

# The Fates of Chicken Nuclear Lamin Proteins during Mitosis: Evidence for a Reversible Redistribution of Lamin B<sub>2</sub> between Inner Nuclear Membrane and Elements of the Endoplasmic Reticulum

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**Abstract.** In chicken, three structurally distinct nuclear lamin proteins have been described. According to their migration on two-dimensional gels, these proteins have been designated as lamins A, B<sub>1</sub>, and B<sub>2</sub>. To investigate the functional relationship between chicken lamins and their mammalian counterparts, we have examined here the state of individual chicken lamin proteins during mitosis. Current models proposing functional specializations of mammalian lamin subtypes are in fact largely based on the observation that during mitosis mammalian lamin B remains associated with membrane vesicles, whereas lamins A and C become freely soluble.

Cell fractionation experiments combined with immunoblotting show that during mitosis both chicken lamins B<sub>1</sub> and B<sub>2</sub> remain associated with membranes, whereas lamin A exists in a soluble form. In situ im-

munolectron microscopy carried out on mitotic cells also reveals membrane association of lamin B<sub>2</sub>, whereas the distribution of lamin A is random. From these results we conclude that both chicken lamins B<sub>1</sub> and B<sub>2</sub> may functionally resemble mammalian lamin B.

Interestingly, immunolabeling of mitotic cells revealed an association of lamin B<sub>2</sub> with extended membrane cisternae that resembled elements of the endoplasmic reticulum. Quantitatively, we found that all large endoplasmic reticulum-like membranes present in metaphase cells were decorated with lamin B<sub>2</sub>-specific antibodies. Given that labeling of these mitotic membranes was lower than labeling of interphase nuclear envelopes, it appears likely that during mitotic disassembly and reassembly of the nuclear envelope lamin B<sub>2</sub> may reversibly distribute between the inner nuclear membrane and the endoplasmic reticulum.

THE nuclear lamina is a meshwork of intermediate filaments apposed to the nucleoplasmic surface of the inner nuclear membrane (1). It is thought to serve skeletal functions important for nuclear envelope integrity (4, 9, 27) and interphase chromatin organization (2, 15, 20). Based on cDNA sequence data, human lamins A and C have been shown to be members of the intermediate filament protein family (6, 24). Moreover, the filamentous structure of the lamina was visualized in vivo and demonstrated in vitro by reconstitution of 10-nm filaments from isolated lamins (1). Lamins have been found in a wide variety of organisms including molluscs, insects, amphibians, birds, and mammals (for review see reference 16). In vertebrates, different lamin subtypes have been characterized (10, 17, 22). In these organisms the nuclear lamina is formed either by a single lamin subtype or by a combination of two or more lamins. Differential expression of lamins during both development and tissue differentiation has been described in amphibians (3, 33), birds (23), and mammals (19, 31).

During open mitosis, when the nuclear envelope disintegrates, lamin filaments are disassembled and the lamins are distributed throughout the whole cytoplasm (10, 32). Disassembly appears to be a consequence of hyperphosphorylation of lamin polypeptides (8, 11, 25, 29). The extensive studies carried out on mammalian lamins revealed characteristic differences between lamin subtypes in the mitotic state. By cell fractionation and sedimentation analysis, it was shown that mammalian lamins A and C exist as free monomers after filament disassembly, while lamin B remains associated with membrane vesicles (8). Dissolution of membranes with nonionic detergents is required to solubilize lamin B. Finally, the membrane association of mitotic mammalian lamin B has been demonstrated directly by an elegant combination of cell fractionation and immunoelectron microscopy (4). Based on these observations, it would appear that lamin B may mark nuclear membrane fragments to be used for envelope reassembly, whereas lamins A and C may serve as focal points for the initiation of lamina reformation

on the surface of telophase chromosomes (11, 27, 28; for review see reference 7). Assuming that this model is of general significance, one would expect to find lamins associated with nuclear envelope-derived membranes during mitosis in other organisms.

In chicken, three lamins have been characterized with the aid of mAbs (22). According to their migration on two-dimensional gels, these proteins have been designated as lamins A, B<sub>1</sub>, and B<sub>2</sub>. They show differential expression during development (23). Whereas lamin B<sub>2</sub> is a quantitatively constant element of the chicken nuclear lamina (except during the pachytene stage when germ cells lack a lamina structure [34, 35]) the relative amounts of lamins A and B<sub>1</sub> vary considerably among different cell types. In most adult cell types, lamins A and B<sub>2</sub> predominate, while lamin B<sub>1</sub> represents a quantitatively minor component (23).

The relationship between mammalian and avian lamins is not clear. Classifications based on isoelectric points would place mammalian lamin A and chicken lamin A in one group, whereas mammalian lamin B and both chicken lamin B subtypes would belong to a separate group. However, by immunological criteria chicken lamin B<sub>1</sub> is related to mammalian lamin B whereas chicken lamin B<sub>2</sub> is related to chicken lamin A as well as to a quantitatively minor mammalian lamin component (22). To learn more about possible functional similarities between the lamin proteins, we have analyzed the state of chicken lamins during mitosis. Using a combination of cell fractionation and immunoblotting, we show here that both chicken lamins B<sub>1</sub> and B<sub>2</sub> remain associated with membranes during mitosis, while lamin A exists in free form in mitotic cells. Furthermore, by immunoelectron microscopic labeling of cryosections with lamin B<sub>2</sub>-specific mAbs, we have been able to observe directly the in situ morphology of lamin-binding membranes in mitotic cells. Based on the appearance of these membranes and quantitative analyses, it appears that during mitotic nuclear envelope disassembly there may exist a reversible flow of lamin B<sub>2</sub> to the endoplasmic reticulum (ER).<sup>1</sup>

## Materials and Methods

### Cell Cultures

Fibroblasts were prepared from 10–11-d chick embryos as described (21). They were cultured in Eagle's medium supplemented with 8% FCS (Flow Laboratories, Ayrshire, Scotland), 2% heat-inactivated chicken serum (Gibco Biocult, Glasgow, Scotland), 10 mM HEPES, pH 7.3, and 0.01 mM thiodiglycol. Chicken erythroblasts (line HD3 A6 ts34 AEV) (12) were a gift from H. Beug, European Molecular Biology Laboratory (EMBL), Heidelberg, FRG. They were grown in suspension in the medium specified above.

To enrich cultures for mitotic cells, nocodazole was added to a final concentration of 0.04 µg/ml for 6 h (for ultrastructural studies) or to 0.05 µg/ml for 8 h (for biochemical studies).

### Antibodies

mAbs specific for chicken lamin A (L3-4B4), lamin B<sub>1</sub> (L-5), and lamin B<sub>2</sub> (L3-5D10 or E-3) have been described previously (22, 23).

### Cell Fractionation

Chicken embryonic fibroblasts (CEFs) grown on plastic petri dishes were

1. *Abbreviations used in this paper:* CEF, chicken embryonic fibroblast; ER, endoplasmic reticulum.

treated with nocodazole. Cells from five petri dishes (13.5 cm in diameter) were mechanically detached, collected, and washed in ice-cold PBS (137 mM NaCl, 3 mM KCl, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 6 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.1). All subsequent steps were carried out on ice. Cells were resuspended in 1 ml of 50 mM Tris-HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 2.5 mM KCl, 1 mM phenylmethylsulfonyl fluoride, 1% Trasylol (Bayer, Leverkusen, FRG), 3 mM β-mercaptoethanol. After incubation for 10 min, cells were broken up in an homogenizer (Dounce/Kontes Glass Co., Vineland, NJ). Cell disruption was monitored by phase-contrast microscopy. 0.1 vol of 2.3 M sucrose in homogenization buffer was added. To one half of the homogenate Triton X-100 was added to a final concentration of 1% and samples were incubated for 10 min. Mitotic cell homogenates were centrifuged for 10 min at 15,000 g. Interphase cell homogenates were subjected to low speed centrifugation (1,500 g for 10 min). The resulting pellets were resuspended in homogenization buffer (see above) to equal the volume of the supernatants. Samples were mixed with concentrated sample buffer for SDS-PAGE (18) to give a 1× solution of sample buffer, heated for 3 min to 100°C, and stored at -20°C until used.

### Gel Electrophoresis and Immunoblotting

SDS gel electrophoresis and immunoblotting were carried out essentially as described (23). Equal volumes of supernatant and pellet fractions were loaded, equivalent to ~10% of one preparation described above. Ascites fluids of mAbs used as primary antibodies were diluted 1:2,000. Secondary antibodies were <sup>125</sup>I-iodinated sheep anti-mouse IgG (Amersham Corp., Arlington Heights, IL) diluted to 0.1 µCi/ml. Filters were autoradiographed using intensifying screens.

### Immunolectron Microscopic Techniques

Cells were fixed as pellets in 4% formaldehyde (freshly prepared from paraformaldehyde) for 10 min at room temperature, followed by fixation in 8% formaldehyde for an additional 50 min on ice. Pellets were infiltrated with 2.1 M sucrose in PBS, frozen in liquid nitrogen, and ultrathin sectioned on a Reichert Ultracut (model FC4, Reichert Jung, Vienna, Austria) at a temperature of ~-100°C, using glass knives prepared as described (14). Sections were transferred with a drop of 2.3 M sucrose in PBS onto Formvar carbon-coated and glow-discharged EM grids. The thawed frozen sections were washed two times for 15 min each in 0.5% ovalbumin (Sigma Chemical GmbH, Munich, FRG), 50 mM NH<sub>4</sub>Cl, 50 mM glycine, 50 mM lysine in PBS, and in 0.2% gelatin in PBS before incubating with antibodies. Incubation with antibodies was for 1 h each. Washes between antibody incubations were for 30 min with six changes of the washing solution. Undiluted cell culture supernatants of mAbs L3-4B4, L-5, and L3-5D10 were used as primary antibodies. As a secondary antibody, affinity-purified rabbit anti-mouse IgG at a concentration of 2 µg/ml was used (rabbit anti-mouse serum was a gift of H. Beug, EMBL). Colloidal gold-conjugated protein A (8 nm) used as a marker was a gift of Y.-D. Stierhof (Hygiene Institut, Universität Tübingen, Tübingen, FRG). All antibody dilutions and washings were done in 0.2% gelatin PBS. After a final wash in H<sub>2</sub>O for 15 min, sections were immersed in 1.8% methyl cellulose (Fluka AG, Buchs, Switzerland) containing 0.3% acidic uranyl acetate, and dried over silica gel.

Quantitation was done according to Griffiths and Hoppeler (13). Contour lengths of membranes were determined by counting intersections using a lattice grid system (13, 39). Gold particles per micrometer were calculated by the formula  $G_L = G/d \times I$ , where  $G$  is the number of gold particles over a membrane,  $d$  is the distance between lines on the lattice grid, and  $I$  is the number of intersections.

### EM Techniques

Cells were fixed in 2% glutaraldehyde (Sigma Chemical GmbH) in 125 mM cacodylate buffer, pH 7.2, for 10 min at room temperature and for further 50 min on ice. After embedding in 2% agarose (Sea Plaque Marine Colloids, Rockland, ME) cells were postfixed for 1 h in 1% OsO<sub>4</sub> in the same buffer. Dehydration, embedding in Epon, and sectioning were done according to standard procedures. All sections were viewed in an Elmiskop (model 1A, Siemens-Allis Inc., Cherry Hill, NJ) at 80 kV.

## Results

The experiments described below were carried out with

CEFs and with an established chicken erythroblast cell line (HD 3 A6 ts 34 AEV) (12). Lamins were detected with mAbs specific for individual chicken lamins A, B<sub>1</sub>, and B<sub>2</sub>.

### Cell Fractionation Experiments

To investigate the state of the lamins during mitosis by cell fractionation, CEFs were arrested in mitosis by treatment with nocodazole, and mitotic cells were collected by mechanical shake-off. Under these conditions mitotic cells represented >95% of the cells harvested. Cells were homogenized in hypotonic buffer. To one half of the homogenate Triton X-100 was added to a final concentration of 1% to dissolve lipid membranes. Particulate fractions were then separated from soluble fractions by centrifugation at 15,000 g for 10 min. The fractions were subjected to SDS-PAGE and the distribution of lamin polypeptides was analyzed by immunoblotting (see Materials and Methods). When interphase cells were fractionated, all three lamins were found in the pellet fraction after low speed centrifugation, irrespective of whether Triton X-100 was present or not (Fig. 1 *a*). This result is consistent with other data showing that lamins isolated from interphase cells exist as insoluble filaments under these extraction conditions (8).

When mitotic cells were fractionated, the bulk of lamin A was found in the soluble fraction, even if the homogenate had not been treated with Triton X-100 (Fig. 1 *b*). More than 80% of lamin A was found to be soluble even after a 45-min centrifugation at 160,000 g (not shown). In contrast, both lamins B<sub>1</sub> and B<sub>2</sub> appeared in the particulate fraction of mitotic homogenates provided that they had not been treated with detergent. Remarkably, both lamins B<sub>1</sub> and B<sub>2</sub> were sedimented quantitatively after low speed centrifugation (1,500 g for 10 min) suggesting an association with relatively large structures (not shown). That this fractionation behavior of lamins B<sub>1</sub> and B<sub>2</sub> is due to an association with membranes became apparent when membrane lipids were solubilized with Triton X-100 before fractionation. Under these conditions, lamins B<sub>1</sub> and B<sub>2</sub> were found exclusively in the soluble fraction (Fig. 1 *b*). Thus both lamins B<sub>1</sub> and B<sub>2</sub> are associated with membranes during mitosis.

### Immunoelectron Microscopic Analysis

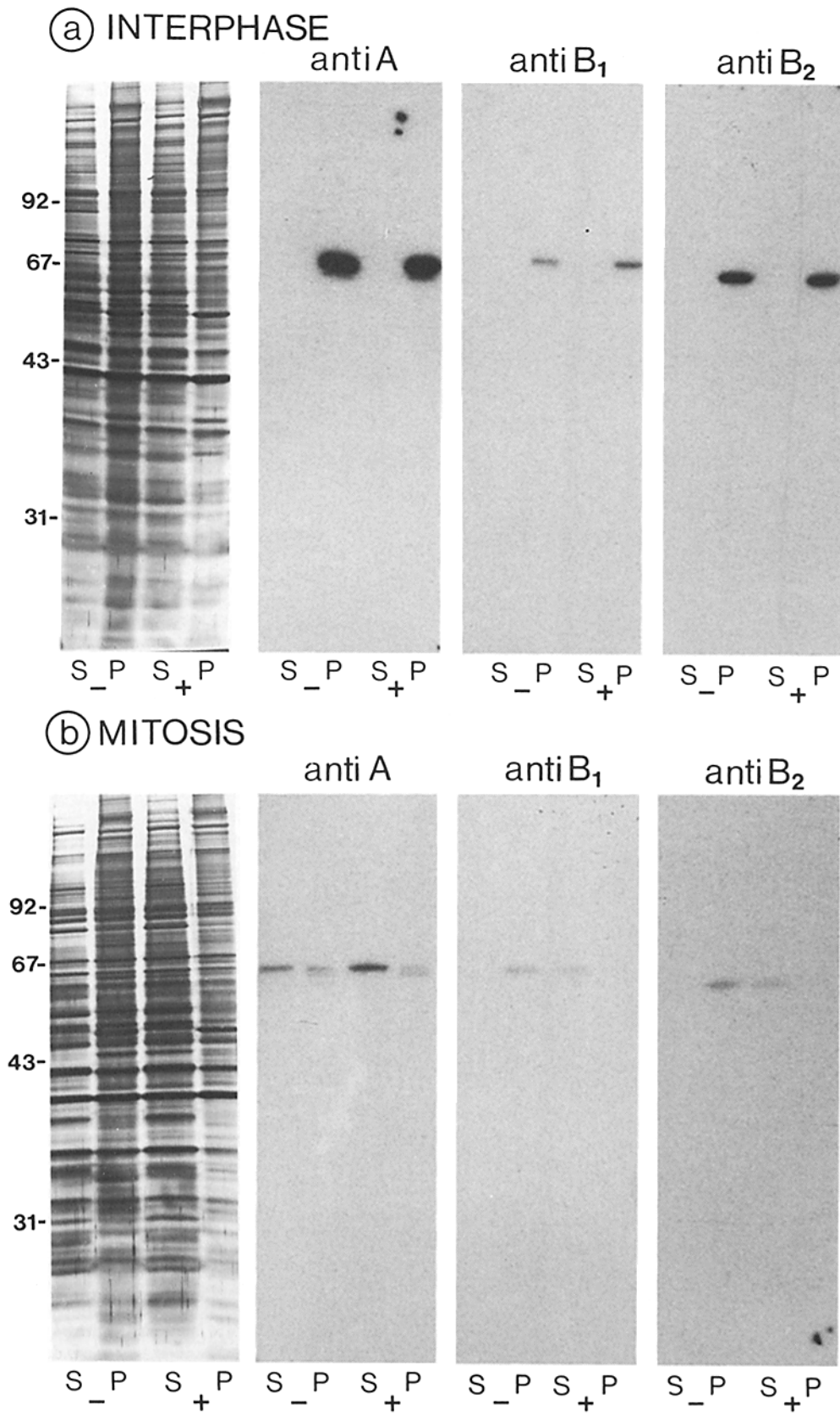
To confirm the differential mitotic distribution of individual chicken lamin proteins by an independent approach, we carried out an ultrastructural analysis. To localize lamin proteins in situ by immunoelectron microscopy, it was first necessary to find fixation conditions allowing the visualization of membrane structures while preserving antigenicity. When standard resin-embedding methods in conjunction with glutaraldehyde and OsO<sub>4</sub> fixation were used, the reactivity of the antilamin antibodies was completely abolished. Therefore we chose a preparation method avoiding glutaraldehyde and OsO<sub>4</sub> fixation. Moreover, we wished to minimize extraction of lipids by dehydrating solvents. With the antibodies used here, immunostaining of ultrathin cryosections of material fixed with high concentrations of formaldehyde (8%) gave the most satisfactory results with respect to both labeling intensity and ultrastructural preservation. Membranes show negative contrast under these conditions (Figs. 2–4). Antibodies were detected at the EM level using protein A-coated colloidal gold in conjunction with a rabbit

anti-mouse IgG as a bridging antibody. Insertion of a bridging antibody leads to an ~1.5-fold increase in the linear dimension of the antibody protein A-gold complex (i.e., 26 nm), thereby decreasing the resolution. However, this step was necessary to achieve a sufficient grain density. The relatively low density of labeling achieved by the technique applied might be explained in part by the fact that mAbs, which will recognize only a single epitope on a protein, were used. Furthermore, on sections of fixed material, epitopes that are not directly exposed to the surface of the section are not accessible to the antibodies. This became apparent when immunogold-labeled cryosections were embedded and cut perpendicular to the original cryosection plane (not shown; see also reference 36). Higher grain densities are observed when isolated nuclei or envelopes are labeled with the indirect immunogold technique (9).

Fig. 2 shows sections of CEFs in interphase (Fig. 2, *a* and *b*) and metaphase (Fig. 2, *c* and *d*) stained with antibodies specific for lamin A (Fig. 2, *a* and *c*) and lamin B<sub>2</sub> (Fig. 2, *b* and *d*), respectively. In sections of interphase cells, gold particles are confined to the nuclear envelope, as expected from the known location of the lamina (Fig. 2, *a* and *b*). No significant differences in the grain distribution are seen with antibodies directed against lamin A or lamin B<sub>2</sub>. Slightly higher nonspecific labeling over the cytoplasm and the nucleoplasm was always observed with antibody L3-4B4 directed against lamin A (Fig. 2 *a*). This was also observed when monolayer cells or sections were stained for indirect immunofluorescence with this antibody (not shown). In mitotic cells most of the lamin B<sub>2</sub>-specific label is found at or close to long membrane cisternae (Fig. 2 *d*). Taking into account the size of the antibody protein A-gold complexes used here this indicates membrane association of the lamin B<sub>2</sub> antigen. The membrane cisternae are located at the periphery of metaphase cells, where they can reach considerable dimensions. In contrast, the lamin A-specific label does not show a preferential association with membranes but is found randomly distributed throughout the cytoplasm (Fig. 2 *c*). These findings thus fully confirm our cell fractionation data.

To see whether the extended membrane cisternae decorated by lamin B<sub>2</sub>-specific antibodies are peculiar to fibroblasts, we analyzed another chicken cell type. Figs. 3 and 4 show sections of erythroblasts in different mitotic phases stained with the lamin B<sub>2</sub>-specific antibody, L3-5D10. During interphase, labeling for lamin B<sub>2</sub> is almost exclusively confined to the nuclear envelope as expected (not shown). In metaphase cells, long membrane cisternae are located predominantly in a narrow zone at the cell periphery (Fig. 3). Some of these membranes extend over as much as one fifth of the cell perimeter (Fig. 3 *a*). Rather unexpectedly, all of these long membrane cisternae, which resemble those of the ER, appear to be decorated by lamin B<sub>2</sub>-specific antibody-gold complexes (Fig. 3, *a* and *b*). Although the labeling appears less dense compared to the labeling of interphase nuclear membranes and shows some variation in that it leaves short areas of the membranes unstained, we could detect label associated with every extended membrane system. Analyzing many areas similar to those shown in Fig. 3 we found not a single exception to this observation.

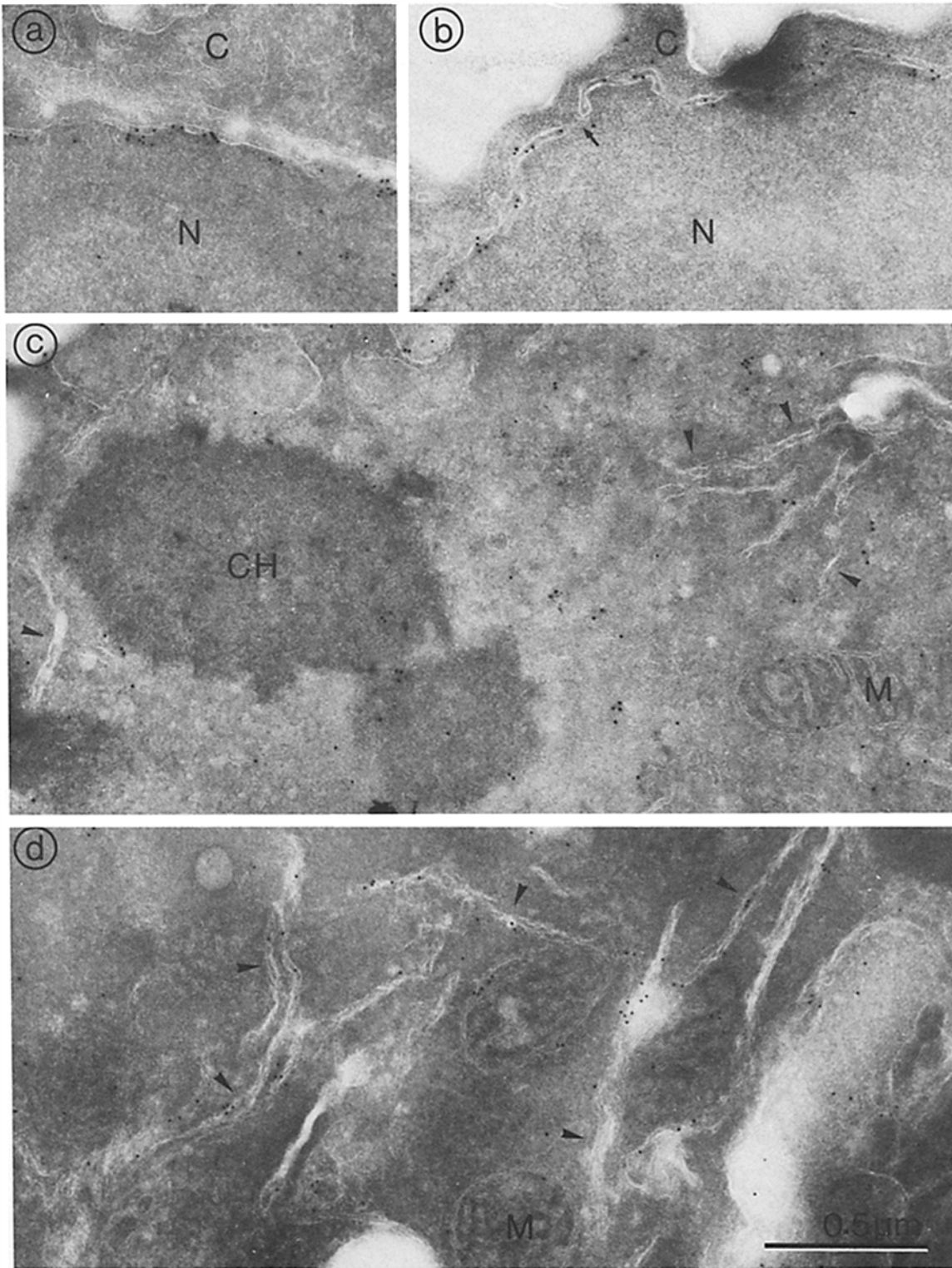
To quantitate these observations we have determined the number of gold particles per unit length for nuclear envelope and ER membranes using a method described by Griffiths



**Figure 1.** Analysis of lamin protein distribution after cell fractionation of CEFs in interphase and mitosis. Cells in interphase (a) or mitosis (b) were fractionated into soluble fractions (S) and particulate fractions (P) in the presence (+) or absence (-) of Triton X-100. Proteins were separated by SDS-PAGE (10% wt/vol polyacrylamide) and either stained with silver or transferred to nitrocellulose. Filters were probed with mAbs L3-4B4 (*anti-A*), L-5 (*anti B<sub>1</sub>*), or E-3 (*anti B<sub>2</sub>*). Molecular masses of marker proteins are as follows: phosphorylase B, 92 kD; BSA, 67 kD; ovalbumin, 43 kD; carbonic anhydrase, 31 kD.

and Hoppeler (13). We find significantly lower numbers of gold particles per unit length for ER membranes in metaphase cells ( $3.9 \pm 1.2$  particles/ $\mu\text{m}$ ) than for nuclear envelope membranes in interphase ( $8.6 \pm 2.9$  particles/ $\mu\text{m}$ ). The

difference is significant at the 0.2% level by *t* test. Assuming equal efficiency of labeling of both membrane types, this would suggest that lamin B<sub>2</sub> becomes distributed over the whole ER membrane system during metaphase. Negligible

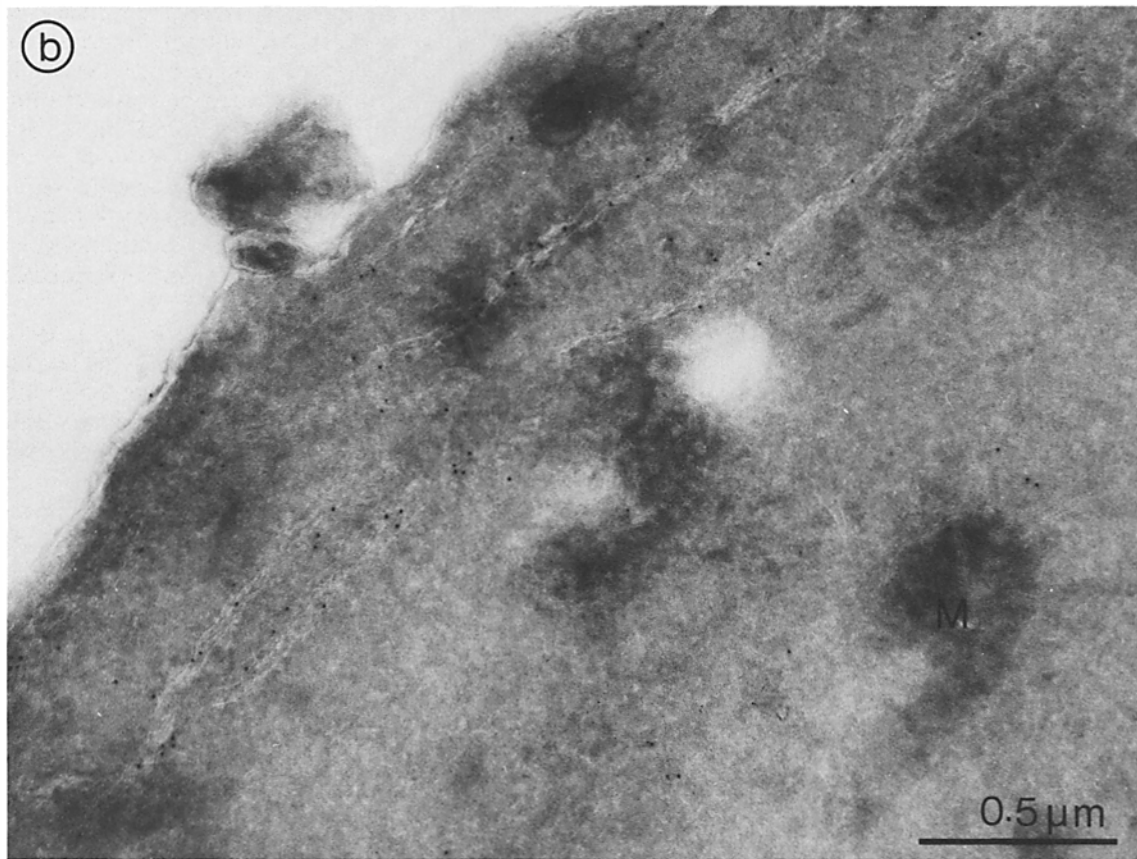
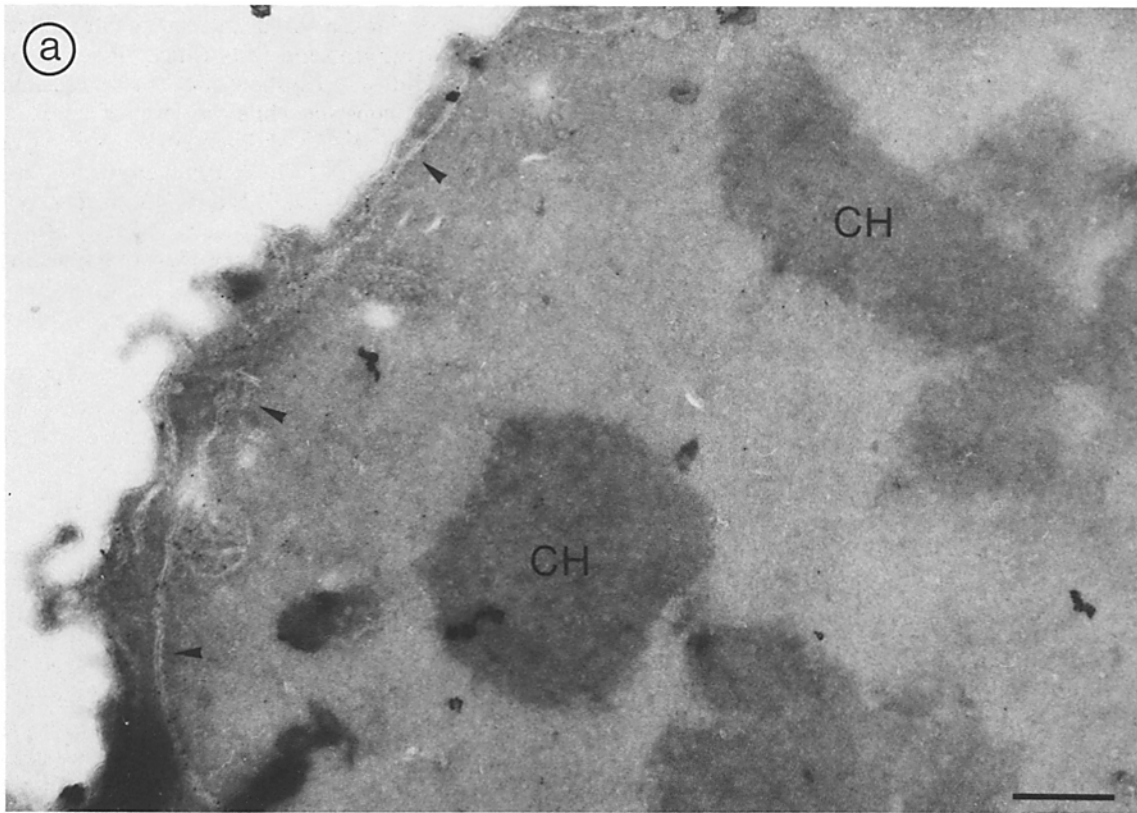


**Figure 2.** Immunoelectron microscopic localization of lamin A and lamin B<sub>2</sub> in CEFs. Ultrathin cryosections of CEFs in interphase (*a* and *b*) and metaphase (*c* and *d*) were stained with mAbs L3-4B4 (anti A) (*a* and *c*) or L3-5D10 (anti B<sub>2</sub>) (*b* and *d*). The arrow in *b* denotes a nuclear pore; arrowheads in *c* and *d* denote some of the nuclear envelope-derived membrane cisternae. C, cytoplasm; N, nucleoplasm; CH, chromosomes; M, mitochondria.

numbers of gold particles were found over the ER and the plasma membrane in interphase cells.

Fig. 4 *a* shows an overview of a cell in telophase at a stage at which the two daughter nuclei have already formed but

still reside in a common cytoplasm. Compared to metaphase cells, membrane cisternae are distributed more evenly throughout the cytoplasm; in particular, they are often seen in the vicinity of the newly formed nuclear envelopes and



**Figure 3.** Immunoelectron microscopic localization of lamin B<sub>2</sub> in chicken erythroblasts in metaphase. Ultrathin cryosections of chicken erythroblasts in metaphase were stained with mAb L3-5D10 (anti B<sub>2</sub>). *b* represents a larger magnification of a field similar to the one shown in *a*. Arrowheads in *a* point to long membrane cisternae. *CH*, chromosomes; *M*, mitochondrion.

tend to form stacks in some areas (Fig. 4 b, arrowheads). Lamin B<sub>2</sub>-specific label is found on the newly formed nuclear envelopes as well as on several membranes of the ER. However, in contrast to the situation in metaphase, a considerable number of very scarcely labeled membranes can be detected at these later stages of mitosis (Fig. 4), indicating that cytoplasmic membranes might be depleted of lamin B<sub>2</sub> during nuclear envelope reformation.

The above immunolabeling experiments were carried out on cells that had been treated with nocodazole to enrich for mitotic cells. To rule out the possibility that the peculiar morphology of the envelope-derived membranes in mitotic cells were artificially induced by drug treatment, we compared the ultrastructure of treated and untreated cells. We used cryosectioning as well as conventional fixation and embedding techniques. In either case, no significant differences could be detected between nocodazole-treated and untreated cells. Fig. 5 shows ultrathin epon sections of untreated (Fig. 5 a) and nocodazole-treated (Fig. 5 b) chicken erythroblasts in metaphase. The extended membrane cisternae found to be characteristic for mitotic cells are clearly visible. Furthermore this type of fixation reveals that the membrane cisternae are partly decorated by ribosomes. Cells in interphase lack the described membrane cisternae (not shown).

## Discussion

Lamins form a family of related proteins. In different vertebrates, a number of lamin subtypes have been characterized (16). In chicken, three lamins have been described with the aid of subtype-specific mAbs. They were named lamins A, B<sub>1</sub>, and B<sub>2</sub> (22). The relationship between lamin subtypes isolated from different species is not clear. According to their isoelectric points, chicken and mammalian lamins A would form a subgroup whereas chicken lamins B<sub>1</sub> and B<sub>2</sub> would be grouped together with mammalian lamin B. By immunological criteria, however, lamin B<sub>2</sub> seems to be more closely related to chicken and mammalian lamins A and to a quantitatively minor, newly discovered mammalian lamin protein, than to mammalian lamin B. On the other hand, chicken lamin B<sub>1</sub> is related to mammalian lamin B (22).

An alternative scheme of classification, which we used here, aims at elucidating the functional role of the different lamin subtypes. As has emerged from the study of mammalian lamins, differences in the subcellular distribution of individual lamin subtypes during mitosis may reflect different arrangements of these polypeptides in the interphase lamina and, presumably, different roles in envelope disassembly and reassembly during mitosis (4, 7, 9, 27). The cell fractionation experiments described here demonstrate that during mitosis chicken lamin A becomes soluble, as previously shown for mammalian lamin A and C (8) and for *Xenopus* lamin L<sub>III</sub> (3, 32, 33). Interestingly, both chicken lamins B<sub>1</sub> and B<sub>2</sub> resemble mammalian lamin B in that they remain associated with membranes throughout cell division. For lamins A and B<sub>2</sub>, these results have been confirmed independently by in situ immunoelectron microscopy. Clearly, in the light of the results presented here, it would be of interest to examine the mitotic distribution of the mammalian homologue of lamin B<sub>2</sub>.

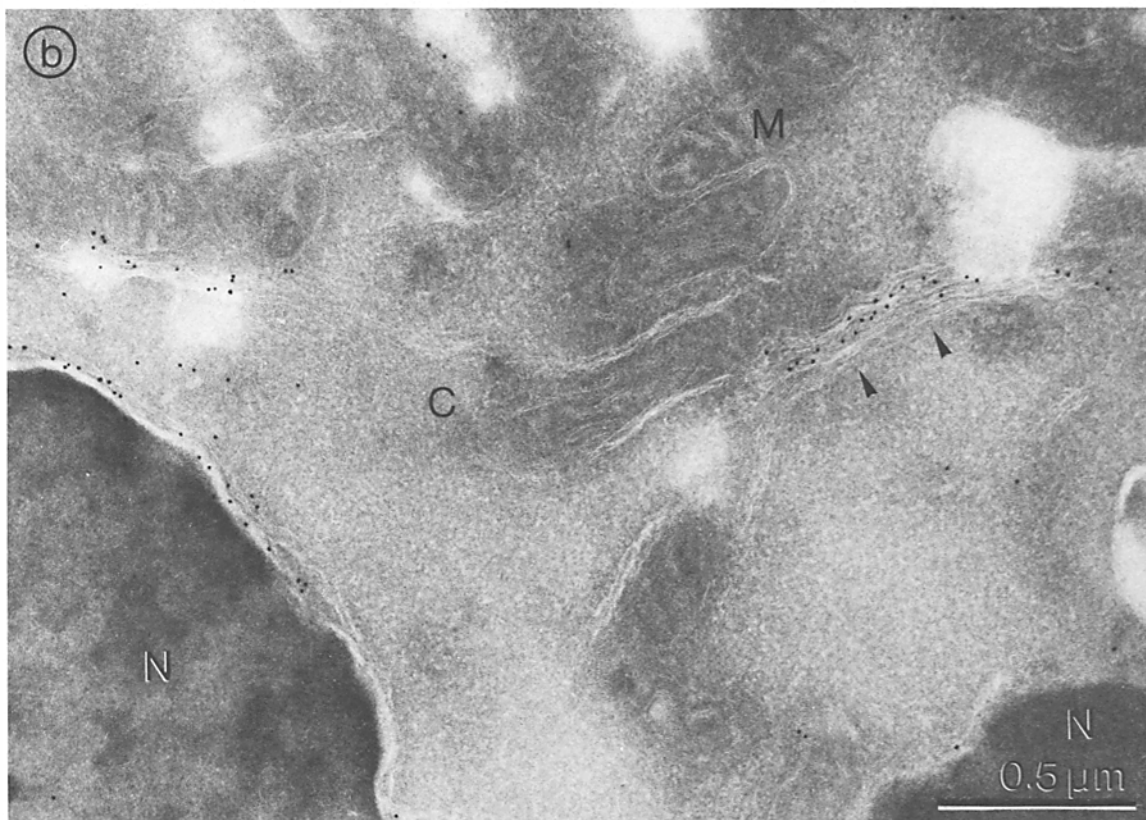
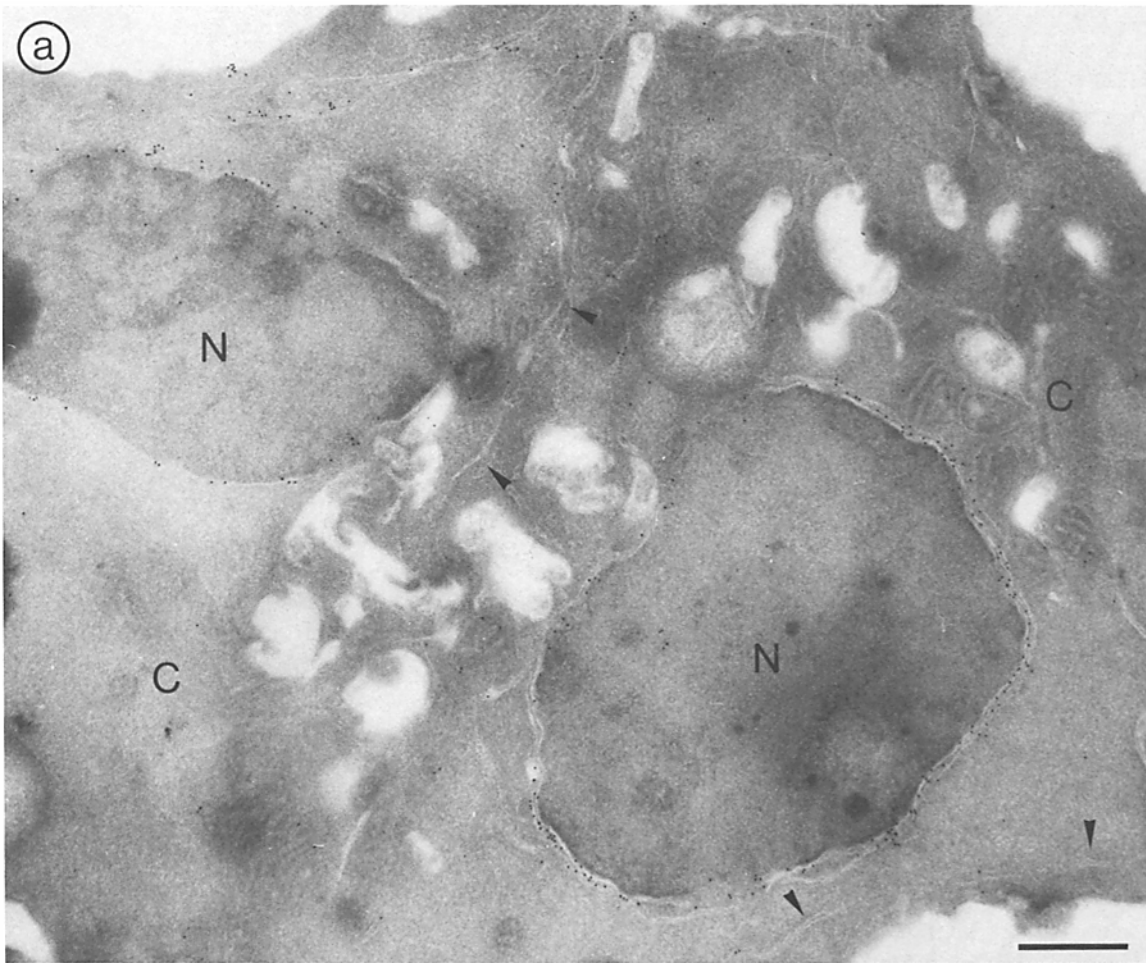
Interestingly, studies on the lamin composition during

early development of chicken (23) and during in vitro differentiation of avian hematopoietic cells (Stick, R., and H. Beug, unpublished observations) show that the level of lamin B<sub>2</sub> remains relatively constant while the level of lamin A varies depending on the state of differentiation. A constant expression of mammalian lamin B concomitant with a variable expression of lamins A and C has also been observed in differentiating embryonal carcinoma cells and early mouse embryos (19, 31). These findings suggest that B-type lamins (as defined by functional criteria [4, 9; this study]) may represent constitutive elements of the lamina structure and may play fundamental roles in the nuclear skeleton. In the light of this hypothesis, however, it remains puzzling that in oocytes and cleavage nuclei in *Xenopus* only one lamin (L<sub>III</sub>) is present (3, 17, 33). L<sub>III</sub> is not membrane associated in eggs nor during mitotic phases in cleavage embryos (3, 32, 33). Sequence analysis suggests that L<sub>III</sub> does not belong to the type A/C lamins (Stick, R., unpublished observations).

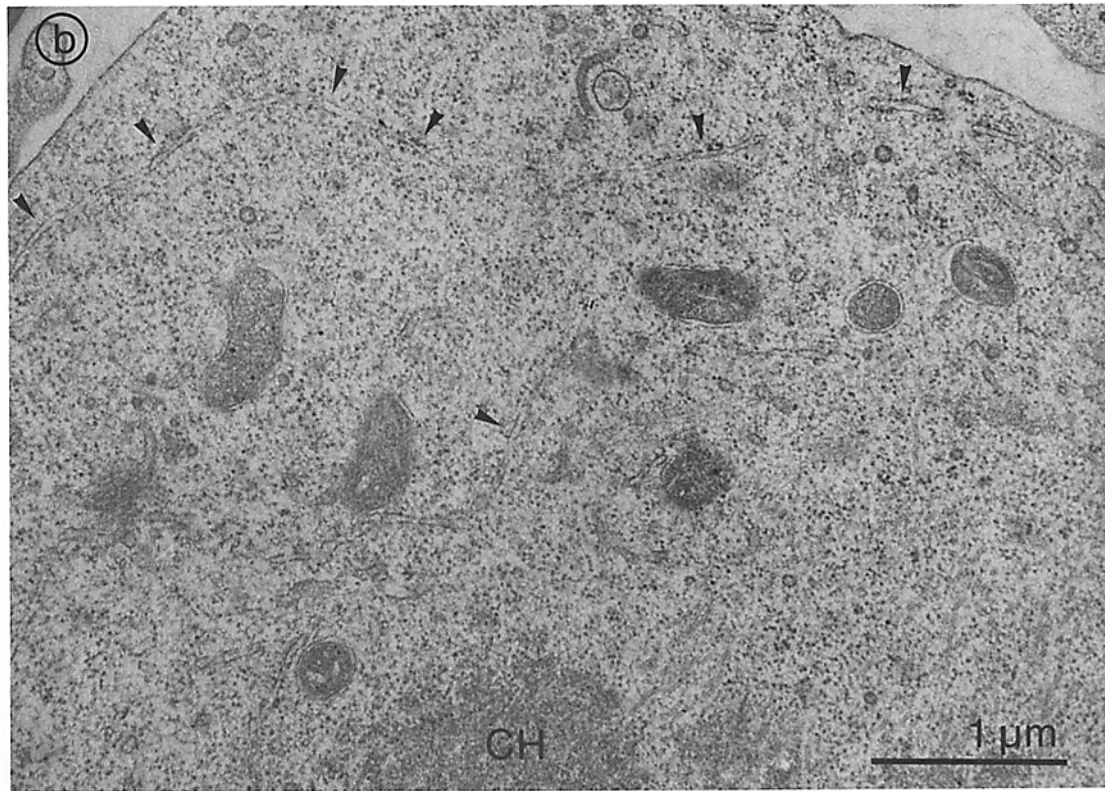
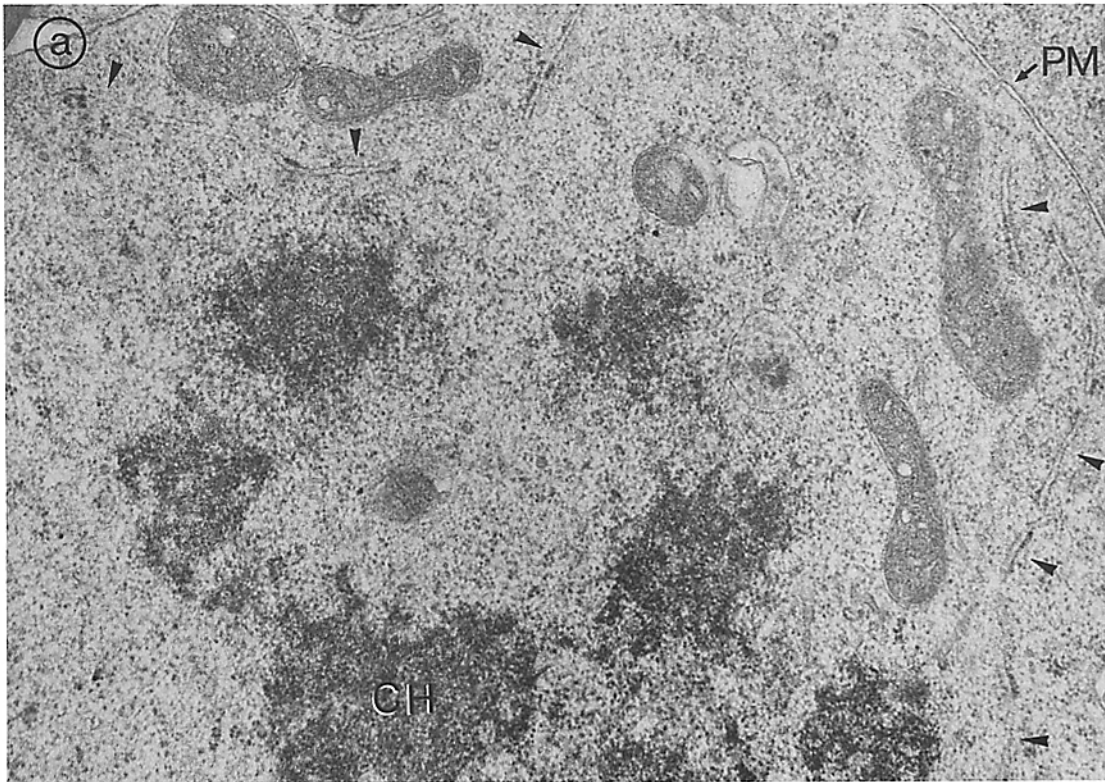
By in situ localization of lamin B<sub>2</sub> at the EM level, we were able to identify the morphology of lamin-binding membranes in mitotic cells. Several authors have described the ultrastructure of mitotic cells (5, 26, 30, 40). All of these reports describe an increased number of membrane cisternae after nuclear envelope fragmentation, suggesting their nuclear origin. Cell type-specific variations in the appearance of these membrane structures have been observed. Fragmentation of the nuclear envelope into small membrane vesicles has been described for rat thyroid epithelial cells (40). In rat thymic lymphocytes, the nuclear envelope surrounds the metaphase chromosomes completely although fenestrated at a few sites, a situation closely resembling mitotic events in amoeba (26). The cell types analyzed here show an intermediate degree of envelope fragmentation. Long membrane cisternae decorated by lamin B<sub>2</sub> antibodies are found at the periphery of metaphase cells. Some of them extend over as much as one fifth of the cell perimeter. Consistent with these cytological findings is the observation that after cell fractionation lamin B-containing mitotic membranes can be pelleted even by low speed sedimentation.

During telophase, the nuclear envelope as well as the cytoplasmic membranes are decorated by lamin B<sub>2</sub> antibodies. This is in agreement with immunofluorescence observations, which showed that a fraction of the lamin polypeptides persist in the cytoplasm for considerable lengths of time after cytokinesis.

The observation that in metaphase cells all ER-like membranes are decorated with lamin B<sub>2</sub>-specific antibodies attracts special attention. It has long been established that a continuity exists in interphase cells between the outer nuclear membrane and the ER; moreover, evidence is accumulating to suggest that diffusion of proteins may also occur to and from the inner nuclear membrane (37). Thus, one possible interpretation of our results would be that during nuclear envelope disassembly lamin B<sub>2</sub> flows from the inner nuclear membrane to the ER. Such a dynamic model is supported by our quantitative analyses of immunolabeling by anti-lamin B<sub>2</sub> antibodies. Firstly, lamin B<sub>2</sub> was found to be distributed over essentially the whole ER system visible in metaphase cells. Secondly, labeling of ER membranes was significantly lower than labeling of nuclear envelope membranes in interphase, as would be expected if, at early stages of mitosis, lamin B<sub>2</sub> became distributed over a larger mem-







**Figure 5.** Comparison of the ultrastructure of nocodazole-treated and untreated mitotic chicken erythroblasts. Chicken erythroblasts were grown in the presence (*b*) or absence (*a*) of nocodazole, fixed with glutaraldehyde and OsO<sub>4</sub>, and embedded in Epon. Some of the extended membrane cisternae characteristic for mitotic cells are marked by arrowheads. *PM*, plasma membrane; *CH*, chromosomes.

**Figure 4.** Immunoelectron microscopic localization of lamin B<sub>2</sub> in chicken erythroblasts in telophase. Ultrathin cryosections of chicken erythroblasts in telophase were stained with mAb L3-5D10 (anti B<sub>2</sub>). Arrowheads in *a* point to long membrane cisternae only very scarcely labeled with the antibody. Arrowheads in *b* mark a stack of ER membranes. *C*, cytoplasm; *N*, nucleoplasm; *M*, mitochondrium.

brane surface. Finally, the progressive appearance of very scarcely labeled cytoplasmic membranes in telophase might be explained by depletion of membrane cisternae as a result of lamin B<sub>2</sub> flowing back to the reforming nuclear envelope. Conceivably this latter result might also be explained by postulating a recruitment of all lamin B<sub>2</sub>-containing membranes to the reforming nuclear envelope with concomitant formation of new cytoplasmic membranes. Though this alternative explanation cannot be rigorously excluded, evidence in favor of de novo synthesis of ER membranes is scarce (for review see reference 38).

In conclusion, the use of subtype-specific antilamin antibodies enabled us for the first time to localize individual lamins in situ at the EM level. Moreover, it was possible to monitor the fate of these proteins during mitosis. From our data we conclude that both chicken lamins B<sub>1</sub> and B<sub>2</sub> may functionally resemble mammalian lamin B, and that mitotic interactions between the inner nuclear membrane and the ER may be highly dynamic. Though the relatively low labeling density achieved with the mAbs used here impedes an extensive quantitative analysis of our data, it is clear that B-type lamin-specific antibodies represent useful markers for studying the fate of the nuclear membranes during the cell cycle.

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