Inner Nuclear Membrane Protein LBR Preferentially Interacts with DNA Secondary Structures and Nucleosomal Linker[†]

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ABSTRACT: The lamin B receptor (LBR) is an integral protein of inner nuclear membrane whose nucleoplasmic amino-terminal domain contributes to the attachment of the membrane to chromatin. Here we analyzed the interactions of a recombinant GST protein containing the amino-terminal domain of the protein with in vitro reconstituted nucleosomes and short DNA fragments. Data show that the LBR amino-terminal domain (AT) binds linker DNA but does not interact with the nucleosome core. Titration and competition studies revealed that the interaction between LBR AT and DNA is saturable, of high affinity (K_D ~4 nM), independent of DNA sequence, and enhanced by DNA curvature and supercoiling. In this respect, LBR amino-terminal domain binding to nucleosomes is similar to that of histone H1 and non histone proteins HMG1/2 which both bind preferentially to linker DNA and present a significant affinity for DNA secondary structures.

Membranes of the nuclear envelope (NE)[1] compartmentalize chromatin in eukaryotic cells. They consist of two concentric bilayers, outer and inner nuclear membranes, connected around the nuclear pore complexes, where nucleocytoplasmic transport takes place. The inner nuclear membrane (INM) and peripheral heterochromatin are tightly bound and the isolation of NEs necessitates the action of DNAses (*1*). The INM attachment to chromatin is mediated by a specific set of integral proteins with amino-terminal domains (AT) facing the nucleoplasm (*2*). Among the best characterized INM proteins are LAP2 and LBR, which contain one and several transmembrane domains, respectively (*3*, *4*).

One way of connection of INM proteins to chromatin is indirect, through the nuclear lamina, a fenestrated meshwork of intermediate filaments apposed between chromatin and INM (*5*). In addition, INM proteins are directly attached to chromatin via specific peptide motifs present in their nucleoplasmic domains (*4*, *6*−*8*).

In vivo and in vitro studies have emphasized the role played by the ~25 kDa amino-terminal domain of LBR in the targeting and attachment of LBR to chromatin. First, after metaphase, LBR is targeted to chromatin in anaphase A, prior to any other components of the NE, including lamins (*9*, *10*). Second, transfection experiments with chimeric constructs have shown that LBR AT is essential for the targeting of LBR to INM after synthesis and then for its retention in this membrane domain (*11*, *12*). Third, the presence of LBR-containing vesicles has been shown to be necessary for the in vitro assembly of nuclear membranes around demembranated sperm chromatin from sea urchin (*13*), Xenopus (*14*, *15*), and CHO mitotic chromosomes (*16*).

Due to the complexity of the chromatin preparations used in previous cell-free studies, the precise target of LBR AT in chromatin has not been identified. Here, we present an in vitro analysis of the interactions of recombinant LBR AT with the nucleosome, the basic unit of chromatin organization, and with short DNA fragments with various secondary structures. Experiments were performed with well-defined components including purified core histones, recombinant H1 histone, and DNA fragments that favor histone octamer positioning in a nonrandom fashion (*17*).

EXPERIMENTAL PROCEDURES

Fusion Proteins. Plasmid encoding a GST fusion protein containing the amino-terminal domain of human LBR (GST-207) has been previously described (*7*). *Escherichia coli* strain TG1 was transformed by standard methods (*18*). The expressed GST fusion protein was purified on glutathione−Sepharose (Pharmacia) according to the manufacturer and then analyzed by denaturing electrophoresis (*19*). The concentration of the protein was estimated by Coomassie blue staining of the gel, before storage at −80 °C.

Antibodies and Western Blotting Procedure. Proteins were resolved by SDS−PAGE on 10 or 12% gels and then by immunoblotting, as previously described (*20*). Nitrocellulose sheets were probed with a 1:150 dilution of polyclonal rabbit antibody R207 directed against the whole amino-terminal

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[1] Abbreviations: AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride; bp, base pair(s); BSA, bovine serum albumin; ECL, enhanced chemoluminescence; GST, gluthatione S-transferase; PAGE, polyacrylamide gel electrophoresis; CHO, Chinese hamster ovary; NE, nuclear envelope; INM, inner nuclear membrane; LBR, lamin B receptor; LAP2, lamina-associated polypeptide 2; HP1, heterochromatin protein 1; HMG1, high mobility group protein 1; MAR, matrix-associated DNA region; AT, amino-terminal domain.

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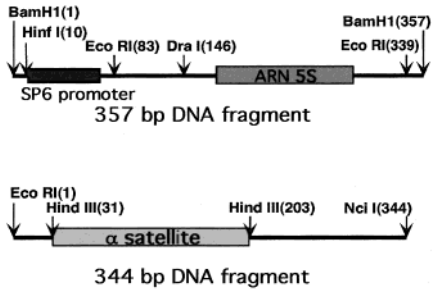

FIGURE 1: Schematic representation of the sequence of the 357 bp and 344 bp DNA fragments. The 357 bp fragment contains the SP6 promoter sequence (black box) linked to the sea urchin 5S RNA gene (gray box). The 344 bp fragment contains the 172 bp repeat of α-satellite DNA (gray box) from african green monkey. Major restriction sites are indicated (arrows).

domain of human LBR (*21*) or with a 1:1500 dilution of a polyclonal goat anti-GST serum (Pharmacia), then incubated with the appropriate secondary antibodies coupled to horseradish peroxidase, and finally revealed with the ECL technique, following the instruction of the manufacturer (Amersham).

DNAs. The 357 bp DNA fragment (see Figure 1), containing the SP6 RNA polymerase promoter linked to a sea urchin 5S RNA gene (*22*), was obtained from a BamHI digest of plasmid pUC[357.4] (*23*). The 146 bp DNA fragment was obtained from the BamHI and DraI double digest of pUC[357.4] and contains the SP6 promoter only. The 344 bp fragment, containing the 172 bp repeat unit of African green monkey α-satellite DNA from the plasmid pFS 522 (*24*), was obtained by sequential digestion with Eco RI and BamHI and then NciI. Dephosphorylation and 5′-end labeling with ^{32}P-ATP and T4 polynucleotide kinase were performed according to standard protocols. Circular DNAs, either relaxed or supercoiled, were obtained from the 357 bp DNA fragment as previously described (*25*).

*Nucleosome Reconstitution***.** Core histones were prepared from duck erythrocyte nuclei as described (26). Duck erythrocyte recombinant H1 was a gift from Dr. A. Hamiche (LBME-CNRS, Toulouse, France). Core histones were assembled on DNA fragments using the salt jump method (*17*, *23*, *26*, *27*). Briefly, DNA (50 μg/mL) and core histones (histone/DNA ratios of 0.25 and 0.4 (w/w) for the 357 bp DNA fragment, and of 1 for the 146 bp DNA fragments) were incubated in 2 M NaCl and 20 mM Tris, pH 7.5, at 37 °C for 10 min, then diluted to 12.5 μg/mL DNA and 0.5 M NaCl with D buffer (20 mM Tris, pH 7.5, 0.2 mM EDTA, 1 mg/mL BSA), and finally dialyzed against 50 mM NaCl, 10 mM Tris, pH 7.5, and 1 mM EDTA at room temperature for 4 h. When needed, H1 histone was added to the 0.5 M NaCl diluted histone and DNA mix, at a ratio H1/core histones = 1.1 (w/w), according to Zivanovic et al. (*26*).

GST-207−DNA Binding Reactions and Mobility Studies. GST-207, at the indicated concentrations, was added to DNA or to histones and DNA mix, in the presence of 0.5 M NaCl, and then dialyzed against 50 mM NaCl, 10 mM Tris, pH 7.5, and 1 mM EDTA at room temperature. Nucleoprotein complexes were analyzed on 5% polyacrylamide gels at an acrylamide-to-bisacrylamide ratio of 29/1 (w/w), in TBE 0.5× or in TGE 1×, i.e., 25 mM Tris-HCl, pH 8.4, 190 mM glycine, and 1 mM EDTA (see legends of Figures 3 and 5). After a 1 h preelectrophoresis, samples were loaded and resolved by a 2−3 h electrophoresis at 50 V. DNA retardation was either visualized by ethidium bromide staining or detected by autoradiography of the dried polyacrylamide gel at −80 °C using BioMax MR film (Kodak) and an intensifying screen.

Micrococcal Nuclease Digestions. Digestions were performed on DNA or chromatin reconstituted in the presence or absence of GST-207 as described above, excepted for dialysis that was performed against 10 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA. Samples, at a DNA concentration of 12.5 μg/mL, were supplemented with 0.5 mM $CaCl_2$ and incubated at 37 °C with 16×10^{-3} units of micrococcal nuclease/mL (Sigma) for 5 min. Deproteinized DNAs were analyzed onto a 4% polyacrylamide gel at an acrylamide-to-bisacrylamide ratio of 19/1 (w/w), in 40 mM Tris-HCl, pH 7.8, 20 mM sodium acetate, and 2 mM EDTA.

Saturation and Competition Experiments. Saturation and competition with DNA and chromatin preparations were performed at equilibrium ($t \geq 3$ h at room temperature) in 20 μL of DN buffer that contains 10 mM Tris, pH 8, 50 mM NaCl, 0.2 mM EDTA, 1 mg/mL BSA and 1 mM AEBSF. For saturation experiments, 80 ng of GST-207 fusion protein was incubated with the indicated concentrations of ^{32}P-labeled 357 bp DNA fragment for 3.5 h and then mixed with 20 μL of glutathione−Sepharose beads (Pharmacia) equilibrated in DN buffer. GST-isolated protein DNA complexes were sedimented at 1000*g* for 5 min and then washed with one volume of DN buffer. The two supernatants were pooled, and the radioactivities of bound (pellet) and unbound (supernatant) DNA fractions were counted. At each DNA concentration, nonspecific binding was measured by omitting fusion protein in the assay, and the appropriate correction was made. Competition experiments were performed by incubating a fixed amount of labeled DNA (required to half-saturate the fusion protein) with increasing amounts of unlabeled competitor DNA or chromatin. Cold competitors were either (i) linear 357 bp DNA fragment naked or reconstituted with core histones, plus or minus H1 histone, or (ii) 357 bp DNA minicircles either relaxed or supercoiled. Molarities of bound (B), free (F), and total (T) fractions were calculated, and the percentage of bound DNA (B/T) was plotted as a function of competitor concentration. Aternatively, data were plotted according to Scatchard (*28*), and dissociation constants (K_D) were measured.

RESULTS

Amino-Terminal Domain of LBR Interacts with Nucleosomal DNA. Figure 2A shows the secondary structure of human LBR which contains a nucleoplasmic amino-terminal domain of 207 amino acids upstream of a hydrophobic domain corresponding to eight putative transmembrane segments. The amino-terminal domain of LBR contains two globular domains, GI and GII, separated by a positively charged loop (*4*, *7*).

Recombinant GST fusion protein containing the 207 first amino acids of human LBR amino-terminal domain was purified from soluble bacterial extracts using glutathione−Sepharose and then analyzed by immunoblotting with antibodies directed either against LBR (Figure 2B, lane

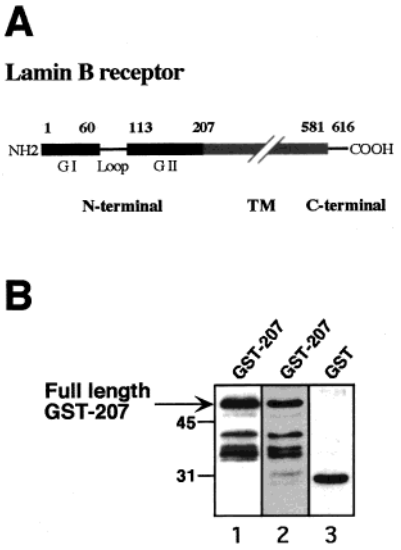

FIGURE 2: Schematic representation of the secondary structure of LBR and electrophoretic analysis of LBR amino-terminal GST-fusion protein. (A) The amino-terminal domain (N-terminal), the transmembrane domains (TM, gray bar) and the carboxyl-terminal domain (C-terminal) of human LBR are shown. Bold black bars refer to two globular domains (GI and GII) of the N-terminal domain that have been described previously (*7*). (B) Soluble extracts from *E. Coli* transformed by plasmids encoding a GST fusion protein containing the amino-terminal domain of human LBR (GST-207, lanes 1 and 2), or GST alone (lane 3), were analyzed by immunoblotting, using affinity-purified anti-LBR antibody R207 (lane 1) or anti-GST serum (lanes 2 and 3). Signals of ~55 and ~29 kDa (full length proteins) were revealed for LBR fusion protein and GST protein, respectively. Lower MW immunoreactive components present in lane 1 and 2 are either proteolytic fragments or partial translation products of GST-207.

1) or GST (Figure 2B, lanes 2). The preparation contained immunoreactive components of the expected mobility (~55 kDa) and also shorter fragments that may correspond either to partially translated products or to proteolytic fragments.

The interaction of LBR fusion proteins with DNA and with nucleosomes reconstituted by the in vitro assembly of core histones onto the 357 bp linear DNA fragment was studied by electrophoresis on a 5% polyacrylamide gel (Figure 3A). When the 357 bp DNA fragment was reconstituted with increasing concentrations of GST-207 (from 1 to 4 μg/mL) in the absence of core histones, the 357 bp DNA signal was progressively shifted (Figure 3A, lanes 2−4). At the highest concentration of GST-207, the naked DNA signal almost disappeared while upper bands were reinforced (Figure 3A, lane 4). When 4 μg/mL of GST protein alone were used in place of GST-207, no shift of naked DNA was observed (Figure 3A, lane 6). The nucleosome preparation used to study the chromatin−LBR interaction was heterogeneous, since several bands were obtained, with a reduced mobility compared to that of naked DNA (Figure 3A, lane 7). The heterogeneity was not due to a mix of mono- and dinucleosomes since, under our experimental conditions, only 30−40% of the DNA was assembled with histones and dinucleosome assembly was reduced (*23*). In fact, the different signals correspond to mononucleosomes with different mobilities, depending upon the distance of the histone octamer from the center of the DNA fragment (*17*). When nucleosome reconstitution was performed in the presence of increasing concentrations of GST-207 (from 1 to 4 μg/mL), each nucleosome band was progressively shifted (Figure 3A, lanes 8−10). At the highest concentration of GST-207, nucleosome bands were hidden by the strong signal due to DNA-GST-207 complexes (Figure 3A, lane 10). By contrast, when 4 μg/mL of GST protein alone was used during reconstitution, no shift of nucleosomes was observed (Figure 3A, lane 12). Therefore the GST moiety of the GST-207 fusion protein is unlikely to play a role in the retardation of the recombinant protein. Similar data were obtained when the same experiments were performed with the amino-terminal domain of recombinant chicken LBR (data not shown). These retardation experiments showed that the amino-terminal domain of LBR binds to DNA as well as to nucleosomes reconstituted with the 357 bp DNA fragment.

LBR Amino-Terminal Domain Binds to Linker DNA. As the histone octamer in nucleosome binds only 146 bp of DNA, nucleosomes reconstituted on 357 bp DNA contain both histone-bound and histone-free DNA, which we will refer to as linker DNA. To determine if the GST-207 binding to chromatin was due to its binding to linker DNA or to the nucleosome core, the following experiments were undertaken. First, the interaction of GST-207 with chromatin was performed with nucleosome reconstituted on a 146 bp DNA fragment, i.e., with a nucleosome that does not contain linker DNA. Data show that nucleosomes assembled on the 146 bp DNA migrated as a single band on polyacrylamide gel (Figure 3B, lane 1) and that the addition of 4 μg/mL of GST-207 did not lead to any retardation of the nucleosome signal while naked DNA was almost entirely complexed to the fusion protein (Figure 3B, lane 2). Moreover, GST-207 does not bind to core histones since no histones were retrieved in pull down experiments performed in the absence of DNA, using glutathione−Sepharose-bound GST-207 (data not shown). Second, nucleosomes reconstituted on the 357 bp DNA fragment in the absence or presence of GST-207 at a concentration of 2 μg/mL were digested with micrococcal nuclease and the size of the DNA fragments obtained after digestion was analyzed by polyacrylamide gel electrophoresis. The data of Figure 3C (lanes 1 and 2) show that in both preparations a ~146 bp fragment was obtained, characteristic of the DNA protection generated by the histone octamer, an additional fragment of ~160 bp being observed in the presence of GST-207 (Figure 3C, lane 1). Micrococcal digestion of naked DNA or GST-207/DNA complexes was rapid and did not lead to any fragment with the nucleosomal length (data not shown). Thus, GST-207 interaction with the nucleosome leads to the protection of an additional ~15 bp located in the linker DNA. From these two types of experiments, we concluded that the LBR AT interaction with chromatin reconstituted with the 357 bp linear fragment is not mediated by the nucleosome core but rather takes place through linker DNA.

Interactions of the Amino-Terminal Domain of LBR with DNA of Different Sequences and Conformations. To further analyze the interactions of LBR AT with DNA, saturation and competition studies were performed with different DNA preparations.

Titration experiments were performed by incubating at room temperature a fixed amount of GST-207 fusion protein (at a concentration of 4 μg/mL, i.e., 75 nM), with increasing concentrations of the ^{32}P-labeled 357 bp DNA fragment under conditions where complexes were soluble. At equilibrium ($t \geq 3$ h), GST-207 was isolated by binding to

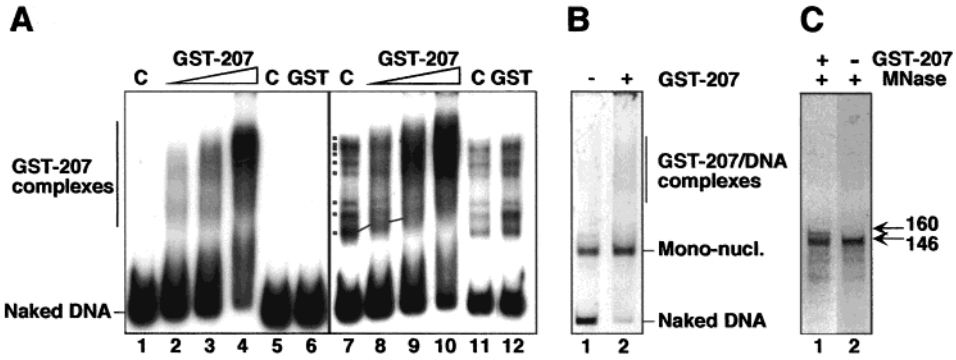

FIGURE 3: Binding of human LBR amino-terminal domain to DNA and nucleosomes. (A) DNA (lanes 1−6) and mononucleosomes (lanes 7−12) were assembled with increasing amounts of GST-207 protein at the following concentrations: 1 μg/mL (lanes and 8); 2 μg/mL (lanes and 9); 4 μg/mL (lanes 4 and 10). GST protein alone was added at 4 μg/mL in lanes 6 and 12. Lanes 1 and 5 refer to DNA control, and lanes 7 and 11 refer to mononucleosomes control. Complexes were resolved on a 5% polyacrylamide gels in TBE 0.5×. Note that DNA and mononucleosomes migration (dots in lane 7) was delayed by the addition of LBR fusion protein (see bars between lanes 7, 8, and 9 for mononucleosomes) but not by the addition of GST protein (lanes 6 and 12). (B) Nucleosomes without linker DNA were reconstituted with a 146 bp DNA fragment (lane 1). After the addition of 4 μg/mL of GST-207 (lane 2), the signal for naked DNA disappeared while the mononucleosomes signal remained intact. TGE buffer instead of TBE was used during electrophoresis. (C) DNA protection from microccocal nuclease digestion of mononucleosomes reconstituted without or with GST-207 at a concentration of 2 μg/mL. Note the protection of 146 bp DNA in lanes 1 and 2 and of an additional 160 bp DNA in the presence of GST-207 in lane 1. Arrows indicate the 146 and 160 bp-digested products.

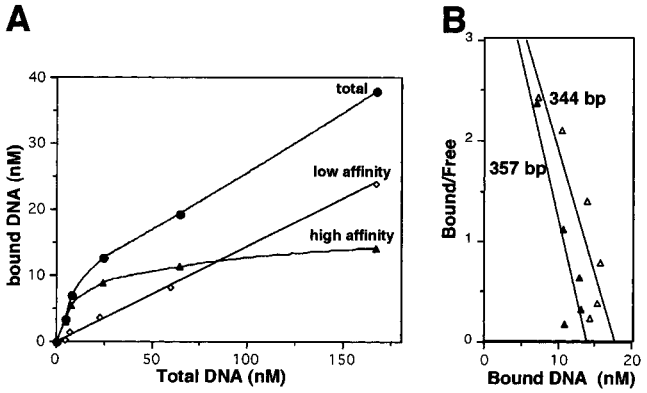

FIGURE 4: Quantification of LBR AT affinity for DNA. (A) Saturation analysis. A total of 80 ng of GST-207 was incubated with increasing amounts of labeled DNA. At equilibrium, DNA bound to fusion protein was retrieved by binding to glutathione−Sepharose beads and counted (total). Nonspecific DNA binding (low affinity) measured in the absence of GST-207 was subtracted from total binding to obtain specific binding (high affinity). (B) Affinity measurement. A total of 80 ng of GST-207 was mixed with 65 ng of either labeled 357 bp or labeled 344 bp fragments under conditions of half-saturation and then incubated with increasing concentrations of cold 357 bp or 344 bp DNA fragments, respectively. Scatchard plots obtained for both DNA fragments are presented. K_D values of ∼4 and ∼6.2 nM at 22 °C were found for 357 and 344 bp DNAs, respectively.

glutathione−Sepharose beads, and radioactivity of bound and free DNA was measured. Figure 4A shows that binding of the linear DNA to GST-207 was saturable. Saturation was obtained with a ∼13 nM concentration of 357 bp DNA, i.e., one molecule of GST-207 per ∼70 bp. When studied under similar conditions, no binding of GST protein to DNA was observed (data not shown).

Competition studies were performed by using a fixed amount of ^{32}P-labeled 357 bp linear DNA and GST-207 under conditions of half-saturation, in the presence of increasing concentrations of DNA competitors. Cold linear 357 bp DNA fragment was the first competitor to be used, and data, expressed as Scatchard plots, are shown in Figure 4B. A linear plot was obtained, showing that the binding is not cooperative and that only one class of DNA binding site was present in LBR amino-terminal domain. A dissociation constant (K_D) at 22 °C of 4 nM ± 1.9 (S.E.) was calculated (mean of 4 experiments).

Several parameters of the interaction between LBR AT and DNA were then studied, including the role of DNA sequence, curvature, and supercoiling. The role of DNA sequence was checked by performing the competition assay with α-satellite 344 bp DNA fragment. A K_D value of 6.25 nM was obtained (Figure 4B), close to that obtained with the 357 bp DNA fragment, suggesting that the DNA sequence plays no role in the interaction. The role of DNA curvature and supercoiling was analyzed by competing the binding of the labeled 357 bp linear DNA fragment with the 357 bp DNA fragment under either a closed circular form (relaxed) or its supercoiled isomer containing two negative superhelical turns, i.e., with a physiological superhelical density. Figure 5A shows that the binding of GST 207 for the 357 bp DNA fragment was significantly modified by the change in DNA conformation, the relative affinity being supercoiled > closed circular > linear. Half-maximal displacement of each DNA species was calculated from these curves to be of 31.6 nM for linear DNA and of 11.4 nM and 7.4 nM (i.e. 3−4 times lower) for closed circular and supercoiled DNA, respectively. These data show that curvature and supercoiling are two factors that enhance the affinity of DNA for LBR AT.

Interaction of the Amino-Terminal Domain of LBR with H1-Containing Nucleosomes. As histone H1 is the major linker binding protein in chromatin, its putative role in the binding of GST-207 to nucleosome was also investigated. Chromatin was reconstituted with the linear 357 bp DNA fragment, core histones, and recombinant H1 histone (*29*), generating complexes with modified mobilities compared to H1-free nucleosomes and DNA−H1 complexes (Figure 5B, right panel, compare lanes 1 and 2). Competition studies show that an 8 mol excess of 357 bp DNA assembled in this chromatin preparation only generated a 40% inhibition of the binding of the labeled linear DNA, compared to the 80% inhibition obtained with the same molar excess of naked 357 bp linear DNA or DNA assembled into mononucleo-

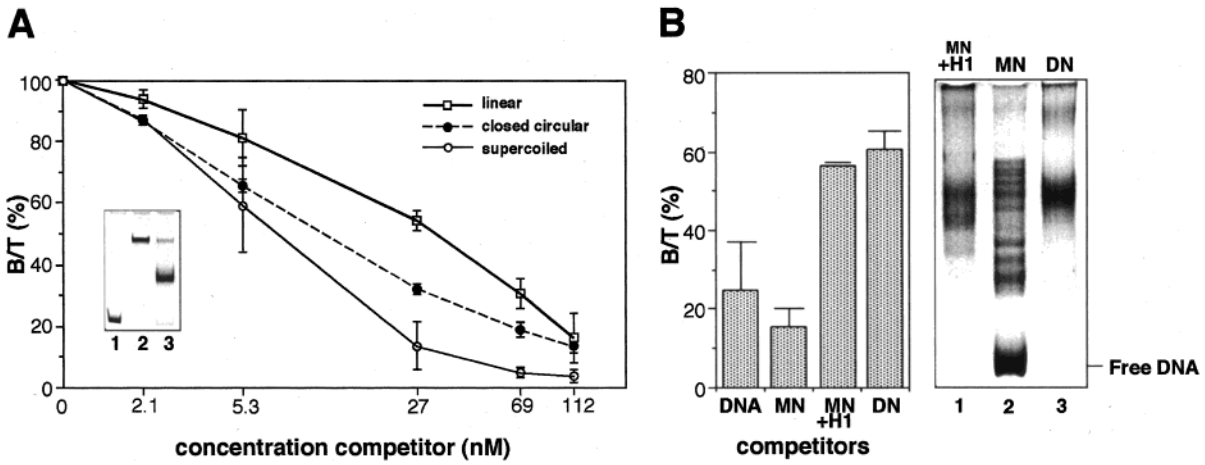

FIGURE 5: Interactions of the amino-terminal domain of LBR with DNA or chromatin. Competition experiments were performed under the conditions described in the legend of Figure 4. (A) DNA fragments of 357 bp either linear, closed circular, or supercoiled (see respective electrophoretic resolution in lanes 1, 2, and 3 of inset) were used as competitors. After correction for nonspecific binding, the percentage of labeled linear 357 bp fragment bound to fusion protein was plotted as a function of the log of the concentration of cold competitor expressed in nM. Half-maximal displacements were of 7.4, 11.4, and 31.6 nM for supercoiled, closed circular, and linear DNA, respectively. (B) Competition experiments were performed with free DNA and chromatin preparations including mononucleosomes (MN), nucleosomes containing H1 (MN + H1), and dinucleosomes (DN): right panel, electrophoretic resolution of the 3 chromatin preparations in TGE buffer; left panel, the figure presents the percentage of labeled linear 357 bp fragment bound to GST-207 in the presence of an 8 mol excess of the same DNA fragment assembled into different chromatin preparations. A control experiment performed with naked DNA is presented in DNA lane. Bars in (A) and (B) represent the standard errors.

somes (Figure 5B, left panel; compare lanes DNA, MN, and MN + H1). The competition generated by nucleosomes containing H1 was similar to that obtained by the same molar excess of 357 bp DNA reconstituted into dinucleosomes that only generate 50 bp of linker DNA (Figure 5B, left panel, lane DN). These data show that the presence of histone H1 on the 357 bp DNA fragment assembled into nucleosome and on the 357 bp naked DNA decreases the binding of the amino-terminal domain of LBR to DNA.

DISCUSSION

We investigated the in vitro interactions of the amino-terminal domain of human LBR with nucleosomes reconstituted with a 357 bp DNA fragment, i.e., containing a nucleosome core and ~200 bp of free DNA. Using band shift assays, we showed that LBR AT recombinant protein induces a significant retardation of both DNA and nucleosomes. The nucleosome retardation was not due to the binding of LBR AT to nucleosome core but rather to its binding to linker DNA in a sequence independent fashion. In this respect, LBR AT is similar to linker DNA binding proteins H1 and HMG1/2 that also induce nucleosome retardation (*30*). However, while LBR AT and HMG1/2 induce a retardation of all nucleosomes whatever their position on the DNA fragment, linker histone H1 is able to accelerate the migration of nucleosomes located in the center of the DNA fragment, likely by bringing linker arms close together (*29*; see also Figure 5B, right panel). Thus, in contrast to histone H1, LBR AT does not seem to generate chromatin compaction.

Titration studies showed that 357 bp linear DNA binding to LBR AT was saturable and noncooperative. Scatchard analysis revealed one type of DNA binding site with a K_D of approximatively 4 nM. This K_D value is of the same order of magnitude as that found for the interactions of histone H1 with naked DNA (*31*) and of A-type lamins for matrix associated DNA regions (MARs) (*32*), measured under similar ionic strength conditions. From these experiments, we calculated that the LBR AT recombinant protein (~55 kDa) covered approximatively 70 bp on DNA, in contrast with the ~15 bp protected from micrococcal digestion by the same protein. Comparatively, the 80 amino acids (~9 kDa) of a single HMG domain in HMG1/2 protein, which contains two of these domains, binds to 20 bp DNA (*33*) and protect a linker DNA fragment of the same size from micrococcal digestion experiments (*30*). The relatively large size of the DNA binding site calculated from the titration studies may be due to a steric binding hindrance by the GST moiety of the fusion protein that does not hinder micrococcal digestion.

Circular and supercoiled conformations of the 357 bp DNA fragment generate a 3−4-fold increase in affinity for LBR AT compared to its linear conformation. Thus, DNA curvature should be one of the factors responsible for the increase in affinity. Furthermore, the difference in affinity for LBR AT between relaxed and supercoiled DNAs could be explained by an increase of the DNA curvature and/or by the formation of a crossover of the two helices in the supercoiled minicircle. At the 50 mM sodium chloride concentration used in our study, a minicircle with a physiological superhelical density can be a mix of molecules with a circular configuration and of molecules with a crossed configuration (*34*). Interestingly, linker histones H1 and the HMG1/2 family of proteins also bind supercoiled DNA (*35*−*37*) as well as cruciform-like structures (*38*−*41*), another form of crossed configuration. The HMG1/2 family of proteins also has the ability to recognize bent DNA structures (*42*). A comparison of the amino-terminal domain of LBR and the HMG box domain, using the protein secondary structure prediction program PSI-BLAST, did not reveal any similarity between both domains.

In the cell nucleus, LBR interacts with peripheral chromatin, which is mostly condensed and transcriptionally inactive. As linker histone H1 may be more likely associated with "silent" chromatin, its putative role in modulating the

affinity of LBR AT for nucleosome was investigated. Mononucleosomes containing H1 were less efficient in binding LBR AT than H1-free nucleosomes and free DNA, showing that histone H1 and LBR AT do not cooperate but rather are in competition for the binding to linker DNA.

Although this study was performed with well-defined reagents, it faced limitations that are inherent to in vitro studies. First, the full length fusion protein preparation contains some lower molecular weight fragments that may also bind DNA (Figure 2B). Second, posttranscriptional modifications of histones (reviewed in ref *43*) and LBR (*44*, *45*) that are present in living cells were absent from recombinant proteins. Attempts will be made in future experiments to try to overcome these limitations.

The LBR-DNA interactions studied here are biologically relevant since, in the cell nucleus, LBR AT faces the nucleoplasm. As LBR AT also binds chromatin proteins of the HP1 family (*6*, *7*), both interactions may cooperate to enhance LBR binding affinity for chromatin. In coordination with lamins and the LAP2 family of integral proteins, which are also chromatin-binding proteins (*32*, *8*), LBR therefore provides additional attachments sites that may be responsible for the strong adhesion of the nuclear envelope to chromatin.

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