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Transcription Modulation by a Rat Nuclear Scaffold Protein, P130, and a Rat Highly Repetitive DNA Component or Various Types of Animal and Plant Matrix or Scaffold Attachment Regions

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The *Xmn*I fragment, a highly repetitive DNA component, and animal and plant matrix or scaffold attachment region (MAR/SAR) were examined for similarity in interaction with nuclear scaffold. As the *Xmn*I fragment bound a 130 kDa scaffold protein (P130) *in vitro,* various types of MAR/SAR fragments could bind 130 and 123 kDa scaffold proteins. The native *Xmn*I and MAR/SAR fragments clearly augmented SV40 promoter-mediated luciferase gene transcription following transient transfection of recombinant plasmids into various types of recipient cells. In contrast, the *Xmn*I fragment methylated at the cytosine base of the unique *Hin*dIII site, and a synthetic variant DNA deficient in base unpairing characteristic of MAR/SAR, could neither bind P130 nor augment this transcription. These two types of genomic regions appeared to have similar properties of interaction with nuclear scaffold, by which the activity of appropriately positioned promoter can be modulated. © 2000 Academic Press

Key Words: **nuclear scaffold protein; highly repetitive DNA component; matrix or scaffold attachment region.**

Highly repetitive DNA components are located in heterochromatinic regions of the interphase nucleus and in the centromeric and telomeric regions of metaphase chromosomes (1–5). We have cloned a highly repetitive DNA component from fragments resulting from digestion by *Hin*dIII of rat genomic DNA recovered from liver nuclear scaffold (6). Since permutation of the *Hin*dIII fragment indicated DNA bend maximally expressed in the *Xmn*I fragment, this fragment was examined for properties of its interaction with nuclear scaffold (7, 8). The *Xmn*I fragment preferentially bound a 130 kDa scaffold protein. Based on this property, this protein was purified and termed as P130. P130 was hyperphosphorylated form of P123 with an apparent size of 123 kDa, and more readily bound the *Xmn*I fragment than P123 (9). The *Xmn*I fragment could be methylated both *in vivo* and *in vitro* on cytosine of the unique *Hin*dIII site. In a Southwestern analysis, the methylated *Xmn*I fragment did not bind P130, and instead bound an 83 kDa polypeptide (10). Therefore, the interaction of the *Xmn*I segments in the genome with nuclear scaffold appeared to be regulated in two distinct fashions: phosphorylation of P123 and dephosphorylation of P130, and methylation and demethylation of the *Hin*dIII site. On the other hand, animal and plant genomes carry a matrix or scaffold attachment region (MAR/SAR) which also interacts with nuclear matrix. MAR/SAR positioned in the vicinity of a promoter can reportedly augment transcription of a reporter gene, when the recombinant construct is integrated into the genome of the host animal, suggesting that MAR/SAR plays roles in modulating transcription of nuclear genes (11–13). In addition, methylation and demethylation of DNA appeared to regulate chromatin remodeling in transcription regulation of nuclear genes (14). We therefore examined whether, like MAR/SAR, the native and methylated *Xmn*I fragment can modulate the activity of transcription through interaction with proteins. We found in the present study that a rat highly repetitive DNA component and various types of MAR/SAR fragments interact similarly with nuclear scaffold and augment SV40 promoter-mediated luciferase gene transcription following transient transfection of recombinant plasmids.

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MATERIALS AND METHODS

Cell lines. All cell lines used were from the Health Science Research Resources Bank (Osaka, Japan). Ac2F and AH66tc (rat hepatoma cell lines), CHO and HeLa S3, and NIH3T3 cells were respectively maintained in monolayer culture with minimum essential medium, Ham F12 medium, and Dulbecco's modified Eagle medium each containing 10% fetal bovine serum and appropriate concentrations of antibiotics under 5% CO_2 and 95% air at 37°C. Culture media (Gibco/BRL) and all other materials were purchased through local distributors.

Plasmids carrying the XmnI and MAR/SAR fragments. pGL-3 promoter vector (Promega Corp., Madison) was used to determine the extent of augmentation of SV40 promoter-mediated luciferase gene transcription by various types of DNA fragments tethered upstream from the stretch encompassing SV40 promoter and the firefly luciferase gene. The genomic fragments used were the 370 bp *Xmn*I fragment (*Xmn*I) (cf. Fig. 2) (7, 8); IgH enhancer 3′-MAR (Cu300) and 5′-MAR (Cu700) (15); heptamer of a 26-bp long base unpairing region of Cu300 (Wild) and octamer of its 25-bp long mutant sequence deficient in base unpairing due to substitution of ATATAT in Wild with CTGCT (Mut) (15); the fragment between nucleotides 564 to 995 of the human interferon β gene (hINFβ) (16); and a plant MAR, ST-LS1, comprising nucleotides 2643 to 3140 of the potato *ST-LS1* gene (17). Plasmid DNA was propagated in *E. coli* DH5 α and the final preparation was obtained by CsCl equilibrium centrifugation.

Methylation of the XmnI fragment. pGL-3-*Xmn*I (20 μg) was incubated with 0.1, 1.0, or 10 units of M.*Alu*I, which specifically methylates the cytosine base of *Hin*dIII site (Takara, Tokyo), at 37°C for 3 h. Aliquots of the plasmids methylated and extracted with phenol/chloroform were digested with *Hin*dIII. Based on the fact that the *Xmn*I fragment has one *Hin*dIII site (cf. Fig. 2), the extent of methylation was determined by amounts of the fragments uncut (370 bp) and cut by *Hin*dIII.

Determination of the extent of augmentation of transcription. Lipofection of the recombinant pGL-3 and pRL-SV40 with Transfectam (Promega Corp.) into cells subcultured in dishes with a diameter of 60 mm, preparation of cell lysate, and determination of luciferase activity using a dual luciferase assay kit on TOPCOUNT (Packard Instrument Co., Inc.) were performed according to the supplier's instructions. The extent of augmentation of transcription was determined as luciferase activity relative to control activity exhibited by cells that received wild type pGL-3 promoter vector and pRL-SV40.

Southwestern analysis. The P fraction prepared from nuclei purified from rat liver was denatured and solubilized in the presence of sodium dodecylsulfate (SDS) and 2-mercaptoethanol at room temperature. Equal volumes of the solubilized P fraction were developed by polyacrylamide gel electrophoresis in the presence of SDS (SDS–PAGE), and processed as described previously (7, 8). The *Xmn*I and MAR/SAR fragments labeled with [α-^{32}P] dCTP were used as probes.

RESULTS

Augmentation of SV40 promoter-mediated transcription. For assay of luciferase, Ac2F cells that received pGL-3-*Xmn*I carrying the *Xmn*I fragment were lysed at various intervals of incubation after lipofection. As shown in Fig. 1, cells at 24 and 36 h of incubation exhibited luciferase activity five to six times the basal activity revealed by cells that received the wild-type pGL-3 promoter vector. pGL-3-Wild carrying a heptamer of a synthetic base unpairing region within IgH 3′-MAR also appeared to augment luciferase production, but to twice control level at comparable periods of incubation. pGL-3-Mut, deficient in base unpairing, exhibited no significant augmentation over the time course examined. These findings clearly demonstrated that the *Xmn*I fragment and the synthetic base unpairing region of MAR/SAR augmented SV40 promoter-mediated luciferase gene transcription probably through interaction of the inserted DNA fragments with proteins. That prolonged incubation of these transfected cells reduced luciferase activity suggested both structural alteration of the recombinant pGL-3 vectors and the importance of timing of assaying for luciferase.

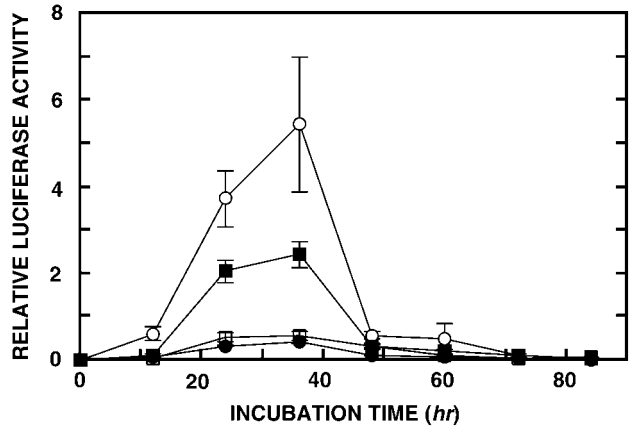

FIG. 1. Induction of luciferase by transient transfection of recombinant plasmids with either the *Xmn*I fragment or the base unpairing region. Ac2F cells that received pGL-3-*Xmn*I (○), pGL-3-Wild (■), pGL-3-Mut (□), or pGL-3 promoter vector (●) were cultured and their lysates were prepared at the indicated intervals. Luciferase activities relative to control are expressed as the mean value of three experiments ± SD.

Requirement of an AT-Rich region with bend center for augmented expression of luciferase. Various mutants each carrying part of the *Xmn*I fragment were prepared and examined for transcription augmentation. As shown in Fig. 2, luciferase activity expressed by pGL-3-*Xmn*I was four times basal activity, confirming the result shown in Fig. 1. pGL-3-X/H carrying the 5′ half of the *Xmn*I fragment exhibited a similar level of luciferase activity. Since pGL-3-Ha/H and pGL-3-D/H, both of which include the region between the sites for *Dra*I and *Hin*dIII, exhibited luciferase activity over ten times control level, the region between the 5′ *Xmn*I and *Hae*III sites appeared to have an inhibitory effect on transcription augmentation by the region between the sites for *Dra*I and *Hin*dIII. The region between the sites for *Dra*I and *Hin*dIII thus appeared to be required for augmentation of luciferase gene transcription. Related to this, the *Xmn*I fragment has three structural features. First, an AT-rich region which is protected in

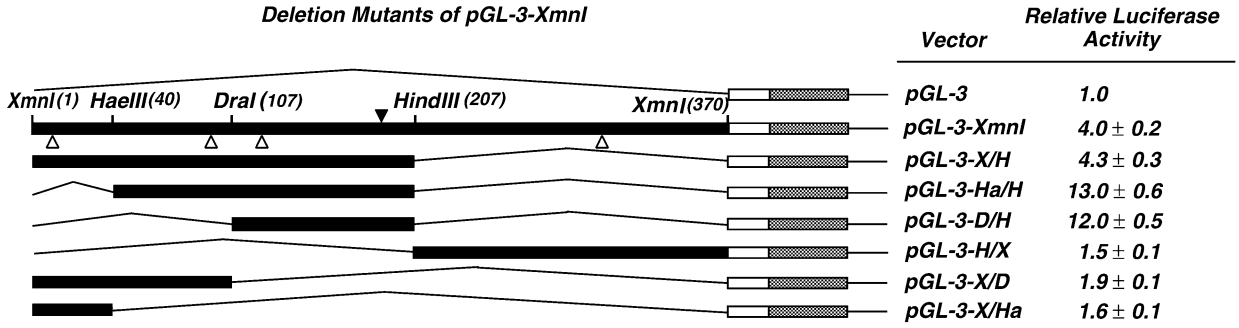

FIG. 2. Requirement of a region in the *Xmn*I fragment for the augmentation of transcription. DNA segments prepared from the *Xmn*I fragment and tethered upstream from the stretch encompassing SV40 promoter (open bar) and the firefly luciferase gene (shaded bar) were illustrated with their relative luciferase activity. Inserted and deleted regions of DNA were illustrated with filled bars and lines, respectively. Recognition sites for restriction endonucleases were indicated together with nucleotide number parenthesized over the length of the *Xmn*I fragment. Filled and open triangles respectively denote positions of the bend center and AT-rich regions.

the complex of the *Xmn*I fragment and purified P130 from digestion by DNaseI is present near the *Dra*I site (18). An ACATAT sequence similar to the ATATAT sequence designated in the IgH3′ MAR as the base unpairing region, which actually augmented transcription in pGL-3-Wild, begins seven bp downstream from the *Dra*I site (cf. Fig. 2). Second, the *Xmn*I fragment forms the DNA bend (6). The bend center was presumed to be present about 20 bp upstream from the *Hin*dIII site. Third, the cytosine base in this *Hin*dIII site is methylated *in vivo* and *in vitro* to control binding of the *Xmn*I fragment and P130 or an 83 kDa polypeptide (10). The region between the *Dra*I and *Hin*dIII sites features all three of these characteristics.

Inability of the methylated XmnI fragment to augment transcription. pGL-3-*Xmn*I methylated by M. *Alu*I *in vitro* was examined for augmentation of transcription. As shown in Fig. 3, luciferase activity revealed by the wild-type pGL-3 promoter vector was not affected by methylation. While Ac2F cells that received the native pGL-3-*Xmn*I induced luciferase to a level comparable to that shown in Fig. 1, the pGL-3-*Xmn*I methylated with increasing amounts of M. *Alu*I clearly reduced luciferase induction. Finally, pGL-3-*Xmn*I maximally methylated with 10 units of M. *Alu*I for 3 h became resistant to digestion with *Hin*dIII and inert with respect to augmentation of luciferase gene transcription. A similarly methylated *Xmn*I fragment did not bind to P130 and bound to an 83 kDa polypeptide in Southwestern analysis (10). If this alteration occurs *in vivo*, then the transcription augmentation by the *Xmn*I fragment is due to the required binding of the *Xmn*I fragment and P130.

Augmentation of transcription by the XmnI and MAR/SAR fragments in various cell lines. Table 1 summarizes levels of luciferase induction during 24-h incubation in five different types of cell lines following transient transfection of recombinant pGL-3 plasmids each carrying either the *Xmn*I fragment or distinct MAR/SAR fragments. In Ac2F cells, pGL-3-Cu700 and pGL-3-*Xmn*I augmented production of luciferase to five to six times control level. This augmentation appeared to be three times stronger than that with pGL-3-hINFβ. In AH66tc cells, pGL-3-Cu700 most strongly enhanced luciferase production (12 times control level). This extent is three to four times higher than those with plasmids carrying other DNA fragments. Moreover, pGL-3-Cu700 yielded twice control level in HeLa S3 cells, in which pGL-3-*Xmn*I induced luciferase activity twice that induced by pGL-3-Cu700. In contrast, pGL-3-hINFβ and pGL-3-Cu300 appeared to equally augment luciferase production in all cell lines tested. This variability in extent of augmentation suggests that proteins interacting with the DNA segments tested are in part intrinsic to individual cell lines.

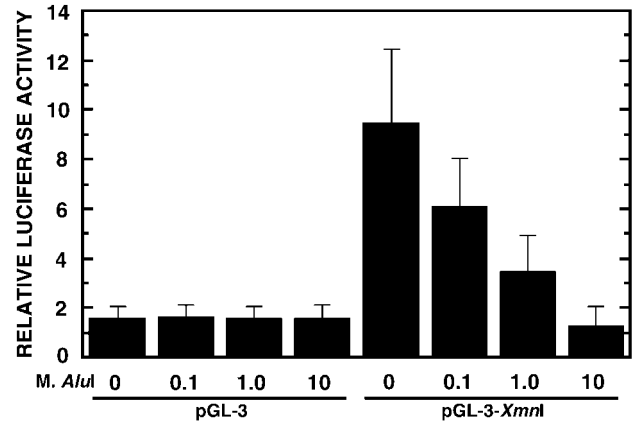

FIG. 3. Suppression of luciferase induction by methylation of pGL-3-*Xmn*I. Lysates were prepared at 24 h of incubation of Ac2F cells transfected with pGL-3-*Xmn*I and pGL-3 promoter vector which were methylated to variable extents, and luciferase activity was assayed.

TABLE 1

Augmentation of SV40 Promoter-Mediated Transcription in Five Different Cell Lines by Various Types of MAR/SAR Fragments

Cell line	MAR/SAR fragment						
	*Xmn*I	Cu300	Cu700	Wild	Mut	hINFβ	ST-LS1
Ac2F	5.1 ± 0.3	1.9 ± 0.4	6.3 ± 0.6	3.3 ± 0.7	1.0 ± 0.4	2.1 ± 0.3	3.6 ± 0.7
AH66tc	3.9 ± 0.3	2.7 ± 0.2	12.0 ± 0.6	3.9 ± 0.2	1.4 ± 0.1	2.8 ± 0.3	4.3 ± 0.2
NIH3T3	3.9 ± 0.4	2.0 ± 0.5	2.6 ± 0.6	6.9 ± 0.5	1.6 ± 0.2	2.8 ± 0.3	2.2 ± 0.4
CHO	5.7 ± 0.9	1.9 ± 0.1	7.0 ± 0.3	3.2 ± 0.2	0.7 ± 0.1	2.5 ± 0.1	2.5 ± 0.2
HeLa S3	5.4 ± 1.0	1.7 ± 0.3	2.5 ± 0.5	3.2 ± 1.0	1.1 ± 0.4	2.5 ± 0.5	2.9 ± 0.2

Note. Various types of cells were transfected with recombinant plasmids each carrying the indicated DNA fragments and cultured for 24 h. Cell lysates were assayed for luciferase activity.

Binding of the MAR/SAR fragments to proteins with sizes similar to P130. Equal amounts of proteins solubilized from the P fraction isolated as scaffold fraction from rat liver nuclei were probed with the seven radioactive DNA fragments with identical specific radioactivity. As can be seen from Fig. 4, with the exception of Mut, all of the probes exhibited signals indicating binding to a polypeptide with an apparent molecular mass of 130 kDa. In addition to this signal, the hINFβ and Cu300 probes appeared to bind to another polypeptide of approximately 120 kDa, a size similar to that of P123 which is the hypophosphorylated isoform of P130. In this regard, under similar conditions the native *Xmn*I probe exhibited signals corresponding to sizes of 130 and 123 kDa. Based on this property of interaction of the *Xmn*I fragment and scaffold proteins, P130 was purified from rat liver nuclear scaffold (8). Therefore, the polypeptide bound with the six DNA probes may be P130 and P123, indicating that P130 and P123 similarly recognize a highly repetitive DNA component and the MAR/SAR fragments examined.

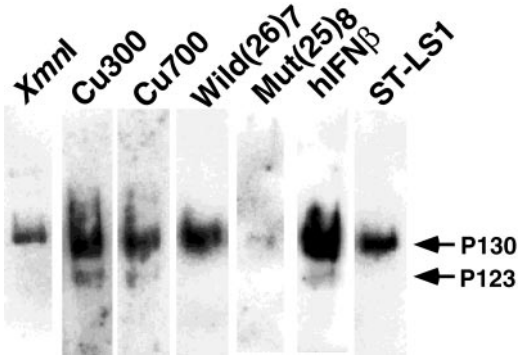

FIG. 4. Binding of the *Xmn*I and MAR/SAR fragments to P130 and P123. Proteins recovered from rat liver nuclear scaffold were developed by SDS–PAGE and probed with DNA fragments indicated. Positions of P130 and P123 were indicated.

DISCUSSION

In the present study, the *Xmn*I fragment, a rat highly repetitive DNA component, augmented the SV40 promoter-mediated luciferase gene transcription determined by transiently expressed luciferase activity in various types of cells. Moreover, the *Xmn*I fragment with the methylated *Hin*dIII site did not augment this transcription. Since Hibino *et al.* have demonstrated that the binding of the *Xmn*I fragment and P130 *in vitro* is inhibited by methylation of the cytosine base at this *Hin*dIII site (10), the suppression of the transcription augmentation by methylation indicates the requirement of P130 for processes of transcription augmentation. Further, as the *Xmn*I fragment bound P130 in a Southwestern analysis, various types of MAR/SAR fragments bound two nuclear scaffold proteins with sizes similar to those of P130 and its isoform, P123. Base unpairing appeared to play principal roles in both the binding of MAR/SAR fragments and P130, and augmentation of luciferase gene transcription. MAR/SAR segments in recombinant plasmids, therefore, can interact with P130, and the interaction of MAR/SAR with P130 appeared to be required for augmentation of luciferase gene transcription. The present study thus for the first time demonstrated the similarity of a highly repetitive DNA component to MAR/SAR in interaction with a scaffold protein, P130, by which appropriately positioned promoter is activated.

An AT-rich sequence was assigned to a base unpairing region necessary for interaction of a MAR/SAR fragment with a nuclear matrix protein, SATB1 (special AT-rich sequence binding-protein 1), in thymus and testis (15). In the present study, P130 also bound DNA having the synthetic ATATAT sequence, but not a variant DNA, indicating that P130 distinct from SATB1 likewise requires base unpairing to recognize its binding site in genomic DNA. We have confirmed that in rat, P130 is present in all tissues examined including thymus (Hibino *et al.*, unpublished observa-

tion). Highly repetitive DNA components are detectable in the centromeric regions in the metaphase nucleus by *in situ* hybridization using a 92-bp long component prepared by digestion of the rat genomic DNA with *Eco*RI (5). We confirmed their centromeric localization using the *Xmn*I fragment (370 bp in size) as a probe (not shown). On the other hand, MAR/SAR segments are reportedly expected to be present at both up- and downstream boundaries of nuclear genes, and are correspondingly distributed at ~100 kb intervals in the genome (19). Therefore, P130 can bind, on the one hand, to highly repetitive DNA components in centromeric regions, and on the other hand, to MAR/SAR segments widely dispersed in stretches of chromosomal DNA. In this context, signals expressed by *in situ* hybridization clearly indicate that a large number of repetitions are present in the centromeric regions, but do not exclude the possibility that single segments of the highly repetitive DNA component are dispersed over stretches of chromosomal DNA. Like the repetitive DNA components in centromeric regions, their dispersed segments, if present in the genome, can be binding sites of P130. P130 thus appears to be an important protein by which chromatins are anchored to nuclear scaffold in most types of cells in rat.

It is unclear at present which subnuclear space is the site for transcription of recombinant constructs transiently transfected. Association of the recombinant construct with nuclear scaffold is considered likely. Alternatively, since different types of cells exhibited variable extents of luciferase activity, it is conceivable that P130 and proteins similar to P130 are also present in a nuclear space from which these proteins might be readily extractable with a high concentration of salt together with other proteins. In either case, the binding of P130 to the *Xmn*I and MAR/SAR fragments might result in circumstances under which proteins required to form the structural and functional basis of chromatins are more efficiently assembled. If this is the case, then the transcription augmentation observed is considered to reflect efficacy of binding of P130 and the highly repetitive DNA component or MAR/SAR on assembly of transcription machinery. Methylation of the *Hin*dIII site in the *Xmn*I fragment appears likely to function to regulate, with the aid of demethylation, reversible appearance and disappearance of binding sites of P130 on chromosomal DNA. Therefore, methylation of the *Hin*dIII site of highly repetitive DNA components might be one of underlying mechanisms of chromatin remodeling in which P130 can play roles presumably together with the methyl-CpG-binding protein 2 (MeCP2) with size of 83 kDa (20). Similar properties have not been reported for MAR/SAR. Detailed characterization of the structure, function, and subnuclear localization of P130 and of the precise distribution of a highly repetitive DNA component over the chromosomal DNA is required to clarify interesting functions of a nuclear scaffold protein, P130.

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REFERENCES

1. Brutlag, D. L. (1980) Molecular arrangement and evolution of heterochromatic DNA. *Ann. Rev. Genet.* **14,** 121–144.
2. Singer, M. F. (1982) Highly repeated sequences in mammalian genomes. *Int. Rev. Cytol.* **76,** 67–112.
3. Masumoto, H., Sugimoto, K., and Okazaki, T. (1989) Alphoid satellite DNA is tightly associated with centromere antigens in human chromosomes throughout the cell cycle. *Exp. Cell Res.* **181,** 181–196.
4. Radic, M. Z., Lundgren, K., and Hamkalo, B. A. (1987) Curvature of mouse satellite DNA and condensation of heterochromatin. *Cell* **50,** 1101–1108.
5. Sealy, L., Hartley, J., Donelson, J., Chalkley, R., Hutchison, N., and Hamkalo, B. (1981) Characterization of a highly repetitive sequence DNA family in rat. *J. Mol. Biol.* **145,** 291–318.
6. Nakamura, K., Ikeda, Y., Iwakami, N., Hibino, Y., and Sugano, N. (1991) Bending of a highly repetitive component in rat nuclear DNA. *Biochem. Int.* **25,** 355–362.
7. Hibino, Y., Nakamura, K., Asano, S., and Sugano, N. (1992) Affinity of a highly repetitive bent DNA for nuclear scaffold proteins from rat liver. *Biochem. Biophys. Res. Commun.* **184,** 853–858.
8. Hibino, Y., Nakamura, K., Tsukada, S., and Sugano, N. (1993) Purification and characterization of nuclear scaffold proteins which bind to a highly repetitive bent DNA from rat liver. *Biochim. Biophys. Acta* **1174,** 162–170.
9. Hibino, Y., Ohzeki, H., Hirose, N., and Sugano, N. (1998) Involvement of phosphorylation in binding of nuclear scaffold proteins from rat liver to a highly repetitive DNA component. *Biochim. Biophys. Acta* **1396,** 88–96.
10. Hibino, Y., Ohzeki, H., Hirose, N., Morita, Y., and Sugano, N. (1998) Involvement of DNA methylation in binding of a highly repetitive DNA component to nuclear scaffold proteins from rat liver. *Biochem. Biophys. Res. Commun.* **252,** 296–301, doi:10.1006/bbrc.1998.9620.
11. Xu, M., Hammer, R. E., Blasquez, V. C., Jones, S. L., and Garrard, W. T. (1989) Immunoglobulin κ gene expression after stable integration. *J. Biol. Chem.* **264,** 21190–21195.
12. Forrester, W. C., van Genderen, C., Jenuwein, T., and Grosschedl, R. (1994) Dependence of enhancer-mediated transcription of the immunoglobulin μ gene on nuclear matrix attachment regions. *Science* **265,** 1221–1225.
13. Jenuwein, T., Forrester, W. C., Fernández-Herrero, L. A., Laible, G., Dull, M., and Grosschedl, R. (1997) Extension of chromatin accessibility by nuclear matrix attachment regions. *Nature* **385,** 269–272.
14. Poljak, L., Seum, C., Mattioni, T., and Laemmli, U. K. (1994) SARs stimulate but do not confer position independent gene expression. *Nucl. Acids Res.* **22,** 4386–4394.

15. Dickinson, L. A., Joh, T., Kohwi, Y., and Kohwi-Shigematsu, T. (1992) A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. *Cell* **70,** 631–645.
16. Klehr, D., Maass, K., and Bode, J. (1991) Scaffold-attached regions from the human interferon β domain can be used to enhance the stable expression of genes under the control of various promoters. *Biochemistry* **30,** 1264–1270.
17. Stockhaus, J., Eckes, P., Blau, A., Schell, J., and Willmitzer, L. (1987) Organ-specific and dosage-dependent expression of a leaf/stem specific gene from potato after tagging and transfer into potato and tobacco plants. *Nucl. Acids Res.* **15,** 3479–3491.
18. Hibino, Y., Tsukada, S., and Sugano, Y. (1993) Properties of a DNA-binding protein from rat nuclear scaffold fraction. *Biochem. Biophys. Res. Commun.* **197,** 336–342, doi:10.1006/bbrc.1993.2480.
19. Schübeler, D., Mielke, C., Maass, K., and Bode, J. (1996) Scaffold/Matrix-attached regions act upon transcription in a context-dependent manner. *Biochemistry* **35,** 11160–11169.
20. Nan, X., Ng, H.-H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., and Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393,** 386–389.