CMLS, Cell. Mol. Life Sci. 62 (2005) 2194–2203
1420-682X/05/202194-10
DOI 10.1007/s00018-005-5198-9

CMLS Cellular and Molecular Life Sciences

Review

RNA uridylyltransferases

R. Aphasizhev

Department of Microbiology and Molecular Genetics, University of California, Irvine, B240-Medical Sciences I, California 92697 (USA), Fax: +1 949 8249394, e-mail: ruslan@uci.edu

Received 10 May 2005; received after revision 7 June 2005; accepted 16 June 2005
Online First 15 September 2005

Abstract. The terminal RNA uridylyltransferases (TUTases) catalyze transfer of UMP residues to the 3′ hydroxyl group of RNA. These activities are widespread among eukaryotes and appear to be involved in a variety of RNA-processing pathways. Recent studies of RNA editing in trypanosomatids have provided the first insights into the biological functions of RNA uridylyltransferases, which had eluded biochemical identification despite 30-year-old evidence of such activities in mammals and plants. Comparative sequence analysis of trypanosomal TUTases and their homologs revealed by large-scale genomic projects demonstrates a significant level of biochemical and structural diversity between putative uridylyltransferases. The conserved catalytic domain has acquired additional protein modules and appears to have adapted to perform functionally distinct tasks of guided U-insertion into mRNA and constrained addition of an oligo[U] tail to guide RNAs. Here I discuss the current knowledge of this novel enzyme family and possible roles of RNA uridylylation in the regulation of gene expression.

Key words. Uridylyltransferase; TUTase; RNA editing; editosome; *Trypanosoma*; *Leishmania*; gRNA; U6 snRNP; cytoplasmic poly(A) polymerase.

Introduction

Just as with another homopolyribonucleotide transferase, poly(A) polymerase, the activity that adds UMP residues to RNA was discovered long before its biological functions could be envisaged. The first reports of template-independent poly(U) polymerase activity in rat liver date back more that 30 years [1, 2]. Subsequent work identified UTP-specific transferase activity associated with cytoplasmic ribosomes [3]. In cowpea (*Vigna unguiculata*), the terminal RNA uridylyltransferase (TUTase) has been characterized as an ~50-kDa polypeptide that catalyzes transfer of single UMP residues to polyribonucleotide substrate [4]. Renewed interest in poly(U) polymerase was sparked by the hypothesis that a terminal uridylyltransferase can participate in initiation of minus-strand synthesis by poliovirus replicase, thus serving as a host factor required for replication of poliovirus [5]. This activity, originally detected in initiation factor 2 (eIF2) preparations from rabbit reticulocyte ribosomal salt wash, appears to be similar to one reported by Milchev and Hadjiolov [3]. Further studies established that in HeLa cells, partially soluble and partially ribosome-associated ~68-kDa factor requires an RNA primer and possesses a template-independent poly(U) polymerase activity [6, 7]. The current model of picornaviral genome replication does not involve TUTase [for a review see ref. 8] and the gene for the host factor has not been identified. The observations that several small cellular RNAs in human cells contain non-encoded uridylyl residues [reviewed in ref. [9] prompted re-examination of possible TUTase functions in HeLa cells and resulted in separation of at least two distinct TUTases.

Post-transcriptional mRNA editing and TUTases

Most current knowledge on TUTases comes from studies of parasitic protists from the order Kinetoplastida,

whose mitochondria post-transcriptionally edit their mRNAs, specifically trypanosomes (type sp. *Trypanosoma brucei*) and Leishmania (type sp. *Leishmania tarentolae*). These organisms possess a single tubular mitochondrion, which contains arguably the most unusually organized genome, the kinetoplastid DNA (kDNA) [10]. The single network of catenated minicircles and maxicircles is positioned within the mitochondrial matrix adjacent to the basal body of the flagellum. Maxicircles are the informational carriers and contain 18 clustered protein-coding genes and two rRNA genes. The transcripts of 12 genes undergo post-transcriptional editing by the insertion and deletion of uridine (U) residues, thereby correcting frame-shifts, creating start or stop codons and, occasionally, contributing more than half of the mRNA coding sequence. The minicircles encode guide RNAs (gRNAs), which are complementary to mature edited sequences [11]. The gRNAs have 3′ non-encoded oligo(U) tails [12]. The editing process is accomplished by concerted action of endonucleases, uridylyltransferase or 3′-5′ U-specific exonuclease and RNA ligase organized in a multi-protein complex of ~1.6 MDa, the editosome. Site specificity and number of added or deleted uridylyl residues are directed by gRNA, likely with participation of additional factors [for reviews see refs. 13, 14].

Trypanosomal TUTase activity, which, similar to that in *V. unguiculata* [4], adds a single UMP residue to ribosomal RNAs, was first found by White and Borst in total cellular extracts of *T. brucei* [15]. This finding attracted particular interest due to the discovery of U-insertion editing in trypanosomal mitochondria just a year earlier [16]. In an attempt to trace putative enzymatic activities that may be responsible for the incorporation of uridilylates into mitochondrial messenger RNAs during the editing reaction, Bakalara et al. [17] demonstrated the presence of TUTase in highly purified mitochondria of *L. tarentolae*. However, in mitochondrial extract, TUTase activity added multiple Us to endogenous RNAs suggesting that the mitochondrial and cytoplasmic TUTases in *L. tarentolae* may be distinct enzymes. This work started a quest for trypanosomal mitochondrial TUTase that lasted well over a decade. From the studies on trypanosomal [18] and *Leishmania* [19, 20] systems, it soon became clear that TUTase activity is associated with heterogeneous high-molecular-weight RNA-protein complexes in both organisms, ranging from 10 to 45S, as determined by velocity sedimentation in glycerol gradients. Development of internal uridine incorporation [21] and full-round in vitro editing assays [22, 23] in the mid-1990s indicated that an ~20S complex was the particle with the highest specific U-insertion/deletion RNA-editing activity. This concept was further strengthened by co-purification of in vitro editing activity with an ~20S particle through conventional chromatography columns and glycerol gradients [24].

Trypanosomal TUTases: a new theme in template-independent transferases

The general chemical mechanism of nucleotide transfer onto the 3′ end of a nucleic acid chain is conserved throughout evolution yet is catalyzed by several distinct enzyme superfamilies. During RNA or DNA polymerization reactions, acidic and/or polar residues in the active site co-ordinate two divalent metal ions, most commonly Mg^{2+}, so as to position the triphosphate moiety of the α-phosphate of the incoming 5′ nucleoside triphosphate for the in-line nucleophilic attack by the 3′ hydroxyl of the growing polynucleotide chain. This results in the formation of a phosphodiester bond and a pyrophosphate leaving group [25]. Although sharing basic catalytic chemistry, the catalytic modules of template-dependant RNA polymerases have been divided into at least five evolutionarily unrelated folds based on sequence and structural comparisons [26]. In contrast, all template-independent RNA polymerases possess a highly conserved catalytic domain and belong to the superfamily of nucleotidyltransferases exemplified by eukaryotic DNA polymerase β (Pol β) [27, 28]. The sequence-based classification distinguishes nine groups within the Pol β superfamily that are involved in a variety of biological processes including mRNA polyadenylation, tRNA processing, antibiotic resistance, signal transduction and many others [27]. The homoribonucleotide polymerases, which are specific for only one species of nucleoside triphosphate, are represented by eukaryotic [29], bacterial [30] and viral poly(A) polymerases [31]. Poly(A) polymerases from different kingdoms belong to distinguishable groups sharing only the most conserved features characteristic of the entire Pol β superfamily. RNA uridylyltransferase activities appear to be restricted to eukaryotic organisms.

Identification of the first gene for a TUTase was achieved by chromatographic enrichment of the major UMP-incorporating activity from purified mitochondria of *L. tarentolae* [32]. The nearly homogenous preparation contained a polypeptide of ~140 kDa, which was used for gene cloning by reverse genetics methods. The single-copy gene encoded a 1120-amino-acid protein that, upon expression in *Escherichia coli*, displayed enzymatic activity virtually identical to that of the highly purified mitochondrial enzyme. This protein has been termed RNA-editing TUTase 1 (RET1). Unlike eukaryotic poly(A) polymerases, recombinant RET1 does not require additional protein factors for robust UMP polymerization activity: in the presence of Mg^{2+} ions, processive polymerization of several-hundred-nucleotide-long products is observed. Substitution of Mg^{2+} with Mn^{2+} relaxes TUTase nucleotide specificity, similar to the extensively documented properties of poly(A) polymerases [33]. Size-fractionation and chemical cross-linking experiments suggested a homotetrameric organization of the enzyme, which is

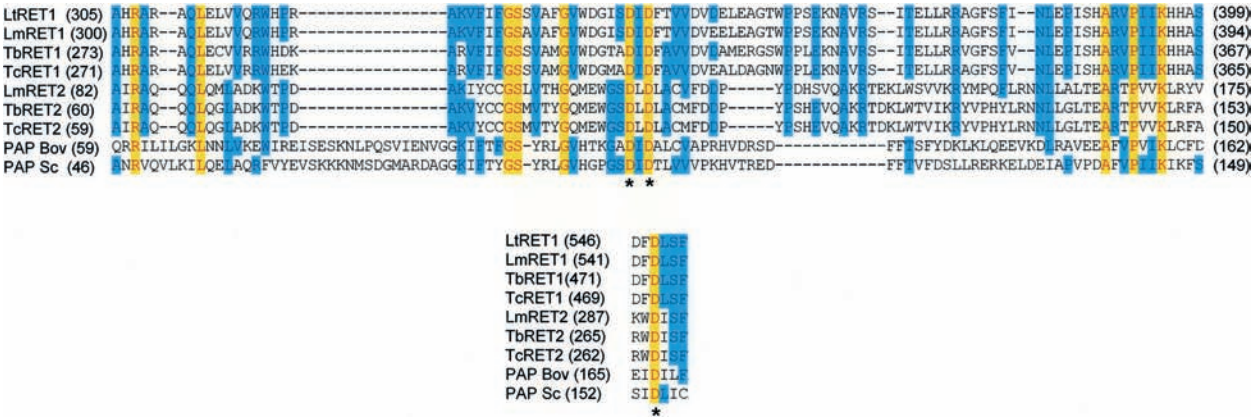

Figure 1. Partial multiple-sequence alignment of RNA-editing TUTases and nuclear poly(A) polymerases. Metal-co-ordinating aspartate residues are indicated by asterisks. Identical residues are red on yellow background; blocks of similar residues are black on blue background.

rather unusual among nucleotidyltransferases. Sequence comparisons revealed a signature motif closely matching the hG[G/S](x9–13)DxD[D/E]h (x stands for any amino acid, h hydrophobic) consensus for the Pol β superfamily signature sequence. Iterative Blast-PSI searches of sequence databases and phylogenetic comparison of the RET1 group with eukaryotic poly(A) polymerases, 2′-5′ oligoadenylate synthetases (OASs), TRF4/5 poly(A) polymerases and archaeal CCA-adding enzymes showed that the RET1 group is an apparently monophyletic assemblage characterized by a set of highly conserved sequence motifs and frequent fusion to a putative nuclease domain [34], albeit nuclease activity has not been detected in RET1 preparations [32]. Alanine-scanning mutagenesis of RET1 TUTase at positions conserved between RET1 and eukaryotic poly(A) polymerases [29] identified three carboxylate residues as crucial for activity: two aspartates of the signature sequence and the third aspartate located 202 amino acids toward the C terminus [35] (fig. 1). In eukaryotic poly(A) polymerases with known three-dimensional structure, the third residue is separated from the signature sequence by ~50 amino acids [29], but all three carboxylates lie in close proximity in three-dimensional space, permitting co-ordination of divalent metal ions [36–38]. The linker sequence forms a loop that contains a cluster of highly conserved amino acids, which has been proposed to bind the 3′ end of the RNA substrate [39]. In RET1, the extra 150-amino-acid insertion (fig. 2) appears to constitute a helix-rich domain essential for function: deletion of this region abolished enzymatic activity of the recombinant protein [35]. The delineation of the catalytic and central domain in RET1 (fig. 2) is based on homology with known structures of eukaryotic poly(A) polymerases [36, 37]. No relationship could be detected between the poly(A) polymerase RNA-binding domain and RET1. The poly(A) polymerase-associated domain, as defined in the PFAM database (http://pfam.wustl.edu), is typically found in many proteins that also contain a catalytic nucleotidyltransferase domain. Overlapping oligomerization and RNA-binding regions in RET1 have been mapped, albeit with low resolution, by sequential C terminus deletions [35]. These regions have no recognizable motifs or similarities with any other proteins beyond kinetoplastid RET1 species, suggesting the presence of a divergent, possibly function-specific, structural fold.

In addition to the processive RET1 enzyme, another TUTase activity has been reported, which has a distributive character: it adds one UMP residue to a single-stranded RNA substrate per RNA-binding event [40]. The first report of editosome purification by conventional chromatographic techniques indicated that a TUTase activity was present in the complex but did not detect the candidate uridylyltransferase among seven major polypeptides [24]. Application of immunoaffinity purification methods and mass spectrometry for analysis of the core RNA-editing complex, the editosome, identified a candidate enzyme in *T. brucei* [41] and *L. tarentolae* [42]. This second TUTase was dubbed RET2. *Leishmania* RET2 shared similarity with RET1 in the catalytic and central

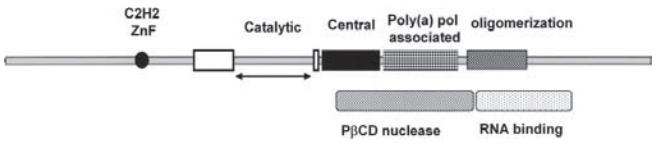

Figure 2. Schematic representation of putative domains in RET1 TUTase. The oligomerization and RNA-binding domains were determined by deletion analysis [35]. The PβCD nuclease region of OAS is indicated according to Rogozin et al. [34]. The catalytic and central domain reflect partial homology to respective regions of eukaryotic poly(A) polymerases [36, 37]. The 150-amino-acid insertion is shown by the arrow. The C2H2 zinc finger and poly(A)-polymerase-associated domains are shown as predicted by searching the Pfam protein families database at http://pfam.wustl.edu/.

domains [42] but, remarkably, was half the size and lacked the zinc finger domain, which is essential for RET1 activity [35]. Thus, mitochondrial terminal uridylyltransferases display significant diversity in structure and function.

Based on the structure of bovine poly(A) polymeraze co-crystallized with ATP in the presence of manganese ions [37], several residues in the catalytic domain have been proposed to form contacts with the adenine base. All these positions are conserved among TUTases and poly(A) polymerases making them unlikely nucleotide selection determinants (fig. 1). A recent high-resolution structure of bovine poly(A) polymerase suggests that nucleotide specificity may be determined by contacts between the middle domain and incoming ATP [38]. The conservation of the catalytic mechanism among nucleotidyltransferases apparently imposes rigid constraints on the evolution of the catalytic domain, whereas nucleotide substrate specificity likely resides in the more divergent central domain.

TUTase functions in mitochondria of trypanosomes

Immunochemical and RNA cross-linking studies of RET1 interactions in mitochondrial extract indicated that nearly one-third of the total amount of gRNA is bound to RET1 or RET1-containing complexes [32]. Sub-stoichiometric amounts of RET1 could be detected in an affinity-purified complex of gRNA-binding proteins, MRP1/MRP2 [43], but also in the ~20S core editing complex [42]. Association of RET1 with MRP1/MRP2 and the 20S editosome depends on RNA, although the nature of the RNA component remains unknown. Glycerol gradient fractionation of mitochondrial extract followed by native gel analysis allowed detection of at least five RET1-containing complexes ranging between 500–1600 kDa, of which one (~650 kDa) was stable enough to withstand partial chromatographic purification [32]. Clearly, RET1 is part of heterogeneous complexes, which may reflect interactions with distinct binding partners involved in facilitating RET1 enzymatic functions. Alternatively, due to high intrinsic affinity for poly(U) RNA, RET1 may remain bound to its substrates, uridylylated RNA molecules, as they enter different functional complexes, e.g. the editosome. These protein-protein and RNA-protein contacts await further investigation. The down-regulation of RET1 expression in *T. brucei* by RNA interference resulted in inhibition of cell growth and mitochondrial insertion/deletion RNA editing [32]. The deleterious effect on mitochondrial translation and thus assembly of respiratory chain complexes is the most likely cause of the RNAi-induced growth phenotype [44]. The steady-state abundance of edited mRNAs decreased dramatically for all transcripts examined, except cytochrome oxidase subunit II mRNA (COII). This transcript is edited by the cis-acting RNA sequence located in the 3′ untranslated region [for references see ref. 45] and apparently lacks the post-transcriptionally added oligo(U) tail – a hallmark feature of trans-acting gRNAs [12].

RET2 has been identified as an integral editosome component in *T. brucei* [46] and *L. tarentoale* [47]. Analysis of RET2 interactions within the editing complex revealed contacts with the zinc-finger-containing protein, TbMP81, which also interacts with RNA-editing ligase 2 (REL2) forming a U-insertion sub-complex [48]. Functions of RET1 and RET2 have been resolved by evaluating in vivo mRNA editing in the respective *T. brucei* RNAi cell lines [47]. RNAi down-regulation of both TUTases had a similar inhibitory effect on in vivo editing of all examined transcripts with the exception of COII mRNA, where the RET2 RNAi consequences were much more profound. Depletion of RET2 also led to a decrease in relative abundance of editosomal proteins TbMP81 and REL2 RNA ligase, although the overall integrity of the 20S editosome was not affected. This is consistent with the notion that components of the U-insertion sub-complex are involved in extensive interactions essential for their successful assembly into the editosome. In any event, the assembly of the U-insertion sub-complex, but not the entire 20S editosome, appears to be dependent on the presence of both TbMP81 [49] and RET2 [47] proteins but not REL2 [50].

The pre-cleaved editing assays that bypass the requirement for initial endonucleolytic cleavage proved instrumental for dissection of individual enzymatic reactions [51]. The 20S editosome partially purified from RET2-depleted mitochondria was virtually inactive in the pre-cleaved U-insertion assay, whereas depletion of RET1 had a minimal effect [47]. The in vitro U-deletion editing activity of the 20S editosome was unaffected in both cases, in agreement with the suggestion that U-deletion and U-insertion pathways are functionally segregated within the editing complex [48, 52, 53]. The most plausible explanation for RNA editing inhibition by RET1 RNAi comes from one set of data demonstrating shortening of the gRNA oligo(U) tail in RET1-depeleted *T. brucei* cells [47]. This may explain the differential inhibitory effect of RET1 and RET2 RNAi on U-insertion into COII mRNA, the only site edited by a guiding sequence lacking an oligo(U) tail. These findings for the first time suggested an essential role for a post-transcriptionally added oligo(U) tail in gRNA function. Stabilization of a hybrid between gRNA and purine-rich pre-mRNA may be important for the initiation of editing [12], particularly for gRNAs with short anchor sequences. Indeed, a study of cytochrome b mRNA interacting with cognate gRNA demonstrated the possibility of such contacts occurring in vitro [54]. Alternatively, the oligo(U) tail may serve as a '3′ tether' following endonucleolytic cleavage maintain-

ing pre-mRNA fragments in close proximity required for efficient U-insertion/deletion and ligation. The oligo(U) tail was not required for full-round in vitro editing [22, 23, 55] but the modification of the 3′ terminal ribose led to inhibition of U-insertion [56]. Stabilization of the mRNA 5′ cleavage fragment-gRNA interaction by extending Watson-Crick base-pairing significantly enhanced in vitro editing assays [51, 57–59]. The exact role of the oligo(U) tail still remains a mystery. It is entirely possible that initial gRNA-mRNA hybrid formation and stabilization of cleavage products both require an oligo(U) tail. In addition, a potential role as binding site for protein factors should not be neglected as several polypeptides from mitochondrial extracts have been shown to bind specifically to poly(U) RNA in vitro [60, 61]. Guide RNAs are primary transcripts with homogenous 5′ ends that can be readily labeled with guanylyltransferase in the presence of [α-^{32}P]GTP [11, 62, 63] indicating that they have a 5′ diphosphate or triphosphate. The 3′ ends of gRNAs are also remarkably uniform in sequence [64]. It is not clear whether precise termination of transcription or 3′-end-processing events are responsible for generation of pre-gRNA substrates for RET1 TUTase. The average oligo(U) tail length has been put at ~15 nucleotides [12] and is strikingly different from the extension profiles of highly purified or recombinant RET1: in vitro this enzyme adds several hundred nucleotides to a single-stranded RNA primer [32]. It appears that RET1 processivity is tightly regulated in vivo in the editing complex by a yet unknown mechanism, which may be coordinated with the exonucleolytic 3′ end processing. Indeed, a candidate processing activity, the U-specific 3′-5′ exonuclease, has been partially purified from *Leishmania* mitochondria [65]. Unlike RNA editing exonuclease 1 (REX1), which is stably associated with the 20S editosome [66], this exonuclease has been chromatographically separated from RNA ligase and RET1 TUTase and characterized as a ~75-kDa protein [65]. The gene for this protein, however, has not been identified, leaving a remote possibility that the observed activity was an indication of REX1 dissociating from the 20S editosome.

Considering the presence of uridylate residues within the poly(A) tails of mitochondrial mRNAs [67, 68] and uridylylation of ribosomal RNAs [69], involvement of RET1 in mitochondrial processes other than RNA editing appears to be plausible. For example, Read's laboratory

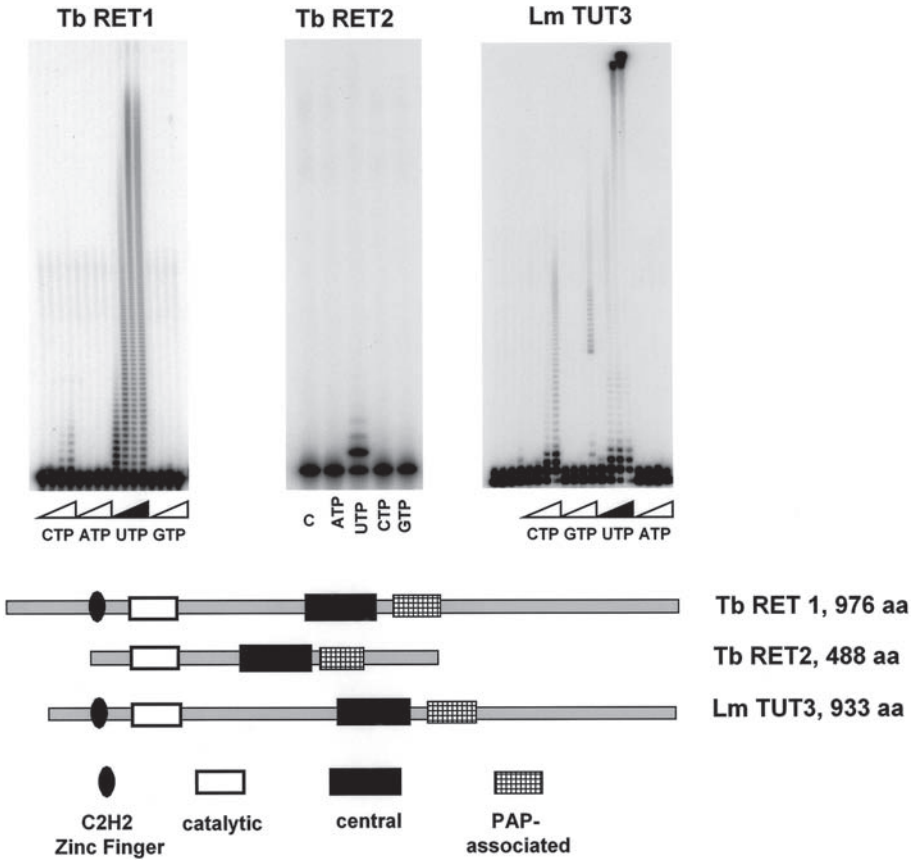

Figure 3. Domain organization and processivity of trypanosomal TUTases. Purified recombinant enzymes were incubated with synthetic 5′-labeled RNA primer in the presence of ribonucleotide triphosphates. Products were separated on a 15% polyacrylamide/urea gel. PAP, poly(A) polymerase.

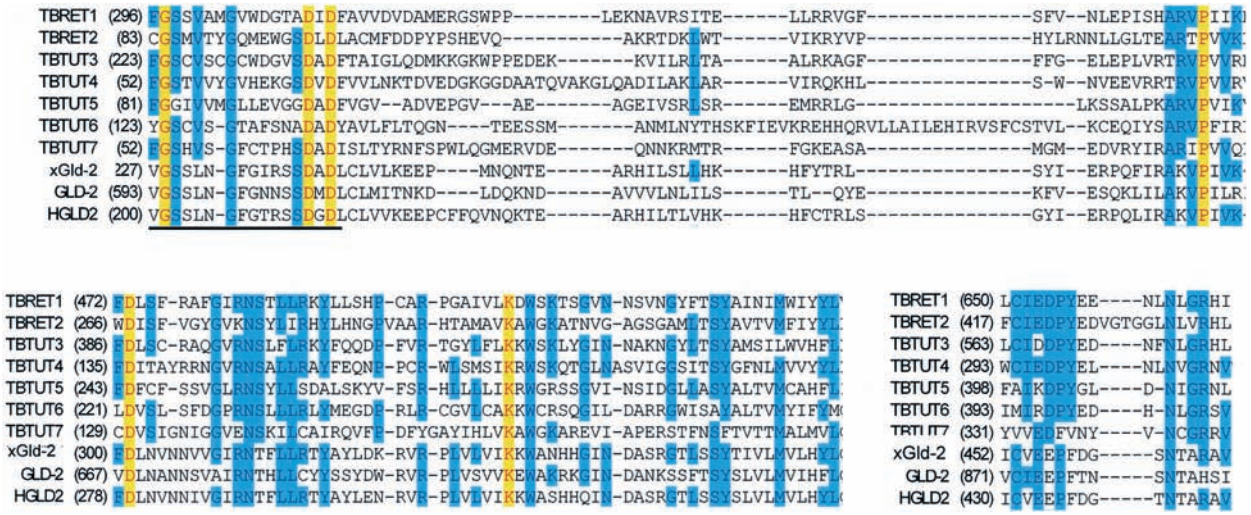

Figure 4. Partial sequence alignment of RNA-editing (RET1, RET2) and putative trypanosomal (TUT3–7) TUTases with cytoplasmic poly(A) polymerase. xGld-2, Gld-2 and hGld2 are cytoplasmic poly(A) polymerases from *Xenopus laevis*, *Caenorhabditis elegans* and humans. The signature sequence of the Pol β superfamily is underlined. Residues are color-coded as in figure 1.

recently demonstrated that UTP polymerization by RET1 is required for UTP-dependent mRNA degradation in *T. brucei* mitochondria [70]. The role of RET1 in this phenomenon is still unknown. When considering possible RET1 targets in mRNA degradation, one may also take into account a robust RNA primer-independent UTP polymerization activity of RET1 [35].

The accuracy and efficiency of RET2-catalyzed U-insertion into mRNA are crucial for the overall fidelity of RNA editing. A study by Igo et al. [58] showed that base-pairing of an extended 5′ cleavage fragment with guiding nucleotides and the thermodynamic stability of the double-stranded region immediately surrounding the editing site contribute to the efficacy of U-insertion editing. The insertion of Us is dictated by the intrinsic selectivity of RET2 for UTP and not by the nature of guiding nucleotides. The base-pairing of newly added Us with the gRNA, however, enhances the insertion of subsequent bases, perhaps by restoring the optimal structure of the RET2 substrate. Ultimately, ligation of correctly extended 5′ cleavage fragment to the 3′ fragment of mRNA is also stimulated by base-pairing with guiding nucleotides [71].

Non-editing TUTases of trypanosomes

U-insertion/deletion RNA editing is a unique process enabling expression of pseudogenes in the mitochondrial genome [72]. This phenomenon is known only in kinetoplastids and provides little clue to the biological roles of UTP-specific transferases in other species. Sequence comparisons and phylogenetic analyses, however, identified TUTase homologs that are predicted to share the UTP specificity to RET1 and RET2. The closest homolog of RET1 and RET2 in the trypanosomal databases is the TUT3 protein from *T. brucei* [73] and *L. major* (fig. 3). This highly processive enzyme is not targeted to the mitochondria and its extension pattern resembles that of RET1. A C2H2 zinc finger which is essential for activity in RET1 is also found in TUT3 (fig. 3). The nucleotide specificity and biological functions of RET1/RET2 homologs in trypanosomatids (figs. 3, 4) remain to be investigated. Remarkably, the closest non-trypanosomal matches represent a recently discovered family of cytoplasmic poly(A) polymerases (fig. 4). The growing list of functions for members of this family includes involvement in germline development in *Caenorhabditis elegans* [74], the S-M checkpoint [75] and adenylation of specific mRNA(s) [76] in *Schizosaccharomyces pombe* and cytoplasmic polyadenylation in *Xenopus laevis* [77]. The common feature of cytoplasmic poly(A) polymerases appears to be lack of a C-terminal RNA-binding domain characteristic for nuclear poly(A) polymerases [74]. Because of low affinity for RNA substrates, members of the Gld-2 family require an RNA-binding factor(s) to bind the specific mRNA(s) and yield robust polyadenylation in the cytoplasm [78]. Similarities between TUTases and cytoplasmic poly(A) polymerases do not end at the sequence homology level: the recombinant CID1 from *S. pombe* is both a poly(A) and poly(U) polymerase in vitro [75]. Given that intracellular ATP and UTP concentrations [79] are both at least ten times higher than their respective apparent Kms for TUTases [35, 73], the availability of substrate is unlikely to dictate the nature of polymerization product in vivo. Considering the virtual absence of transcriptional control in trypanosomes [80], extension of the poly(A) tail, which in other organisms

often promotes translation and mRNA stability [81], may be one of the mechanism for post-transcriptional regulation of gene expression.

As we learn more about non-editing TUTases, questions arise whether kinetoplastid TUTases and their homologs in other Metazoa have conserved or divergent mechanisms of UTP recognition. A search for TUTase inhibitors aimed at the UTP binding site may produce compounds that will block not only RNA editing but also other pathways in trypanosomes, making TUTases attractive drug targets. The crucial question for the prospects of TUTase-based trypanocides comes down to whether human and trypanosomal TUTases can be differentiated at the level of medicinal chemistry. This underlines the importance of identifying and establishing biochemical functions and atomic structures of human TUTases discussed below.

Human uridylyltransferases

The observations that several small cellular RNAs in human cells contain non-encoded uridylyl residues [reviewed in ref. [9] prompted re-examination of possible TUTase functions in HeLa cells and resulted in separation of at least two distinct TUTases [82]. The involvement of RET2 TUTase in guided transfer of genetic information appears to be an exception among uridylyltransferases. Typically, TUTases are involved in the 3′ end processing of RNAs. Several laboratories have demonstrated that incubation of HeLa cell extracts with [α-^{32}P]UTP leads to 3′ end labeling of many short RNAs, including the 5S RNA and U6 snRNA [83–85]. Lund and Dahlberg [83] showed that in approximately 90% of total U6 snRNA, the post-transcriptionally added 3′-terminal uridylyl residue has a 2′,3′ cyclic phosphate. The remainder of the U6 population carries stretches of up to 20 Us [83, 86]. Although human TUTases remain elusive, Benecke's laboratory has characterized two putative activities with different RNA substrate requirements: the non-specific and U6-snRNA-specific TUTases [82]. The latter enzyme catalyzes the regeneration of the 3′ end to restore that of a newly synthesized U6 snRNA transcript: a total of four UMP residues are added. [87]. Such an activity is unlikely to be responsible for addition of the non-coded oligo(U) tails found on a sub-population of U6 snRNAs, but rather may be charged with rebuilding transcriptionally encoded uridines removed by a nuclease specific for the 3′ end of U6 snRNA [88]. The authors note that tight control of U6-specific TUTase activity comes largely from specificity for RNA structure and not template-dependent reactions [87]. The four encoded uridines at the 3′ end are likely to be important for the formation of a stem-loop element required for structural rearrangements of U6 snRNA [89]. The 3′ end structure of newly synthesized or restored U6 snRNA is a binding site for La protein, which in turn is essential for U6 RNA stability and assembly of the U6 snRNP particle [90, 91]. The U6 snRNA lacks a binding site for SM proteins. Instead, formation of the U6 snRNP involves binding of seven Sm-like proteins to the stretch of non-coded uridylyl residues at the 3′ end [92]. The many peculiarities of U6 biogenesis are beyond the scope of this review [see ref. 93] but the presence of a sophisticated system that controls the state of the 3′ end by uridylylation/deuridylylation, thus probably affecting U6 snRNP nuclear retention, assembly and functions, is apparent. Not clear yet is how many TUTases exist in mammalian cells. Work in the author's laboratory identified three non-mitochondrial and thus not involved in RNA editing, UTP-specific enzymes in trypanosomes [R. Aphasizheva et al., unpublished data]. Interestingly, the highest-scoring homolog of editing and non-editing trypanosomal TUTases in the human genome are the recently identified cytoplasmic poly(A) polymerases, including hGld-2 [78] (fig. 4). Therefore, the possibility still remains that human and trypanosomal TUTases are not homologous proteins.

Identification of TUTases in higher eukaryotes and accumulation of knowledge about trypanosomal non-editing TUTases will ultimately answer questions on the evolutionary conservation of UTP specificity and TUTase functions. The intriguing relationship between TUTases and cytoplasmic poly(A) polymerase also remains to be resolved.

Acknowledgements. I thank L. Simpson (UCLA), P. Gershon (UCI) and A. Mushegian (Stowers Institute) for valuable comments on the manuscript. Work in the author's laboratory is supported by NIH grant AI064653.

1 Wilkie N. M. and Smellie R. M. (1968) Chain extension of ribonucleic acid by enzymes from rat liver cytoplasm. Biochem. J. **109:** 485–494
2 Wilkie N. M. and Smellie R. M. (1968) Polyribonucleotide synthesis by subfractions of microsomes from rat liver. Biochem. J. **109:** 229–238
3 Milchev G. I. and Hadjiolov A. A. (1978) Association of poly(A) and poly(U) polymerases with cytoplasmic ribosomes. Eur. J. Biochem. **84:** 113–121
4 Zabel P., Dorssers L., Wernars K. and Van Kammen A. (1981) Terminal uridylyl transferase of *Vigna unguiculata*: purification and characterization of an enzyme catalyzing the addition of a single UMP residue to the 3′-end of an RNA primer. Nucleic Acids Res. **9:** 2433–2453
5 Dasgupta A., Zabel P. and Baltimore D. (1980) Dependence of the activity of the poliovirus replicase on the host cell protein. Cell **19:** 423–429
6 Andrews N. C. and Baltimore D. (1986) Purification of a terminal uridylyltransferase that acts as host factor in the in vitro poliovirus replicase reaction. Proc. Natl. Acad. Sci. USA **83:** 221–225
7 Andrews N., Levin D. and Baltimore D. (1985) Poliovirus replicase stimulation by terminal uridylyl transferase. J. Biol. Chem. **260:** 7628–7635

8 Agol V. I., Paul A. V. and Wimmer E. (1999) Paradoxes of the replication of picornaviral genomes. Virus Res. **62:** 129–147
9 Perumal K. and Reddy R. (2002) The 3′end formation in small RNAs. Gene Expr. **10:** 59–78
10 Lukes J., Guilbride D. L., Votypka J., Zikova A., Benne R. and Englund P. T. (2002) Kinetoplast DNA network: evolution of an improbable structure. Eukaryot. Cell **1:** 495–502
11 Blum B., Bakalara N. and Simpson L. (1990) A model for RNA editing in kinetoplastid mitochondria: 'guide' RNA molecules transcribed from maxicircle DNA provide the edited information. Cell **60:** 189–198
12 Blum B. and Simpson L. (1990) Guide RNAs in kinetoplastid mitochondria have a nonencoded 3′ oligo(U) tail involved in recognition of the pre-edited region. Cell **62:** 391–397
13 Simpson L., Aphasizhev R., Gao G. and Kang X. (2004) Mitochondrial proteins and complexes in *Leishmania and Trypanosoma* involved in U-insertion/deletion RNA editing. RNA **10:** 159–170
14 Stuart K. D., Schnaufer A., Ernst N. L. and Panigrahi A. K. (2005) Complex management: RNA editing in trypanosomes. Trends Biochem. Sci. **30:** 97–105
15 White T. and Borst P. (1987) RNA end-labeling and RNA ligase activities can produce a circular ribosomal RNA in whole cell extracts from trypanosomes. Nucleic Acids Res. **15:** 3275–3290
16 Benne R., Van den Burg J., Brakenhoff J., Sloof P., Van Boom J. and Tromp M. (1986) Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. Cell **46:** 819–826
17 Bakalara N., Simpson A. M. and Simpson L. (1989) The *Leishmania* kinetoplast-mitochondrion contains terminal uridylyltransferase and RNA ligase activities. J. Biol. Chem. **264:** 18679–18686
18 Pollard V. W., Harris M. E. and Hajduk S. L. (1992) Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. EMBO J. **11:** 4429–4438
19 Byrne E., Bringaud F. and Simpson L. (1995) RNA-protein interactions in the ribonucleoprotein T-complexes in a mitochondrial extract from *Leishmania tarentolae*. Mol. Biochem. Parasitol. **72:** 65–76
20 Peris M., Frech G. C., Simpson A. M., Bringaud F., Byrne E., Bakker A. et al. (1994) Characterization of two classes of ribonucleoprotein complexes possibly involved in RNA editing from *Leishmania tarentolae* mitochondria. EMBO J. **13:** 1664–1672
21 Frech G. C., Bakalara N., Simpson L. and Simpson A. M. (1995) In vitro RNA editing-like activity in a mitochondrial extract from *Leishmania tarentolae*. EMBO J. **14:** 178–187
22 Seiwert S. D., Heidmann S. and Stuart K. (1996) Direct visualization of uridylate deletion in vitro suggests a mechanism for kinetoplastid RNA editing. Cell **84:** 831–841
23 Kable M. L., Seiwert S. D., Heidmann S. and Stuart K. (1996) RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. Science **273:** 1189–1195
24 Rusche L. N., Cruz-Reyes J., Piller K. J. and Sollner-Webb B. (1997) Purification of a functional enzymatic editing complex from *Trypanosoma brucei* mitochondria. EMBO J. **16:** 4069–4081
25 Steitz T. A. (1997) DNA and RNA polymerases: structural diversity and common mechanisms. Harvey Lect. **93:** 75–93
26 Iyer L. M., Koonin E. V. and Aravind L. (2003) Evolutionary connection between the catalytic subunits of DNA-dependent RNA polymerases and eukaryotic RNA-dependent RNA polymerases and the origin of RNA polymerases. BMC Struct. Biol. **3:** 1
27 Aravind L. and Koonin E. V. (1999) DNA polymerase beta-like nucleotidyltransferase superfamily: identification of three new families, classification and evolutionary history. Nucleic Acids Res. **27:** 1609–1618
28 Holm L. and Sander C. (1995) DNA polymerase beta belongs to an ancient nucleotidyltransferase superfamily. Trends Biochem. Sci. **20:** 345–347
29 Martin G. and Keller W. (1996) Mutational analysis of mammalian poly(A) polymerase identifies a region for primer binding and catalytic domain, homologous to the family X polymerases and to other nucleotidyltransferases. EMBO J. **15:** 2593–2603
30 Raynal L. C. and Carpousis A. J. (1999) Poly(A) polymerase I of *Escherichia coli*: characterization of the catalytic domain, an RNA-binding site and regions for the interaction with proteins involved in mRNA degradation. Mol. Microbiol. **32:** 765–775
31 Gershon P. D., Ahn B. Y., Garfield M. and Moss B. (1991) Poly(A) polymerase and a dissociable polyadenylation stimulatory factor encoded by vaccinia virus. Cell **66:** 1269–1278
32 Aphasizhev R., Sbicego S., Peris M., Jang S. H., Aphasizheva I., Simpson A. M. et al. (2002) Trypanosome mitochondrial 3′ terminal uridylyl transferase (TUTase): the key enzyme in U-insertion/deletion RNA editing. Cell **108:** 637–648
33 Martin G. and Keller W. (1998) Tailing and 3′-end labeling of RNA with yeast poly(A) polymerase and various nucleotides. RNA **4:** 226–230
34 Rogozin I. B., Aravind L. and Koonin E. V. (2003) Differential action of natural selection on the N and C-terminal domains of 2′-5′ oligoadenylate synthetases and the potential nuclease function of the C-terminal domain. J. Mol. Biol. **326:** 1449–1461
35 Aphasizheva I., Aphasizhev R. and Simpson L. (2004) RNA-editing terminal uridylyl transferase 1: identification of functional domains by mutational analysis. J. Biol. Chem. **279:** 24123–24130
36 Bard J., Zhelkovsky A. M., Helmling S., Earnest T. N., Moore C. L. and Bohm A. (2000) Structure of yeast poly(A) polymerase alone and in complex with 3′-dATP. Science **289:** 1346–1349
37 Martin G., Keller W. and Doublie S. (2000) Crystal structure of mammalian poly(A) polymerase in complex with an analog of ATP. EMBO J. **19:** 4193–4203
38 Martin G., Moglich A., Keller W. and Doublie S. (2004) Biochemical and structural insights into substrate binding and catalytic mechanism of mammalian poly(A) polymerase. J. Mol. Biol. **341:** 911–925
39 Keller W. and Martin G. (2002) Gene regulation: reviving the message. Nature **419:** 267–268
40 McManus M. T., Adler B. K., Pollard V. W. and Hajduk S. L. (2000) *Trypanosoma brucei* guide RNA poly(U) tail formation is stabilized by cognate mRNA. Mol. Cell Biol. **20:** 883–891
41 Panigrahi A. K., Schnaufer A., Ernst N. L., Wang B., Carmean N., Salavati R. et al. (2003) Identification of novel components of *Trypanosoma brucei* editosomes. RNA **9:** 484–492
42 Aphasizhev R., Aphasizheva I., Nelson R. E., Gao G., Simpson A. M., Kang X. et al. (2003) Isolation of a U-insertion/deletion editing complex from *Leishmania tarentolae* mitochondria. EMBO J. **22:** 913–924
43 Aphasizhev R., Aphasizheva I., Nelson R. E. and Simpson L. (2003) A 100-kD complex of two RNA-binding proteins from mitochondria of *Leishmania tarentolae* catalyzes RNA annealing and interacts with several RNA editing components. RNA **9:** 62–76
44 Nebohacova M., Maslov D. A., Falick A. M. and Simpson L. (2004) The effect of RNA interference down-regulation of RNA editing 3′-terminal uridylyl transferase (TUTase) on mitochondrial de novo protein synthesis and stability of respiratory complexes in *Trypanosoma brucei*. J. Biol. Chem. **279:** 7819–7825
45 Golden D. E. and Hajduk S. L. (2005) The 3′-untranslated region of cytochrome oxidase II mRNA functions in RNA editing of African trypanosomes exclusively as a cis guide RNA. RNA. **11:** 29–37

46 Ernst N. L., Panicucci B., Igo R. P. Jr, Panigrahi A. K., Salavati R. and Stuart K. (2003) TbMP57 is a 3′ terminal uridylyl transferase (TUTase) of the *Trypanosoma brucei* editosome. Mol. Cell **11:** 1525–1536

47 Aphasizhev R., Aphasizheva I. and Simpson L. (2003) A tale of two TUTases. Proc. Natl. Acad. Sci. USA **100:** 10617–10622

48 Schnaufer A., Ernst N. L., Palazzo S. S., O'Rear J., Salavati R. and Stuart K. (2003) Separate insertion and deletion subcomplexes of the *Trypanosoma brucei* RNA editing complex. Mol. Cell **12:** 307–319

49 Law J. A., Huang C. E., O'Hearn S. F. and Sollner-Webb B. (2005) In *Trypanosoma brucei* RNA editing, band II enables recognition specifically at each step of the U insertion cycle. Mol. Cell Biol. **25:** 2785–2794

50 Gao G. and Simpson L. (2003) Is the *Trypanosoma brucei* REL1 RNA ligase specific for U-deletion RNA editing, and is the REL2 RNA ligase specific for U-insertion editing? J. Biol. Chem. **278:** 27570–27574

51 Igo R. P., Palazzo S. S., Burgess M. L., Panigrahi A. K. and Stuart K. (2000) Uridylate addition and RNA ligation contribute to the specificity of kinetoplastid insertion RNA editing. Mol. Cell. Biol. **20:** 8447–8457

52 Cruz-Reyes J., Rusche L., Piller K. J. and Sollner-Webb B. (1998) *T. brucei* RNA editing: adenosine nucleotides inversely affect U-deletion and U-insertion reactions at mRNA cleavage. Mol. Cell **1:** 401–409

53 Cruz-Reyes J., Zhelonkina A. G., Huang C. E. and Sollner-Webb B. (2002) Distinct functions of two RNA ligases in active *Trypanosoma brucei* RNA editing complexes. Mol. Cell. Biol. **22:** 4652–4660

54 Leung S. S. and Koslowsky D. J. (2001) Interactions of mRNAs and gRNAs involved in trypanosome mitochondrial RNA editing: structure probing of an mRNA bound to its cognate gRNA. RNA **7:** 1803–1816

55 Byrne E. M., Connell G. J. and Simpson L. (1996) Guide RNA-directed uridine insertion RNA editing in vitro. EMBO J. **15:** 6758–6765

56 Burgess M. L. K., Heidmann S. and Stuart K. (1999) Kinetoplastid RNA editing does not require the terminal 3′ hydroxyl of guide RNA, but modifications to the guide RNA terminus can inhibit in vitro U insertion. RNA **5:** 883–892

57 Cruz-Reyes J., Zhelonkina A., Rusche L. and Sollner-Webb B. (2001) Trypanosome RNA editing: simple guide RNA features enhance U deletion 100-fold. Mol. Cell. Biol. **21:** 884–892

58 Igo R. P. Jr, Lawson S. D. and Stuart K. (2002) RNA sequence and base pairing effects on insertion editing in *Trypanosoma brucei*. Mol. Cell. Biol. **22:** 1567–1576

59 Kapushoc S. T. and Simpson L. (1999) In vitro uridine insertion RNA editing mediated by cis-acting guide RNAs. RNA **5:** 656–669

60 Vanhamme L., Perez-Morga D., Marchal C., Speijer D., Lambert L., Geuskens M. et al. (1998) *Trypanosoma brucei* TBRGG1, a mitochondrial oligo(U)-binding protein that co-localizes with an in vitro RNA editing activity. J. Biol. Chem. **273:** 21825–21833

61 Blom D., Burg J., Breek C. K., Speijer D., Muijsers A. O. and Benne R. (2001) Cloning and characterization of two guide RNA-binding proteins from mitochondria of *Crithidia fasciculata*: gBP27, a novel protein and gBP29, the orthologue of *Trypanosoma brucei* gBP21. Nucleic Acids Res. **29:** 2950–2962

62 Maslov D. A. and Simpson L. (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. Cell **70:** 459–467

63 Sturm N. R. and Simpson L. (1991) *Leishmania tarentolae* minicircles of different sequence classes encode single guide RNAs located in the variable region approximately 150 bp from the conserved region. Nucleic Acids Res. **19:** 6277–6281

64 Thiemann O. H. and Simpson L. (1996) Analysis of the 3′ uridylylation sites of guide RNAs from *Leishmania tarentolae*. Mol. Biochem. Parasitol. **79:** 229–234

65 Aphasizhev R. and Simpson L. (2001) Isolation and characterization of a U-specific 3′-5′ exonuclease from mitochondria of *Leishmania tarentolae*. J. Biol. Chem. **276:** 21280–21284

66 Kang X., Rogers K., Gao G., Falick A. M., Zhou S. and Simpson L. (2005) Reconstitution of uridine-deletion precleaved RNA editing with two recombinant enzymes. Proc. Natl. Acad. Sci. USA **102:** 1017–1022

67 Decker C. J. and Sollner-Webb B. (1990) RNA editing involves indiscriminate U changes throughout precisely defined editing domains. Cell **61:** 1001–1011

68 Read L. K., Myler P. J. and Stuart K. (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. J. Biol. Chem. **267:** 1123–1128

69 Adler B. K., Harris M. E., Bertrand K. I. and Hajduk S. L. (1991) Modification of *Trypanosoma brucei* mitochondrial rRNA by posttranscriptional 3′ polyuridine tail formation. Mol. Cell. Biol. **11:** 5878–5884

70 Ryan C. M. and Read L. K. (2005) UTP-dependent turnover of *Trypanosoma brucei* mitochondrial mRNA requires UTP polymerization and involves the RET1 TUTase. RNA **11:** 763–773

71 Blanc V., Aphasizhev R., Alfonzo J. D. and Simpson L. (1999) The mitochondrial RNA ligase from *Leishmania tarentolae* can join RNA molecules bridged by a complementary RNA. J. Biol. Chem. **274:** 24289–24296

72 Simpson L., Thiemann O. H., Savill N. J., Alfonzo J. D. and Maslov D. A. (2000) Evolution of RNA editing in trypanosome mitochondria. Proc. Natl. Acad. Sci. USA **97:** 6986–6993

73 Aphasizhev R., Aphasizheva I. and Simpson L. (2004) Multiple terminal uridylyltransferases of trypanosomes. FEBS Lett. **572:** 15–18

74 Wang L., Eckmann C. R., Kadyk L. C., Wickens M. and Kimble J. (2002) A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*. Nature **419:** 312–316

75 Read R. L., Martinho R. G., Wang S. W., Carr A. M. and Norbury C. J. (2002) Cytoplasmic poly(A) polymerases mediate cellular responses to S phase arrest. Proc. Natl. Acad. Sci. USA **99:** 12079–12084

76 Saitoh S., Chabes A., McDonald W. H., Thelander L., Yates J. R. and Russell P. (2002) Cid13 is a cytoplasmic poly(A) polymerase that regulates ribonucleotide reductase mRNA. Cell **109:** 563–573

77 Barnard D. C., Ryan K., Manley J. L. and Richter J. D. (2004) Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell **119:** 641–651

78 Kwak J. E., Wang L., Ballantyne S., Kimble J. and Wickens M. (2004) Mammalian GLD-2 homologs are poly(A) polymerases. Proc. Natl. Acad. Sci. USA **101:** 4407–4412

79 Traut T. W. (1994) Physiological concentrations of purines and pyrimidines. Mol. Cell Biochem. **140:** 1–22

80 Clayton C. E. (2002) Life without transcriptional control? From fly to man and back again. EMBO J. **21:** 1881–1888

81 MacDonald P. (2001) Diversity in translational regulation. Curr. Opin. Cell Biol. **13:** 326–331

82 Trippe R., Sandrock B. and Benecke B. J. (1998) A highly specific terminal uridylyl transferase modifies the 3′-end of U6 small nuclear RNA. Nucleic Acids Res. **26:** 3119–3126

83 Lund E. and Dahlberg J. E. (1992) Cyclic 2′,3′-phosphates and nontemplated nucleotides at the 3′ end of spliceosomal U6 small nuclear RNA's. Science **255:** 327–330

84 Reddy R., Henning D., Das G., Harless M. and Wright D. (1987) The capped U6 small nuclear RNA is transcribed by RNA polymerase III. J. Biol. Chem. **262:** 75–81

85 Tazi J., Forne T., Jeanteur P., Cathala G. and Brunel C. (1993) Mammalian U6 small nuclear RNA undergoes 3′ end modifications within the spliceosome. Mol. Cell. Biol. **13:** 1641–1650

86 Rinke J. and Steitz J. A. (1985) Association of the lupus antigen La with a subset of U6 snRNA molecules. Nucleic Acids Res. **13:** 2617–2629
87 Trippe R., Richly H. and Benecke B. J. (2003) Biochemical characterization of a U6 small nuclear RNA-specific terminal uridylyltransferase. Eur. J. Biochem. **270:** 971–980
88 Booth B. L. Jr and Pugh B. F. (1997) Identification and characterization of a nuclease specific for the 3′ end of the U6 small nuclear RNA. J. Biol. Chem. **272:** 984–991
89 Fortner D. M., Troy R. G. and Brow D. A. (1994) A stem/loop in U6 RNA defines a conformational switch required for pre-mRNA splicing. Genes Dev. **8:** 221–233
90 Pannone B. K., Kim S. D., Noe D. A. and Wolin S. L. (2001) Multiple functional interactions between components of the Lsm2-Lsm8 complex, U6 snRNA and the yeast La protein. Genetics **158:** 187–196
91 Xue D., Rubinson D. A., Pannone B. K., Yoo C. J. and Wolin S. L. (2000) U snRNP assembly in yeast involves the La protein. EMBO J. **19:** 1650–1660
92 Achsel T., Brahms H., Kastner B., Bachi A., Wilm M. and Luhrmann R. (1999) A doughnut-shaped heteromer of human Sm-like proteins binds to the 3′-end of U6 snRNA, thereby facilitating U4/U6 duplex formation in vitro. EMBO J. **18:** 5789–5802
93 Will C. L. and Luhrmann R. (2001) Spliceosomal UsnRNP biogenesis, structure and function. Curr. Opin. Cell Biol. **13:** 290–301