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Complete nucleotide sequence of a human c-*onc* gene: Deduced amino acid sequence of the human c-*fos* protein

(differentially expressed gene/proto-oncogene/osteosarcoma virus homolog/*Alu* repeat/5′ untranslated region)

FLIP VAN STRAATEN*, ROLF MÜLLER†, TOM CURRAN, CHARLES VAN BEVEREN, AND INDER M. VERMA

Molecular Biology and Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138

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ABSTRACT **The complete nucleotide sequence of the c-*fos*(human) gene, the human cellular homolog of the oncogene (v-*fos*) of Finkel–Biskis–Jinkins murine osteosarcoma virus, has been determined. The c-*fos*(human) gene contains four discontinuous regions when compared with the v-*fos* gene. Three of the discontinuities are flanked by sequences characteristic of introns, while the fourth discontinuity is due to a deletion of 104 base pairs in the v-*fos* gene. As a consequence of the deletion, the predicted c-*fos*(human) and v-*fos* gene products differ at their carboxyl termini. Transcripts of 2.2 kilobases from the c-*fos*(human) gene have been identified in human cells. The sizes of these transcripts are in close agreement with the size expected from the nucleotide sequence after removal of introns.**

Acquisition of normal cellular sequences by retroviruses imparts to them the ability to induce neoplastic transformation (1). A number of acutely transforming viruses, each carrying a distinct oncogene, have been isolated from a variety of species (1, 2). The normal cellular homologs (c-*onc*) of the viral oncogenes (v-*onc*) have been well conserved in evolution across a broad variety of species. For instance, sequences homologous to v-*src* (the oncogene of Rous sarcoma virus) (3) and v-*abl* (the oncogene of Abelson murine leukemia virus) (4) can be detected in normal human cells (1), as well as in invertebrates (5). Because of their evolutionary conservation, a crucial role of c-*onc* gene-encoded proteins in normal cellular metabolism has often been postulated (6). Many c-*onc* genes are expressed in a variety of tissues and cell lines (7–9). In some instances, expression has been shown to be modulated during development and differentiation (10, 11).

As part of studies concerned with the organization of c-*onc* genes, we have recently isolated and molecularly cloned a biologically active Finkel–Biskis–Jinkins murine osteosarcoma virus (FBJ-MuSV) proviral DNA (12). FBJ-MuSV is a replication-defective transforming virus that induces osteogenic sarcomas *in vivo* and transforms fibroblasts *in vitro* (13, 14). A 55-kilodalton phosphoprotein encoded by the FBJ-MuSV viral genome has been identified as the transforming gene product (15). Sequences homologous to the oncogene (v-*fos*) of FBJ-MuSV have been identified in a number of species (12). The cellular homolog (c-*fos*) of the v-*fos* gene has been molecularly cloned from human cells (16). Here, we present the complete nucleotide sequence of the c-*fos*(human) gene, which can encode a protein of 380 amino acids. When the sequence is compared with that of the c-*fos*(mouse) gene (17), the sequence conservation in the coding domains is found to be approximately 90%.

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MATERIALS AND METHODS

Recombinant DNA. The isolation of the molecular clone pc-*fos*(human)-1 has been described (16). Briefly, Southern blot analysis of an *Eco*RI digest of DNA from the human lymphoblastic cell line (CCRF-CM) revealed a *fos*-specific fragment of about 9 kilobase pairs, which was molecularly cloned in the Charon 30 vector and subsequently subcloned in plasmid pBR322 [pc-*fos*(human)-1]. This plasmid appeared to contain the entire c-*fos*(human) gene.

DNA Sequence Analysis. Cleavage sites for various restriction enzymes within the 9-kilobase-pair insert of pc-*fos*(human)-1 DNA were mapped by using partial digestion of end-labeled DNA (18). The nucleotide sequence of >91% of both strands of the sequence presented in Fig. 2 and of >97% of the data in Fig. 3 was determined by using the partial chemical degradation procedure (19). A summary of the precise procedures used here has been reported (20).

RNA Isolation and Blot Analysis. RNA was isolated from BeWo cells (21) and mouse placenta, selected for poly(A)$^+$ RNA, and analyzed by agarose gel electrophoresis followed by RNA blotting as described (11).

RESULTS

c-*fos*(human) Gene Transcripts. To determine whether the c-*fos*(human) gene is transcriptionally active, we analyzed RNA from several human cell lines. Poly(A)$^+$ RNA isolated from a cell line (BeWo) derived from a malignant gestational choriocarcinoma of human fetal placenta (21) was fractionated by agarose gel electrophoresis and transferred to nitrocellulose paper. Hybridization with a v-*fos*-specific probe revealed a single band of about 2.2 kilobases (Fig. 1, lane 2). This size is similar to that of *fos*-related transcripts found in mouse placenta (lane 3) (11). Transcripts from the c-*fos* gene of 2.2 kilobases have also been observed in RNA isolated from human placenta and fetal membranes (unpublished data).

Nucleotide Sequence of the c-*fos*(human) Gene. The complete nucleotide sequence of the c-*fos*(human) gene is shown in Fig. 2*A*. From the "TATA box" (22) to the presumptive polyadenylylation signal (23), the c-*fos*(human) gene contains 3,415 nucleotides. When compared with the v-*fos* gene sequence (17), the c-*fos*(human) gene has four discontinuous regions. The nucleotides around the ends of the discontinuities are compared with the consensus sequences for RNA splice donor and acceptor sites in Table 1 (24). It is apparent that the first three

Abbreviation: FBJ-MuSV, Finkel–Biskis–Jinkins murine osteosarcoma virus.

* Present address: MRC Laboratory of Molecular Biology, Cambridge, England.
† Present address: European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany.

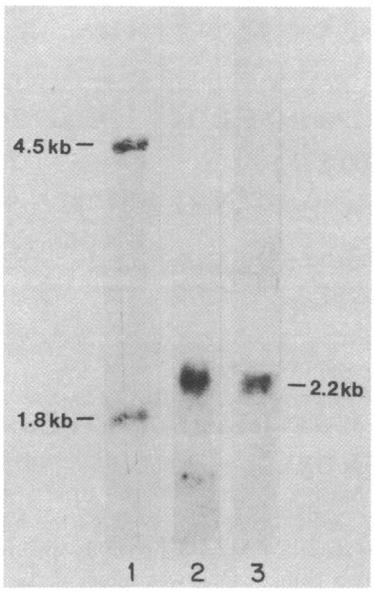

FIG. 1. Comparison of transcripts from the c-*fos*(human) and c-*fos*(mouse) genes. Lanes: 1, poly(A)$^-$ RNA (18S/28S; *ca.* 10^4 cpm) size standard from NIH/3T3 cells metabolically labeled with [^{14}C]uridine; 2, poly(A)$^+$ RNA (30 μg) from BeWo cells; 3, poly(A)$^+$ RNA (20 μg) from 17th day mouse placenta. RNAs were separated on a 1.1% agarose gel, blotted onto nitrocellulose paper, and hybridized to a v-*fos*-specific probe [*Pst* I/*Pvu* II 1.0-kilobase-pair fragment (12)]. Blots were exposed for ≈36 hr.

discontinuous regions contain sequences that agree with the consensus donor splice site at their 5′ ends and acceptor sites at their 3′ ends. However, the fourth discontinuity, located between nucleotides 2,582 and 2,685, does not have an appropriate splice donor or acceptor site (Table 1). This region corresponds to the 104-nucleotide sequence that has been deleted from the v-*fos* gene (17). When the nucleotide sequence of the c-*fos*(human) gene is compared with that of the c-*fos*(mouse) gene (17), a remarkable degree of homology can be noticed. The nucleotide differences between the exons of the two c-*fos* genes are indicated in Fig. 2 and summarized in Table 2. More than 89% of the nucleotides in the exons of the c-*fos*(human) and c-*fos*(mouse) genes are identical. The 5′ and 3′ untranslated regions also show extensive sequence homology (76% homology in the 5′ and 90% homology in the 3′ untranslated regions).

The c-*fos*(human) gene can encode a protein of 380 amino acids. The predicted c-*fos*(human) protein would be initiated at position 289, the first AUG codon in the mRNA (26), and terminated at position 2,729 (Fig. 2). The deduced c-*fos*(human) gene product is hydrophilic and very acidic (38 positive residues versus 50 negative residues) and contains a high proportion of serine residues (>14% of the total amino acids). It contains no glycosylation sites of the form Asn-X-Ser or Asn-X-Thr (27).

A diagram of the c-*fos*(human) gene is shown in Fig. 2*B*. The figure also shows the location of the two *Alu* family repeat sequences (28) present in the 9.0-kilobase-pair pc-*fos*(human)-1 DNA. The position of the beginning of an open reading frame to the right of the second *Alu* repeat sequence is also marked.

The 5′ flanking sequences of mammalian genes have been shown to play a role in gene regulation (22). In the case of the c-*fos*(human) gene, this region has a high proportion of guanosine and cytosine residues (63%), and contains two inverted repeats of 10 base pairs (Figs. 2 and 3). The importance of the region in regulating expression of the c-*fos*(human) gene is not known.

Comparison of the c-*fos*(human) Gene Product with Those of v-*fos* and c-*fos*(mouse). The deduced amino acid sequence of the c-*fos*(human) gene product is compared with the sequences of the predicted v-*fos* and c-*fos*(mouse) gene products (17) in Fig. 4. In the first 332 amino acids, there are 27 amino acid differences between the c-*fos*(human) and v-*fos* gene products and 22 differences between the products of the c-*fos*(human) and c-*fos*(mouse) genes. The remaining 48 amino acids of both the c-*fos*(human) and c-*fos*(mouse) gene products are totally different from those of the v-*fos* protein.

DISCUSSION

Organization of c-*onc* Genes. The kinship between viral oncogenes and their cellular homologs is now well established (1). More than 17 oncogenes that have cellular counterparts have been identified. We have investigated the structure of the human cellular homolog of the transforming gene of FBJ-MuSV. Like other known cellular oncogenes (1), the c-*fos*(human) gene contains intervening sequences. To date, only two c-*onc* genes are known, the cellular homolog of the mouse sarcoma virus oncogene (c-*mos*) (20, 29, 30) and two of the c-*ras* genes, c-*ras*^{Ha}-2 and c-*ras*^{Ki}-2 (31, 32), that are devoid of introns. Cellular sequences acquired by the retroviruses, however, do not contain intervening sequences. Recently, it has been shown that, on infection with α-globin genomic DNA containing an intron inserted into the proviral DNA, the progeny virions contain processed α-globin mRNA linked to viral genomic RNA (33). Thus, retroviruses containing genes or sequences with introns can be transcribed and processed to generate spliced RNA transcripts. The precise mechanism of the acquisition of cellular sequences, however, remains obscure.

Inspection of the sequence of the c-*fos*(human) gene indicates that it is a complete transcriptional unit. If initiation occurs at the purported 5′ cap and terminates at the poly(A) addition signal at position 3,510 (Fig. 2), the c-*fos* gene transcript after splicing of the introns should be about 2.2 kilobases long. This is in agreement with the sizes of c-*fos*(human) gene transcripts detected in human cells (Fig. 1, lane 2). The sizes of c-*fos* transcripts observed in a number of mouse tissues are similar (Fig. 1, lane 3; ref. 11). In contrast to c-*fos*(mouse), the c-*fos*(human) gene has a second poly(A) addition signal at position 3,233 (Fig. 2). Utilization of this poly(A) signal, however, would produce a transcript that is approximately 270 nucleotides shorter than c-*fos*(mouse) mRNA.

Cellular oncogenes are conserved in evolution across a broad variety of species. The human and mouse genomes diverged

FIG. 2 (*on next page*). Nucleotide sequence of the c-*fos*(human) gene. The nucleotide sequence of a 6.5-kilobase-pair region of pc-*fos*(human)-1 DNA was determined. (*A*) The sequence of 3,565 nucleotides encompassing the c-*fos*(human) gene is shown. Signals important for transcription are indicated: TATA-box, approximate 5′-capped nucleotide, and poly(A) addition signal (of which two are indicated). Direct repeats of eight nucleotides each separated by six nucleotides are underlined at positions 35–42 and 49–56. The dotted boxes denote the extremities of the v-*fos* gene (17). The amino acids encoded by each exon are shown above the nucleotide sequence, and the boundaries of each intron are marked by arrows. Wavy arrows indicate the 104 nucleotides deleted in the v-*fos* gene. The nucleotides in the c-*fos*(mouse) gene that differ from the corresponding nucleotides in the c-*fos*(human) gene are indicated below the sequence. (*B*) Diagrammatic sketch delineating the salient features of the c-*fos*(human) gene and adjacent sequences. The trapezoid with the thickened base shown in the fourth exon represents the region of 104 base pairs deleted from the v-*fos* gene (17). TAG at position 2,834 is the termination triplet used in FBJ-MuSV. The positions of two *Alu* family repeat sequences and an open reading frame (ORF) are also indicated. a.a., Amino acid(s).

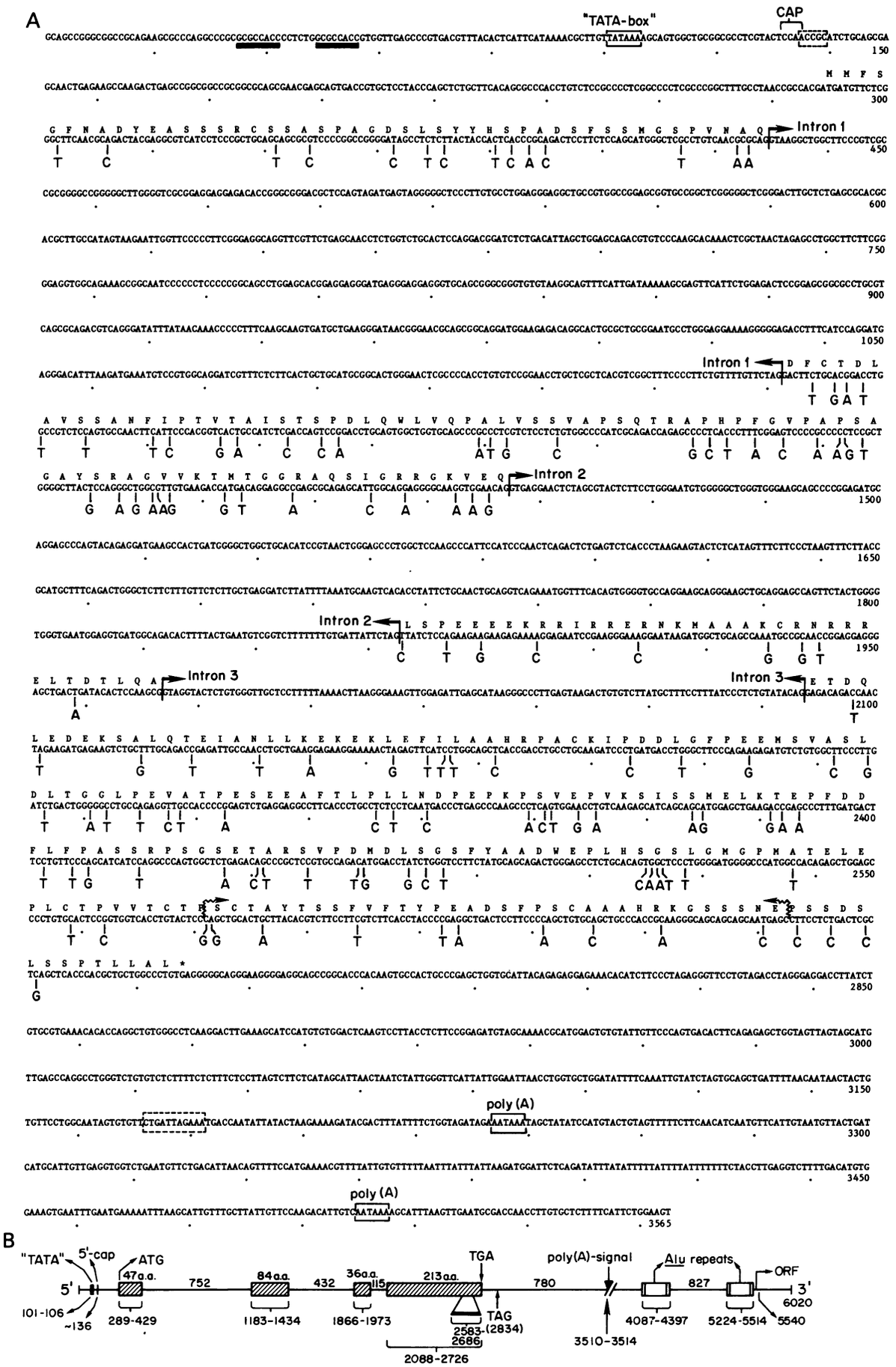

FIG. 2. (*Legend appears at the bottom of the preceding page.*)

Table 1. Sequences of the c-*fos*(human) gene at points of discontinuity with the v-*fos* gene

	Donor site	Acceptor site
Consensus sequence	A-G/G-T-N-A-G	Y-N-Y-Y-Y-N-C-A-G/
Intron 1 (430–1,182)	A-G/G-T-A-A-G	T-T-G-T-T-C-T-A-G/
Intron 2 (1,435–1,865)	A-G/G-T-G-A-G	T-T-A-T-T-C-T-A-G/
Intron 3 (1,974–2,087)	C-G/G-T-A-G-G	T-G-T-A-T-A-C-A-G/
Deletion (2,582–2,685)	C-C/C-A-G-C-T	G-C-A-A-T-G-A-G-C/

Consensus sequences for the 5'-ends (donor sites) and 3'-ends (acceptor sites) of introns found in eukaryotic genes (24) are shown for reference. Nucleotides at the ends of the discontinuities in the c-*fos*(human) gene (versus the v-*fos* gene) are given. The presence of cytosine residues at both extremes of the deletion prevents the exact assignment of the ends. Nucleotides that agree with specified nucleotides of the splice consensus sequences are shown in boldface type; slashes indicate exon–intron junctions or points of discontinuity; Y, thymidine or cytosine.

Table 2. Comparison of c-*fos*(human) and c-*fos*(mouse) gene nucleotide sequences

Region of c-*fos* gene	Nucleotides,* no.	Gaps,† no.		Base changes,‡ no.		% identity
		Mouse	Human	Transition	Transversion	
5' Untranslated to TATA	100	3	1	13	22	54
TATA to ATG	188	1	1	20	18	76
Exon 1	141	0	0	8	6	90
Exon 2	252	0	0	24	16	84
Exon 3	108	0	0	4	5	92
Exon 4						
Before deletion	494	0	0	41	16	88
Deletion	104	0	0	6	4	90
After deletion	41	0	0	2	2	90
Intron 1	753	6	8	82	68	76
Intron 2	431	7	4	61	64	63
Intron 3	114	2	1	14	16	66
3' Untranslated TGA to poly(A)	789	4	4	37	20	90

The nucleotide sequence of the c-*fos*(human) gene shown in Fig. 2 was compared with that of the c-*fos*(mouse) gene (17) by using the ALIGN program (25). A unitary matrix and a gap penalty of three were used. The ATG triplet indicated is that of the first methionine residue in the *fos* gene products (position 289 in Fig. 2; position 284 in figure 3 of ref. 17). The poly(A) signal indicated is the second of the two available in the c-*fos*(human) gene (Fig. 2, position 3,510).

* In the c-*fos*(human) gene.

† Inserted by the ALIGN program to optimize alignment scores.

‡ Separated into transitions (purine–purine or pyrimidine–pyrimidine) and transversions (purine–pyrimidine).

approximately 70 million years ago, yet the coding regions of c-*fos*(human) and c-*fos*(mouse) appear to be ≈90% conserved. Another c-*onc* gene, the c-*mos*(human), shows 77% homology with c-*mos*(mouse) sequences (34). By comparison, the mouse and human α-globin genes show somewhat lower homology than do the c-*fos* genes (≈80% in the exons, 60% in the introns, and ≈70% in the 5' and 3' untranslated regions) (35).

The nucleotide sequence analysis of the c-*fos*(human) gene revealed some other interesting features. Instead of the "CAT-box" located 80 nucleotides upstream from the capped nucleotide of many eukaryotic genes (22), a direct repeat of the sequence G-C-G-C-C-A-C-C separated by six nucleotides (underlined in Fig. 2) can be identified. The 5' flanking sequences are (G+C)-rich (63%), whereas the 3' flanking sequences are (A+T)-rich (61%) (Figs. 2 and 3). Approximately 570 nucleotides downstream from the 3' poly(A) addition signal, there are two *Alu* family sequences (28) separated by 827 nucleotides. The first of the two repeat sequences is bounded by a six-nucleotide direct repeat while the second has a 16-base-pair direct repeat. At least one, and possibly both, *Alu* repeat sequences can be transcribed *in vitro* by RNA polymerase III (36) to yield appropriate size transcripts (unpublished data). About 20 nucleotides to the 3' end of the second *Alu* repeat sequence, there appears to be another open reading frame (ORF in Fig. 2), which begins with a methionine codon ≈100 nucleotides downstream from a potential promoter sequence, T-A-A-T-A-A (data not shown).

Activation of c-*onc* Genes. Acquisition of c-*onc* sequences by retroviruses renders them oncogenic. Yet, c-*onc* sequences themselves are unable to either transform fibroblasts *in vitro* or induce neoplasia *in vivo* (29, 31, 32). Some c-*onc* genes can, however, be activated to transform fibroblasts if they are linked to viral promoters (29, 31, 32). Here we show that the c-*fos*(human) gene contains in its carboxyl-terminal coding domain the 104-bp sequence (positions 2,583–2,686 in Fig. 2) deleted in the v-*fos* gene (17), with the result that the c-*fos*(human) and v-*fos* gene products differ at their carboxyl termini. Preliminary results suggest that the c-*fos*(human) gene is unable to transform fibroblasts *in vitro* (unpublished data). However, if sequences representing the c-*fos* carboxyl terminus are replaced with the v-*fos* carboxyl terminus, the resulting chimeric c-*fos*(human)–v-*fos* gene, which includes the 3' long terminal repeat, is able to transform rat fibroblasts. The converse construct, containing the 5' long terminal repeat, the v-*fos* amino terminus, and the c-*fos*(human) carboxyl terminus, failed to induce transformation (unpublished data). These results suggest that the induction of neoplastic transformation by FBJ-MuSV involves the enhanced expression of a structurally aberrant form of the *fos* protein.

It has recently been reported that a transforming gene isolated from human bladder carcinoma cells, the c-*ras*^{Ha}-1 gene, has acquired its oncogenic potential by a single base change at amino acid 12 with respect to its normal cellular homolog (37, 38). These findings, together with the sequence data presented here, raise the possibility that a mutated form of the c-*fos*(human) gene product may play a role in the multistage process of induction of certain human malignancies.

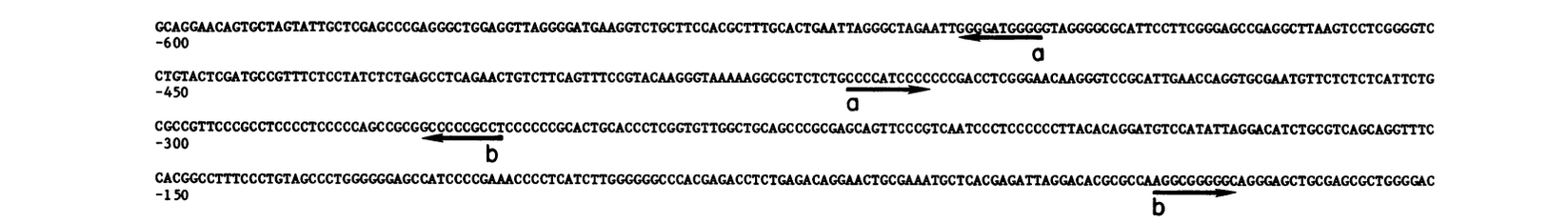

FIG. 3. Nucleotide sequence of the c-*fos*(human) gene 5' flanking region. The sequence of the 600 nucleotides upstream of the sequence given in Fig. 2 is presented. (Nucleotide −1 in this figure is adjacent to nucleotide 1 of Fig. 2.) Two pairs (a and b) of 10-nucleotide inverted repeats are underlined with arrows.

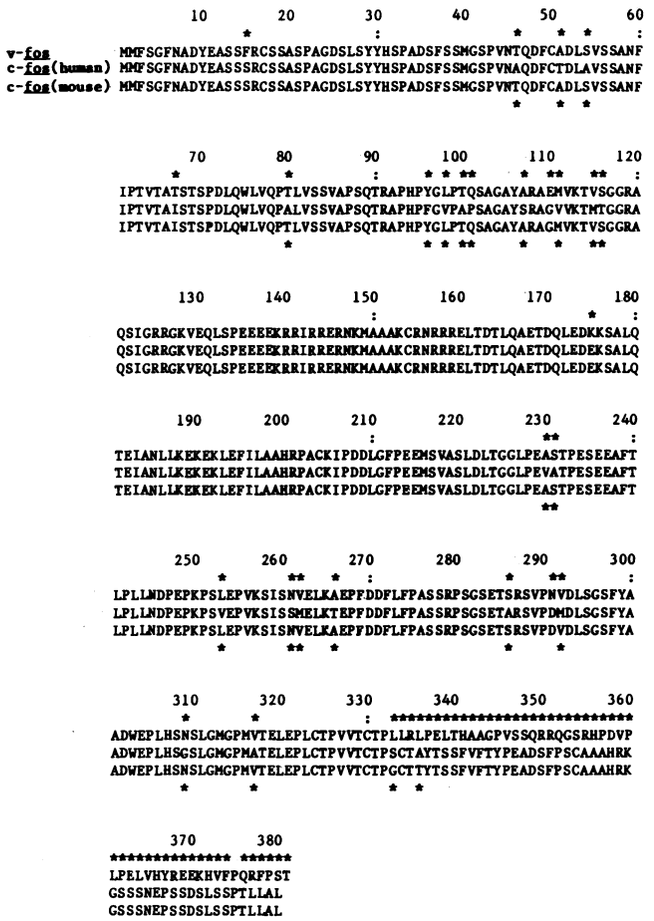

FIG. 4. Comparison of c-*fos*(human), c-*fos*(mouse), and v-*fos* gene products. Amino acids are indicated by the single-letter code; those that differ between the c-*fos*(human) and v-*fos* genes are shown above the sequences, while those that differ between the c-*fos*(human) and c-*fos*(mouse) genes are shown below. The carboxyl-terminal 48 amino acids of the c-*fos*(human) and c-*fos*(mouse) gene products are totally different from those of the v-*fos* protein.

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