9. Typical reactions with the copper-phenanthroline system, see (*7*), were carried out in 800 ml of toluene at 80° to 90°C while bubbling through pure oxygen, which seems to breach safety regulations in most companies or institutions.
10. The boiling point of toluene is 110°C, which means that it is less suitable for the oxidation of, e.g., 1-pentanol, giving valeraldehyde (pentanal) with a boiling point of 103°C.
11. Because the catalyst is already in a separate solid phase, the alcohol must be solubilized; otherwise, reaction rates are very low. See, for instance, T. Mallat and A. Baiker, *Catal. Today* **19**, 247 (1994).
12. Bathophenanthroline disulfonate, or 4,7-diphenyl-1,10-phenanthroline disulfonate, is a nontoxic pale yellow crystalline powder with no odor, CAS: [52746-49-3]. It is moderately soluble in water (>10% w/w). For further product information, see http://129.8.100.52/html/grad-lab/msds/d/4,7-diphenyl-1,10-phenanthroli0. It is commercially available from Pfaltz & Bauer (Waterbury, CT), Acros (Geel, Belgium), Alfa Aesar (Ward Hill, MA), Lancaster Synthesis (Windham, NH), or TCI (Tokyo). It is often used in biomedical kits to determine the iron content (non-heme) of serum or plasma in the diagnosis of iron deficiency anemia, hemochromatosis, and chronic renal disease through colorimetry. Sentinel Diagnostic (Milan, Italy) sells standard kits under the name of "Iron Bato."
13. The initial turnover frequency (in mmol/mmol per hour) is an indication of the speed of the reaction. It denotes the average number of substrate molecules (in mmol) that is converted by each mmol of catalyst in 1 hour. The turnover number (in mmol/mmol) denotes the average number of substrate molecules (in mmol) that 1 mmol of catalyst has converted during the course of the reaction.
14. In a reaction with 2-hexanol, the catalyst solution was recycled five times. Reactivity and selectivity remained >90 and 98%, respectively, of the initial values.
15. The catalyst solutions were prepared by stirring $Pd(OAc)_2$ (0.0112 g, 0.05 mmol) and PhenS* (0.0274 g, 0.05 mmol) overnight in water (50 ml) to give a clear yellow solution. Consecutively, $NaOAc{\cdot}3H_2O$ (0.136 g, 1 mmol) and NaOH were added until pH ~11.5. The purity of the water and the chemicals used may dramatically influence the reaction rate.
16. Standard catalytic experiments were carried out in a closed Hastelloy C autoclave (175 ml). The autoclave was cooled and charged with the following catalyst solution: PhenS*$Pd(OAc)_2$ (0.05 mmol, 0.25 to 0.50%) in water (50 ml), alcohol (10 to 20 mmol), and internal standard (*n*-heptane, *n*-octane, or *n*-dodecane). The autoclave was pressurized with air and heated to 100°C (30 bar) until the reaction was complete. After reaction, the autoclave was cooled to room temperature and depressurized, collecting any volatile material in a liquid nitrogen trap. The mixture was extracted with Et_2O (Et, ethyl), the organic layer was dried over $MgSO_4$, and a different external standard was added (*n*-dodecane or *n*-hexadecane). Samples were analyzed by ^1H nuclear magnetic resonance ($CDCl_3$, 300 MHz) and gas chromatography [a Varian Star 3400 instrument equipped with a carbowax column (50 m by 0.53 mm)]. Ether was used to extract the relatively small amount of alcohol (10 to 20 mmol) from the aqueous phase to obtain good recoveries and more reliable data. In a large-scale process, the aqueous phase would be recycled after decantation of the product, and the use of organic solvent would be superfluous.
17. For identification of the catalyst, see G. J. ten Brink, I. W. C. E. Arends, G. Papadogianakis, R. A. Sheldon, *Appl. Catal. A* **194–195**, 435 (2000).
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Translation of the Edited mRNA for Cytochrome b in Trypanosome Mitochondria

Anton Horváth,[1] Edward A. Berry,[2] Dmitri A. Maslov[1]*

The type of RNA editing found in the kinetoplast-mitochondria of trypanosomes and related protozoa, involving uridylate insertions and deletions, creates translatable messenger RNAs (mRNAs) out of nonsense pre-edited RNAs by correcting encoded defects that vary from simple frameshifts to large "cryptic" regions. However, any evidence for translation of these mRNAs in the kinetoplast has been missing for decades. We identified a kinetoplast-encoded protein, apocytochrome b, whose mRNA is edited in the 5′ region. The determined amino-terminal sequence of the protein coincides with the predicted sequence derived from the edited region, demonstrating that the cognate apocytochrome b mRNA is translated into a functional protein. This finding represents the first direct evidence for a functional translation system in the kinetoplasts.

In trypanosomes and related protozoa from the order Kinetoplastida, mRNAs for several kinetoplast-mitochondrial genes undergo the posttranscriptional process of RNA editing, where additions, and occasionally deletions, of uridylate residues in the pre-edited mRNA produce a corrected reading frame (*1*). Depending on the mRNA species, the extent of this process varies from repairing an encoded frameshift by inserting a few uridylate residues to creating an entire reading frame by the insertion of hundreds of residues (*2, 3*). Small transcripts, termed guide RNAs, serve as the templates for the enzymatic editing machinery by defining the sites of editing and the number of insertions or deletions (*4–6*).

The functional significance of RNA editing is not clear. Although it may represent a mechanism of mitochondrial gene regulation, no direct biochemical proof for translation of edited mRNAs has been found since RNA editing was discovered in trypanosomes more than a decade ago (*2, 7*). The kinetoplast DNA sequence studies showed that, similar to other mitochondria, it encodes components of the mitochondrial ribosomes and subunits of the respiratory complexes, such as cytochrome c oxidase subunits I, II, and III (respiratory complex IV), apocytochrome b (complex III), and adenosine triphosphatase subunit 6 (complex V) (*8*). These complexes were isolated and their subunit composition analyzed by protein microsequencing (*9–11*). All detected subunits turned out to be nuclear encoded, thus leaving the existence of kinetoplast-encoded proteins an open question. Because earlier attempts in several laboratories to demonstrate kinetoplast translation in vivo or to isolate kinetoplast ribosomes were unsuccessful (*7*), these results, taken together, might question the very existence of a functional kinetoplast system of translation. However, the predicted encoded polypeptides were extremely hydrophobic, often with an increased cysteine content, and these properties could make the detection difficult, most likely due to aggregation and abnormal electrophoretic migration. Here, we present a first identification of a kinetoplast-encoded protein, apocytochrome b, a subunit of cytochrome bc_1 (complex III, ubiquinol:cytochrome c oxidoreductase) that is translated from an edited mRNA.

In the trypanosomatid *Leishmania tarentolae* the mRNA for apocytochrome b is edited in the 5′ region by insertion of 39 uridylate residues at 15 sites (Fig. 1) (*12*). The process creates 20 NH_2-terminal in-frame codons, including the initiation triplet AUG. The predicted polypeptide of 44.5 kD contains 63% nonpolar residues, making it extremely hydrophobic. The cytochrome bc_1 complex was purified from *L. tarentolae* kinetoplasts by chromatography on DEAE-sepharose (*13, 14*). The purified enzymatic complex had a specific activity of 81.6 mU/μg. The cytochrome b and c_1 spectra and the heme content were similar to a previously isolated complex from the related trypanosomatid, *Crithidia fasciculata* (*9*). Analysis of the enzyme from *L. tarentolae* by denaturing SDS-polyacrylamide gel electrophoresis (Fig. 2) revealed nine subunits, all of which are nuclear encoded (*14*), similar to the enzyme from *C. fasciculata*. These subunits include three bands in the "core protein" region (bands 1 through 3), the

[1]Department of Biology, University of California, Riverside, CA 92521, USA. [2]Division of Structural Biology, University of California, Berkeley, CA 94720–5230, USA.

*To whom correspondence should be addressed. E-mail: maslov@ucrac1.ucr.edu

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DNA:              ...AAA A G      CG G  AGA     G  A
RNA:     5'- 30 nt - AAAuAuGuuuuuuCGuGuuAGAuuuuuGuuAuuu
protein:                 M  F  F  R  V  R  F  L  L  F

DNA:          A  A   AGAAA   A G  G C TTTA...
RNA:     uuuuuAuuAuuuAGAAAuuuAuGuuGuCuUUUA- 1050 nt-UAA
protein: F  L  L  F  R  N  L  C  C  L  L  - 350 aa -Ter
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Fig. 1. A portion of the gene (DNA), edited mRNA (RNA), and deduced amino acid sequence (protein) (*20*) of apocytochrome b from *Leishmania tarentolae*. The uridylate residues inserted by RNA editing are shown in lowercase. nt, nucleotide; aa, amino acid.

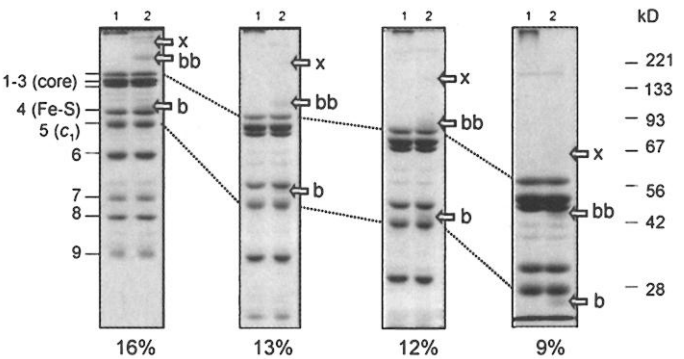

Fig. 2. Subunit composition of the purified cytochrome bc_1 complex of *L. tarentolae* analyzed by electrophoresis in tris–glycine–SDS-polyacrylamide gels of different concentrations. Before loading on gels, the sample (~20 μg of protein) was denatured at 100°C (lane 1) or 37°C (lane 2). Positions of the nuclear encoded subunits are indicated to the left. Positions of the markers in the 9% gel are shown to the right. The arrows indicate three components (*b*, *bb*, and *x*) with abnormal gel migration and sensitivity to heat denaturation.

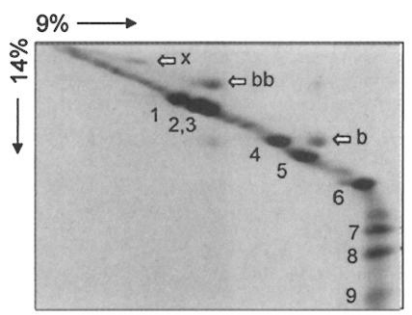

Fig. 3. Two-dimensional gel used for NH_2-terminal sequencing of components *b* and *bb*. Purified cytochrome bc_1 (200 μg of protein) was separated in a first-dimension 9% Tris–glycine–SDS-polyacrylamide gel. The gel slice was incubated in an excess of 1% SDS and 1% β-mercaptoethanol at 37°C for 30 min and polymerized on top of a second-dimension 14% gel. The band designations are as in Fig. 2.

Rieske iron-sulfur protein (band 4), and cytochrome c_1 (band 5). In addition, we detected three substoichiometric components, labeled *b*, *bb*, and *x*, which displayed unusual properties expected of hydrophobic proteins: these components completely precipitated when the complex was denatured at 100°C before loading on the gel, and their relative electrophoretic mobilities were dependent on the gel concentration. For example, component *b* is seen to migrate slower than band 4 in a 16% Tris–glycine–SDS-polyacrylamide gel, but showed a faster mobility than band 5 in a 9% gel (Fig. 2). Both of these properties are related to hydrophobicity and have been reported with mitochondrial cytochrome b from other organisms (*15*). The apparent molecular mass of component *b* estimated by a Ferguson plot (*16*) gave a value of 48 kD, which is close to the predicted mass of the kinetoplast-encoded cytochrome b. For components *bb* and *x*, the corresponding values were too large to be accurately determined, with component *bb* at least twice as big as component *b*.

The abnormal gel mobilities of components *b*, *bb*, and *x* allowed their separation from other subunits by denaturing two-dimensional gel electrophoresis (Fig. 3). A characteristic rectangle formed by components *b* and *bb* in the gel suggested that a partially reversible dimerization or a higher-order oligomerization of *b* takes place during denaturation of the sample. Although the electroblotting efficiency of these components, especially from more concentrated gels, was poor, it was possible to visualize components *b* and *bb* on Coomassie stained polyvinylidene difluoride membranes. To remove an observed NH_2-terminal block of sequence analysis by Edman degradation, the components *b* and *bb* were treated with 0.5 M HCl. This procedure removes NH_2-terminal formyl groups previously found in mitochondrially encoded bovine cytochrome c oxidase subunit II (*17*). Seventeen residues were determined for component *b* and 15 residues for *bb*. Both sequences matched with the predicted NH_2-terminal sequence of apocytochrome b (Fig. 1), indicating that components *b* and *bb* represent the monomeric and oligomeric forms of this protein. These residues are encoded by codons created by editing of the mRNA. The nature of component *x* is unclear; it may represent a stable high molecular weight aggregate of apocytochrome b, but this was not verified by sequence analysis because this component could not be detected on blots.

The previous available evidence for translation of kinetoplast messengers included immunodetection of a few cytochrome c oxidase and NADH dehydrogenase subunits with the sera raised against the predicted peptides (*18, 19*). However, it was not shown whether the detected polypeptides represented the bona fide subunits of the corresponding enzymes. The NH_2-terminal sequencing of apocytochrome b we report is the first direct identification of a kinetoplast-encoded protein that is translated from a cognate edited mRNA. Because this protein was found within an enzymatically active respiratory complex, this finding demonstrates that a functional translation system operates in the kinetoplast-mitochondria of trypanosomes.

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