Identification of a Novel Transcriptional Regulatory Element Common to the p53 and Interferon Regulatory Factor 1 Genes*

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The promoter regions of both the interferon regulatory factor (IRF1) and p53 antioncogenes contain a previously unidentified sequence denoted IPCS, which markedly increases the transcriptional activity of a reporter gene placed under the control of an heterologous promoter in transfected U937 cells. In contrast, transfection of U937 cells with reporter vectors containing p53 and IRF1 promoters with mutated IPCS sites resulted in a 4-fold reduction in the constitutive expression of those two genes. The transcriptional activity of IPCS is strictly correlated with the binding of a novel nuclear factor, IPCS-binding factor (IPCS-BF). IPCS-BF, which is composed of a single polypeptide of 26 kDa, is present constitutively in nuclear extracts of both U937 cells and peripheral blood mononuclear cells from healthy donors. The finding that the pattern of binding of IPCS-BF to the IPCS is unlike that of any known transcription factor and that the IPCS sequence does not exhibit any significant homology with any known binding site present in the database, strongly suggest that IPCS-BF is a novel transcription factor which, by virtue of this ability to regulate the expression of the p53 and IRF1 genes, could play a central role in the control of cell proliferation and/or apoptosis.

Mutations in the p53 gene are frequently detected in a number of different types of human tumors, and the results of numerous studies suggest that inactivation or alteration in the expression of p53 gene is a critical step leading to neoplastic transformation (1, 2). Although the physiological function of p53 protein remains unclear, the results of recent studies suggest that it plays a major role in maintaining the integrity of the genome (3). This function includes the induction of growth arrest at the G1/S check point of the cell cycle and the induction of programmed cell death (apoptosis) in response to various DNA damaging agents (4–6). The wild-type p53 protein acts as a transcriptional activator (7) that binds to and stimulates the expression of various cellular genes involved in cell cycle control (8), DNA repair, and apoptosis (8–11).

The interferon regulatory factor 1 (IRF1) gene was described initially as a positive transcription factor involved in the regulation of the expression of both the IFN-α/β genes (12) and IFN-inducible genes (13, 14), and hence in the cellular response to virus infections. More recently, it has been suggested by several authors that IRF1 acts as a tumor-suppressor gene (15, 16). IRF1 belongs to the IRF family of transcription factors, which includes IRF2, ICSBP, IRF3, and ISGF3γ (17). The IRF1 DNA binding motif is present in at least two different transcriptional regulatory sequences, the positive regulatory domain I involved in the regulation of the expression of the IFN-β gene and the interferon-stimulated responsive element, present in the promoters of genes the expression of which is induced by the type I IFNs (13). Among the genes which contain a binding site for IRF1 in their promoter region, increased transcription of none of these genes can adequately explain the antioncogenic effects of IRF1, with the possible exception of the increase activity of protein kinase R gene, which is involved in the activation of NFkB and the inhibition of cell proliferation (18). It has been shown experimentally, however, that IRF1 plays a role in apoptosis (19, 20), in phenotypic reversion (19, 21), and in cell cycle control (16). In addition, chromosomal deletion or inactivation of one of both copies of the IRF1 gene is frequently detected in human leukemias or in the preleukemic syndrome (22), and more recently the presence of IRF1 has been shown to be essential for “DNA damage induced cell cycle arrest” (23).

The expression of p53 is inducible by a number of different stimuli. This property is due to the presence of several different regulatory elements within the p53 promoter. In particular the p53 promoter contains a genotoxic stress response sequence, extending from −70 to −40 (24), c-Myc-Max heterodimers bind to a basic helix-loop-helix site (25), and a putative binding site for IRF1 (26). More recently, two overlapping binding sites for NF1 and YY1 have been identified in the region which spans positions −227 to −194 of the p53 promoter, indicating that this region is also implicated in the constitutive expression of the p53 gene (27).

Expression of the IRF1 gene is strongly induced by both IFN-α/β and IFN-γ. This property is due to the presence of a regulatory element called IR (inverse repeat) within the IRF1 promoter (28). This sequence includes a GAS element (γ-interferon-activating sequence), which binds the homodimer STAT1/STAT1 following activation by IFN-γ. In addition, the IR can bind the heterodimer STAT1/STAT2 following induction with IFN-α (29). Thus, both the STAT1/STAT2 heterodimer and the STAT1/STAT1 homodimer can bind the IR sequence

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1 The abbreviations used are: IRF1, interferon regulatory factor 1; IFN, interferon; GAS, γ-interferon-activating sequence; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction; BrdUrd, bromodeoxyuridine; IR, IFN response element; IPCS, IRF1 p53 common sequence; BF, binding factor; PBL, peripheral blood leukocyte; luc, luciferase; bp, base pairs.

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and strongly transactivate IRF1 expression (15, 28). Furthermore, several putative Sp1 sites have been identified in the IRF1 promoter, which could be involved in the basal transcriptional activity of this gene (21, 28).

The p53 and IRF1 promoters also contain NFкB sites (28, 30), conferring upon each gene inductibility by a variety of different agents such as oxidative stress, cytokines such as interleukin-1β, or tumor necrosis factor-α. In addition, expression of these two genes is constitutive and ubiquitous in the quasi-totality of untransformed tissues (22). Another common feature of the two genes is the absence of TATA boxes (28, 31, 32).

Our results indicate that the sequence regulated and in their functions, suggesting that under certain circumstances the products of both genes may be required simultaneously within the cell. To test this hypothesis we analyzed the promoter regions of both genes for the presence of common putative regulatory sequences.

We have identified a sequence within the promoter region of the p53 gene which exhibits a strong homology with a sequence overlapping the IR element of the IRF1 promoter. The results of the experiments presented herein show that the sequence contained within the p53 promoter is not inducible, however, by either IFN-α/β or IFN-γ. These studies have led to the identification of a new transcription factor, denoted IPCS-BF (IRF1 p53 common sequence binding factor), which is present constitutively under physiological conditions in normal cells, and the presence of which is necessary for the basal and induced transcriptional activity of both the p53 and IRF1 genes.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Cell Treatments—**U937 cells (ATCC CRL 1593) derived from a human histiocytic lymphoma (33) were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies, Inc.). Cells were treated with recombinant human IFN-α2 (Intron A), a generous gift from Schering-Plough, or IFN-γ purchased from R&D Systems, at the concentrations indicated in the text. Human PBMC were isolated from heparinized blood by density gradient centrifugation on the Ficoll-Paque (Pharmacia Biotech Inc.).

**Nuclear Extracts—**Nuclear extracts were prepared using a modification of the procedure described by Osborn et al. (34). Briefly, 106 cells were washed twice with phosphate-buffered saline, lysed with 20 mM of a buffer containing 10 mM HEPES, pH 7.9, 0.1% Nonidet P-40 (Fluka), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, and the following protease inhibitors: 1 mM phenylmethylsulfonfluoride, 50 μg/ml α-phenylmethylsulfonfluoride, and 5 μg/ml each of leupeptin, pepstatin, aprotonin, and antipain). Samples were incubated for 15 min on ice, and the cellular lysate was vortexed briefly and centrifuged in a microcentrifuge for 10 min at 4 °C. Nuclear pellets were resuspended in 15 μl of extraction buffer (20 mM HEPES, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, together with the same protease inhibitors as described above), and incubated for 15 min at 4 °C, mixed briefly, and centrifuged in a microcentrifuge for 10 min at 4 °C. The supernatant was then added to an equal volume of storage buffer (20 mM HEPES, pH 7.9, 20% glycerol, 0.2 mM EDTA, 0.5 mM DTT, together with the same protease inhibitors as described above) and stored at −70 °C.

**Electrophoretic Mobility Shift Assay—**Synthetic double stranded oligonucleotide probes were labeled with α-32P dCTP (Amersham Corp.) by “filling-in” with Sequenase, and then purified on Sephadex G-50. The protein-DNA binding reactions for the identification of STAT1/STAT1 by “filling-in” with Sequenase, and then purified on Sephadex G-50. The reaction mixture was then electrophoresed on a 6% nondenaturing acrylamide gel. Competition experiments were carried out using a 50-fold molar excess of the unlabeled probe.

The following oligonucleotides (and their complementary strands) were used in these studies: IPCS-IRF1, 5′-AGCGTATTTCCCCCAGA-ATGACGGCAACGGACGAC-3′; IPCS-p53, 5′-AAATCGAGATTCTCTCA-AATGATTTCAC-3′; p53-A, 5′-AAAATGATTTCCAC-3′; IRF1-A, 5′-GAATAGCCGGCAGC-3′; M-385, 5′-AAATCGAGATTCTCTCA-AATGATTTCAC-3′; M-383, 5′-AAATCGAGATTCTCTCA-AATGATTTCAC-3′; M-380, 5′-AAATGATTTTCAC-3′; M-385, 5′-AAATGATTTTCAC-3′; and M-376, 5′-AAATGATTTTCAC-3′.

**Constructions—**The IPS-p53-luc, IPS-p53-A-luc, and M-382-luc were constructed by cloning, respectively, the following synthetic double stranded oligonucleotides: 5′-AAATCGAGATTCTCTCA-AATGATTTCAC-3′, 5′-AAATCGAGATTCTCTCA-AATGATTTCAC-3′, and 5′-AAATGATTTTCAC-3′ in the pGL2-promoter site in the pGL2-basic vector (Promega). The integrity of each construct was verified by sequencing.

**Site-directed Mutagenesis—**Site-directed mutagenesis of the p53 and IRF1 promoters was undertaken using a modification of the procedure described by Ho et al. (35). Briefly, two overlapping fragments of one promoter cloned in pG5l basic vector were amplified in two separate PCRs. The first reaction used a flanking primer that hybridized on the vector at the 5′ end of the insert sequence, and one internal primer that hybridized at the site of the mutation and contained the mismatched base. The second reaction employed one flanking primer that hybridized on the vector at the 3′ end of the insert sequence, and an internal primer that specified the overlap and also contained the mismatched base.

The primers on the vector were: pGL2S, 5′-TTCGATTTAGTGCTT- TACGGC-3′; pGL2AS and 5′-CTCTATTTCATACAGTACCG-3′. The following primers were used for the mutagenesis of the p53 promoter: pGL2MS 5′-CCAAACTGCTCCACAAATTCTGC-3′ and pGL2AS 5′-GGTGAAGACATTTTGGAATCTC-3′ and for the mutagenesis of the IRF1 promoter: IRF1-MS 5′-CTTCCATTTTACCAACGAGCAG-3′ and IRF1-AS 5′-CTCTTTCTGCATCGCCTAC-3′. As the two fragments generated by the first PCR are overlapping, they are “fused” by denaturing and annealing them in a subsequent primer extension reaction. Finally the “fusion” product was amplified by PCR using the flanking primers pGL2S and pGL2AS. The product of this PCR was then digested with XhoI and BglII and subcloned in an empty pG5l vector (Promega Biotech Inc.). The integrity of all these constructs was verified by sequencing.

**Transfection—**U937 cells were transfected with DNA by electroporation. Briefly, cells were washed in phosphate-buffered saline and resuspended in RPMI 1640 medium. Each sample contained 10 μg of DNA with 5 × 106 cells in a volume of 800 μl. Cells were electroporated in a 1-cm electroporation cuvette (Bio-Rad) at 350 V, 960 μF. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies, Inc.). Alternatively, cells were treated with recombinant human IFN-α2 or IFN-γ, at the concentrations and the time indicated in the text. The cells were harvested 8 h after transfection, and the level of luciferase activity was determined. Ten ml of cell suspension were centrifuged for 5 min at 5,000 rpm, and the cell pellet was then lysed with 100 μl of luciferase cell culture lysis reagent (Promega). The lysate was then centrifuged for 5 min at 14,000 rpm, and 30 μl of this supernatant were then assayed for luciferase activity in the presence of 70 μl of reaction buffer containing 20 mM Tricine, 1 mM MgCO3/Mg(OH)2, 2.67 mM MgSO4, 0.1 mM EDTA, 33.3 mM DTT, 470 μM luciferin (Sigma), and 530 μM ATP (Pharmacia). The chemiluminescence produced during the first 10 s of the reaction was determined using a luminometer (Luminometer 1250, BioOrbit). The luciferase activity of each extract was then normalized with respect to protein concentration determined by the Coomassie assay (Pierce). All the data presented in the text represent the mean of at least three independent transfections.

**UV Cross-linking—**The UV cross-linking experiment was performed with minor modifications of a procedure reported previously (36). The binding reaction was first carried out as described for probes IPCS-p53, one differing from the other on the strand where all the T residue were substituted with BrdUrd. The mixture of U937 cell nuclear extract and probes were loaded onto a 6% polyacrylamide gel. After electrophoresis, the gel was covered with Saran Wrap and irradiated on ice with a UV-B source for 30 min. The bound and free DNAs were located by autoradiography, excised, and added to SDS-sample buffer.
buffer. The sample were heated at 90 °C for 5 min and analyzed by SDS-polyacrylamide gel electrophoresis, 10% gel.

RESULTS

Identification of a Sequence Common to the p53 and IRF1 Promoters (IPCS) Which Is Bound by the Same Nuclear Factor—Comparison of the nucleotide sequences of the IRF1 and p53 promoters led to the identification of a 24-bp sequence present within each promoter exhibiting a common sequence homology of approximately 70%. This sequence, denoted IPCS (IRF-1 p53 common sequence), is located at positions −399 to −376, relative to the transcription start site, within the promoter of the p53 gene (IPCS-p53) (32), and at positions −130 to −107 within the IRF1 promoter (IPCS-IRF1). The IPCS encompasses the previously defined IR sequence present within the IRF1 promoter (28) (Fig. 1).

Although the consensus binding sequence of IR has not been precisely defined, the degree of sequence homology shared by the IPCS-p53 and IPCS-IRF1 sequences is such that IPCS-p53 would be expected to be able to bind the STAT1/STAT2 heterodimer and/or STAT1/STAT1 homodimer, the dimerization of which is induced respectively by IFN-α/β and IFN-γ (29). This hypothesis was also supported by the fact that IPCS-p53 strictly respects the GAS consensus sequence (TTNCNNNAA). To test this assumption, EMSAs were carried out using two probes derived from the IPCS element of the p53 and IRF1 genes, termed IPCS-p53 and IPCS-IRF1, respectively. This study was carried out using the IFN-sensitive U937 promonocytic cell line, which constitutively expresses both the IRF1 (37) and p53 gene products (38). Nuclear extracts of untreated U937 cells, or U937 cells treated with 1000 IU of IFN-α or IFN-γ for 1 h, were tested in an EMSA for the presence of complexes able to bind the 33-bp IPCS-IRF1 probe derived from the sequence present within the IRF1 promoter. The IPCS-IRF1 probe was found to bind proteins induced by IFN-α or IFN-γ which, on the basis of the low mobility of the protein-DNA complexes formed (28), are likely to represent STAT1/STAT2 heterodimer and STAT1/STAT1 homodimer respectively (Fig. 2, lanes 2 and 3). In contrast, the 30-bp IPCS-p53 probe, derived from the homologous sequence present within the p53 promoter, was unable, however, to bind any induced complex (Fig. 2, lanes 5 and 6). These results clearly eliminate the hypothesis that the IPCS-p53 sequence acts as an IFN-responsive element. The high degree of similarity between the electrophoretic pattern of two constitutive complexes (denoted complex A and complex B in Fig. 2) detected by the two probes suggests, however, the existence of constitutive transcription factors common to both genes.

Thus, nuclear extracts from untreated U937 cells were analyzed by EMSA in an attempt to identify nuclear factors that could bind to both the IPCS-p53 and IPCS-IRF1 probes. Competition experiments indicated that only complex B, detected with the labeled IPCS-p53 probe, is displaced by a 50-fold excess of either the IPCS-p53 or IPCS-IRF1 unlabeled probes (Fig. 3, lanes 1 and 4), or from U937 cells treated with either 1,000 IU/ml IFN-α for 1 h (lanes 2 and 5) or 1,000 IU/ml IFN-γ for 1 h (lanes 3 and 6). The slight difference in the mobility of complex B between the two probe is due to the 3-bp additional length of the IPCS-IRF1 probe.
nonspecific binding at the top of the gel (Fig. 3). Thus, the constitutive IPCS-BF complex, is the only complex present in the nuclear extracts of U937 cell, which is formed specifically, with both the IPCS-p53 and IPCS-IRF1 probes.

Site-directed Mutagenesis of IPCS and the Determination of the IPCS-BF Minimum Binding Site—To determine the minimal binding site of IPCS-BF, the IPCS-p53 and IPCS-IRF1 probes were radiolabeled and used in EMSA competition experiments with unlabeled probes from the two conserved regions shared by the IPCS-p53 and IPCS-IRF1 sequences (Fig. 4A). The first region contains 16 or 14 bp which encompass the GATTNC motif and which are present in the IPCS-p53 and IPCS-IRF1 probes, respectively (these fragments are termed IPCS-p53B and IPCS-IRF1B). The second region contains 14 or 13 bp, comprising the AAATGA motif, which are present in the IPCS-p53 and IPCS-IRF1 sequences (Fig. 4A). The first region contains 16 or 14 bp which encompass the GATTNC motif and which are present in the IPCS-p53 and IPCS-IRF1 probes, respectively (these fragments are termed IPCS-p53B and IPCS-IRF1B). The second region contains 14 or 13 bp, comprising the AAATGA motif, which are present in the IPCS-p53 and IPCS-IRF1 probes, respectively. These sequences are denoted p53-A and IRF1-A.

The IPCS-BF complex formed on the IPCS-p53 or IPCS-IRF1 probes, with nuclear extracts from U937 cells, is displaced by an excess of the unlabeled p53-A and IRF1-A probes, respectively (Fig. 4B, lanes 3 and 6). Neither the p53B nor IRF1B elements competed, respectively, with the IPCS-p53 and IPCS-IRF1 probes for binding of IPCS-BF (Fig. 4B, lanes 2 and 5). These results indicate that IPCS-BF binds to a sequence containing the AAATGA motif, which is shared by the p53-A and IRF1-A probes. This motif overlaps the 3' end of the IR sequence within the IRF1 promoter, and consequently constitutes a DNA binding sequence independent of IR.

To further characterize the binding of IPCS-BF to IPCS, we synthesized a set of probes containing point mutations (Table I) for use in competition experiments. EMSAs were performed with nuclear extracts from U937 cells using either the p53-A or IRF1-A sequences as a probe. Each position within p53-A was mutated. Unlabeled probes containing point mutations were used as competitors for the binding of IPCS-BF to the p53-A or IRF1-A labeled probes in EMSA reactions (Fig. 5, A and B). Point mutations that compete little or not at all with the labeled p53-A or IRF1-A probes were considered to represent important positions for binding of IPCS-BF to its recognition sequence. The results of these experiments are summarized in Table I. Mutations of any one nucleotide in the AAATG motif significantly decreased the ability of the sequence to compete for the binding of IPCS-BF to either the p53-A or IRF1-A wild-type probes (Fig. 5, A and B). The results of competition analysis show that the adenine at position −384 within the AAATG motif appears to be less important than the other residues for the binding of IPCS-BF. The cytidine at position −376, which is shared by both wild type probes, is also implicated in the binding of IPCS-BF to the p53-A or IRF1-A sequences, albeit in a less critical manner. The results of competition experiments between the p53-A and IRF1-A sequences showed that nucleotides, which are not common to both the p53-A and IRF1-A probes, had no significant effect on the binding of IPCS-BF. Mutation of the nucleotide at position −380 had no effect in competition experiments on the binding of IPCS-BF to the wild-type probes. In addition, none of the wild or mutated
probes displaced any of the nonspecific complexes, confirming the high degree of specificity of the binding of IPCS-BF to DNA.

The results of these studies have shown that the binding characteristics of IPCS-BF to the p53-A or IRF1-A sequences are identical and have led to the identification of the following degenerated motif AAATGRYKKCMMS (IUAP code) for the binding of IPCS-BF.

Identification of IPCS-BF in Different Cells Types—To be sure that the presence of IPCS-BF is not restricted to a particular cell line we looked for the presence of this factor in nuclear extracts from human peripheral blood leukocytes (PBL) from healthy donors. The results of EMSA experiments, using the IPCS-p53 or IPCS-IRF1 sequences as probes, show that the same complexes appear to be present in nuclear extracts from PBL from normal donors (Fig. 6, lanes 2 and 8), as in nuclear extracts from U937 cells (Fig. 6, lanes 1 and 7). Furthermore, the use of IPCS-p53, IPCS-IRF1, p53-A, and IRF1-A as unlabeled probes in competition experiments show that the binding properties of the factor present in nuclear extracts from PBL from normal donors are the same as those described previously for IPCS-BF using nuclear extracts from U937 cells (Fig. 6, lanes 3–6 and 9–12). These results suggest therefore that IPCS-BF is a transcription factor, which is present constitutively in normal human peripheral blood mononuclear cells and in the promonocyte cell line U937.

Estimation of the Number and the Molecular Weight of the Polypeptide Chains Involved in the Binding of IPCS-BF to DNA—To estimate the molecular weight of IPCS-BF and to determine the number of polypeptide chains implicated in the binding of this factor to DNA, we undertook UV cross-linking experiments. This analysis was carried out using nuclear extracts from U937 cells and two probes derived from the p53-A sequence. The respective strand of these probes on which all thymidine residue are substituted by BrdUrd determines the difference between the two probes. To increase the possibility of covalent binding, we carried out these experiments using the p53-A fragment, which contains more thymidine residues than the IRF1-A element. As shown in Fig. 7, only one polypeptide of about 26 kDa was found to bind to the two different p53-A probes. This size was determined from the position of molecular weight markers and subtracting from the molecular weight of the single strand oligonucleotide. The results of these experi-

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**FIG. 5.** A, EMSA analysis using the p53-A probe and nuclear extracts from untreated U937 cells. Competitions reactions were performed with a 50-fold molar excess of point mutated oligonucleotides. Each position from −385 to −376 was individually mutated, and each oligonucleotide was used in a competition reaction with the wild-type sequence as a probe. B, as in A, except that IRF1-A was used as the wild-type probe.

**FIG. 6.** EMSA analysis using IPCS-IRF1 or IPCS-p53 probes and nuclear extracts from U937 cells or from human PBL. Lanes 1, 2, 7, and 8, no competitor; lanes 3 and 9, 50-fold molar excess of IPCS-p53; lanes 4 and 10, 50-fold molar excess of IPCS-IRF1; lanes 5 and 11, 50-fold molar excess of p53-A; lanes 6 and 12, 50-fold molar excess of IRF1-A.
ments suggest that the binding of IPCS-BF to the IPCS sequence involves the interaction of a single polypeptide of 26 kDa with both strands of DNA.

**Functional Analysis of IPCS Sequences**—To test the transcriptional activity of the IPCS sequence, we constructed the following reporter vectors: IPCS-p53-luc, p53-A-luc, and M-382-luc (sequence mutated at position −382) containing, respectively, the IPCS-p53, p53-A, and the M-382 sequences, cloned upstream of the SV40 promoter directing the expression of the luciferase reporter gene (Fig. 8A). U937 cells were then transfected transiently with these constructions (Fig. 8B). The presence of either the IPCS-p53 or p53-A sequence in the reporter vector resulted in a 4–6-fold increase in luciferase activity, compared with cells transfected with the control vector containing the SV40 promoter alone (Fig. 8B). Furthermore, introduction of the M-382 mutation into the IPCS sequence abrogated completely the increase in luciferase activity (Fig. 8B). Thus, these results demonstrate clearly that IPCS possesses a positive transcriptional activity.

To evaluate the role of IPCS in the control of transcriptional activity of the p53 and IRF-1 genes, we have cloned 861 and 616 bp, respectively, of the promoter region of the p53 and IRF-1 genes, immediately upstream of the cDNA of the luciferase reporter gene (pGL-p53-luc, pGL-IRF-1-luc; Fig. 9A). The 3′ end of the p53 promoter is 10 bp downstream of the major p53 transcription initiation site, and the 3′ end of IRF-1 promoter includes the unique IRF-1 transcription initiation site. Four of the nucleotides that constitute the IPCS sequence were mutated in both the p53 and IRF-1 promoters. These mutations comprise the nucleotides at positions −382 to −379 within the

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**FIG. 7. UV cross-linking of IPCS-BF.** EMSA was carried out with IPCS-p53-A probe. The gel was UV-irradiated for 30 min and then autoradiographed to locate the IPCS-IPCS-BF complex. Proteins were extracted and subjected to SDS-polyacrylamide gel electrophoresis. Lane 1, IPCS-p53-A probe containing BrdUrd substitutions in the minus strand; lane 2, IPCS-p53-A probe containing BrdUrd substitutions in the plus strand; lanes 3 and 4, as lanes 1 and 2, respectively, but without UV irradiation. The unbound strand is shown at the bottom of the gel.

**FIG. 8. A, schematic representation of the constructs used in transfection experiments, harboring the oligonucleotides IPCS-p53, p53-A, and M-382 cloned upstream from the SV40-luciferase reporter gene. B, luciferase assays in transiently transfected U937 cells. The level of luciferase activity is expressed in arbitrary luminometer units. U937 cells were transfected with 10 μg of each construct.
p53 promoter, and the nucleotides at positions −110 to −107 within the IRF-1 promoter (Fig. 9A). The level of luciferase expression of the four vectors including the wild-type and mutant p53 and IRF-1 promoters were tested in transient transfection experiments in U937 cells (Fig. 9B). The results of these experiments show that the introduction of these mutations into the IPCS sequence reduces at least 5-fold the levels of luciferase activity relative to the wild-type promoter, for both IRF1 and p53 genes. These results show that IPCS-BF is a transcriptional activator of both the p53 and IRF1 promoters, and suggest that IPCS-BF is implicated in the control of the constitutive transcriptional activity of these promoters. As the IPCS sequence partially overlaps the IFN IR in the IRF1 promoter, we determined whether IPCS-BF plays a role in the induction of the expression of the IRF1 gene by IFNs. Treatment of U937 cells, transfected with either the wild-type or IPCS-mutated IRF1 promoter linked to the luciferase reporter gene, with IFN-α or IFN-γ increased the level of luciferase activity approximately 10- and 4-fold, respectively, compared with cells treated with control preparations (Fig. 10). Although the basal or induced activity of the IRF1 promoter is decreased 4-fold when the IPCS site is mutated, the amplitude of induction by IFN-α or -γ is not affected within IPCS. Thus, mutations affecting the binding of IPCS-BF do not affect the inductibility of the IR sequence by IFN. Furthermore, based on previously published data (28) mutations within what we have defined as the IPCS sequence of the IRF1 promoter do not affect nucleotides within the IR element which are implicated in the binding of inducible factors by IFN-α or IFN-γ. Thus, taken together these results suggest that the IR element and IPCS within the IRF1 promoter are two independent regula-
tory sequences, even though the transcriptional activity of factors formed by the dimerization of the STAT proteins, which are induced by the IFNs, are markedly increased in the presence of IPCS-BF.

**DISCUSSION**

We have identified a novel, highly conserved sequence, denoted IPCS, which is present in the promoter of both the p53 and IRF1 genes. We have shown that a single nucleolar factor, present constitutively in nuclear extracts from both U937 cells and PBL from healthy donors, binds specifically to this sequence. This nucleolar factor denoted, IPCS-BF, is composed of a single polypeptide chain of about 26 kDa, which binds directly to DNA. We have defined the minimal binding site of IPCS-BF, which overlaps and exceeds by at least 4 nucleotides at the 3’ end of the IR sequence of the IRF1 promoter defined previously (28). Point mutation analysis lead to the identification of the nucleotides that are required for IPCS-BF binding. The AAAT-GRYYKCC sequence, which we have identified as the minimal sequence required for IPCS-BF binding, may well exist, however, in a degenerate form, which potentially could well bind AP1 or members of the CREB family of transcription factors. Thus, it is possible that some of the complexities observed in EMSA using nuclear extracts from U937 cells or PBL from normal donors, and the IPCS-p53 or IPCS-IRF1 probes may contain such factors (Figs. 3 and 6). It is highly unlikely, however, that IPCS-BF is in fact AP1 or a member of the CREB family of factors for the following reasons. (i) Competition experiment using mutants M-385 and M-383, which are unable to bind IPCS-BF, show that the first and the third adenines are essential to IPCS-BF binding. However, in these two mutants the putative AP1 and CRE sequences, respectively, RTGASTMA and TGACGTMWW (39, 40), remain intact. (ii) It has been shown experimentally that the adenine in position 4 of the AP1 recognition sequence and the adenine in position 3 of the CRE consensus sequence (RTGASTMA and TGACGTMWW) are indispensable for the binding of these two factors (39, 40), whereas mutation of this base in mutant M-380 of the IPCS sequence does not inhibit the binding of IPCS-BF. (iii) The 3’ adenine in the AP1 consensus sequence can never be a cytosine (39), whereas substitutions of this base do not affect the binding of IPCS-BF. These data indicate that IPCS-BF possesses binding properties quite distinct from those described previously for AP1 or CREB, strongly suggesting that IPCS-BF is not either of these factors. Furthermore, AP1 and the CREB factors are composed of two subunits (39, 40), whereas the data obtained from the UV cross-linking experiments suggest that the binding of IPCS-BF to DNA involves a single polypeptide chain. Furthermore, the properties of IPCS-BF binding to IPCS are not characteristic of any known nuclear factor and the comparison of the IPCS consensus sequence with the binding site data base (Tfsite) did not show any significant homology with any previously described sequence, again suggesting that IPCS-BF is indeed a novel transcription factor.

We have shown that IPCS under the influence of a heterologous promoter is able to increase 4-fold the constitutive transcriptional activity of a reporter gene in transfection experiments in U937 cells. The introduction of point mutations into IPCS, which abolish the binding of IPCS-BF, also reduces reporter gene expression to the level of the control vector in transfectected U937 cells. Thus, this activity is strictly correlated with the binding of IPCS-BF to IPCS. So, IPCS-BF would appear to be a novel transcription factor, which possesses a positive transcriptional activity.

Transient transfection of U937 cells with reporter vectors containing the p53 and IRF1 promoters mutated at the IPCS site, resulted in a 4–5-fold reduction in the constitutive level of expression of the two promoters, compared with the wild-type promoters. Thus, the IPCS site would appear to contribute significantly to the constitutive transcriptional activity of both the IRF1 and p53 genes. As IPCS-BF is present constitutively in nuclear extracts of both U937 cells and in nuclear extracts from PBL from healthy donors, IPCS-BF would appear to play a role in regulating the constitutive expression of the p53 and IRF1 genes under physiological conditions, at least in lymphoid tissue. We have shown that IPCS-BF binds to both the IPCS-p53 and IPCS-IRF1 sequences in an identical manner, strongly suggesting that IPCS-BF plays a role in the transcriptional coregulation of the IRF1 and p53 genes. Furthermore, these data suggest that the appearance of spontaneous mutations within the coding regions of the IPCS-BF gene, or in the IPCS-BF recognition site in the promoter of either the p53 or IRF1 genes, may play a role in neoplastic transformation.

Treatment of U937 cells with IFN-α or IFN-γ results in a 10- to 4-fold increase, respectively, in the transcriptional activity of both the wild- type and IPCS mutated IRF1 promoter. The transcriptional activity of the uninduced or induced IPCS-mutated IRF1 promoter is, however, at least 5-fold lower than either the uninduced or IFN-induced activity of the wild-type IRF1 promoter. Thus, these data suggest that the mutation within the IPCS site does not inhibit the transactivation of the IRF1 promoter by the STAT1/STAT1 homodimer or the STAT2/STAT1 heterodimer. Furthermore, these results indicate that the IR element and the IPCS site are functionally independent, but may act in synergy to enhance the transcriptional activity of the IRF1 promoter.

Thus, our results suggest that IPCS behaves as a constitutive enhancer, which is necessary for the maintenance of the transcriptional activity of both the IRF1 and p53 genes. Furthermore, the constitutive activity of the p53 and IRF1 promoters may result from the concerted action of IPCS with other regulatory elements which confer a basal transcriptional activity upon these promoters. For example, the sequence NF1/YY1 has been reported to confer basal transcriptional activity upon the p53 promoter (27), and the IRF1 promoter contains several SP1 sites which could be responsible at least in part for the basal activity of the IRF1 gene (15, 28).

Sequence homology searches in the data bases, led to the identification the IPCS motif in the promoter regions of the IRF1 and p53 genes of both rats and mice. The highly conserved nature of this sequence at approximately the same position in different species attests to the importance of the IPCS sequence in the transcriptional regulation of the p53 and IRF1 genes. Interestingly, numerous human antioncogenes including Bax, Wt1, mm23H1, Rb, and WAF1, also contain a putative IPCS-BF binding site in their promoter regions. Thus, IPCS-BF may play an important role in the control of cell proliferation and apoptosis.

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