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c-*fos* gene expression in cell revertants from a transformed to a pseudonormal phenotype

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c-*fos* gene expression in two types of mouse sarcoma cells of spontaneous origin and in revertants to pseudonormal phenotype has been investigated. In the latter cells the content of c-*fos* mRNA is similar to that in normal fibroblasts. Activity of transcription factors interacting with the regulatory elements, SRE, DSE and TRE, in the c-*fos* promoter do not correlate with the c-*fos* mRNA concentration. However, experiments with cells transformed with the indicator plasmid, *fos*-CAT, showed that the 600 bp c-*fos* promoter region provides the chloramphenicol acetyltransferase activity correlating with c-*fos* mRNA expression in cell revertants to a pseudonormal phenotype.

Tumorigenicity; c-*fos* oncogene; Transcription factor

1. INTRODUCTION

The c-*fos* gene is an immediate early gene, and serves as a marker of transition from a quiescent to a mitotic state of a cell in response to growth stimuli [1]. Therefore, investigation of the regulation of this gene in cells in response to different growth stimuli may shed some light on characteristic features of cells which differ in their tumorigenic potential. We investigated c-*fos* expression in two lines of spontaneously transformed embryonic fibroblasts of mice, CBA and C3H. Each of these lines was represented by a pair of clones: tumorigenic, and non-tumorigenic, obtained from the first one as a result of reversion from a transformed phenotype to a pseudonormal one in the process of cloning [2]. The clones differed in their tumorigenicity and their response to growth stimuli. Like normal fibroblasts, non-tumorigenic cells possess the property of density-dependent growth arrest in monolayer culture and could be stimulation by different growth factors. Tumorigenic cells do not respond to the stimulation [2].

2. MATERIALS AND METHODS

Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). Transfection of cells was performed by the calcium phosphate-mediated precipitation method [3]. Retardation of cell growth was achieved by cultivation in DMEM with 0.5% embryonal serum for 48 h, followed by serum starvation for 36 h. Stimulation was performed by incubation in 20% FCS or TPA (5 nM). For stable transfection, cells were co-transfected with the plasmids, *fos*-CAT and pSV2neo [4], at a ratio of 10:1. The cells were selected for neomycin resistance in medium containing G418 (500 μg/ml). Individual clones were isolated, amplified, and characterized for the incorporation and expression of the construct by Southern blot analysis and CAT (chloramphenicol acetyltransferase) activity, as described in [5]. The plasmid, *fos*-cat, was constructed by inserting the *Hin*dIII–*Nar*I (600 bp) fragment of the mouse c-*fos* gene and CAT gene (*Cla*I–*Bam*HI) in the PBS plasmid (Stratagene) in the *Hin*dIII–*Bam*HI sites. The control plasmid for testing the transient transfection efficiency contained the LTR on the Rous sarcoma virus and CAT gene. RNA was isolated as described in [6]. Northern blot analysis was performed according to [7]. As a probe, a *Pst*I–*Pst*I fragment of the v-*fos* (1,000 bp) was used. Specific radioactivity of the probe was 5×10^8 cpm/μg.

Oligonucleotides: DSE (5′-[^{32}P]-AGCTTGATGCCCATATTAGGACATCTA), TRE (5′-[^{32}P]-GATCCATGAGTCAGAG), SRE (5′-[^{32}P]-CGGATGTCCATATTAGGACATCTGCGTCAGCAG) were synthesized by standard automated methods and purified by polyacrylamide gel electrophoresis followed by reverse-phase liquid column chromatography (Silasorb-C8, 0–50% gradient of acetonitrile with 20 mM $LiClO_4$). Nuclear extracts were prepared as described in [8]. For the binding assay, typically, 5 μg of protein was incubated with 1 ng (10,000 cpm) of a radiolabeled oligonucleotide. The complexes formed were analyzed by electrophoresis in 5% polyacrylamide gels.

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3. RESULTS AND DISCUSSION

The cells investigated in this work represented two lines of aggressive sarcoma. These cells were obtained from mice fibroblasts, C3H and CBA, by spontaneous transformation in vitro, and then re-cloned 6 times. After each re-cloning, the cells were tested for the ability to produce tumors in singene mice (test for tumorigenicity). Most of the clones were formed by the parent aggressive cells, which were non-sensitive to growth factors and showed no contact inhibition. However, at each recloning about 15% of revertants were obtained which were sensitive to growth factors and showed contact inhibition [2]. These cell lines may be an interesting

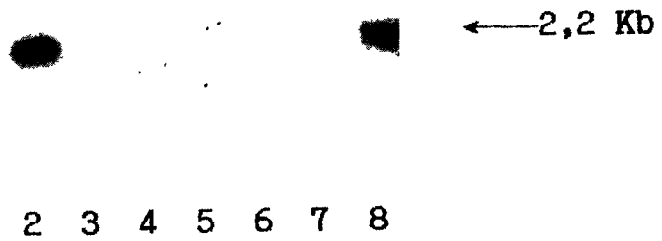

Fig. 1. Determination of c-*fos* mRNA content in cells. (Lanes 1–4) Fibroblasts of mice CBA. (Lanes 5–8) Fibroblasts of mice C3H. (Lanes 1,5) Quiescent non-tumorigenic cells (48 h in the serum-free medium); (2,8) quiescent non-tumorigenic cells incubated with 20% FCS for 30 min; (3,6) non-tumorigenic cells in log-phase of growth; (4,7) tumorigenic cells.

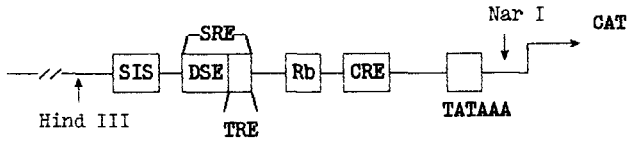

Fig. 2. Regulatory sites of the c-*fos* promoter in the *fos*-CAT plasmid. SIS, PDGF control element; DSE, double symmetry element; SRE, serum response element; TRE, TPA response element; Rb, retinoblastome control element; CAT, the start of the gene coding for chloramphenicol acetyltransferase.

model for investigation of mechanisms of malignant transformation of cells, because they give the possibility of investigating both spontaneous transformation and normalization of the cellular phenotype.

In this study the following cells were investigated: (i) tumor cells, obtained from fibroblasts of mice C3H; (ii) non-tumorigenic revertants obtained from the above cells; (iii) tumor cells, obtained from fibroblasts of mice CBA; (iv) non-tumorigenic revertants, obtained from the above cells (iii). Cells (i) and (iii) have a highly transformed phenotype; cells (ii) and (iv) have a pseudonormal phenotype. Fig. 1 shows the results of assay of c-*fos* mRNA content in the above cells under different conditions, namely in quiescent, stimulated and proliferating non-tumorigenic cells and in malignant cells, which can only be sustained by growing, and die at low concentrations of the serum. After 30 min stimulation of the quiescent cells with embryonal serum or TPA, a considerable amount of the mRNA was detected in the cells (Fig. 1). This result is in accordance with the data on c-*fos* regulation in normal fibroblasts [9]. The content of c-*fos* mRNA in the quiescent pseudonormal cells

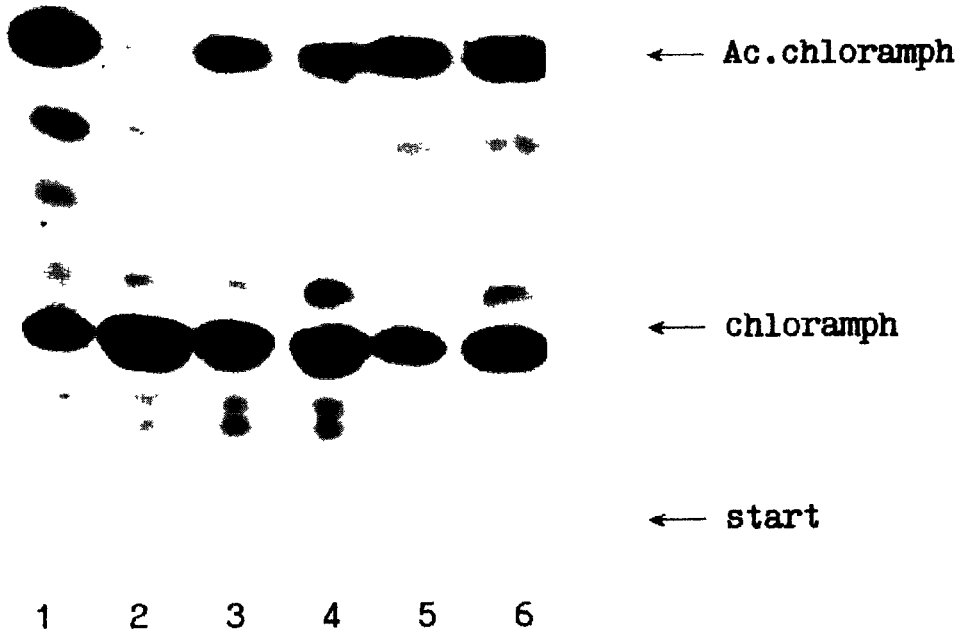

Fig. 3. CAT assay in extracts from C3H cells 48 h after transfection with *fos*-CAT plasmid. (Lane 1) Quiescent non-tumorigenic cells incubated with 20% FCS for 3 h; (2) quiescent non-tumorigenic cells, 48 h in serum-free medium; (3) non-tumorigenic cells in log-phase of growth; (4) non-tumorigenic cells in log-phase of growth incubated with 20% FCS for 3 h; (5) quiescent non-tumorigenic cells incubated with TPA for 3 h; (6) tumorigenic cells.

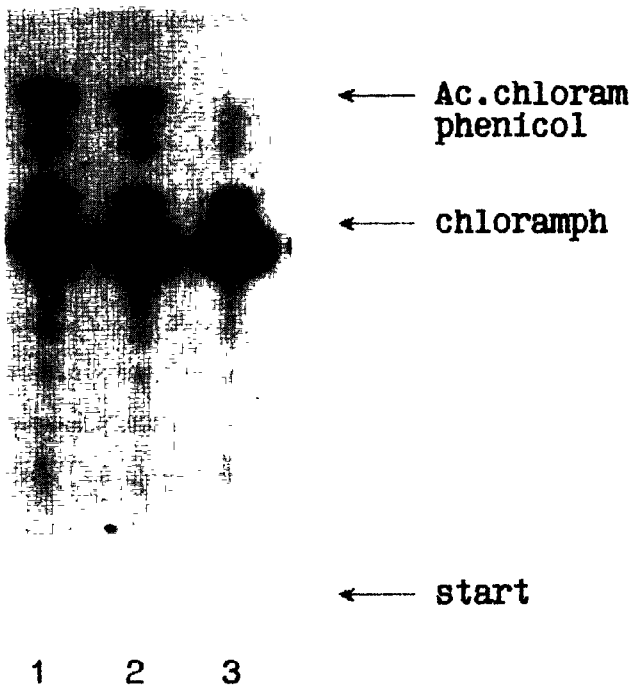

Fig. 4. CAT assay in extracts from C3H cells stably transfected with *fos*-CAT plasmid. (Lane 1) Quiescent non-tumorigenic cells incubated with 20% FCS for 3 h; (2) non-tumorigenic cells in log-phase of growth; (3) quiescent non-tumorigenic cells.

and in the malignant cells was too low to be detected by Northern hybridization under the conditions used.

We tried to find out whether the known regulatory elements of the c-*fos* gene promoter can provide the pattern of regulation of the indicator gene, CAT, similar to that observed for the c-*fos* gene in these cells. To this aim, we have constructed a *fos*-CAT plasmid (Fig. 2). Determination of CAT activity in quiescent and TPA-stimulated cells or FCS cells transiently transfected with the *fos*-CAT plasmid, showed that the selected promoter fragment provides the CAT activity, correlating with the level of c-*fos* RNA in the cells (Figs. 1 and 3). Similar results were obtained with cells stably transfected with the *fos*-CAT plasmid (Fig. 4).

Since the major regulatory sites of the c-*fos* gene promoter region are shown [10–15], we assayed the activity of the transcription factors interacting with these sites in the transformed and non-transformed cell lines. It was found that stimulation of synthesis of c-*fos* mRNA with TPA or embryonic serum in non-tumorigenic cells do not correlate with DSE, SRE and TRE transcription factor activities (Fig. 5). The data shown are for SRE; the same results were obtained for DSE and TRE. An increase in the c-*fos* mRNA concentration was observed 15 min after cell stimulation, while an increase in the factors' activity was detected 6 h later (data not shown).

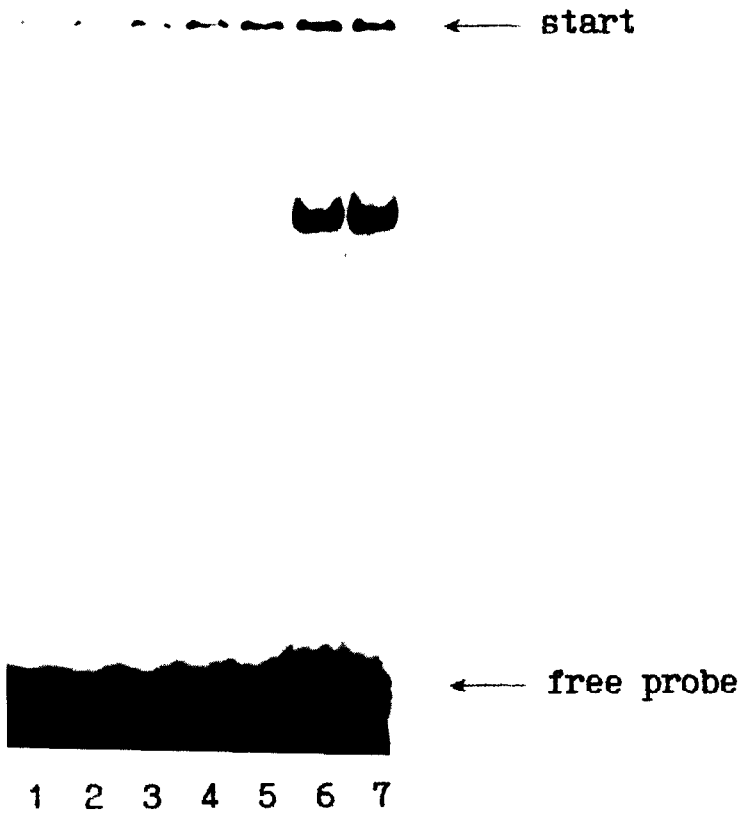

Fig. 5. Gel-retardation of SRE oligonucleotide with nuclear extracts from C3H cells. (Lane 1) Quiescent non-tumorigenic; (2) quiescent non-tumorigenic, incubated with 20% FCS for 30 min; (3) quiescent non-tumorigenic, incubated with 20% FCS for 1 h; (4) quiescent non-tumorigenic, incubated with TPA for 30 min; (5) quiescent non-tumorigenic, incubated with TPA for 1 h; (6) non-tumorigenic in log-phase of growth; (7) tumorigenic.

Despite the different concentrations of c-*fos* mRNA, similar high levels of the factors' activities were found in log-phase growth in transformed and non-transformed cells. The absence of a correlation between c-*fos* transcription and activity of the transcription factors interacting with SRE was demonstrated in the human cell line, A431 [16].

The results indicate that the investigated part of the c-*fos* promoter region is sufficient to provide the observed c-*fos* gene regulation pattern. The absence of correlation between c-*fos* mRNA concentrations and transcription factor activities may be explained either by some modification of the transcription factors affecting the regulation or by the existence of some other transcription factors or by regulation sites besides those tested [17].

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REFERENCES

[1] Curran, T. (1988) FOS: The Oncogene Handbook, pp. 307–325, Elsevier, Amsterdam.
[2] Lavrovsky, V., Guvakova, M. and Lavrovsky, Y. (1992) Eur. J. Cancer 28, 17–21.
[3] Wigler, M., Pellicer, A., Silverstein, S. and Axel, R. (1977) Cell 11, 223–232.
[4] Southern, P. and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327–341.
[5] Gorman, C., Merlino, G., Willingham, M., Pastan, I. and Howard, B. (1982) Proc. Natl. Acad. Sci. USA 79, 6777–6782.
[6] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[7] Lehtola, L., Sistonen, L., Koskinen, P., Lehvaslaiho, H., Di Renzo, M., Comoglio, P. and Alitalo, K. (1991) J. Cell. Biochem. 45, 69–81.
[8] Groot, R., Foulkes, N., Mulder, M., Kruijer, W. and Sassone-Corsi, P. (1991) Mol. Cell. Biol. 11, 192–201.
[9] Sassone-Corsi, P., Sisson, S. and Verma, I.M. (1988) Nature 334, 314–319.
[10] Robbins, P., Horowitz, J. and Mulligan, R. (1990) Nature 346, 668–671.
[11] Wagner, B., Hayes, T., Hoban, C. and Cochran, B.H. (1990) EMBO J. 9, 4477–4484.
[12] Konig, H., Ponta, H., Rahmsdorf, U., Buscher, M., Schonthal, A., Rahmsdorf, H. and Herrlich, P. (1989) EMBO J. 8, 2559–2566.
[13] Show, P., Frash, S. and Nordheim, A. (1989) EMBO J. 8, 2567–2574.
[14] Sassone-Corsi, P., Ransone, L., Lamph, W. and Verma, I.M. (1988) Nature 336, 692–695.
[15] Treisman, R. (1987) EMBO J. 6, 2711–2717.
[16] Prywes, R. and Roeder, R. (1986) Cell 47, 777–784.
[17] Lucibello, F.C., Ehlert, F. and Müller, R. (1991) Nucleic Acids Res. 19, 3583–3591.