RNA editing - a novel RNA processing phenomenon in trypanosome mitochondria **CO-3**

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RNA editing is an apparently posttranscriptional RNA processing phenomenon occuring in the mitochondrion of kinetoplastid protozoa that involves the addition of uridines at specific sites not encoded in the mitochondrial DNA and the deletion of encoded uridines at specific sites. Editing also occurs to correct internal frameshifts in two maxicircle genes, and uridines are also inserted in 3' untranslated regions and poly A tails of mature mRNAs. The extensive editing at the 5' ends of the Cyb, COIII, MURF2 and MURF3 transcripts extends the reading frames by creation of new amino acids and AUG methionine initiation codons, in the case of the former three genes, which are not encoded in the genome. The new amino acids for each gene are conserved in three kinetoplastid species, although the actual pattern of uridine addition and deletion differs for each species. Hybridization analysis indicated that there was no DNA template for the edited sequences either in maxicircle, minicircle or nuclear DNA. The mechanism of RNA editing is unknown. There are no apparent conserved sequences in flanking regions which could possibly function as signals for uridine addition and deletion. The function of RNA editing appears to be the creation of new N-terminal amino acids and methionine initiation codons, which may act as a translational control mechanism for mitochondrial protein synthesis.

The COII and MURF3 genes encode internal frameshifts which are conserved between species. The -1 and +1 frameshifts of the COII and MURF3 genes are respectively corrected by the addition of four uridines and five uridines at several sites. The precise patterns are absolutely conserved in several species, except for T. brucei which does not contain a recognizable MURF3 gene. T. brucei also does not contain recognizable COIII and ORF12 genes, and it was shown by Feagin et al (1988) that a recognizable COIII transcript is created by extensive RNA editing (>50% of the sequence is added by urdine insertion) of a transcript from a region located at the same relative place in the genome as the COIII gene in L. tarentolae, and it is likely that MURF3 and ORF12 transcripts are also created by this mechanism.

We have developed a modified primer extension assay for analyzing the relative abundance of edited and unedited transcripts for specific edited regions. By this method we showed that 89% of the COII transcripts are edited whereas only 36% of the MURF3 transcripts are edited in the frameshift region. Partially edited molecules were not observed within the limits of sensitivty of this method.

In order to demonsrate that edited mRNA is functional in translation, a polyclonal antibody was raised to a synthetic peptide with the predicted COOH-terminal amino acid sequence of the COII protein past the frameshift region. This antibody was able to react with a COII polypeptide of the predicted molecular weight in Western analysis of a mitochondrial lysate. This implies that the edited COII mRNA is translated, and is, in fact, the first evidence for mitochondrial protein synthesis in a kinetoplastid.

Studies are continuing on this interesting phenomenon with the purpose of understanding the mechanism.