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Scaffold/Matrix Attachment Region Elements Interact with a p300-Scaffold Attachment Factor A Complex and Are Bound by Acetylated Nucleosomes

Joost H. A. Martens, Matty Verlaan, Eric Kalkhoven, Josephine C. Dorsman, and Alt Zantema*

Molecular Cell Biology, Centre for Biomedical Genetics, Leiden University Medical Center, Leiden, The Netherlands

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The transcriptional coactivator p300 regulates transcription by binding to proteins involved in transcription and by acetylating histones and other proteins. These transcriptional effects are mainly at promoter and enhancer elements. Regulation of transcription also occurs through scaffold/matrix attachment regions (S/MARs), the chromatin regions that bind the nuclear matrix. Here we show that p300 binds to the S/MAR binding protein scaffold attachment factor A (SAF-A), a major constituent of the nuclear matrix. Using chromatin immunoprecipitations, we established that both p300 and SAF-A bind to S/MAR elements in the transiently silent topoisomerase I gene prior to its activation at G_1 during cell cycle. This binding is accompanied by local acetylation of nucleosomes, suggesting that p300–SAF-A interactions at S/MAR elements of nontranscribed genes might poise these genes for transcription.

Activation of gene expression is thought to be a multistep process involving changes in chromatin structure, as well as site-specific events such as the acetylation of histones (20). An important factor in higher-order organization is the nuclear matrix, a proteinaceous structure that consists of a network of RNPs and other nonhistone proteins and that serves as a scaffold for loops of chromatin (reviewed in reference 5). This matrix has been associated with the regulation of transcription, DNA replication, and RNA processing (44). The DNA regions anchoring the chromosomal DNA to the nuclear matrix (called matrix-associated regions [MARs] or scaffold-attachment regions [SARs] [8]) are composed of AT-rich sequences. They have a unique structure characterized by a narrow minor groove, a high unwinding potential and the formation of hairpin structures (5). S/MARs are located in the introns of several large genes (32, 53) and also at borders of transcription units (23, 47). S/MAR elements have often been implicated in the regulation of gene expression. They are frequently found close to enhancers (37, 48), and they can stimulate gene expression of heterologous reporter genes when integrated into the genome (56) and can regulate chromatin accessibility (21, 30).

Several S/MAR binding proteins have been identified and characterized over recent years. One of the first was the scaffold attachment factor-A (SAF-A), initially identified as a component of RNP particles (35) but later also characterized as a S/MAR-binding protein (17, 18, 24, 36, 52). Other S/MAR binding proteins include ubiquitous, abundant proteins such as SAF-B (50), topoisomerase II (1), histone H1 (28), lamin B1 (42), HMGI/Y (66), and nucleolin (11), but also proteins that are expressed in a more cell-type-specific fashion, such as SatB1 (10) and p114 (63). Even though many of these proteins have been thoroughly characterized biochemically, it is still relatively unclear how they are involved in the gene regulating processes of S/MAR elements.

In recent years much progress has been made in the characterization of the events at the site of transcription initiation. Of the factors involved the transcriptional coactivators p300/CBP were originally described as interaction modules that form a bridge between the basal transcription machinery and upstream transcription factors (25). CBP was cloned by virtue of its binding to CREB (7) and p300 as an adenoviral-E1A associating protein (15). Viral oncogenes such as the adenovirus E1A and simian virus 40 (SV40) large T can interact with p300/CBP and disrupt the interaction of p300/CBP with other factors. The homologous proteins p300 and CBP contain three cysteine-histidine (CH)-rich regions, of which CH3 constitutes the major interaction site for adenovirus E1A. An additional role of p300/CBP became clear when it was found that p300/CBP have intrinsic acetyltransferase activity (4, 45) and associate with other proteins with acetyltransferase activity such as p/CAF (64). The acetylation of histone tails is generally related to transcription activation (57). In addition, acetylation of transcription factors by p300/CBP can lead to either activation or repression of transcription (55).

Here we report a strong interaction between p300 and SAF-A. We show that p300 and SAF-A bind to S/MAR elements and that their binding is disrupted when the viral protein E1A or SV40 large T is present. The binding of p300 and SAF-A to S/MAR elements seems to be restricted to the transcriptional inactive state. However, while no transcription occurs, these S/MAR elements are bound by acetylated histones, suggesting that the presence of p300 at S/MAR elements might realize a localized chromatin state ready for transcription.

MATERIALS AND METHODS

Yeast two-hybrid assay. Yeast two-hybrid screening was performed as described previously (3) with a random-primed human cDNA testis library fused to a VP16 transactivating domain by using the vector pVP16 (61) and the yeast strain Y190 (59). The bait plasmid pMD4-p300 (amino acids [aa] 1570 to 1848) was made by cloning an 834-bp p300 cDNA fragment into pMD4. pMD4 was a

* Corresponding author. Mailing address: MCB, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands. Phone: 31(0)71-5276113. Fax: 31(0)71-5276284. E-mail: zantema@lumc.nl.

gift from M. van Dijk and R. Bernards. Y190, which contains LacZ and HIS3 reporter genes, was transformed with plasmid pMD4-p300 and the pVP16 cDNA library. In the screen 5×10^6 transformants were tested, and the HIS^+ positive colonies were subsequently tested for β-galactosidase activity by colony filter assay. Of the double-positive clones (HIS^+, $LacZ^+$) one contained the coding sequence for aa 633 to 806 of SAF-A.

Cell lines and cell fractionation. The cell lines used are the human osteosarcoma cell line U-2 OS, the adenovirus type 5 E1-transformed human embryonic retinoblastoma (HER) cell line 911 (19), human glioblastoma cell line T98G (54), human diploid foreskin fibroblasts VH10, and SV40 early region transformed VH10 cells (38). Cell fractionation of U-2 OS cells was performed essentially as previously described (17, 22). Briefly, cells were washed with phosphate-buffered saline and resuspended in cytoskeleton (CSK) buffer {100 mM NaCl, 300 mM sucrose, 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] [pH 6.8], 3 mM $MgCl_2$, 1 mM EGTA, and 0.5% Triton X-100 containing phenylmethylsulfonyl fluoride [Sigma] and RNasin [Promega]} and incubated at 4°C for 3 min. Skeletal frameworks were pelleted by centrifugation at $650 \times g$ for 5 min, and the supernatant was removed. After an additional wash with CSK buffer, the skeletal framework was treated with RNase A (Roche Diagnostics) for 20 min at room temperature in a CSK buffer with lower NaCl (50 mM) and no RNasin. Digestion was terminated by the addition of ammonium sulfate to a final concentration of 0.25 M. The remaining nuclear skeleton was pelleted at $1{,}000 \times g$ for 5 min while the RNA-bound fraction stayed in solution. The chromatin-bound fraction was released from the CSK-washed skeletal framework by sonication. After three sonications in a Branson sonifier 250 at 15% output for 20 s, ammonium sulfate was added to a final concentration of 0.15 M, and the remaining nuclear skeleton was pelleted at $1{,}000 \times g$ for 5 min while the supernatant contained the chromatin-bound fraction.

Antibodies and immunoprecipitation assays. The p300 rabbit polyclonal antibodies 1 and 3 were raised against aa 91 to 328 and 1834 to 2049, respectively, and were as previously described (14). A rabbit polyclonal serum against SAF-A was raised against a purified, bacterially produced His-tagged protein containing aa 1 to 243 of SAF-A. The 3G6 mouse monoclonal antibody against SAF-A and the 4D11 mouse monoclonal antibody against hnRNP L were gifts from G. Dreyfuss (13, 49), and the mouse monoclonal antibody M73 against adenovirus type 5 E1A was a gift from E. Harlow (27). N15 rabbit polyclonal serum against p300, C21 antibody against $p90^{rsk1}$, and the C19 antibody against ATF-2 were purchased from Santa Cruz. MN11 is a mouse monoclonal antibody against p300/CBP (9) and was purchased from PharMingen. Rabbit polyclonal antibody antiacetylated histone H3, raised against the N-terminal peptide acetylated at lysine 9 and lysine 14, was purchased from Upstate Biotechnology (catalog number 06-599). The antihistone polyclonal antiserum raised against purified *Drosophila* core histones was a gift from G. E. Chalkley and C. P. Verrijzer (33). Immunoprecipitations were performed as described before (26) in assay buffer (0.1% NP-40–250 mM NaCl–50 mM Tris-HCl [pH 7.5] containing a mixture of protease inhibitors [34]).

DNA constructs. Glutathione *S*-transferase (GST)–p300ADA2, GST-p300CH3, and GST-p300BAIT fusion genes were constructed by cloning digested p300 PCR fragments into pGEX-2T (Pharmacia). GST-E1A is a fusion protein of GST and aa 1 to 90 of adenovirus type 5 E1A (34). The pGem4-U21.1 plasmid with full-length SAF-A (35) was a generous gift of G. Dreyfuss. GST–SAF-A was generated by cloning a SAF-A PCR fragment encoding aa 537 to 806 into pGEX-2T.

GST pull-down analysis. GST fusion protein-coated beads and in vitro-translated proteins were prepared as previously reported (12). In vitro-translated [^{35}S]methionine-labeled proteins were made by a coupled transcription-translation kit (TNT; Promega). For the E1A protein, a plasmid with adenovirus type 5-12S E1A behind a T7 promoter was used; for full-length SAF-A and SAF-A protein fragments a PCR was performed with an upstream oligonucleotide containing the T7 promoter sequence followed by a Kozak sequence (51), and as a downstream primer the sequence at the end of the studied fragment followed by a stop codon. The [^{35}S]methionine-labeled proteins bound to GST fusion proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography. For pull-down assays with whole-cell extracts, the preparation of cell extracts and incubation with beads coated with GST or GST fusion protein were performed as described before (34). Bound proteins were analyzed by SDS-PAGE followed by Western blotting.

Competition assay. GST-p300CH3 and GST-E1A-N-CR1 (aa 1 to 90) were prepared as previously reported (12). GST-E1A was cleaved with thrombin (Sigma) for 2 h at 4°C. The reaction was terminated by addition of phenylmethylsulfonyl fluoride (Sigma); the sample was then dialyzed in assay buffer (0.1% NP-40–250 mM NaCl–50 mM Tris-HCl [pH 7.5] containing protease inhibitors). The purified protein was analyzed by SDS-PAGE, followed by Coomassie staining. In vitro-translated full-length [^{35}S]methionine-labeled SAF-A was incubated for 1 h in assay buffer with GST-p300CH3, after which different concentrations of E1A or bovine serum albumin (BSA; Sigma) were added. After an additional incubation for 1 h, bound proteins were analyzed by SDS-PAGE followed by autoradiography.

Gel filtration chromatography. Cell extracts were prepared as described before (34) and loaded onto a preequilibrated Superose 6HR 10/30 gel filtration column (Pharmacia) controlled by an FPLC System (Pharmacia). Chromatography was performed in assay buffer (0.1% NP-40–250 mM NaCl–50 mM Tris-HCl [pH 7.5] containing protease inhibitors), and the flow rate was 0.4 ml/min. Fractions (1.5 ml) were collected and analyzed by SDS-PAGE and Western blotting with 3G6 against SAF-A, MN11 against p300/CBP, and C21 against $p90^{rsk1}$.

ChIPs. Cells at 90% confluence were cross-linked with 1% formaldehyde for 10 min at room temperature. Cross-linking was quenched with 125 mM glycine, and whole-cell extracts were prepared for use in the chromatin immunoprecipitations. These chromatin immunoprecipitation assays (ChIPs) were performed as described by Kuras and Struhl (41). The average size of fragmented chromatin was ca. 500 bp as analyzed on agarose gels. For immunoprecipitations, the fragmented chromatin from a 15-cm dish was used, except for p300 immunoprecipitates, for which five times more input was used in parallel with the same amount of input for a nonimmune precipitation. DNA from the immunoprecipitates or from the input (10% of a 15-cm dish) was analyzed either by PCR or by real-time PCR by using the Lightcycler quantification method (Roche Diagnostics). For both PCR analyses, either 1/100 (input) or 1/20 (immunoprecipitates) of the DNA was amplified with 50 pmol of the indicated primers in 40-μl reaction mixtures containing 200 μM concentrations of deoxynucleoside triphosphates, 2.7 mM $MgCl_2$, and 0.25 U of AmpliTaq (Perkin-Elmer). After 4 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C were performed. PCR products were electrophoresed on 2% agarose gels containing ethidium bromide, analyzed under UV light, and presented in inversed intensity. The real-time PCR results were presented as the amount of template immunoprecipitated relative to the amount precipitated with a nonimmune antibody. All ChIP experiments presented were performed at least two times.

The primers used for amplification were as follows: TopMII, GCTCACTGT GACCTCTGC and CCCAGCACTTTGTGCAGC; pTop, GAGTGGGGACCA CCTCCAC and CAATCGGAAATCCGCTTCG; Top exon 13, CAGCAGATA GGTCCACTTGG and CCTTACCTTGATTCGTGAAC; Top exon 20, CACC TTTCTCAGGTGGAGCC and GCTGCACACTTTTCTCTACC; TopMI, CCC TGCTGCTAATGGTATGG and CTTCTGAGGAAACGACTTTGG; c-*myc*, GCGAACACAACGTCTTGG and GTTCTCGTCGTTTCCGCAAC; *c-jun*, CTAGGGTGGAGTCTCCATGG and GCTCAACACTTATCTGCTAC; Igκ, CTGCAACAACTTGATAGGAC and GGGTGAATTCTTGATAGCTTTAC; and β-globin, CACAGTCTGCCTAGTACATTAC and GCCCTGAAAGAAA GAGATTAGG.

Reverse transcription-PCR (RT-PCR). RNA was isolated by using the Promega SV RNA Isolation Kit (Promega). cDNA was made by using random hexamers and the Superscript first-strand synthesis system (Gibco-BRL). PCR was performed as described above, and PCR products were analyzed on ethidium bromide-stained agarose gels. The following primers were used: for GAPDH, AATCCCATCACCATCTTCC and ATGAGTCCTTCCACGATA CC; for p300, CGGGATCCGCTGCATCCAGTCTCTG and GCTCTAGATC AAGGGAGGCCCTGTTGCTG; and for topoisomerase I, GAGTGGGGACC ACCTCCAC and GGATAGCGCTCTTCTTCCC.

RESULTS

Interaction between p300 and SAF-A. In a search for proteins involved in the functional regulation of p300, a yeast two-hybrid screen was performed with a p300 fragment that comprises the ADA2 homology domain and CH3 (aa 1570 to 1844). This led to the identification of SAF-A as a p300-associating protein. To test whether p300 and SAF-A associate in vivo, we examined whether endogenously expressed p300 and SAF-A could be coimmunoprecipitated from human cells. As shown in Fig. 1A, SAF-A readily coprecipitated when p300 was immunoprecipitated from a U-2 OS cell extract by using three different antibodies against p300. Since p300 also coprecipitated when SAF-A was immunoprecipitated, we conclude that the endogenous p300 and SAF-A proteins interact in cells. To

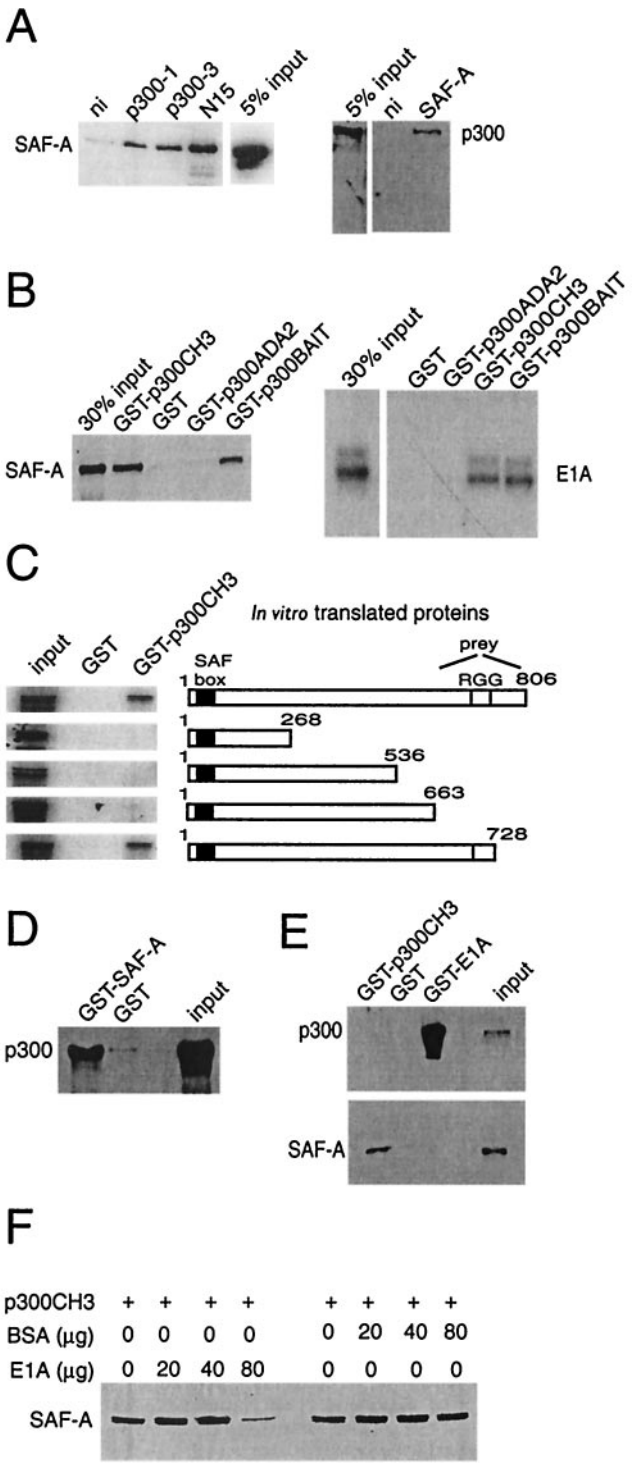

FIG. 1. p300 interacts with SAF-A in vivo and in vitro. (A) Endogenous p300 coimmunoprecipitates with SAF-A. U-2 OS whole-cell extracts were immunoprecipitated with antibodies against p300 (p300-1, p300-3, and N15) or SAF-A and tested on Western blot for coimmunoprecipitated SAF-A or p300. (B) GST pull-down assays. In vitro-translated [^{35}S]methionine-labeled 12S-E1A or SAF-A (aa 633 to 806) retained on GST, GST-p300ADA2 (aa 1573 to 1731), GST-p300CH3 (aa 1726 to 1848), or GST-p300BAIT (aa 1573 to 1848) was visualized by autoradiography. (C) Mapping of the SAF-A–p300 interaction by GST pull-down. In vitro-translated SAF-A subdomains retained on GST-p300CH3 were visualized by autoradiography. (D) GST–SAF-A (aa 537 to 806) retains endogenous p300 from U-2 OS whole-cell extracts as visualized on a Western blot with an anti-p300 antibody. (E) GST pull-down assay. GST-E1A (aa 1 to 90) and GST-p300CH3 retain endogenous p300 or SAF-A, respectively, as visualized on Western blots. (F) Competition assay. Increasing amounts of E1A or BSA were used for competing away in vitro-translated SAF-A retained on GST-p300CH3.

delineate the interaction domain of p300 and SAF-A, in vitro binding studies were performed. Pull-down studies with GST fusion proteins with the p300 bait sequence or its separate ADA2 homology and CH3 domains showed that in vitro-translated SAF-A interacts with the CH3 domain of p300, as does E1A, while neither protein binds to GST or GST-ADA2 (Fig. 1B). The in vitro-translated SAF-A protein used comprises the C-terminal aa 633 to 806, the SAF-A protein domain identified in our yeast two-hybrid screen to interact with p300. GST pull-down studies with in vitro-translated full-length or C-terminally truncated SAF-A protein showed that SAF-A contains a single binding site for p300 localized between aa 663 and 728 (Fig. 1C), a region previously identified as an RNA-binding domain (35). The GST-p300CH3 protein also binds SAF-A from cell extracts (Fig. 1E), while GST–SAF-A (aa 537 to 806) brings down full-length p300 from whole-cell extracts (Fig. 1D).

The binding of E1A and SAF-A to the same domain of p300 might imply a mutually exclusive binding. Indeed, no indication for a trimeric complex of E1A, p300, and SAF-A is found since a pull-down experiment with GST-E1A (aa 1 to 90) brings down only p300 (Fig. 1E). Further evidence for a mutually exclusive binding is provided by the fact that increasing amounts of E1A can compete for binding of in vitro-translated SAF-A to GST-p300CH3, while increasing amounts of BSA have no effect on the binding of SAF-A to GST-p300CH3 (Fig. 1F). Taken together, these results show that SAF-A interacts, both in vivo and in vitro, with p300. Binding occurs through the CH3 domain of p300, precluding E1A from binding to this site simultaneously.

p300–SAF-A binds S/MAR elements. To investigate whether p300 and SAF-A (120 kDa) exist in a multiprotein complex, U-2 OS cell extracts were fractionated on a gel filtration column. Western blotting analysis revealed that the endogenous p300 eluted at ca. 1.5 MDa (Fig. 2A), a finding consistent with previous reports (43). The endogenous SAF-A eluted over a broader range of molecular weights with a large fraction that cofractionated with p300 (Fig. 2A). From these fractions p300 and SAF-A could be readily coimmunoprecipitated (data not shown). As a control, the elution pattern of p90^{rsk1} kinase was analyzed and shown to elute at ca. 90 kDa, indicating that it is not present in a complex with p300 and/or SAF-A.

SAF-A has been identified as both chromatin and RNP bound. To investigate whether p300 binds SAF-A in both locations, we analyzed the presence of p300 and SAF-A via various cellular fractionation methods (17, 22). First, we purified RNP particles in the 45% sucrose fraction by centrifugation of whole-cell extracts over a sucrose gradient (22). In these RNP particles, SAF-A and hnRNP L, another RNP protein, were clearly present, but neither p300 nor histones were detected (Fig. 2B), indicating that p300 does not bind to SAF-A

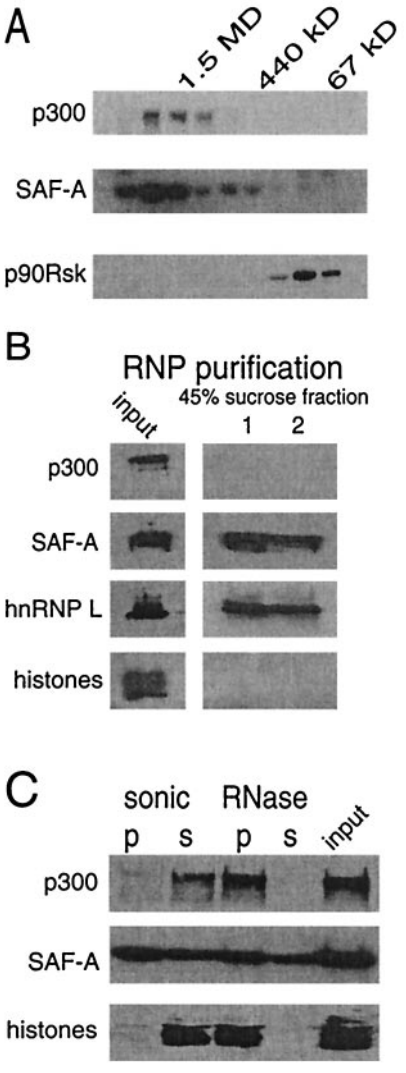

FIG. 2. SAF-A and p300 cofractionate. (A) U-2 OS cell extract was fractionated on a Superose 6 column, and fractions were collected and tested on Western blot for the presence of SAF-A, p300, and the protein kinase $p90^{rsk}$. At the top the elution of markers is indicated by their molecular sizes in kilodaltons. (B) RNP particles were isolated from whole-cell extracts in the 45% sucrose fractions (lanes 1 and 2 represent two independent gradients). The presence of SAF-A, hnRNP L, histones, and p300 was tested by Western blot. (C) U-2 OS nuclear skeleton was separated by two procedures into a chromatin and an RNP-associating fraction. In the sonication procedure, the pellet (p) represents the RNP-associated fraction and the supernatant (s) represent the chromatin-associated fraction. In the RNase procedure, the pellet represents the chromatin-associated fraction and the supernatant represents the RNP-associated fraction. The presence of p300, SAF-A (120 kDa), and histones was detected by Western blotting.

present in hnRNP particles. To determine the presence of p300 and SAF-A in chromatin, we prepared distinct nuclear fractions (17). For this, cells were lysed in an isotonic buffer in the presence of 1% Triton X-100. This treatment released only a small amount of SAF-A, whereas the majority of the protein remained bound to nuclear structures. From this nuclear fraction, which still contained p300, chromatin can be released by sonication and, alternatively, RNA can be released by an RNase treatment. SAF-A was found equally distributed in the nuclear fractions, as described by Fackelmayer et al. (17). However, p300 was mainly found in the chromatin fractions, which were identified by the presence of histone proteins (Fig. 2C). These data indicate that p300 and SAF-A most likely associate in a chromatin-related context.

The binding of SAF-A to S/MAR elements has been intensively studied with the MII S/MAR element of topoisomerase I. In order to study whether both SAF-A and p300 are present at S/MARs, ChIPs on the S/MAR elements of the topoisomerase I gene were performed in U-2 OS cells. Immunoprecipitation of SAF-A brought down both the MI and the MII S/MAR element as detected with primer sets in these elements (Fig. 3A and B). More interestingly, an antibody against p300 also brought down these elements. A nonimmune control serum was used to show the specificity of the immunoprecipitations. The antibodies to SAF-A or to p300 do not immunoprecipitate other parts of the topoisomerase I gene, as shown by the use of two different primer sets, one in the promoter and one in exon 13, that were added as internal controls for the PCR (Fig. 3A and B). Therefore, these data indicate a specific interaction of the p300–SAF-A complex with S/MAR elements in U-2 OS cells.

To test whether the association of p300 and SAF-A with S/MAR elements was gene specific, we used ChIP assays to analyze their binding to several other well-defined S/MAR elements. Binding of SAF-A and p300 was found to occur at the S/MARs of the topoisomerase I (MI and MII [40]), c-*myc* (6), β-globin (29), and Igκ (62) genes (Fig. 3B and C). Since p300 is known to have acetyltransferase activity, we also examined the acetylation status of histone H3 at the different S/MARs. Interestingly, we found the presence of acetylated histone H3 at the S/MAR elements (Fig. 3C). Apart from nonimmune controls, we studied the *c-jun* promoter and found no binding of p300 and SAF-A to this site. The S/MAR elements we tested were located on various chromosomes, and we conclude that the presence of a p300–SAF-A complex on S/MAR elements seems to be a general phenomenon.

Viral oncogenes disrupt p300–SAF-A–S/MAR complexes. Binding of SAF-A to p300 occurs via the p300CH3 domain that also interacts with the adenovirus E1A protein (15). This interaction is mutually exclusive (Fig. 1E and F), suggesting that the binding of SAF-A and p300 may be disrupted in adenovirus-transformed cell lines. To test whether the presence of E1A interfered with the formation of a p300–SAF-A–S/MAR complex, ChIP assays were performed in the adenovirus E1-transformed HER cell line 911 (19). As shown in Fig. 4A, no binding of either p300 or SAF-A to S/MAR elements could be detected in 911 cells, suggesting that disruption of the p300–SAF-A interaction by E1A also disrupts the SAF-A binding to the S/MAR element. The mechanism of this disruption is not established but might include the presence of other histone acetyltransferase (HAT)-containing complexes at S/MAR elements in virally transformed cells, since we found at all S/MAR elements studied the presence of acetylated histone H3 (see also Discussion). As a control, ChIP assays were performed on the *c-jun* promoter, which is active in adenovirus-transformed cells (14). Both p300 and the transcription factor ATF-2 could be detected. Similar results were obtained by

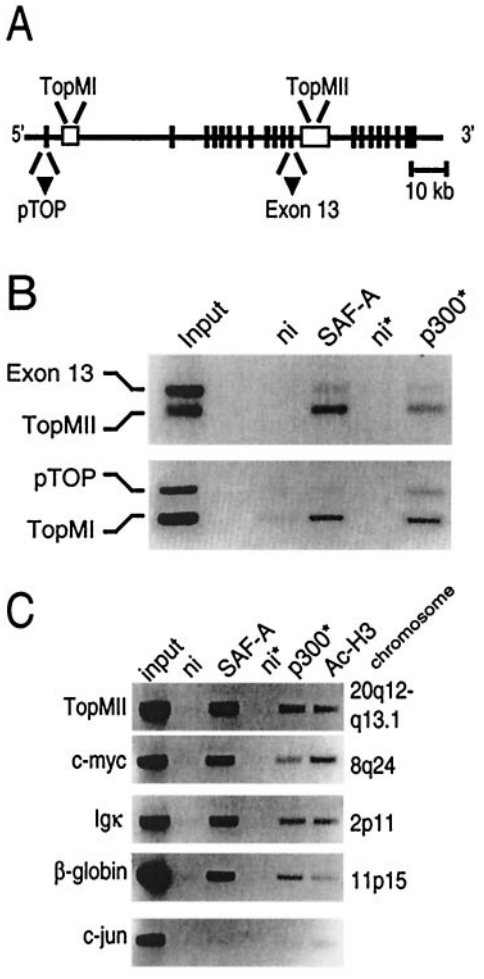

FIG. 3. SAF-A/p300 bind S/MAR elements. (A) Schematic representation of the topoisomerase I gene (39, 53) showing the MI and MII S/MAR elements. Vertical bars represent the exons, open boxes represent the S/MAR elements, and triangles represent the positions of the internal controls in panel B. (B and C) ChIP assays with U-2 OS cells with antibodies against SAF-A, p300, and acetylated histone H3. Nonimmune serum (ni) was used as a control. The DNAs of the immunoprecipitated fractions were isolated, and topoisomerase I sequences in the immunoprecipitated DNA were amplified by PCR. Asterisks indicate that five times more input was used in these assays. TopMI and TopMII are fragments within the S/MAR elements of topoisomerase I; pTOP is a fragment within the promoter region, and exon 13 is a fragment within exon 13 of the topoisomerase I gene. In panel C, additional S/MAR sequences were tested: MII of the topoisomerase I gene (TopMII) and the S/MAR sequences of the c-*myc*, Igκ, and β-globin genes, respectively. As a control for specific amplification, a primer set in the *c-jun* promoter was used. The chromosomal location of the various genes is shown on the right.

using 293 cells, an adenovirus-transformed human embryonic kidney cell line (data not shown). A quantitative comparison of these assays in U-2 OS and 911 cells, based on real-time PCR and with the TopMII S/MAR primer set, is shown in Fig. 4B. Quantification shows that in U-2 OS cells immunoprecipitation of p300 and SAF-A brings down almost 5 and 75 times more MII S/MAR DNA, respectively, than a control immunoprecipitation, whereas in adenovirus-transformed cells in both cases levels comparable to the control are immunoprecipitated.

Like E1A, SV40 large T antigen has also been shown to interact with the CH3 domain of p300 (2, 16) and similarly might reduce the binding of p300 and SAF-A to S/MAR elements. Therefore, we compared VH10 primary human foreskin fibroblasts with their SV40-transformed counterpart (38). The quantified ChIP assays using the TopMII S/MAR primer set with VH10 cells show a strong binding of SAF-A and p300 to the S/MAR element of topoisomerase I (Fig. 4C), while in the SV40-transformed VH10 cells binding of SAF-A and p300 to the S/MAR element is strongly reduced (Fig. 4C). From these results we conclude that the binding of p300 and SAF-A to the MII S/MAR element, as observed in U-2 OS and VH10 cells, is absent in SV40- and adenovirus-transformed cells.

Several lines of evidence indicate that S/MAR elements are involved in the regulation of transcription. In order to investigate whether the binding of a p300–SAF-A complex to S/MAR correlates with active transcription, we studied the expression of the S/MAR-containing gene topoisomerase I. RT-PCR of topoisomerase I mRNA showed a higher level of expression in the adenovirus-transformed cell line compared to the U-2 OS and VH10 cells (Fig. 4D), as described earlier (54). Also, for the SV40-transformed cell line we found increased topoisomerase I expression. These data show expression of topoisomerase I while no p300–SAF-A is bound to the S/MAR elements of topoisomerase I and indicate a possible role for p300–SAF-A complexes at S/MAR elements of genes that are not transcribed.

Cell cycle-dependent disruption of p300–SAF-A–S/MAR complexes. In order to further substantiate the hypothesis that binding of p300–SAF-A to S/MAR elements is restricted to nontranscribed genes, we studied the cell cycle-dependent induction of the topoisomerase I expression in parallel with binding of SAF-A and p300 to the MII S/MAR element in the topoisomerase I gene. For this we used the glioblastoma cell line T98G (see, e.g., reference 58) since these cells can be arrested by serum deprivation and can then be efficiently stimulated to reenter the cell cycle by addition of serum. Moreover, these cells can be density arrested. The mRNA expression of topoisomerase I was studied by RT-PCR and was found to be absent after serum deprivation, while increased expression was found during the first 36 h after serum stimulation (Fig. 5A). After growth for 72 h (when the cells are density arrested), topoisomerase I expression again decreased. In parallel with the expression, the binding of p300 and SAF-A to the S/MAR elements of topoisomerase I was determined by ChIP assays. The quantitative results from real-time PCR are depicted in Fig. 5B. Before serum stimulation a relatively strong binding of the SAF-A and p300 proteins to the MII S/MAR element could be observed. After serum stimulation, the binding of both p300 and SAF-A to the MII S/MAR element was decreased at all time points tested, except at 72 h when again the binding of both p300 and SAF-A to the MII S/MAR element was detected. As a control, a primer set in the topoisomerase I promoter was used, which showed no binding of SAF-A or p300 at any time point. These results clearly show that p300 and SAF-A are not bound to S/MAR elements when the topoisomerase I gene is expressed. To investigate the possible consequence of the presence of the acetyltransferase p300 at

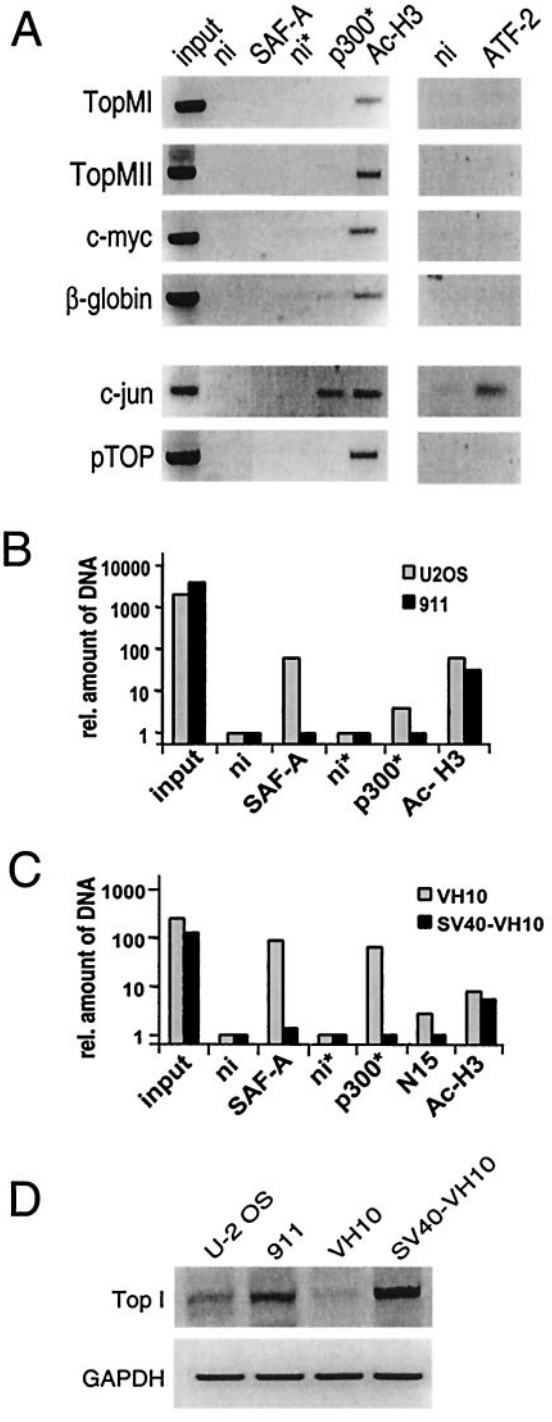

FIG. 4. Absence of binding of SAF-A or p300 to S/MAR elements in virally transformed cells. (A) ChIP assays with adenovirus-transformed 911 cells with a control antibody (ni) and antibodies against SAF-A, p300, acetylated histone H3, and ATF-2. Primer sets in the topoisomerase I promoter (pTOP) and the *c-jun* promoters were used as a control. Asterisks indicate that five times more input was used. (B) Quantification of ChIP results. The relative difference in the amount of precipitated DNA as determined by real-time PCR is presented for the ChIP results found for the MII S/MAR element of topoisomerase I in Fig. 4A (911 cells) and Fig. 3B (U-2 OS cells) on a log scale. Nonimmune values are set at 1. (C) Absence of binding of SAF-A or p300 to S/MAR elements in an SV40-transformed cell line compared to nontransformed VH10 cells. The relative difference in amount of precipitated DNA as determined by real-time PCR is presented for the ChIP results found for the MII S/MAR element of topoisomerase I (TopMII). (D) RT-PCR for the mRNA expression of the topoisomerase I and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) genes in U-2 OS, 911, VH10, and SV40-transformed VH10 cells.

S/MAR elements, we assessed the acetylation of histone H3 during the same time course. As shown in Fig. 5C, acetylated histone H3 was present at the S/MAR elements of the topoisomerase I gene, irrespective of its expression. In contrast, acetylated histone H3 could only be detected in other parts (e.g., the promoter) when the gene was actively transcribed. The presence of acetylated H3 during transcription is most likely due to HAT activities associated with the transcription machinery. Hyperacetylation in the absence of transcription, however, was found to be restricted to S/MAR elements. These findings show that, although SAF-A–p300 may somehow be involved in keeping H3 acetylated at S/MAR elements in the absence of transcription ($T = 0$ h, $T = 72$ h), the complex is dispensable for keeping H3 acetylated.

DISCUSSION

The transcriptional coactivators p300 and CBP were originally described as bridging factors between various transcription factors and the basal transcription machinery. They were later found to contain intrinsic acetyltransferase activity and to associate with other proteins containing acetyltransferase activity, such as p/CAF. Here we identified a novel function of p300, namely, its association with SAF-A, a protein found as a S/MAR-interacting component of the nuclear matrix and as a component of RNP particles (13). Our studies suggest a role for p300 bound to SAF-A at S/MAR elements. SAF-A interacts with S/MARs via its N-terminal SAF box (36) and via its C-terminal domain with p300, allowing binding of SAF-A to both p300 and a S/MAR element. The C-terminal domain of SAF-A also contains the RGG-box, involved in binding to RNA (35), suggesting that RNA and p300 binding to SAF-A are mutually exclusive. Our results showing that p300 is absent from RNP particles support this view.

For the topoisomerase I gene, release of p300–SAF-A from the S/MAR elements by stimulation of the S phase or by viral oncoproteins is accompanied by enhanced transcription. This suggests that the p300–SAF-A complex functions at the S/MAR elements of nontranscribed genes. The presence of p300, together with SAF-A, at S/MAR elements of inactive genes is unlikely to contribute to the inactive state of the gene. Perhaps, p300 at S/MAR elements contributes to the enigmatic feature of S/MAR elements to stimulate transcription of nearby genes (5). One possibility would be that p300 forms a bridge between SAF-A at S/MAR elements and transcription factors already bound to the promoter, enabling a quick complete activation of the gene. However, this would implicate that p300 should be present at the promoter elements of S/MAR-containing genes, which is in contrast to our findings.

Alternatively, the main contribution of p300 or associated proteins to the transcriptionally competent state through S/MAR elements might be by acetylation of histone tails and,

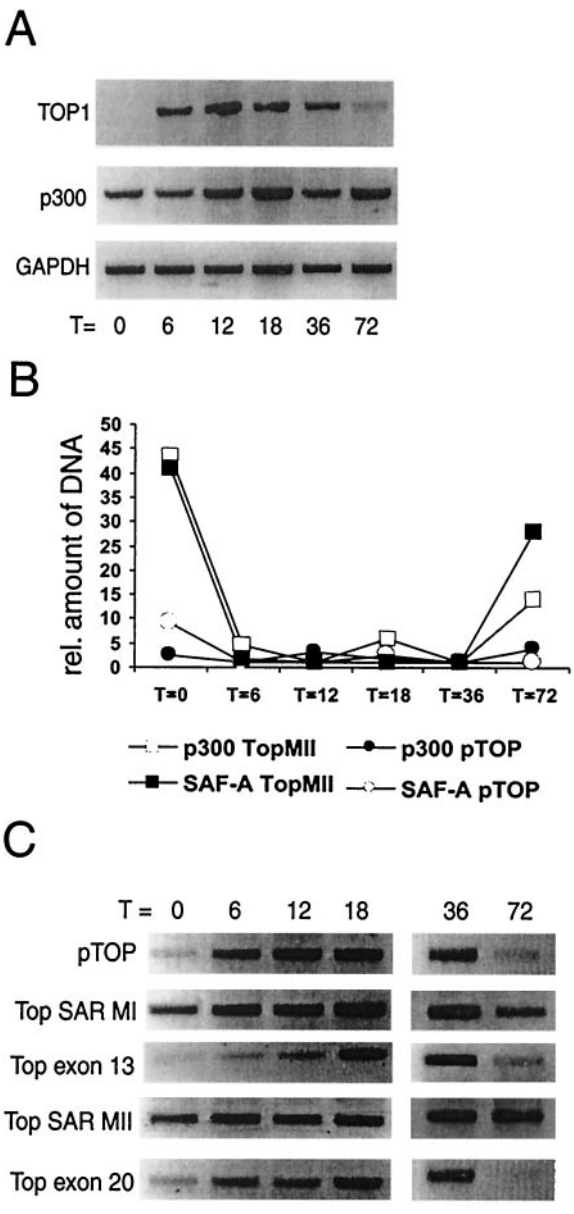

FIG. 5. Inactive topoisomerase I gene has SAF-A, p300, and acetylated histone H3 bound to its S/MAR element. (A) RT-PCR for the RNA expression of the topoisomerase I, p300, and GAPDH genes of serum-arrested T98G cells and after subsequent stimulation by serum for 6, 12, 18, 36, and 72 h, representing growing and subsequently (72 h) density-arrested cells. (B) ChIP assays with the serum-stimulated cells were analyzed with real-time PCR with primers within the topoisomerase I S/MAR element and in the topoisomerase I promoter. Depicted is the relative level of precipitated DNA with antibodies to SAF-A or p300 in comparison to a nonimmune control antibody. (C) ChIP assays with an antibody against acetylated histone H3 show high levels of histone H3 acetylation at S/MAR elements of topoisomerase I when the gene is inactive, in contrast to promoter and exon sequences that only show hyperacetylated histone H3 when the gene is expressed.

indeed, we found acetylated histone H3 at all S/MAR elements studied. An analysis of the topoisomerase I gene during activation showed that the S/MAR elements of the inactive gene are bound by p300–SAF-A and hyperacetylated histones are present at the S/MAR elements but not at other parts of the gene. When transcription occurs, hyperacetylated histones are present throughout the gene, probably due to histone acetylation activities accompanying the elongation by RNA polymerase II (46). The S/MAR-restricted occurrence of acetylation observed here has recently also been shown for the chromosomal integrated immunoglobulin μ enhancer (21). Together, these findings suggest that one of the mechanisms by which S/MAR elements are able to affect transcription of nearby genes is via the generation of an extended domain of histone acetylation.

The presence of viral oncoproteins such as E1A and SV40 large T can disrupt the binding of p300 to the S/MAR elements. Surprisingly, they also disrupt the binding of SAF-A to these elements. However, no effect of the viral oncogenes was observed on the acetylation state of histone H3 at the S/MAR elements. The release of p300–SAF-A from S/MAR elements is unlikely to be a consequence of transcription, since the c-*myc* gene is barely expressed in adenovirus-transformed cells (60; data not shown) and there is hardly any expression of β-globin in the nonerythroid cells used here (data not shown). Another option is that the presence of viral proteins results in SAF-A protein modifications leading to an inability to bind S/MAR elements. Finally, it is also possible that the viral oncogenes induce an altered protein composition at the S/MAR elements localizing acetyltransferases other than p300 to these elements. An altered protein composition at the S/MARs might explain both the absence of binding of SAF-A and the presence of acetylated histones without detectable levels of p300 at S/MAR elements in these cells. However, at present we cannot exclude that other virus-induced mechanisms are involved in releasing p300 and SAF-A from S/MAR elements and in keeping the nucleosomes at S/MAR elements acetylated.

Taken together, our results suggest that p300 not only functions at promoter and enhancer sequences but also at S/MAR elements via association with SAF-A. In non-virally transformed cells and in primary cells S/MAR elements of nontranscribed genes bind p300–SAF-A, while there is only localized histone H3 hyperacetylation at the S/MAR elements. This strongly suggests that in this situation the presence of the p300–SAF-A complex is responsible for the acetylation. The most obvious candidate is p300, but it is certainly also possible that the complex contains other p300-associated HATs such as p/CAF (64) and SRC-1 (31, 65). Association of p300 and SAF-A with S/MAR elements of nontranscribed genes might ensure the presence of localized histone acetylation and poise these chromatin regions for transcription.

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