The Interferon (IFN)-stimulated Gene Sp100 Promoter Contains an IFN-γ Activation Site and an Imperfect IFN-stimulated Response Element Which Mediate Type I IFN Inducibility

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Expression of the nuclear domain-associated proteins Sp100, PML, and NDP52, is enhanced by interferons (IFNs) on the mRNA and protein level. Increase both of Sp100 and PML mRNA is due to enhanced transcription of the corresponding genes which occurs independently of cellular protein synthesis immediately upon IFN-β addition. Here, we describe the molecular cloning and functional analysis of the Sp100 promoter. DNA sequence analysis revealed potential binding sites for several constitutive and IFN-inducible transcription factors. Consistent with the absence of a TATA box and an initiator element, several transcription initiation sites were found.Transient expression studies identified an imperfect IFN-stimulated response element within the first 100 nucleotides upstream of the major transcription start site. This element rendered a heterologous promoter IFN-β-inducible and bound IFN-stimulated gene factor 2 strongly but IFN-stimulated gene factor 3 only weakly. An IFN-γ activation site approximately 500 base pairs upstream of the IFN-stimulated response element was found to bind three IFN-α/β activation factors upon IFN-β induction and conferred both type I and type II IFN inducibility upon a heterologous promoter. These data demonstrate a novel arrangement of a non-overlapping IFN-γ activation site and an IFN-stimulated response element mediating type I IFN inducibility, previously not reported for other IFN-stimulable promoters.

Sp100 is a protein located in novel discrete nuclear domains (“nuclear dots,” NDs) and an autoantigen predominantly in patients with primary biliary cirrhosis (1–5). Experimental evidence suggests a transcriptional transactivating function for this protein (2, 6). Recently, the murine Sp100 gene (mSp100) was identified and found to be highly repetitive and variable in copy number (7, 8). The expression of the Sp100 and mSp100 genes as well as of the genes of two additional ND-associated proteins, PML and NDP52 (9–12) is greatly enhanced by type I (α/β) and type II (γ) interferons (IFNs) (7, 12–15). Similar to Sp100, PML is also autoantigenic in primary biliary cirrhosis (4) and appears to be a transcriptional transactivator (16). It was discovered originally in patients with acute promyelocytic leukemia (APL), a hematopoietic malignancy in which the normal development of myeloid precursors is blocked at the promyelocytic stage. In APL cells the PML protein is fused to retinoic acid receptor-α sequences (17, 18), and its expression leads to a redistribution of both the PML and Sp100 proteins. Since retinoic acid treatment of APL cells leads to normalization of the ND pattern and releases the differentiation block, it was speculated that disruption of the NDs causes APL (9–11). The cell growth and tumor suppressing activities of PML support this speculation (19–22). NDS and their associated proteins appear to play a role not only in tumorigenesis and autoimmunity but also in virus infection as changes in cellular localization are induced by various viral transcription factors (13, 23–26). The autoimmunogenic character and putatively similar function of Sp100 and PML, the modulation of the NDS in tumorigenesis and virus infection, and the IFN-enhanced expression of all known ND proteins prompted us to investigate in more detail the mechanism of the transcriptional regulation of these genes. For Sp100 and PML, we could recently demonstrate that IFN-enhanced expression is mainly due to an increased transcription rate of the corresponding genes (14). However, from these and other studies (15), it has not become clear which factors and mechanisms are involved in IFN inducibility.

Activation of transcription by IFNs involves binding of the ligands to their receptors followed by tyrosine phosphorylation of the receptors, of specific tyrosine kinases, and of latent cytoplasmatic transcription factor subunits called STATs (signal transducers and activators of transcription) (reviewed in Refs. 27–29). The STATs are then assembled to form functional transcription factors which are translocated into the nucleus. Within their promoter regions IFN-α/β-inducible genes contain IFN-stimulated response elements (ISREs) (28) that interact specifically with three major complexes, the IFN-stimulated gene factors ISGF1, ISGF2, and ISGF3. ISGF3, the major primary transcriptional activator (30, 31), consists of three α-subunits, p91 (STAT1α), p84 (STAT1β), and p113 (STAT2) that assemble in the cytoplasm upon phosphorylation (see above) and, together with the ISGF3γ subunit p48, form transcriptionally active ISGF3. ISGF2, identical to IFN regulatory factors...
factor-1 (IRF-1) (32, 33) also binds to the ISRE, but more slowly after IFN addition. Unlike ISGF3, ISGF2 binding requires ongoing protein synthesis (34). Currently, it is believed that ISGF2 functions as a positive transcriptional regulator which maintains IFN-enhanced transcription initiated by ISGF3 (35). ISGF1 is identical or at least contains IFN regulatory factor-2 (IRF-2) (36, 37) and appears to be a constitutive ISRE-binding factor that acts negatively on ISGF2 (36). In contrast to ISGF3, both ISGF1 and ISGF2 need only the ISRE core sequence 5'-TTCCNNTTT-3' for binding (28).

In contrast to type I IFNs, receptor-binding of IFN-γ triggers tyrosine phosphorylation only of STAT1, which leads to homodimerization and subsequent translocation of the active transcription factor into the nucleus (reviewed in Refs. 27 and 20). This factor, IFN-γ activation factor (GAF), and a cognate IFN-γ activation site (GAS) were discovered originally through studies on the IFN-α/β- and IFN-γ-induced transcription of the GBP gene (38–40). Later on, GAS binding was also observed for an IFN-α-inducible factor which was therefore termed IFN-α activation factor (AAF) (41). However, the discovery of the STAT proteins and the elucidation of the mechanisms of their activation strongly suggested that GAF and AAF are identical (42) and that a GAS may render a promoter responsible for both types of IFNs.

Here we describe the cloning and functional characterization of the Sp100 promoter region and the identification of sequence elements to which IFN-induced transcription factors bind. The results demonstrate that IFN-inducible Sp100 gene transcription is similar but not identical to the very well studied IFN-inducible GBP gene and, therefore, represents a unique example of an IFN-regulated promoter.

**EXPERIMENTAL PROCEDURES**

**Isolation of Genomic Sp100 DNA**—For isolation of Sp100 promoter DNA fragments, a genomic library of the Burkitt’s lymphoma cell line BL64 in λ vector EMBl3A (43) was screened with a full-length 32P-labeled Sp100 cDNA probe (2). Positive plaques were further screened with 32P-labeled oligonucleotides from the 5′-end of the Sp100 cDNA (Sp32, 5′-ATGGCAGGTGGGGGCGG-3′; Sp347, 5′-CCACTCTCTGTAGTAGCAGG-3′) according to standard protocols (44). The DNA of one of the positive λ clones was double-digested with SacI and XhoI, and all fragments were ligated into SacI-digested pUC19. A genomic DNA fragment containing the appropriate Sp100 promoter fragment was then identified by hybridization of the bacterial colonies with a fluorescein-11-dUTP-labeled oligonucleotide from the 5′-end of the Sp100 coding region (Sp61, 5′-GGTTAAGGATGAGGCGG-3′) and by enhanced chemiluminescence detection according to the manufacturer’s protocol (Amersham Corp.). The nomenclature of the oligonucleotides corresponds to their 5′-positions in the Sp100-cDNA (2).

**DNA Sequencing and Polymerase Chain Reaction (PCR)**—Double-stranded DNA was sequenced using the Sequenase Version 2.0 kit (U.S. Biochemical Corp.) and 5′-32P-dATP, or by PCR cycle-sequencing with fluorescence dyes and use of a Li-Cor 4000 DNA sequencer. For PCR amplifications, buffers, reaction mixes, and cycling conditions were chosen as described elsewhere (45).

**Preparation of Total and Poly(A)⁺ RNA**—Total cellular RNA was prepared by the urea/LiCl method (46). Poly(A)⁺ RNA was purified from total RNA using Oligotex-dT spin columns (Qiagen).

**Primer Extension Analysis**—Four micrograms of poly(A)⁺ RNA per reaction mixed with 500,000 cpm 32P-labeled primer (specific activity, 3 × 10⁶ cpm/μg) were denatured for 10 min at 90°C in 15 mM KCl, 10 mM Tris/HCl (pH 8.3), 1 mM EDTA, and subsequently annealed for 90 min at 60°C. For primer extension 30 μl of reverse transcriptase mix (30 μl Tris/HCl (pH 8.3), 15 mM MgCl₂, 8 mM dithiothreitol, 0.2 μg/ml RNasin, 2.0 μg/ml actinomycin D, 3 mM dNTPs, 40 units of RNase, 9.5 units of avian myeloblastosis virus reverse transcriptase) were added, and the samples were incubated 80 min at 37°C. The reactions were stopped by adding 105 μl of RNase mix (100 mM NaCl, 10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 100 μg/ml salmon sperm DNA, 20 μg/ml DNase-free RNase A) and incubation for 15 min at 37°C. After phenol extraction and ethanol precipitation, samples were analyzed by electrophoresis on 6% acrylamide (29:1), 7 μm urea gels.

**Cell Culture, Cytokines, and Transient Transfection Assays**—Hela S8, HeLaS2, and HuH7 (47) cells were maintained as monolayers in Dulbecco’s modified Eagle medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. Human IFN-β and -γ were used at concentrations of 1000 and 100 units/ml, respectively. Plasmid DNAs were introduced into cells by the calcium phosphate procedure (44). One picomole of Sp100 promoter CAT construct and 1 μg of a CMV-lacZ construct, adjusted to 10 μg of DNA with pUC19, were precipitated per 6-cm dish. For transfection of thymidine kinase/ISRE- and thymidine kinase/GAS-CAT constructs, 3 pmol of plasmid was used. Calcium phosphate-DNA co-precipitates were left on the cells overnight and removed by exchange of the medium, and cells were then incubated for a further 24 h in Dulbecco’s modified Eagle’s medium with or without IFN.

**RESULTS**

**Cloning and Sequence Analysis of the Sp100 Promoter Region**—A genomic DNA fragment containing promoter sequences of the Sp100 gene was isolated from a genomic phase λ library of the Burkitt’s lymphoma cell line BL64 by hybridization with a 32P-labeled full-length Sp100 cDNA (2). An approximately 10-kilobase pair long restriction fragment of the insert was subcloned into pUC19. The promoter region of this insert, comprising 1114 bp upstream of the translational start codon of the Sp100 gene and 146 bp downstream thereof extending into intron I, was sequenced using synthetic oligonucleotides as primers (Fig. 1). Inspection of the sequence revealed neither a consensus CCAAT or TATA box nor a consensus initiator element that can functionally substitute a TATA box (51). However, we found the sequence element ACTTCN₁₈GCCA at the transcription initiation sites (see below and Fig. 1) which diverges by only one nucleotide from the housekeeping initiator protein 1 (HIP1) binding site (ATTTCN₁₈GCCA) described for several other TATA-less promoters (52). As HIP1 was shown to be sufficient for mediating transcription initiation at the ATTTC and/or GCCA site when combined with at least one binding site for transcription factor Sp1 (52, 53), and as the Sp100 promoter also has a closely spaced Sp1 binding site (Fig. 1), these sequences are probably important for Sp100 promoter activity and transcription initiation. Similarly, potential binding sites for the transcription factor Ap1 (54) and for NF-κB (55) could also play a role in promoter activity (Fig. 1). Three sequence motifs, two consensus GAS located at positions −655 to −647, −811 to −803, and one with similarity to an ISRE at position −162 to −149, which may confer IFN inducibility upon the Sp100 gene, were also identified (Fig. 1). Both GAS motifs exactly match the
consensus sequence (5’-TTCCNNGAA-3’) (56), whereas the ISRE diverges from the consensus sequence (5’-AGTTTC-NNTTTCNY-3’) (28) at three nucleotide positions (216, 215, and 214), one of them (216) being highly conserved. Taken together, computer analysis of the Sp100 promoter sequence revealed several features typical for promoters of housekeeping genes and three potential elements which may be relevant for IFN-enhanced transcription of the Sp100 gene.

Mapping of the Transcription Initiation Sites—A prerequisite for functional analysis of promoters is the mapping of the start sites of transcription. Therefore, primer extension analysis was carried out by reverse transcription of RNA from IFN-β-treated or untreated HEp2 cells, using a 32P-labeled oligonucleotide specific for Sp100 or, as a control, for β-actin as primers. Two distinct size classes of reverse transcription products were observed with RNA from IFN-treated cells (three separate experiments, one example shown in Fig. 2, lane 2) with the Sp100 oligonucleotide indicating several transcription start sites at the potential ACTTC- and GCCA-HIP1 binding region (Fig. 1). In untreated cells, essentially the same type of signals were seen but only after a much longer exposure (Fig. 2, lanes 1 and 1*). Although the sizes of the products were the same in IFN-treated and untreated cells, the major transcription initiation site corresponding to one of the fast migrating fragments was predominant only in IFN-treated cells (Fig. 2, compare lanes 1* and 2). This indicates an IFN-dependent quantitative change in transcription initiation site usage. The correct mapping of the 5’-end of the β-actin mRNA in the control experiment with RNA from IFN-β-treated or untreated HEp2 cells, the lack of reverse transcripts when Escherichia coli tRNA was used as negative control template (Fig. 2, lanes 3, 4, and 5, respectively), and the much stronger Sp100 signals in IFN-treated compared to untreated cells demonstrate that the Sp100 primer extension products reflect true transcription initiation sites. These results were confirmed by RNase protection experiments (data not shown).

Functional Characterization of the Sp100 Promoter—Mapping of the transcription initiation sites provided the basis for the identification of sequence elements regulating the constitutive and IFN-inducible Sp100 promoter activity by transient transfection experiments. Several Sp100 promoter fragments were generated by PCR (Fig. 3A) and cloned blunt end into SmaI-digested vector pGCAT-C (58) upstream of the CAT gene. All constructs, except pGCAT-1091 and 2266, contain the authentic Sp100 translation start codon for expression of CAT fusion proteins (Fig. 3A). Three transient transfection experiments with HuH7 cells and two different DNA preparations (Fig. 3B, transfections 1, 2, and 3) of each CAT construct containing promoter fragments in sense orientation (Fig. 3A) resulted in similar, although slightly fluctuating, amounts of CAT enzyme at levels comparable to that achieved with a reference herpes simplex virus (HSV) thymidine kinase promoter construct (pBLCAT2) (59) (Fig. 3B). Consistent with our previous computer predictions, these experiments indicate that the sequence elements required for constitutive Sp100 promoter activity are located between positions –143 and –14.
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When the same experiments were performed with cells treated after transfection with IFN-β, all fragments in sense orientation with 5′-ends between positions −1104 and −192 and 3′-ends either at position +12 or −14 exhibited much higher promoter activity than in untreated cells (Fig. 3B). Progressive 5′-deletions up to nucleotide position −193 (constructs pGCAT-1116 to −204) gradually reduced IFN-β inducibility from 13.0- to 7.4-fold, but did not completely abolish IFN-β responsiveness. The fragment with the 5′-end at position −143 and the 3′-end at position +12 retained basal promoter activity but lacked IFN inducibility completely. As expected, the promoter fragment tested in reverse orientation (Fig. 3, pGCAT-1116(−)) as well as the vector without insert had neither constitutive nor IFN-inducible promoter activity. These data locate an IFN-β-responsive element within region −192 to −144 of the Sp100 promoter. This is consistent with our previous identification of an ISRE at position −162 to −149 (Fig. 1) presumed to play a role in IFN inducibility. However, the gradual decrease in IFN inducibility associated with the progressive 5′-deletions of the promoter fragments up to the position of the ISRE argues for additional sequence elements upstream of the ISRE required for full IFN-inducibility of the Sp100 promoter.

The Sp100-ISRE Confers IFN-β Inducibility—Transient transfection experiments suggested but did not prove that the ISRE within region −192 to −144 contributes to IFN-β-enhanced Sp100 promoter activity. In order to corroborate this suggestion, HuH7 cells were transfected with CAT plasmids containing in both orientations either a synthetic Sp100 oligonucleotide spanning region −168 to −143 and including the ISRE (5′-TTAATACTTTCACCTCTTTTTCC-3′) or an oligonucleotide containing the ISRE from the IFN-inducible gene ISG15 (60) (5′-GGGAAAGGAAAACCGAAACTGAAGCC-3′) upstream of the HSV thymidine kinase promoter of plasmid pBLCAT2. When the transfected cells were treated with IFN-β, the promoter activities of all four constructs increased from 3.6- to 9.2-fold (data not shown), demonstrating that irrespective of the orientation both oligonucleotides convey IFN inducibility.

Identification of Proteins Binding to the Sp100-ISRE—IFN-β-enhanced Sp100 gene transcription occurs rapidly and is independent of protein synthesis (14). Therefore, we tested in gel mobility shift assays whether ISGF3, the primary transcriptional activator of IFN-α/β-stimulated genes, and/or other proteins bind to the Sp100-ISRE. For these experiments, we used the oligonucleotides containing either the Sp100-ISRE or, as a control, the ISG15-ISRE (see above). The nuclear protein extracts were prepared from untreated Hela S3 cells or from cells treated for 16 h with IFN-γ and IFN-β during the last 30 min to obtain maximal levels of ISGF3 (61). With the Sp100-ISRE, one major DNA-protein complex was evident in untreated cells, its amount strongly increasing upon IFN treatment (Fig. 4, A and B, lanes 1 and 2). Upon longer exposure of the gel, additional DNA-protein complexes became apparent (Fig. 4B, lanes 2 and 4–6). Competition with unlabeled Sp100-ISRE (Fig. 4, A and B, lanes 3) but not with an unrelated oligonucleotide (Sp1-oligo, Fig. 4, A and B, lanes 4) strongly inhibited formation of the major and four minor DNA-protein complexes (one of them can be seen only after much longer exposure; Fig. 4B, lanes 2 and 4), demonstrating that they are due to sequence specific protein binding. When antibodies against ISGF2/IRF1 were added to the binding reactions, the amount of the major DNA-protein complexes drastically decreased, and supershifted ones appeared (Fig. 4, A and B, lanes 6). This demonstrates that ISGF2/IRF1 binds to the Sp100-ISRE and is a component of the major DNA-protein complex. A similar experiment performed with or without antibodies against p84/p91 (both proteins are components of ISGF3) revealed an IFN treatment-dependent DNA-protein complex containing ISGF3 as it supershifted after antibody addition (visible only after long exposure, Fig. 4B, lanes 2 and 5). The oligonucleotide containing the ISG15-ISRE showed higher levels of the same ISGF3-DNA complex in untreated cells, which increased even further after IFN-β treatment (Fig. 4, A and B, lanes 7 and 8). Taken together, these experiments revealed very similar IFN-enhanced complex formation of ISGF2/IRF1 with the Sp100-ISRE and the ISG15-ISRE, much weaker binding of ISGF3 to the Sp100-ISRE, and similar complexes formed by both oligonucleotides (compare Fig. 4A, lane 8, and Fig. 4B, lane 2).

IFN-β-inducible Factors Bind to the Sp100-GAS—The very weak complex formation of the Sp100-ISRE with ISGF3 suggested the participation of additional transcription factors in IFN-enhanced Sp100 promoter activity. This prompted us to investigate whether AAF/STAT1 may be such a factor by testing its binding to the canonical GAS sequences previously identified in the Sp100 promoter region (Fig. 1). In gel mobility shift assays with the Sp100-GAS at position −655 to −647 (Fig. 1), three complexes were detected almost exclusively with extracts from Hela S3 cells treated for 30 min with IFN-β (Fig. 5, lanes 1 and 2). Binding of all three complexes proved to be specific since competition with unlabeled Sp100-GAS but not with Sp1-oligo prevented their formation (Fig. 5, lanes 3 and 5, respectively). As a positive control, an oligonucleotide containing the β-casein-GAS known to bind not only STAT5 but also IFN-induced STAT1 homodimers (63) was used. This oligonucleotide formed two protein complexes corresponding in mobility to the two fast migrating Sp100-GAS complexes and both also formed only when extracts from IFN-treated cells were used (Fig. 5, lanes 9 and 10). The co-migration of the β-casein-GAS and the two fast migrating Sp100-GAS complexes as well
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### A. Sp100 Promoter Constructs

| Construct | -104 | pGCAT-1116 | +12 |
|-----------|------|------------|-----|
| pGCAT-769 | +12  |            |     |
| pGCAT-575 | +12  |            |     |
| pGCAT-291 | +12  |            |     |
| pGCAT-232 | +12  |            |     |
| pGCAT-204 | +12  |            |     |
| pGCAT-155 | +12  |            |     |
| -1101     |      |            |     |
| pGCAT-1091| -1101|            |     |
| pGCAT-266 | -11  | pGCAT-1116 | -110 |
| pGCAT-C   |      |            |     |
| tk-CAT    |      |            |     |

### B. Amount of CAT-protein

| Transfection | - IFN | + IFN | + IFN | + IFN | Factor | S.D. |
|--------------|------|------|------|------|--------|------|
| Transfection #1 | 3.7 | 54.9 | 3.0 | 39.8 | 2.2 | 23.9 | 13.0 | 2.0 |
| Transfection #2 | 5.9 | 68.6 | 3.5 | 46.3 | 2.6 | 29.1 | 12.0 | 1.1 |
| Transfection #3 | 4.2 | 51.0 | 1.8 | 20.8 | 2.0 | 24.9 | 12.1 | 0.5 |

#### FIG. 3. Functional analysis of constitutive and IFN-induced Sp100 promoter activity. A, schematic representation of the Sp100 promoter fragments cloned blunt end into SmaI-digested vector pGCAT-C (plasmid nomenclature according to length of the fragments in base pairs). The antisense orientation of one of the fragments is indicated by a dash. B, CAT protein levels produced (in picograms/200 μl) from each construct in three independent experiments (1, 2, and 3) as determined by ELISA. Mean values of duplicates standardized by the same amount of β-galactosidase enzyme activity (A420 = 2.0) are given. S.D. = standard deviation of fold inducibility measured in the three independent experiments.

as the complete disappearance of the latter by competition with unlabeled β-casein-GAS strongly indicate that they contain common components. These are STAT1α and/or STAT1β as deduced from the disappearance of both Sp100-GAS complexes and the emergence of two supershifted bands after addition of α-p84/p91 antibodies to the binding reaction (Fig. 5, lane 6). Currently, we cannot determine whether the slow migrating Sp100-GAS complex also contains these proteins as the band at the corresponding position in this blot may correspond to a supershifted complex of one of the fast migrating ones or to the third Sp100-GAS complex which did not supershift. The lack of supershifted complexes in the gel mobility shift experiments with antibodies against ISGF3/γ/p48 or ISGF2/IRF1 (Fig. 5, lanes 7 and 8, respectively) indicates that these proteins are not components of any of the Sp100-GAS complexes. Interestingly, with an oligonucleotide containing the second potential promoter fragment (nucleotide position -811 to -803, see Fig. 1) no complex formation could be observed, irrespective of whether protein extracts from untreated or IFN-β-treated cells were used (data not shown). Thus, only one of the two GAS elements appears to contribute to IFN inducibility of the Sp100 promoter.

The Sp100-GAS Renders a Heterologous Promoter IFN-β- and IFN-γ-inducible—The gel mobility shift assays demonstrated that the Sp100-GAS at position -655 to -647 is able to form complexes with three AAFs. To study the functional significance of this finding we cloned the Sp100-GAS oligonucleotide (see legend to Fig. 5) as a monomer and as a trimer upstream of the HSV thymidine kinase promoter of plasmid pBLCAT2 and investigated the IFN inducibility of the corresponding plasmids. Transient transfection experiments of two different preparations of each plasmid revealed that the Sp100-GAS monomer construct was only poorly inducible by IFN-β (1.3-fold), whereas it was strongly inducible by IFN-γ (10.6-fold) (data not shown). In contrast to the monomer construct, however, the Sp100-GAS trimer construct was equally well inducible by IFN-β and γ (10.4- and 9.5-fold, respectively) (data not shown). These data demonstrate that the Sp100-GAS is able to confer IFN-β and IFN-γ responsiveness to a heterologous promoter. The very poor IFN-β inducibility of the Sp100-GAS monomer construct is in good agreement with the transient transfection experiments shown in Fig. 3, where deletion of the region containing the Sp100-GAS did not result in a reduction of the IFN-β inducibility of the corresponding Sp100 promoter construct. Furthermore, our data are consistent with results obtained with other GAS elements in transient transfection experiments (see, for example, Ref. 42). The weak type I IFN responsiveness of promoters containing only one GAS element is believed to result from a shorter lived interaction of AAFs with GAS elements compared to IFN-γ-activated GAFs.

#### DISCUSSION

In this report we have described the isolation and functional characterization of the promoter region of the IFN-inducible Sp100 gene. Heterogeneous transcription start sites differentially used in IFN-treated and untreated cells as well as sequence elements and transcription factors involved in constitutive and IFN-inducible promoter activity were identified. Unlike all other IFN-inducible genes known so far, IFN inducibility of the Sp100 promoter appears to be controlled by the combination of an imperfect ISRE and a GAS separated by more than 400 nucleotides and probably by additional sequence elements.

We found that transcription from the Sp100 promoter is initiated at various sites as described for example for the IFN-inducible GBP and 9–27 genes (64, 65). Indirect evidence has been provided that Sp100 transcription initiation is controlled by the transcription factors HIP1 and Sp1, similar as described for promoters of several housekeeping genes, the ISGF2/IRF1 gene, and for the SV40 late promoter (52). The start sites of transcription for the Sp100 promoter are identical in IFN-treated and untreated cells. However, similar to the situation...
described for the IFN-inducible murine 202 gene (66), we found preferential initiation at a specific site in IFN-treated cells, suggesting that IFN can also modulate the efficiency of transcription initiation at specific sites. As circumstantial evidence suggests that the type of splicing of Sp100 mRNAs may also be affected by IFN (14), it appearsthatIFNmodulates Sp100 gene transcription on the level of transcriptional initiation as well as posttranscriptionally.

With the exception of the GBP gene, all genes known to respond to type I IFNs contain either an ISRE or a GAS but not both in their promoter regions (see Refs. 28 and 67 and references therein). The GBP gene is the only example where an ISRE and an overlapping GAS are both necessary for transcriptional induction by IFN-α/β (40, 41). The IFN inducibility of the Sp100 and GBP promoters appears similar in some but not all aspects. The ISREs of the Sp100 and GBP promoters both diverge from the consensus sequence (a G/C transversion at the second, very highly conserved, position) and, therefore, have a very low affinity for ISGF3 (our data) (40). Unlike the ISRE and GAS elements of the GBP promoter, those in the Sp100 promoter are separated by several hundred nucleotides. Moreover, the GAS motifs of both promoters are different both in sequence and function as reflected by the different DNA-protein complexes formed in gel mobility shift assays (our data) (67). These differences may be in part responsible for the biphasic and monophasic kinetics of Sp100 and GBP gene transcription upon type I IFN treatment, respectively, and the less pronounced induction of Sp100 gene transcription upon IFN-γ treatment (14, 39). In addition, differences in sequences flanking both elements of the two promoters could also play a role as they can alter the specificity and the extent of the IFN response as shown for the ISRE of the 6-16 and (2'-5')oligoadenylate synthetase genes, respectively (65, 68).

Although the Sp100-ISRE diverges from the consensus sequence, it binds the same proteins as the “consensus” ISRE from the ISG15 gene promoter. Five comparable specific DNA-protein complexes, one of them containing ISGF2/IRF1 and one identified as ISGF3, formed with each of the two oligonucleotides. However, as concluded from the intensities of the signals the proteins of these complexes appeared to have higher affinities for the ISG15-ISRE than for the Sp100-ISRE. This is probably due to the T/C transition in the second T-triplet of the Sp100-ISRE affecting both ISRE and ISRE core binding.

ISGF3 and ISGF2/IRF1 were unequivocally identified as components of two of the Sp100-ISRE-protein complexes. The complex migrating below the very faint Sp100-ISRE-ISGF3...
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