Vol. 152, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1988 Pages 264-269

BOVINE LIVER cDNA CLONES ENCODING A PRECURSOR OF THE α-SUBUNIT OF THE MITOCHONDRIAL ATP SYNTHASE COMPLEX

Gail A. M. Breen

Program in Molecular and Cell Biology
The University of Texas at Dallas
P.O. Box 830688
Richardson, TX 75083-0688

Received March 4, 1988

SUMMARY: cDNA clones encoding a precursor of the α-subunit of the mitochondrial ATP synthase complex have been isolated from a bovine liver cDNA library using the α-subunit gene from Saccharomyces cerevisiae as a probe. Analyses of the nucleotide sequence of these cDNA clones reveal that the bovine liver ATP synthase α-subunit is initially synthesized as a precursor with an amino-terminal extension 43 amino acids in length. This aminoterminal presequence contains seven basic residues, no acidic residues, and seven polar uncharged serine and threonine residues. A single mRNA species, approximately 1.85 kb in length, was detected for the ATP synthase α-subunit precursor in both bovine liver and heart.

The ATP synthase complexes of mitochondria, chloroplasts, and bacterial membranes are structurally and functionally similar (for reviews see 1-3). Functionally, the ATP synthase complexes synthesize ATP from ADP and P_i, utilizing energy generated from the electron transport chain. Structurally, the complexes consist of a soluble F_1 region that contains the catalytic sites for ATP synthesis and hydrolysis and a membrane-integrated F_o region that is involved in proton translocation. The F_1 region is composed of five distinct subunits--α, β, γ, δ, and ε in a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$. The catalytic sites are located either on β-subunits or at α-β subunit interfaces. In eucaryotic cells, the ATP synthase complex is composed of subunits encoded by both the nuclear and the mitochondrial genomes. For example, in animal cells and in fungi all of the F_1-subunits are encoded by nuclear genes, synthesized on cytoplasmic ribosomes (often as larger precursors) and imported into mitochondria (for reviews see 4, 5).

In this study we isolate and characterize cDNA clones that encode a precursor of the α-subunit of the bovine liver mitochondrial ATP synthase complex. Determination of the nucleotide sequence of these clones reveals that the bovine liver α-subunit is initially synthesized as a precursor containing an aminoterminal presequence 43 amino acids in length. Northern RNA

0006-291X/88 $1.50

hybridization analyses revealed a single transcript, approximately 1.85 kb in length, for the α-subunit precursor in bovine liver and heart.

MATERIALS AND METHODS

Colonies from a bovine liver cDNA library (6) constructed in the plasmid vector pBR322 were screened (7) using a nick-translated (8) 0.6-kb SalI-HindIII fragment of the ATP synthase α-subunit gene from S. cerevisiae (9) as a probe. Hybridization was carried out at 30 °C for 24 h in a solution containing 50% formamide, 10x Denhardt's (1x = 0.02% polyvinylpyrrolidone/0.02% NaCl/0.02% bovine serum albumin), 6x SSC (1x = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 0.1% SDS, 0.5% NP-40, 100 μg/ml tRNA and 10^6 cpm/ml of ^{32}P-labeled probe. Following hybridization, the filters were washed at 40 °C in a solution of 0.2x SSC plus 0.1% SDS and then exposed to X-ray film at -70 °C with intensifying screens. The nucleotide sequence of the inserts from positively hybridizing clones was determined using either the chemical modification method (10) or the dideoxy chain termination method (11), after subcloning various restriction fragments into pUC18 (12). Northern RNA blot hybridization analyses were carried out as described previously (13, 14).

RESULTS AND DISCUSSION

Isolation of cDNA Clones

A bovine liver cDNA library (6) constructed in the plasmid vector pBR322 was screened by colony hybridization (7) using a fragment of the ATP synthase α-subunit gene from S. cerevisiae (9) as a probe. Sequence data have indicated that the yeast and bovine mitochondrial ATP synthase α-subunit proteins are approximately 67% homologous (9, 15). Screening approximately 60,000 colonies with the yeast probe identified fourteen positively hybridizing clones. Characterization of DNA isolated from these fourteen clones revealed a 1.1 kilobase pair insert. Restriction mapping analyses showed that all of the cDNA inserts had the same cleavage map. In an attempt to obtain cDNA clones with longer inserts, we rescreened the bovine liver cDNA library with the insert from one of the positively hybridizing clones; however, no clones with longer inserts were recovered.

Sequence Analyses of a cDNA Clone

The insert from one of the positively hybridizing clones was subcloned into the plasmid vector pUC18 for nucleotide sequence determination. The nucleotide sequence of the cDNA insert was determined on both strands using the strategy shown in Figure 1. The nucleotide sequence of this cDNA and the deduced amino acid sequence are shown in Figure 2. Analysis of this nucleotide sequence reveals an open reading frame beginning at an ATG codon and extending for 1076 nucleotides. This open reading frame is flanked by 79 bp of noncoding DNA at the 5' end. Comparison of the amino acid sequence of the bovine liver α-subunit predicted from the DNA sequence of this cDNA clone with the amino acid sequence of the bovine heart α-subunit determined by direct

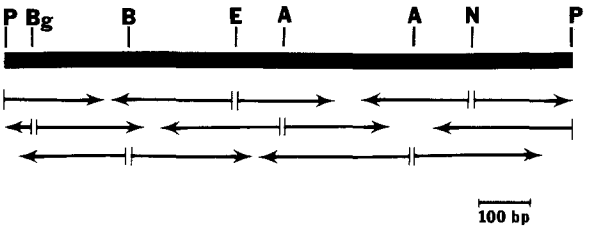

Figure 1. Partial restriction map of the bovine liver ATP synthase α-subunit cDNA and the strategy used for nucleotide sequence determination. Arrows represent the direction and length of the sequence determined in individual experiments. The restriction endonuclease sites are: P, PstI; Bg, BglII; B, BamH1; E, EcoR1; A, AvaII; N, NcoI.

amino acid analyses (15) reveals that the cDNA clone encodes amino acids 1 through 316 of the mature protein. Six amino acid differences were found between the predicted amino acid sequence of the bovine liver α-subunit and that of the bovine heart. These differences occur at amino acid residue 1, where the liver sequence is glutamine while the heart sequence is glutamic acid; residue 8, where the liver sequence is methionine and the heart is valine; residue 101, where the liver sequence is aspartic acid and the heart is glutamic acid; residue 121, where the liver is valine and the heart is isoleucine; residue 125, where the liver is isoleucine and the heart is alanine; and residue 315, where the liver is serine and the heart is alanine. It is not yet known whether these amino acid differences between the liver and heart sequences represent tissue-specific differences between α-subunits or polymorphic differences between animals or sequencing artifacts.

The cDNA also contains an open reading frame in phase with the sequence encoding the mature α-subunit protein beginning with a methionine residue at amino acid minus 43. Examination of the 43 amino acids comprising this amino-terminal presequence reveals that it contains seven basic arginine and lysine residues, no acidic residues, and seven nonpolar serine and threonine residues. The calculated molecular mass of this putative leader peptide is 4595 daltons.

RNA Analyses

Northern blot hybridization analyses were carried out to determine the size and number of the ATP synthase α-subunit precursor transcripts in bovine liver and heart. A single hybridizing species, approximately 1.85 kb in length, was identified in both tissues (Figure 3). The steady-state level of the α-subunit precursor transcript was approximately tenfold more abundant in bovine heart than in bovine liver. A similar ratio was observed previously for the mRNA of the precursor of the β-subunit of the bovine mitochondrial ATP synthase complex (13).

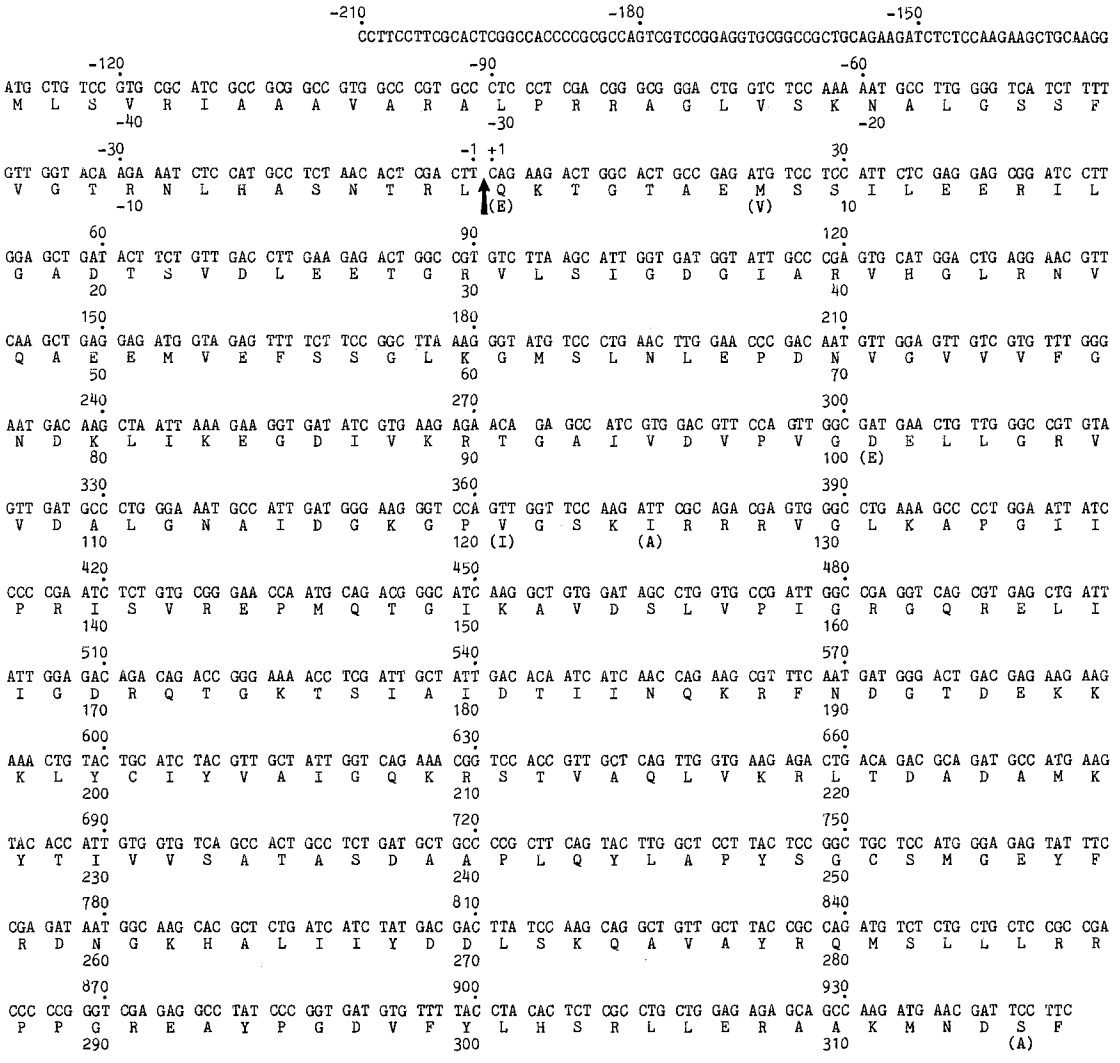

Figure 2. Nucleotide and predicted amino acid sequences of the bovine liver ATP synthase α-subunit precursor. Nucleotides and amino acids are numbered using the following conventions. The glutamine residue found at the aminoterminus of the mature α-subunit and its first corresponding base (C) are designated +1. All downstream amino acids and nucleotides carry (+) signs and all upstream amino acids and nucleotides have (-) signs. The amino acid residues of the bovine heart α-subunit sequence (15) that differ from those of the liver sequence appear below the liver sequence in parentheses.

Comparison of ATP Synthase α-Subunit Sequences

In animal cells and fungi, the α-subunit of the mitochondrial ATP synthase complex is synthesized as a precursor containing a NH_2-terminal leader peptide that is processed during import into mitochondria (9). The sequence of α-subunit leader peptide has now been determined for three diverse species, Bos taurus (this work), S. cerevisiae (9) and Xenopus laevis (16), allowing an evolutionary comparison (see Figure 4). Such a comparison reveals that the α-

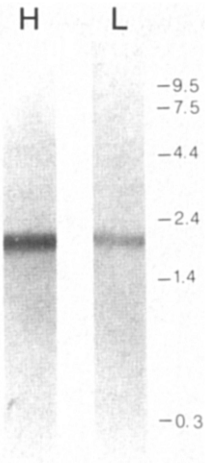

Figure 3. Northern blot hybridization analyses of the ATP synthase α-subunit precursor mRNA. Bovine liver (L) RNA, 36 μg, and heart (H) RNA, 8 μg, were denatured, separated by electrophoresis in 1.2% agarose gels in the presence of 2.2 M formaldehyde and then transferred to nitrocellulose filters. The filters were hybridized with a 900-bp BamH1-PstI fragment of the cDNA insert which was labeled with ^{32}P using the random primer method (19). Size markers are indicated in kb.

subunit leader peptides of the two animal species examined (bovine and Xenopus) are highly homologous (overall homology approximately 68%). Interestingly, the only region of the α-subunit leader peptides that diverges extensively between the two animal species is the region upstream of the putative cleavage site. In contrast, there is little amino acid sequence homology between the α-subunit leader peptides of either animal species and that of the yeast, S. cerevisiae (overall homology approximately 30%). In spite of the lack of sequence homology between the α-subunit leader peptides of animals and fungi, all three sequences have features in common with other

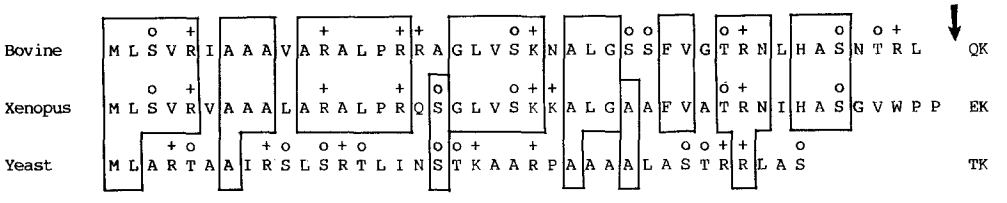

Figure 4. Comparison of the ATP synthase α-subunit presequences from B. taurus, X. laevis, and S. cerevisiae. The ATP synthase α-subunit presequences from bovine liver (bovine, this work), X. laevis oocytes (Xenopus, 16) , and S. cerevisiae (yeast, 9) are indicated. The NH_2-terminal amino acid residue of the mature bovine α-subunit protein (15) and the putative N-terminal amino acid residues of the mature α-subunit proteins of X. laevis and S. cerevisiae are indicated after the arrow. Positively charged lysine and arginine residues are indicated by a +, and hydroxylated serine and threonine residues by a o. Identical amino acid residues are boxed.

presequences that "target" proteins to the mitochondrion--including a preponderance of hydroxylated amino acid residues and a large number of positively charged amino acid residues (for reviews see 17, 18). In all three species, the homology between the predicted amino acid sequences of the α-subunit leader peptides is less than the homology between the mature proteins. For example, the bovine α-subunit presequence is approximately 30% homologous to the α-subunit presequence of S. cerevisiae whereas the two mature subunits are approximately 67% homologous. Similarly, the bovine α-subunit leader peptide is approximately 68% homologous to that of X. laevis oocytes while the mature subunits are 85% homologous.

ACKNOWLEDGMENTS: I thank Dr. R. T. A. MacGillvray, The University of British Columbia, for the generous gift of the bovine cDNA library and Dr. M. Douglas, The University of Texas Southwestern Medical School, for the generous gift of the cloned ATP synthase α-subunit gene from S. cerevisiae. I thank K. Garnett for expert technical assistance and C. Bailey for excellent editorial assistance. This work was supported by a grant from the National Institutes of Health.

REFERENCES

1. Amzel, L. M., and Pedersen, P. L. (1983) Annu. Rev. Biochem. 52, 801-824.
2. Senior, A. E., and Wise, J. G. (1983) J. Membrane Biol. 73, 105-124.
3. Walker, J. E., Saraste, M., and Gay, N. J. (1984) Biochim. Biophys. Acta 768, 164-200.
4. Tzagoloff, A., and Myers, A. M. (1986) Annu. Rev. Biochem. 55, 249-285.
5. Douglas, M., McCammon, M., and Vassarotti, A. (1986) Microbiol. Rev. 50, 166-178.
6. MacGillivray, R. T. A., and Davie, E. W. (1984) Biochemistry 23, 1626-1634.
7. Hanahan, D., and Meselsen, M. (1980) Gene 10, 63-71.
8. Rigby, P., Dieckman, N., Rhodes, C., and Berg, P. (1977) J. Molec. Biol. 113, 237-251.
9. Takeda, M., Chen, W.-J., Saltzgaber, J., and Douglas, M. G. (1986) J. Biol. Chem. 261, 15126-15133.
10. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560.
11. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
12. Yanisch-Perron, C., Vieriera, J., and Messing, J. (1985) Gene 33, 103-119.
13. Breen, G. A. M., Holmans, P. L., and Garnett, K. E. (1988) Biochemistry, in press.
14. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, New York.
15. Walker, J. E., Fearnley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M., and Tybulewicz, V. L. J. (1985) J. Molec. Biol. 184, 677-701.
16. Weeks, D. L., and Melton, D. A. (1987) Proc. Natl. Acad. Sci. USA 84, 2798-2802.
17. Douglas, M. G., McCammon, M. T., and Vassarotti, A. (1986) Microbiol. Rev. 50, 166-178.
18. Schatz, G. (1987) Eur. J. Biochem. 165, 1-6.
19. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.