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Specificity of SAF-A and Lamin B Binding In Vitro Correlates With the Satellite DNA Bending State

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Abstract There is evidence that Matrix Attachment Region (MAR)-binding proteins also bind satellite DNA (satDNA). The aim of the current work was to determine whether the major nuclear matrix (NM) MAR-binding proteins are able to recognize satDNAs of different locations and what DNA structural features are important for the recognition. In nuclei and NM, a number of the same polypeptides were recognized on a southwestern blot when MAR of immunoglobulin κ gene (Ig κ MAR) and pericentromeric (periCEN) satDNA fragments were used. However, the binding decreased dramatically when human and mouse CEN satDNA were used for the probes. After an NM extract was subjected to ion exchange chromatography, the main DNA-binding proteins were identified as SAF-A (scaffold attachment factor A) and lamin B. It was not possible to test the binding of lamin B by gel mobility shift assay (GMSA), but SAF-A showed an ability to distinguish CEN and periCEN satDNA fragments in GMSA. While periCEN fragments have an abnormally slow mobility on electrophoresis, which is a hallmark of bent DNA, CEN satDNA fragments have a normal mobility. A computer analysis was done using the wedge model (Ulanovsky and Trifonov [1987] Nature 326:720–722), which describes how the curved state depends on particular nucleotide sequences. The curved states of the fragments predicted by the model are in good agreement with their ability to be recognized by NM proteins. Thus SAF-A and lamin B are able to recognize conserved structural features of satDNA in the same way that MAR-binding proteins recognize MARs in spite of a lack of a consensus sequence. CEN and periCEN satDNAs are distinguished by proteins in correlation with the helical curvature of these fragments. J. Cell. Biochem. 83: 218–229, 2001.

Key words: nuclear matrix; satellite DNA; heterochromatin; DNA curvature; protein/DNA sequence specific interaction

The nuclear matrix/scaffold (NM) binds particular heterogeneous non-homologous sequences in promoter regions and introns of some genes; these regions are referred to as Matrix or Scaffold Attachment Regions (MARs) [Boulikas, 1995]. MARs are believed to be located at the base of the chromatin loops and to be responsible for the loop attachment to the NM. MARs are relatively rare in the euchromatic regions, occurring on average at every 75 kb. The total number of MARs in the eukaryotic genome is estimated to be no more than 1×10^5, corresponding to about 1% of the genome. A number of proteins that bind MARs selectively in vitro have been found in higher eukaryotes [Boulikas, 1995].

The known MAR-binding proteins (Table I) have the same main binding features as the whole NM. The affinity of binding decreases with decreasing fragment length. MAR-binding proteins recognize the structural features of a sequence rather than the sequence itself [Romig et al., 1992; Tsutsui et al., 1993; Renz and Fackelmayer, 1996]. As a result, MAR-binding proteins can bind fragments specifically without sequence homology in contrast to sequence-specific binding proteins such as transcriptional factors or restriction endonucleases. Usually MAR-binding proteins bind DNA through a narrow minor groove of oligo(dA) islets. Histone H1 and topoisomerase II (TopoII) specifically and cooperatively bind

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TABLE I. MAR- and satDNA-Binding Proteins

Protein	M.w. kDa	MAR-binding	References	SatDNA-binding	References
Topo II	165–180	+	Adachi et al. [1989]	+	Consensus in satDNA, Kas and Laemmli [1992]
SAF-B	150	+	Renz and Fackelmayer [1996]		
SAF-A/SP120/ hnRNP U	120	+	Romig et al. [1992]; Kiledjian and Dreyfuss [1992]; Tsutsui et al. [1993]; von Kries et al. [1994]; Fackelmayer et al. [1994]	+	Lobov et al. [1998], 2000
ARBP/MeCP2	83	+	von Kries et al. [1991]; Buhrmester et al. [1995]	+	Hibino et al. [1998]
Lamins	60–70	+	Luderus et al. [1992], 1994	+	Current paper
Histone H1	35	+	Izaurralde et al. [1989]		
P130/matrin 3	130			+	Hibino et al. [1998]
CENPB	80			+	Masumoto et al. [1989]; Kipling and Warburton [1997]
W-BP/p70	70			+	Harata et al. [1988]. Podgornaya et al. [2000]; Enukashvily et al. [2000]
D1/HMG-Iα	50	+	Disney et al. [1989]; Ashley et al. [1989]	+	Levinger and Varshavsky [1982]; Strauss and Varshavsky [1984]

+ — the protein was fist tested according to its indicated binding capacity, + — the binding capacity was reported later, blank box — no information.

MARs in a narrow minor groove of several consecutive A/T residues. The same has been shown for High Mobility Group (HMG) proteins [Solomon et al., 1986], which compete with histone H1 for MAR binding in vivo [Zhao et al., 1993]. There is a DNA-binding motif TPKRPRGRPKK in HMG-I(Y) called the "AT hook" [Reeves and Nissen, 1990]. Artificial synthetic polipeptides with 10–20 copies of this motif have the ability to bind MARs in vitro and in vivo [Strick and Laemmli, 1995]. D1/HMG-Iα protein appears to be a natural analog of such artificial proteins. Though initial studies tested the binding of D1/HMG-Iα to satellite DNA (satDNA), in vivo, it binds to euchromatic A/T-rich sequences, i.e., MARs [Ashley et al., 1989].

Paradoxically, the main part of the residual NM DNA is satDNA, although MARs were defined according to their ability to be bound to the NM. Highly repetitive DNA components (satDNAs) have been demonstrated to be located preferentially in the heterochromatic region of the interphase nucleus and in the centromeres and subtelomeres of metaphase chromosomes [Manuelidis, 1997; Donev and Djondjurov, 2000]. SatDNA may form the framework of the interphase nucleus and/or metaphase chromosomes [Razin, 1987; Manuelidis, 1997]. Such repetitive components have been shown to have some properties characteristic of bent DNA [Radic et al., 1987; Martinez-Balbas et al., 1990; Hibino et al., 1992].

Alphoid satDNA (α-satDNA) is the main and probably the only class of tandem repeats in the primate centromere (CEN). α-satDNA accounts for 10% of the genome, is built of monomers of 170–172 bp and has been mapped to CEN of all human chromosomes [Willard and Waye, 1987]. It has been shown for chromosomes 7, 21, and X that the CEN region is built of two α-satDNA subclasses [Waye et al., 1987; Wevrick and Willard, 1991; Ikeno et al., 1994; He et al., 1998]. Probably the same is true for the rest of the chromosomes. The CEN and pericentromeric (periCEN) subclasses are named αI and αII, respectively [He et al., 1998]. Fluorescent in situ hybridization (FISH) shows that the αI-array is represented by two dots, 1 per each chromosome, while the α II-array looks like a thread connecting the αI dots [He et al., 1998]. The αI-block apparently represents the CEN kinetochore domain. According to its position, αII could be responsible for the contact between sister chromatids. Analysis of stable dicentric chromosomes led to the conclusion that the mouse minor satellite (satMi) (an analog of human αI) is not necessary for the association of sister chromatids [Vig and Paweletz, 1993]. MARs have been shown to occur in human α-satDNA 25–50 times more often than in euchromatic regions which contain genes that are transcribed [Strissel et al., 1996].

The mouse satMi is built of monomers of 120 bp, representing less than 1% of the genome, and is located in CENs of all mouse chromosomes but Y [Wong and Rattner, 1988]. The monomer of the mouse major satellite (satMa) is 234 bp. SatMa has been mapped to periCEN heterochromatin of all mouse chromosomes and represents 5–10% of the genome [Radic et al., 1987]. The satMa array separates the only arm of the mouse telocentric chromosomes from the satMi array, which is located just in CEN.

PeriCEN satDNAs (α II, satMa) contain few or no "CENP-B box" copies [Wevrick and Willard, 1991; Ikeno et al., 1994; He et al., 1998]. CENP-B is the box TTCGnnnnAnnCGGG (n is any nucleotide) which is recognized by mammalian conserved CENP-B (CEN Protein B) [Masumoto et al., 1989; Kipling and Warburton, 1997]. CENP-B does not bind methylated boxes, which affect protein localization in vivo [Mitchell et al., 1996]. CENP-B is a unique example of a sequence-specific binding protein among MAR- and satDNA binding proteins (Table I).

There is evidence that MAR-binding proteins are also satDNA binding. These proteins are capable of recognizing conserved structural features of satDNA in the same way that they recognize MARs in spite of a lack of consensus. The aim of the current work was to determine whether major NM MAR-binding proteins can recognize satDNAs of different locations and what structural features could be important for recognition.

MATERIALS AND METHODS

Preparation of Nuclei and NM

Mouse livers or human placenta (5 g) were homogenized in buffer TEM (25 mM Tris-HCl pH 7.4, 1 mM EGTA, 3 mM $MgCl_2$, and 1 mM PMSF) containing 350 mM of sucrose and centrifuged at 2500*g* for 15 min at +4°C. The pellet was resuspended in TEM buffer containing 2.2 M sucrose and layered onto a cushion of the same solution. The nuclei were precipitated by centrifugation at 90,000*g* for 45 min at +4°C.

For NM preparations, the nuclei were resuspended in TM buffer (10 mM Tris-HCl pH 7.4 and 5 mM $MgCl_2$) and treated with DNase I (Boehringer Mannheim, Germany) (30 U/1 mg DNA) for 30 min at +20°C. NaCl was added to the solution in drops up to a concentration of 2 M. The insoluble material (NM) was pelleted by centrifugation at 500*g* for 15 min at +4°C [Belgrader et al., 1991].

NM Protein Fractionation by Ion-Exchange Chromatography

NM proteins were solubilized in buffer E (25 mM Tris-HCl pH 9.0, 8 M urea, 5 mM EDTA, 1% 2-mercaptoethanol, 100 mM NaCl, 1 mM PMSF at +4°C). The extract was clarified by centrifugation at 50,000*g* for 15 min at +4°C and loaded onto a 2 ml Q-Sepharose column equilibrated with buffer E. Bound proteins were eluted by a step-wise gradient of 100–1,000 mM NaCl in buffer E with steps of 100 mM.

DNA Probes

The following cloned sequences were used: a 471 bp dimer of the mouse satMa repeating unit [Radic et al., 1987] cloned into pBluescript II KS+ [*Hind*III and *Xba*I], a 362 bp trimer of the mouse satMi repeating unit [Kipling et al., 1994] cloned into pGEM7 [*EcoR*I and *Hind*III], and a 365 bp mouse Ig κ MAR (Matrix Attachment Region from the immunoglobulin *k* gene) from plasmid pAR1 [*BamH*I and *Hind*III] [Tsutsui et al., 1993]. Clones 11-4 (1868 bp) and pN31 (1821 bp) contain 11-mers of α-satellite fragments cloned into pUC119 polylinker. The fragments are located on loci α21-I and α21-II of human chromosome 21, respectively [Ikeno et al., 1994]. The fragments were cut out with the indicated restriction enzymes, end-labeled with T4 DNA polymerase (Boehringer Mannheim, Germany) in the presence of [α-^{32}P]dATP and isolated by agarose gel electrophoresis.

Gel Mobility Shift Assay (GMSA)

The specific DNA-binding activity was revealed by a GMSA [Strauss and Varshavsky, 1984]. The incubation mixture usually contained a 1 ng [α-^{32}P]-labeled DNA-fragment, 5 μl (5 μg of total protein) of Q-Sepharose fraction and 0.1–5 μg of nonspecific competitor DNA. The probes were incubated in a buffer (20 mM Tris-HCl pH 7.4, 0.1 mM EDTA, 0.1% Triton X-100, 1% 2-mercaptoethanol, 2 mM $MgCl_2$, and 5% glycerol) for 50 min. The sample was subjected to 4% polyacrylamide gel electrophoresis (PAGE) (40 mA, 3 h) in TAE buffer [Sambrook et al., 1989]. Gels were dried and used to expose CEA X-ray film (Sweden). The relative mobility of DNA–protein complexes was calculated as the ratio of the complex

mobility (the distance from the start point) to the mobility of an unbound DNA fragment. The autographs were scanned and analyzed with the program Image Tool. The program Microsoft Excel was used for calculations and graphic representation.

Southwestern Blotting

Seven percent SDS–PAGE was carried out as described [Laemmli, 1970]. Southwestern blotting was performed generally as described [von Kries et al., 1991] with some modifications. After electrophoresis, proteins were electrotransferred to a PVDF membrane (Millipore) in buffer containing 25 mM Tris and 190 mM glycine, pH 8.3. The membrane was preincubated in binding buffer (15 mM Tris-HCl pH 7.4, 50 mM NaCl, and 1 mM EDTA) containing 5% non-fat dry milk for 45 min twice, followed by incubation in binding buffer containing 0.1 mg/ml BSA, ^{32}P-labeled DNA fragment and various amounts of competitor DNA (as indicated in figure legends) for 1.5 h at room temperature. The membrane was finally washed three times with binding buffer for 15 min, dried, and used to expose X-ray film (XAR-5, Kodak). E. coli DNA ultrasonicated to an average length of 500 bp was used as a nonspecific competitor.

Immunoblotting

SDS–PAGE and protein transfer were done as indicated above for southwestern blotting. The membrane was preincubated with TBS/Tween-20 (150 mM NaCl, 10 mM Tris-HCl, and 0.05% Tween-20 pH 7.5) containing 3% non-fat dry milk for 1 h. The solution was replaced with the fresh solution containing polyclonal rabbit antiserum against SP120 at 1:800 dilution or with guinea pig serum raised against complexes (1:1,000) and the membrane was incubated for 1.5 h. Both serums recognise same protein SP120/SAF-A/hnRNP-U [Lobov et al., 2000]. Then, the membrane was incubated with biotinylated goat anti-rabbit IgG (Sigma, USA) and avidin-alkaline phosphatase conjugate (Vector Labs, Burlingame, CA) in TBS/Tween-20 with washing between stages. Staining was done with BCIP/NBT (5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium, Sigma) as a substrate. Neither the second antibodies nor streptavidin-alkaline phosphatase added without the first antibodies gave any staining.

PAGE Analysis of Sequence-Directed DNA Curvature

DNA fragments were electrophoresed in 7% PAGE (29:1 acrylamide:bis-acrylamide) at 5 V/cm in 1× TAE buffer (20 mM Tris-acetate, 1 mM EDTA). Electrophoresis was run either at +4°C or at room temperature in the presence of 0.1 μg/ml ethidium bromide added to the samples, gel and running buffer. After electrophoresis at +4°C gels were stained with ethidium bromide. Electrophoretic mobilities were compared to those of a 100 bp ladder (Promega, Germany).

Computer Analysis of Sequence-Directed DNA Curvature

Computer analysis of DNA structure was performed according to the wedge model of Ulanovsky and Trifonov [1987]. The DNA path was calculated using NAMOT2 (Nucleic Acids MOdelling Tool, Los Alamos National Laboratory) software. Pictures are assembled into plates using Adobe Photoshop software.

RESULTS

Specificity of NM Protein Binding on Southwestern Blot

SatMa DNA is the main component of the mouse NM residual DNA so it was used as a probe. In the nuclei and NM, a number of the same polypeptides can be seen on a southwestern blot, though additional bands appear in the nuclei (Fig. 1A). The set of proteins revealed in NM resembled well-known MAR-binding proteins (Table I). Comparison of the Ig κ MAR and satMA binding activity shows that the same set of proteins is revealed when the amount of competitor DNA is minimal and that two of the proteins, p120 and p68, are the most stable in binding (Fig. 1B). When satMa- and Ig κ MAR-containing plasmids were used as a competitor they competed out the complexes with the counterpart fragment to roughly the same extent, with satMa being slightly more efficient (data not shown). The binding capacities of p120 and p68 to satMa and Ig κ MAR were indistinguishable but they were in order of magnitude less when satMi was used as a probe (Fig. 1B). The satMi binding capacity to p120 and p68 was comparable to that of a pUC plasmid fragment of a similar length, i.e., the binding was nonspecific (data not shown).

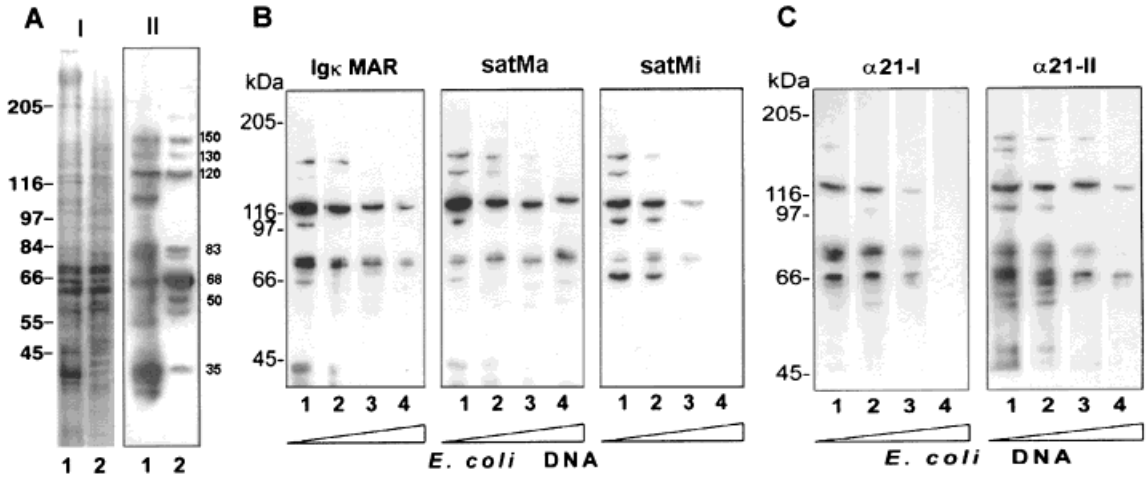

Fig. 1. A mouse liver nuclei (1) and NM (2) proteins (30 μg) were separated by electrophoresis on a 7% SDS-polyacrylamide gel (I), transferred to a PVDF membrane and incubated with an end-labeled satMa fragment in the presence of 100-fold excess of E. coli competitor DNA (II). Molecular weight of marker proteins is given in kDa on the left, molecular weight of the main NM satMa binding proteins is given in kDa on the right. **B** and **C:** Southwestern blotting of mouse NM proteins (30 μg) was done by using 10 ng of end-labeled Ig κ MAR (Ig κ MAR); mouse major (satMa) and minor (satMi) satellites (B); human NM proteins (30 μg) and labeled human alphoid satellite fragments (10 ng) from loci α21-I (α21-I) and α21-II (α21-II) were used in the absence of competitor DNA (1) or in presence of 50- (2), 500- (3), or 5,000- (4) fold excess of E. coli competitor DNA (C). Triangles under the panels indicate the increasing amount of *E. coli* competitor DNA here and everywhere. Molecular weight of marker proteins is given in kDa on the left at each panel.

The difference in binding between satMa and satMi is striking because they share long sequences with 83% homology [Wong and Rattner, 1988]. They differ in their position in the chromosome. The satMa block is located in periCEN while the satMi block is located in the CEN itself. The same positioning is characteristic of the human α21-II and -I blocks of α-satDNA. These α-satDNAs were used to probe the binding specificity of proteins from human NM and showed the same tendency, i.e., p120 and p68 are preferred in binding, but in this case, the binding of p83 [probably ABRP: Attachment Region Binding Protein], von Kries et al., 1991] according to its molecular mass and characteristic twin zone) is more obvious. Still α21-I binding is an order of magnitude less than that of α21-II (Fig. 1C). Thus, on southwestern blots, p120 and p68 are able to discriminate different but very closely related satDNAs.

Protein Identification

The NM extract (Fig. 2A lane E) was subjected to ion exchange column fractionation and the fractions were tested in a southwestern blot (Fig. 2B, satMa*), by immunoblot with antibodies against scaffold attachment factor A (SAF-A) and lamin B (Fig. 2B, SAF-A, lamin B), and by GMSA with the labeled satMa fragment (Fig. 2C). It can be seen that p120 and p68 are able to bind the satMa fragment on the southwestern blot but they go to different fractions (Fig. 2B, satMa*). The immunoblot shows that p120 corresponds to SAF-A and that p68 corresponds to lamin B (Fig. 2B, SAF-A, lamin B). The analysis of these fractions by GMSA shows that lamin B does not bind a satMa fragment directly under the conditions used and it is impossible to obtain specific DNA–protein complexes in contrast to SAF-A (Fig. 2C). The relative mobility of high mobility complexes in the fractions with both SAF-A and lamin B was 0.65±0.003 (Fig. 2C, lower arrow), though it was 0.5±0.003 in the complexes with SAF-A alone (Fig. 3). In subsequent experiments only fraction 2 with SAF-A without lamin B was used.

Specificity of SAF-A Binding in Solution (GMSA)

GMSA with different labeled fragments was used in order to compare the binding ability of SAF-A in solution with immobilized protein. The ability of SAF-A to bind Ig κ MAR and satMA fragments was indistinguishable in GMSA (Fig. 3A) in the same way as it was in the southwestern blot (Fig. 1B). Antibodies against SAF-A caused a hypershift of both Ig κ MAR and satMA protein complexes (Fig. 3B).

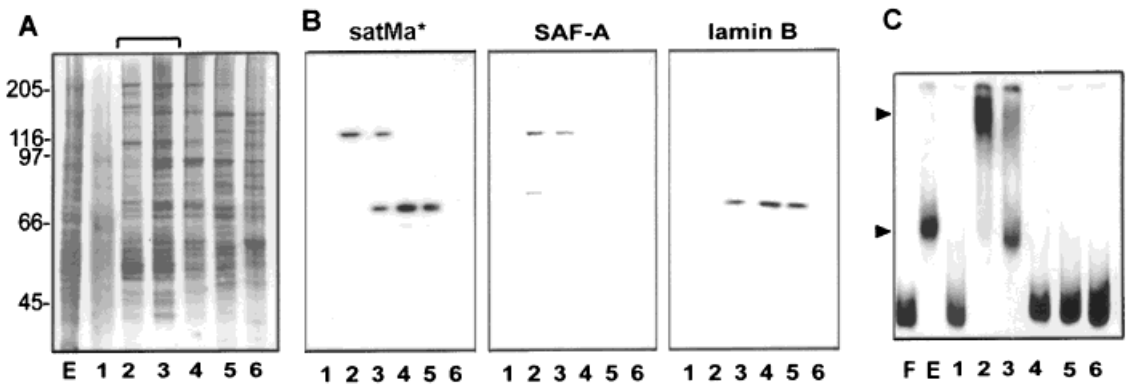

Fig. 2. Mouse NM proteins solubilized in 8 M urea solution were applied to a Q-Sepharose column and eluted by a step-wise gradient of NaCl concentration. Fractions were analyzed by 7% SDS-polyacrylamide gel (**A**), by southwestern blotting with satMa as a probe in the presence of a 500-fold excess of E. coli competitor DNA (**B**, satMa*), by Western blotting with polyclonal antibody against SAF-A (B, SAF-A) or monoclonal antibody against lamin B (B, lamin B) and by GMSA (**C**). GMSA samples contained 0.2 ng labeled satMa, 5 µl of each fraction and 3 µl of extract and a 100 fold excess of competitor DNA. The numbers 1–6 below the panels correspond to the fractions of the same number, E-NM extract prior to chromatography, F-free fragment. Bracket in A indicates the active fractions according to GMSA (C). Arrowheads on C indicate the most prominent complexes.

With the optimization of GMSA conditions, SAF-A binding activity was detected in one complex with a relative mobility of 0.5±0.003 with both the Ig κ MAR and satMA fragments (Fig. 3A and B).

GMSA revealed a striking difference in SAF-A binding to the satMa and satMi fragments (Fig. 3C). The satMi-SAF-A complexes disappeared completely when the amount of competitive DNA was optimal for satMa complexes (Fig. 3C). The efficiency of the satMa end-labeling was higher than that of satMi due to the difference of the end sequences. So densitometry was done and the ratio of the amount of fragments in complexes to the amount of free fragments was estimated. The average values from five experiments are shown (Fig. 3D). In solution SAF-A formed less specific complexes

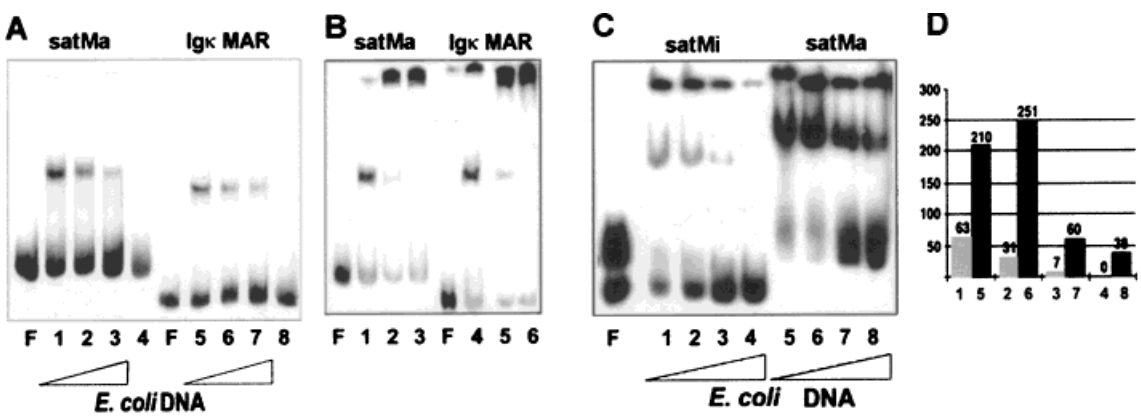

Fig. 3. SAF-A binding specificity in GMSA. **A:** Samples contained 0.2 ng labeled satMa or Ig κ MAR, 5 µl of fraction 2 and non-specific competitor DNA: 200- (1, 5), 400- (2, 6), or 800- (3, 7) fold excess. **Lanes 4**, 8: 20-fold excess of the counterpart plasmid (i.e., MAR for 4, satMa for 8, specific competitor). **B:** Hyper-shift assay. Samples contained 0.2 ng of labeled satMa or Ig κ MAR fragment, 100-fold weight excess of nonspecific competitor DNA, 5 µl of fraction 2 (1.5) and polyclonal antibody against SAF-A (2.3 and 5.6). Serum with antibodies was in final dilution 1:100 (2.5) and 1:200 (3.6). **C:** Comparison of binding specificity of SAF-A for satMa and satMi. Samples contained 0.2 ng of end-labeled major (satMa) or minor (satMi) satellite and 100- (1.5), 200- (2.6), 400- (3.7), or 800- (4.8) fold excess of E. coli competitor DNA. Both free fragments were loaded on the same lane (F). **D:** Densitometry of the bands in panel C, Figure 3, arithmetic mean from five experiments is shown. Ordinate axis shows the ratio of fragment in complexes to the free fragments in relative units. Numbers above the columns are the ratio of the amount of bound fragment to the amount of free fragment multiplied by 100. Grey, satMi; black, satMa. Numbers below lanes-according to Figure 3C.

with satMi. The amount of competitive DNA needed to prevent SAF-A from forming a complex with satMi was about an order of magnitude less than that needed to prevent it from forming a complex with satMa (Fig. 3C and D). These results were similar to those obtained with the southwestern blot (Fig. 1B), and show that SAF-A in solution is able to recognize different satDNAs with similar sequences.

Estimation of Intrinsic Curvature of satDNAs

Both satMa and satMi contain multiple stretches of 3–6 A/T. Since SAF-A is able to distinguish satMa from satMi, it does not simply bind to any AT-rich DNA and/or narrowed minor groove. Bent DNA fragments are known to move slowly in PAGE at +4°C, but at +55°C or in the presence of intercalating stains, such as ethidium bromide, Hoechst 33258 or DAPI (4,6-diamidino-2-phenylindole), they assume a straight state and consequently migrate with a normal fragment mobility [Anderson, 1986]. Although a satMa fragment has an abnormally slow mobility on PAGE, which is a hallmark of bent DNA [Radic et al., 1987], satMi does not (Fig. 4A). The same change of mobility was observed with the α21-II and -I fragments, though the shift of mobility was less dramatic because of their large fragments size (data not shown). The difference in the DNA curvature of these satDNAs may be responsible for the observed difference in SAF-A binding affinity.

A computer analysis was done to obtain further insights into the deviations in DNA structure. The wedge model is the most advanced and most convenient to work with [Ulanovsky and Trifonov, 1987]. The possible curved state of all the fragments used was evaluated by computer analysis according to this model, which allows the DNA axis turn to be estimated from the sequence of the fragment.

The total turn of the DNA axis in degrees was determined as the sum of the local turns between AA and TT dinucleotides. The fragment with a big local turn in one part can be straight in total if the turn in the other part is in the opposite direction. This is just the case with satMi (Fig. 4B, satMi). In spite of the regularity of the oligo(A) blocks, they are positioned out of phase with the DNA helix step and as a result the whole satMi fragment is not bent but acquires the form of a stretched helix. In contrast, the contribution of most oligo(A)

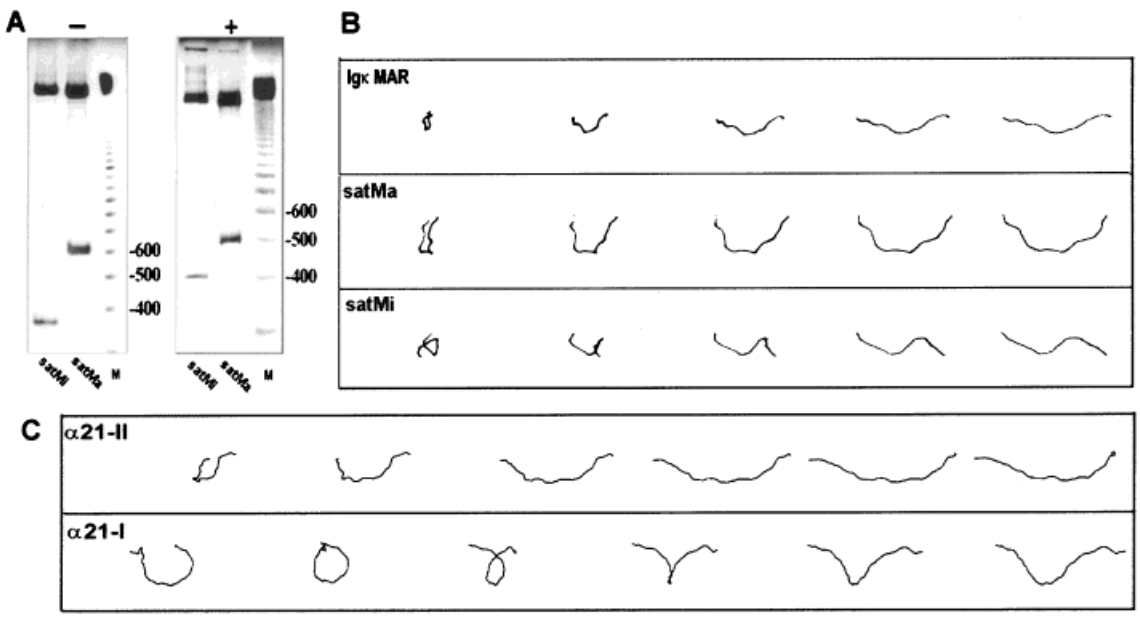

Fig. 4. **A:** Electrophoretic analysis of sequence-directed DNA curvature. SatMi (satMi) and satMa (satMa) fragments were electrophoresed on a 7% polyacrylamide gel at +4°C (–) or in the presence of 1 μg/ml ethidium bromide (+) at room temperature. **Lanes M**, 100-bp marker ladders; fragment size in bp is shown at right. **B:** Computer analysis of three-dimensional DNA structure was performed according to the wedge model of Ulanovsky and Trifonov [1987]. Two-dimensional projections of the helical axis of Ig κ MAR (Ig κ MAR), major satDNA (satMa) and satMi (satMi) DNA fragments are shown. Each consecutive projection is rotated by 20 degrees. Note the non-planar helical shape of the satMi fragment. **C:** Same as in B, but human alphoid fragments α21-I (α21-I) and α21-II (α21-II) used for the analysis.

tracks are additive, resulting in a bent state of the whole satMa fragment. The total turn goes on in the plain of the DNA helix and the fragment acquires the form of an unclosed circle (Fig. 4B, satMa). Ig κ MAR did not show any shift in its mobility in PAGE (data not shown) and the computer analysis did not reveal any strong bending, though the acquired form of the whole fragment resembles satMa more than satMi (Fig. 4B, Ig κ MAR).

The α21-II and -I fragments of α-satDNA show the same tendency (Fig. 4C). The bent state of human α-satDNA fragments has not been shown before. The computer analysis of one of the α-satDNA fragments revealed only a slight deviation from a straight helix [Fitzgerald et al., 1994]. α-satDNAs are known to have a high degree of divergence between subfamily members. Therefore, the structural state of some representatives could differ significantly. The computer analysis according to the wedge model [Ulanovsky and Trifonov, 1987] shows that α21-II is bent as a whole while α21-I is not (Fig. 4C). The curved state of the whole α21-II satDNA fragment is correlated with its higher affinity for lamin B and SAF-A in the same way that the curved state of satMa is correlated with its higher affinity for these proteins (Fig. 4 and 1).

Thus, the binding of SAF-A and lamin B to a satDNA in vitro is more closely correlated with the amount of bending in the satDNA fragment than with the presence of other features such as boxes or a local helix turn.

DISCUSSION

Computer programs that search for MARs take into consideration several local features of the sequence. These include AT-motifs [A(T)nA] and GC-motifs [TG(T)nG], poly(dA) and poly (dTn) tracks, the consensus sequence of TopoII binding or purine-pyrimidin tracks, AT-blocks with regular alternation, signals of kinked DNA, e.g., $(TA)_3(TG)_{3,4}CA$ [Singh et al., 1997; Glasko et al., 2000]. There is no consensus sequence for MARs. However, MARs from distant species are capable of binding to the same NM preparations, so the mechanism of binding is fairly well retained [Cockerill and Garrard, 1986; Mirkovitch et al., 1988; Amati et al., 1990]. The structural features mentioned above are sufficiently preserved to allow sequence recognition by NM proteins.

Cloned scaffold-associated *Drosophila melanogaster* MARs are all bent [Homberger, 1989]. An artificial bent fragment, $(A_5N_5)_{10}$, was found to bind to *D. melanogaster* NM with high affinity. The intrinsic DNA curvature found in MARs has been proposed to be responsible for their specific binding to NM [Homberger, 1989]. On the other hand, it was shown that a bent state is not necessary or sufficient for a sequence to be a MAR [von Kries et al., 1990]. The curve of the DNA helix appears to enhance the sequence affinity to the NM. The attachment of some MARs is developmentally regulated [Mirkovitch et al., 1988] and MARs are composed of boxes for different transcriptional factors [Boulikas, 1995]. So the depth of the curvature may be regulated by these factors depending on the necessity for a MAR to be attached to the NM.

In contrast, the condensed state of heterochromatin is more stable [Wallrath, 1998]. SatDNA, a constituent of heterochromatin, possesses many of the above-mentioned characteristics of MARs. For example, satMa and satMi are A/T rich (64 and 66% respectively). Each satMi monomer bears a TopoII recognition site. Actually, there is much similarity between MARs and satDNA. The repetitive components of satDNA have even been suggested to be NM/scaffold attachment regions (MARs/SARs) [Hibino et al., 1998].

There is a structural similarity between different satDNAs in spite of the high variability of sequences [Beridze, 1986; Saitoh et al., 1989; Martinez-Balbas et al., 1990; Fitzgerald et al., 1994]. Structural features could affect chromatin packing because these structures are recognized by specific proteins [Harata et al., 1988; Saitoh et al., 1989; Hibino et al., 1992; Radic et al., 1992; Podgornaya et al., 2000]. Out of 20 satDNA fragments analysed 17 are A/T rich [Martinez-Balbas et al., 1990]. Poly(dA) and poly(dT) tracks are characteristic of satDNA. They are often positioned in phase with the helix step periodicity of ~10.5 bp [Wu and Crothers, 1984; Ulanovsky and Trifonov, 1987]. As a result, many of these fragments acquire a stable bent mode, which has been shown for satDNA of salamander [Barsacchi-Pilone et al., 1986], hen, pheasant, and turkey [Kodama et al., 1987; Saitoh et al., 1989]; crayfish *Artemia* [Benfante et al., 1989], monkey, and rat [Martinez-Balbas et al., 1990; Nakamura et al., 1991], *D. melanogaster* [Doshi et al., 1991], swan [Fitzgerald et al., 1994],

beetles [Barcelo et al., 1997], and mouse satMa [Radic et al., 1987]. The islets, which are mostly curved, are likely to attract nucleosomes [Hsieh and Griffith, 1988]. The periodicity of such islets leads to the phasing of the nucleosomes, which is a feature of heterochromatin. This could be one of the mechanisms by which satDNA is compacted into heterochromatin [Trifonov and Sussman, 1980; Fitzgerald et al., 1994].

Several proteins that had originally been shown to be MAR-binding proteins have also been shown to be satDNA-binding (Table I). On the other hand, D1/HMG-1a, which is an a-satDNA-binding protein, was found to have a wider specificity, including a specificity for MARs. The binding of P130/matrin 3 to satDNA depends of the methylated state of the satDNA [Hibino et al., 1998] and it could be MAR-specific when a MAR is hypomethylated, although this has not yet been tested. P130/matrin 3 appears to be present on southwestern blots when Ig k MAR is used as a probe (Fig. 1B), although further studies are needed to confirm this. Most of these proteins recognize the structural features of DNA rather than sequence motifs. ARBP was found to be homologous to the rat protein MeCP2, previously identified as a methyl-CpG-binding protein. The recognition of both MAR and satDNA by ARBP/MeCP2 depends on the structure of these DNAs [Weitzel et al., 1997]. P83 (Fig. 1A, II), which is probably ARBP/MeCP2, is the third most specific in satDNAs binding (after SAF-A and lamin B) (Fig. 1C).

The present results show that SAF-A and lamin B can recognize in vitro satDNA fragments with similar sequences according to their bending state. This is what we showed for two pairs of satDNAs with similar sequences but different locations in the chromosomes: mouse satMa-satMi and human α21-II and -I. These results confirm the correlation between protein recognition and the degree of bending of a fragment. Direct experiments could be done only with artificial fragments such as $(A_5N_5)_{10}$ [Homberger, 1989], but not with satDNA fragments because it is impossible to change the sequence and maintain the curvature of the target DNA in order to use it to trace the relationship between protein binding and DNA curvature.

The ability of aggregates of lamins to specifically bind not only MARs but also satDNA of an exact type is especially important with respect of heterochromatin localization in the nuclei. Constitutive heterochromatin formed by satDNA is known to be located on the peripheral undermembrane [Prokofjeva-Belgovskaja, 1986; Boulikas, 1995; Moir et al., 1995]. It has been shown that the DNA-binding of lamins depends on the rod-domain, which is characteristic of the intermediate filament class of proteins [Boulikas, 1995; Moir et al., 1995]. The association of heterochromatic regions with the undermembrane lamina suggests that lamins could interact with satDNA. Our results confirm that this occurs in vitro.

The consequences of these results for the in vivo situation could be as follows. In rodents, satDNA is known to be highly variable, but it is relatively conserved within individual species. In *Mus musculus*, satMa monomers vary <5% [Vissel and Choo, 1989] while satMi monomers vary ~5.6% [Kipling et al., 1994]. So it is possible that the structural features of a fragment in vitro may be reflected in the whole array of the corresponding satDNA in vivo.

The CEN domains of α-I and satMi differ dramatically from those of periCEN α-II and satMa by their ability to be bent and consequently by their ability to be recognized by exact proteins. The satDNA curvature may be the structural foundation for an organization that is more complicated than a merely heavy compact state of interphase heterochromatin. Different proteins could be involved and associated with different areas of heterochromatin and different satDNAs [Manuelidis, 1997; Enukashvily et al., 1999]. MAR-binding proteins also bind periCEN satDNA (Table I). Transcriptionally silent sequences of an exact type, such as periCEN tandem repeats, may actively participate in interphase to organize specific groups of sequences in mammalian nuclei as a result of the specific binding of proteins. This could provide a functional advantage in cementing particular patterns of expression in cells of different lineage [Manuelidis, 1997].

Repetitive DNA sequences, with precise positions in the human genome, are capable of forming a wide variety of unusual DNA structures with simple and complex loopfolding patterns [Catasti et al., 1999]. The loop size in vivo could be a consequence of the degree of fragment curvature observed in vitro. The formation of such stable structures offers a mechanism of unwinding which is advantageous during transcription. These unusual

DNA structures also provide unique protein recognition motifs' that are quite different from a Watson–Crick double helix and that allow MAR-binding proteins to bind. However, the formation of the same unusual DNA structures during replication is likely to cause instability in the lengths of the DNA repeats. Thus, the selective advantage of the unusual structures formed by the DNA repeats in the regulation of gene expression may be offset by the genomic instability caused by the same structures during replication. The repeat number is a critical parameter that helps maintain a balance between the advantage gained from an unusual structure during gene expression and the disadvantage posed by the same structure during replication [Catasti et al., 1999]. SatDNA arrays may compensate for this disadvantage by having structural similarity with MARs and by being situated in close proximity to MARs [Lobov et al., 2000].

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