An upstream negative regulatory element in human granulocyte-macrophage colony-stimulating factor promoter is recognised by AP1 family members

Claudia Gramigni[a], Silvana Penco[a], Giovanna Bianchi-Scarrà[a], Roberto Ravazzolo[a,b], Cecilia Garrè[a,*]

[a]*Department of Clinical and Experimental Oncology, University of Genoa, Viale Benedetto XV, 6, 16132 Genoa, Italy*
[b]*Laboratory of Molecular Genetics, G. Gaslini Institute, Largo G. Gaslini, 5, 16148 Genoa, Italy*

Received 28 September 1998; received in revised form 27 October 1998

Abstract Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine involved in haematopoiesis and host defence. Production of GM-CSF has been detected in tumour cells including the U87MG astrocytoma cell line. Previous studies have been focused on the regulatory role of the proximal region of the GM-CSF promoter. Our studies on the distal region of the promoter in U87MG cells identify a negative *cis* element (−1377/−1298) which contains a AP1-like site able to bind c-*jun* and c-*fos* transcription factors, according to the results of DNA/protein binding assays. Mutagenesis of the AP1-like site eliminates AP1 binding and the negative effect on promoter activity.

Key words: Granulocyte-macrophage colony-stimulating factor; AP1; U87MG; Transcriptional regulation; Negative *cis* element

1. Introduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a glycoprotein cytokine involved in haematopoiesis and host defence, can be produced by different cell types in response to appropriate stimuli. Constitutive expression of GM-CSF has been detected in acute myeloid leukaemia, solid-tumour cells and several cell lines [1], including the U87MG astrocytoma cell line [2].

GM-CSF production is highly controlled both at the transcriptional and at the post-transcriptional level [1]. Transcriptional control is regulated by different transcription factors whose binding sites have been identified in the most proximal (629 bp) promoter region. In addition, a strong cyclosporin A-sensitive enhancer, located 3 kb upstream of the GM-CSF transcriptional start site, was previously described [3]. We previously identified a NF-κB-like binding site, located between −2002 and −1984, acting as a positive transcriptional element in the Mo lymphoblastoid cell line [4]. This same NF-κB-like binding site participates in response to phorbol ester in 5637 cells derived from bladder carcinoma [5]. These observations suggested that important regulatory elements in distal regions of the GM-CSF promoter may significantly contribute to the transcriptional regulation of the gene, in physiological and/or pathological conditions, as well as in a tissue-specific way. In order to continue our investigation on distal promoter elements, we have performed transfection experiments in U87MG cells, derived from the astrocytic cell lineage and expressing GM-CSF. We found a negative regulatory *cis* element (between −1377 and −1298 bp), which contains an AP1-like site. The products of the proto-oncogenes c-*jun* and c-*fos*, members of the AP1 transcription factor family, are leucine zipper proteins able to bind the TPA-responsive element (TRE) TGA(C/G)TCA as Jun/Jun homodimers or Jun/Fos heterodimers.

We report here that the AP1-like site (TGAATCA) in the GM-CSF promoter is able to bind c-*jun* and c-*fos* transcription factors. The substitution of the first nucleotide (T to G) of the core consensus sequence in the above AP1-like site eliminates both the c-*jun* and c-*fos* binding and the negative regulatory effect on promoter activity.

2. Materials and methods

2.1. Cell culture and transient transfections

Cell lines (U87MG, 5637 and HeLa) were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% foetal calf serum, 2 mM glutamine and 0.05 mg/ml gentamicin.

Transfections of U87MG cells were performed by the standard calcium phosphate coprecipitation technique [6] using 10 μg of plasmid pPF20 and equimolar amounts of the other constructs, together with 800 ng of pCMV-β-galactosidase as internal control. Chloramphenicol acetyltransferase (CAT) activity was measured by standard methods [7] after normalising for β-galactosidase activity and protein concentration. All experiments were performed in duplicate.

2.2. GM-CSF mRNA and protein assay

Total cellular RNA was purified according to Chomczynski and Sacchi [8]. RT-PCR was performed as described [9] and the RT-PCR assay of GAPDH mRNA was included in all tested samples as a control.

Assay of GM-CSF protein in cellular conditioned medium was performed by a solid-phase immunoenzymetric assay (EASIA, Medgenix Diagnostics, Fleurus, Belgium) as previously described [10].

2.3. Nuclear extracts and DNase I footprinting

Nuclear extracts from 5637 cells, HeLa cells, both stimulated with 50 ng/ml TPA for 8 h, and U87MG cells were prepared according to the method of Dignam et al. [11] and dialysed against a buffer containing 25 mM HEPES, pH 7.9, 100 mM NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulphonyl fluoride and 10% glycerol. A 3′ end-labelled 224 bp footprinting probe, which includes the 151 bp promoter region from −1416 to −1266, was prepared by cleaving plasmid pCla3 (see Section 2.5) with *Hin*dIII, labelling at the 3′ end with Klenow enzyme and a dNTP mixture containing

*Corresponding author. Fax: (39) (10) 3538978.
E-mail: regar@unige.it

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; TRE, TPA responsive element; CAT, chloramphenicol acetyltransferase; RT-PCR, reverse transcriptase polymerase chain reaction; EMSA, electrophoretic mobility shift assay

PII: S0014-5793(98)01443-4

[α-^{32}P]dCTP, digesting with *Eco*RI and purifying the end-labelled DNA fragment on a 5% polyacrylamide gel. The DNA binding reaction was performed in a final volume of 50 μl containing 25 mM HEPES, pH 7.9, 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.1 M NaCl, 10% glycerol, 2 μg of [poly(dI-dC)], 25 fmol of end-labelled DNA probe and increasing amounts (15, 22.5, 30, 37.5 μg) of nuclear extract or buffer used for dialysis of nuclear extracts. The binding reaction was allowed to proceed at room temperature for 20 min. DNase I (Boehringer, grade I, 1 mg/ml in 50% glycerol) was appropriately diluted (1:100 for samples containing nuclear extract and 1:1000 for samples without extract) immediately before use in an ice-cold buffer containing 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 62.5 mM $MgCl_2$, 10 mM $CaCl_2$ and 1 mg/ml bovine serum albumin. 2 μl was added to the binding mixture and incubated for 1 min (samples with extract) or 30 s (samples without extract) at room temperature. The reaction was stopped with 15 μl of 5% SDS, 125 mM EDTA and 0.7 mg/ml tRNA, and the samples subjected to phenol-chloroform extraction and ethanol precipitation. The digestion products were then analysed by electrophoresis on denaturing 8% polyacrylamide gel containing 7 M urea. An aliquot of the same end-labelled DNA fragment was also subjected to the G+A sequencing reaction [12] and loaded on the same gel for identification of protected sequences.

2.4. Electrophoretic mobility shift assay (EMSA)

Blunt-ended double-stranded oligonucleotides corresponding to the GM-CSF promoter wild-type sequence between −1312 and −1293 (RI) 5′-TTTAGGCTGAATCAGCCTCT-3′ (upper strand) and mutant sequence (mRI) 5′-TTTAGGCGGAATCAGCCTCT-3′ (upper strand) were used as labelled probes or as unlabelled competitors.

Double-stranded oligonucleotide described to bind selectively AP1 (TRE) 5′-GCGCGCTTGATGACTCAGCCGGAA-3′ (upper strand) and 5′-GCGTTCCGGCTGAGTCATCAAGCG-3′ (lower strand), blunt-ended double-stranded oligonucleotides described to bind NF-κB 5′-GTAGGGGACTTTCCGAGCTCGAGATCCTATG-3′ (upper strand) and E2F 5′-TAGTTTTCGCGCTTAAATTTGA-3′ (upper strand) were also used. The oligonucleotides were labelled with T4-polynucleotide kinase and [γ-^{32}P]ATP. The labelled fragments were incubated with 4 μg/assay of non-specific DNA [poly(dI-dC)], in 12.5 mM HEPES, pH 7.9, 50 mM NaCl, 0.5 mM phenylmethylsulphonyl fluoride, 0.5 mM dithiothreitol and 10% glycerol. Nuclear extract (6.5 μg protein/assay) were added to a total volume of 15 μl and the reaction incubated at room temperature for 20 min. Reaction products were resolved on a 5% polyacrylamide gel in 0.25×TBE (22 mM Tris borate, 0.25 mM EDTA). Gels were dried prior to autoradiography at −70°C. Anti-c-*jun* (Santa Cruz Biotechnology) and anti-c-*fos* (Oncogene Science) polyclonal antibodies were used to test for the presence of c-*jun* and c-*fos* in the gel shift complex. The antibodies were pre-incubated with nuclear extract and [poly(dI-dC)] for 15 min at 50 ng, 100 ng, 200 ng for anti-c-*jun* and 100 ng, 250 ng for anti-c-*fos* before the addition of the labelled oligonucleotide in the EMSA binding mixture; the mixture was then incubated for an additional 15 min at room temperature before gel electrophoresis.

2.5. Plasmid construction and mutagenesis

Plasmid pPF2000 [13], which contains the first 2010 bp upstream of the transcription start site, was used to create, by means of PCR, constructs containing the RI element. The pair of oligonucleotides RI-1 5′-GCAGACTCAGACCACAGTGC-3′, corresponding to the sequence located between −1416 and −1397 in the 2010 bp GM-CSF promoter, and RI-2 5′-ACTCCAGGTGCAGTGCCTCT-3′, corresponding to the −1285 to −1266 sequence, were used to generate a PCR product containing the GM-CSF promoter sequence located between −1416 and −1266. In order to facilitate the subsequent cloning into the pBLCAT2 vector, *Bam*HI restriction sites were introduced at the 5′ end of oligonucleotides RI-1 and RI-2. The PCR fragment was digested with *Bam*HI and subcloned in the polylinker of the pBLCAT2 vector, upstream of the thymidine kinase promoter (pCla3).

Site-directed mutagenesis of pPF22 (starting at nucleotide 1377 upstream of the transcription start site) was performed using a two-step PCR procedure with a set of four primers (RI-3, RI-4, E1 and E2), as previously described [14]. Primers RI-3 and RI-4 correspond to the double-stranded mutant oligonucleotide mRI, while primer E2 5′-CTCCCGGAACGACCCCTTCG-3′ corresponds to the promoter sequence between −881 and −862, and E1 5′-GGTGCGGGCCTCTTCGCTAT-3′ corresponds to a sequence of pBLCAT3 vector (281–300). We obtained a 670 bp product with a substitution in the first nucleotide (−1305) of the AP1-like site (GGAATCA). This PCR product (including 516 bp promoter from −1377 to −862 and 154 bp of the pBLCAT3 vector) produced, by digestion with *Nsi*I and *Bam*HI, a fragment of 197 bp that was reinserted in the pPF22 substituting the wild-type fragment (pCla22).

3. Results and discussion

3.1. GM-CSF mRNA expression in U87MG cells

Our preliminary RT-PCR assay of the basal amount of GM-CSF mRNA in U87MG cells showed an undetectable mRNA level compared with the high mRNA level expressed by Mo cells (data not shown). On the other hand, the EASIA GM-CSF protein assay of U87MG cell cultures conditioned medium showed synthesis of about 40 pg/ml, compared with 2000 pg/ml in conditioned medium of the T lymphoid Mo cell line. Taken together, these results confirm that U87MG shows constitutive GM-CSF expression although at a low level, as previously reported [2]; therefore U87MG cells represent a useful model to continue our investigation of transcriptional regulation of this cytokine in different GM-CSF-producing cellular systems.

3.2. Functional analysis of GM-CSF promoter sequences in U87MG cells

The plasmid PF2000, containing the fragment of the GM-CSF promoter from −2010 to +26 fused to the CAT reporter gene and different plasmids containing progressive deletions of the above promoter region (PF20, PF22, PF18, PF12,

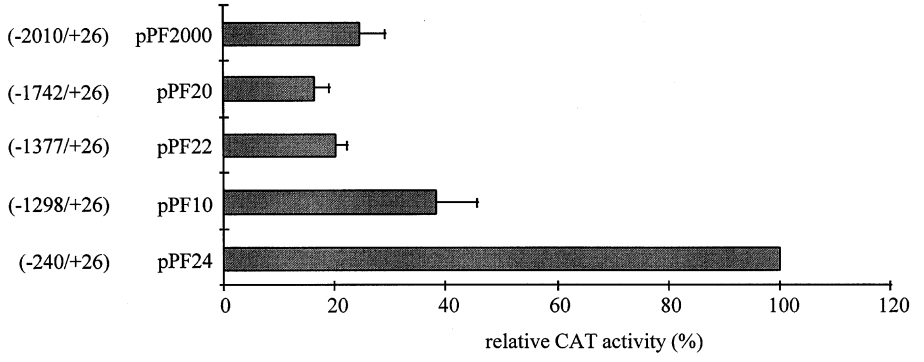

Fig. 1. Functional activity of the GM-CSF promoter constructs in U87MG cells. Values represent the mean ± S.E.M. of CAT activity of at least three independent experiments performed in duplicate. CAT activity of pPF24 is considered 100%.

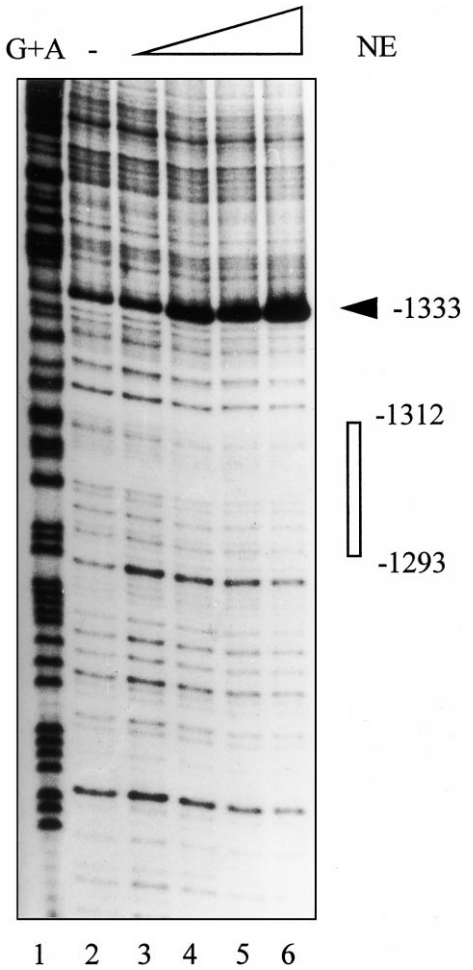

Fig. 2. DNase I footprinting of the GM-CSF promoter sequence between −1416 and −1266. Lane 1, G+A sequence; lane 2, without extract; lanes 3–6, with 15, 22.5, 30, 37.5 μg of U87MG crude nuclear extract, respectively. The arrow indicates the hypersensitive site, the bar indicates the protected region.

PF14, PF4, PF24), as previously described [13], were used in transient transfection experiments in U87MG cells. Fig. 1 shows the results of transfection experiments referred to promoter activity of the pPF24 (−240 to +26 promoter fragment) considered as 100%. We observed the highest promoter activity with the promoter sequence contained in pPF24 and a general repression with the upstream sequence up to −2010. Transfections with intermediate constructs indicated a decrease of activity between pPF2000 (−2010/+26) and pPF20/pPF22 (−1742/+26; −1377/+26) and a significant increase of activity with pPF10 (−1298/+26). This confirmed that the previously described positive element between −2002 and −1984 [5,6] was effective also in this cell type and suggested the presence of negative regulatory elements.

3.3. *The GM-CSF promoter sequence between −1416 and −1266 is recognised by nuclear factors*

A restriction fragment containing the 151 bp promoter sequence between −1416 and −1266 was used as a labelled probe for footprinting analysis with a nuclear extract from U87MG cells, to identify DNA/protein binding sites in the above negative regulatory element. Our results show a hypersensitive site located around −1333, as well as an area of relative protection between −1312 and −1293 (Fig. 2).

A computer analysis of the protected sequence (RI) indicated a potential AP1-like site (TGAATCA) and an upstream partially overlapping E2F-like binding site (TTTAGGCTG). To further characterise the *trans*-acting factors binding to the above described sequence, we synthesised a double-stranded oligonucleotide reproducing the −1312 to −1293 sequence (RI) that corresponds to the DNase I protected region. An EMSA assay with nuclear extract of U87MG cells resulted in the appearance of two retarded complexes, as shown in Fig. 3a, lane 1. Complex I was specifically competed in a dose-dependent manner by the same unlabelled oligonucleotide used as a probe (lanes 2, 3, 4), whereas complex II, which was not competed, was due to non-specific binding. This was also confirmed in EMSA assays when [poly(dI-dC)] was pre-incubated with nuclear extract resulting in the disappearance of complex II (data not shown). Competition with double-stranded oligonucleotides containing AP1, E2F and NF-κB binding sites indicated that the AP1 oligonucleotide was able to compete complex I formation as efficiently as the RI oligonucleotide (lanes 8, 9, 10), whereas the E2F oligonucleotide was a very weak competitor (lanes 5, 6, 7) and NF-κB was unable to compete (data not shown). Complex II was not competed by any of the above oligonucleotides.

3.4. *AP1 family members binding to the RI element*

When we used the AP1 oligonucleotide as a labelled probe in comparison with the RI oligonucleotide (Fig. 3b) we observed that the retarded complexes were identical (lanes 1, 3) and complex I was competed by the RI oligonucleotide (lane 2). Moreover, complex I was abundantly present in nuclear extracts from the 5637 and HeLa cell lines both induced with TPA (lanes 4, 5).

The above results strongly suggest that an AP1 factor binds the RI oligonucleotide and, therefore, we used specific anti-c-*jun* and anti-c-*fos* antibodies in EMSA assays to further support this hypothesis (Fig. 4). When we used the RI oligonucleotide as a labelled probe and the antibodies were pre-incubated with nuclear extract and the [poly(dI-dC)] non-specific competitor, we observed that complex I was significantly reduced with anti-c-*jun* antibody in a dose-dependent manner (Fig. 4a, lanes 3–5). The same effect, although less striking, was observed with the anti-c-*fos* antibody (Fig. 4b). When we used an AP1 consensus oligonucleotide as a labelled probe, we obtained reduction of complex I with both antibodies (Fig. 4c). These results indicate that c-*jun* and c-*fos* proteins are components of complex I.

3.5. *Effect of mutagenesis on AP1 binding and on promoter activity*

We then synthesised a double-stranded mutant oligonucleotide containing a single base substitution (T to G) on the basis of previous data about the bases required for AP1 binding [15]. This mutant oligonucleotide (mRI) was unable to compete binding of complex I (Fig. 5a, lane 3) and, when used as a labelled probe, was unable to form the complex (lanes 4, 5, 6).

The same single base pair mutation that abolished the binding of complex I in EMSA was introduced in a construct containing a promoter fragment from −1377 to +26 (pCla22) that was used in transient transfection experiments

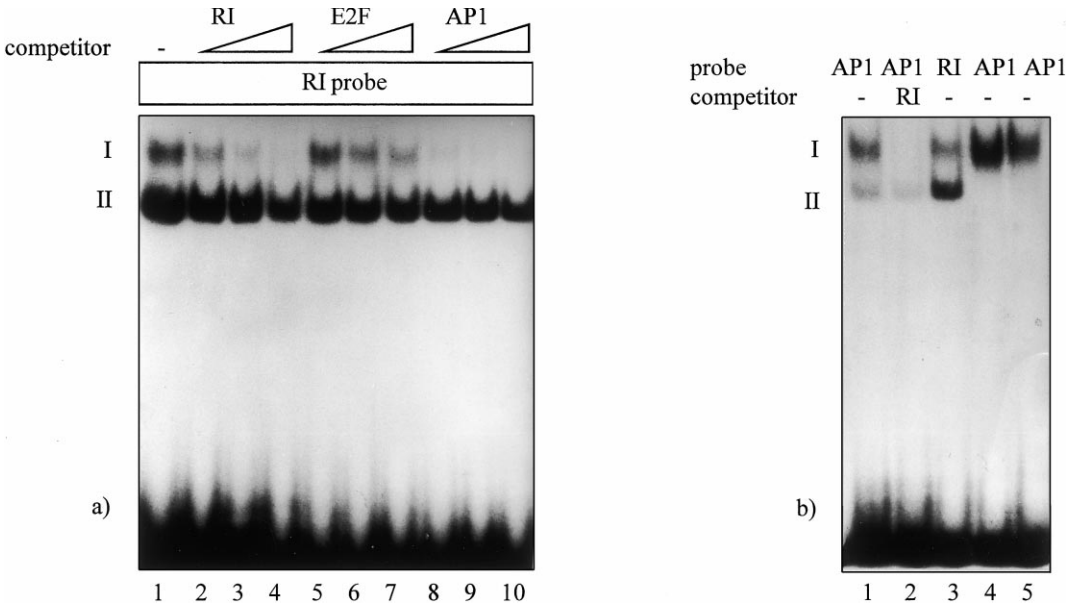

Fig. 3. Electrophoretic mobility shift assays with the RI and AP1 probes. a: The labelled RI oligonucleotide was incubated with 6.5 μg of U87MG nuclear extract: without competitor (lane 1); competition with 100-, 200-, 400-fold molar excess of unlabelled RI oligonucleotide (lanes 2–4) or the same amounts of unlabelled E2F oligonucleotide (lanes 5–7) or of unlabelled AP1 oligonucleotide (lanes 8–10). b: RI and AP1 oligonucleotides were both used as labelled probes as indicated, RI oligonucleotide was used as competitor (400-fold molar excess, lane 2). Nuclear extracts were from U87MG cells (lanes 1–3), TPA-induced (50 ng/ml for 8 h) 5637 cells (lane 4) and HeLa cells (lane 5). I and II indicate retarded complexes.

in comparison with its wild-type counterpart pPF22. Mutagenesis resulted in an increase of promoter activity that was comparable to the activity of a construct (pPF10) in which the negative element was deleted (Fig. 5b). Therefore the negative effect on transcription of the RI element can be suppressed by deletion or by targeted mutation in the AP1-like sequence.

Cytokine expression in the central nervous system has been mainly referred to the role of regulation of the immune re-

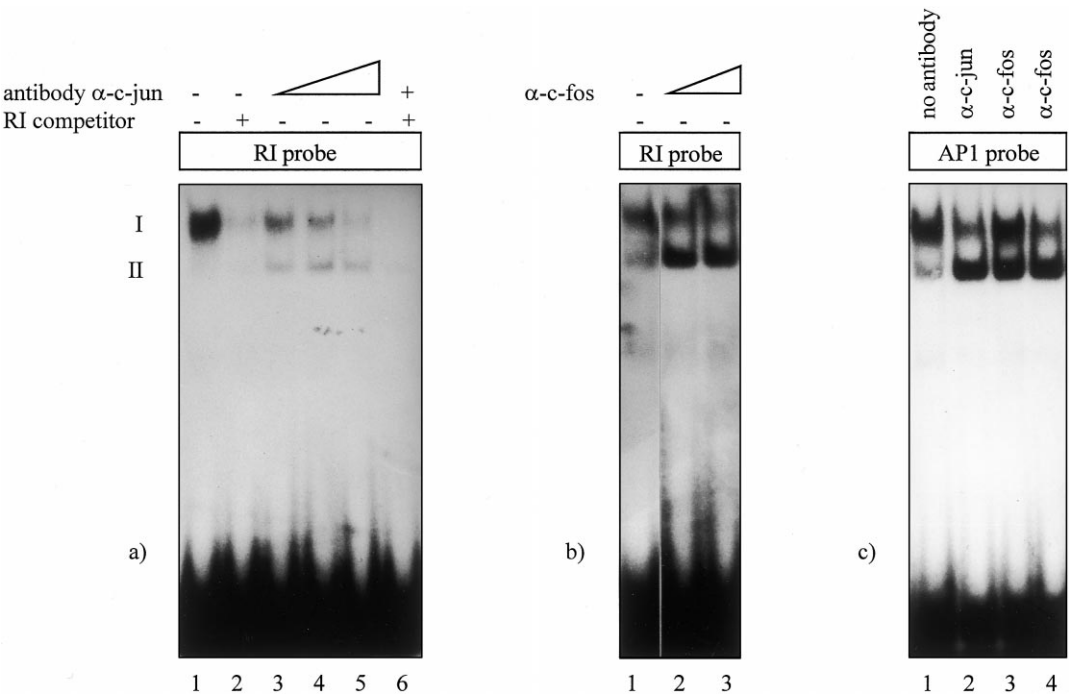

Fig. 4. Electrophoretic mobility shift assays with nuclear extracts from U87MG cells in the presence of anti-c-*jun* and anti-c-*fos* antibodies. a: The assay was performed with RI oligonucleotide as labelled probe in the presence of pre-immune serum (lane 1) or in the presence of 50 ng (lane 3), 100 ng (lanes 4, 6), 200 ng (lane 5) of anti-c-*jun* antibody. A 400-fold molar excess of RI unlabelled oligonucleotide as competitor was included in lanes 2 and 6. b: Assay performed with RI oligonucleotide as labelled probe in the presence of pre-immune serum (lane 1), or in the presence of 100 ng (lane 2) and 250 ng (lane 3) of anti-c-*fos* antibody. c: Assay performed with AP1 oligonucleotide as labelled probe in the presence of pre-immune serum (lane 1), in the presence of 100 ng of anti-c-*jun* antibody (lane 2), 100 ng (lane 3) and 250 ng (lane 4) of anti-c-*fos* antibody.

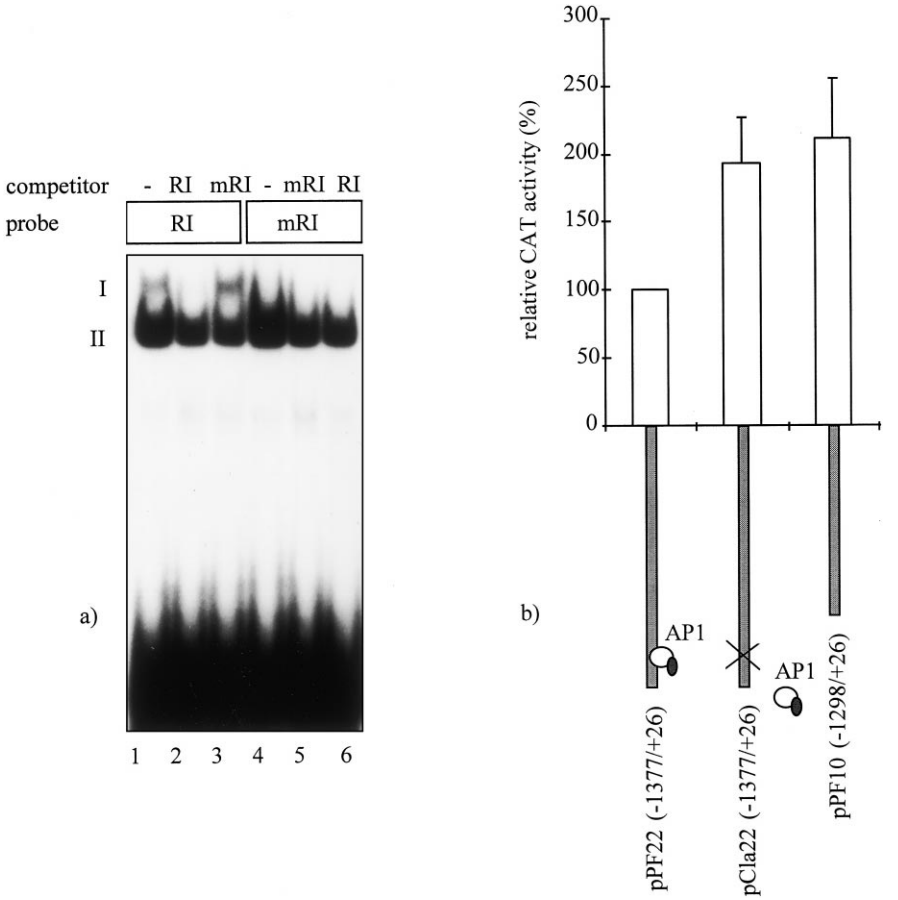

Fig. 5. a: Electrophoretic mobility shift assays with the mutant RI probe (mRI). RI and mRI oligonucleotides were used as labelled probes or unlabelled competitors (400-fold molar excess) as indicated. In all assays nuclear extract from U87MG cells was used. b: Effect of mutagenesis on promoter activity. Functional analysis of the construct (pCla22) containing the T to G mutation at the AP1-like site in comparison with the activity of the wild-type construct (pPF22) and of the construct in which the negative element was deleted (pPF10). Values represent the mean ± S.E.M. of CAT activity of at least three independent experiments performed in duplicate. The activities of pCla22 and pPF10 were related to the activity of pPF22, set at 100%.

sponse, but increasing evidence suggests that some of them, among which GM-CSF, also play an important role in establishing and maintaining the normal homeostatic environment, besides functioning as intercellular signals that orchestrate the response to injury. GM-CSF, locally produced by glial astrocytes, provides an essential element for recruitment and activation of blood-derived monocytes as well as resident brain macrophages. Furthermore, GM-CSF seems to be required for survival and function of microglia since it is able to enhance microglial survival and it is the most effective stimulus promoting morphological differentiation in foetal cultures [16]. The immunocytochemical localisation of GM-CSF, restricted to reactive astrocytes, has been demonstrated in the characteristic lesions of multiple sclerosis and Alzheimer's disease [16].

AP1 was originally described as a transcriptional activator of several genes, induced as part of the cellular immediate early response to serum stimulation or to phorbol esters [17]. However, AP1 has also been reported to behave as a negative *trans*-acting factor of various genes including c-*fos*, osteocalcin, insulin, adipocyte P2, muscle creatine kinase and Myo D [18]. The way by which this negative effect is achieved is not homogeneous and therefore different molecular mechanisms have to be considered. For example, muscle creatine kinase transcription is repressed by AP1 through inhibition of the muscle-specific transactivators myogenin and Myo D mediated by their interaction with c-*jun* and subsequent prevention of DNA binding [19,20]. Repression of the c-*fos* promoter results from direct binding to a TRE element [21] and a similar mechanism seems to be active also on the MHC class I gene [18]. More complex interactions have been described for insulin [22], rat prolactin [23], osteocalcin [24], atrial natriuretic factor [25] and chorionic gonadotrophin α and β [26] promoters. The GM-CSF promoter contains a sequence, named hCLEO, located between −54 and −30, which is a binding site for AP1 in cooperation with other factors such as Elf-1, NF-ATp and NK-κB [27,28]. A similar site, in which AP1 and NF-AT interact in a cooperative fashion, is also present within the cyclosporin A-sensitive enhancer, located 3 kb upstream of the GM-CSF gene [3]. Both these sites act as positive elements in GM-CSF regulation.

The expression of cytokines is tightly controlled in the producing cells with transcriptional initiation starting only after proper stimulation and with several positively and negatively acting factors involved in a concerted regulation at multiple sites of cytokine promoters. Negative regulatory elements and cognate *trans*-acting factors have been identified in most cytokine promoters [29]. Regarding GM-CSF, negatively acting elements have been found in a region containing three copies of the CATT sequence between −65 and −31 [30] and in the

region containing the CK1 and CK2 elements between −114 and −66 [31]. Our results indicate that another more upstream region contains a negative regulatory sequence that interacts with an AP1 factor.

Acknowledgements: This work was supported by the University of Genoa local funds, MURST national projects CNR 96.03082.CT04 and 97.04362.CT14 to C.G., by MURST national project to G.B.-S., by AIRC and CNR-Progetto Finalizzato Biotecnologie to R.R.

References

[1] Gasson, J.C. (1991) Blood 77, 1131–1145.
[2] Tweardy, D.J., Mott, P.L. and Glazer, E.W. (1990) J. Immunol. 144, 2233–2241.
[3] Cockerill, P.N., Bert, A.G., Jenkins, F., Ryan, G.R., Shannon, M.F. and Vadas, M.A. (1995) Mol. Cell. Biol. 15, 2071–2079.
[4] Musso, M., Ghiorzo, P., Fiorentini, P., Giuffrida, R., Ciotti, P., Garrè, C., Ravazzolo, R. and Bianchi-Scarrà, G. (1996) Biochem. Biophys. Res. Commun. 223, 64–72.
[5] Ghiorzo, P., Musso, M., Mantelli, M., Garrè, C., Ravazzolo, R. and Bianchi-Scarrà, G. (1997) FEBS Lett. 418, 215–218.
[6] Chen, C. and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752.
[7] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1994) in: Current Protocols in Molecular Biology (Albright, L.M., Coen, D.M. and Varki, A., Eds.), Vol. 1, p. 9.7.6, John Wiley and Sons, Chichester.
[8] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[9] Ciotti, P., Rainero, M.L., Nicolò, G., Spina, B., Garrè, C., Casabona, F., Santi, P.L. and Bianchi-Scarrà, G. (1995) Melanoma Res. 5, 41–47.
[10] Penco, S., Pastorino, S., Bianchi-Scarrà, G. and Garrè, C. (1995) J. Biol. Chem. 270, 12263–12268.
[11] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475–1489.
[12] Belikov, S. and Wieslander, L. (1995) Nucleic Acids Res. 23, 310.
[13] Fiorentini, P., Musso, M., Penco, S., Giuffrida, R., Pistoia, V., Garrè, C. and Bianchi-Scarrà, G. (1995) Biochem. Biophys. Res. Commun. 214, 1015–1022.
[14] Higuchi, M., Krummel, B. and Saiki, R.K. (1988) Nucleic Acids Res. 16, 7351–7367.
[15] Kataoka, K., Noda, M. and Nishizawa, M. (1996) Oncogene 12, 53–62.
[16] Lee, S.C., Liu, W., Brosnan, C.F. and Dickson, D.W. (1994) Glia 12, 309–318.
[17] Lamph, W.W., Wamsley, P., Sassone-Corsi, P. and Verma, I.M. (1988) Nature 334, 629–631.
[18] Howcroft, T.K., Richardson, J.C. and Singer, D.S. (1993) EMBO J. 12, 3163–3169.
[19] Li, L., Chambard, J.-C., Karin, M. and Olson, E.N. (1992) Genes Dev. 6, 676–689.
[20] Bengal, E., Ransone, L., Scharfmann, R., Dwarki, V.J., Tapscott, S.J., Weintraub, H. and Verma, I.M. (1992) Cell 68, 507–519.
[21] Lucibello, F.C., Lowag, C., Neuberg, M. and Muller, R. (1989) Cell 59, 999–1007.
[22] Inagaki, N., Maekawa, T., Sudo, T., Ishii, S., Seino, Y. and Imura, H. (1992) Proc. Natl. Acad. Sci. USA 89, 1045–1049.
[23] Farrow, K.N., Manning, N., Schaufele, F. and Gutierrez-Hartmann, A. (1996) J. Biol. Chem. 271, 17139–17146.
[24] Schüle, R., Umesono, K., Mangelsdorf, D.J., Bolado, J., Pike, J.W. and Evans, R.M. (1990) Cell 61, 497–504.
[25] McBride, K., Robitaille, L., Tremblay, S., Argentin, S. and Nemer, M. (1993) Mol. Cell. Biol. 13, 600–612.
[26] Pestell, R.G., Hollenberg, A.N., Albanese, C. and Jameson, J.L. (1994) J. Biol. Chem. 269, 31090–31096.
[27] Schreck, R. and Baeuerle, P.A. (1990) Mol. Cell. Biol. 10, 1281–1286.
[28] Tsuboi, A., Sugimoto, K., Yodoi, J., Miyatake, S., Arai, K. and Arai, N. (1991) Int. Immunol. 3, 807–817.
[29] Ye, J. and Young, H.A. (1997) FASEB J. 11, 825–833.
[30] Ye, J., Zhang, X. and Dong, Z. (1996) Mol. Cell. Biol. 16, 157–167.
[31] Coles, L.S., Occhiodoro, F., Vadas, M.A. and Shannon, M.F. (1994) Nucleic Acids Res. 22, 4276–4283.