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Review

Gene transcription in trypanosomes

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Abstract

Trypanosoma brucei and the other members of the trypanosomatid family of parasitic protozoa, contain an unusual RNA polymerase II enzyme, uncoordinated mRNA 5′ capping and transcription initiation events, and most likely contain an abridged set of transcription factors. Pre-mRNA start sites remain elusive. In addition, two important life cycle stage-specific mRNAs are transcribed by RNA polymerase I. This review interprets these unusual transcription traits in the context of parasite biology.

Keywords: RNA polymerases; Trypanosomes; Basal transcription factors

Contents

1. Overview of mRNA synthesis in trypanosomes

The recently completed genome sequencing project for *Trypanosoma brucei* reveals 11 megabase-sized chromosomes that contain clusters of interspersed pre-rRNA, tRNA, small nuclear (sn) RNA, small nucleolar (sno) RNA, short interfering (si) RNA, spliced leader (SL) RNA, and mRNA coding genes [14]. Many clusters contain multiple, co-expressed protein-coding genes, which is a unique aspect of trypanosome biology. Despite this peculiarity, trypanosomes have highly conserved copies of all three eukaryotic RNA polymerase enzymes. Fig. 1 broadly defines the roles of the three RNA polymerases in trypanosomes. *T. brucei* RNA polymerase I (RNAP I) characteristically transcribes the pre-rRNA (18S, 5.8S and 28S) gene cluster. Surprisingly, this trypanosome enzyme also transcribes two mRNAs that are life cycle stage-specific and encode the bloodstream-form variant surface glycoproteins (VSGs) and the procyclic-form procyclins (EPs and GPEETs) [19,36]. *T. brucei* RNA polymerase II (RNAP II) transcribes mRNAs as expected, as well as the trypanosome-specific, cap 4 ($m^7Gpppm^{6,2}AmpAmpCmpm^3Um$)-containing, SL RNA gene [2,17]. Finally, *T. brucei* RNA polymerase III (RNAP III), as predicted, transcribes tRNAs, 5S RNA and the U-rich snRNAs [32].

While the trypanosome RNA polymerases largely resemble their well-studied eukaryotic homologs in transcriptional

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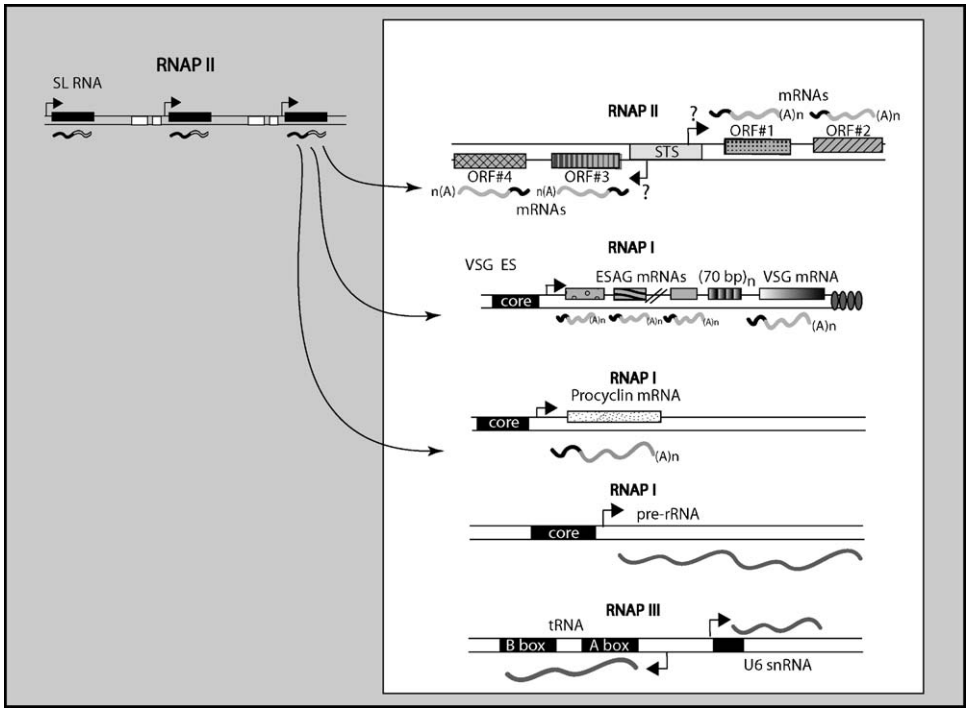

Fig. 1. Schematic overview of trypanosome transcription. All promoter elements, represented by a single black block box include two to four short, ~10 bp sequence-specific elements. The SL RNA promoter elements are white boxes. The grey area labeled STS may contain transcription start sites for pre-mRNAs. The SL (black wavy line) comprises the first 39 nts of every mRNA (grey wavy lines followed by (A)n to signify the poly(A) tail). In mature mRNAs the SL is followed by a 5′ untranslated region, of gene-specific length, an open reading frame, a 3′ untranslated region, also of gene-specific length, and a poly(A) tail. For a single mRNA the 5′ and 3′ UTRs are often heterogeneous, meaning that different SL addition and poly(A) additions sites are utilized during mRNA maturation. The pre-rRNA, which encodes the 18S, 5.8S and 28S rRNA components, the unusual tRNA and snRNA coupled genes, are shown with dark grey wavy lines. The oblong circles show subtelomeric regions.

activity and multisubunit structure, they clearly have assumed parasite-specific demands, perhaps attributable to unique trypanosomatid polymerase domains and/or interacting protein partners. Database mining and biochemical experiments demonstrate that trypanosomes possess homologs of the twelve to fifteen polypeptides within the three human and *Saccharomyces cerevisiae* RNA polymerases [1,23,24,26]. Interestingly, deviations exist; for example, the second largest subunit of the *T. brucei* RNAP I enzyme has a unique amino-terminal domain, the largest subunit of *T. brucei* RNAP II has a unique carboxyl-terminal domain (CTD), and there are two isoforms of the RPB5 and RPB6 subunits, which are usually single proteins shared among all three RNA polymerase in other eukaryotic cells (see Table 1) [15,26,39,42,45].

Similar to other eukaryotic mRNAs, trypanosome mRNAs possess 5′ and 3′ modifications. Integral to the resolution of individual mRNAs from the co-transcribed, polycistronic, open reading frames (ORFs) is a set of coordinated polyadeny-

Table 1
Core RNA polymerase II machinery in *T. brucei*, *Plasmodium falciparum*, *Saccharomyces cerevisiae*, *Homo sapiens*, and *Archaea*

	Subunits	Organism				
		Trypanosoma brucei	*Plasmodium falciparum*	*Saccharomyces cerevisiae*	*Homo sapiens*	*Archaea*
Protein						
RNAP II	RPB 1–12	√	√	√	√	√
	additional isoforms	RPB5z, RPB6z	×	×	×	×
Transcription factor						
TFIIA	2	√	√	√	√	×
TFIIB	1	√	√	√	√	√
TFIID	14 TAFs	×	√ (four homologs)	√	√	×
TBP	1	√	√	√	√	√
TFIIE	2	×	√	√	√	√
TFIIF	2	×	√ (β-subunit)	√	√ (three subunits)	×
TFIIH	10	√ (three homologs)	√ (eight homologs)	√	√	×
SNAPc	5	√ (three homologs)	×	×	√	×

Plasmodium data are derived from PlasmoDB and [7,11]. *Archaea* data are from [46]. *T. brucei* data are from [12,25,35,40]. Yeast and human data are summarized in [21].

lation and capping reactions. To resolve the polycistronic pre-mRNAs into stable, translatable units, trypanosomes have at the ready a prefabricated, highly modified cap (cap 4; $m^7Gpppm^{6,2}AmpAmpCmpm^3Um$) derived from high copy SL RNA gene transcripts. An abundant supply of pre-capped SL RNAs readily allows the parasite to synthesize highly stable mature messages. Specifically, two tandem mRNAs are separated with an intergenic space, and 3′ polyadenylation of the first gene occurs after the second gene receives a cap 4-containing SL RNA sequence on its 5′ end. For example, ORF#1 undergoes 3′ end modification during the capping of ORF#2. While intergenic spacing is variable, capping and polyadenylation occur within ∼200 bp of each other [29,43] (see Fig. 1). This highly unusual set of processing events enables a single round of RNAP II transcription to produce numerous functional mRNAs. Fast growing trypanosomes eschew the laborious co-transcriptional capping process present in many other eukaryotes, which requires a paused RNAP II, recruitment of three enzymatic capping activities, and the formation of a specifically modified CTD within the largest RNAP II subunit. Polycistronic gene transcription also allows trypanosomes to avoid the repetitive transcription reinitiation required of their free-living relatives.

2. Regulation of mRNA production

In the well-studied eukaryotes, and even in eubacteria, transcription initiation is a key regulatory point in controlling the level of gene expression. In the eukaryotic paradigm, both regulated and housekeeping mRNA production is controlled by the sequence-specific DNA binding of proteins to specific *cis*-acting elements. The constellation of *trans*-acting proteins is divided into basal factors and activators, which function together to recruit RNAP II to initiate mRNA synthesis. Transcription initiation is therefore the culmination of coordinated events that include protein modifications, chromatin remodeling, direct protein–DNA interactions, and co-activator assemblies.

In *T. brucei*'s transition from tsetse fly to man, one would imagine that large subsets of genes are activated at the transcriptional level, following the paradigm outlined above. However, studies to date do not support this hypothesis. Polycistronic transcription coupled with the absence of classic RNAP II promoters indicates that transcription initiation is not a significant rate-limiting factor in *T. brucei* mRNA production. Instead, constitutive gene expression appears to occur [10]. As a consequence, multifactorial, rate-limiting initiation complexes, and the associated intricacies, appear simply to be absent in *T. brucei*. This notion is underscored by the lack of easily identifiable co-activators, activators, and basal transcription factors in these parasites.

The transcriptional uniqueness of *T. brucei* should be considered in the light of the primary focus of parasites, which is survival in a host environment. Factors inherent to parasite survival include successful replication in disparate environments, the ability to quickly transition from tsetse fly to mammalian bloodstream, a degree of autonomy from the host, and, of course, evasion of the host immune response. Might the transcriptional idiosyncrasies associated with *T. brucei* aid in its survival? Perhaps the large glucose uptake from host blood [4] is the cost of high-level, constitutive gene expression, at least in bloodstream-form parasites. The continuous supply of transcripts, in turn, may promote a rapid response to environmental stresses and changes, including the parasite's eventual return to the tsetse fly. While this large-scale transcription appears energetically wasteful, it may actually help ensure parasite survival in the face of environmental fluctuation.

In place of differential promoter firing of RNAP II-dependent genes, trypanosomes have a plastic genome that enables them to use gene amplification to affect mRNA production, and therefore, protein abundance. For example, the characteristic subpellicular structure that gives trypanosomes their tapered shape is dependent on the expression of numerous, tandemly reiterated α- and β-tubulin genes. The requirement for abundant SL RNAs to cap each mRNA is met by the reiteration, at the chromosome level, of SL RNA genes.

3. RNAP II transcriptional machinery

Examination of the known RNAP II transcriptional machinery in trypanosomes offers an incomplete picture of RNAP II function in these parasites. The sequencing of three trypanosome genomes, and biochemical studies of SL RNA gene expression, contributes to a developing model of RNAP II transcription. The emergent theme is a minimalist version of higher eukaryotic transcription, coupled with trypanosome-specific factors, as discussed below.

The recently published genomes of *T. brucei*, *T. cruzi*, and *Leishmania major*, nicely outline the transcription factors (TFs) readily identifiable by homology and Pfam domain searches [3,14,25]. While trypanosome homologs are present for all of the subunits of eukaryotic RNAP II, many of the basal transcription factors appear absent (e.g. TFIIB, TFIIF, TFIIE, several of the TFIIH subunits, the largest subunit of TFIIA (α and β proteins), and the TATA binding protein (TBP)-associated factors (TAFs) found within TFIID) [26]. The apparent absence of these factors lends itself to several possible interpretations: (1) highly divergent forms of the basal factors are present, eluding bioinformatic detection; (2) trypanosome-specific proteins take on equivalent roles to their distantly related, well-studied eukaryotic transcription factors; (3) greatly relaxed transcriptional regulation eliminates the need for these factors. Recent biochemical studies are addressing these hypotheses.

Our knowledge of the proteins essential for RNAP II-dependent transcription in *T. brucei* comes mainly from SL RNA gene studies. This gene contains the only defined RNAP II-dependent promoter and is the sole RNAP II-dependent snRNA gene in these parasites. The SL RNA gene promoter is TATA-less, B-recognition element (BRE)-less, and contains a bi-partite proximal sequence element (PSE), and thus resembles the well-studied human U1 snRNA promoter [8,20,30]. U1 snRNA synthesis requires the snRNA-activating protein complex (SNAPc), TBP, TFIIA, TFIIB, TFIIF, TFIIE, along with RNAP II [27]. Trypanosomes utilize a SNAPc for SL RNA synthesis. Interestingly, the trypanosomal SNAPc appears to be a minimalist version, consisting of only three subunits compared to the five

of human SNAPc. The *T. brucei* SNAPc subunits co-purify with divergent forms of the general transcription factor TFIIA and TBP. Reflecting its divergence from other eukaryotic TBPs, trypanosomal TBP is named TRF4 or tTBP [12,38]. Chromatin immunoprecipitation studies demonstrate that TRF4/tTBP associates not only with the SL RNA gene promoter region but also with the 3′ untranslated region of several protein coding genes [38]. This unexpected association of trypanosomal TBP with 3′ untranslated regions of select protein coding genes adds yet another curiosity to trypanosomatid transcription. Recent work from our laboratory has identified a divergent form of the general transcription factor TFIIB that is essential for transcription from the SL RNA gene promoter [35]. This essential trypanosome protein has many of the traits of well-characterized eukaryotic TFIIBs, yet interacts with at least one trypanosome-specific partner. The precise roles of trypanosome SNAPc, tTBP, TFIIA, and TFIIB, as well as their protein–protein and protein–DNA interactions, are as yet undefined.

These functional studies demonstrate that more basal transcription factors are present in trypanosomes than initially suspected from database analysis. For example, the TFIIA-α/β subunits are faintly recognizable as such, causing them to have eluded bioinformatic detection. Furthermore, examination of trypanosome SNAPc suggests that the parasites utilize a trypanosome-specific polypeptide. This polypeptide, tSNAP42, shares only a single motif, namely a myb domain, with the SNAP190 subunit of metazoan SNAPc [12,40]. However, the myb domain is not conserved among trypanosomatid SNAPc. In summary, as our knowledge of trypanosomatid transcription is limited, it is premature to determine if the apparent relaxed transcriptional regulation is related to the apparent loss of various basal transcription factors. A summary of the RNAP II machinery present in trypanosomes derived from biochemical studies and genome sequence analysis is shown in Table 1.

In spite of this, our increased understanding of SL RNA gene transcription indicates that this process mirrors that found at RNAP II-dependent U snRNA genes in higher eukaryotes [22]. Clearly, there are distinctly trypanosomal aspects to SL RNA gene transcription. Trypanosomes are primitive, ancient eukaryotes. As such, they serve as a window into transcriptional evolution, providing a glimpse into how a wide range of eukaryotes customized basal transcription factors to meet their own specific needs.

If trypanosomes ultimately lack several of the basal transcription factors, perhaps we may better decipher their mechanisms of transcription by comparing them to the archaeal model. Archaea, though a separate domain from eubacteria and eukaryotes, utilize a single RNA polymerase that is very similar to eukaryotic RNAP II. In the archaeal transcriptional system, this enzyme is recruited to promoters by merely three basal transcription factors: TBP, TFB (the homolog of TFIIB), and TFE (the homolog of TFIIE). Moreover, in vitro transcription can be reconstituted using only polymerase, TBP, and TFB [46,47]. The bare-bones archaeal systems may serve as a reference when contemplating the lack of factors and other transcriptional quirks of their kinetoplastid relatives.

4. RNAP II-dependent transcription of mRNA-coding genes

The emerging picture of trypanosomatid SL RNA gene transcription parallels our current knowledge of RNAP II-dependent U snRNA gene transcription in other systems. However, models of RNAP II-dependent transcription built from well-studied systems, appear insufficient to explain trypanosome mRNA synthesis.

Characteristic of most, if not all, RNAP II-dependent mRNA coding genes in trypanosomes is their curious chromosomal arrangement. Large numbers of genes are encoded on a single DNA strand that is preceded by a 1–13 kb non-transcribed region referred to as a strand switch (STS) region. The other end of the STS region begins a second set of genes, encoded on the opposite DNA strand (see Fig. 1). Therefore, the polycistronic gene arrays radiate bi-directionally from a STS region. Nuclear run-on data from *L. major* suggest that polycistronic gene transcription originates in the STS region [31]. However, closer inspection of STS regions at multiple sites in the *T. brucei* genome reveals a dearth of typical eukaryotic elements, such as TATA boxes, and no overrepresented ∼10 bp sequence blocks that could function as promoter elements. This may indicate that transcription is randomly initiated or that promoter elements are unusual. We consider these possibilities in the context of what is known about the trypanosome RNAP II-associated transcription factors identified thus far.

TBP plays a universal role in all eukaryotic transcription. TBP is part of the TFIID complex, which associates with mRNA coding gene promoters through a TBP–DNA interaction [21]. TBP from higher eukarya contains two pairs of phenylalanines in the well-conserved carboxyl terminal domain. These amino acids play key roles in TATA box binding [6]. Trypanosomal TBP lacks the first phenylalanine pair, suggesting that the parasite protein binds different DNA sequences within mRNA coding genes.

The basal transcription factor TFIIB plays a role at both RNAP II-dependent mRNA coding genes and snRNA genes in yeast and metazoans [37]. TFIIB has major roles in start site selection and in bridging TBP to RNAP II. It also may orient the transcriptional machinery through its interaction with the BRE that flanks the TATA box. Curiously, many of the conserved residues that direct start site selection and those that interact with the BRE are not conserved in trypanosomal TFIIB [35]. As with TBP, the non-conserved DNA binding residues in the parasite protein may suggest novel protein–DNA interactions within mRNA coding genes.

The CTD of the largest subunit of RNAP II (the 220 kDa RPB1-encoded protein) coordinates the co-transcriptional capping, splicing, and polyadenylation events to craft a eukaryotic mRNA [21]. This is accomplished through differential phosphorylation of the YSPTSPS amino acid repeats that comprise the CTD. The largest subunit of trypanosome RNAP II includes the seven domains important for RBP1 function (domains A–G), but lacks the characteristic CTD repeats [15,42]. Nevertheless, there is a ∼250 amino acid carboxyl terminal region of trypanosome RBP1 containing di-serines that are potentially phosphorylated

[9,17]. The non-standard CTD could imply that the orchestration of transcription is carried out in a fundamentally different manner from other eukaryotes. The function of the unusual trypanosome RPB1 carboxyl terminal domain in mRNA production is a key piece of the transcriptional puzzle missing in these parasites.

5. *T. brucei* exploits RNAP I for its parasitic life style

RNAP I has a single function in most eukaryotic cells: it synthesizes pre-rRNA. This is processed into the 28S, 18S and 5.8S rRNAs, which are found in ribosomes. A most unusual finding in trypanosome biology is that RNAP I also synthesizes the mRNAs encoding the proteins that cover the surface of the parasite. In particular, the blood-stage specific antigen, the VSG, and the insect stage-specific EP and GPEET antigens, require RNAP I for their expression. The EP/GPEET promoter contains three promoter elements within the 150 bp region upstream from the transcriptional start site [5,28,41]. The VSG expression site promoter, as well as metacyclic VSG promoters, require a CA at the transcription start site (CA are −1/+1), and two other short sequences centered at −60 and −36 [18,44]. The pre-rRNA promoter is minimally bipartite, with two essential elements centered at −57 and −27. The proximity of these elements to each other within each of the RNAP I promoters, and to their respective transcriptional start sites, as well as their relatively simple modular structure is reminiscent of the pre-rRNA promoters identified in the well-studied eukaryotes. However, as there appears to be limited sequence conservation among the three trypanosome RNAP I-dependent promoters, it is unclear if they recruit RNAP I using the same set of factors. Nevertheless, one study indicates that EP/GPEET and pre-rRNA promoters compete for transcription factors [28]. Another report using hybrid-promoters suggests that the VSG promoter is more similar to the pre-rRNA promoter than it is to the EP/GPEET promoter [44]. A third study concludes that a sequence stretch, centered 220 bp upstream from the pre-rRNA promoter [28], resembles an essential SL RNA gene promoter element and interacts with a component of the tSNAP complex, which is essential for RNAP II-driven SL RNA gene expression. In total, these studies highlight the complexity of the transcriptional picture at the RNAP I promoters. It is possible that the pre-rRNA and SL RNA genes, which are both constitutively expressed, share common transcription factors, whereas stage-specific genes, specifically the VSG and EP/GPEET genes, require a higher degree of regulation that is directed by unique sets of transcription initiation complexes.

Why is *T. brucei* the only known trypanosomatid family member that has employed RNAP I for mRNA transcription? As a possible consequence of the high genomic instability found in trypanosome genomes, *T. brucei* possibly garnered an RNAP I promoter for expression of its crucial VSG proteins. This line of reasoning predicts that high levels of VSG mRNA synthesis would result from sharing the robust RNAP I, normally dedicated solely to pre-rRNA synthesis. In addition, linking VSG mRNA synthesis to a RNAP I-utilized promoter would allow VSG mRNA to be expressed in a compartmentalized transcriptional factory, as are pre-rRNA genes [34]. Clearly, over expression of VSG mRNA would enable parasites to produce the extremely dense VSG surface coat with limited antigenic complexity that is characteristic of *T. brucei* [13]. It is possible that production of large VSG amounts serves to absorb antibody, either during rapid membrane endocytosis of VSG–antibody complexes, and/or by generating free antigen in the blood (Markus Engstler, pers. commun.). Both mechanisms could effectively protect the parasite from the lethal effects of the host immune system. Indeed, it is the VSG coat that serves as the trypanosome's major defense against the host immune system. The genetic recombination events that first positioned a VSG ORF, and then EP and GPEET ORFs, downstream from a pre-rRNA promoter may have been stabilized by the advantage of quickly producing large amounts of surface proteins in response to environmental changes during the parasite's life cycle. The intracellular *Leishmania* ssp. and *T. cruzi*, appear to have not adopted this remarkable RNAP I activity. Therefore, this gene expression trick may be a relatively new one and thus species-specific; alternatively, the selective pressure on extracellular *T. brucei* may be unique.

6. A dual function for RNAP III transcription in trypanosomes

Trypanosome RNAP III transcribes tRNA and all U-rich snRNA genes. tRNA gene promoters in trypanosomes contain intragenic A and B box *cis*-acting elements and therefore resemble those of other eukaryotes. A subset of trypanosome tRNA gene promoters have a secondary function: their A and B boxes serve as promoter elements in upstream (∼95 bp), and oppositely oriented U3, U6 and 7SL RNA genes [32,33]. In vivo, U6 snRNA expression in *T. brucei* requires three promoter elements. One of these is intragenic (+2/+11), and the second two are the upstream tRNA's A and B boxes (see Fig. 1). This mode of transcriptional regulation is distinct from U6 snRNA gene transcription in both humans and yeast. In humans, a TATA box and proximal and distal sequence elements (PSE and DSE, respectively) are found upstream of the transcriptional start site. Yeast contain an upstream TATA box, an intragenic A box, and a downstream B box. Despite the absence of a TATA box in the *T. brucei* U6 snRNA gene promoter, trypanosomal TBP is integral for the expression of this gene, as shown by RNA interference and chromatin immunoprecipitation studies.

T. brucei U2 snRNA synthesis requires the same three elements as does U6 snRNA expression, but is unique in that a bona-fide tRNA gene is not adjacent to this promoter, although A and B boxes remain [16]. In most eukaryotes, U2 snRNA is transcribed by RNAP II, but α-amanitin sensitivity studies reveal it is RNAP III-dependent in trypanosomes, as are all U-rich snRNAs.

The neighboring, divergent tRNA and snRNA gene arrangement is not restricted to *T. brucei*. Indeed, such an arrangement is found across the Trypanosomatidae family, including *Leishmania*, *Leptomonas*, and *Crithidia*. Perhaps this unique arrange-

ment of shared promoter elements between tRNA and U-rich snRNA genes is yet another reflection of simplified transcriptional regulation in these parasites.

7. Summary

A review of *T. brucei* transcription underscores the unique nature these parasites have employed in their gene expression, such as the shared gene promoters associated with RNAP III-dependent transcription, the utilization of RNAP I for protein expression, and the how an unusual CTD functionally contributes to the RNAP II enzyme. Importantly, such a review also highlights our lack of answers to many long-standing questions, including that of differential VSG mRNA expression and transcription initiation of RNAP II-dependent pre-mRNAs. Ultimately, a greater understanding of these trypanosomatid transcriptional traits will allow for greater insight into the parasite lifecycle, which hopefully will translate into better therapeutic development.

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