

Purification of Terminal Uridyltransferase from the *Crithidia* Kinetoplast-Mitochondrion

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Terminal uridylyltransferase is a candidate responsible for a post-transcriptional RNA editing process of mitochondrial transcripts in kinetoplastid protozoans. The activity was solubilized by detergent lysis of mitochondria of *Crithidia fasciculata* isolated by the Renografin density gradient floatation centrifugation, and the enzyme was partially purified using chromatography on phosphocellulose, DEAE-cellulose and Sephadex G200 columns. The major activity of the purified enzyme was to add more than 30 uridylates to the 3'-OH end of guide RNA molecules, required magnesium cations and acted in a UTP dependent manner.

Keywords: kinetoplastid protozoan/ RNA editing / guide RNA

Several species of mitochondrial mRNA in kinetoplastid protozoans such as *Crithidia*, *Leishmania*, and *Trypanosoma* are extensively edited after transcription. The location of editing domains, number of editing sites within a single domain and number of uridine (U) residues to be added or deleted at each editing site are very specific to individual mRNA. As to the mechanism of RNA editing, a model that small RNA, named guide RNA (gRNA), specify the sequence alternation has been proposed, based on the finding that the intergenic regions of maxicircle contain some sequences that can pair with edited mRNA sequences if G:U pairs are allowed. This model was experimentally supported by detection of small RNA species with poly(U) tails that can hybridized to the corresponding regions of the maxicircles and minicircles[1, 2].

In the current model of the editing pathway, the

gRNA also serves as the U donor for the editing process via the poly(U) tails of gRNA molecules. This model, which draws analogies from RNA splicing, proposes that editing occurs through multiple rounds of trans-esterification which predicts mRNA-gRNA chimeric molecules as the reaction intermediates. Such chimeric RNAs could also be formed by a mechanism that utilizes endonuclease and RNA ligase. Both chimeric models propose that the role of uridylyltransferase (TUTase) activity is to add the non-coded poly(U) tails to the gRNAs. In the enzyme cascade model, on the other hand, TUTase would be also responsible for uridylate addition to the liberated 3' end in the editing sites which was generated by specific endonuclease for the editing reaction[1, 2]. To examine the function of TUTase in the editing reaction, I first attempted to purify the enzyme.

For assaying TUTase activity, enzyme fraction was

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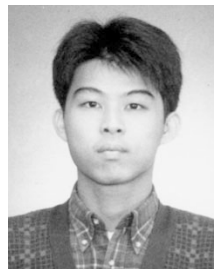
Scope of research

The following is the current major activities of this facility.

With emphasis on regulatory mechanisms of gene expression in higher organisms, the research activity has been focused on analysis of signal structures at the regulatory regions of transcriptional initiation and of molecular mechanisms involved in post-transcriptional modification by the use of eukaryotic systems appropriate for analysis. As of December 1994, studies are concentrated on the molecular mechanism of RNA editing in mitochondria of kinetoplastids.



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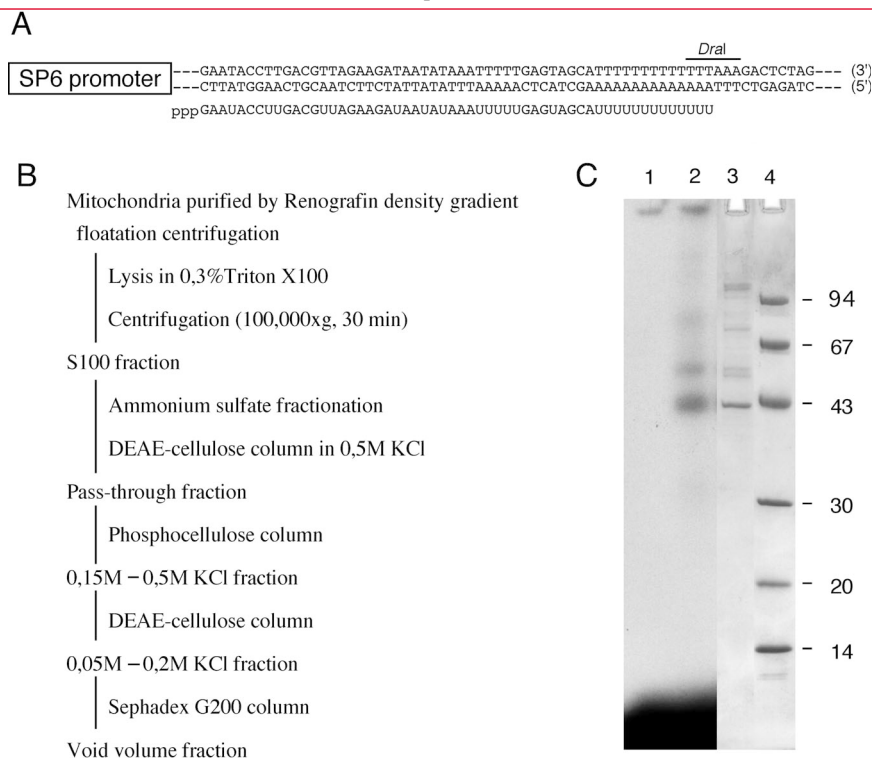


Figure 1. (A) The sequence of Cyb-I gRNA. Below the sequence of the structural gene for Cyb-I gRNA downstream of the promoter for SP6 RNA polymerase in pKS920, the sequence of the transcript on the *DraI*-digested plasmid is shown. (B) Purification procedures of TUTase from purified mitochondria of *C. fasciculata*. (C) Identification of proteins interacted with ^{32}P -labelled Cyb-I gRNA by the UV irradiation method. ^{32}P -labelled Cyb-I gRNA was incubated with the Sephadex G-200 fraction (lane 2). After UV irradiation, the reaction mixture was digested with RNase A and subjected to SDS-PAGE. Lane 1 is of no enzyme fraction control. The Sephadex G-200 fraction was resolved by gradient SDS-PAGE (10-20%), and stained with Coomassie Brilliant Blue (lane 3). By the side of the marker lane (lane 4), molecular weights ($\times 10^3$) of marker are given.

incubated in the reaction mixture containing 0.02M Tris-HCl, pH 7.9, 0.1mM EDTA, 1mM DTT and 0.05mM UTP in the presence of ^{32}P -labelled gRNA (Cyb-I gRNA). The gRNA was synthesized *in vitro* on *DraI*-digested pKS920 carrying the promoter for SP6 RNA polymerase and the structural gene for gRNA responsible for the RNA editing process on the 5'-terminal region of the transcript of the apocytochrome b cryptogene in the presence of ($\alpha^{32}\text{P}$)UTP (Figure 1A). The reaction mixtures were electrophoresed on a polyacrylamide-urea gel and the activity for transfer of uridylates from UTP to the 3'-OH end of the RNA was determined.

Crithidia cells were grown in the brain heart infusion medium containing hemin. After treated with digitonin, the cells were disrupted in a Polytron. The kinetoplast-mitochondrial fraction was isolated by Renografin isopycnic floatation centrifugation, lysed in 0.05M Tris-HCl, pH7.5, 0.1mM EDTA and 1mM DTT containing 0.3% Triton X-100 and centrifuged for 30 min at 100,000xg. The procedures to purify TUTase from the S100 fraction are summarized in Figure 1B.

The Sephadex G200 fraction showed a major band at 43kDa in molecular weight and a few faint bands at 50, 75, and 100 kDa in SDS-polyacrylamide gel electro-

phoresis (SDS-PAGE) (Figure 1C, lane 3). TUTase certainly recognizes the 3'-OH end of gRNA. To identify the molecular weight of TUTase, the enzyme fraction was incubated with ^{32}P -labelled Cyb-I gRNA to form the TUTase-gRNA complexes. After UV irradiation to form the covalent linkage between TUTase and gRNA in the complexes, the reaction mixtures were digested with RNase, and subjected to SDS-PAGE. The result of analysis is shown in Figure 1C, lanes 1 and 2. The 43 kDa protein was mainly labelled with ^{32}P and the 50 kDa protein was faintly labelled. Therefore, the 43 kDa or 50 kDa protein would be TUTase or a subunit of TUTase which interacted with 3'-OH of gRNA. The major activity of the purified enzyme was to add more than 30 uridylates to the 3'-OH end of gRNA molecules, required magnesium cations and acted in a UTP dependent manner.

References

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