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No potential conflicts of interest were disclosed.

Key words

Trypanosoma; ribosomal RNA; pre-18S rRNA processing; UTP10; U3 snoRNA.

ABSTRACT

Ribosome biosynthesis, best studied in opisthokonts, is a highly complex process involving numerous protein and RNA factors. Yet, very little is known about the early stages of pre-18S rRNA processing even in these model organisms, let alone the conservation of this mechanism in other eukaryotes. Here we extend our knowledge of this process by identifying and characterizing the essential protein TbUTP10, a homolog of yeast U3 small nucleolar RNA-associated protein 10 - UTP10 (HEATR1 in human), in the excavate parasitic protist *Trypanosoma brucei*. We show that TbUTP10 localizes to the nucleolus and that its ablation by RNAi knock-down in two different *T. brucei* life cycle stages results in similar phenotypes: a disruption of pre-18S rRNA processing, exemplified by the accumulation of rRNA precursors, a reduction of mature 18S rRNA, and also a decrease in the level of U3 snoRNA. Moreover, polysome profiles of the RNAi-induced knock-down cells show a complete disappearance of the 40S ribosomal subunit, and a prominent accumulation of the 60S large ribosomal subunit, reflecting impaired ribosome assembly. Thus, TbUTP10 is an important protein in the processing of 18S rRNA.

INTRODUCTION

Ribosomes, large ribonucleoprotein complexes that perform protein synthesis, are essential for all living cells. The biogenesis of ribosomes is a very complex process that involves multistep pre-ribosomal RNA (pre-rRNA) cleavage and numerous modifications that are followed by the stepwise assembly of ribosomal proteins around completely processed rRNAs. The large ribosomal subunit (LSU or 60S) contains three rRNA molecules – 25/28S, 5.8S and 5S, while the small subunit (SSU or 40S) assembles around 18S rRNA. This process is very well studied and quite conserved throughout the eukaryotes (Klinge et al. 2012; Wilson and Doudna 2012).

Generally, ribosomes have a similar function in bacteria, archaea and eukaryotes. However, among these supergroups there are important differences in ribosome size, structure, composition and the rRNA:protein ratio (Melnikov et al. 2012). Classically-shaped eukaryotic 80S ribosomes are composed of 60S LSU and 40S SSU, together containing more than 70 ribosomal proteins and 4 rRNAs. Processing of rRNAs and ribosome biosynthesis are carefully orchestrated processes that begin in the nucleolus, continue in the nucleoplasm and are completed in the cytoplasm (Tschochner and Hurt 2003).

The rRNA genes are organized in conserved repeated clusters, usually present in dozens to hundreds of copies. The precursor for 25S/28S, 18S and 5.8S rRNA is transcribed in the nucleolus by RNA polymerase I as a single 35S pre-rRNA transcript, while the precursor for 5S rRNA is generated by RNA polymerase III in the nucleoplasm. The 35S precursor, best studied in *Saccharomyces cerevisiae*, has an ETS/18S/ITS1/5.8S/ITS2/28S/ETS order, where ITS and ETS stand for the internal and external transcribed spacer, respectively. It is co-transcriptionally modified by multiple pseudouridylations and 2'-O-ribose methylations. The entire rRNA maturation process

involves several small nucleolar (sno) RNAs, as well as many proteins including endo- and exonucleases operating in large complexes (Venema et al. 1999).

The late stages of SSU and LSU maturations are well understood, whereas we know relatively little about the early stages of 18S rRNA processing. This pathway initiates with the assembly of a large ribonucleoprotein (RNP) complex called the SSU processome, which is involved in the early-stage cleavage of the 18S rRNA transcript at positions A0, A1 and A2 (Bernstein et al. 2004, Dragon 2002). The SSU processome is constituted of ribosome assembly factors UtpA, UtpB, UtpC and the U3 small nucleolar RNP (Dragon 2002). The first subcomplex that associates with the pre-rRNA is the heptameric multi-protein UtpA complex, which includes UTP10 (Pöll et al. 2014; Sun et al. 2017). At the initiation of rRNA transcription, SSU processome subunits UTP8, UTP9 and UTP17 bind to the 5' end of the nascent pre-rRNA, whereas UTP4, UTP5, UTP10 and UTP15 interact with nucleotides downstream of the 5' ETS region. Furthermore, other UtpA and UtpB subunits bind to the 5' ETS, subsequently recruiting the U3 snoRNP (formed by U3 snoRNA and Nop56, Nop58, Snu13, Nop1 and Rrp9 proteins), which also associates with the 5' ETS. Finally, as transcription proceeds, the processome is assembled around a folded 18S rRNA (Hunziker et al. 2016; Sun et al. 2017; Chaker-Margot 2018). Depletion of individual UtpA subunits hints at a role of this complex as the initiator of pre-ribosome assembly by binding to the nascent pre-rRNA and then recruiting UtpB and the U3 snoRNA (Pérez-Fernández 2007). Importantly, a subset of SSU processome components link RNA polymerase I transcription with pre-rRNA processing (Gallagher et al. 2004).

In the early diverging eukaryotic lineages represented almost invariably by protists, many fundamental cellular processes exhibit unusual features, with rRNA processing being no exception. *Trypanosoma brucei*, the causative agent of African sleeping sickness, is one such eukaryote belonging to the diverged supergroup Excavata (Hampl et al. 2009) and the

best studied representative of this clade. In this flagellate, the 28S rRNA is divided into two large LSUα and LSUβ fragments plus four small srRNA1-4 molecules, while its 18S rRNA is larger than in most other eukaryotes (Campbell et al. 1987, White et al. 1986). While the processing of pre-rRNA in yeast is initiated by cleavage of the 5´ ETS, in trypanosomes the processing initiates by the endonucleolytic separation of 18S rRNA from the rest of the precursor transcript (Michaeli 2011). Moreover, SSU processing is unique as two U3 snoRNA-crosslinkable 5' ETS sites are required for SSU rRNA maturation in *T. brucei* (Hartshorne 1998; Hartshorne and Toyofuku 1999; Hartshorne et al. 2001).

Remarkably, about 140 snoRNAs were found in *T. brucei* to play a role in rRNA maturation, most of them being involved in modification, only about 20 in rRNA processing (Gupta et al. 2010). However, our knowledge of the protein factors participating in rRNA processing and ribosome biogenesis in *T. brucei* and other unicellular eukaryotes remains fragmentary at best and only several proteins have been studied so far. In particular, it was shown that the depletion of NOG1 or the LSU protein L5 lead to the accumulation of rRNA precursors and defect in 60S biogenesis (Jensen et al. 2003; Umaer et al. 2014). On the other hand, the depletion of exoribonuclease XRNE leads to the accumulation of aberrant 18S and 5.8S rRNAs (Sakyiama et al. 2013). Finally, proteins TbNOB1 and TbPNO1 play a role in specific 18S rRNA 3′ end cleavage (Kala et al. 2017) and the U3 snoRNA-associated protein NOP1 was shown to be connected with trypanosome-specific rRNA processing events that generate small rRNA fragments. Interestingly, while silencing of NOP1 and NOP56 did not affect the level of snoRNAs, depletion of their partner NOP58 from the U3 snoRNP complex led to the destabilisation of several snoRNAs, including U3 (Barth et al. 2008). The genome of *T. brucei* contains many other homologs of the processome factors (Michaeli 2011), but no experimental data is available about the initial steps of its rRNA processing.

In this report, we have identified a homolog of the yeast UTP10 UtpA subunit, also known as BAP28 in zebrafish and HEATR1 in humans. We show that TbUTP10 is a nucleolar protein that is required for growth of procyclic (PS) and bloodstream stages (BS) of *T. brucei*, which infect the tsetse fly vector midgut and mammalian bloodstream, respectively. TbUTP10 is involved in the processing and maturation of pre-18S rRNA, its depletion resulting in U3 snoRNA depletion and an abnormal polysome profile, thus impairing ribosome assembly. These results highlight a conserved role of TbUTP10 in the pre-18S rRNA maturation process of extremely evolutionary distant eukaryotes.

RESULTS

Data mining and structural modelling reveal a putative *T. brucei* homolog of UTP10/BAP28/HEATR1

A structural homology search using HHpred (Söding et al. 2005) revealed the presence of a potential homolog of *UTP10/Bap28/HEATR1* in the *T. brucei* genome (**Fig. 1A-C**), annotated as Tb927.9.2900 in the TriTrypDB genome database (Aslett et al. 2009), which we name *TbUTP10*. This homology is not readily apparent by overall sequence similarity (**Suppl. Fig. 1**). However, the N-terminus of the protein is highly conserved in comparison to UTP10 homologs from yeast, zebra fish and humans (**Fig. 1A**). The C-terminus of opisthokont UTP10 consists of a HEAT (Huntington, elongation A subunit, TOR) repeat, an iteration of two α-helices linked by a short loop (Turi et al. 2018). The amino acid sequence similarity of the C-terminus of TbUTP10 with its putative opisthokont homologs is low (**Suppl. Fig. 1**), precluding determination if this motif is present in the trypanosomatid homolog. To overcome this problem, a Swiss-Model (Biasini et al. 2014) prediction of the tertiary structure of the

TbUTP10 C-terminus using the HEAT repeat of the 104 kDa centrosomal protein as a template was performed, indicating UTP10 ends with a HEAT repeat as in the other homologs (**Fig. 1B**).

TbUTP10 encodes a much larger protein (287 kDa) than its opisthokont homologs in yeast (200 kDa), zebrafish (242 kDa) and human (236 kDa) (Dragon et al. 2002; Azuma et al. 2006; Prieto et al. 2007). The alignment of TbUTP10 with these sequences reveals several insertions that account for this increase in size (**Suppl. Fig. 1**). We checked all the extra sequences of *T. brucei* protein (which are mainly around 20 aa long, the longest one being 57 aa) using protein domains identifier – InterProScan (http://www.ebi.ac.uk/interpro/search/sequence-search), but didnt find any functional domains that might point to additional roles of this protein in *T. brucei* in comparison to orthologues in other systems.

Maximum likelihood-based phylogeny (**Fig. 1C**) of the UTP10 proteins recovered some of the main eukaryotic lineages, such as the opisthokonts, green algae including plants, dinoflagellates, chromerids, stramenopiles and kinetoplastids. It also revealed increased rates of evolution and high level of divergence in most clades, which results in a low overall branching support especially in terminal branches. Kinetoplastids form a monophyletic and robustly supported group with internal branching corresponding to the accepted kinetoplastid phylogeny with *Bodo saltans* as a sister clade to the parasitic trypanosomatids (**Fig. 1C**).

TbUTP10 is localized in the nucleolus

In order to determine its localisation in the PS and BS *T. brucei*, the TbUTP10 gene was *in situ* C-terminally tagged with a tandem Ty epitope and enhanced yellow fluorescent protein (eYFP) tag. In both life cycle stages protein expression was high enough to record the signal directly in live cells. The advantage of live-cell imaging is that it avoids potential artefacts,

which can be caused by fixation. In order to take pictures of moving trypanosomes after staining, we used a previously described technique, in which the cells are covered by a thin sheet of 1% agarose and directly observed by confocal microscopy (Huang et al. 2014). TbUTP10 protein is localised in the nucleolus in both PS and BS cells (**Fig. 1D**). The individual channels are shown in **Suppl. Fig . 5**. This suggests that TbUTP10 function may be similar that of its opisthokont homologs.

Ablation of TbUTP10 severely affects fitness

T. brucei expressing the Ty-eYFP tagged TbUTP10 were electroporated with the p2T7-177 construct for tetracycline-inducible expression of a ~500 bp double stranded RNA targeting TbUTP10. The efficiency of RNAi was verified by Western blot analysis detecting TbUTP10-Ty-eYFP with an antibody recognizing the Ty epitope (**Figs. 2A and B; insets**). In both PS and BS *T. brucei*, a significant decrease of the tagged protein was observed after 2 days of RNAi induction, and it was almost undetectable after day 4.

Upon TbUTP10 depletion, growth inhibition was observed in both PS and BS RNAi-cell lines (**Figs. 2A and B**). However, this phenotype was more prominent in BS cells, where growth was already inhibited by day 2 after RNAi induction and then completely stopped after day 5, suggesting that TbUTP10 is an essential protein in this life stage. This hypothesis is further supported by the knock-out of a single *TbUTP10* allele (sKO) replaced with a hygromycin resistance marker (**Suppl. Fig. 2A**). Three independent attempts to prepare a *TbUTP10* double knock-out cell line failed, providing indirect evidence that the target protein is indeed essential.

TbUTP10 is directly involved in pre-18S rRNA processing

198 Since we hypothesized that TbUTP10 plays a role in 18S rRNA biosynthesis, we next
199 investigated the impact of its depletion on pre-18S rRNA processing. More specifically, we
200 predicted that ablation of TbUTP10 will cause the accumulation of pre-18S rRNA precursors,
201 indicating an error in early stages of maturation.

202 Three different oligonucleotide probes (**Table 4**) were used in the Northern blot
203 analysis. The position of these probes, as well as the sizes of all predicted precursors and the
204 final product are depicted in **Fig. 3A**. The three cleavages at positions A0, A1, and A2 are
205 required for the production of the mature 18S rRNA and consequently the 40S ribosomal
206 subunit (Michaeli 2011). If TbUTP10 was involved in pre-18S rRNA processing, an increase
207 of the 9.6 kb-long full-length precursor, and a concomitant decrease of the 2.2 kb-long final
208 product was expected to occur upon TbUTP10 silencing. The intensities of the respective
209 RNA species were quantified by densitometry and any changes (in percent) were calculated in
210 comparison to RNA from control parental cell lines, 7SL RNA was used as a normalized
211 control (**Figs. 3B, C; Suppl. Fig. 2B and 3**). The latter were chosen for comparison instead of
212 uninduced RNAi cell lines to avoid any confounding effects of potential RNAi leakage.

213 The comparative analysis confirmed our hypothesis. On day 4 after RNAi induction of
214 PS cells (**Fig. 3B**), the 5'ETS probe revealed a significant increase of the full-length
215 precursor, as well as that of the 3.7 kb intermediate (we did not detect the 3.3 kb intermediate
216 in our analysis). The increase of the 9.6 kb-long precursor and a parallel decrease of the
217 smaller precursors was confirmed by the ITS1 probe. Finally, the SSU probe showed that the
218 largest precursor accumulates, while the other RNA species bands decrease. The fully
219 processed 2.2 kb-long product decreased by approximately 30% on day 4, meaning that
220 production of the mature 18S rRNA is impaired. In contrast, signal of the LSU probe
221 remained unaltered, showing that the defect in rRNA processing is only SSU specific.

The results obtained in the BS TbUTP10-RNAi cells are similar (**Fig. 3C)**. All three probes showed an accumulation of the full-length precursor and a concomitant decrease of the 3.7 kb precursor upon the down-regulation of TbUTP10. The decrease of the mature 2.2 kb SSU rRNA product is less pronounced in the BS than in the PS cells, but the overall trend is the same. Moreover, the sKO cells showed an even more pronounced increase of the 9.6 kb-long 5'ETS precursor, with the ITS1 and SSU precursors also increased and the mature product decreased by about 20% (**Suppl. Fig. 2B and 3**). In conclusion, Northern blot analysis of full-length precursor and mature 18S rRNA upon ablation of TbUTP10 by two alternative approaches and in two different life cycle stages is consistent and shows that TbUTP10 is specifically involved in the 18S rRNA processing as it was already described in other organisms.

TbUTP10-depleted cells have an abnormal polysome profile

Since TbUTP10 is localised in the nucleolus and its depletion affects maturation of the pre-18S rRNA transcript, we next investigated whether its RNAi-mediated depletion results in an altered polysome profile, reflecting a defect in ribosome biogenesis. Based on the previously described processing phenotype, we predicted a decrease of the 40S SSU, which contains mature 18S rRNA, and a consequent decrease in the 80S ribosome. The amount of cells required for this experiment limited its application to the PS RNAi cell line. The lysates from parental cells and RNAi induced cell line were separated by 10 to 50% sucrose gradient centrifugation and the fractions were monitored by absorbance at 254 nm to detect the protein component of ribosomes (**Fig. 4**). Three biological replicates for each cell line had a very consistent pattern (**Figs. 4A and B)**, allowing us to analyse their overlay using peak annotations (**Fig. 4C)**.

The collective polysome profile of the TbUTP10-depleted *T. brucei* sample revealed that the 40S ribosomal subunit peak was completely missing. This was confirmed by hybridizing RNA isolated from individual fractions with SSU (18S) and LSU (28S) probes which showed a disappearance of the signal from fractions 3 and 4 corresponding to the 40S peak (**Suppl. Fig. 4**). Furthermore, it showed that the 60S subunit accumulated substantially, which may have been due to it not being incorporated into the 80S ribosome because of lack of availability of 40S subunits. This result is congruent with the 80S ribosome peak being much narrower than in the parental control. These results indicate that upon TbUTP10 down-regulation, the resulting 18S rRNA processing defect results in a decrease of the 40S SSU, ultimately impairing ribosomal assembly.

TbUTP10 silencing affects U3 snoRNA

As mentioned above, in yeast and other organisms UTP10 binds to U3 snoRNA and its associated proteins, thereby contributing to the maturation of 18S rRNA. Hence, we wondered whether the depletion of TbUTP10 will have an effect on the U3 snoRNA level. For that aim, the signal of U3 oligonucleotide probe was compared with the 7SL RNA signal, which was used as a loading control (**Fig. 5**). The intensities of the respective RNA species were quantified by densitometry and compared with RNA obtained from the control parental cell lines (**Figs. 5A, B; Suppl. figure 2C and 3**). Indeed, we observed a decrease of U3 snoRNA level in each TbUTP10-depleted cell line.

DISCUSSION

Ribosomes are crucial components of all cells, as they are responsible for translation of mRNAs into proteins. Their biosynthesis is a highly complex process that requires the action of numerous RNA and protein factors. Because of its ubiquity, this process is likely to be

highly conserved, yet one would also expect lineage-specific novelties in highly divergent eukaryotes.

In agreement with this supposition, the ribosome of *T. brucei* has its share of unique features, which include its 28S rRNA split into 6 fragments (White et al. 1986) and the existence of unusual inter-subunit bridges, as observed by cryo-electron microscopy (Hashem et al. 2013). Furthermore, trypanosomatid 18S rRNA is very long compared to other organisms (Campbell et al. 1987), with significant differences in pre-18S rRNA processing (Michaeli et al. 2011). However, virtually nothing is known about the proteins mediating early stages of this process, motivating us to undertake functional analysis of TbUTP10, a protein known to be involved in 18S rRNA processing in opisthokonts (Dragon et al. 2002; Turi et al. 2018; Azuma et al. 2006). Here, we have shown that the gene *Tb927.9.2900* in the excavate protist *T. brucei* is a distantly related homolog of yeast *UTP10*, zebrafish *BAP28* and human *HEATR1*, and therefore have renamed it *TbUTP10*. Due to its significant divergence from its opisthokont homologs, TbUTP10 had to be identified based on a tertiary structure prediction (Söding et al. 2005). Interestingly, TbUTP10 is larger than its opisthokont homologs, which may be an adaptation to the longer length of its 18S rRNA substrate. We have shown that TbUTP10 is a nucleolar protein, which was also confirmed in the Tryptag protein localisation database for both N and C terminally tagged protein in PS stage (http://tryptag.org/?query=Tb927.9.2900). Northern analysis of knock-out and knock-down cell lines confirmed that it is involved in pre-18S rRNA processing. Although the band quantifications did not consistently exhibit the trend in all 18S rRNA intermediate precursors, there is a clear accumulation of full-length precursors and a decrease of the final 18S rRNA product. Combined, these results confirmed that UTP10 has a conserved function in excavates and opisthokonts.

Polysome analysis of TbUTP10-depleted PS trypanosomes revealed that the 40S SSU peak completely disappeared, which is consistent with the requirement of 18S rRNA for its maturation. The 60S LSU exhibited prominent accumulation, likely because it cannot be paired with the 40S SSU to form the 80S ribosome. Still, a residual amount of 40S SSU allowed the assembly of 80S ribosomes, albeit with reduced abundance. It is likely that this limited yet ongoing ribosomal assembly allowed continued proliferation of PS cells at a reduced growth rate. However, its dramatic impact on *T. brucei* BS growth and the profound change in the polysome profile of PS cells indicates an essential function of TbUTP10 in ribosome biogenesis. Additional evidence for the indispensability of TbUTP10 was a repeated failure to create a double knock-out cell line.

These observations are in agreement with biochemical studies performed in yeast and point to a conserved function. Association of UTP10 with pre-18S rRNA processing was first discovered in *S. cerevisiae*, where it co-purified with the SSU processome that specifically associated with the U3 snoRNA and also played a role in pre-rRNA transcription (Dragon et al. 2002). It was proposed that U3 snoRNP recruitment to the processome is enhanced by a UTP10-U3 snoRNA 3' domain interaction (Hunziker et al. 2016). Our results demonstrate that the depletion of TbUTP10 is associated with the decreased level of U3 snoRNA, which was also observed in cells down-regulated for Nop58, a protein that belongs to the U3snoRNP complex (Barth et al. 2008). It is not an unexpected result, since recent structural studies revealed that these proteins actually seem to function next to each other (Kornprobst et al. 2016; Sun et al. 2017; Barandun et al. 2017). Indeed, UTP10 deletion inhibited the early pre-rRNA processing steps in the 18S rRNA maturation, causing the accumulation of its precursors, although it had only mild effects on rRNA transcription and 25S or 5.8S rRNA synthesis. Depletion of Bap28 and HEATR1 in zebrafish and mammals, respectively, results in increased p53-dependent apoptosis in the central nervous system, leading to abnormal brain

and organ development and subsequent death of zebrafish embryos (Turi et al. 2018; Azuma et al. 2006).

In *T. brucei*, the essentiality of this protein (labelled under previous name Tb09.160.1560) was already indicated in the genome wide RNAi screen for all studied libraries - bloodstreams, procyclics as well as differentiated ones (Alsford et al. 2011). The growth defect following its depletion was more pronounced when TbUTP10 was depleted in BS. We speculate that this life cycle stage requires a more rapid flux of ribosome biogenesis due to faster proliferation of BS compared to PS. This hypothesis would agree with observation of increased HEATR1 levels in certain cancer types, which are also highly proliferative (Wu et al. 2014). Taken together, our study showed that TbUTP10 has a conserved function in eukaryotes. Its importance for rapidly proliferating BS possibly parallels the effects of upregulation of HEATR1 in cancer cells.

MATERIALS AND METHODS

Phylogenetic analysis

Eukaryotic homologues of yeast UTP10 were identified using BLAST against the custom database with the representative sampling of eukaryotic lineages as well as HHPred in case of *T. brucei*. The dataset was aligned using E-INS-i algorithm implemented in MAFFT 7 (Katoh and Standley 2013), the ambiguously aligned and/or gap-rich regions were manually extracted using SeaView 4 (Gouy et al. 2010). The resulting dataset contained 48 taxa and 799 amino acid positions. The phylogeny of UTP10 was inferred using Maximum likelihood under the LG+F+R5 model (5 categories of variable sites with relaxed distribution) as implemented in

IQTree 1.5 (Nguyen et al. 2015). This particular model was chosen as the best fitting based on AIC and BIC scores in a modeltest implemented in IQTree. Ultra-fast bootstrap values as a mean of branching support were assessed from 50,000 replications.

T. brucei cell lines, cultivation and growth curves

T. brucei PS SMOXP9 strain (Poon et al. 2012) was grown at 27°C in SDM-79 medium (Brun and Schönenberger 1979) supplemented with 10% fetal bovine serum (FBS), while BS 427 - single marker strain (Wirtz et al. 1999) was kept at 37°C and 5% CO_2 in HMI-9 medium supplemented with 10% FBS. Additionally, antibiotics were added to the media according to the selection markers present (**Tables 1 and 2**). For growth measurements, BS and PS cells were induced at a density of 2 x 10^5/ml and 3 x 10^6/ml, respectively, counted daily using a Z2 Particle Counter (Beckman Coulter) and diluted back to the initial density as appropriate. Representative growth curves are shown, with the experiment repeated three times to ensure reproducibility.

Generation of cell lines

Generation of cell lines expressing tagged TbUTP10. In PS cells, TbUTP10 was endogenously C-terminally tagged with a tandem Ty epitope and eYFP tag using recently developed tagging tools for trypanosomes (Dean et al. 2015). The pPOTv2 vector was a template for PCR with ~100 bp primers (2900_Ty_Fwd and 2900_Ty_Rev; see **Table 3** for all primer sequences), of which the 5'-most 80 bp represented homologous flanks for recombination into the *TbUTP10* locus. The PCR product that was directly electroporated into SMOXP9 cell line, and clones were selected using hygromycin.

In BS cells, three attempts of this rapid technique failed. Therefore, we decided to extend the flanking homologous regions to about 500 bp and performed a separate amplification of the

left (primers A-5U2900-F and A-5U2900-YFP-R primers) and right (primers B-3U2900-Hyg-F and B-3U2900-R) homology arms, plus the Ty+eYFP tags and hygromycin^{R} cassette from the pPOTv2 vector (primers C-YFP_Hyg-F and C-YFP_Hyg-R). These PCR products were fused into a final product (primers D-Nest-2900-F and D-Nest-2900-R), which was purified and subsequently electroporated into BS 427 cells.

Generation of RNAi and single knock-out cell lines. A 515 bp fragment of the TbUTP10 gene was PCR amplified from genomic DNA (primers 2900_p2T7_Fw and 2900_p2T7_Rv) and cloned into the p2T7-177 vector (Wickstead et al. 2002) via the XhoI and BamHI restriction sites. The linearized p2T7-177 vector was subsequently electroporated into both tagged cell lines and clones were selected with phleomycin. PS cell and BS cell clones were induced with 1 μg/ml tetracycline. Single knock-out of TbUTP10 in the BS 427 strain was generated using the fusion PCR method (Merritt and Stuart 2013), with the hygromycin resistance marker cassette flanked by TbUTP10's 5′- and 3′-untranslated regions to facilitate homologous recombination. These constructs were sequentially electroporated into the cells.

Localisation of TbUTP10 protein in live cells

In order to visualize TbUTP10, 5 x 10^6 live PS or BS cells were stained with 1 μl of 20 μM MitoTracker Red CMXRos (Molecular Probes) and two drops of Nuc Blue Live Cell Stain (Molecular Probes), incubated for 15 min at their respective cultivation conditions, spun down and resuspended in Iscove's Modified Dulbecco's Medium. The cells were subsequently immobilized under a sheet of 1% agarose and observed under an OlympusFluoViewFV1000 confocal microscope as described elsewhere (Huang et al. 2014).

Western blot analysis

Cell lysates were prepared in Laemmli sample buffer at a concentration allowing the loading of 5 x 10^6 cells per lane and separated on a 3-10 % gradient SDS-PAGE gel. The proteins were subsequently transferred onto a PVDF membrane by electro-blotting at 20V overnight to ensure transfer of large molecular weight proteins. Membranes were blocked with 5% (w/v) non-fat milk prepared in PBS with 0.5% (v/v) Tween 20 and probed with monoclonal mouse α-Ty antibody (1:2,000; Sigma Aldrich) overnight at 4°C. The membrane was subsequently incubated with secondary α-mouse polyclonal antibody conjugated with horseradish peroxidase (1:1,000) (Sigma) and visualized using Clarity western ECL substrate (Bio-Rad). Monoclonal anti-α-tubulin antibody produced in mouse (1:1000; Sigma T9026) was used as a loading control.

Northern blot analysis

Total RNA was isolated using TriReagent (MRC), precipitated, and resuspended in RNase-free water. For the analysis of small rRNAs, 5 μg of total cellular RNA was separated on a denaturing 8% polyacrylamide/8M urea gel, electroblotted to Zeta-Probe membrane and cross-linked to the membrane with UV light. For the analysis of bigger rRNAs, 10 μg were loaded on a 1% agarose-formaldehyde gel. Samples were blotted overnight to a Zeta-Probe membrane by capillarity action and subsequently UV cross-linked as described previously (Vondrušková et al. 2005). The probes (**Table 4**) were radiolabelled with [γ^{32} P] dATP using polynucleotide kinase (New England Biolabs) and hybridization with the probe was performed overnight at 45°C (U3 snoRNA and 7SL RNA probes) or 50°C (the other probes). The membrane was exposed to a Fuji Imaging phosphor screen and scanned in a Phoshoimager (Amersham) for signal detection.

Sucrose density gradient sedimentation

The polysome profile analysis was performed as described previously (Fleming et al. 2016). Fifty mL shaking cultures were grown overnight at 27 °C to mid-log phase. Cells were collected by centrifugation and all subsequent steps were carried out at 4 °C. Cells were washed twice in 15 mL of ice-cold PBS supplemented with 100 µg/mL cyclohexamide and final pellet suspended in 780 µL of polysome buffer (10 mM Tris-HCl pH 7.5, 300 mM KCl, 10 mM MgCl2) supplemented with 100 µg/mL cyclohexamide, 2 mM DTT, and 1X Protease inhibitor cocktail. Cells were lysed by addition of NP-40 to a final concentration of 0.25% and incubated on ice for 10 minutes. Lysates were clarified by centrifugation at 15,000 g for 10 minutes. One mg equivalent of OD260 units was loaded on each 10–50% sucrose gradient prepared in lysis buffer and centrifuged at 36,600 RPM for 2 hours at 4 °C in a Beckman SW41Ti rotor. A gradient fractionator (ISCO UA-6 UV Vis with Type 11 optical unit) was used to record UV profiles.

ACKNOWLEDGEMENTS

We thank Keith Gull and Sam Dean (University of Oxford) for providing the pPOTv2 plasmid, Moritz Niemann (Universität Bern) for advice on using the HHpred database and Zhenqui Huang (Arizona State University) for help with the cell immobilisation technique. Support from the Czech Grant Agency (16-18699S to JL and 17-24036S to HH), ERC CZ LL1601 and the ERD Funds, project OPVVV 16_019/0000759 are kindly acknowledged. Authors have no conflict of interest to declare.

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Table 1: Antibiotics mixture compositions for different cell lines.

Used T. brucei strains	**Added antibiotics**
PS wt (SMOX P9)	Puromycin
PS TbUTP10 YFP+Ty tagged RNAi cell line (clone 4)	Puromycin, Hygromycin, Phleomycin
BS wt (427)	G418
BS TbUTP10 YFP+Ty tagged RNAi cell line (clone 2)	G418, Hygromycin, Phleomycin
BS sKO-Hygro	G418, Hygromycin

Table 2: Concentrations of all antibiotics in respective media.

	Concentrations in medium	
Used antibiotics	**PS**	**BS**
G418	-	2.5 µg/ml
Puromycin	0.5 µg/ml	
Phleomycin	2.5 µg/ml	2.5 µg/ml
Hygromycin	25 µg/ml	2 µg/ml
Tetracycline (for RNAi inductions only)	1 µg/ml	1 µg/ml

Table 3: DNA oligonucleotides used as PCR primers

Primers	Sequence
Procyclic tagging	

2900_Ty_Fwd	TCGTTGTGGAGCAGGCGCGGAGGTTGTGTGGACATCTTTCATCCATTACGGGCCAAGATGTCCTGTATGCGATGGGTTCCACTAGTGTGAGCAAGG
2900_Ty_Rev	TCTTGACTTAGAGAGCTACCTCACCGAGATGTCATCGTGTACATGTACTCCAAGGCACACCCTTCCCATTGCAGCGGGTACTATTCCTTTGCCCTCGGAC
Bloodstream tagging	
A-5U2900-F	CATGCGGCAGATCTCATAGC
A-5U2900-YFP-R	ACTAGTGTGAGCAAGGGGAACCCATCGCATACAGG
B-3U2900-Hyg-F	GTCCGAGGGCAAAGGAATAGTACCCGCTGCAATGGGAAGGG
B-3U2900-R	GAGAAAAATAAAGAAGGGA
C-YFP_Hyg-F	ACTAGTGTGAGCAAGGGCGAG
C-YFP_Hyg-R	CTATTCCTTTGCCCTCGGAC
D-Nest-2900-F	GCTCAACAGCATCAGGATCA
D-Nest-2900-R	CACCAACACCACATCTGTCAA
RNAi primers	
2900_p2T7_Fw	CGACTCGAGACATGGCGCGTACTTTTACC
2900_p2T7_Rv	CGAGGATCCAGACAGTCACGCAACAGCAC
Bloodstream knock-out	
A-5U2900-F	CCCCCTACACTGTTCACACC
A-5U2900-Hyg-R	GGTGAGTTCAGGCTTTTTCATTTGCTCAAGCAGTTACTTA
B-3U2900-Hyg-F	GTCCGAGGGCAAAGGAATAGTACCCGCTGCAATGGGAAGG

B-3U2900-R	ACCAAAACCTTTGTCCCTCA
C-Hyg-F	ATGAAAAAGCCTGAACTCACC
C-Hyg-R	CTATTCCTTTGCCCTCGGAC
D-Nest-2900-F	TGCAGGCTCCCTACTACAGC
D-Nest-2900-R	AAGAGGGGGAAAAGGTAGCA

649

650

651 **Table 4**: DNA oligonucleotide probes used for Northern blot hybridization.

Probe	Sequence of the probe
5'ETS	5'-AGTGTAAGCGCGTGATCCGCTGT-3'
SSU	5'-GGCTAAGTCCTTGAAACAAGCA-3'
ITS1	5'-GGTTGCATACTGTGCAATTATACATGC-3'
LSU	5'-GTCCTGCCACACTCAGGTCTGA-3'
U3 snoRNA	5´-TGC CGT TCA TCG AAC-3`
7SL RNA	5´-CAACACCGACACGCAACC-3`

652

653

654 **FIGURE LEGENDS**

655

656 **Fig. 1. *T. brucei Tb927.9.2900* gene encodes a putative *UTP10/Bap28/HEATR1* homolog**

657 **A**. Comparison of the N-terminus of TbUTP10 (Tb) and its homologs in zebrafish *Danio*

658 *rerio* (Dr), human (Hs) and yeast *Saccharomyces cerevisiae* (Sc). The complete alignment is

659 shown in **Suppl. Fig. 1**.

660 **B.** Model of the TbUTP10 C-terminal HEAT domain built by SWISS MODEL using

661 Centrosomal protein of 104 kDa as a template.

C. Maximum likelihood topology of TbUTP10 eukaryotic homologues constructed using LG+F+R5 model as implemented in IQTree. Numbers at branches correspond to the ultrafast bootstrap branching support inferred from 50000 replicates in IQTree. The dataset consisted of 48 taxa and 799 amino acid positions. See relevant part of Materials and Methods for details. Kinetoplastids are shown in grey box. The only three species of opisthokonts (dashed box) in which homologues of TbUTP10 were studied are indicated with a star.
D. TbUTP10 protein is localized in the nucleolus of both PS (left) and BS (right) *T. brucei*. Localization of TbUTP10-Ty-GFP (in green) in the nucleolus. Mitochondria are stained in red (MitoTracker) and DNA in blue (Nuc Blue Live Cell Stain). Scale bar: 5 μm.

Fig. 2. TbUTP10 protein is essential for normal growth of both PS (left) and BS (right) *T. brucei.*
Cumulative growth curves of TbUTP10 RNAi (ind = RNAi induced with tetracycline) in PS cells (**A**) and BS cells (**B**) are shown, as compared to the non-induced controls (non-ind). Western blot analysis of TbUTP10-Ty-GFP levels during a RNAi time course in PS (**A** – inset) or BS (**B** - inset) probed with anti-Ty antibody; anti-α-tubulin antibody signal shown as a loading control. dpi, days post induction. Representative growth curves are shown, with the experiment repeated three times to ensure reproducibility.

Fig. 3. TbUTP10 is involved in the processing of pre-18S rRNA
A. First 18S rRNA processing steps in *T. brucei* (adapted from Michaeli 2011). The asterisk shows the position of the first cleavage, B1 (top). Coloured bars indicate the positions of the 5' ETS, SSU and ITS1 probes used for detection of the precursors in the pre-18S rRNA processing pathway. The respective lengths of full-length precursors (9.6 kb), intermediates

(3.7 kb, 3.3 kb, 2.6 kb) and the final product (2.2 kb) (bottom) are also given. Please note that sizes are not to scale in this diagram.

Northern blot analysis of parental strain (P) vs. PS TbUTP10 RNAi cells **(B)** and BS TbUTP10 RNAi cells (**C**), induced for 1 to 4 days (1 to 4). The colours in the panels correspond to the probes from **Fig. 3A.**

The graphs show percentage of band intensity at a given control compared to the parental control of each RNA species depicted for both cell line: PS RNAi (**B**) and BS RNAi (**C**). 7SL rRNA was used as a normalized control, with the changes in RNA levels being calculated by normalisation of the wild type and RNAi-induced or sKO signal. A clear trend of accumulation of the full length precursors (FLP) and decrease of final products (FP) are shown by arrows, as well as the values for each intermediate product (IP). Representative Northern blots are shown, with the experiment repeated three times to ensure reproducibility.

Fig. 4. Polysome profiling analysis of PS TbUTP10 RNAi cell line

A. Parental PS cell line -P (solid black line); **B.** PS TbUTP10 RNAi cell line (dashed line). Peaks corresponding to 40S, 60S, 80S and the polysomes are indicated.

C. Overlay of polysome profiling analysis of parental versus RNAi induced cells (the profiles to the right in panels A and B are compared as representative examples).

Fig. 5. TbUTP10-silencing affects U3 snoRNA

Total RNA was separated on a denaturing 8% polyacrylamide/8M urea gel (top) and Northern blot analysis of parental strain (P) vs. PS TbUTP10 RNAi cells (**A**) and BS TbUTP10 RNAi cells (**B**) induced for 1 to 4 days (I1 to I4) was performed. U3 oligonucleotide probe was compared to 7SL RNA.

711 The band intensity compared to the parental control of each rRNA species is depicted (in
712 percentage) for both cell line: PS RNAi (**A**) and BS RNAi (**B**). 7SL RNA was used as a
713 normalized control, with changes in the rRNA levels being calculated by normalisation of the
714 wild type and the RNAi-induced signal to 7SL rRNA. Note the decrease of the level of U3
715 snoRNA in both TbUTP10-depleted cell line. Representative Northern blots are shown, with
716 the experiment repeated three times to ensure reproducibility.
717
718 **Suppl. Fig. 1: Complete alignment of TbUTP10** (1, on top) with homologs from
719 opisthokont species (2, Bap28 from zebrafish *Danio rerio*; 3, human HEATR1; 4, UTP10
720 from yeast *Saccharomyces cerevisiae*).
721
722 **Suppl. Fig. 2: Single knockout of TbUTP10 gene leads to accumulation of pre-18S rRNA**
723 **precursors, decrease of final 18S product and affects the level of U3 snoRNA**
724 **A.** Verification of sKO strain by PCR. Agarose gel-resolved PCR products of primer pair (A-
725 5U2900-F and B-3U2900-R) flanking the genomic integration site of the constructs from
726 genomic DNA isolated from parental strain (P) and single knockout (sKO). The size of each
727 amplicon is indicated.
728 **B.** Northern blot analysis of parental strain (P) vs. BS sKO cells. The colours in the panels
729 correspond to the probes from **Fig. 3A.** The graphs show percentage of band intensity at a
730 given control compared to the parental control of each RNA species depicted for BS sKO.
731 **C.** Northern blot analysis of parental strain (P) vs. BS sKO cells (**C**). U3 oligonucleotide
732 probe was compared to 7SL RNA.
733 The band intensity compared to the parental control of each rRNA species is depicted (in
734 percentage) for BS sKO. 7SL RNA was used as a normalized control, with changes in the

735 rRNA levels being calculated by normalisation of the parental strain and sKO signal to 7SL
736 rRNA. Note the decrease of the level of U3 snoRNA.
737
738 **Suppl. Fig. 3: Northern blot analysis quantification**
739 The tables show percentage of band intensity at a given control compared to the parental
740 control of each RNA species depicted for each cell line: PS RNAi (**A**), BS RNAi (**B**), BS sKO
741 (**C**). A clear trend of accumulation of the full length precursors (FLP) and decrease of final
742 products (FP) are shown by arrows, as well as the values for each intermediate product (IP).
743
744 **Suppl. Fig. 4: Polysome profiling fractions of PS TbUTP10 RNAi cell line assayed by**
745 **hybridisation with SSU and LSU probes**
746 A. Parental PS cell line -P (solid black line); B. PS TbUTP10 RNAi cell line (dashed line).
747 Peaks corresponding to 40S, 60S, 80S and the polysomes are indicated.
748 Below each ribosome profile is shown Northern blots of RNA isolated from the each depicted
749 sucrose gradient fractions (as in Fig. 4) hybridized with the oligonucleotide probes specific
750 for SSU (18S) and LSU (28S).
751
752 **Suppl. Fig. 5: TbUTP10 protein is localized in the nucleolus of both PS (top) and BS**
753 **(bottom) stages of *T. brucei*.**
754 Localization of TbUTP10-Ty-GFP (in green) in the nucleolus (**A**). DNA in blue (Nuc Blue
755 Live Cell Stain) (**B**), mitochondria are stained in red (MitoTracker) (**C**), overlay (**D**) and light
756 microscopy of living cells embedded in 1% agarose (**E**) . Scale bar: 5 μm.
757