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LBP-p40 Binds DNA Tightly through Associations with Histones H2A, H2B, and H4

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Laminin binding protein precursor p40 (LBP-p40) was long believed to be located exclusively in the cytoplasm. We recently reported localization of epitope-tagged LBP-p40 to the nucleus tightly associated with nuclear structure as well as on ribosomes. In this paper, we analyze the interaction of LBP-p40 with DNA and nuclear proteins *in vitro*. LBP-p40 was found to bind to a double-stranded DNA cellulose column at moderate salt. However, when mixed with a high salt nuclear extract, LBP-p40 was eluted from the DNA cellulose column only at higher salt. An LBP-p40 affinity column indicated that both histone H1 and in particular the core histones associate with LBP-p40. Using recombinant core histone molecules fused with glutathione *S*-transferase (GST), we demonstrate that histones H2A, H2B, and H4 are capable of interacting with LBP-p40, whereas H3 is not. These results suggest that association of LBP-p40 with histones H2A, H2B, and H4 confers tight binding of LBP-p40 to chromatin DNA in the nucleus.

Key Words: **LBP-p40; DNA-binding protein/histones; chromatin structure.**

A cDNA encoding a 40kDa (exactly 32,817Da) polypeptide was isolated as a sequence corresponding to an antigen rich in cancer cells (1–3) and identified as a laminin binding protein precursor, LBP-p40, based on sequence homology with a previously reported 67 kDa laminin binding protein (4). However, this nomenclature evoked a controversy concerning the function of LBP-p40. Several reports indicated that LBP-p40 was localized on the 40S ribosome (5–7) casting doubt on its presumed function as a laminin receptor (8). Two homologues of LBP-p40 referred to as YST-1 and YST-2 were cloned in Saccharomyces cerevisiae, an organism that has no laminin. Disruption of either YST-1 or -2 resulted in retarded yeast growth and incomplete polysome formation, while disruption of both genes was lethal (9). These findings supported the view that LBP-p40 functions in translation. However, other studies indicated that LBP-p40 may be the Sindvis virus receptor (10), a positional marker in embryonic eye development (11, 12), and/or a precursor of the classic 67kDa laminin receptor (13). However, rigorous data on the biological significance of the 67kDa laminin receptor could not be obtained, while the structure and function of the integrin type laminin receptor have been more extensively investigated (14). More recently, Rieger *et al.* (15) reported that LBP-p40 interacts with prion protein in eukaryotic cells. Clearly, the function of LBP-p40 awaits further elucidation. We encountered LBP-p40 by a quite different approach. To analyze nuclear architectures, we have generated numerous monoclonal antibodies against nuclear proteins. One monoclonal antibody, M108, recognized a common 40 kDa protein on cytoplasmic particles, the nuclear envelope, and mitotic chromosomes (16). The antigen appeared to be tightly associated with both nuclear matrix and chromatin DNA in the interphase nucleus (17). The protein was isolated from cytoplasmic particles and identified as LBP-p40 (18). Then, we reported that epitope-tagged LBP-p40 migrated to the nucleus as well as to 40S ribosomes, and that LBP-p40 could be extracted from the nucleus by sequential treatment with DNase I and high salt-detergent in the same manner as an endogenous nuclear p40 (18). McCaffery, *et al.* also reported that a monoclonal antibody against LBP-p40 immunohistochemically labeled an antigen in the nucleus as well as in cytoplasmic particles (12). Similarly, YST proteins were first isolated as DNA-binding proteins from crude nuclear extract (9, 19). These findings suggest that LBP-p40 may have a physiological function in the nucleus, in addition to its role in translation. The discovery of DNA-binding ability in

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ribosomal proteins (20) has led to recent studies that demonstrated extraribosomal functions for many ribosomal proteins. Here, we have shown that recombinant human LBP-p40 binds a DNA cellulose column and that the binding is augmented by the association of LBP-p40 with the core histones, H2A, H2B, and H4. Our results suggest a novel function for LBP-p40 within chromatin in the nucleus.

MATERIALS AND METHODS

Polyclonal antibodies. Rabbit anti-recombinant LBP-p40 antibody was obtained by immunized rabbits with purified recombinant LBP-p40. Rabbit anti-mouse LBP-p40 antibody was generated from rabbits immunized with cytoplasmic LBP-p40 purified from Ehrlich tumor cells. Rabbit anti-histone H1 antibody was obtained by immunizing rabbits with histone H1 (Boehringer).

Preparation of nuclei. Nuclei were prepared from Ehrlich tumor cells by modifying the procedure of Muramatsu et al. (21). The cells suspended in 20 vol (of the cell pellet) of 10 mM Tris–HCl (pH 7.5), 10 mM NaCl, 1.5 mM $MgCl_2$ were sit on ice for 10 min and centrifuged at 600 g for 10 min. The swollen cells were resuspended in the same volume of 10 mM Tris–HCl (pH 7.5), 10 mM NaCl, 1.5 mM $MgCl_2$, 0.3% NP 40, 0.2% sodium deoxycholate. The mixture was homogenized for 10 strokes in a glass-teflon homogenizer. The homogenate was centrifuged at 1200 g for 5 min to sediment the crude nuclei. The pellet was resuspended in a 10-fold volume (of the original cell volume) of 0.25 M sucrose and 3.3 mM $CaCl_2$ layered over an equal volume of 0.88 M sucrose and centrifuged at 1200 g for 10 min. The pellet contained purified nuclei.

Preparation of nuclear extract. In a modification of a procedure previously described (22, 23), the purified nuclei were suspended with DNase I (200 μg/ml, Sigma) in 50 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM$CaCl_2$, 1mM phenylmethysulfonyl fluoride (PMSF); incubated at room temperature for 30 min; and centrifuged at 10000 g for 5 min. The pellet was washed in 150 mM NaCl, 50 mM Tris–HCl (pH7.5), 1 mM PMSF, and then centrifuged as described above. The pellet was resuspended in 500 mM NaCl, 1% NP 40, 2 mM EDTA, and 1 mM PMSF in 50 mM Tris-HCl (pH 9.0), incubated at 4°C for 1 h and recentrifuged at 10000 g for 5 min, resulting in a supernatant referred to as high-salt detergent supernatant. Finally, the extract was diluted with salt-free 50 mM Tris–HCl (pH 7.5) containing PMSF to adjust the salt concentration to 150 mM. Nuclear extract eluted from the DNA cellulose column with 0.5 M NaCl Nuclear extract described above was applied to a double strand DNA cellulose column (0.5 g) equilibrated in 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, and 1 mM PMSF. The column was washed with 0.3 M NaCl in the same buffer and the proteins were eluted in the same buffer containing 0.5 M NaCl. We called this fraction the 0.5 M NaCl-eluted nuclear extract.

Construction of the LBP-p40 protein expression plasmid. The human LBP-p40 cDNA was obtained by PCR from a HeLa cDNA library (18). The cDNA was cloned in the PET21a vector at *Nde*I site, and transfected into the *E. coli* strain BL21DE3 (Novagen). The cDNA sequence obtained from the positive isolates completely matched the LBP-p40 cDNA reported previously (2).

Purification of recombinant LBP-p40. The transformant was grown at 37°C to 0.6 of an optical density at 600 nm, IPTG (isopropyl-1-thio-beta-D-galactopyranoside) was added to a final concentration of 1 mM, and the culture was grown additional 3 h. Cells were collected by centrifugation, suspended in 200 ml of buffer A (50 mM Tris–HCl, pH 7.5, 30 mM NaCl), and disrupted by ultrasonication. The sonicate was centrifuged at 8000 g for 20 min, and the pellet was resuspended in 200 ml of buffer A containing 1 M sucrose. The suspension was centrifuged as above. Then the pellet was resuspended in 200 ml of buffer A containing 2% Triton X-100, 10 mM EDTA and incubated overnight at 4°C. The solution was sedimented at 8000 g for 20 min, and the pellet was dissolved in 20 ml of buffer B (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM DTT) containing 8 M urea. The solution was dialyzed against buffer B containing 4 M urea, then buffer with 1 M urea, and finally against urea-free buffer B. Insoluble material was pelleted at 100,000 g for 30 min and the supernatant was saved. The supernatant was applied to a 5 ml HiTrap Q column (Pharmacia) equilibrated with buffer B from the FPLC system (Pharmacia). The column was washed with 100 ml of buffer B and bound proteins were eluted with a 40-ml linear 100–600 mM NaCl gradient in buffer C (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1 mM DTT). LBP-p40 was found in 300–350 mM NaCl elution positions, which were pooled and loaded onto a gel filtration column (Hiload 16/25; Pharmacia) equilibrated in buffer C containing 500 mM NaCl. LBP-p40 eluted with an apparent molecular mass of 40 kDa. For the final step, LBP-p40 containing fractions were dialyzed against buffer B and fractionated on a FPLC Mono Q column (1 ml; Pharmacia). After the column was washed with buffer B, proteins were eluted with a 20 ml linear 100–600 mM NaCl gradient in buffer C. The p40 fraction in each step was confirmed by Western blotting using the anti-LBP-p40 antibody directed against cytoplasmic LBP-p40. The elution pattern of recombinant LBP-p40 through each column was completely consistent with native LBP-p40 of Ehrlich tumor cells.

Purification of histone H1 and core histones. Histone H1 and core histones were purified by modifying the procedure of Simon and Felsenfeld (24). Nuclei from Ehrlich tumor cells were prepared as described above, disrupted by sonication in 50 mM Tris–HCl (pH 7.5) buffer containing 80 mM NaCl, and sedimented by centrifugation at 10,000 g for 20 min. The pellet was sit on ice for 30 min after being suspended in 50 mM Tris–HCl (pH 7.5) buffer containing 300 mM NaCl, 1% NP-40, 1 mM EDTA and 1mM PMSF and centrifuged at 10,000 g for 20 min. The pellet was washed with the same buffer and incubated overnight at 4°C in 100 mM phosphate buffer (pH 6.8) containing 650 mM NaCl and 1 mM PMSF. The suspension was centrifuged at 15000 g for 20 min and the supernatant was loaded onto a hydroxylapatite column. The column was washed with 100 mM phosphate buffer (pH 6.8) containing 650 mM NaCl. Histone H1 was eluted with 100 mM phosphate buffer containing 700 mM NaCl and among core histones, H2A and H2B were mainly eluted with 100 mM phosphate buffer (pH 6.8) containing 1.25 M NaCl. Neither H3 nor H4 could be efficiently isolated by this procedure. Purified histones were dialyzed against the elution buffer.

LBP-p40 affinity column. An LBP-p40 affinity column was prepared by coupling 2.5 mg of purified recombinant LBP-p40 with 1 ml of CN-Br activated Sepharose. Approximately 100 μg of core histones and 50 μg of histone H1 were loaded onto the column equilibrated with 50 mM Tris–HCl (pH 7.5) buffer containing 50 mM NaCl. Proteins were eluted with 50 mM Tris–HCl (pH 7.5) containing 100, 300, or 500 mM NaCl.

Elution of LBP-p40 from the DNA cellulose column. One hundred micrograms of LBP-p40 and approximately 100 μg of histone H1 or core histones were loaded onto a DNA cellulose column equilibrated with 50 mM Tris–HCl (pH 7.5) buffer containing 50 mM NaCl. The column was washed with the same buffer, and the proteins were eluted with 100, 300, and 500 mM NaCl buffer.

Glutathione S-transferase (GST)-fusion protein production. GST-core histone DNAs (25) were kindly provided by Dr. R. G. Roeder (Rockefeller Univ.). GST and GST fusion protein were overproduced in *E. coli* strain BL21 DE3 (Novagen). Cultures of 400 ml were grown at 37°C to an optical density at 600 nm. The IPTG was added to a final concentration of 1 mM and growth was continued for 3 h. Cells were pelleted and suspended in 20 ml of buffer D (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA). All the buffers included 1 mM PMSF. The suspension was sonicated, and NP-40 was added to a final concentration of 1%. The suspension was incubated at 4°C for

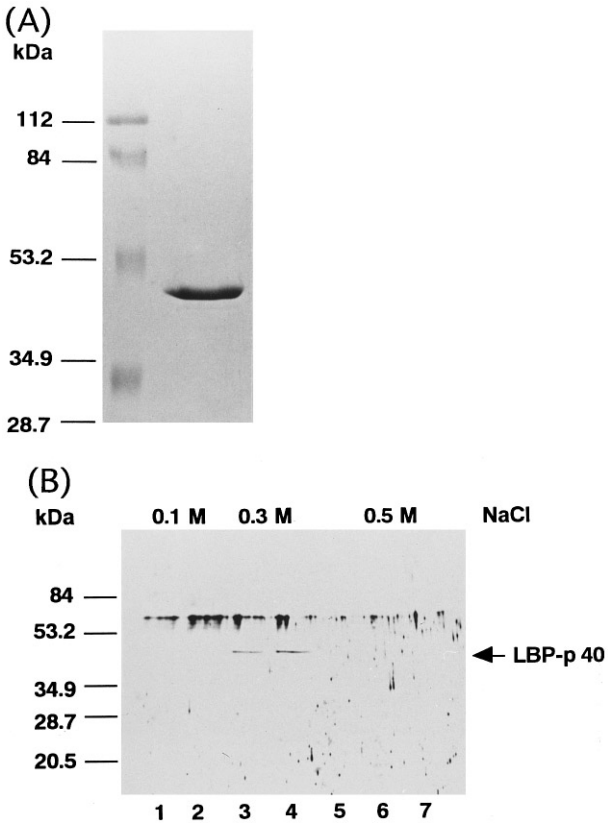

FIG. 1. Binding of purified recombinant LBP-p40 to a DNA cellulose column. (A) LBP-p40 purified from the final Mono Q column fraction step was analyzed on a 10% SDS-PAGE gel and stained with Coomassie blue. (B) Fractions eluted with 100 (lanes 1 and 2), 300 (lanes 3 and 4), and 500 (lanes 5 and 6) mM NaCl from the DNA cellulose column were immunoblotted with anti-recombinant LBP-p40 antibody.

1 h and debris was pelleted at 12,000 g for 10 min. The supernatant was mixed with 0.3 ml of glutathione-CL4B resin (Pharmacia) at room temperature for 1 hr and centrifuged at 500 g for 5 min. The resin was washed with 10 ml of buffer D. GST-proteins were eluted with 10 mM glutathione, 50 mM Tris–HCl (pH 8.0) containing 300 mM NaCl. The protein containing fractions were dialyzed against 50 mM Tris–HCl (pH 7.5) buffer containing 300 mM NaCl and 1 mM EDTA.

In vitro binding assays. Twenty microliters of a 50% slurry of glutathione-CL4B resin was washed five times with 50 mM Tris–HCl (pH 7.5) buffer containing 0.2% NP-40 and 300 mM NaCl. Resins were then mixed with proteins in 500 μl of the same buffer at 4°C for 1 h and washed five times with 1 ml of the same buffer. Bound proteins were then resuspended in Laemmli's sample buffer and analyzed on SDS–polyacrylamide gels.

Immunoblot analysis. A protein-transferred nitrocellulose membrane was blocked with 5% skim milk, and then incubated for 16 h at 4°C with a primary antibody in 5% skim milk, followed by 1 hr incubation at room temperature with alkaline phosphatase-conjugated goat anti-rabbit IgG in 5% skim milk. Immunoreactivity was detected using the bromochloroindoyl phosphate/nitroblue tetrazolium color development substrate (Gibco, BRL). Alternatively, after incubation with a primary antibody, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody in 0.1% Tween 20 in PBS for 1 h at room temperature and then developed by ECL reagent (Amersham).

RESULTS

Binding of LBP-p40 to DNA cellulose. Recent nuclear localization of LBP-p40 led us to investigate its potential in binding DNA. Highly purified, recombinant LBP-p40 (Fig. 1A) was loaded on single-stranded and double-stranded DNA cellulose columns. Fig. 1B shows the protein profile from a double-stranded DNA column. At 100 mM NaCl LBP-p40 was found to be retained on both types of columns, and was then eluted with 300 mM NaCl-containing buffer.

Nuclear proteins stabilize DNA binding of LBP-p40. As shown in Fig. 1B, purified recombinant LBP-p40 does not bind to DNA cellulose in NaCl higher than 300 mM. However, given that nuclear extraction of endogenous LBP-p40 requires 500 mM NaCl (18), we hypothesized that other nuclear proteins present in the high-salt nuclear extract may interact with LBP-p40 to increase its binding affinity for DNA. We tested this hypothesis by mixing purified recombinant LBP-p40 with 0.5M NaCl DNA cellulose eluates derived from crude nuclear extract and examining the DNA binding properties of LBP-p40 with the DNA cellulose column. Interestingly, in the presence of such nuclear proteins LBP-p40 was not eluted with 300 mM NaCl but instead required 500 mM NaCl-containing buffer (Fig. 2). This finding suggests that high salt nuclear extract includes DNA-associated protein factors that interact with LBP-p40 and stabilize its DNA binding.

Identification of DNA binding cofactors of LBP-p40. The major protein components of the 0.5 M NaCl DNA cellulose eluate derived from crude high salt nuclear extract were histone H1 and core histones, both of which were eluated from an LBP-p40 affinity column with 500 mM NaCl (data not shown). We conclude that LBP-p40 is capable of associating with histone H1 and with the core histones. These interaction capabilities of LBP-p40 were further examined by testing individual core histones expressed as GST-fusion proteins. Bind-

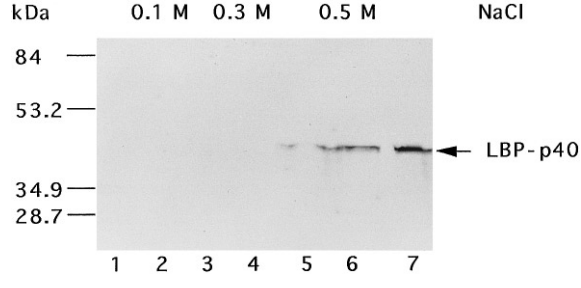

FIG. 2. The 0.5 M NaCl-eluted nuclear extract conferred tight binding of LBP-p40 to the DNA cellulose column. Recombinant LBP-p40 and the 0.5 M NaCl-eluted nuclear extract were loaded onto the DNA cellulose column. Fractions eluted with 100 (lanes 1 and 2), 300 (lanes 3 and 4), and 500 (lanes 5–7) mM NaCl from the DNA cellulose column were subjected to Western blotting using anti-recombinant LBP-p40 antibody.

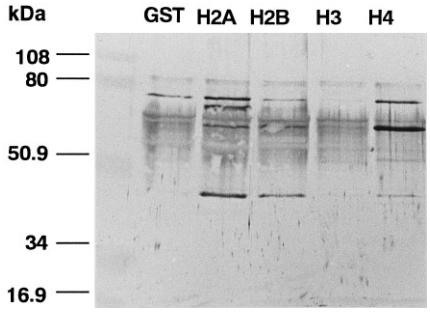

FIG. 3. Binding of LBP-p40 with core histones. One microgram of recombinant LBP-p40 was mixed with 2 μg of GST or 1 μg of each GST-core histone and precipitated with glutathione-CL4B resin. Proteins eluted from the washed beads were immunoblotted with anti-recombinant LBP-p40 antibody.

ing assays at 300mM NaCl revealed that purified recombinant LBP-p40 interacts specifically histones H2A, H2B, and H4 but not with H3 (Fig. 3). We imagine that LBP-p40 may associate with histone-containing chromatin through multiple possible interaction surfaces.

Histones stabilize LBP-p40 binding to DNA cellulose. To confirm that LBP-p40 interactions with histones are relevant to LBP-p40 DNA binding characteristics, we repeated the double-stranded DNA cellulose chromatography experiments in the presence of homogeneous preparations of histone H1 or the core histones. When LBP-p40 was loaded onto the column together with histone H1, washed with 50 mM NaCl, and eluted with 100, 300, and 500 mM NaCl, LBP-p40 was detected in the 300 mM NaCl fractions (Fig. 4A) as seen with LBP-p40 alone (Fig. 1B). However, when LBP-p40 and the core histones were loaded on the column together, LBP-p40 was detected in fractions eluted with 500 mM NaCl but not with 300 mM NaCl (Fig. 4B). In summary, these data indicate that LBP-p40 binding to DNA is stabilized by core histones via interactions with H2A, H2B, and H4; a potential interaction between LBP-p40 and the linker histone H1, however, does not appear to modify their DNA binding characteristics.

DISCUSSION

In this paper, we report the binding of LBP-p40 to DNA cellulose, potential associations of LBP-p40 and histones H1, H2A, H2B, and H4, and apparent stabilization of LBP-p40 binding to DNA via interactions with the core histones, H2A, H2B, and H4. LBP-p40 itself bound the DNA cellulose column in the presence of 0.1 M NaCl, and eluted from the column with 0.3M NaCl. These findings suggest that LBP-p40 weakly associated with DNA, and to our knowledge is the first evidence that LBP-p40 binds DNA. Since LBP-p40 has been considered the 67kDa laminin receptor precursor or a 40S ribosome component, DNA-binding activity of this protein has never been investigated. However, recently, DNA-binding motifs were found in a number of ribosomal proteins (26, 27). Yst 1 and 2, homologues of mammalian LBP-p40, were cloned in *S. cerevisiae* (9, 19). These DNA-binding proteins were first isolated from the yeast nucleus using a single-stranded DNA cellulose column. The Yst 1 was bound by antisera against the DNA-binding protein fraction that was eluted from the DNA cellulose column by 2 M NaCl after washing with 0.05 M NaCl, and was originally called NAB1 (Nucleic Acid Binding protein 1) (19). These results appear to be consistent with our findings regarding the DNA-binding properties of LBP-p40. The DNA-binding experiments of LBP-p40 were performed using purified recombinant LBP-p40. During the LBP-p40 isolation steps, large aggregates of the protein were often generated. We carefully eliminated such contaminants through gel-filtration and ion-exchange chromatography, and collected the molecule eluted from each column with the elution pattern completely identical to the endogenous LBP-p40 present in the cytoplasm of mammalian cells. To further rule out possible effects of either modifications or alterations in

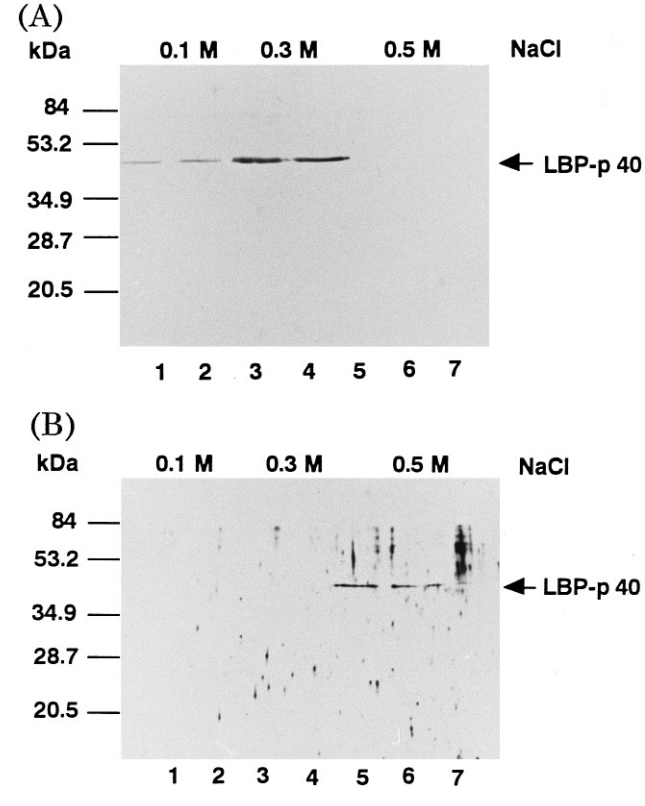

FIG. 4. Histones modify binding of LBP-p40 to the DNA cellulose column. Histone H1 (A) or core histones (B) mixed with LBP-p40 were loaded onto the DNA cellulose column. The columns were washed with 50 mM NaCl buffer. Proteins eluted with 100 (lanes 1 and 2), 300 (lanes 3 and 4), 500 (lanes 5–7) mM NaCl were immunoblotted with anti-recombinant LBP-p40 antibody.

the recombinant protein, we isolated endogenous LBP-p40 from the cytoplasm of Ehrlich tumor cells (18) and investigated the association of the protein with the DNA cellulose column. The elution pattern of endogenous LBP-p40 was identical to that of the recombinant LBP-p40. Sequence analysis of LBP-p40 has not revealed a DNA binding motif and thus the protein domain required for DNA binding or the type of interactions with nucleic acid remain unclear. Guo et al. have shown that the region of LBP-p40 thought to bind laminin also binds heparin (29). This region is called peptide G and consists of N-IPCNNKGAHSV GLMWWMLAR-C. Since many nucleic-acid binding proteins interact with heparin, peptide G may be a candidate DNA-binding region of LBP-p40 (9). Using the LBP-p40 affinity column, histone H1 and the core histones were shown to associate with LBP-p40, with the core histones binding under more stringent conditions. *In vitro* binding assays using recombinant core histones demonstrated that H2A, H2B bind to LBP-p40 with high affinity, H4 with lower-affinity, whereas H3 does not bind LBP-p40. The amino acid sequence of H3 is not extraordinarily different from the other core histones. Indeed the homology of H2B, H3, and H4 with H2A1 was 20.71, 23.45, and 22.73%, respectively, using GENETYX-MAC. Neither HMG-1/2 purified from a calf thymus nor recombinant EBNA-1 of Epstein-Barr virus were associated with LBP-p40. These observations indicate that the association of LBP-p40 with some of histones is other nuclear proteins like eIF-4 may also associate with LBP-p40 (8), the 0.5 M NaCl-eluted DNA binding nuclear proteins used here contained mainly histone H1 and core histones. The reason that we employed the 0.5 M NaCl-eluted nuclear extract is that LBP-p40 was extracted by high-salt (0.5 M NaCl) detergent (2% NP-40) treatment from isolated nuclei of mammalian cells (17). It remains to be elucidated whether or not LBP-p40 itself binds chromatin DNA without association with histones. However, since epitope-tagged LBP-p40 was extracted from isolated nuclei by 0.5 M NaCl–2% NP-40 treatment (18), it is likely that LBP-p40 in the nucleus firmly binds chromatin DNA through association with core histones. Several histone binding proteins have been identified as follows; in *Xenopus laevis*, nucleoplasmin binds H2A and H2B, and N1/N2 binds H3 and H4 (30–32). These factors are molecular chaperones that control the binding of histones and DNA to form the nucleosome. Although the mammalian homologues for nucleoplasmin and N1/N2 have not yet been identified, nucleosome assembly factors have been isolated in other organisms and shown to bind histones. Spt6p associated with H3 and H4 in *Saccharomyces cerevisiae* (33), and its homologues were identified in *Caernohabditis elegance* and humans (34, 35). ATP-independent nucleosome assembly factor, NAP-1, (36) binds histone octamers, preferentially those containing H2A and H2B. LBP-p40 has no sequence-similarities with these histone-binding factors so far identified, and the ability to bind with the particular combination of histones H1, H2A, H2B, and H4 appears unique.

All the core histones did not appear to associate with LBP-p40 in the nucleus. When 100 μg of core histones were mixed with 100 μg of LBP-p40 in Fig. 4, we calculated from densitometrical assay that 3 to 4 μg of LBP-p40 bound to core histones. Considering the molecular weight of LBP-p40 and core histone octamer, the molar ratio of LBP-p40-bound core histones was approximately 12.5 to 16% of total core histones. Since the minimal 657 bp scaffold-associated region is present between 5 kb histone genes from the analysis of *Drosophila* histone gene cluster (37), the ratio of scaffold-associated region to total genome is calculated more than 13.4%. This ratio appears to be consistent to that of core histones complexed with LBP-p40. Indeed, we already reported that LBP-p40 appeared to be localized in nuclear matrix region in the nucleus (17, 18). Taken together, LBP-p40 may bind to the core histones localized in nuclear matrix or scaffold-associated region in the nucleus.

What could be the biological significance of the association of LBP-p40 with the core histones? There have been no reports on the relationship of LBP-p40 with nuclear proteins nor suggestions on the involvement of LBP-p40 in the nuclear architecture. However, we recently succeeded in reducing the cellular expression of LBP-p40 by the use of antisense RNA (38). Decrease expression of endogenous LBP-p40 resulted in cell-cycle arrest at the S-phase and finally in apoptotic cell death. We also found that the isolated nuclei in the antisense LBP-p40 clones was highly sensitive to micrococcal nuclease (Kaneda; unpublished observation). These findings suggest that LBP-p40 may play an essential role in maintaining nuclear structures. In this paper, we have shown that LBP-p40 binds DNA tightly in vitro through histone association. These observations support the involvement of LBP-p40 in nuclear architecture, providing a possible mechanism. LBP-p40 may thus be involved in stabilizing chromatin through multiple associations with the protein and nucleic acid components of the nucleosome. An *in vitro* nuclear assembly system would assist in further investigating the precise role of LBP-p40 in the nucleus.

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