Expression of Human Fibroblast Growth Factor 2 mRNA Is Post-transcriptionally Controlled by a Unique Destabilizing Element Present in the 3'-Untranslated Region between Alternative Polyadenylation Sites*

(Received for publication, February 1, 1999, and in revised form, April 19, 1999)

Christian Touriol, Antonin Morillon, Marie-Claire GenSac, Hervé Prats, and Anne-Catherine Prats

From INSERM U397, Endocrinologie et Communication Cellulaire, Institut Louis Bugnard, Centre Hospitalier Universitaire de Rangueil, Avenue Jean Pouthès, 31403 Toulouse Cedex 04, France

Fibroblast growth factor 2 (FGF-2) belongs to a family of 18 genes coding for either mitogenic differentiating factors or oncogenic proteins, the expression of which must be tightly controlled. We looked for regulatory elements in the 5823-nucleotide-long 3'-untranslated region of the FGF-2 mRNA that contains eight potential alternative polyadenylation sites. Quantitative reverse transcription-polymerase chain reaction revealed that poly(A) site utilization was cell type-dependent, with the eighth poly(A) site being used (95%) in primary human skin fibroblasts, whereas proximal sites were used in the transformed cell lines studied here. We used a cell transfection approach with synthetic reporter mRNAs to localize a destabilizing element between the first and second poly(A) sites. Although AU-rich, the FGF-2-destabilizing element had unique features: it involved a 122-nucleotide direct repeat, with both elements of the repeat being required for the destabilizing activity. These data show that short stable FGF-2 mRNAs are present in transformed cells, whereas skin fibroblasts contain mostly long unstable mRNAs, suggesting that FGF-2 mRNA stability cannot be regulated in transformed cells. The results also provide evidence of a multilevel post-transcriptional control of FGF-2 expression; such a stringent control prevents FGF-2 overexpression and permits its expression to be enhanced only in relevant physiological situations.

* This work was supported in part by grants from the Association pour la Recherche sur le Cancer, the Ligue Nationale contre le Cancer, and the Conseil Régional Midi-Pyrénées and by European Community Biotechnology Program Contract 94/99-181 (Subprogram Cell Factory, Actions de Recherches Concertées). The costs of publication of this article were defrayed in part by the payment of page charges. This advertisement must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of successive fellowships from the Ministère de l’Education Nationale et de la Recherche and from the Ligue Nationale contre le Cancer.
§ Present address: Inst. de Biologie Physico-Chimique, CNRS UPR 9073, 13, rue Pierre et Marie Curie, 75005 Paris, France.
¶ To whom correspondence should be addressed. Tel.: 33-561-32-21-42; Fax: 33-561-32-21-41; E-mail: pratsac@rangueil.inserm.fr.
1 The abbreviations used are: FGF-2, fibroblast growth factor 2; nt, nucleotide(s); UTR, untranslated region; ARE, AU-rich element; RT-PCR, reverse transcription-polymerase chain reaction; CAT, chloramphenicol acetyltransferase; GM-CSF, granulocyte/macrophage colony-stimulating factor; PBS, phosphate-buffered saline.
alternative length-modifying polyadenylation sites, prompted us to determine its regulatory role in FGF-2 isoform expression. In this report, we show that poly(A) site utilization varies with cell type, and we identify a destabilizing element between the first and second polyadenylation sites of FGF-2 mRNA. These observations suggest that regulation of FGF-2 expression occurs at the level of RNA stability, conditioned by use of the poly(A) site.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—PCRs were performed using the complete FGF-2 cDNA as a template and the primer couple RTA1/PA1rev, RTA2/PA2rev, or RTA8/PA8rev, hybridizing upstream from the first, second, or eighth poly(A) site, respectively (Table I). The resulting fragments were subcloned into the EcoRI site of the vector Bluescript pKS. The corresponding plasmids, pKS-PA1, pKS-PA2, and pKS-PA8, were digested by XcmI + MscI, AvrII, and DraI and religated to obtain an internal deletion, giving the plasmids pKS-PA1A, pKS-PA2A, and pKS-PA8ΔΔ, respectively.

The constructs pCAT-A0, p5′-CAT-A0, p5′-CAT-A1, and p5′-CAT-A8 were described previously (14). pCAT-A0 and p5′-CAT-A8 were called pKS-CAT-pA and p5′-CAT-A7, respectively (see Fig. 2A). The BspEI-SmaI fragment of p5′-CAT-A1 was introduced into pCAT-A0 digested by BspEI + SmaI to construct pCAT-A1; pCAT-A2 was obtained by insertion of the BamHI-Klenow-BspEI fragment from plasmid pCAT-DOG, containing the complete FGF-2 3′-UTR sequence downstream from CAT, into pCAT-A0 (BamHI site at position 3441 of FGF-2 cDNA) (14). Plasmid pCAT-A3 was obtained by subcloning the PstI-Klenow-AvrII fragment from pSCT-DOG (AvrII and PstI sites at positions 3115 and 4228 of FGF-2 cDNA, respectively) into plasmid pCAT-A2. Plasmid pCAT-A4 was obtained by subcloning the NsiI-Klenow-AvrII fragment from pSCT-DOG (AvrII and NsiI sites at positions 3115 and 5403 of FGF-2 cDNA, respectively) into plasmid pCAT-A2. Plasmid pCAT-A8 was constructed by introducing the pSCT-DOG-XhoI-Klenow-BspEI fragment containing the long 3′-UTR sequence into plasmid pCAT-A0 (pKS-CAT-pA) (14). Plasmids of the 5′-UTR series were obtained by subcloning the BspEI-XhoI fragments obtained from plasmids of the pCAT series into p5′-CAT-A0.

Plasmid pCAT-3′-inverted was obtained by subcloning the Klenow fragment-treated XhoI-MscI fragment of pSCT-DOG (containing the entire 3′-UTR) into the Smal site of dephosphorylated pCAT-A0 (see Fig. 3A). The pCAT-3′-GM-CSF and pCAT-3′-GM-ΔAU constructs were obtained by amplifying DNA fragments by PCR using oligonucleotide primers CAT-RT5′ and GMrev (Table I) from plasmids pCMV-CAT-GM-ΔAU(+) and pCMV-CAT-GM-ΔAU(−), respectively (kindly provided by G. Huez). These PCR fragments were digested by BspEI before cloning into the BspEI-Smal sites of pCAT-A0.

Plasmids pCAT-A2A1, pCAT-A2A2, and pCAT-A2A3 were obtained by subcloning the SpeI-Klenow-BspEI, NsiI-Klenow-BspEI, and EcoRV-BspEI fragments of pCAT-A2, respectively, between the BspEI-Smal sites of pCAT-A0 (see Fig. 3B). Plasmids pCAT-dest208 and pCAT-dest334 were obtained by subcloning the SpeI-Klenow-XhoI and AvrII-Klenow-XhoI fragments of pCAT-A2, respectively, into pCAT-A0 digested by SmaI plus XhoI (AvrII, SpeI, NsiI, and EcoRV sites at positions 5114, 3233, 2726, and 1932 from the 5′-end of FGF-2 cDNA, respectively). The BspEI-XhoI fragments from the pCAT series plasmids were subcloned into plasmid p5′-CAT-A0 to obtain the corresponding 5′ CAT plasmids.

Plasmid pCAT-destΔA and pCAT-destAS were obtained by digestion of plasmid pCAT-dest by AvrII and SpeI, respectively, followed by religation (see Fig. 4). Plasmid pCAT-ΔAdest was obtained by digestion of pCAT-A8 by AvrII plus BamHI, followed by Klenow treatment and religation.

In Vitro Transcription—DNAs were obtained either by plasmid linearization or by PCR amplification using T3 tail-containing primers (for antisense RNAs). Transcription was performed with T7 or T3 RNA polymerase using transcription kits provided by Ambion Inc.: the mMESSAGE mMACHINETM kit was used for capped mRNAs, and the MAXScriptTM or the MEGAScriptTM kit for uncapped and/or labeled RNAs. The resulting transcripts were precipitated by ethanol, resuspended in 20 μl of ethidium bromide staining on agarose gel, and their integrity was verified. The templates for T3 transcription of antisense RNAs against the dest element were obtained by PCR using primers RTA2 and PA2revT3 (Table I), followed by AvrII, SpeI, or AvrII digestion (A51, A52, and A53, respectively) (see Fig. 4).

**Human Skin Fibroblast Cultivation**—A piece of human skin obtained from the plastic surgery department of Rangueil Hospital (Toulouse, France) was washed for 15 min at 20 °C in six antibiotic/PBS baths (15 ml): 1) penicillin/streptomycin (Life Technologies, Inc.) at a dilution of 1:50; 2) gentamycin (Life Technologies, Inc.) at a dilution of 1:750; 3) Bastrim (80 mg/ml; Roche Molecular Biochemicals) at a dilution of 1:800; 4) Cytox (2 mg/ml; Bayer) at a dilution of 1:1000; 5) Fortum (333 mg/ml; Glaxo Wellcome) at a dilution of 1:1000; and 6) amphotericin (Life Technologies, Inc.) at a dilution of 1:100. The skin was cut into 1-mm² pieces, which were left to dry in a Petri dish for 10 min. Dulbecco's modified Eagle's medium plus 10% fetal calf serum and penicillin/streptomycin were added, and cultivation was pursued for 3–4 weeks until fibroblasts grew from the skin pieces, before trypsinization and seeding into new dishes (P0). The fibroblasts could then be used for seven passages.

**Cell Transfection by RNA—** COS-7, HeLa, and SK-Hep-1 cells (see Ref. 24) were transfected by the DMRIE-C method (Life Technologies, Inc.). Briefly, 100 μl of serum-free medium containing 2 pmol of RNA (1–10 μg depending on RNA size) was mixed with 100 μl of serum-free medium containing 10 μl of DMRIE-C reagent. After addition of 0.8 ml of serum-free medium, the mixture was added to PBS-washed cells and incubated for 14 h at 37 °C.

Primary human skin fibroblasts were electroporated using a Bio-Rad apparatus. PBS-washed cells were scraped, resuspended in 10% fetal calf serum-containing medium, and centrifuged at 1000 rpm for 10 min. The pellet was washed with PBS and centrifuged twice. Cells were resuspended in serum-free medium at a final concentration of 2.5 × 10⁶ cells/ml. 400 μl of cell suspension was mixed with 2 pmol of RNA (1–10 μg) and transferred to a 4-mm electroporation cuvette. An electric shock of 260 V/950 microfarads was applied, and then the cells were seeded in medium-containing dishes and incubated at 37 °C for 12–16 h.

Seven dishes (5 cm in diameter) were transfected with 1 μg of RNA/dish to measure mRNA stability. After incubation for 2 h, the medium was removed; the cells were washed with PBS, and fresh medium was added. The cells were harvested either immediately or after increasing periods of time (7.5–360 min).

CAT activity determinations were performed as described previously (14). Luciferase activity was measured using the Promega luciferase assay system.

**Quantitative Reverse Transcription-Polymerase Chain Reaction**—The cDNAs were synthesized using the Superscript™ preamplification system (Life Technologies, Inc.) according to the manufacturer's instructions. The reverse transcription reaction was carried out using 1 μg of total RNA and 50 ng of random hexamers in a final volume of 20 μl. Variable amounts of internal standard RNAs synthesized from the pKS-PA1, pKS-PA2ΔΔ, and pKS-PA8ΔΔ plasmids (see above) were added to the reactions as described previously (24) to quantify the different regions of the FGF-2 mRNA 3′-UTR.

PCR was performed with the primer couple RTA1/PA1rev, RTA2/PA2rev, or RTA8/PA8rev, hybridizing upstream from the first, second, or eighth poly(A) site, respectively. The resulting fragments were 400–500 bp long.
Alternative Polyadenylation and Destabilization of FGF-2 mRNA

An RNA-destabilizing Element Is Present between the First and Second Polyadenylation Sites of the FGF-2 mRNA—The observed variations in the 3′-UTR length due to cell type-specific alternative polyadenylation prompted us to look for regulatory elements in the 5823 nt-long 3′-UTR of FGF-2 mRNA. To avoid interference from the alternative polyadenylation process and to analyze the expression of one mRNA species at a time, cell transfection was performed using in vitro transcribed, capped, and polyadenylated mRNAs (25). This procedure has been previously validated for mRNA half-life analysis in a study of the GM-CSF ARE (26).

COS-7 cells were transfected with CAT mRNAs bearing five of the different FGF-2 mRNA 3′-UTRs (measuring 83, 2390, 3073, 4446, and 5823 nt, respectively), fused or not to the leader region of FGF-2 mRNA (Fig. 2A). CAT mRNA expression was first analyzed by CAT activity measurement (Fig. 2B). CAT-A1 mRNA with the shortest 3′-UTR was efficiently expressed, in comparison to the CAT-A0 control, whereas CAT expression from A2, A3, A4 and A8 mRNAs was very inefficient. The same profiles were obtained in the presence of the FGF-2 mRNA leader (Fig. 2B, 5′-UTR). This suggested the existence of an inhibitory element in the FGF-2 mRNA 3′-UTR between the first and second poly(A) sites.

The half-lives of the different CAT mRNAs were measured to determine whether this inhibitory element was an RNA-destabilizing element or a translational silencer (Fig. 2C). This analysis revealed a CAT-A1 mRNA half-life (110 min) similar to the CAT-A0 mRNA half-life (126 min), whereas a drastic shortening of the half-life was measured for CAT-A2, CAT-A3, CAT-A4, and CAT-A8 mRNAs (26, 25, 18, and 14 min, respectively). This demonstrated that the inhibitory element located between the first and second poly(A) sites was a destabilizing element. The same destabilizing effect was observed in the presence of the FGF-2 mRNA 5′-region, even though the 5′-UTR mRNAs were slightly more stable than their CAT counterparts (Fig. 2C).

CAT mRNAs half-lives were also measured in skin fibroblasts and HeLa and SK-Hep-1 cells (Fig. 2, D–F). The destabilizing element clearly shortened the mRNA half-life by 4-fold in all these cell types, as in COS-7 cells, thus indicating that the activity of the FGF-2 mRNA-destabilizing element was not cell type-specific.

The FGF-2 mRNA-destabilizing Element Involves a 122-nt Tandem Repeat Located Upstream from the Second Poly(A) Site—Prior to further characterization of the FGF-2 mRNA-destabilizing element, the possibility of a nonspecific destabilizing effect due to the large size of the 3′-UTR was considered by analyzing the effect of the 5823 nt-long 3′-UTR in a reverse orientation (Fig. 3A, CAT-3′-inverted) on mRNA stability. The results showed that the CAT-A8-inverted mRNA, like the control CAT-A0, was four times more stable than the CAT-A8 mRNA, both in COS-7 cells and in skin fibroblasts, so the hypothesis of nonspecific effects generated by the unusual length of the 3′-UTR could be ruled out.

The efficiency of the FGF-2 mRNA-destabilizing element was also compared with that of the well characterized GM-CSF ARE. COS-7 cells and skin fibroblasts were transfected with CAT mRNAs bearing the 3′-UTR of the GM-CSF mRNA, with or without its ARE (Fig. 3A, CAT-3′-GM-CSF and CAT-3′-GM-ΔAU). Measurement of the mRNA half-life showed that the GM-CSF ARE was able to reduce the CAT mRNA half-life, under our conditions, by a factor of 3 (Fig. 3A), in accordance with the report of Rajagopalan and Malter (26). The FGF-2 mRNA-destabilizing element, which could shorten the mRNA half-life by a factor of 4, could thus be considered an efficient destabilizing element.
The destabilizing element was then more precisely localized by a deletion approach (Fig. 3B). RNA transfection of human skin fibroblasts showed that removal of the 208 nt upstream from the second poly(A) site abolished the destabilizing effect (Fig. 3B, CAT-A2). However, this 208-nt-long fragment, when directly inserted 3' of the CAT gene, was not sufficient to generate mRNA destabilization (CAT-dest208). In contrast, a fragment corresponding to the 334 nt upstream from the second poly(A) site was sufficient to induce the destabilizing effect (Fig. 3B, CAT-dest334). Removal of this fragment (called DEST hereafter) from the complete FGF-2 3'-UTR also abolished RNA destabilization (Fig. 4C, A8dest).

Examination of the DEST nucleotide sequence revealed the existence of two 122-nt-long direct repeats with 88% identity, 79% of which consisted of A and U residues. However, each of these repeats contained only a single AUUUA motif (Fig. 4A), whereas AREs described in proto-oncogene and lymphokine mRNAs always contain reiterated AUUUA motifs (23).

Deletions were therefore performed within the DEST element, and RNA transfection was carried out in skin fibroblasts as described above. The deletion of a 57-nt-long AluI/III fragment corresponding to the 3'-part of the upstream repeat did not affect RNA destabilization (Fig. 4B, destA), whereas the deletion of a 126-nt-long SpeI fragment, which removed a complete repeat, abolished the destabilizing effect (Fig. 4C, destAS).
An alternative strategy was to cotransfect CAT mRNA with an excess of antisense RNAs targeting different parts of the DEST element. The results given in Fig. 4 show that an antisense RNA targeting the complete DEST element was able to prevent RNA destabilization, whereas an antisense RNA directed against the CAT sequence had no effect (Fig. 4, AS3 and ASCAT). Furthermore, shorter antisense RNAs directed against part or all of the downstream repeat were also able to abolish the destabilizing effect (AS1 and AS2).

These data show that the DEST element involves the two AU-rich direct repeats. Apparently both repeats, except for the 3'-part of the upstream repeat, are required and are responsible for the entire destabilizing effect of the long FGF-2 mRNA 3'-UTR.

**DISCUSSION**

We show in this report that the length of the FGF-2 mRNA 3'-UTR is conditioned by a process of alternative polyadenylation, specific to the cell type. The proximal poly(A) sites seem to be preferentially used in three transformed cell lines, whereas an antisense RNA directed against the CAT sequence had no effect (Fig. 4, B and D, AS3 and ASCAT). Furthermore, shorter antisense RNAs directed against part or all of the downstream repeat were also able to abolish the destabilizing effect (AS1 and AS2).

These data show that the DEST element involves the two AU-rich direct repeats. Apparently both repeats, except for the 3'-part of the upstream repeat, are required and are responsible for the entire destabilizing effect of the long FGF-2 mRNA 3'-UTR.

We provide the first evidence that a member of the ever-increasing FGF family is regulated at the level of mRNA stability by a cis-acting element, the presence of which is controlled by alternative polyadenylation. Regulation of FGF-2 mRNA stability has been reported only in *Xenopus*, in which the *gfg* antisense RNA that induces FGF-2 mRNA destabilization by a process of RNA editing is involved (27). However, the mammalian *gfg* mRNA, although present in cells, has never been shown to affect FGF-2 mRNA stability (28). Our results suggest that mammalian FGF-2 mRNA stability is regulated by a process similar to that controlling other cytokines and involving AU-rich elements.

The AU-rich FGF-2 mRNA-destabilizing (DEST) element described here is unique. It does not contain the tandem AUUUA motifs described for most proto-oncogenes, cytokines, and growth factor mRNAs or the long U-rich region enhancing the destabilizing effect of AREs found in c-fos mRNA, for example

**Fig. 3. Characterization of the FGF-2 mRNA-destabilizing element.** A, COS-7 cells (middle panel) and skin fibroblasts (right panel) were transfected with CAT mRNAs bearing either the complete 5823 nt in the correct orientation (CAT-A8) or in the antisense orientation (CAT-3' inverted) or the GM-CSF AU-rich 3'-UTR, complete (CAT-3' GM-CSF) or ARE-deleted (CAT-3' GM-ΔAU). The constructs are schematized in the left panel. B, skin fibroblasts were transfected with CAT mRNAs bearing different deletions derived from CAT-A2 (middle panel) and CAT-A2 (right panel) constructs. CAT-A0, CAT-A1, and CAT-A2 mRNAs were used in Fig. 2. The CAT-A2 constructs 5'CAT-A2A1, 5'CAT-A2A2, and 5'CAT-A2A3 contain deletions of 210, 720, and 1510 nt, respectively, located just upstream from the second poly(A) site. CAT-dest208 and CAT-dest304 contain the 208- and 334-nt fragments located just upstream of the second poly(A) site, respectively. Half-life measurements were obtained as described in the legend to Fig. 2 and are presented as autoradiograms, histograms, and values. The time between transfection (after RNA removal) and cell harvesting is indicated for each point. The transfected cell type (A) and the presence or absence of the FGF-2 5'-leader (B) are indicated on top of each panel.

No study of FGF-2 mRNA half-life had been carried out up to now because of the technical difficulty of detecting the mRNA on Northern blots. Furthermore, the presence of several mRNA species resulting from alternative polyadenylation rendered interpretation very complex and prevented the expression of homogenous mRNAs after DNA transfection. The development and optimization of the RNA transfection procedure described here, novel for primary cells, were crucial in studying the expression of a single mRNA species in the absence of alternative polyadenylation.

We provide the first evidence that a member of the ever-increasing FGF family is regulated at the level of mRNA stability by a cis-acting element, the presence of which is controlled by alternative polyadenylation. Regulation of FGF-2 mRNA stability has been reported only in *Xenopus*, in which the *gfg* antisense RNA that induces FGF-2 mRNA destabilization by a process of RNA editing is involved (27). However, the mammalian *gfg* mRNA, although present in cells, has never been shown to affect FGF-2 mRNA stability (28). Our results suggest that mammalian FGF-2 mRNA stability is regulated by a process similar to that controlling other cytokines and involving AU-rich elements.

The AU-rich FGF-2 mRNA-destabilizing (DEST) element described here is unique. It does not contain the tandem AUUUA motifs described for most proto-oncogenes, cytokines, and growth factor mRNAs or the long U-rich region enhancing the destabilizing effect of AREs found in c-fos mRNA, for example
In fact, sequence comparison revealed homology of the FGF-2 DEST element to the interleukin-2 and vascular endothelial growth factor AREs, which are also unusual (29, 30). However, neither of these destabilizing elements contains the 122-nt-long direct repeat observed for FGF-2 (Fig. 4). Evidence is provided here that the presence of AUUUA motifs is not sufficient to destabilize FGF-2 mRNA: the 3'-UTR contains 15 AUUUA motifs outside the DEST element, which do not influence mRNA decay (Fig. 4) (13). Furthermore, the two AUUUA motifs located in the 5'-part of each repeat in the DEST element are unable to generate RNA instability by themselves and require the presence of tandem repeats for RNA destabilization.

This raises the question of protein involvement in the regulation of FGF-2 ARE activity. The ARE-mediated destabilization mechanism, although still mechanistically unclear, involves RNA/protein interactions. At least 10 ARE-specific binding proteins have been identified to date, with different affinities for specific RNA sequences (23). Nine of them are able to bind to the c-myc ARE, and six of them are able to bind to poly(U); two of these proteins, AU-B and AU-C, are unable to bind to the c-myc ARE or poly(U). Interestingly, the AU-B protein binds to the interleukin-2 ARE (31). Furthermore, the inactivation of the vascular endothelial growth factor ARE, involved in hypoxia-induced mRNA stabilization, is correlated with the binding of three proteins with molecular masses of 17, 28, and 32 kDa (30). This supports the hypothesis that the FGF-2 DEST element could also be regulated by cellular protein binding. The requirement of both elements of the repeat for RNA destabilization suggests that the potential regulatory protein(s) has two binding sites in the DEST element; one possibility would be the cooperative binding of two protein molecules, only active as a dimer, to the DEST element.

There are few examples in the literature of regulation involving processes of alternative polyadenylation coupled with RNA stability. One interesting case of inducible stability dependent on polyadenylation is provided by the glutaminase mRNA. This mRNA is expressed with two forms of 3'-UTR, the longer of which contains a pH-responsive stability element (32). Vascular endothelial growth factor mRNA stability is regulated by a hypoxia-controlled ARE located between two alternative polyadenylation sites (33). The FGF-2 DEST element, located between the first and second poly(A) sites, could ensure a rapid turnover of the FGF-2 mRNA in cells presenting polyadenylation at A2 or downstream. The coupled polyadenylation and destabilization of FGF-2 mRNA in such a case could enable the cell to provide a rapid regulation change in response to exogenous stimuli.

An interesting hypothesis is provided by the results in Fig. 1, which show that the shortest mRNA (cleaved at A1) is present in the three transformed cell lines (28–100%), but constitutes only 4.5% of the FGF-2 mRNA in primary skin fibroblasts.
These results suggest that the FGF-2 mRNA can be destabilized in skin fibroblasts, but is stable in transformed cells, where it is devoid of the destabilizing element. We have shown in a previous report that FGF-2 expression is transcriptionally regulated in normal skin fibroblasts, but constitutively expressed in transformed cell lines (SK-Hep-1 and HeLa) as well as in skin fibroblasts transformed by SV40 large T antigen (34). The present data once again suggest that FGF-2 expression cannot be regulated in transformed cells, a feature that could be a cause or a consequence of the transformed phenotype.

All these observations indicate that although FGF-2 expression undergoes transcriptional control, it is mostly regulated post-transcriptionally at three levels: mRNA polyadenylation, stability, and translation. The existence of several post-transcriptional regulations for a given mRNA renders the control of expression very stringent, which permits its expression only under relevant conditions. It also provides the possibility for untransformed cells to very rapidly modulate the level of FGF-2 expression in response to exogenous stimuli. The unlocking of these regulations may be one of the parameters responsible for cell transformation.

Acknowledgments—We thank R. Courret for pictures and D. Warwick for English proofreading. We also thank Prof. Costagliola for human skin samples and G. Huez for plasmids pCMV-CAT-GM-AU(+ and pCMV-CAT-GM-AU(−).

REFERENCES

1. Mason, I. J. (1994) Cell 76, 547–552
2. Yamaguchi, T. P., and Rossant, J. (1995) Curr. Opin. Genet. Dev. 5, 485–491
3. Smallwood, P. M., Munoz-Sanjuan, I., Tong, P., Macke, J. P., Hendry, S. H. C., Gilbert, D. J., Copeland, N. G., Jenkins, N. A., and Nathans, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9850–9857
4. Hoshikawa, M., Ohbayashi, N., Yonamine, A., Konishi, M., Ozaki, K., Fukui, S., and Itoh, N. (1998) Biochem. Biophys. Res. Commun. 244, 187–191
5. Ohbayashi, N., Hoshikawa, M., Kinzura, S., Yamasaki, M., Fukui, S., and Itoh, N. (1998) J. Biol. Chem. 273, 18161–18164
6. Wagner, J. A. (1991) Curr. Top. Microbiol. Immunol. 165, 95–118
7. Coffin, J. D., Florkiewicz, R. Z., Neumann, J., Mart-Hopkins, T., Dorn, G. W., Lightfoot, P., German, R., Howles, P. N., Kier, A., O’Toole, B. A., Sasse, J., Gonzalez, A. N., Baird, A., and Doetschman, T. (1995) Mol. Biol. Cell 6, 1861–1973
8. Yanagisawa-Miwa, A., Uchida, Y., Nakamura, F., Tomaru, T., Kido, H., Kamijo, T., Sugimoto, T., Kaji, K., Utsuyama, M., Kurashima, C., and Ito, H. (1992) Science 257, 1401–1403
9. Kandel, J., Bossy-Wetzel, E., Radavanyi, F., Klagsbrun, M., Folkman, J., and Hanahan, D. (1991) Cell 66, 1095–1104
10. Coudrec, B., Prats, H., Bayard, F., and Amalric, F. (1991) Cell Regul. 2, 699–708
11. Quarto, N., Talarico, D., Florkiewicz, R., and Rifkin, D. B. (1991) Cell Regul. 2, 699–708
12. Florkiewicz, R. Z., and Sommer, A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3978–3981
13. Prats, H., Kughad, M., Prats, A.-C., Klagsbrun, M., Lelias, J. M., Liaisun, P., Chalon, P., Tauber, J. P., Amalric, F., Smith, J. A., and Caput, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1386–1388
14. Arnaud, E., Touriol, C., Boutonnet, C., Genesa, M. C., Vagner, S., Prats, H., and Prats, A.-C. (1999) Mol. Cell. Biol. 19, 505–514
15. Bugler, B., Amalric, F., and Prats, H. (1991) Mol. Cell. Biol. 1, 573–577
16. Briggstock, D. R., Sasse, J., and Klagsbrun, M. (1991) Growth Factors 4, 189–196
17. Bikfalvi, A., Klein, S., Pintucci, G., Quarto, N., Mignatti, P., and Rifkin, D. B. (1991) J. Cell Biol. 129, 233–243
18. Prats, A.-C., Vagner, S., Prats, H., and Amalric, F. (1992) Mol. Cell. Biol. 12, 4796–4805
19. Vagner, S., Genesa, M. C., Maret, A., Bayard, F., Amalric, F., Prats, H., and Prats, A.-C. (1995) Mol. Cell. Biol. 15, 35–44
20. Bensaid, M., Malecze, F., Prats, H., Bayard, F., and Tauber, J. P. (1989) Exp. Eye Res. 45, 801–813
21. Knee, R. S., Pitcher, S. E., and Murphy, P. R. (1994) Biochem. Biophys. Res. Commun. 205, 577–583
22. Wickens, M., Anderson, P., and Jackson, R. J. (1997) Curr. Opin. Genet. Dev. 7, 220–232
23. Jarzembowski, J. A., and Malter, J. S. (1997) Prog. Mol. Subcell. Biol. 18, 141–172
24. Vagner, S., Touriol, C., Galy, B., Audigier, S., Genesa, M. C., Amalric, F., Bayard, F., Prats, H., and Prats, A.-C. (1996) J. Cell Biol. 135, 1391–1402
25. Gallie, D. R. (1991) Genes Dev. 5, 2108–2116
26. Rajagopalan, L. E., and Malter, J. S. (1996) J. Biol. Chem. 271, 19871–19876
27. Kimelman, D., and Kirschner, M. W. (1989) Cell 59, 687–696
28. Murphy, P. R., and Knee, R. S. (1994) Mol. Endocrinol. 8, 652–659
29. Henics, T., Sanfridson, A., Hamilton, B. J., Nagy, E., and Rigby, W. F. (1994) J. Biol. Chem. 269, 5377–5383
30. Claffey, K. P., Shih, S. C., Mullen, A., Dzienski, S., Cusick, J. L., Abrams, K. R., Lee, S. W., and Deitmar, M. (1998) Mol. Biol. Cell 9, 469–481
31. Behjatine, P. R., Petryniak, B., June, C. H., Thompson, C. B., and Lindsten, T. (1991) Mol. Cell. Biol. 11, 3288–3295
32. Hansen, W. R., Barsic-Tress, N., Taylor, L., and Curthoys, N. P. (1996) Am. J. Physiol. 271, P126–P131
33. Levy, A. P., Levy, N. S., Wegner, S., and Goldberg, M. A. (1995) J. Biol. Chem. 270, 13333–13340
34. Galy, B., Marett, A., Prats, A.-C., and Prats, H. (1999) Cancer Res. 59, 165–171