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1 **TbUTP10, a protein involved in early stages of pre-18S rRNA processing in**
2 ***Trypanosoma brucei***

3
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20
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22
23 Key words

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

25

26 **ABSTRACT**

27

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

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51 **INTRODUCTION**

52 Ribosomes, large ribonucleoprotein complexes that perform protein synthesis, are essential
53 for all living cells. The biogenesis of ribosomes is a very complex process that involves
54 multistep pre-ribosomal RNA (pre-rRNA) cleavage and numerous modifications that are
55 followed by the stepwise assembly of ribosomal proteins around completely processed
56 rRNAs. The large ribosomal subunit (LSU or 60S) contains three rRNA molecules – 25/28S,
57 5.8S and 5S, while the small subunit (SSU or 40S) assembles around 18S rRNA. This process
58 is very well studied and quite conserved throughout the eukaryotes (Klinge et al. 2012;
59 Wilson and Doudna 2012).

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

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

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

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

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

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

108 Remarkably, about 140 snoRNAs were found in *T. brucei* to play a role in rRNA
109 maturation, most of them being involved in modification, only about 20 in rRNA processing
110 (Gupta et al. 2010). However, our knowledge of the protein factors participating in rRNA
111 processing and ribosome biogenesis in *T. brucei* and other unicellular eukaryotes remains
112 fragmentary at best and only several proteins have been studied so far. In particular, it was
113 shown that the depletion of NOG1 or the LSU protein L5 lead to the accumulation of rRNA
114 precursors and defect in 60S biogenesis (Jensen et al. 2003; Umaer et al. 2014). On the other
115 hand, the depletion of exoribonuclease XRNE leads to the accumulation of aberrant 18S and
116 5.8S rRNAs (Sakyama et al. 2013). Finally, proteins TbNOB1 and TbpNO1 play a role in
117 specific 18S rRNA 3' end cleavage (Kala et al. 2017) and the U3 snoRNA-associated protein
118 NOP1 was shown to be connected with trypanosome-specific rRNA processing events that
119 generate small rRNA fragments. Interestingly, while silencing of NOP1 and NOP56 did not
120 affect the level of snoRNAs, depletion of their partner NOP58 from the U3 snoRNP complex
121 led to the destabilisation of several snoRNAs, including U3 (Barth et al. 2008). The genome
122 of *T. brucei* contains many other homologs of the processome factors (Michaeli 2011), but no
123 experimental data is available about the initial steps of its rRNA processing.

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

132

133

134 **RESULTS**

135

136 **Data mining and structural modelling reveal a putative *T. brucei* homolog of** 137 **UTP10/BAP28/HEATR1**

138 A structural homology search using HHpred (Söding et al. 2005) revealed the presence of a
139 potential homolog of *UTP10/Bap28/HEATR1* in the *T. brucei* genome (**Fig. 1A-C**), annotated
140 as Tb927.9.2900 in the TriTrypDB genome database (Aslett et al. 2009), which we name
141 *TbUTP10*. This homology is not readily apparent by overall sequence similarity (**Suppl. Fig.**
142 **1**). However, the N-terminus of the protein is highly conserved in comparison to UTP10
143 homologs from yeast, zebra fish and humans (**Fig. 1A**). The C-terminus of opisthokont
144 UTP10 consists of a HEAT (Huntington, elongation A subunit, TOR) repeat, an iteration of
145 two α -helices linked by a short loop (Turi et al. 2018). The amino acid sequence similarity of
146 the C-terminus of TbUTP10 with its putative opisthokont homologs is low (**Suppl. Fig. 1**),
147 precluding determination if this motif is present in the trypanosomatid homolog. To overcome
148 this problem, a Swiss-Model (Biasini et al. 2014) prediction of the tertiary structure of the

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

152 *TbUTP10* encodes a much larger protein (287 kDa) than its opisthokont homologs in
153 yeast (200 kDa), zebrafish (242 kDa) and human (236 kDa) (Dragon et al. 2002; Azuma et al.
154 2006; Prieto et al. 2007). The alignment of TbUTP10 with these sequences reveals several
155 insertions that account for this increase in size (**Suppl. Fig. 1**). We checked all the extra
156 sequences of *T. brucei* protein (which are mainly around 20 aa long, the longest one being 57
157 aa) using protein domains identifier – InterProScan
158 (<http://www.ebi.ac.uk/interpro/search/sequence-search>), but didnt find any functional domains
159 that might point to additional roles of this protein in *T. brucei* in comparison to orthologues in
160 other systems.

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

168

169 **TbUTP10 is localized in the nucleolus**

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

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

180

181 **Ablation of TbUTP10 severely affects fitness**

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

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

196

197 **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.

222 The results obtained in the BS TbUTP10-RNAi cells are similar (**Fig. 3C**). All three
223 probes showed an accumulation of the full-length precursor and a concomitant decrease of the
224 3.7 kb precursor upon the down-regulation of TbUTP10. The decrease of the mature 2.2 kb
225 SSU rRNA product is less pronounced in the BS than in the PS cells, but the overall trend is
226 the same. Moreover, the sKO cells showed an even more pronounced increase of the 9.6 kb-
227 long 5'ETS precursor, with the ITS1 and SSU precursors also increased and the mature
228 product decreased by about 20% (**Suppl. Fig. 2B and 3**). In conclusion, Northern blot
229 analysis of full-length precursor and mature 18S rRNA upon ablation of TbUTP10 by two
230 alternative approaches and in two different life cycle stages is consistent and shows that
231 TbUTP10 is specifically involved in the 18S rRNA processing as it was already described in
232 other organisms.

233

234 **TbUTP10-depleted cells have an abnormal polysome profile**

235 Since TbUTP10 is localised in the nucleolus and its depletion affects maturation of the pre-
236 18S rRNA transcript, we next investigated whether its RNAi-mediated depletion results in an
237 altered polysome profile, reflecting a defect in ribosome biogenesis. Based on the previously
238 described processing phenotype, we predicted a decrease of the 40S SSU, which contains
239 mature 18S rRNA, and a consequent decrease in the 80S ribosome. The amount of cells
240 required for this experiment limited its application to the PS RNAi cell line. The lysates from
241 parental cells and RNAi induced cell line were separated by 10 to 50% sucrose gradient
242 centrifugation and the fractions were monitored by absorbance at 254 nm to detect the protein
243 component of ribosomes (**Fig. 4**). Three biological replicates for each cell line had a very
244 consistent pattern (**Figs. 4A and B**), allowing us to analyse their overlay using peak
245 annotations (**Fig. 4C**).

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

256

257 **TbUTP10 silencing affects U3 snoRNA**

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

266

267 **DISCUSSION**

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

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

273 In agreement with this supposition, the ribosome of *T. brucei* has its share of unique
274 features, which include its 28S rRNA split into 6 fragments (White et al. 1986) and the
275 existence of unusual inter-subunit bridges, as observed by cryo-electron microscopy (Hashem
276 et al. 2013). Furthermore, trypanosomatid 18S rRNA is very long compared to other
277 organisms (Campbell et al. 1987), with significant differences in pre-18S rRNA processing
278 (Michaeli et al. 2011). However, virtually nothing is known about the proteins mediating early
279 stages of this process, motivating us to undertake functional analysis of TbUTP10, a protein
280 known to be involved in 18S rRNA processing in opisthokonts (Dragon et al. 2002; Turi et al.
281 2018; Azuma et al. 2006). Here, we have shown that the gene *Tb927.9.2900* in the excavate
282 protist *T. brucei* is a distantly related homolog of yeast *UTP10*, zebrafish *BAP28* and human
283 *HEATR1*, and therefore have renamed it *TbUTP10*. Due to its significant divergence from its
284 opisthokont homologs, TbUTP10 had to be identified based on a tertiary structure prediction
285 (Söding et al. 2005). Interestingly, TbUTP10 is larger than its opisthokont homologs, which
286 may be an adaptation to the longer length of its 18S rRNA substrate. We have shown that
287 TbUTP10 is a nucleolar protein, which was also confirmed in the Tryptag protein localisation
288 database for both N and C terminally tagged protein in PS stage
289 (<http://tryptag.org/?query=Tb927.9.2900>). Northern analysis of knock-out and knock-down
290 cell lines confirmed that it is involved in pre-18S rRNA processing. Although the band
291 quantifications did not consistently exhibit the trend in all 18S rRNA intermediate precursors,
292 there is a clear accumulation of full-length precursors and a decrease of the final 18S rRNA
293 product. Combined, these results confirmed that UTP10 has a conserved function in excavates
294 and opisthokonts.

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

305 These observations are in agreement with biochemical studies performed in yeast and
306 point to a conserved function. Association of UTP10 with pre-18S rRNA processing was first
307 discovered in *S. cerevisiae*, where it co-purified with the SSU processome that specifically
308 associated with the U3 snoRNA and also played a role in pre-rRNA transcription (Dragon et
309 al. 2002). It was proposed that U3 snoRNP recruitment to the processome is enhanced by a
310 UTP10-U3 snoRNA 3' domain interaction (Hunziker et al. 2016). Our results demonstrate
311 that the depletion of TbUTP10 is associated with the decreased level of U3 snoRNA, which
312 was also observed in cells down-regulated for Nop58, a protein that belongs to the U3snoRNP
313 complex (Barth et al. 2008). It is not an unexpected result, since recent structural studies
314 revealed that these proteins actually seem to function next to each other (Kornprobst et al.
315 2016; Sun et al. 2017; Barandun et al. 2017). Indeed, UTP10 deletion inhibited the early pre-
316 rRNA processing steps in the 18S rRNA maturation, causing the accumulation of its
317 precursors, although it had only mild effects on rRNA transcription and 25S or 5.8S rRNA
318 synthesis. Depletion of Bap28 and HEATR1 in zebrafish and mammals, respectively, results
319 in increased p53-dependent apoptosis in the central nervous system, leading to abnormal brain

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

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

332

333

334

335

336 MATERIALS AND METHODS

337 Phylogenetic analysis

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

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

348

349 ***T. brucei* cell lines, cultivation and growth curves**

350 *T. brucei* PS SMOXP9 strain (Poon et al. 2012) was grown at 27°C in SDM-79 medium (Brun
351 and Schönenberger 1979) supplemented with 10% fetal bovine serum (FBS), while BS 427 -
352 single marker strain (Wirtz et al. 1999) was kept at 37°C and 5% CO₂ in HMI-9 medium
353 supplemented with 10% FBS. Additionally, antibiotics were added to the media according to
354 the selection markers present (**Tables 1 and 2**). For growth measurements, BS and PS cells
355 were induced at a density of 2 x 10⁵/ml and 3 x 10⁶/ml, respectively, counted daily using a Z2
356 Particle Counter (Beckman Coulter) and diluted back to the initial density as appropriate.
357 Representative growth curves are shown, with the experiment repeated three times to ensure
358 reproducibility.

359

360 **Generation of cell lines**

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

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

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

375 *Generation of RNAi and single knock-out cell lines.* A 515 bp fragment of the
376 TbUTP10 gene was PCR amplified from genomic DNA (primers 2900_p2T7_Fw and
377 2900_p2T7_Rv) and cloned into the p2T7-177 vector (Wickstead et al. 2002) via the XhoI and
378 BamHI restriction sites. The linearized p2T7-177 vector was subsequently electroporated into
379 both tagged cell lines and clones were selected with phleomycin. PS cell and BS cell clones
380 were induced with 1 µg/ml tetracycline. Single knock-out of TbUTP10 in the BS 427 strain
381 was generated using the fusion PCR method (Merritt and Stuart 2013), with the hygromycin
382 resistance marker cassette flanked by TbUTP10's 5'- and 3'-untranslated regions to facilitate
383 homologous recombination. These constructs were sequentially electroporated into the cells.

384

385 **Localisation of TbUTP10 protein in live cells**

386 In order to visualize TbUTP10, 5 x 10⁶ live PS or BS cells were stained with 1 µl of 20 µM
387 MitoTracker Red CMXRos (Molecular Probes) and two drops of Nuc Blue Live Cell Stain
388 (Molecular Probes), incubated for 15 min at their respective cultivation conditions, spun down
389 and resuspended in Iscove's Modified Dulbecco's Medium. The cells were subsequently
390 immobilized under a sheet of 1% agarose and observed under an OlympusFluoViewFV1000
391 confocal microscope as described elsewhere (Huang et al. 2014).

392

393 **Western blot analysis**

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

404

405 **Northern blot analysis**

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

417

418 **Sucrose density gradient sedimentation**

419 The polysome profile analysis was performed as described previously (Fleming et al. 2016).
420 Fifty mL shaking cultures were grown overnight at 27 °C to mid-log phase. Cells were
421 collected by centrifugation and all subsequent steps were carried out at 4 °C. Cells were
422 washed twice in 15 mL of ice-cold PBS supplemented with 100 µg/mL cyclohexamide and
423 final pellet suspended in 780 µL of polysome buffer (10 mM Tris-HCl pH 7.5, 300 mM KCl,
424 10 mM MgCl₂) supplemented with 100 µg/mL cyclohexamide, 2 mM DTT, and 1X Protease
425 inhibitor cocktail. Cells were lysed by addition of NP-40 to a final concentration of 0.25% and
426 incubated on ice for 10 minutes. Lysates were clarified by centrifugation at 15,000 g for 10
427 minutes. One mg equivalent of OD260 units was loaded on each 10–50% sucrose gradient
428 prepared in lysis buffer and centrifuged at 36,600 RPM for 2 hours at 4 °C in a Beckman
429 SW41Ti rotor. A gradient fractionator (ISCO UA-6 UV Vis with Type 11 optical unit) was
430 used to record UV profiles.

431

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439

440 **REFERENCES**

441 Alsford S, Turner DJ, Obado SO, Sanchez-Flores A, Glover L, Berriman M, Hertz-Fowler C,
442 Horn D. 2011. High-throughput phenotyping using parallel sequencing of RNA
443 interference targets in the African trypanosome. *Genome Res.* **21**: 915-924.

444 Aslett M, Aurrecoechea C, Berriman M, Brestelli J, Brunk BP, Carrington M, Depledge DP,
445 Fischer S, Gajria B, Gao X, Gardner MJ, Gingle A, Grant G, Harb OS, Heiges M,
446 Hertz-Fowler C, Houston R, Innamorato F, Iodice J, Kissinger JC, Kraemer E, Li W,
447 Logan FJ, Miller JA, Mitra S, Myler PJ, Nayak V, Pennington C, Phan I, Pinney DF,
448 Ramasamy G, Rogers MB, Roos DS, Ross C, Sivam D, Smith DF, Srinivasamoorthy
449 G, Stoeckert CJ Jr, Subramanian S, Thibodeau R, Tivey A, Treatman C, Velarde G,
450 Wang H. 2009. TriTrypDB: a functional genomic resource for the Trypanosomatidae.
451 *Nucleic Acids Res* **38**: D457-462.

452

453 Azuma M, Toyama R, Laver E, Dawid IB. 2006. Perturbation of rRNA Synthesis in the
454 bap28 Mutation leads to apoptosis mediated by p53 in the zebrafish central nervous
455 system. *J Biol Chem.* **281**: 13309–13316.

456

457 Barandun J, Chaker-Margot M, Hunziker M, Molloy KR, Chait BT, Klinge S. 2017.
458 The complete structure of the small-subunit processome. *Nat Struct Mol Biol* **24**: 944-
459 953.

460

461 Barth S, Shalem B, Hury A, Tkacz ID, Liang XH, Uliel S, Myslyuk I, Doniger T, Salmon-
462 Divon M, Unger R, Michaeli S. 2008. Elucidating the role of C/D snoRNA in rRNA
463 processing and modification in *Trypanosoma brucei*. *Eukaryot Cell* **7**: 86-101.

464

465 Bernstein KA, Gallagher Jennifer EG, Mitchell BM, Granneman S, Baserga SJ. 2004. The
466 small-subunit processome is a ribosome assembly intermediate. *Eukaryot Cell* **3**:1619–
467 1626.

468

469 Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F, Gallo
470 Cassarino T, Bertoni M, Bordoli L, Schwede T. 2014. SWISS-MODEL: modelling
471 protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids*
472 *Res* **42**: W252-258.

473

474 Brun R, Schönenberger M. 1979. Cultivation and in vitro cloning or procyclic culture forms
475 of *Trypanosoma brucei* in a semi-defined medium. Short communication. *Acta Trop*
476 **36**: 289-292.

477 Campbell DA, Kubo K, Clark CG, Boothroyd JC. 1987. Precise identification of cleavage
478 sites involved in the unusual processing of trypanosome ribosomal RNA. *J Mol Biol*
479 **196**: 113–124.

480

481 Chaker-Margot M. 2018. Assembly of the small ribosomal subunit in yeast: mechanism and
482 regulation. *RNA* **24**: 881-891.

483

484 Dean S, Sunter JD, Wheeler Richard J, Hodgkinson I, Gluenz E, Gull K. 2015. A toolkit
485 enabling efficient, scalable and reproducible gene tagging in trypanosomatids. *Open*
486 *Biol* **5**: 140197.

487

488 Dez C, Dlakić M, Tollervey D. 2007. Roles of the HEAT repeat proteins Utp10 and Utp20 in
489 40S ribosome maturation. *RNA* **13**: 1516-1527.

490

491 Dragon F, Gallagher JE, Compagnone-Post PA, Mitchell BM, Porwancher KA, Wehner KA,
492 Wormsley S, Settlege RE, Shabanowitz J, Osheim Y, Beyer AL, Hunt DF, Baserga SJ.

493 2002. A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA
494 biogenesis. *Nature* **417**: 967-970.

495 Fleming IM, Paris Z, Gaston KW, Balakrishnan R, Fredrick K, Rubio MA, Alfonzo JD. 2016.
496 A tRNA methyltransferase paralog is important for ribosome stability and cell division
497 in *Trypanosoma brucei*. *Sci Rep* **6**: 21438.

498

499 Gallagher JE, Dunbar DA, Granneman S, Mitchell BM, Osheim Y, Beyer AL, Baserga SJ.
500 2004. RNA polymerase I transcription and pre-rRNA processing are linked by specific
501 SSU processome components. *Genes Dev* **18**: 2506-2517.

502

503 Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user
504 interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* **27**:
505 221-224.

506

507 Gupta SK, Hury A, Ziporen Y, Shi H, Ullu E, Michaeli S. 2010. Small nucleolar RNA
508 interference in *Trypanosoma brucei*: mechanism and utilization for elucidating the
509 function of snoRNAs. *Nucleic Acids Res* **38**: 7236-7247.

510

511 Hampl V, Hug L, Leigh JW, Dacks JB, Lang BF, Simpson AG, Roger AJ. 2009.
512 Phylogenomic analyses support the monophyly of Excavata and resolve relationships
513 among eukaryotic "supergroups. *Proc Natl Acad Sci USA* **106**: 3859-3864.

514

515 Hartshorne T, Toyofuku W, Hollenbaugh J. 2001. *Trypanosoma brucei* 5'ETS A'-cleavage is
516 directed by 3'-adjacent sequences, but not two U3 snoRNA-binding elements, which

517 are all required for subsequent pre-small subunit rRNA processing events. *J Mol Biol*
518 **313**: 733-749.

519

520 Hartshorne T, Toyofuku W. 1999. Two 5-ETS regions implicated in interactions with U3
521 snoRNA are required for small subunit rRNA maturation in *Trypanosoma brucei*.
522 *Nucl. Acids Res* **27**: 3300-3309.

523

524 Hartshorne T. 1998. Distinct regions of U3 snoRNA interact at two sites within the 5' external
525 transcribed spacer of pre-rRNAs in *Trypanosoma brucei* cells. *Nucleic Acids Res* **26**:
526 2541-2553.

527

528 Hashem Y, des Georges A, Fu J, Buss SN, Jossinet F, Jobe A, Zhang Q, Liao HY, Grassucci
529 RA, Bajaj C, Westhof E, Madison-Antenucci S, Frank J. 2013. High-resolution cryo-
530 electron microscopy structure of the *Trypanosoma brucei* ribosome. *Nature* **494**: 385–
531 389.

532

533 Huang Z, Kaltenbrunner S, Šimková E, Staněk D, Lukeš J, Hashimi H. 2014. Dynamics of
534 mitochondrial RNA-binding protein complex in *Trypanosoma brucei* and its petite
535 mutant under optimized immobilization conditions. *Eukaryot Cell* **13**: 1232-1240.

536 Hunziker M, Barandun J, Petfalski E, Tan D, Delan-Forino C, Molloy KR, Kim KH, Dunn-
537 Davies H, Shi Y, Chaker-Margot M, Chait BT, Walz T, Tollervey D, Klinge S. 2016.
538 UtpA and UtpB chaperone nascent pre-ribosomal RNA and U3 snoRNA to initiate
539 eukaryotic ribosome assembly. *Nature Commun* **7**: 12090.

540

541 Jensen BC, Wang Q, Kifer CT, Parsons M. 2003. The NOG1 GTP-binding protein is required
542 for biogenesis of the 60 S ribosomal subunit. *J Biol Chem* **278**: 32204-32211.
543

544 Kala S, Mehta V, Yip CW, Moshiri H, Najafabadi HS, Ma R, Nikpour N, Zimmer SL,
545 Salavati R. 2017. The interaction of a *Trypanosoma brucei* KH-domain protein with a
546 ribonuclease is implicated in ribosome processing. *Mol Biochem Parasitol* **211**: 94-
547 103.
548

549 Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7:
550 Improvements in performance and usability. *Mol Biol Evol* **30**: 772–780.
551

552 Klinge S, Voigts-Hoffmann F, Leibundgut M, Ban N. 2012. Atomic structures of the
553 eukaryotic ribosome. *Trends Biochem Sci* **37**: 189-198.
554

555 Kornprobst M, Turk M, Kellner N, Cheng J, Flemming D, Koš-Braun I, Koš M, Thoms M,
556 Berninghausen O, Beckmann R, Hurt E. 2016. Architecture of the 90S Pre-ribosome:
557 A Structural View on the Birth of the Eukaryotic Ribosome. *Cell* **166**: 380-393.
558

559 Melnikov S, Ben-Shem A, Garreau de Loubresse N, Jenner L, Yusupova G, Yusupov M.
560 2012. One core, two shells: bacterial and eukaryotic ribosomes. *Nat Struct Mol Biol*
561 **19**: 560-567.
562

563 Merritt C, Stuart K. 2013. Identification of essential and non-essential protein kinases by a
564 fusion PCR method for efficient production of transgenic *Trypanosoma brucei*. *Mol*
565 *Biochem Parasitol* **190**: 44–49.

566

567 Michaeli S. 2011. rRNA biogenesis in trypanosomes. In: *Nucleic Acids and Molecular*
568 *Biology*, vol. 28; ed. A. Bindereif,: 123-148.

569

570 Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective
571 stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*
572 **32**: 268–274.

573

574 Pérez-Fernández J, Román A, De Las Rivas J, Bustelo XR, Dosil M. 2007. The 90S
575 preribosome is a multimodular structure that is assembled through a hierarchical
576 mechanism. *Mol Cell Biol* **27**: 5414-5429.

577

578 Pöll G, Li S, Ohmayer U, Hierlmeier T, Milkereit P, Perez-Fernandez J. 2014. *In vitro*
579 reconstitution of yeast tUTP/UTP A and UTP B subcomplexes provides new insights
580 into their modular architecture. *PLoS ONE* **9**: e114898.

581

582 Poon SK, Peacock L, Gibson W, Gull K, Kelly S. 2012. A modular and optimized single
583 marker system for generating *Trypanosoma brucei* cell lines expressing T7 RNA
584 polymerase and the tetracycline repressor. *Open Biol* **2**: 110037.

585

586 Prieto JL, McStay B. 2007. Recruitment of factors linking transcription and processing of pre-
587 rRNA to NOR chromatin is UBF-dependent and occurs independent of transcription in
588 human cells. *Genes Dev* **21**: 2041-2054.

589

590 Sakiyama J, Zimmer SL, Ciganda M, Williams N, Read LK. 2013. Ribosome biogenesis
591 requires a highly diverged XRN family 5'->3' exoribonuclease for rRNA processing in
592 *Trypanosoma brucei*. *RNA* **19**: 1419-1431.

593

594 Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology
595 detection and structure prediction. *Nucleic Acids Res* **33**: W244-248.

596

597 Sun Q, Zhu X, Qi J, An W, Lan P, Tan D, Chen R, Wang B, Zheng S, Zhang C, Chen X,
598 Zhang W, Chen J, Dong MQ, Ye K. 2017. Molecular architecture of the 90S small
599 subunit pre-ribosome. *eLife* **6**, e22086.

600

601 Tschochner H, Hurt E. 2003. Pre-ribosomes on the road from the nucleolus to the cytoplasm.
602 *Trends Cell Biol* **13**: 255-263.

603

604 Turi Z, Senkyrikova M, Mistrik M, Bartek J, Moudry P. 2018. Perturbation of RNA
605 polymerase I transcription machinery by ablation of HEATR1 triggers the
606 RPL5/RPL11-MDM2-p53 ribosome biogenesis stress checkpoint pathway in human
607 cells. *Cell Cycle* **17**: 92-101.

608

609 Umaer K, Ciganda M, Williams N. 2014. Ribosome biogenesis in African trypanosomes
610 requires conserved and trypanosome-specific factors. *Eukaryot Cell* **13**: 727-737.

611

612 Venema J, Tollervey D. 1999. Ribosome synthesis in *Saccharomyces cerevisiae*. *Ann Rev*
613 *Genet* **33**: 261-311.

614

615 Vondrušková E, van den Burg J, Zíková A, Ernst NL, Stuart K, Benne R, Lukeš J. 2005.
616 RNA interference analyses suggest a transcript-specific regulatory role for
617 mitochondrial RNA-binding proteins MRP1 and MRP2 in RNA editing and other
618 RNA processing in *Trypanosoma brucei*. *J Biol Chem* **280**: 2429-2438.
619

620 White TC, Rudenko G, Borst P. 1986. Three small RNAs within the 10 kb trypanosome
621 rRNA transcription unit are analogous to Domain VII of other eukaryotic 28S rRNAs.
622 *Nucleic Acids Res* **14**: 9471–9489.
623

624 Wickstead B, Ersfeld K, Gull K. 2002. Targeting of a tetracycline-inducible expression
625 system to the transcriptionally silent minichromosomes of *Trypanosoma brucei*. *Mol*
626 *Biochem Parasitol* **125**: 211–216.
627

628 Wilson DN, Doudna Cate JH. 2012. The structure and function of the eukaryotic ribosome.
629 Cold Spring Harb Perspect Biol 4.
630

631 Wirtz E, Leal S, Ochatt C, Cross GA. 1999. A tightly regulated inducible expression system
632 for conditional gene knock-outs and dominant-negative genetics in *Trypanosoma*
633 *brucei*. *Mol Biochem Parasitol* **99**: 89–101.
634

635 Wu ZB, Qiu C, Zhang AL, Cai L, Lin SJ, Yao Y, Tang QS, Xu M, Hua W, Chu YW, Mao Y,
636 Zhu JH, Xu J, Zhou LF. 2014. Glioma-associated antigen HEATR1 induces functional
637 cytotoxic T lymphocytes in patients with glioma. *J Immunol Res* 131494.
638
639

640 **Table 1:** Antibiotics mixture compositions for different cell lines.

Used <i>T. brucei</i> 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

641

642

643

644 **Table 2:** Concentrations of all antibiotics in respective media.

Used antibiotics	Concentrations in medium	
	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

645

646

647

648 **Table 3:** DNA oligonucleotides used as PCR primers

Primers	Sequence
Procylic tagging	

2900_Ty_Fwd	TCGTTGTGGAGCAGGCGCGGAGGTTGTGTGGACATCTTT CATCCATTACGGGCCAAGATGTCCTGTATGCGATGGGTT CCACTAGTGTGAGCAAGG
2900_Ty_Rev	TCTTGACTTAGAGAGCTACCTCACCGAGATGTCATCGTG TACATGTACTCCAAGGCACACCCTTCCCATTGCAGCGGG TACTATTCCTTTGCCCTCGGAC
Bloodstream tagging	
A-5U2900-F	CATGCGGCAGATCTCATAGC
A-5U2900-YFP-R	ACTAGTGTGAGCAAGGGGAACCCATCGCATAACAGG
B-3U2900-Hyg-F	GTCCGAGGGCAAAGGAATAGTACCCGCTGCAATGGGAA GGG
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	CCCCCTACACTGTTACACC
A-5U2900-Hyg-R	GGTGAGTTCAGGCTTTTTTCATTTGCTCAAGCAGTACTTA
B-3U2900-Hyg-F	GTCCGAGGGCAAAGGAATAGTACCCGCTGCAATGGGAA GG

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'-GTCCTGCCCACTCAGGTCTGA-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.

662 **C.** Maximum likelihood topology of TbUTP10 eukaryotic homologues constructed using
663 LG+F+R5 model as implemented in IQTree. Numbers at branches correspond to the ultrafast
664 bootstrap branching support inferred from 50000 replicates in IQTree. The dataset consisted
665 of 48 taxa and 799 amino acid positions. See relevant part of Materials and Methods for
666 details. Kinetoplastids are shown in grey box. The only three species of opisthokonts (dashed
667 box) in which homologues of TbUTP10 were studied are indicated with a star.

668 **D.** TbUTP10 protein is localized in the nucleolus of both PS (left) and BS (right) *T. brucei*.
669 Localization of TbUTP10-Ty-GFP (in green) in the nucleolus. Mitochondria are stained in red
670 (MitoTracker) and DNA in blue (Nuc Blue Live Cell Stain). Scale bar: 5 μ m.

671

672 **Fig. 2. TbUTP10 protein is essential for normal growth of both PS (left) and BS (right)**
673 *T. brucei*.

674 Cumulative growth curves of TbUTP10 RNAi (ind = RNAi induced with tetracycline) in PS
675 cells (**A**) and BS cells (**B**) are shown, as compared to the non-induced controls (non-ind).
676 Western blot analysis of TbUTP10-Ty-GFP levels during a RNAi time course in PS (**A** –
677 inset) or BS (**B** - inset) probed with anti-Ty antibody; anti- α -tubulin antibody signal shown as
678 a loading control. dpi, days post induction. Representative growth curves are shown, with the
679 experiment repeated three times to ensure reproducibility.

680

681 **Fig. 3. TbUTP10 is involved in the processing of pre-18S rRNA**

682 **A.** First 18S rRNA processing steps in *T. brucei* (adapted from Michaeli 2011). The asterisk
683 shows the position of the first cleavage, B1 (top). Coloured bars indicate the positions of the
684 5' ETS, SSU and ITS1 probes used for detection of the precursors in the pre-18S rRNA
685 processing pathway. The respective lengths of full-length precursors (9.6 kb), intermediates

686 (3.7 kb, 3.3 kb, 2.6 kb) and the final product (2.2 kb) (bottom) are also given. Please note that
687 sizes are not to scale in this diagram.

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

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

698

699

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

701 **A.** Parental PS cell line -P (solid black line); **B.** PS TbUTP10 RNAi cell line (dashed line).

702 Peaks corresponding to 40S, 60S, 80S and the polysomes are indicated.

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

705

706 **Fig. 5. TbUTP10-silencing affects U3 snoRNA**

707 Total RNA was separated on a denaturing 8% polyacrylamide/8M urea gel (top) and

708 Northern blot analysis of parental strain (P) vs. PS TbUTP10 RNAi cells (A) and BS

709 TbUTP10 RNAi cells (B) induced for 1 to 4 days (I1 to I4) was performed. U3

710 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 μ m.

757