Journal of Cellular Biochemistry 76:360–367 (2000)

Differential Role for Sp1/Sp3 Transcription Factors in the Regulation of the Promoter Activity of Multiple Cyclin-Dependent Kinase Inhibitor Genes

Alfredo Pagliuca, Pasquale Gallo, and Luigi Lania*

Department of Genetics, Molecular and General Biology, University of Naples "Federico II," 80134 Naples, Italy

Abstract Cyclin-dependent kinase inhibitors play a significant role in cell cycle progression and in cellular differentiation and their expression is regulated in different cellular settings. GC-rich regions in the promoter sequences of the cyclin-dependent kinase inhibitor genes p15*INK4B* and p21*CIP1/WAF1* mediate the transcriptional response of these genes to extracellular stimuli. Similar GC-rich sequences in the promoter of the p15*INK4A* and p16*INK4B* gene can be targeted for transcriptional inactivation by methylation of cytosine residues. GC-rich regions represent putative target sites for binding of the ubiquitously expressed Sp1 and Sp3 transcription factors. Using a combination of functional and biochemical studies, we analyzed the potential role of the Sp1 and Sp3 factors in the regulation of CDKI p15, p16, and p21 promoter activities. Using transient reporter gene assays, we determined that Sp1 is a strong activator of these promoters, whereas Sp3 functions as a weak transactivator. We have identified multiple protein-binding sites in the proximal promoter sequences of these genes by footprinting analysis. Some of these sites are bound by Sp1 and Sp3, as demonstrated by gel-shift experiments using Sp1/Sp3-specific antibodies, permitting the demonstration that a differential role exists for Sp1 and Sp3 in the regulation of the activity of these promoters. J. Cell. Biochem. 76:360–367, 2000.

Key words: transcription regulation; Sp1-Sp3 proteins; CDKI regulation; CG-box

CDKI encoding genes play a pivotal role in the control of eukaryotic cell cycle, and are therefore subjected to different levels of regulation in order to exert their growth arrest activity in different cellular settings and biological phenomena. Such regulation can be achieved by either posttranscriptional mechanisms or control of gene transcription [reviewed by Morgan, 1995]. The promoter of the p21*CIP1/WAF1* gene represents a versatile element in coupling differentiation stimuli to induction of transcription in cell culture, with a relatively small GC region that mediates induction by a variety of agents such as butyrate, nerve growth factor (NGF), and transforming growth factor-β (TGFβ) [Yan and Ziff, 1997; Nakano et al., 1997; Datto et al., 1995]. Similarly, a GC region was implicated in the induction of the p15*INK4B* gene by TGF-β [Li et al., 1995], highlighting the existence of common elements in the regulation of two functionally related genes, yet belonging to different families of CDK inhibitors. The CDKI p16 is closely related to p15 and is encoded by the *CDKN2A/INK4A* gene that lies next to the *INK4B* gene on human chromosome 9p21. This locus is frequently targeted by inactivating mutations in several neoplastic samples [Swafford et al., 1997; Liew et al., 1999]. Alternatively, inactivation of this gene can also occur via methylation of GC sequences in the promoter region [Fournel et al., 1999; Tanaka et al., 1999; Liew et al., 1999; Lee et al., 1997; Drexler, 1998]. Thus, it appears that GC-rich sequences are cis elements that play a role in the basal and enhanced transcription of the genes encoding the CDKI p15, p16, and p21.

The Sp family of proteins comprise ubiquitous and tissue-restricted transcription factors that bind GC-rich DNA sequences and other

Grant sponsor: Associazone Italiana per la Ricerca sul Cancro (AIRC); Grant sponsor: Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST).

*Correspondence to: Luigi Lania, Dipartimento di Genetica, Biologia Generale e Molecolare, Università Federico II, Via Mezzocannone 8, 80134 Naples, Italy. E-mail: lania@biol.dgbm.unina.it

Received 11 October 1999; Accepted 13 October 1999

related GT and GA motifs through their zinc-finger domains [reviewed in Lania et. al., 1997]. The ubiquitously expressed and closely related Sp1 and Sp3 factors have been found to regulate the promoters of several genes, including cell-cycle regulated genes, with Sp1 defined as a potent cooperative transcriptional activator and Sp3 as a weaker transactivator or a repressor [Majello et al., 1994; Birnbaum et al., 1995; Lania et. al., 1997]. In addition, Sp1-binding sites appear to play a critical role in the maintenance of the methylation-free CpG island [Brandeis et al., 1994; Macleod et al., 1994]. It appears that Sp1 plays an important role in controlling putative cell cycle-regulated genes and is required to prevent methylation of CpG islands.

On the basis of these assumptions, we have studied the effect of Sp1 and Sp3 on the promoters of the CDKI encoding genes p21*CIP1/WAF1*, p16*INK4A*, and p15*INK4B* by transient transfection assays, in vitro footprinting and electrophoretic mobility shift assay (EMSA). Confirming and extending previous results on the p21 and p15 promoters [Datto et al., 1995; Li et al., 1995], we find that the sequences implicated in the TGF-β response are avidly bound by Sp1 and Sp3 factors, allowing for transactivation of these promoters by Sp1 and, to a lesser extent, Sp3. In addition, we have identified multiple GC and GT boxes in the proximal promoter region of the p16*INK4A* promoter that are bound in vitro by Sp1 and Sp3, allowing for Sp-mediated activation. It is intriguing that these different CDKI gene promoters are regulated in a similar fashion by the Sp1/ Sp3 transcription factors.

MATERIALS AND METHODS

Reporter Plasmids

p15-luciferase plasmid (p15GL3) was constructed by polymerase chain reaction (PCR), using genomic DNA from HeLa cells to amplify a DNA target corresponding to residues +42 to −113 of the p15 sequences (Genbank N.S75756). The PCR product was cloned into pGL3 (Promega). p16-luciferase reporter was constructed by PCR on human genomic DNA using primers based on the sequence deposited as Genbank N.X94154. The resulting PCR product was cloned into the *Bgl*II *Hin*dIII sites of pGL3 to obtain p16-LGL and corresponds to region from −650 to +254; this construct was used to derive p16GL by subcloning the *Sma*I-*Hin*dIII fragment into pGL3 and corresponds to sequences from +1 to +254. p21-luciferase constructs: nucleotide residues from −108 to +11 of the p21 promoter sequence deposited as Genbank N. U24170 were amplified from p21WWP and cloned into the *Bgl*II and *Hin*dIII sites of pGL3 to obtain p21GL. Details of the primer sequences and PCR parameters are available upon request. Effector plasmids: pPac expression plasmids for Sp1 and Sp3 have been previously described [Majello et al., 1994].

Transient Transfections

Drosophila Schneider cells were grown at 25°C in Schneider medium (Gibco-Life Technologies) supplemented with 10% heat-inactivated fetal calf serum (FCS) and transfected as previously described [Majello et al., 1994, Pagliuca et al, 1998]. At 48 h after addition of the precipitates, the cells were harvested and extracts assayed for luciferase activity in a TD20 luminometer (Turner designs) using the Luciferase Assay System (Promega). The values obtained were normalized for the protein content by the Bradford assay (Bio-Rad).

Nuclear Extracts, EMSA, and Footprinting

Nuclear extracts were prepared using subconfluent HeLa cell cultures as previously described [Pagliuca et al 1998]. The p21 region from −108 to +11 was end-labeled and used in footprinting assays. For EMSAs, two p21-specific probes were used; the GC probe derived from the *Sma*I DNA fragment (from −108 to −61) and the MP probe (from −61 to +15). Probes for p15 analysis were derived from the p15GL3 plasmid, for footprinting analysis the p15GL3 was digested with *Mlu*I, followed by end-labeling and cutting with *Sma*I; and for EMSA analysis p15GL3 was digested with *Eco*RI-*Sma*I and end-labeled. To obtain the EMSA probes for the p16 promoter, the p16GL plasmid was cut with *Hin*dIII and *Sac*II, labeled, and purified; the footprinting p16 probe was obtained by labeling the NheI-end generated by cutting p16GL with *Hin*dIII and *Nhe*I. The EMSA conditions were as described [Pagliuca et al., 1998], and the antisera against Sp1 and Sp3 were from Santa Cruz Technology. The footprinting reactions were performed with Core Footprinting Assay Kit (Amersham Pharmacia Biotech) and the reactions were resolved on a 8%acrylamide/8 M urea gel.

RESULTS

Sp1 and Sp3 Differentially Activate the p15, p16, and p21 Promoters

To analyze the role of the GC-rich regions in the promoter regions of the p15, p16, and p21 genes as putative target sites for transcriptional regulation mediated by Sp factors, we proceeded to clone relevant portions of the promoters into the luciferase reporter vector pGL3 (Promega). Given the high frequency of putative Sp-binding sites in the promoters to be analyzed, we chose to clone only the more proximal regions, including the relevant elements such as the TATA box and the start site for p21 and the Inr element for p15. Reporter plasmids p21GL, p15GL, p16LGL and p16GL were then cotransfected along with expression vectors pPacSp1 and pPacSp3 in *Drosophila* SL2 cells, which lack mammalian Sp factors and are therefore instrumental for Sp activity assay [Majello et al., 1994]. The p16LGL construct was compared with p16GL for the transcriptional response to Sp factors, and being the two constructs very similar in their behavior (data not shown), we chose to continue the analysis of the smaller construct p16GL, containing the multiple transcription start sites reported for p16 promoter [Hara et al., 1996]. As shown in Figure 1, Sp1 is a potent activator of these reporter constructs; even at very low ratios of effector/reporter, the activity of the promoters is raised 7- to 30-fold in the Sp1 cotransfected samples. Conversely, when Sp3 was tested, the p15, p21, and p16 reporters were very weakly activated (up to 3 fold), even at relatively high effector/reporter ratios (Fig. 1).

Multiple Protein Binding Sites Exist in the p15, p16, and p21 Constructs

To assess whether the Sp1-mediated activation of the CDKI promoters tested could be the result of direct binding in mammalian cells, we proceeded to the biochemical analysis by in vitro binding assays with HeLa cell nuclear protein extract. First, we sought to identify cis elements that could act as binding sites in our experimental constructs. To this end, we performed in vitro footprinting analysis on the three promoter fragments. The footprinting pattern for p15 showed a sharp protection of GC boxes comprised in the region from approximately residue −90 up to the Inr element (Fig. 2), and in an E-box element in position −36 that represents a putative binding site for the bHLH family of transcription factors. The p21 promoter fragment was widely protected from DNase digestion in a region with 86% GC content previously identified as responsible for the transcriptional induction of these genes by TGF-β [Datto et al., 1995; Li et al., 1995] and in other relevant elements such as the TATA box, a downstream GC box and two E-boxes near the 3′ end of the construct (Fig. 3).

In our analysis of the p16 construct, we found that its more 5′ region is scarcely protected in our assay, whereas further downstream of the transcriptional start sites, in the region from +60 to the 3′ end, protected areas were observed that correspond to GC and GA sequences (Fig. 4). Apart from the putative Sp-binding sites, the footprinting pattern of the three constructs also comprises: (1) regions with a different binding specificity, and (2) regions whose sequence does not lead to evident binding proteins yet are protected in our assays and represent a clue for the identification of additional transcription factors involved in the regulation of these genes.

Both Sp1 and Sp3 Bind the p15, p16, and p21 Promoters

We next proceeded to define whether the putative binding sites found in the footprinting assay might bind Sp1/Sp3 factors by EMSA in the presence of specific Sp1/Sp3 antisera. To this end, we chose to analyze the promoter fragments of p15 and p16,which displayed the higher density of putative Sp binding sites, namely the residues from −113 to −24 in the p15 promoter and from +115 to +207 in the p16 promoter. The p21 promoter was analyzed using two separate probes: the GC probe spanning residues from −108 to −63, and the MP probe from −62 to +11.

Confirming the data from the footprinting experiments, we observed multiple retarded bands in all cases (Fig. 5). These retarded bands were further analyzed either by competition with unlabeled oligonucleotide or by preincubation of the HeLa cell nuclear extracts with antibodies directed against Sp1 or Sp3 proteins, respectively. The result of this analysis is that both Sp1 and Sp3 appear to be involved in binding to the p15, p16, and p21 probes.

Incubation of the p15 *Sma*I-EcoRI fragment (−113/−24) with the nuclear extract results in an EMSA pattern that displays three retarded

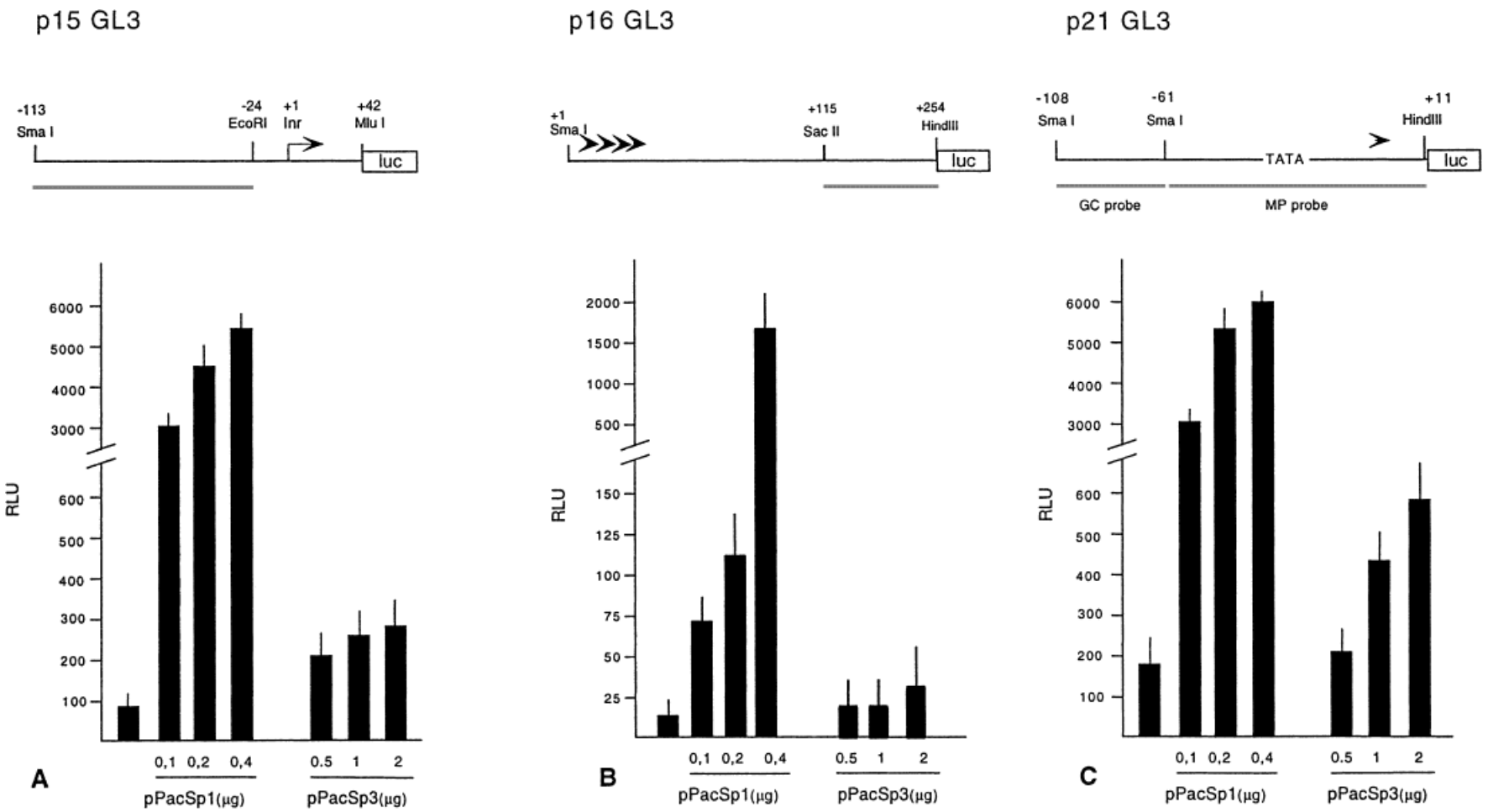

Fig. 1. Differential effect of Sp1 and Sp3 on the activity of the p15, p16, and p21 promoters. **Top:** Schematic description of the reporter constructs, with the transcription start sites indicated by arrowheads and the region chosen for the subsequent EMSA analysis indicated by shaded bar. **Bottom:** Relative luciferase unit (RLU) values of the *Drosophila* SL2 cell samples transfected with 1 µg of the p15 (**A**), p16 (**B**), and p21 (**C**) reporter constructs, either alone or with increasing amounts of Sp1 and Sp3 expression vectors, as indicated. The DNA content of the transfection mixture was kept constant by the addition of the empty expression vector. Lines at top of columns indicate the standard deviation.

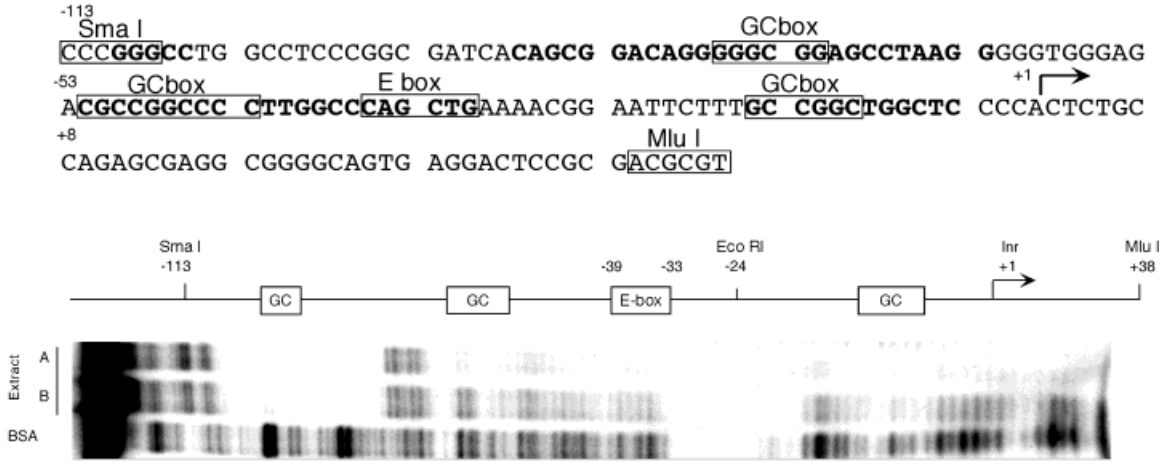

Fig. 2. Footprinting analysis of the p15 promoter. **Top:** Nucleotide sequences of the p15 promoter probe; residues in bold correspond to the regions protected by DNase I digestion. **Bottom:** Footprinting pattern with HeLa cell nuclear extracts with 2 and 4 units, respectively, of DNase I (**lanes A and B**) or with bovine serum albumin (BSA), as indicated.

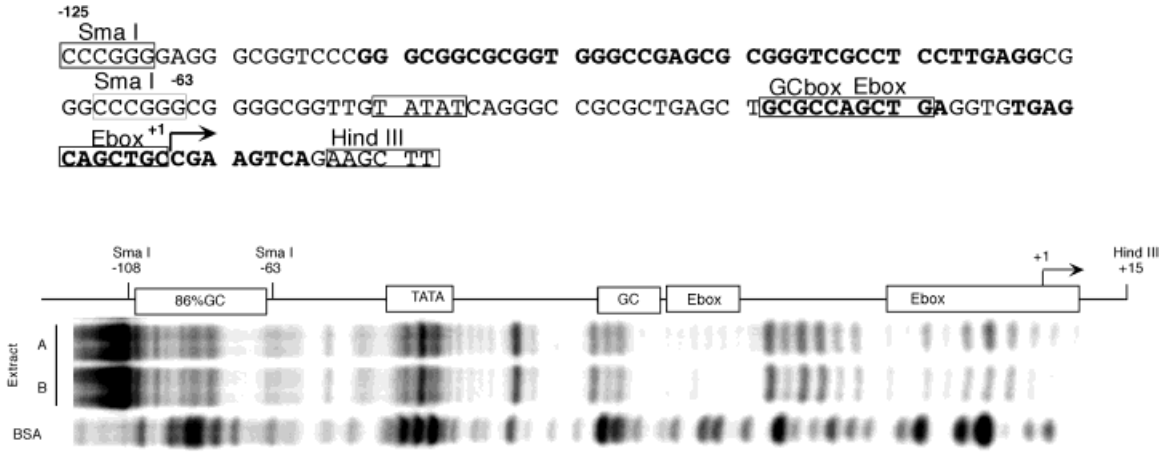

Fig. 3. Footprinting analysis of the p21 proximal promoter sequences. **Top:** Nucleotide sequences of the probe with the residues in bold corresponding to the regions protected by DNase I digestion. **Bottom:** Footprinting pattern of the p21 proximal promoter sequences with conditions as described in the legend of Fig. 2.

bands: two supershifted by the anti-Sp3 antibody, and the remaining supershifted by the anti-Sp1 antibody (Fig. 5A). A similar experiment with the p16 *Sac*II-*Hin*dIII probe (+115/+254) led to similar results, except for the appearance of two weaker bands that were neither supershifted with antibodies against Sp1/Sp3 proteins nor competed with a cold Sp1 consensus oligonucleotide (Fig. 5B). Confirming previous published data [Li et al., 1995; Datto et al., 1995], we found that the residues comprised in the p21 promoter fragment are bound by Sp1 and Sp3 with high affinity; similar patterns were observed with both p21- derived probes, with a lower band and an upper doublet characteristic of experiments in which the Sp1/Sp3 factors are involved (Fig. 5C,D). Inclusion of specific anti-Sp1 and anti-Sp3 antibodies demonstrated that the p21 GC probe spanning residues from −108 to −63 forms DNA complexes with both Sp1 and Sp3 (Fig. 5C). The p21 MP probe from −62 to +11 (Fig. 5D) also forms specific DNA-complexes as demonstrated by competition with a molar excess of the unlabeled probe (lane D2) and are likely due to Sp factor binding, as they can also be competed with an Sp-consensus oligonucleotide (lane D3).

DISCUSSION

Sp1/Sp3 binding sites are found in the promoter sequences of several mammalian genes,

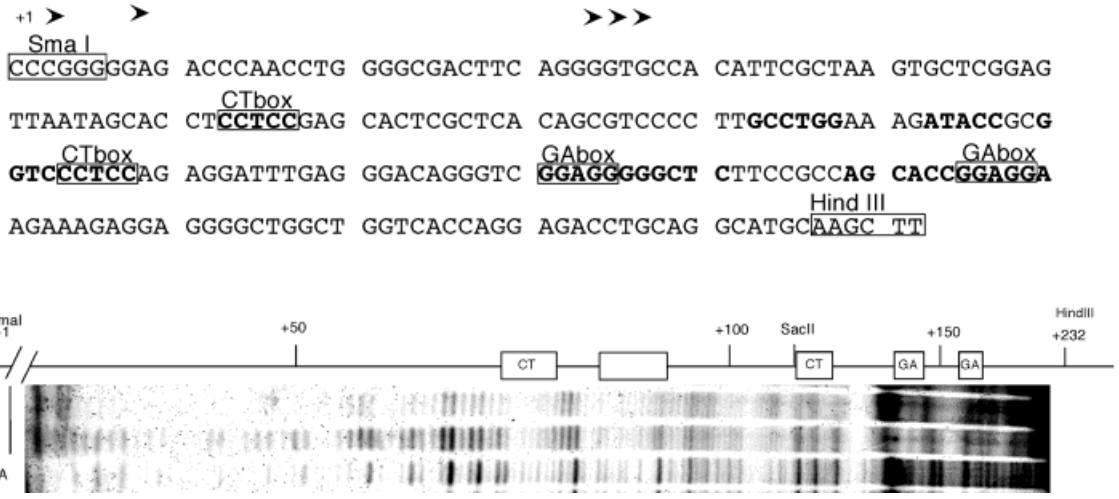

Fig. 4. Footprinting analysis of the minimal p16 promoter sequences. **Top:** Nucleotide sequence of the probe used in the footprinting experiment is reported. Residues in bold correspond to the regions protected by DNase I digestion. **Bottom:** Footprinting using conditions described in the legend of Fig. 2.

including those encoding the CDKI proteins p15, p16, and p21. In this report, we have addressed, with different biochemical and functional approaches, the issue of the potential role of these sequences in the transcriptional regulation of these genes. We find a good correlation between the functional reporter gene assays and the biochemical in vitro binding assays for the p15, p16, and p21 promoters, permitting the determination that Sp1/Sp3 proteins bind their cognate sites in the proximal promoter of the p15 and p21 gene and in the region 3′ to the transcriptional start site of the p16 gene. This binding allow these genes for transcriptional activation by Sp1 and to a much lesser extent by Sp3 protein. Thus, these two ubiquitous transcription factors appear to exert distinct influence on the transcription of these genes playing an important role in regulating cell-cycle progression.

Another point of interest is the relevance of Sp1 and Sp3 in the basal level of transcription of the p16 promoter. Methylation of the cytosine residues in the Sp1/Sp3 target sequences can inhibit transactivation by Sp1 family members [Mancini et al., 1999]. Methylation of the cytosine residues accounts for several cases of transcriptional inactivation of the tumor suppressor gene *INK4A* and *INK4B* [Brandeis et al., 1994; Klangby et al., 1998; Swafford et al., 1997]. The ability of Sp1 factor to act as an efficient transactivator of the p16 gene expression prompts for further analysis of the cis elements that mediate this phenomenon, in particular with point mutagenesis and methylation analysis.

The presence of different factors interacting with common binding sites raises the cogent question of how a promoter is regulated by diverse factors with similar DNA-binding specificity. Interestingly, the region encompassing the Sp1/Sp3-binding sites within the proximal promoter of p21/WAF/CIP1 has shown to be the minimal region for mediating upregulation of this promoter by TFG-β, phorbol ester, okadaic acid, progesterone, geranylgeranyltransferase, and the histone deacetylase inhibitor TSA [Datto et al., 1995; Biggs et al., 1996; Adnane et al., 1998, Owen et al., 1998; Sowa et al., 1997]. A recent reports showed that Sp1 but not Sp3 is the mediator of the TGF-β signal [Li et al., 1998]. Conversely, it has been reported that Sp3 is the putative mediator of the stimulation of the INK4B gene by calcium in primary keratinocytes [Prowse et al., 1997]. Geranylgenaryltransferase I inhibitor GGTI-298 upregulates Sp1 transcriptional activity [Adnane et al., 1998], and progesterone regulates p21 promoter activity through functional cooperation between Sp1 and CBP/p300 [Owen et al., 1998]. Finally, it has recently been reported that Sp3, but not Sp1, mediates the transcriptional activation of p21 gene promoter by TSA [Sowa et al., 1999]. Clearly, these reports strongly suggest that Sp1 and Sp3 transcriptional activities are differentially modulated in different cellular settings. A challenging issue would be to determine how different signal transduction

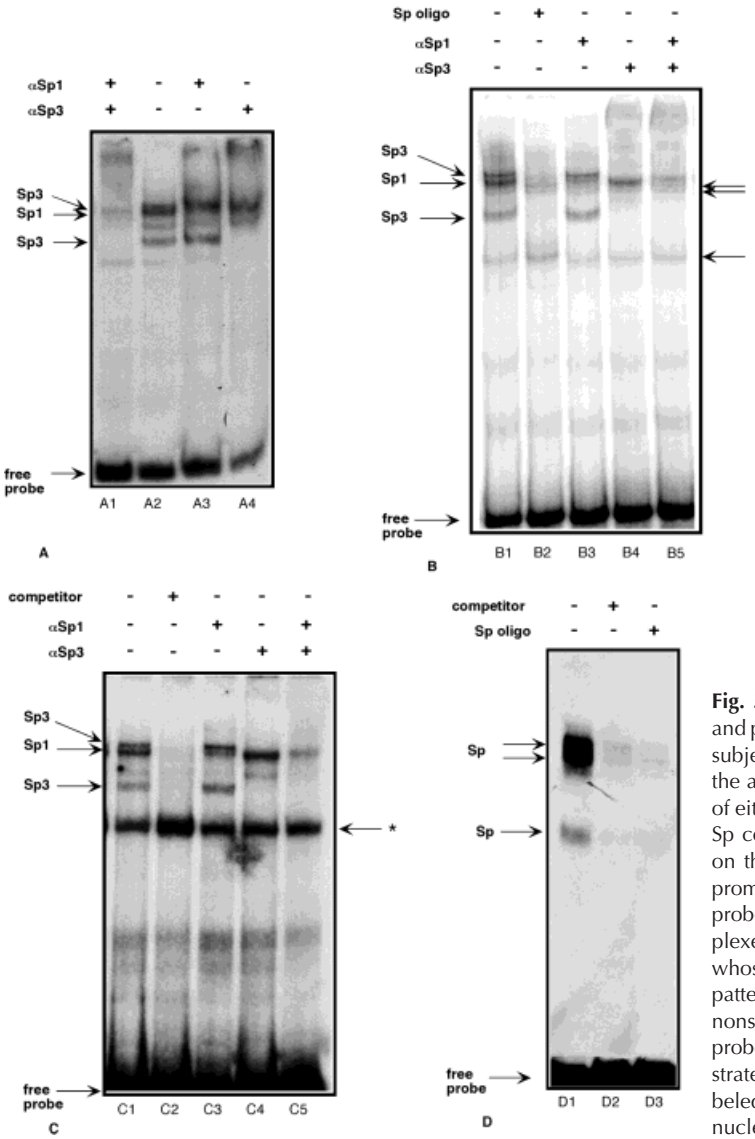

Fig. 5. Binding of Sp1/Sp3 proteins to the p15, p16, and p21 promoters. The probes evidenced in Fig. 1 were subjected to EMSA analysis with preincubation, before the addition of the radiolabeled probe, with antibodies of either excess of unlabeled probe (competitor) or cold Sp consensus oligonucleotides (Sp oligo), as indicated on the top of the panels. **A:** EMSA pattern of the p15 promoter probe. **B:** EMSA pattern of the p16 promoter probe. Arrows on the right indicate protein-DNA complexes that do not arise from Sp factors binding and whose nature has not been further investigated. **C:** EMSA pattern of the p21 MP probe. An asterisk indicates a nonspecific complex. **D:** EMSA pattern of the p21 GC probe. The complexes formed are specific, as demonstrated by competition with a molar excess of the unlabeled probe (**lane D2**) and with an Sp-consensus oligonucleotide (**lane D3**).

pathways exploit two ubiquitously expressed factors such as Sp1 and Sp3 to obtain inducible expression of their dedicate target genes.

ACKNOWLEDGMENTS

We thank Miss R. Terracciano for technical help. The work performed by A.P. was in partial fulfillment of his doctoral thesis. This work was supported by a grant from the Associazone Italiana per la Ricerca sul Cancro (AIRC) and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) (to L.L.).

REFERENCES

Adnane J, Bizouarn FA, Qian Y, Hamilton AD, Sebti SM. 1998. p21(WAF1/CIP1) is upregulated by the geranylgeranyltransferase I inhibitor GGTI-298 through a transforming growth factor beta- and Sp1-responsive element: involvement of the small GTPase rhoA. Mol Cell Biol 18:6962–6970.

Biggs JR, Kudlow JE, Kraft AS. 1996. The role of the transcription factor Sp1 in regulating the expression of the WAF1/CIP1 gene in U937 leukemic cells. J Biol Chem 271:901–906.

Birnbaum MJ, van Wijnen AJ, Odgren PR, Last TJ, Suske G, Stein GS, Stein JL. 1995. Sp1 trans-activation of cell cycle regulated promoters is selectively repressed by Sp3. Biochemistry 34:16503–16508.

Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H. 1994. Sp1 elements protect a CpG island from de novo methylation. Nature 371:435–438.

Datto MB, Yu Y, Wang XF. 1995. Functional analysis of the transforming growth factor beta responsive elements in the WAF1/Cip1/p21 promoter. J Biol Chem 270:28623–28628.

Drexler HG. 1998. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. Leukemia 12:845–859.

Fournel M, Sapieha P, Beaulieu N, Besterman JM, MacLeod AR. 1999. Down-regulation of human DNA-(cytosine-5) methyltransferase induces cell cycle regulators p16(ink4A) and p21(WAF/Cip1) by distinct mechanisms. J Biol Chem 274:24250–24256.

Hara E, Smith R, Parry D, Tahara H, Stone S, Peters G. 1996. Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. Mol Cell Biol 16:859–867.

Klangby U, Okan I, Magnusson KP, Wendland M, Lind P, Wiman KG. 1998. p16/INK4a and p15/INK4b gene methylation and absence of p16/INK4a mRNA and protein expression in Burkitt's lymphoma. Blood 91:1680–1687.

Lania L, Majello B, De Luca P. 1997. Transcriptional regulation of the Sp family proteins. Int J Biochem Cell Biol 29:1313–1323.

Lee YY, Kang SH, Seo JY, Jung CW, Lee KU, Choe KJ, Kim BK, Kim NK, Koeffler HP, Bang YJ. 1997. Alterations of p16INK4A and p15INK4B genes in gastric carcinomas. Cancer 80:1889–1896.

Li JM, Nichols MA, Chandrasekharan S, Xiong Y, Wang XF. 1995. Transforming growth factor beta activates the promoter of cyclin-dependent kinase inhibitor p15INK4B through an Sp1 consensus site. J Biol Chem 270:26750–26753.

Li JM, Datto MD, Shen X, Hu PP-C, Yu Y, Wang XF. 1998. Sp1, but not Sp3, functions to mediate promoter activation by TGF-β through canonical Sp1 binding sites. Nucleic Acids Res 26:2449–2456.

Liew CT, Li HM, Lo KW, Leow CK, Chan JY, Hin LY, Lau WY, Lai PB, Lim BK, Huang J, Leung WT, Wu S, Lee JC. 1999. High frequency of p16INK4A gene alterations in hepatocellular carcinoma. Oncogene 18:789–795.

Macleod D, Charlton J, Mullins J, Bird AP. 1994. Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. Genes Dev 8:2282–2292.

Majello B, De Luca P, Hagen G, Suske G, Lania L. 1994. Different members of the Sp1 multigene family exert opposite transcriptional regulation of the long terminal repeat of HIV-1. Nucleic Acids Res 22:4914–4921.

Mancini DN, Singh SM, Archer TK, Rodenhiser DI. 1999. Site-specific DNA methylation in the neurofibromatosis (NF1) promoter interferes with binding of CREB and SP1 transcription factors. Oncogene 18:4108–4119.

Morgan DO. 1995. Principles of CDK regulation. Nature 374:131–134.

Nakano K, Mizuno T, Sowa Y, Orita T, Yoshino T, Okuyama Y, Fujita T, Ohtani-Fujita N, Matsukawa Y, Tokino T, Yamagishi H, Oka T, Nomura H, Sakai T. 1997. Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. J Biol Chem 272:22199–22206.

Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. 1998. Progesterone regulates transcription of the p21(WAF1) cyclin-dependent kinase inhibitor gene through Sp1 and CBP/p300. J Biol Chem 273:10696–10701.

Pagliuca A, Cannada-Bartoli P, Lania L. 1998. A role for Sp and helix-loop-helix transcription factors in the regulation of the human Id4 gene promoter activity. J Biol Chem 273:7668–7674.

Prowse DM, Bolgan L, Molnar A, Dotto GP. 1997. Involvement of the Sp3 transcription factor in induction of p21Cip1/WAF1 in keratinocyte differentiation. J Biol Chem 272:1308–1314.

Sowa Y, Orita T, Minamikawa S, Nakano K, Mizuno T, Nomura H, Sakai T. 1997. Histone deacetylase inhibitor activates the WAF1/Cip1 gene promoter through the Sp1 sites. Biochem Biophys Res Commun 241:142–150.

Sowa Y, Orita T, Minamikawa-Hiranabe S, Mizuno T, Nomura H, Sakai T. 1999. Sp3, but not Sp1, mediates the transcriptional activation of the p21/WAF1/Cip1 gene promoter by histone deacetylase inhibitor. Cancer Res 59:4266–4270.

Swafford DS, Middleton SK, Palmisano WA, Nikula KJ, Tesfaigzi J, Baylin SB, Herman JG, Belinsky SA. 1997. Frequent aberrant methylation of p16INK4a in primary rat lung tumors. Mol Cell Biol 17:1366–1374.

Tanaka T, Iwasa Y, Kondo S, Hiai H, Toyokuni S. 1999. High incidence of allelic loss on chromosome 5 and inactivation of p15INK4B and p16INK4A tumor suppressor genes in oxystress-induced renal cell carcinoma of rats. Oncogene 18:3793–797.

Yan GZ, Ziff EB. 1997. Nerve growth factor induces transcription of the p21 WAF1/CIP1 and cyclin D1 genes in PC12 cells by activating the Sp1 transcription factor. J Neurosci 17:6122–6132.