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ATP Synthase from Bovine Mitochondria: Sequences of Imported Precursors of Oligomycin Sensitivity Conferral Protein, Factor 6, and Adenosinetriphosphatase Inhibitor Protein†

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ABSTRACT: Oligomycin sensitivity conferral protein (OSCP), factor 6 (F6), and ATPase inhibitor protein are all components of the ATP synthase complex of bovine mitochondria. They are encoded in nuclear DNA. Complementary DNA clones encoding the precursors of these proteins have been isolated from a bovine library by using mixtures of synthetic oligonucleotides as hybridization probes, and their DNA sequences have been determined. The deduced protein sequences show that the OSCP, F6, and inhibitor proteins have N-terminal presequences of 23, 32, and 25 amino acids, respectively. These presequences are not present in the mature proteins. It is assumed that they serve to direct the proteins into the mitochondrial matrix. The cDNA clones have also been employed as hybridization probes to investigate the genetic complexity of the three proteins in cows and humans. These experiments indicate that the bovine and human inhibitor and bovine F6 proteins are encoded by single genes but suggest the possibility of the presence in both species of more than one gene (or pseudogenes) for the OSCP.

Oligomycin sensitivity conferral protein (OSCP), factor 6 (F6), and ATPase inhibitor protein are all associated with the ATP synthase (F_1F_0 ATPase) of bovine mitochondria. This multisubunit enzyme is a complex of about 13 different polypeptides (Stiggal et al., 1978; Lutter et al., 1987; Walker et al., 1987b) that is anchored in the inner mitochondrial membrane by an intrinsic membrane sector F_0. The rest of the enzyme, the F_1 complex, which contains the catalytic sites of the enzyme [for reviews see Senior (1978) and Cross (1981)], is in the mitochondrial matrix and is apparently attached to F_0 by a stalk (Senior, 1971; Knowles et al., 1972) composed of, at least in part, the OSCP (MacLennan & Tzagoloff, 1968) and F6 (Fessenden-Radan, 1972). The inhibitor, a small basic protein, binds to F_1 and may well have a physiological role in modulating its activity (Pullman & Monroy, 1963; Asami et al., 1970; Ernster et al., 1973; van de Stadt & van Dam, 1974). Two of the subunits of ATP synthase, ATPase-6 and A6L, both intrinsic membrane proteins, are products of the mitochondrial genome (Fearnley &

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Table I: Synthetic Oligonucleotides Used for Isolation of Bovine cDNAs for OSCP, F6, and ATPase Inhibitor Protein

protein	protein sequence	oligonucleotide, 3′–5′	complexity	T_{min} (deg)
OSCP	MGGMIV	TACCCNCCNTAC-TA$^{R}_{T}$CA	48	48
F6	DMNTP	CTRTACTTRTGN-AARGG	32	44
inhibitor	KHHENE	TTYGTGCTRCTY-TTRCT	32	42

Walker, 1986). The remainder, including OSCP, F6, and the inhibitor, are nuclear gene products. Mitochondrial proteins encoded in nuclear DNA are synthesized on cytoplasmic ribosomes, often as precursors with an N-terminal presequence that specifies entry to the mitochondrion. The presequences are not present in the mature proteins; they are removed during entry into the mitochondrion (Schatz & Butow, 1983; Hay et al., 1984). In order to study the nature of the precursors of a closely associated group of proteins, we are investigating the precursors of the subunits of the ATP synthase complex. As described below, we have cloned the cognate complementary DNAs of the OSCP, F6, and inhibitor proteins using as hybridization probes synthetic mixed oligonucleotides 17 bases in length with sequences deduced from the known protein sequences of the mature subunits (Ovchinnikov et al., 1984; Grinkevich et al., 1984; Fang et al., 1981; Dianoux et al., 1984; Runswick et al., 1986). The probes for the OSCP, F6, and inhibitor contained 48, 32, and 32 unique sequences, respectively. The nucleotide sequences of the cloned cDNAs have been determined and the protein sequences of the subunits deduced from them. They show minor differences to the published protein sequences. All three proteins have an N-terminal extension. These presequences of the OSCP, inhibitor, and F6 proteins are 23, 25, and 32 amino acids in length, respectively, and are weakly related to each other. They also show some relationship to the two different precursors, P1 and P2, of the bovine dicyclohexylcarbodiimide-reactive proteolipid, a hydrophobic subunit of ATP synthase. These latter proteins have longer presequences of 61 and 68 amino acids, respectively (Gay & Walker, 1985a).

The availability of the cDNA clones has also allowed us to make a preliminary investigation of the bovine and human genes for the OSCP, F6, and inhibitor proteins. These experiments were carried out because two mitochondrial proteins, the proteolipid subunit of ATP synthase and the ADP/ATP translocase, have each been shown to be encoded by two expressed genes in both the human and bovine genomes (Gay & Walker, 1985b; Walker et al., 1987a; M. R. Dyer, S. J. Powell, A. L. Cozens, and J. E. Walker, unpublished results). The experiments described here provide evidence for single genes in both species for inhibitor and of a single bovine gene for F6; they suggest the possibility of greater complexity for OSCP genes.

Materials and Methods

Chemical Reagents. Nucleotide monomers were purchased from Cruachan Chemicals, Livingstone, Scotland, U.K. The sources of other chemicals and biochemicals have been given previously (Gay & Walker, 1985a,b).

Oligonucleotide Synthesis. Mixed oligonucleotides (summarized in Table I) with sequences corresponding to the protein sequences in the OSCP, F6, and inhibitor proteins were synthesized by a solid-phase phosphotriester method (Gait et al., 1982; Sproat & Bannwarth, 1984). They were purified by gel electrophoresis in a 20% polyacrylamide gel, in the presence of ethidium bromide (2 μg/mL). Bands were visualized in UV light, excised, placed in a dialysis bag containing water, and dialyzed against water for 18 h at 25 °C. Gel was removed from the retentate by ultrafiltration through a Millex filter (0.4 μm). The filtrate was extracted with equal volumes of butan-1-ol and then diethyl ether. The aqueous layer was dried by rotary evaporation in vacuo and the purified oligonucleotide mixture redissolved in H_2O (400 μL). A unique oligonucleotide primer 17 bases in length and complementary to the F6 cDNA was synthesized with an Applied Biosystems 380B synthesizer. It was diluted without further purification to 0.2 pmol/μL in water, and 2 μL of the resulting solutions was used in each sequencing reaction.

Screening the cDNA Library. The cDNA library derived from bovine heart and liver RNA (Gay & Walker, 1985a) was screened with radiolabeled oligonucleotides as described elsewhere (Gay & Walker, 1985a,b).

In the case of ATPase inhibitor protein, recombinants were transferred to Whatman 541 filter papers (Gergen et al., 1979). Hybridization was performed at 37 °C for 16 h, and subsequent washing steps were performed at 42 °C. For isolation of OSCP and F6 clones, recombinants were transferred to Pall membranes (Gay & Walker, 1985b). Hybridization with oligonucleotide was performed for 18 h at 5 °C below the minimum melting temperatures of the probes estimated to be 48 (OSCP), 44 (F6), and 42 °C (inhibitor). Subsequent washing steps were performed at these temperatures, then at 2 °C higher, and finally at 4 °C above these temperatures.

DNA Sequence Analysis. Inserted cDNAs were excised from the positively hybridizing plasmids with *Eco*RI and *Bam*HI and then recloned into bacteriophages M13-mp8 and -mp9 that had been previously digested with appropriate enzymes. DNA sequences of M13 clones were then determined by the dideoxy chain-termination method of Sanger et al. (1979) as modified by Biggin et al. (1983), using the universal primer LMB2 (Duckworth et al., 1981). In the case of F6, an internal primer was also employed (see Figure 3). The DNA sequences of each of the three different cDNAs were fully determined in both senses of the DNA. They were compiled with computer programs DBAUTO and DBUTIL (Staden, 1982) and analyzed with ANALYSEQ (Staden, 1984).

Preparation of DNA. As soon as possible after slaughter of the animal, a bovine liver was cut into approximately 1-cm cubes, frozen in liquid nitrogen, and then transported back to the laboratory. Frozen tissue (5 g) was broken into small pieces with an Omnimix homogenizer and then resuspended in a buffer (50 mL) containing 0.34 M lithium acetate, 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, 0.1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl), pH 8.0, and 2% lithium dodecyl sulfate. The suspension was mixed on a rotary wheel for 10 min first with 0.5 volume of phenol, and then with an additional 0.5 volume of chloroform for a further 10 min. The solution was centrifuged at 2000 rpm in an IEC benchtop centrifuge for 10 min at 4 °C. The aqueous layer was removed and the extraction procedure repeated a further 3 times. The DNA was precipitated at −20 °C by addition of sodium acetate to a final concentration of 0.1 M (pH 5.0) and 2.5 volumes of ethanol. The suspension was centrifuged at 4 °C for 20 min at 2000 rpm. The pellets were dried in vacuo and redissolved gently on a rotary wheel in 10 mL of buffer containing 10 mM Tris·HCl, pH 8.0, and 1 mM EDTA. The solution was treated at 37 °C for 1 h with pancreatic ribonuclease A (Sigma, type 1-A; final concentration 200 μg/mL). Sodium dodecyl sulfate was added (final

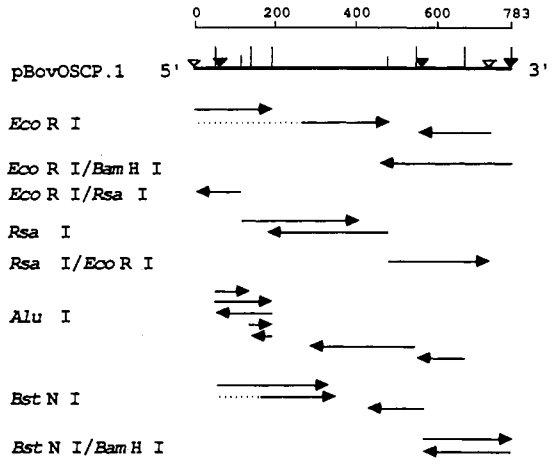

FIGURE 1: Sequence analysis of cDNA clones encoding the precursor of the bovine mitochondrial ATP synthase subunit OSCP. The thick line represents the inserted cDNA in the isolate pBovOSCP.1. The insert is flanked by an *Eco*RI site (▽) and a *Bam*HI site (↓) in the polylinker of the vector. It also contains an internal *Eco*RI site. The horizontal arrows represent the DNA sequences determined in fragments from various digests of the insert. Restriction enzyme sites: ▼, *Bst*NI; tall vertical lines, *Alu*I; short vertical lines, *Rsa*I. The dotted lines indicate that the sequences between the primer and the start of the continuous arrow were not determined in that particular experiment. The scale is in bases.

concentration 0.1%) and the solution mixed gently. The preparation was then digested at 37 °C for 2 h with proteinase K (400 μg/mL) and then extracted 3 times as before with 0.5 volume each of phenol and chloroform. Finally, the DNA was precipitated as above in the presence of sodium acetate and ethanol. The yield of DNA was 8.7 mg.

Human DNA was prepared from a placenta by a similar procedure.

Southern Blots. Samples of bovine and human DNA (20 μg/gel slot) were digested at 37 °C for 2.5–3.0 h in 400 μL of buffer with various restriction endonucleases, including *Bam*HI, *Eco*RI, *Pst*I, *Kpn*I, *Sac*I, *Xho*I, and *Nco*I (all from New England Biolabs). The DNA was precipitated at −20 °C with ethanol and sodium acetate (final concentration 0.3 M). Electrophoresis of digests and transfer of fragments (Southern, 1975) to Hybond-N membranes (Amersham International PLC) was performed essentially as described by Southern (1975). After electrophoresis (25 mA, 18 h) in a 0.7% high gelling temperature (HGT) agarose gel (17 cm × 20 cm, total vol 200 ml) the DNA was denatured by shaking the gel for 1 h in a solution containing 0.5 M sodium hydroxide and 1.5 M sodium chloride. The gel was washed with a buffer containing 0.5 M Tris·HCl, pH 8.0, and 1.5 M sodium chloride. DNA was transferred overnight onto Hybond-N membranes (Amersham International PLC) in 20 × SSC (3 M sodium chloride, 0.3 M sodium citrate). DNA was fixed to the membranes by illumination with UV light. "Prime cut" probes radiolabeled with ^{32}P were made as described by Farrell et al. (1983).

Prehybridization of membranes was carried out for 2–3 h at 56 °C by shaking the membranes in a solution containing 6 × SSC, 5 × Denhardt's solution [which contains 5 mg/mL each polyvinyl pyrrolidone, bovine serum albumin (fraction V), and Ficoll], sonicated salmon sperm DNA (100 μg/mL), and 0.5% sarkosyl. Hybridization was performed overnight at 65 °C in the presence of the same solution containing 10% dextran sulfate and the radiolabeled probe. Then filters were washed twice at 65 °C in 2 × SSC and twice in 0.2 × SSC (both solutions containing 0.5% sarkosyl). Autoradiography was performed at −70 °C for 24–120 h with preflashed film (Fuji RX 150).

Results and Discussion

Cloning and DNA Sequence Analysis. (A) OSCP. From the screening experiments of the cDNA library with the mixture of 32 oligonucleotides, two positively hybridizing recombinants pBovOSCP.1 and pBovOSCP.2 were isolated which proved to contain sequences encoding the OSCP. The

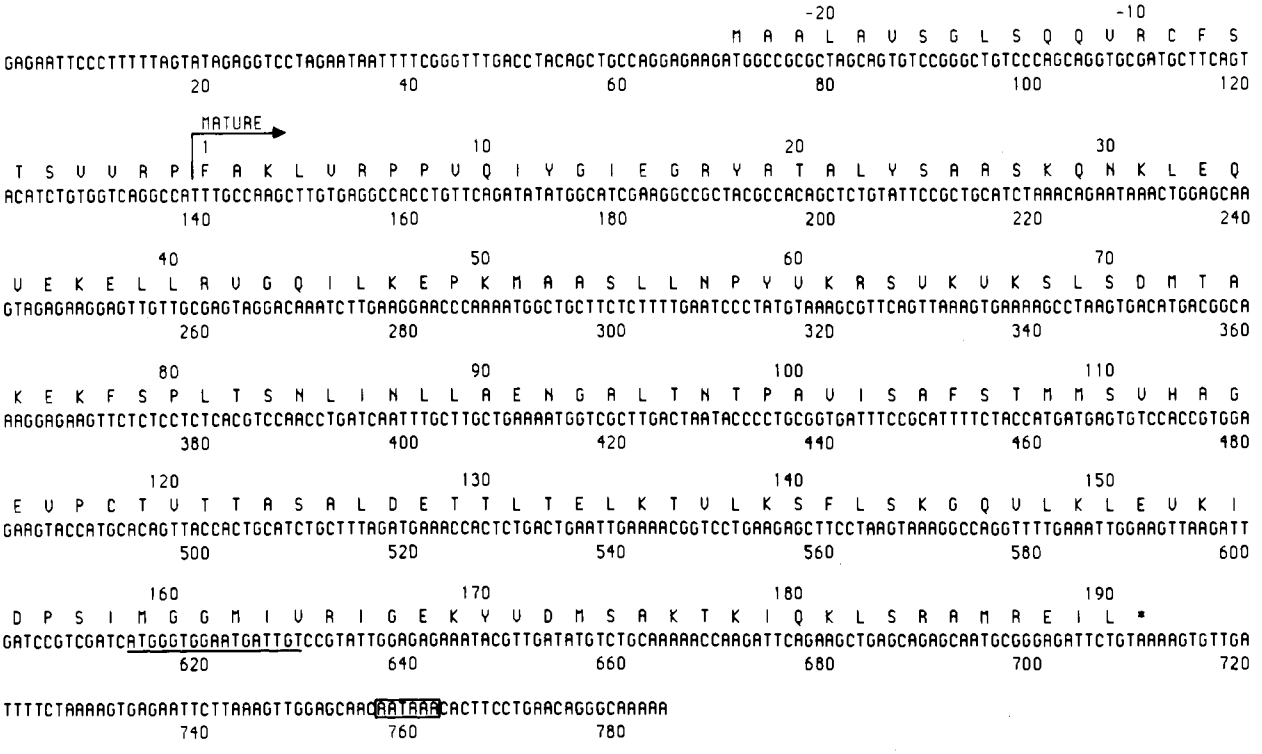

FIGURE 2: DNA sequence of a cDNA encoding the precursor of OSCP, a subunit of bovine mitochondrial ATP synthase. The proposed import sequence runs from amino acids −23 to −1 and the mature protein from 1 to 190. The underlined DNA sequence is the region to which the oligonucleotide probe hybridized, and the boxed sequence is a potential signal for polyadenylation (Proudfoot & Brownlee, 1975).

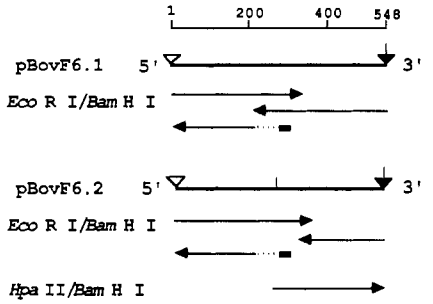

FIGURE 3: Sequence analysis of cDNAs encoding the precursor of the bovine mitochondrial ATP synthase subunit F6. The thick lines represent two independent isolates, pBovF6.1 and pBovF6.2. The inserts are flanked by *Eco*RI (▽) and *Bam*HI (↓) sites in the polylinker of the vector. pBovF6.2 is shorter at its 5′ end than pBovF6.1 and starts at base 14 of pBovF6.1; at its 3′ end it differs from pBovF6.1 in so far as it contains the sequence ATAC after the T residue at position 519 in pBovF6.2. The veritcal bar in pBovF6.2 represents an *Hpa*II site and the thick horizontal bar a synthetic sequencing primer. Otherwise, for meaning of symbols, see legend to Figure 1.

sequence of pBovOSCP.1 was determined fully in both senses of the DNA as described in Figures 1 and 2. Only the 5′ end sequence of pBovOSCP.2 has been characterized; it starts at base 53 and is identical with pBovOSCP.1, at least up to base 193.

(B) F6. Four independent isolates, pBovF6.1–4, containing sequences coding for F6 were identified, and two of them, pBovF6.1 and pBovF6.2, were fully characterized and used to establish the cDNA sequence as described in Figure 3. Clone pBovF6.3 was characterized at its 5′ end only. It extends from base 78 in the sequence presented in Figure 4 at least up to base 323. The isolate pBovF6.4 was characterized only at the 3′ end of the insert; from the poly(A+) tail it is identical in a 3′–5′ direction with the sequence in Figure 4 at least up to base 206.

(C) Inhibitor. Three independent isolates encoding the inhibitor pBovIn.1–3 were identified, and the first two were characterized (see Figure 5) and were used to establish the cDNA sequence presented in Figure 6. The third isolate pBovIn.3 has been characterized only at the 5′ end and starts at base 13; it is identical with the sequence presented in Figure 6 at least up to base 326.

Mature Protein Sequences. The sequences of the cDNAs described in the previous section and in Figures 2, 4, and 6 encode the bovine mitochondrial OSCP, F6, and inhibitor subunits of ATP synthase, respectively. The protein sequences deduced from the cDNA sequences for the OSCP and inhibitor proteins differ slightly from the published sequences, which were determined by direct protein sequence analysis. Of the five differences in the sequence of the OSCP, three of them are acid to amide changes; at positions 15, 127, and 187 Ovchinnikov et al. (1984) found glutamine, asparagine, and glutamine, whereas the DNA sequence gives glutamic acid, aspartic acid, and glutamic acid, respectively. At positions 129 and 142, two further differences are found; the protein sequence has alanine and lysine at these positions whereas threonine and serine, respectively, are encoded in the cDNA.

The two published versions of the protein sequence of the ATPase inhibitor disagree over amide assignments at positions 29, 30, and 61. Frangione et al. (1981) found glutamic acid, glutamine, and glutamic acid, and Dianoux et al. (1984) propose glutamine, glutamic acid, and glutamine, whereas the amino acids encoded in the cDNA are all glutamic acid residues. Mass spectrometric analyses of peptides derived from the inhibitor protein (Runswick et al., 1986) agree with the assignments based upon the cDNA sequence.

The protein sequence encoded in the cDNA for F6 agrees exactly with the directly determined protein sequence of Grinkevich et al. (1984), and in their independent protein sequence analysis Fang et al. (1984) also obtained the same sequence except that they observed both threonine and phenylalanine at position 62; the assignment from the cDNA sequence is threonine. On the basis of their observation of this ambiguity in protein sequence and of other ambiguities in other preparations of F6, and also because of the presence of multiple forms of F6 with different chromatographic properties, Fang et al. (1984) propose the presence of allotypic variants of F6 in the bovine population. While this may explain the sequence differences observed for the F6 and raises the possibility that the differences in sequence between our data and those of Ovchinnikov et al. (1984) for OSCP may arise for similar reasons, it should be noted that the protein sequence analysis of the inhibitor protein by mass spectrometry (Runswick et al., 1986) employed inhibitor protein isolated from several bovine hearts; no sequence heterogeneities were observed in these experiments that would explain the differences observed by Frangione et al. (1981) on the one hand and by Dianoux et al. (1984) on the other. In relation to the chromatographic heterogeneities of F6, it should be remembered that the α, β, and δ subunits of bovine F_1 ATPase and the ATPase inhibitor protein all have frayed N-termini (Walker et al., 1985; Runswick et al., 1986) and that therefore preparations of these

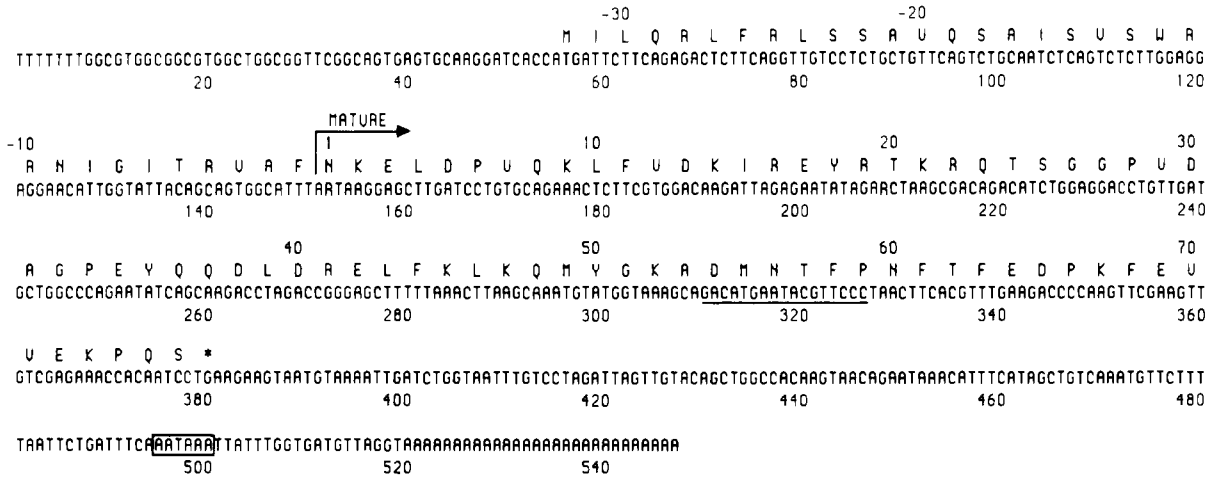

FIGURE 4: DNA sequence of a cDNA encoding the precursor of F6, a subunit of bovine mitochondrial ATP synthase. Amino acids −32 to −1 correspond to the proposed mitochondrial import sequence, and the mature F6 protein is amino acids 1–76. The underlined and boxed DNA sequences have the same meaning as in Figure 2.

Table II: Molecular Weights of Bovine Mitochondrial OSCP, F6, and ATPase Inhibitor Proteins and of Their Precursors

	M_r		
	mature		precursor, from
subunit	from gels	from sequence	sequence
OSCP	21 000	20 968	23 350
F6	9000	9071	12 532
inhibitor	10 000	9582	12 301

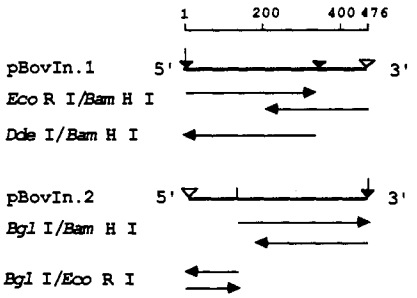

FIGURE 5: Sequence analysis of cDNA clones encoding the precursor of bovine mitochondrial ATPase inhibitor protein. The thick lines represent the inserts in the recombinants pBovIn.1 and pBovIn.2. pBovIn.2 is shorter at its 5′ end than pBovIn.1 and starts at base 13. ▽ and ↓ represent *Eco*RI and *Bam*HI sites, respectively, flanking the inserts in the polylinker of the vector. ▼ is a *Dde*I site. The small vertical bar in pBovIn.2 is a *Bgl*I site. For meaning of other symbols see legend to Figure 2.

proteins are also chromatographically heterogeneous.

In Table II the molecular weights of the three proteins calculated from their sequences are compared with values derived by gel electrophoresis.

Protein Presequences. The protein sequences predicted from the cDNA sequences of the OSCP, F6, and the ATPase inhibitor extend beyond the N-terminal ends of the mature proteins. In each cDNA only one in-phase ATG condon is found near to the 5′ ends. This is presumed to be the initiation codon. In the inhibitor cDNA the proposed initiation codon is immediately preceded by the sequence TGA (a potential stop codon), and the triplet TAA (also a potential stop codon) is found upstream and in phase in the OSCP cDNA. Therefore, taking into account the two assumptions that, first, the cDNAs extend up to near the 5′ end of the message and that, second, the DNA sequences themselves in the 5′ region are not artifactual, having arisen, as sometimes happens, for example, during cDNA synthesis and cloning, these are the only possible initiation codons. In the 5′ sequence of the F6 cDNA the only in-phase ATG codon is the one designated as the initiator in Figure 4. In this case no in-phase potential stop codons are found upstream of it, and the formal possibility exists that the coding sequence for the precursor extends beyond the end of the sequence determined in the cDNA. However, the sequence relationship between the F6 and OSCP precursors (see below) is against this. In the case of the inhibitor the view that the 5′ end of cDNA is not artifactual is supported by direct sequencing with an appropriate primer on total bovine heart poly(A+) RNA. These generated sequence data are consistent with those obtained from the cDNA clone (N. J. Gay, unpublished results). Other in vitro experiments to attempt to demonstrate that the presequences of OSCP, F6, and inhibitor can direct import of proteins into mitochondria are in progress.

The three presequences have properties in common with those found in other mitochondrial proteins encoded in nuclear genes. For example, they all have a net basic charge; the OSCP, F6, and inhibitor presequences contain two, four, and three arginine residues, respectively, and no acidic amino acids. This net positive charge, it has been proposed (Viebrock et al., 1982), helps to drive the precursors into mitochondria aided by the proton potential gradient across the inner mitochondrial membrane.

The presequences of OSCP and the inhibitor are related to each other. In the region near to the start of the mature proteins they are also related to those of the P1 and P2 proteolipids of ATP synthase and of cytochrome oxidase subunit IV (Figure 7). In general, import sequences have been found to show little sequence homology (von Heijne, 1986), but it has been proposed that they share the common structural feature of being folded into an amphipathic α-helix with charged and hydrophobic faces which can interact with the mitochondrion. So they are thought to play a crucial role in transporting proteins into mitochondria (Roise et al., 1986; von Heijne, 1986).

In order to examine the presequences of F6, OSCP, and the inhibitor protein for the presence of sequences that might form such structures, residues 1–18 of each presequence were plotted onto helical wheels (Schiffer & Edmundson, 1967; Figure 8). Should these sequences be folded into α-helices, they would all have a hydrophobic face, this feature being particularly evident in the F6 presequence. In all three cases, positive charges and the amino group of the N-terminal methionine residues would be on the opposing face.

Number of Genes for OSCP, F6, and ATPase Inhibitor Protein. The dicyclohexylcarbodiimide-reactive proteolipid, a membrane subunit of bovine ATPase synthase, is encoded by two different genes that are expressed at different levels in various bovine tissues (Gay & Walker, 1985b). A similar situation is found in humans, and a number of spliced pseu-

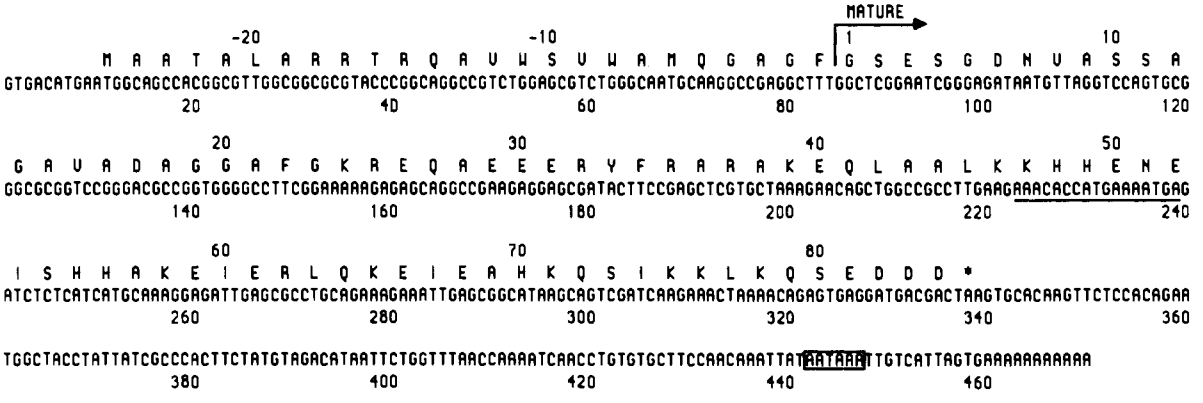

FIGURE 6: DNA sequence of a cDNA encoding the precursor of bovine mitochondrial ATPase inhibitor protein. The proposed mitochondrial import sequence is amino acids −25 to −1, and the mature inhibitor protein is amino acids 1–84. For meaning of underlined and boxed sequences see legends to Figures 2 and 4.

FIGURE 7: Relationships between presequences of imported bovine mitochondrial proteins. Shaded residues indicate homologies between the precursors of the OSCP and inhibitor. Boxed residues are homologies in at least three out of the five sequences displayed. The mature proteins follow on from the five sequences. References: cytochrome oxidase subunit IV (Lomax et al., 1984); P1 and P2 (Gay & Walker, 1985b).

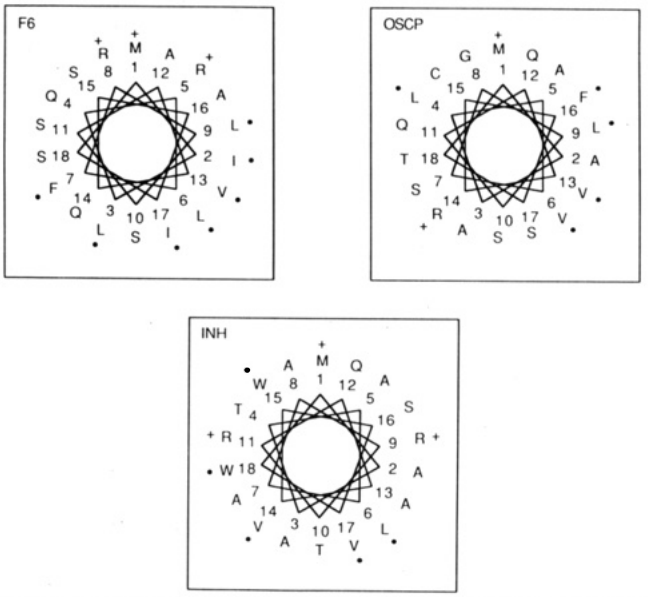

FIGURE 8: Presequences of bovine F6, OSCP, and ATP inhibitor plotted on helical wheels: (+) positively charged and (•) hydrophobic amino acids. The numbering starts at the proposed initiator methionine residues of the preproteins.

dogenes for this protein have been detected in both the bovine and human genomes (M. R. Dyer and J. E. Walker, unpublished results). These findings, and a more recent finding of at least two expressed bovine (Walker et al., 1987a) and three expressed human genes for mitochondrial ADP/ATP translocase (S. J. Powell, A. L. Cozens, M. J. Runswick, and J. E. Walker, unpublished results; Battini et al., 1987), stimulated interest in investigating the number of genes for OSCP, F6, and the inhibitor protein. Therefore, digests of human and bovine DNA were hybridized with radioactive probes derived from the corresponding cDNAs. These experiments yielded simple patterns of hybridization when bovine DNA was hybridized with probes for the inhibitor and F6 (Figure 9). They are consistent with unique bovine genes. Hybridization of human DNA with the same two probes was less informative; no significant hybridization between human DNA and bovine F6 probe was detected under the conditions employed in three independent experiments, and the inhibitor probe detected a single band (at about 3 kilobases) in the *Eco*RI digest only. In similar experiments using a probe derived from the bovine cDNA for OSCP, several bands hybridized in bovine DNA (Figure 9) and complex patterns of hybridization with *Sac*I, *Nco*I, *Hin*dIII, and *Eco*RI digests of human DNA were also obtained with the same bovine probe (data not shown). This is consistent with the presence of multiple genes (or pseudogenes) for OSCP but is not proof of their presence. An alternative explanation is that hybridization with several bands arises because of the presence of intervening sequences in the gene over the region covered by the cDNA probe. The issue can be resolved by characterization of the genes.

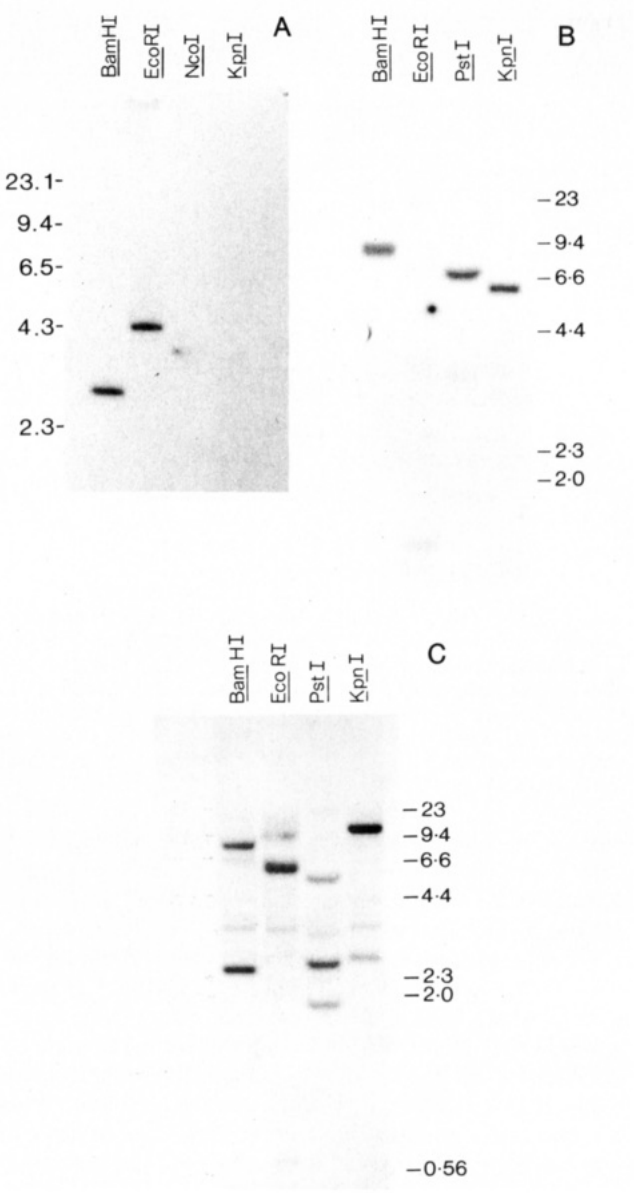

FIGURE 9: Hybridization of digests of bovine and human DNA with probes derived from bovine cDNAs for OSCP, F6, and inhibitor. The hybridization probe for the inhibitor was an M13 clone containing the entire sequence; that for F6 was either a fragment containing bases 78–356 (hybridization with human DNA) or an *Eco*RI–*Bam*I fragment, bases 1–548 (hybridization with bovine DNA); the probe for OSCP was either an *Eco*RI–*Bam*HI partial digestion fragment (bases 1–783; with human DNA) or a *Hin*dIII–*Eco*RI fragment (bases 143–738; with bovine DNA). For other experimental details see Materials and Methods. (A) Bovine DNA probed with inhibitor cDNA: (B) bovine DNA probed with F6 cDNA: (C) bovine DNA hybridized with OSCP probe. The data obtained with human DNA are not shown but are described in the text.

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