INTRODUCTION

In eukaryotic cells, the nuclear envelope is a complex organelle which separates nucleoplasmic materials from the cytoplasm. The nuclear envelope consists of three major components, the nuclear lamina, the inner and outer nuclear membranes, and the nuclear pore complexes (reviewed by Gerace and Burke, 1980; Gant and Wilson, 1997). While the outer nuclear membrane faces the cytoplasm and is continuous with the peripheral rough and smooth endoplasmic reticulum, the inner nuclear membrane is tightly associated with the nuclear lamina and contains specific lamina- and chromatin-binding integral membrane proteins. The inner and outer nuclear membranes are connected at the nuclear pore complexes (reviewed by Georgatos et al., 1994; Gerace and Foisner, 1994). The nuclear lamina is a filamentous protein meshwork of 10 nm-diameter intermediate filaments located between the inner nuclear membrane and chromatin. Possible functions of the nuclear lamina include providing a structural framework of the nuclear envelope and anchoring sites at the nuclear periphery for interphase chromatin (Gerace and Burke, 1980; McKeon, 1991; Nigg, 1992; Moir et al., 1991).

In vertebrates, the nuclear lamina is mainly composed of a polymeric assembly of subunit proteins, termed lamins (McKeon, 1991; Nigg, 1992; Moir et al., 1991). In mammals, lamin proteins are classified into A-type and B-type lamins on the basis of their amino acid sequences and biochemical properties. Four lamins, A, B1, B2 and C, are commonly found in somatic cells, and two additional lamins, C2 and B3, are found in mammalian germ cells. B-type lamins maintain an association with nuclear membrane vehicles during M-phase, while A-type lamins are solubilized into the cytoplasm at the beginning of mitosis (reviewed by Nigg, 1992; Moir et al., 1991). Several studies have shown that A-type and B-type lamins directly bind to chromosome/chromatin (Glass and Gerace, 1990; Höger et al., 1991; Yuan et al., 1991; Glass et al., 1992, 1993; Taniura et al., 1995) and DNA (Shoeman and Traub, 1990; Luderus et al., 1992, 1994), suggesting that nuclear lamins play a role in organizing chromatin at the nuclear envelope. In addition to lamin, recent studies have found that the nuclear lamina also contains a number of minor lamina-associated proteins (Georgatos et al., 1994; Gerace and Foisner, 1994). Several inner nuclear membrane proteins which bind to the lamina have been characterized, and their primary sequences have been determined. These include otefin (p53) (Padan et al., 1990), p58/lamin B receptor (LBR) (Worman et al., 1990; Ye and Worman, 1994), lamina-associated polypeptides (LAP) 1 (Senior and Gerace, 1988; Martin et al., 1995) and LAP2 (Foisner and Gerace, 1993; Furukawa et al., 1995) in higher eukaryotes. These proteins are specifically distributed along the inner nuclear membrane and are tightly associated with nuclear lamins. Of these proteins, p58/LBR and LAP2 directly bind to chromosome/chromatin and DNA in vitro (Foisner and Gerace, 1993; Ye and Worman, 1994; Pyrpasopoulou et al., 1996; Furukawa et al., 1997, 1998). More recent studies have found that p58/LBR specifically interacts with human HP1-type chromodomain proteins, of which their homolog in Drosophila is involved in position-effect variegation (Ye and Worman, 1996; Ye et al., 1997). Thus, it is likely that inner nuclear membrane proteins play a role in the regulation of chromatin structure and function.

SUMMARY

Lamina-associated polypeptide (LAP) 2, which directly interacts with B-type lamins and chromosomes, is an integral membrane protein specifically distributed along the inner nuclear membrane of the nuclear envelope. The chromatin- and lamin-binding activity of LAP2 suggests that LAP2 plays an important role in targeting mitotic vesicles to chromosomes and reorganizing the nuclear structure at the end of mitosis. Here I identified a LAP2 interacting protein, termed L2BP1 (LAP2 binding protein 1). The rat L2BP1 cDNA sequence is predicted to encode a protein of 89 amino acids which turns out to be a rat homolog of mouse and human BAF (Barrier-to-Autointegration Factor). L2BP1 is distributed diffusely throughout the nucleus in interphase cells. It is, however, highly concentrated at the chromosomes during the M-phase. Further, the L2BP1 binding domain of LAP2 overlaps its chromosome-binding region. These findings suggest that L2BP1 is a candidate mediator of LAP2-chromosome interaction at the end of mitosis.

Key words: Nuclear envelope, Nuclear lamina, Lamin, LAP2, BAF, Chromosome, Chromatin
attachment of chromatin to the nuclear envelope, and are part of the nuclear envelope architecture along with lamin proteins.

LAP2 is one such integral membrane protein which binds directly to both B-type lamins and chromosomes modulated by mitotic phosphorylation, and was originally identified and cloned in the rat (Foisner and Gerace, 1993; Furukawa et al., 1995). A number of different isotypes of LAP2 in mouse and human that vary in primary sequence (LAP2/TMPO α-ζ) (LAP2 = TMPO β), arise from a single gene by alternative splicing (Harris et al., 1994; Berger et al., 1996). These different LAP2/TMPO isotypes are expressed at different times depending on the tissue/cell type and developmental stage (Ishijima et al., 1996; Alsheimer et al., 1998), suggesting that the protein isotypes are involved in tissue- and development-specific nuclear organization. At least three subdomains in the LAP2 protein have been functionally identified. The 53 amino acid region between residues 244 and 296 is involved in the translocation of LAP2 to the nucleus as well as in DNA binding (Furukawa et al., 1997). The 85 amino acid region between residues 1 and 85 can directly associate with metaphase chromosome (Furukawa et al., 1998). The 76 amino acid region between residues 298 and 373 has been identified as the B-type lamin binding region (Furukawa et al., 1995, 1998).

Microinjection of this lamin-binding region into mammalian cells did not have any effect on reassembly of the nuclear envelope, but inhibited nuclear growth and progression into the S-phase (Yang et al., 1997). Further, a previous immunofluorescence study showed that LAP2 accumulates at the surface of chromosomes prior to the assembly of nuclear B-type lamins at the nuclear envelope during late anaphase (Foisner and Gerace, 1993). These results imply that LAP2 may be involved in two independent steps of nuclear architecture reformation at the end of mitosis, that is, initial targeting of the nuclear membranes to the chromosome, and the subsequent rearrangement of the dynamic structures of the nuclear lamina.

In the present study, in order to identify a protein with which the LAP2 protein interacts during nuclear architecture reformation, a yeast two-hybrid screen using the nucleoplasmic domain of LAP2 as a bait, was carried out. The molecular characterization of a small nuclear protein, termed L2BP1, is described. L2BP1 represents a non-histone chromosomal protein and may participate as targeting sites on chromosomes for membrane traffic in the first step of nuclear architecture reformation.

MATERIALS AND METHODS

Screening of cDNA encoding rat L2BP1

A genetic screen using the two-hybrid system was performed as previously described (Durfee et al., 1993; Harper et al., 1993). The bait plasmid pAS-LAP2 nucleoplastic domain (ΔC1) (Fig. 7) was transformed into the Saccharomyces cerevisiae Y190 strain. This strain was transformed with the cDNAs in the 9.5/10.5-day mouse cDNA library in the VP16-activation domain vector (Vojtek et al., 1993). 5.5×10⁶ transformants were analyzed as described by Harper et al. (1993). To obtain the cDNA of the full-length L2BP1 mRNA, a cDNA library was constructed in the λZAPII vector (Stratagene, La Jolla, CA, USA) using cDNAs generated from poly(A)⁺ mRNA of rat FRSK cell, and screened with the mouse L2BP1 clone 1 cDNA fragment (Fig. 1). The nucleotide sequence was determined in both directions by the dideoxy sequencing method using an Applied Biosystems 320 Genetic Analyzer (Foster, CA, USA).

Construction of plasmids for LAP2 and L2BP1 and expression in yeast two-hybrid vectors

To generate LAP2 mutant fragments encoding the ΔC4, ΔNC6, ΔNC7, and ΔNC8 fragments (Fig. 7) for the yeast two-hybrid assay, clone 4b, ΔC1, ΔC2, and ΔC3 of LAP2, respectively, were digested with XhoI. A BamHI linker was added, and then the construct was digested with BamHI. The other LAP2 fragments were generated as previously described (Furukawa et al., 1995). Members of a set of LAP2 fragments were inserted into the BamHI site of a pACT vector to produce a GAL4-activation domain-fusion protein. To construct a GAL4-DNA-binding-domain-ρat L2BP1-fusion protein expression vector, rat L2BP1 in pBluescript vector was digested with XhoI, a BglII linker was added, and then a polymerase chain reaction was performed using the primer TGCGGATCCATGACAACC-TCTAAAAGCAC and T7 primers. After gel purification, this fragment was digested with BamHI and BglII, and subcloned into the BamHI site of the pAS vector.

The yeast two-hybrid assay for interaction between L2BP1 and LAP2 was performed according to a slight modification of the procedure described by Durfee et al. (1993). The Y190 strain carrying pAS-L2BP1 was transformed with each pACT-LAP2 mutant construct by the lithium acetate procedure. The transformants were subsequently tested for β-galactosidase activity by both the plating method using 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), and the liquid method using O-nitrophenyl-β-D-galactoside as previously described (Furukawa et al., 1998).

Immunofluorescence study to determine the localization of L2BP1

The rat L2BP1 cDNA fragments obtained above were cloned into an influenza hemagglutinin (HA) epitope-tagged eukaryotic expression vector driven by the cytomegalovirus enhancer and promoter (Furukawa and Hotta, 1993). The epitope-tagged expression vectors were transfected into MOP8 cells in 10 cm dishes containing coverglasses by the calcium phosphate coprecipitation method (Furukawa et al., 1990). For immunolabeling of endogenous L2BP1, anti-L2BP1-epitope-specific antibodies were produced against the N-terminal 17 amino acid sequence between amino acid residues 4 and 20 of the L2BP1 protein in rabbits (Fig. 4). After the antibodies were affinity-purified in a peptide-conjugated column, they were used to determine the localization of L2BP1 in rat FR5K cells. Production and purification of anti-L2BP1-epitope-specific antibodies were carried out at Quality Controlled Biochemicals, Inc. (Hopkinton, MA, USA). The localization of chimerical and endogenous L2BP1 proteins was detected by immunofluorescence using an anti-HA-epitope-specific monoclonal antibody (Boehringer Mannheim Co., Mannheim, Germany) and anti-L2BP1-epitope-specific antibody, respectively, as described previously except that 4% paraformaldehyde was used for fixation (Furukawa and Hotta, 1993).

Cell fractionation

Rat FR5K cells were harvested from cell cultures using a scraper, and then washed in excess volumes of 4°C phosphate-buffered saline. After centrifugation at 800 g for 10 minutes, the pelleted cells were suspended in two packed-cell volumes of buffer A (10 mM Hepes, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT), and lysed by 10 strokes in a Dounce homogenizer at 4°C. The crude nuclear pellet and cytoplasmic material were separated by low speed centrifugation at 800 g for 10 minutes.

In vitro binding assay with LAP2 and L2BP1

In vitro binding was performed as previously described (Furukawa and Kondo, 1998). To generate test proteins, the BamHI fragment from the pVP16-L2BP1 clone 1 plasmid was linked to thioredoxin-His tag sequences in the sense direction (THL2-SD), or in the anti-sense direction (THL2-AD) (Fig. 3), at the BamHI site of the pET32 vector (Novagen, Madison, WI, USA). The sense and anti-sense directions of
the L2BP1 clone 1 sequence are predicted to encode 10 kDa of L2BP1 and 6.2 kDa proteins, respectively. Glutathione agarose bound with glutathione S-transferase (GST) alone, or GST-L2BP1 AC1 protein, was incubated with bacterial lysate containing thioredoxin-His tagged L2-SD or L2-AD proteins. The bound proteins were eluted from glutathione agarose with a 2% sodium dodecyl sulfate (SDS) solution.

**Northern analysis**

Poly(A)+ RNA of rat FRSK cells, mouse NIH3T3 cells, and human HeLa cells was purified using the guanidium thiocyanate method and Oligotex-dT30 resins (Furukawa and Hotta, 1993). The electrophoresed mRNA were transferred and immobilized on a nylon membrane by deionized formamide, 1% SDS, 5× Denhardt’s reagent, and 50 μg/ml salmon sperm DNA for at least 12 hours. Unbound labeled probe was removed by washing with 0.1× SSC containing 0.1% SDS at 65°C.

**Western blotting**

Protein samples which were obtained by the in vitro binding assays and cell fractionation were electrophoresed on SDS-polyacrylamide gels (Laemmli, 1970), and then analyzed by western blotting as described by Towbin et al. (1979).

**RESULTS**

**Identification of proteins binding with LAP2**

To identify proteins that interact with LAP2, a yeast two-hybrid screen which uses GAL4 recognition sites to regulate the expression of both His3 and LacZ was carried out with the nucleoplasmic domain of LAP2 as a bait. The cDNAs of a mouse embryo day 9.5/10.5 cDNA library were fused with the VP16-acidic-transactivation domain (VP16), and screened. Colonies that grew on yeast drop-out medium lacking Leu, Trp, His, and containing 3-amino-1,2,4-triazole and which became blue when assayed by X-Gal colony plate assay, were selected. Seven positive colonies were obtained out of the 5.5×10⁶ colonies screened. Southern blotting, restriction mapping and partial sequencing indicated that these clones were derived from two different mRNAs. Four clones were found to contain three similar cDNAs (Fig. 1A), encoding a protein which was termed L2BP1 (LAP2 Binding Protein 1), while the other three clones encoded B-type lamin gene (Furukawa and Kondo, 1998).

To confirm the length and the expression of the L2BP1 mRNA, Northern blot analysis of poly(A)+ RNA from rat FRSK cells, mouse NIH3T3 cells, and human HeLa cells was performed under high stringency conditions using the 520 bp fragment of mouse L2BP1 clone 1 cDNA as the probe (Fig. 2). The L2BP1 mRNA was present in all 3 cell lines, and the blot revealed a ~0.8 kb hybridized species in all three mammalian cultured cell lines.

To isolate the full length L2BP1 cDNA, the cDNA library of rat FRSK cells was screened using the 520 bp fragment of mouse L2BP1 clone 1 cDNA as a probe. Three independent clones were obtained from the screening. Complete sequence analysis of these cDNAs indicated that these clones were identical and were derived from same mRNA. The largest showed an approximately 750 bp cDNA insert, suggesting that it was nearly full length. The rat L2BP1 cDNA sequence is

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**Fig. 1.** Isolation and sequencing of cDNAs which encode L2BP1. (A) Alignment of partial length mouse L2BP1 cDNAs (clones 1, 11 and 26) isolated by yeast two-hybrid screening, compared with the full length rat L2BP1 cDNA clone (rat L2BP1) isolated by nucleotide screening. Major restriction sites and the large open reading frame (open box) are indicated. (B) cDNA sequence and deduced amino acid sequence of rat L2BP1. The deduced L2BP1 protein sequence of the large ORF is indicated by the three-letter code. Base pairs and amino acids are numbered separately. The underlined regions indicate boundary in-frame stop codons. The poly(A)+ additional sequence is indicated in italic letters. The GenBank accession number for rat L2BP1 is AB024333.
predicted to encode a polypeptide of 89 amino acids with a calculated molecular mass of 10044, bound by in-frame stop codons (Fig. 1B).

To test the specificity of the interaction between L2BP1 and LAP2, co-transformation of pairwise combinations of pVP16-L2BP1 clone 1 plasmid and GAL4-DNA-binding domain (GD)-fusion LAP2 nucleoplasmic domain (ΔC1) (Fig. 7) or non related GD-fusion protein plasmids, as well as GD-rat L2BP1 plasmid and GAL4-activation domain-fusion protein plasmids, into the Y190 yeast strain, was carried out (Table 1).

The transformants were then assayed for β-galactosidase activity. In both cases, only the combination of LAP2 and L2BP1 was positive for β-galactosidase activity. Interaction between L2BP1 and LAP2 was further confirmed by an in vitro binding assay using glutathione S-transferase (GST)-fused LAP2 ΔC1 and thioredoxin-His (TH)-fused L2BP1 (Fig. 3). Glutathione agarose bound to purified GST-fused LAP2 nucleoplasmic domain was incubated with two different bacterial lysates containing TH:L2-SD or TH:L2-AD (see Materials and Methods) (Fig. 3A). The bound L2BP1 was analyzed by western blotting with anti-His-specific monoclonal antibody. The GST-fused LAP2 AC1 efficiently binds TH:L2BP1-SD, but not TH:L2BP1-AD (Fig. 3B). Therefore, the interaction between LAP2 and L2BP1 is direct and specific.

**Intracellular distribution of L2BP1 during cell division**

To analyze the localization of the protein product of L2BP1 cDNA in a cell, anti-L2BP1-specific polyclonal antibodies were generated against the 17-residue synthetic peptide containing amino acids 4-20 of the rat L2BP1 open reading frame (ORF) (Fig. 4A). Following affinity purification in a peptide-conjugated column, the anti-L2BP1-peptide-specific antibodies were used for western blotting. Examination of total extracts of rat FRSK cells by immunoblotting showed that the antibodies specifically recognized a ~11 kDa protein (Fig. 4B, lane 4) which was not present in the corresponding preimmune sera (data not shown). This value is roughly consistent with the 10 kDa protein predicted from the rat cDNA of L2BP1. The antibodies also recognized the TH:L2-SD fusion protein in total bacterial lysate (Fig. 4C), but not TH:L2-AD (data not shown). Both the bacterial-expressed and endogenous L2BP1 protein bands disappeared when the anti-L2BP1-specific antibodies were pre-incubated with an excess amount of the immunizing peptide (Fig. 4C and D, lanes 8 and 10, respectively).

To determine the localization of L2BP1 protein in a cell, subcellular fractionation was performed with the anti-L2BP1-peptide-specific antibodies. The L2BP1 protein was found in the nuclear fraction, but not in the cytoplasmic fraction of rat FRSK cells (Fig. 4B, lanes 5 and 6). Immunofluorescence staining of rat FRSK cells with the antibodies showed that L2BP1 protein is present in the nucleus. To further validate the nuclear localization of L2BP1, an influenza hemagglutinin (HA) epitope-tagged version of the rat L2BP1 cDNA was transfected into mouse MOP8 cells (Fig. 5B). Immunofluorescence microscopy with an anti-HA-specific monoclonal antibody revealed that the ectopically expressed HA-tagged L2BP1 is localized in the nucleus.

To further examine the behavior of L2BP1, the localization of L2BP1 during the M-phase in rat FRSK cells was studied.

**Table 1. Analysis of the specific interaction between L2BP1 and LAP2**

<table>
<thead>
<tr>
<th>Gal4-DNA-binding domain-fusion</th>
<th>GD</th>
<th>GD-LAP2(ΔC1)</th>
<th>GD-lamC</th>
<th>GD-SNF1</th>
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<tr>
<td>VP16-activation domain-fusion</td>
<td>VP-L2BP1</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Gal4-activation domain-fusion</td>
<td>GA</td>
<td>GA-LAP2(ΔC1)</td>
<td>GA-SNF1</td>
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The Y190 strain was co-transformed with plasmids of the indicated pairwise combination. β-Galactosidase activity was determined by a plating method. Individual transformants were grown on synthetic plates lacking leucine and tryptophan. Positive and negative color reactions on the plates are indicated by + (positive) and – (negative), respectively.
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by confocal immunofluorescence microscopy using the anti-L2BP1-peptide-specific antibodies (Fig. 6). While L2BP1 was localized diffusely throughout the nucleus in interphase cells (Fig. 5A), L2BP1 accumulated throughout the entire chromosomes during prometaphase (Fig. 6A), metaphase (Fig. 6B), and anaphase (Fig. 6C). These results indicate that L2BP1 is a nuclear protein and a component of the chromosome.

Fig. 3. Analysis of in vitro binding of L2BP1 to LAP2. (A) To generate test L2BP1 protein, the BamHI fragment from the mouse L2BP1 clone 1 (Fig. 1) was linked to thioredoxin-His tag sequences in the sense direction (TH:L2-SD), or in the anti-sense direction (TH:L2-AD). (B) The interaction of TH:L2-SD protein (lanes 2 and 4-6) and TH:L2-AD protein (lanes 1 and 3) with LAP2 was examined using an in vitro binding assay. A LAP2 mutant comprising residues 1-398 was fused to GST at the N terminus (Fig. 7). A mixture of these proteins was probed with anti-His-tag-specific monoclonal antibody after electrophoretic fractionation on SDS-polyacrylamide gel (13%). Bacterial lysates containing TH:L2-AD (lane 1) and TH:L2-SD (lanes 2 and 5) before incubation with GST-LAP2 or GST alone are shown. The precipitated protein samples from TH:L2-AD and TH:L2-SD incubated with GST-LAP2 are shown in lanes 3 and 4, respectively. The result of the control sample of TH:L2-SD incubated with GST alone is shown in lane 6. The positions of proteins derived from L2BP1 cDNA are marked as TH:L2-SD and TH:L2-AD, respectively. GST alone is not able to bind to TH:L2-SD (lanes 5 and 6).

Fig. 4. Detection of L2BP1 protein in rat FRSK cultured cells by western blot analysis. (A) The amino acid sequence (one letter code) of the N-terminal peptide used as the antigen for the production of antibodies, is underlined. (B) Total cell extract (lanes 1 and 4), cytoplasmic fraction (lanes 2 and 5) and crude nuclear fraction (lanes 3 and 6) were prepared from rat FRSK cells. Protein samples (50 µg) were electrophoresed on SDS-polyacrylamide gels (17%). Western blotting with affinity-purified anti-L2BP1-peptide-specific antibodies is shown in lanes 1-3. Proteins visualized by staining with Coomassie blue are shown in lanes 4-6. (C and D) Competition experiments for anti-L2BP1-peptide-specific antibodies were carried out using bacterial lysate containing TH:L2-SD (C) and crude nuclear fraction (D) by western blotting. The diluted antibodies were preincubated with (+) and without (−) 0.5 mM immunizing peptide. The migration positions of the L2BP1 and TH:L2-SD proteins are marked as L2BP1 and TH:L2-SD, respectively.

Sequences involved in interaction of LAP2 with L2BP1

We previously demonstrated that multiple regions of the large LAP2 nucleoplasmic domain are required for nuclear rim localization with independent targeting by the first (residues 1-296) and the second (residues 298-409) halves of its N terminus (Furukawa et al., 1995). The targeting function of LAP2 could be involved in its resultant binding to a nuclear component (Furukawa et al., 1997, 1998). To obtain insight into the function of L2BP1 in the nuclear rim localization of LAP2, I investigated which sequences of LAP2 bind to L2BP1 in a yeast 2-hybrid system. A series of deletion mutants of LAP2 were constructed, and the binding of LAP2 mutants to L2BP1 was detected by activation of a LacZ reporter gene (Fig. 7).

Seven LAP2 deletion mutants extending from the N or C terminus were analyzed (Fig. 7, ΔC1 and ΔN). A high level of β-galactosidase activity was induced in yeast cells containing the mutants comprised of more than the first 195 residues from the N terminus (ΔC1-3), as in yeast cells containing the full-length LAP2 (Fig. 7, FL). However, β-galactosidase activity was not induced in yeast cells containing ΔC4, which was comprised of only the first 67 residues. Furthermore, in the N-terminal deletion experiment of LAP2 sequences, the β-galactosidase gene was not activated in yeast cells containing the mutants lacking the first 195 residues from its N terminus (ΔN2-4).

Five deletions extending from both the N and C termini of LAP2 were also examined (Fig. 7, ΔNC3-8). Of these, the construct containing residues 68-195 was the minimal sequence that was sufficient for interacting with L2BP1, based...
on the N- or C-terminal deletion studies described above. The ΔNC8 mutant which contains amino acid residues 67-195 of LAP2, as well as ΔNC6 and 7, did induce a high level of β-galactosidase activity. Hence, this region alone appears to be important for interacting with L2BP1. Furthermore, the ΔNC5 mutant which lacked the first 137 residues of ΔNC6 did not activate the β-galactosidase gene, suggesting that the region of LAP2 between residues 67 and 137 is important for L2BP1 binding.

**DISCUSSION**

cDNA cloning and function of L2BP1

In this study, L2BP1 was identified as a protein which specifically interacts with LAP2. Sequencing of the cDNA clones revealed that L2BP1 is a small acidic protein (pI=5.7) of 89 amino acids. This protein was bound to chromatin during the M-phase of mitosis.

After the cloning and sequencing of L2BP1 was completed, the cDNA cloning of BAF (Barrier-to-Autointegration Factor) from mouse NIH 3T3 cells and human cells was reported (Lee and Craigie, 1998). The amino acid sequence of mouse and human BAF is 98% and 97% identical, respectively, to rat L2BP1 (Fig. 8). Thus, they are homologous proteins. BAF was identified as a cellular trans-acting factor involved in protecting the reverse-transcribed Molony murine leukemia viral DNA against autointegration. Although its original cellular function remains unclear, Lee and Craigie (1994, 1998) clearly showed that mouse BAF directly and avidly binds DNA with no apparent sequence specificity. Furthermore, it was shown that the BAF protein is present in solution as a dimer, and that a high concentration of BAF forms a precipitable macromolecular complex with naked substrate DNAs (Lee and Craigie, 1998). Thus, it was suggested that intramolecular bridging by BAF compacts the viral DNA, and subsequent intermolecular interaction among BAF-DNA complexes results in the formation of a network.

In this study, it was clearly demonstrated that L2BP1/BAF is a nuclear protein (Figs 4 and 5). The behavior of L2BP1/BAF reveals localization throughout the nucleus in interphase cells, while it moves to the condensed chromosomes and remains with the chromosome through the M-phase (Fig. 6). Immunofluorescent images of chromosomes showed that L2BP1/BAF is distributed over the entire chromosomal region. Protein-protein interaction between L2BP1s was also confirmed by the yeast two-hybrid method (data not shown). It is likely that intramolecular and intermolecular bridging by
Fig. 7. Analysis of binding of LAP2 mutant proteins to L2BP1 in the yeast 2-hybrid system. The interaction of a series of deletion mutants of LAP2 with L2BP1 was examined in a yeast two-hybrid assay for β-galactosidase activity. Deletion mutants of LAP2 were fused to the Gal4-activation domain, and L2BP1 was fused to the Gal4-DNA-binding domain at its N termini. The horizontal line indicates the LAP2 sequence present in each construct. Full-length (FL), C-terminal (ΔC), N-terminal (ΔN), and N/C-terminal (ΔNC) deletions are shown in the diagram. β-Galactosidase activity was measured by both the liquid and the X-gal plate methods. Enzyme units in extracts and color phenotypes on plate are shown in the right column. Positive and negative color reactions on the plates are indicated by B (positive) and W (negative), respectively. nt, not tested. The open and filled bars indicate the predicted transmembrane sequence and a region particularly important sequence for binding with L2BP1 in the diagram, respectively. The hatched bar indicates the common 186 amino acid sequence of the LAP2 family, the first 186 amino acids sequence at the N terminus is also shown. The horizontal line indicates the Gal4-activation construct which does not have insert in plasmid showed 0.38 unit and negative color in the liquid and the X-gal plate.

L2BP1/BAF to DNA may function in cellular genomic DNA organization. These results strongly suggest that L2BP1/BAF is a basic component of chromatin and plays a role in the formation of high-order chromosomal structure during the cell cycle.

Interaction between LAP2 and L2BP1

LAP2 is a 53 kDa nuclear envelope polypeptide which binds to mitotic chromosome, B-type lamin and DNA (Foisner and Gerace, 1993; Furukawa et al., 1997, 1998; Furukawa and Kondo, 1998). Further, these binding domains in the LAP2 protein have been mapped in detail by biochemical and molecular genetic methods (Furukawa et al., 1997, 1998). The chromosome-binding domain of LAP2 is located between amino acid residues 1 and 85. The B-type lamin and DNA binding domains, which exist at amino acid residues 298-373 and 244-296, respectively, are distinct from the chromosome-binding domain of LAP2. The chromosome-binding activity of LAP2 is thought to be important for initial contact between the nuclear membrane vesicles and the mitotic chromosome at the beginning of nuclear envelope reassembly (Foisner and Gerace, 1993). In this study, it was demonstrated that L2BP1 directly binds to LAP2 in vitro (Fig. 3), and that the L2BP1 binding domain of LAP2 as determined by a yeast two-hybrid assay is located between amino acid residues 67 and 137 (Fig. 7). Thus, the L2BP1-binding domain of LAP2 overlaps the chromosome-binding domain of LAP2. Interestingly, L2BP1 is distributed at the chromosomes during the M-phase (Fig. 6). Therefore, L2BP1 may function as a mediator for LAP2-chromosome interaction at the end of mitosis.

A number of LAP2 related proteins (LAP2/TMPO α-ζ) produced by alternative splicing from a single gene have been described in mammalian cells (Harris et al., 1994; Berger et al., 1996). Of the proteins which make up the mouse LAP2 family, the first 186 amino acids sequence at the N terminus is a common domain (Berger et al., 1996) (Fig. 7). The second LAP2 protein group, LAP2α and ζ, lacks the hydrophobic transmembrane sequence as well as the B-type lamin binding domain at the C terminus of the original LAP2 protein (LAP2β). A recent study on human LAP2α showed that LAP2α is distributed throughout the interior of the nucleus except the nucleoli, and is clearly distinct from the nuclear rim localization of LAP2β (Dechat et al., 1998). Thus, localization of the LAP2α protein is not specific to the nuclear envelope. However, Dechat et al. (1998), demonstrated that LAP2α is a component of the karyo- and cyto-skeleton in vivo, and that it directly interacts with chromosomes in vitro. Further, LAP2α moves to the surface of chromosomes earlier than the majority of lamin proteins during post-mitotic nuclear reassembly. Thus, LAP2α, as LAP2β, may play a role in the reassembly of the nuclear structure during mitosis. These suggest that the common first 186 amino acids sequence of the LAP2 family, which contains the L2BP1 binding site, plays a major role in associating with chromatin and chromosome. It is thus possible that interaction between L2BP1 and LAP2α is required to confer the function of chromosomal and nuclear organization in the nuclear dynamics of the mammalian cell cycle.

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