MOLECULAR AND CELLULAR BIOLOGY, Aug. 1988, p. 3244–3250
0270-7306/88/083244-07$02.00/0

Vol. 8, No. 8

Determinants That Contribute to Cytoplasmic Stability of Human c-*fos* and β-Globin mRNAs Are Located at Several Sites in Each mRNA

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Received 11 December 1987/Accepted 3 May 1988

We have analyzed the contributions to cytoplasmic stability in an mRNA species with a very short half-life (human c-*fos*) and an mRNA species with a very long half-life (human β-globin). When the human c-*fos* promoter was used to drive the expression of human c-*fos*, β-globin, and chimeric DNAs between c-*fos* and β-globin in transfected cells, a pulse of mRNA synthesis was obtained following induction of transcription by refeeding quiescent cells with medium containing 15% calf serum. The mRNA half-life was determined by using Northern (RNA) blot analysis of mRNAs prepared at various times following the pulse of transcription. Under these conditions human c-*fos* mRNA exhibited a half-life of 6.6 min and human β-globin mRNA exhibited a half-life of 17.5 h. Replacement of the 3′ end of the c-*fos* mRNA with the 3′ end of the β-globin mRNA increased the half-life of the resultant RNA from 6.6 to 34 min. The reciprocal chimera had a half-life of 34.6 min compared with the 17.5-h half-life of β-globin mRNA. These results suggest that sequences which make a major contribution to mRNA stability reside in the 3′ end of either or both molecules. A chimera in which the 5′ untranslated region of globin was replaced by part of the 5′ untranslated region of *fos* led to destabilization of the encoded mRNA. This construct produced an mRNA with a half-life of 6.8 h instead of the 17.5-h half-life of globin. This result suggests that additional determinants of stability reside in the 5′ end of these mRNA molecules. Substitution of part of the 5′ untranslated region of *fos* by the 5′ untranslated region of β-globin yielded an mRNA with stability similar to *fos* mRNA. These results suggest that interactions among sequences within each mRNA contribute to the stability of the respective molecules.

Regulation of mRNA stability is likely to play an important role in controlling gene expression in a broad range of contexts in eucaryotic cells. Analysis of the structural features which contribute to mRNA stability has been attempted only quite recently, however. The technical issues presented by the use of [^{3}H]uridine labeling (3, 11, 25) or chemical inhibitors of transcription (26, 28, 29, 32) to create a pulse-chase scenario in which mRNA half-lives can be measured are a significant limitation in such studies. To address this question, we have turned to the use of an inducible eucaryotic promoter, c-*fos*, which produces a short pulse of mRNA synthesis in response to an inductive stimulus (10, 14). Using this system, we have examined the stability determinants of the human c-*fos* and β-globin mRNAs by analyzing chimeric constructs which contain different segments of each of these mRNAs transcribed under the control of the c-*fos* promoter. Using Northern (RNA) blotting to analyze the level of cytoplasmic RNA present at various times after stimulation of the *fos* promoter, we have obtained a measurement of mRNA stability for each chimeric RNA, as well as for the parental RNAs. These results suggest that the RNA structures which contribute to the long half-life of the β-globin mRNA and the short half-life of the c-*fos* mRNA are not located at a single site in either mRNA molecule. These data are most compatible with the view that a number of sites or structures on each mRNA molecule or an interaction between the two ends of each mRNA contribute to its overall stability.

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MATERIALS AND METHODS

Cells and tissue culture. All cells were grown in Dulbecco modified Eagle medium with 2 mM glutamine. The medium was supplemented with 10% calf serum for growth (GIBCO Laboratories, Grand Island, N.Y.), 5% platelet-poor plasma (see below) for growth factor deprivation, or 15% calf serum for growth factor refeeding. Transfections were selected in 0.5 mg of active G418 per ml 2 days after the transfection procedure (31). Cells were maintained in this concentration of G418 until initial freezing for maintenance of the cell line. Cells were thawed into the medium with 0.25 mg of G418 per ml for approximately 12 h and then placed in 0.5 mg of G418 per ml. Experiments were all performed in the absence of G418. All experiments were performed on cells which were between 30 and 100% confluent. The *fos* mRNA does not show complete induction in cells below 30% confluence as described by Rollins et al. (24).

Platelet-poor plasma. Human plasma obtained from the American Red Cross was spun at 25,000 × *g* for 20 min to remove platelets and fat from the top. It was heated to 56°C for 30 min and centrifuged at 14,000 × *g* for 20 min. The supernatant was dialyzed against 0.15 M NaCl (6,000-molecular-weight cutoff) overnight and recentrifuged at 30,000 × *g* for 1.5 to 2 h. The fat was again aspirated off the top, and the supernatant was filter sterilized and stored in aliquots at −20°C.

Construction of *fos*-globin junction for GLO5 and derivative plasmids (see Fig. 1b). GLO5 was constructed to produce globin RNA (with the globin capping sequence) from the *fos* promoter. The junction was created by using a mutagenic oligonucleotide from nucleotides −16 to −4 of *fos* connected to nucleotides −4 to +8 of globin. The complementary DNA

sequence between the *fos* and globin regions of the mutagenic oligonucleotide were made to loop out and become degraded upon transformation. Single-stranded M13-*fos*-globin DNA was purified and annealed to the phosphorylated mutagenic oligonucleotide and to a universal M13 priming oligonucleotide. For the annealing reaction, 20 pmol of phosphorylated mutagenic oligomer, 20 pmol of unphosphorylated universal primer, and 1 pmol of single-stranded M13-*fos*-globin template were incubated at 65°C for 10 min in 20 mM Tris (pH 7.5)–10 mM $MgCl_2$–50 mM NaCl–1 mM dithiothreitol in a total volume of 10 μl. After 5 min at room temperature the tube was placed on ice. The DNA was then incubated overnight at 15°C with Klenow fragment to extend the second strand started by the oligonucleotides and with T4 DNA ligase to covalently bond the ends. An equal volume of extension-ligation mixture (20 mM Tris [pH 7.5], 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM each deoxynucleoside triphosphate, 1 mM ATP, 3 U of T4 DNA ligase, 2.5 U of Klenow fragment of DNA polymerase I) was added to the annealing reaction mixture, mixed, and incubated overnight at 15°C. This DNA mixture was used to transform JM101 that was supplemented with exponentially growing JM101 and plated on soft agar. Forty-eight M13 plaques were grown up as minipreps, the supernatant was saved as a virus stock, and the bacteria were treated with normal miniprep procedures to isolate the replicative form of each virus. The DNAs were first screened by digestion with *Pst*I, the site for which was contained in the looped sequence. Fourteen of these had lost the *Pst*I site and were further checked by fine restriction mapping on acrylamide gels. The version that was used for the construction of GLO5 and derivative plasmids (GLOFOS7 and GLOFOS8) was sequenced and shown to be exactly as expected.

Construction of plasmids. Chimeric constructs between human c-*fos* and β-globin were designed so that breakpoint sites would minimally disrupt the *fos* and globin proteins. Human c-*fos*, FOSGLO4, and GLOFOS7 all encode intact c-*fos* proteins. Human β-globin (GLO5) and FOSGLO3 both encode intact β-globin proteins. GLOFOS8 encodes a hybrid protein in which 29 amino acids have been removed from the C terminus of globin and replaced by 12 amino acids when connected to the 3′ end of *fos*. In no case was an open reading frame introduced or splicing interrupted. In each case the single cytoplasmic RNA was the expected size on the basis of comparison with 28S and 18S rRNA bands on the photographs of the gels prior to transfer. All constructs used the poly(A) addition signal carried on the 3′ end of that mRNA. For human c-*fos*, GLOFOS7 and GLOFOS8, the poly(A) signal from the human c-*fos* gene was used. For GLO5, FOSGLO3, and FOSGLO4, the poly(A) signal from the human β-globin gene was used. Standard DNA manipulations were carried out as described by Maniatis et al. (18). PUCFOS1 was constructed by the ligation of pUC19 cut with *Sal*I and *Pst*I with the 3.9-kilobase-pair (kb) *Xho*I-*Nsi*I fragment of human c-*fos* (5, 33). FOSGLO3 was constructed by ligation of the 3.4-kb *Nae*I-*Hin*dIII fragment of PUCFOS1 with the 3.13-kb *Nco*I (blunt)-*Hin*dIII fragment of human β-globin (8). FOSGLO4 was constructed by ligation of the 6.44-kb *Ssp*I (partial)-*Hin*dIII fragment of PUCFOS1 with the 1.8-kb *Bst*XI (blunted)-*Hin*dIII fragment of human β-globin. GLO5 was constructed by ligation of the 0.7-kb *Apa*I-*Bam*HI fragment of deleted M13-*fos*-globin (see above and Fig. 1b) to the 5.42-kb *Apa*I-*Bam*HI (partial) fragment of FOSGLO3. PUCFOS10 was constructed by the ligation of pUC19 cut with *Hin*dIII and *Sal*I with the 5.2-kb *Hin*dIII-*Xho*I fragment of human c-*fos*. GLOFOS7 was constructed by ligation of the 3.26-kb *Nco*I (blunted)-*Hin*dIII fragment of GLO5 with 4.4-kb *Nae*I (partial)-*Hin*dIII fragment of PUCFOS10. GLOFOS8 was constructed by ligation of the 4.3-kb *Bst*XI (blunted)-*Hin*dIII fragment of GLO5 with the 1.4-kb *Ssp*I (partial)-*Hin*dIII fragment of PUCFOS10.

Transfection. Plasmid DNA was transfected into NIH 3T3 cells by using the calcium phosphate coprecipitation technique (23). Since the plasmids did not carry the neomycin resistance gene (Tn*5*), each was cotransfected at a molar ratio of 10:1 with pSV2neo (31). After transfection the cells were washed well with serum-free medium and either split 1/5 into medium if confluent or nearly confluent or placed in medium at 37°C. Two days later the cells were selected in G418. No G418-resistant colonies were observed in mock-transfected controls. After selection in G418, roughly 24 colonies from each transfection were cloned out. Each cell line was grown up in three 100-mm tissue culture dishes. One dish of each colony was frozen immediately, and the other two were used to screen the cell lines for induction of the *fos* promoter-driven mRNA. Both of these were fed media containing 5% platelet-poor plasma for 1 to 2 days. One was then fed media containing 15% calf serum to stimulate the *fos* promoter. Total cellular RNA was isolated and run on a formaldehyde gel for Northern blot analysis to determine the extent of *fos*-induced mRNA in that cell line.

RNA gel and transfer to Northern Blot. Total RNA (for screening cell lines) was isolated by a modification of the method of Chirgwin et al. (4). Cytoplasmic RNA was isolated for experiments as described by Maniatis et al. (18). RNA was run in a denaturing agarose gel system containing formaldehyde as described in the literature accompanying the GeneScreen Plus (New England Nuclear Corp., Boston, Mass.) filter membrane onto which it was electrotransferred.

Hybridization and analysis of blots. A section of the globin gene, a 2.6-kb piece of genomic DNA from *Nco*I to *Pst*I, was used as probe for specific detection of constructs GLO5 (human β-globin), FOSGLO3, FOSGLO4, and GLOFOS8. To distinguish exogenous human c-*fos* RNA from endogenous mouse c-*fos* RNA, we used a 0.39-kb *Nae*I fragment of human c-*fos* DNA as probe for human c-*fos* and GLOFOS7. This *Nae*I-*Nae*I fragment was chosen because it diverged (by approximately 15%) from the corresponding mouse gene and was high in G+C content. It encodes 255 bases that appear in the mature mRNA, including a 5-base-pair insertion. When hybridized at 72°C and washed under stringent conditions (0.1× SSCPE [20× SSCPE is 3 M NaCl, 0.3 M sodium citrate, 0.3 M KH_2PO_4, plus 20 mM EDTA] at 60°C), this fragment will anneal specifically to human c-*fos* RNA and not to mouse c-*fos* RNA. Baked blots were prehybridized overnight in 1 M NaCl–10% dextran sulfate–1% sodium dodecyl sulfate (SDS) in a rotating H_2O bath at 63°C, except for blots to be hybridized with the human c-*fos* probe, which were prehybridized at 72°C. For hybridization the prehybridization mixture was replaced with the fresh mixture described above plus 100 μg of salmon sperm DNA per ml and probe, which had been boiled together for 10 min. Hybridization was performed for 18 to 24 h as described for the prehybridization. DNA was labeled by using OLB (oligo labeling buffer) as described by Feinberg and Vogelstein (7) with the following modification. DNA was not isolated from the gel, but was run in an agarose gel made with low-melting-point agarose. The reaction was performed in the presence of agarose. All probes were run in agarose, except for the 0.39-kb human c-*fos* DNA, which was isolated from the gel because of its small size. Blots were washed as follows: twice at room temperature for 20 min each in 2× SSCPE–1%

SDS, twice at 55°C for 30 min each in 2× SSCPE–1% SDS, twice at 55°C for 30 min each in 0.5× SSCPE–1% SDS, and then twice at 55°C for 30 min each in 0.25× SSCPE–0.5% SDS. Blots hybridized with human c-*fos* DNA were further washed twice at 60°C for 30 min each in 0.1× SSCPE–0.5% SDS. The blots were then rinsed in 1× SSCPE briefly, wrapped in Saran Wrap, and exposed to film. The cytoplasmic RNA signal was quantified by using an Ultroscan Scanning Densitometer (LKB Instruments, Inc., Rockville, Md.). Film was preflashed such that the optical density of the film increased roughly 0.15 optical density units at 540 nm relative to unflashed film to ensure linearity of the signal (15).

Method for determination of half-lives. The half-life of the RNA ($t_{1/2}$) was defined as $t_{1/2} = 0.693/k$, where $k = 2.303 \times$ slope of the best-fit line through the datum points of the $\log_{10}$ of the concentration of the reactant plotted against time (19). The slope of the best-fit line through such datum points was used to calculate the k value in the above equation to determine the half-lives of the respective RNAs. Half-lives and standard errors of half-lives (see Fig. 6) were calculated by using these slopes, the errors of the best-fit lines, and the above equation. The t statistic was calculated by dividing the half-life of a given RNA by its standard error. Using this value and the degrees of freedom in the model (1 less than the number of datum points), the P value was determined from a t-value table (the distribution of t) in Snedecor and Cochran (30). If $P < 0.05$, the model of decay of cytoplasmic RNA with time was considered acceptable.

RESULTS

Determination of half-lives. The structures of the chimeras constructed between human c-*fos* and human β-globin genes are diagrammed in Fig. 1a. Each is under transcriptional control of the human c-*fos* promoter. GLO5 (human β-globin) was constructed so that transcription from the globin capping site would be driven from the *fos* promoter. The construct was made via an M13 intermediate in which the *fos*-globin junction was created by using an oligonucleotide (Fig. 1b). Sequencing confirmed that the *fos*-globin junction of GLO5 was 4 bases upstream of the globin capping site and that the *fos* capping site had been removed, thus producing an intact β-globin mRNA transcribed under the control of the *fos* promoter. The chimeras GLOFOS7 and GLOFOS8, derived from GLO5, share transcriptional initiation sequences with GLO5 and hence give rise to chimeric mRNAs with 5′ β-globin sequences and 3′ *fos* sequences transcribed under the control of the *fos* promoter. All chimeras constructed give rise to mRNAs with intact open reading frames (see legend to Fig. 1a).

Each construct (human c-*fos*, GLO5 [human β-globin], FOSGLO3, FOSGLO4, GLOFOS7, and GLOFOS8 [Fig. 1a]) was separately cotransfected into BALB/c-3T3 cells along with pSV2neo (31). Individual clones were screened for transcription of the introduced DNA in response to refeeding of quiescent cells (cells maintained in media containing 5% platelet-poor plasma) with medium containing 15% calf serum. For each construct a highly inducible clone was identified which gave rise to an mRNA species of the expected size. The effectiveness of the shutoff of transcription driven by the constructs under our experimental conditions can be assessed by the data presented in Fig. 2 and 3. In Fig. 2, levels of endogenous c-*fos* mRNA have been determined by quantitative Northern blotting experiments. The highest level of c-*fos* mRNA occurs 35 min after serum

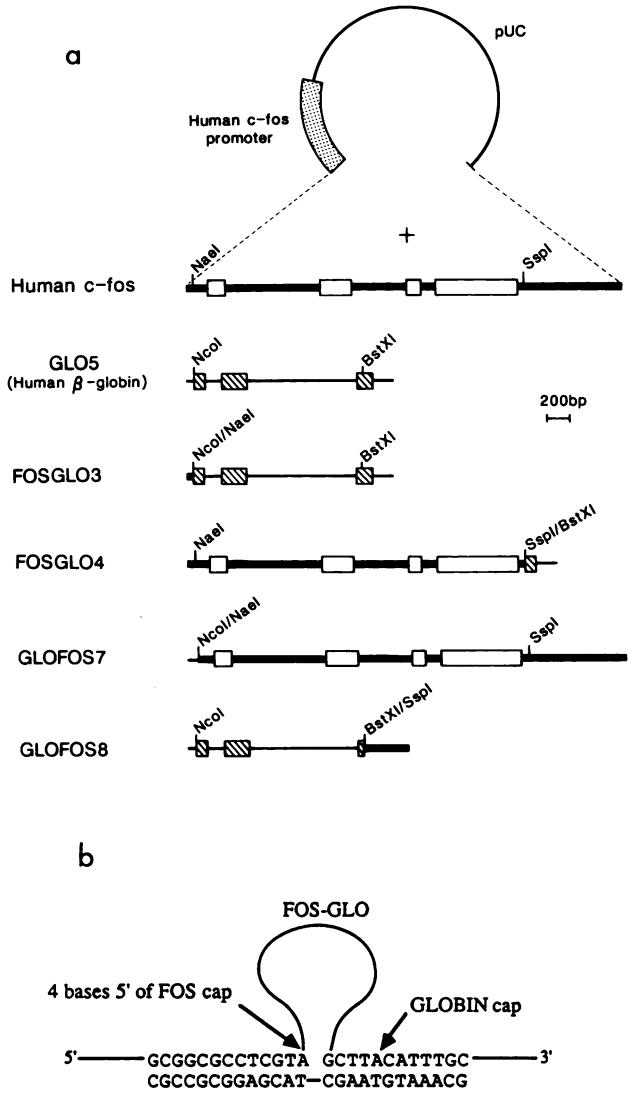

FIG. 1. (a) Genomic, unspliced versions of human c-*fos*, GLO5, FOSGLO3, FOSGLO4, GLOFOS7, and GLOFOS8. Symbols: ▬, *fos* 5′ and 3′ untranslated regions and intervening sequences; ▭, *fos* protein-coding regions; ——, globin 5′ and 3′ untranslated regions and introns; ▨, globin protein-coding sequences. The scale is shown. (b) Construction of M13 intermediate for construction of GLO5. The upper line represents the sense strand of M13 containing the junction between the *fos* promoter and the 5′ region of globin, including the part to be deleted (looped line). The lower line is a 25-mer that was made to span exactly the region of *fos* and globin which was to be deleted.

stimulation, and this mRNA level returns to background levels by 60 min poststimulation. These results indicate that this mRNA is produced in a rapid pulse following serum stimulation and that the shutoff of transcription is rapid and complete. The results shown in Fig. 3, with the introduced human c-*fos* mRNA, demonstrate that the introduced mRNA sequences are transcribed in an analogous pulse. The results shown in Fig. 4 and 5, as well as the results of all additional experiments performed in the course of determining mRNA stability, demonstrate similar kinetics for the induction and shutoff of transcription from *fos*-driven constructs. Translation of an RNA may affect its stability (9, 13, 17). Chimeric mRNAs are likely to be translated, since they

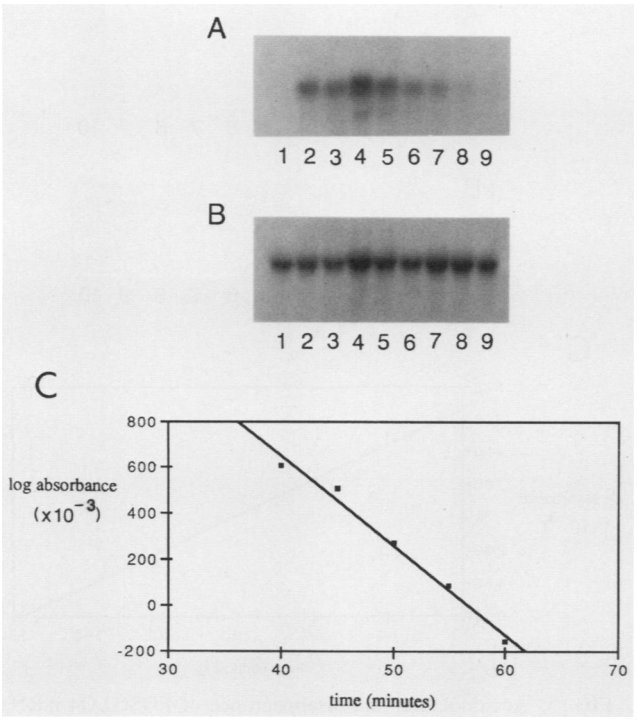

FIG. 2. Accumulation and disappearance of mouse c-*fos* mRNA. Northern blot of cytoplasmic RNAs extracted from 1C6 cells (harboring exogenous human c-*fos*) after 0, 25, 30, 35, 40, 45, 50, 55, or 60 min (lanes 1 to 9, respectively) of refeeding with medium containing 15% calf serum after 2 days of growth arrest in 5% plasma. (A) Blot hybridized with mouse c-*fos* DNA under normal conditions. (B) Same blot rehybridized with rat α-tubulin. (C) Best-fit line of the normalized mouse c-*fos* absorbances plotted against time.

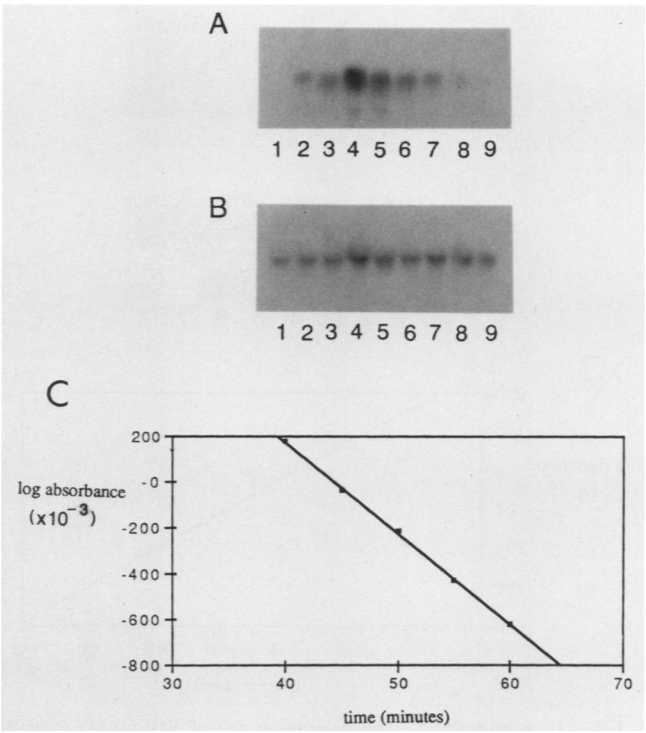

FIG. 3. Accumulation and disappearance of human c-*fos* mRNA in 1C6 cells. Northern blot of cytoplasmic RNAs extracted from 1C6 cells after 0, 25, 30, 35, 40, 45, 50, 55, or 60 min (lanes 1 to 9, respectively) of refeeding with media containing 15% calf serum after 2 days of growth arrest in 5% plasma. (A) Blot hybridized with the 0.39-kb *Nae*I fragment of human c-*fos* at 72°C and washed with 0.1× SSCPE at 60°C. (B) Same blot rehybridized with rat α-tubulin. (C) Best-fit line of the normalized *fos* absorbances plotted against time.

are associated with polysomes and can be dissociated from polysomes by puromycin treatment (data not shown), which causes a functional dissociation of the mRNA from the ribosomes as demonstrated by Blobel and Sabatini (2) and Blobel (1).

To measure mRNA half-lives, quantitative Northern blotting experiments for each construct were performed. Preliminary experiments were used to identify the appropriate intervals for mRNA sampling for each construct. mRNA half-lives were then determined by pulse induction of the c-*fos* promoter and extraction of cytoplasmic RNA at a series of subsequent time points. Each Northern blot was initially hybridized to a probe specific for the introduced DNA (Fig. 2A to 5A) and subsequently rehybridized with rat α-tubulin cDNA (16) as a normalizing control (Fig. 2B to 5B). The normalized band of highest intensity was taken as the starting time point, and the normalized band of lowest intensity above the constitutive level was taken as the final time point. Representative experiments are shown in Fig. 2 to 5. The data obtained are shown beside the schematized mRNA for each construct in Fig. 6 and summarized in Table 1. The half-life of the endogenous mouse c-*fos* transcript was determined by rehybridization with mouse c-*fos* blots from other experiments for which an appropriate time course had been used (Fig. 6; Table 1). For each construct the data consistently fit first-order kinetics of decay with a high degree of statistical confidence (Fig. 2C to 5C; Table 1).

Effect of 3′ end on mRNA stability. To assess the effects of the 3′ ends of these mRNAs on their half-lives, we made the following comparisons (Fig. 6). In comparing human c-*fos* and FOSGLO4, we determined that the replacement of part of the 3′ untranslated region of *fos* with the 3′ untranslated region of globin plus a small stretch at the end of its third exon changed the half-life of the resultant mRNA to 34 min from 6.6 min in c-*fos* (a fivefold effect). In the reciprocal chimera, in which a portion of the 3′ untranslated region of *fos* replaced the 3′ end of globin (3′ untranslated region plus some coding sequences), comparing GLO5 (human β-globin) and GLOFOS8, the effect was even more dramatic. The 3′ untranslated region of *fos* destabilized the basic globin mRNA from one having a half-life of 17.5 h to one having a half-life of 34.6 min (a 30-fold effect).

Effect of 5′ end on mRNA stability. To consider the effects of the 5′ untranslated regions of *fos* and globin on mRNA stability, we made comparisons of (i) GLO5 and FOSGLO3 and (ii) human c-*fos* and GLOFOS7 (Fig. 6). In the former case, the entire globin 5′ untranslated region was replaced by the first 40 bases of the *fos* mRNA, including its cap. This replacement destabilized the globin mRNA from one having a half-life of 17.5 h to one having a half-life of 6.8 h (a 2.5-fold effect). In the latter case, replacement of those 40 bases of *fos* with the entire 50-base 5′ untranslated region of globin has no apparent effect. Since the half-lives of these mRNAs are very short, it cannot be determined whether they are statistically different.

Effect of coding sequences and adjacent sequences on mRNA stability. To assess the input of the body of the mRNA on the inherent stability, two comparisons between

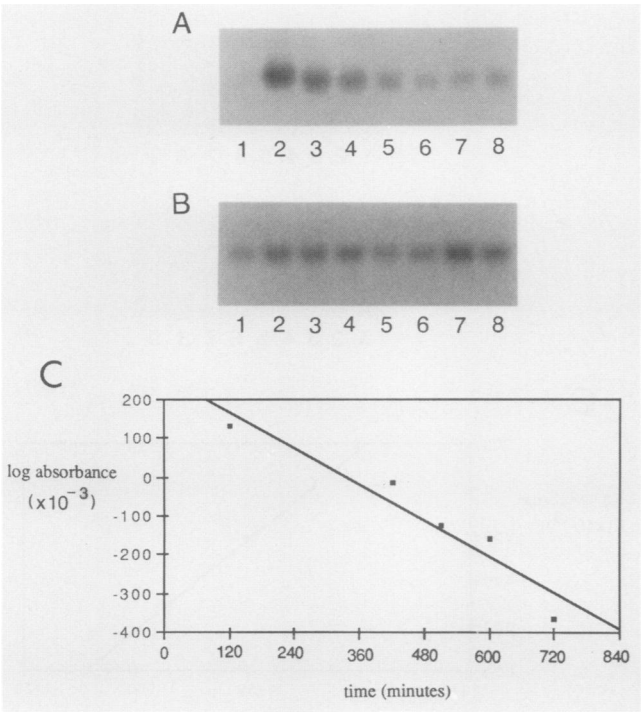

FIG. 4. Accumulation and disappearance of FOSGLO3 mRNA in 3b1a cells. Northern blot of cytoplasmic RNAs extracted from 3b1a cells after 0, 120, 420, 510, 600, 720, 1,230, or 1,590 min (lanes 1 to 8, respectively) of refeeding with medium containing 15% calf serum after 2 days of growth arrest in 5% plasma. (A) Blot hybridized with the 2.6-kb *Nco*I-*Pst*I segment of human β-globin. (B) Same blot reprobed with rat α-tubulin. (C) Best-fit line of the normalized globin absorbances plotted against time.

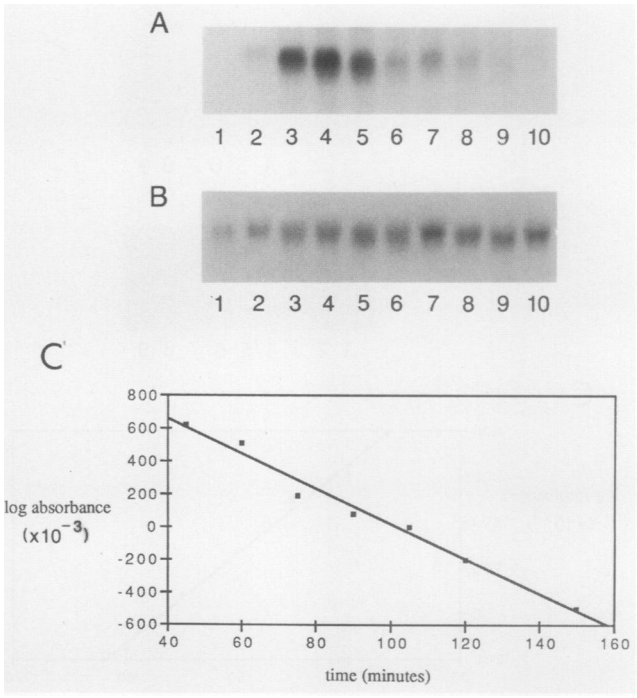

FIG. 5. Accumulation and disappearance of FOSGLO4 mRNA in 5a1 cells. Northern blot of cytoplasmic RNAs extracted from 5a1 cells after 0, 15, 30, 45, 60, 75, 90, 105, 120, or 150 min (lanes 1 to 10, respectively) of refeeding with medium containing 15% calf serum after 2 days of growth arrest in 5% plasma. (A) Blot hybridized with the 2.6-kb *Nco*I-*Pst*I segment of human β-globin. (B) Same blot reprobed with rat α-tubulin. (C) Best-fit line of the normalized globin absorbances plotted against time.

constructs can be made (Fig. 6). In comparing FOSGLO3 and FOSGLO4, it is mostly the coding regions of these hybrids that are transposed. A 12-fold destabilizing effect is conferred by the coding sequences of *fos* plus a small amount of its 3′ untranslated region, since the half-life of FOSGLO4 is 34 min and the half-life of FOSGLO3 is 411 min (6.8 h). In comparing GLOFOS7 and GLOFOS8, the same transposition has been made. In this case there is only a 2.5-fold destabilizing effect conferred by *fos* coding sequences plus a small amount of its 3′ untranslated region. This region of *fos* changes the half-life of GLOFOS8, which is 34.6 min, to 13.3 min for GLOFOS7. This result strongly suggests that it is the context of the sequence and/or the actual junctions that were destroyed that are influential in determining RNA stability.

DISCUSSION

We have demonstrated the utility of expressing DNAs from the c-*fos* promoter for the purpose of systematically studying RNA stability. This method provides an accurate approach to the analysis of the stability of any gene under these conditions. The half-life of 14.0 ± 5.1 min measured for c-*fos* mRNA in this study compares well with values determined by other investigators. Rahmsdorf et al. (22) measured the half-life of mouse c-*fos* mRNA in NIH 3T3 cells after stimulation of serum-starved cells to be 9 min by using actinomycin D.

There are two alternative views of the basis for mRNA stability in eucaryotic cells. At one extreme, mRNA stability may be determined by discrete sequence elements within each mRNA molecule. Models of mRNA stability which are based on a single dominant recognition motif for mRNA degradation would predict that chimeras in which such sequence elements are moved from one context to another will have their stability determined exclusively by those sequence elements. According to this view, if the stability of an mRNA molecule were determined by a single such sequence, then chimeras should have the stability of one parent mRNA or the other. The results presented here are not consistent with this view. Intermediate values for stability between β-globin and c-*fos* are observed for all of the chimeras tested. Unless the junction sequences created by the formation of the chimeras include sequences important in determining mRNA stability, the determination of mRNA stability for c-*fos* and β-globin by a single sequence within each mRNA molecule does not appear likely. At the other extreme, two more interpretations of the data remain possible. One is that a number of discrete sequences contribute to the overall stability of a given mRNA. The other is that interaction between sequences within an mRNA molecule contribute to the determination of stability. Our results are compatible with either of these two models.

Our data do indicate that sequences in the 3′ untranslated region of c-*fos* or β-globin or both make major contributions to determining mRNA stability. These results are consistent with the data obtained by Shaw and Kamen (27), who identified a 51-bp sequence in the 3′ untranslated region of human granulocyte-monocyte colony-stimulating factor mRNA, which destabilizes rabbit β-globin mRNA. However, these authors suggest that the sequence AUUUA is the primary element determining a short mRNA half-life be-

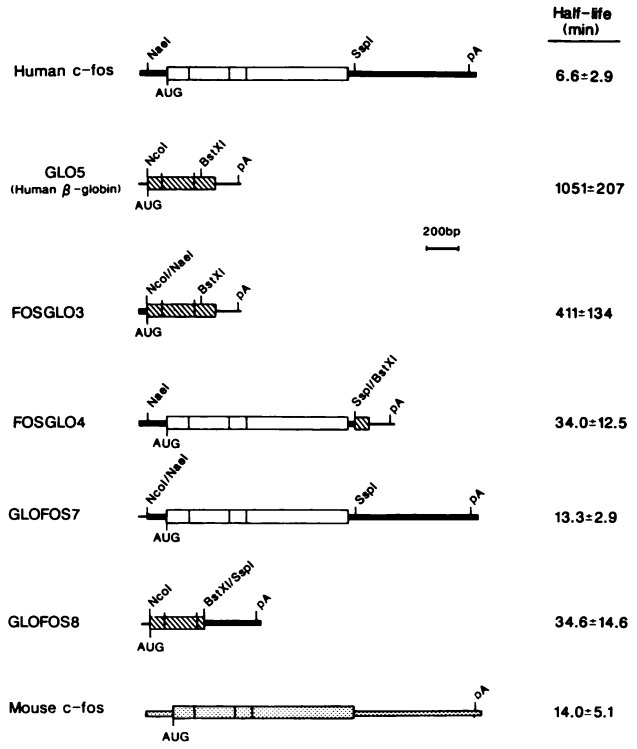

FIG. 6. Schematic drawing of RNA structures of human c-*fos*, GLO5, FOSGLO3, FOSGLO4, GLOFOS7, and GLOFOS8 accompanied by their average half-lives (see Table 1). Symbols: ▬, 5′ and 3′ untranslated regions of *fos*; ▭, *fos* protein-coding sequences, with exon boundaries marked; ——, 5′ and 3′ untranslated regions of globin; ▨, globin protein-coding sequences, exon boundaries marked. ▩, mouse c-*fos*. Thin blocks represent 5′ and 3′ untranslated regions; thick blocks represent coding regions with exon boundaries marked. The scale is shown. Half-lives are given in minutes beside each structure. Data on a given cell line tested in a state of growth arrest and in a state of growth were averaged together, since there was no difference.

TABLE 1. mRNA half-lives

Construct	Cell line	Half-life (min) ± SD[a]	P^b
Human c-*fos*	1C2	9.0 ± 0.8	<0.001
	1C6	3.3 ± 0.1	<0.001
	1C6	7.5 ± 0.2	<0.001
GLO5 (human β-globin)	2d1	914 ± 145	<0.001
	2d1	950 ± 150	<0.005
	2d1	1,289 ± 364	<0.025
FOSGLO3	3c4b	583 ± 94	<0.025
	3c4b	258 ± 48	<0.005
	3b1a	384 ± 65	<0.005
	3b1a	420 ± 39	<0.001
FOSGLO4	5a1	27.4 ± 2.5	<0.001
	5a1	26.1 ± 1.8	<0.001
	5d3	49.3 ± 8.1	<0.001
	5d3	48.4 ± 0.0	<0.001
GLOFOS7	A3a	11.3 ± 2.2	<0.005
	A3a	16.7 ± 3.7	<0.01
GLOFOS8	C3b	52.8 ± 8.8	<0.001
	C3b	39.9 ± 11.0	<0.01
	C3b	23.2 ± 1.8	<0.001
	A2c	22.3 ± 4.8	<0.01
Mouse c-*fos* (endogenous)	C3b	12.0 ± 0.5	<0.025
	BALB/c/3T3	15.8 ± 0.8	<0.001
	A3a	13.1 ± 2.8	<0.01
	1C6	7.7 ± 0.6	<0.001
	1C2	21.5 ± 3.1	<0.001

[a] Average half-lives and standard deviations are as follows: human c-*fos*, 6.6 ± 2.9 min; GLO5, 1,051 ± 207 min; FOSGLO3, 411 ± 134 min; FOSGLO4, 34.0 ± 12.5 min; GLOFOS7, 13.3 ± 2.9 min; GLOFOS8, 34.6 ± 14.6 min; mouse c-*fos*, 14.0 ± 5.1 min.

[b] $P \leq 0.05$ is significant.

cause this sequence appears from one to eight times in the 3′ untranslated region of many lymphokine mRNAs with short half-lives. AUUUA occurs three times in the 3′ untranslated region of c-*fos*, consistent with the suggestion of Shaw and Kamen (27). The presence of AUUUA on human c-*fos* and chimeric RNAs (GLOFOS7 and GLOFOS8) does seem to have a dramatic effect. However, this motif also is present in the 3′ untranslated region of human β-globin mRNA and chimeric RNAs (FOSGLO3 and FOSGLO4), as well a number of other long-lived mammalian β-globin mRNAs. The presence of this motif in these stable mRNAs indicates that the presence of the sequence AUUUA alone cannot account for the stability characteristics of an mRNA.

Our results also support the presence of sequences contributing to the stability of c-*fos* and β-globin mRNAs in regions other than the 3′ untranslated region. Several studies support the contribution of sequences outside the 3′ untranslated region to mRNA stability in other systems. For example, Yen et al. (34) identified a 13-base pair sequence at the 5′ end of the coding region of β-tubulin genes which is responsible for the autoregulation of β-tubulin mRNA levels by reducing mRNA stability in the presence of excess β-tubulin monomers. Rabbitts et al. (21) and Eick et al. (6) show the involvement of the 5′ end of the *myc* mRNA in determination of stability. Data supporting interactions between widely separated sequences within an mRNA molecule contributing to stability have been presented by Jones and Cole (12) and Piechaczyk et al. (20) for the c-*myc* mRNA. In addition, our data suggest that sequences which consist mostly of coding regions play a role in the determination of mRNA stability.

Our data are not inconsistent with the view that several specific sequences contribute to the short half-life of the c-*fos* mRNA, perhaps by serving as direct targets for mRNA degradation. However, chimeras in which sequences are reciprocally exchanged do not necessarily exhibit reciprocal half-lives. These findings suggest the possibility that context (involvement in secondary or tertiary structure) has a significant effect on specific sequences which signal mRNA degradation.

In addressing these issues, a more comprehensive panel of chimeric constructs in which additional regions of the two genes are exchanged would help to decide between the models discussed above and to identify, if possible, specific signals for mRNA degradation. Such studies would be the precursor to more precise point mutation studies created by in vitro mutagenesis to target specifically nucleotides crucial in forming structures or sequences determining mRNA stability.

ACKNOWLEDGMENTS

We thank Brent Cochran for providing human c-*fos* DNA. We thank Ann Kitchen and John Townes, who helped to supply the

platelet-poor plasma. The technical assistance of Dorothy Fallows and Joan Lane is greatly appreciated. We thank Al Baldwin, Brent Cochran, Jim Cunningham, Michelle Hoffman, Tim Hunt, Allan Jacobson, Robert Levenson, Randall Smith, and Tim Tully for helpful discussions, ideas, and readings.

This study was supported by Public Health Service grant CA-17575 to D.E.H. from the National Cancer Institute.

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