Mitochondrial Proteins Interacting with Guide RNAs

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In mitochondria of kinetoplastid protozoa, many of mRNA transcripts of structure genes are modified within coding regions in a posttranscriptional processing characterized by the insertion and, less frequently, the deletion of uridine residues. This process, known as RNA editing, involves small RNA molecules, guide RNAs, which specify the sequence information required. The aim of this study was to identify mitochondrial proteins that are in direct physical contacts with gRNA molecules, thereby possibly responsible for the RNA editing reaction. Using an ultraviolet light-induced cross-linking technique and a gel retardation assay, seven proteins with apparent molecular weights ranging from 20 to 67 kDa in size were identified. Four species of the proteins were purified with DEAE-cellulose and phosphocellulose column chromatographies. One of them probably contacts with 3' poly(U)n tails of the gRNA molecules because the binding of the protein was sensitive to the presence of oligo (U)n.

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Many mitochondrial transcripts in kinetoplastid protozoa such as *Crithidia*, *Leishmania*, and *Trypanosoma* undergo remarkable posttranscriptional processing named RNA editing that is required for creation of functional mRNA. The location of editing domains, number of editing sites within a single editing domain and number of uridine residues to be added or deleted at each editing site are very specific to individual mRNAs, thus creating initiation and termination codons and extending open reading frames. The sequence information for editing is probably contained in small RNAs termed guide RNAs (gRNAs) which are complementary to edited mRNAs. Two models, the enzyme cascade model and the transesterification model, have been proposed for the mechanism of this process. Both models propose initial base pairing-mediated recognition of the preedied mRNA sequence immediately 3' of the region whose editing the gRNA directs with the 5 to 15 nucleotide 5' anchor sequence of gRNA. Thus, all gRNAs have an anchor region, but these differ in sequence between individual gRNAs. All gRNAs described to date are of similar size, averaging about 60 nucleotides, including a posttranscriptionally added 3' oligo(U)n tail of approximately 10 to 15 nucleotides. Guide RNA and mRNA are thought to interact during editing in a ribonucleoprotein (RNP) complex. The minimal function of such a complex is presumably maintain the proximity of the mRNA 5' and 3' fragments. A more extensive function for the complex, perhaps involving catalysis, gRNA selection, or developmental regulation is also possible. The aim of this study was to identify mitochondrial proteins that are in direct physical contact with gRNA molecules, thereby possibly responsible for RNA editing.

To identify mitochondrial proteins that form stable, direct contacts with gRNA molecules, I used two different techniques, an ultraviolet (UV) light-induced cross-link

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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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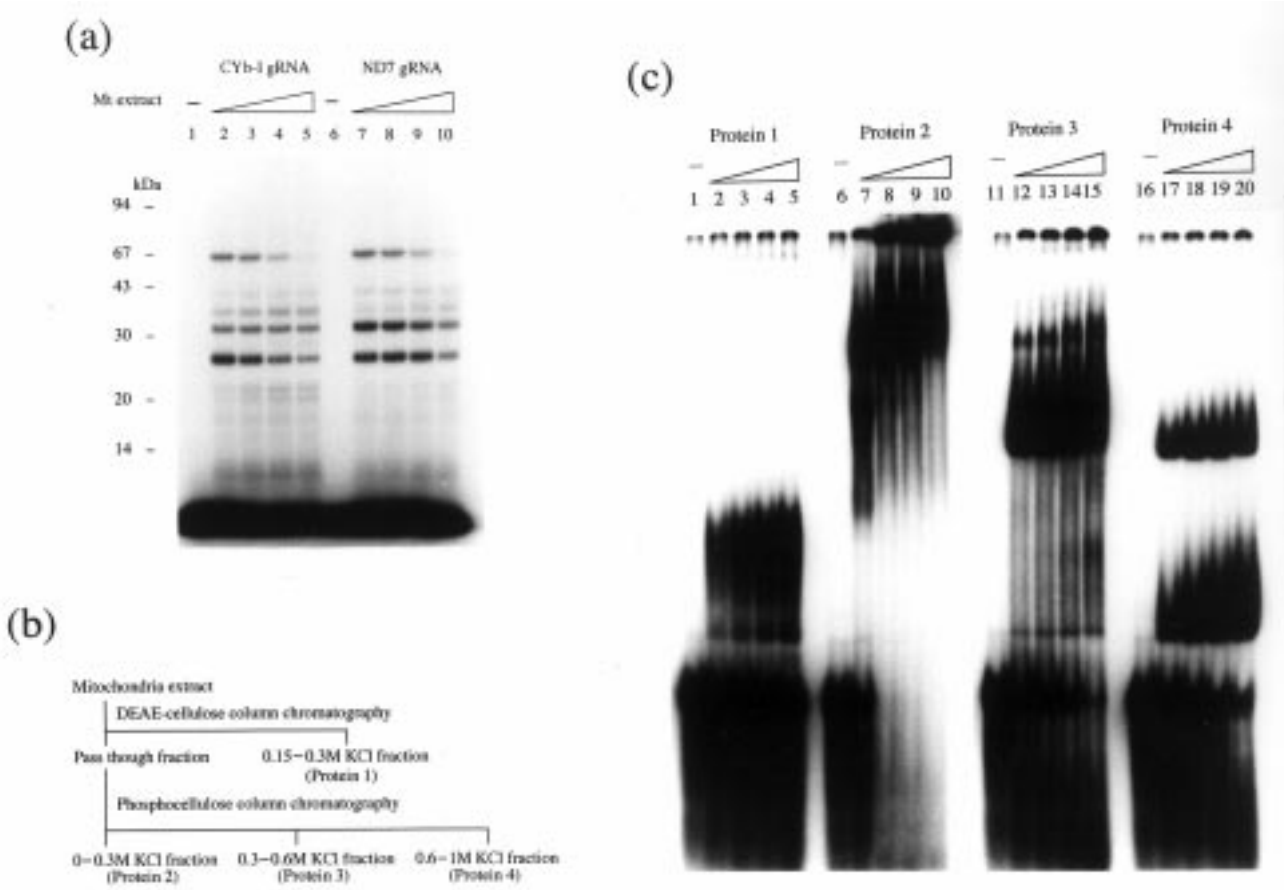

*F*ig. 1 (a) Protein cross-linking of ^{32}P-labeled CYb-I and ND7 gRNA with mitochondrial proteins of *C. fasciculata*. (b) Purification procedures of proteins interacted with CYb-I gRNA. (c) Patterns of a gel retardation assay with ^{32}P-labeled CYb-I gRNA and the purified protein fractions.

ing technique and a gel retardation assay. ^{32}P-Labeled gRNA molecules were incubated with the S100 mitochondrial extract of *C. fasciculata* in low monovalent cation concentration and subsequently irradiated at 254 nm. The reaction was performed in the presence of a defined concentration of tRNA molecules to account of non-specific RNA-protein interactions. Two gRNA substrates used in this study are specific for different editing domains of *C. fasciculata*, CYb-I and ND7 mRNAs and were labeled with (α^{32}P)UTP by *in vitro* transcription. The transcripts contain a few vector-derived extrabases at their 5' end and have 3' oligo(U) tails of about 10 nucleotides. The cross-linked samples were extensively digested with ribonuclease, and the proteins were separated on a SDS-polyacrylamide gel. Proteins cross-linked to the gRNAs were detected by the radioactive oligonucleotides which remained to be covalently attached after the ribonuclease treatment while the none-cross-linked protein were stripped from the RNA by the denaturing conditions in electrophoresis. Seven mitochondrial proteins were identified with apparent molecular weights of 20, 22, 26, 32, 37, 40, and 65kDa [Fig. 1(a)]. The two different gRNA substrates gave essentially an identical cross-linking pattern, indicating the assembly of the same RNP complex upon mitochondrial extract addition. Several major ribonucleoprotein complexes which can be resolved on 4% native polyacrylamide gels are formed during incubation of *in vitro*-transcribed gRNA with the mitochondrial extract. I made an attempt to purify the individual proteins interacted with gRNAs. The S100 extract was applied on a DEAE-cellulose column and separated to the pass through fraction and the absorbed fraction. The absorbed proteins were eluted with 0.4M KCl after washing the column with 0.15 M KCl. The pass through fraction was applied onto a phosphocellulose column and eluted with three steps, 0.3M, 0.6M and 1M KCl [Fig. 1(b)]. The separated protein fractions were further purified with hydroxyapatite column chromatography. Patterns of the gel retardation assay with the purified proteins and ^{32}P-labeled CYb-I gRNA are shown in Fig. 1(c). Protein IV probably contacts with 3' poly(U)n tails of the gRNA molecules because the binding of the protein was sensitive to the presence of oligo (U)n.

References

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