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ARTICLES

Immunolocalization of Detergent-Susceptible Nucleoplasmic Lamin A/C Foci by a Novel Monoclonal Antibody

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Abstract The A-type lamins are localized in the interior of the nucleus as well as on the nuclear periphery. In this study, we have characterized a monoclonal antibody LA-2F9 produced against recombinant rat lamin A which stains a subpopulation of various cell types in a pattern of small nucleoplasmic foci that are unusually susceptible to mild detergent/salt extraction. The specific reactivity of mAb LA-2F9 towards lamins was confirmed by immunoblotting of HeLa and C3H10T½ whole cell lysates and nuclear lysates. The epitope for LA-2F9 was narrowed down to amino acid residues 268–278 (SAKLDNARQSA). To check whether the appearance of lamin foci was cell-cycle-dependent, C3H10T½ cells were serum-starved and then refed to trigger cells to enter the G_1 phase of the cell-cycle. The intensity of staining increased 3.5-fold within 6 h of refeeding, when the maximum number of cells were labeled with LA-2F9. We also checked whether the LA-2F9 foci colocalized with nuclear proteins known to be distributed in small foci such as hnRNPs, snRNPs, SC-35, and p80 coilin, but did not find evidence of colocalization. Our studies suggest that LA-2F9 has a novel and specific reactivity towards detergent-susceptible lower order lamin structures that are likely to be assembly intermediates. J. Cell. Biochem. 91: 730–739, 2004.

Key words: nuclear lamina; nuclear matrix; lamin A; nucleoplasmic foci

The lamins are the major components of the nuclear lamina, a fibrous structure situated beneath the inner nuclear membrane which is required to maintain nuclear morphology and integrity. There are two classes of lamins, the B-type lamins which are found in nearly all somatic cells, and the A-type lamins which are expressed only in differentiated cells [reviewed by Moir et al., 1995; Gant and Wilson, 1997; Stuurman et al., 1998]. Lamins have been proposed to be essential for the organization of nuclear functions. There is significant evidence that an intact nuclear lamina is required for DNA replication [Moir et al., 1994; Ellis et al., 1997], as well as RNA polymerase II-dependent transcription [Spann et al., 2002]. In addition to the peripheral lamina, lamins A/C form intranuclear speckles in interphase cells that colocalize with RNA splicing factor compartments, and have been proposed to mediate the spatial organization of transcription and RNA splicing [Jagatheesan et al., 1999; Kumaran et al., 2002]. The presence of lamins in the interior of the nucleus has been well documented in earlier studies [Goldman et al., 1992; Bridger et al., 1993; Hozák et al., 1995; Neri et al., 1999]. Mutations in the human lamin A gene (*LMNA*) cause debilitating diseases that mainly affect muscle, cardiac, adipose and neuronal tissues, and also cause premature aging [reviewed by Hutchison, 2002; Mounkes et al., 2003].

The lamins belong to the intermediate filament family of proteins and, like other members of the family, contain a central α-helical coiled coil domain, flanked by flexible amino and carboxy terminal domains. During mitosis, phosphorylation of essential serine residues on either end of the rod domain results in depolymerization of the lamina into dimers and tetramers, and the lamina is reassembled towards late telophase and in early G_1 phase of the cell-cycle. GFP-lamin A and B have been

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observed to form a veil of fluorescence in the nucleoplasm of interphase cells in addition to the typical nuclear rim pattern, which is however not as stable as the peripheral lamina as it can be easily extracted by detergent-containing buffers [Broers et al., 1999; Moir et al., 2002]; and more sensitive nucleoplasmic structures, likely to be assembly intermediates, have been identified in early G_1 cells by FRAP analysis [Moir et al., 2002]. But it is not clear whether the veil of fluorescence observed with GFP-lamins can be identified as a distinct lamina structure by biochemical or immunochemical methods.

In this report, we describe the properties of a monoclonal antibody, mAb LA-2F9 produced against recombinant rat lamin A which stains a subpopulation of various cell types in a pattern of small nucleoplasmic foci. The susceptibility of these foci to detergent/salt extraction together with the increased intensity of staining by LA-2F9 in early G_1 cells suggests that this monoclonal antibody specifically recognizes lower order lamin structures that may be assembly intermediates.

MATERIALS AND METHODS

Lamin Antibodies

mAb LA-2F9 was isolated in a screen for mouse monoclonal antibodies raised to recombinant rat lamin A which has been described in detail previously [Jagatheesan et al., 1999]. Antibodies to recombinant rat lamins used in this study and characterized earlier are mAb LA-2B3 which stains the nuclear periphery and mAb LA-2H10 that recognizes intranuclear lamin A speckles [Jagatheesan et al., 1999]. All three mAbs are of the IgM subtype.

Cell Culture

C3H10T½ mouse fibroblasts or HeLa cells were grown on coverslips in petri dishes containing Dulbecco's minimal medium supplemented with 10% fetal bovine serum. C3H10T½ mouse fibroblasts were growth-arrested by incubation in medium containing 0.5% fetal bovine serum for 72 h.

Immunofluorescence Microscopy

Cells were washed with phosphate-buffered saline (PBS) and then fixed by treatment with 3.5% formaldehyde for 15 min followed by 0.5% (v/v) Triton X-100 for 6 min at room temperature. Cells were then incubated with 0.5% gelatin in PBS for 1 h followed by incubation with first antibody for 1 h and then FITC-conjugated second antibody for 1 h at room temperature for single labeling experiments. For double labeling experiments, fixed cells were incubated with the first primary antibody (to the nuclear marker) followed by species-specific biotinylated second antibody and avidin-Cy3, and then with LA-2F9 and FITC-conjugated anti-mouse IgM antibody. The sources of the primary antibodies to nuclear marker proteins used in dual labeling experiments were as follows. A rabbit polyclonal antibody to p80 coilin was a gift from Dr. A. Lamond (University of Dundee, Scotland) and a mouse monoclonal antibody (IgG subtype) that recognizes hnRNP A2/B1 was kindly provided by Dr. G. Dreyfuss (University of Pennsylvania, USA). Mouse monoclonal antibodies to SC-35 and U1 snRNP 70 kD (both IgG subtypes) were from Sigma Chemicals Co. (St. Louis, MO) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Samples were mounted in Vectashield (Vector Laboratories, Burlingame, CA) containing 1 μg/ml DAPI. There was no cross-reactivity of fluorescent second antibodies in control experiments in which either primary antibody was omitted. Antibody conjugates were from Jackson Immunoresearch Laboratories (West Grove, IN), Molecular Probes (Eugene, OR), or Vector Laboratories. Samples were viewed on an Olympus BX60 fluorescence microscope with a cooled camera device, and images were analyzed using ImagePro software. Confocal laser-scanning immunofluorescence microscopy (CLSM) was carried out for dual stained samples and cell-cycle studies with LA-2F9 on a Meridian Ultima scan head attached to an Olympus IMT-2 inverted microscope fitted with a 100×, 1.3 NA or 60×, 1.4 NA objective lens, with excitation at 515, 488, and 351–364 nm (Argon-ion laser). Image analysis, including crossover subtraction and 2D quantitation of LA-2F9 fluorescence for cell-cycle studies was done using DASY master program V4.19 (Meridian Instruments, Inc., Okemos, MI). Images were assembled using Adobe Photoshop 5.0.

DNA Synthesis Assay

DNA synthesis was measured by a standard assay as follows. C3H10T½ cells were plated on coverslips and incubated with BrdU at a final

concentration of 100 μM for 30 min (pulse label) or 15 μM for 2–24 h (cumulative label). For dual labeling studies, cells were rinsed with PBS, fixed with 3.5% formaldehyde for 5 min, permeabilized with 0.2% Triton X-100 for 15 min, and blocked in 10% horse serum and 0.2% Triton X-100 for 1 h. Samples were then stained with mAb LA-2F9 for 1 h followed by FITC-conjugated anti-mouse IgM antibody for 1 h. After rinsing with PBS, cells were post-fixed with 3.5% formaldehyde for 5 min, DNA was denatured with 2 M HCl containing 0.5% Triton X-100, and 0.5% Tween-20 for 30 min, followed by neutralization with 1 mg/ml sodium borohydride. The samples were incubated with anti-BrdU mouse monoclonal antibody (Sigma Chemicals Co.) for 1 h, followed by anti-mouse IgG Cy3 conjugate (Chemicon International, Temecula, CA) and mounted in Vectashield containing DAPI.

Epitope Mapping

Various deletion mutants of lamin A were constructed and expressed in *E. coli*, and the bacterially expressed lamin A fragments were tested for their reactivity against mAb LA-2F9 by immunoblot analysis as described earlier [Jagatheesan et al., 1999]. Peptides were synthesized on pins using a Multipin noncleavable peptide kit from Chiron Mimotopes (Clayton, Australia) and used to determine the mAb LA-2F9 epitope by an ELIZA assay as described by the manufacturer.

Immunoblot Analysis

Samples of whole cell lysates, purified nuclei, liver nuclear envelopes, and recombinant lamin A were separated by SDS–PAGE, and then electroblotted onto nitrocellulose membrane filters. Immunodetection was carried out using undiluted monoclonal antibody supernatants and alkaline phosphatase-conjugated secondary antibodies. Rat liver nuclear envelopes were also separated by two-dimensional IEF–SDS–PAGE by the method of O'Farrell [1975], and immunoblotted as above. Rat liver nuclear envelopes were obtained by nuclease treatment and salt extraction of purified rat liver nuclei as described [Kaufman et al., 1983]. These preparations of nuclear envelopes were enriched for nuclear lamins, and were particularly well suited for the separation of A- and B-type lamins in IEF gels.

Extraction of Nuclei

For extraction with CSK buffer, cells plated on coverslips were rinsed twice with CSK buffer (10 mM PIPES pH 6.8, 300 mM sucrose, 100 mM NaCl, 1 mM EGTA, 1 mM PMSF) and then treated with CSK buffer containing 0.5% Triton X-100 for 5 min on ice as described [Nickerson et al., 1992]. The samples were then washed with CSK buffer and PBS, fixed with formaldehyde, and stained as above or used for Western blots. For extraction with TM buffer, cells plated on coverslips were rinsed twice with TM buffer (50 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$) and then incubated for 10 min on ice in TM buffer containing 0.4% Triton X-100, 0.5 mM $CuCl_2$, and 0.2 mM PMSF as described [De Conto et al., 2000]. Cells were rinsed and incubated with DNaseI (20 U/ml) or RNase A (20 μg/ml) or in the absence of nuclease for 20 min at 37°C in TM buffer. The samples were then treated with 2 M NaCl for 5 min on ice, washed with TM buffer, fixed with formaldehyde, and stained as described above.

RESULTS

Immunoreactivity and Epitope-Mapping of mAb LA-2F9

mAb LA-2F9 was isolated in a screen for monoclonal antibodies against recombinant rat lamin A. The specific reactivity of mAb LA-2F9 towards lamins was checked by immunoblotting of HeLa and C3H10T½ whole cell lysates and nuclear lysates, as well as liver nuclear envelope preparations. As shown in Figure 1A, LA-2F9 recognized proteins migrating at the position of lamin A (70 kDa) and lamin C (62 kDa), and also stained recombinant lamin A, and there was no cross-reactivity with any other proteins in the range of 200–14,000 Da. A well-characterized antibody to lamin A, LA-2B3, showed similar reactivity. To verify the specific reactivity of LA-2F9 towards A-type lamins, immunoblotting was carried out with samples of nuclear envelopes separated by two-dimensional IEF–SDS–PAGE. By this procedure, the A-type lamins migrate as a series of spots of pI 6.9–7.1 due to differential phosphorylation [Ottaviano and Gerace, 1985] and are well-separated from the B-type lamins which migrate at pI 5.6–5.8. mAb LA-2F9 exclusively reacted with A-type lamin isoforms as shown in Figure 1B.

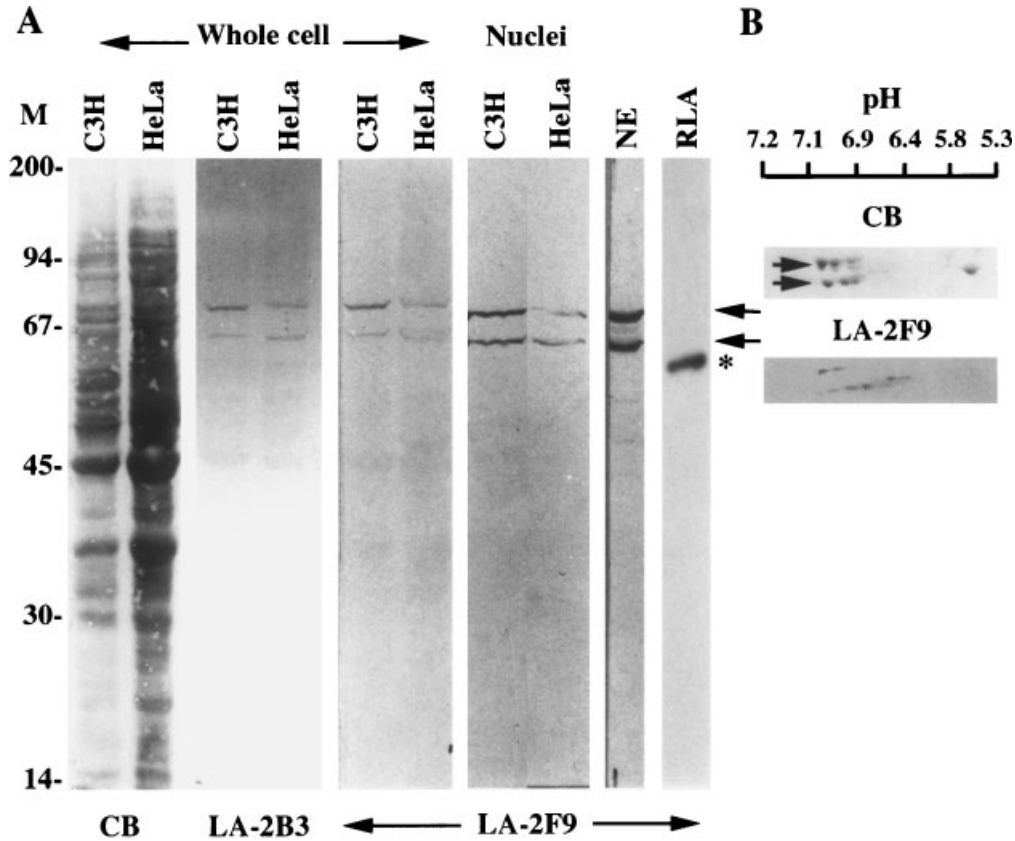

Fig. 1. Western blot analysis of cell fractions. **A**: C3H10T½ and HeLa whole cell lysates (about 1×10^6 cells per lane) and nuclear lysates, rat liver nuclear envelopes (NE) and recombinant lamin A (RLA) were resolved by 10% SDS–PAGE, transferred to nitrocellulose and blotted with mAb LA-2F9 or LA-2B3. A representative Coomassie blue-stained gel of whole cell lysates is also shown (CB). **B**: NE (from 3×10^6 nuclei) were separated by two-dimensional IEF–10% SDS–PAGE and stained with Coomassie blue (CB), or immunoblotted with mAb LA-2F9. The pH gradient of the IEF run is indicated. Molecular mass markers (M) are: myosin, 200 kD; phosphorylase b, 94 kD; albumin, 67 kD; ovalbumin, 45 kD; carbonic anhydrase, 30 kD; and lysozyme, 14 kD. The positions of endogenous lamins A and C are marked by arrows and that of RLA by an asterisk.

To determine the epitope recognized by LA-2F9, the epitope region was initially identified by immunoblotting of bacterially expressed deletion fragments of lamin A. It was narrowed down to residues 247–291 since the antibody reacted with segment 171–291 but not with 171–246 (see Table I). A series of overlapping peptides spanning this region were then synthesized and their binding to mAb LA-2F9 was determined by an ELIZA assay. The epitope for LA-2F9 was narrowed down to residues 268–278 (SAKLDNARQSA) which was the shortest peptide sequence that showed reactivity. A BLAST search indicated that this sequence was not present in any proteins other than the A-type lamins.

Immunolocalization of Lamins With Specific mAbs

Immunofluorescent staining of various cell types such as HeLa and C3H10T½ with mAb

TABLE I. Epitope Analysis of mAb LA-2F9

Sequence	Reactivity
Deletion constructs[a]	
26–610	+
26–355	+
355–572	–
171–625	+
26–171	–
171–291	+
291–355	–
171–246	–
Peptides[b]	
248–261 LRAQHEDQVEQYKK	–
258–271 QYKKELEKTYSAKL	–
260–270 KKELEKTYSAK	–
264–274 EKTYSAKLDNA	–
268–278 SAKLDNARQSA	+
266–279 TYSAKLDNARQSAE	+
268–281 SAKLDNARQSAERN	+
270–283 KLDNARQSAERNSN	–
272–285 DNARGSAERNSNLV	–
278–291 AERNSNLVGAAHEE	–

[a]Deletion fragments of lamin A protein were tested by immunoblot analysis with mAb LA-2F9.
[b]Synthetic peptides were tested by ELIZA assays with mAb LA-2F9.

LA-2F9 gave a pattern of small nuclear foci in a subpopulation of the cells without labeling the nuclear rim as shown in Figure 2. There was considerable variation in the intensity of the signal within a population of cells. When optical sectioning was carried out at 0.5 μm intervals with stained cells viewed by CLSM, foci could be seen throughout the nucleus but excluding the nucleolus (data not shown). Cells stained with mAb LA-2B3 gave the typical nuclear rim pattern for lamins and mAb LA-2H10 labeled large internal nuclear speckles as observed earlier for these antibodies [Jagatheesan et al., 1999]. Both LA-2B3 and LA-2H10 stained all cells in the population with similar intensity, unlike LA-2F9. It may be noted that the intranuclear staining by mAb LA-2F9 was revealed after 1 h of incubation with the antibody and did not require a long period of incubation. Harsher methods of fixing such as methanol at −20°C partially distorted the LA-2F9 staining pattern (data not shown). When HeLa cells were stained with LA-2F9 that had been preincubated with the epitope-containing peptide (residues 268–281) no labeling was seen, whereas preincubation of LA-2F9 with a non-epitope peptide (residues 272–285) did not affect staining. This confirmed the specificity of immunolabeling of cells by LA-2F9. A similar pattern of staining was observed with other cell types such as

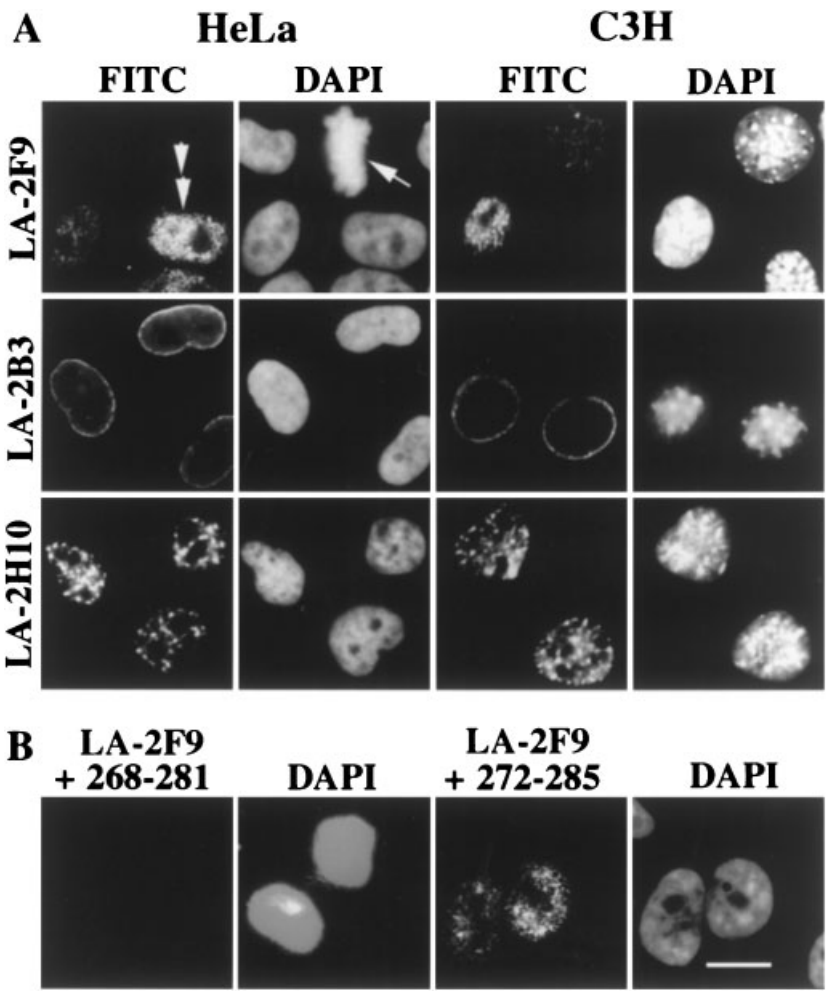

Fig. 2. Immunolocalization of lamins by fluorescence microscopy. **A**: Formaldehyde-fixed HeLa or C3H10T½ cells were stained with the indicated mAbs to lamins A/C, and counterstained with DAPI. A nucleus with strong staining by LA-2F9 is indicated by a double arrowhead. The arrow indicates a metaphase cell that is not stained by LA-2F9. **B**: HeLa cells were stained with mAb LA-2F9 that had been preincubated with pin-peptides 268–281 (epitope) or 272–285 (non-epitope) for 1 h at 4°C. Bar, 10 μm.

C2C12 mouse myoblasts and MCF-7 epithelial cells (data not shown).

Sensitivity of Nucleoplasmic Foci to Nuclear Extraction Procedures

The treatment of cultured cells with detergent and DNaseI and RNase A followed by salt extraction results in the removal of >95% of chromatin and yields an insoluble network comprised primarily of lamins and tightly bound proteins [reviewed by Nickerson, 2001]. Both internal lamin speckles (recognized by LA-2H10) and nuclear rim lamin structures are not visibly altered by this extraction procedure in most cell types [Nickerson et al., 1992; Jagatheesan et al., 1999]. Certain nuclear matrix components have been reported to be sensitive to RNase A [Nickerson, 2001], which might be due to the RNA-dependent association of the nuclear mitotic apparatus protein (NuMA) with lamins reported recently [Barboro et al., 2002]. On the other hand, the veil of fluorescence observed within nuclei of cells expressing GFP-lamin A disappears upon extraction of cells with detergent and salt [Moir et al., 2002]. To check the susceptibility of LA-2F9 foci, we extracted C3H10T½ cells with CSK buffer containing 0.5% Triton X-100 as described [Nickerson et al., 1992] followed by mild washing conditions, and stained the sample with LA-2F9. The pattern of small foci which were observed routinely in about 60% of cells disappeared in the extracted cells and less than 5% of cells were stained (Fig. 3A). Further treatment with nucleases did not cause additional changes or unmasking of epitopes (data not shown). Extraction of cells under milder conditions with detergent in TM buffer

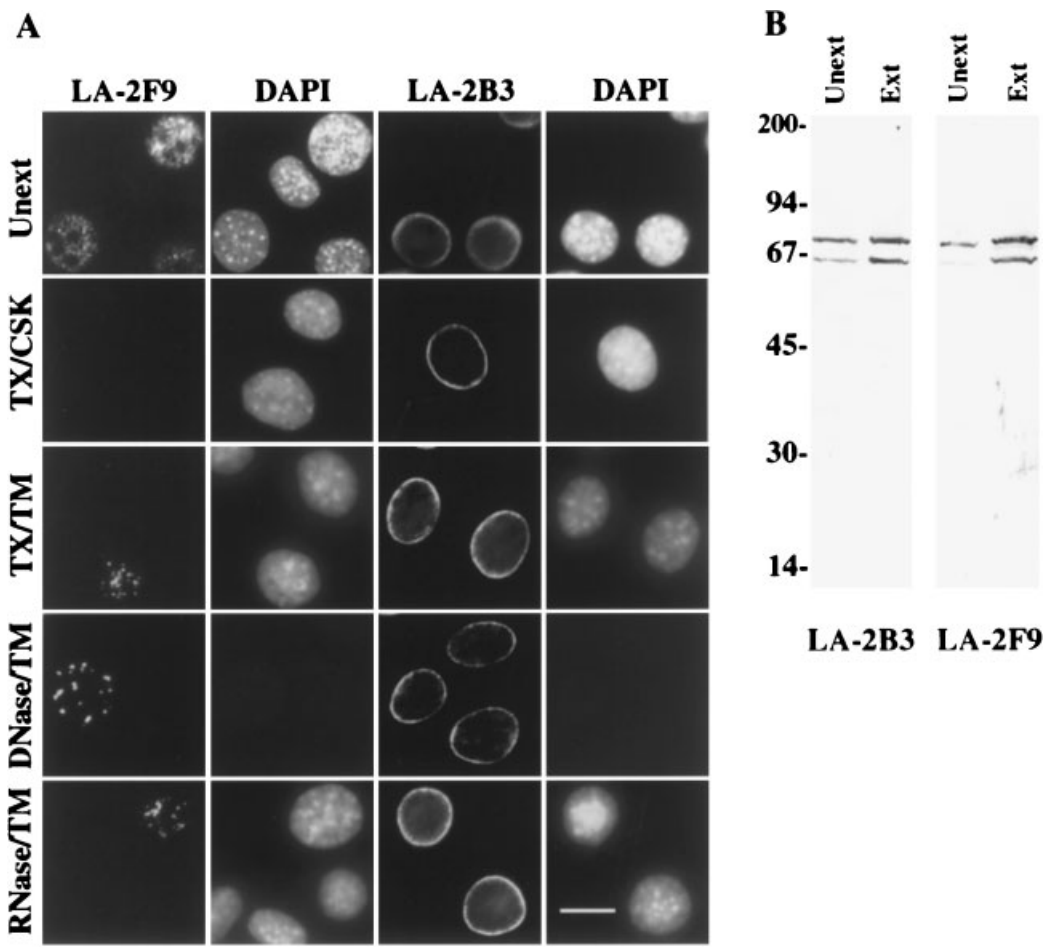

Fig. 3. Immunolocalization of lamins in extracted cells. **A**: C3H10T½ cells were extracted with detergent in CSK buffer (TX/CSK) or with detergent in TM buffer (TX/TM), or DNaseI (DNase/TM), or RNase A (RNase/TM), fixed with formaldehyde and stained with mAb LA-2F9 or LA-2B3 and DAPI. Unextracted cells (unext) are also displayed. Bar, 10 μm. **B**: Western blot analysis of extracted and unextracted cells with LA-2B3 and LA-2F9. Molecular mass markers are described in the legend to Figure 1.

followed by salt (0.1 M or 2 M NaCl or 0.25 M $(NH_4)_2SO_4$) as described by De Conto et al. [2000] depleted lamin foci in a lower percentage of cells and staining in the form of few bright fluorescent spots could be seen in about 25% of the cells, suggesting that the lamin structures recognized by LA-2F9 had aggregated in these cells (possibly due to higher concentrations) and become insoluble. A reduction in the Triton X-100 concentration from 0.4 to 0.1% in TM buffer caused a milder effect of partial aggregation of LA-2F9 foci in most cells (data not shown). Depletion of LA-2F9 foci was also observed upon extraction with TM buffer containing an RNase A inhibitor (data not shown) suggesting that the foci were not associated with RNA. Treatment of cells with DNaseI or RNase A in TM buffer after detergent extraction also resulted in aggregates in about 25% of cells and did not cause any additional changes (Fig. 3A). Formaldehyde-crosslinking prior to salt extraction did not prevent the collapse of LA-2F9 foci. The typical nuclear rim staining of lamins with LA-2B3 was preserved in cells extracted with detergent in TM buffer or CSK buffer (Fig. 3A). Similar susceptibility to extraction with detergent was observed with HeLa cells (data not shown). Thus, the lamin foci stained by LA-2F9 are unusually susceptible to detergent, salt-extraction and are likely to correspond to lower order structures rather than typically stable lamin structures. When extracted and unextracted samples were analyzed by Western blotting, no significant differences in intensity or mobility of bands were discernible between the blots stained with LA-2F9 and LA-2B3 (shown in Fig. 3B). This indicates that LA-2F9 recognizes the total A-type lamin population when the proteins are in a denatured state after SDS–PAGE and it is not possible to detect a specific LA-2F9-reactive fraction by Western blotting. We suggest that the population of lamin protein recognized by LA-2F9 in immunofluorescence assays might be a small fraction of the total A-type lamins in the cell. This is supported by the pattern of GFP-lamin A fluorescence in which the veil of internal fluorescence is substantially less than the nuclear rim fluorescence [Moir et al., 2002].

Cell-Cycle-Dependent Appearance of Lamin Foci

Since staining of lamin foci by LA-2F9 occurred only in a subpopulation of cells, we checked whether the appearance of lamin foci was cell-cycle-dependent. We were particularly interested in the G_1 phase since it was previously reported that the veil of GFP-lamin A fluorescence was maximal in early G_1 cells [Moir et al., 2002]. C3H10T½ cells were serum-starved for 72 h and then refed with serum-containing medium to trigger cells to enter the G_1 phase of the cell-cycle. At various time points after refeeding, cells were stained with LA-2F9 and a 2D quantitation of immunofluorescence was carried out. The number of stained cells decreased from 60% in a proliferating population to 23% upon quiescence. Further, positive quiescent cells had a 2-fold lower intensity of staining compared to dividing cells. Importantly, the signal increased 3.5-fold within 6 h of refeeding, when cells were in G_1 phase of the cell-cycle. At this time-point, 77% of cells were labeled with LA-2F9. Both the intensity of signal and number of cells stained by LA-2F9 decreased by 17 h and remained relatively constant thereafter. This is shown graphically in Figure 4. Cells were also labeled with BrdU to give an indication of the number of cells that were synthesizing DNA and values are given in Figure 4. In dual labeling experiments with anti-BrdU antibody and LA-2F9 it was observed that approximately 40% of cells

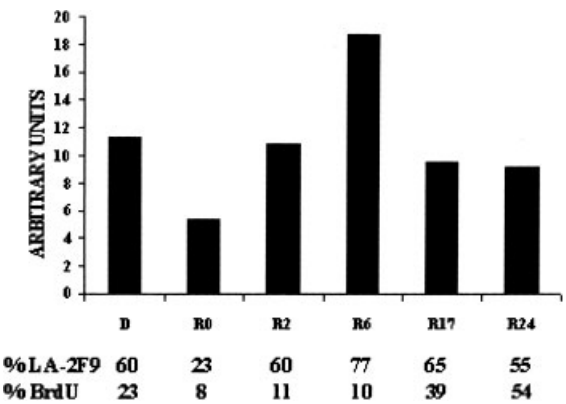

Fig. 4. Quantitative analysis of cell-cycle dependent LA-2F9 staining. C3H10T½ cells were serum-starved for 72 h and then refed with serum-containing medium for 0–24 h. Dividing cells (D), quiescent cells (R0), and cells refed for 2, 6, 17, and 24 h (R2-24) were fixed, stained with LA-2F9, and viewed by confocal microscopy. Quantitation of fluorescence intensities of LA-2F9 staining was carried out and the average intensity of staining of n = 50 cells per timepoint has been plotted in arbitrary units. The percentage of cells positive for LA-2F9 and BrdU at each timepoint is indicated (pulse label for dividing cells, cumulative label for 0–24 h refed cells). The data is representative of three separate experiments.

that had incorporated BrdU were also positive for LA-2F9 staining (Fig. 5). Hence, although lamin foci stained with LA-2F9 were maximal in early G_1 cells, they continued to be present in later stages of the cell-cycle also. However, mitotic cells were never observed to be stained by LA-2F9 (Fig. 2A), unlike the typical uniform staining of the mitotic cytoplasm by many antibodies to lamins.

We investigated the possibility that LA-2F9 lamin foci might colocalize with nuclear proteins that are distributed in specific compartments or foci. Dual labeling studies were carried out with LA-2F9 and antibodies to hnRNP A2/B1, splicing factors SC-35 and U1 snRNP 70 kD. However, there was no significant colocalization of LA-2F9 foci (less than 5%) with these proteins, as shown in Figure 5. LA-2F9 also did not colocalize with p80 coilin which is found in Cajal bodies or with DNA replication centers that had incorporated BrdU. The absence of significant colocalization with SC-35 confirms that the lamin foci detected by mAb LA-2F9 are distinct from lamin speckles that colocalize extensively with splicing factor compartments and are stained by mAb LA-2H10 [Jagatheesan et al., 1999]. (Dual staining studies with mAbs LA-2F9 and LA-2H10 were not attempted as both mAbs were of the IgM subtype.) Unlike splicing factor compartments, LA-2F9 foci were not affected by the transcriptional inhibitor α-amanitin (data not shown). Although this is not an exhaustive study, our present results suggest that lamin foci stained with mAb LA-2F9 do not colocalize with the nuclear compartments that have been analyzed and are unlikely to be involved in their functions.

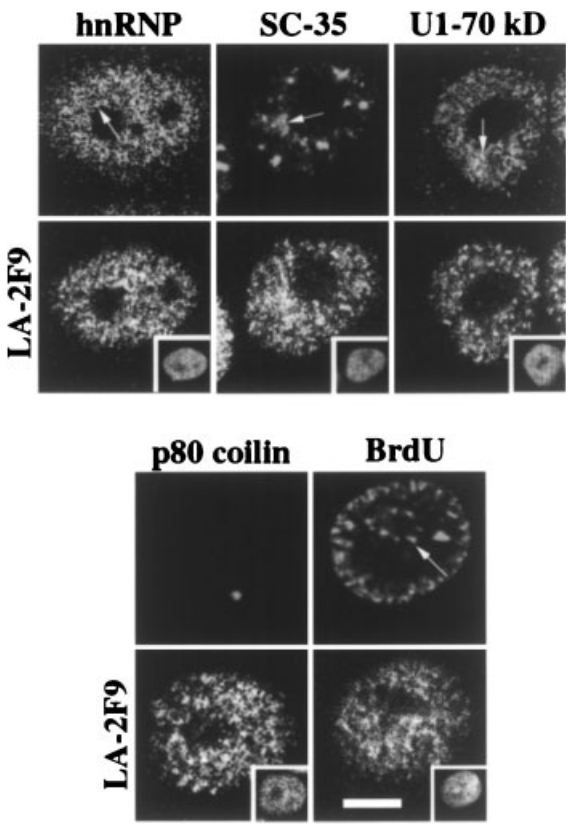

Fig. 5. Localization of lamin A/C foci and nuclear markers. Formaldehyde-fixed HeLa cells were stained with mAb LA-2F9 and antibodies to the indicated nuclear antigens, and viewed by confocal microscopy. Single sections of 0.5 μm have been displayed. Samples stained with DAPI are shown as insets in the LA-2F9 panels. Arrows indicate sporadic colocalized foci (less than 5% total LA-2F9 foci per cell). Bar, 5 μm.

DISCUSSION

The most distinctive lamin structure that is generally observed is the typical nuclear rim network underlying the inner nuclear membrane, which is stable to extraction with detergents and nucleases. However, it is becoming increasingly evident that lamins can adopt various structures in the interior of the nucleus. These may be large foci that colocalize with RNA splicing factor compartments [Jagatheesan et al., 1999] or DNA replication centers [Moir et al., 1994], or a uniformly dispersed stable nucleoplasmic network [Hozák et al., 1995; Neri et al., 1999], or small foci that are likely to contain lamin assembly intermediates, which are more conspicuous in the G_1 phase of the cell-cycle [Bridger et al., 1993], especially in GFP-lamin A-expressing cells [Broers et al., 1999; Moir et al., 2002] and cells microinjected with purified lamins [Goldman et al., 1992]. The basis for the reported variations in lamin structures is not known. But based on the observed intermediates in lamin filament assembly in vitro [Stuurman et al., 1998], it can be hypothesized that these structures arise from regulated alterations in protofilament interactions, which can in turn give rise to differential accessibility of antibody epitopes, as suggested earlier [Jagatheesan et al., 1999].

In this study, we have described the characteristics of a novel monoclonal antibody to recombinant lamin A that specifically recognizes lamins A/C and stains a subpopulation of growing cells in a pattern of small foci. These structures are more prominent in G_1 cells but do persist in other phases of the cycle, and are

highly susceptible to extraction with detergent. These properties are similar to those reported for GFP-lamin A, which forms a veil of fluorescence in interphase cells and more sensitive (mobile) structures in early G_1 cells [Moir et al., 2002], which have been proposed to be intermediates in lamina assembly. The detection of these unstable structures after G_1 phase suggests that a population of lower order lamin filaments might persist in interphase. Consistent with this, Barboro et al. [2002] have observed 3 and 4 nm lamin protofilaments as constituents of a thin fibrillar web within the nuclei of rat hepatocytes, which are nondividing cells. The absence of reactivity of mAb LA-2F9 with the nuclear rim suggests that its epitope, which has been narrowed down to amino acid residues 268–278 (SAKLDNARQSA) in this study, is inaccessible in the peripheral network. This antibody also does not label RNA splicing factor compartments or DNA replication centers. Further, our dual staining studies indicate that LA-2F9 lamin foci do not colocalize with hnRNP A2/B1 complexes and are thus unlikely to be directly associated with RNA. This is supported by the observation that depletion of these foci by detergent, salt-extraction is not prevented by an RNase A inhibitor. Surprisingly, LA-2F9 does not label mitotic cells, wherein the lamins are depolymerized to dimers and tetramers. One possibility is that its epitope might be masked by a posttranslational modification such as phosphorylation in mitotic cells.

The exclusive reactivity of LA-2F9 towards more sensitive and possibly lower order structures is consistent with the suggestion that the assembly states and functions of the lamins at the nuclear periphery and in the nucleoplasm may be different [Moir et al., 2002]. Recent advances in the field have strengthened the view that nucleoskeletal proteins play an essential role in many nuclear functions [reviewed by Shumaker et al., 2003]. Although the elements that control the different polymeric states of lamins are not known at present, their identification would be important for understanding the role of lamins in many nuclear processes.

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