Biochimica et Biophysica Acta, 1184 (1994) 139–141

BBABIO 40291

Short Sequence-Paper

Sequence of a mouse embryo cDNA clone encoding proteolipid subunit 9 (P1) of the mitochondrial H^+-ATP synthase

Lajos Pikó *, Donna E. Nofziger, Linda M. Western [1] and Kent D. Taylor

Developmental Biology Laboratory, VA Medical Center, 16111 Plummer Street, Sepulveda, CA 91343, USA

(Received 14 September 1993)

Key words: ATPase; Proteolipid; Subunit 9 (P1); cDNA; Nucleotide sequence

A cDNA clone to an abundantly expressed mRNA in cleavage stage mouse embryos has been sequenced and identified as encoding subunit 9 (P1) of the mitochondrial H^+-ATP synthase. The deduced amino acid sequence of the mature subunit 9 protein differs in a single residue from the corresponding rat, ovine, bovine and human subunits.

The mitochondrial H^+-ATP synthase complex (F_1F_0-ATPase; complex V of the mitochondrial oxidative phosphorylation system) is composed of two principal sectors: an integral membrane-bound segment, F_0, which is concerned with proton translocation and F_1, the catalytic portion of the enzyme. In mammals, the F_0 segment consists of the proteolipid subunit 9 (dicyclohexylcarbodiimide [DCCD]-binding protein; a homologue of subunit c of bacterial ATP synthase) which is encoded by the nuclear genome and subunits 6 and 8 that are derived from mitochondrial genes [1,2]. The amino acid sequence of subunit 9 has been highly conserved in H^+-ATPase complexes from a variety of sources [3].

In the bovine genome, the subunit 9 protein of ATP synthase is encoded by two different nuclear genes, designated P1 and P2. The mRNAs derived from the two genes produce identical mature protein subunits but differ in the amino acid sequence of the import signal peptide, which is removed during entry into the mitochondrion, and have divergent 5′ and 3′ untranslated regions (UTRs) [4,5]. Both mRNAs have been detected in all bovine tissues examined but their expression differs according to the embryonic origin of the tissue: the P1:P2 mRNA ratio is about 1:1 in mesoderm derivatives (heart, muscle, kidney) and about 1:3 in endoderm derivatives (liver, intestine, lung) and brain [4]. Two different cDNAs homologous to the bovine P1 and P2 gene transcripts have been identified also in cDNA libraries from rat [6], human [7,8] and ovine [9] tissues. Although the signal peptides of the precursor polypeptides encoded by these cDNAs vary, the sequence of the 75-amino-acid mature subunit 9 protein has been found to be identical in all of these species.

Here we report the nucleotide sequence of a cDNA clone (originally designated clone A9) isolated from a two-cell mouse cDNA library and selected for study because of its high level of expression in cleavage-stage mouse embryos [10]. A GenBank data search identified this cDNA as the mouse homologue of the bovine P1 gene for subunit 9 of the mitochondrial ATP synthase.

The two-cell cDNA library was originally constructed in pUC8 vector by G-C tailing [10]. The cDNA insert studied here was subcloned into pGEM4Z vector (Promega) and this clone was used for nucleotide sequencing and the production of hybridization probes. Sequencing was carried out in part by the chemical degradation method [11] and in part by thermal cycle sequencing using appropriate primers and the Vent(exo$^-$) DNA polymerase according to the supplier's protocol (New England Biolabs).

Fig. 1 shows the nucleotide sequence of the mouse subunit 9 (P1) cDNA clone in comparison to a rat subunit 9 (P1) cDNA [6]. Although the mouse cDNA is truncated at both termini, it contains an open reading frame of 408 nucleotides capable of encoding a signal peptide of 61 amino acids and a mature subunit protein of 75 amino acids (Fig. 2). The mouse and rat cDNAs exhibit a high degree of nucleotide homology

* Corresponding author. Fax: +1 (818) 8959554.
[1] Present address: Syva Co., Palo Alto, CA 94304, USA.
The sequence data reported in this paper have been submitted to the GenBank, EMBL and DDBJ Nucleotide Sequence Databanks under accession number L19737.

SSDI 0005-2728(93)E0198-Y

```
                                                          ↓Precursor
MOUSE                                                     AAAATGCAGACCACC   15
RAT   GTGTGCTGTGGGCCGAGCAGGGGCTGCAGGGGAGTGGGAGTGCAGATTGA.............G   65

MOUSE AAGGCACTGCTCATTTCTCCAGCTCTGATTGGCTCCTGTACTAGGGGTCTAATCAGGCCTGTGTC   80
RAT   .....................T......C...........C.....................   130

MOUSE TGCCTCCCTCCTGAGCAGACCAGAGGCCCCATCTAAGCAGCCTTCCTGCAGCAGCTCCCCTCTCC  145
RAT   ..............T...........T.........AA...........T.............   195

                                                          ↓Mature
MOUSE AGGTGGCCCGACGGGAATTCCAGACCAGTGTCATTTCCCGGGACATCGACACAGCAGCCAAGTTC  210
RAT   .......A.....................T.............T.................T   260

MOUSE ATTGGTGCTGGGGCCGCCACAGTTGGTGTGGCTGGATCAGGAGCTGGCATTGGCACAGTGTTTGG  275
RAT   .....................................................G.........   325

MOUSE TAGCTTGATTATTGGCTATGCCAGGAACCCATCTCTCAAGCAGCAGCTCTTCTCCTATGCCATTC  340
RAT   ..............................G.....................................C.  390

MOUSE TGGGGTTTGCCCTGTCTGAGGCCATGGGACTCTTCTGTTTGATGGTCACCTTCCTCATCCTCTTC  405
RAT   ....C.....................G.....................G.................   455

          ↓3'UTR
MOUSE GCCATGTGAGGCTCCCTGGGGTCACCCAGCCGTCCCTGCTGCTTTGACTCCATGCCAGTCCTAGT  470
RAT   ..............A...........G.....................................G..  520

MOUSE GCTGGAGTCTACTGAGCTTTACCATTAAA                                        499
RAT   .......G.G.....................CTACGTTTCTCT                        561
```

Fig. 1. Nucleotide sequence of a mouse embryo cDNA clone encoding subunit 9 (P1) of the H^+-ATPase complex. The clone contains an open reading frame coding for the full-length precursor polypeptide but is truncated in the 5′ and 3′ UTRs. The mouse sequence is shown in comparison to a rat subunit 9 (P1) cDNA sequence [6]. In the two terminal regions where the mouse cDNA is truncated, the rat sequence is shown. For the remainder of the rat cDNA, dots indicate nucleotides identical to those in the mouse sequence. The initiation and termination codons for the precursor polypeptide are overlined; the putative polyadenylation signal ATTAAA is underlined. The arrows indicate the beginning of the sequence coding for the precursor polypeptide and the mature subunit protein, respectively.

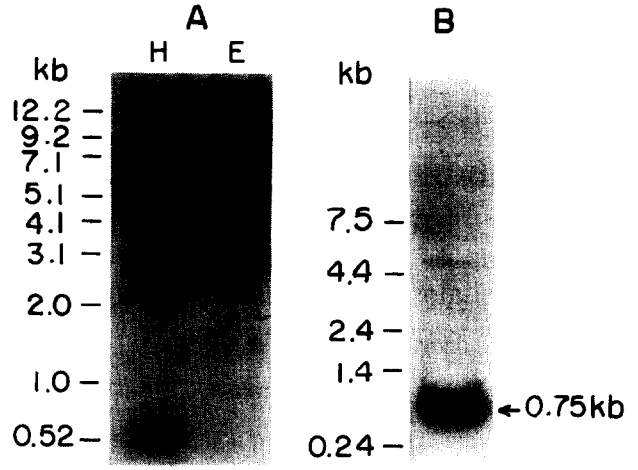

Fig. 3. (A) Southern blot analysis of the mouse H^+-ATPase gene. Samples of 5 μg mouse liver nuclear DNA were digested with *Eco*RI (lane E) or *Hin*dIII (lane H), electrophoresed on a 0.7% agarose gel, and blotted onto a nitrocellulose filter. The blot was hybridized with 10 ng/ml probe DNA labeled with ^{32}P to a specific activity of about $5 \cdot 10^6$ dpm/ng using random priming of the mouse subunit 9 (P1) cDNA template. (B) Northern blot hybridization of a poly(A)$^+$ RNA fraction (60 ng/lane) from mouse F9 embryonal carcinoma cells with a ^{32}P-labeled cRNA probe (about $1.6 \cdot 10^6$ dpm/ng) to mouse subunit 9 (P1) cDNA. For procedures see Refs. 14 and 15.

of about 94% in the region coding for the signal peptide and in the 3′UTR and 96.4% in the region coding for the mature subunit.

The amino acid sequence (as deduced from the nucleotide sequence) of the full-length precursor polypeptide of the mouse subunit 9 (P1) is shown in Fig. 2 in comparison to other mammalian polypeptides derived from the P1 gene. The amino acid sequence of the import signal peptide in the mouse differs in three residues from the rat sequence, seven from the ovine, eight from the bovine and fourteen from the human. With respect to the mature subunit, the mouse sequence shows a single amino acid change (from alanine to threonine) at position 129 as compared to the other species. Thus the mouse appears to be the first mammalian species reported so far showing a variance in the sequence of the mature subunit 9 protein.

```
          ↓Precursor
MURINE    MQTTKALLISPALIRSCTRGLIRPVSASLLSRPEAPSKQPSCSSSPLQVA   50
RAT       ...........V.........................K...C.......
OVINE     ....G.....................F.....I..V...Y..G.....
BOVINE    ....G.....................F.....IQ.V...Y..G.....
HUMAN     ...AG..F.......C...........F.NS.VNS.....Y.NF.....

                    ↓Mature
MURINE    RREFQTSVISRDIDTAAKFIGAGAATVGVAGSGAGIGTVFGSLIIGYARN  100
RAT       ..................................................
OVINE     .........V........................................
BOVINE    .........V........................................
HUMAN     .........V........................................

MURINE    PSLKQQLFSYAILGFALSEAMGLFCLMVTFLILFAM                136
RAT       ...........................A.......
OVINE     ...........................A.......
BOVINE    ...........................A.......
HUMAN     ...........................A.......
```

Fig. 2. The deduced amino acid sequence of the mouse subunit 9 (P1) precursor protein is shown in comparison to the rat [6], ovine [9], bovine [4] and human [8] P1 polypeptides. The dots denote residues identical to those in the mouse protein. The arrows indicate the first amino acid residue of the precursor polypeptide and the mature subunit, respectively.

Hybridization of *Eco*RI- and *Hin*dIII-digested mouse nuclear DNA with a mouse subunit 9 (P1) cDNA probe yielded three to four major and several minor bands (Fig. 3A) suggesting that the mouse subunit 9 gene of ATP synthase also occurs in multiple copies. In the bovine [5], ovine [9] and human [12] genomes, the subunit 9 gene family includes several pseudogenes in addition to the two expressed genes (P1 and P2).

In F9 mouse embryonal carcinoma cells, Northeren blot analysis revealed a single poly(A)$^+$ RNA band of the expected molecular weight (about 750 nucleotides, including the poly[A] tail) hybridizing with the subunit 9 (P1) probe (Fig. 3B). Dot hybridization experiments ([10]; and Taylor and Pikó, unpublished data) indicate that mRNA hybridizing with the subunit 9 (P1) probe is moderately prevalent in mouse oocytes but increases greatly in abundance in the early embryo from the two-cell stage onward, coincident with a pronounced fine structural differentiation and increase in respiratory activity of the mitochondria [13]. Whether the P1

gene alone or both the P1 and P2 genes are expressed at these early stages of development is as yet unkown.

This work was supported by Public Health Service research grant HD-19691 from the National Institute of Child Health and Human Development.

References

1 Hatefi, Y. (1985) Annu. Rev. Biochem. 54, 1015–1069.
2 Futai, M., Noumi, T. and Maeda, M. (1989) Annu. Rev. Biochem. 58, 111–136.
3 Sebald, W. and Hoppe, J. (1981) Curr. Top. Bioenerg. 12, 2–64.
4 Gay, N.J. and Walker, J.E. (1985) EMBO J. 4, 3519–3524.
5 Dyer, M.R., Gay, N.J. and Walker, J.E. (1989) Biochem. J. 260, 249–258.
6 Higuti, T., Kuroiwa, K., Kawamura, Y., Morimoto, K. and Tsujita, H. (1993) Biochim. Biophys. Acta 1172, 311–314.
7 Farrell, L.B. and Nagley, P. (1987) Biochem. Biophys. Res. Commun. 144, 1257–1264.
8 Higuti, T., Kawamura, Y., Kuroiwa, K., Miyazaki, S and Tsujita, H. (1993) Biochim. Biophys. Acta 1173, 87–90.
9 Medd, S.M., Walker, J.E. and Jolly, R.D. (1993) Biochem. J. 293, 65–73.
10 Taylor, K.D. and Pikó, L. (1987) Development 101, 877–892.
11 Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
12 Dyer, M.R. and Walker, J.E. (1993) Biochem. J. 293, 51–64.
13 Pikó, L. and Chase, D.G. (1973) J. Cell Biol. 58, 357–378.
14 Taylor, K.D. and Pikó, L. (1990) Mol. Reprod. Dev. 26, 111–121.
15 Taylor, K.D. and Pikó, L. (1991) Mol. Reprod. Dev. 28, 319–324.