Volume 15 Number 4 1987 Nucleic Acids Research

Posttranscriptional regulation of c-fos mRNA expression

Hans Jobst Rahmsdorf, Axel Schönthal, Peter Angel, Margarethe Litfin, Ulrich Rüther* and Peter Herrlich

Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, and Universität Karlsruhe, Institut für Genetik, Postfach 3640, D-7500 Karlsruhe 1 and *European Molecular Biology Laboratory, Postfach 102209, D-6900 Heidelberg, FRG

Received September 25, 1986; Revised December 17, 1986; Accepted January 19, 1987

ABSTRACT

The transient induction of c-fos mRNA and protein suggests that regulation occurs not only by transcriptional activation but also at the level of turnover of the gene product. Here we present evidence for the rapid turnover of c-fos mRNA and some of the requirements for its specific degradation. The half life of induced mature cytoplasmic c-fos mRNA is 9 min in both serum-starved and growing primary human fibroblasts and in NIH 3T3 cells. A structure present at the 3' end of the c-fos mRNA molecule is involved in its low stability since the substitution or the removal of the untranslated 3' portion prolongues the RNA life time. The rapid turnover of fos mRNA requires, in addition, continued protein synthesis. Treatment of cells with cycloheximide stabilizes c-fos mRNA. Washing out cycloheximide reestabilishes the rapid turnover. Both changes occur with lag periods of less than 17 minutes.

INTRODUCTION

c-fos is the normal cellular homologue of the transforming gene of the FBJ and FBR murine osteosarcoma viruses (1,2). Both the viral and cellular fos-proteins are localized in the cell nucleus (3). Their molecular function is unknown. c-fos has been implicated in the control of cellular proliferation, differentiation and cellular stress. Support for the first idea comes from the increased levels of c-fos transcripts during the transition of fibroblasts from Go to S (4,5,6,7,8). A correlation between c-fos expression and differentiation has been suggested by the tissue-specific expression of c-fos (9,10), by the triggering of differentiation by fos gene constructs transfected

into embryonal carcinoma cells (11,12), and by its induction during in vitro differentiation of various cell types (13,14,15,16,17,18). That c-fos is a putative stress gene is suggested by the inducibility by ultraviolet light (UV; 8). There is evidence for both transcriptional and post- transcriptional modulation of c-fos RNA levels. Upon stimulation of serum-deprived mouse fibroblasts by serum or PGDF, the rate of c-fos transcription is enhanced dramatically within 15 minutes and returns to prestimulation levels at 45-60 minutes past stimulation (6). 5'flanking sequences have been defined which mediate the serum response (19). Mature c-fos mRNA can be detected in treated cells within minutes from addition of an inducer, reaches a maximum level at 30 minutes and then disappears with an estimated half life of 10-20 minutes (4,5,6,7,8). Antibodies against the fos protein precipitate several highly modified species of c-fos protein all of which are subjected to rapid turnover (3,5,7,15). The apparent rapid turnover of c-fos mRNA and protein suggests rigid control and a defined brief action of the gene product.

Inhibitors of protein synthesis lead to a large overaccumulation of fos mRNA (5,8,15,20,21). In view of the rapid turnover of fos mRNA one possible mechanism leading to overproduction could be the prolongation of fos mRNA half-life in the presence of protein synthesis inhibitors. Such a mechanism has been shown to be active in macrophages (20). In murine fibroblasts inhibitors of protein synthesis lead to enhanced and prolonged transcription when the fos-gene is activated by growth factors (21).

We address here the mechanism of specific c-fos mRNA turnover and the question whether transcriptional activation is the only mechanism by which inhibitors of protein synthesis lead to fos overaccumulation in fibroblasts. We show, that in fibroblasts

inhibitors of protein synthesis transform fos mRNA into a stable RNA species. Furthermore we show that a portion of the normal 3'untranslated portion of c-fos m-RNA is essential for its rapid degradation.

MATERIALS AND METHODS

Cells and culture conditions

Primary human fibroblasts (NFB München) were from a skin biopsy of a young healthy man and cultured at 37C, 5% CO_2 in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 µg/ml, P.S.). The fibroblasts were starved at about 80% confluency by discarding the complete medium, washing the cells once with a phosphate buffered salt solution (PBS), and leaving the cells in DMEM, 0.5% FCS, P.S. for 40 to 48 hours. The starvation procedure decreased ^{3}H-thymidine incorporation to about 10% without leading to detectable cell death. NIH 3T3 cells were grown in DMEM, 10% FCS, P.S..

Essential materials and plasmids

The radiochemicals and the nick translation kit were from Amersham England. Oligo (dT) cellulose was from Collab. Res., Lexington, Ma. TPA and Actinomycin D were from Sigma, München. Fos expression was probed with a 1 kb PstI fragment of the FBJ murine osteosarcoma virus, cloned into PBR322 (22). We thank Dr.R.Müller, Heidelberg, for providing us with this clone. Actin levels were determined with a mouse α-actin cDNA clone obtained from Dr.M.E.Buckingham (23).

RNA purification

Cellular RNA was purified by one of the following methods

1. Cells were lysed in 7 M urea, 2% SDS, 0.75 M NaCl, 1mM EDTA, 10 mM Tris HCl pH 8.0. The nucleic acids were extracted once with phenol/chloroform (1:1), followed by several extractions with

chloroform/isoamylalcohol (24:1). The RNA was centrifuged through a cushion of 5.7 M CsCl, 0.1 M EDTA, after addition of 0.4 g CsCl/ml (24). The RNA pellet was dissolved in 10 mM Tris HCl pH 7.5, precipitated with ethanol and used for dot blot or northern blot analysis. Poly(A)+ RNA was purified by passing total RNA through oligo-dT-cellulose.

2. Cells were washed with ice cold PBS, sampled in 10 ml ice cold STE (0.1 M NaCl, 20mM Tris HCl pH 7,4, 10mM EDTA), lysed with 0.5% SDS, homogenized with an ultrathorax for 30 sec. and incubated with proteinase K (300 µg/ml) at 37C for 30 min. After phenol-chloroform extraction, the NaCl concentration was adjusted to 0.5M, and the RNA adsorbed overnight to Oligo-dT- cellulose. Oligo-dT-cellulose was washed several times with high salt buffer (0.3M NaCl, 10 mM Tris HCl pH 7.5, 5 mM EDTA, 0.1% SDS), the poly(A)+ RNA was eluted with water, and precipitated with ethanol after addition of 10µg tRNA/ml.

Dot-Blot hybridization and quantitation, Northern Blot hybridization

For dot blot hybridization the required amount of total RNA was dried in a vacuum centrifuge, taken up in 20µl 50% formamide, 6% formaldehyde, 1xTBE (90mTris HCl pH8.0, 90mM boric acid, 2.5mM EDTA), heated at 37C for 15 minutes and then at 65C for 3 minutes, diluted with 180µl 10xSSC and spotted onto nitrocellulose filters. For Northern Blot hybridization RNA's were separated on 1% agarose gels under denaturing conditions according to Kazmaier et al. (25). After RNA transfer onto nitrocellulose, the filters were washed in 6xSSC, 0.05% SDS, baked at 80C for 2 hours and prewashed twice at 65C in 4xSSC, 0.02% bovine serum albumin, ficoll and polyvinyl pyrollidone each, 6.7mM NaH_2PO_4, 10mM Na_2HPO_4, 0.05% $Na_4P_2O_7$ and 0.1% SDS. 20µg/ml small denatured salmon sperm DNA were added to the second prewash. The hybridization mix contained 3-5x10^6 cpm/ml of nick

translated cDNA, 4xSSC, 10mM EDTA, 0.1% SDS and salmon sperm DNA (20µg/ml). Hybridization was at 65C for 20 hours. The filters were washed four times at 65C in 2xSSC, 1xSSC, 1xSSC and 0.5xSSC containing 6.7mM NaH_2PO_4, 10mM Na_2HPO_4, 0.05% $Na_4P_2O_7$ and 0.1% SDS, dried and exposed at -80C with an intensifier screen.

Dot blot quantitation was done by scanning autoradiograms in the linear exposure range of the film in a vertical and horizontal direction with a Joyce-Loebel densitometer. Northern blots and S1-gels were scanned in the vertical direction. Hybridization of the probe with yeast tRNA was substracted as background. Accurate determination and application of identical RNA-amounts was checked by hybridization with "control clones" (K7 (26), mouse actin), by hybridization against total radioactive cDNA synthesized on mRNA (27) and by staining the gel and nitrocellulose filters with ethidium bromide.

Metallothionein-c-fos and mouse mammary tumor virus-c-fos chimeric genes and gene transfection

The chimeric genes p19/1, p48/3 and p76/21 have been described (12). Briefly, the constructs contain: the pSV2-neo vector (28); the human metallothionein IIA promoter region (-770 to +75,(29)); and the mouse c-fos gene, in which the EcoR1-site in the first intron was destroyed. p19/1 contains the whole c-fos coding region including the translation stop codon and the polyA addition site (see fig.3). p48/3 was created by deletion of a SalI - BclI fragment of 478 bp in the 3'-noncoding region of c-fos. In p76/21 the 3'noncoding region of c-fos was exchanged by the 3'LTR of pFBJ-2 (22,30). p28/34 was constructed by deleting all fos 3'sequences between 2800 (SalI) and 3413 (MstII) and cloning the deleted gene in a BamH1 site behind the long terminal repeat of mouse mammary tumour virus (MMTV-LTR, 31). For transfection, 5µg of plasmid DNA were precipitated with Ca-phosphate onto 10^6 3T3 cells. p28/34 which does not contain

the neo-markers, was cotransfected with pSV2-neo. After 24 hours cells were trypsinized, respread at 1/5 density and neomycin resistant colonies selected with G418 (300µg/ml). Single colonies were isolated and expanded in DMEM containing G418.

RESULTS

1. Specific turnover of cytoplasmic c-fos mRNA

Upon induction of c-fos in primary human fibroblasts, mRNA levels reached a maximum within 30-60 minutes. Then further accumulation ceased and the mRNA disappeared decreasing to 50% of maximum in about 10 to 15 minutes. Similar kinetics of c-fos mRNA disappearance were observed in serum-starved human fibroblasts after serum, UV or TPA induction or in growing human fibroblasts after TPA or UV induction (TPA and serum data shown in fig.1). The half life of c-fos mRNA was determined accurately by blocking further transcription with actinomycin D and isolating RNA at various times thereafter. The dose of actinomycin used blocked transcription totally (see fig. 2a, lane 3). Microdensitometer tracings of Northern blot resolutions were plotted. The t1/2 of RNA transcribed from the endogenous c-fos gene of primary human fibroblasts or of 3T3 cells was 9-10 minutes (figs.2b and 3). Thus RNA decay is the major determinant of c-fos mRNA levels at times beyond 60 minutes after induction and the rate of c-fos transcription must be quite low then.

The rapid degradation is specific for c-fos mRNA since other RNA species such as metallothionein II A transcripts, actin and collagenase RNAs remained stable over the period examined (actin shown in fig.2a).

2. Superinduction of c-fos mRNA

Combined treatment of cells with an inducer of c-fos and inhibitor of translation enhanced c-fos mRNA accumulations many fold (5,8,15,20,21). Examples of superinduction using either

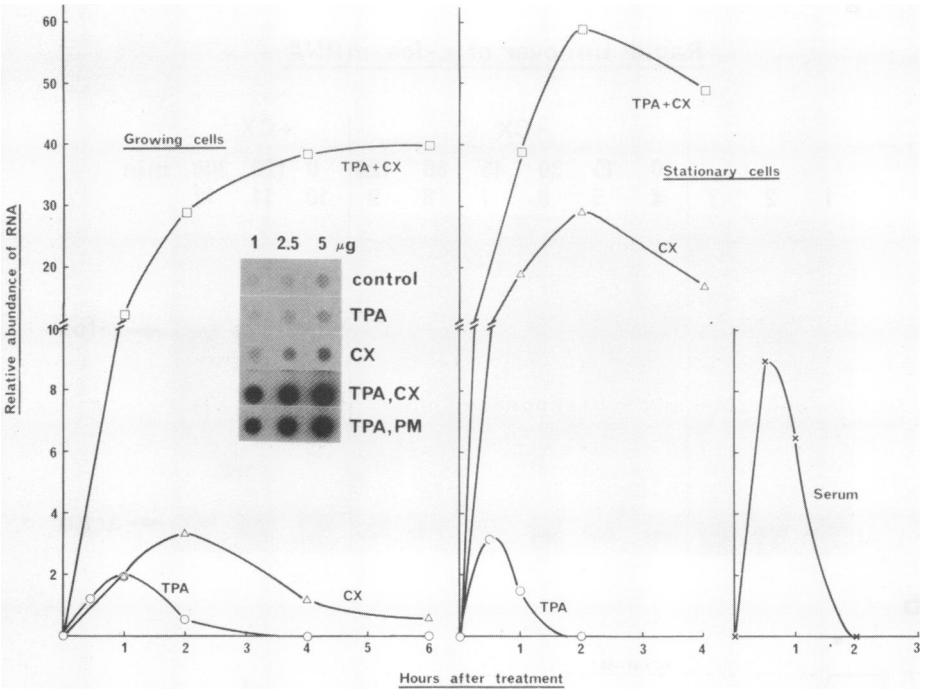
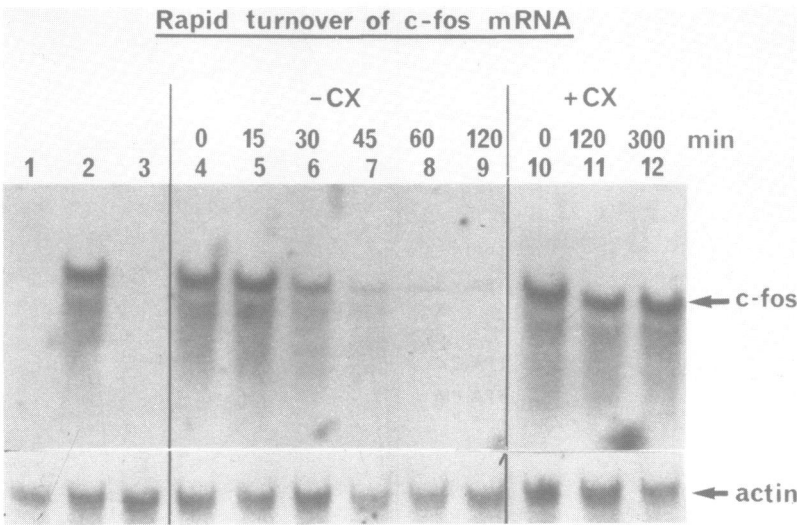


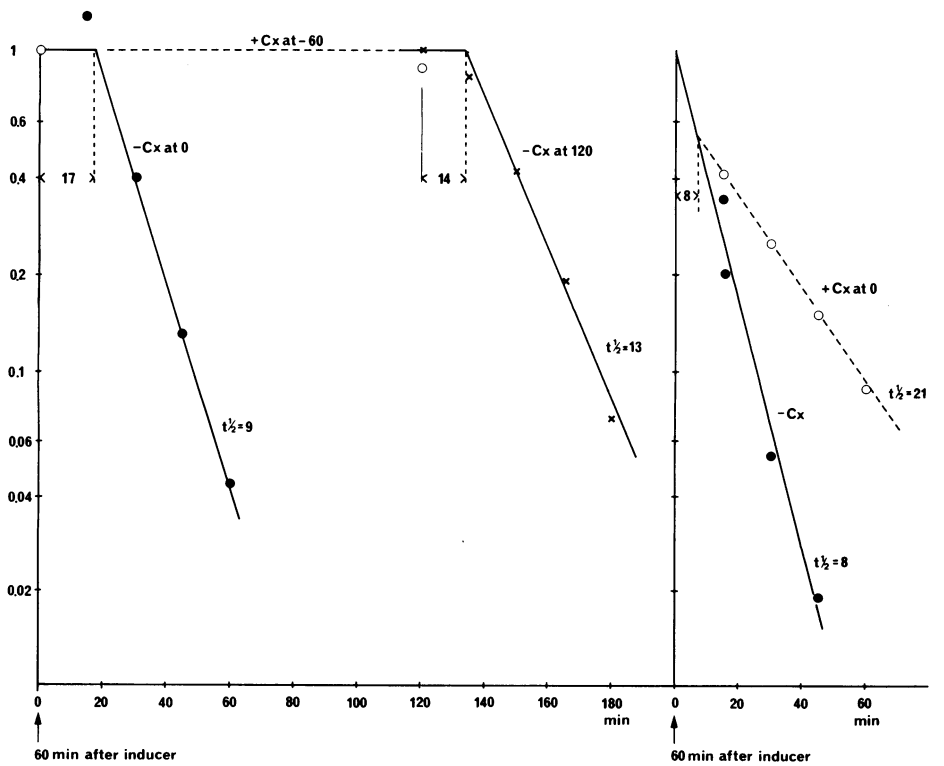
Fig.1
c-fos induction in primary human fibroblasts
Normal human fibroblasts were treated in the logarithmic growth phase or after serum starvation (40 hours, 0.5% serum) with TPA (20ng/ml), cycloheximide (CX, 10µg/ml), both agents together, or with fetal calf serum (10%). Total RNA was prepared and probed in dot blots with the nicktranslated 1kb viral PstI fos-fragment isolated from pfos-1 (22). Numbers for the fos mRNA amount were derived by densitometric evaluation of the dots and are arbitrary. Hybridization to yeast tRNA was substrated as background. The insert shows an experiment with puromycin (PM, 100µg/ml) in comparison with other conditions. Preparation of the RNAs was at 4 hours after treatment.

cycloheximide or puromycin are shown in fig.1. Superinduction occurred in various cell types irrespective of the transcriptional inducer (not shown). These include mouse spleen lymphocytes, mouse fibroblasts and F9 embryonal carcinoma cells, and inducing agents such as serum, TPA, UV and LPS (lymphocytes).

a


Rapid turnover of c-fos mRNA
-CX
+CX
0 15 30 45 60 120 0 120 300 min
1 2 3 4 5 6 7 8 9 10 11 12
c-fos
actin

b


+Cx at -60
-Cx at 0
-Cx at 120
17
14
8
t½=9
t½=13
+Cx at 0
t½=21
-Cx
t½=8
1
0.6
0.4
0.2
0.1
0.06
0.04
0.02
0 20 40 60 80 100 120 140 160 180 min
0 20 40 60 min
60 min after inducer
60 min after inducer

The addition of cycloheximide alone also caused enhanced c-fos mRNA levels (fig.1).

The enhanced fos mRNA levels accumulating in the presence of cycloheximide could be due to an increased transcriptional rate, to lack of shut-off of transcription or to a block of RNA degradation. Greenberg and co-workers have shown, that inhibitors of protein synthesis in the absence of serum lead only to a marginal increase of c-fos gene transcription in 3T3 fibroblasts (21). This agrees with our findings in human fibroblasts, where cycloheximide increased RNA polymerase density at the fos gene by

Fig.2
Rapid change in c-fos RNA turnover rates after removal (2a and 2b left panel) or addition (2b right panel) of cycloheximide
Primary human fibroblasts were starved in DMEM containing 0.5% FCS for 40 hours.
Fig.2a and 2b left panel: c-fos RNA was induced by treating the cells with 10µg/ml cycloheximide and 20% FCS. After 60' some of the plates were washed 5 times with warm DMEM, 0.5% FCS containing 5µg/ml actinomycin D, but no cycloheximide, and incubated further with this medium. Other plates were washed and incubated under identical conditions, but with a medium containing 10µg/ml CX. RNA was prepared at 0', 15', 30', 45', 60' and 120' after actinomycin D addition (lanes 4-9 fig 2a), respectively, in the case of CX presence, at 0', 120' and 300' (lanes 10-12). Lane 1: untreated cells; Lane 2: cells treated for 1 hour with 20% FCS and 10µg/ml CX; Lane 3: cells treated as in lane 2 but in the presence of 5µg/ml actinomycin D. Equal amounts of RNA were separated on agarose gels. The Northern blots were probed with nick-translated c-fos DNA and reprobed with actin cDNA. Fig.2b left panel shows the kinetics of fos mRNA turnover. After densitometric scanning the fraction of non degraded fos mRNA was plotted as a function of the duration of actinomycin D treatment in the absence (●———●) or presence (o------o) of CX. x———x shows fos RNA levels in cells which had been induced for 1 hour with serum and CX and then incubated with serum, CX and actinomycin D (5µg/ml) for another 2 hours. Then CX has been removed and the decay of fos RNA was followed.
Fig.2b right panel: c-fos RNA was induced by treating the cells with 20% FCS for 1 hour. Then actinomycin D (5µg/ml) was added to the plates and half of the plates received 10µg/ml CX. Decay of c-fos mRNA was followed in the absence (●———●) and in the presence (o-----o) of CX.

about 1.5-fold as determined in nuclear run on experiments, (results not shown). In the presence of serum, protein synthesis inhibitors enhanced and prolonged transcription at the fos gene several fold (21).

To determine whether increased transcription was the only mechanism by which inhibitors of protein synthesis lead to fos mRNA accumulation, we measured the half-life of c-fos mRNA in the presence and absence of cycloheximide after having stopped further transcription with actinomycin D (fig.2). In the presence of actinomycin D, cycloheximide indeed stabilized c-fos mRNA (fig.2a). No degradation could be detected in the 5 hour period examined. This prolongation of the half life contributes thus significantly to the superinduction and the longer lasting plateau levels of c-fos mRNA observed (fig.1). We note that cycloheximide alone led to higher levels of c-fos RNA in serum-starved primary human fibroblasts, than in cycling cells (fig.1). A minor increase in transcriptional rate in serum starved cells (not shown) seems too low to explain the differences. Confluent murine C3H 10T1/2 fibroblasts also contain a much higher fos mRNA level than logarithmically growing cells (32).

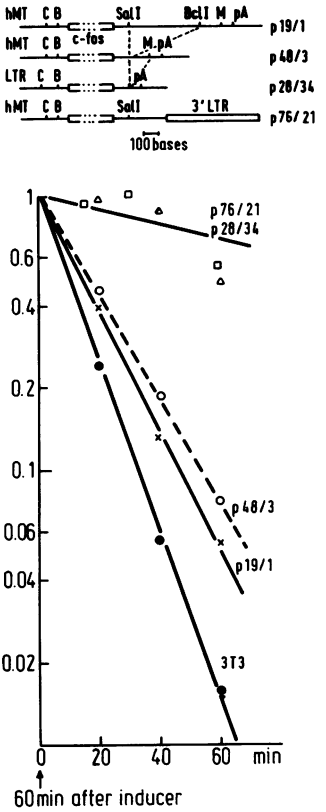
3. Rapid changes of c-fos mRNA decay rates upon addition or removal of cycloheximide

The curves in fig.1 showing accumulation of c-fos mRNA after TPA treatment with or without cycloheximide appear to deviate very early. Since inhibition of RNA decay as a major effect of cycloheximide, the change is thus probably established very rapidly. The time course of the change in decay rate was determined by quantitating c-fos mRNA at various times after removal or addition of cycloheximide. It took less than 17 minutes after washing out the inhibitor to reestablish the "9-minute-turnover" irrespective of the time the cells had been

kept in cycloheximide (one and three hours in fig.2b). Reestablishment of rapid turnover was possible although no further transcription was permitted for 2 hours. To perform the reverse experiment we needed to induce in the absence of any inhibitor for 60 minutes. Then actinomycin D was added alone or in combination with cycloheximide. The effect of cycloheximide was visible with brief kinetics similar to the wash-out experiment (8 min in fig.2b). The rate of decay was not decreased to the same extent, however, as in those conditions where cycloheximide was present from the beginning (addition together with the inducer).

4. The 3'untranslated portion of c-fos mRNA is important for its rapid turnover

To define what distinguishes c-fos mRNA from other RNA species in the degradation process, we introduced chimeric gene constructs into 3T3 cells and followed the fate of RNA after induction. Replacing the 5'flanking sequence, c-fos promoter and the first 73 nucleotides of transcribed sequence by corresponding portions of the metallothionein II A gene (12) did not alter significantly the turnover rate of c-fos mRNA: the half lifes of serum-induced endogenous transcript and of cadmium-induced RNA from the transfected gene were similar (fig.3). Certain changes in the DNA coding for the 3' untranslated part of c-fos mRNA, however, prolonged the half life. While a metallothionein II A promoter c-fos construct with a deletion from SalI to BclI(p48/32, +2800 to +3278) did not alter the half life, further deletion up to the MstII site (p28/34, +2800 to +3413) stabilized the mRNA. Also a substitution of all sequences beyond the SalI site by FBJ sequences including the viral LTR(p76/21) gave rise to RNA species with half lifes of about two hours (fig.3). This suggests that 3'-untranslated sequences between +3278 and +3413 are important for the rapid turnover of fos RNA.



The 3'untranslated portion of c-fos mRNA is important for its rapid turnover.

Fig.3

The c-fos 3'untranslated region of the mRNA is necessary for its rapid destruction

The MT/fos and LTR/fos chimeric gene constructs with different 3' ends (see Material and Methods) were transfected into NIH 3T3 cells and single colonies were grown up. They were treated in the late-log phase with 20% serum (3T3), with $3x10^{-6}$M $CdCl_2$ (p19/1, p48/3, p76/21) or with 10^{-7}M dexametasone (p28/34) for one hour. Then 20µg/ml actinomycin D were added to the cultures, and RNA was prepared. 20µg/ml actinomycin D inhibited serum-mediated fos induction completely when given together with serum. Equal amounts of RNA were separated on agarose gels and the amount of c-fos RNA was probed with nick-translated fos DNA. After densitometric scanning c-fos RNA amounts were calculated as fraction present at the time of actinomycin D addition. $CdCl_2$ treatment did not induce the endogenous 3T3 gene, so that the RNA transcribed from the transfected gene could be measured directly. In addition, the levels of RNA transcribed from the transfected gene were determined by S_1-analysis using a Eco RI-BamHI fragment

of the human MT-promoter region (-67 to +73) or for p28/34, a Eco RI - ACCI fragment labeled at the ACCI site. The human metallothionein probe does not hybridize with the murine metallothioneins. RNA Northern blot and S_1-mapping determination gave similar results. To ascertain equal loading we reprobed the RNA's with an actin-cDNA. hMT: human metallothionein promoter; LTR: mouse mammary tumor virus LTR; 3'LTR: 3'LTR of FBJ-2; c-fos: translated region of the mouse c-fos gene; C: start of transcription; B: BamH I; M:Mst II; pA: poly(A) addition signal. ● ——— ● 3T3; x———x p19/1; o-----o p48/3; △ ——— △ p76/21; □——— □ p28/34.

DISCUSSION

The specific degradation of c-fos mRNA shows the following features: The turnover is of similar rate (around 9 min t 1/2) in several cell types and growth conditions. Induction of c-fos by serum, TPA, UV or Cadmium (endogenous resp. transfected chimeric gene) does not alter the turnover rate significantly. The turnover is dependent on ongoing protein synthesis. Exchange of the c-fos promoter and the first 73 nucleotides has no effect on the turnover of the mRNA. Also removal of 3'-untranslated sequences between the Sal I (+2800) and the Bcl I site (+3278) did not change the turn over rate. Further deletion up to the Mst II site (+3413) or substitution of the sequences beyond the Sal I site stabilized the mRNA indicating a role of the untranslated portion in the degradation process.

The data can explain in vivo observations using these or similar mutants. In transgenic mice, the replacement mutant (p76/21) caused constitutive levels of c-fos mRNA in several tissues (U.Rüther, unpublished), presumably by stabilization of the mRNA, while p19/1 and p48/3 did not. In cell culture, constructs corresponding to p76/21 caused the morphological transformation of 3T3 cells, while p48/3 did not. In an extensive analysis using a viral-cellular chimeric fos gene, transforming ability was gained by deleting a 67 bp sequence 3' of the Bcl 1

site (33,34). Thus, it is likely that in vitro transformation is linked to the stabilization of c-fos mRNA. One could argue, that the rapid turnover of c-fos mRNA is a necessity without which transformation is unavoidable.

Involvment of the untranslated 3' end of c-fos mRNA in the degradation process parallels similar conditions reported on hsp 70 RNA. A mutant in the 3' end turned stable (35). Recently Shaw and Kamen (36) showed, that an AT rich sequence in the 3'-untranslated region of several unstable RNA's is responsible for this rapid turnover (37,38,39).

c-fos mRNA becomes totally stable upon addition of cycloheximide or of other inhibitors of protein synthesis, including puromycin. This shows that protein synthesis inhibitors affect similar steps in fibroblasts and in macrophages (20) and that stimulation of transcription (21) is not the only mechanism by which inhibitors of protein synthesis lead to cfos mRNA overaccumulation. The turnover process responds rapidly to addition or removal of the inhibitor by rate changes in the order of minutes.

Several interpretations of our results are feasible. For example a labile protein component is required for rapid c-fos mRNA turnover. This component is present in growing and stationary cells (compare the rates of fos mRNA degradation in growing and stationary cells in fig.1 and in the presence and absence of 20% serum in fig.3); it needs for its action the sequences between the Bcl I and the Mst II site of the 3'-untranslated fos region; and it disappears rapidly in the presence of protein synthesis inhibitors. The finding that cycloheximide did not stabilize as well when given to serum treated cells as when given together with serum (compare fig.3b left panel and right panel) may suggest the participation of another component in the degradation process, which is serum

induced. Also the fact that the serum induced cfos mRNA transcribed from the endogenous gene is turned over somewhat more rapidly than the Cd induced mRNA transcribed from the transfected gene 19/1 (fig.3) points into this direction. Could this second component be the fos protein? We note that Meijlink et al (34) gained transformation also (and mRNA stabilization perhaps) when deleting the 3'most portion of the fos translated region. Also hsp 70 protein seems to control its mRNA turnover (40). There is at present no clue as to a possible role for the RNase L system in mRNA turnover. Oligoadenylate synthetase which produces a cofactor of the system is in fact serum-induced (41).

An alternative interpretation assumes a recognition sequence in the untranslated 3' portion used by a constitutive RNase when the RNA is actively translated. The recognition structure is not formed when translation stops or when sequences between the Bcl I site and the Mst II site are deleted. Ribosome free fos mRNA (as present in serum starved cells treated simultanously with serum and cycloheximide) does not form the recognition structure, so that the RNA is completely stable (fig.2b, left panel). When ribosomes are arrested on the mRNA by cycloheximide (as it happens when serum stimulated cells are treated with cycloheximide), some of the mRNA molecules might form the recognition structure; then the mRNA is not completely stabilized (fig.2b, right panel).

These hypotheses lead to testable predictions. Amino acid analogues inactivating the function of newly synthesized protein, should not affect c-fos RNA turnover if the RNase were a stable enzyme. Point mutation in cfos could define a role of fos protein in turnover. No mRNA turnover enzyme has yet been isolated. Knowledge of a recognition structure may promote such an attempt.

ACKNOWLEDGEMENTS

We are grateful to Dr.Rolf Müller for valuable suggestions and criticisms, to Drs.Helmut Ponta, Isabel Roditi, Marita Büscher and Michael Karin for discussions and to Dr.Michael Kazmaier who helped in one of the experiments.

REFERENCES

1. Finkel, M.P., Biskis, B.O. and Jinkins, P.B. (1966) Science, 151, 698-701.
2. Verma, I.M., Curran, T., Müller, R., van Straaten, F., Mac Connell, W.P., Miller, A.D. and Van Beveren, C (1984) Cancer Cells, 2, 309-321.
3. Curran, T., Miller, A.D., Zokas, L. and Verma, I.M. (1984) Cell, 36, 259-268.
4. Cochran, B.H., Reffel, A.C. and Stiles, C.D. (1983) Cell, 33, 939-947.
5. Müller, R., Bravo, R., Burckhardt, J. and Curran, T. (1984a) Nature, 312, 716-720.
6. Greenberg, M.E. and Ziff, E.B. (1984) Nature, 311, 433-438.
7. Kruijer, W., Cooper, J.A., Hunter, T. and Verma, I.M. (1984) Nature, 312, 711-716.
8. Angel, P., Rahmsdorf, H.J., Pöting, A. and Herrlich P. (1985) Cancer Cells, 3, 315-319.
9. Müller, R., Slamon, D.J., Tremblay, J.M., Cline, M.J. and Verma, I.M. (1982) Nature, 299, 640-644.
10. Müller, R., Verma, I.M. and Adamson, E.D. (1983) EMBO J., 2, 679-684.
11. Müller, R. and Wagner, E.F. (1984) Nature, 311, 438-442.
12. Rüther, U., Wagner, E.F. and Müller, R. (1985) EMBO J., 4, 1775-1781.
13. Gonda, T.J. and Metcalf, D. (1984) Nature, 310, 249-251.
14. Müller, R., Müller, D. and Guilbert, L. (1984b) EMBO J., 3, 1887-1890.
15. Mitchell, R.L., Zokas, L., Schreiber, R.D. and Verma, I.M. (1985) Cell, 40, 209-217.
16. Müller, R., Curran, T., Müller, D. and Guilbert, L. (1985) Nature, 314, 546-548.
17. Curran, T. and Morgan, J.I. (1985) Science, 229, 1265-1268.
18. Kruijer, W., Schubert, D. and Verma, I.M. (1985) Proc. Natl. Acad. Sci. USA, 82, 7330-7334.
19. Treisman, R. (1985) Cell, 42, 889-902.
20. Mitchell,R.L., Henning-Chubb,C., Huberman,E., Verma,I.M. (1986) Cell 45, 497-504.
21. Greenberg,M.E., Hermanowski,A.L., Ziff,E.B. (1986) Molec. and Cell. Biol. 6, 1050-1057.

22.Curran, T., Peters, G., Van Beveren, C., Teich, N.T. and Verma, I.M. (1982) J.Virol., 44, 674-682.
23.Minty, A.J., Caravatti, M., Robert, B., Cohen, A., Daubas, P., Weydert, A., Gros, F. and Buckingham, M.E. (1981) J. Biol. Chem., 256, 1008-1014.
24.Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W.J. and Goodman, H.M. (1977) Science, 196, 1313-1319.
25.Kazmaier, M., Brünning, E. and Ryffel, G.U. (1985) EMBO J., 4, 1261-1266.
26.Angel, P., Pöting, A., Mallick, U., Rahmsdorf, H.J., Schorpp, M. and Herrlich, P. (1986) Mol. Cell. Biol. 6, 1760-1766.
27.Rahmsdorf, H.J., Harth, N., Eades, A.-M., Litfin, M., Steinmetz, M., Forni, L. and Herrlich, P. (1986) J. Immunol., 136, 2293-2299.
28.Southern, P.J. and Berg, P. (1982) J. Molec. Appl. Gen., 1, 327-334.
29.Karin, M., Haslinger, A., Holtgreve, H., Richards, R.I., Krauter, P., Westphal, H.M. and Beato, M. (1984), Nature, 308, 513-519.
30.Van Beveren, C., van Straaten, F., Curran, T., Müller, R. and Verma, I.M. (1984) Cell, 32, 1241-1255.
31.Ponta, H., Ball,R., Steinmetz, M. and Groner,B. (1985). EMBO J. 4, 3447-3453.
32.Shuin.T., Billings,P.C., Lillehaug,J.R., Patierno,S.R., Roy-Burman,P. and Landolph, J.R. (1986). Cancer Res. 46, 5302-5311.
33.Miller, A.D., Curran, T. and Verma, I.M. (1984) Cell, 36, 51-60.
34.Meijlink, F., Curran, T., Miller, A.D. and Verma, I.M. (1985) Proc. Natl. Acad. Sci. USA, 82, 4987-4991.
35.Simcox, A.A., Cheney, C.M., Hoffman, E.P. and Shearn, A. (1985) Mol. Cell. Biol., 5, 3397-3402.
36.Shaw,G. and Kamen,R. (1986). Cell 46, 659-667.
37.Raj, N.B.K. and Pitha, P.M. (1981) Proc. Natl. Acad. Sci. USA, 78, 7426-7430.
38.Cochran, B.H., Zullo, J., Verma, I.M. and Stiles, C.D. (1984) Science, 226, 1080-1082.
39.Dani, Ch., Mechti, N., Piechaczyk, M., Lebleu, B., Jeanteur, Ph. and Blanchard, J.M. (1985), Proc. Natl. Acad. Sci. USA, 82, 4896-4899.
40.DiDomenico, B.J., Bugaisky, G.E. and Lidquist, S. (1982), Cell, 31, 593-603.
41.Zullo, J.N., Cochran, B.H., Huang, A.S. and Stiles, C.D. (1985), Cell, 43, 793-800.