Interaction of SP100 with HP1 proteins: A link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment

Jacob-S. Seeler, Agnès Marchio, Delphine Sitterlin, Catherine Transy, and Anne Dejean*

Unité de Recombinaison et Expression Génétique, Institut National de la Santé et de la Recherche Médicale U163, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15

ABSTRACT The PML/SP100 nuclear bodies (NBs) were first described as discrete subnuclear structures containing the SP100 protein. Subsequently, they were shown to contain the PML protein which is part of the oncogenic PML-RARα hybrid produced by the t(15;17) chromosomal translocation characteristic of acute promyelocytic leukemia. Yet, the physiological role of these nuclear bodies remains unknown. Here, we show that SP100 binds to members of the heterochromatin protein 1 (HP1) families of non-histone chromosomal proteins. Further, we demonstrate that a naturally occurring splice variant of SP100, here called SP100-HMG, is a member of the high mobility group-1 (HMG-1) protein family and may thus possess DNA-binding potential. Both HP1 and SP100-HMG concentrate in the PML/SP100 NBs, and overexpression of SP100 leads to enhanced accumulation of endogenous HP1 in these structures. When bound to a promoter, SP100, SP100-HMG and HP1 behave as transcriptional repressors in transfected mammalian cells. These observations present molecular evidence for an association between the PML/SP100 NBs and the chromatin nuclear compartment. They support a model in which the NBs may play a role in certain aspects of chromatin dynamics.

The PML/SP100 nuclear bodies (NBs), also variously referred to as Kr-bodies (1), ND10 (2) or PODs (3), have received much attention because they represent the first example of a subnuclear "organelle" whose integrity is compromised in a human cancer. First described as discrete subnuclear structures containing the SP100 autoantigen (4–6), they were then shown to contain the PML protein that is part of the leukemogenic PML-RARα fusion protein associated with acute promyelocytic leukemia (for review, see refs. 7–9). Strikingly, NB structure is disrupted in acute promyelocytic leukemia cells but is restored by retinoic acid (RA), the ligand for the retinoic acid receptor α (RARα). This restoration correlates with the therapeutic effect of RA in this type of leukemia (1, 3, 10). The normal function of the NBs remains obscure because, unlike other nuclear structures defined by proteins with an identified physiological activity, the function of all presently known components of the NBs is unknown. Besides PML and SP100, these now include the *s*mall *u*biquitin-related *mo*difier SUMO1/PIC1 protein (11–13), as well as the interferon-induced protein ISG-20 (14). Apart from being disaggregated in an RA-reversible manner in acute promyelocytic leukemia cells, a number of additional observed phenomena suggest that the NBs perform critical cellular functions. These structures respond to environmental stimuli such as heat-shock (2), interferons (15–17), and are the specific subnuclear targets for DNA tumor viral early gene products (18–21). The finding that DNA virus replication commences in close proximity to the NBs further suggests that the NBs contain critical factors for the viral replication cycle and by extension, for cellular growth control as well. This finding is further substantiated, in this regard, by the nearly complete disappearance of NBs during cell division (4).

With the aim to further investigate the role of the PML/SP100 NBs, we searched for additional protein components of these structures. Here, we show that SP100 interacts *in vivo* and *in vitro* with members of the HP1 (heterochromatin protein 1) family of non-histone chromosomal proteins. Further, we describe a variant cDNA encoding a SP100 protein that contains a HMG-1-like domain. Our data thus suggest a possible involvement of NB components in the control or maintenance of chromatin or heterochromatin architecture.

MATERIALS AND METHODS

cDNA Constructions. All cDNA constructions were made according to standard methods (22) and verified by sequencing. The SP100-HMG cDNA (GenBank accession no. AF056322) clone was isolated from a cDNA library derived from the ZR-75–1 human breast cancer cell line (a gift from Malcolm Parker, Imperial Cancer Research Fund, London). Mammalian expression constructs were made in vector pSG5 (Stratagene). Influenza hemaglutinin (HA-antigen) fusion constructs were derived from cDNAs first cloned into the yeast shuttle vector pACT2 (CLONTECH). Deletion mutations were created by using convenient restriction sites and/or by PCR with appropriate primer pair combinations and verified by sequencing. cDNAs for *Drosophila* HP1 (dHP1) (23) and human HP1γ (24) were gifts from T. C. James (Trinity College, Dublin, Ireland) and H. Worman (Columbia University, New York), respectively. A cDNA for HP1β/M31 was obtained by reverse transcription–PCR from BALB/c mouse liver mRNA (25). Details of all constructions are available on request.

Yeast Two-Hybrid Screening. A cDNA encoding amino acids 1–480 of SP100 (6), here called SP100A, was fused to LexA in vector pBTM116 (26) and challenged with a human liver-derived cDNA library in vector pACT2 (27) in yeast strain L40 (CLONTECH). Interacting clones were selected by growth on minimal medium lacking leucine, tryptophan, and histidine (−LWH) supplemented with 5 mM 3-aminotriazole.

Biochemistry: Seeler *et al.*

Proc. Natl. Acad. Sci. USA 95 (1998)   7317

After plasmid rescue, putative interacting clones were retested for interactions with a GAL4 DNA-binding domain (DBD) fusion of SP100A (vector pAS2, CLONTECH) and with a GAL4 DBD fusion of an unrelated protein (GAL4 DBD-HBV X) as negative control. LacZ reporter activity was determined as described (27).

***In Vitro* Binding Experiments.** Glutathione *S*-transferase (GST) fusions of SP100A(1–480), NDP52, HP1α(1–191) (WT), HP1α(113–191), and HP1α(1–112) were created by cloning of the appropriate insert into either pGEX2T or pGEX3X (Pharmacia). A cDNA encoding amino acids 233–280 of the murine SP100 (28) was obtained by reverse transcription-PCR of mRNA from BALB/c mouse liver and cloned into pGEX3X. Proteins were expressed in strain BL21(pLysS) grown at 30°C and purified on glutathione Sepharose beads (Pharmacia) by standard methods. ^{35}S-methionine-labeled *in vitro*-translated proteins were prepared by using the T7-TNT coupled reticulocyte lysate system (Promega). Binding experiments ("GST pull-downs") were carried out as described (29).

Transfections and Reporter Assays. HeLa cells were transfected with the indicated reporter and effector plasmids by using the calcium phosphate precipitation method (22). Chloramphenicol acetyltransferase (CAT) assays were performed as described (30), quantified by PhosphorImager analysis (IMAGEQUANT) of thin-layer chromatograms and normalized for transfection efficiency by using pCH110 and measuring β-galactosidase activity. GAL4–DBD fusions were made by using M1, a mammalian expression vector under the control of the SV40 early enhancer (31). The TK reporter, a gift from Norbert Lehming (Max Delbrück Laboratorium, Cologne, Germany), was made by cloning five GAL4 (17 mer) sites into the polylinker of BLCAT2. The β-globin–CAT reporter was a gift from P. Chambon (ICBM, Strasbourg, France). Each experiment was performed at least three times with similar results.

Antibodies and Immunofluorescence Staining. Polyclonal antisera against SP100 and PML were raised in rabbits against GST-fusion proteins. Antisera against HP1α were raised in mice against a GST-HP1α fusion. Sera were incubated with GST-coupled glutathione beads before use to remove anti-GST reactivity. The 12CA5 mAb (Boehringer Mannheim) was used against the HA-tag; however, in Fig. 4*Q*, the HA-tag was detected by using rabbit serum SC802 (Santa Cruz Biotechnology). Immunofluorescence staining of HeLa cells grown on coverslips was carried out as described (18). Confocal laser scanning microscopy was carried with a Leica SM microscope, and acquired digital images were processed by using ADOBE PHOTOSHOP v.3.1 software (San Jose, CA).

RESULTS

SP100 Interacts with Members of the HP1 Family. To gain a better understanding of the possible function(s) of the PML/SP100 NBs, we wished to identify putative cellular interacting partners of the SP100 protein. To this end, we undertook a yeast two-hybrid screen (32) of a human liver-derived cDNA library by using a LexA DBD fusion of the first-cloned SP100, here called SP100A (6), as bait. Two cDNAs encoding members of the HP1 family of non-histone chromosomal proteins were isolated (Fig. 1*A*). The first, encoding HP1α (33), was isolated as a full-length species, whereas the second, HP1γ (24), represented a partial, 5′-truncated cDNA encoding amino acids 36–173 of this protein. Apart from the original HP1 protein, whose cDNA was isolated from *Drosophila* (here called dHP1, ref. 23), the HP1 family also contains a third human homolog, HP1β, that is identical to the murine M31 (or mMOD2) gene product (25). We thus tested M31/HP1β as well as dHP1 for interaction with
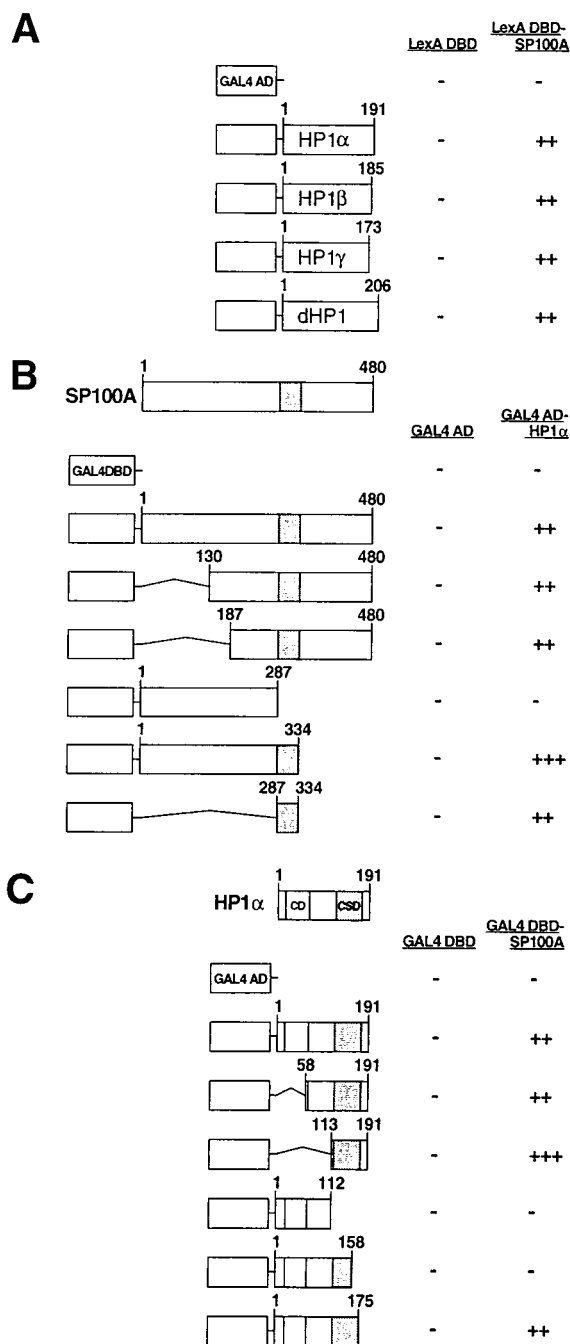

FIG. 1. Yeast two-hybrid interaction experiments. (*A*) A LexA DBD fusion of SP100A interacts with GAL4 AD fusions of human (HP1α, −β, −γ) and *Drosophila* (dHP1) heterochromatin 1 proteins. The indicated expression plasmids were cotransformed into yeast strain L40 and assayed for β-galactosidase reporter activity. (*B*) SP100A and the indicated deletion derivatives, fused to the GAL4 DBD, were expressed in yeast strain Y-190 together with the GAL4 AD (negative control) or a GAL4 AD fusion of HP1α. (*C*) GAL4 AD fusions of HP1α and deletions were transformed into yeast with the GAL4 DBD alone (control) or a GAL4 DBD fusion of SP100A. Interactions were quantified by using β-galactosidase reporter activity, such that "−" corresponds to <1, "+" to 1–10, "++" to 10–100, and "+++" to >100 β-galactosidase units. The deduced interaction domains of each protein are shaded grey. All interactions also were verified against GAL4 DBD or −AD fusions of unrelated proteins (data not shown).

SP100 in the yeast two-hybrid assay and found they interacted as efficiently as HP1α and HP1γ (Fig. 1*A*).

To map the interaction domains of the SP100 and HP1 proteins, we performed a deletion analysis of each protein for

assay in the yeast two-hybrid system. For this analysis, SP100A and its deletion derivatives were fused to the GAL4 DBD and transformed into yeast strain Y190 together with a GAL4-activation domain (GAL4 AD) fusion of HP1α. These experiments revealed a central region of SP100A, between residues 287 and 334, as sufficient for the interaction with HP1α (Fig. 1*B*). Similar experiments carried out with SP100A deletions also revealed the N-terminal 186 aa to be required for SP100-SP100 dimerization (data not shown) and thus suggesting that HP1α-binding and dimerization occur through distinct SP100 protein domains.

To map the SP100-interacting domain of HP1α, the latter and its deletion derivatives were fused to the GAL4 AD and cotransformed with a GAL4 DBD fusion of SP100. A region between residues 113 and 191 of HP1α was found to be sufficient for SP100 interaction (Fig. 1*C*). This same region also has been shown to mediate HP1α-HP1α dimerization (ref. 34 and data not shown). HP1 proteins share two highly conserved globular domains, an amino-terminal chromo domain (CD) and a carboxy-terminal chromo shadow domain (CSD), which span residues 22–66 and 123–175 of HP1α, respectively (35, 36). Thus, our results implicate the region containing the carboxy-terminal CSD, and not the CD, in the interaction with SP100.

A Variant of the NB Component SP100 is an HMG-1-like Protein. The possible significance of an interaction between SP100 and the non-histone chromosomal proteins of the HP1 family was to be unexpectedly underscored by the following results. From the results of Western and Northern blot analyses, it appeared likely that SP100 exists in multiple isoforms (refs. 5 and 37, and data not shown). The first described SP100A cDNA (6) encodes a protein of 480 aa, whereas a second variant, called SP100B, contains additional 3′ sequence, thus encoding a protein of 688 aa (37) (Fig. 2*A*). However, neither of these variants contains significant homologies to other known proteins. In the course of a study aimed at isolating additional SP100 splicing variants that might encode known protein motifs, we have isolated from a cDNA library derived from human breast cancer cells, a cDNA species consisting of sequences encoding amino acids 1–684 of SP100B fused to an HMG-1 (high mobility group 1), homologous peptide. Joining the SP100B portion to the HMG-1-like portion is a short sequence encoding 14 aa (given in **bold** in Fig. 2*B*). This variant cDNA thus encodes a protein of 879 aa, which we named SP100-HMG, with a predicted molecular mass of 104 kDa. The existence of the SP100-HMG mRNA was confirmed by the isolation of several independent cD-

NAs—some of them corresponding to minor splice variants of the largest isolate—as well as by Northern blot and reverse transcription-PCR analyses performed on mRNAs from different cell types. The description of this work will be presented in detail elsewhere (unpublished data). The high degree of conservation between the HMG domain of SP100-HMG, HMG-1, and HMG-2 is shown in the amino acid alignment in Fig. 2*B*. The HMG-1-like domain that spans amino acids 699–879 of the SP100-HMG protein exhibits 87% identity and 93% similarity with human HMG-1 but lacks 24 N-terminal residues, including the first 10 aa of the conserved HMG A-box. Gel retardation analysis with this HMG-1-like domain on cruciform DNA probes (38) confirmed that this molecule retains significant DNA-binding activity (data not shown). Thus, besides the interaction of SP100 with HP1 proteins, the existence of the SP100-HMG variant further supports a potential association of SP100 NB components with chromatin.

SP100 and SP100-HMG Associate with HP1 *in Vitro*. To confirm that the interactions between SP100 and HP1 proteins observed above are not merely specific to yeast cells, we performed a series of GST "pull-down" experiments. In a first set of experiments (Fig. 3*A*, lanes 1–6), bacterially produced GST fusion proteins, immobilized on glutathione beads, of SP100A, HP1α(1–191) full-length, the CSD-containing HP1α(113–191), the CD-containing HP1α(1–112), and the unrelated NDP52 control protein, were incubated with *in vitro*-translated, ³⁵S-methionine-labeled SP100A. In agreement with the yeast two-hybrid experiments, labeled SP100A was retained by beads containing SP100 (lane 3), the full-length (lane 4), and amino-terminally deleted, CSD-containing HP1α proteins (lane 5) but not the carboxy-terminally deleted CD-containing HP1α protein (lane 6) nor the NDP52 control protein (lane 2). The same set of GST fusion proteins incubated with the radiolabeled, variant SP100-HMG protein produced identical results (lanes 7–12), suggesting that the additional C-terminal sequences of SP100-HMG interfere neither with SP100 dimerization nor with HP1α interactions. In a reciprocal experiment (lanes 13–17), we wished to determine whether the previously mapped HP1α interaction domain of SP100, spanning residues 287–334, also functioned *in vitro*. Additionally, we took the opportunity to investigate whether the murine form of SP100, mSP100, could potentially interact with HP1α. For this purpose, the partial murine sequence homologous to the human SP100A(287–334), namely mSP100(233–280) (28), (see amino acid alignment in Fig. 3*B*), was fused in-frame to GST and expressed in bacteria. As seen in Fig. 3*A*, radiolabeled HP1α efficiently bound the
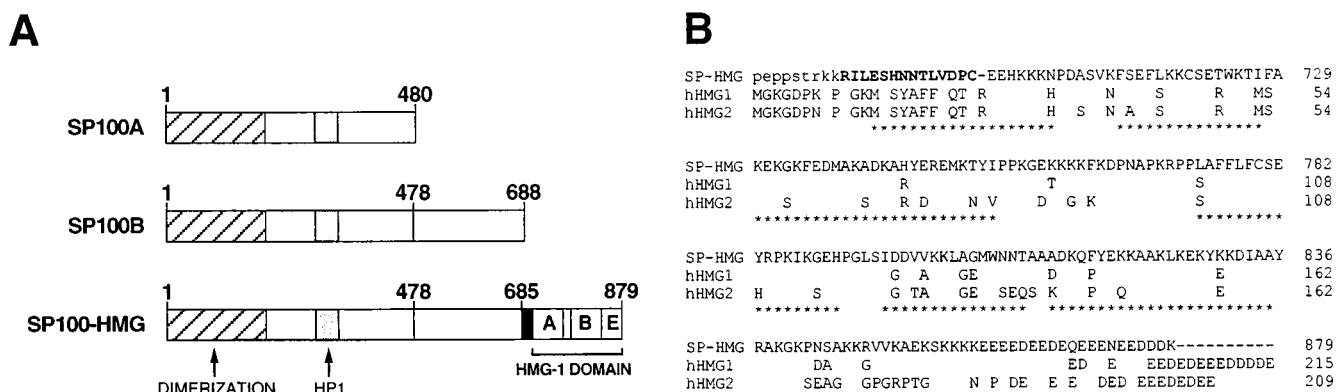
A

B

Fig. 2. (*A*) Schematic diagram of SP100A (GenBank accession no. M60618), SP100B (accession no. U36501), and SP100-HMG (accession no. AF056322) proteins discussed in this study. The dimerization and HP1-binding domains as well as the position of the HMG-1-like domain containing the conserved A and B boxes and acidic domains (E) are indicated. (*B*) Amino acid alignment of the HMG-1 domain of SP100-HMG with the two human homologs, HMG-1 and HMG-2. Only residues differing from SP100-HMG are given. Sequence in lower case corresponds to SP100B-derived residues; sequence in bold is neither SP100B-derived nor homologous to HMG-1. Asterisks indicate the extent of conserved α-helices in the HMG-1 and -2 proteins.
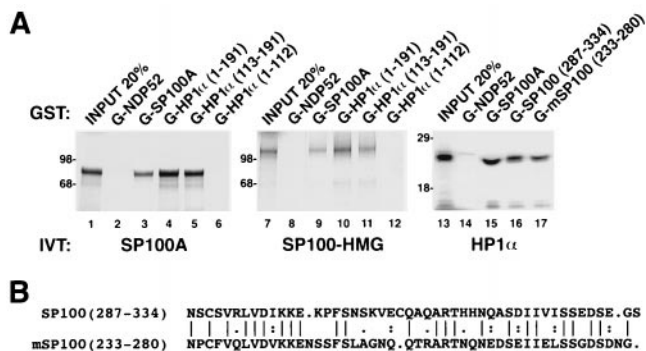
FIG. 3. SP100 homodimerizes and interacts with HP1α *in vitro.* (*A*) The indicated GST fusion proteins coupled to glutathione Sepharose beads were incubated with ³⁵S-labeled *in vitro* translated SP100A (lanes 2–6), SP100-HMG (lanes 8–12), and HP1α (lanes 14–17) and washed, eluted, and subjected to electropheresis and autoradiography to reveal the bound radiolabeled proteins. Twenty percent of the input for each radiolabeled protein is shown in lanes 1, 7, and 13. (*B*) Amino acid alignment of the human [SP100(287–334)] and murine [mSP100(233–280)] HP1-binding domains of SP100. Identical residues are joined by lines, conservative substitutions by dots, and periods indicate alignment gaps.

human full-length SP100A (lane 15) and SP100(287–334) (lane 16) as well as the murine mSP100(233–280) (lane 17) but not the NDP52 control protein (lane 14). These results suggest the existence of direct physical interactions between SP100 proteins and the CSD of HP1α. They further suggest that SP100–HP1 interactions are not restricted to primates.

SP100-HMG and HP1 Colocalize with SP100 in the NBs. We then wished to determine whether the above observed protein–protein interactions correspond to visible subnuclear complexes. Because SP100A concentrates in specific NBs (1–6), we investigated first whether its variant form, SP100-HMG, and then the HP1 proteins might similarly be associated with these subnuclear structures. For this purpose, HA antigen-tagged fusion constructs of SP100-HMG as well as HP1α and its CD- and CSD-deletion derivatives were transfected into HeLa cells. 12CA5 mABs were used to detect the transfected proteins whereas rabbit antisera against recombinant SP100 or PML were used as markers for the NBs.

As shown in Fig. 4*A–C*, overexpressed SP100-HMG is targeted to the NBs, which are revealed here by staining with an anti-PML antiserum. The targeting of SP100-HMG thus was found to be indistinguishable from that of SP100A (data not shown), suggesting that the N-terminal, SP100A-specific, rather than the SP100B- and/or HMG-1-like portions of the protein, contain the targeting determinant(s).

Coimmunofluorescence staining of cells transfected with HA-tagged HP1α by using anti-HA and anti-SP100 antibodies revealed a specific accumulation of this protein in the NBs, in addition to a nucleoplasmic diffuse signal (Fig. 4, *D–F*). Transfection of HA-tagged HP1β/M31, HP1γ, and dHP1 produced similar staining patterns (data not shown). We also found that an HP1α protein deleted for the CD (HP1α 58–191) retained the ability to target the NBs (Fig. 4, *G–I*). In contrast, partial truncation of the CSD (HP1α 1–158) abolished the speckled pattern and rendered HP1α entirely nucleoplasmic diffuse (Fig. 4, *J–L*). These data thus extend our previous findings of the interactions between SP100 and HP1 proteins and substantiate the importance of the HP1 CSD for the formation of a SP100/HP1 complex *in vivo.*

To next visualize the endogenous HP1α protein, we raised a murine antiserum against bacterially expressed GST-HP1α. The reactivity of this serum against HP1α was verified in Western blot and immunoprecipitation experiments by using both HeLa cell extracts and *in vitro*-synthesized HP1α protein
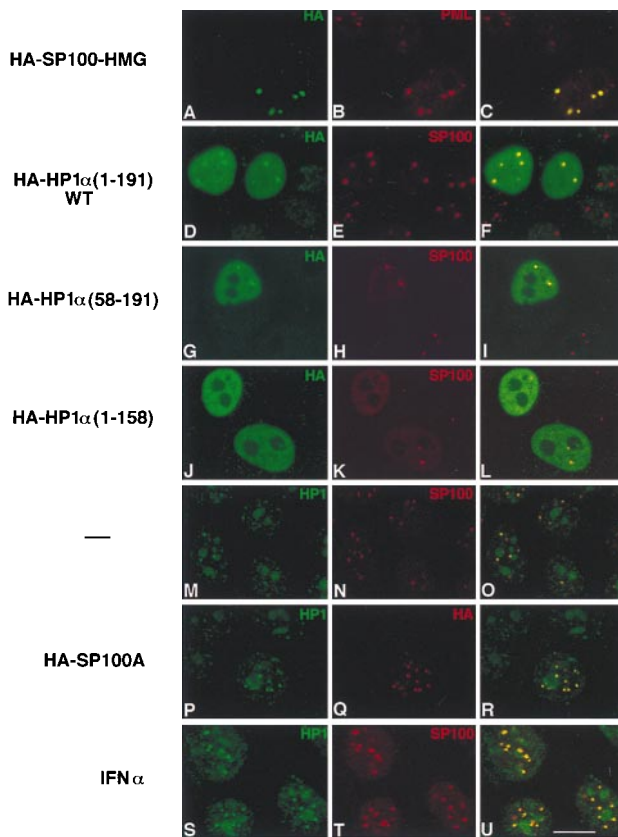
FIG. 4. SP100-HMG and HP1α are targeted to the PML/SP100 NBs. HeLa cells were transfected with plasmid constructs expressing HA-tagged SP100-HMG (*A–C*), HP1α(1–191) (*D–F*), the CSD-containing HP1α(58–191) (*G–I*), and the CD-containing HP1α(1–158) (*J–L*) or SP100A (*P–R*) proteins, or were left untransfected (*M–O*), or were treated with 200 units/ml interferonα for 16 hr (*S–U*), as indicated. Cells in *A–L* were permeabilized with TritonX-100 after fixation with formalthyde, whereas cells in *M–U* were briefly extracted with TritonX-100 before formalthyde fixation to remove excess soluble proteins. The overexpressed proteins were revealed with antibodies against the HA tag (HA; 12CA5 mAb in *A, D, G,* and *J* or the SC802 rabbit polyclonal serum in *Q*). Native NB components were visualized with rabbit sera against PML (*B*) or SP100 (*E, H, K, N,* and *T*). A murine polyclonal serum ("HP1" in *M, P,* and *S*) was used to stain endogenous HP1α. The secondary antibodies used were conjugated to fluorescein isothiocyanate- (*Left*, green) or to TexasRed (*Center*, red). Confocal overlay of red and green panels yields yellow in the *Right* panels (*C, F, I, L, O, R,* and *U*). (Scale bar, in *U,* = 10 μm.)

(data not shown). When HeLa cells were first fixed with paraformaldehyde and then permeabilized with Triton X-100 (Sigma) coimmunofluorescence staining with anti-HP1α and anti-SP100 sera showed the distribution of HP1α to be nuclear diffuse, with some accumulation in the nucleoli; only a small proportion of cells revealed a significant association of HP1 signal with the SP100 NB signal (not shown). However, when cells were extracted briefly in a Triton X-100-containing buffer before fixation to remove soluble proteins (18), the visibility of colocalization between endogenous HP1α and SP100 proteins in NBs was greatly enhanced (Fig. 4, *M–O*). In cells transiently transfected with SP100A, an even stronger NB-specific HP1α signal was obtained (Fig. 4, *P–R*). Transfection of a SP100-HMG expression vector similarly led to the enhanced accumulation of endogenous HP1α in the NBs (not shown). Enhanced HP1α staining in the NBs also was obtained when SP100 synthesis was induced by interferon (Fig. 4, *S–U*), showing that increased NB size and number is correlated with increased HP1 signal (cf. Fig. 4, *A–C* with *G–I*). These results indicate that a detectable portion of HP1α protein is readily
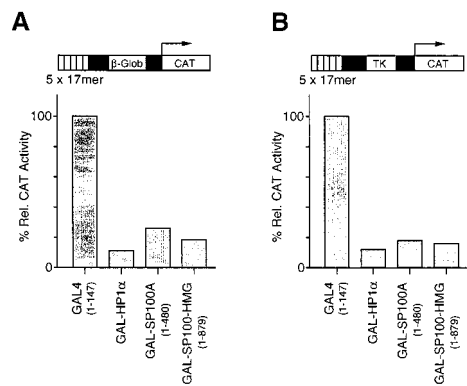
FIG. 5.   HP1α, SP100A, and SP100-HMG behave as transcriptional repressors when tethered to DNA. The GAL4 DBD alone, or GAL4 DBD fusions of HP1α, SP100A, or SP100-HMG were transfected into HeLa cells together with a plasmid containing five GAL4-binding sites fused to the β-globin (*A*) or the thymidylate kinase (*B*) minimal promoters upstream of the CAT reporter gene. CAT reporter activity was normalized for transfection efficiency to an internal β-galactosidase control and expressed as a percentage of the activity obtained with the GAL4 DBD alone. Transfections were carried out with 3 μg each of reporter and effector plasmids.

present in the NBs and that enhancement of SP100 synthesis, either by transfection or by interferon induction, results in an increased accumulation of HP1 in these structures.

Trancriptional Repression by SP100 and HP1 Proteins. Our finding, first, of an association between SP100 proteins with HP1 non-histone chromosomal proteins, and second, of the possibly DNA binding SP100-HMG variant, suggested a transcriptional effect of these proteins. Further, the involvement of dHP1 in position effect variegation in *Drosophila*, likely via heterochromatin-mediated silencing mechanisms, suggested a transcriptional repressing activity (39). Indeed, one of the hallmarks of heterochromatin is that it constitutes a transcriptionally repressive environment. Thus, we wished to establish whether the SP100 and HP1 proteins in this study could potentially act as repressors. To this end, we prepared fusions of HP1α, SP100A, and SP100-HMG with the GAL4 DBD for transfection into HeLa cells together with a CAT reporter plasmid bearing five GAL4-binding sites upstream of either the TK or the β-globin minimal promoter. As seen in Fig. 5, expression of all three proteins as GAL4 DBD fusions resulted in a significant, eight- to twentyfold, decrease in the activity from both the β-globin (Fig. 5*A*) and the TK (Fig. 5*B*) reporters, compared with that obtained with the GAL4 DBD alone. In no case, was repression observed in the absence of GAL4 sites in the reporter plasmids (data not shown). A transcriptional repressing effect of HP1 has been noted (40); we suggest here that SP100A and SP100-HMG proteins also can function as repressors when tethered to DNA.

DISCUSSION

In the present work, we have shown that the NB-associated SP100 protein complexes with members of the HP1 family of non-histone chromosomal proteins, both *in vitro* and *in vivo*, and that these complexes are seen to concentrate in the PML/SP100 NBs. Our results are extended by the discovery of a SP100 splice variant, SP100-HMG, characterized by the presence of potential DNA-binding domains, the HMG boxes. These findings raise the interesting possibility that the PML/SP100 NBs may affect chromatin structure.

HP1 proteins are believed to represent an important structural component of heterochromatin, a suggestion supported by their involvement in position effect variegation (reviewed in refs. 41–43) as well in the proper functioning of the centromere

during mitosis (44). HP1 proteins are targeted by several other cellular proteins, including the lamin B receptor (24), the transcriptional cofactors TIF1α and -β (40), as well as the origin recognition complex, ORC (45). Interactions of HP1 with SWI/SNF complex member BRG-1 and with a RAD-54-like protein called HP-BP-38 also have been reported (40). Although the precise functional significance of these interactions remains to be determined, these data nonetheless suggest that HP1 proteins might represent important targets for cellular regulators of transcription, DNA replication or repair.

The existence of the variant SP100-HMG protein suggests the possibility of a direct association of a NB protein with chromatin by DNA binding through the HMG box motifs. These motifs are found in the canonical HMG-1 and -2 non-histone chromosomal proteins, and mediate nonsequence-specific DNA binding with a marked preference for bent, single-stranded or cruciform, i.e., non-B DNA structures (reviewed in refs. 46–48). In addition, several transcription factors, such as UBF (49), LEF-1 (50), and SRY (51), also contain HMG box motifs. Interestingly, these proteins exhibit both sequence-specific and nonspecific interactions with DNA, mediated by the HMG box motifs. It thus would be interesting to know whether SP100-HMG also might be capable of sequence-specific DNA binding and hence possibly represent a bona fide transcription factor.

A more indirect association of SP100 proteins with chromatin also might be provided by interactions with HP1. Alternatively, or in addition, HP1 may be recruited to the NBs, i.e., away from chromatin. Indeed, our data suggest that enhanced SP100 expression correlates with increased accumulation of HP1 in the NBs. However, the quantity of HP1 in the NBs, as well as the amount necessary to influence chromatin structure by HP1 depletion, remains to be established. The investigation of the possible influence of SP100 proteins on gene expression via chromatin structural mechanisms, such as position effect variagation, is in progress.

The observed transcriptional repressing effect of the DNA-bound SP100 and HP1 proteins could be interpreted as a possible consequence of the involvement of these proteins in the establishment or stabilization of a repressive, heterochromatin-like structure. The presence of homo- and heteromeric interaction interfaces in both SP100 and HP1 may facilitate the formation of large protein complexes that could impede the access of the transcriptional machinery to the DNA template.

Taken together, our results provide an important foundation for the further investigation of the physiological role of the NBs and their relationship with processes surrounding cell growth, differentiation, and neoplasia.

1. Weis, K., Rambaud, S., Lavau, C., Jansen, J., Carvalho, T., Carmo-Fonseca, M., Lamond, A. & Dejean, A. (1994) *Cell* **76,** 345–356.
2. Maul, G. G., Yu, E., Ishov, A. M. & Epstein, A. L. (1995) *J. Cell. Biochem.* **59,** 498–513.
3. Dyck, J. A., Maul, G. G., Miller, W. H., Jr., Chen, J. D., Kakizuka, A. & Evans, R. M. (1994) *Cell* **76,** 333–343.
4. Ascoli, C. A. & Maul, G. G. (1991) *J. Cell Biol.* **112,** 785–795.
5. Xie, K., Lambie, E. J. & Snyder, M. (1993) *Mol. Cell Biol.* **13,** 6170–6179.

6. Szostecki, C., Guldner, H. H., Netter, H. J. & Will, H. (1990) *J. Immunol.* **145,** 4338–4347.
7. Grignani, F., Fagioli, M., Alcalay, M., Longo, L., Pandolfi, P. P., Donti, E., Biondi, A., Lo Coco, F., Grignani, F. & Pelicci, P. G. (1994) *Blood* **83,** 10–25.
8. Lavau, C. & Dejean, A. (1994) *Leukemia* **8,** 1615–1621.
9. Warrell, R. P., de Thé, H., Wang, Z.-Y. & Degos, L. (1993) *N. Engl. J. Med.* **329,** 177–189.
10. Koken, M. H., Puvion-Dutilleul, F., Guillemin, M. C., Viron, A., Linares-Cruz, G., Stuurman, N., de Jong, L., Szostecki, C., Calvo, F., Chomienne, C., *et al.* (1994) *EMBO J.* **13,** 1073–1083.
11. Boddy, M. N., Howe, K., Etkin, L. D., Solomon, E. & Freemont, P. S. (1996) *Oncogene* **13,** 971–982.
12. Sternsdorf, T., Jensen, K. & Will, H. (1997) *J. Cell Biol.* **139,** 1621–1634.
13. Müller, S., Matunis, M. J. & Dejean, A. (1998) *EMBO J.* **17,** 61–70.
14. Gongora, C., David, G., Pintard, L., Tissot, C., Hua, T. D., Dejean, A. & Mechti, N. (1997) *J. Biol. Chem.* **272,** 19457–19463.
15. Guldner, H. H., Szostecki, C., Grötzinger, T. & Will, H. (1992) *J. Immunol.* **149,** 4067–4073.
16. Lavau, C., Marchio, A., Fagioli, M., Jansen, J., Falini, B., Lebon, P., Grosveld, F., Pandolfi, P. P., Pelicci, P. G. & Dejean, A. (1995) *Oncogene* **11,** 871–876.
17. Stadler, M., Chelbi-Alix, M. K., Koken, M. H., Venturini, L., Lee, C., Saib, A., Quignon, F., Pelicano, L., Guillemin, M. C., Schindler, C. & de The, H. (1995) *Oncogene* **11,** 2565–2573.
18. Carvalho, T., Seeler, J. S., Öhman, K., Jordan, P., Pettersson, U., Akusjärvi, G., Carmo-Fonseca, M. & Dejean, A. (1995) *J. Cell Biol.* **131,** 45–56.
19. Doucas, V., Ishov, A. M., Romo, A., Juguilon, H., Weitzman, M. D., Evans, R. M. & Maul, G. G. (1996) *Genes Dev.* **10,** 196–207.
20. Everett, R. D. & Maul, G. G. (1994) *EMBO J.* **13,** 5062–5069.
21. Korioth, F., Maul, G. G., Plachter, B., Stamminger, T. & Frey, J. (1996) *Exp. Cell Res.* **229,** 155–158.
22. Ausubel, F., R. Brent, R. E. Kingston, D. D. More, J. G. Seidman, J. A. Smith, K. Struhl (1995) *Current Protocols in Molecular Biology* (Wiley, New York), 3rd Ed.
23. James, T. C., Eissenberg, J. C., Craig, C., Dietrich, V., Hobson, A. & Elgin, S. C. (1989) *Eur. J. Cell Biol.* **50,** 170–180.
24. Ye, Q. & Worman, H. J. (1996) *J. Biol. Chem.* **271,** 14653–14656.
25. Singh, P. B., Miller, J. R., Pearce, J., Kothary, R., Burton, R. D., Paro, R., James, T. C. & Gaunt, S. J. (1991) *Nucleic Acids Res.* **19,** 789–794.
26. Hollenberg, S. M., Sternglanz, R., Cheng, P. F. & Weintraub, H. (1995) *Mol. Cell. Biol.* **15,** 3813–3822.
27. Sitterlin, D., Tiollais, P. & Transy, C. (1997) *Oncogene* **14,** 1067–1074.
28. Weichenhan, D., Kunze, B., Zacker, S., Traut, W. & Winking, H. (1997) *Genomics* **43,** 298–306.
29. David, G., Alland, L., Hong, S.-H., Wong, C.-W., DePinho, R. A. & Dejean, A. (1998) *Oncogene*, in press.
30. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
31. Sadowski, I., Bell, B., Broad, P. & Hollis, M. (1992) *Gene* **118,** 137–141.
32. Fields, S. & Song, O. (1988) *Nature (London)* **340,** 245–247.
33. Saunders, W. S., Chue, C., Goebl, M., Craig, C., Clark, R. F., Powers, J. A., Eissenberg, J. C., Elgin, S. C., Rothfield, N. F. & Earnshaw, W. C. (1993) *J. Cell Sci.* **104,** 573–582.
34. Ye, Q., Callebaut, I., Pezhman, A., Courvalin, J. C. & Worman, H. J. (1997) *J. Biol. Chem.* **272,** 14983–14989.
35. Aasland, R. & Stewart, A. F. (1995b) *Nucleic Acids Res.* **23,** 3163–3173.
36. Koonin, E. V., Zhou, S. & Lucchesi, J. C. (1995) *Nucleic Acids Res.* **23,** 4229–4233.
37. Dent, A. L., Yewdell, J., Puvion-Dutilleul, F., Koken, M. H., de The, H. & Staudt, L. M. (1996) *Blood* **88,** 1423–1426.
38. Bianchi, M. (1988) *EMBO J.* **7,** 843–849.
39. Paro, R. (1990) *Trends Genet.* **6,** 416–421.
40. Le Douarin, B., Nielsen, A. L., Garnier, J. M., Ichinose, H., Jeanmougin, F., Losson, R. & Chambon, P. (1996) *EMBO J.* **15,** 6701–6715.
41. Grigliatti, T. (1991) in *Functional Organization of the Nucleus*, eds. Hamkalo, B. A. & Elgin, S. C. R. (Academic, San Diego), pp. 588–628.
42. Elgin, S. C. (1996) *Curr. Opin. Genet. Dev.* **6,** 193–202.
43. Singh, P. B. (1994) *J. Cell Sci.* **107,** 2653–2668.
44. Kellum, R. & Alberts, B. M. (1995) *J. Cell Sci.* **108,** 1419–1431.
45. Pak, D. T., Pflumm, M., Chesnokov, I., Huang, D. W., Kellum, R., Marr, J., Romanowski, P. & Botchan, M. R. (1997) *Cell* **91,** 311–323.
46. Landsman, D. & Bustin, M. (1993) *BioEssays* **15,** 539–546.
47. Laudet, V., Stehelin, D. & Clevers, H. (1993) *Nucleic Acids Res.* **21,** 2493–2501.
48. Baxevanis, A. D. & Landsman, D. (1995) *Nucleic Acids Res.* **23,** 1604–1613.
49. Jantzen, H. M., Admon, A., Bell, S. P. & Tjian, R. (1990) *Nature (London)* **344,** 830–836.
50. Travis, A., Amsterdam, A., Belanger, C. & Grosschedl, R. (1991) *Genes Dev.* **5,** 880–894.
51. Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Münsterberg, A., Vivian, N., Goodfellow, P. & Lovell-Badge, R. (1990) *Nature (London)* **346,** 245–250.