Journal of Cell Science 113, 3473-3484 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS1581

Lamina-associated polypeptide 2α binds intranuclear A-type lamins

Thomas Dechat[1], Barbara Korbei[1], O. Anthony Vaughan[2], Sylvia Vlcek[1], Christopher J. Hutchison[2] and Roland Foisner[1,*]

[1]Department of Biochemistry and Molecular Cell Biology, Vienna Biocenter, University of Vienna, A-1030 Vienna, Austria
[2]The Department of Biological Sciences, The University of Durham, Durham DH1 3LE, UK
*Author for correspondence (e-mail: foisner@abc.univie.ac.at)

Accepted 18 July; published on WWW 13 September 2000

SUMMARY

The nucleoskeletal protein lamina-associated polypeptide 2α (LAP2α) contains a large, unique C terminus and differs significantly from its alternatively spliced, mostly membrane-integrated isoforms, such as LAP2β. Unlike lamin B-binding LAP2β, LAP2α was found by confocal immunofluorescence microscopy to colocalize preferentially with A-type lamins in the newly formed nuclei assembled after mitosis. While only a subfraction of lamins A and C (lamin A/C) was associated with the predominantly nuclear LAP2α in telophase, the majority of lamin A/C colocalized with LAP2α in G_1-phase nuclei. Furthermore, selective disruption of A-type lamin structures by overexpression of lamin mutants in HeLa cells caused a redistribution of LAP2α. Coimmunoprecipitation experiments revealed that a fraction of lamin A/C formed a stable, SDS-resistant complex with LAP2α in interphase cells and in postmetaphase cell extracts. Blot overlay binding studies revealed a direct binding of LAP2α to exclusively A-type lamins and located the interaction domains to the C-terminal 78 amino acids of LAP2α and to residues 319-566 in lamin A/C, which include the C terminus of the rod and the entire tail common to lamin A/C. These findings suggest that LAP2α and A-type lamins cooperate in the organization of internal nuclear structures.

Key words: Lamin, Lamina-associated polypeptide, Mitosis, Nuclear matrix, Nuclear reassembly

INTRODUCTION

The nuclear lamina, outer and inner nuclear membranes and nuclear pore complexes represent the major components of the nuclear envelope (NE), which forms the boundary of the nucleus in eukaryotic cells and structurally and functionally separates nucleoplasm and cytoplasm. The nuclear lamina is a dense filamentous network underlying the inner nuclear membrane and is mainly composed of intermediate filament (IF)-type proteins, the lamins. Like all IF proteins, the lamins have a central α-helical rod domain flanked by nonhelical N-terminal head and C-terminal tail domains. Two lamin monomers form coiled-coil interactions via their rod domains, resulting in dimers, the basic subunit in lamin filaments (for recent reviews, see Goldberg et al., 1999b; Stuurman et al., 1998). According to their primary sequence, biochemical properties and expression patterns, the lamins are grouped into two classes: A- and B-type lamins. In mammalian cells the major B-type lamins are lamin B1 and B2, which are encoded by two separate genes, and at least one B lamin is constitutively expressed in all somatic cells. They are farnesylated at their C termini and are tightly associated with the inner nuclear membrane in interphase cells and with membrane vesicles in mitotic cells (reviewed in Gant and Wilson, 1997; Moir et al., 1995). A-type lamins are derived from a single gene by alternative splicing and are predominantly expressed in terminally differentiated cells. The two main representatives are lamin A and its smaller splice variant lamin C, which is missing approx. 90 amino acids at the C terminus. During the onset of mitosis A-type lamins become soluble and are dispersed throughout the cytoplasm in metaphase cells.

Apart from their function as a structural framework providing mechanical stability for the nucleus, the lamins have also been implicated in several other cellular processes. Based on their ability to bind to DNA (Baricheva et al., 1996; Luderus et al., 1992; Rzepecki et al., 1998) and to chromosomes and chromatin (Belmont et al., 1993; Glass et al., 1993; Glass and Gerace, 1990; Goldberg et al., 1999a; Hoger et al., 1991; Taniura et al., 1995), lamins have been proposed to anchor chromatin at the NE and to affect higher order chromatin organization (reviewed in Gotzmann and Foisner, 1999). Additionally, lamins might play a role in DNA replication (Ellis et al., 1997; Gant et al., 1999; Gotzmann and Foisner, 1999; Spann et al., 1997) and apoptosis (reviewed in Gruenbaum et al., 2000). Since lamins reversibly dissociate from chromosomes during mitosis in a phosphorylation-dependent manner they might also be involved in the cell cycle-dependent dynamics of nuclear structure, but their specific role is not entirely clear (reviewed in Foisner, 1997; Gant and Wilson, 1997; Marshall and Wilson, 1997).

Whereas the peripheral nuclear lamina has long been known, there is an increasing number of reports showing lamins in the

nuclear interior. Prelamin A (Lutz et al., 1992) and ectopically expressed lamin A/C (Goldman et al., 1992; Pugh et al., 1997) were transiently found in nucleoplasmic foci during their assembly into the nuclear lamina. Intranuclear lamin structures have also been observed in G_1 phase cells (Bridger et al., 1993) and in association with DNA replication centers in S phase cells (Moir et al., 1994). In addition to these more transient structures, stable intranuclear lamin structures that persist throughout all stages of interphase have been observed using special preparation techniques (Hozak et al., 1995; Neri et al., 1999). Furthermore, stably expressed lamin A fused to the green fluorescence protein (GFP) was found in an extensively branched, intra- and transnuclear network (Broers et al., 1999) and a new monoclonal antibody to lamin A was described to stain nuclear speckles containing RNA splicing factors (Jagatheesan et al., 1999).

Lamin-binding proteins are fundamentally involved in determining the specific localization and functions of the lamin structures (Gerace and Foisner, 1994; Gotzmann and Foisner, 1999; Ye et al., 1998). Among those are the integral membrane proteins, namely lamina-associated polypeptides (LAP) 1 (Foisner and Gerace, 1993; Martin et al., 1995) and p58/lamin B receptor (LBR) (Worman et al., 1990), and proteins not integrated into the membrane, like Young Arrest (YA) (Goldberg et al., 1998) and otefin (Ashery-Padan et al., 1997). In addition, a group of related lamin binding proteins, which share a homologous sequence motif (LEM domain) approx. 40 amino acids long in their N-termini (Lin et al., 2000), have been implicated in diverse functions of nuclear organization (for a recent review, see Dechat et al., 2000). The LEM domain proteins comprise the membrane protein emerin (Manilal et al., 1996), which has been linked to an inherited disease called Emery Dreifuss Muscular Dystrophy, MAN1 (Lin et al., 2000; Paulin-Levasseur et al., 1996), and up to six mammalian LAP2 isoforms (α, β, γ, ε, δ and ξ), which are derived from a single gene by alternative splicing and include transmembrane proteins as well as nonmembrane proteins (Berger et al., 1996; Dechat et al., 1998; Foisner and Gerace, 1993; Furukawa et al., 1995; Harris et al., 1994). Additional putative embryonic forms of LAP2 isoforms have been discovered in *Xenopus* oocytes (Gant et al., 1999; Lang et al., 1999) and similar proteins might be present in mammalian cells but have not been discovered yet.

Most of these lamina-associated proteins have been identified by their localization at the nuclear periphery and/or their tight association with a nuclear lamina/matrix fraction in subcellular fractionation studies. Only in a few cases have the interactions of these proteins been analyzed in more detail. The high molecular weight isoforms of LAP1, LAP1A and 1B were found to specifically bind to A- and B-type lamins in vitro (Foisner and Gerace, 1993), and were expressed together with lamin A/C only in differentiated cells (Martin et al., 1995). The smallest LAP1 isoform, LAP1C, and emerin showed a lamin A/C-dependent accumulation at the NE (Powell and Burke, 1990; Sullivan et al., 1999). LBR and LAP2β, which is the largest transmembrane protein of the LAP2 isoforms, bind preferentially to lamin B (Foisner and Gerace, 1993; Furukawa and Kondo, 1998; Furukawa et al., 1995; Meier and Georgatos, 1994; Worman et al., 1988). Microinjection of recombinant LAP2β fragments containing the lamin B-binding region into G_1 cells (Yang et al., 1997) or addition of the fragment to in vitro nuclear assembly reactions (Gant et al., 1999) inhibited nuclear growth, so it has been suggested that LAP2β might control nuclear lamina dynamics. All the interactions between lamins and associated proteins described above are restricted to the nuclear periphery, as all the lamin binding proteins are integral constituents of the inner nuclear membrane. Potential interaction partners for intranuclear lamins are histones H2A and H2B, as they have been found to interact with lamins in vitro (Goldberg et al., 1999a; Taniura et al., 1995). The physiological significance of these interactions, however, remains to be determined.

LAP2α, the non-membrane isoform of the LAP2 family, is another candidate for an intranuclear lamin-binding component. It was identified as part of the internal nucleoskeleton and has been implicated in nuclear structure organization during the cell cycle (Dechat et al., 1998; Vlcek et al., 1999). LAP2α only shares its 187-residue N terminus with the other LAP2 isoforms and contains a unique, functionally different C terminus 506 amino acids long. The lamin B-binding domain in the LAP2β isoform is absent in LAP2α, leading to the prediction that LAP2α and β have different lamin-binding properties. Here, we demonstrate that LAP2α colocalizes specifically with intranuclear lamin A/C structures in early G_1 cells and possibly in S-phase cells. Furthermore we show by coimmunoprecipitation and blot overlay assays that a subfraction of A-type lamins forms a tight complex with LAP2α in interphase cells and that the C-terminal domain of LAP2α binds directly to the C terminus of lamin A/C.

MATERIALS AND METHODS

Cell culture and synchronization

NRK and HeLa cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 10 mM Hepes, pH 7.0, and 50 μg/ml penicillin and streptomycin (all from Life Technologies, Paisley, UK) at 37°C in a humidified atmosphere containing 5% CO_2. Medium was supplemented with 100 μg/ml G418 (Life Technologies) and 200 μg/ml hygromycin B (Boehringer Mannheim, Germany) for stable HeLa Tet-On (Clontech Laboratories, Palo Alto, CA, USA) cell clones transfected with a construct encoding myc-tagged full-length LAP2α (Vlcek et al., 1999). Expression of exogenous LAP2α was induced for at least 15 hours upon addition of 2 μg/ml doxycycline (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to the medium. To obtain metaphase-arrested cells, subconfluent HeLa cell cultures were incubated for 12-14 hours in medium containing 0.2 μg/ml nocodazole (Calbiochem-Behring Corp., La Jolla, CA, USA). Weakly attached mitotic cells were harvested by mechanical shake-off and incubated for 1 hour in medium containing 20 μM cytochalasin B (Sigma-Aldrich Chemie GmbH) and 0.2 μg/ml nocodazole. Interphase cells were harvested with a cell scraper.

Expression of GFP-lamin mutants and immunofluorescence microscopy

HeLa cells grown on coverslips to 20% confluency were transfected with pEGFP-Lamin B1Δ2+, a derivative of pGEX-Xlamin B1Δ2+ (Ellis et al., 1997), using calcium phosphate. Alternatively, a human lamin A mutant missing the N-terminal 33 residues was inducibly expressed in HeLa cells by removal of tetracycline from the culture medium upon cotransfection of the lamin-mutant-expressing plasmid phLA-N33 (a gift of Dave Gilbert, University of Syracuse) and the transactivator plasmid pMR101/tTa (a gift of Bernard Precious, St Andrew's University, Scotland; Precious et al., 1995).

For immunofluorescence microscopy, cells grown on coverslips

were fixed with ice-cold 1:1 (v/v) methanol:acetone for 10 minutes and processed for immunolabeling, or fixed with 3.7% formaldehyde in PBS for 20 minutes at room temperature followed by incubation in 50 mM NH_4Cl in PBS and in PBS plus 0.1% Triton X-100 for 5 minutes each. For immunolabeling, samples were incubated in PBS plus 0.2% gelatin for 30 minutes and with primary and secondary antibodies in PBS/gelatin for 1 hour each at room temperature. Primary antibodies used were hybridoma supernatants containing antibodies to LAP2α (Dechat et al., 1998) and to lamin A/C (JOL 2 and JOL 3; both diluted 1:2; Dyer et al., 1997), or the following affinity-purified antibodies: rabbit anti-myc (diluted 1:50; a gift of M. Toegel and F. Propst, University of Vienna), guinea pig anti-lamin B (10 μg/ml; Ottaviano and Gerace, 1985), guinea pig anti-lamin A/C (diluted 1:100; Foisner and Gerace, 1993), goat anti-lamin A/C (N-18; diluted 1:100; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), mouse anti-lamin A (133A2; diluted 1:1000; Sasseville and Raymond, 1995), mouse anti-lamin B (Ab-1; diluted 1:30; Calbiochem-Behring Corp.), anti-NuMa (undiluted; Calbiochem-Behring Corp.) and rabbit anti-lamin C (diluted 1:100). The polyclonal rabbit antibody against lamin C was raised to the last eight amino acids of lamin C, including an N-terminal lysine as a linker (KHHVSGSRR). The peptide was coupled to keyhole limpet haemocyanin through primary amino groups using glutaraldehyde. The resulting protein-peptide conjugate was dialyzed overnight at 4°C against PBS, and used to immunize a rabbit. Immune serum was screened by indirect immunofluorescence microscopy and affinity purified against 10 mg of the lamin C peptide conjugated to CH-Sepharose 4B. The antibody recognizes the lamin C-specific tail domain of recombinant lamin C, but no other part of recombinant lamin C or any other recombinant lamins on immunoblots. The antibody detects a single band migrating at 65 kDa in immunoblots of nuclei isolated from HeLa, human dermal fibroblasts and SW13 cells. In a majority of human cell lines and primary human fibroblasts the antibody stains the NE exclusively (R. S. Venables, S. McLean, D. Luny, E. Moteleb, S. Morley, R. A. Quinlan, E. B. Lane and C. J. Hutchison, unpublished data).

Secondary antibodies used were donkey anti-mouse IgG conjugated to FITC, donkey anti-goat IgG conjugated to Cy3, goat anti-mouse IgG and goat anti-rabbit IgG conjugated to Texas Red (all from Jackson Immuno-Research, West Grove, PA, USA), donkey anti-guinea pig IgG conjugated to Texas Red (Accurate Chemicals & Scientific Corp., Westbury, NY, USA), and goat anti-rabbit IgG conjugated to Alexa Fluor 488 (Molecular Probes, Leiden, Netherlands). DNA was stained with 1 μg/ml Hoechst dye 33258 (Calbiochem-Behring Corp.) for 10 minutes. Samples were mounted in Mowiol and viewed in a Zeiss Axiovert microscope and a Zeiss LSM confocal laser scanning microscope.

Immunoprecipitation

1×10^8 nocodazole-arrested mitotic or interphase HeLa cells were lysed in 500 μl KHM buffer (50 mM Hepes, pH 7.4, 78 mM KCl, 10 mM EGTA, 8.4 mM $CaCl_2$, 4 mM $MgCl_2$) containing 1% Triton X-100, 20 μM cytochalasin B, 1 mM dithiothreitol (DTT), 500 μg/ml DNase, 200 μg/ml RNase (both Boehringer Mannheim), the protease inhibitors PMSF (1 mM), benzamidine (2 mM), and aprotinin, leupeptin and pepstatin (2 μg/ml each), and for mitotic cell lysates the phosphatase inhibitors calyculin A (0.1 μM), okadaic acid (0.1 μM), β-glycerophosphate (0.5 mM), and orthovanadate (1 mM) (Sigma-Aldrich Chemie GmbH, Life Technologies, and Calbiochem-Behring Corp.). After a 5 minute incubation at room temperature, 0.5% SDS was added and samples were incubated for 15 minutes at room temperature and diluted fivefold with KHM buffer plus supplements, yielding a final concentration of 0.1% SDS and 1% Triton X-100. 500-800 μl of cell lysate were mixed with 25 μl Protein G-Sepharose beads (Sigma-Aldrich Chemie GmbH), centrifuged for 3 minutes at 1000 rpm (Heraeus Megafuge 1.0R), and supernatants were mixed with 50 μl Protein G-Sepharose beads that were preincubated with either monoclonal antibody to LAP2α (Dechat et al., 1998) or hybridoma medium containing 10% FCS overnight. Following incubation for 4 hours at 4°C by end-over-end rotation, the beads were pelleted through 30% sucrose at 1000 rpm for 3 minutes at 4°C (Heraeus Megafuge 1.0R), washed with buffer and prepared for immunoblotting.

For the preparation of postmitotic cell lysates, mitotic HeLa cells were suspended in KHM buffer supplemented with 1 mM DTT, 20 μM cytochalasin B, protease inhibitors and RNase and DNase, and homogenized on ice in a metal ball cell crusher (EMBL, Heidelberg, Germany) using a 2.08 mm metal ball. After an incubation at 37°C for 5 minutes, phosphatase inhibitors, 0.5% SDS and 1% Triton X-100 were added and samples were processed for immunoprecipitation as described above. Mitotic lysates were prepared by adding phosphatase inhibitors prior to cell lysis and by centrifugation of cell lysates at 2000 rpm (Heraeus Megafuge) for 5 minutes at 4°C.

Preparation of recombinant LAP2α and lamin C polypeptides

The construction of bacterial pET23a-derived (Novagen Inc., Madison, WI, USA) expression vectors coding for different LAP2α fragments and of pGEX-2T-derived (Pharmacia Biotech, Vienna, Austria) plasmids encoding lamin C fragments has been described previously (Dyer et al., 1997; Vlcek et al., 1999). His-tagged LAP2α proteins and GST-tagged lamin C proteins were expressed in *E. coli* BL21 (DE3) by induction with 0.4 mM isopropyl-β-D-thiogalactopyranoside for 2-4 hours at 37°C. Bacteria were harvested by centrifugation at 4000 rpm for 5 minutes (Heraeus Megafuge 1.0R), frozen in 1/10 of the original culture volume of Hepes buffer (20 mM Hepes, pH 7.4, 100 mM NaCl, 1 mM DTT, protease inhibitors), thawed and lysed by addition of 0.1 mg/ml lysozyme, 0.1% Triton X-100, 10 mM $MgCl_2$, 50 μg/ml DNase and 20 μg/ml RNase, and incubation for 30 minutes at 30°C. After centrifugation, pellet fractions were solubilized in Hepes buffer containing 7 M urea and soluble fractions were stored at –20°C.

Lamin C cDNA in pBluescribt KS+ (a gift of G. Krohne, Würzburg, Germany) and LAP2α cDNAs in pET23a plasmids were transcribed in vitro using T7 polymerase (Promega Corp., Madison, WI, USA); RNAs were translated in vitro using the Rabbit Reticulocyte Lysate (Promega) and [^{35}S]methionine (NEN Life Science Products Inc., Boston, MA, USA), according to the manufacturer's instructions.

Purification and labeling of rat lamin A/C

Lamin A/C was purified from rat liver as previously described in Foisner and Gerace (1993) using phosphocellulose ion exchange chromatography. Lamin A/C fractions were desalted into 10 mM sodium borate, pH 8.5, containing 1 M urea on a Bio-Gel P-6 column (BioRad, Hercules, CA, USA), and incubated with one IODO-BEAD (Pierce Chemical Company, Rockford, IL, USA) and 0.5 mCi $Na^{125}I$ (NEN Life Science Products Inc.) for 15 minutes at room temperature. The reaction was stopped by applying the reaction mix onto a Bio-Gel P-10 (BioRad) gel filtration column in 10 mM sodium borate, pH 8.5, and 1 mM DTT. Fractions were analyzed in a gamma counter (Packard Instrument Company, Meriden, CT, USA) and used for the overlay assay.

Gel electrophoresis, immunoblotting and blot overlay assay

Recombinant lamin and LAP2 proteins, purified rat vimentin (Foisner et al., 1988), or salt-washed NE fractions (SWNE) from rat liver (Foisner and Gerace, 1993) were separated by SDS-PAGE (Laemmli, 1970) and stained with Coomassie Blue or transblotted onto nitrocellulose (0.2 μm; Schleicher and Schuell Inc., Dassel, Germany) in 48 mM Tris-HCl, pH 9.4, 39 mM glycine, using the Mini Transblot system (BioRad). Nitrocellulose membranes were stained with PonceauS, washed in PBST (PBS, 0.05% Tween 20) and incubated in overlay buffer (10 mM Hepes, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 2 mM EGTA, 0.1% Triton X-100, 1 mM DTT) for 1 hour with three

changes. After blocking with 2% BSA (w/v) in overlay buffer, membranes were probed either with whole reticulocyte lysate containing in vitro-translated ^{35}S-labeled proteins, diluted 1:50, or with ^{125}I-labeled lamin A/C (0.5 μg/ml) in overlay buffer plus 1% BSA (w/v) and 1 mM PMSF for 3 hours at room temperature. After extensive washing in overlay buffer, nitrocellulose was air dried, and bound proteins were detected by autoradiography.

For the immunological detection of transblotted proteins the Protoblot Immunoscreening System (Promega) or the SuperSignal detection system (Pierce Chemical Company) were used. Primary antibodies used were anti-LAP2α antibodies (hybridoma supernatants, undiluted), anti-lamin B (Ab 1, diluted 1:50), anti-lamin A/C (1E4; diluted 1:1000; Loewinger and McKeon, 1988) and antiserum to LAP2 (diluted 1:1000; gift of Larry Gerace, La Jolla, USA).

RESULTS

Colocalization of intranuclear lamin A/C structures with LAP2α

We have recently reported that LAP2α and A-type lamins may transiently colocalize during postmitotic nuclear assembly in NRK cells using a guinea pig antiserum to A-type lamins (Dechat et al., 1998). To make sure that the intranuclear localization of lamin A/C in G_1 nuclei was not a peculiarity of the guinea pig antiserum, we analyzed the redistribution of both lamins and LAP2α during nuclear assembly in more detail by confocal double immunofluorescence microscopy using monoclonal antibodies to LAP2α and a series of different available antisera to lamins (Fig. 1). Using a goat antiserum (N18) to lamin A/C, we found that in early telophase most of the lamins were still cytoplasmic, whereas the majority of LAP2α was located around and between decondensing chromosomes (Fig. 1A, row a). A small subfraction of lamin A/C, however, seemed to colocalize with LAP2α structures in the newly formed nucleus. In later stages of assembly lamin A/C accumulated in the nucleus and colocalized with LAP2α at the nuclear envelope and in intranuclear speckles (row b). In G_1 nuclei, both LAP2α and A-type lamins remained punctuate throughout the nucleus (row c). Superimposition of the LAP2α and lamin images revealed that lamin A/C were not only found in the nuclear interior together with LAP2α (yellow), but were also located in a LAP2α-independent nuclear rim (red), suggesting that G_1 nuclei contained both intranuclear as well as peripheral A-type lamin structures. In S-phase cells, the peripheral staining of lamin A/C became more prominent, while LAP2α

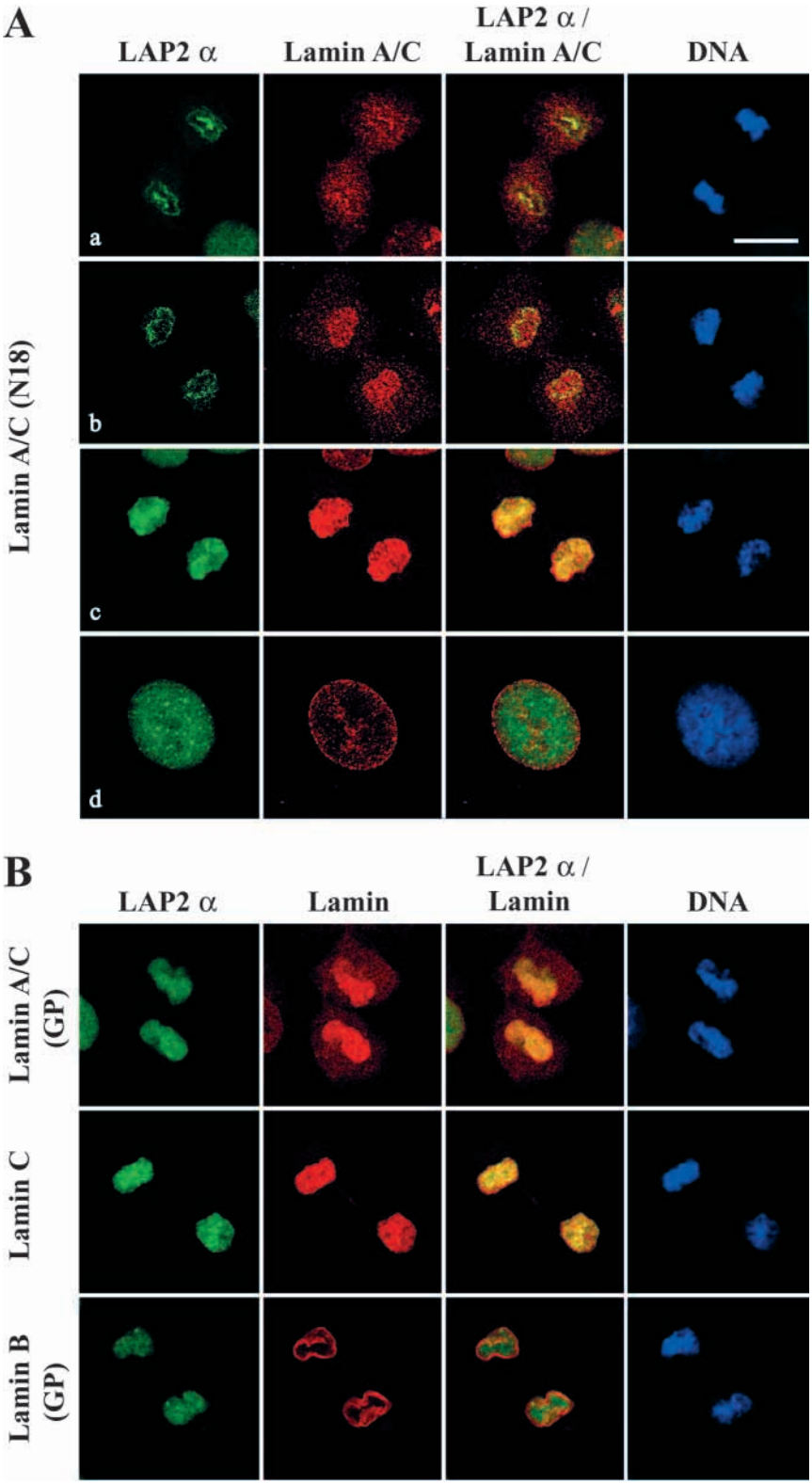

Fig. 1. Cellular distribution of LAP2α and A and B-type lamins in NRK cells at various postmetaphase cell cycle stages (a-d). NRK cells were processed for immunofluorescence microscopy using (A) goat antiserum (N18) to lamin A/C, (B) guinea pig antiserum (GP) to lamin A/C or lamin B, or rabbit antiserum to lamin C, and monoclonal antibody 15/2 to LAP2α. DNA was stained with Hoechst dye. Confocal images are shown and green and red images were merged to visualize colocalization. See text for details. Bar, 10 μm.

remained uniformly distributed throughout the nucleus (row d). Due to the different staining intensities of intranuclear LAP2α and lamin A/C in S-phase cells it was impossible to identify a potential colocalization of these structures in merged images. Lamin structures detected within the nucleus (row d, red structures) most probably represent invaginations of the nuclear membrane (Fricker et al., 1997). To confirm the colocalization of LAP2α and lamin A/C in G_1-phase cells, we tested different lamin antibodies in NRK (Fig. 1B) and HeLa (Fig. 2) cells. All antisera used, including goat antiserum (N-18), a guinea pig antiserum to lamin A/C (GP; Dechat et al., 1998; Ottaviano and Gerace, 1985), and a rabbit antiserum to lamin C (R. S. Venables and C. J. Hutchison, unpublished) detected similar structures in G_1 nuclei, which colocalized with intranuclear LAP2α in both cell lines. Using a stable HeLa cell clone expressing myc-tagged full-length LAP2α (Vlcek et al., 1999), we were able to perform double-labeling of the cells using monoclonal antibodies JOL 3 to lamin A/C (Dyer et al., 1997) or 133A2 to lamin A (Sasseville and Raymond, 1995) and an antiserum to the Myc-tag for detection of LAP2α (Fig. 2B). In all cases, intranuclear A-type lamins colocalizing with LAP2α were clearly detected in G_1-phase cells. In contrast, double staining of G_1 nuclei of NRK (Fig. 1B) and HeLa (Fig. 2B) cells with antibodies to lamin B and LAP2α revealed that lamin B was exclusively located in a nuclear rim, independent of intranuclear LAP2α structures. Thus, we concluded that during postmitotic nuclear assembly, at least up to late G_1 phase, LAP2α may preferentially bind to A-type lamins and help to establish nuclear structure. From these immunofluorescence results, we saw no clear evidence for intranuclear LAP2α-lamin A/C complexes during S phase. However, as shown below, biochemical results suggested that such complexes might exist.

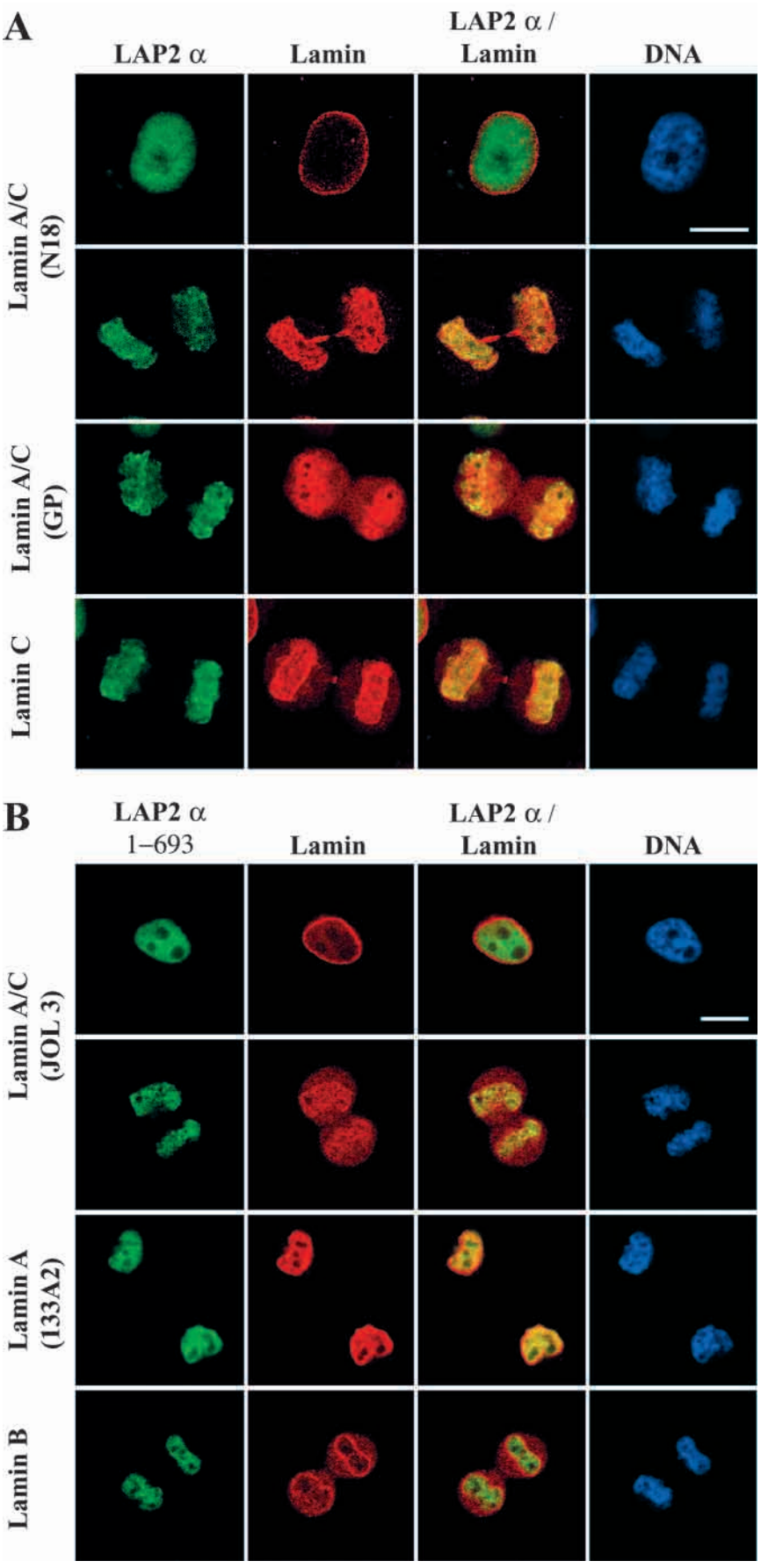

Fig. 2. Cellular distribution of LAP2α and A- and B-type lamins in HeLa cells (A) and in stable HeLa cell clones expressing Myc-tagged, full length LAP2α (1-693) (B) at various post-metaphase cell cycle stages. Antibodies used are goat antiserum (N18) or guinea pig antiserum (GP) to lamin A/C or rabbit antiserum to lamin C, and monoclonal antibodies 15/2 to LAP2α (A), JOL 3 to lamin A/C, 133A2 to lamin A, or Ab-1 to lamin B, and polyclonal anti-Myc antibody to detect Myc tagged LAP2α 1-693 (B). DNA was stained with Hoechst dye. Confocal images are shown and green and red images were merged to visualize colocalization. See text for details. Bar, 10 μm.

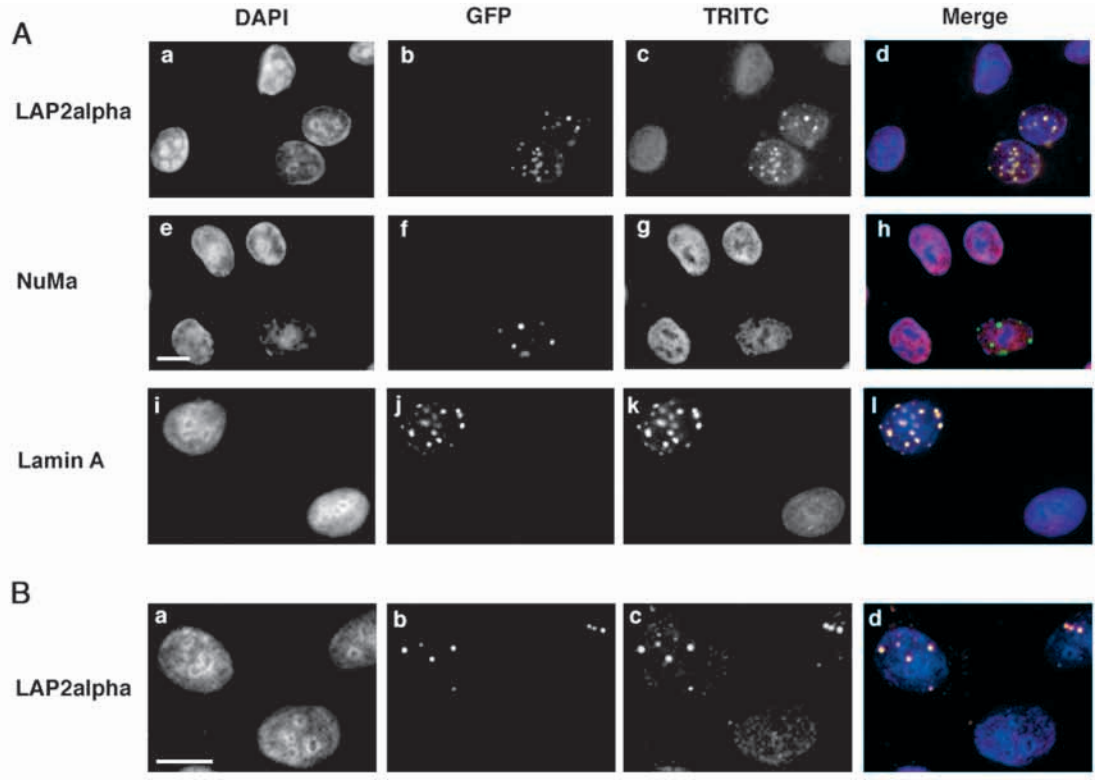

Fig. 3. Redistribution of LAP2α by overexpression of GFP-lamin B1Δ2+ mutants (A) or GFP-lamin AΔN33 (B). GFP-lamin mutants were transiently expressed in HeLa cells and samples were processed for immunofluorescence microscopy 72 hours after transfection using monoclonal antibodies to LAP2α, or to NuMa or to lamin A (JOL 4). Double fluorescence confocal images depicting mutant lamin by the GFP signal and the respective proteins by TRITC-coupled secondary antibodies are shown. DNA was stained with DAPI and images were merged to visualize colocalization. Bars, 10 μm.

Lamin B mutant-dependent relocalization of cellular lamin A caused selective redistribution of LAP2α

To obtain further support for a potential interaction of LAP2α with A-type lamins in interphase nuclei, we transiently expressed a mutant *Xenopus* lamin B1 (lamin B1Δ2+, Fig. 3A) or a human lamin A mutant (ΔN33 lamin A, Fig. 3B) in HeLa cells. These mutants have previously been shown to promote lamina disassembly in in vitro assembled sperm nuclei (Ellis et al., 1997) and to cause a selective redistribution of A-type lamins in mammalian cells (O. A. Vaughan and C. J. Hutchison, unpublished data). Upon transient expression of C-terminal GFP fusion proteins of these lamin mutants in HeLa cells, the ectopic proteins were detected in intranuclear aggregates in the transfected cells (Fig. 3A, GFP panels b,f,i). Immunofluorescence microscopy using antibodies to lamin A/C revealed that all of the endogenous lamin A/C, which was found in a nuclear rim and in intranuclear foci in untransfected cells, was entirely redistributed to the nuclear aggregates of the GFP-lamin B1 mutants in transfected cells (Fig. 3, row lamin A, i-l). In contrast, endogenous lamins B1 and 2 were found to be unaffected and were retained exclusively at the nuclear rim (O. A. Vaughan and C. J. Hutchison, unpublished data). To analyze the effects of the selective lamin A/C redistribution on the cellular localization of LAP2α, we stained transfected cell cultures with LAP2α-specific antibodies. Similar to lamin A/C, a significant amount of LAP2α was redistributed into the GFP-lamin mutant and lamin A/C-containing nuclear aggregates, leaving only a faint uniform nuclear staining (Fig. 3A,B, panels c). In untransfected control cells surrounding the GFP-lamin-expressing cells, LAP2α was uniformly distributed throughout the entire nucleus. These data clearly demonstrated that the selective disruption of endogenous A-type lamin structures caused a severe redistribution of cellular LAP2α into GFP-lamin mutant/lamin A/C aggregates. This observation is consistent with a direct or indirect association of LAP2α with cellular A-type lamins. To exclude the possibility that disruption of the cellular lamin A/C structures affect other nuclear matrix-type proteins in a general, more unspecific manner, we investigated the distribution of NuMa upon transient expression of the GFP-lamin B1 mutant. Unlike LAP2 α, NuMa was not relocalized to intranuclear GFP-lamin B1/lamin A aggregates, but remained distributed throughout the nucleus as in nontransfected control cells (Fig. 3A, panel g). Thus, disruption of lamin A structures selectively affected LAP2α and suggested a direct or indirect physical link of these proteins in nuclear structures.

LAP2 α and lamin A/C form a stable complex in interphase cells and during nuclear reassembly after mitosis

To detect the potential association of LAP2α with lamin A/C at the biochemical level, we performed coimmunoprecipitations from asynchronously growing cells using monoclonal antibodies to LAP2α. As lysis of cells in Triton X-100-containing buffers did not solubilize significant amounts of lamins and LAP2α (data not shown; compare Dechat et al., 1998) or caused unspecific precipitation of protein complexes, we used 0.5% SDS to solubilize the majority of endogenous proteins and performed immunoprecipitation upon dilution of the soluble fraction to 0.1% SDS. Immunoblot analysis of the LAP2α immunoprecipitates showed that a substoichiometric amount of lamin A/C were coprecipitated with LAP2α (Fig. 4A, left lane α), while lamin B was not detected in these samples. Control precipitations using an unrelated IgG did not bring down significant amounts of any of these proteins (Fig. 4A, lane K). Thus, we concluded that, unlike lamin B, a

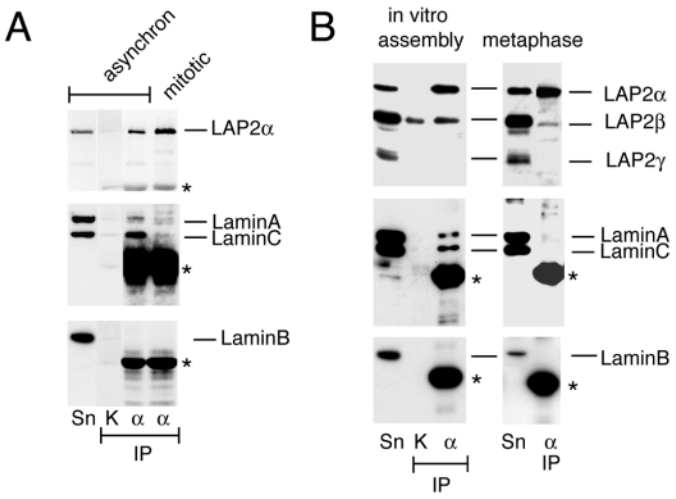

Fig. 4. Coimmunoprecipitation of LAP2α and lamin A/C. (A) Asynchronously growing HeLa cells or nocodazole-arrested mitotic cells were lysed in buffers containing 1% Triton X-100 and 0.5% SDS and diluted to 0.1% SDS. Soluble fractions (Sn) were processed for immunoprecipitation using LAP2α-specific antibody 15/2 (α) or serum IgG (K) coupled to Protein G-beads, and immunoprecipitates were analyzed by immunoblotting using monoclonal antibodies 15/2 to LAP2α, 1E4 to lamin A/C and Ab-1 to lamin B. (B) Nocodazole-arrested HeLa cells were lysed in KHM buffer and cell lysate was either immediately mixed with phosphatase inhibitors (metaphase) or after 10 minutes incubation at room temperature (in vitro assembly). Samples were supplemented with 1% Triton X-100 and 0.1% SDS and immunoprecipitation was performed as above. Immunoblots of LAP2α immunoprecipitates using LAP2 antiserum, monoclonal antibodies 1E4 to lamin A/C and Ab-1 to lamin B are shown. Asterisks indicate the immunoglobulin heavy chain.

subfraction of lamin A/C formed a tight complex with LAP2α in interphase nuclei, which was even stable under the partially denaturing conditions of 0.1% SDS. As a control, we precipitated LAP2α from nocodazole-arrested mitotic cells (Fig. 4A, right lane α), when LAP2α and A-type lamins have been shown to become soluble and to be dispersed throughout the cytoplasm (Dechat et al., 1998). Neither lamin A/C nor lamin B were found to coprecipitate with LAP2α in mitotic cells, indicating that the stable LAP2α-lamin A/C complex dissociates during metaphase.

As we have previously described that LAP2α reassociated with chromosomes very early during postmitotic reassembly (Dechat et al., 1998), we were interested to test whether LAP2α-lamin A/C complexes are also formed during an in vitro nuclear assembly reaction. Nocodazole-arrested mitotic cells were mechanically broken in buffers without Triton, and cell lysates were incubated for 15 minutes at 30°C to allow partial nuclear assembly. Upon addition of Triton X-100 and SDS, LAP2α was immunoprecipitated from soluble fractions. Immunoblot analyses of the precipitated proteins with anti-LAP2 antiserum, which detects all isoforms of LAP2, and with lamin antibodies, showed that lamin A/C, but not lamin B, coprecipitated with LAP2α (Fig. 4B). A small amount of LAP2β found in LAP2α precipitates is likely to be contamination, as control precipitations using an unrelated IgG brought down a similar fraction of LAP2β. In contrast, lamins were not detected in control precipitates using unrelated IgG (Fig. 4B, lane K). LAP2α, precipitated from metaphase cell lysates, did not contain any coprecipitating lamins or LAP2β (Fig. 4B, metaphase). Therefore, we concluded that a small, substoichiometric fraction of lamin A/C, but not lamin B, bound to LAP2α during the very early stages of nuclear assembly. The association of lamin A/C with LAP2α structures was apparently mediated by high-affinity interactions since the complex was stable in 0.1% SDS. Experiments performed in the absence of SDS revealed that LAP2α-lamin A/C complexes formed during the assembly reaction were insoluble and precipitated unspecifically (data not shown).

LAP2 α interacts directly with A-type lamins in vitro via the C-terminal α-specific region

Our experiments indicated that a subfraction of cellular lamin A/C formed a tight complex with LAP2α in interphase cells. To test whether this association was caused by a direct interaction of LAP2α with A-type lamins or was mediated by other proteins in the complex, we performed in vitro binding studies using blot overlay assays. Full-length LAP2α was expressed and radioactively labeled in an in vitro translation reaction using reticulocyte lysate and [^{35}S]methionine. The labeled protein (Fig. 5, left lane) was overlaid onto transblotted fractions of salt-washed rat liver nuclear envelopes containing lamins A, B1 and C. Although all the lamins were clearly detected in the Triton X-100/salt-insoluble and in the urea-soluble fractions, lamin A/C were predominantly detected in

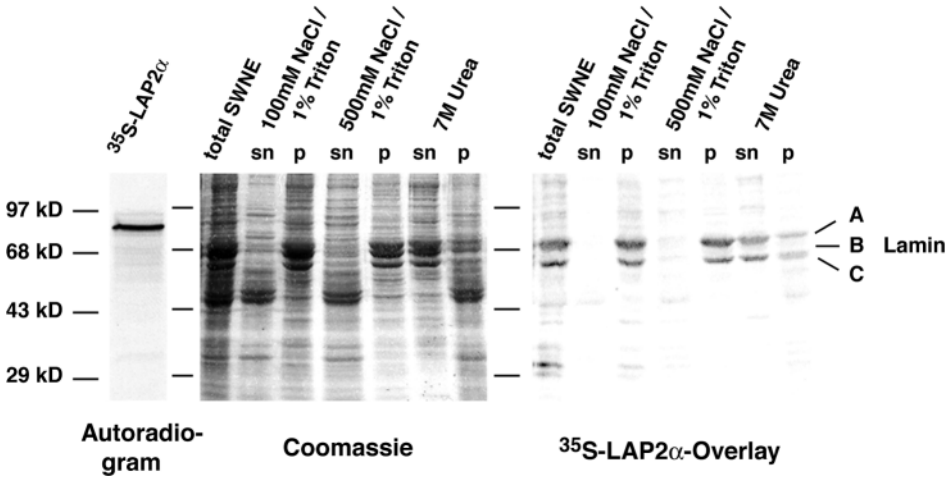

Fig. 5. Blot overlay assay of LAP2α onto fractions of rat liver nuclei. Total salt-washed nuclear envelopes prepared from rat liver (SWNE) or soluble (sn) and insoluble (p) fractions of SWNE following extraction with salt/Triton X-100 or urea-containing buffers, were separated by SDS-PAGE and stained with Coomassie Blue or transblotted to nitrocellulose and overlaid with in vitro translated ^{35}S-labeled full-length LAP2α (shown in the first lane). Radioactive proteins and bound LAP2α were detected by autoradiography.

the autoradiogram of the overlaid blot (Fig. 5). These data clearly showed that LAP2α interacted preferentially with A-type lamins, which is consistent with the immunofluorescence and the immunoprecipitation studies. However, since the reticulocyte lysates are highly concentrated nuclear extracts, it could not be excluded that an unlabelled protein from the lysate mediated this interaction.

To address this question and to narrow down the domain of LAP2α, which mediates the association with A-type lamins, we performed blot overlay assays using purified lamins and transblotted recombinant LAP2α fragments covering different regions of the protein. We overlaid lamin A/C, which had been purified from rat liver nuclei by column chromatography and subsequently labeled in vitro using ^{125}I, onto recombinant full-length LAP2α (1-693) and the N-terminal fragment (LAP2α 1-187), common to all LAP2 isoforms. As shown in Fig. 6B, labeled lamins bound exclusively to the full-length protein, but did not interact at all with the N-terminal fragment, although it was used at the same concentration as the full-length protein. This experiment clearly showed that LAP2α bound directly to lamin A/C and suggested that the α-specific C-terminal region of LAP2α mediated this interaction.

In order to confirm this hypothesis and to narrow down the potential lamin interaction domain in the α-specific domain of LAP2α, in vitro translated and ^{35}S-labeled lamin C was overlaid onto transblotted recombinant LAP2α fragments containing different regions of the α-specific domain (Fig. 6C). As expected, labeled lamin C bound to full-length LAP2α (1-693) and to the entire α-specific C-terminal domain (188-693). Since N-terminal LAP2α fragments 1-254, 1-414 and 1-615 did not interact with lamin C, the potential interaction domain was assigned to the C-terminal 78 amino acids of LAP2α (Fig. 6A). Accordingly, the smallest C-terminal LAP2α fragment (410-693), containing the putative lamin interaction domain, bound lamin C, although the signal was weaker when compared to those obtained with the full-length protein and the entire α specific domain. To demonstrate the reliability and the specificity of the solid-phase overlay assays, we performed

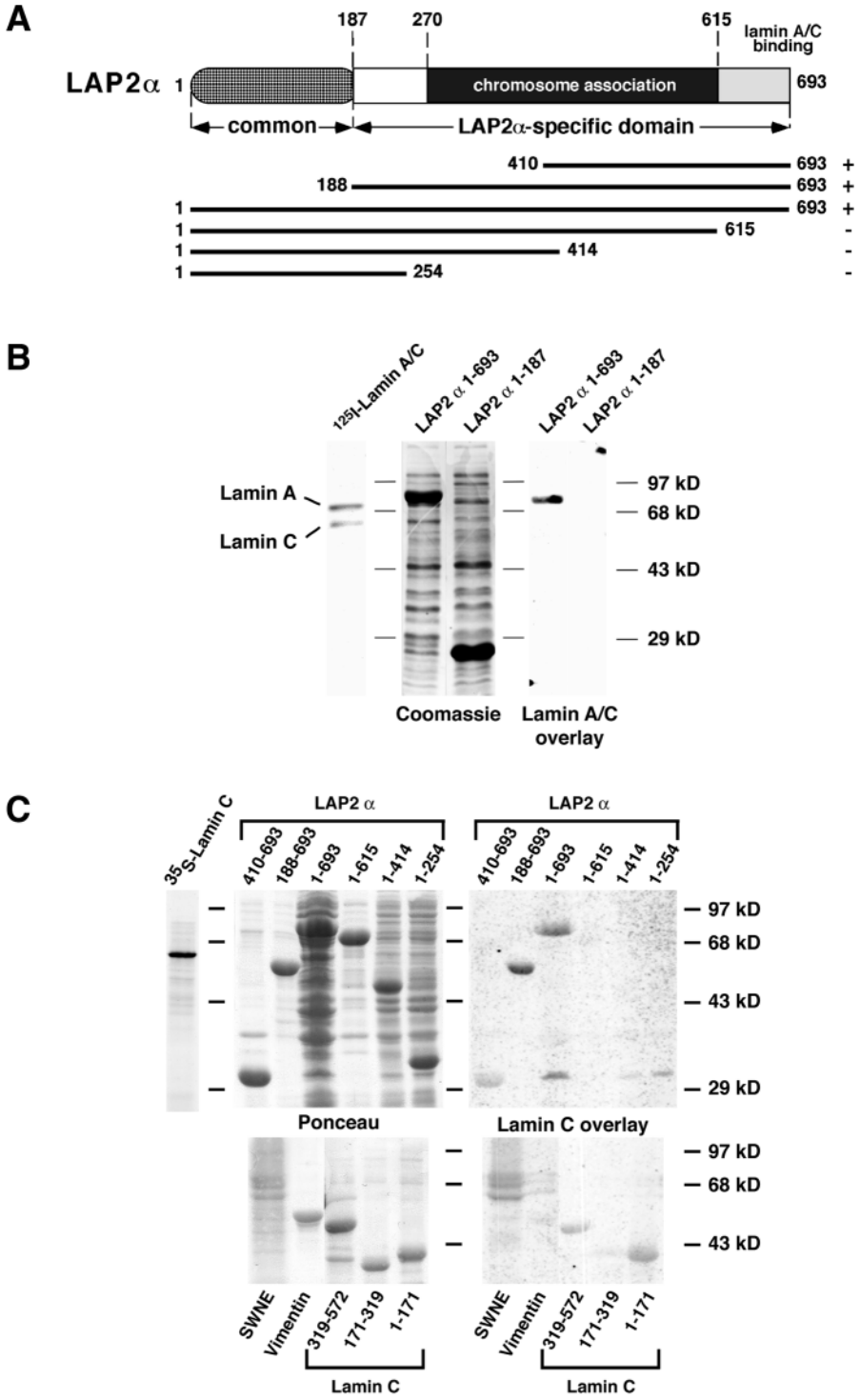

Fig. 6. Interaction of LAP2α with lamin A/C is mediated by its C-terminal 78 amino acids. (A) Schematic drawing of LAP2α and LAP2α fragments used for binding studies and localization of molecular domains in the polypeptide. Numbers denote amino acid positions; +, interaction, –, no interaction with lamins A or C. (B) Lamin A/C were purified from rat liver nuclear envelopes, labeled with ^{125}I, and overlaid onto transblotted recombinant full-length LAP2α or LAP2 common domain 1-187. Coomassie Blue-stained gels of proteins and autoradiograms of overlaid blots are shown. (C) In vitro translated ^{35}S-labeled lamin C was overlaid onto transblotted recombinant LAP2α fragments indicated (upper panels) or onto transblotted salt-washed nuclear envelope fractions of rat liver (SWNE), vimentin and recombinant lamin C fragments (lower panels). Ponceau S stains of blots and autoradiograms of overlays are shown. Note that the approx. 34 kDa band in lanes 1-693, 1-414 and 1-254 in the autoradiogram represents bacterial proteins binding unspecifically to lamin C.

various control experiments and tested lamin-lamin and lamin-vimentin interactions (Fig. 6C, lower panel). While labeled lamin C bound to lamins in the salt-washed nuclear envelope fraction from rat liver nuclei (SWNE), we could not detect any interaction with the cytoplasmic intermediate filament protein vimentin. Furthermore, lamin C bound only recombinant lamin C fragments covering the N-terminal (1-171) and C-terminal (319-572) domains, which contain both N- and C-terminal regions of the α-helical coiled-coil region of lamins known to be important for intermediate filament assembly (Quinlan et al., 1995), while a fragment representing the central part of the rod domain (171-319) did not bind.

The C-terminal domain of lamin C mediates the interaction with LAP2α

Having shown that the C terminus of LAP2α interacts with A-type lamins, we wanted to narrow down the interaction domain in the lamin A/C polypeptide chains. When radiolabeled, in vitro-translated full-length LAP2α was overlaid onto recombinant lamin C fragments, we observed binding only to the C-terminal region of lamin A/C (319-572), while no significant signal above background could be observed with the N-terminal region (1-171) and the central rod domain (171-319) (Fig. 7A). Control experiments revealed that the α-specific C-terminal region of LAP2α showed the same binding properties as full length LAP2α, and that both proteins did not interact with the cytoplasmic intermediate filament protein vimentin (Fig. 7B). Thus, the LAP2α interaction domain of A-type lamins can be restricted to a C-terminal region of the proteins 250 amino acids long, which is present in both lamin A/C (Fig. 7C). It is unlikely that the 6 extreme C-terminal, lamin C-specific or the 98 lamin A-specific amino acids are involved in this interaction as both lamin A/C showed a similar interaction in vitro.

DISCUSSION

In this study we show that LAP2α binds preferentially to A-type lamins by in vivo and in vitro experiments. We further demonstrate that the lamin A/C interaction domain of LAP2α can be narrowed down to the extreme C terminus of the protein, which is unique to LAP2α.

Differential lamin binding properties of LAP2α and LAP2β

LAP2β, the best characterized membrane-anchored isoform of the LAP2 family, has been found to bind exclusively to B-type lamins (Foisner and Gerace, 1993). The lamin B interaction domain of LAP2β was mapped to amino acids 298-370 (Furukawa et al., 1998), a region that is also found in its entire length in the LAP2β-related isoforms LAP2ε and LAP2δ and in parts in LAP2γ and LAP2ζ, while it is completely absent in LAP2α (Dechat et al., 2000). Thus most of the LAP2 isoforms, which are transmembrane components, may bind with different affinities to B-type lamins and mediate the association of the membrane with the peripheral lamina. In contrast, LAP2α, which is less closely related to the other

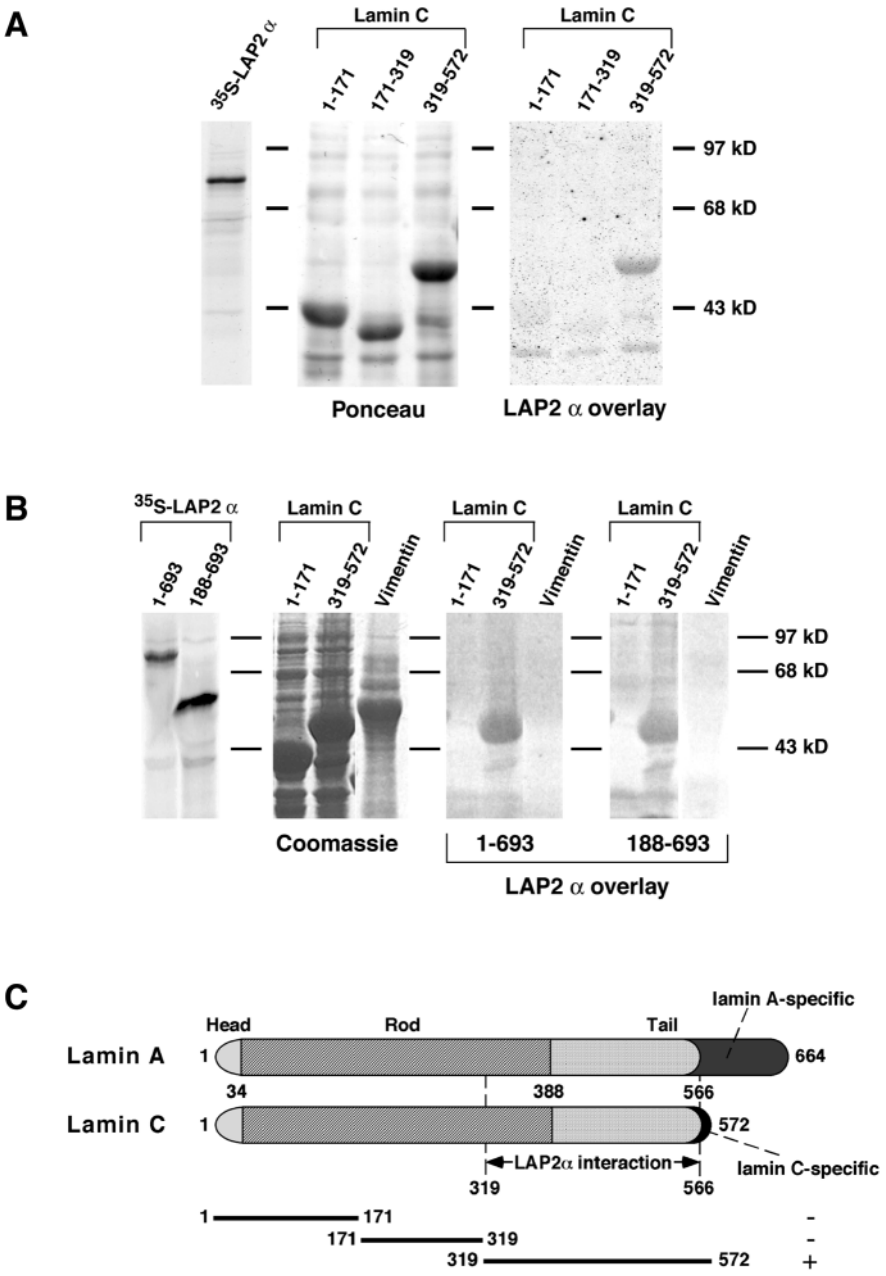

Fig. 7. LAP2α binds to the C terminus of lamin A/C. In vitro translated ^{35}S-labeled recombinant LAP2α or the LAP2α-specific domain (188-693) were overlaid onto transblotted recombinant lamin C fragments as indicated (A) or onto transblotted lamin fragments and vimentin (B). Coomassie Blue-stained SDS-gels of samples and autoradiograms of overlays are shown. (C) Schematic drawing of lamin A/C, depicting molecular domains, LAP2α interaction region, and recombinant lamin fragments used. +, binding, –, no binding to LAP2α; numbers indicate positions of amino acids in lamin polypeptides.

isoforms and does not contain the lamin B-binding domain of LAP2β, was not expected to possess similar lamin binding properties. Indeed, we were unable to detect a significant colocalization of LAP2α with lamin B-type structures throughout the cell cycle, nor could we find any direct in vitro interaction of these proteins. Instead, LAP2α bound specifically to lamin A/C. This interaction is most likely mediated by LAP2α's extreme C terminus, which is unique for the protein. These findings are also consistent with a failure to detect interactions of LAP2β with A-type lamins in previous experiments (Foisner and Gerace, 1993).

Thus, one can conclude that the LAP2 protein family includes proteins interacting with either A or B-type lamins. In accordance with the cellular localization of the proteins, the B-type lamin-binding LAP2 isoforms are membrane proteins, whereas the isoform that binds A-type lamins is located throughout the nucleus.

Specificity of LAP2α-lamin A/C interactions

A valid concern with solid-phase overlay binding studies is the reliability and significance of the observed interactions for specific processes in vivo. To address this issue, we performed a series of in vivo studies, demonstrating a direct or indirect link between LAP2α and lamin A/C but not lamin B, and we included numerous control experiments in our in vitro binding assays.

The observed specific colocalization of lamin A/C with LAP2α at specific cell-cycle stages in two cell lines by immunofluorescence microscopy, using four different antisera and three different monoclonal antibodies to different lamins, is in our opinion strong evidence for an association of LAP2α with A-type lamins in vivo. Furthermore, the described redistribution of LAP2α together with A-type lamins into nuclear aggregates upon selective disruption of lamin A/C structures by expression of a lamin mutants in HeLa cells, also provided strong evidence for a structural and/or functional link of LAP2α to A-type lamins, and suggested that these proteins cooperate in the organization of the nucleus in interphase. Coimmunoprecipitation of LAP2α and A-type lamins from interphase cell lysates or from postmetaphase lysates during postmitotic nuclear assembly, but not from clear metaphase lysates, showed at the biochemical level that at least a subfraction of lamin A/C was associated with LAP2α structures. Interestingly, these complexes were extremely stable and resistant to denaturation and solubilization even in 0.1% SDS, suggesting that the interactions were of high affinity. This hypothesis is also consistent with our observations that as soon as lamins begin to assemble higher order structures after mitosis, they immediately become highly insoluble in Triton X-100 containing buffers and tend to sediment by their own. Due to this extremely high chemical stability of lamin complexes we had to use the rather stringent conditions in our coprecipitation studies. Thus, it is very likely that we only detect a subfraction of LAP2α-bound lamin A/C in this assay and that additional, less stable pools of cellular complexes of lamins and LAP2α may exist.

As we encountered similar problems due to the insolubility of lamin complexes in the in vitro binding studies using recombinant or purified lamin proteins (data not shown), we used solid-phase overlay techniques to detect a direct interaction of A-type lamins with LAP2α. Based on the following control experiments we consider these assays to reflect specific interactions. Firstly, in vitro translated proteins from reverse-transcribed cDNA as well as purified lamins from rat liver tissue revealed identical results in the overlay assays. Secondly, LAP2α bound to lamin A/C, but not to lamin B or to cytoplasmic vimentin, all of which contain a molecular domain organization similar to members of the intermediate filament protein family. Thus, unspecific interactions of LAP2α with coiled coil domains common to all intermediate filament proteins can be excluded. Thirdly, unspecific sticking of the proteins to larger amounts of transblotted proteins is very unlikely, as on the same blot protein fragments at similar concentrations that often differed only by the presence or absence of a small region (compare LAP2α 1-693, 1-615 and 1-414 in Fig. 6C) clearly revealed different binding affinities. Finally, in vitro translated lamin C only bound lamin fragments containing the N- and C-terminal regions of the lamin rod section, which are known to be important for intermediate filament assembly (reviewed in Fuchs and Weber, 1994; Quinlan et al., 1995).

Potential functions of LAP2α-lamin A/C interactions

Intranuclear lamin structures have been described (Broers et al., 1999; Hozak et al., 1995; Jagatheesan et al., 1999), but nuclear lamin interaction partners are not clearly defined. In vitro binding studies have shown that histones H2A and H2B bind to C-terminal regions of A- and B-type lamins (Goldberg et al., 1999a; Taniura et al., 1995), but the significance and specific role of these interactions for lamin function(s) remain to be determined.

Apart from histones, LAP2α is the only known interaction partner of intranuclear lamin A/C. At present, one can only speculate on the specific function(s) of this interactions. Considering the association of LAP2α with chromosomes at very early stages of post-mitotic nuclear reassembly (Vlcek et al., 1999) and the subsequent translocation of A-type lamins to nuclear LAP2α structures, LAP2α might target a subfraction of A-type lamins to intranuclear sites, where the proteins assemble into stable nucleoskeletal structures. Our experiments with dominant negative mutant lamins, showing that the observed disruption of endogenous lamin A structures also caused a relocalization of LAP2α, would argue for a function of lamin A in targeting LAP2α to specific intranuclear sites. However, the nuclear localization of LAP2α in cells lacking lamin A/C (our unpublished data) indicated that other interactions of LAP2α with nuclear proteins might also be important for nuclear targeting of LAP2α.

It is not completely clear whether LAP2α-lamin A/C complexes exist only transiently during nuclear assembly, or whether they exist throughout interphase. Immunofluorescence microscopy did not reveal clear intranuclear lamin structures in S-phase cells and significant colocalization with LAP2α was not detectable in these cells. If intranuclear lamin structures exist in S-phase cells, one has to assume that the antibodies might not have access to these structures due to the bulky chromatin or replication-specific changes in nuclear structure. The identification of LAP2α-lamin A/C complexes in SDS-solubilized fractions of asynchronously growing cells would argue for the existence of such complexes throughout interphase, although one cannot exclude that only a subpopulation of cells in a specific cell cycle stage contain

these complexes. The relocalization of LAP2α upon disruption of endogenous lamin A structures by transiently expressed lamin mutants in S-phase cells makes it very likely, however, that LAP2α and lamin A/C exist in the same supramolecular complex also in S-phase nuclei.

The specific functions of LAP2α and lamins in the nucleus remain unclear, but recent findings that mutations in one allele of the lamin A gene can be linked to the autosomal dominant form of Emery Dreifuss Muscular Dystrophy (EDMD; Bonne et al., 1999), to dilated cardiomyopathy (Fatkin et al., 1999), and to Dunnigan-type partial lipodystrophy (Cao and Hegele, 2000; Shackleton et al., 2000), suggest fundamental functions of lamin A in the structural organization of chromatin or in controlling gene expression (for reviews, see Gruenbaum et al., 2000; Wilson, 2000). Similarly, targeted disruption of the lamin A gene and loss of expression of lamin A/C in mice severely affected nuclear structure and led to muscular dystrophy (Sullivan et al., 1999). Thus, it is very intriguing to speculate that the functions of those proteins that interact with A-type lamins, such as LAP2α, may also be impaired in autosomal dominant EDMD and in lamin-deficient mice and may contribute to the observed phenotype.

We wish to thank Jürgen Steiner for help with the in vitro translation experiments. Furthermore, we are grateful to Crafford A. Harris and Mary Anne Talle, R. W. Johnson Pharmaceutical Res. Inst., Raritan, New Jersey, USA for providing original LAP2 cDNAs and monoclonal antibodies. We also thank Larry Gerace, The Scripps Research Institute, La Jolla, USA for providing the guinea pig antisera to lamin A/C and to lamin B, and the antiserum to LAP2β; Yves Raymond, Institute du Cancer de Montreal, Canada, for antibody 133A2; and Martin Toegel and Fritz Propst, University of Vienna, Austria, for their generous gift of affinity-purified anti-Myc antibody; Georg Krohne, University Würzburg, Germany, for providing lamin C cDNA; Dave Gilbert, University of Syracuse, Sicily, for the lamin mutant expression plasmid phLA-N33; and Bernard Precious, St Andrew's University, Scotland, for the transactivator plasmid pMR101/tTa. This study was supported by grants from the Austrian Science Research Fund (FWF) No. P13374 to R.F. and from the Wellcome Trust (No 052339) to O.A.V.

REFERENCES

Ashery-Padan, R., Weiss, A. M., Feinstein, N. and Gruenbaum, Y. (1997). Distinct regions specify the targeting of otefin to the nucleoplasmic side of the nuclear envelope. *J. Biol. Chem.* **272**, 2493-2499.

Baricheva, E. A., Berrios, M., Bogachev, S. S., Borisevich, I. V., Lapik, E. R., Sharakhov, I. V., Stuurman, N. and Fisher, P. A. (1996). DNA from Drosophila melanogaster beta-heterochromatin binds specifically to nuclear lamins in vitro and the nuclear envelope in situ. *Gene* **171**, 171-176.

Belmont, A. S., Zhai, Y. and Thilenius, A. (1993). Lamin B distribution and association with peripheral chromatin revealed by optical sectioning and electron microscopy tomography. *J. Cell Biol.* **123**, 1671-1685.

Berger, R., Theodor, L., Shoham, J., Gokkel, E., Brok-Simoni, F., Avraham, K. B., Copeland, N. G., Jenkins, N. A., Rechavi, G. and Simon, A. J. (1996). The characterization and localization of the mouse thymopoietin/lamina-associated polypeptide 2 gene and its alternatively spliced products. *Genome Res.* **6**, 361-370.

Bonne, G., Di Barletta, M. R., Varnous, S., Becane, H. M., Hammouda, E. H., Merlini, L., Muntoni, F., Greenberg, C. R., Gary, F., Urtizberea, J. A. et al. (1999). Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy [In Process Citation]. *Nat. Genet.* **21**, 285-288.

Bridger, J. M., Kill, I. R., O'Farrell, M. and Hutchison, C. J. (1993). Internal lamin structures within G_1 nuclei of human dermal fibroblasts. *J. Cell Sci.* **104**, 297-306.

Broers, J. L., Machiels, B. M., van Eys, G. J., Kuijpers, H. J., Manders, E. M., van Driel, R. and Ramaekers, F. C. (1999). Dynamics of the nuclear lamina as monitored by GFP-tagged A-type lamins. *J. Cell Sci.* **112**, 3463-3475.

Cao, H. and Hegele, R. A. (2000). Nuclear lamin A/C R482Q mutation in canadian kindreds with dunnigan-type familial partial lipodystrophy [In Process Citation]. *Hum. Mol. Genet.* **9**, 109-112.

Dechat, T., Gotzmann, J., Stockinger, A., Harris, C. A., Talle, M. A., Siekierka, J. J. and Foisner, R. (1998). Detergent-salt resistance of LAP2alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. *EMBO J.* **17**, 4887-4902.

Dechat, T., Vlcek, S. and Foisner, R. (2000). Lamina-associated polypeptide 2 isoforms and related proteins in cell cycle-dependent nuclear structure dynamics. *J. Struct. Biol.* **129**, 335-345.

Dyer, J. A., Kill, I. R., Pugh, G., Quinlan, R. A., Lane, E. B. and Hutchison, C. J. (1997). Cell cycle changes in A-type lamin associations detected in human dermal fibroblasts using monoclonal antibodies. *Chromosome Res.* **5**, 383-394.

Ellis, D. J., Jenkins, H., Whitfield, W. G. and Hutchison, C. J. (1997). GST-lamin fusion proteins act as dominant negative mutants in Xenopus egg extract and reveal the function of the lamina in DNA replication. *J. Cell Sci.* **110**, 2507-2518.

Fatkin, D., MacRae, C., Sasaki, T., Wolff, M. R., Porcu, M., Frenneaux, M., Atherton, J., Vidaillet, H. J., Jr., Spudich, S., De Girolami, U. et al. (1999). Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease [see comments]. *N. Engl. J. Med.* **341**, 1715-1724.

Foisner, R. (1997). Dynamic organisation of intermediate filaments and associated proteins during the cell cycle. *BioEssays* **19**, 297-305.

Foisner, R. and Gerace, L. (1993). Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. *Cell* **73**, 1267-1279.

Foisner, R., Leichtfried, F. E., Herrmann, H., Small, J. V., Lawson, D. and Wiche, G. (1988). Cytoskeleton-associated plectin: in situ localization, in vitro reconstitution, and binding to immobilized intermediate filament proteins. *J. Cell Biol.* **106**, 723-733.

Fricker, M., Hollinshead, M., White, N. and Vaux, D. (1997). Interphase nuclei of many mammalian cell types contain deep, dynamic, tubular membrane-bound invaginations of the nuclear envelope. *J. Cell Biol.* **136**, 531-544.

Fuchs, E. and Weber, K. (1994). Intermediate filaments: structure, dynamics, function, and disease. *Annu. Rev. Biochem.* **63**, 345-382.

Furukawa, K., Fritze, C. E. and Gerace, L. (1998). The major nuclear envelope targeting domain of LAP2 coincides with its lamin binding region but is distinct from its chromatin interaction domain. *J. Biol. Chem.* **273**, 4213-4219.

Furukawa, K. and Kondo, T. (1998). Identification of the lamina-associated-polypeptide-2-binding domain of B-type lamin. *Eur. J. Biochem.* **251**, 729-733.

Furukawa, K., Pante, N., Aebi, U. and Gerace, L. (1995). Cloning of a cDNA for lamina-associated polypeptide 2 (LAP2) and identification of regions that specify targeting to the nuclear envelope. *EMBO J.* **14**, 1626-1636.

Gant, T. M., Harris, C. A. and Wilson, K. L. (1999). Roles of LAP2 proteins in nuclear assembly and DNA replication: Truncated LAP2beta proteins alter lamina assembly, envelope formation, nuclear size, and DNA replication efficiency in *Xenopus laevis* extracts. *J. Cell Biol.* **144**, 1083-1096.

Gant, T. M. and Wilson, K. L. (1997). Nuclear assembly. *Annu. Rev. Cell Dev. Biol.* **13**, 669-695.

Gerace, L. and Foisner, R. (1994). Integral membrane proteins and dynamic organization of the nuclear envelope. *Trends Cell Biol.* **4**, 127-131.

Glass, C. A., Glass, J. R., Taniura, H., Hasel, K. W., Blevitt, J. M. and Gerace, L. (1993). The alpha-helical rod domain of human lamin A/C contains a chromatin binding site. *EMBO J.* **12**, 4413-4424.

Glass, J. R. and Gerace, L. (1990). Lamin A/C bind and assemble at the surface of mitotic chromosomes. *J. Cell Biol.* **111**, 1047-1057.

Goldberg, M., Harel, A., Brandeis, M., Rechsteiner, T., Richmond, T. J., Weiss, A. M. and Gruenbaum, Y. (1999a). The tail domain of lamin Dm0 binds histones H2A and H2B. *Proc. Natl. Acad. Sci. USA* **96**, 2852-2857.

Goldberg, M., Harel, A. and Gruenbaum, Y. (1999b). The nuclear lamina: molecular organization and interaction with chromatin. *Crit. Rev. Eukaryot. Gene Expr.* **9**, 285-293.

Goldberg, M., Lu, H., Stuurman, N., Ashery-Padan, R., Weiss, A. M., Yu, J., Bhattacharyya, D., Fisher, P. A., Gruenbaum, Y. and Wolfner, M. F. (1998). Interactions among Drosophila nuclear envelope proteins lamin, otefin, and YA. *Mol. Cell. Biol.* **18**, 4315-4323.

Goldman, A. E., Moir, R. D., Montag-Lowy, M., Stewart, M. and Goldman, R. D. (1992). Pathway of incorporation of microinjected lamin A into the nuclear envelope. *J. Cell Biol.* **119**, 725-735.

Gotzmann, J. and Foisner, R. (1999). Lamins and lamin-binding proteins in functional chromatin organization. *Crit. Rev. Eukaryot. Gene Expr.* **9**, 257-265.

Gruenbaum, Y., Wilson, K. L., Harel, A., Goldberg, M. and Cohen, M. (2000). Nuclear lamins: Structural proteins with fundamental functions. *J. Struct. Biol.* **129**, 313-323.

Harris, C. A., Andryuk, P. J., Cline, S., Chan, H. K., Natarajan, A., Siekierka, J. J. and Goldstein, G. (1994). Three distinct human thymopoietins are derived from alternatively spliced mRNAs. *Proc. Natl. Acad. Sci. USA* **91**, 6283-6287.

Hoger, T. H., Krohne, G. and Kleinschmidt, J. A. (1991). Interaction of Xenopus lamins A and LII with chromatin in vitro mediated by a sequence element in the carboxyterminal domain. *Exp. Cell Res.* **197**, 280-289.

Hozak, P., Sasseville, A. M., Raymond, Y. and Cook, P. R. (1995). Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *J. Cell Sci.* **108**, 635-644.

Jagatheesan, G., Thanumalayan, S., Muralikrishna, B., Rangaraj, N., Karande, A. A. and Parnaik, V. K. (1999). Colocalization of intranuclear lamin foci with RNA splicing factors. *J. Cell Sci.* **112**, 4651-4661.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lang, C., Paulin-Levasseur, M., Gajewski, A., Alsheimer, M., Benavente, R. and Krohne, G. (1999). Molecular characterization and developmentally regulated expression of Xenopus lamina-associated polypeptide 2 (XLAP2). *J. Cell Sci.* **112**, 749-759.

Lin, F., Blake, D. L., Callebaut, I., Skerjanc, I. S., Holmer, L., McBurney, M. W., Paulin-Levasseur, M. and Worman, H. J. (2000). MAN1, an inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin [In Process Citation]. *J. Biol. Chem.* **275**, 4840-4847.

Loewinger, L. and McKeon, F. (1988). Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO J.* **7**, 2301-2309.

Luderus, M. E., de Graaf, A., Mattia, E., den Blaauwen, J. L., Grande, M. A., de Jong, L. and van Driel, R. (1992). Binding of matrix attachment regions to lamin B1. *Cell* **70**, 949-959.

Lutz, R. J., Trujillo, M. A., Denham, K. S., Wenger, L. and Sinensky, M. (1992). Nucleoplasmic localization of prelamin A: implications for prenylation- dependent lamin A assembly into the nuclear lamina [published erratum appears in *Proc. Natl. Acad. Sci. USA* 1992 Jun 15;89(12):5699]. *Proc. Natl. Acad. Sci. USA* **89**, 3000-3004.

Manilal, S., thi Man, N., Sewry, C. A. and Morris, G. E. (1996). The Emery-Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. *Hum. Mol. Genet.* **5**, 801-808.

Marshall, I. C. B. and Wilson, K. L. (1997). Nuclear envelope assembly after mitosis. *Trends Cell Biol.* **7**, 69-74.

Martin, L., Crimaudo, C. and Gerace, L. (1995). cDNA cloning and characterization of lamina-associated polypeptide 1C (LAP1C), an integral protein of the inner nuclear membrane. *J. Biol. Chem.* **270**, 8822-8828.

Meier, J. and Georgatos, S. D. (1994). Type B lamins remain associated with the integral nuclear envelope protein p58 during mitosis: implications for nuclear reassembly. *EMBO J.* **13**, 1888-1898.

Moir, R. D., Montag-Lowy, M. and Goldman, R. D. (1994). Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. *J. Cell Biol.* **125**, 1201-1212.

Moir, R. D., Spann, T. P. and Goldman, R. D. (1995). The dynamic properties and possible functions of nuclear lamins. *Int. Rev. Cytol.* **162B**, 141-182.

Neri, L. M., Raymond, Y., Giordano, A., Capitani, S. and Martelli, A. M. (1999). Lamin A is part of the internal nucleoskeleton of human erythroleukemia cells. *J. Cell. Physiol.* **178**, 284-295.

Ottaviano, Y. and Gerace, L. (1985). Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* **260**, 624-632.

Paulin-Levasseur, M., Blake, D. L., Julien, M. and Rouleau, L. (1996). The MAN antigens are non-lamin constituents of the nuclear lamina in vertebrate cells. *Chromosoma* **104**, 367-379.

Powell, L. and Burke, B. (1990). Internuclear exchange of an inner nuclear membrane protein (p55) in heterokaryons: in vivo evidence for the interaction of p55 with the nuclear lamina. *J. Cell Biol.* **111**, 2225-2234.

Precious, B., Young, D. F., Bermingham, A., Fearns, R., Ryan, M. and Randall, R. E. (1995). Inducible expression of the P, V, and NP genes of the paramyxovirus simian virus 5 in cell lines and an examination of NP-P and NP-V interactions. *J. Virol.* **69**, 8001-8010.

Pugh, G. E., Coates, P. J., Lane, E. B., Raymond, Y. and Quinlan, R. A. (1997). Distinct nuclear assembly pathways for lamin A/C lead to their increase during quiescence in Swiss 3T3 cells. *J. Cell Sci.* **110**, 2483-2493.

Quinlan, R., Hutchison, C. and Lane, B. (1995). Intermediate filament proteins. *Protein Profile* **2**, 795-952.

Rzepecki, R., Bogachev, S. S., Kokoza, E., Stuurman, N. and Fisher, P. A. (1998). In vivo association of lamins with nucleic acids in *Drosophila melanogaster*. *J. Cell Sci.* **111**, 121-129.

Sasseville, A. M.-J. and Raymond, Y. (1995). Lamin A precursor is localized to intranuclear foci. *J. Cell Sci.* **108**, 273-285.

Shackleton, S., Lloyd, D. J., Jackson, S. N., Evans, R., Niermeijer, M. F., Singh, B. M., Schmidt, H., Brabant, G., Kumar, S., Durrington, P. N. et al. (2000). LMNA, encoding lamin A/C, is mutated in partial lipodystrophy [In Process Citation]. *Nat. Genet.* **24**, 153-156.

Spann, T. P., Moir, R. D., Goldman, A. E., Stick, R. and Goldman, R. D. (1997). Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. *J. Cell Biol.* **136**, 1201-1212.

Stuurman, N., Heins, S. and Aebi, U. (1998). Nuclear lamins: their structure, assembly, and interactions. *J. Struct. Biol.* **122**, 42-66.

Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C. L. and Burke, B. (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* **147**, 913-920.

Taniura, H., Glass, C. and Gerace, L. (1995). A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. *J. Cell Biol.* **131**, 33-44.

Vlcek, S., Just, H., Dechat, T. and Foisner, R. (1999). Functional diversity of LAP2a and LAP2β in postmitotic chromosome association is caused by an a-specific nuclear targeting region. *EMBO J.* **18**, 6370-6384.

Wilson, K. L. (2000). The nuclear envelope and disease. *Trends Cell Biol.* **10**, 125-129.

Worman, H. J., Evans, C. D. and Blobel, G. (1990). The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. *J. Cell Biol.* **111**, 1535-1542.

Worman, H. J., Yuan, J., Blobel, G. and Georgatos, S. D. (1988). A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA* **85**, 8531-8534.

Yang, L., Guan, T. and Gerace, L. (1997). Lamin-binding fragment of LAP2 inhibits increase in nuclear volume during the cell cycle and progression into S phase. *J. Cell Biol.* **139**, 1077-1087.

Ye, Q., Barton, R. M. and Worman, H. J. (1998). Nuclear lamin-binding proteins. In *Subcellular Biochemistry: Intermediate filaments*, (ed. H. Herrmann and J. R. Harris), pp. 587-610. London, UK: Plenum Publishing.