A Thymocyte Factor SATB1 Suppresses Transcription of Stably Integrated Matrix-Attachment Region-Linked Reporter Genes†

Terumi Kohwi-Shigematsu,*,‡ Karin Maass,§ and Jürgen Bode§

Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, 70A-1118, 1 Cyclotron Road, University of California, Berkeley, California 94720, and GBF, Gesellschaft für Biotechnologische Forschung mbH, Genregulation und Differenzierung/Genetik von Eukaryonten, Mascheroder Weg 1, D-38124 Braunschweig, Germany

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ABSTRACT: SATB1 specifically recognizes and binds to specialized genomic regions with an ATC sequence context with high base-unpairing propensity. Such base-unpairing regions (BURs) are typically identified within nuclear scaffold- or matrix-attachment regions (S/MARs). SATB1 is a homeodomain protein and is predominantly expressed in thymocytes. We obtained BHK cell lines expressing low levels of SATB1 by stable transfection and investigated its effect on stably integrated MAR-linked SV40 enhancer/promoter-driven luciferase reporter genes. For this study, both naturally occurring and synthetic MARs, as well as an AT-rich non-MAR control, were tested. Previous studies demonstrated that MAR sequences augment transcription of the linked reporter luciferase gene. Here, we show that SATB1 dramatically reduces the high levels of MAR-linked luciferase gene transcription. Transcription was virtually abolished for a reporter gene surrounded by two MARs at the 5′ and 3′ ends of the gene, which otherwise confer the highest level of transcriptional augmentation. On the other hand, SATB1 did not affect expression of an AT-rich non-MAR-linked luciferase gene or of endogenous housekeeping genes. This study shows that SATB1 acts as a strong transcriptional suppressor on a reporter gene linked to MARs when it is stably integrated into chromatin.

Genomic DNA sequences with a high affinity for the preparation of nuclear matrices *in vitro* are operationally designated as scaffold- or matrix-attachment regions (SARs or MARs; in this report, we employ MAR for simplicity). The nuclear matrix is the skeletal framework of the nucleus which remains insoluble after extraction of nuclei with high salt (Berezney & Coffey, 1974), and its ultrastructure and components have been the subjects of active research (Nickerson et al., 1995, 1997; Ferraro et al., 1996). Although definitive proof is yet to be provided, MARs are considered strong candidates for the genomic sites at which eukaryotic chromosomes are attached to the nuclear matrix during interphase *in vivo*, thereby functionally separating chromatin into independent topological domains [reviewed in Nelson et al. (1986), Gasser and Laemmli (1987), and Bode et al. (1995, 1996)]. MARs appear to have biological significance. For example, in the case of the immunoglobulin heavy chain gene (IgH) enhancer, both flanking MARs were found to be essential for its B cell specificity in transgenic mice (Forrester et al., 1994), and these MARs have also been reported to be responsible for chromatin accessibility (Jenuwein et al., 1997). MARs surrounding the immunoglobulin κ gene are needed for B-cell-specific demethylation of the gene (Kirillov et al., 1996).

Various MAR sequences contain specific regions, mostly not more than 200 base pairs in length, that exhibit an unusually high unwinding property by stable base-unpairing. When MARs are placed under negative superhelical strain, it is these base-unpairing regions (BURs) that unwind and relieve the strain (Kohwi-Shigematsu & Kohwi, 1990). For example, both the 5′ and the 3′ MARs surrounding the immunoglobulin heavy chain (IgH) enhancer contain such BURs. Particularly in the BUR of the 3′ MAR, a core unwinding element is present that continues to be base-unpaired under negative supercoiling even in the presence of Mg^{2+}, the condition under which the rest of plasmid DNA becomes double-stranded. When the core unwinding element was mutated, the entire 3′ MAR lost its unwinding propensity and remained completely as double-stranded DNA under negative superhelical strain (Kohwi-Shigematsu & Kohwi, 1990). When 25 base pairs containing the core unwinding element were concatemerized [referred to as wild-type $(25)_7$], it readily became base-unpaired under negative superhelical strain and augmented transcriptional activity in stably transfected cells. This concatemer bound with high affinity to the nuclear matrix, and therefore, it is operationally defined as a MAR. In contrast, if a similar sequence with a mutated core unwinding element was concatemerized [referred to as mutated $(24)_8$], it lost its base-unpairing property, bound poorly to the nuclear matrix, and did not affect the reporter gene transcription in stably transfected cells (Bode et al., 1992). The high unwinding property of genomic sequences is apparently an important characteristic of functional MAR elements.

The nuclear matrix has been shown to provide the sites for active transcription [reviewed in Stein et al. (1995)] and replication (Razin et al., 1986; Vaughn et al., 1990; Hozák et al., 1993) to take place in nuclei. Because MARs are thought to be the genomic site at which chromatin is anchored onto the nuclear matrix, proteins that bind to

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* Corresponding author. Telephone: (510) 486-4983. Fax: (510) 486-4545. E-mail: terumiks@lbl.gov.

‡ University of California.

§ Gesellschaft für Biotechnologische Forschung mbH.

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sequences within MARs may be important for regulatory functions such as transcription and replication. In fact, MARs are frequently colocalized with regulatory sequences (Cockerill & Garrard, 1986; Gasser & Laemmli, 1986; Cockerill et al., 1987; Jarman & Higgs, 1988). SATB1 was previously cloned on the basis of its unusual capability of distinguishing BUR from those similarly AT-rich DNAs that resist unwinding (Dickinson et al., 1992). SATB1 binds the synthetic MAR wild-type $(25)_7$ with high affinity with the dissociation constant K_d in the range of 1 nM (Dickinson & Kohwi-Shigematsu, 1995), while it does not bind to mutated $(24)_8$, which is an AT-rich, non-MAR sequence. SATB1 contains a MAR-binding domain (Nakagomi et al., 1994) and a recently identified divergent homeodomain (Dickinson et al., 1997). The isolated MAR-binding domain specifically binds to the BUR region of MAR segments. BURs consist of ATC sequence stretches in which one strand exclusively consists of well-mixed A, T, and C but lacks G. When such ATC sequences are clustered, DNA readily unwinds under negative superhelical strain (Kohwi-Shigematsu & Kohwi, 1990; Dickinson et al., 1992). The combined action of the homeodomain and the MAR-binding domain further increases the specificity and enables SATB1 to target the core unwinding element for binding. Therefore, SATB1 is designed to recognize the key structural element of MARs. The homeodomain protein SATB1 is a cell-type specific protein which is predominantly expressed in thymocytes. Homeodomain proteins are known to participate in transcriptional regulation [reviewed in Gehring (1987)].

Here, we examined whether SATB1 has either transcriptional suppression or an enhancement effect on gene expression mediated by BURs. If so, SATB1 may be a new type of transcriptional regulatory protein that regulates multiple-gene transcription because BURs, a specialized sequence context, are presumably associated with multiple genes in a given cell. To test this hypothesis, we have studied the activity of SATB1 on MAR-linked reporter genes in stably transfected cells.

EXPERIMENTAL PROCEDURES

Plasmids. For pMSG-AT1146, the cDNA (pAT1146) encoding human SATB1 (Dickinson et al., 1992) was excised from pBluescript by *Hin*dIII/*Bam*HI treatment, blunt-ended by Klenow polymerase, and cloned between the *Nhe*I/*Sal*I sites of the pMSG (Pharmacia) polylinker. In this position, the gene is under the control of the inducible MMTV promoter/enhancer and linked to a gpt selector gene. The construction of a series of pLu-based plasmids (SV40 promoter-driven luciferase gene construct), i.e., pLu, pLu-E, pLu-E-P, pLu$(25bp)_7$, and pLu$(24bp)_8$, was previously described (Klehr et al., 1991; Bode et al., 1992; Dietz et al., 1994) and was used for supertransfection experiments (below). E corresponds to a 2.2 kb MAR fragment found upstream of the human interferon-β (huIFN-β) gene (Klehr et al., 1991; Bode & Maass, 1988), and P represents a 1.8 kb MAR upstream of the potato ST-LS1 gene (Stockhaus et al., 1987; Klehr et al., 1991). Both E and P are *Eco*R1 fragments. The E fragment was adapted by linkers and cloned into the *Bam*H1 site of plasmid Lu which is upstream of the SV40 promoter. The P fragment was cloned into the Nru 1 site of plasmid Lu downstream of the luciferase gene after being blunt-ended by Klenow polymerase. Both wild-type $(25)_7$ and mutated $(24)_8$ sequences were excised from a Bluescript vector as a *Bam*HI-*Sal*I fragment and cloned into plasmid Lu at the *Bam*HI and *Sal*I restriction sites upstream of the SV40 promoter (Bode et al., 1992).

Cell Culture and Stable Gene Transfer. BHK cells were grown as described (Bode et al., 1986). The above pMSG derivative (pMSG-AT1146) and unmodified pMSG were transfected in parallel in order to create the recipient cell lines for expression studies of MAR vectors and MAR-free controls. The transfection protocol has been optimized to yield low copy numbers (Klehr et al., 1991, 1992). This has been achieved by omitting carrier DNA and by transferring linearized DNA. Selection for the expression of the *Escherichia coli* gpt gene encoding xanthine-guanine-phosphoribosyl transferase [XGPRT; see Mulligan and Berg (1981)] was in a medium (DME-gpt) obtained by adding 14 μg/mL hypoxanthine, 0.2 μg/mL aminopterin, 10 μg/mL thymidine, 250 μg/mL xanthine, and 25 μg/mL mycophenolic acid to DME containing 10% dialyzed FCS. Media were replaced every 3 days with fresh DME-gpt. After 2 weeks, the resulting clones were isolated and provided with fresh DME-gpt or cultivated as a mixture under the same conditions. After another 2 weeks, cultivation was continued with regular DME/FCS medium. The clonal mixture and individual clones thereof were used for supertransfection (see the example in Figure 2).

Supertransfection with MAR Plasmids and Controls. MAR plasmids pLu-E, pLu-E-P, pLu$(25bp)_7$, and pLu$(24bp)_8$ and a MAR-free control (pLu) were transfected in parallel into the BHK cell lines harboring either pMSG or pMSG-SATB1 and selected by 1000 μg/mL neomycin analogue G-418 as previously described (Klehr et al., 1991, 1992). The luciferase gene activity was subsequently determined from the mixture of G-418 resistant clones as described below.

Average Copy Numbers. All data from stable expression experiments were referenced to the number of integrated copies, which was determined as described before (Klehr et al., 1992).

RT-PCR. Total RNA from 10^6 cells was lysed in 100 μL of RNazol solution (Cinna/Biotecx, Friendswood, TX), followed by phenol/chloroform extraction and 2-propanol precipitation according to the manufacturer's protocol. Reverse transcription of total RNA (0.125, 0.5, and 2 μg each) in a total volume of 30 μL was performed at 42 °C for 1 h, using the following mixture: 10 ng each of primers SATB1 (nucleotide positions relative to the translational start site 984−1002, 5′-CACTGCATACTGG-3′; nested SATB1-1 cDNA primer) and GAPDH (nucleotide positions 340−359, 5′-GAGATGATGACCCTTTTGGC-3′; nested GAPDH-1 cDNA primer) and 50 units of reverse transcriptase (StrataScript RT RNase H reverse transcriptase, Stratagene) in a single tube in 1×RT buffer [50 mM TRIS (pH 8.3), 75 mM KCl, and 3 mM $MgCl_2$] containing 10 mM DTT, 500 μM dNTP (Ultrapure dNTP set, Pharmacia), and 20 units of RNAsin (Promega).

For polymerase chain reaction, the above mixture was divided equally into two tubes, one for SATB1 and the other for GAPDH amplification. Each reaction was carried out in 50 μL of 1×PCR buffer [10 mM TRIS (pH 8.3), 50 mM KCl, 0.001% gelatin, and 1.5 mM $MgCl_2$] containing 5 μL of the above-described cDNA sample, 200 μM dNTPs, 2.5 units of Taq polymerase (Promega, Madison, WI), 100 ng of each set of unlabeled primer, and 1 μg of radiolabeled primer. For GAPDH cDNA amplification, two primers [sense primer at nucleotide positions 4−22 (5′GTGAAG-

GTCGGTGTCAACGG-3′) and antisense primer at nucleotide positions 286−303 (5′-GAAGACGCCAGTAGACTC-3′)] were used (Baier et al., 1993). For SATB1, two primers [sense primer at nucleotide positions −13 to 7 (5′-GACAGTGACTGAGTATGGATC-3′) and antisense primer at nucleotide positions 480−497 (5′-ATGTGACCACATGATACA-3′)] were used. One nanogram of each of the SATB1 and GAPDH sense primers was kinased prior to being used with [γ-^{32}P]ATP and T4 polynucleotide kinase in order to radiolabel the PCR products synthesized in each round of amplification. Amplifications of divided cDNA samples representing approximately 0.125, 0.5, and 2 μg of total RNA were carried out separately for 21, 29, and 35 cycles, respectively, using the RoboCycler Gradient 40 Temperature Cycler (Stratagene). The temperature cycles were set as follows: 95 °C for 1 min, 50 °C for 2 min, and 72 °C for 1 min. One-fifth of the PCR products were resolved by agarose gel electrophoresis, dried, visualized directly by autoradiography, and quantified by laser scanning densitometry.

Luciferase Tests. Expression levels were determined for regularly grown cells, or cells that had been exposed to 2 mM dexamethasone for 16 h, to boost the expression directed by the MMTV promoter/enhancer. For this purpose, phenol red and calf serum had to be omitted from the medium. Extracts were directly prepared from a defined number of cells. Usually, 10^5−10^8 cells were lysed in 300−1000 μL of extraction buffer [0.1 M KH_2PO_4 (pH 7.8) and 1 mM dithiothreitol]. The bioluminescence of 10−50 μL was quantified in a Berthold Biolumat model LB9500c apparatus by integrating the output over the first 10 s and correcting this value for the number of cells in the assay.

Northern Blots. Pyruvate kinase (PK) expression was determined in parallel with luciferase on Northern blots. Total cellular RNA was prepared from two 69 cm^2 culture dishes by the guanidinium thiocyanate method (Chomczynski & Sacchi, 1992). RNA (10 μg per lane) was separated by formaldehyde−agarose gel electrophoresis as described (Shübeler et al., 1996). The gel was successively washed in water (one time) and 10× SSC (two times) and blotted onto GeneScreenPlus (Dupont) with 10× SSC, prehybridized, and hybridized with probes that had been labeled by random priming (rediprime, Amersham). Luciferase was probed by a 655 bp *Eco*RI fragment excised from pAGLu and pyruvate kinase by a 600 bp *Pst*I fragment from Pyrkin PkPBS.

Western Blot. The preparation of cell extracts and the following Western blot analysis were performed as previously described (Dickinson & Kohwi-Shigematsu, 1995).

RESULTS AND DISCUSSION

Establishment of Cell Lines Stably Expressing SATB1. Since MARs are capable of augmenting the transcriptional activity of linked reporter genes in stably transfected cells [reviewed in Bode et al. (1995, 1996)], we investigated the effect of SATB1 on a reporter luciferase gene (Lu) in this experimental system. We have chosen cells that do not normally express SATB1 so that any effect on transcription of a reporter gene could be unambiguously attributed to newly introduced SATB1. During the course of this study, it became evident that cultured cells (NIH3T3, HeLa, BHK, and L cells) stably transfected with a SATB1-expressing construct driven by the SV40 enhancer/promoter could not be maintained due to cell death. For those cells that survived the selection procedure, the SATB1 gene was either deleted or rearranged as shown by abnormally sized messages on Northern blots (data not shown). Overexpression of SATB1 in transfected cells is apparently toxic. This led us to establish a stable cell line in which SATB1 expression remains at a relatively low level. To this end, a pMSG-derived expression construct was stably introduced into BHK cells in which the SATB1 gene was driven by a dexamethasone-inducible MMTV promoter. The individual clones transfected with pMSG-AT1146 and selected for gpt expression by mycophenolic acid were shown to maintain the intact transgene by Southern blots (data not shown). Also, Western blot revealed a full-size SATB1 protein (86 kDa) migrating as 103 kDa on SDS−PAGE (representative data for an isolated cell clone are shown in Figure 1B). We have examined the mycophenolic acid-selected clonal mixture and four individually isolated clones for SATB1 expression. Before induction with dexamethasone, the expression levels were low and ranged far below that of housekeeping genes such as pyruvate kinase and β-actin. Since the SATB1 message virtually escaped detection on Northern blots using 10 μg of total RNA, we chose an RT-PCR protocol for quantification using an appropriate set of primers for the human SATB1 gene. Quantitative RT-PCR measurements were carried out using RNA from human erythroleukemia (K562) cells as a reference. K562 cells express a small amount of SATB1 corresponding to 0.003% of the total protein as opposed to 100-fold more in thymocytes (Dickinson & Kohwi-Shigematsu, 1995). We also used RNA from human promyelotic leukemia (HL60) cells that express a significantly higher level of SATB1 than K562, but its mRNA is still barely detectable on Northern blots under the above conditions. At 35 cycles, the RT-PCR signals appear to be saturated for both RNAs prepared from K562 and HL60 cells. We have chosen 29 cycles of amplification to examine the RT-PCR signals for various amounts of RNA prepared from individually cloned BHK cells stably transfected with SATB1 and control untransfected BHK cells. These measurements demonstrated that SATB1-transfected BHK cells contained a significantly higher level of SATB1 message than K562 cells, and the expression level was almost comparable to that for HL60 cells (Figure 1A, top panel, lanes 4 and 10−13). No SATB1 signal arose from BHK cells mock-transfected with the pMSG plasmid at 29 cycles (Figure 1A, top panel, lanes 7−9), and it remained undetected even under the maximum 35 cycles of PCR amplification used in the RT-PCR measurement (data not shown). BHK cells transfected with pMSG-pAT1146 showed similarly low levels of SATB1 expression when examined either as individually isolated clone cells or as a clonal mixture (data not shown).

As an internal control that allows sample-to-sample comparison and normalization of expression levels, we used a "housekeeping" gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The expression level of GAPDH is known to remain relatively constant among different cell lines (Baier et al., 1993). At 21 cycles of amplification at which the signal for the GAPDH expression level is still far from reaching saturation, the BHK mRNA levels appear to be unchanged between BHK control cells and SATB1-expressing BHK cells (Figure 1A, bottom panels, lanes 7−12). The data show that, at least for this housekeeping gene, SATB1 did not affect its expression.

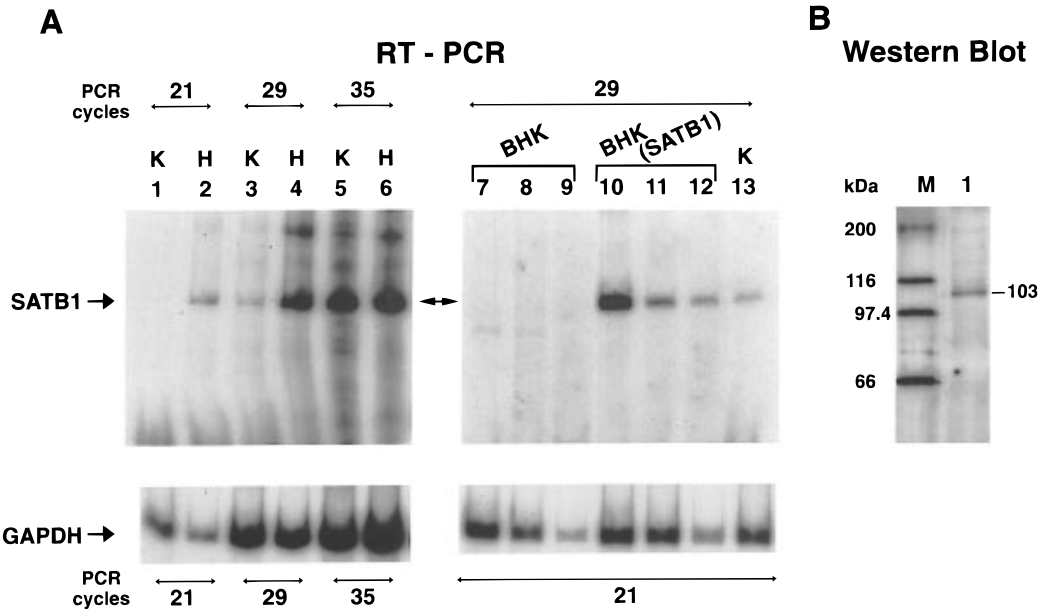

FIGURE 1: Analysis of the BHK cells stably transfected with the SATB1 gene. (A) The level of SATB1 message in a representative isolated clone of SATB1-transfected BHK cells [BHK(SATB1)] was estimated by quantitative RT-PCR in comparison to that in HL60 and K562 cells. The top two panels represent RT-PCR amplification for the SATB1 gene. The number of PCR cycles and cells from which RNA (2 μg) was isolated are designated on top of each lane (abbreviations are K for K562 cells and H for HL60 cells). The left panel shows determination of the optimal PCR cycle (29 cycles) at which a SATB1-amplified band of 510 bp is seen for both K562 and HL60 cells, but at a significantly different intensity (lanes 3 and 4). At a saturation level (35 cycles), the intensity of the band for these two cells becomes equal (lanes 5 and 6). The right panel shows a comparison of the SATB1 gene with PCR of 29 cycles between BHK control cells and BHK(SATB1) cells using 0.125 μg (lanes 9 and 12), 0.5 μg (lanes 8 and 11), and 2 μg (lanes 7 and 10) along with 2 μg of RNA from K562 cells (lane 13). The bottom two panels represent RT-PCR results of the GAPDH gene for the same RNA as indicated on top of each lane. The exact number of PCR cycles for the GAPDH gene are indicated below the lanes. The radiolabeled RT-PCR products were separated on a 1.5% agarose gel, dried, and exposed to an X-ray film. (B) Forty micrograms of proteins extracted from BHK(SATB1) cells (lane 1) was subjected to Western blotting using anti-SATB1 antibody. The full-length SATB1 migrates as a 103 kDa protein on SDS−PAGE. Lane M shows protein size markers in kilodaltons.

Dexamethasone treatment increased the mRNA levels approximately 3-fold (data not shown); however, this level of induction did not change the overall outcome of the results described below. The SATB1-expressing BHK cells showed no apparent difference in growth rate at least for three months after full selection. The relatively low level of SATB1 expression in stably transfected cells, as shown in Figure 1A, was critical for maintaining cell viability to allow us to determine the effect of SATB1 on MAR-linked reporter genes. It should be noted, however, that SATB1 stably transfected cells could not be maintained indefinitely, as they tend to eventually lose a full-length SATB1.

SATB1 Suppresses the Expression of Stably Transfected MAR Constructs. A mycophenolic acid-selected clonal mixture and individually cloned BHK cells stably pretransfected with either the pMSG-SATB1 vector or a pMSG control were submitted to a second round of transfections with constructs that contain the SV40 promoter-driven luciferase gene (Lu) linked to a single MAR at the 5′ end of the reporter gene [$(25)_7$Lu, E-Lu], two flanking MARs (E-Lu-P), an AT-rich, non-MAR sequence [$(24)_8$-Lu], or a control, pLu (see Figure 2A for a map). For this secondary transfection, selection was for G418 resistance. All subsequent analyses were performed with the G418-selected clonal mixture instead of individually isolated clones. E, P, and $(25)_7$ represent MAR sequences, where E corresponds to a major portion of a MAR found upstream from the human interferon-β gene (Bode & Maass, 1988), P represents a MAR upstream of the potato ST-LS1 gene (Dietz et al., 1994), and $(25)_7$ refers to a synthetic MAR element. In this paper, we refer to wild-type $(25)_7$ as $(25)_7$ and mutated $(24)_8$ as $(24)_8$ for convenience. The synthetic MAR, $(25)_7$, was prepared by multimerization (seven times) of 25 bp derived from a MAR 3′ of the IgH enhancer that contains a core unwinding element; the mutated version $(24)_8$ was prepared in a manner similar to that of $(25)_7$ except that the core unwinding element was mutated to make a nonunwinding variant (Kohwi-Shigematsu & Kohwi, 1990; Bode et al., 1992). We confirmed our earlier finding (Bode et al., 1992) in which authentic MAR sequences including naturally occurring MARs as well as the synthetic MAR, but not the mutated AT-rich sequences, augmented the luciferase reporter gene activity in stably transfected cells in the absence of SATB1 expression. The gene activity was measured by both the luciferase activity (relative light units, solid bar) (Figure 2B) and the mRNA level by Northern blots analysis (Figure 2C). In BHK cells containing pMSG-p1146 that are expressing SATB1, the luciferase gene activity was dramatically reduced in every case of MAR-linked reporter gene constructs. This strong suppression effect of SATB1 on MAR-linked reporter genes was observed either when the mycophenolic acid-selected clones (SATB1-expressing BHK cells containing pMSG-p1146) were examined as a pool or when isolated clones were individually grown and examined (Figure 2B, hatched bars). For the luciferase gene surrounded by two MARs (E-Lu-P), the gene activity was virtually abolished (Figure 2B,C, hatched bars). Because the MAR-linked reporter gene activity was examined as a G418-selected clonal mixture, this strong suppression of reporter gene activity in the E-Lu-P construct suggests that SATB1 is capable of suppressing MAR-linked reporter genes regardless of integration sites in the genome under this experimental condition. On the other hand, the low levels of luciferase gene activity of pLu or $(24)_8$-pLu remained

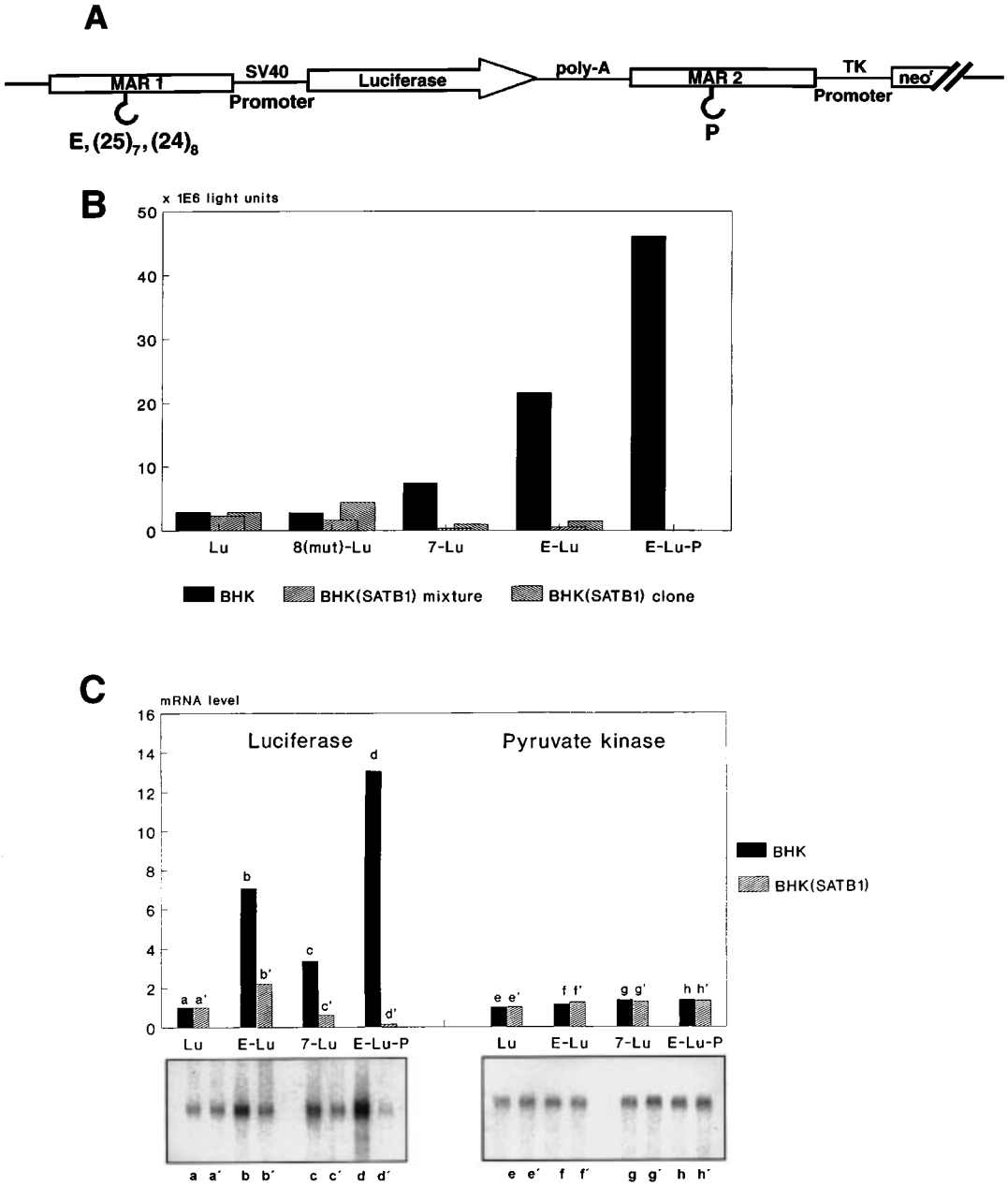

FIGURE 2: SATB1-mediated transcriptional suppression of stably expressed S/MAR constructs. (A) The map for the MAR-linked luciferase gene constructs. (B) Luciferase measurements on a reporter construct (Lu) and its derivatives. The indicated single- or double-MAR constructs [$(25)_7$-Lu, E-Lu, and E-Lu-P] or controls [Lu and $(24)_8$−Lu], respectively, were transfected into three populations of BHK cells. Population 1 (solid bars) contained stably integrated copies of the pMSG vector plasmid. Population 2 (darker hatched bars) represents the mycophenolic acid-selected clonal mixture of BHK cells transfected with pMSG-AT1146, and population 3 refers to a representative cloned cell line derived from population 2 (lighter hatched bars). Subsequent analyses were performed with a G418-selected clonal mixture stably transfected with MAR-linked reporter gene constructs. Both cell populations 2 and 3 expressed similarly low levels of SATB1 from an integrated pMSG-AT1146 construct, and the results of the luciferase reporter gene activity were similar as indicated by related but distinguishable forms of cross-hatching. (C) Expression levels deduced from Northern blots. The mRNAs from the same series of transfectants were quantified by successive hybridizations with a probe for luciferase and for a housekeeping gene (pyruvate kinase). Data are shown for BHK populations 1 and 2 transfected with MAR-linked reporter gene constructs or the control construct (pLu). The intensity of hybridized signals was quantitated by laser densitometer scanning of short-exposure autoradiographs of the dried Northern blots shown below the bar diagram. The bar diagram represents the mRNA levels relative to that of BHK cells transfected with pLu. The results shown in panels B and C are representative data of at least four independent sets of experiments. The standard deviation for the extent of suppression of the reporter gene due to SATB1 expression remained within ±20%.

unaffected by the presence of SATB1 (Figure 2B,C). This is supported by previous analyses according to which SATB1 binds only to $(25)_7$ but not to $(24)_8$ (Dickinson et al., 1992), clearly distinguishing $(25)_7$, with high unwinding potential and high affinity to nuclear matrix, from its similarly AT-rich mutant, $(24)_8$, which lacks both unwinding property and binding affinity to the nuclear matrix. These data show that, in these transfected cells, SATB1 has a suppression effect on MAR-linked reporter genes. It is noteworthy that the MAR-linked reporter gene construct that normally conferred the highest transcriptional augmentation is most severely affected in the presence of SATB1.

In addition to GAPDH, we investigated the level of expression of another housekeeping gene, pyruvate kinase, as a control. In all stably transfected cells, either in the presence or in the absence of SATB1, the pyruvate kinase gene mRNA level remained unchanged (Figure 2, bottom panel). These data, together with the result for GAPDH,

confirms that SATB1 does not affect expression of housekeeping genes. These data also show that the suppression effect on MAR-linked reporter gene activity which is ascribed to SATB1 is not due to cell death. The stably transfected SATB1-expressing BHK cells which we isolated grow at a rate similar to that of control BHK cells at least during the first three months after selection, and the transcriptional suppression effect by SATB1 is apparently selective.

We have also performed transient expression experiments to examine the effect of SATB1 on MAR-linked reporter genes. However, it was difficult to draw a clear conclusion from such studies. Although we detected a SATB1 suppression effect, the extent of suppression varied greatly (anywhere from 10 to 50% suppression) among independently performed experiments. Therefore, the full repressing effect appears to require integration of the transfected DNAs. Some residual repression in the transient experiments may depend on a transient association of the transfected DNAs with the nuclear matrix. A higher variability within this series of experiments may depend both on the nature of this association and on the difficulties in controlling the variability which is intrinsic to this technique.

Although their number is still small, there are other proteins known to share similar binding specificity with SATB1. This includes nucleolin (Dickinson & Kohwi-Shigematsu, 1995), breast cancer-associated protein p114 (Yanagisawa et al., 1996), and a B cell factor Bright (Herrscher et al., 1995). Bright is expressed in mature B cells as opposed to SATB1 which is expressed in immature T cells. In contrast to SATB1, Bright enhances the CAT gene if it is linked to the IgH enhancer including the surrounding MARs (Herrscher et al., 1995). There is also a report that nucleolin is a transcriptional suppressor (Yang et al., 1994).

Our findings that a homeodomain protein SATB1 strongly suppresses transcription of MAR-linked reporter genes have important biological implications. SATB1, which is predominantly expressed in thymocytes, may have a specific role in T cell development through regulation of gene expression by binding to the BURs of MAR segments. We have recently shown that SATB1 binds to specialized genomic segments containing ATC sequences *in vivo* in thymocytes (I. deBelle, S. Cai, and T. Kohwi-Shigematsu, manuscript in preparation). Important questions such as whether SATB1 binds to a selected group of ATC sequences in thymocytes to regulate a specific set of genes must await further study.

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