MINIREVIEW

RNA Polymerase Transcription Machinery in Trypanosomes

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Transcription is a fundamental biological process employed by all living organisms to decode their genetic information. The information stored in genomic DNA is copied into RNA molecules by polymerization of ribonucleotide building blocks, which ultimately gives rise to different classes of transcripts. mRNAs encode polypeptides, rRNAs drive the macromolecular protein-synthesis machinery, and tRNAs act as adaptor molecules to assemble amino acids into proteins. Synthesis of specific transcripts is influenced by environmental and internal cell signals, which in turn are pivotal for the control of cellular regulatory networks.

Trypanosomes are unicellular parasitic protozoa, members of the order Kinetoplastidae, which diverged early during evolution. They cause a wide range of debilitating diseases in humans and domestic animals. *Trypanosoma brucei*, known as the African trypanosome, is transmitted by tsetse flies in sub-Saharan Africa (15). Infection fulminates into African sleeping sickness in humans and nagana in animals (3). *T. brucei* is a digenetic parasite that cycles as a procyclic form in the digestive tract of the tsetse vector and as an extracellular bloodstream form in its mammalian host. During its complex life cycle, the parasite passes through five successive morphologically distinct forms (39). Parasites change from the procyclic form, which is characterized by a procyclic-specific surface coat, through two morphologically distinct forms in the fly and then they emerge as long, slender bloodstream forms, covered with a variant surface glycoprotein coat. Once inside the mammalian host, the long slender bloodstream form actively divides and establishes parasitemia. In the late phases of infection, the morphology of the parasite changes to nondoing short stumpy forms, which are ready to be taken up by the insect during a blood meal. The bloodstream form, with a rudimentary mitochondrion, is perfectly adapted to utilize the abundant supply of glucose from the mammalian blood and generate sufficient energy by glycolysis. The insect form, on the other hand, has a functional mitochondrion and generates most of its energy by respiration. These necessary metabolic adaptations depend upon a precise orchestration of numerous metabolic and cell biological activities.

Studies of trypanosomes have uncovered several unusual biological phenomena (6). Notable among them are trans splicing of a 39-nucleotide spliced leader (SL) RNA at the 5′ end maturation. RNA editing, used to produce mitochondrial mRNA, requires extensive alterations of primary transcripts by guide RNAs. Although guide RNA-dependent RNA editing is uniquely observed in trypanosomes, trans splicing has subsequently been observed in several other lower eukaryotes, including nematodes, trematodes, euglenoids, and chordates. Therefore, studies of trypanosomes are expected to uncover cryptic mechanistic components of eukaryotic biology and reveal exotic cellular processes.

RNA POLYMERASE II: FUNCTION AND STRUCTURE

Eukaryotes employ three different RNA polymerases (pol), (RNA pol I, II, and III) to transcribe their nuclear DNA. Each enzyme is responsible for transcription of different types of RNA (2). RNA pol II synthesizes pre-mRNAs and a subset of the U-rich short RNAs that function in splicosomes and are involved in the processing of pre-mRNAs into translatable mRNAs. The clear link between the two types of RNA pol II-dependent RNAs is that their 5′ ends are capped, which requires the addition of an m7G to the 5′ triphosphate end of the primary transcript. The cotranscriptional capping of RNAs is facilitated by protein-protein interactions between RNA pol II and the capping enzymes (49). Additional cotranscriptional activities require RNA pol II to function with multiple proteins. These cotranscriptional activities are assigned to specific subunits of RNA pol II and to specific domains within these subunits. Therefore, a detailed understanding of trypanosome RNA pol II subunit composition will help explain how this enzyme functions to produce both pre-mRNAs and SL RNAs in trypanosomes.

RNA pol II contains 12 subunits, designated as RPB1 to RPB12. RPB1, RPB2, RPB3, and RPB11 are the functional and structural homologs of eubacterial core subunits of RNA polymerase (34). These four trypanosome protein subunits follow the paradigm and likely function as the basic components of the enzyme.

RPB1, the largest subunit of the *T. brucei* enzyme, is probably the most interesting component of the RNA pol II complex (16, 55). First, there are two nonallelic genes that code for this polypeptide. These genes are referred to as pol II A and pol II B. They differ in four amino acids, in regions that are
known to function in DNA binding in other enzymes. However, these differences are likely strain specific, as parasites from different serodemes have different numbers of amino acid substitutions in the two nonallelic gene copies. The importance, if any, of four RNA pol II largest subunit genes in *T. brucei* is unclear, as there is no corollary for this in related trypanosomes. Second, the largest subunit of trypanosome pol II resembles the β′ subunit of eubacterial RNA polymerase in eight regions, domains A to H. This is consistent with a common function for this polypeptide with the corresponding subunit in other eukaryotic pol II enzymes. The nonstructured carboxyl end of the polypeptide deviates from the heptapeptide repeat of YSPTSPS of varying length that is characteristic of yeast and mammalian proteins (34). The role of the heptapeptide repeat is to modulate cotranscriptional processes, including capping, splicing, elongation, and polyadenylation. This is achieved by coordinated kinetic alterations in the phosphorylation of the serines and threonines in the repeating YSPTSPS motif. The trypanosome protein extends beyond the eubacterial-like eight domains and trails with a nonrepetitive sequence. Although there is evidence that the trypanosome carboxyl-terminal domain is phosphorylated, we do not know how these modifications contribute to RNA pol II function (11).

The additional components of eukaryotic pol II are RPB4 to RPB10 and RPB12. These components of RNA pol II, along with general transcription factors, generally mirror eubacterial sigma factors in function. Precisely, RPB4 to RPB10 and RPB12 contribute to the enzyme’s ability to respond to activators to bind tightly with promoter regions, properly initiate RNA transcripts, and ensure efficient and accurate RNA synthesis.

Interestingly, trypanosomes have two isoforms of RPB5 and -6, designated RPB5 and -5z and RPB6 and -6z (12, 30, 33, 43). Trypanosome RNA pol II contains a single isoform of each of these two proteins (RPB5 and RPB6), although it is unclear if there is a minor form of the enzyme that possesses the “z” isoform of either polypeptide.

The elegant 12-subunit crystal structure of yeast RNA pol II shows that RPB6 interacts with RPB7 (4). Since there are two isoforms of RPB6 in trypanosomes, there is the possibility of a specific RPB6 interaction with RPB7. RPB7 is a component of the RPB4/7 heterodimer that interacts with the core enzyme to facilitate specific transcription initiation.

In yeast and mammalian cells, RPB4 and RPB7 are essential for the initiation of transcription but not for RNA chain elongation (5). The RNA pol II enzymes isolated from two laboratories, the Bellofatto and Pays laboratories, likely contain the full complement of 12 subunits, although in both preparations only a subset of the proteins was identified by mass spectrometric analysis (9, 13). Both enzyme preparations contained the noncore subunits, RPB4 and RPB7, implying that the enzymes should be initiation and elongation competent. In one set of experiments, this was confirmed by in vitro elongation and specific start site selection at the SL RNA gene promoter (9). However, it is possible that the limited number of cofractionating polypeptides that copurified with the enzyme preparation contributed to these activities.

**GENERAL TRANSCRIPTION FACTORS IN TRYPANOSOME RNA POL II-DEPENDENT TRANSCRIPTION**

A pivotal regulatory point of gene expression in many organisms is transcription initiation. This requires the formation of a preinitiation complex, composed of multiple proteins that interact with RNA pol II (see Fig. 1). The proteins recognize a promoter, unwind a short region of adjacent DNA, and initiate RNA synthesis at a specific nucleotide. Classic in vitro analysis of basal transcription, in which regulation is stripped away, revealed a small set of proteins, TFIIA, -B, -D, -E, -F, and -H, that participate in the recruitment and positioning of RNA pol II at gene promoters (20). Using a combination of biochemistry and bioinformatics to study the SL RNA gene promoter, the only described RNA pol II-dependent promoter in trypanosomes, we have constructed a partial list of the homologs of these proteins. The impact of these discoveries on the current understanding of trypanosome biology is only beginning to be unveiled.

Biochemical studies of RNA pol II function in trypanosomes have been limited to transcriptional studies of the SL RNA gene (Fig. 1). The role of candidate general transcription factors is assessed by complementary protein depletion and add-back studies, as well as protein-promoter interaction studies at the SL RNA gene promoter. In this way, trypanosome homologs of TFIIA, TATA-binding protein (TBP), and TFIIH have been uncovered.

TFIIB is a single polypeptide factor required for the association of RNA pol II to the preinitiation complex. The crystal structure of yeast TFIIB, complexed with TBP and DNA, reveals that TFIIB closely associates with DNA and contains a complex surface potentially available for regulatory protein interactions (45). The salient features of TFIIB include an N-terminal zinc finger domain, followed by a direct repeat in the C-terminal region. Trypanosome TFIIB contains the zinc finger domain typical of TFIIB but lacks the C-terminal direct repeat. Indeed, extensive biochemical analysis was necessary to confirm the identity of *T. brucei* TFIIB (47, 52). We have recently demonstrated that the unusual C-terminal region is important for RNA pol II recruitment and/or function at the SL RNA gene promoter (D. Wah and V. Bellofatto, unpublished data).

TBP, the universal transcription factor, is involved in transcription by all three RNA polymerases in eukaryotes (21).
The N-terminal regions of TBPs are variable, whereas the C-terminal regions are relatively conserved and form two domains that are highly related to each other. TBP folds into a saddle-shaped structure with the inner surface binding and bending DNA. TBP functions with many different partners, for example, as a component of TFIID in RNA pol II-dependent transcription, to interact with activators and other regulatory factors that control transcription. The quest for the trypanosome TBP protein began with PCR-based gene amplification and cDNA library screening with probes containing sequences conserved among known TBP genes. The trypanosome TBP was not uncovered until the Tritryps database (http://www.genedb.org/) was complete and the Tschudi/Ullu laboratory identified a protein containing TBP the C-terminal signature domain (50). As expected for a functional TBP, this T. brucei protein, called TRF4, was essential for cell viability and was recruited to the SL RNA gene promoter. Subsequently, two laboratories biochemically purified T. brucei TBP as a tightly associated factor of tSNAP (10, 53). The significant divergence in the trypanosome TBP may indicate functional variation in its role in transcription (22, 58). Specifically, the trypanosome protein lacks two of the four important phenylalanines that are responsible for bending the DNA on either side of the TATA box. As it is unclear how TBP alters DNA structure at non-TATA sites, we cannot predict how the trypanosome TBP interacts with DNA in a TATA-element-less genome. In addition, the trypanosome TBP does not appear to be a member of a TFIID-like complex. Indeed, the TBP-associated factor (TAF) proteins that comprise yeast TFIID do not have obvious homologs in the Tritryps database. Nevertheless, the trypanosome TBP may function as a universal transcription factor as it interacts with DNA in a TATA-element-less genome.

TFIIF is a component of the RNA pol II basal transcription machinery and functions in both transcription initiation and elongation. In mammals and yeast, the protein complex is comprised of nine polypeptides, present as two subcomplexes (57). The core complex, the larger of the two subcomplexes, contains two helicases, XPB and XPD, and two regulatory proteins, p44 and p52. The second subcomplex, called the cyclin-activating kinase complex, contains CDK7-cyclin H and the Mat1 protein. The trypanosome TFIIF core complex has been partially characterized by two groups (32, 33). The XPD subunit was identified by bioinformatics, and then tightly associated proteins were analyzed using TAP-tagged, XPD-containing T. brucei cell lines. It was confirmed that the trypanosome TFIIF core contains the expected XBP, p52, and p44 subunits. This four-subunit complex, albeit along with stoichiometric amounts of other copurified proteins, was able to restore RNA pol II-dependent SL RNA transcription in an in vitro assay. Moreover, RNA interference knockdown of XPD resulted in cell death. Therefore, trypanosome RNA pol II likely utilizes TFIIF as a basal transcription factor.

Interestingly, there appear to be two closely related but distinctly different XBP subunits in the Tritryps genomes. In T. brucei only one of these proteins, XBP-105 kDa, cofractionated with the functional TFIIF. As TFIIF is also known to participate in RNA pol I-driven transcription, it is intriguing to speculate that the XPB-89 kDa protein might function in a second TFIIF complex in trypanosome RNA pol I transcription (25).

An important step in the transition of RNA pol II from transcription initiation to elongation requires the transcription factor TFIIS. TFIIS helps RNA pol II escape the pauses that the enzyme undergoes 20 to 30 nucleotides after transcription initiation (18). TFIIS contains three domains, of which the central and C-terminal domains are essential for protein function. These domains insert into the RNA pol II active site and participate in the release from pausing. Mammals and yeast encode a single TFIIS; however, the Tritryps organisms encode three or four versions of this protein. In T. brucei two proteins, annotated as TFIIS1-1 and -2-1, contain the canonical three domains, including a zinc ribbon in domain III. Interestingly, one of the trypanosome polypeptides (TFIIS2-1) has an amino-terminal extension that contains a PWPP motif. A third TFIIS-like protein, annotated as TFIIS2-2, resembles a C-terminally truncated TFIIS, missing TFIIS domains II and III. All three proteins localize to the nucleus; hence, it is tempting to speculate that they assist in RNA pol II-dependent pre-mRNA synthesis (J.-P. Daniels, B. Wickstead, and K. Gull, personal communication).

The only small RNA synthesized by RNA pol II in trypanosomes is the m7GpppG-capped SL RNA. The SL RNA gene promoter is a simple tripartite structure, situated within 100 bp upstream from the AACU (1-1 to +4) transcription start site. The TSNAP complex (tSNAPc), along with TBP, TFIIF, and TFIIF, is essential for SL RNA synthesis. This complex is trimeric, containing tSNAP50, tSNAP42, and tSNAP26. TSNAP50 is orthologous to the human SNAP50 subunit of hSNAPc, which is required for RNA pol II-dependent U snRNA expression. The additional two subunits of tSNAP are sufficiently different from hSNAPc subunits; tSNAP26 resembles a truncated version of hSNAP43, and tSNAP42 is unique to trypanosomes.

TFIIF, a heterodimeric transcription factor, functions in RNA pol II transcription by stabilizing the binding of TBP to DNA. TFIIF is thought to be an antirepressor, working to block proteins that interfere with preinitiation complex formation. A T. brucei protein homologous to the small subunit of TFIIA biochemically fractionates with tSNAPc (10, 53). The T. brucei homolog of the large subunit remains elusive in both biochemical and bioinformatic analysis.

**GENERAL TRANSCRIPTION FACTORS IN TRYPANOSOME RNA POL I-DEPENDENT TRANSCRIPTION**

In most eukaryotes, RNA pol I is recruited to simple promoters, containing a single upstream element, located ~100 bp from the transcription start site, and a core element that straddles the start site. These promoters are used exclusively for rRNA gene expression and enable the enzyme to transcribe a 45S pre-rRNA, which is then processed into 18S, 5.8S, and 28S rRNA. Two protein complexes, SL1 and UBF, are essential for RNA pol I recruitment to the rRNA promoter.

A salient feature of T. brucei gene expression is that the genes for the major surface glycoproteins that coat both procyclic and blood form parasites are transcribed by RNA pol I. Thus, this enzyme has a dual function: it makes 45S rRNAs as
well as a subset of mRNAs. RNA pol I in blood form *T. brucei* functions in two subnuclear locales: variant surface glycoprotein mRNAs are transcribed in a discrete ‘expression site body’ and 45S rRNA are transcribed in the nucleolus (42). T. brucei RNA pol I enzyme is a ~12-subunit complex, as are other eukaryotic RNA pol I enzymes (51). Biochemical and bioinformatic analyses have uncovered the RPA1, RPA2, RPC40, RPB5z, RPB6z, RPB8, RPC19, RPB10z, and RPA12 subunits (44, 60).

*T. brucei* RNA pol I possesses its own sets of RPB5, RPB6, and RPB10 paralogs, called RPB5z, -6z, and -10z. While the RPB5z subunit differs from the canonical subunit by sequence insertions, RPB6z contains an amino-terminal deletion along with a short internal insertion. Bioinformatic analysis predicts a similar ‘specialization’ of RNA polymerase complexes in other lower eukaryotes (12).

*T. brucei* RNA pol I also appears to have a trypanosome-specific component, called RPA31, which copurified with RNA pol I in the studies conducted by two separate groups (43, 60). Recently, the Günszl laboratory showed that *T. brucei* RPA31 is essential for in vitro RNA pol I transcription and cell viability (43). It is tempting to speculate that the unusual components of trypanosome RNA pol I are related to this enzyme’s role in pre-mRNA transcription in *T. brucei*. However, it appears that pre-mRNA synthesis by RNA pol I is restricted to *T. brucei*, although all members of the Triryp group have RPA31 and RPB5z, -6z, and -10z orthologs.

**GENERAL TRANSCRIPTION FACTORS IN TRYPANOSOME RNA POL III-DEPENDENT TRANSCRIPTION**

RNA pol III functions to transcribe small RNAs in eukaryotic cells, including the 5S rRNA, tRNAs, snoRNAs, and a subset of the U-rich snRNAs. This important enzyme and its associated factors have not been characterized in detail in trypanosomes. Extensive studies in other eukaryotic systems have described RNA pol III as a 17-subunit enzyme, of which nine subunits form the core enzyme (19). Bioinformatic analysis has uncovered trypanosome homologs of many of the RNA pol III subunits (30). It is of note that the RPC17 subunit appears to be missing in trypanosomes. This is an interesting finding as RPC17, unique to RNA pol III and highly conserved between yeast and humans, is essential for cell viability (17).

RNA pol III shares several subunits with either RNA pol I, pol II, or both, and it contains some exclusive polypeptides. The RPB5 and RPB6 subunits are the same in all three enzymes. *T. brucei* RNA pol III contains a single version of the two RPB5 isoforms: specifically, the form present in RNA pol II and not in RNA pol I. It is important to discover which of the RPB6 and RPB10 paralogs reside in the trypanosome RNA pol III enzyme. Additionally, it will be interesting to see if the division of RPB5, RPB6, and RPB10 paralogs is the same in all trypanosomes or in *T. brucei* alone, where pol II shares the functions of pre-mRNA synthesis with RNA pol I (37).

RNA pol III in yeast and mammals is recruited to tRNA genes by TFIIIB and TFIIIC and to 5S rRNA genes by TFIIA, TFIIIB, and TFIIIC. Only one RNA pol III transcription factor, namely BRF, a component of TFIIIB, has been described in trypanosomes (1, 14, 27, 53). Although, other RNA pol III transcription factors remain elusive, they likely exist, as trypanosome tRNA gene promoters contain the 10-bp internal promoter elements (A and B boxes), to which transcription factors TFIIIB and TFIIIC normally bind. Interestingly, several trypanosome tRNA genes reside antiparallel and upstream from other RNA pol III-dependent genes, namely the U6 snRNA (40). The U6 snRNA requires the tRNA gene promoter in addition to its own intragenic element (41). This curious finding allows us to speculate that trypanosome RNA pol III is likely recruited to the tRNA-U6 snRNA gene pair by a set of transcription factors that are more elaborate than those found in other organisms.

The discovery of TFIIIA, a nine zinc finger domain transcription factor, facilitated our understanding of how zinc finger motifs mediate protein-nucleic acid interactions that are crucial for cellular functions (31). TFIIIA is a dual function protein. It works in 5S rRNA transcription initiation by binding to DNA and coordinating the assembly of TFIIIC and TFIIIB at the promoter. It also works as a nuclear/cytoplasmic shuttle by binding 5S rRNA and escorting it into the cytoplasm as part of the 7S RNP (56).

**EPIGENETIC CONTROL OF TRANSCRIPTION**

Epigenetic control is defined as regulation of gene expression by chromatin structure and modifications. It is now appreciated as being central to cell cycle progression as well as pathogenesis in trypanosomes. Trypanosomes have the expected complement of core histones, namely H2A, H2B, H3, and H4. They also contain variants of H2, H3, and H4, namely H2Az, H2BV, and H3V. Like most trypanosome genes that are orthologous to those defined in characterized systems, the trypanosome histones also diverge substantially from those in well-studied organisms (8, 23, 28, 36). Small group modifications, mainly acetylation and methylation that occur on lysines and arginines, are present and reflect the importance of the histone code throughout evolution. However, mass spectrometric analysis indicates that these modifications in trypanosomes are less abundant and thus simpler than in other organisms (23). The paucity of modifications reflects the dearth of histone-modifying enzymes, deduced in part by biochemical studies and in part by bioinformatic analysis.

Recent studies have begun to shed light on the effects of epigenetic control on the trypanosome cell cycle. The activity of DOT1B (a H3-K76-Me3-specific methylase) is important for the developmental transformation of blood form *T. brucei* to tsetse form procyclics (29). The role of DOT1B is further highlighted as an enzyme that modulates the progression of procyclic cells through mitosis, although no such role has been elucidated in blood forms. The epigenetic control of genes is crucial for cell cycle progression (G2/M transition), and a previous study showed that it is regulated by at least one histone deacetylase (DAC), the 63.5-kDa DAC4 protein in *T. brucei* (26). This study also hinted at a role for two additional deacetylases, DAC1 and -3, which were found to be essential for cell viability.

Finally, chromatin packaging, regulated in part by epigenetic mechanisms, also seems to directly contribute to gene expression in trypanosomes. There are two rather distinct observations that led to this conclusion. First, position-dependent gene
expression occurs, in some cases as a function of the life cycle stage of *T. brucei*. The experiments showed that both variant surface glycoprotein-expression site (VSG-ES) and RNA polymerase-driven reporter genes, engineered into different chromosomal locations, were differentially repressed as a function of their location relative to telomeres and to silenced genes (24). Second, monoallelic transcription of VSG is coupled with its expression at subtelomeric regions that are positioned at ‘expression site bodies’ in the blood form stages (42). Our understanding of the extent of epigenetic control in trypanosomes awaits further genetic, biochemical, and cell biological studies.

**SUMMARY**

Unprecedented discoveries have been the norm in trypanosome research. In the past, reviewers have drawn comparisons with its expression at subtelomeric regions that are positioned under ‘expression site bodies’ in the blood form stages (42). Our understanding of the extent of epigenetic control in trypanosomes is rudimentary and has not evolved to incorporate all the higher eukaryotes? Or does it indicate that the trypanosomes are highly evolved, having done away with most proteins in higher eukaryotes? Whether they play any role in the epigenetic control of gene expression in *T. brucei* is yet to be verified.

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