Two distinct roles for terminal uridylyl transferases in RNA editing

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Gene expression in the mitochondria of kinetoplastid protists like the human pathogen Trypanosoma brucei occurs via a remarkable pathway involving both the deletion of encoded uridines (Us) and the insertion of extra Us at defined positions within mRNAs synthesized from “cryptogenes” in the maxicircle DNA (see refs. 1 and 2 for recent reviews). In some cases, >50% of the nucleotides present in the mature mRNA are generated by RNA editing events. U addition and removal create sense from nonsense, producing ORFs that encode the proteins involved in oxidative phosphorylation and other mitochondrial functions. Many of these changes are developmentally regulated, occurring only in the procyclic (insect) stage or the bloodstream (mammalian) form. Although the overall mechanism by which this extraordinary phenomenon occurs has been sketched out (3–6), filling in the details has been difficult because of the low abundance and complexity of the editing apparatus, the low efficiency of in vitro editing assays, the lack of assays for processive editing, and difficulties in assigning functions to specific proteins. The article by Aphasizhev et al. (7) in this issue of PNAS provides important information regarding the function of two of the key enzymes involved in this fascinating process.

The insertion and deletion of Us into kinetoplast mRNAs occur through the concerted action of a series of enzymes (Fig. 1) (see references in ref. 2). Specificity is provided by small RNA molecules termed guide RNAs (gRNAs), which base-pair to preedited mRNAs just downstream of editing sites (8). gRNAs have three functional domains: an anchor region, which anneals to the substrate; a guiding region, which directs the insertion or deletion of Us residues; and an oligo(U) tail, which is added posttranscriptionally and is thought to help tether the purine-rich 5′ cleavage fragment to the rest of the complex. Binding of the gRNA targets cleavage of the mRNA immediately upstream of the anchor duplex by an editing endonuclease, creating a 5′ cleavage fragment that is the substrate for either a 3′ terminal uridylyl transferase (TUTase) in the case of U insertion or a 3′ to 5′ exonuclease for U deletions. The number of Us added or deleted is determined by both the sequence of the gRNA and the specificity of the editing enzymes; the new 3′ end of the upstream fragment pairs with the guiding region, extending the anchor duplex and directing ligation of the two mRNA fragments. Editing of a given message occurs with an overall 3′ to 5′ polarity and usually involves multiple gRNAs that act sequentially. Often gRNA binding sites are created by prior editing events and are presumably accessed upon dissociation of the previous gRNA.

Editing is catalyzed by a multisubunit complex, termed the “editosome.” The size of this macromolecular complex varies, depending on the isolation conditions, but it generally sediments at 19–25S in glycerol gradients and contains between 7 and 21 polypeptides (9–13), only some of which have been directly linked to the editing reaction (see refs. 1 and 2). A number of editosome components have now been identified through sequencing and/or mass spectrometric analysis of proteins present in native complexes, and their genes have been cloned based on sequences present in the Leishmania major and T. brucei genome databases or via PCR using degenerate primers (13–16). This information has made it possible to augment conventional biochemical fractionation through purification of complexes containing individual tandem affinity purification-tagged proteins (15, 16), and the availability of antibodies raised against native complexes or recombinant proteins has facilitated affinity purification, immunodepletion, and coimmunoprep-

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Trypanosomes have two terminal uridylyl transferases, each playing a distinct role in RNA editing.

Thus, it is now clear that there are two TUTases in trypanosome mitochondria and that each plays a distinct role in RNA editing.

RET1 and RET2 are present in separate complexes (7, 20). There are also two RNA ligases implicated in editing, REL1 (TbMP52/band IV) and REL2 (TbMP48/band V), and it has been suggested that REL1 is required for deletion editing, whereas REL2 is required for U insertion (21, 26). However, other studies suggest that the functions of REL1 and REL2 may not be mutually exclusive (19, 22, 24). In addition, subcomplexes containing a subset of editosome components have been observed, particularly upon depletion of individual editosome components (7, 9, 15, 17, 22, 23, 27), raising a number of interesting questions regarding the mechanics of trypanosome editing. How is the switch between insertion and deletion editing affected, given that insertion and deletion sites are interspersed and often directed by the same gRNA? Are there separate complexes for insertion and deletion, or does editing occur within a single complex in which the substrate flips back and forth between insertion and deletion sites? How is the substrate shuttled among the endonuclease, TUTase or exonuclease, and ligase active sites? What roles might be played by other components identified in these complexes? How is editing regulated during development? The answers to these questions will await further dissection and eventual reconstitution of functional editing complexes from purified components.

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