Hum Genet (1987) 75:339–344

Human Genetics

Human elongation factor 1α: a polymorphic and conserved multigene family with multiple chromosomal localizations

Ghislain Opdenakker[1]*, Yofre Cabeza- Arvelaiz[1], Pierre Fiten[1], Roger Dijkmans[1], Jo Van Damme[1], Guido Volckaert[1], Alfons Billiau[1], August Van Elsen[2], Bernadette Van der Schueren[3], Herman Van den Berghe[3], and Jean-Jacques Cassiman[3]

[1]Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium
[2]Department of Genetics, University of Antwerp, B-2610 Wilrijk, Belgium
[3]Center for Human Genetics, University of Leuven, B-3000 Leuven, Belgium

Summary. One of the genes activated in human melanoma cells by the tumor-promoting phorbol ester is that of the elongation factor 1α. A cDNA clone containing the complete 3′-end untranslated region and the nucleotide sequences coding for 227 carboxyterminal amino acids was isolated. Computer-assisted comparison with known sequences of elongation factors from other species revealed homologies up to 73% and 63% on amino acid and nucleotide sequences, respectively. Northern blot analysis of mRNA from unstimulated and phorbol ester-treated cells showed a 3- to 5-fold increase in cytoplasmic elongation factor 1α mRNA after phorbol ester induction. When compared with the phorbol ester-inducible single-copy gene transcripts coding for the tissue-type plasminogen activator, the cellular mRNA content of elongation factor 1α is 30 times higher. By Southern blot analysis experiments on human genomic DNA, a multi-gene family was found showing polymorphisms in restriction endonuclease fragment lengths (RFLP). Several polymorphisms were studied more extensively in the population on more than 100 DNA samples from normal individuals and in three-generation families. In situ hybridization of the cDNA probe to normal human metaphase chromosomes showed multiple chromosomal localizations of the elongation factor gene(s), with peak hybridization on the chromosomes 1, 2, 4, 5, 6, 7, and 15. The estimate of the gene copy number in humans is more than ten copies per (haploid) genome.

Introduction

It has previously been shown by Ashino-Fuse et al. (1984) and Opdenakker et al. (1983, 1985, 1986) that the human melanoma cell line (Bowes) can be stimulated by tumor-promoting phorbol esters to produce increased amounts of poly(A)-rich mRNA in general and of tissue-type plasminogen activator specifically. However, little is known about the precise mechanism of action of the phorboid tumor promoters. We aimed at gathering information on the sequence of events and the cellular reprogramming functions leading from phorbol ester-receptor activation to the enhanced synthesis and secretion of proteins such as tissue-type plasminogen activator. A crucial step towards the understanding of this reprogramming is the cloning and identification of cDNA sequences corresponding to specific tumor promoter-regulated mRNAs. The cloning of one of these phorbol ester-regulated genes, i.e., the tissue-type plasminogen activator gene, has recently been described by several groups. Yang-Feng et al. (1986) have furthermore mapped the PLAT locus to human chromosome 8p12 by in situ hybridization.

We have now isolated by positive differential screening a cDNA clone corresponding to another phorbol ester-induced mRNA. Nucleotide sequence analysis and homology search showed that a gene for one of the human elongation factors (EF-1α) had been characterized. Furthermore, it was suggested by Southern blot analysis that, in contrast with the tissue-type plasminogen activator, human EF-1α is managed by a polymorphic and conserved multigene family with multiple chromosomal localizations.

Materials and methods

Cells and treatment of the cells

The melanoma cell line Bowes was grown in modified minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum, nonessential amino acids, and 1.35 mg/ml sodium bicarbonate. Extensive kinetic studies on the induction by phorbol esters of tissue-type plasminogen activator protein (Ashino-Fuse et al. 1984) and mRNA (Opdenakker et al. 1983, 1985) showed that optimal mRNA induction was obtained by treatment of the cells for 6 h with 100 ng/ml 12-O-tetradecanoyl-phorbol 13-acetate.

Dot-blot and Northern blot analysis

Messenger RNA was extracted from phorbol ester-treated and untreated human melanoma cells (Opdenakker et al. 1982). Dilution series (1.1–90 μg) of total cytoplasmic RNA were subjected to dot-blot hybridization experiments on nitrocellulose membranes and hybridized to labeled cDNA probes of EF and, as a control, that of tissue-type plasminogen activator.

For Northern blot analysis equal amounts (10 μg) of poly(A)-rich RNA were first separated in agarose gels and further blotted onto nitrocellulose membranes prior to hybrid-

* *Present address and address for offprint requests:* Department of Molecular Biophysics and Biochemistry, Kline Biology Tower, Yale University, 260 Whitney Avenue, New Haven, CT 06511, USA

ization. Prehybridization and hybridization conditions are essentially as described by Thomas (1980) except that dextran sulphate was lowered to 5%.

The human tissue-type plasminogen activator cDNAs used in the present study were $pABX_2$ (Harris et al. 1986) and pGVHTPA. For Southern blot analysis we have used either the whole recombinant plasmid pGVHTPA, a derivative from pt-PA 21 (Harris et al. 1986), or its inserted *BglII* fragment. For dot-blot and Northern blot analysis of t-PA mRNA we used either this *BglIII* fragment or the 3′ end *PstI* cDNA fragment from $pABX_2$. Human EF cDNA probe was always the 3′ end fragment as indicated in the manuscript.

Molecular cloning and nucleotide sequence analysis

Poly(A^+)RNA from phorbol ester-stimulated cells was isolated by affinity chromatography on oligo(dT)-cellulose by the method of Aviv and Leder (1972). After precipitation in ethanol, the RNA was size-fractionated on linear sucrose gradients. cDNA synthesis was done with the 20S fraction by oligo(dT)-priming using the method of Gubler and Hofmann (1983). After polyethyleneglycol precipitation-mediated fractionation (Opdenakker et al. 1984), cDNAs longer than 1000 base pairs (bps) were cloned in *PstI*-cleaved pBR322 by d(G)-d(C) homopolymer tailing. cDNA libraries, subcultured in microtiter plates were inoculated on hybridization membranes, and the ordered colonies sequentially screened with radiolabeled cDNA probes prepared from mRNA from unstimulated and phorbol ester-stimulated melanoma cells. With these probes, about 20 phorbol ester-induced sequences could be identified in a library containing about 10000 recombinants.

The plasmid pAB 48K-5 was cleaved with *PstI,* and the inserted fragment isolated after separation through a low-melting-point agarose gel. Restriction fragments of the cDNA were subcloned in the dual-sequencing vector pGV451, a derivative of pCSV31 (Volckaert et al. 1984). Randomly chosen subclones and subsequently subclones identified by colony hybridization with fragments deduced from these sequences were sequenced by modification of the chemical degradation technique of Maxam and Gilbert (1980).

DNA preparation

A micromethod for the preparation of DNA of high relative molecular mass was adapted from Scott et al. (1984). Briefly, $0.7–1.0 \times 10^7$ leukocytes were isolated from 5–10 ml peripheral blood by gradient centrifugation in ficoll/hypaque (Lymphoprep, Nyegaard). Cells were transferred to 1.5-ml Eppendorf vials, washed twice with phosphate-buffered saline, and resuspended in 0.5 ml 0.3 *M* Tris-HCl buffer (pH 8.0) containing 0.1 *M* NaCl, 0.2 *M* sucrose, and 0.05 *M* EDTA. The cells were lysed by the addition of 125 µl lysis buffer (0.5 *M* Tris-HCl pH 9.2, 0.25 *M* EDTA, 2.5% sodium dodecyl sulphate) and incubated for 15 min at 60°C; proteins were then digested for 16 h at 37°C by the addition of 62.5 µl proteinase K at 1 mg/ml in water. After the addition of 70 µl 5 *M* NaCl the lysate was subsequently extracted twice with water-saturated phenol and once with chloroform/isoamyl alcohol (24/1) and ether. DNA was precipitated with ethanol for 20 min at −20°C, washed once with 70% ethanol, dissolved in 100 µl 10 m*M* Tris-HCl (pH 7.4), and stored at 4°C. DNA concentrations and purity were determined spectrophotometrically (A_{260}/A_{280}) on a dilution series of one-tenth of the sample.

Southern blot analysis

Purified genomic DNA samples (10 µg/lane) were digested according to conditions recommended by manufacturers with either *Bam*HI, *Eco*RI, or *Hind*III (Boehringer) at 37°C overnight and electrophoresed in a 0.8% agarose gel in 40 m*M* Tris-acetate pH 7.4/1 m*M* EDTA. Phage λ DNA preparations, digested with *Hind*III or *Eco*RI/*Hind*III (Boehringer), were included as molecular size markers. Gels were run at 40 V for 18–20 h and then stained with ethidium-bromide and photographed. The gels were denatured with 0.5 *M* NaOH/1.5 *M* NaCl for 45 min and neutralized with 1 *M* Tris-HCl pH 7.2, 1.5 *M* NaCl, and 0.001 *M* Na_2EDTA, and the DNA fragments were transferred to Hybond^N membranes (Amersham International) using the method first described by Southern (1975). After transfer, filters were rinsed in 2 × standard saline citrate (SSC 1 × = 150 m*M* NaCl/15 m*M* sodium citrate pH 7.0) and then irradiated with a standard ultraviolet transilluminator for 5 min.

Nucleic acid hybridization

Filters were prehybridized for 16 h at 45°C in 50% Formamide, 5 × SSC: 10 × Denhardt's solution (Denhardt's solution: 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 50 m*M* Na-phosphate pH 6.8, 10% dextran sulphate, and 100 µg/ml denatured sonicated herring sperm DNA. Filters were hybridized overnight at 45°C in 10 ml of the same solution with addition of $1–2 \times 10^{-6}$ cpm/ml ^{32}P probe labeled by the method of Feinberg and Vogelstein (1983) to a specific activity of 1×10^9 dpm/µg. Filters were washed at 45°C once with 2 × SSC for 30 min, twice with 2 × SSC, 0.1% SDS for 30 min, and finally once with 0.1 × SSC, 0.1% SDS for 30 min. Filters were then exposed to preflashed Agfa Gevaert Curix RP II film with two intensifying screens at −70°C for 3–7 days.

In situ hybridization

Chromosomal in situ hybridization was performed according to the method of Harper and Saunders (1981) on mitotic chromosome preparations from human peripheral blood lymphocyte cultures. The ^3H-labeled probe with specific activity of 6×10^7 cpm/µg DNA was used at a concentration of 200 ng/ml. The slides, coated with nuclear track emulsion NTB2 (Kodak), were exposed for 4–6 days at 4°C. Secondary to the development, a G-banding was performed.

Results

Cloning of phorbol ester-induced sequences

In order to clone ds-cDNAs of phorbol ester-induced transcripts, poly(A)-rich RNA was prepared from phorbol ester-treated cells, and ds-cDNA was synthesized. After polyethyleneglycol-facilitated size-fractionation and tailing with terminal deoxynucleotidyltransferase in the presence of dCTP, the cDNA was reannealed into *Pst*I-cleaved and dG-tailed pBR322. About 10000 transformed *E. coli* colonies were obtained and subcultured in 96-well microtiter plates. Replicas on appropriate membrane supports were made to allow for differential screening with radioactive cDNA probes. A single

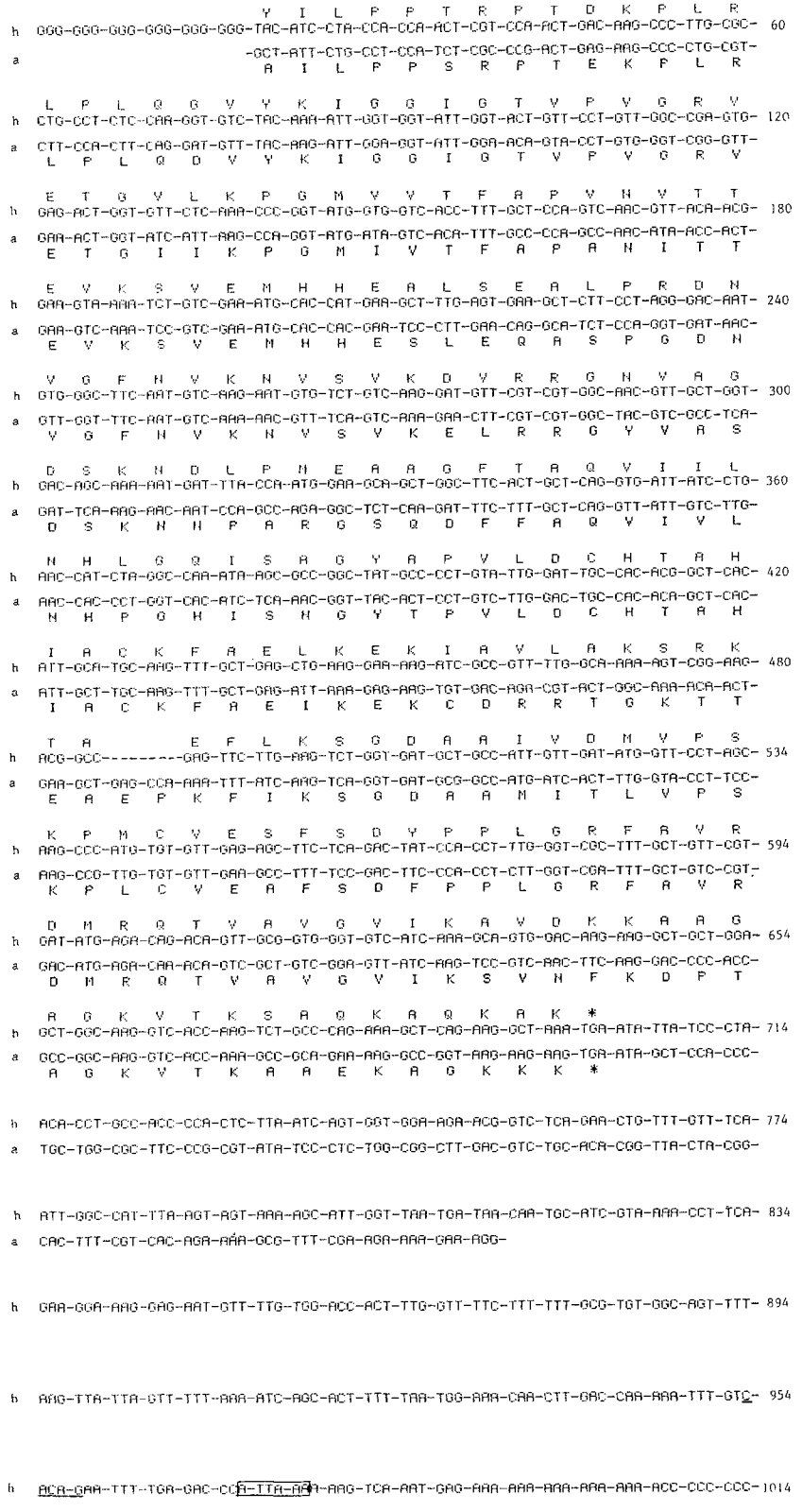

Fig. 1. Comparison of the human *(h)* pAB 48K-5 nucleotide and amino acid sequences with those of the *Artemia (a)* EF 1α. The human cDNA sequence entails a 3′ poly(A)$_{16}$-sequence, preceded by a nonclassical polyadenylation signal ATTAAA *(box)*. At position 954 the signal CACAG is also found *(underlined)*

membrane was then subsequently hybridized with probes derived from unstimulated and from stimulated cells. By this technique we isolated 20 different but possibly related plasmids which hybridized to a greater extent with cDNA derived from phorbol-ester-treated but not from untreated cells.

The inserted cDNA from one plasmid, pAB 48K-5, was subcloned and sequenced by the method of Maxam and Gilbert until sufficiently overlapping sequences could be aligned. Comparison of the nucleotide sequence to those recorded in the EMBL DNA data bank (release of October 1985) revealed homology with EF-1α of *Artemia* (Fig.1). When allowing for six-nucleotide deletion in the human (or insertion in the *Artemia*) sequence at nucleotide number 487, overall homologies of 63% and 73% were found on nucleotide and on

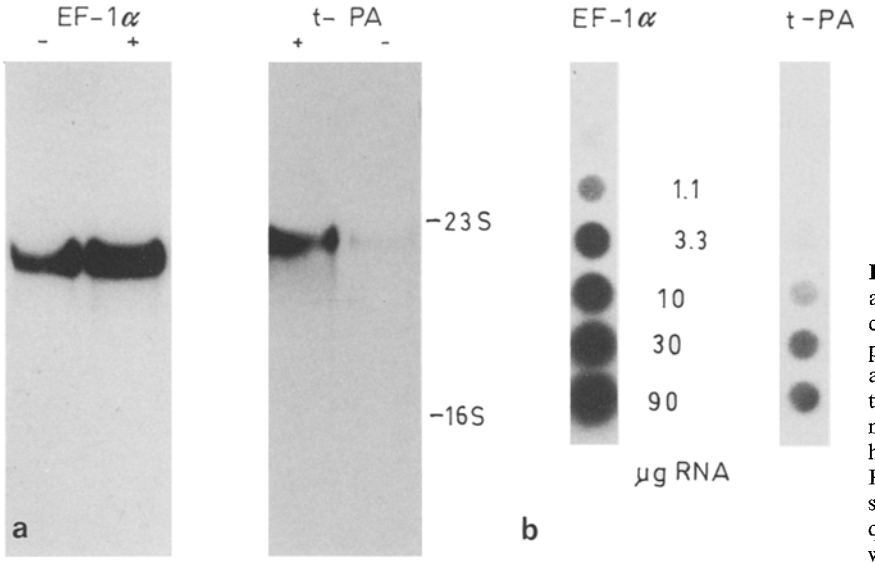

Fig. 2a, b. Northern blot (**a**) and dot-blot (**b**) analysis of EF-1α and t-PA mRNA. Confluent cultures of melanoma cells were treated with phorbol ester (+) or left untreated (−). Equal amounts of poly(A^+)RNA (Northern blot) or of total cytoplasmic RNA (dot blot) were applied to nitrocellulose membranes and subsequently hybridized to t-PA and EF-1α cDNA probes. Ribosomal RNA from *E. coli* was run as size standardization in Northern blot analysis. For quantitative comparison in dot blot assays, probes with identical specific activities were used

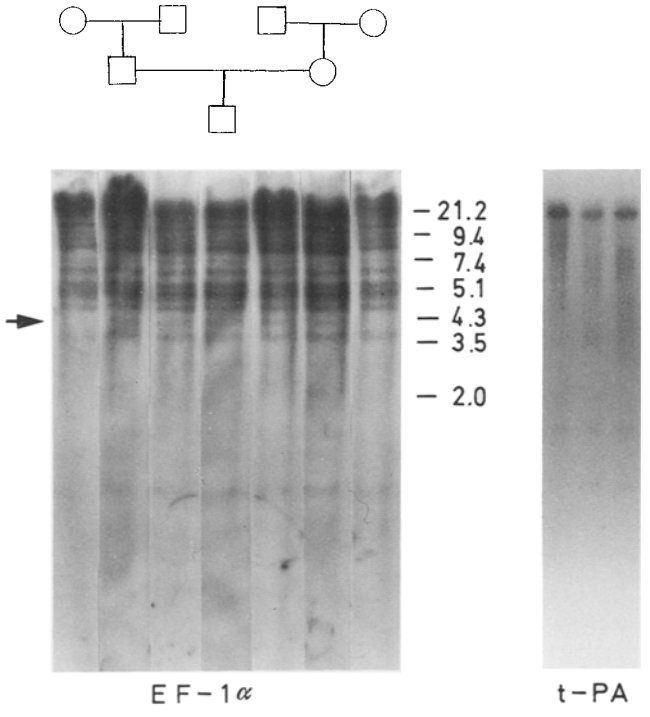

Fig. 3. Southern blot analysis of EF-1α DNA. Preparations of 10 μg genomic DNA of 10 individuals were digested with *Bam*HI, separated on agarose gels, and blotted onto nylon membranes. Subsequently, the samples from 7 individuals *(left)* of one family were hybridized to the EF-1α cDNA probe, and an RFLP at 4.1 kb was detected *(arrow)*. On *top*, the pedigree and at the *right*, a molecular-size standardization in kb is indicated. For comparison, genomic DNA preparations from 3 unrelated individuals *(right)* were hybridized to a 1200 3′-end cDNA, coding for tissue type plasminogen activator (t-PA)

amino acid sequences, respectively. The cDNA clone also encompasses the poly(A)-tail, preceded by a nonclassical polyadenylation signal ATTAAA at position 972 (Wickens and Stephenson 1984). At position 954 the sequence CACAG, which is known to interact with the small nuclear ribonucleoprotein U4 (Berget 1984), is found.

Table 1. Polymorphisms detected with the 1.0 kbp elongation factor 1α cDNA probe. All band sizes are in kilobases. *N*, size of the Caucasian population analysed

Enzyme	Constant bands	Allele	Length	Frequency	*N*
*Bam*HI	18	A1	4.1	0.74	53
	Range: 20–0.6 kb	A2	Absent	0.26	
	(varying intensity)	B1	0.5	0.04	53
		B2	Absent	0.96	
*Eco*RI	24	C1	8	0.13	25
	Range: 22–1.3 kb	C2	Absent	0.87	
	(varying intensity)	D1	1.3	0.04	25
		D2	Absent	0.96	
*Hind*III	24	E1	8.2	0.13	23
	Range: 22–0.9 kb	E2	Absent	0.87	

Characterization of cytoplasmic RNA by hybridization to pAB 48K-5 cDNA

Messenger RNA from untreated and phorbol ester-treated cells was purified by affinity chromatography. Equal amounts of each were separated by electrophoresis on agarose gels, blotted onto nitrocellulose membranes, and hybridized to human EF-1α and t-PA probes. Figure 2a shows that both mRNAs were induced by phorbol esters. However, whereas t-PA mRNA was enhanced up to 20-fold, those of EF-1α were increased only 2- to 5-fold. From the relative mobilities in agarose gels, EF-1α mRNA and t-PA mRNA could be estimated to have about the same molecular size. Figure 2b shows a typical dot-blot assay, allowing us to estimate relative amounts of EF-1α and t-PA mRNA in the cells. It appears from several independent experiments that EF-1α mRNA is 30 times more abundant than t-PA mRNA.

Analysis of genomic DNA hybridizable to pAB 48K-5 cDNA

Figure 3 shows the result of a Southern-blot hybridization analysis with the EF-1α-cDNA probe on DNA from seven members of three consecutive generations of a single family. For comparison, a similar analysis with a probe for the t-PA gene, a typical single-copy gene, is included. In contrast

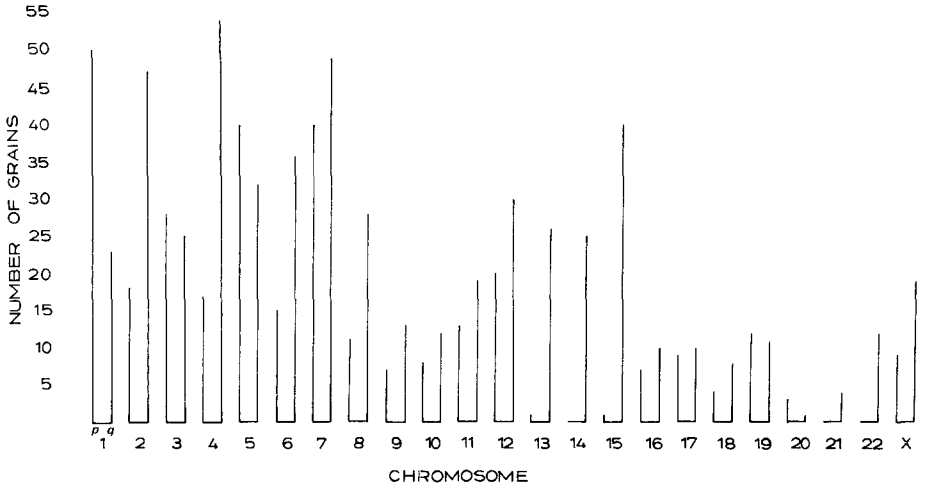

Fig. 4. In situ hybridization of EF-1α cDNA to human metaphase chromosomes. Diagram showing grain distribution in 426 metaphases. The *abscissa* represents the two arms of the chromosomes; the *ordinate* gives the number of grains

with the low number of bands detected with the t-PA probe, the pAB 48K-5 cDNA probe hybridized to 19 fragments of *Bam*HI-digested DNA. Eighteen of these occurred invariably in DNA of all individuals examined; a 4.1-kb fragment occurred in five of seven individuals. A more accurate estimate of the occurrence of this and other polymorphisms of the EF-1α gene was obtained by restriction analysis using *Bam*HI, *Eco*RI, and *Hind*III on DNA of randomly chosen individuals (Table 1). The frequency of occurrence of the polymorphic bands differed widely, the *Bam*HI 4.1-kb band occurring with the highest frequency. With respect to the less frequently occurring polymorphic bands, there was complete concordance between the observed 0.5 kb *Bam*HI fragment and the presence of 1.3 kb *Eco*RI polymorphic fragment and similarly between the 8 kb *Eco*RI RFLP and the 8.2 kb *Hind*III fragments. Table 1 also shows the number of invariant bands detected by the probe in genomic DNA digested with different enzymes, i.e., 18 invariant bands with *Bam*HI and 24 invariant bands with either *Eco*RI or *Hind*III. Such a large number of constant bands detected by this probe suggests that the sequences occur in multiple copies.

Chromosomal mapping of pAB 48K-5 sequences by in situ hybridization

In situ hybridization with the pAB 48K-5 cDNA probe was done on 426 metaphase chromosome preparations obtained from cultures of normal human peripheral blood leukocytes. The frequency histogram of Fig. 4 shows the presence of EF-1α sequences on a large number of chromosomes, predominantly on the short arms of chromosomes 1, 5, and 7 and on the long arms of chromosomes 2, 4, 5, 6, 7, and 15. Control experiments using probes with similar specific activities from single-copy genes resulted in single chromosomal signals.

Discussion

Among the genes reprogrammed by the tumor-promoting phorbol esters, that of the human EF-1α was identified by nucleotide sequence analysis and homology search by computer analysis. The inducibility by phorboids was furthermore confirmed by Northern blot analysis of cytoplasmic mRNA, identifying EF-1α mRNA as an ~20S mRNA. Comparison of cellular EF-1α mRNA content with plasminogen activator (t-PA) mRNA content by dot-blot analysis showed the presence of ~30 times more EF-1α mRNA than t-PA mRNA. Since the latter is the product of a typical single-copy gene, mapped to human chromosome 8p12 (Yang-Feng et al. 1986), the abundance of EF-1α mRNA could be the result of the transcription of several EF-1α gene copies. Genomic Southern blot analysis with the partial cDNA probe showed on autoradiography multiple high molecular weight bands with all enzymes tested, a typical feature of multigene families. All restriction enzymes in this study recognizing hexanucleotide sequences showed RFLPs. Probably the multigene family will consist of multiple functional genes and/or pseudogenes. On all Southern blot analyses, bands of varying intensities were observed; it is not yet clear if this is due to amplification effects on some gene(s) or pseudogene(s) or to weaker hybridization because of lesser nucleotide sequence homologies between the probe and the restriction endonuclease fragments.

The gene copy number was estimated by dot-blot hybridization of genomic DNA with probes of t-PA and EF-1α. Both cDNA probes used had comparable length and specific activities. When tested in dilution series of equal amounts of genomic DNA, the signal with the EF-1α probe exceeded more than ten times that with the t-PA probe, indicating that multiple copies of EF-1α-related sequences exist in the (haploid) human genome. Unpublished work on the cloning of human genomic EF-1α DNA sequences yielded copy numbers exceeding 100 (data not shown). Also the finding of multiple chromosomal localizations is in agreement with the existence of multiple genes.

The nucleotide sequence analysis of the partial cDNA reveals a 227-amino acid carboxyterminal open reading frame, followed by a complete 3′-untranslated region. Instead of the classical AATAAA polyadenylation signal, the signal ATTAAA, present at a frequency of 12%, initiates 13 nucleotides further polyadenylation (Wickens and Stephenson 1984). Homologies with the EFs of other species, as outlined in the studies of van Hemert et al. (1985), were also found. The human EF-1α genes are significantly inducible by phorbol esters, but less than the t-PA-gene. It must be emphasized that basal (uninduced) levels of EF-1α mRNA by far exceed those of t-PA. For comparison see the Northern blot analysis in Fig. 2.

From the results of our studies, a general picture emerges of the genomic organization of the repeated human EF-1α

sequences. Since these genes play a role in translational processes, it was not surprising to find them activated after phorbol ester treatment, a process where transcription and translation in general and for specific genes in particular (Opdenakker et al. 1983, 1986) are enhanced. It is not yet known if the phorbol ester-regulated genes are all activated through similar promotor regions or if the existence of more than 100 copies of the EF-1α-related genes could lead to, e.g., transcription by physical proximity of at least 100 other genes.

Our findings on the existence of multiple copies were extended in the mapping of EF-1α genes to multiple chromosomes. Only few studies revealed similar findings. Recently, Floyd-Smith et al. (1985) mapped the human β-tubulin genes and Tripputi et al. (1986), the human histone genes to multiple chromosomes. All these multigene families share common characteristics: they have highly interspecies-conserved sequences, are multiple, and are functional in the essential machinery of replication, transcription, and translation. Their DNA sequences can be classified as repetitive DNAs.

It still remains an unanswered question how these well-conserved genes have become multigene families throughout evolution. Although duplication, deletion, or insertion processes may have been involved, these theoretical possibilities have to be substantiated by more experimental evidence [e.g., genomic cloning and mapping of different (pseudo)genes].

Because of their high copy number, these sequences might provide a high recombination rate, useful for DNA-mediated gene transfer in mammalian cells. Such an approach has already been used successfully in yeast with ribosomal DNA as the recombinogenic gene (Orr-Weaver et al. 1981).

Acknowledgements. The authors wish to thank the following for financial support: Geconcerteerde Onderzoeksacties (GOA, Belgium), the Cancer Fund of the A.S.L.K. (General Savings and Retirement Fund), and the National Bank of Belgium. G.O. is a senior research assistant, R.D. a research assistant, and J.V.D. and G.V. are research associates of the Belgian N.F.W.O. Y.C.A. holds a fellowship from the International Cultural Cooperation Program of Flanders through the Venezuelan Ministry of Coordination and Planification. G.O. is an honorary CRB fellow of the Belgian American Educational Foundation and a recipient of a N.A.T.O. research grant. The excellent technical assistance of Magda Dehaen and editorial work of Christiane Callebaut are acknowledged.

References

Ashino-Fuse H, Opdenakker G, Fuse A, Billiau A (1984) Mechanism of the stimulatory effect of phorbol 12-myristate 13-acetate on cellular production of plasminogen activator. Proc Soc Exp Biol Med 176:109–118

Aviv H, Leder P (1972) Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408–1412

Berget SM (1984) Are U4 small nuclear ribonucleoproteins involved in polyadenylation? Nature 309:179–182

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Floyd-Smith GA, de Martinville B, Francke U (1985) A beta-tubulin expressed gene M40 (TUBB) is located on human chromosome 6 and two related pseudogenes are located on chromosomes 8 (TUBBP1) and 13 (TUBBP2). Cytogenet Cell Genet 40:629

Gubler U, Hoffman BJ (1983) A simple and very efficient method for generating cDNA libraries. Gene 25:263–269

Harper ME, Saunders GF (1981) Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma 83:431–439

Harris TJR, Patel T, Marston FAO, Little S, Emtage JS, Opdenakker G, Volckaert G, Rombauts W, Billiau A, De Somer P (1986) Cloning of cDNA coding for human tissue-type plasminogen activator and its expression in *Escherichia coli*. Mol Biol Med 3:279–292

van Hemert FJ, Amons R, Pluijms WJM, van Ormondt H, Möller W (1984) The primary structure of elongation factor EF-1α from the brine shrimp *Artemia*. EMBO J 3:1109–1113

Maxam A, Gilbert W (1980) Sequencing DNA by labeling the end and breaking at bases: DNA segments, end labeling, cleavage reactions, polyacrylamide gels and strategies. Methods Enzymol 65:499–560

Opdenakker G, Weening H, Collen D, Billiau A, De Somer P (1982) Messenger RNA for human tissue plasminogen activator. Eur J Biochem 121:269–274

Opdenakker G, Ashino-Fuse H, Van Damme J, Billiau A, De Somer P (1983) Effects of 12-*O*-tetradecanoylphorbol 13-acetate on the production of mRNAs for human tissue-type plasminogen activator. Eur J Biochem 131:481–487

Opdenakker G, Dijkmans R, Volckaert G, Billiau A, De Somer P (1984) Use of polyethyleneglycol-induced precipitation to improve the efficiency of cDNA cloning. Arch Intern Physiol Biochim 92:B48–B49

Opdenakker G, Billiau A, Volckaert G, De Somer P (1985) Determination of tissue-type plasminogen-activator mRNA in human and non-human cell lines by dot-blot hybridization. Biochem J 231:309–313

Opdenakker G, Bosman F, Van Damme J, Billiau A (1986) Messenger RNA for a phorbol-ester induced 48,000 dalton protein from human melanoma cells. Biochem Biophys Res Commun 136:122–129

Orr-Weaver TL, Szostak JW, Rothstein RJ (1981) Yeast transformation: a model system for the study of recombination. Proc Natl Acad Sci USA 78:6354–6358

Scott RW, Vogt TF, Croke ME, Tilghman SM (1984) Tissue-specific activation of a cloned α-fetoprotein gene during differentiation of a transfected embryonal carcinoma cell line. Nature 310:562–567

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Thomas PS (1980) Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205

Tripputi P, Emanuel BS, Croce CM, Green LG, Stein GS, Stein JL (1986) Human histone genes map to multiple chromosomes. Proc Natl Acad Sci USA 83:3185–3188

Volckaert G, De Vleeschouwer G, Blöcker E, Frank R (1984) A novel type of cloning vector for ultrarapid chemical degradation sequencing of DNA. Gene Anal Techn 1:52–59

Wickens M, Stephenson P (1984) Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3′ end formation. Science 226:1045–1051

Yang-Feng T, Opdenakker G, Volckaert G, Francke U (1986) Human tissue-type plasminogen activator gene located near chromosomal breakpoint in myeloproliferative disorder. Am J Hum Genet 39:79–87

Received August 11, 1986 / Revised October 20, 1986