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Spatial Distribution of Lamin A and B1 in the K562 Cell Nuclear Matrix Stabilized With Metal Ions

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Abstract When the nucleus is stripped of most DNA, RNA, and soluble proteins, a structure remains that has been referred to as the nuclear matrix, which acts as a framework to determine the higher order of chromatin organization. However, there is always uncertainty as to whether or not the nuclear matrix, isolated in vitro, could really represent a skeleton of the nucleus in vivo. In fact, the only nuclear framework of which the existence is universally accepted is the nuclear lamina, a continuous thin layer that underlies the inner nuclear membrane and is mainly composed of three related proteins: lamins A, B, and C. Nevertheless, a number of recent investigations performed on different cell types have suggested that nuclear lamins are also present within the nucleoplasm and could be important constituents of the nuclear matrix. In most cell types investigated, the nuclear matrix does not spontaneously resist the extraction steps, but must rather be stabilized before the application of extracting agents. In this investigation, by immunochemical and morphological analysis, we studied the effect of stabilization with different divalent cations (Zn^{2+}, Cu^{2+}, Cd^{2+}) on the distribution of lamin A and B1 in the nuclear matrix obtained from K562 human erythroleukemia cells. In intact cells, antibodies to both lamin A and B1 mainly stained the nuclear periphery, although some immunoreactivity was detected in the nuclear interior. The fluorescent lamin A pattern detected in Cu^{2+}- and Cd^{2+}-stabilized nuclei was markedly modified, whereas Zn^{2+}-incubated nuclei showed an unaltered pattern of lamin A distribution. By contrast, the distribution of lamin B1 in isolated nuclei was not modified by the stabilizing cations. When chromatin was removed by nuclease digestion and extraction with solutions of high ionic strength, a previously masked immunoreactivity for lamin A, but not for lamin B1, became evident in the internal part of the residual structures representing the nuclear matrix. Our results indicate that when metal ions are used as stabilizing agents for the recovery of the nuclear matrix, the distribution of both lamin A and lamin B1 in the final structures, corresponds to the pattern we have very recently reported using different extraction procedures. This observation strengthen the concept that intranuclear lamins may act as structural components of the nuclear matrix. J. Cell. Biochem. 75:36–45, 1999.

Key words: nuclear matrix; divalent cations; nuclear lamins; protein distribution

The original definition of nuclear matrix is a biochemical one that refers to those proteins and structures that remain after nuclei are extracted with buffers of high ionic strength, followed by digestion with nucleases to remove most of the protein and DNA [Berezney and Coffey, 1974]. The nuclear matrix is composed of three distinct domains: an outer lamina, an inner fibrogranular network, and residual nucleoli [Berezney, 1984]. Biochemically, these structures consist mostly of nonhistone pro-

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teins and RNA [Berezney et al., 1995; Nickerson et al., 1995; Martelli et al., 1996]. Evidence has demonstrated that this insoluble network could be involved in DNA replication, RNA synthesis and processing, anchoring of DNA loops through the interaction of specific sequences, regulation of gene expression, and a plethora of other functions [for a review, see Berezney, 1991]. The nuclear lamina is a 10- to 50-nm-thick fibrous protein network lining the nucleoplasmic face of the inner nuclear membrane [for a review, see Moir et al., 1995]. In most mammalian cell types, the lamina is composed of two major types of polypeptides: the acidic B-type lamins (B1 and B2) and the neutral A-type lamins (lamins A and C in mammals)[Moir et al., 1995]. Even though lamins have been localized to the nuclear periphery, they have also been found within the nucleoplasm during certain phases of the cell cycle (G1 and S) or in some pathological conditions [e.g., Moir et al., 1994; Machiels et al., 1995; Hozak et al., 1995]. In particular, Hozak et al. [1995] showed that in HeLa cells, lamin A forms part of a diffuse skeleton that ramifies throughout the nuclear interior. Internal lamin A is undetectable in whole cells or in isolated nuclei, because it is buried in dense chromatin and inaccessible to antibodies. However, lamin A becomes accessible when chromatin is removed by nuclease digestion and electroelution. We have very recently extended these observations using K562 human erythroleukemia cells, in which we demonstrated that both lamin A and B1 are constituents of the internal nuclear matrix, and lamin A, but not B1, is associated with some types of nuclear inclusions such as coiled and GATA bodies [Neri et al., 1999a].

It should be emphasized that lamins share considerable sequence homology with intermediate filament proteins [Raymond and Gagnon, 1988]. Moreover, an intermediate filament-like skeleton is seen in chromatin-depleted nuclei prepared using conditions close to the physiological [Jackson and Cook, 1988; Hozak et al., 1994]. Thus, lamins could be a fundamental component of this network. Even if a nuclear matrix has been prepared from many tissues and cell lines, its existence in vivo is still under debate. This is mainly because isolated nuclei are subjected to extensive manipulations before the final structure is obtained and there is a substantial risk of creating in vitro artifacts [Cook, 1988; Jack and Eggert, 1992; Pederson, 1998]. In particular, depending on the cell type as well as on the isolation procedure, the inner network and/or nucleolar remnants can be absent, while the outer lamina is always present [Stuurman et al., 1992a]. Therefore, in some cases, to preserve the inner fibrogranular network and the nucleolar remnants, it is necessary to "stabilize" isolated nuclei by either physical (mild heat exposure) or chemical (sodium tetrathionate, Ca^{2+}, Cu^{2+}) treatment [Evan and Hancock, 1985; Martelli et al., 1991; Stuurman et al., 1992b; Mattern et al., 1996; Lebkowski and Laemmli, 1982a,b; Lewis and Laemmli, 1982; Lewis et al., 1984]. In our series of recent studies, we have started to systematically investigate the effects of different stabilizing procedures on the spatial distribution of nuclear matrix polypeptides [Neri et al., 1995, 1997a,b].

We have very recently demonstrated the influence on isolated K562 erythroleukemic nuclei of millimolar concentrations of metal ions such as Zn^{2+}, Cu^{2+}, and Cd^{2+}. All exerted stabilizing effects on the distribution of two polypeptides (240-kDa NuMA, topoisomerase IIα) [Neri et al., 1999b], which are supposed to play an important structural role at the matrix level [Neri et al., 1997b,c]. By contrast, the spatial distribution of an RNP antigen was modified by the exposure to metal ions [Neri et al., 1999b].

This article reports our data concerning the influence of the above mentioned metal ions on the intranuclear distribution on lamin A and B1. We have found that all these divalent cations maintain a spatial distribution of the two proteins similar to the localization we have previously obtained using different stabilizing agents and extraction protocols [Neri et al., 1999a]. These data reinforce the contention that lamins A and B1 are important structural components of the nucleoskeletal network.

MATERIALS AND METHODS

Cell Culture

K562 human erythroleukemia cells were grown in RPMI-1640 medium supplemented with 10% newborn calf serum. The cells were seeded at a density of 10^5/ml and used 4 days later, when they reached a density of 10^6/ml.

Cell Cycle Analysis

To evaluate the cell cycle, 5×10^5 cells were fixed in 1 ml cold 70% ethanol at 4°C for 1 h.

The cells were centrifuged, washed in phosphate-buffered saline (PBS), resuspended in 0.4 ml PBS, and treated with 0.5 µg/ml RNase (Type I-A; Sigma Chemical Co., St. Louis, MO) for 1 h at 37°C. After the addition of 40 µg/ml of propidium iodide (Sigma) samples were analyzed by FACScan (Becton Dickinson, San Jose, CA).

Antibodies

The monoclonal antibody recognizing the A-type of nuclear lamins (133A2) belongs to the IgG3 class, while the monoclonal recognizing lamin B_1 (119D5-F1) belongs to the IgG1 class. Their specificity has been described in previously published papers [Collard et al., 1992; Machiels et al., 1995; Hozak et al., 1995].

Isolation of Nuclei and Preparation of Nuclear Matrix

Cells were washed once in PBS, and resuspended to 1.5×10^7/ml in 10 mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$, 1.0 mM PMSF, 1 µg/ml each of aprotinin and leupeptin (TM-2 buffer, temperature = 10°C). After 5 min at 0°C, Triton X-100 was added to 0.5% (w/v) and cells were sheared by one passage through a 22-gauge needle fitted to a 30-ml plastic syringe. Nuclei were sedimented at 400 g for 8 min and washed twice in TM-2 buffer. They were resuspended to 2 mg DNA/ml in 10 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 5 mM $MgCl_2$ plus protease inhibitors as above (STM-5 buffer). They were stabilized for 30 min at 0°C in STM-5 buffer containing 2 mM $ZnCl_2$, $CuSO_4$, or $CdCl_2$. In some cases nuclei were incubated in STM-5 for 30 min at 0°C. After two washes in STM-5 buffers, they were digested for 60 min at 0°C with 50 IU DNase I/mg DNA (Sigma). An equal volume of 4 M NaCl in 10 mM Tris-HCl, pH 7.4, 0.2 mM $MgCl_2$, 1 mM PMSF (LM buffer) was added, followed by 8 vol of 2 M NaCl in LM buffer. Structures were sedimented at 1,500*g* for 10 min and washed once in LM buffer.

SDS-PAGE and Western Blotting Analysis

Protein from 1×10^7 nuclei or from an equivalent number of nuclear matrices was dissolved in electrophoresis sample buffer [Laemmli, 1970], separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to 0.2-µm nitrocellulose sheets for Western blotting analysis. After saturation with 4% bovine serum albumin (BSA) and 5% normal goat serum (NGS) in PBS for 1 h at 37°C, the sheets were incubated overnight at 4°C with the primary antibodies in PBS containing 4% BSA, 5% NGS. After washing in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20 (TBST buffer), the sheets were incubated for 30 min at 25°C with peroxidase-conjugated anti-mouse IgG (Sigma) diluted 1:3,000 in TBST buffer. After washing as above, antibody binding was revealed by enhanced chemiluminescence (ECL) (Boehringer, Mannheim, Germany). Densitometric analysis was performed on the Molecular Analyst GS670 (Bio-Rad, Hercules, CA).

Immunofluorescent Staining

Cells in PBS, nuclei in STM-5, and matrices in TM-2, respectively, were plated onto 0.1% poly-L-lysine-coated glass slides and adhesion was allowed to proceed for 30 min at 37°C for cells or at room temperature for nuclei and nuclear matrices. Whole cells were fixed in freshly prepared 4% paraformaldehyde in PBS for 30 min at room temperature and then permeabilized for 10 min at room temperature in PBS containing 0.5% Triton X-100. Isolated nuclei and matrix samples were fixed without any additional treatment. Isolated nuclei were fixed with the same concentration of paraformaldeheyde as above, prepared in STM-5 buffer, while for matrix preparations, the fixation buffer was TM-2. These buffers were supplemented with 50 mM sodium cacodylate, pH 7.4. After several washes with PBS, nonspecific binding of antibodies was blocked by a 30-min incubation at 37°C with PBS, 2% BSA, 5% NGS. Slides were incubated for 3 h at 37°C with the appropriate primary antibody diluted in PBS, 2% BSA. Slides were then washed three times in PBS and reacted with fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody (Sigma), diluted 1:50 in PBS, 2% BSA, 5% NGS for 1 h at 37°C, and mounted as previously described [Neri et al., 1994].

Confocal Laser Scanning Microscope (CLSM) Analysis

Samples were imaged by a LSM410 (Zeiss, Oberkochen, Germany). This confocal system was coupled with a 25-mW multiline Argon ion laser as light source. To reveal the FITC signal, a 488-nm wavelength was selected with a band-pass filter. The laser power was tuned at 10 mW

to obtain the highest light stability, and the laser beam was attenuated to 30% of transmission with a neutral density filter to limit bleaching of FITC fluorescence. Samples were observed with a ×100 1.4 numerical aperture planapochromat objective lens. In the detection path, the emitted fluorescent light was focused on a back pinhole in front of the detector, a photomultiplier tube (PMT). To block any unwanted contribution signal, when observing FITC, a 515-nm OG longpass filter has been inserted before the PMT, as a barrier filter. The PMT was set at 864 mV. Images were acquired, frame by frame, with a scanning mode format of 512 × 512 pixels. Pixel values were recorded in the range of 0–255 (8 bits) as previously described [Neri et al., 1997b].

Image Processing Analysis

Digitalized optical sections were transferred from the CLSM to the graphics workstation Indigo Irix XS24 (Silicon Graphics, Mountain View, CA) and stored on the graphics workstation with a scanning mode format of 512 × 512 pixels and 256 grey levels. The image processing was performed using the ImageSpace software (Molecular Dynamics, Sunnyvale, CA). To reduce the unwanted background noise generated by the photomultiplier signal amplification, all the image stacks were treated with a three-dimensional filter (gaussian filtering) that was carried out on each voxel, with a mask of 3 pixel in the x, y, and z direction (3 × 3 × 3). Photographs were taken by a digital video recorder Focus ImageCorder Plus (Focus Graphics, Foster City, CA), using 100 ASA TMax black and white film (Kodak Limited, Rochester, NY).

Statistical Analysis

Data are the mean from three different experiments and are expressed as mean ± standard error. The asterisk indicates significant differences ($P < 0.001$) in a Student's paired t-test. All the other differences were found to be not significant, with $P > 0.05$.

RESULTS

Cell Cycle Analysis

Because intranuclear expression of lamin A has previously been demonstrated to be most easily detectable during the G1 phase of the cell cycle [Bridger et al., 1993], our study was performed on a population highly enriched in G1 resting cells. Therefore, K562 cells were cultured for 4 days without any medium change. As shown in Table I,cell cycle analysis showed that 87.1% of cells were in G1, 7.8% in S-phase, and 5.1% in G2/M.

Immunoblotting Analysis

By immunoblotting analysis, we determined whether the antigens studied in this investigation were retained in the nuclear matrices prepared from isolated nuclei stabilized with different metal ions. As presented in Figure 1, the antibody to lamin A stained a band with a molecular mass of approximately 68–70 kDa. In all types of nuclear matrices, obtained from nuclei stabilized at 0°C by Zn^{2+}, Cu^{2+}, or Cd^{2+} ions, all of the antigen was retained in the final structures.

Similar data were obtained with the monoclonal antibody to lamin B1, which stained a band of 66–67 kDa (Fig. 1). In fact, also in this case, all the antigen was retained in the final matrices independently of the exposure of isolated nuclei to various stabilizing ions.

The results of the densitometric analysis corroborated the visual inspections of the blots, demonstrating a complete recovery of the two antigens in the matrix structures (Table II).

CLSM Analysis of Lamin A Distribution in Intact Cells, Isolated Nuclei, and Nuclear Matrices

Intact cells showed a peripheral distribution of lamin A defining the nuclear boundary as confirmed by DAPI staining (data not shown). Details obtained by confocal microscopy demonstrated that the the peripheral lamina was not uniformly thick (Fig. 2A). In fact, grains connected by thinner segments appeared to be the constituents of the outer lamina. Nevertheless, in intact cells we were able to observe the presence of several spots, differing in size and shape, distributed in the inner nuclear do-

TABLE I. Percentage Distribution in the Various Phases of the Cell Cycle of K562 Cells Cultured for 4 Days Without Any Change of Medium*

Phase of cell cycle	%
G1	87.1 ± 6.9
S	7.8 ± 2.4
G2/M	5.1 ± 1.8

*The results are the mean from three different experiments ± SD.

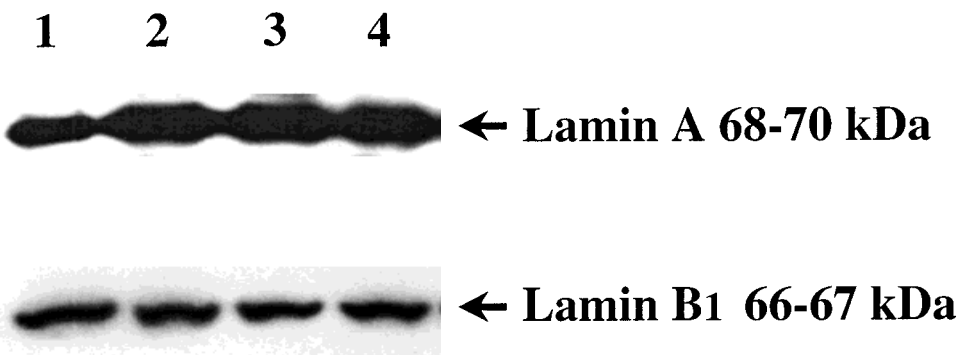

Fig. 1. Western blotting analysis for lamin A and lamin B. **Lane 1**, isolated nuclei; **lane 2**, Zn^{2+}-stabilized matrix; **lane 3**, Cu^{2+}-stabilized matrix; **lane 4**, Cd^{2+}-stabilized matrix.

TABLE II. Densitometric Analysis of Fluorograms Showing the Recovery of Lamin A and B1 in Isolated Nuclei and in the Various Types of Nuclear Matrices*

Fraction	Lamin A	Lamin B1
Nuclei	41.5 ± 3.7	35.4 ± 2.9
Zn^{2+} matrix	48.3 ± 4.2	34.8 ± 3.4
Cu^{2+} matrix	48.5 ± 4.5	34.5 ± 3.1
Cd^{2+} matrix	47.6 ± 3.8	35.2 ± 3.0

*Data are expressed in arbitrary units, as a representative of four separate experiments ± SD.

mains. These spots were sometimes connected with peripheral lamin A.

Incubation at 0°C of isolated nuclei in a buffer containing only Mg^{2+} induced the appearance of additional spots of the grains enlarged in size (Fig. 2B), clearly demonstrating their connection with the nuclear periphery. If the incubation was performed in the presence of Zn^{2+} ions, the peripheral lamina was similar to that at 0°C (cf. Fig. 2B and 2C). More fluorescent grains were detectable in the inner nucleus. In most cases a large and irregular internal spot was observed as a very brilliant structure. If the stabilization was effected at 0°C in the presence of Cu^{2+} ions, the peripheral lamina was less defined because the nuclear interior was stained by a diffuse punctate fluorescence accompanied by numerous (5–7 per nucleus) spots (Fig. 2D). This fluorescence pattern surrounded the nucleoli as detected by phase-contrast microscopy (not shown), that appeared as negative areas.

If incubation at 0°C was performed in the presence of Cd^{2+} ions, the entire nucleus was homogeneously decorated by a very fine staining, except for nucleoli (Fig. 2E).

Stabilization of nuclear matrices with Zn^{2+} resulted in a very regular fluorescent immunostaining characterized by dots at both the nuclear periphery and interior. The outer lamina was not clearly separated, and no masses were present in the interior (Fig. 2F). In nuclear matrices stabilized by Cu^{2+} ions, the fluorescent signal was dispersed over all the nuclear interior (Fig. 2G), except for nucleolar remnants. As a consequence of this distribution the peripheral staining was not easily distinguishable. Furthermore, large fluorescent masses could be seen in the nuclear interior together with smaller granules. Structures prepared from nuclei kept in the presence of Cd^{2+}, showed a completely different immunostaining, similarly to what observed in isolated nuclei (cf. Fig. 2E and 2H). Also in this case nucleolar remnants were detectable as negative areas.

CLSM Analysis of Lamin B1 Distribution in Intact Cells, Isolated Nuclei, and Nuclear Matrices

In intact cells, the peripheral lamina appeared as a circle constituting fluorescent segments of different length interrupted by small gaps (Fig. 3A). A distinctive feature of the segments was the presence of rounded enlargements. At least one thick filamentous structure was present within the nucleus. Depending on the angle of incidence of the optical section, this structure could be seen connected with the periphery or as an isolated spot in the inner nucleus. Serial sequenced stacks of confocal sections demonstrated the continuity of filaments with the nuclear periphery (not shown).

In nuclei kept at 0°C with Mg^{2+} ions only, the immunofluorescent signal at the periphery was thicker with still appreciable round fluorescent granules. The filamentous structures were very

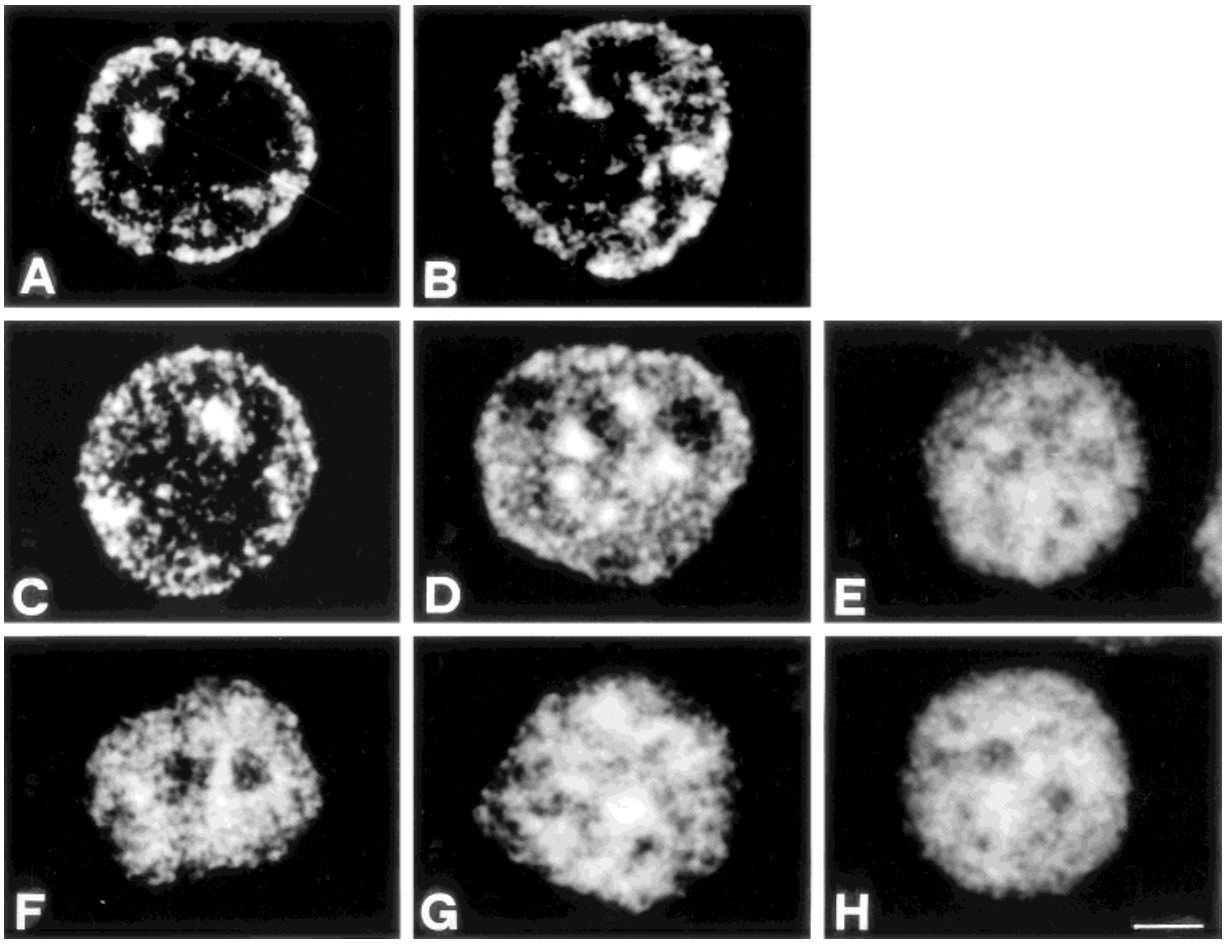

Fig. 2. Single optical section taken at the equatorial plane of intact cell **(A)**, isolated nuclei **(B–E)**, and nuclear matrices **(F–H)** immunodecorated with anti-lamin A antibody. Nuclei in STM-5 buffer were incubated at 0°C (B), at 0°C in the presence of Zn^{2+} (C), at 0°C in the presence of Cu^{2+} (D), at 0°C in the presence of Cd^{2+} (E). Nuclear matrices were stabilized by Zn^{2+} (F), Cu^{2+} (G), or Cd^{2+} (H). Scale bar = 2.5 μm.

evident and similar to that of intact cells, connected with the periphery and passing through the nuclear interior. Isolated spots in the inner nucleus were also clearly seen (Fig. 3B). This fluorescent pattern was well maintained in nuclei incubated with all the divalent cations (Fig. 3C–E).

When Zn^{2+} was employed as the stabilizing agent, the peripheral lamina appeared as a continuous ring characterized by the presence of small rounded dots. The internal filamentous structures were less apparent (Fig. 3F). In matrices stabilized by Cu^{2+} ions, the peripheral lamina was thick and connected with the center of the nucleus by large and brilliant filamentous structures (Fig. 3G). In samples stabilized by Cd^{2+}, the fluorescent pattern was quite similar, even though the peripheral staining was thicker and more continuous (Fig. 3H).

Quantitative Analysis

Table III shows the results of quantitative analysis performed on the samples analyzed, and immunostained for lamin A and B1; 300 cells, nuclei, and nuclear matrices were manually counted and the staining patterns examined in three separate experiments. A statistical test was performed, by comparing the number of structures exhibiting the typical immunofluorescent pattern identified in intact cells with the number of those showing a different pattern. The data showed that significant differences were detected in several samples after different treatments, thus demonstrating that the changes in the distribution of antigens we have illustrated in CLSM analysis section were indeed present in the great majority of isolated nuclei and matrices.

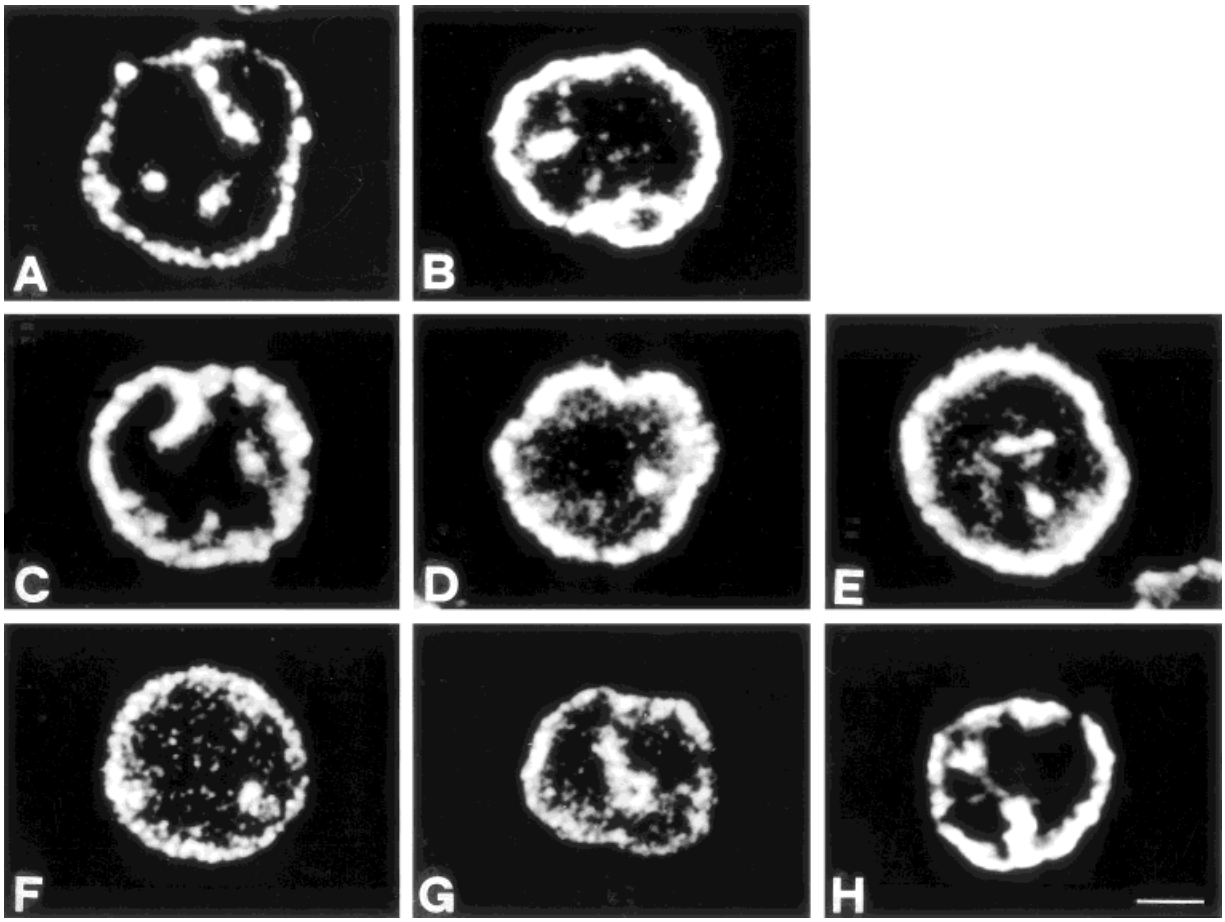

Fig. 3. Single optical section taken at the equatorial plane of intact cell **(A)**, isolated nuclei **(B–E)**, and nuclear matrices **(F–H)** immunodecorated with anti-lamin B1 antibody. Nuclei in STM-5 buffer were incubated at 0°C (B), at 0°C in the presence of Zn^{2+} (C), at 0°C in the presence of Cu^{2+} (D), at 0°C in the presence of Cd^{2+} (E). Nuclear matrices were stabilized by Zn^{2+} (F), Cu^{2+} (G), or Cd^{2+} (H). Scale bar = 2.5 μm.

DISCUSSION

A key issue in the nuclear matrix field is to determine the extent to which the stabilizing procedures commonly employed for the isolation of nucleoskeletal structures may influence the subnuclear distribution of proteins with a likely structural role [Berezney et al., 1995; Nickerson et al., 1995; Martelli et al., 1996].

As far as divalent cations are concerned, Laemmli and coworkers first suggested that metalloprotein interactions could be important to maintain the organization of mammalian cell nuclei, similarly to chromosome scaffold [Lebkowski and Laemmli, 1982a,b; Lewis and Laemmli, 1982; Lewis et al., 1984].

Following our original very recent observations on the intranuclear presence of lamins A and B1 in K562 erythroleukemia cells [Neri et al., 1999a] and on the stabilizing effects on nuclear matrix network caused by Zn^{2+}, Cu^{2+}, and Cd^{2+} ions in the same cell line [Neri et al., 1999b], we decided to analyze the behavior of both lamin A and B1 in nuclei and nuclear matrix exposed to millimolar concentrations of these divalent cations.

As indicated by immunoblotting analysis, all the stabilizing ions produced essentially the same results in that all of lamin A and B1 originally present in isolated nuclei were recovered in the final structures.

In intact cells, antibodies to lamin A mainly stained the nuclear periphery, but some immunoreactivity was also detected in the nuclear interior. This pattern was quite well conserved in preparations of isolated nuclei, kept at 0°C in the presence of either Mg^{2+} ions alone or Mg^{2+}

TABLE III. Percentage of Cells, Isolated Nuclei, and Matrices Displaying an Immunofluorescent Staining Different From the Typical Pattern Described for Intact Cells†

	Lamin A	Lamin B1
Cells	2.7 ± 1.1	3.8 ± 1.2
Nuclei 0°C	2.9 ± 1.3	5.2 ± 1.9
Nuclei 0°C + Zn^{2+}	7.5 ± 1.9	6.4 ± 1.7
Nuclei 0°C + Cu^{2+}	72.7 ± 6.6*	6.8 ± 1.9
Nuclei 0°C + Cd^{2+}	89.1 ± 9.9*	5.9 ± 1.6
Matrix + Zn^{2+}	83.3 ± 8.4*	6.7 ± 1.8
Matrix + Cu^{2+}	82.1 ± 8.5*	7.1 ± 2.0
Matrix + Cd^{2+}	91.8 ± 10.5*	7.8 ± 2.1

†See the Results section for each of the antibodies employed in this study. Data are the mean from three different experiments ± SD; 300 cells, isolated nuclei, or matrices were manually counted and examined. For each of the antibodies, the staining patterns of isolated nuclei and matrices were compared with the immunofluorescent staining displayed by intact cells, that served as a control.
*Significant differences ($P < 0.01$). All other differences were found to be not significant with $P > 0.05$.

plus Zn^{2+}. By contrast, when either Cu^{2+} or Cd^{2+} was present, a previously masked immunofluorescent staining was detected.

This peculiar behavior of lamin A after exposure to Cu^{2+} and Cd^{2+} is different from what we have observed in nuclei stabilized by heat or incubated with Cu^{2+} in a buffer containing spermine-spermidine instead of Mg^{2+} [Neri et al., 1999a]. Indeed, in those cases no unmasking of fluorescence was observed in nuclei. It should be remembered that our ultrastructural observations have previously shown that Zn^{2+}, Cu^{2+}, or Cd^{2+} caused the same modifications in isolated nuclei, i.e., a clustering of interchromatin granules and an enlargement of interchromatin spaces. However, the accessibility of the antibody to intranuclear lamin A was enhanced only by treatment with either Cu^{2+} or Cd^{2+}. The different behavior of lamin A in isolated nuclei stabilized by either Cu^{2+} or Cd^{2+}, when compared with Zn^{2+}-exposed nuclei, might be an indication of the different molecular targets of the metals. So far, however, these targets remains to be identified. The stabilizing effect of Zn^{2+} might be due to the presence of nuclear proteins with zinc finger domains such as the multifunctional transcription factor YY1 [McNeil et al., 1998; Bushmayer and Atchison, 1998], ZNF74 (an RNA-binding protein that belongs to a large subfamily containing a Kruppel-associated box repressor motif)[see Grondin et al., 1997], and AKAP 95 (a protein that interacts with the regulatory subunit of type II cAMP-dependent protein kinase)[see Coghlan et al., 1994]. As far as Cu^{2+} or Cd^{2+} ions are concerned, information about the molecular mechanisms leading to stabilization of the inner matrix are very scarce [see Chiu et al., 1993].

When chromatin was removed by extraction with high salt buffers, followed by digestion with DNase I, the masked fluorescent lamin A staining became evident also in structures stabilized by Zn^{2+}. The immunolabeling given by the antibody to lamin A was quite similar in all types of nuclear matrices and appeared reminiscent of that we have previously observed in nuclear matrix/scaffold prepared by different isolation protocols [Neri et al., 1999a].

Different results were given by the antibody to lamin B1; indeed, in intact cells, it immunodecorated the nuclear periphery and some internal thick filamentous structures. This aspect was quite well conserved in all types of nuclei as well as in nuclear matrices and no dramatic unmasking of the fluorescence was detected.

These observations appear particularly interesting and need to be emphasized because it is now well established that several nuclear matrix components change their subnuclear distribution depending on the techniques employed for preparing nucleoskeletal structures [Neri et al., 1994, 1995, 1997b,c]. However, we have demonstrated by immunofluorescent staining and CLSM analysis that some matrix constituents, such as NuMA and topoisomerase IIα, are resistant to different stabilizing treatments and also for this reason may be fundamental constituents of an intranuclear skeleton [Neri et al., 1997b, 1999b]. Interestingly, our morphological results about NuMA are in good agreement with the recent data reported by Gueth-Hallonet et al. [1998]. These investigators were able to demonstrate that transient overexpression of NuMA in HeLa cells resulted in the appearance of ordered lattices that can fill the nucleus and that are stable to detergent extraction. By contrast, overexpression of NuMA constructs truncated at residue 2005 or 2030 in the tail domain caused a drastic reorganization of nuclear components, including DNA and his-

tone H1. Also overexpression of a construct lacking the head and much of the coiled-coil region had marked consequences on nuclear organization.

As far as intranuclear lamins are concerned, their possible role in the organization of the internal nuclear matrix was investigated in sperm pronuclei assembled in normal or in lamin B3-depleted extracts. Analysis with field emission in lens scanning electron microscopy in the nuclear matrix of controls showed a dense array of interconnected filaments that could be labeled with anti-lamin B3 antibodies. By contrast, nuclear matrices prepared from lamin-depleted nuclei contained poorly organized or aggregated filaments, not labeled by the antibody. These results suggested that lamin proteins are also part of an intranuclear filament network and are important for its correct assembly [Zhang et al., 1996). Moreover, the importance of intranuclear lamins is also underlined by the fact that they are not only present in neoplastic cell lines but have also been detected in either normal human fibroblasts [Bridger et al., 1993] or *Drosophila* cells [Berrios and Fisher, 1998].

Therefore, in our opinion, the fact that intranuclear lamin A and B1 are resistant to various isolation protocols, as demonstrated by morphological investigations, reinforces the contention that they serve as structural components of the internal nucleoskeleton. Thus, we can infer from these data that Zn^{2+}, Cu^{2+}, and Cd^{2+} ions are effective stabilizing agents for intranuclear lamins and they may represent a useful tool to further investigate their functions.

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