Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 377–381, January 1988
Biochemistry

Two distinct genes for ADP/ATP translocase are expressed at the mRNA level in adult human liver

(cDNA library/pEX1 vector/HeLa cells/multiple mRNAs)

JANE HOULDSWORTH* AND GIUSEPPE ATTARDI

Division of Biology, California Institute of Technology, Pasadena, CA 91125

Contributed by Giuseppe Attardi, September 25, 1987

ABSTRACT **Several clones hybridizing with a bovine ADP/ATP translocase cDNA were isolated from an adult human liver cDNA library in the vector pEX1. DNA sequence analysis revealed that these clones encode two distinct forms of translocase. In particular, two clones specifying the COOH-end-proximal five-sixths of the protein exhibit a 9% amino acid sequence divergence and totally dissimilar 3′ untranslated regions. One of these cDNAs is nearly identical in sequence to an ADP/ATP translocase clone (hp2F1) recently isolated from a human fibroblast cDNA library [Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S. & Baserga, R. (1987) *J. Biol. Chem.* 262, 4355–4359], with three amino acid changes and a few differences in the 3′ untranslated region. Another clone isolated from the pEX1 library contains a reading frame encoding the remaining, NH_2-end-proximal, 37 amino acids of the translocase. This sequence differs significantly (14% amino acid sequence divergence) from the corresponding segment of hp2F1, and the 5′ untranslated regions of the two clones are totally dissimilar. RNA transfer hybridization experiments utilizing the clones isolated from the pEX1 library revealed the presence in HeLa cells of three distinct mRNA species. The pattern of hybridization and the sizes of these mRNAs suggest a greater complexity of organization and expression of the ADP/ATP translocase genes in human cells than indicated by the analysis of the cDNA clones.**

The ADP/ATP translocase is an integral protein of the inner mitochondrial membrane that carries out the exchange between the extramitochondrial and intramitochondrial ADP and ATP, linking the processes of ATP production to those of ATP utilization (1). This protein can thus exert a control on the rate of oxidative phosphorylation, as well as on the rates of the energy-consuming processes. Because of its central role in cellular energy metabolism, the ADP/ATP translocase is likely to be a highly regulated protein to fit the varying demands of the cell in relationship to functional, developmental, and tissue-specific factors. Indeed, evidence coming from an analysis of the immunological reactivity of the translocator protein extracted from different tissues (2, 3) and of the tissue specificity of anti-translocase autoantibodies found in some heart (4) and liver diseases (5) has strongly suggested the existence of multiple isoforms of this protein. These findings fit in well with the emerging concept of a developmental and tissue-specific control of the apparatus of oxidative phosphorylation. The isolation from a human fibroblast cDNA library of a clone for an ADP/ATP translocase that is growth-regulated has recently been reported (6).

In this paper, we report on the isolation and structural characterization of cDNAs for the ADP/ATP translocase from an adult human liver cDNA library. Evidence is presented that two different genes for this protein, one of which may correspond to the gene identified in human fibroblasts (6), are expressed at the mRNA level in the adult human liver.†

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MATERIALS AND METHODS

Isolation of ADP/ATP Translocase cDNA Clones. A library that had been constructed by subcloning *Pst* I fragments 400–1600 base pairs (bp) long from an adult human liver cDNA library (7) in the plasmid vector pEX1 (8) was screened by colony hybridization (9), using a nick-translated 1.2-kilobase (kb) cDNA (T10.1BE9) of the bovine ADP/ATP translocase (gift of J. Walker, Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K.) as a probe. For this purpose, replicate filters were washed at 68°C for 1 hr in 0.1× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate)/0.5% $NaDodSO_4$ and then incubated in 40% (vol/vol) formamide in 5× SSC/0.2% $NaDodSO_4$/20 mM sodium pyrophosphate/1× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin) containing 0.1 mg of sonicated denatured salmon sperm DNA per ml, at 37°C for 2 hr (10). Hybridization was then carried out at 37°C for 15 hr in the same solution containing 2×10^5 cpm of ^{32}P-labeled probe per ml. The filters were washed at 37°C twice in 40% (vol/vol) formamide in 5× SSC/0.2% $NaDodSO_4$ for 30 min, twice in 2× SSC/0.2% $NaDodSO_4$ for 30 min, and twice in 1× SSC/0.2% $NaDodSO_4$ for 30 min. Positive clones were rescreened and then subcultured in large scale for characterization by restriction mapping, using standard procedures (10).

DNA Sequencing. The nucleotide sequence of the isolated clones was determined by using both the Maxam–Gilbert method (11) and the dideoxynucleotide chain-termination method (12). In the latter case, standard techniques were used for cloning in M13 mp18 and M13 mp19 phages (13). Avian myeloblastosis virus reverse transcriptase (14), 2′-deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate (15), and 7-deaza-2′-deoxy-GTP (instead of 2′-deoxy-GTP) (16) were used in the dideoxy sequencing reactions.

RNA Transfer Hybridization Analysis. Cytoplasmic polysomal RNA was extracted from exponentially growing HeLa cells (maintained in suspension in Eagle's phosphate medium) as previously described (17, 18). Polyadenylylated RNA was isolated by double passage over an oligo(dT)-cellulose column (19). Samples of denatured RNA (3 μg per lane) were electrophoresed in a 1.1% agarose/5 mM CH_3HgOH gel in 50 mM sodium borate, pH 8.2/0.1 mM EDTA (19) and transferred to Zeta-Probe membranes (Bio-

Abbreviation: nt, nucleotide(s).

*Present address: Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

†These sequences are being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession nos. J03591 for clone pHAT3, J03592 for clone pHAT8, and J03593 for clone pHAT14).

Rad) by electroblotting. The filters were washed, pretreated, and then incubated for hybridization as described above, except for the addition of 10% dextran sulfate to the prehybridization and hybridization mixtures. Hybridization was carried out at 37°C for 15 hr with various formamide concentrations, as indicated below, depending on the probe used. ^{32}P-labeled probes were prepared by random-primed labeling of isolated inserts (20) to a specific activity of 2 × 10^8 cpm/μg. Filters were washed at 37°C, as detailed above.

RESULTS

Isolation and Characterization of Human ADP/ATP Translocase cDNA Clones. Out of 150,000 colonies screened of the human cDNA library, using a cDNA for the bovine ADP/ATP translocase as a probe, 26 positively hybridizing clones were isolated. As judged by insert size and restriction map analyses, these represented seven different clones, two nonoverlapping (pHAT3 and pHAT14) and five overlapping (pHAT5, pHAT8, pHAT22, pHAT27, and pHAT32) (Fig. 1). The nucleotide sequence of the inserts of pHAT14 and of the five overlapping clones was determined by using both the Maxam-Gilbert method (11) and the dideoxynucleotide chain-termination method (12), that of the insert of pHAT3, by the latter method, as described in the legend of Fig. 1.

The nucleotide sequence of the insert of pHAT14 (190 bp) revealed a 3'-truncated reading frame of 111 nucleotides (nt), and, upstream of this, an untranslated region of 79 nt (Fig. 2). The occurrence of a *Pst* I site at the 3' end of the reading frame indicated that this insert derived from *Pst* I cleavage of a longer insert during subcloning of the library (see *Materials and Methods*). A comparison with the derived amino acid sequence of the cDNA clone for the ADP/ATP translocase from human fibroblasts (6) showed that the reading frame of pHAT14 encodes the NH_2-end-proximal 37 amino acids of a related protein.

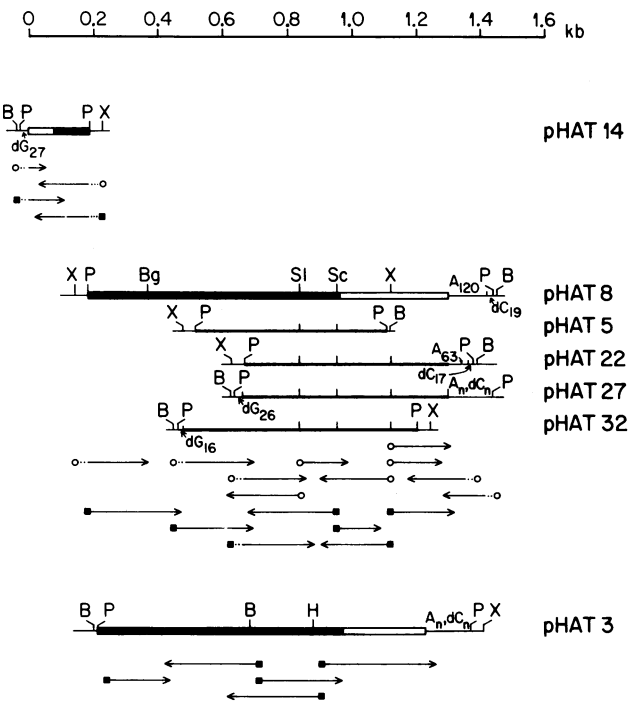

FIG. 1. Partial restriction map and sequencing strategy of the inserts of the ADP/ATP translocase cDNA clones. The inserts have been aligned on the basis of the sequence comparison with the human translocase clone hp2F1 (6). The scale above the cDNA inserts indicates the nucleotide position (in kb) relative to the 5' end of the pHAT14 cDNA insert. For pHAT14, pHAT8, and pHAT3, the black bar represents the protein-encoding region, the open bar, a 5' or 3' untranslated region, and the lines, portions of the clones pertaining to the poly(A), poly(dG), and poly(dC) tails and to vector sequences. For the clones that overlap pHAT8, the thick line represents the protein-encoding and the untranslated regions of the cDNA, the thin line, the tails and vector sequences. The arrows represent the direction and extent of sequencing from the indicated site. ○, Starting sites (5' end) for sequencing by the Maxam–Gilbert method (11); and ■, by the dideoxynucleotide chain termination method (12). B, *Bam*HI; P, *Pst* I; X, *Xba* I; Bg, *Bgl* II; Sl, *Sal* I; Sc, *Sac* I; H, *Hin*dIII.

pHAT8, with an insert size of 1235 bp [including the poly(A) tail], was the longest clone isolated (Fig. 1). The inserts of clones pHAT5, -22, and -32 were found to correspond to the regions from nt 440 to nt 1025, from nt 592 to nt 1285, and from nt 403 to nt 1125 of the insert of pHAT8, respectively (in the numbering system of Fig. 2); pHAT27 had an insert that appeared to be identical to that of pHAT22, but in the inverted orientation. The cDNA of pHAT8 contained a 5'-truncated reading frame of 789 nt and a 3' untranslated region of 326 nt (Fig. 2). Here too, the occurrence of a *Pst* I sequence at the 5' end of the reading frame pointed to the derivation of the insert of pHAT8 from *Pst* I cleavage of a longer insert during the subcloning of the library. A comparison with the sequence of the ADP/ATP translocase from human fibroblasts (6) showed that the reading frame of pHAT8 encodes a major portion of a related protein immediately downstream of the segment encoded in pHAT14. The 3' untranslated region of pHAT8 did not exhibit a typical polyadenylylation signal (21).

The insert of clone pHAT3 was 1018 bp long, excluding the poly(A) tail, and contained a 5'-truncated reading frame of 761 nt and a 3' untranslated region of 257 nt (Fig. 2). Here again, the occurrence of a *Pst* I sequence at the 5' end of the reading frame indicated that the insert of clone pHAT3 derived from *Pst* I cleavage of a longer insert. The deduced amino acid sequence showed that this cDNA clone also codes for a major portion of a protein related to the ADP/ATP translocase from human fibroblasts (6), from amino acid 47 to the COOH end. The 3' untranslated region of pHAT3 exhibited a typical polyadenylylation signal (21) 13 nt upstream of the poly(A) tail (Fig. 2).

Comparison of the ADP/ATP Translocase Clones with hp2F1. The protein segments encoded in the pHAT14 and pHAT8 cDNAs are clearly distinct from the protein encoded in hp2F1, the clone for ADP/ATP translocase isolated from a human fibroblast cDNA library (6) (Figs. 2 and 3). In particular, the reading frame of pHAT14 shows a 79% nucleotide sequence and an 86% amino acid sequence similarity to the corresponding portion of the reading frame of hp2F1. The great majority (65%) of the nucleotide changes are silent. Furthermore, the 5' untranslated region of pHAT14 is totally dissimilar from the 5' untranslated region of hp2F1. The reading frame of pHAT8 shows a 78% nucleotide sequence and a 91% amino acid sequence similarity to the corresponding region of the hp2F1 reading frame. Here too, the majority (82%) of the nucleotide substitutions are silent. The 3' untranslated region of pHAT8 is also very different in sequence from that of pH2F1.

The insert of pHAT3 corresponds to the segment of the hp2F1 cDNA from nt 137 to the 3' end, including a portion of the poly(A) tail (Fig. 2). In contrast to the inserts of pHAT14 and pHAT8, the sequence of the insert of pHAT3 is almost identical to that of the hp2F1 cDNA. In particular, in the reading frame, four nucleotide differences were found between hp2F1 and pHAT3. These discrepancies are located at nt 197, 332, 485, and 699 (in the numbering system of Fig. 2). At nt 197 in pHAT3 there is a G, while in hp2F1 an A is observed. This change also results in an amino acid change, from Glu in hp2F1 to Gly in pHAT3. It is noted that, in the translocases from maize (22), *Neurospora* (23), yeast (24), and bovine heart (25), a Gly residue is conserved at this position. At nt 332 and 485, the base substitutions result in

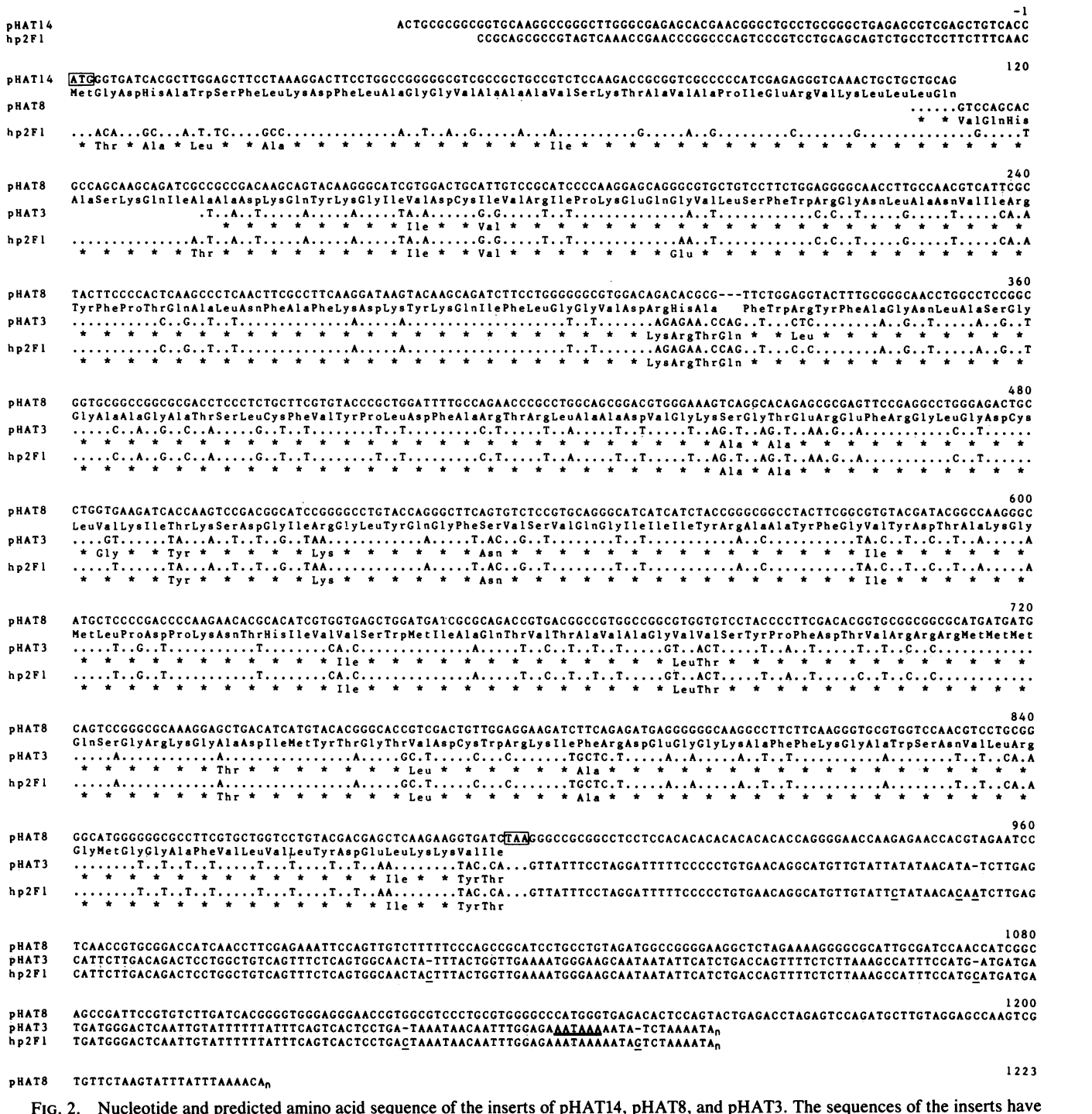

FIG. 2. Nucleotide and predicted amino acid sequence of the inserts of pHAT14, pHAT8, and pHAT3. The sequences of the inserts have been aligned with the sequence of hp2F1 (6). A one-codon gap has been introduced in the reading frame of pHAT8 at positions 322–324, to align it with the reading frames of pHAT3 and hp2F1. The initiation codon of pHAT14 and the termination codon of pHAT8 are boxed. In the reading frames, nucleotides identical to those of pHAT14 and pHAT8 are indicated by dots; identical amino acids are indicated by asterisks. The recognition site for polyadenylylation (21) in the 3′ untranslated region of pHAT3 is doubly underlined. In the 3′ untranslated region of hp2F1, nucleotide substitutions or insertions as compared to the homologous region of pHAT3 are underlined.

corresponding codon changes: Arg and Val in hp2F1 to Leu and Gly, respectively, in pHAT3. The nucleotide difference at position 699 does not result in an amino acid substitution. A number of nucleotide differences (seven substitutions or deletions/insertions) were observed in the 3′ untranslated regions of hp2F1 and pHAT3 (Fig. 2).

Comparison of the Human and Bovine ADP/ATP Translocase Sequences. Fig. 3 shows the complete, directly determined amino acid sequence of the ADP/ATP translocase from bovine heart (25) and, underneath it, the deduced sequences of pHAT14, pHAT8, hp2F1, and pHAT3. One amino acid gap at position 108 had to be introduced in the sequence of pHAT8 to align it with the bovine sequence. In the homologous maize (22), *Neurospora* (23), and yeast (24) protein sequences, one amino acid gap is also observed in this position. Interestingly, this gap is not present in hp2F1 nor in pHAT3 (Fig. 2). The protein coding sequence of pHAT14, 37 residues long, exhibits four differences from the corresponding segment of the bovine protein (89% similarity). The 262 amino acid stretch encoded in pHAT8 differs from the corresponding segment of the bovine protein at 26 positions (90% similarity). A similar divergence from the

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                                                                    50
Bovine      SDQALSFLKDFLAGGVAAAISKTAVAPIERVKLLLQVQHASKQISAEKQ
pHAT14      MG.H.W.............V................
pHAT8                                          .........A.D..
hp2F1       MT.A....A.....................................T..D..
                                                  (pHAT3)└──→

                                                                   100
Bovine      YKGIIDCVVRIPKEQGFLSFWRGNLANVIRYFPTQALNFAFKDKYKQIFL
pHAT8       ....V..I........V..............................
hp2F1       ................EV..............................
(pHAT3)                 (G)

                                                                   150
Bovine      GGVDRHKQFWRYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGKGAA
pHAT8       ......A-.....................................SGT
hp2F1       ....KRT.....................................AG.
(pHAT3)           (L)

                                                                   200
Bovine      QREFTGLGNCITKIFKSDGLRGLYQGFNVSVQGIIIYRAAYFGVYDTAKG
pHAT8       E...R...D.LV..T....I.......S....................
hp2F1       E...R...D.LV..Y....IK.....................I......
(pHAT3)           (G)

                                                                   250
Bovine      MLPDPKNVHIIVSWMIAQTVTAVAGLVSYPFDTVRRRMMMQSGRKGADIM
pHAT8       .......T..V.............V.......................
hp2F1       .......T..VI............T...................T...
(pHAT3)

                                                                   298
Bovine      YTGTVDCWRKIAKDEGPKAFFKGAWSNVLRGMGGAFVLVLYDEIKKFV
pHAT8       ...........FR...G.........................L..YI
hp2F1       ....L.......R...G.........................YT
(pHAT3)
```

FIG. 3. Comparison of the deduced amino acid sequences of the inserts of the human ADP/ATP translocase cDNA clones pHAT14, pHAT8, hp2F1, and pHAT3 with the amino acid sequence of the bovine ADP/ATP translocase (25). The amino acid residues are shown in standard single-letter symbols. Identical residues are indicated by dots. Below the hp2F1 sequence, the residues that differ in pHAT3 are shown in parentheses.

bovine sequence is exhibited by the protein encoded in the human translocase cDNA clone hp2F1 (6) (90% similarity) and by that encoded in pHAT3 (89% similarity). Between the predicted sequences for the two human ADP/ATP translocase proteins identified here (encoded in pHAT8 and pHAT3), there is 91% similarity in the common segment.

The amino acid differences on the whole tend to be in clusters and do not occur in regions that are highly conserved. The latter regions include the six hydrophobic segments that are postulated to traverse the membrane as α-helices and that are part of three repeated domains. The whole polypeptide is thought to derive from an ancestral gene by two duplication events (26).

Identification of ADP/ATP Translocase mRNAs in HeLa Cells. Polyadenylylated RNA was isolated from HeLa (human) cells and subjected to RNA transfer hybridization analysis, using the inserts of the pHAT clones as probes. In view of the evidence of the existence of multiple isoforms of ADP/ATP translocase cDNAs, relatively low-stringency conditions were used in the hybridizations. The results of these experiments are shown in Fig. 4. When pHAT8 was used as a probe, three RNA species reacting with the probe were detected (Fig. 4*a*). The same three bands, with similar relative intensities, were detected by hybridization with probes derived from clones overlapping with pHAT8 (pHAT5, pHAT22, and pHAT32) (Fig. 4*b*). The most strongly hybridizing band (b) corresponds to an RNA with a size of ≈1450 nt, as estimated by comparison of its migration with that of the mitochondrial RNA markers (Fig. 4*b*). Of the two other bands, one corresponds to an RNA with a size of ≈1600 nt, and the other represents an RNA species of ≈1300 nt.

When pHAT3 was used as a probe in 40% formamide, it hybridized exclusively to the c band (Fig. 4*b*). In 30% formamide, pHAT3 still hybridized predominantly to the c band, but one could detect a weak hybridization to the b band and an even weaker reaction with the a band (Fig. 4*a*). The pattern did not change appreciably when the percentage of formamide was

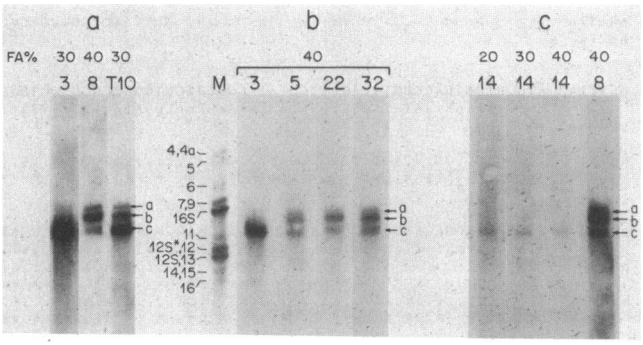

FIG. 4. Hybridization analysis of HeLa cell mRNA. Polyadenylylated RNA was isolated from HeLa cells, fractionated by electrophoresis in a 1.1% agarose/5 mM CH_3HgOH gel (3 μg per lane), transferred to a nylon membrane, and then hybridized at 37°C with ^{32}P-labeled cDNA probes for ADP/ATP translocase. In *a*, 3 × 10^6 cpm of probe was used for the first lane (30-hr exposure), 6 × 10^6 cpm for the second lane, and 6 × 10^6 cpm for the third lane (17-hr and 30-hr exposure, respectively); in *b*, 5 × 10^6 cpm per lane (7-hr exposure) was used; and in *c*, 1.5 × 10^6 cpm per lane (22-hr exposure) was used. Mitochondrial RNA species labeled *in vitro* (27) were used as molecular weight markers (lane M). The sizes of 16S rRNA, RNA11, 12S* rRNA, and 12S rRNA are 1559, ≈1200, ≈1045, and 954 nt (27, 28). The percent (vol/vol) formamide (FA) present during hybridization is indicated at the top; 3, 5, 8, 14, 22, 32 indicate cDNA probes from pHAT3, pHAT5, pHAT8, pHAT14, pHAT22, and pHAT32, respectively, and T10 indicates a cDNA for bovine ADP/ATP translocase. Bands a, b, and c are discussed in the text.

lowered (to 20% and 10%) (not shown). It is also interesting that the bovine cDNA probe T10 reacted predominantly with the c band, and, in order of decreasing intensity, with the b and a bands (Fig. 4*a*). The insert of pHAT14 was found to hybridize exclusively with band c, the reaction being in general weak and decreasing with increasing formamide concentration (Fig. 4*c*). The fairly high background, possibly due to the poly(dG) tail, prevented the detection of a possible minor hybridization with the b and a species.

DISCUSSION

The cDNA library used in this work was constructed by cutting out the cDNA inserts of another library (7) with *Pst* I and inserting the fragments into *Pst* I-cleaved pEX1 (8). As a result, some of the original cDNA inserts were cut at internal *Pst* I sites. The *Pst* I sites at the 5′ end of pHAT3 and pHAT8 and at the 3′ end of pHAT14 were generated in this way. The human translocase cDNA clone hp2F1 recently identified (6) has two *Pst* I sites, and it is from the second site that pHAT3 was found to extend towards the 3′ end. In pHAT8, the *Pst* I site at the same position has been lost due to mutation, while the first site is retained, and it is from this site that pHAT8 extends towards the 3′ end; pHAT14 extends also from this site towards the 5′ end (Fig. 1).

The main conclusion of this work is that two genes for the ADP/ATP translocase are expressed at the mRNA level in adult human liver. In fact, the protein-encoding sequences of pHAT8 and pHAT3 are clearly distinct (23% divergence in nucleotide sequence and 9% divergence in amino acid sequence). Furthermore, the 3′ untranslated regions of the two inserts are totally different. The reading frame and 3′ untranslated region of pHAT3 are nearly identical to those of the human cDNA hp2F1, which is expressed in fibroblasts (6). However, the differences are probably too many to be explained by polymorphism. Although one cannot exclude this possibility, the alternative explanation that the inserts of pHAT3 and hp2F1 derive from two distinct genes seems more likely.

pHAT14 is the most intriguing among the clones isolated from the pEX1 library. In principle, its insert could derive

from the same liver mRNA from which the insert of pHAT8 or that of pHAT3 derives. The insert of pHAT8 was found to react strongly with HeLa cell mRNA b, suggesting that this may be the RNA from which that insert derives. If it is so, the failure of pHAT14 to hybridize with mRNA b would argue against the possibility that the inserts of pHAT8 and pHAT14 have a common origin. On the other hand, if the inserts of pHAT3 and hp2F1 derive from the same mRNA, the insert of pHAT14 must have a different origin. In fact, the reading frame of pHAT14 is clearly distinct from the corresponding portion of the reading frame of hp2F1 (21% divergence in nucleotide sequence and 14% in amino acid sequence). Furthermore, its 5′ untranslated region is totally dissimilar from the 5′ untranslated region of hp2F1. Therefore, either the inserts of pHAT14 and pHAT3 share the same origin, distinct from that of hp2F1, or the cDNA of pHAT14 derives from an mRNA different from that (those) generating the pHAT3 and hp2F1 cDNAs. The observation that pHAT14 hybridizes with the HeLa cell mRNA c with which pHAT3 exhibits a strong reaction would support the idea that the two clones derive from the same cDNA. However, pHAT14 hybridizes with mRNA c much less strongly than expected for the size of its insert, and its reaction with this RNA decreases with increasing formamide concentration, despite its high G+C content (68%). These two facts argue more in favor of the interpretation that the insert of pHAT14 originates from a liver mRNA distinct from that from which pHAT3 derives. In fact, this mRNA may not be present at all in HeLa cells, the reaction of pHAT14 with HeLa cell mRNA c being due to cross-hybridization. This interpretation would imply that three different genes for the ADP/ATP translocase (corresponding to the cDNAs of pHAT14, pHAT8, and pHAT3) are expressed in adult human liver. Further work is needed to clarify this issue. Whatever the relationship of pHAT14 to pHAT3 is, the available evidence suggests that at least three different genes for the ADP/ATP translocase are expressed in different human tissues.

The analysis of the translocase-specific mRNAs in HeLa cells has revealed three discrete mRNA species, with sizes of 1300, 1450, and 1600 nt. The different relative intensities of the signals produced by hybridization of these mRNA species with pHAT3, pHAT8, and pHAT14 presumably reflect in part different degrees of sequence similarities and in part differences in G+C content of the three inserts. In fact, the insert of pHAT3 has a G+C content of 46.5%, that of pHAT8, 60.5%, and that of pHAT14, 68%.

The shortest mRNA (species c), which showed in the present work the strongest hybridization with pHAT3, is smaller than the mRNA from a human promyelocytic leukemia cell line, which was previously reported to be the only mRNA hybridizing with hp2F1 (6). The latter species was estimated to be ≈1500 nt in length and was assumed to correspond to the 1228 bp of the complete or near-complete cDNA isolated from human fibroblasts plus a poly(A) tail. If the missing segment of the cDNA of pHAT3 is similar in size to the equivalent region of the insert of hp2F1 (205 bp, including a 69-nt 5′ untranslated segment and a 136-nt coding stretch), and if the mRNA corresponding to pHAT3 is indeed the species c detected in HeLa cells, this mRNA would have a poly(A) tail of an unusually short size (≈70 residues). On the other hand, it is possible that the mRNA species c expressed in HeLa cells has a shorter 5′ or 3′ untranslated region compared to hp2F1. This would imply a complexity of the genes for the ADP/ATP translocase even greater than so far suspected. In previous work (29), two distinct mRNAs with sizes of 1200 and 1500 nt were detected in bovine tissues (heart, kidney, liver, and uterus) by using ADP/ATP translocase cDNA clones isolated from a bovine heart library as probes. It was postulated that these two mRNAs differ in the lengths of their 5′ untranslated region. A remarkable feature of these mRNAs is their very short 3′ untranslated region (26 or 27 nt).

The 1600-nt mRNA species (a), which hybridizes to a moderate extent with pHAT8 and very weakly with pHAT3 and with the bovine ADP/ATP translocase clone T10, very probably also codes for an ADP/ATP translocase. The relatively weak hybridization of this RNA species with the ADP/ATP translocase-specific clones, as compared to mRNA species b and c, could reflect a lower amount of an RNA similar in sequence to that from which the cDNA of pHAT8 or pHAT3 derives, or the presence of an RNA with a more divergent sequence of the protein coding region. Its larger size may be due to a longer 5′ and/or 3′ untranslated region, as compared to pHAT8, pHAT3, and hp2F1. In any case, the presence of this mRNA in HeLa cells points to the possible existence of another gene for the ADP/ATP translocase or to a different transcription or RNA processing pathway.

It is clear from the results reported here that two, and very probably more, genes for the ADP/ATP translocase exist and are expressed in the human genome. This work thus provides a basis, at the gene structure level, for the previous immunological evidence of tissue specificity of the ADP/ATP translocase (2–5). Further work on the organization of the ADP/ATP translocase genes and on the pattern of their expression should reveal the significance of the complexity of the genetic control of this key protein in relationship to functional, developmental, and tissue-specific factors.

We are greatly indebted to Keith Stanley for providing us with the human cDNA library in the vector pEX1 and to John Walker for the bovine ADP/ATP translocase clone T10.1BE9. The technical assistance of Ms. Arger Drew is gratefully acknowledged. These investigations were supported by National Institutes of Health Grant GM-11726 to G.A. and a Damon Runyon-Walter Winchell Cancer Fund Fellowship to J.H.

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