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A Highly Amplified Mouse Gene Is Homologous to the Human Interferon-Responsive Sp100 Gene Encoding an Autoantigen Associated with Nuclear Dots

THILO GRÖTZINGER,[1] KIRSTEN JENSEN,[1] HANS H. GULDNER,[1] THOMAS STERNSDORF,[1] CARIN SZOSTECKI,[1] MANFRED SCHWAB,[2] LARISSA SAVELYEVA,[2] BERTHOLD REICH,[1] AND HANS WILL[1]*

Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, 20251 Hamburg,[1] and Deutsches Krebsforschungszentrum, 69120 Heidelberg,[2] Germany

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In human cells, three proteins are currently known to colocalize in discrete nuclear domains (designated nuclear dots): Sp100, a transcription-activating protein autoantigenic primarily in patients with primary biliary cirrhosis; PML, a tumor suppressor protein involved in development of acute promyelocytic leukemia; and NDP52, a protein of unknown function. Here we report sequence similarities between the Sp100 protein and a putative protein encoded by a highly amplified mouse gene which is visible as an inherited homogeneously staining region (HSR) on chromosome 1 of some mouse populations. By in situ hybridization, the Sp100 gene was mapped to locus 2q37, the syntenic region of the HSR on mouse chromosome 1. Unlike the highly amplified mouse gene, Sp100 was found to be a single-copy gene and showed no restriction fragment length polymorphisms. Sequence similarities in the promoter regions and similar exon-intron organizations of the two genes were revealed. As for Sp100, steady-state levels of the mRNAs of the HSR-encoded genes could be greatly increased by interferon (IFN) treatment. As in human cells, IFN treatment led to an enlargement in both size and number of nuclear dots in mouse cells as visualized by immunofluorescence staining with autoimmune sera from patients with primary biliary cirrhosis. These data indicate that a gene located in the inherited HSR of mice, designated mSp100, is homologous to the human Sp100 gene, has a similar gene organization, and responds similarly to IFN treatment.

We have recently characterized a nuclear autoantigen, designated Sp100, against which antibodies are found primarily in sera of patients with primary biliary cirrhosis (PBC) (31, 33). The expression of Sp100 is strongly increased by interferons (IFNs), viral infection, and transformation (13), which may be relevant for Sp100 autoantigenicity and PBC etiology. Sequence similarities with several viral proteins (30) and experimental evidence suggest that the Sp100 protein may function as a transcriptional transactivator (32, 42). By indirect immunofluorescence staining, the Sp100 autoantigen has been located in discrete nuclear domains (nuclear dots [NDs]) in the nuclei of a variety of human cell types and tissues (31, 35). Two transcription-regulatory proteins, the cell-encoded PML protein (10, 16, 40) and the herpes simplex virus type 1 latency-regulating protein ICP0 (21), were recently also shown to colocalize with these nuclear domains. Association of ICP0 with NDs leads to a displacement of the Sp100 and PML proteins and may be important for the life cycle and latency of herpes simplex virus type 1 (21). Interestingly, aberrant expression of the PML gene in acute promyelocytic leukemia as a result of fusion with the retinoic acid receptor alpha gene disrupts the NDs and induces a microspeckled nuclear and cytoplasmic expression pattern of PML and Sp100 (10, 16, 40). It has been speculated that this reorganization of the nuclear domains may cause acute promyelocytic leukemia. This hypothesis is based on the observation that after therapy with retinoic acid, nuclear domains are reorganized and the differentiation block in the leukemic cells is relieved. Lately, it has been reported that PML itself has a tumor suppressor function (15, 19, 22) and is overexpressed in various tumors (15, 35). Moreover, relocation of the PML protein to virus-induced structures has been described after infection of cells with adenoviruses. This phenomenon was speculated to be associated with functional inactivation of PML (25). Recently, it was found that a third protein, designated NDP52, colocalizes with Sp100 and PML in NDs, changes its cellular localization upon viral infection, and increases in amount after IFN treatment (17). A region at the C terminus of NDP52 with sequence similarities to a LIM protein interaction domain was speculated to mediate interaction of ND proteins. All three ND-associated proteins, Sp100, PML, and NDP52, seem to have a coiled-coil domain which may be important for their cellular localization and functions. The aim of our studies is the identification of the ND-associated proteins in mice in order to be able to study the function of the nuclear domains in an animal model.

Here we report a significant sequence similarity of the Sp100 protein with a protein deduced from a mouse cDNA mapping to a highly amplified gene of chromosome 1 (11). The corresponding chromosomal region is visible in different species of the genus *Mus* as an inherited homogeneously staining region (HSR) of variable size (1, 41). HSRs are designated according to their uniform staining pattern after G banding and are distinct chromosomal sites which always contain highly amplified DNA sequences. HSRs drew general interest when they were found to contain amplified genes mediating drug resistance to cells (6, 8, 20) or amplified oncogenes whose overexpression contributes to tumorigenesis (2, 27). In addition to these somatic types of HSRs, several populations of European wild mice have one hereditary HSR (37) evident as a polymorphism of chromosome 1 (between bands C5 and D). Molecular

* Corresponding author. Mailing address: Heinrich-Pette-Institut, Martinistr. 52, 20251 Hamburg, Germany. Phone and fax: 40-48051-221.

analysis revealed in the HSR a cluster of long-range repeats (~1,810 copies per haploid genome) which are about 100 kb in length (24). In HSR^- genomes, a similar cluster which contains between 3 and 50 copies exists at the same site on chromosome 1 (1), suggesting that the HSR evolved by in situ amplification of the low-copy-number cluster (36). In mice with high-copy-number clusters, five or six corresponding poly(A)$^+$ transcripts were found, while in mice with low-copy-number clusters, only a single HSR-derived transcript was detected (39). Whether one or several proteins are produced from these transcripts is unknown. So far, neither a selective advantage nor an altered phenotype is evident for HSR^+ mice compared with HSR^- mice. However, a functional significance of these hereditary HSRs is likely because HSR sequences are transcribed, and an increased number and amount of HSR-specific transcripts are observed concomitant with gene amplification (11, 39).

Here, we present data which indicate that the mouse gene family located in the long-range repeat cluster is homologous to the human Sp100 gene, has a similar exon-intron organization, expresses several transcripts in cultured cells, and has retained IFN responsiveness.

MATERIALS AND METHODS

PCR amplification, cloning, and DNA sequencing. PCR was performed with 1 μg of genomic DNA or 50 ng of linearized genomic plasmid p52/1 in a 100-μl reaction mixture containing 50 mM Tris-HCl (pH 9.1), 3.5 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 150 μg of bovine serum albumin per ml, 0.2 mM deoxynucleoside triphosphates, 20 pmol of each primer, and a mixture of 2.5 U of *Taq* DNA polymerase (Boehringer) and 0.08 U of *Pfu* DNA polymerase (Stratagene) (5). DNA fragments were amplified for 30 cycles in a Robocycler (Stratagene) as follows: 30 s at 99°C, 1 min at the appropriate annealing temperature (t^a), and the appropriate time (t) at 68°C. t^a and t are given below for each primer pair.

For localization of introns I to IV of the Sp100 gene, four pairs of primers were used: for intron I, Sp32(+) (5′-ATGGCAGGTGGGGGCGG-3′) and Sp103(−) (5′-CATCTCATTTGCTACTGG-3′) (t^a = 55°C, t = 2 min); for intron II, Sp106(+) (5′-CCATCTTCCTGCACACAGCC-3′) and Sp193(−) (5′-GAATA CAATGTCATAGAGCAGCC-3′) (t^a = 64°C, t = 8 min); for intron III, Sp142(+) (5′-GTTCACGGAAGACCAGGGTG-3′) and Sp446(−) (5′-GAAT TAAATCGGGGTATTCCTG-3′) (t^a = 60°C, t = 2 min); and for intron IV, Sp417(+) (5′-TCAACATGCAGGAATACCCCG-3′) and Sp528(−) (5′-GAC CTCTCCTCCCTCTCTTC-3′) (t^a = 61°C, t = 2.5 min). Primers are named according to the positions of the 5′ nucleotides in the Sp100 cDNA sequence (30). The PCR products were blunt-end cloned into the *Sma*I site of vector pUC19 according to standard protocols (26), and exon-intron boundaries were sequenced by using a Sequenase version 2.0 DNA sequencing kit (U.S. Biochemical) and pUC19 primers pUC268(+) (5′-GGGAAGGGCGATCGGTG-3′) and pUC560(−) (5′-CATTAGGCACCCCAGGC-3′).

The complete coding region of the 1.3-kb HSR cDNA (11) was PCR generated by amplifying separately the 5′ part (nucleotide positions 3 to 180 [11]) present in plasmid pMmHSRc-10E (11) with primers HSR-5′ (5′-CGGGATC CCCAGACAGCTGTGGAAG-3′) and HSR-3′E (5′-ACGGTCTCGGAG GCTCTC-3′) (t^a = 60°C, t = 20 s) and the 3′ part (nucleotide positions 181 to 920) present in plasmid pMmHSRc-3 (11) with primers HSR-5′E (5′-GAG AGCCTCCGAGACCGTGAATTCATCACTGGTAAAATG-3′) and HSR-3′ (5′-CCATCGATCATGAAACTGTTGCCACATTG-3′) (t^a = 55°C, t = 45 s). In a second PCR with primers HSR-5′ and HSR-3′ (t^a = 60°C, t = 1 min 10 s), the two fragments were fused via the overlapping HSR-3′E sequence introduced in the 3′ HSR fragment in the first amplification by primer HSR-5′E.

Fluorescence in situ hybridization. Preparation of metaphase spreads of Epstein-Barr virus-immortalized diploid lymphoblastic cells and Giemsa staining of chromosomes were performed by using standard cytogenetic techniques. Pretreatment of slides for fluorescence in situ hybridization, probe labeling, hybridization, and fluorescence detection of hybridized probes were done according to published methods (4). The Sp100-specific probe (plasmid p52/1) was labeled with biotin-16-dUTP (Boehringer) and detected with fluorescein isothiocyanate-avidin (Gibco/BRL). The digoxigenin-labeled chromosome 2-specific satellite probe (Oncor) was detected with rhodamine-labeled antidigoxigenin antibodies (Boehringer). Suppression of repetitive DNA sequences was achieved by preannealing with Cot-1 DNA (Gibco/BRL). Chromosome spreads were visualized by fluorescence microscopy, using an Axiophot microscope (Zeiss) equipped with a cooled charge-coupled camera (Photometrics KAF 1400-50) controlled by a Macintosh IIfx computer. Images were recorded with filter sets for 4′,6-diamidino-2-phenylindole, fluorescein, and rhodamine and processed to pseudo-colored images by using Gene Join software, developed by R. Rand, Yale University. The final images were produced by taking photographic slides from the screen.

Preparation of genomic DNA and Southern blotting. Genomic DNA preparations and Southern blotting experiments were carried out as described previously (26).

Preparation of total and poly(A)$^+$ RNA and Northern (RNA) blotting. Total RNA was prepared by the LiCl-urea method as described previously (3). Poly(A)$^+$ RNA was prepared from total RNA by using Oligotex spin columns (Qiagen) as instructed by the manufacturer and then used for Northern blotting as described previously (26).

Cells, cytokines, and indirect immunofluorescence microscopy. NIH 3T3, BALB/c 3T3, and simian virus 40 (SV40)-transformed BALB/c 3T3 mouse cells (9) were maintained in Dulbecco's modified Eagle medium (Gibco/BRL) supplemented with 10% fetal calf serum. Mouse IFN-α/β (Sigma) was used at a concentration of 1,000 U/ml for the times indicated. Indirect immunofluorescence microscopy was performed as described elsewhere (13). Briefly, cells were grown on chamber slides (Nunc) in Dulbecco's modified Eagle medium and treated as indicated. Fixation of cells was performed for 10 min in ice-cold acetone. Human PBC serum or rabbit anti-Sp100 antiserum was diluted 1:100 in phosphate-buffered saline (PBS; 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 140 mM NaCl, 2.6 mM KCl [pH 7.3]) and incubated on cells for 1 h at room temperature. For detection, dichlorotriazinyl amino fluorescein-conjugated goat anti-human, goat anti-rabbit, or goat anti-rat immunoglobulin G was diluted 1:200 in PBS and incubated as described above.

Bacterial expression of full-length HSR protein and immunization. The bacterial expression vector pQE-11 (Qiagen) was double digested with the restriction enzymes *Bam*HI and *Hin*dIII, and the PCR fragment containing the complete coding sequence of the 1.3-kb HSR cDNA was digested with *Bam*HI only (a unique *Bam*HI site was previously introduced at the 5′ end of the DNA fragment by PCR, using primer HSR-5′). Vector and fragment were ligated via the *Bam*HI site, the *Hin*dIII site was filled in with Klenow enzyme, and the construct was recircularized by blunt-end ligation. Isopropylthiogalactopyranoside (IPTG)-induced expression of the protein and Ni-nitrilotriacetic acid-agarose purification via a His_6 tag at the N terminus of the recombinant fusion protein were done according to the Qiagen protocol. Immunization of animals and booster injections with the protein either in polyacrylamide or together with Freund's adjuvant were performed as described previously (14). A synthetic peptide corresponding to amino acid sequence EVNMEKYPDLNLLRR of the protein encoded in the 1.3-kb cDNA of the mouse HSR (positions 100 to 114) was modified at the amino-terminal end with a cysteine residue, coupled to ovalbumin, and used as an antigen for immunization.

RESULTS

Sequence similarity of Sp100 and HSR-encoded murine protein. A search in the GenEMBL nucleic acid sequence data bank, using the cDNA sequence coding for the nuclear autoantigen Sp100, revealed a DNA sequence identity of 49.6% to a murine 1.3-kb cDNA (data not shown) mapped to a naturally occurring, inherited HSR (11). The murine cDNA contains an open reading frame for a putative protein of 208 amino acids (aa) (11). Protein sequence alignment of the complete putative mouse protein with the Sp100 sequence (aa 28 to 238) resulted in 38.5% identical and 17.8% similar amino acids (Fig. 1). The similarity is particularly striking between aa 35 and 145 of the Sp100 protein and between aa 9 and 117 of the murine protein. Within this region, the percentages of identity and similarity were 55.0 and 20.7, respectively.

The high sequence similarity in a subregion prompted us to determine whether this subregion corresponds to specific exons of the Sp100 gene which may be more conserved than other exons. Therefore, we analyzed and compared the genomic organization of the 5′ end of the Sp100 gene with that of the coding region of the murine cDNA sequence. PCR analysis of a 10-kb genomic Sp100 DNA fragment cloned in pUC19 (p52/1) and of genomic DNA isolated from placental tissue revealed that the region with the highest sequence similarity begins at the 5′ border of exon III and ends at the 3′ border of exon IV (Fig. 1 and 2A). Our comparative analysis with genomic sequences for the HSR-encoded gene available in GenBank (accession numbers L16452 to L16460) indicates that exons III and IV of Sp100 correspond in size exactly to exons II and III, respectively, of the mouse gene (Fig. 1).

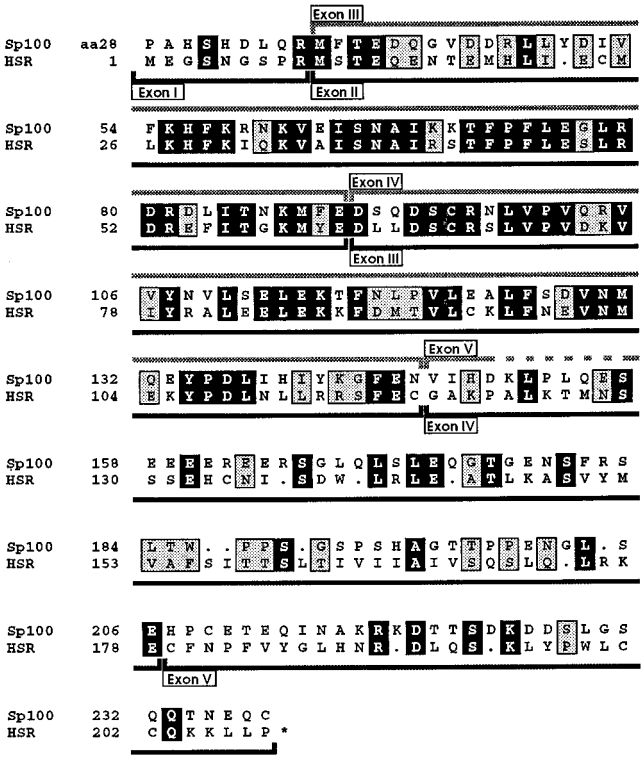

FIG. 1. Protein sequence alignment of Sp100 and the putative HSR-encoded murine protein. Amino acid sequences of the Sp100 protein and that of the murine protein encoded by the 1.3-kb HSR cDNA are shown. Identical and similar amino acids are displayed in black and gray boxes, respectively. Dots denote gaps artificially introduced for optimal alignment. Gray and black bars above and below the sequences indicate exon borders of the Sp100 and HSR genes, respectively. Amino acids were considered similar as specified in reference 7.

Significant but reduced sequence similarity is also observed downstream of exon IV, which ends abruptly at the 3′ border of exon IV of the mouse gene (Fig. 1). Beginning with exon V of the mouse gene, 11 aa of both proteins show no sequence similarity, whereas downstream thereof, significant sequence similarity is seen again. In GenBank, three alternative sequences are given for exon V of the gene family in the HSR. Only two of them contain sequences with homology to exon V and the 3′ untranslated region of the 1.3-kb cDNA (GenBank accession numbers L16457 and L16459; the corresponding protein sequence is aligned with that of Sp100 in Fig. 1). The third genomic sequence, designated alternative exon V (GenBank accession number L16460), obviously is derived from a different copy of the gene family, as it is not present in the 1.3-kb cDNA. Taken together, these data indicate that the HSR cDNA-encoded mouse protein and the Sp100 autoantigen share an evolutionarily highly conserved domain encoded by two adjacent exons, and additional less conserved sequences are located downstream thereof. The striking similarity in the exon-intron organization at the 5′ regions of the corresponding genes suggests that the two genes are related and have evolved from a common ancestral gene. A search for sequences shared by a promoter fragment of the Sp100 gene (12) and by one copy of the gene family (GenBank accession number L16452) revealed 67% sequence identity in a stretch of 105 nucleotides located at a small and identical distance from the translational start codon of the corresponding proteins (Fig. 2B). Included in this region is a sequence element with similarities to an IFN-stimulated response element (ISRE) (18). These data suggest that the two genes also have common transcription regulatory elements.

Localization of the Sp100 gene to human chromosome 2q37. To study whether the sequence similarity between the human Sp100 and mouse HSR-encoded protein is due to synteny of the loci of the corresponding genes, chromosomal localization studies were performed. By in situ hybridization with plasmid p52/1 and chromosome 2-specific satellite DNA as probes, the Sp100 gene was located on the long arm of chromosome 2 (Fig. 3b) and precisely mapped to position 2q37 by G banding (Fig. 3a and b). The mouse HSR was previously mapped to position 5.3 ± 2.1 centimorgans distal to the *Inha* locus and proximal to the *Bcl-2* locus on chromosome 1 (termed *D1Lub1*) and cosegregated with the loci *Acrg*, *Sag*, and *Akp-3* (36). According to previously published comparative mapping data, the region of mouse chromosome 1 containing the inherited HSR is syntenic with the region of human chromosome 2 (23) to which we mapped the Sp100 gene. On the basis of our own as well as published mapping data (11), we conclude that the human Sp100 gene is homologous to the mouse HSR-encoded gene family. Moreover, since we did not find any significant hybridization of Sp100 cDNA with mouse chromosomal DNA in Southern blots under stringent conditions (data not shown),

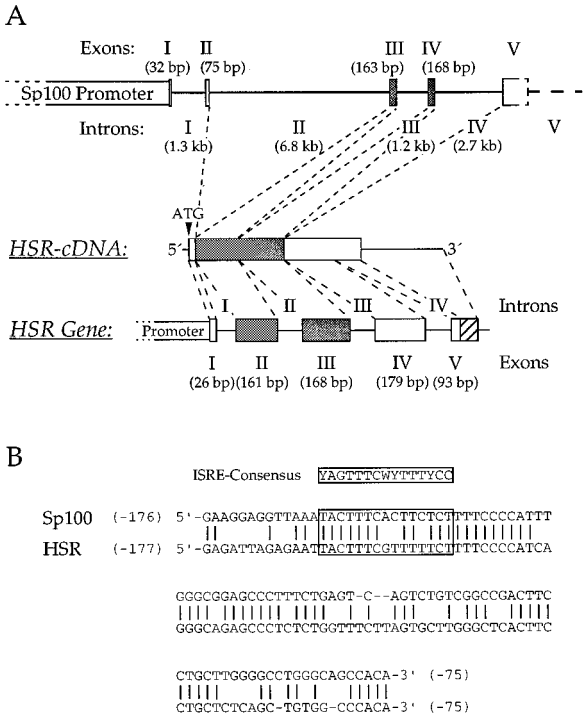

FIG. 2. Schematic presentation of the organization of the Sp100 and HSR genes. (A) Exon-intron boundaries of both genes are depicted relative to the 1.3-kb HSR-derived cDNA. The shaded boxes represent regions with the highest sequence similarity. Exon-intron organization of the HSR gene was taken from reference 39 and from the corresponding sequences deposited in GenBank. The sizes of the HSR gene-specific introns are not drawn to scale, as their lengths were only roughly estimated (39). Sp100 intron sizes were determined by amplification and analysis of the PCR fragments on agarose gels. (B) Sequence alignment of an Sp100 and an HSR promoter fragment. The first nucleotide of the translation start codon of each protein was designated position +1. Between the two sequences (105 bp), homology of 67% was identified. For optimal alignment, only three and two artificial gaps had to be introduced (indicated by dashes) into the Sp100 and HSR promoter sequences, respectively. An ISRE consensus sequence (18) and similar sequences in the Sp100 and HSR promoter regions are boxed.

FIG. 3. Mapping of the Sp100 gene to human chromosome 2q37. (a) G-banded metaphase spreads of a human lymphocyte. The arrow indicates band 2q37, which corresponds to the Sp100 gene position; the arrowhead shows the centromere region of chromosome 2. (b) The same partial metaphase chromosomes to which a 10-kb DNA fragment encompassing the 5′ portion of the Sp100 gene (plasmid p52/1) labeled with biotin-16-dUTP and chromosome 2-specific satellite DNA labeled with digoxigenin were cohybridized. The Sp100 probe was detected with fluorescein isothiocyanate-avidin (arrow), and the satellite DNA was detected with rhodamine-labeled antidigoxigenin antibodies (arrowhead). The chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (blue). Green signals label the terminal portion (2q37) of the two chromatids of the long arm of chromosome 2.

the existence of an additional mouse gene with a high DNA sequence similarity to the Sp100 gene elsewhere in the mouse genome which may have escaped in situ hybridization analysis is excluded.

The Sp100 gene is a single-copy gene. The inherited HSR in mice is polymorphic and contains a gene about 100 kb in size and of variable copy number (between approximately 3 and 1,810 copies). Some of the amplified genes contain rearrangements which have occurred during amplification (39). If amplification is an inherent property of both the mouse and the human genes, the Sp100 gene should also be a variable multicopy gene and show restriction fragment length polymorphisms. To investigate this possibility in detail, the copy number of the Sp100 gene was determined by Southern blotting. For quantitation of the copy number, serial dilutions of *Stu*I-digested plasmid p52/1 corresponding to 125, 25, 5, and 1 gene copy of chromosomal DNA (Fig. 4) were run in parallel with 10 μg of *Stu*I-digested placental genomic DNA of a healthy individual. Hybridization of the blot with a radiolabeled 1.7-kb Sp100-specific *Stu*I promoter fragment contained in plasmid p52/1 and semiquantitative evaluation revealed that the Sp100 protein is encoded in a single-copy gene (Fig. 4B). A similar experiment performed with another *Stu*I fragment comprising exons I and II and intron I of the Sp100 gene corroborated this conclusion (data not shown).

To analyze whether the Sp100 gene copy number differs between PBC- and non-PBC patients and whether gene rearrangements and mutations occur in vivo, genomic DNAs from peripheral lymphocytes of 33 PBC and 55 non-PBC patients

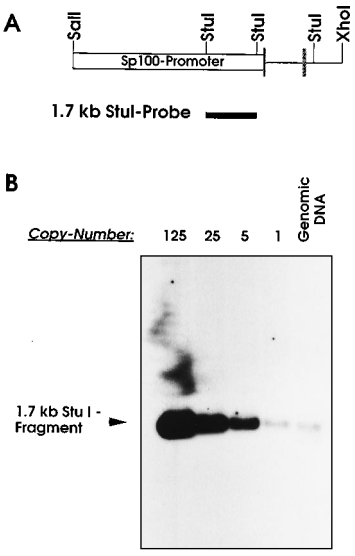

FIG. 4. The Sp100 gene is a single-copy gene. (A) Schematic presentation of plasmid p52/1 (12 kb). A *Sal*I-*Xho*I genomic DNA fragment was cloned into the *Sal*I site of pUC19. Gray bars represent exons I and II of the Sp100 gene. Approximate positions of the *Stu*I sites are depicted. The black bar represents the 1.7-kb *Stu*I DNA fragment used as a probe for the Southern blot in panel B. (B) Gene copy number analysis by Southern blotting. The gel was loaded with 2.34 ng, 468 pg, 93.5 pg, and 18.7 pg of plasmid p52/1 DNA digested with *Stu*I, corresponding to 125, 25, 5, and 1 copy of a 1.7-kb DNA fragment in 10 μg of genomic DNA (lane labeled genomic DNA). To each sample dilution, 10 μg of mouse genomic DNA digested with *Bam*HI was added as a carrier. The blot was hybridized with the ^{32}P-labeled Sp100 DNA promoter fragment isolated from plasmid p52/1 by *Stu*I restriction and gel purification.

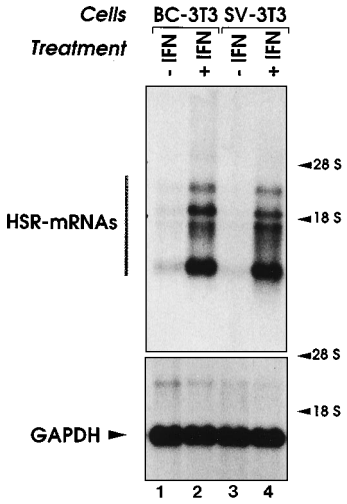

FIG. 5. Northern blot analysis of HSR mRNAs. (A) Normal BALB/c 3T3 cells (BC-3T3) and BALB/c 3T3 cells transformed with SV40 (SV-3T3) were treated for 6 h with mouse IFN-α/β (+ IFN) or left untreated (− IFN). Five micrograms of poly(A)$^+$ RNA was loaded per lane and hybridized with ^{32}P-labeled full-length HSR cDNA. (B) As a control, the blot was rehybridized with a ^{32}P-labeled rat glyceraldehyde-3-phosphate dehydrogenase cDNA (GAPDH) (38).

were analyzed by *Hin*dIII restriction enzyme digestion and Southern blotting, using full-length Sp100 cDNA as a hybridization probe. No differences were detected with any of these samples, as both the intensities of the hybridization signals and the banding patterns of all DNAs from the PBC and non-PBC patients were identical (data not shown). These results indicate that the Sp100 gene is highly conserved and not amplified in the PBC and non-PBC patients studied.

Similar to Sp100, HSR-mRNA levels are greatly increased after IFN treatment. We have previously reported that the steady-state levels of the Sp100 transcript are increased after IFN treatment (13) or viral transformation (29) of human cells. Therefore, we were interested in examining whether the same is true for HSR-derived transcripts. This possibility was analyzed by Northern blot analysis, using RNAs from untransformed and SV40-transformed BALB/c 3T3 cells (9) with or without prior IFN treatment and ^{32}P-labeled mouse HSR-specific cDNA as a probe. Analogously to HSR$^+$ mouse livers (11), several transcripts were observed in untransformed BALB/c 3T3 cells (Fig. 5, lane 1). Transformation of these cells with SV40 did not increase the low levels of these RNAs (Fig. 5, lane 3). However, a drastic increase of the levels of all these transcripts was observed upon IFN treatment, irrespective of transformation (Fig. 5, lanes 2 and 4). Taken together, these data demonstrate that levels of RNAs hybridizing with the 1.3-kb HSR-derived cDNA are increased after IFN treatment of cells whereas they are not upregulated after transformation with SV40. Thus, IFN-mediated elevation of RNA levels is a common biological characteristic of both the human Sp100 gene and the homologous mouse gene.

HSR-derived proteins and nuclear domains in NIH 3T3 mouse cells. The existence of the HSR-derived mouse protein was predicted from the 1.3-kb cDNA but had not been experimentally demonstrated. By expressing in *Escherichia coli* a recombinant protein corresponding to the complete coding region of the 1.3-kb cDNA, we aimed to produce antisera in rabbits and rats for detection of the HSR protein(s) in mouse cells (see Materials and Methods). Despite intensive efforts, we could obtain no high-quality antisera which would have allowed visualization of the HSR protein(s) in immunoblots or by immunostaining of cells with or without prior IFN treatment. Moreover, an attempt to produce antisera against an ovalbumin-conjugated synthetic polypeptide derived from the homology region (see Materials and Methods) for detection of the endogenous HSR-encoded protein(s) was unsuccessful, although high titers of antibodies against the peptide were obtained as detected by enzyme-linked immunosorbent assay. These results suggest that the protein encoded by the HSR-derived 1.3-kb cDNA is either not synthesized or has low immunogenicity or that all antigenic sites are hidden under the conditions tested. Alternatively, the 1.3-kb cDNA cloned may have mutations introduced during cDNA synthesis or gene amplification in vivo or may reflect only a subpopulation of the 1.3-kb mRNAs transcribed from a rare or unique copy of the mutated HSR gene. In both cases, detection of the corresponding protein would be difficult.

To circumvent these problems and to study whether anti-Sp100 antibodies immunologically cross-react with HSR-derived protein(s) in mouse cells, antisera raised against the full-length recombinant Sp100 protein and an N-terminal fragment thereof (aa 1 to 253) containing the homology region were used for immunostaining and immunoblot studies. Surprisingly, also none of these sera showed any significant staining of mouse cells or an immunoreactive protein band on immunoblots with mouse cell proteins (data not shown), although they were strongly positive in similar experiments with human cells. These results suggest that the HSR-derived protein, if expressed at all or at sufficient levels, is not immunologically cross-reactive with anti-Sp100 antibodies produced against recombinant Sp100 proteins.

Since autoimmune sera frequently recognize conformation-dependent epitopes which cannot be produced by immunization with recombinant proteins (34), we also used 20 ND-staining autoimmune sera from PBC patients for detection of HSR-derived protein(s) in mouse NIH 3T3 cells (with or without prior IFN treatment). Of the 20 sera, 16 were anti-Sp100 and anti-PML autoantibody positive, 3 had anti-Sp100 autoantibodies only, and 1 had anti-PML autoantibodies only (28). All sera containing both anti-PML and anti-Sp100 autoantibodies or only anti-PML autoantibodies stained ND structures

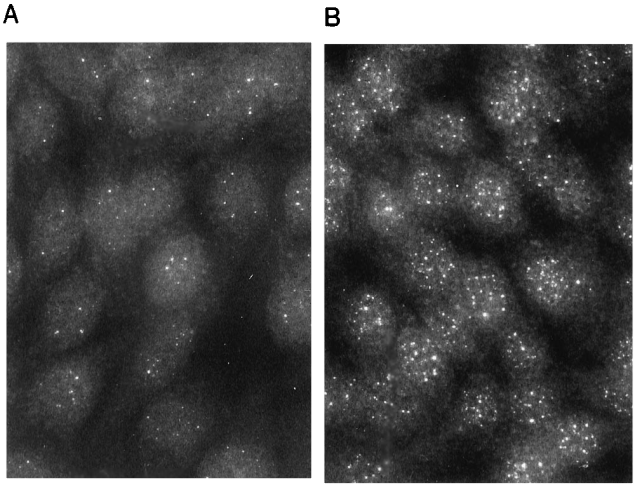

FIG. 6. Immunofluorescence staining of NIH 3T3 cells with a PBC serum containing anti-Sp100 and anti-PML autoantibodies. Cells were left untreated (A) or treated with mouse IFN-α/β for 14 h (B). Both the size and the number of the NDs increase after IFN treatment.

in the NIH 3T3 cells (Fig. 6A) indistinguishable from NDs in nuclei of human cells (13), whereas those with anti-Sp100 autoantibodies alone did not. Furthermore, the ND staining was strongly enhanced (larger and brighter NDs) after pretreatment of the cells with IFN (Fig. 6B). These data indicate that mouse cells contain nuclear domains whose size and number can be enhanced by IFN similarly as described for human cells (13). As none of the human autoimmune sera detected IFN-inducible mouse proteins in immunoblots (data not shown), the ND-associated mouse proteins recognized could not be unequivocally identified.

DISCUSSION

In this study, we presented several lines of evidence which demonstrate that the IFN-responsive human Sp100 gene is homologous to a highly amplified mouse gene (hereafter referred to as mSp100) visible as HSR on chromosome 1 in some mouse strains.

Strong evidence for the homology of the Sp100 and mSp100 genes was provided by their chromosomal mapping to syntenic loci. By Southern blot analysis, the Sp100 protein was found to be encoded by a single-copy gene. Semiquantitative evaluation of the intensity of the signals of the in situ hybridization study was consistent with this analysis. However, somatic amplification of the Sp100 gene occurring in nonlymphocytic cells or in minor subpopulations of lymphocytes or other cells is not excluded by our study. Moreover, amplified Sp100 genes could exist in humans of other ethnic origins not yet analyzed.

The highly variable copy number of the mSp100 gene compared with the bona fide single-copy human Sp100 gene is an intriguing observation. Why this difference between the two genes exists is currently enigmatic. Amplification of the mSp100 gene is associated with increased levels of the corresponding mRNAs (11, 39) and presumably also with enhanced expression of the protein(s). Amplification of the mSp100 gene in mice is probably a result of environmental factors, infection processes, or mutation events as known or supposed for most other expressed amplified genes. In most cases, amplification of genes is associated with enhanced expression of one or several proteins which fulfill important functions in cell proliferation or metabolism of xenobiotic agents. The lack of amplification of the human Sp100 gene could indicate that human cells are not exposed to those factors which trigger amplification of the mSp100 gene in mice. Alternatively, mechanisms other than amplification may allow modulation of human Sp100 gene expression.

Despite the amplification of the mSp100 gene, the levels both of the corresponding mRNAs and of mRNAs from the single-copy Sp100 gene increased to similar extents after IFN treatment. This observation provides further evidence for common biological characteristics of the two genes and raises new questions. Why do the amounts of all mSp100 transcripts rise after IFN treatment of the cells? If it is true that all mRNAs except the 3.0-kb transcript are transcribed only from rearranged genes in HSR^+ mice (as proposed previously [39]), our data indicate that all transcribed mSp100 genes have retained IFN responsiveness. Responsible for this is most likely the potential ISRE identified in the mSp100 gene promoter (Fig. 2B). A similar ISRE present and active in the homologous region of the Sp100 promoter (12) strongly supports this assumption.

So far, only one mRNA, 1.3 kb in length, expressed from the mSp100 gene has been characterized by cDNA cloning and sequencing (11). Interestingly, in mice with a low copy number of the mSp100 gene, a transcript of 3.0 kb is synthesized instead of the 1.3-kb mRNA, and five or six transcripts ranging in size from 4.8 to 1.3 kb are detected in HSR^+ mice (11, 39). The absence of the 1.3-kb mSp100-specific mRNA in HSR^- mice but its presence as even the most dominant species in our cell lines, which are derived from HSR^- BALB/c mice, raises the question of whether the mSp100 gene is as highly amplified in immortalized cells as in HSR^+ mice and whether the 1.3-kb mRNA is expressed from a C-terminally rearranged gene copy. Northern hybridization studies revealed that all mSp100-specific transcripts identified so far, including the 1.3-kb mRNA, have common 5′ sequences and that the larger transcripts contain additional unknown sequences transcribed from the 3′ end of the mSp100 gene family (39). The lack of Northern hybridization signals with mouse RNA, using full-length human Sp100 cDNA as a probe, excludes the existence of long stretches of identical DNA sequences at the 3′ end of the mSp100 gene. When Southern blotting was performed with chromosomal DNA from other animals, the most significant cross-hybridization was observed with DNA from monkeys, less was observed with DNA from pigs, and none was observed with DNA from mice (data not shown). These data indicate a drastic divergence of the Sp100 DNA sequence from sequences of the homologous genes of rodents and other mammals. Furthermore, they suggest a rapid evolutionary change of DNA sequences of the ancestor of the Sp100 gene.

So far, despite intensive efforts, no mSp100 proteins have been identified experimentally. A potential coiled-coil domain in the mSp100 protein region encoded by exon III which corresponds to that of exon IV of human Sp100 is predicted (data not shown and reference 16). Since putative coiled-coil domains were also detected for PML and NDP52 (16, 17), such a domain may be a common feature of all ND-associated proteins and could play a role in protein-protein interaction, structure, and/or cellular localization. Formally, we could not demonstrate whether the mSp100 proteins are ND associated. The ND staining observed with the human autoimmune sera is presumably primarily due to anti-PML autoantibodies. However, autoantibodies cross-reactive with mSp100 proteins which may be present only in PBC sera positive both for anti-PML and anti-Sp100, as well as autoantibodies to other ND-associated antigens, may also cause ND staining. Our demonstration of the existence of ND domains in mouse cells and the potential coiled-coil domain common to all ND proteins suggests that mSp100 proteins are components of these structures, though some may be aberrantly expressed from rearranged genes and not locate in NDs. The isolation of additional cDNAs and antisera against new recombinant mSp100 proteins will provide the tools necessary to characterize the types and locations of the mSp100 proteins expressed as well as the compositions and functions of the mouse nuclear domains.

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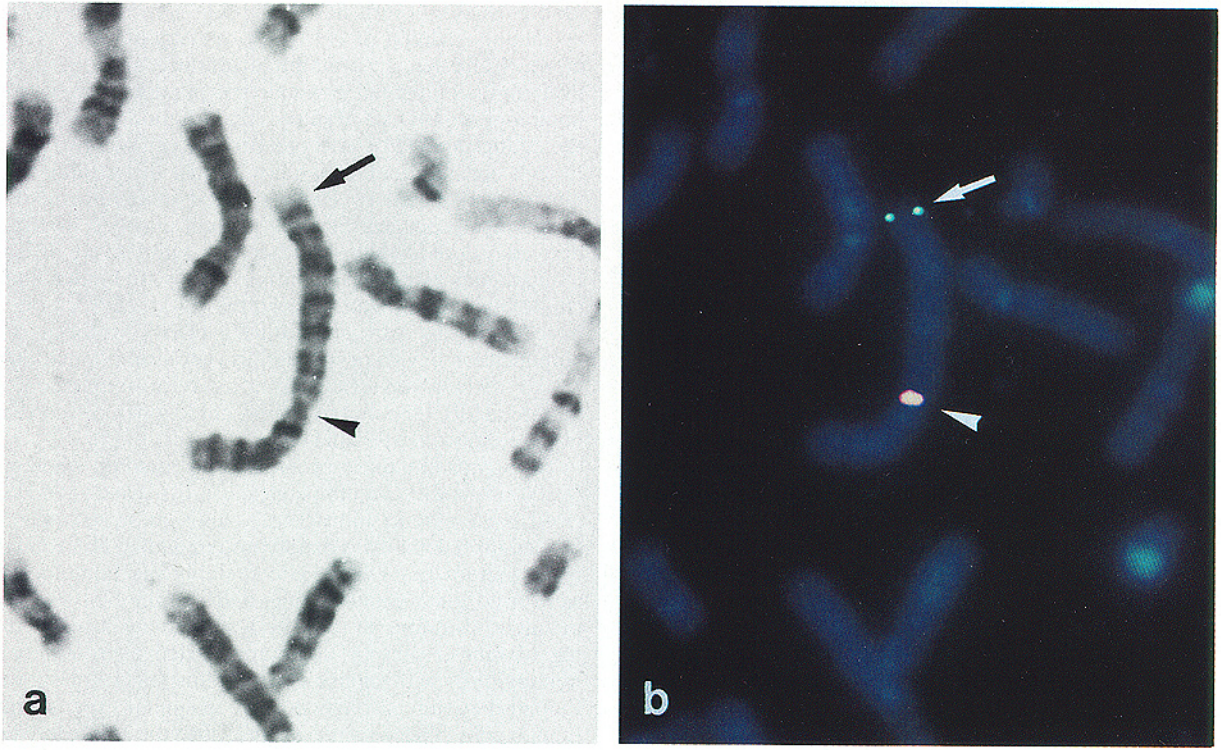
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