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Two genes encoding the bovine mitochondrial ATP synthase proteolipid specify precursors with different import sequences and are expressed in a tissue-specific manner

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Two cDNAs encoding different precursor proteins of the same mature proteolipid subunit of mitochondrial ATP synthase have been cloned from a bovine cDNA library. The hybridisation probe was a mixture of 17-mer oligonucleotides containing 256 discrete sequences. The coding sequences of the two cDNAs differ in 25 silent positions of codons and the 3′ non-coding sequences are only weakly related. The precursor sequences, which direct the import of the proteolipid into the mitochondrion, are 61 and 68 amino acids long. They are related to each other in regions which probably are recognition signals for the processing protease. The corresponding genes are expressed differently in various tissues in a way that reflects their embryonic origin.

Key words: ATPase/proteolipid/mitochondrial import/tissue specificity

Introduction

The mitochondrial ATP synthase complex (F_1F_0-ATPase; reviewed by Senior, 1979) is anchored in the inner membrane of the organelle by an intrinsic membrane sector, F_0. The proteolipid [or dicyclohexylcarbodiimide (DCCD) binding protein] is an essential element for the function of the proton channel in this membrane enzyme complex (Sebald and Hoppe, 1981). As its name implies it is a hydrophobic protein; in bovine mitochondria it is 75 amino acids in length (Sebald and Hoppe, 1981). In mammals (Anderson *et al.*, 1981, 1982), *Aspergillus nidulans* (Turner *et al.*, 1979), *Neurospora crassa* (Jackl and Sebald, 1975) (but not *Saccharomyces cerevisiae*; Macino and Tzagoloff, 1979) it is a nuclear gene product. It is presumed that it is synthesised on free ribosomes as a longer precursor, and imported into the mitochondrion in a post-translational process that involves removal of a segment from the N-terminal region of the precursor (Schatz and Butow, 1983; Hay *et al.*, 1984). In the *N. crassa* pre-proteolipid this import sequence is 66 amino acids long (Viebrock *et al.*, 1982).

In the course of studies of genes for subunits of bovine mitochondrial ATP synthase, we have cloned cDNAs corresponding to precursors of the bovine mitochondrial proteolipid. We employed as hybridisation probe a mixture of 256 oligonucleotides, 17 bases long, with sequences predicted from a segment of the known protein sequence of the mature proteolipid (Sebald and Hoppe, 1981). Thereby we have characterised two different cDNA species. DNA sequence analysis reveals that they encode exactly the same mature proteolipid (with 25 changes in silent first and third positions of the codons) but differ substantially both in the DNA sequence encoding the processed import sequence and in the 3′ non-coding regions. The import sequences are sufficiently conserved, however, to suggest that they both direct the protein into mitochondria.

By hybridisation of diverged regions of the two cDNAs with RNA derived from different tissues, it appears that the two genes are expressed in a tissue specific manner.

Results and Discussion

Cloning and DNA sequence analysis

In the first screening of the cDNA library with the mixture of 256 oligonucleotides, five independent positively hybridising clones P1.3, P2.1, P2.2, P2.4 and P2.5 were isolated. Subsequently, DNA sequence analysis showed that four of these isolates were derived from the same RNA sequence, whereas one, P1.3, was related to them but clearly derived from a different RNA species. The genes corresponding to these RNAs have been named P1 and P2. Both groups of isolates (summarised in Figure 1) contained sequences encoding regions of the C-terminal end of the proteolipid; none contained cDNA covering its entire sequence. In order to obtain clones containing longer cDNA inserts, the library was re-screened with probes derived from the inserts of P1.3 and P2.4. This second round of screening produced an isolate P2.14, which proved to contain an essentially full length cDNA insert. A third round of screening with the insert of P1.17 as probe was required to produce full length isolates of P1, namely P1.53, P1.59 and P1.63.

DNA sequence analysis showed that the P1 and P2 inserted cDNAs contain open reading frames encoding proteins of 136 and 143 amino acids, respectively (Figure 2). The two proteins are identical in sequence in their C-terminal 77 amino acids; all but the first two amino acids of this sequence are the sequence of the bovine ATP synthase proteolipid subunit. Therefore, the two proteins encoded in the P1 and P2 cDNAs are different pre-

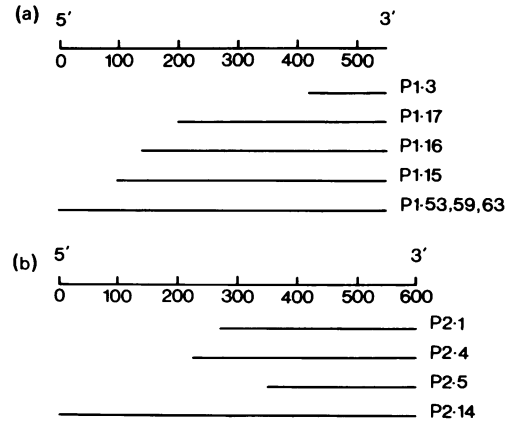

Fig. 1. Extent of inserts in cDNA clones encoding bovine proteolipids. The scale is in bases. All clones isolated extended from within the region corresponding to the 3′ poly(A) tails of the mRNAs. In the diagrams the 3′ end is the junction between 3′ non-coding region and poly(A) tails. **(a)** and **(b)** are isolates corresponding to P1 and P2, respectively.

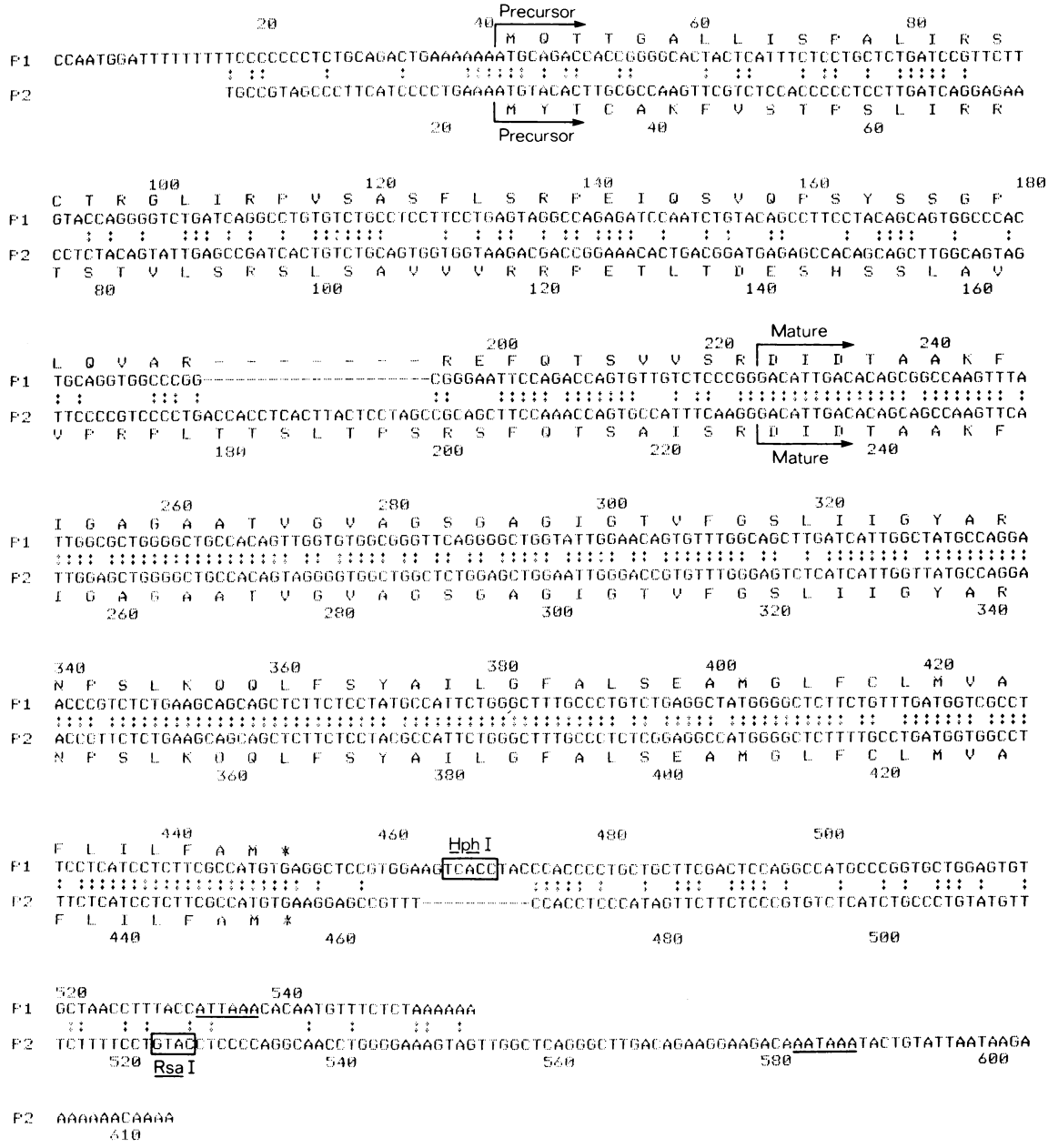

Fig. 2. DNA sequences of bovine cDNAs and derived protein sequences of P1 and P2 pre-proteolipids. The nucleotide sequences were aligned with the computer program NUCALN (Wilbur and Lipman, 1983). The numbers refer to the nucleotide sequences and colons denote identities. Dashes indicate insertions in both nucleotide and protein sequences. The sites of initiation of translation and of processing of the pre-proteolipids are shown. The underlined sequences are likely to be signals for addition of poly(A) to the 3′ end of the message (Proudfoot and Brownlee, 1976). The *Hph*I site in P1 and the *Rsa*I site in P2 were employed to produce specific prime cut probes from the diverged 3′ ends of the two cDNAs (see Figure 6).

cursors of this subunit. To produce the mature subunit, 61 and 68 amino acids respectively are removed from the P1 and P2 precursors (see Figure 3).

As the conservation of the mature protein sequence implies, the corresponding coding sequences are also highly conserved, differing from each other only in 25 silent first and third positions of codons (Figure 2). In contrast, the 3′ non-coding regions of the P1 and P2 cDNAs are much less related, 39 bases being conserved. The P2 mRNA is somewhat longer than P1 in this region [157 and 109 bases respectively, excluding poly(A) tails]. It should be noted that the six consecutive A residues at the 3′ end of P1 are specified in the gene sequence (N.J. Gay and J.E. Walker, unpublished results). In two out of seven isolates examined, the three terminal A residues are replaced by the sequence CTC; it is assumed that this is an allelic polymorphism. The sequence of P1 and P2 cDNAs both contain polyadenylation signals near to their 3′ ends (see Figure 2) (Proudfoot and Brownlee, 1976) and given that the mRNAs are polyadenylated it seems to be reasonable to assume that they will be translated and the protein products produced. However, direct proof for this is at present lacking. The coding regions corresponding to the pre-sequences are much less conserved than those for the mature protein (see Figure 2); 82 bases out of 202 are identical, and conserved bases are clustered. The extent of the 5′ non-coding region has not yet been established with absolute certainty. However, it is clear from size estimates of 570 ± 15 bp and 630 ± 15 bp for the P1 and P2 mRNAs made by Northern hybridisations, that the sequences of the cDNAs cannot extend much

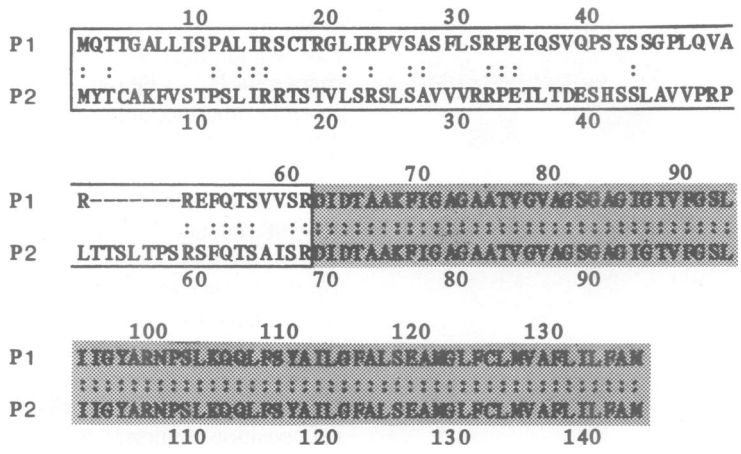

Fig. 3. Alignment of protein sequences of the two proteolipid precursors. The computer program PRTALN (Wilbur and Lipman, 1983) was employed. The dots indicate identities. The alignment requires an insertion in P1 seven amino acids long (indicated by dashes). The pre-sequences are boxed and the sequence of mature proteolipid is shaded.

beyond the determined sequences.

The processed pre-sequences

The pre-sequence of P1 is 61 amino acids in length and that of P2 seven amino acids longer. Thus, they are approximately the same length as the pre-sequence of the *N. crassa* proteolipid (66 amino acids). The bovine proteolipid pre-sequences are significantly related to each other and also much more weakly to the pre-sequence of the *N. crassa* pre-proteolipid. The relationship between the two bovine pre-sequences is also evident from a comparison of their hydrophobicity profiles (Figure 4). The similarity found suggests that they have related secondary structures. In common with the *N. crassa* pre-sequence, the bovine precursors contain a relatively large number of basic residues; P1 has seven, P2 nine (plus one histidine) and *N. crassa* 12. Both P1 and P2 also contain two glutamic acid residues, one of them, Glu-34, being in equivalent positions in both sequences; in addition, P2 contains an aspartic acid residue.

In the case of the *N. crassa* pre-proteolipid it has been suggested that the highly charged pre-sequence helps to solubilise the lipophilic mature protein in an aqueous environment. In addition, it has been proposed that the large net positive charge of the protein helps to drive it across the inner mitochondrial membrane, aided by the net negative electrochemical potential inside (Viebrock *et al.*, 1982). The observations reported here with the bovine pre-proteolipid sequences are consistent with this idea.

Strictly conserved residues in P1 and P2 pre-sequences group in three local environments (see Figure 3), around residues 10 – 15, 32 – 34 and 59 – 68 (P2 numbering). The first two of these clusters contains a conserved proline residue which may be important in the formation of bends in the secondary structure of the pre-sequence. The third cluster is found immediately before the mature protein sequence, this region being related also to the pre-sequence of other bovine ATPase subunits (J.E. Walker *et al.*, unpublished work) and of cytochrome oxidase subunit IV (Lomax *et al.*, 1984), it probably represents a recognition site for a proteolytic processing enzyme. However, the pre-sequences are remarkably diverse. A common feature is that cleavage occurs at one or two residues to the C-terminal side of an arginine residue. A Lys-Arg sequence is associated with the recognition site for processing of *N. crassa* pre-proteolipid and the precursor of cytochrome c peroxidase (Kaput *et al.*, 1982) contains a Lys-Arg sequence.

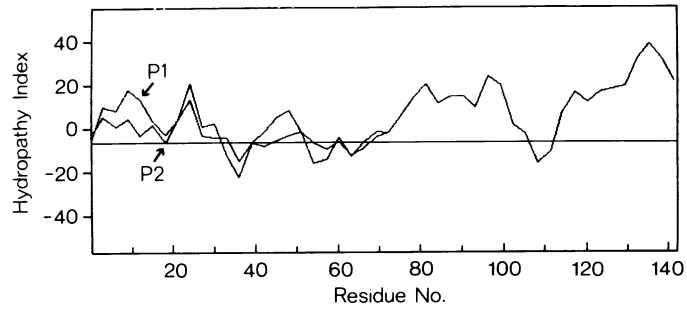

Fig. 4. Comparison of the hydrophobic profiles of pre-proteolipids bovine P1 and P2. The computer program HYDROPLOT, based upon the algorithm of Kyte and Doolittle (1982), was employed with a window 11 amino acids in length.

(a)	P1(17-24)	S	C	T	R	G	L	I	R
(b)	P2(17-24)	R	T	S	T	V	L	S	R
(c)	P1(25-32)	V	S	A	S	F	L	S	R
(d)	P1(54-61)	F	Q	T	S	V	V	S	R
(e)	P2(25-32)	L	S	A	V	V	V	R	R
(f)	P2(61-68)	F	Q	T	S	A	I	S	R
(g)	CO IV	I	S	T	S	V	C	V	R

Fig. 5. Related sequences within the pre-sequences of the bovine proteolipids and cytochrome oxidase subunit IV (Lomax *et al.*, 1984). In **(d)**, **(f)** and **(g)** cleavage occurs on the C-terminal side of the conserved arginine residue. In **(a)** – **(c)** and **(e)** the sequences may represent additional sites for recognition by a processing protease.

The *N. crassa* protein is processed in two stages. The first cleavage takes place at an internal site in the pre-sequence, also characterised by a Lys-Arg recognition sequence (Schmidt *et al.*, 1984). A similar site is also found in the middle of the cytochrome c peroxidase pre-sequence, which also may be processed in two stages (Kaput *et al.*, 1982). The bovine P2 pre-sequences contain two Arg-Arg sequences (see Figure 3). The sequence of the region preceding one of these (P2, 31 – 32) is related to the equivalent sequence in P1 and also to the sequences preceding the cleavage sites at the start of the mature protein (see Figure 5). So it may be that the bovine pre-proteolipids are also cleaved in an analogous fashion to their *N. crassa* counterpart.

Destination of the two precursors

It could be argued *a priori* that the two pre-sequences might serve to direct the proteolipid subunit to different organelles, for

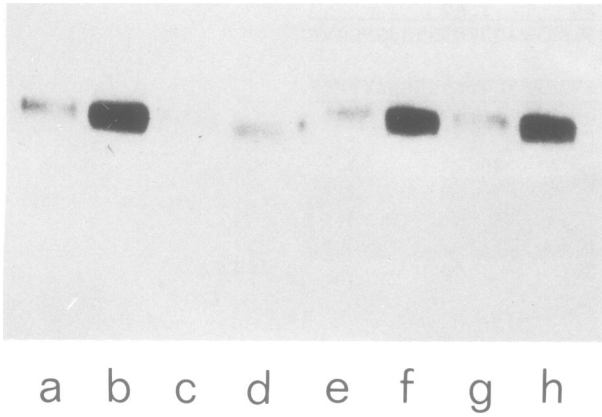

Fig. 6. Northern blot analysis of poly(A)$^+$ RNA from heart and liver with P1 and P2 specific hybridisation probes. **(a)–(d)** were hybridised with a P1-specific probe (residues 459–558 of P1 sequence in Figure 2) and **(e)–(h)** with a P2-specific probe (residues 526–615 of P2 sequence in Figure 2). **(a)** 0.37 μg of poly(A)$^+$ heart RNA **(b)** 1.8 μg poly(A)$^+$ heart RNA **(c)** 0.4 μg poly(A)$^+$ liver RNA and **(d)** 2.0 μg poly(A)$^+$ liver RNA. **(e)–(h)** are duplicates of **(a)–(d)**.

instance one to the mitochondrion, the other to coated vesicles. This would imply that coated vesicles (or some other organelle) contain an H^+-ATPase with a mature proteolipid subunit, and possibly other subunits, identical to that in the mitochondrion. Whilst it has been shown that coated vesicles (Forgac *et al.*, 1983; Stone *et al.*, 1983) and other secretory vesicles (Apps *et al.*, 1980; Gluck *et al.*, 1982; Hutton and Peshavaria, 1982; Okhuma *et al.*, 1982) contain an H^+-ATPase there is no evidence to show that any of these enzymes is an F_1F_0-ATPase, let alone that it has at least one subunit identical to that of the mitochondrial complex. Indeed, the available evidence is contrary to this hypothesis, all known secretory H^+-ATPases being relatively insensitive to the effect of DCCD.

It seems more likely that both precursors enter mitochondria, a supposition supported by the significant relationship of the two precursor sequences (see Figure 3). Further experiments are required to determine if they are able to enter the same mitochondria (for example isolated from a particular tissue).

Variable expression of the P1 and P2 genes in different tissues

Initially we examined the representation of the two transcripts

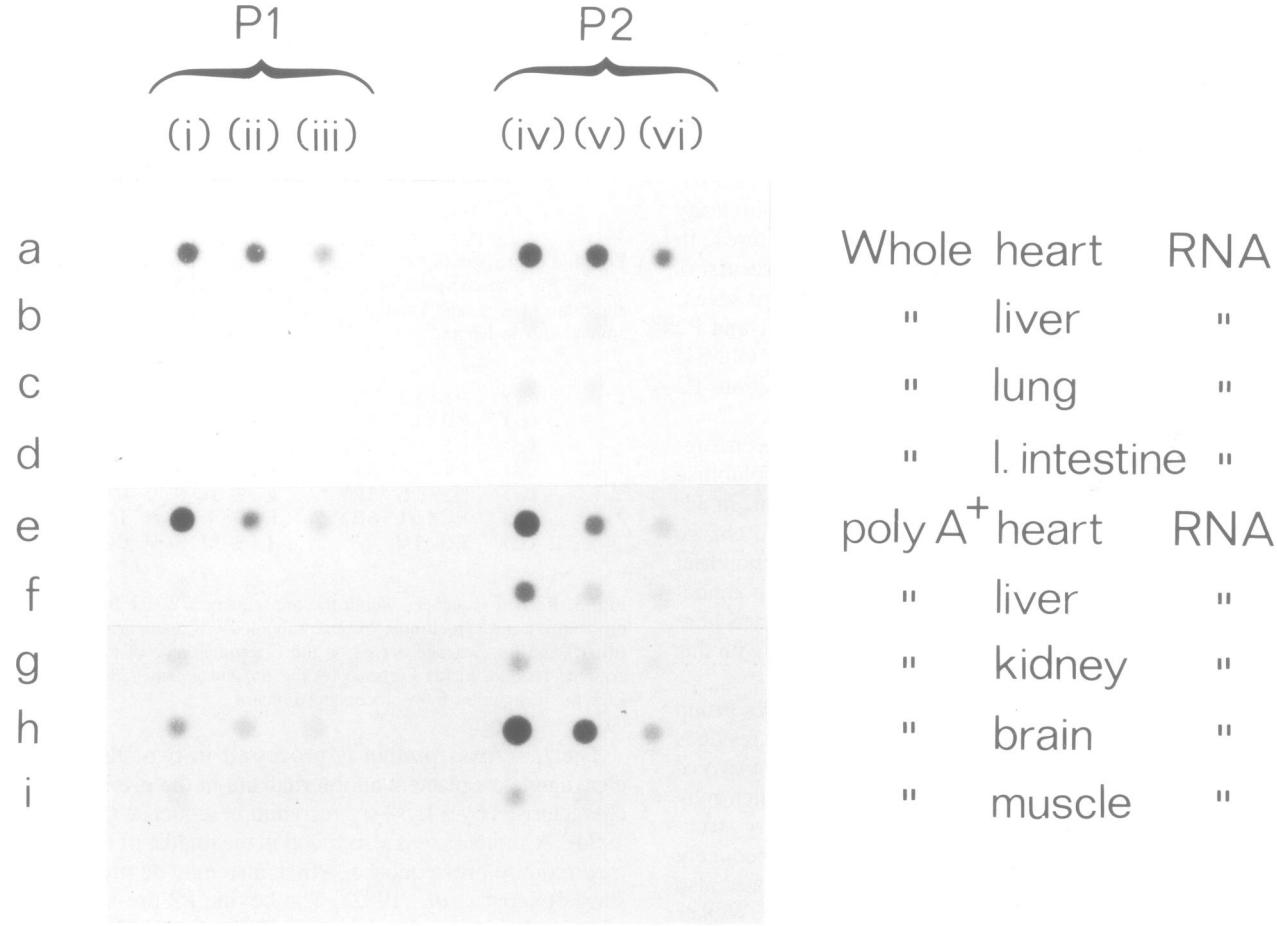

Fig. 7. Dot blot analysis of RNA from various bovine tissues using P1 and P2 specific probes. **(ii)** and **(iii)** and **(v)** and **(vi)** are doubling dilutions of the samples applied in **(i)** and **(iv)**. **(a)** whole heart RNA (12.5 μg); **(b)** whole liver RNA (27.5 μg); **(c)** whole lung RNA (5 μg); **(d)** whole intestine RNA (2 μg); **(e)** poly(A)$^+$ heart RNA (1 μg); **(f)** poly(A)$^+$ liver RNA (1.3 μg); **(g)** poly(A)$^+$ kidney RNA (0.3 μg); **(h)** poly(A)$^+$ brain RNA (1.4 μg); **(i)** poly(A)$^+$ skeletal muscle RNA (0.4 μg).

in poly(A)$^+$ RNA isolated from bovine heart and liver. Northern blot analysis using P1- and P2-specific probes, derived from the highly diverged 3'-untranslated regions of the two cDNAs (see Figure 2), revealed that the two RNA species were present in both RNA samples (Figure 6). However, the ratios of expression (P1:P2) were found to be distinctly different. Densitometric analysis of the autoradiograms shows that this ratio is 1:3 for RNA from liver and 1:1 for RNA from heart. To extend this analysis, RNA was prepared from a variety of other tissues and the P1:P2 ratio in these tissues was determined using both dot blots (Figure 7) and Northern blots (not shown). A ratio similar to that in liver was also found in intestine, lung and brain, whilst a ratio similar to that in heart was found in kidney and muscle. It should be noted that these experiments do not provide any information about the absolute levels of each RNA in any given cell of each tissue, but do show that one or other of the two genes is being subjected to tissue-specific regulation or that P1 transcripts are being subjected to tissue-specific degradation.

Tissue-specific expression and myopathies

Although this is the first demonstration of duplicated genes encoding the same mammalian mitochondrial protein, tissue-specific expression of nuclear-encoded mitochondrial subunits is evident from studies of mitochondrial myopathies. The best defined example is of a myopathy associated with a defect in the respiratory complex, ubiquinol cytochrome c reductase of skeletal muscle mitochondria, whereas in the same individual, clinical investigations indicated no dysfunction in heart or central nervous system (Kennaway *et al.*, 1984); also the complex functions normally in mitochondria in fibroblasts or peripheral blood leukocytes (V. Darley-Usmar, personal communication).

Tissue-specific supernumerary subunits also appear to be associated with mammalian cytochrome oxidase (Kadenbach *et al.*, 1982). Also, Cumsky *et al.* (1985) have detected iso-subunit V species for cytochrome c oxidase in yeast. No function has been attributed to these subunits; all catalytic function appears to residue in subunits I – II of cytochrome oxidase and so in this respect these tissue-specific subunits are quite distinct from the case of the proteolipid; it is essential for function of the H$^+$-ATPase proton channel.

Materials and methods

Oligonucleotide synthesis

A mixed oligonucleotide with the sequence 5' ATNARRAANGCNACCAT 3' was synthesised by a solid-phase phosphotriester method (Gait *et al.*, 1982; Sproat and Bannwarth, 1983). This corresponds to the protein sequence MVAFLI found near to the C-terminal of the bovine proteolipid sequence. The mixture contains a total of 256 discrete sequences.

cDNA synthesis and cloning

This was performed as described previously (Gay and Walker, 1985).

Screening of the cDNA library

Recombinants were grown on TYE agar supplemented with ampicillin (100 μg/ml). 60 000 recombinants were grown on each 14 cm diameter Petri dish. The colonies were transferred to Pall Biodyne 'A' nylon membrane filters and then the plasmid DNA was amplified (Clewell, 1972) by placing the filters on TYE agar supplemented with chloramphenicol (200 μg/ml) for 12 – 24 h at 37°C. Then the filters were treated with a solution of 0.5 M NaOH, 1.5 M NaCl for 15 min (two changes) followed by buffer containing 0.5 M Tris-Cl, pH 7.5, 1.5 M NaCl for 10 min, and finally for 2 min with 2 × SSC (1 × SSC contains 0.15 M NaCl and 0.015 M tri-sodium citrate). The filters were dried and baked at 80°C to immobilise bound DNA. For hybridisation with oligonucleotides, the filters were first treated at 40°C for 3 h with a pre-hybridisation solution containing 5 × Denhardt's solution, 6 x SSC, 0.5% N-lauryl sarcosine, and boiled, sonicated, salmon testis DNA (100 μg/ml). Hybridisations were performed for 18 – 24 h at the minimum melting temperature of the oligonucleotide minus 5°C (the melting temperature of a short oligonucleotide in 6 × SSC is ~4°C for each G:C base pair and 2°C for each A:T base pair). The filters were washed in the same solution plus ^{32}P-radiolabelled oligonucleotide. They were then washed in 6 × SSC for 2 h (three changes) at the minimum melting temperature, dried and autoradiographed with a screen at −70°C.

For hybridisation with 'prime cut' type probes, the filters were treated at 65°C for 1 h with a pre-hybridisation solution containing 5 × Denhardt's solution, 2 × SSC, 0.5% N-lauryl sarcosine and salmon testis DNA (100 μg/ml). Hybridisation was performed at 65°C for 18 h in the same solution supplemented with 10% dextran sulphate. Finally, the filters were washed in a solution containing 0.2 × SSC and 0.5% N-lauryl sarcosine.

Biodyne 'A' filters may be re-probed many times with no appreciable loss of signal. The filters are prepared again for pre-hybridisation by boiling for 10 min in 10 mM Tris-Cl, pH 7.5. We have found that Biodyne 'A' filters give a superior signal/background ratio when compared with both Whatman 531 paper (Gergen *et al.*, 1979) and nitrocellulose filters.

^{32}P labelling of oligonucleotides and 'prime cut' probes

Oligonucleotides were labelled with [γ-^{32}P]ATP and polynucleotide kinase to a specific activity of 3000 Ci/mmol. 'Prime cut' probes were prepared as described previously (Farrell *et al.*, 1983).

'Northern' and 'dot' blot analysis

Samples of total and poly(A)$^+$-containing RNA were prepared from bovine tissues by the method of Chirgwin *et al.* (1979). Tissues were lyophilised, chopped finely and homogenised in 4 M guanidinium thiocyanate. RNA from the homogenate was pelleted by centrifugation (35 000 *g*, 16 h) through a cushion of 5.7 M CsCl/0.1 M EDTA. Poly(A)$^+$-containing RNA was prepared by the method of Aviv and Leder (1972).

Northern blot analysis was performed by the method of Thomas (1980). 'Dot' blots were prepared with the aid of a 'Hybri-dot' appratus (Bethesda Research Laboratories). Hybridisation was carried out as described previously (Gay and Walker, 1985). After hybridisation the radioactive areas on the filters were cut out and the total radioactivity in each dot or band determined by scintillation counting. The intensities of dots on the autoradiographs were measured by densitometry.

DNA sequence analysis

This was accomplished using the method of Sanger *et al.* (1977) as modified by Biggin *et al.* (1983).

Computer analysis of DNA and protein sequences

The DNA sequences were compiled with the aid of the computer programs DBAUTO and DBUTIL (Staden, 1982) and then analysed for various features with ANALYSEQ (Staden, 1984). DNA sequences of P1 and P2 were aligned with NUCALN (Wilbur and Lipman, 1983) and protein sequences with PRTALN (Wilbur and Lipman, 1983).

Acknowledgements

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References

Anderson,S., Bankier,A.T., Barrell,B.G., de Bruijn,M.H.L., Coulson,A.R., Drouin,J., Eperon,I.C., Nierlich,D.P., Roe,B.A., Sanger,F., Schreier,P.H., Smith,A.J.H., Staden,R. and Young,I.G. (1981) *Nature*, **290**, 457-465.

Anderson,S., de Bruijn,M.H.L., Coulson,A.R., Eperon,I.C., Sanger,F. and Young,I.G. (1982) *J. Mol. Biol.*, **156**, 683-717.

Apps,D.K., Pryde,J.G., Sutton,R. and Phillips,J.H. (1980) *Biochem. J.*, **190**, 273-282.

Aviv,H. and Leder,P. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1408-1412.

Biggin,M., Gibson,T. and Hong,G.F. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2963-3965.

Chirgwin,J.M., Przbyla,A.F., MacDonald,R.J. and Rutter,W.J., (1979) *Biochemistry (Wash.)*, **18**, 5294-5299.

Clewell,D.B. (1972) *J. Bacteriol.*, **110**, 667-670.

Cumsky,M.G., Ko,C., Trueblood,C.E. and Poyton,R.O. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2235-2239.

Farrell,P.J., Deininger,P.L., Bankier,A. and Barrell,B.G. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1565-1569.

Forgac,M., Cantley,L., Wiedenmann,B., Altstiel,L. and Branton,D. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1300-1303.

Gait,M.J., Matthes,H.W.D., Singh,M., Sproat,B.S. and Titmas,R.C. (1982) *Nucleic Acids Res.*, **10**, 6243-6254.

Gay,N.J. and Walker,J.E. (1985) *Biochem. J.*, **225**, 707-712.

Gergen,J.P., Stern,R.H. and Websink,P.C. (1979) *Nucleic Acids Res.*, **7**, 2115-2136.

Gluck,S., Kelly,S. and Al-Awqati,Q. (1982) *J. Biol. Chem.*, **257**, 9230-9233.

Hay,R., Bohni,P. and Glasser,S. (1984) *Biochim. Biophys. Acta*, **797**, 65-87.

Hutton,J.C. and Peshvaria,M. (1982) *Biochem. J.*, **204**, 161-170.

Jackl,G. and Sebald,W. (1975) *Eur. J. Biochem.*, **54**, 97-106.
Kadenbach,B., Hartmann,R., Glanville,R. and Buse,G. (1982) *FEBS Lett.*, **138**, 236-238.
Kaput,J., Goltz,S. and Blobel,G. (1982) *J. Biol. Chem.*, **257**, 15054-15058.
Kennaway,N.G., Buist,N.R.M., Darley-Usmar,V.M., Papadimitrou,A., Dimauro,S., Kelley,R.I., Capaldi,R.A., Blank,N.K. and D'Agostino,A. (1984) *Pediatr. Res.*, **18**, 991-999.
Kyte,J. and Doolittle,R.F. (1982) *J. Mol. Biol.*, **157**, 105-132.
Lomax,M.I., Bachman,N.J., Nasoff,M.S., Caruthers,M.H. and Grossman,L.I. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6259-6299.
Macino,G. and Tzagoloff,A. (1979) *J. Biol. Chem.*, **254**, 4617-5623.
Okhuma,S., Moriyama,Y. and Takano,T. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 2758-2762.
Proudfoot,N.J. and Brownlee,G.G. (1976) *Nature*, **263**, 211-214.
Sadler,I., Suda,K., Schatz,G., Kaudewitz,F. and Haid,A. (1984) *EMBO J.*, **3**, 2137-2143.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Schatz,G. and Butow,R.A. (1983) *Cell*, **32**, 316-318.
Schmidt,B., Wachter,E., Sebald,W. and Neupert,W. (1984) *Eur. J. Biochem.*, **144**, 581-588.
Sebald,W. and Hoppe,J. (1981) *Curr. Top. Bioenerg.*, **12**, 1-64.
Senior,A.E. (1979) in Capaldi,R.A. (ed.), *Membrane Proteins in Energy Transduction*, Dekker Inc., New York and Basel, pp. 233-276.
Sproat,B.S. and Bannwarth,W. (1983) *Tetrahedron Lett.1*, **24**, 5771-5774.
Staden,R. (1982) *Nucleic Acids Res.*, **10**, 4731-4751.
Staden,R. (1984) *Nucleic Acids Res.*, **12**, 521-538.
Stone,D.K., Xie,X.S. and Racker,E. (1983) *J. Biol. Chem.*, **258**, 4059-4062.
Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5201-5205.
Turner,G., Imam,G. and Küntzel,H. (1979) *Eur. J. Biochem.*, **97**, 565-571.
Viebrock,A., Perz,A. and Sebald,W. (1982) *EMBO J.*, **5**, 565-571.
Wilbur,W.J. and Lipman,D.J. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 726-730.

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