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Mini-review

Mitochondrial RNA editing in trypanosomes: Small RNAs in control

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ABSTRACT

Mitochondrial mRNA editing in trypanosomes is a posttranscriptional processing pathway thereby uridine residues (Us) are inserted into, or deleted from, messenger RNA precursors. By correcting frameshifts, introducing start and stop codons, and often adding most of the coding sequence, editing restores open reading frames for mitochondrially-encoded mRNAs. There can be hundreds of editing events in a single pre-mRNA, typically spaced by few nucleotides, with U-insertions outnumbering U-deletions by approximately 10-fold. The mitochondrial genome is composed of ~50 maxicircles and thousands of minicircles. Catenated maxi- and minicircles are packed into a dense structure called the kinetoplast; maxicircles yield rRNA and mRNA precursors while guide RNAs (gRNAs) are produced predominantly from minicircles, although varying numbers of maxicircle-encoded gRNAs have been identified in kinetoplastids species. Guide RNAs specify positions and the numbers of inserted or deleted Us by hybridizing to pre-mRNA and forming series of mismatches. These 50–60 nucleotide (nt) molecules are 3′ uridylated by RET1 TUTase and stabilized via association with the gRNA binding complex (GRBC). Editing reactions of mRNA cleavage, U-insertion or deletion, and ligation are catalyzed by the RNA editing core complex (RECC). To function in mitochondrial translation, pre-mRNAs must further undergo post-editing 3′ modification by polyadenylation/uridylation. Recent studies revealed a highly compound nature of mRNA editing and polyadenylation complexes and their interactions with the translational machinery. Here we focus on mechanisms of RNA editing and its functional coupling with pre- and post-editing 3′ mRNA modification and gRNA maturation pathways.

1. Introduction

The discovery of RNA editing in *Trypanosoma brucei* by Rob Benne and co-workers [1] paved the way to understanding a fundamentally novel mechanism of information transfer between RNA molecules and illuminated a much greater coding capacity of the mitochondrial genome than had been predicted from DNA sequencing. Twelve mitochondrial genes that seemed nonfunctional or nonexistent were identified as cryptogenes whose transcripts must be post-transcriptionally decoded (edited) by inserting or deleting Us in order to produce open reading frames. Initially perceived as a challenge to the central dogma of molecular biology, the editing phenomenon stimulated search for a template ultimately leading to the discovery of guide RNAs (gRNAs) [2]. By allowing for wobble G–U, in addition to canonical Watson–Crick base-pairing, short (50–60 nt) mitochondrial transcripts have been recognized as complementary to edited sequences and, therefore, likely carriers of genetic information. Partial annealing of gRNAs and pre-edited mRNAs immediately suggested a mechanism by which the location and extent of U-insertions and U-deletions are determined [2]. The site selection for gRNA binding is accomplished via short region of complementarity between gRNA's 5′ "anchor" region and mRNA; the rest of gRNA forms an imperfect duplex along the mRNA by either bulging out uridines in mRNA, or adenines and guanosines in gRNA. Unpaired Us in the pre-mRNA are removed, and unpaired purines in the gRNA specify insertion of an equal number of Us into opposing mRNA positions (Fig. 1). In massively (pan) edited mRNAs editing events proceed sequentially in 3′–5′ polarity along the mRNA and require multiple overlapping gRNAs [3]. The overall fidelity of the editing process is astonishingly low with the bulk of the mRNA population represented by partially-edited or miss-edited transcripts, which raises the problem of how correctly edited mRNAs are selected for ribosome binding and translation.

The narrow phylogenetic distribution of U-insertion/deletion editing, which is limited to kinetoplastids protozoans, is indicative of its origination within a particular lineage rather than being a trait shared with the common ancestor of eukaryotes. There are,

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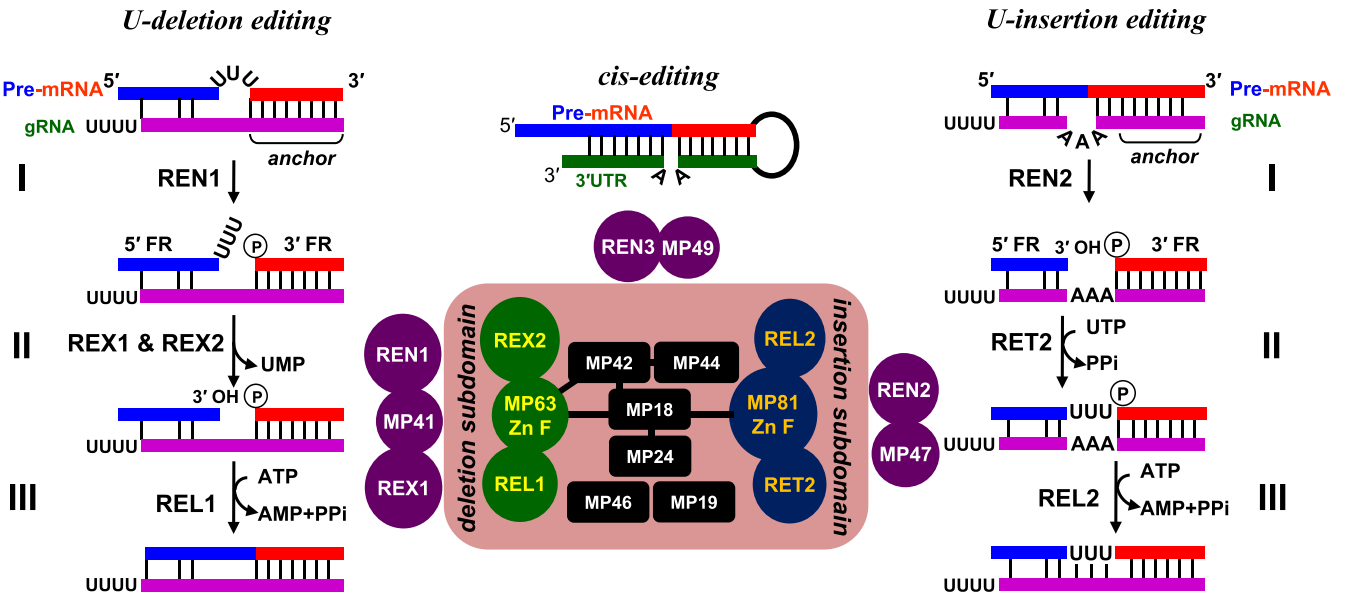

Fig. 1. The RNA editing core complex catalyzes elementary RNA editing reactions. Direct protein–protein interactions within core complex are depicted by black bars. Roman numerals signify three elementary steps of RNA editing: mRNA cleavage, U-deletion or insertion and mRNA ligation. MP: mitochondrial protein (structural and/or RNA binding components); REX: RNA editing exonuclease; REN: RNA editing endonuclease; REL: RNA editing ligase; RET: RNA editing TUTase; anchor: 5–15-nt long double-stranded region formed by the 5′ portion of the gRNA and pre-edited mRNA.

however, signs that RNA editing may have arisen consequential to acquisition of novel functions by primordial cellular enzymatic modules involved in DNA/RNA repair and RNA interference. The sheer complexity of the editing machinery and its intertwinement with pre- and post-editing mRNA polyadenylation and translation raise exciting questions about how RNA editing systems appeared and became fixed in evolution [4,5].

2. Basic mechanism of U-insertion/deletion mRNA editing and activities of the core editing complex

The "enzymatic cascade" model [2] was confirmed by reproducing elementary reactions and complete editing cascade in a single site with synthetic mRNA and gRNA as substrates and crude mitochondrial extract as the source of editing complexes [6,7]. Further studies of purified RNA editing core complex (RECC, also referred to as the ~20S editosome) identified specific components responsible for each enzymatic step and revealed substrate specificities of individual enzymes (reviewed in Ref. [8], Table 1). Remarkably, the information transfer from gRNA to mRNA does not involve an RNA-dependent recognition of the incoming UTP as would be the case in a typical template-copying polymerization reaction. Instead, intrinsic substrate specificities of key enzymes, such as UTP selection by RET2 terminal uridyltransferase (TUTase) [9], are responsible for the overall fidelity of editing.

Editing is initiated by an endonucleolytic pre-mRNA cleavage at the first unpaired nucleoside adjacent to the continuous 'anchor' duplex, which is a bulged out uridine at the deletion site or typically a purine base in the insertion site (Fig. 1). Cleavage reaction generates 5′ and 3′ mRNA cleavage fragments that are presumably tethered by hybridization with gRNA and, most likely, RNA-protein contacts with the RNA editing core complex and/or the gRNA binding complex (GRBC [10], reviewed below). The importance of maintaining mRNA cleavage fragments bound to gRNA has been illustrated in "pre-cleaved" assays that recapitulate a three-RNA hybrid product of an endonucleolytic cleavage (Fig. 1, step II). Specifically, introduction of extended complementarity regions between gRNA and both cleavage fragments stimulated U-insertion, U-deletion and RNA ligation reactions [11–14].

The asymmetrical structures of U-deletion and U-insertion sites are distinguished by RNase III-type endonucleases, REN1 [15] and REN2 [16], respectively. The third endonuclease (REN3) apparently targets the COII mRNA that contains a *cis*-acting guide RNA-like element in its 3′ untranslated region (UTR) [17,18]. Remarkably, while most RNase III catalytic domains form homodimers with two active sites and cleave both strands in the double-stranded RNA, only the pre-mRNA strand is cleaved during editing. To account for a single cleavage event, Carnes et al. suggested that editing

Table 1
Components of the RNA editing core complex from *T. brucei*. Two alternative nomenclatures are used in the current literature to describe RECC subunits [85,102]. Gene identification numbers are provided according to TriTrypDB 5.0 database release (http://tritrypdb.org/tritrypdb/).

Alternative names for proteins	Gene ID	Proposed function
MP81/KREPA1	Tb927.2.2470	Structural, U-insertion subdomain organizer
MP63/KREPA2	Tb927.10.8210	Structural, U-deletion subdomain organizer
MP42/KREPA3	Tb927.8.620	Structural
MP24/KREPA4	Tb927.10.5110	Structural, RNA binding
MP19/KREPA5	Tb927.8.680	Structural
MP18/KREPA6	Tb927.10.5120	Structural, RNA binding
REN1/KREN1	Tb927.1.1690	Insertion site specific endonuclease
REN2/KREN2	Tb927.10.5440	Deletion site specific endonuclease
REN3/KREN3	Tb927.10.5320	Cis-editing site specific endonuclease
MP46/KREPB4	Tb927.11.2990	Structural, heterodimer with endonuclease
MP44/KREPB5	Tb927.11.940	Structural, endonuclease
MP49/KREPB6	Tb927.3.3990	Structural, part of KREN3 module
MP47/KREPB7	Tb927.9.5630	Structural, part of KREN2 module
MP41/KREPB8	Tb927.9.5630	Structural, part of KREN1 module
REX1/KREX1	Tb927.7.1070	U-specific exonuclease
REX2/KREX2	Tb927.10.3570	U-specific exonuclease
REL1/KREL1	Tb927.9.4360	RNA ligase
REL2/KREL2	Tb927.1.3030	RNA ligase
RET2/KRET2	Tb927.7.1550	TUTase