

Review

Transcription in kinetoplastid protozoa: why be normal?

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Abstract

Transcription in the kinetoplastid protozoa shows substantial variation from the paradigms of eukaryotic gene expression, including polycistronic transcription, a paucity of RNA polymerase (RNAP) II promoters, no qualitative regulated transcription initiation for most protein-coding genes, transcription of some protein-coding genes by RNAP I, an exclusive subnuclear location for VSG transcription, the dependence of small nuclear RNA gene transcription on an upstream tRNA gene, and the synthesis of mitochondrial tRNAs in the nucleus. Here, we present a broad overview of what is known about transcription in the kinetoplastids and what has yet to be determined.

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1. Introduction

The order kinetoplastida contains important pathogens of livestock, humans and plants (*Trypanosoma*, *Leishmania* and *Phytomonas*). These unicellular organisms represent an early branch in the eukaryotic evolutionary tree and possess a plethora of biochemical, genetic and morphological features that are either unique to the group (e.g. mitochondrial minicircles, uridine insertion/deletion editing, and glycosomes) or that are used to a greater extent than in most other organisms (e.g. glucosyl phosphatidylinositol (GPI) anchoring of membrane proteins, bent DNA, polycistronic transcription, *trans* splicing, and fragmented rRNA). From a standpoint of cellular differentiation, these diverse protozoa range from free-living cells that are capable of encystation to forms that are dependent on one or two host organisms. These developmental stages display distinct morphologic and metabolic characteristics consistent with a highly regulated level of differential protein expression.

Unlike most other organisms, this regulation is not determined qualitatively at the level of transcription initiation, with the exception of a set of variant surface glycoprotein (VSG) genes expressed exclusively in the metacyclic trypano-

mastigote stage [1]. Transcriptional rates through a polycistron do not appear to vary greatly, and protein levels may be determined very crudely by gene copy number. A feature of the kinetoplastid genome is that genes for many abundant proteins are found in multicopy tandem arrays. Post-transcriptional events, such as mRNA processing and stability, are among the varied mechanisms for differential control of protein levels in this group of organisms [2]. Nevertheless, kinetoplastid protozoa possess a variety of non-standard mechanisms for the synthesis of different RNA molecules (Fig. 1). Unexpected transcriptional features were first encountered in *Trypanosoma brucei*, where VSG mRNA was shown to be synthesized by an α -amanitin-insensitive RNA polymerase (RNAP) [3]. It was a further curiosity that different VSG mRNAs had the same 5'-end sequence, which was not found in the gene. This sequence became known as the spliced leader (SL, a.k.a. miniexon) and was ultimately found to be present at the 5'-end of all mRNAs [4]. The SL was synthesized by an α -amanitin-sensitive RNAP [5], indicating the synthesis of a single mRNA by two distinct RNAPs. Furthermore, the SL and VSG exons originated from two different chromosomes. Joining of the two distinct exons is achieved by *trans* splicing [6]. The necessity for *trans* splicing is now appreciated as the mechanism for maturing the pre-mRNAs generated by polycistronic transcription of VSG [7] and α -amanitin-sensitive housekeeping genes [8]. By this process, the mature mRNA obtains a 7-methyl guanosine (m⁷G) cap from the SL RNA as well as a poly-A tail in a coupled process [9].

Abbreviations: ESB, expression site body; GRNA, guide RNA; J, β -D-glucosyl-hydroxymethyluracil; m⁷G, 7-methyl guanosine; RNAP, RNA polymerase; SL, spliced leader; SnRNA, small nuclear RNA; SnoRNA, small nucleolar RNA; TBP, TATA-binding protein; VSG, variant surface glycoprotein.

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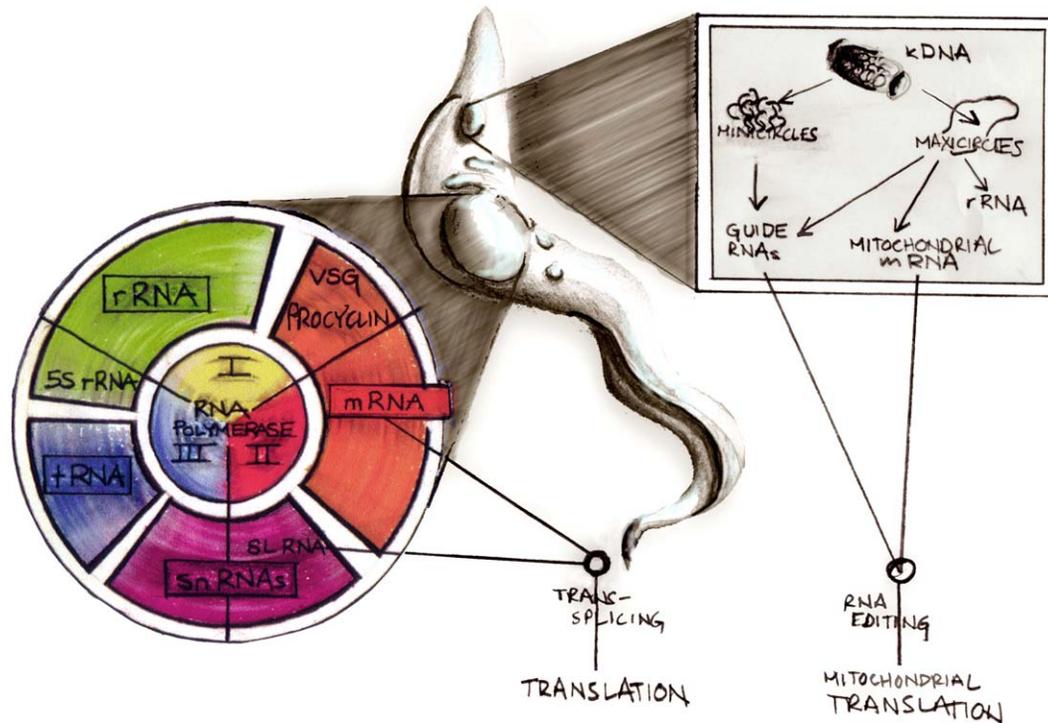


Fig. 1. The spectrum of kinetoplastid transcription. Superimposed on a promastigote form is a wheel of nuclear transcription with the three classes of RNAP, I (yellow), II (red) and III (blue) shown in the center. The transcription of different classes of genes is indicated as the outer edge overlapping the different classes of RNAP. The synthesis of distinct classes of RNAs by different RNAPs is indicated by blended colours: protein-coding mRNA (orange), snRNAs (purple) and rRNA (green). tRNA synthesized in the nucleus is trafficked through the cytosol to the mitochondrion. In the mitochondrion, transcription of mRNA, rRNA and gRNA is performed by at least one nuclear-encoded mitochondrial RNAP. Maturation of nuclear and mitochondrial mRNA by *trans* splicing or RNA editing is indicated.

The extent to which polycistronic transcription occurs in the kinetoplastids is evident from the diametric structure of chromosome 1 in *Leishmania major*, where 50 adjacent open reading frames (ORFs) are oriented unidirectionally on one strand and the remaining 29 ORFs on the opposite strand [10] (see Ref. [11] for the structure of other *Leishmania* chromosomes). A rationale for gene order in the kinetoplastids is not evident. With a few exceptions, such as the pyrimidine biosynthetic gene cluster [12], kinetoplastid polycistrons do not appear to encode functionally related proteins as found in bacterial and nematode operons [13]. It is possible that a high rate of recombination among chromosomes in the kinetoplastids has resulted in the shuffling of the gene order. A schematic of discontinuous transcription and the production of mature mRNA by *trans* splicing is presented in Fig. 2. The recent evidence for transcription initiation by RNAP II in the strand-switch region of *L. major* chromosome 1 [14] will resolve the hunt for this elusive event, but does not necessarily eliminate the possibility of low background levels of non-specific transcription of protein-coding genes by RNAP II.

Here, we summarize the current state of knowledge on kinetoplastid transcription in general and emphasize new developments and transcriptional strategies that are unique to the trypanosomes. Previous detailed reviews of topics in kinetoplastid gene expression [15–18] cite many of the original research publications that are omitted here due to space limitations.

2. Basal transcription

There is increasing experimental and bioinformatic evidence for conservation of the eukaryotic basal transcription machinery and signals in the kinetoplastid protozoa. Of the synthetic machinery, the standard four classes of RNAP have been identified in the kinetoplastid protozoa. The three classes of eukaryotic nuclear RNAP (I, II and III) have been identified by chromatography and largest-polypeptide subunit composition [19]. A summary of the known core nuclear promoter structures and interacting factors is shown in Fig. 3. Furthermore, a mitochondrial RNAP has been identified that generates mRNAs from the maxicircle DNA [20].

The conservation of *cis* signals is evident in genes for small RNAs in kinetoplastids. Consensus internal promoter elements for RNAP III have been identified in 5S rRNA and tRNA genes [21,22], as has a conserved external element for RNAP II-transcribed small nuclear RNA (snRNA) genes upstream of the SL RNA gene [23]. The biochemical characterization of *trans*-acting proteins that bind to the promoter elements is in its early stages. There is a remarkable dearth of orthologous transcription factors in the kinetoplastid databases [24]. Nonetheless, a conserved subunit of the general transcription factors for all three RNAPs [25], the TATA-box-binding protein (TBP), was revealed in genome sequencing projects from multiple kinetoplastid species (e.g. *T. brucei* AQ947932).

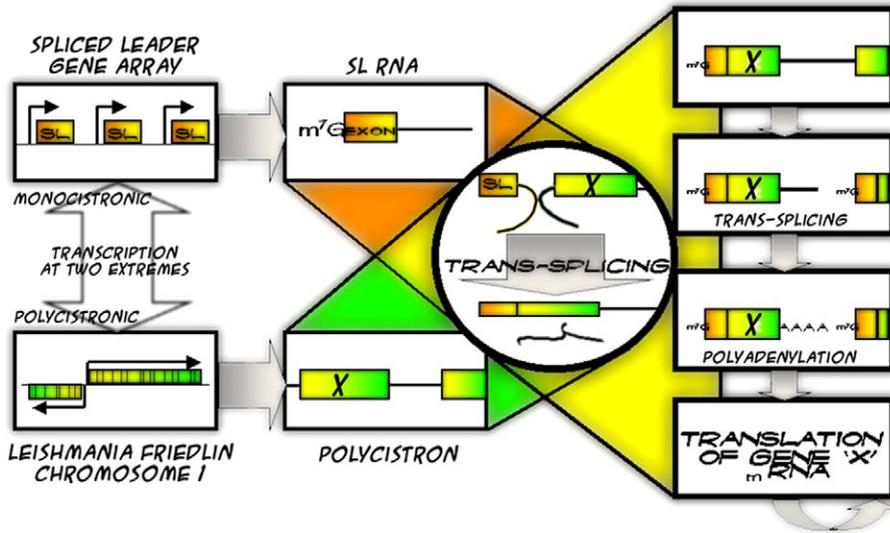


Fig. 2. Schematic of discontinuous transcription and the production of mature mRNA by *trans* splicing. Transcription of short discrete tandemly repeated genes (SL RNA) and polycistronic protein-coding regions (exemplified by *L. major* chromosome 1 [10]) by RNAP II is indicated at the left. The primary transcripts from the two classes of gene are shown in the center intersecting with steps in the *trans* splicing pathway (right panel). The bimolecular *trans* splicing reaction resembles the unimolecular *cis* splicing pathway in many aspects, including the initial cleavage of the SL RNA splice donor site, the formation of 5'-2' phosphodiester bond on a branch-point A residue, cleavage at the pre-mRNA splice acceptor site and ligation of the two exons. Differences between the two processes include the formation of a Y-branched intermediate (rather than a lariat-like intermediate) and the coupled polyadenylation of the upstream mRNA.

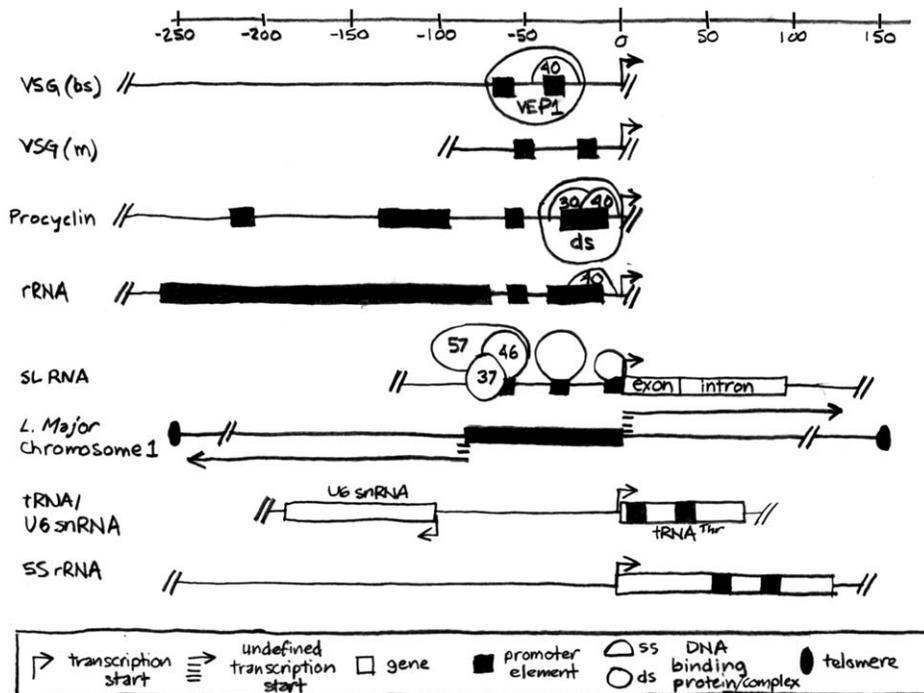


Fig. 3. The structure of promoter elements and transcription units in representative kinetoplastid protozoa. DNA sequence elements that are necessary for transcription initiation (black boxes) are shown to the left of the transcription start site (arrow); stable RNAs from each transcription unit are shown as open boxes. For the RNAP I-transcribed genes, circles represent double-strand DNA-binding proteins, and semi-circles represent single-strand binding proteins. The RNAP I and III structures are derived from publications on *T. brucei*. The RNAP II structures are derived from studies on *L. seymouri* (SL RNA) and *L. major* (protein-coding genes).

3. RNA polymerase I

In most eukaryotic cells, the role of RNAP I is the transcription of rRNA genes. In contrast, RNAP I assumes a broader significance in *T. brucei* because it performs the transcription of two sets of genes that include the developmentally regulated major surface antigens of both the insect (procyclin) and mammalian (VSG) stages of the parasite [26]. The coupling of transcription initiation and the capping of primary transcripts appear to be restricted to snRNAs and the SL RNA [27,28], while m⁷G-cap acquisition by protein-coding exons is achieved by the *trans* splicing reaction. Thus, *trans* splicing circumvents the dependency of primary RNA synthesis by RNAP II and allows this function to be assumed by other RNAPs, such as RNAP I. The two largest subunits of RNAP I have been identified and characterized [29,30], and there is an *in vitro* transcription assay available for rRNA, VSG and procyclin promoters [31].

3.1. rRNA

Transcription of kinetoplastid rRNA genes is performed by an RNAP I that is resistant to 1 mg/ml α -amanitin [32]. The kinetics of rRNA transcription in *T. brucei* [7] is similar to that of other eukaryotes, although the mature large subunit rRNA shows multiple fragmentation. The transcription start sites have been mapped in several diverse kinetoplastids [33]. In *T. brucei*, the primary transcript is not capped; 20% of the 5'-ends possess triphosphates, and 80% have a 5'-hydroxyl [34]. The general structure of the rRNA gene promoter resembles that of other eukaryotes in having a bipartite core structure in the -70 to -10 region and an upstream core element. Mapping of the rRNA gene promoter elements in *Leishmania donovani* has allowed the development of a tetracycline-inducible system for *Leishmania* [35]. A protein that binds to the *T. brucei* rRNA gene core promoter has been identified in gel-shift assays [36]. The notion that rRNA gene promoters are species specific in most cases holds true for the kinetoplastids (see [37] for references and exceptions). In this regard, it is notable in *Trypanosoma cruzi* that promoter activity may correlate with the two major genetic subdivisions [38]. At the other end of the process, transcription termination downstream of the *Leishmania infantum* rRNA gene has been associated with tandem repeats that contain Chi-like elements [39].

3.2. Protein-coding genes (VSG and procyclin transcription units)

Bloodstream trypomastigotes of the African trypanosome *T. brucei* are covered with a dense coat (5×10^6 molecules) of VSG that is changed stochastically during antigenic variation. Similarly, the insect-form midgut procyclic trypomastigotes are coated with 2.5×10^6 carbohydrate-rich molecules termed EP- and GPEET-procyclics (also designated formerly as procyclic acidic repetitive proteins or PARP [40]). Both classes of surface molecules are unusual because

the developmentally regulated levels of mRNA are synthesized by RNAP I. The forces that have driven the characterization of RNAP I transcription units include the following: a mechanistic understanding of immune evasion by antigenic variation in the African trypanosomes, in which a single expression site from a repertoire of about 20 is transcriptionally active in bloodstream forms [41,42]; the switch from metacyclic VSG to bloodstream VSG usage; and the mutually exclusive switch from expression of the VSG gene to the procyclin genes in insect stages of the parasite. A likely transcription terminator for RNAP I has been identified downstream of the last gene in one of the procyclin transcription units [43].

The VSG gene expressed in bloodstream trypomastigotes is present at the end of a long (~50 kb) transcription unit that contains 8–10 other genes, termed expression-site-associated genes (ESAGs), including the transferrin receptor and serum-resistance-associated (SRA) gene [44]. In contrast, the VSG gene expressed in metacyclic trypomastigotes is monocistronic [45], and the procyclin genes are co-transcribed with four other genes [46]. All the evidence indicates that the bloodstream VSG and procyclin promoters are constitutively active with downregulation of transcriptional activity (1) in the 'opposing' cell type, and (2) in bloodstream forms of the 19 'inactive' VSG expression sites. The silencing of inactive VSG expression sites is effective for the complete transcription unit as it affects inserted promoters [47–49] and may be delineated upstream by repetitive 50-bp elements [50]. Transcription does initiate at promoters of inactive VSG expression sites [51,52] and procyclin genes [51,53] in bloodstream forms. Such stage-inappropriate transcription appears to be attenuated by the efficiency of RNA elongation [53,54], and some control of transcription initiation is suggested by a 5- to 10-fold reduction of procyclin promoter activity in bloodstream trypomastigotes [53,55]. Thus, bloodstream VSG and procyclin expression levels may be controlled cumulatively as much as 1000-fold at the levels of transcription initiation and elongation, mRNA stability, and translation efficiency [56–58].

Metacyclic VSG genes, which are transcribed for a short time in the Tsetse fly salivary-gland stage and during early infection of the bloodstream, appear to be regulated more conventionally by transcription initiation [59]. Support for this conclusion includes the lack of detectable precursor transcripts for metacyclic VSG in procyclic trypomastigotes [1].

There is strong evidence that the expression site core promoter region plays a role in the silencing and activation of the VSG expression site [60]. The promoter regions of the VSG and procyclin promoters have been characterized extensively. There is a bipartite core promoter structure for which the elements are interchangeable, even with components of the rRNA promoter [61]. Proteins binding to the core promoter elements have been mapped by several groups. Both upstream and downstream elements of the VSG promoter were necessary for the binding of a double-strand specific protein [62], while a 40-kDa single-strand specific

protein bound to the non-coding strand of the downstream element [36,62]. This 40-kDa protein also bound to G-rich sequences in the rRNA and procyclin promoters [36], the procyclin terminator, and the telomeric repeats [63]. In addition, other proteins have been implicated in binding to the procyclin promoter. An activity with double-strand specificity, which is not shared with the VSG and rRNA promoters, bound to the core (–30) promoter [64]. In contrast, factors with single-strand specificity were detected binding to both –60 and –30 core elements [65]. The number and identity of transcription factors associated with RNAP I transcription in kinetoplastids is clearly complex. Detection (or lack thereof) of specific DNA-binding proteins can be influenced by the length of target DNA [64] used in the gel mobility shift assays. The identification of conserved RNAP I transcription factors in the kinetoplastid databases will undoubtedly speed the resolution of which proteins are involved in transcription of the protein-coding genes.

Transcription of the VSG by RNAP I suggested the possibility that the mono-allelic expression of a single telomeric copy could be controlled by inclusion in the nucleolus, the normal site of rRNA synthesis by RNAP I. Experimental evidence indicated that this was not the case [66,67]. Rather, the active expression site has been identified in a discrete, non-nucleolar location termed the expression site body (ESB) [68]. Signals that target or retain the VSG expression site in the ESB are not known. Possible candidates include histone acetylation and modification by chromatin-remodeling complexes. Consistent with this idea, histone acetylases and deacetylases are essential in *T. brucei* [69]. The role of chromatin structure in kinetoplastid gene expression has been reviewed elsewhere [17,70]. Another possible mechanism for epigenetic effects is the modification of thymine to β -D-glucosyl-hydroxymethyluracil (termed base J) [71]. Found predominantly in repeated DNA, there is a correlation between increased levels of base J in inactive expression sites and decreased levels of base J in active expression sites in bloodstream forms [72], suggesting that silencing could result from a gradient effect. Base J is absent from procyclic form DNA, indicating that it is not involved in the control of the formerly active bloodstream expression site after differentiation to the procyclic form [73]. The pathway for base J synthesis is well understood, as are the recognition determinants of a J-binding protein ([74] and references therein).

4. RNA polymerase II

4.1. Genes that encode housekeeping proteins

Transcription of housekeeping genes such as tubulin and actin is undertaken by an RNAP II that is sensitive to α -amanitin ($ID_{50} = 2\text{--}10 \mu\text{g/ml}$). The organization and transcription of housekeeping genes as polycistrons followed by rapid *trans* splicing of the pre-mRNA have prevented direct detection of the 5'-end of primary transcripts synthesized by RNAP II. In the absence of easily identifiable promoters, it

has been proposed that RNAP II may either initiate transcription randomly within a stretch of DNA or that there may be a few distantly spaced promoters ([1,17], S.M. Beverley, personal communication). Under either of these scenarios, control of transcript abundance may be determined by *cis* elements affecting RNA processing and stability [75]. A potential promoter has been identified within the *T. brucei* *hsp70* locus; however, it failed to drive transcription of a luciferase reporter gene in transient transfection assays [76]. The identification and DNA-structure analysis of strand-switch regions in the *L. major* chromosomes 1 and 3 [11,77] suggest candidate regions for promoters. Although at least one copy of the *L. major* strand-switch region on chromosome 1 was essential for cell viability, it was not necessary for transcription of a distant hygromycin-resistance marker [78]. Despite this observation, nuclear run-on analysis using strand-specific capture templates indicates a hole in transcription and increased bi-directional transcription from the strand-switch region [14]. The gold standard for proving transcription initiation, the identification of triphosphates on the primary transcript, will be difficult to demonstrate. A low level of transcription (~10%) over the entire chromosome [14] may explain the hygromycin resistance detected by Dubessay et al. [78]. As one would not expect to detect a minor promoter acting at 10% efficiency of the promoter defined by the UV-irradiation assay, the data do not indicate whether the background transcription is due to specific or non-specific initiation.

Transcription termination of RNAP II at the end of polycistronic regions has not been characterized. Candidate termination elements are likely to be found in the region between the RNAP II-transcribed microtubule-associated repetitive protein (MARF) gene and the RNAP I-transcribed procyclin genes in *T. brucei* [79]. At the downstream end of the procyclin polycistron there is overlapping antisense transcription, and presumably transcription termination, of opposing RNAP II-transcribed genes [46]. Similarly, regions where tRNA genes are interspersed among protein-coding genes [80] could contain transcription termination elements for RNAP II. However, the transcription of a CAT gene inserted between the U3 snRNA and 7SL RNA genes [80] suggests that this region is accessible to a presumed RNAP II.

In addition to stability issues, post-transcriptional control of mRNA levels may include splicing efficiency as evidenced by nucleotide elements within the intergenic regions of a *Leishmania* cysteine proteinase gene [81] that are removed during *trans* splicing.

4.2. SL RNA

The SL RNA genes are transcribed by an RNAP II [23,82] that is sensitive to α -amanitin ($ID_{50} = 8\text{--}15 \mu\text{g/ml}$). This slightly higher level of resistance than for the complex that transcribes protein-coding genes could be explained by variation in subunit composition of the RNAP II. The human RNAP II promoters for mRNA and snRNA synthesis are not

functionally interchangeable due to differential interaction with signals downstream of snRNA genes [83]. By that token, alternative subunit assembly could occur at a promoter for non-processive transcription of short genes such as the SL RNA gene, in contrast to a promoter that drives processive elongation through polycistronic protein-coding genes. In resolving pre-mRNAs by *trans* splicing, the SL RNA provides the mRNA with a cap 4 structure [84] that comprises an inverted m⁷G cap, 2'-*O*-ribose methylations of nts 1–4, and base methylations of nt 1 and nt 4. It is currently debated whether the complete cap 4 modification is co-transcriptional [28] or post-transcriptional [85]; preliminary results suggest that cap 4 or primary SL sequence may have a role in mRNA translation in *Leishmania tarentolae* (G.M. Zeiner, N.R. Sturm and D.A. Campbell, *J. Biol. Chem.* 278 (2003) 38269–75).

Transcription initiation of the SL RNA gene has been studied extensively in five kinetoplastid species [23]. There are typically 100–200 tandem head-to-tail SL RNA genes in each genome, whose transcription represents 6% of total cell RNA synthesis. The promoters for transcription are upstream of the gene and, while the precise promoter configuration varies from organism to organism, the critical *cis* elements consist of two elements at –60 and –30, and in most cases, a –5 element. The upstream element resembles the proximal sequence element (PSE) of snRNA genes in humans [23,86]. The best-characterized transcription factor in trypanosomatids binds to the –60 element of *Leptomonas seymouri* [87]. Termed PBP-1, it is a 122-kDa complex that consists of three subunits: an uncharacterized protein of 36 kDa, a dileucine-repeat subunit of 46 kDa that can be cross-linked to the promoter, and a 57-kDa polypeptide that is related to the human protein SNAP50, which is in the SNAPc complex that binds to the human snRNA gene PSE [88]. Preliminary results in *L. tarentolae* suggest that TBP is a component of the complex that binds to the –60 promoter element (S. Thomas, N.R. Sturm and D.A. Campbell, unpublished data). A second complex in *L. seymouri*, PBP-2, binds to the essential –30 element [89], and there is evidence for a protein interaction with the start-site proximal (initiator) element [90].

Downstream of the intron, all SL RNA genes possess a run of 5–31 thymidines that has been proposed to serve as a termination element. Mutagenesis of the T-tract showed that a minimum of six Ts are necessary for staggered termination on the *L. tarentolae* SL RNA gene [91]. The resulting primary SL RNA transcript thus contains a poly-U tail of heterogeneous length that is removed in an Sm-protein-binding/stem-loop III-dependent manner [92]. While termination of RNAP II transcription on the vertebrate U2 snRNA gene requires a downstream element, termination on the SL RNA gene appears to resemble rho-independent termination in bacteria.

4.3. Other small RNAs

Small nucleolar (sno) RNAs are transcribed by an α -amanitin-sensitive RNAP [93,94]. Many of the kinetoplas-

tid snoRNA genes are clustered and transcribed as longer precursors [93–95], and some can function as vehicles for RNA interference and antisense silencing of nucleolar transcripts [96]. Thus, the question is raised whether they constitute discrete transcription units containing multiple snoRNAs, or whether they are co-transcribed with a dissimilar class of genes. Are they within the introns (*cis* splicing) or transcribed spacers (*trans* splicing) of protein-coding genes, as found for some snoRNAs in other organisms [97]?

5. RNA polymerase III

The kinetoplastid RNAP III shows an intermediate level of resistance to α -amanitin (ID₅₀ = 150 μ g/ml) and recognizes the three classes of promoter defined for higher eukaryotes [98]. The first consensus eukaryotic promoter sequence to be identified in kinetoplastids was the RNAP III class 1 promoter (box A and box C elements) within the 5S rRNA gene [21]. The class 2 promoter (box A and box B elements) was found conserved within tRNA genes [22]. Preliminary identification of proteins that bind to the kinetoplastid tRNA gene promoter elements has been reported [99].

The class 3 promoter (distal- and proximal-sequence elements) typified by the mammalian U6 snRNA gene has not been found in kinetoplastids. In contrast, there is a dependence of all U-rich (U1–U6) snRNA (and other small RNA) gene transcription on the class 2 promoter of an upstream tRNA gene that is oriented in the opposite direction [18]. While the snRNA genes contain control regions just upstream (U1) or downstream (U2 and U4) of the transcription start site, all small RNA genes transcribed in this manner are found 95–99 nt upstream and on the opposite strand to the tRNA or tRNA-like promoter elements [18]. Thus, transcription of the U1–U5 genes by RNAP III in trypanosomes represents a major difference from mammals, where the corresponding genes are transcribed by RNAP II [83]. In mammals it has been proposed that the four Us in a minimal Sm-binding site, RAUUUUGG, would terminate transcription by RNAP III [83], hence the need to employ RNAP II. In trypanosomes this hypothetical limitation may have been circumvented by the absence of canonical Sm-binding sites in some U-snRNAs [100] or overcome by the evolution of a mechanism to suppress termination of RNAP III on U1 and U5 genes that contain four Us in the Sm-binding site [101,102].

Transcription of U1–U5 by RNAP III raises the issue of how the kinetoplastid snRNAs acquire their initial m⁷G cap, which is obtained co-transcriptionally during synthesis by RNAP II in other eukaryotes. The capping enzymes guanylyltransferase and N⁷G-methyltransferase in mammalian cells are associated with the RNAP II conserved-terminal-domain heptad repeats [103]. Hypermethylation of RNAP III transcripts can be induced artificially in yeast [104]; thus, an analogous mechanism may be critical for kinetoplastid RNAP III transcripts. Kinetoplastid U snRNA hypermethylation may be a one- or two-step reaction, occurring in the

cytoplasm, as in vertebrates, or in a subnucleolar body, as in yeast [105], or via an altogether different pathway that remains to be determined.

6. Mitochondrial transcription

The second transcription-competent organelle of the kinetoplastid cell is the single mitochondrion, which contains two classes of DNA molecules. Maxicircles range in size from 35 to 50 kb and are present at about 10–30 copies per cell, representing the mitochondrial genome. Minicircles range in size from 0.8 to 1.6 kb, are present at about 30 000–50 000 copies per cell, and contain the genes for guide RNAs (gRNA) that are used as templates in the uridine insertion/deletion RNA editing of maxicircle transcripts [106]. Mapping primary maxicircle transcripts has identified the 12S and 9S mitochondrial rRNA subunits and four other unassigned transcripts [107]. Similarly, the minicircle-derived gRNAs have been shown to possess 5'-triphosphates and are thus primary transcripts [108]; however, no consensus promoter elements for a mitochondrial (mt) RNAP have been identified for minicircles or maxicircles. While no mtRNAP is encoded in the maxicircle, a nuclear-encoded single-subunit mtRNAP is responsible for synthesis of maxicircle mRNA [20]. It remains to be determined whether this mtRNAP is also responsible for transcription of minicircle gRNAs.

While the mitochondrial genomes of some organisms, e.g. plants and *Tetrahymena*, may contain an incomplete complement of tRNAs, the kinetoplastid mitochondrial genomes contain no tRNA genes [109]. All kinetoplastid tRNAs are transcribed in the nucleus and imported into the mitochondrion using a variety of targeting signals [109,110].

7. Experimental aspects of transcription

A number of technical advances have been important for transcriptional studies in kinetoplastids. In vivo transcription studies were dependent on the development of a means (i.e. electroporation) to introduce recombinant DNA into the cell artificially and on the development of artificial plasmids that are maintained stably by drug selection. Artificial plasmids that are maintained as multicopy, catenated forms in *Leishmania* and *T. cruzi*, and linear artificial chromosomes have been the most useful for transcription studies. No specific promoters appear necessary for transcription initiation of the episome's selectable-marker gene; transcription appears to continue in a random run-around manner on both strands [111], although potential regulatory elements may be present [112]. In vitro assays that recapitulate transcription in vivo have been developed for the kinetoplastid RNAP I [31], RNAP II [113], and RNAP III [114]. Such assays provide the basis for depletion/restoration and reconstitution experiments with putative transcription factors.

As with RNAP I, the uncoupling of transcription initiation from RNA processing (capping and *cis* splicing) means that

exogenous RNAPs can be effective in kinetoplastids. To this end, bacteriophage T3 and T7 RNAPs have been used successfully for the production of RNA and protein [115]. Most significant for this application is the ability to regulate T7 RNAP transcription by the tetracycline operator–repressor system [116], thus allowing the controlled expression of toxic products. A second major role for this approach is the regulated production of double-stranded RNA-directed mRNA degradation (dsRNA interference; RNAi) in ‘knock-down’ experiments [117]. RNAi has become the popular standard for assaying protein function in *T. brucei*; however, it does not appear to work in *Leishmania* [118].

8. Perspectives

The emerging details of transcriptional mechanisms by the three RNAPs in kinetoplastids have revealed similarities to, and substantial departures from, the paradigms of eukaryotic transcription. With a focus on the control of surface antigen gene expression and transcription by RNAP I, important topics to be addressed include the characterization of transcription factors specific for VSG and the identity, targeting signals and composition of the ESB. In addition, chromatin structure, histone acetyl transferases, and base J may play a role in transcription/silencing of the VSG expression site. For RNAP II, key questions to be answered include the similarity of the SL RNA gene transcription factor complex with the SNAPc complex from humans; the mechanism of transcription initiation in strand-switch regions between protein-coding polycistrons; and determining what, if any, interactions occur between the conserved-terminal-domain of RNAP II and RNA processing factors in the RNAP complexes assembled for short or processive transcription. The major mechanistic models to be derived for RNAP III include how a single set of class 2 promoter elements direct transcription of pairs of opposing small RNA genes, and how the m⁷G-capping machinery that is usually bound to the RNAP II conserved-terminal-domain associates with RNAP III. Undoubtedly, there will be further surprises as we learn more about the details of transcriptional regulation in these organisms.

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