Interaction between an Integral Protein of the Nuclear Envelope Inner Membrane and Human Chromodomain Proteins Homologous to Drosophila HP1*

(Received for publication, March 26, 1996, and in revised form, April 19, 1996)

Qian Ye‡ and Howard J. Worman§
From the Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

At the nuclear envelope in higher eukaryotic cells, the nuclear lamina and the heterochromatin are adjacent to the inner nuclear membrane, and their attachment is presumably mediated by integral membrane proteins. In a yeast two-hybrid screen, the nucleoplasmic domain of lamin B receptor (LBR), an integral protein of the inner nuclear membrane, associated with two human polypeptides homologous to Drosophila HP1, a heterochromatin protein involved in position-effect variegation. LBR fusion proteins bound to HP1 proteins synthesized in vitro translation and present in cell lysates. Antibodies against LBR also co-immunoprecipitated HP1 proteins from cell extracts. LBR can interact with chromodomain proteins that are highly conserved in eukaryotic species and may function in the attachment of heterochromatin to the inner nuclear membrane in cells.

In higher eukaryotic cells, a portion of the transcriptionally inactive heterochromatin is adjacent to the inner nuclear membrane (1, 2). The nuclear lamina is also adjacent to the inner nuclear membrane, but because the lamina is discontinuous (3), the heterochromatin can presumably interact directly with the membrane. During nuclear envelope reassembly at the end of mitosis, vesicles that derive from the inner nuclear membrane also bind to chromosomes in a lamin-independent fashion (4, 5). In Saccharomyces cerevisiae, an organism in which a lamina has not been identified, transcriptionally silent regions of the genome are similarly localized to the nuclear envelope, further suggesting a direct interaction between the chromatin and the inner nuclear membrane (6, 7). Several integral proteins have been localized to the inner nuclear membrane that presumably mediate the attachment of the lamina and chromatin. One of these proteins is LBR,1 or the lamin B receptor, a protein previously shown to bind to B-type laminins (8, 9) and to double-stranded DNA (9). LBR was first characterized in birds (8, 10) and subsequently in mammals (9, 11, 12). Human LBR has a nucleoplasmic, amino-terminal domain of 208 amino acids followed by eight putative transmembrane segments (9). We now demonstrate that the nucleoplasmic domain of LBR binds to human chromodomain proteins homologous to Drosophila HP1, a heterochromatin protein involved in position-effect variegation (13, 14).

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screening—Screening was performed as described (15, 16). DNA encoding amino acids 1 to 208 of human LBR was amplified by the polymerase chain reaction using the Gene Amp Kit (Perkin-Elmer Corp.) with custom oligonucleotide primers (Genset, Paris, France) and a cDNA template (9). Amplified DNA was cloned into the GAL4 DNA binding domain fusion vector pGBT9 (provided by S. Fields, State University of New York at Stony Brook) to produce pGBT9-LBR. S. cerevisiae strain Y190 (provided by S. J. Elledge, Baylor College of Medicine, Houston, TX) was co-transformed with pGBT9-LBR, and approximately 10⁶ recombinants of a HeLa cell cDNA library in the GAL4 activation domain fusion vector pGADGH (Clontech). The positive pGADGH-derived plasmids isolated in this screen were rescued and used to again co-transform yeast strain Y190 with pGBT9-LBR to confirm the interactions.

DNA Sequencing and Sequence Analysis—DNA sequencing was performed using the Sequenase Version II Kit (U. S. Biochemical Corp.). Sequences were analyzed using the Wisconsin Package (Genetics Computer Group, Inc., Madison, WI) and the computer facilities of the National Cancer Institute's Frederick Biomedical Supercomputing Center.

Binding Assays Using In Vitro Translated Proteins—For in vitro transcription-translation, cDNA inserts were cloned into pBFT4 (supplied by J. Licht, Mt. Sinai School of Medicine, New York). In vitro translation was performed using the TNT T3 Coupled Reticulocyte Lysate System (Promega) with [35S]methionine (Amersham). In binding experiments, 20 μl of in vitro translated lysate were added to 200 μl of binding buffer (150 mM NaCl, 20 mM Na-Hepes (pH 7.4), 10% glycerol, 0.05% Nonidet P-40) with 20 μl of glutathione-Sepharose (Pharmacia Biotech Inc.) coupled to equal amounts (3-5 μg) of glutathione S-transferase (GST) or GST-LBR fusion protein (9). GST proteins were produced and purified as described (17). Suspending solutions were incubated at 4 °C with rotation for 2 h. After incubation, the Sepharose was washed 5 times with binding buffer, and bound proteins were then eluted with 4% SDS and analyzed by autoradiography of 12.5% SDS-polyacrylamide slab gels (18). In assays using different salt or detergent concentrations, the binding buffers contained the concentrations indicated.

Binding of Proteins from Cell Lysates to GST Fusion Proteins—HeLa cell lysates were prepared as described below (see Immunoprecipitation), and 400 μl of lysate were incubated with 5 μg of the GST-LBR amino-terminal domain fusion protein or GST coupled to glutathione-Sepharose (20 μl). Incubation was for 2 h at 4 °C with rotation. After incubation, the Sepharose was washed 5 times with 150 mM NaCl, 20 mM Na-Hepes (pH 7.4), 10% glycerol, 0.05% Nonidet P-40. The bound proteins were then eluted with 4% SDS and analyzed by autoradiography of 12.5% SDS-polyacrylamide slab gels (19). In assays using different salt or detergent concentrations, the binding buffers contained the concentrations indicated.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U26311 and U26312.

‡ Supported in part by student research fellowships from the American Liver Foundation.
§ Supported by American Cancer Society Grant CB-119 and a Silberberg Award from Columbia University. To whom correspondence should be addressed: Dept. of Medicine, College of Physicians and Surgeons, Columbia University, 630 West 168th St., 10th Floor, Rm. 508, New York, NY 10032. Tel.: 212-305-8156; Fax: 212-305-6443; E-mail: warnan@columbia.edu.

1 The abbreviations used are: LBR, lamin B receptor; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin.
cDNA inserts were cloned into plasmid pQE31, and polyhistidine fusion proteins were expressed and purified according to the manufacturer’s instructions (Qiagen, Chatsworth, CA).

Immunoprecipitation—Two 100-mm Petri dishes of 293 T cells were grown to 90% confluency in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 2 mM l-glutamine (Life Technologies, Inc.). Cells were washed 3 times with phosphate-buffered saline, harvested by scraping with a rubber policeman, and collected by centrifugation at 500 x g in 10 mM Tris-HCl (pH 7.4), 1 mM MgCl₂, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF). The cells were then incubated on ice for 15 min and broken in a Dounce homogenizer (30 strokes with a tip sonicator). The suspension was centrifuged at 15,000 rpm for 30 min at 4 °C, and the pellets were resuspended in 400 μl of 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 2% BSA, 1 mM dithiothreitol, 0.2 mM PMSF containing either 0.5% or 1% Nonidet P-40 and then disrupted with a rubber policeman, and collected by centrifugation at 500 x g.

Using the nucleoplasmic, amino-terminal domain of LBR as bait, we performed a yeast two-hybrid screen of a HeLa cell cDNA library. Screening of approximately 10⁶ recombinant bait, we performed a yeast two-hybrid screen of a HeLa cell encoded HP1Hs and we showed that it encoded the major portion of a novel protein that was highly homologous to human HP1 proteins. The human protein encoded by ATBP8132 is 98% identical in amino acid sequence with mouse chromodomain protein M32 (22). A human protein 100% identical with M31, another mouse chromodomain protein, has been termed HP1Hs (19, 22). HP1Hs is 80% and 76% similar to M31 and M32, respectively. In keeping with the previous nomenclature used for human HP1 homologues (19), we have termed the protein encoded by ATBP8132 HP1Hs. HP1-like chromodomain proteins are conserved in a wide range of eukaryotic species (23). In particular, HP1Hs is 38% identical and HP1Hs is 29% identical with SWI6 of Schizosaccharomyces pombe (24). Drosophila polycomb, which differs from HP1 for most of its sequence, also contains the chromodomain (25), which spans from amino acids 24 to 60 in HP1 and 20 to 56 in HP1Hs (Fig. 1).

To confirm the interactions detected in the yeast two-hybrid assay, we showed that the amino-terminal domain of LBR bound to HP1Hs and HP1Hs in vitro (Fig. 2a). Purified GST-LBR amino-terminal domain fusion protein was coupled to glutathione-Sepharose. HP1Hs (Fig. 2a, lanes 1–3) and HP1Hs lacking its first 17 amino acids (Fig. 2a, lanes 4–6), synthesized in reticulocyte lysates, bound to the LBR fusion protein but not to GST coupled to glutathione-Sepharose in buffer containing 150 mM NaCl. The amount of HP1Hs that bound to the amino-terminal domain of LBR started to decrease slightly at NaCl concentrations greater than 0.5 M, but significant binding was still observed in buffers containing 1.0 M (Fig. 2b). Binding of HP1Hs to the amino-terminal domain of LBR was also observed in the presence of up to 1% of the nonionic detergent Nonidet P-40 but the denaturing detergent SDS essentially abolished the interaction (Fig. 2c). Binding of HP1Hs to the LBR fusion protein was also not influenced by excessive amounts of BSA, non-fat milk, and/or DNA (data not shown).

RESULTS

Using the nucleoplasmic, amino-terminal domain of LBR as bait, we performed a yeast two-hybrid screen of a HeLa cell cDNA library. Screening of approximately 10⁶ recombinant clones led to the isolation of 10 that grew on the appropriate selection medium and gave detectable β-galactosidase activity. Plasmids isolated from these clones were used to again transform yeast. Two only, termed ATBP115 and ATBP8132, remained positive for β-galactosidase activity when co-transformed with the plasmid that encoded the LBR fusion protein but not the DNA binding domain of GAL4 alone.

Sequencing of the cDNA insert of clone ATBP8115 showed that it encoded HP1Hs (19). Sequencing of the cDNA insert of clone ATBP8132 showed that it encoded the majority of a novel human protein with 65% sequence similarity to HP1Hs. The ATBP8132 cDNA overlapped with two nucleotide sequences of unknown function in GenBank™ and alignment of the translated sequences with those of homologous polypeptides in Drosophila (13, 14, 21), mouse (22), and humans (19, 22) allowed us to deduce the protein’s likely amino terminus. This protein, which we termed HP1Hs (see below), and HP1Hs were highly homologous to Drosophila melanogaster HP1 (Fig. 1), a chromodomain protein localized to heterochromatin (13).

Fig. 1. Primary structures of HP1Hs, HP1Hs, and D. melanogaster (Dm) HP1. Identical or conservative amino acids are shown as white on black. Conservative amino acid substitutions used are: aliphatic hydrophobic, L/I/V/M/A; aromatic hydrophobic, F/Y; basic, R/K/H; acidic, E/D; aliphatic alcoholic, S/T; polar amides, Q/N. The cDNA insert of clone ATBP8132 starts at the G of the initiation codon of HP1Hs and extends in the 3’ direction to a polyadenylate tail. The LBR amino-terminal domain GST fusion protein was also not influenced by excessive amounts of BSA, non-fat milk, and/or DNA (data not shown).
with SDS, separated by SDS-PAGE, and examined by autoradiography. The pellets were washed with SDS, separated by SDS-PAGE, and proteins were eluted with SDS, separated by SDS-PAGE, and transferred to nitrocellulose for immunoblotting with the anti-LBR antibodies. The same proteins recognized in cell extracts bound only to the fusion protein that contained the amino-terminal domain of LBR. b, autoantibodies used to detect HP1 proteins in the cell extracts and bound fractions recognize HP1Hs and HP1Hs. The Coomassie Blue-stained gel shows HP1Hs (lane 1) and HP1Hs (lane 2) polyhistidine fusion proteins. The immunoblot shows that the autoantibodies recognized the HP1Hs (lane 1) and HP1Hs (lane 2) polyhistidine fusion proteins. Migrations of molecular mass standards are indicated in kilodaltons at the left of each panel.

The amino-terminal domain of LBR binds to HP1Hs and HP1Hs in vitro. a, the autoradiogram shows [35S]-labeled HP1Hs (lane 1) and HP1Hs lacking its first 17 putative amino acids (lane 4) synthesized by in vitro translation. HP1Hs (lanes 2 and 3) or HP1Hs (lanes 5 and 6) were incubated with either GST (lanes 2 and 5) or the amino-terminal domain of LBR as a GST fusion protein (lanes 3 and 6) coupled to glutathione-Sepharose. Glutathione-Sepharose was then pelleted by centrifugation and washed, and the bound proteins were eluted with SDS, separated by SDS-PAGE, and examined by autoradiography. HP1Hs and HP1Hs bound to the LBR fusion protein (lanes 3 and 6) but not to GST (lanes 2 and 5), b, a standard amount of [35S]-labeled HP1Hs, 10% of which is shown (lane 1), was used in each binding assay. HP1Hs was incubated with glutathione-Sepharose alone (lane 2) or 3–5 μg of GST coupled to glutathione-Sepharose (lane 3) in binding buffer containing 150 mM NaCl. HP1Hs was also incubated with 3–5 μg of LBR amino-terminal domain GST fusion protein coupled to glutathione-Sepharose (lanes 4–8) in buffers containing the NaCl concentrations indicated above each lane. Glutathione-Sepharose was then washed with buffer containing the indicated NaCl concentration, and the bound proteins were eluted with SDS and examined as above. c, a standard amount of [35S]-labeled HP1Hs, 10% of which is shown (lane 1), was used in each binding assay. HP1Hs was incubated with glutathione-Sepharose alone (lane 2) or 3–5 μg of GST coupled to glutathione-Sepharose (lane 3) in binding buffer containing 0.05% Nonidet P-40. HP1Hs was also incubated with 3–5 μg of LBR amino-terminal domain GST fusion protein coupled to glutathione-Sepharose in buffers containing detergents at the concentrations indicated above each lane (lanes 4–8). Glutathione-Sepharose was washed with buffer containing the indicated detergent type and concentration, and the bound proteins were eluted with 4% SDS and examined as above. Migrations of molecular mass standards are indicated in kilodaltons at the left of each panel.

proteins recognized HP1Hs and HP1Hs expressed from the cDNAs isolated in the two-hybrid screen (Fig. 3b).

Anti-LBR antibodies also co-immunoprecipitated HP1 proteins from cell extracts (Fig. 4). Antibodies from serum of a patient with primary biliary cirrhosis that recognized LBR (9, 20) immunoprecipitated LBR from extracts of 293 T cells (Fig. 4a, lanes 1 and 3). No detectable LBR was present in immunoprecipitates of LBR when control antibodies from another patient with primary biliary cirrhosis were used (Fig. 4a, lane 2). Two polypeptides of approximately 30 kilodaltons that were recogn
nized by anti-HP1 antibodies in cell extracts (Fig. 4b, lane 1) were present in immunoprecipitates obtained with the experimental antibodies (Fig. 4b, lane 3) but not in immunoprecipitates obtained with the control antibodies (Fig. 4b, lane 2). The serum-containing anti-LBR antibodies did not recognize HP1 proteins on immunoblots (Fig. 4c), making it unlikely that the anti-LBR antibodies directly immunoprecipitated HP1 proteins. Serum from both the control and experimental patients also contained antimitochondrial antibodies (Fig. 4c and Ref. 20), a useful internal control for immunoprecipitation, and immunoblotting (data not shown) showed that both immunoprecipitates contained the major mitochondrial autoantigen pyruvate dehydrogenase E2 (26). These experiments strongly suggested that HP1 proteins and LBR were associated in human cells.

**DISCUSSION**

The present results show that human chromomdomain proteins homologous to Drosophila HP1 interact with LBR, an integral membrane protein of the inner nuclear membrane. This interaction can contribute to the association of the heterochromatin with the inner nuclear membrane in higher eukaryotic cells. The specificity of this interaction is demonstrated by the fact that the amino-terminal domain of LBR identified only two clones from approximately 10<sup>6</sup> in a yeast two-hybrid screen, each of which respectively contained the near full-length sequences of HP1<sub>Hs</sub> and HP1<sub>Ms</sub>. Direct interactions between HP1<sub>Hs</sub> and the amino-terminal domain of LBR also occur in vitro under stringent conditions utilizing various salt and detergent concentrations. A LBR fusion protein can also extract HP1 proteins from cell lysates, and antibodies that immunoprecipitate LBR can co-precipitate HP1 proteins.

In *D. melanogaster*, HP1 functions as a suppressor of position-effect variegation. If an active genetic locus in Drosophila euchromatin is translocated near constitutive heterochromatin, transcription from the locus is variably repressed leading to variegation of gene expression (27). Position-effect variegation is associated with altered chromatin structure, and Drosophila heterozygous HP1 mutants demonstrate suppression of position-effect variegation presumably because heterochromatin stabilization is defective (14, 25, 28). Drosophila HP1 has been directly localized to heterochromatin (13, 29), as have been HP1 homologues from mammals (30) and fission yeast (31). The interaction of HP1-type chromodomain proteins with LBR may therefore explain, at least in part, the long-observed association of the heterochromatin with the inner nuclear membrane.

Chromatin is a dynamic structure, and heterochromatin packaging changes dramatically at different stages of the cell cycle. Similarly, the nuclear envelope undergoes profound structural changes during mitosis. LBR undergoes phosphorylation catalyzed by p34<sup>cdc2</sup> protein kinase in mitosis when the inner nuclear membrane breaks down into vesicles that dissociate from the lamina and the chromatin (32). It is phosphorylated by different protein kinases in interphase when the membrane is associated with these structures (33, 34). Phosphorylation of LBR and phosphorylation of HP1 proteins (35) may therefore be responsible for some of the alterations in chromatin organization and nuclear structure which occur at various times during the cell cycle.

Acknowledgments—We thank J.-C. Courvalin for invaluable advice and production of anti-LBR antibodies, P. Küssel and X. Zhang for help with some of the work, W. C. Earnshaw, S. J. Elledge, S. Fields, and J. Licht for reagents, and S. C. R. Elgin for helpful discussions.

**REFERENCES**

1. Blobel, G. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 8527–8529
2. Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber, H., and Sedat, J. (1984) Nature 308, 414–421
3. Paddy, M. R., Belmont, A. S., Saumweber, H., Agard, A. D., and Sedat, J. W. (1990) Cell 62, 89–106
4. Newport, J. W., Wilson, K. L., and Dunphy, W. G. (1990) J. Cell Biol. 111, 2247–2259
5. Chaudhary, N., and Courvalin, J.-C. (1993) J. Cell Biol. 122, 295–306
6. Cockell, M., Palladino, F., Laroche, T., Kyrion, G., Lui, C., Lustig, A. J., and Gasser, S. M. (1995) J. Cell Biol. 129, 909–924
7. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M., and Grunstein, M. (1995) Cell 80, 583–592
8. Worman, H. J., Yuan, J., Blobel, G., and Georgatos, S. D. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 8531–8534
9. Ye, G., and Worman, H. J. (1994) J. Biol. Chem. 269, 11306–11311
10. Worman, H. J., Evans, C. D., and Blobel, G. (1990) J. Cell Biol. 111, 1534–1542
11. Courvalin, J.-C., Lassoued, K., Worman, H. J., and Blobel, G. (1990) J. Exp. Med. 172, 961–967
12. Schuler, E., Lin, F., and Worman, H. J. (1994) J. Biol. Chem. 269, 11312–11317
13. James, T. C., and Elgin, S. C. R. (1986) Mol. Cell. Biol. 6, 3862–3872
14. Eissenberg, J. C., James, T. C., Foster-Hartnett, D. M., Hartnett, T., Negan, V., and Elgin, S. C. R. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9923–9927
15. Chien, C., Bartel, P. L., Stengelzahn, R., and Fields, S. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 9578–9582
16. Durfee, T., Biedner, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilburn, A., Lee, W.-H., and Elledge, S. J. (1993) Genome Dev. 7, 555–569
17. Smith, D. B., and Johnson, K. S. (1988) Genes (Amst.) 67, 31–40
18. Laemmli, U. K. (1970) Nature 227, 680–685
19. Saunders, W. S., Chue, C., Goebl, M., Craig, C., Clark, R. F., Powers, J. A., Eissenberg, J. C., Elgin, S. C. R., Rothfield, N. F., and Earnshaw, W. C. (1993) J. Cell Sci. 104, 573–582
20. Nickowitz, R. E., Wozniak, R. W., Schaffner, S., and Worman, H. J. (1994) Gastroenterology 106, 193–199
21. Clark, R. F., and Elgin, S. C. R. (1992) Nucl. Acids Res. 20, 6607–6607
22. Singh, P. B., Miller, J. R., Pearce, J., Kotthiy, R., Burton, D. R., Paro, R., James, T. C., and Gaunt, S. J. (1991) Nucl. Acids Res. 19, 789–794
23. Asplund, R., and Steward, A. F. (1995) Nucl. Acids Res. 23, 3168–3173
24. Lorentz, A., Oestermann, K., Fleck, O., and Schmidt, H. (1994) Gene (Amst.) 143, 139–143
25. Paro, R., and Hogness, D. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 263–267
26. Coppol, R. L., McNelis, I. L., Surh, C. D., Van de Water, J., Spithill, T. W., Whittingham, S., and Gershwin, M. E. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 7317–7321
27. Muller, H. J. (1930) Genet. 22, 299–334
28. Wallrath, L. L., and Elgin, S. C. R. (1995) Genes Dev. 9, 1263–1277
29. James, T. C., Eissenberg, J. C., Craig, C., Eaton, T., Elgin, S. C. R. (1989) Eur. J. Cell Biol. 50, 170–180
30. Wreggett, K. A., Hill, F., James, T. S. H. Hutchings, A., Butcher, G. W., and Singh, P. B. (1994) Nucleic Acids Res. 22, 99–103
31. Ekwall K., Jaworowski, J.-P., Lorentz, A., Schmidt, H., Cranston, G., and Allshire, R. (1995) Science 269, 1429–1431
32. Courvalin, J.-C., Segi, N., Blobel, G., and Worman, H. J. (1992) J. Biol. Chem. 267, 19035–19038
33. Aptebaum, J., Blobel, G., and Georgatos, S. D. (1990) J. Biol. Chem. 265, 4181–4184
34. Simos, G., and Georgatos, S. D. (1992) EMBO J. 11, 4027–4036
35. Eissenberg, J. C., Ge, Y. W., and Hartnett, T. (1994) J. Biol. Chem. 269, 21315–21321
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\textit{J. Biol. Chem.} 1996, 271:14653-14656.
doi: 10.1074/jbc.271.25.14653

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