ATP Synthase from Bovine Mitochondria: Complementary DNA Sequence of the Import Precursor of a Heart Isoform of the α Subunit†,‡

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ABSTRACT: The α-subunit of ATP synthase from mitochondria is a major component of the extrinsic membrane sector of the enzyme. It is encoded in nuclear DNA. A family of overlapping complementary DNA clones encoding its precursor has been isolated from a bovine library by using in the first instance a mixture of 128 synthetic oligonucleotides designed on the basis of the known protein sequence, and the sequence of the full-length cDNA has been determined. The deduced protein sequence shows that the α-subunit of ATP synthase has a presequence of 43 amino acids that is not present in the mature protein. Presumably it directs the protein into the mitochondrial matrix and is removed during the import process. The encoded protein sequence is also longer by one amino acid at its C-terminal end than the protein isolated from F_1-ATPase, but this alanine residue may have been removed artifactually during release of the F_1-ATPase particle from the inner mitochondrial membrane. With the exception of one uncertainty caused by an ambiguity at one position in the nucleotide sequence, the mature protein sequence encoded in the cDNA is exactly the same as the sequence determined previously by direct analysis of the protein isolated from bovine heart mitochondria [Walker et al. (1985) *J. Mol. Biol. 184*, 677-701]. The cDNA sequence differs in 158 nucleotides over a region of alignment of 1097 nucleotides from a partial cDNA for the α-subunit that has been isolated from a bovine cDNA derived from liver RNA [Breen (1988) *Biochem. Biophys. Res. Commun. 152*, 264-269]. These differences cause only ten changes in amino acid sequence over the regions that can be compared, and most of the changes in nucleotide sequence are in silent codon positions. Therefore, it seems to be very probable that these two cDNA isolates are derived from two different bovine genes, one of which is expressed in heart and the other in liver. The presence in both cows and humans of more than one gene (including possibly pseudogenes) for the α-subunit of ATP synthase is indicated by the hybridization of five to six bands in restriction digests of their DNA with probes taken from the cDNA described in this paper. Thus, the α-subunit of ATP synthase appears to belong to a group of major mitochondrial proteins in mammals, each of which has multiple genes subject to different regulation in various tissues. Other examples are the dicyclohexylcarbodiimide-reactive proteolipid subunit of ATP synthase [Gay & Walker (1985) *EMBO J. 4*, 3519-3524] and the transport protein ADP/ATP translocase [summarized in Powell et al. (1989) *Biochemistry 28*, 866-873].

Mitochondrial ATP synthase is in the inner membrane of the organelle and in cows is a complex of approximately 13 different polypeptides (Walker et al., 1987a). Two of them, both intrinsic membrane proteins, are the products of overlapping genes in mitochondrial DNA (Fearnley & Walker, 1986), whereas the remainder of the subunits are nuclear gene products that are imported into the organelle. In common with other mitochondrial proteins encoded in the nucleus, their mRNAs are translated on cytoplasmic ribosomes usually, but not invariably, as precursors with N-terminal presequences to direct the proteins into the organelle (Schatz & Butow, 1983). It has been found that the dicyclohexylcarbodiimide-(DCCD-) reactive proteolipid, a membrane component of ATP synthase, has two distinct import precursors (Gay & Walker, 1985). They are the products of two different genes in both cows and humans (Gay & Walker, 1985a; Farrell & Nagley, 1987; Dyer & Walker, 1989; Dyer et al., 1989a), and the bovine genes are expressed at different levels in various tissues.

A second example of a major mammalian mitochondrial protein with more than one expressed gene is the transport protein ADP/ATP translocase. Two different bovine cDNAs that code for isoforms have been characterized; one is expressed predominantly in heart and the other in smooth muscle (Walker et al., 1987a; Powell et al., 1989), and in addition three different human genes for the translocase have been detected (Battini et al., 1987; Necklemann et al., 1987; Houldsworth & Attardi, 1988; Cozens et al., 1989). Their expression also appears to be regulated differently in various tissues. There are other indications of multiple gene families subject to tissue-specific regulation for a number of other mammalian mitochondrial proteins. For example, immunological studies of the electron-transfer complex cytochrome *c* oxidase showed the presence in various rat tissues of different isoforms of some of its subunits (Kuhn-Nentwig & Kadenbach, 1985), and Southern blotting experiments on digests of bovine and human DNA with cDNA probes for other components of ATP synthase have demonstrated in at least three cases the presence in both genomes of several related sequences for each of these components. Examples are the oligomycin sensitivity conferral protein (Walker et al., 1987b) and two membrane components, subunits b and d (Walker et al., 1987c; J. E. Walker et al., unpublished results). However, these latter experiments do not provide definitive proof of more than one expressed gene in each case as they are unable to distinguish

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between expressed genes and related pseudogenes. In the cases of the inhibitor and F6 subunits of ATP synthase (Walker et al., 1987b) and the mitochondrial phosphate carrier protein (Runswick et al., 1987), only one or two hybridizing fragments have been observed in Southern blots conducted on human and bovine DNA.

As described in the present paper it is now evident that the α-subunit of bovine ATP synthase has at least two different expressed genes. We have characterized from a bovine library a number of overlapping cDNA clones for this protein, including one that encodes the entire mature subunit and its mitochondrial import sequence. Except for a single ambiguity, the sequence of the mature α-subunit encoded therein is identical with the protein sequence determined by direct sequence analysis of the protein isolated from bovine heart mitochondria (Walker et al., 1985). These clones are distinctly different in sequence from a partial clone for the α-subunit isolated from a bovine cDNA library derived from mRNA prepared from liver (Breen, 1988).

Materials and Methods

Reagents. The sources of chemicals, biochemicals, and enzymes used in experiments described in this paper have been given previously (Gay & Walker, 1985a,b; Walker et al., 1987b,c).

Oligonucleotide Synthesis. A mixture of 128 oligonucleotides with the sequence 3′ C·T·Y·C·T·T/C·G·T·Y·C·A·N·C·G·N·C·A 5′ was synthesized by a solid-phase phosphotriester method (Gait et al., 1982; Sproat & Bannwarth, 1983). This corresponds to the protein sequence EEQVAV, residues 439–444 in the protein sequence of the α-subunit of bovine mitochondrial ATP synthase (Walker et al., 1985). It has an estimated minimal dissociation temperature, T_d, of 48 °C as calculated by the "rule of thumb" that each G·C and A·T base pair contributes 4 and 2 °C, respectively (Suggs et al., 1981). The mixture was purified as described earlier (Walker et al., 1987b). Eight unique oligonucleotides, each 17 bases in length, were employed as primers in the sequence analysis of cDNA clones of the α-subunit (see Figure 1). They were synthesized with the aid of an Applied Biosystems 380B synthesizer and were used as described previously (Walker et al., 1987b) without further purification.

Screening the cDNA Library. The cDNA library was prepared from mRNA isolated mainly from bovine heart but also containing a minor amount of mRNA from liver and was cloned into the plasmid vector pUC8 (Gay & Walker, 1985b). It was screened with radiolabeled mixtures of synthetic oligonucleotides (Gay & Walker, 1985a,b). DNA in recombinants was transferred to Pall nylon membranes and after fixation was hybridized for 18 h at 43 °C (i.e., 5 °C below the minimum dissociation temperature) with the oligonucleotide mixture in the presence of a buffer containing 90 mM Tris·HCl, pH 7.8, 0.9 M sodium chloride, 5 × Denhardt's solution, 0.1 mg of boiled sonicated salmon sperm DNA, and 0.5% sarkosyl. Subsequent washing steps were performed successively at 43, 45, and finally 48 °C in 6 × SSC (0.9 M sodium chloride, 0.09 M sodium citrate). In order to isolate a clone coding for the entire α-subunit and its precursor, three further successive rounds of rescreening of the cDNA library had to be carried out. In these experiments "prime-cut" probes (Farrell et al., 1983) were employed as explained under Results and Discussion.

DNA Sequence Analysis. The excision, purification, and recloning into M13mp8 and -mp9 vectors of inserts from positively hybridizing recombinants have been described before

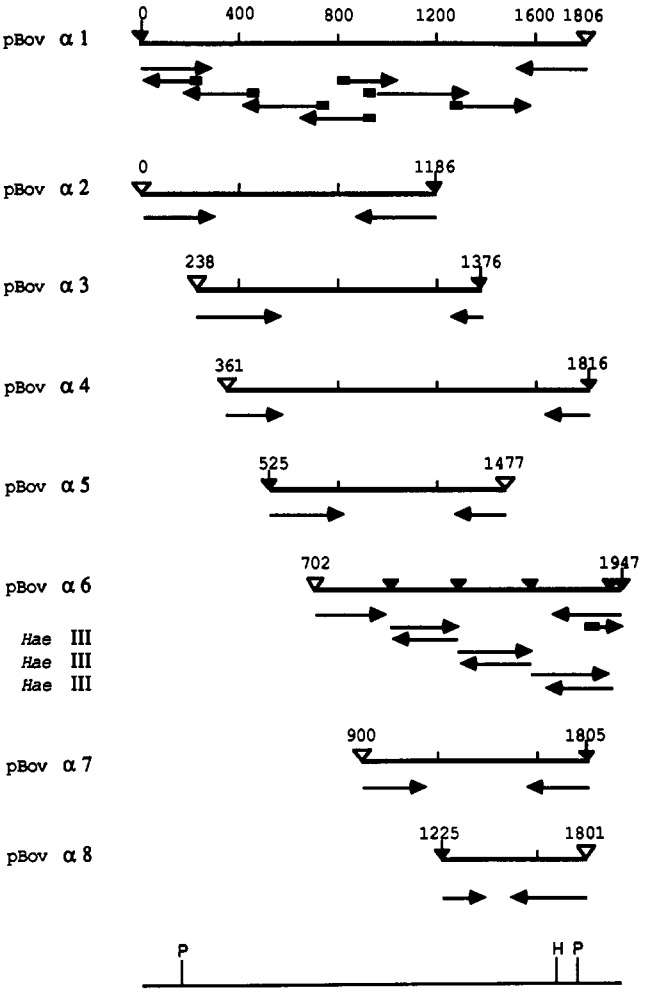

FIGURE 1: Sequence analysis of cDNA clones encoding the precursor of the α-subunit of bovine mitochondrial ATP synthase. The thick lines represent the inserted DNAs in the isolates pBov α1–8. Each insert is flanked by an *Eco*RI (▽) and a *Bam*HI (↓) site in the polylinker of the vector. The horizontal arrows represent the DNA sequences that were determined, and synthetic oligonucleotide primers employed in the sequencing of the inserts in pBov-α1 and pBov-α6 are shown by black boxes. The locations of *Hae*III sites (▼) that were important in the sequencing of pBov-α6 are also indicated. The scale along the top is in bases, and along the bottom are shown the positions of sites for restriction enzymes P (*Pst*I) and H (*Hin*dIII) that were employed in the digestion of bovine and human genomic DNA (see Figure 5).

(Walker et al., 1987b). DNA sequences were determined by the dideoxy method (Sanger et al., 1977) as modified by Biggin et al. (1983). Five internal primers as well as the flanking primer LMB2 (Duckworth et al., 1981) were employed. In the sequencing of the insert from the recombinant pBov-α6, which contains bases 702–1947 of the complete sequence, the excised insert was digested with the restriction enzyme *Hae*III. The resultant fragments were cloned into M13mp8 that had been previously digested with *Sma*I, and their sequences were determined as above. The sequence of the cDNA of the precursor of the α-subunit was determined fully in both senses. Sequences were compiled with the aid of the computer programs DBAUTO and DBUTIL (Staden, 1982) and analyzed with ANALYSEQ (Staden, 1985).

Preparation of DNA. Bovine DNA was obtained from liver (Walker et al., 1987b), and human DNA was prepared from fresh samples (ca. 20–30 mL) of human blood to which 5% EDTA (5 mL) had been added, as follows. After removal of plasma, red blood cells were lysed by addition of buffer (up to 50 mL) containing Tris·HCl (10 mM), magnesium chloride (5 mM), and sodium chloride (10 mM), pH 7.6, and white

cells were recovered by centrifugation. They were washed again with the same solution and then resuspended in a further 2 mL of the buffer. Lysis of white cells was carried out by shaking them gently overnight at 42 °C in a solution (15 mL) containing Tris·HCl (10 mM, pH 7.6), EDTA (10 mM), sodium chloride (50 mM), SDS (0.2%), and proteinase K (200 μg/mL; Gross-Bellard et al., 1973). DNA was collected by spooling it on a syringe needle. Then it was extracted twice with a mixture (pH 8.0) of phenol (3 volumes) and chloroform–isoamyl alcohol (1 volume; 24:1 v/v), washed twice with chloroform–isoamyl alcohol, and finally precipitated with propan-2-ol, rinsed twice with ethanol, and dried briefly in vacuo.

Hybridization with Human and Bovine Genomic DNA. This was performed as described previously (Walker et al., 1987b) by the method of Southern (1975). Samples of bovine and human DNA (20 μg/slot) were digested with the restriction enzyme *Bam*HI, *Hin*dIII, *Pst*I, or *Sac*I (all purchased from New England Biolabs). The digests were hybridized with radioactively labeled prime-cut probes (Farrell et al., 1983). Conditions for prehybridization, hybridization, washing, and autoradiography have been given before (Walker et al., 1987b).

Results and Discussion

Cloning and DNA Sequence Analysis. The cDNA library was screened with the mixture of 128 oligonucleotides representing amino acids 439–444 of the α-subunit of bovine mitochondrial ATP synthase. This yielded a single positively hybridizing clone. Partial DNA sequence analysis identified sequences coding for the C-terminal region of the α protein, and the insert in this partial clone (called α-cd28) extended from approximately nucleotides 1460–1800 (Figure 2). Its sequence has not been characterized further. A prime-cut probe was prepared from the entire insert in this initial isolate, and this was then used to rescreen the library in order to try to identify clones containing inserts coding for longer stretches of the α-subunit. From this second round of screening isolates pBov-α6, -α7, and -α8 were obtained (see Figure 1). The first of these contained the longest insert of the three and was found to extend the available cDNA sequence in a 5′ direction. In a further round of rescreening of the library a fragment containing the 5′ region of pBov-α6 was employed in the preparation of a prime-cut probe. This fragment was bases 702–879 and was released from the clone by digestion with *Eco*RI (restriction site in polylinker flanking its 5′ end) and *Sca*I (see Figure 2 for location of this site). From this experiment isolates pBov-α2, -α3, -α4, and -α5 were recovered. Again they extended the available sequence in a 5′ direction. Isolate pBov-α2 was found to contain sequences beyond the N-terminal of the mature α-subunit, but at its 3′ end contained DNA coding only as far as amino acid 346. Therefore, in order to isolate a full-length clone containing both 5′ and 3′ noncoding regions as well as the coding region, a third round of rescreening was undertaken, this time employing as prime-cut probe bases 1–219. This probe was excised from isolate pBov-α2 with the enzymes *Eco*RI and *Hin*cII, the former site again being in the polylinker flanking the 5′ end of the insert. This experiment yielded isolate pBov-α1, and it was found to contain the sequence in the 5′ noncoding region, the sequence coding for the entire mature α-subunit of ATP synthase, and an N-terminal precursor and also to be terminated at its 3′ end by poly(A). DNA sequences of the inserts in these various clones were determined as explained in Figure 2, and thereby the entire sequence of the cDNA coding for the import precursor of the protein could be compiled in both senses of the DNA.

The final sequence presented in Figure 2 is 1947 nucleotides in length. It is terminated at its 3′ end by the sequence $(A)_{35}$ corresponding to poly(A) in the original mRNA. Sixteen bases to the 5′ side of this run of A residues is the sequence ATTAAA, which can serve as a polyadenylation signal (Proudfoot & Brownlee, 1976). Only one clone that was characterized, namely, pBov-α6 (see Figure 1), extended in a 3′ direction as far as base 1947; all other clones terminated in the vicinity of base 1805, and poly(A) tracts were observed after bases 1801 (pBov-α8), 1805 (pBov-α7), 1806 (pBov-α1), and 1816 (pBov-α4). Overlapping polyadenylation signals ATTAAA (bases 1780–1785) and AATAAA (bases 1785–1790) are probably responsible for this heterogeneity. Otherwise, apart from this variation in the site of addition of poly(A), and a local ambiguity observed at base 1590 (see below), the sequences derived from the various clones are entirely concordant.

Deduced Protein Sequences. The protein sequence for the mature α-subunit of ATP synthase derived from the cDNA sequence agrees entirely with that determined on the protein isolated from bovine heart mitochondria by direct protein sequence analysis (Walker et al., 1985), with the exception of one ambiguity. This ambiguity is at amino acid 481 where glycine was detected during direct protein sequence analysis. In cDNA clones pBov-α1, -α6, and -α8 the codon AGC (Ser) was observed, whereas in clones pBov-α4 and -α7 GGC (Gly) was found, in agreement with the protein data. Since the RNA used in the preparation of the cDNA library was obtained from a single animal, it seems likely that this difference has arisen by mutation during either the propagation of clones in the library or subcloning into M13 vectors.

The cDNA sequence suggests that the precursor of the mature α-subunit is extended at both its N- and C-terminals. The N-terminal residue of the mature protein is pyrrolidonecarboxylic acid. As discussed previously (Walker et al., 1985), according to Orlowski and Meister (1971) this residue could be formed enzymically by the cyclization of either N-terminal glutamine or glutamic acid. Alternatively, it could arise artifactually by acid-induced cyclization during protein purification. However, in other cases such as in many IgG heavy chains, where N-terminal pyrrolidonecarboxylic acid is found in the protein, the amino acid encoded in the cognate DNA is glutamine (Kabat et al., 1987), and this is so in the case of the α-subunit of ATP synthase also (see Figure 2). The N-terminal residue of the mature protein is preceded by a sequence of 43 amino acids up to and including the first in-phase methionine residue. The corresponding ATG codon is assumed to be the translational initiator. The N-terminal extension has a net positive charge of +4 (including the α-amino group), and in this respect it is similar to mitochondrial import sequences that have been observed in other proteins. Putative import sequences now have been determined in four different α-subunits of ATP synthase (see Figure 3). Comparison of these sequences shows as expected that they all have a basic character and none contains an acidic residue.

According to the cDNA sequence the C-terminus of the mature α-subunit is extended by one amino acid, an alanine residue. No evidence for the presence of this residue was obtained during the protein sequencing experiments, and all of the available data showed that the isolated polypeptide chains terminate at the previous residue, glutamic acid 509 (Walker et al., 1985). These chains were isolated from F_1-ATPase, the extrinsic membrane sector of ATP synthase, which was released from the membrane-bound enzyme with chloroform. Recently, this procedure has been shown to release

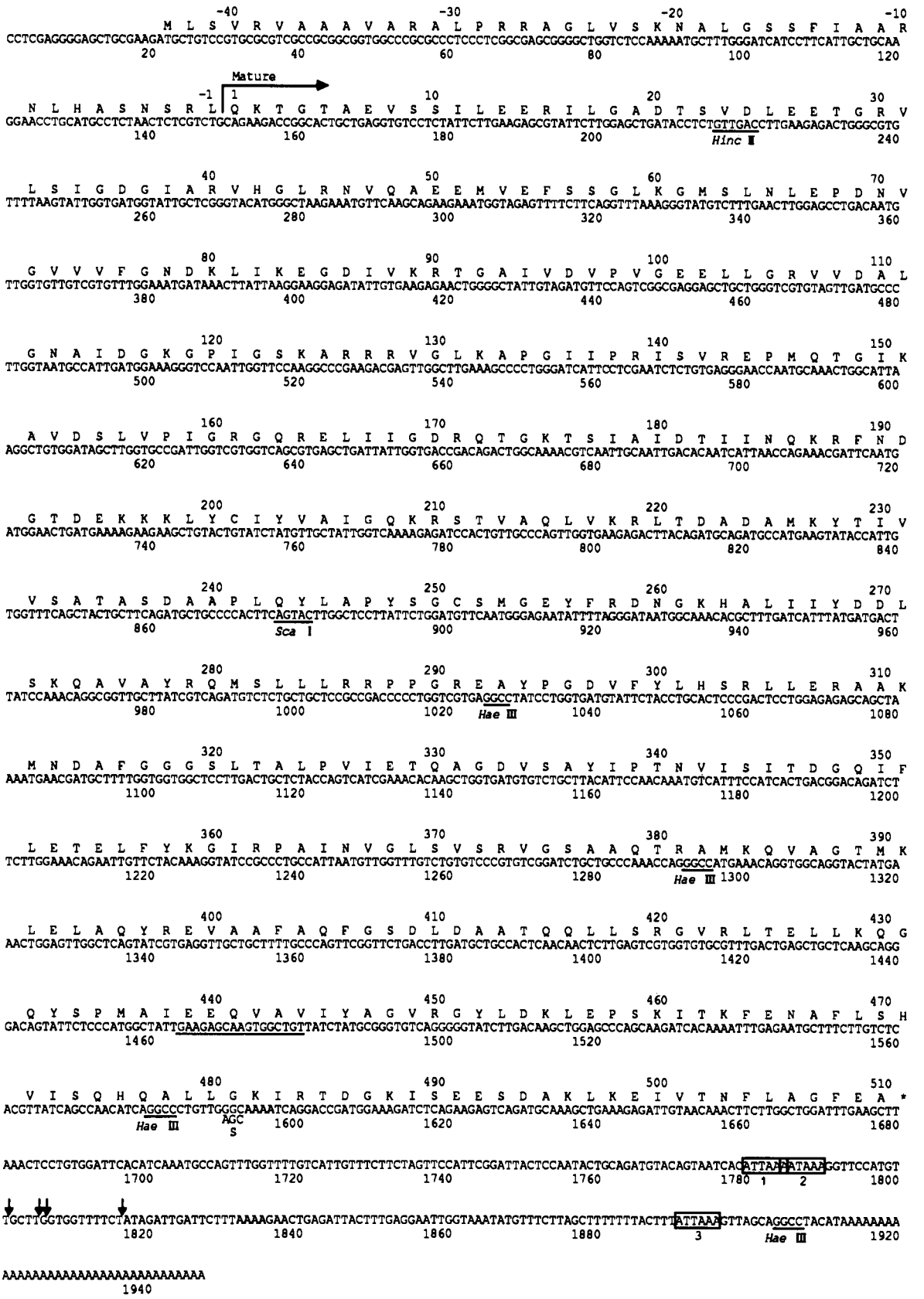

FIGURE 2: DNA sequence of a family of overlapping cDNAs encoding the heart isoform of the import precursor of the α-subunit of bovine mitochondrial ATP synthase. The position corresponding to the N-terminal pyrrolidone carboxylic residue of the mature protein isolated from bovine heart mitochondria is indicated. The mature protein is numbered from 1 to 509, residue 510 being absent from the α-subunit, and the mitochondrial import sequence is numbered from −1 to −43. An ambiguity in the protein sequence derived from the cDNA sequence is shown at amino acid 481 with the alternative codons; glycine was found at this position by direct protein sequence analysis (Walker et al., 1985). Potential polyadenylation signals 1, 2, and 3 are boxed. The first two overlap by one base. The sites of addition of poly(A) in different isolates are shown by vertical arrows. The positions of restriction sites are indicated by underlining. These were employed for generation of fragments for sequence analysis (*Hae*III) and for the preparation of various prime-cut probes used in the isolation of extended clones (*Hinc*II and *Sca*I; see text for details). Also underlined is the protein sequence that was used to design the oligonucleotide probe.

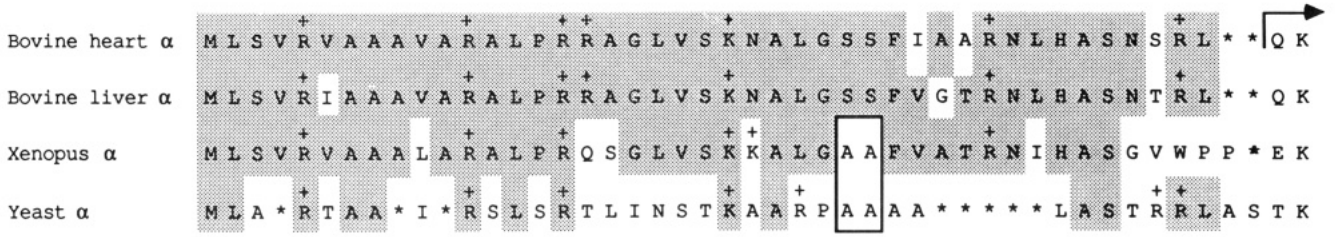

FIGURE 3: Comparison of putative import presequences of the α-subunits of mitochondrial ATP synthase from various species. Amino acids that are identical in two or more species are shaded or boxed, and basic amino acids are indicated by a + sign. Stars indicate the positions of insertions that have been introduced to improve alignment of sequences. The position of the N-terminus of the bovine heart α-subunit is indicated by an arrow (Walker et al., 1985). The sequences are taken from the following sources: bovine heart, this work; bovine liver, Breen (1988); *Xenopus laevis*, Weeks and Melton (1987); yeast, Takeda et al. (1986).

at the same time proteases that degrade the N-terminal regions of the α-, β-, and δ-subunits, giving rise to the observed frayed N-terminals in these proteins (Walker et al., 1985). In contrast, the fraying is absent from the same subunits in the intact ATP synthase complex (J. E. Walker et al., unpublished work). Thus, it is possible that the C-terminal alanine residue predicted from the cDNA sequence to be present in the α-chains is also removed by proteolytic action during release of F_1-ATPase with chloroform. This suggestion can be tested by determination of the C-terminal residue in the α-chains isolated from ATP synthase rather than from F_1-ATPase.

Different Isoforms of the α-Subunit in Bovine Heart and Liver. The sequence of the cDNA encoding the α-subunit of mitochondrial ATP synthase described above was determined almost entirely from two isolates, pBov-α1 and pBov-α6 (see Figure 1). The sequences determined in these two clones agree exactly, and there is no doubt that they are both derived from the same transcript. The sequences determined in other isolates are less extensive, but they too agree exactly with the sequences determined in isolates α1 and α6, and so the compilation of the partial sequences into a single complete sequence is justified. It is noteworthy that the amino acid sequence of the mature α-subunit of bovine mitochondrial ATP synthase encoded in the clones is in exact agreement with that determined by direct protein sequence analysis on material isolated from bovine heart mitochondria (with the exception of the ambiguity noted above).

Recently, the DNA sequence of a partial cDNA clone isolated from a bovine liver library has been described (Breen, 1988). This cDNA encodes the import sequence and residues 1–316 of the mature α-subunit of ATP synthase. However, the encoded partial protein sequence differs from that determined on the protein isolated from bovine hearts in five amino acids, and the mitochondrial import sequences differ in five additional positions (see Figure 4). Alignment of the nucleotide sequence of the liver isolate with that described in the present paper (see also Figure 4) shows more extensive differences. Over the 1097 nucleotides of sequence alignment, they differ in 158 bases (or 14.4%). In the coding sequences all but 10 of these differences are found in silent codon positions, and in the 5′ noncoding regions further changes occur. These differences are too extensive to be attributable to different alleles within the bovine population as was proposed as a possible explanation of the amino acid differences between the bovine heart protein sequence and the partial protein sequence derived from the bovine liver cDNA sequence (Breen, 1988). It is now apparent that it is much more likely that the two sequences derive from two different expressed bovine genes. This should become more evident when the sequence of the liver cDNA clone has been completed since the 3′ noncoding regions of duplicated genes often have diverged much more than coding sequences. This is certainly so in the case of the bovine (Gay & Walker, 1985a) and also the human P1 and P2 genes (Dyer & Walker, 1989) encoding the DCCD-reactive proteolipid subunit of ATP synthase and also for the human T1, T2 (Cozens et al., 1989), and T3 genes (Battini et al., 1987) and the bovine T1 and T2 genes (Powell et al., 1989), all encoding isoforms of mitochondrial ADP/ATP translocase. It is also of considerable practical importance since should the sequences of the 3′ regions of the cDNAs differ as anticipated, they would provide probes to distinguish between the transcripts for the two genes in various tissues, as has been possible in the other examples quoted above (Gay & Walker, 1985a; Powell et al., 1989). In the preliminary Northern analyses that have been described the probe was derived from the coding sequence of the liver cDNA for the α-subunit of ATP synthase (Breen, 1988), and so it would be incapable of distinguishing between transcripts derived from the two related bovine genes, or indeed probably from any additional expressed bovine genes that there may be for the α-subunit.

Number of Bovine and Human Genes for the α-Subunit of Mitochondrial ATP Synthase. In a preliminary attempt to estimate the number of sequences related to the coding region of the bovine α-subunit, we have performed hybridization experiments on digests of both human and bovine genomic DNA, using as hybridization probes sections of the sequence taken from the 5′ region of the cDNA described in this paper. These experiments (see Figure 5) demonstrate the presence in both genomes of between three and six hybridizing bands, depending upon the restriction enzyme employed. At present, it is unknown whether each of these bands represents a different gene; the probe employed in the experiments was 218 base pairs in length, and so it is possible that some of the bands arise from different exons in the same gene. From the cDNA sequences it is clear that there are two bovine genes, and from the hybridization experiments it is possible that there are more in both the bovine and human genomes, but this requires further investigation. Under similar conditions of hybridization other subunits of ATP synthase appear to have more than one gene, whereas others seem to have single genes. In the first group are the dicyclohexylcarbodiimide-reactive proteolipid (Gay & Walker, 1985a; Dyer & Walker, 1988) and possibly the oligomycin sensitivity conferral protein, subunit b and subunit d (Walker et al., 1987b,c). The second group includes the F6 and inhibitor proteins (Walker et al., 1987b) and the γ-subunit (Dyer et al., 1988b). These suggestions have to be viewed with caution for several reasons. First, the number of bands that are detected depends upon the experimental conditions that are employed in hybridization experiments. For example, in the case of the β-subunit of ATP synthase Breen et al. (1988) have proposed that there is a single bovine gene, whereas we have observed at least four different sequences in bovine DNA that hybridize with its cDNA and we have characterized a closely related pseudogene (J. E. Walker et al., unpublished observations). Second, the difficulty in making

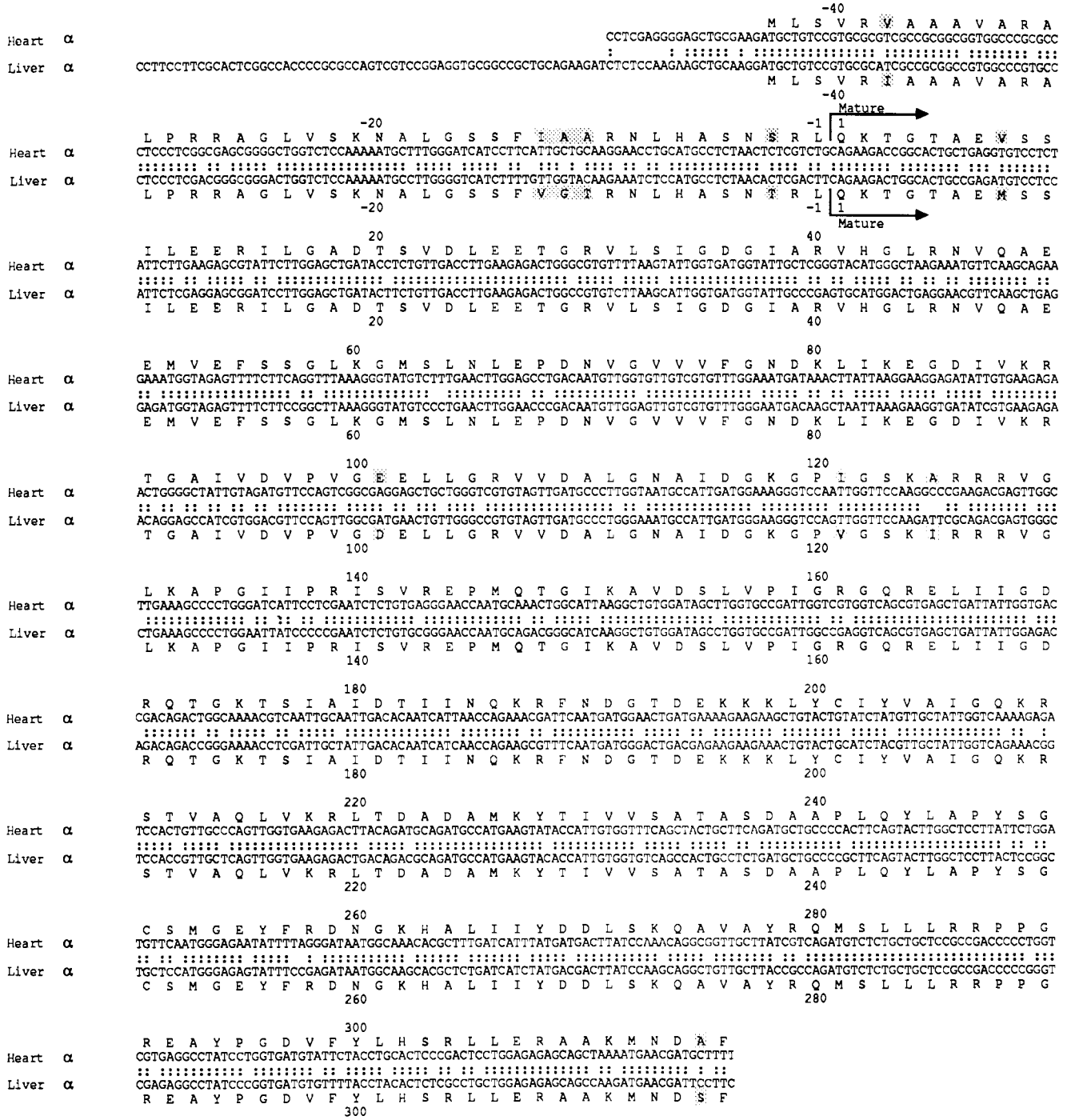

FIGURE 4: Comparison of cDNA sequences and encoded proteins of heart and liver isoforms of the α-subunit of ATP synthase from bovine mitochondria. The entire partial sequence from the liver clone is shown aligned with the corresponding part of the heart sequence. The analysis of the heart isoform is described in the present paper, and the sequence of the partial clone for the liver isoform is taken from Breen (1988). The mature bovine heart protein sequence is described by Walker et al. (1985), and the position of its N-terminal end is indicated. The ten differences in amino acid sequence in the region over which the proteins can be compared are shaded. Colons correspond to identities in nucleotide sequence. The nucleotide alignment was produced with the aid of the computer program NUCALN (Wilbur & Lipman, 1983).

estimates of the number of genes by hybridization experiments is that they could fail to detect sequences coding for homologous proteins where the DNA sequences have diverged extensively.

Tissue-Specific Expression and Myopathies. The work described above on the α-subunit of ATP synthase is the third example established at the DNA level of different levels of expression in various tissues of a mammalian mitochondrial protein. Earlier examples are the bovine DCCD-reactive proteolipid subunit of ATP synthase (Gay & Walker, 1985a) and the bovine ADP/ATP translocase (Walker et al., 1987a; Powell et al., 1989). However, as discussed before (Gay & Walker, 1985) other well-studied examples of duplicated genes encoding the same mitochondrial protein being expressed differently in some tissues are provided by the mitochondrial myopathies (Morgan-Hughes, 1986; Wallace, 1986; Clark et al., 1987; Capaldi, 1988). Among these conditions it has been found, for example, that, within the same individual, normal functional respiratory complexes are found in some tissues and that activity of one of them can be completely absent in skeletal muscle. Thus, it seems likely that the study of the expression of mitochondrial proteins such as the α-subunit and the DCCD-reactive proteolipid subunit of ATP synthase and the ADP/ATP translocase has more general interest and may have

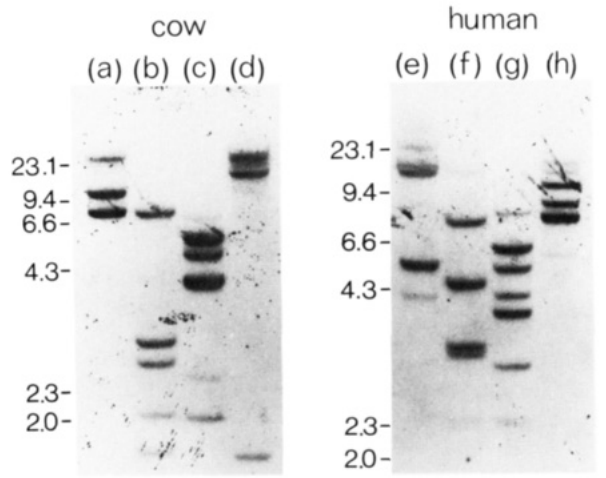

FIGURE 5: Hybridization of restriction digests of bovine and human DNA with a probe taken from the bovine heart cDNA for the α-subunit of mitochondrial ATP synthase. The enzymes employed are as follows: tracks a and e, *Sac*I; tracks b and f, *Pst*I; tracks c and g, *Hin*dIII; tracks d and h, *Bam*HI. A prime-cut probe was employed. This extended from a *Bam*HI site in the polylinker sequence at the 5′ end of the clone containing the precursor of the α-subunit to the *Hin*cII site at nucleotide 218 (see Figure 2). After hybridization the filters were washed in 0.2 × SSC at 65 °C. The positions of DNA markers (produced by digestion of DNA from bacteriophage λ with *Hin*dIII) are shown at the left-hand side of each gel.

a bearing on the understanding of this group of diseases.

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