Biochimica et Biophysica Acta, 1144 (1993) 229–231

BBABIO 40277

Short Sequence-Paper

Cytochromes c_1 of kinetoplastid protozoa lack mitochondrial targeting presequences

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(Received 16 June 1993)

Key words: Mitochondrion; Cytochrome *c* reductase; Cytochrome c_1; cDNA; Targeting presequence; (*C. fasciculata*); (*B. caudatus*)

We have used the polymerase chain reaction to amplify cDNA fragments that encode the amino-terminal sequences of cytochrome c_1 from two distantly related kinetoplastid species, *Crithidia fasciculata* and *Bodo caudatus*. Cloning and sequencing of these fragments have revealed that these proteins lack conventional mitochondrial targeting presequences.

Cytochrome c_1 is a membrane-associated subunit of the cytochrome *c* reductase complex. The eukaryotic cytochromes c_1 thus far examined are nuclear encoded and are translated on cytoplasmic ribosomes as precursor proteins with amino-terminal extensions that vary in length (84 to 61 amino acids) depending on the species (for review see Refs. 1 and 2). These extensions, or presequences, have been implicated in mitochondrial targeting, import, and sorting [3,4]. Prior to assembly of the functional protein complex, the presequence is removed by processing proteases to give the mature-sized cytochrome c_1 protein [5]. We have cloned cDNA fragments encoding the amino-terminal regions of *Crithidia fasciculata* and *Bodo caudatus* cytochrome c_1 and have determined that both proteins lack conventional presequences. The mature *C. fasciculata* cytochrome c_1 protein is formed by the removal of a single methionine residue from the amino terminus of the precursor protein.

Total RNA was isolated from *C. fasciculata* and *B. caudatus* cells using the SDS/hot phenol method described in Adler, et al. [6]. Poly(A)$^+$ RNA was selected by oligo(dT) cellulose column chromatography [7], and first strand cDNA was synthesized using an oligo(dT)$_{15}$ primer and AMV reverse transcriptase as directed by the manufacturer (Promega). Three deoxyoligonucleotides were purchased from Oligos Etc. (Guilford, CT): JP-1 (5′-CAG TTT CTG TAC TTT ATT G-3′), corresponding to part of the miniexon sequence of *C. fasciculata* [8], WP-2 (5′-NAC RTC YTT NGC CAT YTG-3′), a degenerate sequence deduced from a conserved cytochrome c_1 protein sequence [9], and OP-1 (5′-CAA GGC GAA GAT GTA GTC AG-3′), a sequence determined from a genomic clone of *B. caudatus* cytochrome c_1 [9].

A cytochrome c_1 fragment was amplified from *C. fasciculata* cDNA by polymerase chain reaction using 100 μM concentrations of oligonucleotides JP-1 and WP-2 and 400 μM dNTPs as described in Priest and Hajduk [9]. The extension protocol was 5 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min; 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min; and one cycle of 72°C for 15 min. A DNA band of approx. 750 bp was purified from a 1.25% Seaplaque GTG agarose gel (FMC) and was reamplified using the same conditions except that the initial 5 cycles at 37°C were omitted. The reamplified product was digested with *Pst*I and a fragment of approx. 500 bp was purified from a 4% polyacrylamide gel. This fragment was ligated into *Eco*RV/*Pst*I digested Bluescript SK$^+$ plasmid (Stratagene) and used to transform DH5α *E. coli* cells (BRL) [7]. Using oligonucleotides JP-1 and OP-1 and the conditions and protocol described above, a cytochrome c_1 fragment was also amplified from *B. caudatus* cDNA. A DNA band of approx. 430 bp was purified from a 1.25% Seaplaque GTG agarose gel and was reamplified using the same conditions and exten-

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The sequence data in this paper have been submitted to the GenBank Data Library under the accession numbers L13602 (*C. fasciculata*) and L13603 (*B. caudatus*).

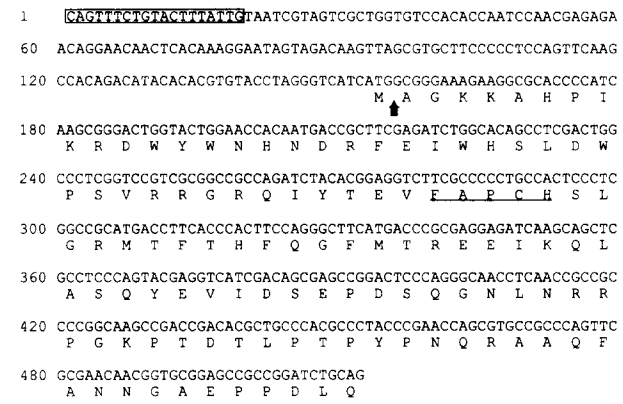

Fig. 1. Nucleotide and deduced amino acid sequence of *C. fasciculata* cytochrome c_1 cDNA. The sequence of the miniexon primer (JP-1) is boxed. The arrow indicates the mature amino terminus of the protein as determined by peptide sequencing [9]. The heme binding site sequence consists of the underlined amino acid residues beginning at nucleotide position 279 [9]. For the remainder of the known protein coding sequence see Priest and Hajduk [9].

sion protocol. The reamplified product was purified on a 1.25% Seaplaque GTG agarose, ligated into Bluescript SK$^+$ plasmid using *Bam*HI linkers [10], and used to transform DH5α cells.

Both strands of the resulting cDNA clones were sequenced using the chain termination technique (Sequenase version 2.0, USB Corp). The cDNA sequence of the *C. fasciculata* cytochrome c_1 clone is shown in Fig. 1. This clone contains only one in-frame ATG initiation codon between the end of the mini-exon sequence at nucleotide position 19 and the beginning of the heme binding site sequence at position 279. This methionine codon is located immediately adjacent to the sequence that encodes the mature amino terminus of the protein [9]. Thus we conclude that the *C. fasciculata* cytochrome c_1 lacks a conventional mitochondrial targeting presequence and that a single methionine is removed from the precursor protein to form the mature cytochrome c_1. As in the *C. fasciculata* clone,

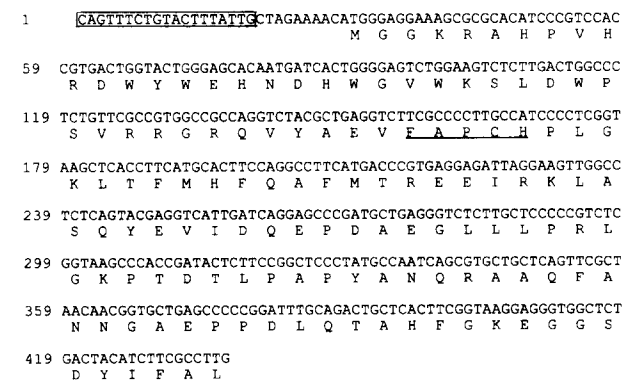

Fig. 2. Nucleotide and deduced amino acid sequence of *B. caudatus* cytochrome c_1 cDNA. The sequence of the miniexon primer (JP-1) is boxed. The heme binding site sequence consists of the underlined amino acid residues beginning at nucleotide position 155 [9].

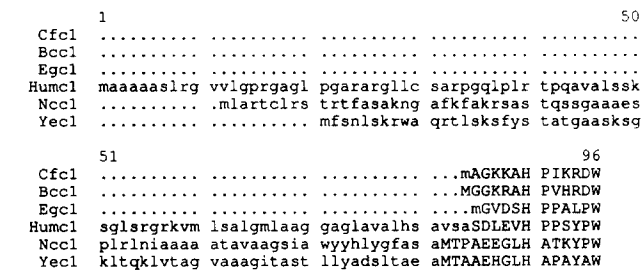

Fig. 3. Amino-terminal sequence alignment of *C. fasciculata* (Cfc1) and *B. caudatus* (Bcc1) cytochromes c_1 with those of *E. gracilis* (Egc1) [11], human (Humc1) [13], *Neurospora crassa* (Ncc1) [14], and yeast (Yec1) [15]. Residues known to be removed by processing are indicated in lower-case letters. The mature amino terminus has not yet been determined for the *B. caudatus* protein.

the sequence of the *B. caudatus* cytochrome c_1 cDNA clone shown in Fig. 2 contains only one in-frame ATG methionine initiation codon between the end of the mini-exon primer and the beginning of the heme binding site sequence. Based on sequence homology, the mature amino terminus of the *B. caudatus* protein probably begins at the glycine immediately adjacent to this methionine. Since there is not sufficient sequence in the 5′ untranslated region of the *B. caudatus* clone to encode a signal peptide, the observed absence of a presequence is not a PCR artifact.

In Fig. 3 the deduced amino-terminal sequences of these two cDNA clones are aligned with those of several representative eukaryotic cytochromes c_1. The relative proximity of the amino termini of the *C. fasciculata* and *B. caudatus* cytochromes c_1 to the mature amino termini of the other eukaryotic proteins supports the conclusion that the kinetoplastid proteins lack conventional presequences. Surprisingly, the sequence alignment also shows that the absence of a presequence is a feature shared with the primitive protozoan flagellate *Euglena gracilis*. Like the kinetoplastid cDNA sequences, the *E. gracilis* cDNA sequence reported by Mukai et al. [11] contains only one ATG initiation codon which is located immediately upstream of the codon for the mature amino-terminal residue. The lack of a presequence implies that the cytochrome c_1 proteins of the kinetoplastids and the euglenoids are imported into the mitochondrion along a "non-conservative" import pathway [12]. We are currently studying this import in vitro.

This work was supported by National Institutes of Health Grant AI21401 and AI28780 (to S.L.H.) and Postdoctoral Fellowship AI08259 (to J.W.P.). S.L.H. is a Burroughs Wellcome Scholar in Molecular Parasitology.

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