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## Binding of Matrix Attachment Regions to Lamin Polymers Involves Single-Stranded Regions and the Minor Groove

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**Chromatin in eukaryotic nuclei is thought to be partitioned into functional loop domains that are generated by the binding of defined DNA sequences, named MARs (matrix attachment regions), to the nuclear matrix. We have previously identified B-type lamins as MAR-binding matrix components (M. E. E. Ludérus, A. de Graaf, E. Mattia, J. L. den Blaauwen, M. A. Grande, L. de Jong, and R. van Driel, *Cell* 70:949–959, 1992). Here we show that A-type lamins and the structurally related proteins desmin and NuMA also specifically bind MARs in vitro. We studied the interaction between MARs and lamin polymers in molecular detail and found that the interaction is saturable, of high affinity, and evolutionarily conserved. Competition studies revealed the existence of two different types of interaction related to different structural features of MARs: one involving the minor groove of double-stranded MAR DNA and one involving single-stranded regions. We obtained similar results for the interaction of MARs with intact nuclear matrices from rat liver. A model in which the interaction of nuclear matrix proteins with single-stranded MAR regions serves to stabilize the transcriptionally active state of chromatin is discussed.**

The current view is that eukaryotic chromatin is divided into topologically constrained loops of tens to hundreds of kilobases. These loops are generated by the binding of specialized DNA sequences to an intranuclear framework, known as the nuclear matrix (4) or nuclear scaffold (37). The loop organization of chromatin may be important not only for the compaction of the chromatin fiber but also for the regulation of gene expression. It has been postulated that each loop represents an independent unit of transcription and replication, being insulated from regulatory influences of neighboring loops (for reviews, see references 7, 15, and 54).

The DNA sequences that are held responsible for fastening the chromatin to the nuclear matrix have been named MARs or SARs (for matrix or scaffold attachment regions) (for reviews, see references 40 and 46). They have been implicated as such on the basis of their high affinity for binding to the nuclear matrix in vitro (10, 37). MARs have been found in the genomes of many different species, often located near important regulatory sequences (10, 18). Some MARs are found at the boundaries of transcription units, where they may delimit the ends of an active chromatin domain (26, 35, 39). It has been shown that certain MARs augment the transcriptional activity of a linked gene in stably transfected cells (5, 32, 41, 49) or in transgenic mice (8, 58). Although these findings indicate that MARs participate in the control of gene expression, their specific role remains unclear.

It is anticipated that the physiological role of MARs is directly related to their binding to the nuclear matrix. In vitro studies have shown that this binding is reversible, of high affinity, and evolutionarily conserved. To date, several matrix proteins that specifically interact with MARs in vitro have been identified. In higher eukaryotes, these include the enzyme

topoisomerase II (1), attachment region binding protein (ARBP) (57), scaffold attachment factor A (SAF-A) (47), lamin B (36), a 120-kDa protein (SP120) (53), and a thymus-specific MAR-binding protein, SATB1 (16).

To gain insight into the physiological roles of these different MAR-matrix interactions, it is important to unravel their molecular details. While still almost nothing is known about the DNA binding domains of the various MAR-binding proteins, knowledge about the sequence and structure of MARs is rapidly increasing. MARs have a length of 300 to 1,000 bp and are typically about 70% A+T rich. They are preferentially bound and cleaved by the enzyme topoisomerase II in vitro (1, 44) and in vivo (28). Although there is no consensus sequence for MARs, they share several sequence characteristics, such as oligo(dA) and oligo(dT) tracts (18) and ATATAT boxes (10). Structural features of MARs include DNA bending (25), a narrow minor groove (1, 38), and a strong potential for base unpairing under superhelical strain (6, 33). The last unwinding property was shown to be important for binding to the nuclear matrix and for augmentation of gene expression in stable transformants (6). Also, the significance of a narrow minor groove for the interaction of MARs with matrix proteins has been reported (1, 16, 27). DNA bending, on the other hand, does not appear to be essential for matrix binding (3, 57).

In this study we investigated the interaction between MARs and lamins in molecular detail. We show that like B-type lamins (36), A-type lamins can bind MARs specifically. Detailed binding studies revealed that the interaction of MARs with polymers consisting of A- plus B-type lamins from rat liver is saturable, of high affinity, and evolutionarily conserved. Two different types of interaction related to different structural features of MARs, i.e., one involving the minor groove of double-stranded MAR DNA and one involving single-stranded regions, have been discerned. The results are discussed in terms of a model for the role of MARs in gene expression.

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## MATERIALS AND METHODS

**Purification of A- and B-type lamins.** A total lamin preparation consisting of lamins A, B, and C was isolated from rat liver nuclear envelopes, as described by Aebi et al. (2). Nuclear envelopes were isolated according to the method of Kaufmann and Shaper (30) in the presence of the reducing agent dithiothreitol (final concentration of 20 mM). The lamin preparation was stored at a concentration of 0.2 to 0.3 mg of protein per ml in lamin buffer (20 mM Tris-HCl [pH 9.0], 500 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 2% Triton X-100) at  $-20^{\circ}\text{C}$ .

The procedure for the purification of A- and B-type lamins was based on that of Aebi et al. (2). All steps were conducted at  $4^{\circ}\text{C}$  unless indicated otherwise. For the purification of A-type lamins, 1 ml of the total lamin preparation was mixed with 24 ml of 6 M urea–20 mM Tris-HCl (pH 9.0) and passed over a 1-ml DEAE-cellulose column (Whatman DE-52). The flowthrough fraction, which was free of B-type lamins and contained approximately 70% of the loaded amount of A-type lamins, was concentrated on a 1-ml phosphocellulose column (Whatman p11). Elution of the latter column with buffer U1 (6 M urea, 20 mM Tris-HCl [pH 9.0], 500 mM KCl, 2 mM EDTA, 1 mM dithiothreitol) yielded a pure A-type lamin fraction containing lamins A and C in a 1:1 ratio at a protein concentration of 0.1 to 0.2 mg/ml.

For the purification of B-type lamins, 1 ml of the total lamin preparation was mixed with 24 ml of 6 M urea–20 mM Tris-HCl (pH 9.0) and passed over a 1-ml phosphocellulose column (Whatman p11). The flowthrough material, which was enriched in B-type lamins, was passed over a DEAE-cellulose column (Whatman DE-52). This column was washed at room temperature with buffer U2 (8.5 M urea, 10 mM MES [morpholineethanesulfonic acid] [pH 6.5], 10 mM glycine, 0.5 mM EDTA, 1 mM dithiothreitol) to remove A-type lamins and subsequently eluted with buffer U1. This yielded a pure lamin B fraction with a protein concentration of 0.1 to 0.2 mg/ml. Purified A-type and B-type lamin preparations were stored in buffer U1 at  $-20^{\circ}\text{C}$ .

**MAR binding procedures.** MAR binding procedures were performed essentially as described before (36). Restriction fragments of a pBR322 plasmid containing the *Drosophila* histone MAR (37) were prepared and end labeled with [ $\alpha$ - $^{32}\text{P}$ ]dATP (New England Nuclear) by using the Klenow fragment of *Escherichia coli* polymerase I. Unincorporated nucleotides were removed by spun-column chromatography with Sephadex G-50. Isolated lamins (0.5 to 1  $\mu\text{g}$  of protein), either total lamin preparation in lamin buffer or purified A- or B-type lamins in buffer U1, were dialyzed for 2 h at room temperature against binding buffer (10 mM Tris-HCl [pH 7.4], 50 mM NaCl, 2 mM EDTA). Under these conditions aggregation of lamins takes place. Binding buffer (to a final volume of 50 to 100  $\mu\text{l}$ ), radioactively labeled restriction fragments of the MAR-containing plasmid (15,000 cpm in 5 ng of DNA), and competitor DNA (types and concentrations as indicated in Results) were added, and binding was carried out for 3 h at  $37^{\circ}\text{C}$ . Lamin binding of DNA fragments was analyzed by a sedimentation procedure or a filter-binding assay. Where indicated, restriction fragments were incubated with the drug distamycin A (Sigma) or chromomycin (Sigma) (for concentrations, see Fig. 8) for 90 min at  $37^{\circ}\text{C}$  prior to addition of the DNA to the lamin aggregates. Stock solutions of the drugs were in 50% dimethyl sulfoxide and were freshly diluted in binding buffer before use.

For the sedimentation procedure, radiolabeled MAR fragments and vector fragments were usually added to the same binding assay. After binding, lamin aggregates were sedi-

mented in a microcentrifuge (30 min at  $10,000 \times g$ ), and radioactive DNA fragments in the pellet and supernatant were purified, size separated on a 1.2% agarose gel, and visualized by autoradiography.

For the filter binding assay, MAR fragments and vector fragments were gel purified, labeled, and added to separate binding assays. After incubation with lamin aggregates the mixture was filtered over a nitrocellulose filter (Schleicher & Schuell) which was equilibrated in binding buffer and mounted in a dot blot apparatus. Following filtration of the samples, the filter was washed four times with binding buffer, and the amount of radioactivity that remained on the filter was determined by scintillation counting. Lamin-independent background binding of DNA fragments to the filter was determined in parallel incubations without lamin protein.

MAR binding to desmin and NuMA was measured exactly as was MAR binding to lamin. Desmin from chicken stomach (Boehringer) was solubilized in lamin buffer at a protein concentration of 0.25 mg/ml. The human NuMA protein was expressed and purified from SF9 cells infected with a recombinant baculovirus carrying the cDNA encoding the full-length human NuMA polypeptide. The baculovirus-derived NuMA protein was solubilized in 8 M urea–20 mM Tris (pH 8.0)–2 mM EDTA–1 mM dithiothreitol–150 mM guanidine-HCl, purified by gel filtration chromatography, and adjusted to a concentration of 0.15 mg/ml. Both proteins were induced to aggregate by dialysis for 2 h at room temperature against binding buffer. MAR binding was analyzed by the filter binding assay described above.

**Electron microscopy of lamin aggregates.** A preparation consisting of rat liver lamins A, B, and C (0.2 to 0.3 mg of protein per ml of lamin buffer) was dialyzed for 2 h at room temperature against binding buffer. Four volumes of binding buffer were added, and the dialyzed preparation was incubated for 3 h at  $37^{\circ}\text{C}$ . Lamin aggregates that had formed were adsorbed to Alcian Blue (0.5 mg/ml; Serva)-pretreated carbon-coated grids, negatively stained with 1% uranyl acetate, and examined under a Philips EM 420 electron microscope operated at 80 kV.

## RESULTS

**MARs bind to A- and B-type lamins.** We have previously shown that aggregates consisting of rat liver lamins A, B, and C specifically bind MARs (36). Furthermore, by means of a DNA-binding protein blot assay we have identified lamin B as a MAR-binding protein. No conclusions, however, could be drawn about the MAR binding capacity of lamins A and C. The fact that these A-type lamins did not bind MARs in the DNA-binding protein blot assay is not conclusive, as this could be due to denaturation by sodium dodecyl sulfate (SDS). For the identification of MAR binding domains of lamins, it is important to establish whether MAR binding is a property of lamins in general or is restricted to B-type lamins. Consequently, we isolated A- and B-type lamins in the absence of SDS and compared their MAR binding properties.

A- and B-type lamins were purified from rat liver nuclear envelopes by ion-exchange chromatography, on the basis of the procedure of Aebi et al. (2). The purified A-type lamin fraction contained lamins A and C in approximately equal amounts, while in the B-type lamin fraction exclusively lamin B could be detected (Fig. 1). We investigated MAR binding to these isolated proteins. Purified A-type lamins and purified B-type lamins, solubilized in a buffer containing 500 mM KCl and 6 M urea, were induced to aggregate by dialysis against binding buffer of low ionic strength without urea. Aggregates that had

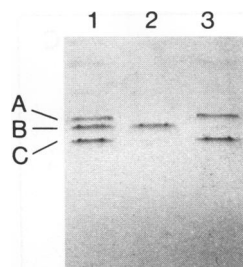


FIG. 1. Purification of A- and B-type lamins. B-type lamins (lane 2) and A-type lamins (lane 3) were purified from a preparation consisting of rat liver lamins A, B, and C (lane 1) as described in Materials and Methods. Proteins were separated on an SDS-10% polyacrylamide gel and stained with Coomassie blue.

formed were incubated with radiolabeled restriction fragments of a pBR322 plasmid in which the *Drosophila* histone MAR had been cloned (37), in the presence of different amounts of *E. coli* competitor DNA. Lamin-bound restriction fragments were separated from unbound fragments by centrifugation and analyzed by electrophoresis and autoradiography (Fig. 2). We observed specific binding of the MAR-containing fragment to both A- and B-type lamins. This fragment bound to these lamin proteins over a large concentration range of *E. coli* competitor DNA, whereas the non-MAR pBR322 fragments

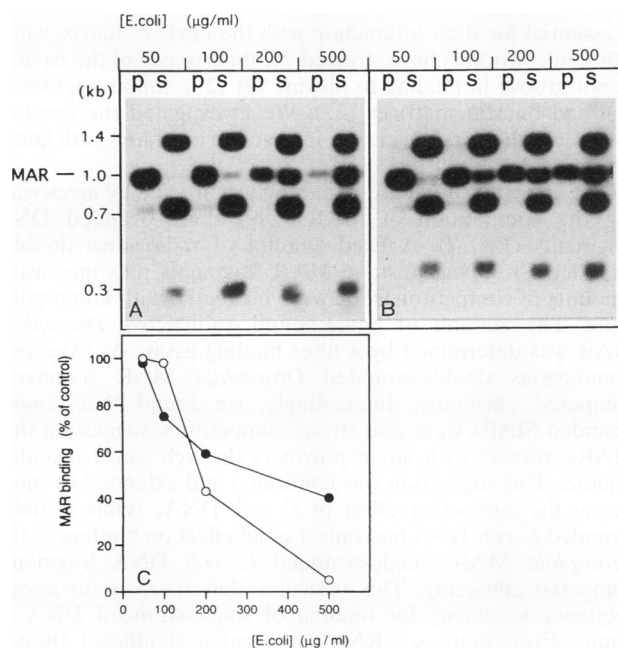


FIG. 2. Specific MAR binding to A- and B-type lamins. Purified A-type lamins (A) and purified B-type lamins (B) in buffer U1 were dialyzed against binding buffer to induce aggregation. Aggregated lamin (1 µg) was incubated in 50 µl of binding buffer with 50 ng of radioactively labeled restriction fragments of a pBR322 clone containing the *Drosophila* histone MAR and with different concentrations (50 to 500 µg/ml) of *E. coli* DNA sonicated to an average size of between 0.2 and 1.2 kb. After binding, lamin aggregates were pelleted and radioactive DNA fragments in the pellet (p) and supernatant (s) were purified and analyzed by agarose gel electrophoresis and autoradiography. Binding values obtained for A-type lamins (○) and B-type lamins (●) are quantified (C).

did not. The amount of *E. coli* DNA that is required to displace the MAR fragment from the lamin aggregates is a measure of the relative strength of the interaction. We thus found that the affinity of MAR binding to purified A-type lamins is somewhat lower than the affinity of MAR binding to B-type lamins. The latter affinity, in turn, is somewhat lower than the affinity measured previously for aggregates consisting of A- plus B-type lamins (36). The finding that A-type lamins as well as B-type lamins show specific MAR binding suggests that general features of the lamin molecule, rather than subtype-specific ones, mediate binding.

**MARs bind to different coiled-coil proteins.** Lamins belong to the family of intermediate filament proteins (for reviews, see references 19 and 48). These proteins consist of a central  $\alpha$ -helical rod domain flanked by nonhelical ends. Among the different intermediate filament proteins the secondary structure of the central rod domain is highly conserved. Intermediate filament proteins can self-assemble into long polymers in which the rod domains are dimerized to coiled-coil molecules.

Recently, a nuclear protein that is structurally related to intermediate filament proteins has been characterized (13, 59). This protein, named NuMA, is a constituent of the nuclear matrix and plays an important role during mitosis (12). Elements of its primary structure are similar to those of intermediate filament proteins. Therefore, NuMA is predicted to form coiled-coil polymers. Another similarity between most intermediate filament proteins and NuMA is the occurrence of S/TPXX amino acid motifs in their globular end domains. This motif is frequently found in gene-regulatory proteins and is supposed to contribute to DNA binding (52).

To establish whether common features of these structurally related proteins play a role in MAR binding, we compared MAR binding properties of lamins with those of NuMA and the intermediate filament protein desmin. Binding of a purified, radioactive MAR fragment and a purified, radioactive pBR322 fragment was measured as a function of the *E. coli* competitor DNA concentration (Fig. 3). The proteins were present in aggregated form. We found that like lamins, desmin and NuMA specifically bound MARs: over a large concentration range of competitor *E. coli* DNA these proteins showed a higher affinity for the MAR fragment than for the pBR322 fragment. MAR binding to desmin and NuMA was, however, of lower affinity than was MAR binding to lamin. The *E. coli* concentrations that were required to displace 50% of bound MAR fragments from desmin and NuMA aggregates were, respectively, 130 and 150 µg/ml, while 500 µg of *E. coli* DNA per ml was required to displace the same fraction of MARs from aggregates consisting of rat liver lamins A, B, and C. These *E. coli* concentrations correspond to excesses in weight over radiolabeled MAR fragments of 130, 150, and 500, respectively. Also, for another intermediate filament protein, keratin from bovine hoof, specific though low-affinity binding of MARs has been observed (50).

The observation that lamins, other intermediate filament proteins, and NuMA specifically bind MARs suggests that common features, such as the coiled-coil structure and S/TPXX motifs, are involved in the recognition of MARs. In addition, the higher binding affinity of lamins suggests that lamin-specific sequences promote binding.

**MAR-lamin interaction is specific, saturable, and evolutionarily conserved.** We analyzed MAR binding to lamin aggregates in more detail. First, the ultrastructure of lamin aggregates as they were formed under MAR binding conditions was studied. A preparation consisting of rat liver lamins A, B, and C in lamin buffer (20 mM Tris-HCl [pH 9.0], 500 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 2% Triton X-100) was

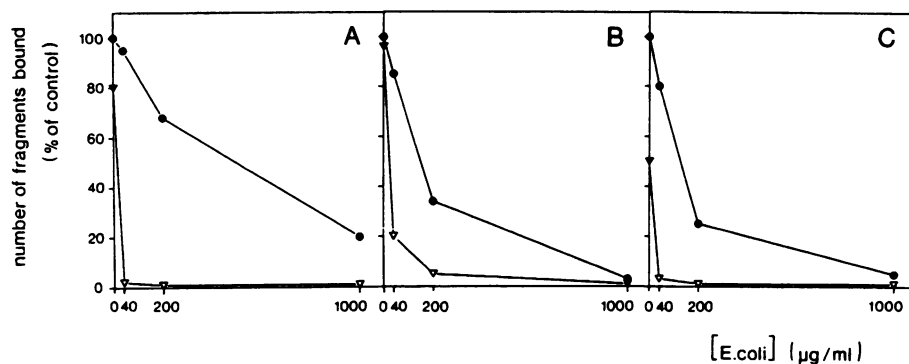


FIG. 3. Specific MAR binding to lamin, NuMA, and desmin. Preparations of lamins A, B, and C from rat liver (A), NuMA from baculovirus-infected SF9 cells (B), and desmin from chicken stomach (C) in lamin buffer were dialyzed against binding buffer to induce protein aggregation. Protein aggregates (0.5  $\mu$ g per assay) were incubated in 50  $\mu$ l of binding buffer with 50 ng of gel-purified, radioactively labeled *Drosophila* histone MAR fragments (●) or pBR322 fragments (▽) in the presence of different concentrations (0 to 1,000  $\mu$ g/ml) of sonicated *E. coli* DNA. After binding, protein aggregates were pelleted and the amount of protein-bound restriction fragments was determined by scintillation counting. Binding is expressed as the percentage of the total amount of input restriction fragments per assay. Shown are the means of determinations of two independent experiments done in duplicate.

dialyzed against binding buffer of low ionic strength and pH 7.4 to induce aggregation. Four volumes of binding buffer were added, and incubation was continued for 3 h at 37°C. Finally, lamin aggregates were visualized by electron microscopy after negative staining. We found that branched, paracrystal-like polymers with diameters of between 10 and 70 nm had formed (Fig. 4). The polymers showed a regular axial repeat of  $24 \pm 1$  nm, which is characteristic for lamin paracrystals (2). All lamin aggregates that were present on the grids had this same filamentous appearance. The structure of the polymers was not influenced by binding of MAR fragments (not shown).

Titration experiments (Fig. 5) revealed that MAR binding to these paracrystal-containing lamin preparations is saturable, noncooperative, and of high affinity. Under saturating conditions, one MAR molecule was bound per 45 lamin dimers. This stoichiometry is consistent with the notion that MARs contain multiple protein binding sites per molecule (3, 25, 47, 57). The hyperbolic rather than sigmoidal shape of the binding curve indicates that binding is not cooperative; this is similar to what we have observed for MAR binding to intact nuclear matrices and shells (36). Scatchard analysis revealed one type of high-affinity binding site with a dissociation constant ( $K_d$ ) of approximately 3.5 nM (Fig. 5, inset).

Figure 6 shows the results of our investigation of binding to lamin of different MAR and non-MAR sequences. We studied competition of the *Drosophila* histone MAR with the MAR of the mouse immunoglobulin  $\kappa$  light chain locus (10, 11), the 5' MAR of the chicken lysozyme gene (38), the 5' MAR of the rat glutamate dehydrogenase gene (14), and the synthetic polynucleotides poly(dA-dT) and poly(dA)·poly(dT). We found that random non-MAR DNA, i.e., pBR322 fragments, competed only poorly. The synthetic A+T-rich non-MAR DNAs competed significantly, while all MAR fragments were very efficient competitors. Significant binding of synthetic A+T-rich polynucleotides has also been reported for intact nuclear matrices (27) and for the MAR-binding proteins SAF-A (47), histone H1 (27), SP120 (53), and topoisomerase II (1). The MAR-binding protein SATB1, on the other hand, shows a much stronger sequence specificity (16). The competition data furthermore show that lamin polymers from rat liver are able to bind different MAR sequences from divergent species. Together with our previous observation that lamins derived from cell types other than rat liver also specifically

bound the *Drosophila* histone MAR (36), these findings indicate that the interaction between MARs and lamins is evolutionarily conserved.

**MARs bind to lamin through single-stranded regions and the minor groove.** Characteristic structural features of MARs include a narrow minor groove and a strong potential for base unpairing under superhelical strain. Bode and coworkers (6, 31) have demonstrated that the unwinding potential of MARs is essential for their interaction with the nuclear matrix, while Käs and coworkers have stressed the importance of the narrow minor groove in binding to histone H1 (27), topoisomerase II (1), and nuclear matrices (27). We investigated the involvement of both properties in the interaction of MARs with lamin polymers.

The importance of base unpairing was studied by investigating the competition of MARs with single-stranded DNA fragments (Fig. 7). A fixed amount of radioactive double-stranded *Drosophila* histone MAR fragments plus increasing amounts of competitor DNA were incubated with lamin polymers. The amount of lamin-bound, radioactive *Drosophila* MAR was determined by a filter binding assay. As expected, homologous double-stranded *Drosophila* MAR fragments competed efficiently. Interestingly, we found that single-stranded MARs were also strong competitors, suggesting that MARs interact with lamin polymers through single-stranded regions. This suggestion was confirmed and extended by measuring the competing effect of *E. coli* DNA. While double-stranded *E. coli* DNA had only a small effect on binding of the *Drosophila* MAR, single-stranded *E. coli* DNA fragments competed efficiently. This indicates that there is no strong sequence specificity for binding of single-stranded DNA to lamin. Even more so, RNA also had a significant though smaller competing effect. The experiments show that MARs interact with lamin polymers through single-stranded regions. The unpairing property of MARs thus plays an important role in their specific interaction with lamins, just as it does in their binding to the nuclear matrix (6, 31).

To test whether lamins interact with the minor groove of MARs, as was found by Käs et al. (27) for matrix binding, we employed the drugs distamycin and chromomycin as competitors. These drugs selectively bind the minor groove of double helical A+T- and C+G-rich sequences, respectively (55, 56). We found that distamycin inhibited binding of the *Drosophila*

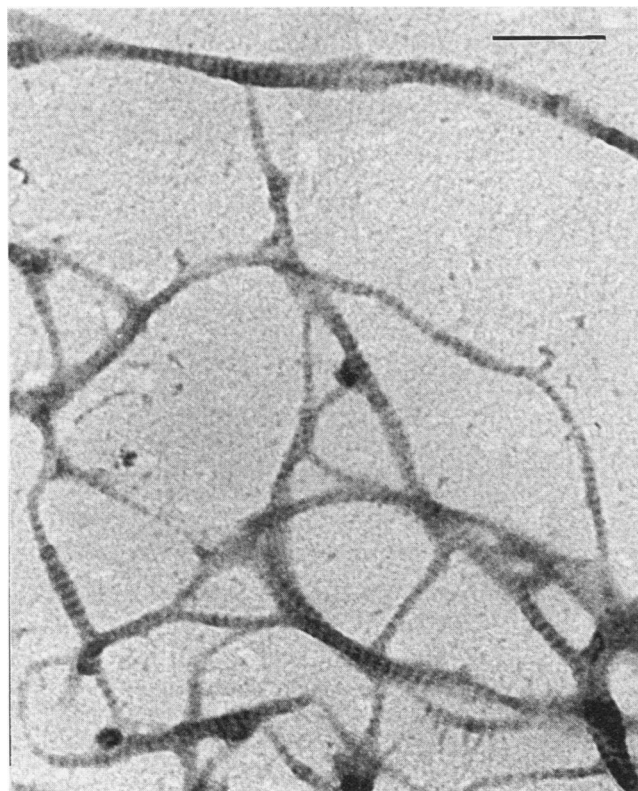


FIG. 4. Formation of paracrystal-like lamin polymers under MAR binding conditions. A preparation consisting of rat liver lamins A, B, and C in lamin buffer (20 mM Tris-HCl [pH 9.0], 500 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 2% Triton X-100) was dialyzed for 2 h at room temperature against MAR binding buffer (10 mM Tris-HCl [pH 7.4], 50 mM NaCl, 2 mM EDTA). Four volumes of binding buffer were added, and incubation was continued for 3 h at 37°C. Lamin aggregates were negatively stained with 1% uranyl acetate. Bar, 250 nm.

histone MAR considerably (Fig. 8). At a saturating concentration of 50  $\mu$ M distamycin, binding was reduced by 70%. Chromomycin had a much smaller effect, maximally suppressing binding by 15%. These results indicate that lamins bind the narrow minor groove of A+T-rich MAR sequences and to a lesser extent also the minor groove of C+G-rich regions.

Since the conformational effect of distamycin on the double helix structure is very small (34), and since distamycin does not affect binding of single-stranded MARs (results not shown), we conclude that at least two types of MAR-lamin interaction exist: one involving single-stranded regions of MARs and one involving the minor groove of duplex regions. Importantly, we found that MAR binding to nuclear matrices from rat liver was affected in the same way by single-stranded DNA, distamycin, and chromomycin as was binding to lamin (results not shown). This implies that the MAR-matrix interaction also involves single-stranded MAR regions and the minor groove.

## DISCUSSION

In this report we show that like lamin B (36), A-type lamins and other coiled-coil proteins specifically interact with MARs. The interaction between MARs and lamin polymers shows many similarities to the interaction of MARs with the nuclear matrix: it is saturable, noncooperative, and evolutionarily conserved. Two different types of MAR-lamin interaction,

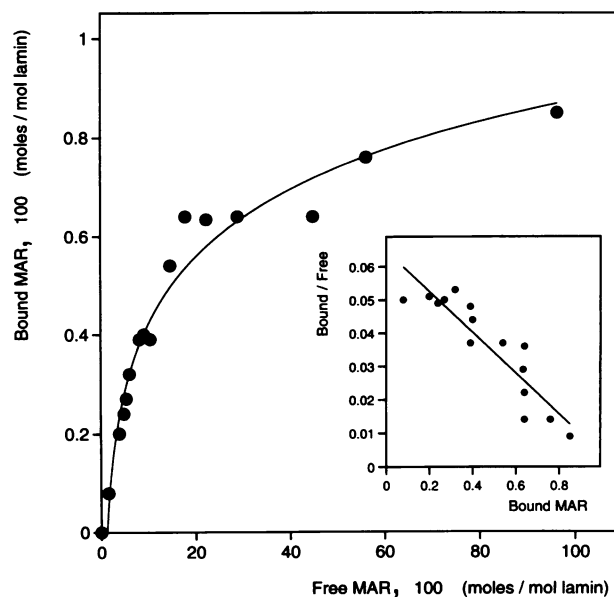


FIG. 5. Saturable, high-affinity MAR binding to lamin polymers. Lamin polymers (1  $\mu$ g of protein) consisting of rat liver lamins A, B, and C were incubated in the presence of 50  $\mu$ g of sonicated *E. coli* DNA per ml with increasing amounts of gel-purified, radioactively labeled restriction fragments containing the *Drosophila* histone MAR or with radioactively labeled pBR322 fragments. The amount of radioactive DNA fragments that bound to the lamin polymers was determined by a filter binding assay. Shown are values for MAR-specific binding. These values were obtained after subtraction of binding values measured for pBR322 fragments from those observed for MAR fragments. In the inset, MAR binding values are displayed in a Scatchard plot.

related to different structural features of MARs, can be discerned: one involving the minor groove and one involving single-stranded regions.

By means of a DNA-binding protein blot assay we have previously identified lamin B as a MAR-binding protein (36). Because of the use of the denaturing agent SDS, no conclusions about the MAR binding capacity of lamins A and C could be drawn. In order to analyze the MAR binding properties of these A-type lamins, we purified A-type and B-type lamins from rat liver nuclear envelopes in the absence of SDS. We found that under these conditions B-type lamins as well as A-type lamins show specific MAR binding. This indicates that general features of the lamin molecule, rather than subtype-specific ones, mediate MAR binding.

This conclusion can be extended further since we found that the structurally related, nonlamin proteins desmin and NuMA also specifically bind MARs, albeit with lower affinity. Desmin is a member of the intermediate filament protein family, to which lamins also belong (for reviews, see references 19 and 48). NuMA is a recently characterized constituent of the nuclear matrix which has an important role during mitosis (12, 13, 59). Intermediate filament proteins and NuMA are built according to a common tripartite structure. They possess a central  $\alpha$ -helical rod domain flanked at both sides by nonhelical domains. Intermediate filament proteins, and presumably also NuMA, self-assemble into long polymers in which the  $\alpha$ -helices are dimerized to coiled-coil molecules. Another similarity between most intermediate filament proteins, including lamins, and NuMA is the occurrence of S/TPXX amino

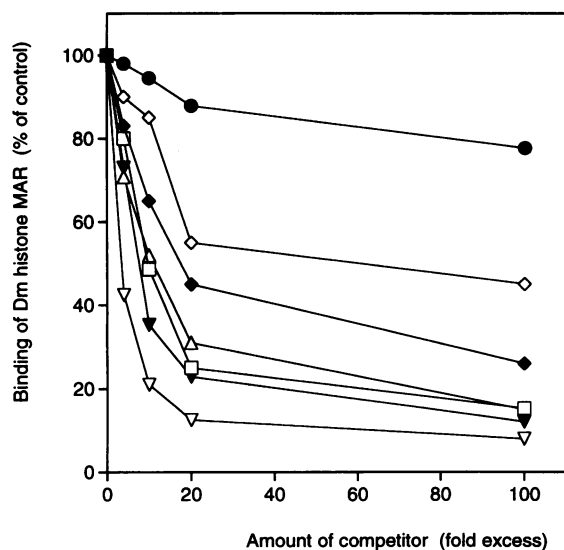


FIG. 6. Binding of different MAR sequences to lamin polymers. Lamin polymers (1  $\mu$ g of protein) consisting of rat liver lamins A, B, and C were incubated in 100  $\mu$ l of binding buffer with 50 ng of gel-purified, radioactively labeled restriction fragments containing the *Drosophila* (Dm) histone MAR, 5  $\mu$ g of sonicated *E. coli* DNA, and increasing amounts of unlabeled competitor DNA fragments [ $\nabla$ , *Drosophila* histone MAR (1.0-kb fragment);  $\square$ , mouse immunoglobulin  $\kappa$  MAR (999-bp fragment);  $\triangle$ , chicken lysozyme gene 5' MAR (446-bp fragment);  $\blacktriangledown$ , rat glutamate dehydrogenase 5' MAR (1.2-kb fragment);  $\bullet$ , pBR322 fragment (754 bp);  $\diamond$ , poly(dA-dT) (sheared to an average length of between 0.3 and 1.2 kb);  $\blacklozenge$ , poly(dA)  $\cdot$  poly(dT) (sheared to an average length of between 0.3 and 1.2 kb)]. The amount of radioactive *Drosophila* histone MAR fragments that bound to the lamin polymers was measured by a filter-binding assay. Binding is expressed as the percentage of control binding measured in the absence of competitor fragments. Shown are the means of determinations of three independent experiments done in duplicate.

acid motifs in their globular end domains. Whereas the physiological relevance of MAR binding to cytoplasmic intermediate filament proteins is difficult to envisage, it is conceivable that the MAR binding capacity of lamins and NuMA has a physiological function. Conclusive evidence concerning this important point awaits *in vivo* DNA-protein cross-linking studies. Nevertheless, the conservation of MAR binding properties among lamins, other intermediate filament proteins, and NuMA suggests that common features, such as the coiled-coil structure and S/TPXX motifs, are involved in MAR recognition. S/TPXX motifs are frequently found in gene-regulatory proteins and are believed to contribute to DNA binding (52). The dimer (SPKK)<sub>2</sub> has been shown to bind preferentially to the minor groove of A+T-rich DNA (9). Similarly, S/TPXX sequences have been found to mediate the specific binding of the chromatin-associated proteins histone H1 (51) and the high-mobility group I protein (45) to the minor groove of A+T-rich DNA. Interestingly, specific binding of chromatin to A- and B-type lamins has also been observed (21, 22, 24). Chromatin binding sites have been mapped to both the  $\alpha$ -helical rod domain (21) and the C-terminal tail domain (20, 24) of lamins.

We found that the interaction between MARs and lamins has many similarities to the interaction between MARs and the nuclear matrix. Like the MAR-matrix interaction, MAR binding to paracrystal-like polymers of rat liver lamins A, B, and C was saturable, noncooperative, and evolutionarily conserved.

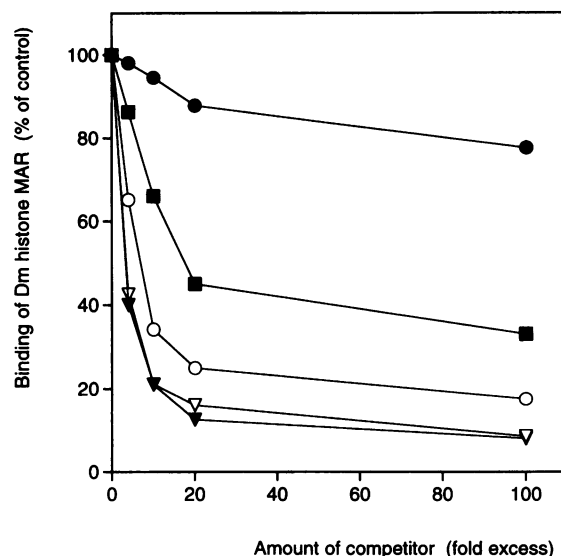


FIG. 7. Inhibition of MAR-lamin interaction by single-stranded nucleic acid. Lamin polymers (1  $\mu$ g of protein) consisting of rat liver lamins A, B, and C were incubated in 100  $\mu$ l of binding buffer with 50 ng of gel-purified, radioactively labeled restriction fragments containing the *Drosophila* (Dm) histone MAR, 5  $\mu$ g of sonicated *E. coli* DNA, and increasing amounts of competitor nucleic acid fragments ( $\blacktriangledown$ , double-stranded *Drosophila* histone MAR;  $\nabla$ , single-stranded *Drosophila* histone MAR;  $\bullet$ , sonicated double-stranded *E. coli* DNA;  $\circ$ , sonicated single-stranded *E. coli* DNA;  $\blacksquare$ , total RNA from HeLa cells). The amount of radioactive *Drosophila* histone MAR fragments that bound to the lamin polymers was measured by a filter binding assay. Binding is expressed as the percentage of control binding measured in the absence of competitor fragments. Shown are the means of determinations of three independent experiments done in duplicate.

Scatchard analysis revealed one type of MAR binding site with a  $K_d$  of approximately 3.5 nM. This is similar to the  $K_d$  of 1.5 nM reported for MAR binding to intact nuclear matrices (14). We have shown previously that nuclear matrices contain two or more different classes of MAR binding sites (36). The same was observed for nuclear shells, which are matrix-derived structures that are highly enriched with lamins. This more complex character of MAR binding to nuclear shells compared with that observed with *in vitro*-assembled lamin polymers could be due to the presence of additional MAR-binding proteins in the shells, as well as to a difference in structure between the nuclear lamina and *in vitro*-assembled lamin polymers. Consistent with the notion that MARs contain multiple protein binding sites per molecule (3, 27, 47, 57), we observed that under saturating conditions, one MAR molecule was bound per 45 lamin dimers. The latter number does not necessarily correspond to the number of lamin binding sites per MAR molecule, since not every one of these 45 lamin dimers in the paracrystal-like aggregates may be accessible for MAR binding. Finally, like MAR-matrix interaction, MAR-lamin interaction was found to be evolutionarily conserved. We have reported previously that the *Drosophila* histone MAR specifically binds to lamins from various species (36). Here we show that different MARs have common binding sites on polymers of rat liver lamins.

We found that synthetic A+T-rich non-MAR DNA also bound to lamin polymers, albeit with a somewhat lower affinity than MAR DNA. Also, intact nuclear matrices (27) and the



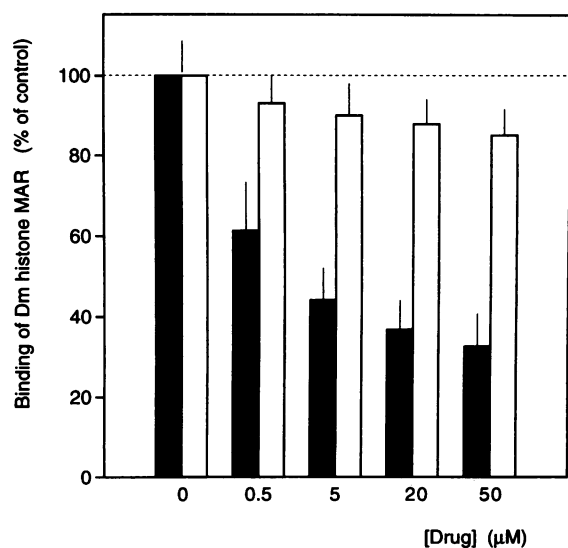


FIG. 8. Suppression of MAR-lamin interaction by distamycin and chromomycin. Gel-purified, radioactively labeled restriction fragments containing the *Drosophila* (Dm) histone MAR were incubated for 90 min at 37°C with the indicated concentrations of distamycin (shaded boxes) and chromomycin (open boxes). Polymerized rat liver lamins A, B, and C (1 μg) were added, and binding was carried out in binding buffer for 3 h at 37°C in the presence of 50 μg of sonicated *E. coli* DNA per ml. The amount of radioactive *Drosophila* histone MAR fragments that bound to the lamin polymers was measured by a filter binding assay. Binding is expressed as the percentage of control binding measured for MAR fragments that had been preincubated in the absence of a drug.

MAR-binding proteins SAF-A (47), topoisomerase II (1), SP120 (53), and histone H1 (27) have been reported to bind A+T-rich non-MAR DNA. So far, only for the MAR-binding protein SATB1 has a much stronger sequence specificity been observed (16). It is likely that the limited sequence selectivity of MAR-lamin interaction reflects the multiple modes of interaction that we discuss in this paper.

Competition experiments with single-stranded DNA fragments revealed that MARs bind to lamin polymers via single-stranded regions. The base sequences of these unpaired regions are of only minor importance since MAR-lamin interaction was almost as efficiently inhibited by single-stranded *E. coli* DNA fragments as by homologous single-stranded MAR fragments. Even RNA had a significant competing effect. We found that this is not the only type of interaction between MARs and lamin. Experiments using the drugs distamycin and chromomycin revealed that lamins also interact with the minor groove of A+T-rich duplex regions and to a lesser extent with the minor groove of G+C-rich regions. Interestingly, we found that MAR binding to nuclear matrices was affected in the same way by single-stranded DNA, distamycin, and chromomycin as was binding to lamin polymers (results not shown). This implies that, like MAR-lamin interaction, MAR-matrix interaction involves single-stranded MAR regions and the minor groove. In direct agreement with this finding, Kay and Bode (31) recently reported a significant competition effect of single-stranded DNA on the binding of MARs to nuclear matrices.

The importance of local base unpairing in MARs has been suggested by others before. Probst and coworkers presented the first evidence that matrix-associated DNA regions expose

single-stranded sites (43) and that these sites might be involved in matrix binding (42). By employing the probe chloroacetaldehyde, which specifically modifies unpaired DNA, Kohwi-Shigematsu and Kohwi (33) have recently demonstrated that MARs are characterized by a strong potential for extensive base unpairing when subjected to superhelical strain. Specific sequence motifs were identified as nucleation sites for unwinding. The unwinding property of MARs has been shown to be important for binding to the nuclear matrix and essential for augmentation of the transcription activity of a linked gene in stable transformants (6). To our knowledge, binding of single-stranded DNA has been studied for two other MAR-binding matrix proteins besides lamin. The thymus-specific protein SATB1 specifically interacts with A+T-rich double-stranded MAR sequences with a high unwinding potential but has no affinity for single-stranded DNA (16). The autonomously replicating sequence (ARS)-binding protein ABF-1 from *Saccharomyces cerevisiae*, on the other hand, recognizes the T-rich single strand of the ARS consensus sequence (23). This interaction is sequence specific, in contrast to what we observed for the interaction of lamins with single-stranded DNA. Minor groove binding has been reported for the MAR-binding proteins SATB1 (16), topoisomerase II (1), and histone H1 (27).

Käs et al. (27) found that histone H1 shows no preferential interaction with denatured MAR sequences. This striking difference in affinity for single-stranded MARs between histone H1 and matrix proteins like lamins may be the clue to one important function of MARs. Along the lines of earlier models (16, 27), we propose that the interaction of nuclear matrix proteins with single-stranded MARs serves to stabilize the transcriptionally active state of chromatin. The current view is that chromatin is divided into independent functional loop domains which are generated by attachment of MARs to the nuclear matrix. In transcriptionally silent chromatin, the chromatin fiber is compacted by histone H1 via its association with nucleosomal linker DNA. Histone H1 molecules interact with each other in a cooperative fashion, presumably via head-to-tail interactions. We predict that inactive chromatin MARs are predominantly double helical and interact with matrix proteins mainly via their minor groove. In transcriptionally active chromatin the 30-nm fiber is in a more open conformation, presumably because of depletion of histone H1 (for a review, see reference 17). Once a chromatin loop has been activated and transcription has started, negative supercoils are generated upstream of the transcription complex. As predicted by the findings of Kohwi-Shigematsu and Kohwi (33), this negative supercoiling will induce stable base unpairing of adjacent matrix-bound MAR sequences. This unpairing of MARs, in turn, will prevent rebinding of released histone H1, as follows from the data of Käs et al. (27). At the same time, binding of the unpaired MARs with matrix proteins such as lamins that have a high affinity for single-stranded DNA will be promoted (6, 31; also this study). Analogous to the model presented by Käs et al. (29), based on the assumption that histone H1-histone H1 interactions in chromatin are highly cooperative, we speculate that histone H1 depletion of MARs will keep adjacent chromatin regions in a histone H1-depleted and thus unfolded conformation.

A similar concept has been proposed by Bode et al. (6) on the basis of their crucial finding that the unpairing property of MARs is important for matrix binding as well as for the augmentation of the transcription activity of a linked gene in stable transformants. On the basis of our present findings, we propose that the interaction of matrix proteins with the minor groove of double-stranded MARs allows the initial building up



of superhelical strain in adjacent chromatin loops at the onset of transcription. The interaction of matrix proteins with unwound MARs may subsequently serve to maintain this situation during ongoing transcription. Our results show that lamins and intact nuclear matrices also recognize non-MAR single-stranded nucleic acids. It is possible that this interaction is also involved in chromatin loop organization during transcription, assuming that unpairing of MARs can spread to neighboring sequences. Alternatively, this interaction could relate to other functions of the nuclear matrix, such as binding of transcription and replication complexes.

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