Molecular Cell
Previews

Marked for Translation: Long A/U Tails as an Interface between Completion of RNA Editing and Ribosome Recruitment

Laurie K. Read,[1,*] Sara L. Zimmer,[1] and Michelle L. Ammerman[1]
[1]Department of Microbiology and Immunology, SUNY Buffalo School of Medicine, Buffalo, NY 14214, USA
*Correspondence: lread@buffalo.edu
DOI 10.1016/j.molcel.2011.03.013

The mechanism by which ribosomes select translatable mRNAs from the complex mixture of incompletely edited mRNAs in trypanosome mitochondria has remained a mystery. In this issue of *Molecular Cell*, Aphasizheva and colleagues (Aphasizheva et al., 2011) reveal a role for long 3′ A/U tails in signaling ribosome recruitment to a fully edited, translatable mRNA.

Gene expression in trypanosome mitochondria entails a remarkable posttranscriptional RNA editing process in which most messenger RNAs (mRNAs) are extensively remodeled by insertion and deletion of uridine (U) residues to create open reading frames (ORFs). In many cases, almost half of the nucleotides in the mature mRNA result from posttranscriptional U insertion. As a result, the steady-state mRNA population in these organelles is exceedingly complex. It is comprised of (1) pre-edited mRNAs that have not yet begun editing, (2) fully edited mRNAs that have completed editing, and (3) a highly heterogeneous mixture of partially edited mRNAs that are edited to varying degrees at their 3′ ends and unedited at their 5′ ends, and which are presumably in the process of being edited. Moreover, six of 18 mitochondrial mRNAs contain encoded ORFs and so do not undergo editing; these are termed "never edited." The complex nature of the mitochondrial RNA population raises an important question—in the face of such a complicated morass of mRNAs, how do mitochondrial ribosomes select only those that contain complete ORFs and are thus suitable for translation? Such a mechanism is presumably critical because translation of pre-edited or partially edited RNAs would result in a massive number of truncated proteins that would compromise cell function. In this issue of *Molecular Cell*, Aphasizheva et al. (2011) report that the long 3′ A/U extension on fully edited RPS12 mRNA mediates recruitment of this ORF-containing mRNA to mitochondrial ribosomes, and they reveal a role for a pentatricopeptide repeat (PPR) protein in coordinating synthesis of these long A/U tails.

It has long been known that edited and never-edited mRNAs occur in two populations with short (~20 nt) and long (~200 nt) 3′ extensions (Bhat et al., 1992). Etheridge et al. (2008) showed that long tails consist of A/U heteropolymers in an ~7:3 A:U ratio and presented limited data suggesting that long A/U extensions are synthesized (Figure 1, step 3) only after editing is completed and an ORF has been created (Figure 1, step 2). PPR proteins mediate multiple aspects of organellar RNA processing and gene expression in fungi, mammals, and plants (Delannoy et al., 2007). Trypanosomes contain a relatively large number of PPR proteins (~30), second only to plants, and one of these has been implicated in the synthesis of long 3′ tails on edited and never-edited RNAs. The Schneider and Koslowsky laboratories showed that PPR1 (here renamed KPAF1) is essential in insect stage *T. brucei* and that its depletion leads to selective loss of long tailed mitochondrial mRNA populations (Mingler et al., 2006; Pusnik et al., 2007). Subsequently, KPAF1 and another PPR protein here named KPAF2, were found associated with the mitochondrial poly(A) polymerase (KPAP1) (Etheridge et al., 2008). Together, these observations suggested that KPAF1, and potentially KPAF2, may play a role in the synthesis of long 3′ tails on mitochondrial RNAs.

It was suspected that synthesis of long A/U tails was catalyzed by KPAP1 and RET1 terminal uridylyl transferase. To explore a potential effect of KPAF1 in this process, Aphasizheva et al. (2011) perform an elegant series of in vitro experiments using an RNA corresponding to the 3′ end of edited mitochondrial RPS12 mRNA and combinations of recombinant KPAP1, RET1, and KPAF1/KPAF2 (KPAF1/2, which form a heterotetramer when coexpressed in *E. coli).* In binary mixing experiments, KPAP1 and RET1 compete for access to edited mRNA 3′ ends. Addition of KPAF1/2 affects these two enzymes differently, slightly stimulating the low processivity of KPAP1 but inhibiting the normally highly processive RET1. Strikingly, when the three components, KPAF1/2, KPAP1, and RET1, are coincubated, long extensions of ~200 nt are produced. Modest U incorporation by RET1 is absolutely required for synthesis of A-rich long A/U tails. Thus, KPAF1/2 modulates the competing activities of KPAP1 and RET1 in a manner that leads to synthesis of 3′ tails with lengths and compositions similar to those observed in vivo (Figure 1, step 3, yellow circle).

What is the function of long 3′ A/U extensions? In vivo, the proportion of a given mRNA with long or short 3′ tails is developmentally regulated in a transcript specific fashion (Bhat, et al., 1992). Long tails are generally correlated with the life-cycle stage in which a translation product is expected to be functional. Thus, mRNA 3′ tail length has been postulated to regulate trypanosome mitochondrial gene expression (Bhat, et al., 1992), possibly by impacting mRNA stability or translation as it does in other systems.

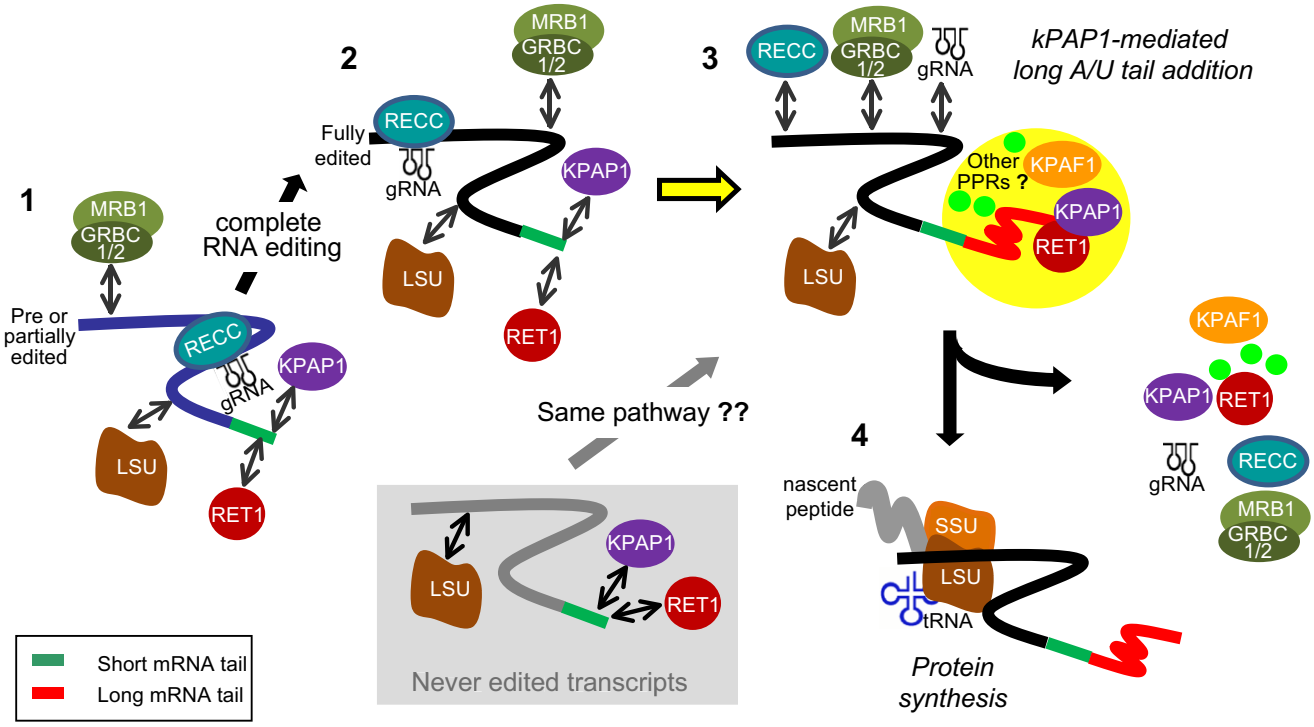

Figure 1. Long A/U Tail Addition Modulated by KPAF1 Serves as an Interface between RNA Editing and Translation

Step 1: RNA editing catalyzed by the RNA editing core complex (RECC) and directed by gRNAs may take place in association with ribosomal large subunit (LSU), editing accessory factors (GRBC1/2; MRB1), and nucleotidyltransferases (KPAP1; RET1). Double-headed arrows represent the spatially and temporally ambiguous nature of these interactions. Step 2: Editing is completed and the mRNA contains an ORF. Step 3: Completion of editing triggers the PPR protein, KPAF1, and potentially KPAF2 and/or other PPR proteins, to coordinate long 3′ A/U tail synthesis (yellow circle). Step 4: RNA editing and processing factors are released from the fully edited, long-tailed mRNA. Ribosomal small subunit (SSU) and tRNA are recruited to the mRNA and protein synthesis commences. Gray box: It is unknown what triggers addition of long A/U tails on never-edited transcripts or whether long tail addition is required for the assembly of a translating ribosome on never-edited mRNAs.

mRNA stability in trypanosome mitochondria is apparently regulated only by short 3′ tails (Kao and Read, 2005; Etheridge et al., 2008), suggesting that the function of long tails might be in translation. To explore this possibility, Aphasizheva et al. (2011) examine the association of various RNA populations with translating ribosomes, defined here as the small percentage of ribosomes that cosediment with the majority of $tRNA^{cys}$. Remarkably, mRNAs with long tails are enriched 4-fold in fractions containing translating ribosomes compared to input mitochondrial RNA. The same fractions are depleted of short-tailed mRNAs, KPAF1/2, RNA editing factors, and guide RNAs (gRNAs) that provide the sequence information for editing. These data strongly suggest that mRNAs bearing long A/U extensions are preferentially recruited to ribosomes for translation after the completion of editing (Figure 1, step 4). A role for A/U extensions in promoting translation is also suggested by labeling experiments in KPAF1 knockdown cells in which a global decrease in mitochondrial translation is coincident with depletion of long A/U tails on several mRNAs.

Using trypanosomes expressing tagged small or large ribosomal subunits (SSUs and LSUs, respectively), the authors further explore the coordination of RNA editing, mRNA 3′ tail addition, and translation. LSU pull-downs are highly enriched in short tailed mRNAs, RNA editing factors, gRNAs, gRNA stabilizing proteins, and RET1. This suggests that mRNA editing and 3′ tail addition may occur in association with the LSU (Figure 1, steps 1–3). Additional studies are needed to fully understand the temporal associations between mRNAs, editing and modification factors, and LSU, as well as the proportions of these factors that are LSU associated. Long-tailed mRNAs, while also present with LSUs, are highly enriched in SSU pull-downs. Thus, once editing is completed and the long tail is synthesized, RNA editing and processing factors apparently disassociate from mRNAs, and SSU and transfer RNA (tRNA) are recruited (Figure 1, step 4).

These studies provide an important piece in the puzzle of trypanosome mitochondrial gene expression. However, several questions remain. For example, how does KPAF1/2 recognize translatable RNAs to effect long tail synthesis? Indeed, the restriction of long tail synthesis to fully edited RNAs, as opposed to partially edited RNAs, remains to be demonstrated as a general phenomenon. KPAF1/2 association with fully edited RNAs could somehow be triggered by completion of the editing process. However, such a mechanism cannot be invoked for the six never-edited RNAs that also receive long A/U extensions (Figure 1, gray box). Second, what is the molecular mechanism by which KPAF1/2 modulates tail

length and composition and how is this process regulated in a transcript specific manner during the trypanosome life cycle? Third, how does the long A/U tail mediate recruitment of SSU and tRNA to mRNAs? Fourth, is there a role for additional mitochondrial PPR proteins in mRNA 3′ tail synthesis? Both ribosome-associated and non-ribosome-associated PPR proteins may function in this process and/or its regulation. Finally, in plants and mammals, PPR proteins regulate translation through associations with ribosomes and mRNA 5′ untranslated regions (e.g., Delannoy et al., 2007; Davies et al., 2009; Prikryl et al., 2011). Whether PPR proteins have a more widespread function in mRNA 3′ tail synthesis, and thereby stability and/or translation, will be an exciting topic of future research.

REFERENCES

Aphasizheva, I., Maslov, D., Wang, X., Huang, L., and Aphasizhev, R. (2011). Mol. Cell *42*, this issue, 106–117.

Bhat, G.J., Souza, A.E., Feagin, J.E., and Stuart, K. (1992). Mol. Biochem. Parasitol. *52*, 231–240.

Davies, S.M., Rackham, O., Shearwood, A.M., Hamilton, K.L., Narsai, R., Whelan, J., and Filipovska, A. (2009). FEBS Lett. *583*, 1853–1858.

Delannoy, E., Stanley, W.A., Bond, C.S., and Small, I.D. (2007). Biochem. Soc. Trans. *35*, 1643–1647.

Etheridge, R.D., Aphasizheva, I., Gershon, P.D., and Aphasizhev, R. (2008). EMBO J. *27*, 1596–1608.

Kao, C.-Y., and Read, L.K. (2005). Mol. Cell. Biol. *25*, 1634–1644.

Mingler, M.K., Hingst, A.M., Clement, S.L., Yu, L.E., Reifur, L., and Koslowsky, D.J. (2006). Mol. Biochem. Parasitol. *150*, 37–45.

Prikryl, J., Rojas, M., Schuster, G., and Barkan, A. (2011). Proc. Natl. Acad. Sci. USA *108*, 415–420.

Pusnik, M., Small, I., Read, L.K., Fabbro, T., and Schneider, A. (2007). Mol. Cell. Biol. *27*, 6876–6888.