and the exosome compete for intron-containing mRNAs [4]. This results in the degradation of more than half of all intron-containing mRNAs before they can enter the spliceosomal machinery [4]: a high energetic price to ensure mRNA quality. There is good evidence for the existence of a similar system in trypanosomes from a recent transcriptome-wide analysis of trypanosome mRNA decay pathways [5].

Only two genes in *Trypanosoma brucei* contain *cis*-introns. However, the parasites encounter another problem of mRNA quality control instead: the accumulation of di- and oligocistronic mRNAs precursors due to inefficient *trans*-splicing. Trypanosomes have an unusual way of transcription: tens to hundreds of genes are co-transcribed and subsequently processed to mature mRNAs by the addition of the capped, 39 nucleotide long mini-exon from the spliced leader mRNA to the 5′ end. This *trans*-splicing is coupled to the polyadenylation of the mRNA from the upstream gene [6]. Like *cis*-splicing, *trans*-splicing is not 100% efficient. Some splice sites are missed, resulting in the formation of di- or oligocistronic mRNA molecules that are present in the nucleus and partially even in the cytoplasm [7–9]. An accumulation of oligocistronic mRNAs is potentially harmful: mRNAs encoded by neighbouring genes are not usually related to each other and are likely to contain mixed regulatory elements. The consequence would be changes in the post-transcriptional regulation of gene expression. There is some evidence for the presence of an active mechanism to keep unspliced mRNAs in the nucleus, as partially processed tubulin mRNAs are more concentrated in the nucleus than in the cytoplasm [10]. Moreover, the half-life of tubulin dicistrons is significantly shorter than the half-life of mature tubulin mRNA, indicating that an active mechanism for the removal of unspliced mRNAs may exist in trypanosomes [7]. Trypanosomes have an exosome that

* Corresponding author. Present address: Biozentrum, Julius-Maximilians Universität Würzburg, Am Hubland, 97074 Würzburg, Germany.
E-mail address: susanne.kramer@uni-wuerzburg.de (S. Kramer).

http://dx.doi.org/10.1016/j.molbiopara.2016.02.009

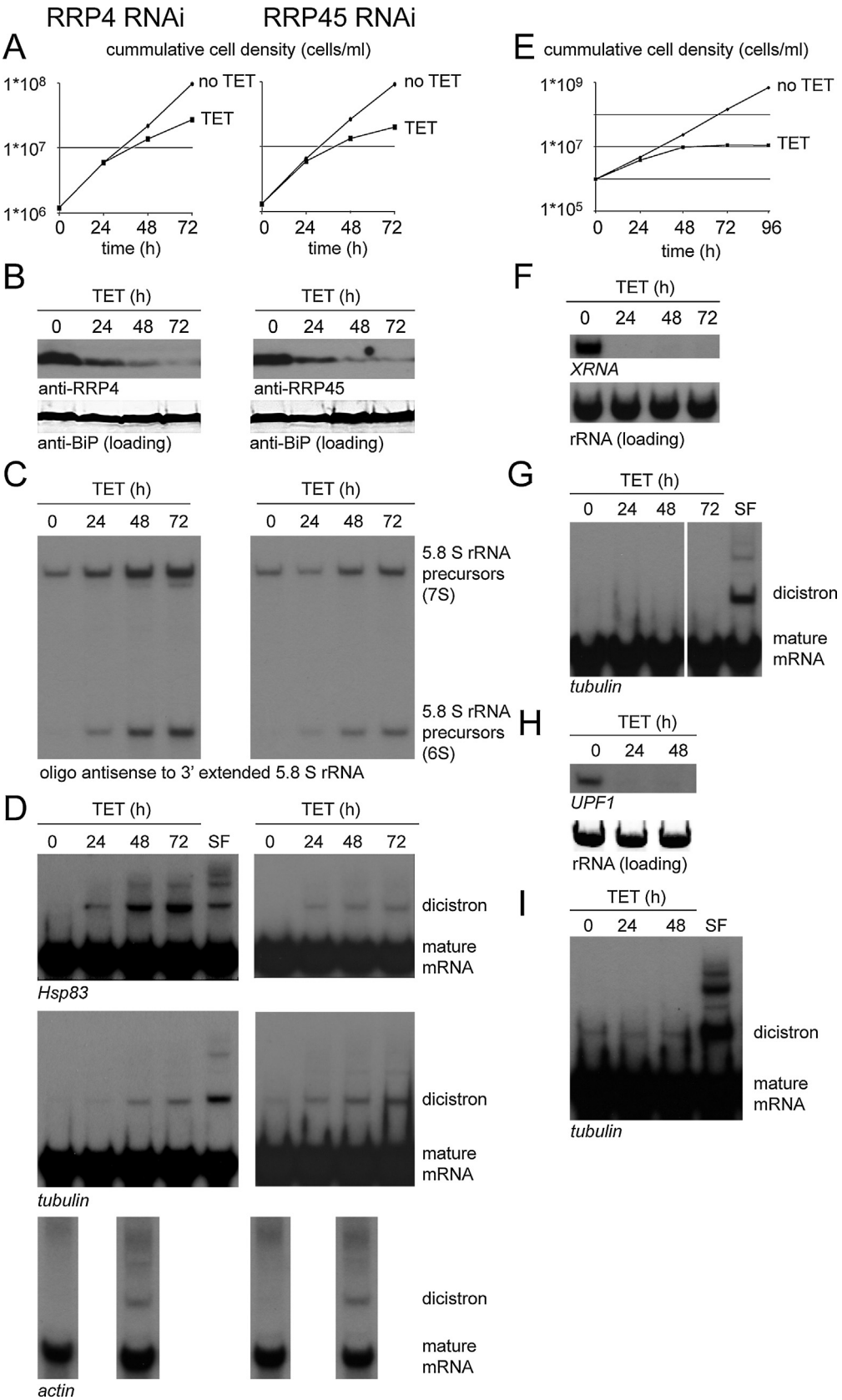

Fig. 1. Inducible RNAi depletion of RRP4, RRP45, XRNA and UPF1.
RNAi was induced by tetracycline (TET). All experiments shown in this figure, with the exception of the actin northern blot in (D) were also done with a second RNAi clone, with similar results (data not shown). Northern and western blots were done according to standard procedures. All northern blots were loaded with equal amounts of total RNA.
(A) Growth in the absence and presence of RNAi depletion of RRP4 (left) or RRP45 (right). (B) Western blots: RRP4 and RRP45 proteins were detected on a western blot at different time-points after RNAi induction using previously described polyclonal antiserum [11]. BiP served as loading control. (C) Northern blots: detection of 5.8S