Molecular Cloning of a cDNA for a Human ADP/ATP Carrier Which Is Growth-Regulated*

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We have identified in a human cDNA library a clone (hp2F1) whose cognate RNA is growth-regulated. The insert has been sequenced and the nucleotide sequence shows a strong homology to the nucleotide sequences of the ADP/ATP carrier cDNA and gene, respectively, isolated from *Neurospora crassa* and *Saccharomyces cerevisiae*. The putative amino acid sequence of hp2F1 shows an 87% homology to the amino acid sequence of the ADP/ATP carrier from beef heart mitochondria. We conclude that the insert of hp2F1 contains the full coding sequence of a human ADP/ATP carrier.

The steady-state RNA levels of the ADP/ATP carrier are growth-regulated. They increase when quiescent cells are stimulated by serum, platelet-derived growth factor, or epidermal growth factor, but not by platelet-poor plasma or insulin. RNA levels of the ADP/ATP carrier decrease instead when growing HL-60 cells are induced to differentiate by either phorbol esters or retinoic acid.

The ADP/ATP carrier (ATP/ADP translocase, ADP/ATP translocator protein, adenine nucleotide translocase) is the most abundant protein in mitochondria and catalyzes the exchange of adenine nucleotides across the mitochondrial inner membrane (1). This exchange is essential to the transfer of energy from oxidative phosphorylation to extramitochondrial processes. We have isolated from a human cDNA library (2) and sequenced a clone which contains the full coding sequence of a human ADP/ATP carrier.

The human clone (hp2F1) was isolated by screening the Okayama-Berg library (2) with an insert from a clone, p2F1, that was originally identified in a Syrian hamster cDNA library (3) as a growth-regulated gene, *i.e.* as a cDNA clone whose cognate RNA levels were increased when G₀ cells were stimulated by serum.

The purposes of the present paper are 1) to show the full nucleotide sequence of the cDNA for a human ADP/ATP carrier and compare it to the nucleotide sequence of the ADP/ATP translocator of *Saccharomyces cerevisiae* (4) and to the amino acid sequence of the ADP/ATP carrier from beef heart mitochondria (5), and 2) to confirm and extend the observation that the steady-state RNA levels of the ADP/ATP carrier are growth-regulated.

EXPERIMENTAL PROCEDURES

Cell Cultures—BALB/c/3T3 fibroblasts were plated in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics (high serum medium). When cells were semi-confluent the high serum medium was removed and low serum medium (containing 1% calf serum instead of 10% fetal calf serum) was added to the cultures. Five days later the cells were quiescent (less than 1% of cells incorporated [³H]thymidine following a 24-h incubation) and were then treated with fresh high serum medium, or PDGF¹ (Collaborative Research) or EGF (Collaborative Research), or insulin (Sigma), or platelet-poor plasma, prepared from human blood as described by Ross *et al.* (6). Except for fetal calf serum, growth factors were added to the conditioned low serum medium. Eight hours after addition of growth factors, the cells were washed with ice-cold phosphate-buffered saline, and harvested with a rubber policeman, and total cytoplasmic RNA was extracted. HL-60 cells were grown as described by Rovera *et al.* (7).

Screening of the cDNA Library—The insert derived from the original hamster p2F1 clone (3) was nick-translated with [³²P]dCTP (8) to a specific activity of 2–4 × 10⁸ cpm/μg and used as probe. Approximately 800,000 recombinants of an Okayama-Berg library derived from SV40-transformed human fibroblasts (2) were screened at high density according to the procedure described by Hanahan and Meselson (9). Twelve positive clones were identified after primary screening. After secondary and tertiary screening four clones with inserts of different lengths were isolated and grown in large scale for further analysis. The longest one, pA₁A₃A, was sequenced, and is now referred to as the human cDNA clone of p2F1, or hp2F1.

DNA Sequencing—DNA sequence analysis was performed utilizing both the dideoxy-chain termination (10) and chemical (11) methods. The GenBank from Palo Alto, CA and the EMBL data bank were searched for nucleotide sequence homology. The National Biomedical Research Foundation data bank was searched for protein homologies.

RNA Extraction and Blotting—Total cytoplasmic RNA was extracted as previously described (3). Blotting was carried out as described by Thomas (12). Hybridization and autoradiography were carried out by standard procedures (12). The human 2F1 clone was nick-translated (8) to a specific activity of 4–5 × 10⁸ cpm/μg. The amount of RNA blotted on each filter was constantly monitored by hybridizing the filters to probes for genes that are not expressed in a cell cycle-dependent manner and/or a probe for the S-phase specific gene histone H3, as previous described (3, 13–15).

Materials—PDGF and EGF (receptor grade) were purchased from Collaborative Research.

RESULTS

Sequence of the cDNA for a Human ATP/ADP Translocase—Fig. 1 shows the complete nucleotide sequence of the p2F1 clone isolated from a human cDNA library (2). It consists of 1228 residues, somewhat shorter than the RNA band detected on RNA blots by the appropriate 2F1 probe, which gives a size of 1.5 kilobases (3). However, the discrepancy could be easily abrogated by the addition of a poly(A) tail. The sequence has an open reading frame of 894 nucleotides,

                                                                                                120
CCGCAGCGCCGTAGTCAAACCGAACCCGGCCCAGTCCCGTCCTGCAGCAGTCTGCCTCCTTCTTTCAACATGACAGATGCCGCATTGTCCTTCGCCAAGGACTTCCTGGCAGGTGGAGTG
                                                                MetThrAspAlaLeuSerPheAlaLysAspPheLeuAlaGlyGlyVal

                                                                                                240
GCCGCAGCCATCTCCAAGACGGCGGTAGCGCCCATCGAGCGGGTCAAGCTGCTGCTGCAGGTGCAGCATGCCAGCAAGCAGATCACTGCAGATAAGCAATACAAAGGCATTATAGACTGC
AlaAlaAlaIleSerLysThrAlaValAlaProIleGluArgValLysLeuLeuLeuGlnValGlnHisAlaSerLysGlnIleThrAlaAspLysGlnTyrLysGlyIleIleAspCys

                                                                                                360
GTGGTCCGTATTCCCAAGGAGCAGGAAGTTCTGTCCTTCTGGCGCGGTAACCTGGCCAATGTCATCAGATACTTCCCCACCCAGGCTCTTAACTTCGCCTTCAAAGATAAATACAAGCAG
ValValArgIleProLysGluGlnGluValLeuSerPheTrpArgGlyAsnLeuAlaAsnValIleArgTyrPheProThrGlnAlaLeuAsnPheAlaPheLysAspLysTyrLysGln

                                                                                                480
ATCTTCCTGGGTGGCGTGGGACAAGAGAACCCAGTTTTGGCGGCTACTTTGCAGGGAATCTGGCATCGGGTGGTGCCGCAGGGGCCACATCCCTGTGTTTTGTGTACCCTCTTGATTTTGCC
IlePheLeuGlyGlyValGlyGlnGluAsnProValLeuAlaAlaThrLeuGlnGlyIleTrpHisArgValValProGlnGlyProHisProCysValPheValTyrProLeuAspPheAla

                                                                                                600
CGTACCCGTCTAGCAGCTGATGTGGGTAAAGCTGGAGCTGAAAGGGAATTCCGAGGCCTCGGTGACTGCCTGGTTAAGATCTACAAATCTGATGGGATTAAGGGCCTGTACCAAGGCTTT
ArgThrArgLeuAlaAlaAspValGlyLysAlaGlyAlaGluArgGluPheArgGlyLeuGlyAspCysLeuValLysIleTyrLysSerAspGlyIleLysGlyLeuTyrGlnGlyPhe

                                                                                                720
AACGTGTCTGTGCAGGGTATTATCATCTACCGAGCCGCCTACTTCGGTATCTATGACACTGCAAAGGGAATGCTTCCGGATCCCAAGAACACTCACATCGTCATCAGCTGGATGATCGCA
AsnValSerValGlnGlyIleIleIleTyrArgAlaAlaTyrPheGlyIleTyrAspThrAlaLysGlyMetLeuProAspProLysAsnThrHisIleValIleSerTrpMetIleAla

                                                                                                840
CAGACTGTCACTGCTGTTGCCGGGTTGACTTCCTATCCATTTGACACCGTTCGCCGCCGCCATGATGATGCAGTCAGGGCGCAAAGGAACTGACATCATGTACACAGGCACGCTTGACTGC
GlnThrValThrAlaValAlaGlyLeuThrSerTyrProPheAspThrValArgArgArgMetMetMetGlnSerGlyArgLysGlyThrAspIleMetTyrThrGlyThrLeuAspCys

                                                                                                960
TGGCGGAAGATTGCTCGTGATGAAGGAGGCAAAGCTTTTTTCAAGCGGTGCATGGTCCAATGTTCTCACAGAGGCATGGGTGGTGCTTTTGTGCTTGTCTTGTATGATGAAATCAAGAAGTAC
TrpArgLysIleAlaArgAspGluGlyGlyLysAlaPhePheLysGlyAlaTrpSerAsnValLeuArgGlyMetGlyGlyAlaPheValLeuValLeuTyrAspGluIleLysLysTyr

                                                                                                1080
ACATAAGTTATTTCCTAGGATTTTTCCCCCTGTGAACAGGCATGTTGTATTCTATAACACAATCTTGAGCATTCTTGACAGACTCCTGGCTGTCAGTTTCTCAGTGGCAACTACTTTACT
Thr***

                                                                                                1200
GGTTGAAAATGGGAAGCAATAATATTCATCTGACCAGTTTTCTCTTAAAGCCATTTCCATGCATGATGATGATGGGACTCAATTGTATTTTTTATTTCAGTCACTCCTGACTAAATAACA

ATTTGGAGAAATAAAAATAGTCTAAAATAn

FIG. 1. Nucleotide sequence of clone hp2F1 which contains the full coding sequence of a human ADP/ATP carrier protein. The initiation and termination codons and the polyadenylation signal are *underlined*. Below the nucleotide sequence is the deduced amino acid sequence.

from residue 70 to residue 963, yielding a putative amino acid sequence of 298 residues. The putative amino acid sequence is also shown in Fig. 1 below the nucleotide sequence.

The AUG initiation codon at nucleotide 70 is preceded by a canonical purine (A, which is the most frequent) at position −3 (16). The termination codon nucleotides (964–966) are UAA. The polyadenylation signal AATAAA (17, 18) extends from nucleotide 1210 to 1215. The presence of characteristic initiation and polyadenylation sequences and the homologies to other ATP/ADP translocases (see below) support the possibility that this is a full length (or nearly full-length) cDNA, containing the entire coding sequence.

Comparison of the Sequence of hp2F1 with the Bovine and Yeast Sequences of ATP/ADP Translocase—The complete nucleotide sequences of the ADP/ATP carrier of *S. cerevisiae* (4) and *Neurospora crassa* (19) have been published. Fig. 2 shows a comparison of the coding sequences of hp2F1 and the ATP/ADP translocase from *S. cerevisiae*. The homology in the coding sequence is 47%. The deduced amino acid sequences are also given; the homology here is 51%.

The complete amino acid sequence of the ADP/ATP carrier from beef heart mitochondria has also been published (5) and was compared with the putative amino acid sequence (derived from the nucleotide sequence) of the hp2F1 clone. The homology extends to 266 out of 297 residues, or 89.6%, and no gaps are necessary for the alignment. The clusters of positive charges noted by Aquila *et al.* (5) are conserved in the human sequence. Of the four regions considered to be the putative transmembrane hydrophobic sequences (5, 19, 20), the first and the second are perfectly conserved (residues 72–92- and 111–134). The third region (residues 175–195) has one substitution, isoleucine for valine, and the last one (208–232) has four substitutions (2 residues are inverted), none of them affecting the charge of the segment, but two of them involving an exchange of tyrosine for valine.

The ADP/ATP Carrier Is Growth-regulated—The p2F1

clone was originally isolated from a Syrian hamster cDNA library as a growth-regulated gene, *i.e.* as a cDNA whose cognate RNA increased when G_0 cells were stimulated by serum (3). Subsequently, it was found to be induced by phytohemagglutinin both in human peripheral blood mononuclear cells (13) and in purified T lymphocytes (21), and by serum in 3T3 cells (22), in human diploid fibroblasts (23), and in rat 3Y1 cells (24). The levels of ADP/ATP carrier mRNA are therefore increased by mitogens in at least five different cell types from four different species. They are also increased by infection of nonpermissive cells with adenovirus under conditions resulting in stimulation of cellular DNA synthesis (22). We have extended these studies to investigate which growth factors in the serum are reponsible for increasing RNA levels of the ADP/ATP carrier. BALB/c/3T3 cells were used and total cellular RNA was prepared from quiescent cells and from cells 8 h after exposure to growth factors. The results are shown in Fig. 3. Serum (*lane b*), platelet-derived growth factor (PDGF, *lane c*), and epidermal growth factor (EGF, *lane e*) all increase the steady-state levels of ADP/ATP carrier mRNA, whereas platelet-poor plasma (*lane d*) and insulin (*lane f*) fail to do so.

Levels of ADP/ATP Carrier mRNA in HL-60 Cells—HL-60 cells are a human promyelocytic leukemia cell line (25) in which the promyelocytes can be induced to differentiate by treatment with various agents (reviewed by Koeffler, 26). TPA (12-*O*-tetradecanoylphorbol-13-acetate) induces terminal differentiation in HL-60 cells along the monocyte-macrophage pathway (7), whereas retinoic acid induces differentiation along the granulocyte pathway (26). Exponentially growing HL-60 cells were treated either with TPA (20 ng/ml, Fig. 4) or retinoic acid (10^{-6} M, Fig. 5) and followed for various lengths of time. Cytoplasmic RNA was extracted at the times indicated and RNA blots were hybridized to the hp2F1 probe. Figs. 4 and 5 show that both TPA and retinoic acid cause a decrease in the mRNA levels of ATP/ADP translocase. With

```
                                                      .         .              60                 .              .
----------------ATGACAGATGCCGCATTGTCCTTCGCCAAGGACTTCCTGGCAGGTGGAGTGGCCGCAGCCATCTCCAAGACGGCGGTAGCGCCCATCGAGCGGGTCAAGCTGCTG
                MetThrAspAlaAlaLeuSerPheAlaLysAspPheLeuAlaGlyGlyValAlaAlaAlaIleSerLysThrAlaValAlaProIleGluArgValLysLeuLeu
ATGTCTCACACAGAA CACACAGAC CAGCAG CACA    GTGT   TATG C C TTT T   TG G      GT CC T   T A  G A  T
MetSerHisThrGluThrGlnThrGlnSerHis    GlyVal   Met      Ser      Ala      GlyAla         T A   G A  T

                              120                 .                     .                  180                .               .
CTGCAGGTGCAGCATGCCAGC---AAGCAGATCACTGCAGATAAGCAATACAAAGGCATTATAGACTGCGTGGTCCGTATTCCCAAGGAGCAGGAAGTTCTGTCCTTCTGGCCGCGGTAAC
LeuGlnValGlnHisAlaSer   LysGlnIleThrAlaAspLysGlnTyrLysGlyIleIleAspCysValValArgIleProLysGluGlnGluValLeuSerPheTrpArgGlyAsn
A    AAT  AG A AG TGCTT A   GG T GTTG   CA GG    G A T   T TCAAGA G CGG CTC TGA GTA G    G    A G
Met    Asn  GluGluMetLeu   GlySerLeu   ThrArg       Leu      PheLys   ThrAlaThrHisGluGlyIleVal

                              240                .                     .                 300                  .
CTGGCCAATGTCATCAGATACTTCCCCACCCAGGCTCTTAACTTCGCCTTCAAAGATAAATACAAGCAGATCTTCCTGGGTGGTGTGGACAAGAGAACCCAGTTTTGGCGCTACTTTGCA
LeuAlaAsnValIleArgTyrPheProThrGlnAlaLeuAsnPheAlaPheLysAspLysTyrLysGlnIlePheLeuGlyGlyValAspLysArgThrGlnPheTrpArgTyrPheAla
ACC      TC C G T    G    G GTT       C  ATT  TC --- GT A TAC ACAGAG C CGATGG A GCCAAG GG     T
Thr        Leu                        Ile   Ser---Leu      SerTyrAspArgGlu   AspGlyTyrAlaLysTrp

                     360                  .                     .              420                      .
GGGAATCTGGCATCGGGTGGTGCCGCAGGGGCCCACATCCCTGTGTTTGTGTACCCTCTTGATTTTGCCCGTACCCGTCTAGCAGCTGATGTGGGT------AAAGCTGGAGCTGAAAGG
GlyAsnLeuAlaSerGlyGlyAlaAlaGlyAlaThrSerLeuCysPheValTyrProLeuAspPheAlaArgThrArgLeuAlaAlaAspValGly------LysAlaGlyAlaGluArg
A    TTTC T    A G T TGTTG G TCTA    A TTCTG C AC AAG G GT    G   CTA GGGTTCT GT AACCT GC   A
       Phe         GlyLeu   Leu      Ser   Tyr               G        AlaArgGlySer  SerThrSerGln

   .                          480                   .                     .              540                .
GAATTCCGAGGCCTCGGTGACTGCCTGGTTAAGATCTACAAATCTGATGGGATTAAGGGCGTGACCAAGGCTTTAACGTGTCTGTGCAGGGTATTATCATCTACCGAGCCGCCTACTTC
GluPheArgGlyLeuGlyAspCysLeuValLysIleTyrLysSerAspGlyIleLysGlyLeuThrGlnGlyPheAsnValSerValGlnGlyIleIleIleTyrArgAlaAlaTyrPhe
C G TAAT  AT GCTA  GTGTATAAG  CACTG  A G C TGTT  T    GT G GTGCCC A TT   C TG  A  GTCTG   T
Gln  Asn    Leu  ValTyrLys  ThrLeu  Thr    LeuLeu      Arg   ValPro    Leu      Val     GlyLeu

        .                        600                .                     .              660              .
GGTATCTATGACACTGCAAAGGGAATGCTTCCGGATCCCAAGAACACTCACATCGTCATCGGATGATCGCACAGACTGTC---ACTGCTGTTGCCGGGTTGACTTCCTATCCATTT
GlyIleTyrAspThrAlaLysGlyMetLeuProAspProLysAsnThrHisIleValIleSerTrpMetIleAlaGlnThrVal---ThrAlaValAlaGlyLeuThrSerTyrProPhe
CT G  C TT TTC  CCTG   GTT ACGGGGCTCTAGAGGGGTC T TG TGC CTT CC ATT GGTTGG   ATT  CATG G   TTCCACTG G  G      C G
Leu      SerPhe   ProVal   LeuThrGlyAlaLeuGluGlySerPheValAlaSerPheLeuLeuGlyTrp   Ile   MetGly   SerThrAla      Leu

   .                          720                   .                     .              780                  .
GACACCGTTCGCCGCCGCATGATGATGCAGTCAGGGCGCAAAGGAACTGACATCATGTACACAGGCACGCTTGACTGCTGGCGGAAGATTGCTCGTGATGAAGGAGGCAAAGCTTTTTTC
AspThrValArgArgArgMetMetMetGlnSerGlyArgLysGlyThrAspIleMetTyrThrGlyThrLeuAspCysTrpArgLysIleAlaArgAspGluGlyGlyLysAlaPhePhe
T G  AA AA AA G      ACT G C AG CC---------  A  GAC TG T G    TTAA    T AGA A G C CGT TTC G
   Thr            GlnThr---------   Lys  Asp  Ala      Leu      ValGlnLys   AlaTyrSerLeu

   .                          840
AAGGGTTGCATGGTCCAATGTTCTCAGAGGCATGGGTGGTGCTTTTGTGCTTGTCTTGTATGATGAAATC-------------------AAGAAGTACACATAA
LysGlyTrpSerAsnValLeuArgGlyMetGlyGlyAlaPheValLeuValLeuTyrAspGluIle-------------------LysLysTyrThr***
CTGTG TG  CA AT T   AG C C CA   GG  CA CTCA   C  C GT GCAACTCATAATGTTTGGC  A  A T AG G
CysGlyAla   IlePhe   ValAlaAla   Gly  IleSer      GlnLeuGlnLeuIleMetPheGly      PheLys***
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FIG. 2. **A comparison of the nucleotide and putative amino acid sequences of the hp2F1 clone (*rows 1* and *2*) and the corresponding sequences of the yeast gene for ADP/ATP carrier (*rows 3* and *4*).** For the latter, only the non-identical residues are shown. The *S. cerevisiae* sequence is from Adrian *et al.* (4).
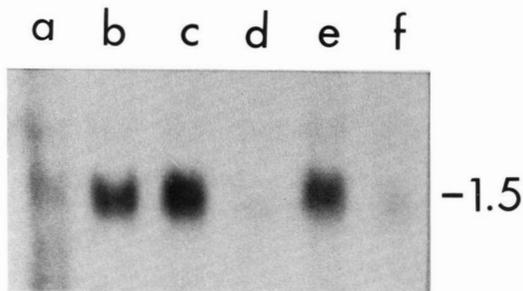

FIG. 3. **RNA levels of ADP/ATP carrier in 3T3 cells treated with different growth factors.** RNA was extracted from quiescent BALB/c/3T3 cells (*lane a*) or from cells treated for 8 h with 10% fetal calf serum (*lane b*), PDGF (2 units/ml, *lane c*), 5% platelet-poor plasma (*lane d*), EGF (20 ng/ml, *lane e*), or insulin (10 µg/ml, *lane f*). RNA blots (20 µg/lane) were hybridized to a radioactive hp2F1 probe as described under "Experimental Procedures."
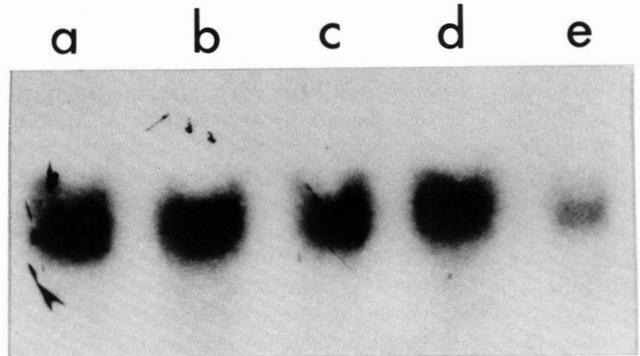

FIG. 5. **RNA levels of ADP/ATP carrier in HL-60 cells after treatment with retinoic acid.** Same conditions as in Fig. 4, except that retinoic acid (10^{-6} M) was used to induce differentiation. *Lanes a, b, c, d,* and *e,* 0, 4, 8, 30, and 70 h after treatment, respectively.
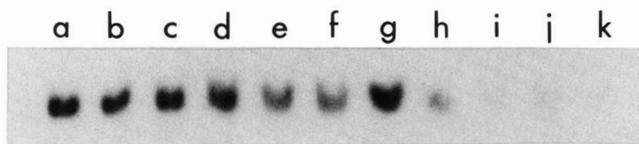

FIG. 4. **RNA levels of ADP/ATP carrier in HL-60 cells after treatment with TPA.** Exponentially growing HL-60 cells were treated with 20 ng/ml TPA. The cells were harvested at the times (after TPA) indicated, and RNA blots were prepared as described (12). The blots were hybridized to nick-translated hp2F1 probe. The lanes are, respectively, *a*, 0; *b*, 15 min; *c*, 30 min; *d*, 1 h; *e*, 2 h; *f*, 4 h; *g*, 8 h; *h*, 24 h; *i*, 48 h; *j*, 72 h; and *k*, 190 h after TPA.

TPA, the signal becomes negligible by 48 h, whereas with retinoic acid, a faint band is still visible at 70 h. In both cases, cell proliferation ceases by 24 h. (Data not shown, but see Fig. 3 in Ferrari *et al.* (27) in which the signal for histone H3 (a marker of cell proliferation) disappears within 8 h of exposure to the differentiating agents. The blots shown in Figs. 4 and 5 are the same used in Figs. 3 and 4 of Ferrari *et al.* (27) except that the probe is different.)

The data thus far available on the regulation of the ATP/ADP carrier mRNA levels by growth factors and other growth-regulated conditions are summarized in Table I.

DISCUSSION

The two major points of this paper are 1) the full coding sequence of a human ADP/ATP carrier; and 2) the observa-

TABLE I

Regulation of ADP/ATP carrier RNA levels under different growth conditions

	Reference
1. Increased by:	
Platelet-derived growth factor	This paper
Epidermal growth factor	This paper
Serum (in several cell types)	3, 23, 24, 31
Phytohemagglutinin in:	
Human peripheral blood cells	13
Purified T lymphocytes	21
Adenovirus infection	22
2. Increased by serum in the presence of cycloheximide	31
3. Not increased by insulin or platelet poor plasma	This paper
4. Decreased by TPA or retinoic acid in HL-60 cells	This paper

tion that the steady-state levels of the ADP/ATP carrier are growth-regulated.

There are in the literature three published sequences of the ADP/ATP carrier: the nucleotide sequence of the cDNA from *N. crassa* (19), the nucleotide sequence of the (intronless) gene from *S. cerevisiae* (4), and the amino acid sequence from beef heart mitochondria (5). The homology of our cDNA clone hp2F1 with the nucleotide sequence of the yeast ADP/ATP carrier is only 47%. However, this protein does not seem to be strictly conserved in the various species. For instance, the amino acid sequence of *Neurospora* ADP/ATP carrier is homologous to the corresponding protein in beef heart mitochondria in only 148 out of 313 residues, and there are gaps in the sequences (19). The homologies between the carriers from yeast and beef heart mitochondria extend to 156 out of 309 amino acids (4).

When the derived amino acid sequence of our cDNA clone hp2F1 is compared to the amino acid sequence of the ADP/ATP carrier from beef heart mitochondria, the homology is 89.6% (266 out of 298 residues). The homology is also strong in the four regions that have been proposed as transmembrane hydrophobic sequences and that are largely conserved from *Neurospora* to beef (4). Here, the homology between hp2F1 and beef heart mitochondria ADP/ATP carrier extends to 84 out of 92 amino acids or 91%. It is reasonable to conclude that the insert of hp2F1 represents a sequence coding for a human ADP/ATP carrier. We insist in saying *a* rather than *the* human carrier, because Southern blot analysis reveals mutliple bands with enzymes that cut the cDNA only once. Preliminary evidence seems to indicate pseudogenes (data not shown) but we cannot rule out a gene family. hp2F1 also has some homology to the brown fat uncoupling protein of rat (28) whose C-terminal end has strong homologies to the ADP/ATP carrier.

p2F1 was originally isolated by differential screening of a cDNA library from Syrian hamster cells (3). The clone was isolated as a cDNA whose cognate RNA was growth-regulated, *i.e.* the steady-state levels of RNA increased when quiescent cells were stimulated by serum. This observation has been repeatedly confirmed in different types of cells from different species (see Table I), and has been extended in this paper. PDGF and EGF, but not insulin or platelet-poor plasma, increase the steady-state RNA levels of ADP/ATP carrier. This is especially interesting since insulin is an anabolic hormone, yet it does not increase the carrier RNA levels that are increased, instead, by mitogens. In addition, at least in HL-60 cells, differentiation, which involved cessation of cell proliferation, results in a decrease in the carrier RNA levels. This is at variance with vimentin, a cytoskeletal protein whose

mRNA levels in HL-60 cells are decreased by retinoic acid, but increased by phorbol esters (27).

Furthermore, RNA levels of the ADP/ATP carrier are increased by serum in the presence of concentrations of cycloheximide that completely suppress protein synthesis (29). This indicates that its induction, like that of c-*myc* (30), c-*fos* (31, 32), p53 (29), and several other growth-regulated genes (29, 33), does not require *de novo* protein synthesis, *i.e.* the products of other growth factor-inducible genes, suggesting that the ADP/ATP carrier gene responds directly to the signal generated by the interaction of the growth factors with their receptors.

What is the meaning of the growth regulation of ADP/ATP carrier RNA? At first glance, it may seem trivial. However, the fact that insulin is ineffective and that the carrier RNA levels are also increased when cellular DNA synthesis is stimulated by adenovirus (22) seem to indicate a non-trivial role of the ADP/ATP carrier in the mitogenic process. Indeed, other proteins involved in the general metabolism of the cell can be growth-regulated. For instance, triose-phosphate isomerase has been considered for some time a major cell proliferation specific isozyme (34). Matrisian *et al.* (35) screened a cDNA library in much the same way as we screened it for the isolation of the p2F1 clone, *i.e.* looking for cDNAs whose cognate RNAs were growth-regulated. Of the five cDNAs they studied, one was actin and the other four coded for glycolytic enzymes: lactate dehydrogenase, glyceralde-hyde-3-phosphate dehydrogenase, enolase, and triose-phosphate isomerase. All were induced by both serum and EGF.

Recently, Glaichenhaus *et al.* (36) found increased levels of RNA for subunit II of mitochondrial cytochrome oxidase in cells transformed by viral or cellular oncogenes, independently of the extent of cell proliferation, and Hertel *et al.* (37) found that ATP is needed for the internalization of the EGF receptor. Interestingly, it has been suggested that an ADP/ATP carrier may be located not only in the mitochondrial membrane, but also in the Golgi membrane, where it would be necessary for the phosphorylation of transported proteins (38). Finally, it should be remembered that the RAS2 gene of *S. cerevisiae* (which has strong homologies to the ras oncogenes) is required for gluconeogenic growth and proper response to nutrient limitations (39). Perhaps the energy producing machinery of the cell plays a larger role in the mitogenic process than hitherto believed. Undoubtedly, the ADP/ATP carrier RNA levels respond with great sensitivity to conditions that affect cell growth.

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