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Review

Unique Aspects of rRNA Biogenesis in Trypanosomatids

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Trypanosomatids are protozoan parasites that cycle between an insect and a mammalian host. The large-subunit rRNA of these organisms undergoes unique processing events absent in other eukaryotes. Recently, small nucleolar RNAs (snoRNAs) that mediate these specific cleavages were identified. Trypanosomatid rRNA is rich in RNA modifications such as 2′-*O*-methylation (Nm) and pseudouridylation (Ψ) that are also guided by these snoRNAs. A subset of these modifications is developmentally regulated and increased in the parasite form that propagates in the mammalian host. Such hypermodification contributes the temperature adaptation and hence infectivity during cycling of the parasite. rRNA processing and modification should be considered promising drug targets for fighting the diseases caused by these parasites.

Trypanosomatids

Trypanosomatids are single-cell flagellated parasites that include *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* species, and are the causative agent of African sleeping sickness, Chagas' disease and leishmaniasis, respectively. These parasites cycle between an insect and a mammalian host, and hence, must adapt to changes involving a temperature difference of almost 10°C. In the mammalian host, the slender bloodstream form (BSF) transforms to a quiescent stumpy form, which is the infectious form that is able to infect the insect vector. Following transmission to the fly vector, the parasite transforms to the procyclic form (PCF). After ~2 weeks in the fly, the parasites migrate to the salivary gland where they appear as the metacyclic infective form and the cycle is completed. The basic molecular machineries that are essential for life, such as translation and RNA splicing, need to be adjusted to function efficiently in these two different hosts.

Major changes in gene expression takes place during cycling between the two hosts [1]. As these parasites lack mechanisms for transcriptional regulation, the robust regulation of gene expression lies in mRNA stability and protein synthesis [2]. Thus, the ribosome must be a key factor in this translation regulation. Here, we summarize the recent advances in understanding of the complex **ribosomal RNA (rRNA)** (see Glossary) processing pathway and how rRNA modifications contribute to adaptation of the parasite while cycling between its two hosts.

rRNA Processing Machinery in Eukaryotes, and the Twist in Trypanosomatids

rRNA processing is a complex process that involves the action of **small nucleolar RNAs (snoRNAs)** and protein factors such as nucleases, AAA-type ATPases, helicases, and GTPases. The processing of rRNA starts in the nucleolus, proceeds in the nucleoplasm, and is completed in the cytoplasm [3]. The entire 35S **pre-rRNA** (in yeast) assembles cotranscriptionally with *trans*-acting factors and early binding ribosomal proteins of the small subunit (SSU) rRNA to form the 90S (SSU) processome, containing the external and internal transcribed spacers, ETS and ITS, respectively (Box 1) [4]. The pre-rRNA undergoes extensive site-specific base modifications and cleavage at positions A0, A1, and A2 (Figure 1A). First, separation of the pre-18S SSU from the pre-large subunit (LSU) rRNA takes place [5] and 5′-ETS is degraded by exonucleolytic activities [6]. The pre-40S particle translocates to the cytoplasm, where final maturation occurs [7,8]. Next, the LSU precursor is cleaved at either the A3 position or at B1. The last step involves cleavage at positions C1 and C2, to generate the 5.8S and 25S rRNA [9,10] (Figure 1A).

In trypanosomatids, the processing is more complex. The LSU is further cleaved to two large fragments (LSUα and LSUβ), as well as four discrete small ribosomal RNAs (srRNA 1,2,4, and 6) (Figure 1A) [11–14]. In *T. brucei*, three predominant rRNA processing intermediates were identified (Figure 1A).

Highlights

Processing of the large rRNA subunit is unique in trypanosomatids and results in cleavage into two large and four small RNA fragments; the processing events are mediated by snoRNAs.

The last small RNA (srRNA1) to be liberated during processing is the first to be transcribed.

The abundance of a given snoRNA can indicate its function; 16 abundant snoRNAs are involved in trypanosome-specific rRNA cleavage pathways, and the others are involved only in rRNA modification.

Six of these abundant snoRNAs direct methylation in the vicinity of the protein exit tunnel of the ribosome, and these modifications serve as check-points to monitor proper rRNA processing.

Trypanosomatids have a rich repertoire of 2′-*O*-methylation (Nm) and pseudouridines (Ψ) on their rRNA, and a subset of these sites are hypermodified in the bloodstream form.

The hypermodified positions are essential for growth, especially at elevated temperature, and may also control the triggering of innate immunity in the mammalian host.

rRNA modification, especially by Ψ, may control stage-specific translation of mRNAs.

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Box 1. LSU rRNA Processing Complexes in Other Eukaryotes

In yeast, over 99 proteins are associated with different steps of 60S ribosomal subunit maturation. Recent insight into the assembly of pre-60S was obtained by cryo-EM of these processing complexes. After cleavage at the A2 site, the 40S and 60S subunits undergo separate processing steps [69]. The 60S pre-LSU subunit folds into six conserved secondary structure domains (I–VI) [69]. Incorporation of ribosomal (r)-proteins into large subunits in yeast cells is hierarchical. Most r-proteins cotranscriptionally associate with pre-rRNA. However, early binding of the proteins to pre-ribosomes is relatively weak and becomes stronger as assembly proceeds. Formation of very early 60S subunit assembly intermediates occurs during and after cleavage at the A2 site. Twelve factors (Ytm1, Erb1, Nop7, Rlp7, Cic1, Nop15, Has1, Drs1, Rpf1, Pwp1, Nop12, and Rrp1) and 11 r-proteins were initially implicated in rRNA processing [69]. The middle stage of LSU maturation includes remodeling events that trigger exclusion of the ITS2 spacer. ITS2 removal is initiated by the endonuclease Las1 by cleavage at the C2 site in ITS2. Then, pre-ribosomes transit from the nucleolus to the nucleoplasm. In the early biogenesis pathway, the GTPase Nog1 also assembles into pre-ribosomes [70]. Construction of the peptidyl-transferase center (PTC) also appears to be essential to initiate removal of ITS2. Five factors – Nsa2, Nog2, Dbp10, Nug1, and Rsa4 – bind at or adjacent to the PTC before C2 cleavage [69]. All of these factors have been identified in trypanosomatids (Table 1, Figure 2). Recent cryo-EM structures demonstrate that export factors are distributed across the intersubunit surface [69]. The last steps of LSU maturation occur in the cytoplasm. These include formation of the 3′-end of 5.8S rRNA, release of the factors, and binding of r-proteins. Five NTPases (Drg1, Ssa1, Efl1, and Lsg1, and the phosphatase Yvh1) were found in yeast to be involved in release and recycling of different factors from cytoplasmic pre-ribosomes. However, of these factors, Drg1, Efl1, and Lsg1 were identified in trypanosomatids (Table 1). Proteins associated with SSf1 that were shown to be important for the early steps of the pre-60S assembly include Dbp6p, Dbp7p, Dbp9p, Npa1p, Npa2p, Nop8p, and Rsa3p [71]. Npa1p, Npa2p, Nop8p, Rsa3p, and Dbp6p form a discrete complex which might function as a scaffold to mediate topological rearrangements by RNA helicases and organize the pre-60S ribosomes [71]. This complex, with the exception of Dbp7 and Dbp9, appears to be missing in trypanosomatids. Thus, it is not clear at this point if the missing factors are indeed absent, or whether they are divergent and thus cannot be identified by bioinformatics or by the presence of peptides in purified complexes (Table 1).

The first cleavage of the 9.6 kb pre-rRNA takes place at B1, then, cleavages take place at sites A′, A0, A1, and A2 [15,16]. The pre-SSU (3.7 kb) is further processed to yield the mature SSU. Although the LSU cleavages were reported almost three decades ago, until recently we knew very little regarding the machinery that is involved in these special cleavages. Recent studies identified trypanosome-specific snoRNAs that are directly involved in the processing, as well as identifying **RNA modifications** that are essential for proper processing [17–19]. However, we still do not know if any trypanosomatid-specific factors are associated with these unique cleavages, nor why such extra processing events developed in trypanosomatids.

snoRNAs Involved in Trypanosome rRNA Processing

snoRNAs are known to participate in the pre-rRNA cleavages (Figure 1B). Five major snoRNAs were reported to function in the processing of SSU: U3 and U14, which are present in both higher and lower eukaryotes, U22, U17 (snR30 in yeast), and snR10, which is present only in yeast [9]. U3 **snoRNP** is the first to bind to the nascent pre-rRNA transcript, and to organize the active processing complex [4]. In yeast and mammals, U3 was shown to be essential for cleavage at the A0 site followed by cleavage at A1, and then at A2 [9,20]. Utp24/hUTP24, a U3-associated endonuclease was proposed to cleave at sites A1 and A2, but the enzyme cleaving at site A0 is not known. A Utp24 homolog was identified in trypanosomatids (Table 1, Figure 2). Trypanosome U3 resembles its homologs [21]. snR30 is involved in SSU processing and governs cleavages at sites A0, A1, and A2 [22,23]. Functional data are still lacking to demonstrate its function in trypanosome rRNA processing. Two other conserved snoRNAs involved in SSU processing are U14 and U22; these snoRNAs do not exist in trypanosomes [17,18,24].

Two major snoRNAs were shown to participate in LSU processing, MRP and U8. MRP directs cleavage at A3 [9]. A trypanosome homolog to MRP was identified that carries the essential domains, but also includes an additional domain (P10) [25]. However, its function in rRNA processing has not yet been demonstrated. In metazoans, U8 is involved in cleavage at ITS2 [26,27]. No homolog of U8 has been found in trypanosomes [17,18,24].

Glossary

2′-*O*-methylation (Nm): RNA modification with methylation on the 2′-carbon of the sugar.
C/D snoRNA: snoRNAs carrying C and D boxes and guide Nm modification.
CRISPR methodology: the 'clustered regularly interspaced short palindromic repeat' is a genome-editing approach used to delete or mutate genes in the genome.
Cryo-EM: cryogenic electron microscopy that is used to determine the 3D structure of a complex, such as ribosomes at high resolution.
Expansion segments (ESs): domains within rRNA whose sequence and structure are not conserved among species and whose function is unknown.
H/ACA snoRNA: snoRNAs that carry the H and ACA boxes and guide Ψ formation.
pre-rRNA: the precursor of rRNA (pre-rRNA) is transcribed by polymerase I and is processed by removal of external transcribed spacer (ETS) and internal transcribed spacer (ITS) to release LSU, SSU, and 5.8S rRNA. In trypanosomes, additional processing exists for the LSU rRNA which is cleaved to LSUα and β, and the small rRNA (sr) 1, 2, 4, and 6.
Ribosomal RNA (rRNA): the large-subunit ribosome carries the large subunit rRNA (LSU), 5.8S and 5S rRNA; the small-subunit ribosome contains only the small subunit (SSU).
RNA modifications: modifications generated by enzymes that covalently change the chemical composition of the sugar or the base, such as methylation of the sugar, or by 180° rotation of the base in case of pseudouridylation (Ψ).
RNA-seq: high-throughput sequencing that determines the coverage and level of RNA transcripts.
RNA walk: a method that detects the interaction site between a small RNA and its target. The method requires UV cross-linking and the ability to amplify the target RNA. The interaction site cannot be amplified, and this identifies the interaction domain.
Small nucleolar RNA (snoRNA): small nucleolar RNA that either

The failure to detect snoRNA present in other eukaryotes mentioned above, and the unique processing events involved in LSU processing, suggest that trypanosomes may possess trypanosome-specific snoRNAs. Indeed, defects in rRNA processing were examined under silencing of NOP1/NOP58, the **C/D snoRNA** core proteins [25] and CBF5, the **H/ACA snoRNA** core protein in *T. brucei* [28]. The defects observed in the silenced cells indicate that C/D and H/ACA snoRNAs operate in the processing of both SSU and LSU rRNA.

The first snoRNAs shown to be involved in trypanosome specific rRNA processing were **TB11Cs2C1** and TB11Cs2C2. TB11Cs2C1 interacts both with the SSU and at a position upstream of A0 in the ETS, and despite the lack of structural resemblance to U14, may nevertheless function as an U14 ortholog (Figure 1B) [29]. **snoRNA interference (snoRNAi)** of TB11Cs2C2 indicates its functions in releasing sr2 and sr6 [24]. **RNA-seq** performed on small RNP particles of *T. brucei* identified additional highly abundant snoRNA species [17]. Four of these RNAs were studied using snoRNAi followed by examining rRNA processing defects as a result of the snoRNA depletion (Figure 1B). The bioinformatic prediction of their interaction domains was examined by '**RNA walk**', a method that was developed by our group and which enables mapping of the snoRNA–rRNA duplex upon *in vivo* UV induction of psoralen cross-links [30]. TB10Cs4C4 is likely to be the functional homolog of U8 since it is involved in cleavage at ITS2 (Figure 1B). TB6Cs1C3 was shown to direct cleavage at ITS6 for liberating sr2 and sr6, and TB9Cs2C1 was shown to be involved in cleavage at the boundaries of LSUα and LSUβ as well as ITS6 (depicted in Figure 1B) [17]. RNA-seq libraries prepared from postribosomal supernatants revealed additional abundant snoRNAs [18]. The function of ten abundant snoRNAs was recently elucidated by snoRNAi and following their rRNA processing defects [18]. TB9Cs2C5 silencing reduced the level of sr1 and sr4. TB9Cs3C3 silencing specifically affects the liberation of sr1. snoRNAi of TB10Cs4C3 affected methylation on SSU, but also 5.8S rRNA and sr4 processing, thus coordinating processing events at the 5′ and 3′ ends of the LSU precursor [18]. snoRNAi of TB11Cs3C2 only affected the liberation of sr1 [18] (Figure 1B). Thus, snoRNPs are directly involved in guiding the processing of srRNAs by interacting with flanking intronic spacers.

As opposed to the snoRNAs discussed above, snoRNAs TB8Cs1C1, TB8Cs1C3, TB9Cs3H2, and TB9Cs2C3 do not interact with rRNA intronic spacers but guide RNA modification on the LSU rRNA [18]. Silencing of these snoRNAs affected the cleavage of sr1, suggesting that liberation of sr1 is a key regulated event in rRNA processing (Figure 1B). Surprisingly, the positions guided by these snoRNAs are restricted to a ribosomal region that spans between the peptide exit tunnel and the sr1 site in the mature ribosome, with most modifications closely surrounding the protein exit tunnel but also near the peptidyltransferase center (PTC) [18] (Figure 3A,B, Key Figure). These observations imply that rRNA modifications serve as check-points that condition the liberation of sr1 on proper rRNA folding. The high abundance of the snoRNA guiding these modification ensures that those critical positions are modified on every ribosome [18]. The ribosome exit tunnel was proposed also by others to serve as a check-point in ribosome biogenesis [31]. Our study showed for the first time that snoRNAs guiding methylation can also affect rRNA processing. The absence of methylation may affect the binding of r-proteins and impair proper folding of the rRNA. The processing defects revealed under silencing of the different snoRNAs involved in LSU processing support the notion that the cleavage of sr2, -4 and -6 takes place before the cleavage of sr1. Indeed, studies in yeast suggested that the r-proteins are incorporated into the ribosome in a hierarchical manner during early, middle, or late biogenesis steps [32,33]. These assembly steps begin at the solvent-exposed side, followed by exit tunnel formation, and are completed by the formation of an intersubunit interface [32,33]. The recently reported cryogenic electron microscopy (**cryo-EM**) structures of *T. cruzi* and *Leishmania donovani* ribosomes suggested a similar dogma in trypanosome ribosome biogenesis, whereby sr-2, -4 and -6 are assembled, and potentially processed, at early biogenesis steps, and sr1 is assembled at later stages [34,35] (Box 2).

It is tempting to speculate that several trypanosomatid-specific snoRNAs may include protein factor(s) that function in directing cleavages to specific and unique sites. For instance, the trypanosome genome lacks genes encoding factors involved in ITS1 cleavage, such as Rrp17 [19], which was shown

mediates processing and/or RNA modification.
snoRNA interference (snoRNAi): the nuclear RNA interference pathway that enables the silencing of snoRNAs.
snoRNP: small RNA protein complexes that are localized in the nucleolus and carry either the C/D or H/ACA snoRNAs.
TB11Cs2C1: the snoRNA nomenclature designates the position of the RNA on the genome and indicates on which chromosome it is located, on which cluster within the chromosome the snoRNAs is found, as well as the position within the cluster (from 5′ to 3′) and whether it is a C/D or H/ACA snoRNA (indicated as C or H respectively). For instance, TB11Cs2C1 is a C/D snoRNAs located on chromosome 11, in cluster 2, and is the first C/D snoRNA in the cluster.

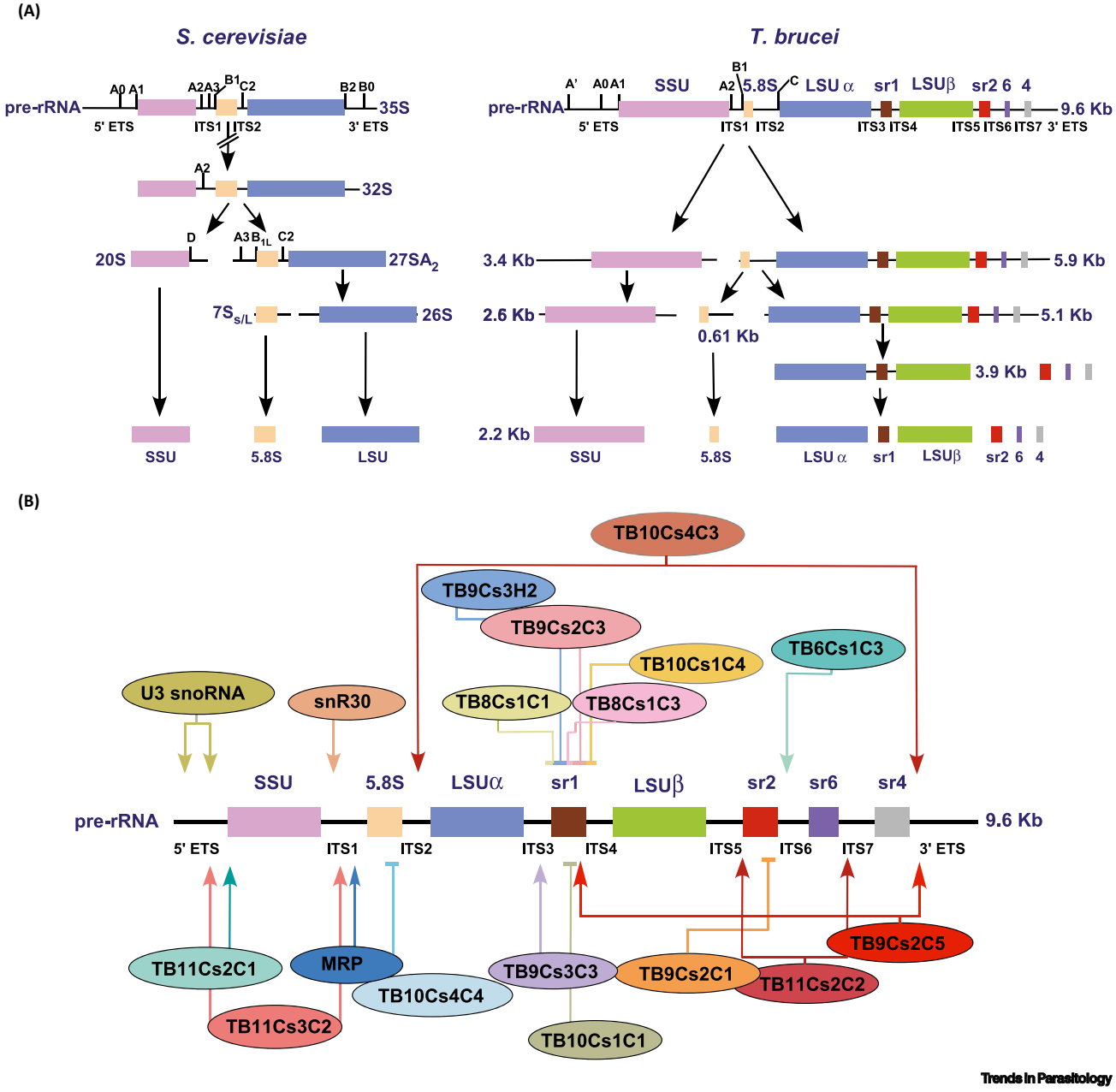

Figure 1. pre-rRNA Processing Pathway.

(A) Schematic representation depicting the pre-rRNA processing pathway in *Saccharomyces cerevisiae* (left) and *Trypanosoma brucei* (right). The positions of the major rRNA cleavage sites within large subunit (LSU), small subunit (SSU), 5.8S, internal transcribed spacer (ITS), and external transcribed spacer (ETS) are indicated. Below, the different intermediates and products produced from these processing events are shown. The SSU is colored in pink, 5.8S in orange, and LSU in blue. (B) Small nucleolar (sno)RNAs involved in *T. brucei* pre-rRNA processing. Schematic representation of *T. brucei* rRNA domains affected by depletion of individual snoRNAs. Closed arrows indicate snoRNAs that directly interact with ITS and/or ETS sequences and orchestrate rRNA processing events. Inhibitory symbol indicates those snoRNAs that affect the liberation of small ribosomal RNAs (srRNAs) and guide essential RNA modifications.

to be involved in A3 cleavage in ITS1 [32], and this factor might be replaced by a trypanosome-specific snoRNP protein. It will therefore be of great interest to characterize the protein constituents of each of the snoRNPs involved in the rRNA processing described above.

SGD ID	*T. brucei* ID	*L. tarentolae*				*S. cerevisiae*			
		UTP18	SSF1	RLP24	ARX1	UTP18	SSF1	RLP24	ARX1
SSU factors [39]									
BMS1	Tb927.11.9080	+	+	-	-	-	-	-	+
DRS1	Tb927.5.1560	-	+	-	-	-	-	-	-
EMG1	Tb927.8.5040	-	+	-	-	-	-	-	-
ENP1	Tb927.6.1900	-	+	-	-	-	-	-	-
ENP2	Tb927.7.700	-	+	-	-	-	-	-	-
G3P3	Tb927.6.4300	+	+	+	+	-	-	-	-
GRP78	Tb927.11.11290	+	+	+	+	-	-	-	-
IMP3	Tb927.11.13930	+	+	-	-	-	-	-	-
IMP4	Tb927.6.2780	+	+	-	-	-	-	-	-
KRE33	Tb927.5.2530	+	+	-	-	+	-	-	-
KRR1	Tb927.6.4350	-	+	-	-	-	-	+	-
MAK21#	Tb927.11.2120	-	+	-	-	-	+	-	-
MPP10	Tb927.3.3590	+	+	-	-	+	-	-	-
NOC2#	Tb927.10.12430	-	+	-	-	-	+	+	-
NOP1	Tb927.10.7500	+	+	+	+	+	+	+	-
NOP12#	Tb927.9.13280	-	+	-	-	-	+	+	-
NOP56	Tb927.8.3750	+	+	+	-	+	+	+	-
NOP58#	Tb927.9.5320	+	+	+	-	+	+	-	-
PNO1	Tb927.9.11840	+	+	-	-	+	-	-	-
RCL1	Tb927.9.14410	-	+	-	-	-	-	-	-
ROK1	Tb927.11.13810	-	+	-	-	-	-	-	-
RPS7B	Tb927.9.3990	+	+	+	+	-	-	-	-
RPS13	Tb927.2.5910	+	+	+	+	-	-	-	-
RRP12	Tb927.11.2510	-	+	-	-	-	-	-	-
RRP5	Tb11.v5.0391	+	+	-	-	+	+	+	-
RRP8	Tb927.1.1120	-	+	-	-	-	+	+	-
RRP9	Tb927.10.2700	+	+	-	-	-	-	-	-
SOF1	Tb927.9.11250	-	+	-	-	-	-	-	-
UTP1	Tb11.v5.0563	+	+	-	-	+	-	-	-
UTP10	Tb927.9.2900	+	+	-	-	-	-	-	-

Table 1. The *Trypanosoma brucei* Homologs of Yeast pre-rRNA Processing Factors Present in the SSU and LSU Processome[a]

(Continued on next page)

SGD ID	*T. brucei* ID	*L. tarentolae*				*S. cerevisiae*			
		UTP18	SSF1	RLP24	ARX1	UTP18	SSF1	RLP24	ARX1
UTP11	Tb11.v5.0481	+	+	-	-	+	-	-	-
UTP12	Tb927.7.4220	+	+	-	-	+	-	-	-
UTP13	Tb927.11.460	+	+	-	-	+	-	-	-
UTP14	Tb927.10.14330	-	+	-	-	+	-	-	-
UTP15	Tb927.8.1980	+	+	-	-	+	-	-	-
UTP18	Tb927.10.9920	+	+	-	-	+	-	-	-
UTP21	Tb927.11.10480	+	+	-	-	+	-	-	-
UTP3	Tb927.11.8050	+	+	-	-	-	-	-	-
UTP6	Tb927.9.15330	+	+	-	-	+	-	-	-
UTP7	Tb927.8.2600	-	+	-	-	-	-	-	-
YRA1	Tb927.2.4710	-	+	-	-	-	-	-	-
Early pre-LSU factors [31,40]									
EBP2	Tb927.9.15060	-	+	+	-	-	+	-	-
ERB1	Tb927.11.6790	-	+	+	+	-	+	+	-
FKBP4	Tb927.10.16100	+	+	+	+	-	-	-	-
HAS1	Tb927.10.6260	-	+	-	-	-	-	-	-
MAK16	Tb927.7.3380	-	+	-	-	-	+	+	-
NIP7	Tb927.10.11990	-	+	+	-	-	+	+	+
NOP16	Tb927.8.4290	-	+	+	-	-	-	-	-
NOP2	Tb927.4.3840	-	+	+	-	-	+	+	+
NSA1	Tb927.11.12030	-	+	+	-	-	+	+	-
PUF6	Tb927.3.2470	-	+	-	-	-	+	+	+
RPF1	Tb927.11.1050	-	+	-	-	-	+	-	-
RRP14	Tb927.11.5810	-	+	-	-	-	-	-	-
SSF1	Tb927.3.2830	+	+	-	-	-	+	+	-
YTM1	Tb927.4.3850	-	+	+	-	-	-	-	-
Common factors of early and late pre-LSU [31,40]									
MRT4	Tb927.10.10010	-	+	+	+	-	+	+	+
NOG1	Tb927.11.3120	-	+	+	-	-	+	+	+
NSA2	Tb927.10.7710	-	+	+	-	-	+	+	+
RRP1	Tb927.8.5490	-	+	-	-	-	+	+	-
Common factors of SSU and early pre-LSU [31,39,40]									
BRX1	Tb927.10.14680	-	+	+	-	-	-	-	-

Table 1. *Continued*

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SGD ID	*T. brucei* ID	*L. tarentolae*				*S. cerevisiae*			
		UTP18	SSF1	RLP24	ARX1	UTP18	SSF1	RLP24	ARX1
Common factors of SSU, early and late pre-LSU [31,39,40]									
RPF2	Tb927.7.270	-	+	+	-	-	+	+	+
RRS1	Tb927.6.2050	-	+	+	-	-	+	-	-
Late pre-LSU factors [31,40]									
ARX1	Tb927.10.14790	+	+	+	+	-	-	-	+
GBLP	Tb927.11.11360	+	+	+	+	-	-	-	-
LHP1	Tb927.10.2370	+	+	+	+	-	-	-	-
MTR4	Tb927.10.7440	+	+	-	-	-	+	-	-
NOG2	Tb927.7.7450	-	+	+	-	-	-	+	+
NOP53	Tb927.9.13340	-	+	-	-	-	-	-	-
NUG1	Tb927.11.2640	-	-	+	-	-	-	-	-
RLP24	Tb927.11.6360	-	+	+	-	-	+	+	+
RLP7	Tb927.7.1730	+	+	+	+	-	+	+	+
RSSA1	Tb927.11.10910	+	+	+	+	-	-	-	-
RSA4	Tb927.8.5990	-	+	+	-	-	-	-	-
SDA1	Tb927.5.2660	-	+	-	-	-	-	+	+
SPB1	Tb927.2.4550	-	+	+	-	-	-	-	-
TSR1	Tb927.8.1410	-	+	-	-	-	+	-	-
Other known pre-rRNA factors [77,78]									
CKA1	Tb927.2.2430	-	+	-	-	-	-	-	-
CKA2	Tb927.9.14430	-	+	-	-	-	-	-	-
ESF1[$]	Tb927.10.7810	-	+	-	-	-	-	-	-
ESF2[$]	Tb927.5.1080	-	+	-	-	-	-	-	-
DBP8[#]	Tb927.10.10380	-	+	-	-	-	-	-	-
CKB1[#]	Tb927.11.10820	-	+	-	-	-	-	-	-
GNO1[#]	Tb927.7.5640	-	+	-	-	+	+	-	-
SNU13[$#]	Tb927.9.5150	+	+	+	-	-	-	-	+
MRD1[$]	Tb927.8.4170	-	+	-	-	-	+	-	-
UTP24[$]	Tb927.10.15810	-	+	-	-	-	-	-	-
DBP4[$]	Tb927.5.4270	-	+	-	-	-	-	-	-
DHR2[$]	Tb927.4.2000	+	+	+	-	-	-	-	-
FYV7[$]	Tb927.11.14710	-	+	-	-	-	-	-	-

Table 1. *Continued*

(Continued on next page)

SGD ID	*T. brucei* ID	*L. tarentolae*				*S. cerevisiae*			
		UTP18	SSF1	RLP24	ARX1	UTP18	SSF1	RLP24	ARX1
FAL1[$]	Tb927.11.8770	+	+	-	-	-	-	-	-
UTP2[$]	Tb927.2.2950	-	+	-	-	-	-	-	-
NOP7[#]	Tb927.9.8200	-	+	-	-	-	+	+	+
FPR3[#]	Tb927.10.15590	+	-	-	-	-	-	-	-
FPR4[#]	Tb927.10.15590	+	+	+	+	-	-	-	-
DBP7[#]	Tb927.3.620	-	+	-	-	-	-	-	+
NOP4[#]	Tb927.11.16020	-	+	-	-	-	+	-	-
SPB4[#]	Tb927.9.10960	-	+	-	-	-	-	-	-
NOC3[#]	Tb11.v5.0274	-	+	-	-	-	+	-	-
PRP43[#]	Tb927.5.1150	-	+	-	-	-	-	-	-
MAK5[#]	Tb927.10.9780	-	+	-	-	-	-	-	-
DBP9[#]	Tb927.11.4980	-	+	+	-	-	+	+	-
CBF5[$#]	Tb927.10.170	+	+	+	-	-	+	-	+
DBP1[$]	Tb927.10.14550	+	+	+	+	-	-	-	-
DBP10[#]	Tb927.4.2630	-	+	-	-	-	+	-	-
DBP2[#]	Tb927.8.1510	+	+	+	+	-	-	-	-
DIM1[$#]	Tb927.6.1610	-	+	-	-	-	-	-	-
DRG1[#]	Tb927.6.3790	-	+	-	-	-	-	-	-
Efl1[#]	Tb927.10.4560	+	+	+	+	-	-	-	-
ERP2[#]	Tb927.11.15230	+	-	-	-	-	-	-	+
GAR1[$#]	Tb927.4.470	-	+	-	-	-	-	-	-
KRI1[$]	Tb927.5.840	-	+	-	-	-	-	-	-
LSG1[#]	Tb927.5.4310	-	+	-	-	-	-	+	+
NAF1[$#]	Tb927.2.3160	-	-	-	-	-	+	-	-
NHP2[$#]	Tb927.4.750	-	+	-	-	-	-	-	-
NMD3[#]	Tb927.7.970	-	+	+	+	-	-	+	+
NOB1[$]	Tb927.11.10860	-	+	-	-	-	-	-	-
PUF7[$]	Tb927.11.14960	-	+	-	-	-	-	-	-
PWP1[#]	Tb927.8.6770	-	+	-	-	-	+	-	+
RIO2[$]	Tb927.6.2840	-	+	-	-	-	-	-	-
RRB1[#]	Tb927.11.3070	-	+	-	-	-	-	-	-
SHQ1[$#]	Tb927.11.16370	-	-	-	-	-	-	-	-

Table 1. *Continued*

(Continued on next page)

SGD ID	*T. brucei* ID	*L. tarentolae*				*S. cerevisiae*			
		UTP18	SSF1	RLP24	ARX1	UTP18	SSF1	RLP24	ARX1
TIF6[#]	Tb11.v5.0246	+	+	+	+	-	+	+	+
XRN1[$#]	Tb927.7.4900	-	+	-	-	-	+	-	+
RRP3[$]	Tb11.v5.0277	-	+	-	-	-	-	-	-

Table 1. Continued

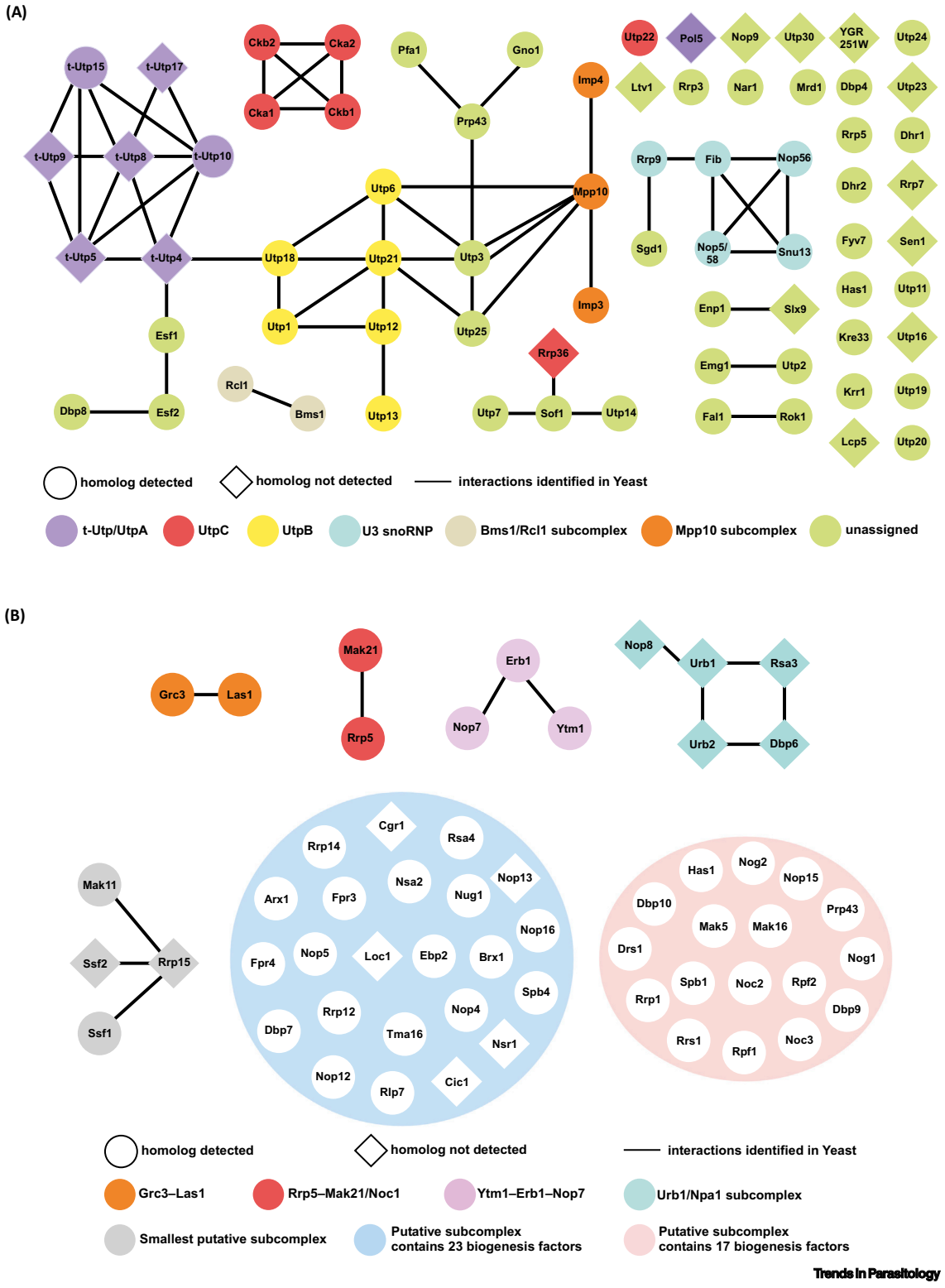
[a]The annotations for yeast factors involved in pre-rRNA processing were derived from [31,39,40,77,78]. A reciprocal BLASTp [79] was performed on each *S. cerevisiae* protein against the *T. brucei* and *L. tarentolae* nonredundant proteins database and compared with peptides detected by liquid chromatography tandem mass spectrometry (LC-MS/MS) of each factor shown. The presence or absence of individual factors is indicated by '+' or '–'. The purification of UTP18, SSF1, RLP24, and ARX1 associated factors was performed in duplicate. The complete list of these purifications will be provided upon request. The identity of the yeast genes and the list of factors associated with yeast proteins (UTP18, SSF1, RLP24, and ARX1) were obtained from SGD[i] and BioGRID[ii], respectively. The nomenclature of *T. brucei* homologs was derived from TriTrypDB[iii]. ECM16(Tb927.6.4600), SGD1(Tb927.6.2650), UTP20(Tb927.10.3790), UTP22(Tb927.10.13180), EFL1(Tb927.3.2170), MDN1(Tb927.1.880), UTP25(Tb927.7.4340), NAR1(Tb927.10.10650), GRC3(Tb927.8.1260), LAS1(Tb927.8.5820), and NOP15 (LtaP09.0110) also exist in trypanosomatids. The symbols '#' and '$' indicate that these factors were also detected in LSU and SSU processome, respectively.

Why Are So Many snoRNAs Involved in Trypanosomatid rRNA Processing?

Trypanosomatid rRNA is characterized by long **expansion segments (ESs)**, sequences present in the rRNA that are not conserved in evolution in structure, length, or sequence (see Figure S1 in the supplemental information online) [36]. rRNA fragments are excised from the trypanosomatid rRNA to shorten the rRNA and to compensate for these expansion segments [36]. But why does trypanosomatid rRNA possess longer expansion segments, including trypanosomatid-specific ones? For example, ES6S, which has been implicated in the recruitment of the eukaryotic initiation factor 3 [35], and ES7S, are the most significantly enlarged segments as compared with other eukaryotic rRNAs. Since translational regulation appears to be a prominent regulatory mechanism of gene expression in trypanosomatids, binding of additional proteins and translation factors to the ribosome is required, and such expansion segments may assist in this complex regulation [37]. Thus, the specific needs for translational regulation, especially when cycling between the hosts, may have led to longer expansion segments to enable binding to a large number of translation regulators [38]. Additional rRNA processing events may have developed to remove parts of the rRNA in order to prevent excessive length. This imposed new requirements for processing, leading additionally to the appearance of expansion segments, which assist in both the processing and stabilization of the fragmented LSU. But what comes first, the chicken or the egg? Did the expansion of the domains emerge to meet the needs of regulating translation, thereby requiring new processing events, with subsequent changes then introduced to stabilize the fragmented rRNA? Is it possible that the needs for both regulation and structural stability were met by the expansions, which then required the additional processing of the LSU rRNA?

rRNA Processing Factors: Factors Present in Other Eukaryotes Are Missing in Trypanosomatids

Many of the trypanosome snoRNAs involved in rRNA processing are unique to these organisms. We therefore asked whether this is also reflected in the protein factors involved in rRNA processing. To this end, we searched the *T. brucei* genome for yeast homologous proteins involved in rRNA processing. In addition, we purified proteins associated with complexes isolated by affinity selection of factors associated with either the SSU (UTP18) [39] or the LSU processome (Ssf1, Rlp24, and Arx1) that are involved in early, middle, and late processing stages, respectively [31,40]. The purification was performed using *Leishmania tarentolae*, and the selected proteins were identified by mass-spectrometry. The trypanosomatid proteins homologous to yeast are listed in Table 1. Among the SSU 90S processome are the 19 factors from the UTP family of proteins. Eight of these UTP proteins were not identified in trypanosomatids (indicated in triangles in Figure 2A) [22,23].



(See figure legend at the bottom of the next page.)

Most of the factors implicated in LSU processing (in yeast) were identified in trypanosomatids, except for the factors indicated by diamonds in Figure 2B, suggesting that, despite major changes in the pattern of rRNA processing, the factors known to participate in LSU processing are present in trypanosomatids. However, we have been unable, as yet, to identify trypanosome-specific protein factors involved in the unique trypanosomatid cleavages. Such factors may be revealed when the protein constituents of trypanosome-specific snoRNPs are identified. We expect trypanosomatid-specific factors to exist because snoRNPs mediating cleavages are found in larger complexes compared with snoRNPs known to only guide modification [18].

C/D snoRNA and Nm Modification in Trypanosomatids

2′-*O*-methylation (Nm) is guided by C/D snoRNAs and mediated by the RNA-associated methyltransferase, NOP1/fibrillarin. C/D snoRNAs harbor C and D boxes, and many have additional box sequences (C′ and D′ boxes) (Figure 3C). The C/D snoRNAs have regions of complementarity to 10–22 nt sequences in pre-rRNA. According to the +5-guiding rule, the methylated nt on the rRNA is the complement to the fifth nucleotide upstream of the D or D′ box (Figure 3C). Each C/D snoRNP binds NOP56, NOP58, SNU13, and NOP1/fibrillarin [41]. It was shown that trypanosome snoRNAs are no exception, and bind all four proteins [25].

It was suggested that ~100 Nm sites exist on trypanosomatid rRNA [42]. The first trypanosomatid C/D snoRNA was discovered in *Leptomonas* [43]. Later, snoRNAs and reiterated gene clusters encoding C/D snoRNA species were identified in *T. brucei* [44]. The snoRNAs are encoded within clusters containing both C/D and H/ACA snoRNAs [45]. The first whole-genome search in *T. brucei* identified 57 C/D genes [45], and 63 C/D genes were identified in *Leishmania major* [46]. Interestingly, ~40% of the Nms are species-specific modifications that do not have counterparts in yeast, humans, or plants, and 40% of these species-specific predicted modifications are located in unique positions outside of the highly conserved, richly modified domains in other eukaryotes (Figure S1) [45,46]. One characteristic feature of trypanosomatid rRNA modifications and their guide RNAs is that, in contrast to the yeast and human genomes, the number of predicted Nms exceeds the number of Ψs. Hypermethylation exists in plants and thermophiles, which helps sustain ribosome function at high temperatures [47]. We suggested that hypermethylation in the parasite may enable it to cope with the temperature shifts during cycling between the two hosts. Indeed, we demonstrated higher expression of C/D snoRNAs in the BSF [25]. Nevertheless, no modifications were detected that are strictly stage-specific [25]. Most recently, we used two genome-wide sequencing methods, RibOxi-seq [48] and 2′-*O*-Me-seq [49], to map the Nms in *T. brucei*, and the mapping data support ~90% of the sites previously predicated [17].

Recent studies in mammals demonstrated that Nm levels are not homogeneous across rRNA [49–52]. RiboMeth-seq data showed that some specific positions in yeast rRNA are only partially methylated. Such differential rRNA modifications may function to fine tune and regulate protein synthesis [49–53]. Nm plasticity controls the intrinsic translational capabilities of human ribosomes [51]. For instance, reduction in the level of Nms affected the translation of mRNA having an internal ribosome entry site (IRES) [51].

The function of Nms in stabilizing the ribosome structure may explain why ribosomes of BSF cells carry hypermodified Nm positions. The role of Nms in ribosome function was studied in yeast by their ablation from within functional domains such as the subunit interface, the PTC, and the decoding center (Figure S1) [54–56]. At least two or three sites need to be changed simultaneously to observe an impact on cell growth and protein synthesis. This observation led to the notion that clusters of Nm sites, rather than individual ones, are essential for ribosome function [54–56]. However, in our recently published study, we demonstrated that, in *T. brucei,* depletion of a single snoRNA guiding modification on the LSU can have a dramatic effect on rRNA processing. This was the first study to link RNA modification with rRNA biogenesis [18].

Figure 2. The Proposed *Trypanosoma brucei* Small Subunit (SSU) and Large Subunit (LSU) Processome Interactome Based on Data from Yeast. (A) SSU processome interactome. The scheme and designation of the subcomplex were derived as described in [77]. The factors whose homologs were identified in *T. brucei* and *Leishmania tarentolae* are indicated in circles, and those that are absent are denoted in diamonds. (B) LSU processome interactome. The interactions are based on [78]. The different subcomplexes are colored.

Key Figure

Small Nucleolar (sno)RNA Guiding rRNA Modifications

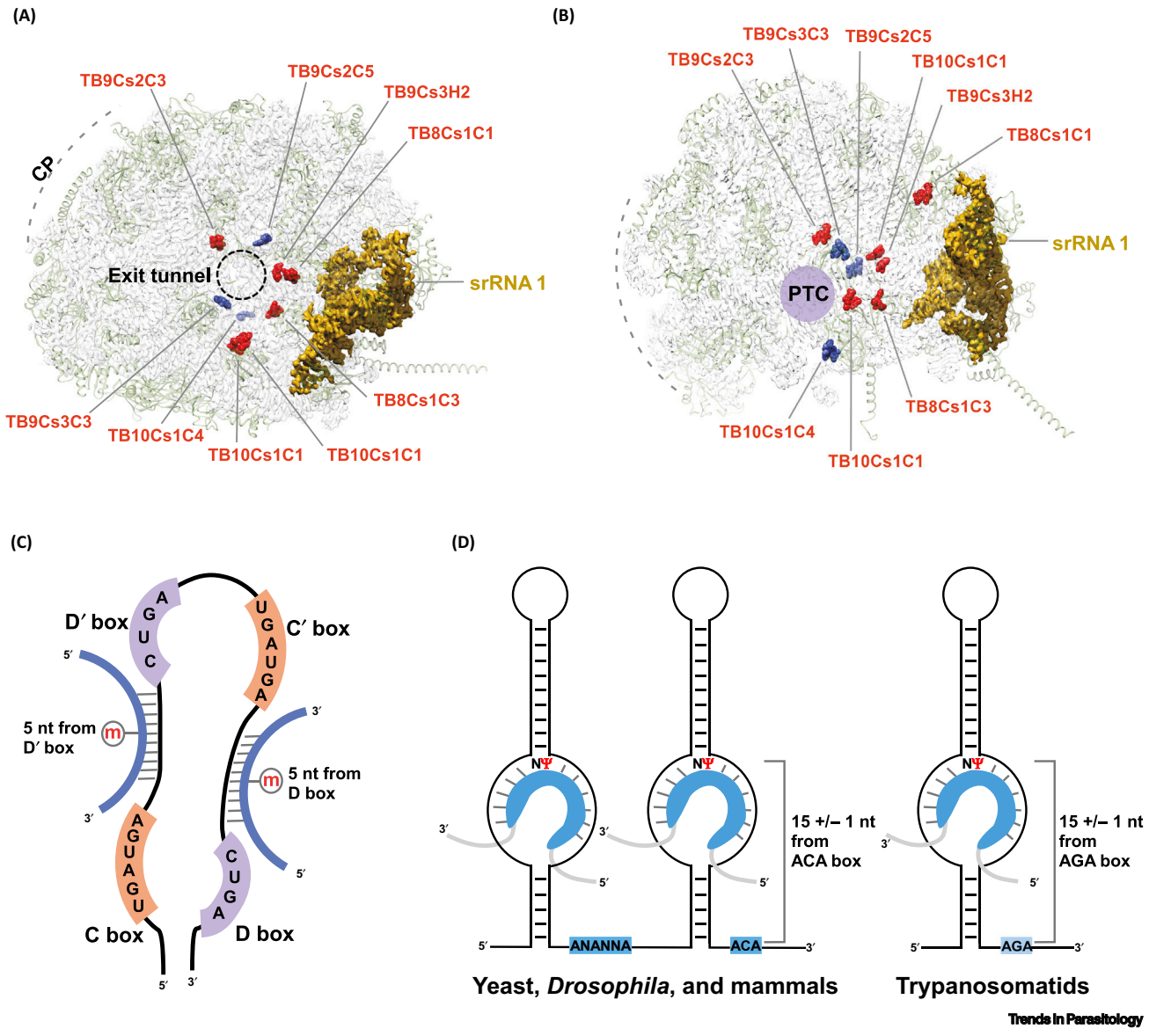


Figure 3. (A) 3D localization of rRNA modifications guided by snoRNAs that affect small ribosomal RNA (srRNA)1 processing, spanning the protein exit tunnel to the site of srRNA1 localization. A view from the surface of a solvent-exposed ribosome demonstrating the proximity of seven rRNA modifications to the protein exit tunnel. The protein exit tunnel and central protuberance (CP) are indicated. RNA modifications are colored in red to indicate those residues whose cognate snoRNA function only to guide modification, and blue for residues whose snoRNA also interacts with intergenic regions in the pre-rRNA. (B) A side view indicating the proximity of the rRNA modifications to srRNA1 in the mature ribosome. rRNA is shown as a solid surface, and r-proteins are indicated as cartoons. srRNA1 is colored dark yellow. CP and the peptidyltransferase center (PTC) are indicated. (C) Schematic representation of the structure of C/D snoRNA. The conserved boxes are shown. The target RNA is colored in blue and base pairs continuously with the snoRNA. The methylated residue (colored in red) is always 5 nt from the D box or D′ box. (D) Schematic representation of the structure of H/ACA snoRNA in eukaryotes (left) and in trypanosomatids (right). The conserved H and ACA boxes are shown. The target RNA is colored in blue and base pairs discontinuously with the snoRNA. The pseudouridine (Ψ) residue is colored in red and is always 14–16 nt from the ACA/AGA box.

Box 2. The Unique Aspects of the Trypanosomatid Ribosomes Revealed by Cryo-EM

The recent cryo-EM studies demonstrated how the six LSU fragments are assembled in order to form the large-subunit ribosome [34,35]. The most critical interactions are those between 5.8S rRNA and the 5′ end of LSU-α, as well as the interaction between the 3′ end of LSU-α and the 5′ end of LSU-β. These two sets of rRNA contacts are mediated by expansion segment ES3L of 5.8S rRNA [35].

The studies showed that sr1 is anchored to the rRNA scaffold via interactions with sr2, and with two anchoring proteins, eL19 and eL34. Sr2 interacts with both LSU-α and LSU-β and the uL3 protein. The *T. cruzi* sr3 (corresponding to *T. brucei* sr6) is anchored via its interaction with eL33 protein. sr4 contains an ES (srRNA4-ES) and makes contact with the rRNA via its interaction with sr2, and uL3. Based on *T. cruzi* structural data, it was proposed that sr2 and sr3 are positioned by their anchoring proteins. sr1 and sr4 are assembled via contacts with sr2 [35]. It was also suggested that the expansion domains stabilize the growing scaffold and the trypanosomatid-specific ESs; notably, the kinetoplastid-specific domain (KSD) stabilizes sr2–4. The processing pathway described in this review supports the notion that sr1 and sr4 processing are linked, but also suggests that sr1 assembly is the final step of processing [18,34,35].

The rich repertoire of C/D snoRNAs was also described in *L. major*, in which 80 C/D snoRNAs were found. Fifteen are predicted to function in trypanosomatid-specific rRNA processing and are related to the *T. brucei* snoRNAs described above. Species-specific snoRNAs were also identified [57]. In addition to their role in stabilizing the RNA, Nms are also known to induce suppression of innate immunity, and rRNA carrying some such modifications prevent Toll-like receptor (TLR) activation [58].

Box 3. Differential Modification on Eukaryotic Ribosomes and Its Implications for Translation

rRNA modifications are introduced at different stages of ribosome biogenesis, stabilizing the secondary and tertiary structure and ensuring efficient and accurate translation [72]. rRNA modifications can also represent an important source of ribosome heterogeneity that may regulate ribosome function in response to environmental and developmental cues, and in diseases. In addition to the most abundant 2′-*O*-methylation (Nm) and pseudouridylation (modification by Ψ), stand-alone enzymes introduce additional base modifications. The enzymes introducing several base methylations such as (m^7G), ($m^6{}_2A$), (m^1A), (m^5C) were identified [73]. Studies in yeast indicate that removal of several Ψs in H69, or in PTC, caused a significant reduction in the translation rate and fidelity affecting stop-codon termination and reading-frame maintenance [54–56]. In addition, rRNA modifications were shown to regulate translation of specific subsets of mRNAs. For instance, ablation of rRNA pseudouridylation alters the affinity of the ribosome for specific mRNAs containing internal ribosome entry sites (IRESs) [74]. The mechanism by which rRNA modifications influence ribosome function lies in their property of expanding the topological potential of specific nucleotides. For example, Nms stabilize helices by increasing base-stacking, and Ψ enables greater hydrogen bond formation and increases the rigidity of the sugar–phosphate backbone. rRNA modifications are also proposed to take part in communication between distant regions of the ribosome [73]. For instance, depletion of snoRNA affects the dynamics of rRNA folding and ribosome assembly [54–56]. However, loss of modifications may also affect the binding of ribosomal proteins [75].

It has been proposed that the constitutively modified positions, which are generally the most conserved, serve to stabilize the core of the ribosome, while the positions that are less conserved contribute to fine-tuning of ribosome function for optimal translation accuracy [51]. It is possible that subpopulations of differently modified ribosomes perform specific functions and regulate translation of specific proteins. Variations in the extent of modification of specific sites in rRNAs were observed under normal growth conditions, but certain modifications were found to change in response to a changing environment [49–53]. Ribosome heterogeneity, due to partial modifications, may selectively adapt the ribosome to translate specific subsets of mRNA. So far, ribosome heterogeneity was attributed to differential binding of ribosomal or RNA-binding proteins. This heterogeneity was shown to control stem cell development and cancer progression as a result of preferential translation of specific mRNAs [76]. In the future, it will be of great interest to examine whether ribosome subpopulations, characterized by particular rRNA modification patterns as described in trypanosomatids, are utilized to drive specific translation of mRNAs during cycling of the parasite. The differential modification may affect the binding of ribosomal and/or RNA-binding proteins that were shown to contribute to 'specialize' the ribosome [76].

Thus, the hypermodified sites in BSF may serve two functions: adjusting the ribosome to optimize function in the mammalian host, and reducing innate immunity, enabling enhanced infection in the host (Box 3).

H/ACA snoRNAs and Pseudouridylation in Trypanosomatids

In most eukaryotes, the H/ACA snoRNAs guiding pseudouridylation (Ψ) consist of two hairpin domains connected by a single-stranded hinge, the H domain, and a tail region, the ACA box (Figure 3D). Two short rRNA recognition motifs of the snoRNA base-pair with rRNA sequences flanking the uridine to be converted to Ψ [41,59]. The Ψ is always located 14–16 nt upstream of the H- box or ACA-box of the snoRNA. In trypanosomatids, all the H/ACA snoRNAs (except snR30) are single-hairpin RNAs, and in addition, these guide RNAs carry an AGA rather than an ACA box (Figure 3D) [17,19,45,46]. Mutations introduced into the AGA box showed that this box is essential for the stability of the guide RNA [60]. Four proteins bind to the H/ACA snoRNA. CBF5 in yeast (dyskerin in humans) is the pseudouridine synthase. Silencing of *T. brucei CBF5* resulted in marked reduction in the level of H/ACA snoRNA [28]. Other proteins that bind to the complex are GAR1, NOP10, and NHP2 [28]. Mutations introduced into the trypanosome H/ACA snoRNA indicated that the size and structure of the apical loop cannot tolerate modifications [60].

Genome-wide Ψ-analysis performed on *T. brucei* rRNA by Ψ-seq in PCF and BSF parasites identified 68 Ψs on rRNA, which are all guided by snoRNAs [61]. The small RNome of both life cycle stages was determined, and 83 H/ACA snoRNAs were identified. We observed elevation of 21 Ψ modifications in the BSF as a result of increased levels of the guiding snoRNAs. Overexpression of snoRNAs guiding modification on H69 provided a slight growth advantage to PCF parasites at 30°C [61]. Interestingly, these modifications are predicted to significantly alter the secondary structure of the LSU rRNA, suggesting that hypermodified positions may contribute to the adaption of ribosome function during cycling between the two hosts. We recently demonstrated that many H/ACA snoRNAs have a dual function, and can potentially guide modification on more than a single target [62]. This mechanism involves conformational change of the pseudouridylation pocket such that it can guide the modification on a nonribosomal substrate using nonconventional rules. Thus, many of the elevated snoRNAs in BSF may guide the modifications on both small nuclear (sn)RNA and rRNA, and impact simultaneously both translation and splicing [62]. Note, dual guidance by H/ACA snoRNA can also take place under different stress conditions, as was demonstrated in yeast [63].

It was always puzzling why so many of the genes encoding H/ACA are found as solitary genes and not in clusters [17]. This genomic organization may be due to the need to individually regulate the levels of the snoRNAs during the developmental cycle. The mechanism of the upregulation of pre-snoRNA in BSF is currently unknown and may involve epigenetic regulation or stabilization of the pre-snoRNA transcripts in BSF. RNA-binding proteins that bind the 3′ noncoding information are likely to regulate the stability of the pre-snoRNA transcript in BSF [64]. snoRNA precursors that are *trans*-spliced and polyadenylated [65] are dually polyadenylated by poly(A) polymerase I (PAP1) and PAP2, which polyadenylate mRNAs [64]. The dual polyadenylation is also required for recruiting the processing machinery that liberates the individual snoRNAs from the precursor.

In *Leishmania,* 81 H/ACA snoRNAs were identified that are predicted to not only guide modification on rRNA and snRNA, but also on tRNAs as per the conventional guiding rules. The comparison between *L. major* and *T. brucei* snoRNAs revealed that, in 48% of the cases, H/ACA snoRNAs are true homologs, since these guide the same modifications in both species, and the snoRNAs share a high level of sequence similarity. However, the remaining 52% of snoRNAs guide the same Ψ but share little sequence similarity. This may hint at the mechanism by which new snoRNAs can be generated, by copying an existing snoRNA and 'changing' the pseudouridylation pocket [57].

The hyperpseudouridylation in BSF rRNA occurs mainly at two regions – (i) H69, and (ii) H39, H89, H90, and H92 – involved in important catalytic activities of the ribosome. H69 forms an important ribosomal intersubunit bridge and is also known to be involved in ribosome recycling [66]. Another essential

position that is hypermodified is U1167, which is located on the A-site finger forming one of the transient intersubunit bridges during ribosome rotation and SSU head swiveling; hence, it may have an impact on the translation efficiency/rate. Thus, the hypermodifications by Ψ and Nm are likely to help the trypanosomatid ribosomes function in the mammalian host. However, the modification may also regulate stage-specific translation (Box 3). Studies are currently underway in our group to investigate the effect on translation of depleting single Ψs and Nms. Depleting single Ψs by perturbation of the snoRNA guiding the modification is a challenge because H/ACA snoRNAs are notoriously difficult to silence [62,67] due to their poor accessibility to small interfering (si)RNA, as their stem–loop structure is covered by proteins. In addition, CRISPR-Cas9 knockout is only possible for solitary snoRNAs, and so far, knockout of such snoRNAs is lethal, suggesting that they have essential functions. In addition, and unlike in most eukaryotes, the snoRNAs guiding Ψ on rRNA also guide modifications on snRNAs [62], and thus, it is impossible to relate the essential nature of the snoRNAs to their effect solely on the ribosome. Nevertheless, dual guiding on both the ribosome and spliceosome is a very unique mechanism whereby these parasites regulate the function of these two essential processes.

Another intriguing possibility is that the hypermodifications may also change the affinity of ribosomes to mRNA, and thus may enable the preferential translation of developmentally regulated mRNA species. Our recent finding demonstrates that Ψ not only affects the strength of RNA–RNA interactions but can also regulate protein–RNA binding [62], raising the intriguing possibility that hypermodification, or any change in modification, may alter the binding of ribosomal or other RNA-binding proteins that may regulate stage-specific translation (Box 3). The relatively high abundance of Ψs may also affect innate immunity, since pseudouridylation was shown to affect the innate immune signaling [68]. For instance, RNA carrying Ψs efficiently bind RIG-1 but failed to trigger the canonical RIG-I changes that induce signaling [68]. However, we cannot rule out the possibility that hypermodified RNAs are also differentially recognized by other RNA sensors to suppress innate immunity.

Concluding Remarks

The complex trypanosomatid ribosome processing pathway is still a mystery. It is not known why multiple fragmentations are needed, and whether these are a consequence of changes that took place to accommodate the many RNA-binding proteins that may regulate translation. Much recent progress was made in identifying the snoRNAs that mediate these fragmentations, and how their action coordinates ribosome assembly. However, the protein composition of these corresponding snoRNPs has not been characterized. The study of trypanosome rRNA modification demonstrates for the first time that Ψs and Nms are developmentally regulated, and that these modifications are hypermodified in the BSF (see Outstanding Questions). Moreover, the trypanosome system is so far the only system in which ablation of a single specific modification can affect growth, especially at elevated temperatures. rRNA modification may have an impact on stage-specific mRNA translation. The ability to manipulate these modifications opens up the opportunity to examine the role of individual modifications on infectivity and on the ability of the parasites to transform from one stage to another, as well as to investigate how such changes affect stage-specific translation. The unique processing of trypanosome rRNA, the rich repertoire of the Nms and Ψs and their essentiality, places rRNA biogenesis as a promising drug target to fight the devastating diseases caused by these parasites.

Outstanding Questions

Why are trypanosomatid H/ACA snoRNAs composed of a single hair-pin loop structure with an AGA box, unlike most eukaryotes? Does the answer to this question lie in the fact that many of these H/ACA snoRNAs are dual guiders?

What are the RNA-binding proteins that bind to the trypanosome-specific snoRNPs involved in rRNA processing, and are these related to processing factors that we failed to identify in the trypanosomatid genome?

How are the snoRNAs developmentally regulated? Is this regulation based on RNA-binding proteins that bind to pre-snoRNAs in order to stabilize the RNA and possibly to recruit the processing machinery that is linked to dual polyadenylation of the precursor?

Is snoRNA gene transcription during cycling between the hosts affected by epigenetic marks?

How does hypermodification on rRNA affect translation? Do these differential rRNA modifications help in preferential binding of RNA-binding proteins to the ribosome? Is r-protein binding developmentally regulated and dependent on rRNA modification?

Does the hypermodification of rRNA and snRNA in BSF relate to the parasite fighting innate immunity?

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SUPPLEMENTAL INFORMATION

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.pt.2019.07.012.

RESOURCES

[i]www.yeastgenome.org/

[ii]https://thebiogrid.org/

[iii]http://tritrypdb.org/tritrypdb/

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