Trends in
Parasitology

Forum

From a bimodal to a multi-stage view on trypanosomes' differential RNA editing

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Significant variations in the abundance of mitochondrial RNA processing proteins and their target RNAs across trypanosome life stages present an opportunity to explore the regulatory mechanisms that drive these changes. Utilizing omics approaches can uncover unconventional targets, aiding our understanding of the parasites' adaptation and enabling targeted interventions for differentiation.

Adaptive stage-specific mitochondrial RNA processing in trypanosomes

Trypanosoma brucei adapts to diverse environmental conditions as it transitions between the mammalian host and tsetse fly vector during its intricate life cycle. These changes are often triggered by environmental cues, such as temperature, pH, and nutrient availability, which are different in the mammal's bloodstream and the tsetse fly's gut [1]. *T. brucei* responds to these cues by altering the expression of specific genes, including genes involved in the expression of mitochondrial proteins. A key feature in *T. brucei* is mitochondrial RNA editing, which involves inserting and deleting uridine residues as guided by mitochondrial genome-encoded gRNAs [2]. This is crucial for properly functioning mitochondrial proteins as it places most mitochondrial mRNAs in the correct reading frame.

The extent and nature of RNA editing can vary significantly between the life cycle stages, reflecting the organism's metabolic needs. The shift between glycolysis in the mammalian host, where oxygen is abundant, and oxidative phosphorylation, where oxygen might be limiting, necessitates different mitochondrial enzymes requiring different RNA processing patterns. The process is regulated by mitochondrial RNA-processing complexes and factors, including a holoenzyme of ~45 proteins for RNA editing and nearly 30 additional proteins involved in mitochondrial RNA processing (Box 1).

Therefore, the parasite integrates signals from its environment and coordinates a network of regulatory mechanisms to ensure that RNA processing syncs with its metabolic needs and life cycle stages. However, these complex interactions among the various molecular components of the RNA processing machinery and the mechanisms of the parasite's differential RNA editing remain unclear.

Key questions focus on the role of RNA-editing machinery in adaptive editing during *T. brucei* differentiation. This includes exploring potential correlations between changes in life stage and variations in the expression pattern and dynamic assembly of the RNA-editing machinery components in response to environmental shifts. Additionally, identifying upstream signals and potential master regulators of the editing process is crucial to unraveling these biological mysteries.

Variations in editing holoenzyme proteins in the *T. brucei* life cycle

While RNA-editing proteins remain the same between mammalian bloodstream forms (BFs) and insect procyclic forms (PFs) of *T. brucei*, mutational experiments reveal differential functions of editing proteins, such as KREPA3 and KREPB5, between BF and PF stages [2]. Despite identical point mutations, distinct outcomes in terms of growth, editing, and editing holoenzyme protein integrity emerge. The stage-specific function of editing proteins aligns with variations in edited mitochondrial transcripts within the electron transport chain (ETC) complex between BF and PF stages, albeit with an unidentified mechanism. These studies utilized monomorphic cell lines, which are less suited for differentiation research since they represent one form of the parasite, limiting the study of stage transitions.

Monomorphic versus pleomorphic

T. brucei's life stage transition involves a nuanced process beyond simple BF-to-PF

Box 1. Mitochondrial RNA-processing complexes and factors

The editing holoenzyme encompasses both catalytic and non-catalytic components, including the RNA-editing catalytic complex (RECC) of 21 proteins, the non-catalytic RNA-editing substrate-binding complex (RESC) of 21 proteins, and the RNA-editing helicase 2 complex (REH2C) of three proteins [2]. The ~800 kDa modular RECC complex involves U insertion/deletion subcomplexes and structural proteins. Three RECC isoforms, featuring RNase III endonucleases, recognize and cleave insertion and deletion sites specified by gRNAs. Both RESC and REH2C subcomplexes interact with RNA-editing substrates and the catalytic RECC complex. Additionally, coordination with nearly 30 other mitochondrial mRNA-processing complexes and factors – such as the 5′ pyrophosphate processome (PPsome), mitochondrial 3′ processome (MPsome), kinetoplast polyadenylation complex (KPAC), and auxiliary factors – is crucial before mRNA translation. RESC plays a pivotal role in orchestrating both pre- and postediting events, interacting with 5′ and 3′ modification complexes and auxiliary factors. Together, these modular complexes, working harmoniously, define the sophisticated machinery of the mitochondrial RNA-processing complexes and factors in *T. brucei*. Coordinated efforts of RECC and RESC ensure the stability of guide RNAs and the precise processing of RNA-editing substrates, while the involvement of REH2C and auxiliary factors adds finesse to the dynamic orchestration of RNA editing in *T. brucei*.

differentiation. Instead, the dividing BF long slender (LS) form first differentiates into a non-dividing BF stage called short stumpy (SS), a pre-adaptation stage crucial for survival in the insect vector [1]. However, parasites cultured in laboratory conditions may lose their pleomorphic LS-to-SS differentiation capacity, converting into monomorphic cells while still retaining an asynchronous BF-to-PF differentiation capacity. Most comparative genomic studies focus on monomorphic cell lines, limiting data to BF and PF stages. Yet, a critical question remains: do the RNA-editing proteins, mitochondrial RNA-processing complexes, and factors undergo modifications during the intermediate differentiation time points from BF to PF?

Modifications in RNA editing and mitochondrial RNA processing proteins during differentiation

A 2016 study by Dejung *et al*. analyzed protein expression profiles of pleomorphic *T. brucei* AnTat1.1 during SS to PF differentiation at various time points [3]. A total of 207 protein profiles underscores the importance of protein regulation at each stage relative to the SS form. Focusing on examining 72 editing holoenzyme and mitochondrial RNA processing proteins and their associated factors during life stage transitions, one can uncover dynamic changes in this intricate process. Roughly 28 proteins demonstrate notable changes in abundance during differentiation (Table 1, and Table S1 in the supplemental information online). However, what significance could the alterations in the abundance of editing holoenzyme and mitochondrial RNA-processing complexes and factors during the differentiation process hold?

Classification of the editing holoenzyme and mitochondrial RNA processing proteins and their associated factors

The proteins listed in the table display distinct expression patterns, categorizing

Table 1. The list of mitochondrial RNA-processing complexes and factors undergoing significant up/downregulation during the differentiation from SS to PF stage[a][a]Gray, not regulated; black, significantly upregulated; white, significantly downregulated. Proteins were regulated if their fold change was greater than 2 and their *P* value was less than 0.05. Adopted from [3].

TriTryp ID	Assigned	Complex name	Time points of differentiation from Bf to PF								
			LS	SS	2 h	4 h	6 h	12 h	24 h	48 h	PF
Tb927.10.7910	RESC19	RESC									
Tb927.7.3950	KRET1	MPsome									
Tb927.2.1860	RESC9	RESC									
Tb927.11.7900	KRBP16	Auxiliary									
Tb927.8.8180	RESC11A	RESC									
Tb927.2.2470	KREPA1	RECC									
Tb927.10.1730	RESC17	RESC									
Tb927.10.9720	KREAP1	Auxiliary									
Tb927.8.620	KREPA3	RECC									
Tb927.10.9000	MPSS2	MPsome									
Tb927.11.8870	KREH1	Auxiliary									
Tb927.4.1500	KREH2	REH2C									
Tb927.9.12770	KPAF3	KPAC									
Tb927.10.10830	RESC13	RESC									
Tb927.1.1330	MEAT1	Auxiliary									
Tb927.6.2140	KH2F2	REH2C									
Tb927.7.1550	KRET2	RECC									
Tb927.7.800	RESC10	RESC									
Tb927.9.4360	KREL1	RECC									
Tb927.6.1200	RESC16	RESC									
Tb11.02.5390	RESC4	RESC									
Tb927.1.3010	RESC18	RESC									
Tb927.9.7260	RESC14	RESC									
Tb927.11.2990	KREPB4	RECC									
Tb927.4.4160	RESC12	RESC									
Tb927.11.9150	MPSS1	MPsome									
Tb927.6.1680	KH2F1	REH2C									
Tb927.11.14380	KPAF2	KPAC									

[a]Gray, not regulated; black, significantly upregulated; white, significantly downregulated. Proteins were regulated if their fold change was greater than 2 and their *P* value was less than 0.05. Adopted from [3].

them into four groups. The initial group – including RESC19, KRET1, RESC9, KRBP16, RESC11A, KREPA1, RESC17, and KREAP1 – shows upregulation at various differentiation time points but not in the PF stage, suggesting their role as regulatory elements in *T. brucei* differentiation. A second group – featuring KREPA3, MPSS2, KREH1, KREH2, KPAF3, and RESC13 – displays upregulation both during differentiation and in the PF stage, possibly serving as dual-purpose regulatory elements. The third group – including, MEAT1, KH2F2, KRET2, RESC10, KREL1, RESC16, RESC4, RESC18, and RESC14 – exhibits significant upregulation solely in the PF stage, indicating potential involvement in PF growth and life support. The final group – with proteins like KREPB4, RESC12, MPSS1, KH2F1, and KPAF2 – is downregulated at any stage compared to the SS parasite, speculatively acting as checkpoints in the *T. brucei* life stage transition and potentially influencing the suppression of other differentiation regulatory proteins within the mitochondrial RNA-processing proteins.

The class-specific functions of these proteins appear more complementary than shared, aligning with their known protein functions. Studying the dynamic interactions of the editosome with RNA and the associated mitochondrial RNA processing proteome is expected to enhance our understanding of various RNA-editing mechanisms. This adaptive response underscores the parasite's ability to navigate diverse host and vector environments, highlighting the dynamic nature of its life cycle.

While analyzing the protein abundance alterations within the *T. brucei* editing machinery and associated proteins across life stages offers a promising entry point for investigating regulatory differences, it is important to note that functional shifts in proteins may not always align with changes in abundance. For example, although KREPA3 and KREH2 activities vary between the BF and PF stages [2,4], correlating fluctuations in protein abundance during BF-to-PF differentiation (Table 1), additional regulatory mechanisms, such as post-translational modifications [5] and differential interactions of RNA-binding proteins [6], likely contribute to regulating the activity of editing proteins in *T. brucei*.

Functional studies of editing machinery and insights from *in vivo* and *in vitro* differentiation

Monitoring the levels of edited and pre-edited mRNA substrates during life-stage transition enables exploration of the dynamics of editing holoenzyme activity regulation. In *in vivo* differentiation, the levels of edited mRNA substrates increase from stumpy BF to PF [1]. Relative edited mRNA levels increase ranges from two- to more than 100-fold, with the cytochrome mRNAs (COII, CYb, and COIII) showing the highest relative increase. Conversely, during *in vitro* differentiation, only a handful of substrates, predominantly cytochromes, show elevated levels of edited transcripts. This suggests that BF-to-PF differentiation can happen despite suboptimal levels of some edited mRNA substrates and the stimuli regulating their editing rate are absent in *in vitro* differentiation conditions.

Cold shock is recognized as a key signal for cellular differentiation. It specifically induces the editing of COII and COIII (of complex IV), while CYb (of complex III) editing is insensitive to it [1]. Interestingly, cold shock induces the expression of COIV mRNA (of complex IV) but not RISP mRNA (of complex III), as nuclear-encoded proteins of these respiratory complexes. This specificity suggests that the regulation of the activity of the editing machinery is both selective and synchronous. Unlike the cold shock, other known signals involved in differentiation, such as low glucose concentration and the inhibition of the RDK1 gene (repressor of differentiation kinase), induce little to no editing of the COII. However, when these signals are combined with cold shock, there is a notable enhancement in the editing of COII mRNA. Active recruitment of COII mRNA by the RESC subcomplex is proposed as a step in COII editing regulation through observing a strong COII mRNA–RESC association during differentiation and PF. This observation aligns with the upregulation of RESC13 during differentiation and PF as shown in Table 1.

The specificity and responsiveness of the RNA-editing regulatory process to complex signals suggest a stepwise regulation mechanism. This mechanism allows for nuanced modulation of editing enzyme activity across different mRNA substrates, providing a more sophisticated regulatory approach than a simple binary on/off model. Such fine-tuning underscores the intricate control mechanisms that govern mRNA editing in response to cellular differentiation signals, highlighting the complex interplay between environmental stimuli and genetic regulation in cellular processes.

Uncovering potential controlling mechanisms in RNA editing during differentiation through omics data analysis

While omics data often undergo general differential expression analysis, examining specifically the mitochondrial proteome and genes/proteins related to mRNA editing is crucial for a comprehensive understanding of the dynamics of mitochondrial RNA-processing complexes and factors. In-depth omics analyses, focusing on these specific areas, may reveal additional insights into the regulation of editing.

Graphing abundances of each timepoint in the form of boxplots, which include median data (abundances), reduces outlier sensitivity, aiding trend identification. Boxplots of data from [3] reveal notable temporal changes in mitochondrial mRNA-

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Figure 1. Comparative expression profiles of mitochondrial mRNA processing and kinase activity proteins over time. Box plots showing expression profile of protein members from: (A) mitochondrial mRNA processing Gene Ontology (GO) groups other than editing proteins, and (B) kinase activity GO groups. Each boxplot represents the abundance of the protein in different time points with four replicates for each; the height of the box corresponds the distribution of log2 abundances, while the line inside the box denotes the median of the dataset. (Data source: [3].)

processing proteins (those other than proteins listed in Table 1 and Table S1), with some displaying switch-like patterns during early and late differentiation (Figure 1A). The selection of these proteins was guided by their Gene Ontology (GO) annotations, with those having relevant GO terms to mitochondrial mRNA processing being included in the analysis. While their direct involvement in *T. brucei* editing is not reported, studies hint at their potential roles. For instance, Tb927.2.6100 impacts kDNA maintenance; its knockdown causes kinetoplast shrinkage and mRNA loss [7]. Limited reports exist on Tb927.10.10120 and Tb927.10.380, despite their intriguing expression patterns. They have been detected in association with essential editing components of the RNA-editing machinery in *T. brucei* [8,9]. Tb927.11.5500 (KRIPP1) and Tb927.11.1250 are reported to be key components of the mitochondrial small subunit (SSU) ribosomal protein complex and are crucial for mRNA processing and translation regulation. Their roles involve modulating the stability of mRNA poly(A/U) tails, influencing gene expression in the parasite [10]. In general, all these proteins warrant future analysis to confirm their regulatory roles.

Potential key kinases in controlling mitochondrial RNA editing during differentiation

Based on GO annotations on data derived from [3], GO terms related to kinase activity were significantly different between BF and PF proteomes. Among these kinases, several exhibit noteworthy expression trends during differentiation, with a few being downregulated and the majority being significantly upregulated (Figure 1B). Amongst these, certain kinases primarily modulate metabolic pathways, and thus, their influence on differentiation regulation may be indirect. These include pyruvate phosphate dikinase (Tb927.11.6280) and phosphoenolpyruvate carboxykinase (Tb927.2.4210), which are enzymes involved in gluconeogenesis. However, existing literature provides some indications of a potential regulatory role in differentiation for other kinases, which warrant further investigation for validation. The role of homoserine kinase (HSK) (Tb927.6.4430) in the PF has been demonstrated, where it potentially serves as a source of homoserine for threonine production [11]. Additionally, given that transcription factors are widely recognized as targets for small ubiquitin-like modifier (SUMO) modification, coupled with research findings demonstrating consistent SUMOylation of HSK in *T. brucei*, hints at its potential additional regulatory role or mechanism mediated by SUMOylation. Mevalonate kinase (Tb927.4.4070) is also considered a dual-purpose protein. In addition to its established role in isoprenoid synthesis, it is thought to potentially regulate the host cell's signaling, a crucial factor in the parasite's invasion process [12]. Pyruvate kinase (PYK) (Tb927.10.14140) has been reported as having RNA-binding activity and undergoing post-translational modifications (PTMs) in *T. brucei*. This variation in PYK's PTMs, as an RNA-binding protein, could potentially influence the expression of other proteins involved in diverse metabolic pathways and differentiation processes [13]. Generally, never-in-mitosis A (NIMA)-related kinases or NEK family serine/threonine-protein kinases are known to be involved in various cellular processes, including cell cycle regulation. While there is no information on the specific role of Tb927.2.2120 (NEK22) in *T. brucei*'s differentiation, its boxplot showing a downregulation similar to that of Tb927.4.5310 (NEK12.2) – a kinase identified as potential regulator of differentiation and reported to repress the transition from BF to PF [14] – suggests a possible regulatory role. Furthermore, CK2A2 (Tb927.2.2430) has been proven essential in the stress response. Considering that differentiation is often induced by environmental stress, CK2 could be a potential regulator of this process [15]. Given the potential regulatory roles for these kinases, further analysis could offer deeper insights into trypanosomes' functions and differentiation regulation.

Concluding remarks

Due to its adaptable life cycle, *T. brucei* requires sophisticated regulation of the editing machinery and RNA editing. Omics data show variations in the abundance of mitochondrial RNA-processing complexes and factors across different parasite life stages, which may serve to stabilize and recruit the substrate mRNAs. It is crucial to understand the dynamics of remodeling of mitochondrial RNA-processing complexes and factors, particularly during life stage transitions, by using cell lines that model these transitions, to identify key differentiation steps. This involves identifying signals that control differential RNA editing, the molecular basis of temperature-sensitive regulation, and exploring the potential role of post-transcriptional modifications. The intricate orchestration of *T. brucei* RNA editing, finely attuned to environmental shifts, underscores the organism's adaptive capabilities. Research into mitochondrial RNA binding and processing proteins, as well as phosphorylation cascade proteins – particularly those with expression profiles similar to editing proteins – could identify potential regulatory mechanisms. Future studies, emphasizing advanced genetic tools and high-throughput approaches, hold the potential to unravel the regulatory networks that govern the specificity and efficiency of RNA editing. This understanding not only advances trypanosome biology but also hints at targeted interventions.

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Declaration of interests

The authors declare no competing interests.

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