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# Activation of the Transforming Potential of the Human *fos* Proto-Oncogene Requires Message Stabilization and Results in Increased Amounts of Partially Modified *fos* Protein

WILLIAM M. F. LEE,[1]* CHIN LIN,[2] AND TOM CURRAN[3]

*Department of Radiation Oncology and the Cancer Research Institute, Department of Medicine,[1] and Howard Hughes Medical Institute,[2] University of California San Francisco, San Francisco, California 94143, and Department of Molecular Oncology, Roche Institute of Molecular Biology, Hoffmann-La Roche, Nutley, New Jersey 07110[3]*

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**The requirements for activation of the transformation potential of the human c-*fos* proto-oncogene were investigated. Recombinant plasmids containing the Moloney murine leukemia virus long terminal repeat directing transcription of the c-*fos* coding region and either the authentic c-*fos* 3′ untranslated region (UTR) or the 3′ UTR from human c-*myc* were inefficient at inducing transformation. In contrast, a recombinant that substituted most of the c-*fos* 3′ UTR with the 3′ portion of the simian virus 40 T-antigen gene transformed cells well. This difference in transformation efficiency appeared to be due to significantly higher levels of *fos* mRNA and protein expressed from the transforming recombinant. This, in turn, was due to the much greater stability of its mRNA compared with those from the poorly transforming recombinants containing the c-*fos* or c-*myc* 3′ UTR. Thus, the 3′ UTR of the human c-*fos* mRNA is responsible for its rapid degradation and limits the steady-state levels of transcript and protein. Cells transformed by the activated human c-*fos* plasmids contained increased amounts of partially modified c-*fos* protein (c-Fos). This form of c-Fos turned over much more rapidly than the highly modified form of c-Fos induced by serum stimulation.**

The *fos* proto-oncogene (c-*fos*) is the cellular homolog of the oncogene (v-*fos*) carried by the FBJ and FBR murine sarcoma viruses (10, 11, 14). In a majority of cell types, the level of c-*fos* expression is normally low but can be induced to high levels by a variety of agents including mitogenic stimuli (5, 18, 20, 26; for a review, see reference 5a). The primary translation product of the human and murine c-*fos* genes contains 380 amino acids and has a predicted molecular mass of 41 kilodaltons (37, 39), but migrates on sodium dodecyl sulfate-polyacrylamide gels with an apparent molecular mass (unmodified) of 55 kilodaltons (8). The apparent molecular mass increases to 62 kilodaltons as a consequence of posttranslational modification, primarily serine and threonine phosphorylation (8, 26). Some of the enzymes involved in this modification are stimulated by agents such as cyclic AMP and phorbol esters that induce c-*fos* expression (2, 6). c-*fos* protein (Fos) exists in a stable complex with a 39-kilodalton protein (p39) that is coimmunoprecipitated with anti-Fos antibodies (12, 13, 17). Recently, p39 has been identified as the product of the *jun* proto-oncogene (33a). Clues to the function of the c-*fos* protein may be found in its ability to bind DNA, its association with chromatin (32, 33), and its participation in a protein complex that binds to a putative regulatory region upstream of an adipocyte-specific gene (15). More recently, the Fos complex has been shown to bind to the same oligonucleotide sequence as the transcription factor AP1/c-*jun* (16, 31). These data suggest that Fos functions in the regulation of gene transcription and perhaps serves a role in signal transduction processes, linking cell surface stimuli to long-term responses.

The human c-*fos* gene has not been implicated directly in the pathogenesis of any naturally occurring tumors, and its ability to transform cells in culture remains to be established. The murine c-*fos* gene, however, has been shown to transform rat fibroblasts (23), but only after enhancement of transcription and removal of inhibitory sequences located within a 67-base-pair (bp) segment in the 3′ untranslated region (UTR) of the mature c-*fos* gene and message (22, 23). The mechanism by which these 3′ sequences prevent transformation of fibroblasts is unknown. However, they have been shown to destabilize c-*fos* mRNA (30), and one could infer that this leads to lower steady-state levels of the mRNA and protein. In the current study, we examined the requirements for transformation of rat fibroblasts by the human c-*fos* gene in vitro and characterized the effect of 3′ sequences on mRNA stability and protein levels.

## MATERIALS AND METHODS

**Plasmids.** The 9-kilobase *Eco*RI-*Eco*RI human c-*fos* clone (7, 39) formed the basis for all subsequent modifications. We replaced all c-*fos* sequences 5′ to the *Hae*II site in exon 1 (about 55 bp 5′ to the initiation ATG [39]) with a partial Moloney murine leukemia virus (MLV) long terminal repeat (LTR) that contains all of U3 and part of R to the *Kpn*I site, where a polylinker (containing *Bam*HI and *Sal*I sites) is inserted. The resulting plasmid, MLV*fos*, is shown in Fig. 1 and contains the MLV enhancer and promoter directing transcription of the c-*fos* gene. Substitution of most of the 3′ UTR of MLV*fos* with segments from other genes was accomplished by removing c-*fos* sequences 3′ to the *Avr*II site (about 110 bp 3′ to the termination codon [39]) and replacing them with the 3′ portions of the human c-*myc* gene (from the *Nsi*I site, about 70 bp 3′ to the c-*myc* termination codon [3]), the simian virus 40 (SV40) T-antigen gene (from the *Hinc*II site just 5′ to the polyadenylation signal [35]), and the human β-globin gene (from the *Bam*HI site in exon 2 [28]). Each of these replacement 3′ gene fragments contained the polyadenylation signal(s) of the respective gene and was subcloned first into a pUC18 or pSP65 vector to generate 5′ *Xba*I and 3′ *Eco*RI sites which facilitated subcloning (*Avr*II-

* Corresponding author.

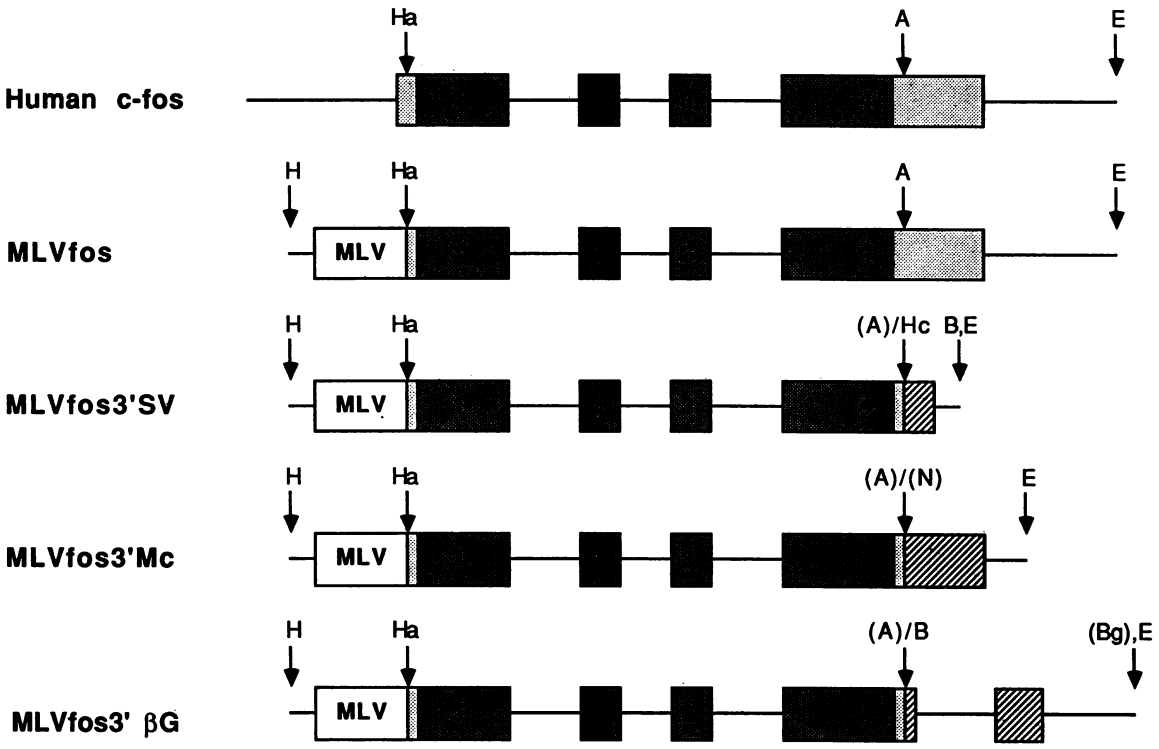


FIG. 1. Recombinant human c-*fos* genes. The diagrams represent the recombinant human c-*fos* clones used in the experiments. Human c-*fos* depicts the relevant part of the *Eco*RI-*Eco*RI genomic clone used in subsequent manipulations; MLV*fos* is the recombinant in which the MLV promoter-enhancer replaces human c-*fos* transcriptional control elements; MLV*fos*3'SV is a derivative of MLV*fos* in which part of the 3' UTR of human c-*fos* has been replaced by a similar element from the SV40 T-antigen gene; MLV*fos*3'Mc is similar to the previous construction except that the replacement used part of the 3' UTR of the human c-*myc* gene; and in MLV*fos*βG, the replacement used part of the 3' end of the human β-globin gene. The details of the constructions are given in Materials and Methods. Darkly shaded boxes represent the coding portions of the four c-*fos* exons (numbered); lightly shaded boxes represent the noncoding portions of c-*fos* exons; unshaded boxes represent portions of the MLV LTR (labeled); and hatched boxes indicate the 3' elements of other genes used to replace part of the 3' UTR of c-*fos*. Relevant restriction sites are indicated by arrows: H, *Hin*dIII; Ha, *Hae*II; A, *Avr*II; E, *Eco*RI; Hc, *Hin*cII; B, *Bam*HI; N, *Nsi*I; and Bg, *Bgl*II. Sites lost in cloning are surrounded by parentheses. The drawings are not to exact scale.

and *Xba*I-cut fragments have complementary ends) between the *Avr*II and *Eco*RI sites of MLV*fos*. The resulting plasmids, MLV*fos*3'Mc, MLV*fos*3'SV, and MLV*fos*3'βG (Fig. 1) resembled MLV*fos* in having the same transcription control element and in encoding the normal human c-*fos* protein but produced transcripts that differed at their 3' ends.

To test the effect of the 3' UTR of human c-*fos* and c-*myc* mRNA on message stability, we created fusion genes containing these segments at their 3' ends. MLVβ-globin (see Fig. 6) was constructed by inserting the partial MLV LTR described above upstream to the 2.9-kilobase *Bam*HI-*Bgl*II fragment of the human β-globin gene (contains about 20 bp of exon 2, intron 2, exon 3, and 3'-flanking sequences) that had been subcloned first into the *Bam*HI site of a pUC vector to generate a 3' *Eco*RI site. Substitution of the 3' end of the MLV β-globin gene was accomplished by removing the *Eco*RI fragment (the β-globin *Eco*RI site is in exon 3, about 190 bp 5' to the polyadenylation signal) and replacing it with the *Avr*II-*Eco*RI 3' human c-*fos* fragment or the *Nsi*I-*Eco*RI 3' human c-*myc* fragment; this produced MLVβ-globin3'*fos* and MLVβ-globin3'*myc*, respectively (See Fig. 6).

**Cells and transfection.** Rat 208F fibroblasts (29) were grown in Dulbecco modified Eagle medium containing 10% fetal calf serum, penicillin-streptomycin, and 5% $CO_2$. Transfection of these cells was performed as previously described (23). In transformation assays, 20 μg of recombinant c-*fos* plasmid DNA was cotransfected with 2 μg of a plasmid that can confer G418 resistance. After transfection, the cells were split equally between plates for focus assay (in the presence of $10^{-6}$ M dexamethasone) and for selection at 400 μg of G418 (GIBCO Laboratories, Grand Island, N.Y.) per ml. The efficiency of transformation by the various c-*fos* recombinants was determined by dividing the number of foci by the number of G418-resistant colonies. This figure agrees well with the percentage of G418-resistant colonies that contain cells appearing transformed.

**RNA and protein analysis.** Total cellular RNA was isolated from cells with either guanidinium isothiocyanate (4) or urea-LiCl (1), and Northern (RNA) analysis was performed by glyoxal denaturation (21). Radiolabeled probe was prepared by random priming with the Klenow fragment of *Escherichia coli* DNA polymerase I and calf thymus random oligonucleotide primer (Pharmacia Fine Chemicals, Piscataway, N.J.). *fos* probe was generated with cloned rat c-*fos* cDNA (6). Immunoprecipitation of *fos* proteins was performed as previously described, with [$^{35}$S]methionine for metabolic labeling (8).

**Actinomycin D treatment.** The half-lives of various mRNA species were measured by treating cells with actinomycin D at a final concentration of 10 μg/ml of medium. After 10 min was allowed for the actinomycin D to act (time zero), RNA was harvested from the cells at various intervals. To measure the half-life of the endogenous rat c-*fos* mRNA, cells

TABLE 1. Efficiency of transformation by human c-*fos* recombinant genes

| Plasmid | % Transformation[a] | | |
|---|---|---|---|
| | Expt 1 | Expt 2 | Expt 3 |
| MLV*fos* | 3 | 6 | 4 |
| MLV*fos*3'SV | 63 | 91 | 79 |
| MLV*fos*3'Mc | | 5 | 3 |
| MMV[b] | 20 | | |
| FBJ (v-*fos*) | 95 | | |

[a] Efficiency is defined as the number of foci formed divided by the number of G418-resistant colonies after transfected cells were divided equally for focus-forming assay and G418 selection.

[b] The murine c-*fos*–FBJ v-*fos* chimeric gene with transforming activity (23).

were placed first in defined medium (Dulbecco modified Eagle medium containing insulin, transferrin, albumin, and penicillin-streptomycin) for 48 h and subsequently stimulated with medium containing 10% fetal calf serum. After 30 min of serum exposure, when c-*fos* mRNA expression is near maximum, actinomycin D treatment was begun and RNA samples were collected as described previously.

## RESULTS

**Transformation of fibroblasts by human c-*fos*.** MLV*fos* (Fig. 1) transformed rat 208F fibroblasts very poorly compared with the FBJ provirus or MMV, a recombinant murine c-*fos* in which most of the 3' UTR of murine c-*fos* had been replaced with the equivalent segment of the FBJ v-*fos* gene with its 3' LTR (23) (Table 1). To determine whether this poor transforming activity was due to sequences in the 3' UTR of the human c-*fos* gene, we replaced most of this region with the majority of the 3' UTR from the human c-*myc* gene (MLV*fos*3'Mc) or with a portion of the 3' UTR from the SV40 T-antigen gene (MLV*fos*3'SV) (Fig. 1). These replacement segments contain sequences required for polyadenylation of the recombinant c-*fos* transcripts. When these recombinant genes were tested for transforming activity, we found that MLV*fos*3'Mc was as ineffective as MLV*fos* in transforming 208F cells, whereas MLV*fos*3'SV was much more effective. In a direct comparison, MLV*fos*3'SV appeared to be more efficient than MMV and nearly as efficient as the FBJ provirus in transforming 208F cells (Table 1). These data showed that the 3' UTR of the human c-*fos* gene also contains sequences that inhibit c-*fos* transforming activity despite augmented transcription and that replacement of this region with the 3' portion of some genes, but not of others, unveils this activity. The cells transformed by the human c-*fos* protein are distinguished easily from parental 208F cells in being refractile and forming a latticelike network of cells, but they appear less transformed than those containing a FBJ v-*fos* gene. Their altered growth properties are also less striking in that they frequently form less distinct and shallower foci.

**c-*fos* mRNA in transfected cells.** To examine whether the differential transformation efficiencies of the various recombinant human c-*fos* genes are due to differences in their level of expression in the transfected cells, we collected pools of hundreds of G418-resistant 208F colonies obtained by cotransfection of each of the recombinants with a plasmid conferring resistance to G418. RNA was isolated from these pools, and expression of c-*fos* mRNA was measured by Northern analysis (Fig. 2). The transcripts generated by MLV*fos*3'Mc and MLV*fos*3'SV were substantially shorter than and could be distinguished by Northern analysis from

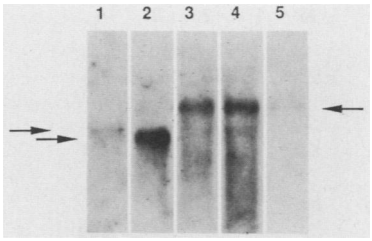


FIG. 2. Transcripts from the various human c-*fos* recombinant genes. Rat 208F cells were cotransfected with each of the various recombinant genes and a neomycin resistance gene. Transfectants were selected with G418, and hundreds of colonies were pooled. Total cellular RNA was isolated from the mass cultures, and 10-μg samples were denatured with glyoxal, resolved by electrophoresis, and blotted to a nylon membrane which was probed with radiolabeled full-length rat c-*fos* cDNA. The autoradiograph shows the results for RNA from 208F cells transfected with MLV*fos*3'Mc (lane 1), with MLV*fos*3'SV (lane 2), and with MLV*fos* (lane 3), from untransfected 208F cells that were placed in quiescence medium for 48 h and stimulated with 10% fetal calf serum for 30 min (lane 4), and from randomly growing, untransfected 208F cells (lane 5). Arrows indicate the position of the messages from the various c-*fos* genes.

endogenous rat c-*fos* mRNA (MLV*fos*3'Mc mRNA was 120 to 500 bases shorter and MLV*fos*3'SV mRNA was 420 to 680 bases shorter; Fig. 2, lanes 1 and 2 compared with lanes 4 and 5). Cells transfected with either of these two recombinants expressed only the transfected gene at appreciable levels, leading us to infer that the *fos* mRNA found in cells transfected with MLV*fos* (which produces a message that is about 90 bases shorter than and indistinguishable from the endogenous c-*fos* mRNA by Northern analysis; Fig. 2, lane 3) is derived mostly from the recombinant gene. Cells transfected with MLV*fos* or MLV*fos*3'Mc produced more c-*fos* mRNA than randomly growing parental 208F cells (Fig. 2, lane 5) but much less than cells transfected with MLV*fos*3'SV. The last recombinant produced as much *fos* mRNA as 208F cells that had been brought to quiescence and stimulated maximally with serum (Fig. 2, lane 4). The data in Fig. 2 were confirmed with other batches of pooled, transfected 208F cells, and we consistently found that expression of *fos* mRNA is highest in MLV*fos*3'SV-transfected and serum-stimulated 208F cells, moderate to low in MLV*fos*-transfected and MLV*fos*3'Mc-transfected cells, and minimal in unstimulated 208F cells.

**c-*fos* protein (Fos) in transfected 208F cells.** If the translational efficiency of the various *fos* messages is similar, the differences in the steady-state levels of mRNA found in these pooled, transfected cells should translate into differences in the level of c-*fos* protein (Fos). Indeed, we found that the level of protein produced by cells transfected with MLV*fos*3'SV was high (Fig. 3, lane 4), that the level of protein produced by cells transfected with MLV*fos* or MLV*fos*3'Mc was significantly lower (lanes 2 and 3), and that each produced more Fos than randomly growing 208F cells (lane 1).

We examined cells transformed by recombinant human c-*fos* for their degree of posttranslational modification and turnover of Fos. Pulse-chase experiments (an example is shown in Fig. 4) revealed that in 208F cells stimulated with serum (lane 1), most of the Fos was chased rapidly into modified forms with higher apparent molecular weights. In contrast, MLV*fos*3'SV- and MMV-transformed cells (lanes 2 and 3) modified much less of their *fos* protein. The half-life of Fos in serum-stimulated 208F cells was estimated to be

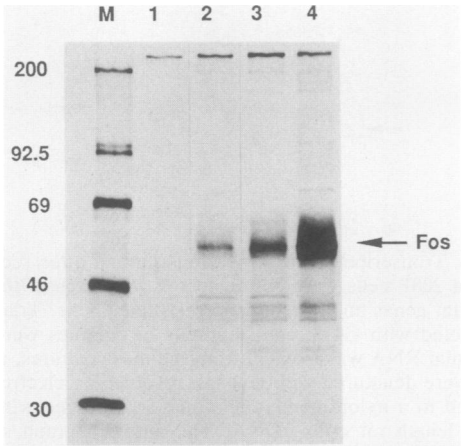


FIG. 3. Fos levels produced by the various human c-*fos* recombinant genes. The cells used in Fig. 2 were labeled with [$^{35}$S] methionine for 15 min, and Fos was immunoprecipitated. Lane M, Marker lane with molecular weight standards ($\times 10^3$) labeled; lane 1, 208F cells transfected with a neomycin resistance gene; lane 2, cells containing MLV*fos*3′Mc; lane 3, cells containing MLV*fos*; lane 4, cells containing MLV*fos*3′SV. The Fos band is indicated.

around 2 h; in MLV*fos*3′SV- and MMV-transformed cells, turnover was much more rapid, and their half-lives were estimated to be around 30 min or less. These findings are consistent with the trend toward less modification and more rapid turnover of Fos in *fos*-transformed 208F cells (8, 26), with the changes being most marked in cells transformed by the human protein. The amount of p39 coprecipitated with a given amount of *fos* protein appeared to be similar in serum-stimulated 208F and MMV-transformed cells but very low in MLV*fos*3′SV-transformed cells. However, conclusions about the comparative turnover rates and degree of modification of Fos and the amount of p39 coprecipitated in MLV*fos*3′SV-transformed rat cells should be drawn with caution, because differences may reflect species differences in the protein made (Fig. 4, lane 1, rat; lane 2, human; and lane 3, mouse).

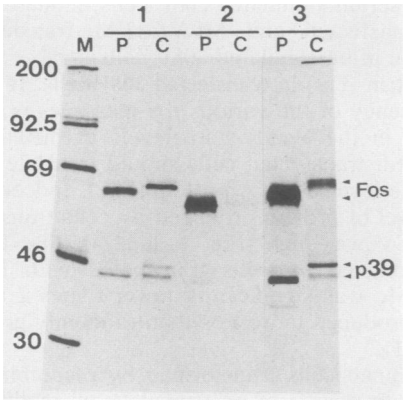


FIG. 4. Posttranslational modification and turnover of Fos. *fos* protein was immunoprecipitated from cells either immediately after labeling with [$^{35}$S]methionine for 15 min (P) or after a 2-h chase with cold methionine (C). Lane 1, 208F cells stimulated with serum; lane 2, MLV*fos*3′SV-transformed 208F cells; and lane 3, MMV-transformed 208F cells. The autoradiograph for lane 3 was exposed five times longer than that for lanes 1 and 2. Lane M is as defined in the legend to Fig. 3.

**Stability of recombinant c-*fos* transcripts.** The difference in c-*fos* mRNA levels in MLV*fos*3′SV-transfected cells compared with MLV*fos*- and MLV*fos*3′Mc-transfected cells could be due to differences in their rates of transcription or to posttranscriptional mechanisms. The first possibility seems highly unlikely, since the transcriptional control elements of the three recombinant human c-*fos* genes are identical and elements affecting transcription have not been described in the 3′ ends of the SV40 T-antigen, c-*myc*, or c-*fos* gene. We examined the second possibility by measuring the half-lives of the messages generated by each of the recombinants. For this analysis, we initially selected individual clones of transfected cells which displayed transformed morphology and presumably expressed higher levels of *fos* mRNA. The cells were treated with actinomycin D to inhibit RNA polymerase II-mediated transcription, and RNA was harvested after various intervals. For comparison, we also measured the decay of the c-*fos* message transcribed from the endogenous rat gene after serum stimulation. MLV*fos*3′SV mRNA was stable, with a half-life of over 3 to 6 h (extrapolated from densitometry of autoradiographs in Fig. 5; data not shown). In these same cells, rat c-*fos* mRNA was unstable and had a half-life of 15 to 30 min (Fig. 5B). MLV*fos* and MLV*fos*3′Mc transcripts disappeared with similarly short half-lives (Fig. 5A and C). To demonstrate that the stabilizing effect of the 3′ end of the SV40 T-antigen gene is not a unique property of this sequence, we substituted the 3′ SV40 T-antigen gene sequence with the 3′ end of the human β-globin gene (MLV*fos*3′βG in Fig. 1). Transcripts from this recombinant in 208F cells were also relatively stable (Fig. 5D). These half-life estimates only serve to give an overall indication of the turnover rates of the various mRNAs, but probably belie the complexities of the actual degradative process, which appears not to proceed with simple first-order kinetics.

In additional experiments (not shown), we found the stabilities of these different *fos* transcripts to be independent of the level of *fos* message found in the cells (transfectants expressing medium or high levels of these recombinants metabolized the message at similar rates) and of recent serum stimulation (transfectants stimulated with serum to induce expression of the endogenous gene metabolized the message from the transfected gene at the same rate as unstimulated cells did). We also observed no influence of the continuous high-level expression of an exogenous c-*fos* gene (e.g., MLV*fos*3′SV) on the profile of endogenous c-*fos* induction by serum or on the stability of its message. This argues against a major autoregulatory effect of Fos in the systems studied here and suggests that the turnover rates of the different *fos* mRNAs are intrinsic properties that do not vary under the conditions tested.

**3′ UTR of human c-*fos* determines message stability.** Stabilization of the message by substituting the 3′ end of the c-*fos* mRNA with SV40 T-antigen or β-globin mRNA segments could be explained by the presence of destabilizing sequences in the 3′ end of the c-*fos* message or could be due to disruption of destabilizing interactions between the c-*fos* 3′ end and other parts of the *fos* mRNA. In the former case, but probably not in the latter, the presence of the c-*fos* (or c-*myc*) 3′ end in an otherwise stable heterologous message should impart instability. Therefore, we transfected into cells a truncated β-globin gene fused to an MLV LTR and two derivatives that substitute the 3′ end of β-globin with the 3′ end from human c-*fos* and c-*myc* (Fig. 6). Each of these three recombinants produced two transcripts that differed in size by about 900 bases (Fig. 7) (probably representing

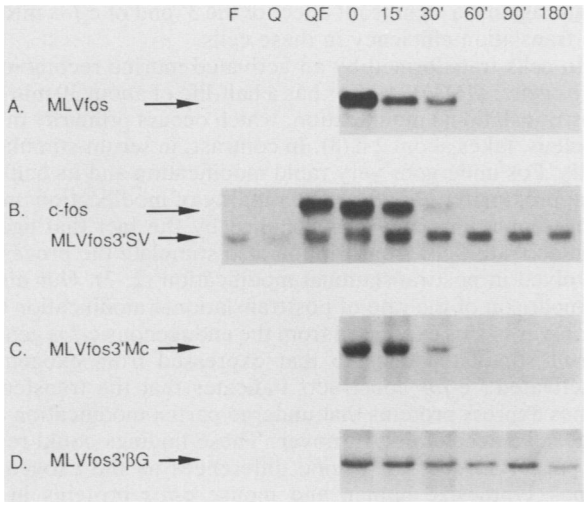


FIG. 5. Stability of messages from recombinant and endogenous c-*fos* genes. Individual clones of 208F cells transfected with each of the recombinant human c-*fos* genes (Fig. 1) were selected on the basis of transformed morphology. The cells were grown in regular medium (except for those transfected with MLV*fos*3'SV, see below) and treated with 10 μg of actinomycin D per ml. Starting 10 min later (time zero), total cellular RNA was isolated at 0, 15, 30, 60, 90, and 180 min. The RNA samples (10 μg) were denatured with glyoxal, resolved by electrophoresis, and blotted to nylon membranes which were probed with radiolabeled rat c-*fos* cDNA. These autoradiograms show the decay in the level of message from the genes after actinomycin D treatment. The cells were transfected with MLV*fos* (A), MLV*fos*3'SV (B), MLV*fos*3'Mc (C), and MLV*fos*3'βG (D). Arrows indicate the positions of the messages from the various genes. To compare the decay of these recombinant c-*fos* messages with the decay of the mRNA from the endogenous rat c-*fos* gene, the cells in panel B (which express *fos* mRNA only from the transfected gene when randomly growing [F] or rendered quiescent with defined medium [Q]) were brought to quiescence in defined medium for 48 h and stimulated with 10% fetal calf serum for 30 min (at which time maximal endogenous c-*fos* expression is observed [QF]). At this time, they were treated with 10 μg of actinomycin D per ml, and RNA samples were harvested as described above.

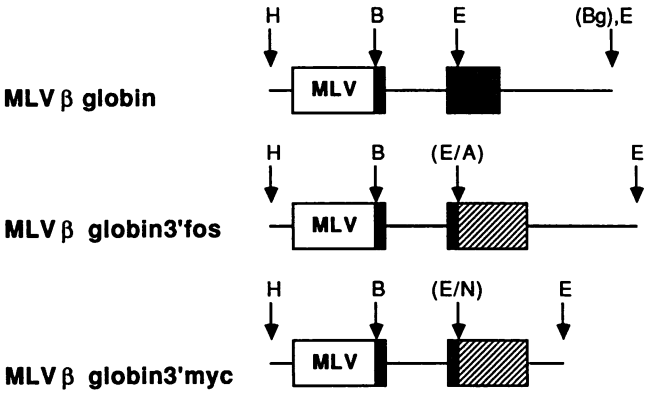


FIG. 6. Recombinant MLVβ-globin genes. A partial human β-globin gene (from the *Bam*HI site in exon 2 to the *Bgl*II site 3' to the gene) was brought under the transcriptional control of a partial MLV LTR (MLVβ-globin). The 3' portion of the β-globin gene was replaced with the 3' end of the human c-*fos* gene (MLVβ-globin3'*fos*) or with the 3' end of the human c-*myc* gene (MLVβ-globin3'*myc*). The conventions used in this figure are similar to those in Fig. 1, except that the darkly shaded boxes represent human β-globin exons and the hatched boxes represent the 3' end of the human c-*fos* or c-*myc* gene. Relevant restriction sites are indicated by arrows: H, *Hin*dIII; A, *Avr*II; E, *Eco*RI; N, *Nsi*I; B, *Bam*HI; and Bg, *Bgl*II. Sites lost in cloning are surrounded by parentheses. Details of the subcloning procedures are outlined in Materials and Methods. The drawings are not to exact scale.

spliced and unspliced forms of the β-globin moiety of the messages), and while the MLVβ-globin transcripts were stable (Fig. 7A; the dark upper band seen in the 30-min lane was not seen in other experiments), transcripts from MLVβ-globin3'*fos* and MLVβ-globin3'*myc* were much less stable (Fig. 7B and C). These data demonstrate that the 3' ends of the normal c-*fos* and c-*myc* messages destabilize the fusion message and, therefore, bear sequences that impart instability to covalently linked mRNA.

## DISCUSSION

The 3' UTR of the human c-*fos* gene and mRNA inhibits transformation despite transcriptional augmentation by a retroviral LTR, and transforming activity is revealed by replacing this segment with the 3' region of the SV40 T-antigen gene. The 3' UTR previously has been found also to inhibit the transformation activity of the murine c-*fos* gene, and a 67-bp segment within this region has been identified as being the responsible element (22). A clue to the mechanism behind this inhibitory effect was provided by the finding that 3' c-*fos* sequences are responsible for the transient nature of c-*fos* mRNA accumulation after serum stimulation (36) and by studies on the GM-CSF gene and message suggesting that A+U-rich sequences contained within the 3' UTR of certain mRNAs (including that of c-*fos*) impart mRNA instability (34). More recently, murine c-*fos* mRNA has been shown to be stabilized by removal of sequences at the 3' end of the message (30). A similar situation exists for human c-*fos* mRNA. Hybrid Fos-encoding mRNAs are substantially more stable when the 3' element comes from genes that produce relatively stable transcripts, but not when the 3' element comes from an unstable mRNA (e.g., c-*myc* [19]). Furthermore, the 3' end of human c-*fos* mRNA imparts instability on an otherwise stable transcript. These results showed that elements within the 3' UTR of the human c-*fos* transcript confer instability on the entire mRNA. The stabilities of the different recombinant Fos-encoding hybrid mRNAs determine their steady-state levels which, in turn, dictate the levels of normal human Fos produced. Since Fos can transform rat fibroblasts by sustained augmented expression (23), the effect of 3' c-*fos* substitutions on message stability can account for the transforming potential of our altered c-*fos* gene, MLV*fos*3'SV.

Our studies on the turnover of human recombinant c-*fos* and rat endogenous c-*fos* transcripts led us to examine their stabilities under a variety of conditions. The experiments performed on cells expressing the various transfected genes indicated that the recombinant and endogenous *fos* mRNAs have stabilities that do not vary appreciably at different levels of Fos expression or after serum stimulation and, thus, appear to be intrinsic properties. Furthermore, our ability to elicit similar levels of rat c-*fos* expression by serum stimulation of parental and transfected 208F cells indicates that sustained high-level Fos expression does not affect significantly the induction of endogenous c-*fos* expression. Thus, under the conditions tested, there is no apparent autoregulatory effect of Fos.

Our studies did not define the destabilizing element within the human c-*fos* 3' UTR. However, A+U-rich sequences

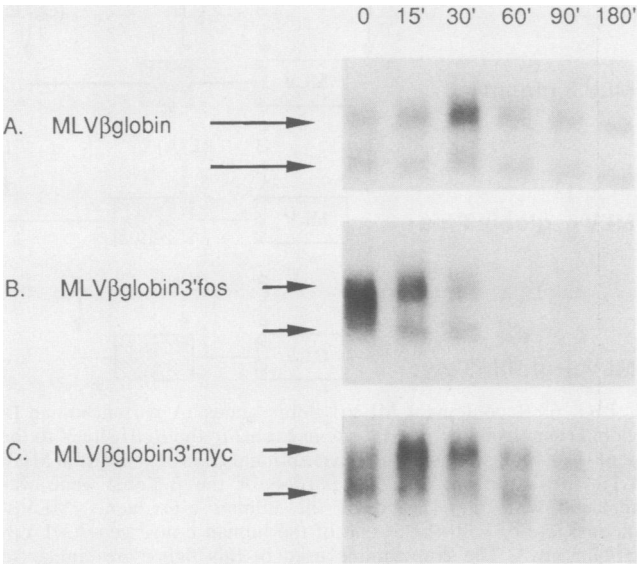


FIG. 7. Stability of mRNAs from the recombinant MLVβ-globin genes. 208F cells cotransfected with each of the three MLVβ-globin recombinants (Fig. 6) and a neomycin resistance gene were selected with G418, and hundreds of colonies were pooled. The cells were treated with 10 μg of actinomycin D per ml, and starting 10 min later (time zero), total cellular RNA was isolated at 0, 15, 30, 60, 90, and 180 min. The RNA samples (10 μg) were denatured with glyoxal, resolved by electrophoresis, and blotted to nylon membranes. These were probed with radiolabeled DNA representing the 3′ ends of the genes used to transfect the cells. These autoradiograms show the decay in the level of message from the genes after actinomycin D treatment. (A) The cells were transfected with MLVβ-globin, and the blot was probed with the radiolabeled *Eco*RI-*Bgl*II fragment of the human β-globin gene; (B) the cells were transfected with MLVβ-globin3′*fos*, and the blot was probed with the radiolabeled *Avr*II-*Eco*RI fragment of the human c-*fos* gene; (C) the cells were transfected with MLVβ-globin3′*myc*, and the blot was probed with the radiolabeled *Nsi*I-*Eco*RI fragment of the human c-*myc* gene. Arrows indicate the positions of the messages from the various genes; each produces two sizes of message compatible with spliced and unspliced forms of the β-globin part of the message.

and the AUUUA motif contained in this region are potential candidates (as suggested by Shaw and Kamen [34]). Both reside in the region corresponding to the 67-base transformation inhibitory sequence of murine c-*fos* (22), and the human and murine genes differ in this region by only two nucleotides (22, 39). The 3′ end of the c-*myc* mRNA, which also inhibits transformation when attached to a Fos-encoding message, has a region strongly resembling the A+U-rich stretch of c-*fos* (3, 19), whereas the 3′ sequences of the SV40 T-antigen and β-globin mRNAs do not (28, 35). Because the 3′ ends of c-*myc* and c-*fos* impart instability on an attached heterologous (partial β-globin) message, the destabilizing influence of these 3′ UTRs appears to be intrinsic to these sequences and is not due to their interactions with the remainder of the c-*fos* mRNA, e.g., by formation of specific secondary structures.

It has been claimed that c-*fos* expression may be regulated at the level of translation and that the 3′ end of the mRNA may play a role in this regulation (24). Our studies in rat fibroblasts show a direct correlation between steady-state *fos* mRNA levels expressed from several different *fos* constructs and the level of Fos produced and are in agreement with previous work (9, 25–27) that suggests a close concordance between c-*fos* mRNA and protein levels. These data argue against a significant effect of the 3′ end of c-*fos* mRNA on translation efficiency in these cells.

In cells transformed by an activated murine recombinant c-*fos* gene, MMV (24), Fos has a half-life of about 30 min and posttranslational modification, which occurs primarily in the nucleus, takes about 2 h (8). In contrast, in serum-stimulated cells, Fos undergoes very rapid modification and its half-life is approximately 2 h (26). The rapidity of modification under these conditions may be explained by the fact that agents that activate c-*fos* transcription also stimulate the processes involved in posttranslational modification (2, 7). Our direct comparison of the rate of posttranslational modification and turnover of Fos expressed from the endogenous c-*fos* gene in serum-stimulated cells to that expressed from exogenous "activated" c-*fos* constructs indicates that the transfected genes express proteins that undergo partial modification and that have accelerated turnover. These findings could result in part from species-specific differences as the exogenous genes synthesize human and mouse c-*fos* proteins in rat cells. However, our results are consistent with the suggestion that extracellular stimuli promote the modification process (2, 9) and that cells which constitutively overproduce Fos from transfected genes lack the stimuli required for rapid modification. Conceivably, cellular transformation by the *fos* oncogene may be a consequence of prolonged exposure of the cell to partially modified *fos* proteins, suggesting that posttranslational modification fulfills a crucial regulatory function. Indeed, one of the effects of the various mutations and deletions that have occurred in the FBJ and FBR murine sarcoma virus v-*fos* genes is a reduction in the extent of posttranslational modification (2, 8, 37, 38).

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## LITERATURE CITED

1. **Auffray, C., and F. Rougeon.** 1980. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. Eur. J. Biochem. **107**:303–314.
2. **Barber, J. R., and I. M. Verma.** 1987. Modification of *fos* proteins: phosphorylation of c-*fos*, but not v-*fos*, is stimulated by 12-tetradecanoyl-phorbol-13-acetate and serum. Mol. Cell. Biol. **7**:2201–2211.
3. **Battey, J., C. Boulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder.** 1983. The human c-*myc* oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell **34**:779–787.
4. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
5. **Cochran, B. H., J. Zullo, I. M. Verma, and C. D. Stiles.** 1984. Expression of the c-*fos* oncogene and a newly discovered "r-*fos*" is stimulated by platelet-derived growth factor. Science **226**:1080–1082.

5a. **Curran, T.** 1988. The *fos* oncogene, p. 307–326. *In* E. P. Reddy, A. M. Skalka, and T. Curran (ed.), The oncogen handbook. Elsevier, Amsterdam.

6. **Curran, T., M. B. Gordon, K. L. Rubino, and L. C. Sambucetti.** 1987. Isolation and characterization of the c-*fos* (rat) cDNA and analysis of post-translational modification in vitro. Oncogene **2**:79–84.

7. **Curran, T., W. P. MacConnell, F. van Straaten, and I. M. Verma.** 1983. Structure of the FBJ murine osteosarcoma virus genome: molecular cloning of its associated helper virus and the cellular homolog of the v-*fos* gene from mouse and human cells. Mol. Cell. Biol. **3**:914–921.
8. **Curran, T., A. D. Miller, L. Zokas, and I. M. Verma.** 1984. Viral and cellular *fos* proteins: a comparative analysis. Cell **36**:259–268.
9. **Curran, T., and J. I. Morgan.** 1986. Barium modulates c-*fos* expression and post-translational modification. Proc. Natl. Acad. Sci. USA **83**:8521–8524.
10. **Curran, T., G. Peters, C. Van Beveren, N. M. Teich, and I. M. Verma.** 1982. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J. Virol. **44**:674–682.
11. **Curran, T., and N. M. Teich.** 1982. Candidate product of FBJ murine osteosarcoma virus oncogene: characterization of a 55,000 dalton phosphoprotein. J. Virol. **42**:114–122.
12. **Curran, T., and N. M. Teich.** 1982. Identification of a 39,000 dalton protein in cells transformed by FBJ murine osteosarcoma virus. Virology **116**:221–235.
13. **Curran, T., C. van Beveren, N. Ling, and I. M. Verma.** 1985. Viral and cellular *fos* proteins are complexed with a 39,000-dalton cellular protein. Mol. Cell. Biol. **5**:167–172.
14. **Curran, T., and I. M. Verma.** 1984. The FBR murine osteosarcoma virus. I. Molecular analysis and characterization of a 75,000 Da *gag-fos* fusion product. Virology **135**:218–228.
15. **Distel, R. J., H.-S. Ro, B. S. Rosen, D. L. Groves, and B. M. Spiegelman.** 1987. Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-*fos*. Cell **49**:835–844.
16. **Franza, B. R., Jr., F. J. Rauscher, III, S. F. Josephs, and T. Curran.** 1988. The Fos-complex and Fos-related antigens recognize sequence elements that contain AP-1 sites. Science **239**: 1150–1153.
17. **Franza, B. R., L. C. Sambucetti, D. R. Cohen, and T. Curran.** 1987. Analysis of Fos protein complexes and Fos-related antigens by high-resolution two-dimensional gel electrophoresis. Oncogene **1**:213–221.
18. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–438.
19. **Jones, T. R., and M. D. Cole.** 1987. Rapid cytoplasmic turnover of c-*myc* mRNA: requirement of the 3′ untranslated sequences. Mol. Cell. Biol. **7**:4513–4521.
20. **Kruijer, W., J. S. Cooper, T. Hunter, and I. M. Verma.** 1984. Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. Nature (London) **312**: 711–716.
21. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange Proc. Natl. Acad. Sci. USA **74**:4835–4838.
22. **Meijlink, F., T. Curran, A. D. Miller, and I. M. Verma.** 1985. Removal of a 67 base pair sequence in the noncoding region of the proto-oncogene *fos* converts it to a transforming gene. Proc. Natl. Acad. Sci. USA **82**:4987–4991.
23. **Miller, A. D., T. Curran, and I. M. Verma.** 1984. c-*fos* protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. Cell **36**:51–60.
24. **Mitchell, R. L., L. Zokas, R. D. Schreiber, and I. M. Verma.** 1985. Rapid induction of the expression of proto-oncogene *fos* during human monocyte differentiation. Cell **40**:209–217.
25. **Morgan, J. I., and T. Curran.** 1986. Role of ion flux in the control of c-*fos* expression. Nature (London) **322**:552–555.
26. **Muller, R., R. Bravo, J. Burckhardt, and T. Curran.** 1984. Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature (London) **312**:716–720.
27. **Muller, R., T. Curran, D. Muller, and L. Guilbert.** 1986. Wounding a fibroblast monolayer results in the rapid induction of the c-*fos* proto-oncogene. EMBO J. **5**:913–917.
28. **Poncz, M., E. Schwartz, M. Ballantine, and S. Surrey.** 1983. Nucleotide sequence analysis of the δβ-globin gene region in humans. J. Biol. Chem. **258**:11599–11609.
29. **Quade, K.** 1979. Transformation of mammalian cells by avian myelocytomatosis virus and avian erythroblastosis virus. Virology **98**:161–165.
30. **Rahmsdorf, H. J., A. Schonthal, P. Angel, M. Litfin, U. Ruther, and P. Herrlich.** 1987. Post-transcriptional regulation of c-*fos* mRNA expression. Nucleic Acids Res. **15**:1643–1659.
31. **Rauscher, F. J., L. C. Sambucetti, T. Curran, R. J. Distel, and B. M. Spiegelman.** 1988. A common DNA binding site for *fos* protein complexes and transcription factor AP-1. Cell **52**:471–480.
32. **Renz, M., B. Vernier, C. Kurz, and R. Muller.** 1987. Chromatin association and DNA binding properties of the c-*fos* proto-oncogene product. Nucleic Acids Res. **15**:277–292.
33. **Sambucetti, L. C., and T. Curran.** 1986. The Fos protein complex is associated with DNA in isolated nuclei and binds to DNA cellulose. Science **234**:1417–1419.

33a. **Rauscher, F. J., D. R. Cohen, T. Curran, T. J. Bos, P. K. Vogt, D. Bohmann, R. Tjian, and B. R. Franza, Jr.** 1988. Fos-associated protein (p39) is the product of the *jun* proto-oncogene. Science **240**:1010–1016.

34. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659–667.
35. **Tooze, J. (ed.).** 1981. DNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Treisman, R.** 1985. Transient accumulation of c-*fos* RNA following serum stimulation requires a conserved 5′ element and c-*fos* 3′ sequences. Cell **42**:889–902.
37. **van Beveren, C., F. van Straaten, T. Curran, R. Muller, and I. M. Verma.** 1983. Analysis of FBJ-MuSV provirus and c-*fos* (mouse) gene reveals that viral and cellular *fos* gene products have different carboxy termini. Cell **32**:1241–1255.
38. **van Beveren, C., S. Enami, T. Curran, and I. M. Verma.** 1984. FBR murine osteosarcoma virus. II. Nucleotide sequence of the provirus reveals that the genome contains sequences derived from two cellular genes. Virology **135**:229–243.
39. **van Straaten, F., R. Muller, T. Curran, C. van Beveren, and I. M. Verma.** 1983. Complete nucleotide sequence of a human c-*onc* gene: deduced amino acid sequence of the human c-*fos* protein. Proc. Natl. Acad. Sci. USA **80**:3183–3187.