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Elongation factor-1 messenger-RNA levels in cultured cells are high compared to tissue and are not drastically affected further by oncogenic transformation

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ABSTRACT

Copy-DNA clones covering the complete coding sequence of human Elongation Factor-1γ mRNA have been isolated and characterized. The expression of Elongation Factor-1 in a variety of cell lines and a number of tissues shows a large increase in Elongation Factor-1 mRNA going from tissue to cultured cells (20-fold). Messenger-RNA levels for Elongation Factor-1α, -1β and -1γ increase in parallel suggesting coordinate regulation of the expression of these genes. Oncogenic transformation in vitro does not strongly affect Elongation Factor-1 mRNA levels.

INTRODUCTION

The Elongation Factor-1 (EF-1) complex is built up of four subunits: α, β, γ and δ. EF-1α is the protein carrying aminoacyl-tRNA's to 80S ribosomes under hydrolysis of GTP. The GDP/GTP exchange activity of this complex is catalyzed by the β- and δ-subunit (1). The EF-1γ protein has a molecular mass of about 50 kD and a low solubility in non-denaturing aqueous buffers, in which it forms a strong complex with EF-1β in the ratio of 1:1. Other features of EF-1γ are that it can easily associate with tubulin (2) and serve as a major substrate for MPF, in vivo as well as in vitro (3,4). Interestingly the N-terminal region of EF-1γ resembles somewhat the N-terminal region of certain amino-acyl-tRNA synthetases (5) and one of the eucaryotic synthetases, valyl-tRNA-synthetase, is described to form a complex with a heavy molecular weight form of EF-1 (6,7). The high molecular weight form of the synthetase reportedly dissociates when its EF-1γ related N-terminus is removed by protease (8). Another hint for a new role of EF-1γ, namely the control of translation originates from Lew et al. who show that EF-1γ is highly overexpressed in certain colon and pancreas carcinomas (9). Studies in which differential screening of normal and cancerous tissues was used have shown that not only EF-1γ but also EF-1α is highly expressed in certain tumors (10). These findings prompted us to compare EF-1 mRNA expression in: 1. normal tissue, 2. untransformed vs. transformed cells in culture and 3. resting vs. actively dividing cells in culture. To this end we used probes for human EF-1α (11), EF-1β (12) and EF-1γ. For the latter purpose a full-length cDNA clone of human EF-1γ was isolated and sequenced.

MATERIALS AND METHODS

Isolation of EF-1γ cDNA clone

In order to amplify EF-1γ cDNA by the Polymerase Chain Reaction (PCR) two short peptide sequences conserved between Artemia and Xenopus laevis EF-1γ were used to synthesize corresponding degenerate oligonucleotides. Copy-DNA was made from total RNA isolated from human culture fibroblasts using the 3′ oligonucleotide. PCR amplification was performed by 30 cycles of 1′ 94°C, 1′ 46°C, 3′ 74°C using both oligonucleotides. The product was phosphorylated and ligated into pUC120. Sequencing (Sanger et al.) and cloning were performed in this vector. A human lambda gt10 cDNA library was plated on *E.coli* C600hfl (10^5 plaques) and filter hybridized on nitrocellulose as described in (13) with the above mentioned PCR-fragment. Overlapping clones containing the complete coding sequence were isolated.

Cells and tissues

Adenovirus transformed baby rat kidney (BRK) cells were obtained by transfection of primary cultures of kidneys of 6- to 7-day old WAG/RIJ rats (14). Clonal cell lines RICC16 and XHOC5 were isolated from cultures transformed with constructs containing the transforming E1 region of Adenovirus 5 (Ad5) and Adenovirus 12 (Ad12) respectively. Adenovirus transformed normal rat kidney (NRK) were derived from the NRK clone 49F. Transformed NRK cells NR24 and NX12 were isolated after transfection of NRK with pSV-neo constructs containing the E1 region of Ad5 or Ad12 respectivily. Adenovirus transformed cell lines were kindly provided by Dr. A.J.van der Eb. Muscle, liver and adipose tissues were taken from healthy adult rats, immidiately frozen in liquid nitrogen and stored at −80°C until needed.

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  1 MAAGTLYTYPENWRAFKALIAAQYSGAQVRVLSAPPHFHFGQTNRTPEFL  50
  1 MAGGTLYTYPDNWRAYKPLIAAQYSGFPIKVASSAPEFQFGVTNKTPEFL  50
  1 MVAGKLYTYPENFRAFKALIAAQYSGAKLEIAKS...FVFGETNKSDAFL  47

 51 RKFPAGKVPAFEGDDGFCVFESNAIAYYVSNEELRGSTPEAAAQVVQWVS 100
 51 KKFPLGKVPAFEGKDGFCLFESSAIAHYVGNDELRGTTRLHQAQVIQWVS 100
 48 KSFPLGKVPAFESADGHCIAESNAIAYYVANETLRGSSDLEKAQIIQWMT  97

101 FADSDIVPPASTWVFPTLGIMHHNKQATENAKEEVRRILGLLDAYLKTRT 150
101 FSDSHIVPPASAWVFPTLGIMQYNKQATEQAKEGIKTVLGVLDSHLQTRT 150
 98 FADTEILPASCTWVFPVLGIMQFNKQATARAKEDIDKALQALDDHLLTRT 147

151 FLVGERVTLADITVVCTLLWLYKQVLEPSFRQAFPNTNRWFLTCINQPQF 200
151 FLVGERITLADITVTCSLLWLYKQVLEPSFRQPFGNVTRWFVTCVNQPEF 200
148 YLVGERITLADIVVTCTLLHLYQHVLDEAFRKSYVNTNRWFITLINQKQV 197

201 RAVLGEVKLCEKMAQFDAKKFAETQPKKDTPRKEKGSREEKQ.KPQAERK 249
201 RAVLGEVKLCDKMAQFDAKKFAEMQPKKETPKKEKPAKEPK..KEKEEKK 248
198 KAVIGDFKLCEKAGEFDPKKYAEFQAAIGSGEKKKTEKAPKAVKAKPEKK 247

250 EEKKAAAPAPEEEMDECEQALAAEPKAKDPFAHLPKSTFVLDEFKRKYSN 299
249 KAAPTPAPAPEDDLDESEKALAAEPKSKDPYAHLPKSSFIMDEFKRKYSN 298
248 E...VPKKEQEEPADAAEEALAAEPKSKDPFDEMPKGTFNMDDFKRFYSN 294

300 EDTLSVALPYFWEHFDKDGWSLWYSEYRFPEELTQTFMSCNLITGMFQRL 349
299 EDTLTVALPYFWEHFDKEGWSIWYAEYKFPEELTQAFMSCNLITGMFQRL 348
295 NEE.TKSIPYFWEKFDKENYSIWYSEYKYQDELAKVYMSCNLITGMFQRI 343

350 DKLRKNAFASVILFGTNNSSSISGVWVFRGQELAFPLSPDWQVDYESYTW 399
349 DKLRKTGFASVILFGTNNNSSISGVWVFRGQDLAFTLSEDWQIDYESYNW 398
344 EKMRKQAFASVCVFGEDNDSSISGIWVWRGQDLAFKLSPDWQIDYESYDW 393

400 RKLDPGSEETQTLVREYFSWEGAFQHVGKAFNQGKIFK 437
399 RKLDSGSEECKTLVKEYFAWEGEFKNVGKPFNQGKIFK 436
394 KKLDPDAQETKDLVTQYFTWTGT.DKQGRKFNQGKIFK 430
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Figure 1. Amino acid sequence comparison of human EF-1γ (upper), Xenopus laevis EF-1γ (middle) and Artemia EF-1γ (lower). Homology assessment: | denotes identical aminoacids, : denotes very similar aminoacids, . denotes similar aminoacids.

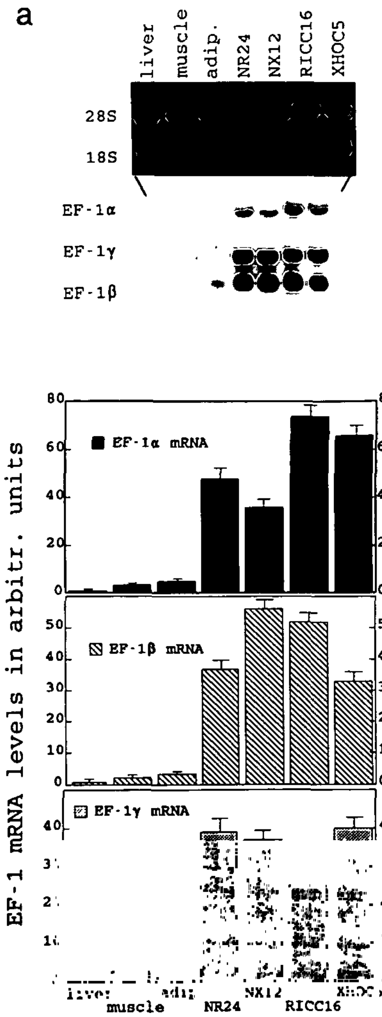

Figure 2. Comparison between EF-1 expression levels in rat tissues and rat transformed culture cells. Twenty-five μg of total RNA isolated as described in materials and methods was subjected to electrophoresis through 1% agarose and after northern blotting hybridized with EF-1 cDNA probes. Liver, muscle and adip. (adipose) are rat tissue RNA's, NR24 and NX12 are adeno transformed NRK cells, RICC16 and XHOC5 are adeno transformed BRK cells a. 25 μg of total RNA quantified by absorbance (260 nm) measurement was electrophoresed through 1% agarose and stained by ethidiumbromide. Sample autoradiographs of EF-1α, EF-1β and EF-1γ probes hybridized to Northern blots of 25 μg of total RNA are shown. b. Band density evaluations of autoradiographs. Standard deviation from the mean of three experiments is shown. Values are in arbitrary units.

Cell culturing and RNA isolation

The cells were cultured in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and harvested prior to confluency. Total RNA of human primary fibroblasts, other cultured cells and rat tissues was isolated using the Nonidet-NP40 lysis procedure with phenol/SDS extraction (15) or the LiCl/urea procedure (16).

Northern blotting, hybridization and quantification

RNA was quantified by measuring the absorbance at 260 nm and its concentration and integrity was confirmed by electrophoresis and ethidiumbromide staining. Prior to electrophoresis the required amount of RNA was dissolved in sample buffer (50% formamide, 2.2M formaldehyde, 20mM morpholinopropanesulphonic acid (MOPS) buffer pH=7.0) and incubated at 57°C for 10 minutes. RNA electrophoresis was done in 1% agarose gels in MOPS-buffer (20mM MOPS, 5mM sodium-acetate, 1mM EDTA, pH=7.0) to which 2.2M formaldehyde was added. Southern and Northern blotting on nitrocellulose were performed according to standard procedures as described in (13). EF-1α, -1β and -1γ probes were prepared by nick translation of corresponding cDNA. Overnight hybridization was carried out (in 50% formamide, 5×SSPE, 1% SDS, 5×Denhardt's and 200 μg of salmonsperm DNA) at 42°C (13). After hybridization blots were washed (3×10 min.) with 3×SSC, 0.5%SDS at 52°C and 1×10 min. with 1×SSC, 0.5%SDS at 60°C. Autoradiographs of Northern blots on Kodak XAR film were quantified by scanning with a Joyce-Loebl densitometer. All results were calculated from duplicate and triplicate experiments or discarded when RNA samples showed signs of degradation.

RESULTS

Elongation factor-1γ sequence

From the isolated EF-1γ cDNA's an open reading frame coding for a protein 437 amino acids in length with a calculated molecular mass of 50 kD can be derived (see fig.1). The human EF-1γ protein has a homology of 60% compared with the Artemia

EF-1γ sequence (17) and 85% compared with the Xenopus laevis EF-1γ sequence (18). The overall homology is of the same order as the homology observed between EF-1β proteins of different species (12), although the conservation of the latter is mainly confined to the C-terminal domain, while in EF-1γ the homology is spread more evenly over the sequence. The MPF-phosphorylation site which is phosphorylated in Xenopus EF-1γ (position 230 (19)) is conserved at the same position in the human protein sequence. Hybridization of Southern blots of human chromosomal DNA digested with several restriction enzymes suggested the presence of several (pseudo-) genes by the number of bands (results not shown). Since several EF-1γ cDNA clones were sequenced and no polymorphisms were seen it can be concluded that only one or a few genes of identical coding sequence are responsible for the major fraction of the transcripts. On Northern blot analysis of total human and rat RNA only a single hybridizing mRNA species with a length of 1500 nucleotides was seen.

EF-1 expression levels

Total RNA from different tissue types and culture cells was hybridized with ^{32}P-labeled human EF-1γ, EF-1β and EF-1α cDNA probes labeled by nick-translation. From figure 2 it can be concluded that the expression level is quite different when normal rat tissues are compared to cultured rat kidney cells transformed by adenovirus. On average EF-1γ, EF-1β and EF-1α mRNA levels are 20-fold higher in transformed culture cells than in normal tissue cells. To investigate whether the elevated EF-1 mRNA levels in the adenovirus transformed rat cells were due to the transformation process a comparison was made between the parental cells and derived transformed cell lines. Cell panels were: primary human foreskin fibroblasts (VH10) and an SV40 immortalized cell line derived from VH10 (VH10sv) (20); normal rat kidney cells (NRK), baby rat kidney cells (BRK) and their adenovirus 5 transformed counterparts NR24, XHOC5 and the adenovirus 12 transformed counterparts NR24 and RICC16 respectively. As can be seen from fig. 3 the overall expression levels of EF-1 mRNA's do not exhibit the pronounced differences in expression comparable to the differences observed between tissues and cultured cells, as shown in fig.2. Transformation of BRK and NRK cells with either the highly oncogenic adenovirus 12 (NR24 and RICC16) or the less oncogenic adenovirus 5 (NX12 and XHOC5) did not have major effects on EF-1 mRNA expression. However, there are minor variations in the amount of EF-1α, EF-1β or EF-1γ mRNA depending on the type or treatment of the cultured cells. Especially, when a comparison is made between the parental NRK and BRK cells versus their adenovirus transformed counterparts it is seen (fig.3) that the EF-1γ:EF-1β ratios are significantly increased. It should be noted that the labeled cDNA probes used in the northern blot assays of fig.2 and fig.3 had different specific activities, resulting in apparent discrepancies in the EF-1 mRNA ratios of the two sets of experiments. In other words only changes in ratios in one Northern blot assay are believed to be significant. We also examined whether actively dividing cells express different levels of EF-1 mRNA's as compared with non-dividing cells. For that, primary human fibroblasts were grown to confluency and kept in that state for 72 hrs. In addition the same cells were grown to half confluency. The EF-1 mRNA levels measured in both samples showed no significant differences in expression levels (results not shown).

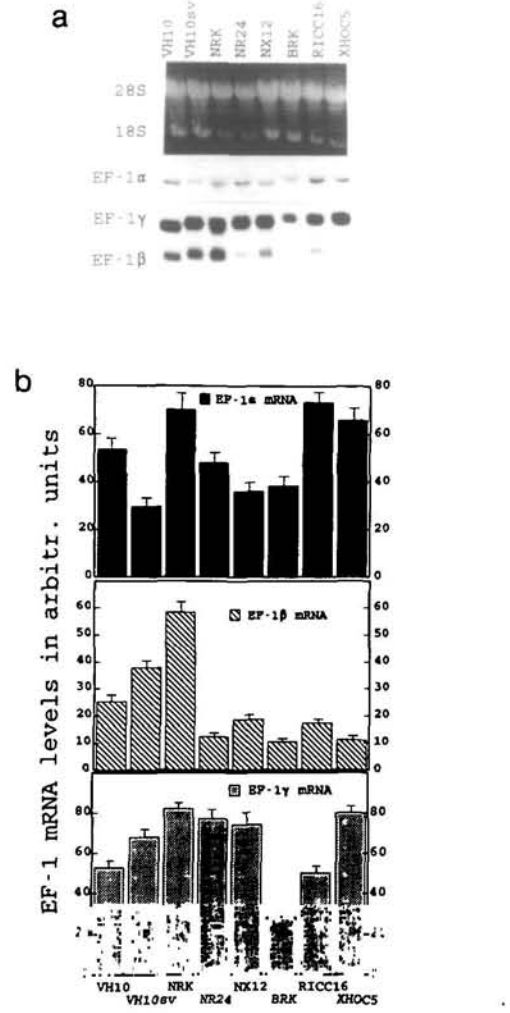

Figure 3. Comparison of EF-1 expression levels in untransformed and transformed VH10, NRK and BRK cells. Ten μg of total RNA isolated as described in materials and methods was subjected to electrophoresis through 1% agarose and after Northern blotting hybridized with EF-1 cDNA probes. VH10 is a human primary fibroblast. VH10sv is VH10 immortalized by SV40 virus. NRK is normal rat kidney cell. BRK is baby rat kidney. NR24 and NX12 are adeno transformed NRK. RICC16 and XHOC5 are adeno transformed BRK. a. 10 μg of total RNA quantified by absorbance (260 nm) measurement was electrophoresed through 1% agarose and stained by ethidiumbromide. Sample autoradiographs of EF-1α, EF-1β and EF-1γ probes hybridized to Northern blots of 10 μg of total RNA are shown. b. Band density evaluations of autoradiographs. Standard deviations from mean values over three experiments are indicated. Values are in arbitrary units.

DISCUSSION

As seen from fig.2 levels of mRNA for EF-1α, EF-1β and EF-1γ increase strongly on comparison of tissues with cultured cells. This increase occurs coordinately rather than being confined to only one of these messengers. This suggests a common mechanism switching the expression of these three elongation factor genes from a low expression to a high expression state. The effect of oncogenic transformation on the level of mRNA for each of the three subunits of EF-1 is fairly complex and seems to depend on the type of virus (adeno5 or adeno12) and the particular EF-1 subunit concerned (fig.3). At this stage we only

remark that in cell culture the relative changes in individual mRNA levels before and after transformation (fig.3) are considerably less than those seen on comparing tissues with cultured cells (fig.2). The reason for the opposite changes in EF-1γ:EF-1β mRNA ratio after transformation of NRK and BRK is unknown and these effects of 'fine tuning' require further study and substantiation (fig.3). The data of fig.2 and fig.3 should be compared to those in two recent reports describing high levels of EF-1α and EF-1γ mRNA in naturally occurring tumors. One report has described an overexpression of EF-1γ mRNA in pancreatic and colon tumours relative to normal adjacent tissue (9). In a related study a high level of EF-1α was found on comparison of colon, breast, lung and gastric tumours relative to normal tissue (10). Our results show that taking cells out of their natural habitat is sufficient to yield high EF-1α, EF-1β and EF-1γ mRNA levels (fig.2) without necessarily any further increase in mRNA level for each of the subunits of EF-1 on transformation (fig.3). Therefore it looks that oncogenic transformation in vivo takes place in two stages: first a rise (or derepression) of protein synthetic factor mRNA as a result of a loss of contact inhibition, followed by the actual transformation process. In cultured cells the first step occurs spontaneously on taking cells out of the tissue and exposing them to growth stimulating factors, thereby obviating the need for a further increase of EF-1 mRNA on infection of such cells with oncogenic viruses. (A recent report by Tatsuka et al. [Nature **359**, 333–336 (1992)] indicates that a high expression of EF-1α results in an increased susceptibility of cells to undergo oncogenic transformation.) Surprisingly the amount of EF-1 mRNA is not influenced by a temporary arrest of cell devision as observed when mRNA levels of actively dividing primary fibroblasts were compared to those found in confluent cultures. A similar invariance of these expression levels has been observed for EF-1γ by Lew et al. (9) who showed that cell-lines sensitive to growth inhibition by retinoic acid did not exhibit a decreased EF-1γ expression after treatment with this agent. It is remarkable that the increase of the level of EF-1γ mRNA, found on comparison of tissue to cultured cells, being transformed or not (our results), is of the same order as when going from healthy tissue to tumour tissue (21). In both comparisons the increase is up to 20-fold. Finally it would be desirable to determine whether the protein levels of each of the four subunits of EF-1 under conditions as studied here correlate with the EF-1 mRNA levels and are followed by concomitant increases or decreases in protein synthetic activity.

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