Trends in Parasitology

Review

Sending the message: specialized RNA export mechanisms in trypanosomes

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Export of RNA from the nucleus is essential for all eukaryotic cells and has emerged as a major step in the control of gene expression. mRNA molecules are required to complete a complex series of processing events and pass a quality control system to protect the cytoplasm from the translation of aberrant proteins. Many of these events are highly conserved across eukaryotes, reflecting their ancient origin, but significant deviation from a canonical pathway as described from animals and fungi has emerged in the trypanosomatids. With significant implications for the mechanisms that control gene expression and hence differentiation, responses to altered environments and fitness as a parasite, these deviations may also reveal additional, previously unsuspected, mRNA export pathways.

Highlights

mRNA export is a highly complex process at the core of gene expression controlling mRNA abundance. Steps include maturation, quality control ensuring that mRNAs are correctly coding, and the export process itself.

Trypanosomes have distinct mechanisms for producing mRNA which sets them apart from animals and fungi. These include *trans*-splicing, absence of introns within open reading frames and polycistronic transcription. These require distinct processes to produce mature mRNAs, which are near identical in structure to higher eukaryotes.

We describe how distinct mechanisms for mRNA processing have resulted in changes to mRNA export mechanisms, encompassing nuclear pore complex composition and organization, mRNA maturation, and quality control mechanisms.

Delivering a coded message from the nucleus: opisthokonts versus kinetoplastids

All cellular life relies on RNA, and consequently RNA-related processes are highly conserved, including the basic features of transcription and translation. With the major innovation of eukaryogenesis being evolution of the nuclear envelope (NE), which separates transcription from translation, both new challenges and opportunities for RNA metabolism emerged. These include the potential for extensive post-transcriptional processing events, which, for mRNA, includes splicing, polyadenylation, and nucleoside modifications throughout, together with a requirement for an export pathway. mRNA export and processing are coupled in eukaryotes as a multistep process that essentially safeguards the translational apparatus from aberrant mRNAs encoding potentially toxic products.

Trypanosomes are obligatory parasites of invertebrates, vertebrates, and/or vascular plants and cause major public health and economic impact. Their lineage arose from very early separation from the main eukaryotic line and likely shortly following radiation from the Last Eukaryotic Common Ancestor (LECA) [1–3]. Trypanosomes (members of the Kinetoplastida class within the phylum Euglenozoa) deviate from canonical mechanisms for many aspects of their biology and gene expression especially, with perhaps the headline features being polycistronic transcription together with ***trans*-splicing** (see Glossary) and the near absence of *cis*-splicing [4–6]. Early studies intimated that control of individual genes through promoter activity is lacking in trypanosomes due to polycistronic transcription, arguing for control mechanisms focused on mRNA turnover and elements within the 3′ untranslated region. This is, however, likely an oversimplification as there are multiple steps between transcription and translation, most of which are shared between essentially all eukaryotes (Figure 1).

mRNA processing and export, as mapped in animals and fungi (both members of the Opisthokonta eukaryotic supergroup), is supported by multiple complexes, amongst which are the EJC (exon-junction complex), **CPSF (cleavage and polyadenylation specificity factor)**,

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TREX (transcription and export), and **TREX-2**, as well as the **NPC (nuclear pore complex)** (Figure 1). Components of each of these complexes are present across the eukaryotic lineage, but several complexes are overall poorly conserved in other lineages such as trypanosomes. Hence, despite the core aspects of RNA metabolism, there has been at least one billion years since the eukaryotic lineage arose and expanded, offering considerable scope for diversity to have evolved between these processes in different lineages. Here we discuss divergence within the NPC and mRNA processing factors that lead to highly distinct mechanisms for sending mRNA to the cytoplasm in trypanosomes and speculate as to their origins and purpose (Figure 2 and Table 1).

The pore is the core

After transcription, processing, and maturation, most RNAs are transported from the nucleus through NPCs, macromolecular protein assemblies embedded within the NE which facilitate selective transport between the nucleoplasm and cytoplasm [7]. This transport process is highly complex with evidence for lineage-specific mechanisms, of which kinetoplastids and opisthokonts provide exemplars (Figure 2 and Table 1); amongst these are structural and compositional changes within the NPC, NPC-associated RNA-processing complexes, and transport factors [8].

NPCs are octagonal structures composed of ~30 different proteins termed nucleoporins (Nups), present in multiple copies to comprise approximately 500 total proteins per NPC (Figure 2) [9–11]. Substructures within the NPC, such as a proteinaceous membrane ring anchoring the NPC to the NE and a core structural scaffold attest to a modular evolution and functionality (Figure 2). The scaffold anchors a class of Nups that contain disordered regions of phenylalanine glycine (FG) and related dipeptide repeats. These proteins are primarily responsible for the selective permeability barrier of the NPC. Transport factors, variously called karyopherins (yeast), or importins and exportins (animals) facilitate transport by virtue of specific interactions with FG-repeats, while these same regions exclude non-karyopherin-bound proteins [7,12,13]. The scaffold also anchors a subset of asymmetrically positioned peripheral Nups (Figure 2) [14], which include nucleoplasm oriented FG-Nups, as well as the **nuclear basket**, which interacts with nuclear mRNA processing complexes which constitute an RNA export platform (Table 1) [15–18]. The NPC thus acts as an interacting platform, especially for peripheral substructures, to provide a hub for multiple steps in gene expression.

Trypanosomes as models for studying evolution of RNA processing and export

The trypanosome NPC appears to be of similar overall architecture and complexity to that of the opisthokonts, including having an evolutionarily conserved cohort of transport factors [19,20]. Thus, trypanosomes offer an excellent system through which to compare various mRNA processing steps, and many of the divergent features place greater reliance on post-transcriptional mechanisms than in opisthokonts. More recently, roles for mRNA-binding proteins (RBPs) are being uncovered, a significant number of which are lineage-specific [21,22]. Some RBPs mediate expression of entire cohorts of mRNAs, acting as master regulators [23–25]. It is formally possible that trypanosome transcription and mRNA-processing mechanisms are simply reduced compared with opisthokonts and reflect an absence of control over the environment, negating any need for complex responses to improve fitness [26]. However, several other trypanosome cellular systems, including the lamin [27], the kinetochore [28], and the endocytic pathway [29], exhibit incomplete retention of metazoan machinery but have emerged as having alternate components, rather than simple reduction. We suggest that this is also the case for gene expression.

From transcription to quality control and export

In opisthokonts, protein-coding RNAs are transcribed exclusively by RNA Pol II, with mRNA export being initiated cotranscriptionally (Figure 1). Several protein factors associate with the

Glossary

CPSF (cleavage and polyadenylation specificity factor) complex: recognizes a hexanucleotide AAUAAA motif in pre-mRNA polyadenylation signals for cleavage. Conserved across eukaryotes, although incompletely characterized in most lineages. Target of benzoxaborole drugs (such as acosiborole) in many parasites.

Mex67: an mRNA export factor associated with a bewildering set of synonyms. It is known as NXF1 or TAP in mammalian systems, and forms a heterodimer with Mtr2, which is known as NXT1 or p15 in mammals. Both contain an NTF2 domain important for their heterodimerization and association with NPCs.

Nuclear basket: a subcomplex of the NPC that faces the nucleoplasm. It has considerable influence on chromatin and gene expression as well as organization of late mRNA processing and export. It has multiple configurations across eukaryotes.

Nuclear pore complex (NPC): a large proteinaceous assembly that mediates bidirectional transport of proteins and RNA between the cytoplasm and nucleoplasm. Multiple nucleocytoplasmic transport pathways utilize this exclusive channel. Conserved, with notable variations, across eukaryotes.

Ran: a small GTPase of the Ras superfamily and a master regulator, and energy source, driving many NPC-mediated transport pathways. It is conserved across eukaryotes.

***trans*-splicing:** a process in which mRNAs are resolved from polycistronic mRNAs, and a short exon, transcribed elsewhere in the genome, is spliced to the 5′-end. The mechanism is similar to that of conventional *cis*-splicing.

TREX (transcription export) complex: a multiprotein complex that plays a major role in the coupling of many steps during mRNA biogenesis, including mRNA transcription, processing, and nuclear export. It is partly conserved across eukaryotes.

TREX-2 (three prime repair exonuclease 2) complex: a platform that binds many components of the nuclear mRNA processing machinery. Only partial conservation across eukaryotes is observed.

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

nascent message to form ribonucleoprotein complexes (RNPs). RNA export is integrated with mRNA biogenesis and processing; amongst these factors are several TREX complexes. TREX is comprised of two different cohorts; THO components Tho2, Hpr1, Mft1, and Thp2 [30], together with two TREX-specific components Sub2 and Yra1, which act as adaptors for mRNA export proteins [31]. The THO/TREX complex mediates transcription elongation in yeast, splicing of mRNAs in vertebrates, and cotranscriptional recruitment of the mRNA export machinery (Figure 1) [30,32]. Beyond opisthokonts and plants, the evolutionary conservation of THO/TREX complex proteins, their functions and pathways become more difficult to decipher.

The DEAD box helicase Sub2 (UAP56 in vertebrates) is the only evolutionarily conserved TREX-complex protein and has been characterized in diverse protists, including *Plasmodium, Toxoplasma*, and trypanosomes [33–35], suggesting a very high level of conservation [33–36]. Silencing of Sub2 results in the accumulation of polyA mRNA in the nucleus of trypanosomes and decreased translation [34,35]. *Toxoplasma* Sub2 is also heavily involved in export as disruption using CRISPR blocks mRNA export [33]. Additional TREX complex proteins are either so diverged as to be undetectable or absent in trypanosome and Apicomplexa genomes [34,35]. Thus, our appreciation of the players and processes underlying mRNA processing, from the point of transcription through to export to the cytoplasm, remains incomplete in protists, albeit with the clear indication that Sub2 at least has conserved functions, and potentially with backfilling replacing the absent TREX subunits [34,35]. What remains unclear is if this represents a secondary loss or later evolution of Sub2-interacting proteins, as recently demonstrated in a proteomic study in the American trypanosome *Trypanosoma cruzi,* where several kinetoplastid-specific proteins were found to be involved in RNA processing and splicing in addition to more evolutionarily conserved factors [37]. Although these newly identified kinetoplastid-specific factors may perform analogous functions to THO/TREX components, they are yet to be functionally interrogated.

As a prelude to export in opisthokonts, Sub2 is displaced by the mRNA export factors **Mex67** and Mtr2. These remodeled complexes are now export competent, but pause at the nuclear basket and engage a quality control checkpoint. This is facilitated by the TREX2 complex, which is tethered to the nuclear periphery via the nuclear basket Nups Mlp1/Mlp2 (Figures 1 and 2 and Table 1) [38,39]. In this context it is relevant that Tpr, the vertebrate nuclear basket Nup, is not simply a passive interaction platform for TREX2 but rather an integral part of the complex itself whose disruption leads to abnormal transcription and export [40]. The TREX2 component Sac3 provides a scaffold for Thp1, Sem1, Cdc31, and Sus1 [41]. Animal Sac3 differs from the yeast ortholog in that animal Sac3 shuttles between sites of active transcription and the NPC while binding directly to Mex67, thus facilitating intranuclear translocation of mRNPs from transcription sites to the NPC in preparation for export [38,42].

Figure 1. Quick start guide to control of eukaryotic gene expression. (A) Steps in the standard model of transcription and translation. Above the arrow are the processes taking place that may be regulated by one or more mechanism. Below the arrow are the molecules that encode biological information. Critically, all of these steps, with the exception of control of transcription (in red), are common between trypanosomes and other eukaryotes. (B) Highly simplified view of mRNA maturation and nuclear export pathways. Multiple complexes are shown in shades of blue that are responsible for the splicing, folding, and processing of mRNAs, and which are associated with the nuclear pore complex (NPC). Precise distinctions between these complexes is difficult, as the composition of complexes varies dynamically, with many proteins being shared and/or acting to link complexes. Association with the NPC, by the transcription export (TREX) complexes, is a critical aspect of the export process. (C) Simplified scheme for trypanosome polycistronic transcription. Top is an example chromosomal region containing several protein coding sequences, with arrows showing direction of RNA Pol II transcription, and illustrating convergent and divergent strand switch regions (SSRs). The leftmost cistron is transcribed as a single contiguous primary RNA transcript as illustrated in Figure 1C, and then resolved into individual mRNAs following *trans*-splicing of a 5′ spliced leader (SL) and polyadenylation. Turnover and other processes regulate the copy number of the mRNA, and additional mechanisms, including translational efficiency also contribute to differential protein levels. All of these processes are discussed in detail in the text. Abbreviations: EJC, exon junction complex; QC, quality control; PABP, polyA-binding protein; SAGA, Spt-Ada-Gcn5 acetyltransferase.

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Figure 2. An illustration of the differences between protein and mRNA export in opisthokonts versus trypanosomes. (A) A comparison of the nuclear pore complex (NPC) structure in opisthokonts versus trypanosomes. The arrangement of the major subcomplexes of the NPC are highlighted. The membrane, inner, and outer rings are structural components that act as a scaffold for the nuclear basket and phenylalanine glycine (FG) repeat containing nucleoporins (Nups) that maintain the permeability barrier of the NPC. The NPC in opisthokonts differs from that in trypanosomes in having a well-recognized mRNA export platform on the cytoplasmic side of the NPC which is evolutionarily divergent in trypanosomes. In addition, the nuclear basket is smaller in trypanosomes. and the mechanism of anchoring the NPC to the nuclear envelope is also divergent. (B) A comparison of transcription and export between opisthokonts and trypanosomes. Opisthokonts have individual RNA Pol II promoters for each gene while trypanosomes have single RNA Pol II-like promoter elements at the beginning of each polycistronic transcription unit comprised of several nonfunctionally related tandem genes. Individual mRNAs from each *polycistronic* transcription unit are resolved by *trans*-splicing. mRNA export in opisthokonts relies on an ATP-dependent DEAD box helicase [ribonucleoprotein (RNP) modelers] to drive directionality of mRNA transport from the nucleus into the cytoplasm. In trypanosomes, it has been postulated that mRNA export is dependent on the GTPase Ran, a radical departure from opisthokonts, concomitant with a lack of an obvious dedicated cytoplasmic mRNA export platform.

TREX2 functions as a staging post for both mRNA processing and for export proteins to interact and facilitate association and repositioning of actively transcribed genes to NPCs in conjunction with the transcription coactivator SAGA (Spt-Ada-Gcn5 acetyltransferase) [43] (Figure 1B). The SAGA complex is comprised of ~20 subunits and, due to the presence of Gcn5, was initially considered as a histone acetyltransferase. However, SAGA also contains a histone deubiquitinase and subunits interacting with transcriptional activators and the general transcription machinery, indicating coordination of a broad range of functions [44]. Just as most THO/TREX components are either cryptic or absent from trypanosomes, there is scant evidence for SAGA components,

Table 1. The NPC components involved in mRNA export[a,b]

Major secondary structures	Trypanosomes	Yeast	Vertebrates	NPC subcomplex
β-propeller, coiled coil	TbNup76	Nup82	Nup88	Cytoplasmic (opisthokonts), possibly cytoplasmic and nucleoplasmic (trypanosomes)
FG-repeats, putative ZnFs	TbNup149	–	–	Part of the TbNup76 complex
FG-repeats	TbNup140	–	–	
α-solenoid (tryps only), FG-repeats	TbNup158[d]	Nup116[c]/Nup100[c]	Nup98	Cytoplasmic and nucleoplasmic, and also Nup82 complex (Yeast)
Coiled coil, FG-repeats	TbNup62	Nsp1	Nup62	Inner ring and cytoplasmic Nup82/88 complex (inner ring only in trypanosomes)
β-propeller, FG-repeats, coiled coil	–	Nup159	Nup214	Cytoplasmic – Nup82/88 complex
FG-repeats	–	Nup42	Nlp1	
Auxiliary factors	–	Dbp5	DDX19	Docks on the Nup82/88 complex (unknown in trypanosomes)
	–	Gle1	Gle1	
	TbGle2	Gle2	Rae1	
	IP6[b]	IP6	IP6	
FG-repeats, ZnFs (Nup153 only)	–	Nup60[c]/Nup1[c]	Nup153	Nucleoplasmic
FG-repeats	–	Nup2	Nup50	
Coiled coil	TbNup110 (110 kDa)	–	–	Nuclear basket
	TbNup92 (92 kDa)	–	–	
	–	Mlp1 (218 kDa)	TPR (267 kDa)	
	–	Mlp2 (195 kDa)	–	

[a]Abbreviation: bIP6, inositol hexakisphosphate.
[b]A comparison of the components of the mRNA export platform between trypanosomes and opisthokonts. Trypanosomes have a Nup82 ortholog but lack several components of the NPC mRNA export platform as represented in most taxa. Instead, they have species-specific proteins that may reflect an unusual mode of gene regulation. Additionally, trypanosomes have different nuclear basket proteins that are half the molecular weight of those of opisthokonts.
[c]Represents yeast-specific gene duplications for which there is a single ortholog in other lineages.
[d]TbNup158 orthologs are represented as two separate polypeptides in most eukaryotes, with an N-terminal FG-repeat and a C-terminal α-solenoid region that are auto-proteolyzed into two distinct proteins. TbNup158 is a single protein containing both domains.

and the recent *T. cruzi* immuno-isolation proteomic study amongst others supports the apparent absence of several canonical orthologs of SAGA components [37].

Thus, it is possible that trypanosomes have a wholly divergent system for processing mRNA, supported by a considerable cohort of kinetoplastid-specific proteins interacting with an evolutionarily preserved Sub2 and other conserved components of the mRNA processing system. Indeed, evidence suggests a tightly coupled system stretching from transcription to translation, supported by evidence that trypanosomes can initiate mRNA export cotranscriptionally [45]. Surprisingly, there is no quality control checkpoint at the trypanosome NPC prior to export of mRNA through the NPC in trypanosomes [40]. Blocking *trans*-splicing, and thus faithful resolution of individual mRNAs from polycistronic mRNAs, does not initiate a 'pause' at the NPC, instead allowing export of nonspliced, nonresolved mRNAs [45].

Notably, trypanosomes have significant species-specific differences in NPC architecture which are focused mainly around the mRNA export machinery, especially the nuclear basket and cytoplasmic mRNA export platforms [19,20,46]. The nuclear basket proteins Tpr and Mlp1/Mlp2 in opisthokonts respectively range from 200 to 270 kDa [47–49], but in trypanosomes these are represented by two proteins of 92 kDa and 110 kDa, suggesting significant evolutionary divergence of this important NPC subcomplex (Table 1) [19,20,46]. Unlike other nuclear basket

Nups, TbNup92 uniquely has a C-terminal BRCT domain; however, it intimately associates with the mitotic spindle and spindle organizer at mitosis, and is a functional analog of yeast Mlp2 [50], which similarly relocates to spindle organizers in a cell-cycle-dependent manner [46,50]. TbNup110 is essential for cellular growth in bloodstream form of *T. brucei* [51] and, analogous to Mlp1, extends circa 40 nm into the nucleoplasm from the NPC [20]. However, the function of TbNup110 in quality control of RNA export is unexplored.

mRNA transport factors in canonical organisms

After pausing at the nuclear basket, mRNA export through the NPC in opisthokonts occurs in a matter of milliseconds. This transport is mediated by non-karyopherin transport factors Mex67 and Mtr2 [52,53]. Mex67 is a multidomain protein with a cargo-binding domain consisting of an RNA recognition motif, a leucine-rich repeat (LRR) which mediates interactions with RNA and auxiliary RNA processing proteins, an NPC-binding domain consisting of an NTF2 domain required to form a heterodimer with Mtr2, and a C-terminal ubiquitin-associated (UBA) domain mediating interactions with FG-Nups [54,55]. The final steps of mRNA export and remodeling in opisthokonts are performed by Nups located on the cytoplasmic face of the NPC [15,17,56] (Figure 2 and Table 1).

The main component of the cytoplasmic mRNA export platform in yeast is the Nup82 complex, a tetrameric assembly comprised of Nup82, Nup159, Nsp1, and Dyn2 (dynein light chain) [15]. Nup82 and Dyn2 are purely structural proteins, while Nsp1 and Nup159 also carry FG repeats. The DEAD box RNA helicase Dbp5 and an RNA export mediator Gle1 associate with Nup159 and together they remodel mRNPs exiting the NPC in an ATP-dependent manner [16,18,56–61]. This allows the Mex67–Mtr2 complex to disengage from export cargo and recycle into the nucleus, providing both directionality and energy to drive mRNA export [52,53,62,63].

Trypanosomes lack the canonical NPC mRNA export platform

Trypanosomes have orthologs of most of the major transport factors present in opisthokonts [19,64], suggesting at some level a high degree of evolutionary conservation. As such it is presumed (albeit unproven) that most of these homologs function as in higher eukaryotes. Indeed, the main mRNA export factor, Mex67, and its partner, Mtr2, are conserved [65–67]. Given this, it is significant to find major differences in mRNA transport mechanisms associated with the trypanosome NPC [20]. Remarkably, orthologs of yeast Nup159, Gle1, and Dbp5 are absent from the trypanosome NPC, the key components of the animal and fungal cytoplasmic RNA export platform [15–18,56,57,59,68].

In yeast the Nup82 complex is anchored over the central NPC channel by the outer ring complex [15,69], an asymmetric position crucial for driving unidirectional ATP-dependent mRNA export [70,71]. Nup159 can be distinguished from all other FG-Nups due to the presence of a unique N-terminal β-propeller, that acts as an interaction platform for Dbp5 (Table 1) [17]. TbNup76 appears to be the trypanosome ortholog of Nup82 and forms a complex with two large FG-Nups, TbNup140, and TbNup149 (Figure 3). The genes encoding the two proteins are adjacent in kinetoplastid genomes and separated by an unusually small (122 bp) intergenic region [19]. Neither TbNup140 nor TbNup149 has a β-propeller, consistent with the absence of Dbp5 from trypanosomes [18]. TbNup140 contains ~100 FG dipeptides spanning ~120 kDa, with an N-terminal 20 kDa coiled-coil motif acting as the NPC anchor. TbNup149 has considerably fewer FG-Nups but is built from three repetitive segments in *Trypanosoma brucei* (Figure 3). The ortholog is larger in *T. cruzi* and *Leishmania major* (170 kDa and 382 kDa respectively). The standout features of TbNup149 are three zinc-finger (ZF)-like motifs that are well conserved between the kinetoplastids [20] (Figure 3). Hence, the entire architecture of this NPC subcomplex is remodeled to a remarkable degree and precludes the presence of a canonical mRNA export mechanism.

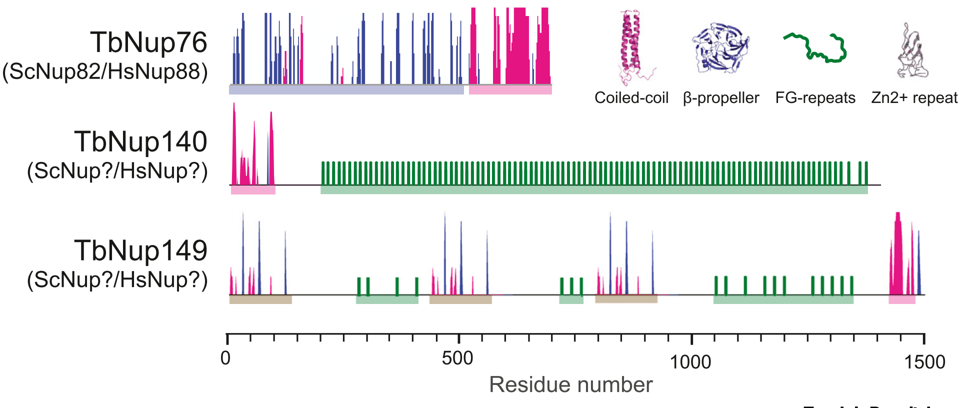

Figure 3. Components of the TbNup76 complex, the trypanosome mRNA export platform. The TbNup76 complex comprises the β-propeller, coiled-coil protein, Nup76, the structural ortholog of Nup82 in yeast (*Saccharomyces cerevisiae* Nup82 or ScNup82) and verterbrate Nup82 (*Homo sapiens* Nup88 or HsNup88). TbNup76 forms a complex with two large phenylalanine glycine (FG)-nucleoporins (Nups) with no obvious orthologs in opisthokonts. TbNup149 appears to have three putative zinc fingers (Zn^{2+}).

Significantly, this configuration is restricted to trypanosomes as *Euglena gracilis*, which has a Dbp5 ortholog, appears to have a more conventional mRNA export mechanism, similar to opisthokonts [72]. The absence of the Nup159–Dbp5 system removes the ATP-mediated steps from mRNA export, which asks the question of how mRNA export is powered and mRNPs remodeled in the absence of ATP as an energy source.

Typically, nucleocytoplasmic transport is mediated by two classes of soluble protein, both of which are characterized by their specific affinity for the NPC, allowing them to rapidly transit themselves and their cognate cargoes between the nucleoplasm and cytoplasm. The first class contains karyopherins, responsible for the import and export of proteins and the export of noncoding RNAs [rRNA, miRNA, tRNA, and small nucleolar (sno)RNA]. The second class contains non-karyopherin nuclear transport factor 2 (NTF2) type transport factors, which import **Ran** (NTF2 itself) and export mRNA (Mex67 with its heterodimeric partner, Mtr2) [73]. A RanGTP/GDP gradient represents the vectoral driver of nucleocytoplasmic transport [73]. RanGTP is involved in the export of proteins from the nucleus through cooperative interactions with exportins (Figure 2). Once in the cytoplasm, Ran-bound GTP is rapidly hydrolyzed to RanGDP through interaction with Ran GTPase-activating protein (RanGAP) and the cofactor Ran-binding protein 1 (RanBP1). A conformational change allows Ran to be released from the exportin complex and to bind NTF2, whose major purpose is to actively import RanGDP back into the nucleus to be reactivated into RanGTP, thus maintaining the gradient. By contrast, directionality of bulk mRNA export in opisthokonts is independent of Ran, relying instead on the ATP-dependent Dbp5 path (Figure 2).

Remarkably, immunoisolation of trypanosome Mex67 recovers stoichiometric quantities of Ran, RanBP1, and a putative Ran GTPase-activating protein, even though it is well established that neither yeast nor vertebrate Mex67 or Mtr2 can bind Ran [20,74,75]. Thus, the interaction of trypanosome Mex67–Mtr2 with Ran is highly atypical. Moreover, once on the cytoplasmic side of the NPC, it is likely that as-yet unknown factors interact with the emerging mRNA [45]. This, coupled with the absence of discernible orthologs of TREX2 complex components suggests that the trypanosome nuclear basket cannot function analogously to opisthokonts. It is, however, interesting that unspliced mRNA is tethered to RNA granules peripheral to the cytoplasmic side of the NPC, hinting at a mechanism for quality control of trypanosome mRNA processing [37].

Increased complexity of mRNA export factors in metazoa and trypanosomes

The frequency of alternative splicing increased with diversification of cell types in multicellular organisms [76]. Multicellular organisms (metazoa) also have additional Mex67 variants (so named nuclear exchange factor or NXF in metazoa), some of which are themselves generated as splice variants and exhibit tissue specificity [55,77–80]. NXF1 is highly expressed in all mammalian tissues, whereas the other paralogs in humans, mice, fruit flies, and nematodes tend to be expressed at lower levels, are tissue-specific and/or developmentally regulated [55]. Humans and mice have at least four NXF gene products: NXF1,2,3, and 5 in humans and NXF1,2,3, and 7 in mice. NXF1 and NXF2 predominantly localize to the nucleoplasm and display mRNA export activities, while NXF3, NXF5, and NXF7 are mainly cytoplasmic, highlighting potential functional differences [55,81]. NXF2 is expressed in testes and neurons [82,83], while NXF3 is expressed mainly in testes [77]. NXF3 lacks the C-terminal UBA domain required for direct interactions with the NPC [54,84], instead having a novel nuclear export signal that allows it to bind the karyopherin nuclear export factor XPO1 and so compensates *in cis* for loss of the canonical NPC targeting domain [77,85]. Last, NXF5 and NXF7 localize to neurons and associate with translating ribosomes, stress granules, and P-bodies [80,82,86–88]. Fruit flies also have four NXFs: NXF1,2,3 and 4, of which only NXF1 is essential and responsible for mRNA export [89]. This suggests that NXF1 is the global mRNA exporter in metazoa while NXF2, NXF3, NXF5, and NXF7 have tissue-specific functions, some of which remain cryptic. Importantly, NXF1, 2, and 3 form heterodimers with NXT1 (nuclear transport factor 2-like export factor 1), the metazoan ortholog of Mtr2, which facilitates NPC localization and translocation [52,79,84,90–92].

Multiple trypanosome paralogs of Mex67 have also recently been identified and characterized [93]. Unlike metazoa, these paralogs are not relatively minor splice variants, but encoded by separate genes, are structurally diverse and have discrete functions. Immunoisolation of TbMtr2 demonstrated an interaction with TbMex67, which has been well characterized; TbMex67 has a noncanonical N-terminal CCCH ZF that is essential and appears, thus far, unique to trypanosomes [67]. Additionally, TbMtr2 interacts with TbMex67b and TbMex67-like or TbMex67L. All three have a NTF2-like domain in addition to the typical LRR domains found in Mex67, while TbMex67 also has a C-terminal UBA. Significantly, neither TbMex67b nor TbMex67L retain the UBA domain, and while not unique to trypanosomes (mammalian also NXF3 lacks this domain), this indicates a distinct modality separating TbMex67 from TbMex67b/TbMex67L [54,84]. Despite this, Mex67b still interacts with the trypanosome splicing machinery [37]. TbMex67L is considerably larger than TbMex67 and TbMex67b due to an extended N-terminal domain.

The genes encoding TbMex67 and TbMex67b are near to each other on chromosome 11, indicative of a gene duplication event. Moreover, this chromosomal region, including the syntenic arrangement of TbMex67 and TbMex67b genes, is conserved throughout the kinetoplastids, and phylogenetic reconstruction indicates that TbMex67 and TbMex67b are more closely related than they are to Mex67L. Orthologs of TbMex67 and TbMex67b are recovered from all kinetoplastids, including the free-living bodonid, *Bodo saltans*, but TbMex67L is not, albeit retaining a presence within all other kinetoplastids, indicating a more recent addition to the repertoire than diversification of TbMex67/TbMex67b. TbMex67 and TbMex67b localize to the nucleolus as well as to NPCs at the NE periphery, consistent with roles in RNA export, while TbMex67L localizes exclusively to the perinucleolar foci in a manner reminiscent of Pol I [94], suggestive of a role specific to rRNA processing. Affinity capture of TbMex67 and TbMex67b coisolates NPC components while TbMex67L does not, instead coisolating with ribosome biogenesis proteins and ribosomal proteins [93]. Thus, trypanosomes are the first unicellular organisms to have multiple orthologs of Mex67 identified, two of which appear to be involved in RNA export and one with a specialized role at the nucleolus and ribosomal biosynthesis.

Mex67 is also involved in the transport of certain noncoding RNA

In opisthokonts, Mex67/Mtr2 function with XPO1, the most abundant export factor that mediates rRNA export, with involvement in 60S and 40S ribosomal subunits and 5S rRNA [95,96]. These additional Mex67 activities appear conserved in trypanosomes, with export of 60S and 40S subunits partially dependent on TbMex67 and TbMtr2. Defects in processing 60S rRNA and aberrations in ribosome assembly occur after silencing [97,98], while TbMex67/TbMtr2 interacts with protein components of the 5S RNP [97,98]. TbMex67 and TbMtr2 are also involved in tRNA export [99], a role fulfilled by exportin-T (XPOT) in opisthokonts [73]. Silencing XPOT does not perturb tRNA export in trypanosomes [99], but rather knockdown of TbMex67 (partially) and TbMtr2 (fully) blocks tRNA export [99]. As only TbMtr2 fully blocks tRNA translocation, this suggests roles for at least one of the additional TbMex67 paralogs in this pathway. It was also recently shown that silencing of two inner ring FG-Nups TbNup62 (Table 1) and TbNup53a directly affected tRNA export, suggesting that these two specific Nups are part of the tRNA export pathway in trypanosomes [100].

Evolutionary divergence in mRNA export mechanisms

Despite making strides in deciphering trypanosome RNA export pathways, we have neither a clear mechanistic paradigm nor an understanding of how the various complexes coordinate. eIF4AIII is a conserved nucleocytoplasmic shuttling RNA DEAD-box helicase and in trypanosomes depends on TbMex67b for function in RNA export [101]. Although eIF4AIII locates at the cytoplasmic side of trypanosome NPCs, it is also present in the nucleoplasm and cytoplasm, and knockdown of TbMex67b leads to nuclear accumulation of eIF4AIII, indicating a functional interaction [101]. Further, DRBD18, an essential and abundant *T. brucei* RNA-binding protein associates with TbMex67 and TbMtr2 *in vivo*, probably through interactions with TbMtr2 [102]. RNAi knockdown of DRBD18 leads to partial nuclear retention of mRNA and an export block of a subset of mRNAs, but has no effect on the export of tRNA [102].

Additional complexity in trypanosomes involves XPO1, which exports only some mRNAs in trypanosomes [103]. Further, leptomycin B treatment of *T. cruzi,* an XPO1 inhibitor, leads to a partial accumulation of a subset of mRNAs, specifically those encoding HSP70, the RNA-binding proteins TcUBP1/TcUBP2, and polyA-binding protein PABP1 [103]. XPO1 is involved in the export of some mRNAs in vertebrates, as well as those viral RNAs bypassing the surveillance system that prevents normal export of unspliced RNAs. Lacking an RNA-binding domain itself, XPO1 relies on interactions with additional proteins to export different classes of mRNA, for example, human antigen R (HuR) and eIF4E- and NXF3-dependent mRNA export [104–108]. Whether such adaptors are present in trypanosomes with similar functions remains to be established, but the observation of only partial blockade to mRNA export following XPO1 silencing in trypanosomes suggests that this is highly likely.

In fungi Crm1, the ortholog of vertebrate XPO1, also mediates export of the large ribosomal subunit, a pathway that depends on Nmd3, an adaptor protein that recruits XPO1 to the 60S subunit in the nucleus in preparation for transport [109,110]. Nmd3 is extremely well conserved and present in both eukaryotes and archaea [111], and TbNmd3 regulates both mRNA and rRNA nuclear export via an XPO1-linked pathway [112]. Silencing TbNmd3 leads to upregulation of procyclin-associated gene transcripts [112] which are transcribed by RNA Pol I [113]. Interestingly, silencing or inhibiting TbXPO1 with leptomycin B, or silencing TbMex67, has a similar phenotype. Considering the evolutionarily conserved relationship between Nmd3 and XPO1, this provides further support for divergence as procyclin-associated genes are mRNAs and not rRNAs, albeit Pol I transcripts, suggesting crossover in Nmd3 function between mRNA and rRNA metabolism in trypanosomes. Presently, we have snapshots of several processes, but lack a holistic view.

Does divergence in mRNA metabolism provide therapeutic opportunities?

Common wisdom suggests that a route to specificity for developing therapeutics against trypanosomes can be achieved via targeting their divergent mechanisms. Given both the essentiality of mRNA processing and – as discussed earlier – significant evidence for divergence, this should be a fertile space for drug discovery. One exciting new example of such a drug is acosiborole, a new therapeutic entering the pipeline for treatment of trypanosomiasis. The target of this drug is CPSF73, a component of the mRNA maturation machinery. Specificity apparently arises from minor divergences within the site in CPSF73 that acosiborole binds [114,115]. Despite this, CPSF73 arose in archaea, indicating an origin predating eukaryogenesis, and is generally very highly conserved across the eukaryotes. Clearly then, mechanistic divergence is not a prerequisite for therapeutic intervention; targeting of mRNA export indeed offers considerable therapeutic potential.

Concluding remarks

Eukaryotic gene expression involves multiple near-ubiquitous processes, many of which have been inherited directly from prokaryotes. Additionally species-specific proteins and pathways are involved that likely arose as adaptations to the specific biology of each organism. For trypanosomes, current understanding has indicated those areas where mRNA processing/export pathways are clearly modified, and which have probably arisen due to polycistronic transcription and the consequences of that mode of gene expression, though many aspects of these processes remain poorly understood (see Outstanding questions). mRNA export and processing are Ran-dependent in trypanosomes, representing a fundamental distinction to how the pathways are controlled compared with the canonical pathways of opisthokonts and most other lineages. It is presently unclear if Ran-dependent RNA export represents the ancestral state, which would unite all export under a Ran umbrella, or arose during evolution of the Discoba, the trypanosome lineage. Multiple Mex67 paralogs likewise could represent a basal configuration, but the absence of the canonical cytoplasmic mRNA export/QC platform is clearly a secondary loss. Given many additional examples of highly distinct nuclear functions restricted to kinetoplastids, including the lamina, nuclear basket and kinetochores, together with novel proteins interacting with mRNA processing pathways, how these divergent systems integrate will provide significant insights into the origins of the nucleus and eukaryogenesis itself.

Outstanding questions

What is the detailed architecture of the trypanosomatid NPC, and how does this impact RNA export mechanisms?

How do the multiple Mex67 paralogs act in concert with the GTPase Ran to control RNA export? And what is the mechanism of their specificity?

What are the functions of the lineage-specific proteins implicated within mRNA export?

How do interactions between chromatin and the divergent trypanosome nuclear basket mediate mRNA processing, activation or repression of specific genes?

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Declaration of interests

The authors declare no competing interests.

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