Integral Membrane Proteins of the Nuclear Envelope Are Dispersed throughout the Endoplasmic Reticulum during Mitosis

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Abstract. We have analyzed the fate of several integral membrane proteins of the nuclear envelope during mitosis in cultured mammalian cells to determine whether nuclear membrane proteins are present in a vesicle population distinct from bulk ER membranes after mitotic nuclear envelope disassembly or are dispersed throughout the ER. Using immunofluorescence staining and confocal microscopy, we compared the localization of two inner nuclear membrane proteins (lamina-associated polypeptides 1 and 2 [LAP1 and LAP2]) and a nuclear pore membrane protein (gp210) to the distribution of bulk ER membranes, which was determined with lipid dyes (DiOC$_6$ and R6) and polyclonal antibodies. We found that at the resolution of this technique, the three nuclear envelope markers become completely dispersed throughout ER membranes during mitosis. In agreement with these results, we detected LAP1 in most membranes containing ER markers by immunogold electron microscopy of metaphase cells. Together, these findings indicate that nuclear membranes lose their identity as a subcompartment of the ER during mitosis. We found that nuclear lamins begin to reassemble around chromosomes at the end of mitosis at the same time as LAP1 and LAP2 and propose that reassembly of the nuclear envelope at the end of mitosis involves sorting of integral membrane proteins to chromosome surfaces by binding interactions with lamins and chromatin.

THE nuclear envelope (NE)[1] is a specialized subcompartment of the ER that forms the nuclear boundary in eukaryotes. It consists of inner and outer membranes, nuclear pore complexes (NPCs), and the nuclear lamina (for review see Gerace and Burke, 1988; Nigg, 1992; Georgatos et al., 1994; Rout and Wente, 1994). The outer nuclear membrane is continuous with peripheral rough and smooth ER and appears to be biochemically and functionally similar to bulk ER membranes. In contrast, the inner nuclear membrane contains specific proteins that are not detected in the peripheral ER (for review see Gerace and Foisner, 1994) and is lined by the nuclear lamina. Inner and outer nuclear membranes are connected by a specialized "pore membrane" that is adjacent to NPCs (see Wozniak et al., 1989).

The nuclear lamina contains mainly a polymeric assembly of intermediate filament-type proteins called nuclear lamins. Four major lamin isotypes have been described in mammalian cells, lamins A, B$_1$, B$_2$, and C (for review see

Nigg, 1992). The lamina is thought to serve as a structural framework for the NE and an anchoring site at the nuclear periphery for interphase chromosomes (for review see Nigg, 1992; Georgatos et al., 1994). In vitro studies have indicated that chromatin interacts directly with lamins (Burke, 1990; Glass and Gerace, 1990; Hoger et al., 1991; Yuan et al., 1991; Taniura et al., 1995) and some minor lamina-associated proteins (see below).

Two integral membrane proteins have been identified in the nuclear pore membrane of higher eukaryotic cells, gp210 (Gerace et al., 1982; Wozniak et al., 1989) and POM121 (Hallberg et al., 1993). These proteins are speculated to have a role in anchoring the NPC to the pore membrane and in nucleating NPC assembly (Gerace et al., 1982; Wozniak et al., 1989). Several integral membrane proteins restricted to the inner nuclear membrane also have been characterized in higher eukaryotes: lamina-associated polypeptide (LAP)1 (Senior and Gerace, 1988; Martin et al., 1995), LAP2 (Foisner and Gerace, 1993; Furukawa et al., 1995), p58/lamin binding receptor (LBR) (Worman et al., 1988, 1990), and otefin (Padan et al., 1990). LAP1 and LAP2 are tightly associated with the lamina, as indicated by their resistance to extraction with a combination of nonionic detergent and high salt (Senior and Gerace, 1988; Foisner and Gerace, 1993). In vitro binding studies have suggested that LAP1 and LAP2

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1. *Abbreviations used in this paper*: LAP, lamina-associated polypeptide; LBR, lamin binding receptor; NE, nuclear envelope; NPC, nuclear pore complex; NRK, normal rat kidney; GFP, green fluorescent protein.

(Foisner and Gerace, 1993), as well as p58/LBR (Worman et al., 1988), directly interact with lamins and that LAP2 (Foisner and Gerace, 1993) and p58/LBR (Ye and Worman, 1996) associate with chromatin. LAP1C and LAP2 each contain a single predicted transmembrane segment and a large nucleoplasmic domain (Furukawa et al., 1995; Martin et al., 1995). In contrast, p58/LBR, which is homologous to yeast sterol C14 reductase (see Georgatos et al., 1994), has eight predicted transmembrane segments. Although functions of specific integral membrane proteins of the inner nuclear membrane have not been determined, it is likely that certain lamin-binding integral membrane proteins have a role in the attachment of lamin filaments to the inner nuclear membrane and in the structure or higher-order arrangement of lamin filaments. They also could play a role in reassembly of the NE at the end of mitosis.

The interphase ER comprises a continuous network of cisternae and tubular membranes extending to the periphery of the cell from the NE (see Warren and Wickner, 1996). During mitosis, the ER undergoes disassembly to vesicles and membrane tubules/cisternae to allow the partitioning of ER membranes to the daughter cells (Warren and Wickner, 1996). In higher eukaryotes, the NE breaks down during mitotic prophase into a form that cannot be structurally distinguished from disassembled elements of the peripheral ER (e.g., Porter and Machado, 1960; Robbins and Gonatos, 1964; Roos, 1973; Zeligs and Wohlman, 1979). The NE is reassembled during late anaphase by a process that involves the association of membrane vesicles and cisternae with the surfaces of the fused chromosome masses coupled with membrane fusion (Robbins and Gonatas, 1964; Roos, 1973; Zeligs and Wollman, 1979). Pore complexes are gradually inserted into the NE, beginning in late anaphase and continuing until early G1 (Maul, 1977). Immunofluorescence microscopy has shown that certain integral membrane proteins of the inner nuclear membrane, LAP1, LAP2 (Foisner and Gerace, 1993), and p58/LBR (Chaudhary and Courvalin, 1993), accumulate at the surfaces of chromosomes during the early stages of NE reformation in late anaphase. However, the mechanism of their reassembly is controversial, in part because the exact fate of integral membrane proteins of the NE during mitosis is unknown (discussed in Gerace and Foisner, 1994).

Two different models have been proposed to explain the disassembly and reformation of nuclear membranes during mitosis. In one model, nuclear membrane proteins are released into specific NE-derived vesicles distinct from bulk ER membranes as a result of NE disassembly and are reassembled in the NE by selective targeting of the NE-specific vesicles to the chromosome surfaces (Wilson and Newport, 1988; Chaudhary and Courvalin, 1993; Maison et al., 1993). In a second model, nuclear membrane proteins become dispersed throughout all ER membranes during mitosis and are sorted to the reforming NE by diffusion through a functionally continuous ER coupled with binding to specific sites at the chromosome surfaces (discussed in Gerace and Foisner, 1994). Distinguishing between these models is important for understanding the mechanisms for assembly and maintenance of the NE and other ER subdomains during the cell cycle.

To directly investigate the fate of nuclear membranes during mitosis, we have used laser-scanning confocal microscopy and electron microscopy to compare the localizations of NE-specific integral membrane proteins to the distribution of bulk ER membranes during mitosis. Our results demonstrate that integral proteins from both the inner nuclear membrane and nuclear pore membrane become dispersed throughout ER membranes during mitosis and are not restricted to a subpopulation of ER vesicles. This indicates that nuclear membranes lose their identity as a discrete subcompartment of the ER during mitosis and strongly supports a model in which the sorting of specific membrane proteins to the NE at the end of mitosis is driven by binding interactions at chromosome surfaces.

Materials and Methods

Cell Culture

All cell lines (COS-7, CV-1, normal rat kidney [NRK], NIH3T3, and HeLa; American Type Culture Collection, Rockville, MD) were grown in DME (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FBS (Hyclone Laboratories, Logan, UT) and 100 U/ml penicillin-streptomycin (GIBCO BRL). Cultures were maintained at 37°C in the presence of 5% CO_2. To obtain mitotically enriched cell populations for confocal fluorescence microscopy, NRK cells growing on coverslips were incubated in medium containing 2 mM thymidine (Sigma Chemical Co., St. Louis, MO) for 11 h to accumulate cells in S phase. The thymidine-containing medium was replaced by normal growth medium for an additional 6 h, and cells were then processed for microscopy. To obtain metaphase cells for immunogold electron microscopy, NRK cells growing on 150-cm^2 plates were incubated in medium containing 2 mM thymidine for 11 h. The thymidine medium was removed, and 4 h later cells were incubated in medium containing 0.6 μg/ml nocodazole for a further 6 h. Mitotic cells were then collected by mechanical shake-off.

Antibodies

LAP1, LAP2, and gp210 were detected with the RL13 (Senior and Gerace, 1988), RL29 (Foisner and Gerace, 1993; Furukawa et al., 1995), and RL20 (Greber et al., 1990) monoclonal antibodies, respectively. The relevant hybridomas were grown in EXCELL 300 serum-free medium (JRH Biosciences, Lenexa, KS), and monoclonal antibodies were purified from the culture supernatants by ammonium sulfate precipitation followed by chromatography on a protein G column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). LAP1 and LAP2 were also detected with affinity-purified rabbit polyclonal antibodies raised against residues 1–320 of LAP1C and residues 298–373 of LAP2. Immunofluorescence labeling of interphase and mitotic cells with these antibodies gave results identical to those obtained with the monoclonal antibodies. These fragments of LAP1C and LAP2 were expressed in *Escherichia coli* as GST fusion proteins and purified from soluble bacterial lysates by chromatography on a glutathione-Sepharose matrix (Pharmacia LKB Biotechnology, Inc.). Rabbits were immunized with the purified fusion proteins as described previously (Melchior et al., 1995). To prepare affinity matrices for purifying anti-LAP1C (1–320) and anti-LAP2 (298–373) antibodies, the purified GST fusion proteins were coupled to CNBr-activated Sepharose (Pharmacia LKB Biotechnology, Inc.) at 0.5–1.0 mg/ml according to the manufacturer's instructions. Antisera were first preadsorbed against GST-agarose to remove the anti-GST antibodies, and the resulting sera were then used to obtain specific affinity-purified antibodies with the affinity matrices as described (Melchior et al., 1995).

Lamin A was detected by an antipeptide polyclonal antibody raised against a synthetic peptide comprising residues 572–585 of human lamin A. This was obtained by coupling the peptide to Keyhole lymphet hemocyanin and immunizing a rabbit as described previously (Melchior et al., 1995). The antibody specifically recognizes lamin A in Western blot analysis of isolated rat liver NEs. The polyclonal anti-ER antiserum was the gift of Dr. Daniel Louvard (Curie Institute, Paris, France). It was obtained by immunizing rabbits with EDTA and salt-stripped canine pancreas rough microsomes, and it recognizes four ER membrane proteins with apparent molecular masses of 29, 58, 66, and 91 kD (Louvard et al.,

1982). Dr. Michael Jackson (R.W. Johnson Pharmaceutical Institute, La Jolla, CA) provided a rabbit polyclonal antiserum against calnexin (Ware et al., 1995); Dr. Stephen Fuller (European Molecular Biology Laboratory, Heidelberg, Germany) provided an antibody against a 12–amino acid peptide composing the COOH terminus of protein disulphide isomerase (Vaux et al., 1990); and Dr. Marilyn Farquhar provided a rabbit polyclonal antiserum against α-mannosidase II (Velasco et al., 1993).

Immunofluorescence Microscopy

The localization of all proteins was examined by indirect immunofluorescence microscopy. NRK cells growing on glass coverslips were enriched in mitotic populations by a thymidine presynchronization (see above). Cells were fixed in 4% formaldehyde in PBS for 6 min at room temperature, permeabilized with 0.2% Triton X-100 in PBS for another 6 min, and treated with PBS containing 0.2% gelatin. Cells were then incubated with primary antibodies diluted in PBS/gelatin for 1 h at room temperature, washed in PBS, incubated for another 40 min at room temperature with secondary antibodies diluted in PBS/gelatin, and washed in PBS. Monoclonal antibodies RL13 (mouse), RL20 (mouse), and RL29 (hamster) were used at 25 μg/ml and were detected with rhodamine-conjugated goat anti–mouse IgG or rhodamine-conjugated goat anti–hamster IgG (Molecular Probes, Inc., Eugene, OR). Polyclonal antisera against the ER (Louvard et al., 1982), α-mannosidase II (Velasco et al., 1993), protein disulphide isomerase (Vaux et al., 1990), and calnexin (Ware et al., 1995) were used at a dilution of 1:200 and stained with rhodamine or fluorescein-conjugated goat anti–rabbit IgG (Molecular Probes, Inc.). For some of the experiments, after the secondary antibody incubation the ER was labeled by DiOC6 or R6 (Molecular Probes, Inc.) at 0.5 μg/ml for 5 min at room temperature. The specimens were mounted in slowfade antifade solution (Molecular Probes, Inc.) and examined with a laser-scanning confocal microscope (model MRC-600; BioRad Labs, Hercules, CA).

Immunogold Electron Microscopy

To carry out double immunogold labeling of LAP1 and ER membranes in mitotic cells, populations of metaphase-enriched NRK cells (see above) were permeabilized in PBS containing 60 μg/ml digitonin for 5 min on ice and then fixed in 4% formaldehyde in PBS with 1 mM MgCl2 for 20 min at 4°C. The cells were washed in PBS and incubated in PBS for 3 h at 4°C with a mixture of the primary antibodies diluted in PBS and 0.5% BSA (RL13 IgG [anti-LAP1] at 25 μg/ml and anti-ER antiserum [Louvard et al., 1982] at 1:100). The cells were then washed in PBS and incubated overnight at 4°C with a mixture of goat anti–mouse IgG conjugated with 10-nm colloidal gold (for labeling LAP1) and goat anti–rabbit IgG conjugated with 5-nm colloidal gold (for labeling the ER) diluted in PBS and 0.5% BSA. After another wash in PBS, the cells were fixed in 2% glutaraldehyde for 30 min at 4°C, washed thoroughly, and then postfixed with 1% OsO4 for 1 h at room temperature. The samples were dehydrated and embedded in Epon 812 resin as described (Guan et al., 1995). Sections were stained with 2% uranyl acetate for 1 min. Micrographs were recorded with an electron microscope at 80 kV (model 600; Hitachi America Ltd., Brisbane, CA). As a nonspecificity control for the gold-coupled antibodies, some samples were incubated in PBS and 0.5% BSA instead of the primary antibody solution before being incubated with the gold-coupled secondary antibodies. Only cells showing clearly defined mitotic chromosomes were analyzed.

Results

Localization of Integral Proteins of the NE in Relation to Bulk ER Membranes during Mitosis by Confocal Microscopy

We have investigated the fate of integral membrane proteins of the NE during mitosis by immunofluorescence staining and laser-scanning confocal microscopy of mitotic NRK cells to analyze whether nuclear membrane proteins are restricted to a subcompartment of the ER during mitosis as they are during interphase. In this study, we compared the localization of proteins of both the inner nuclear membrane and nuclear pore membrane to the distribution of bulk ER membranes. The ER was detected with two fluorescent dyes that selectively label the ER, DiOC6 (Lee and Chen, 1988; Terasaki and Reese, 1992) and R6 (Terasaki and Reese, 1992), as well as with several polyclonal antibodies against ER proteins (see below).

DiOC6 and a polyspecific anti-ER antibody raised against salt and EDTA-extracted rough microsomes (Louvard et al., 1982) gave essentially coincident patterns of fluorescence labeling in interphase and metaphase NRK cells (Fig. 1, A and B, respectively), where arrays of tubular and vesicular membrane elements were seen in the optical sections. DiOC6 and a polyclonal antibody against protein disulfide isomerase, an abundant soluble protein of the ER lumen (Vaux et al., 1990), also gave nearly coincident labeling patterns in interphase and metaphase cells (Fig. 1, C and D, respectively). Similar results were obtained by double labeling of cells with DiOC6 and an antibody against another major soluble protein of the ER lumen, calnexin (Ware et al., 1995; data not shown). Finally, both DiOC6 and R6 gave coincident staining patterns in interphase and metaphase cells (Fig. 1, E and F, respectively). These data indicate that under our staining conditions, DiOC6, R6, and the polyspecific anti-ER antibody selectively label the ER in NRK cells. We have used these three staining reagents interchangeably to detect the ER throughout this study.

We next compared the localizations of LAP1, an integral protein of the inner nuclear membrane, and ER membranes during interphase and mitosis (Fig. 2). In interphase cells, LAP1 was localized in a nuclear rim pattern, whereas the ER membranes were seen as an extensive reticular/vesicular network extending throughout the cell (Fig. 2 A). As expected, overlap between interphase NE and ER labeling occurred at the nuclear rim (Fig. 2 A, Merge). By prometaphase, when the NE has disassembled, LAP1 appeared to be localized throughout the entire ER; nearly every membrane structure labeled with the ER probe also was labeled with the LAP1 antibody (Fig. 2 B, compare LAP1 and ER). Moreover, there was a roughly uniform distribution of LAP1 throughout ER membranes (i.e., the relative intensity of labeling of the various membrane structures is similar with both the LAP1 and ER probes). Dispersion of LAP1 throughout ER membranes appears to occur very close to the time of nuclear lamin depolymerization, since all late prophase cells that we examined by double immunofluorescence staining contained LAPs and lamin in the portions of the NE that remained assembled (Yang, L., and L. Gerace, unpublished observations).

The dispersion of LAP1 throughout ER membranes persisted through metaphase (Fig. 2 C) and mid-anaphase (Fig. 2 D, left cell). In late anaphase, when nuclear membranes begin to assemble around chromosomes, LAP1 became segregated from bulk ER membranes at the periphery of the chromosome masses (Fig. 2 D, right cell). Furthermore, in some late anaphase cells where LAP1 association with chromosomes was apparent, the LAP1 remaining in the peripheral ER was not uniformly localized throughout, but appeared to be locally concentrated in certain ER elements (Fig. 2 D, right cell). A similar phenomenon was seen for LAP2 (see below; data not shown).

DiOC6 | **anti-ER** | **Merge**

A

B

DiOC6 | **anti-PDI** | **Merge**

C

D

DiOC6 | **R6** | **Merge**

E

F
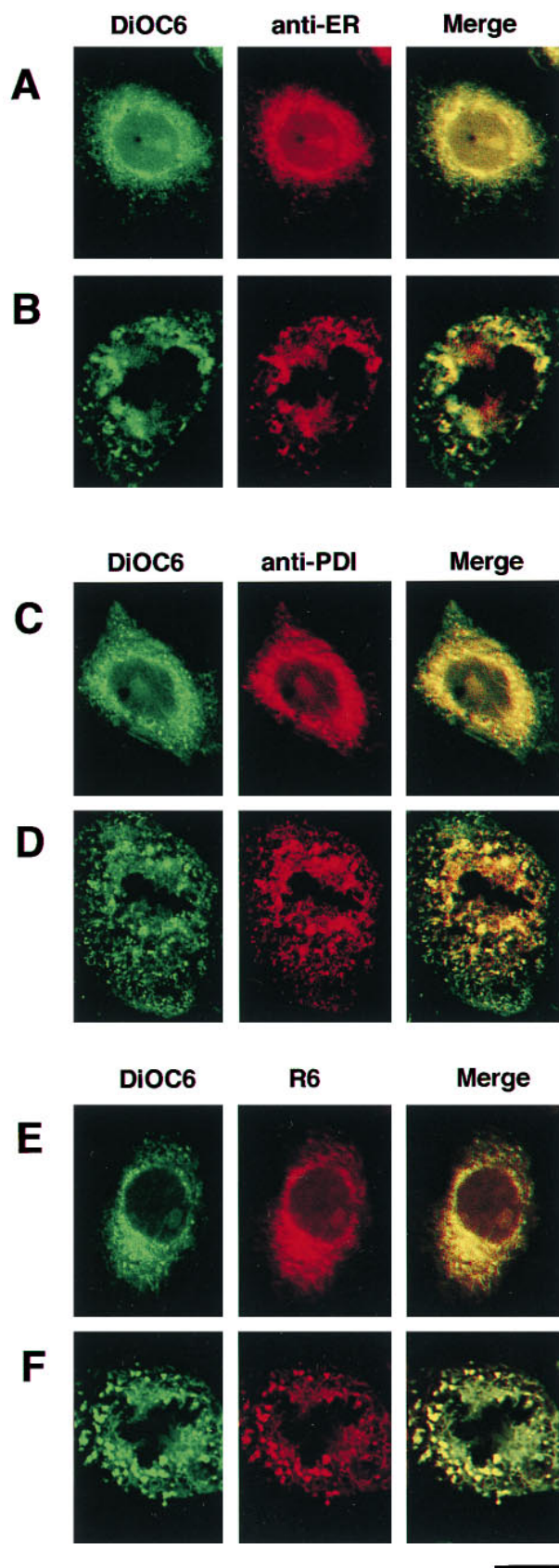
Figure 1. Localization of ER membranes with antibodies and fluorescent dyes. NRK cells were fixed and labeled for indirect immunofluorescence microscopy with a polyspecific anti-ER antibody (*A* and *B*) or anti–protein disulphide isomerase antibody (*anti-PDI*, *C* and *D*) and were then counterstained with $DiOC_6$. Alternatively, the fixed cells were double stained with $DiOC_6$ and R6 (*E* and *F*). Shown are images of the labeling with $DiOC_6$ (*left column*), antibodies or R6 (*center column*), and the merge of the two fluorescent channels (*right column*). Examples of interphase (*A*, *C*, and *E*) and metaphase (*B*, *D*, and *F*) are presented. Bar, 10 μm.
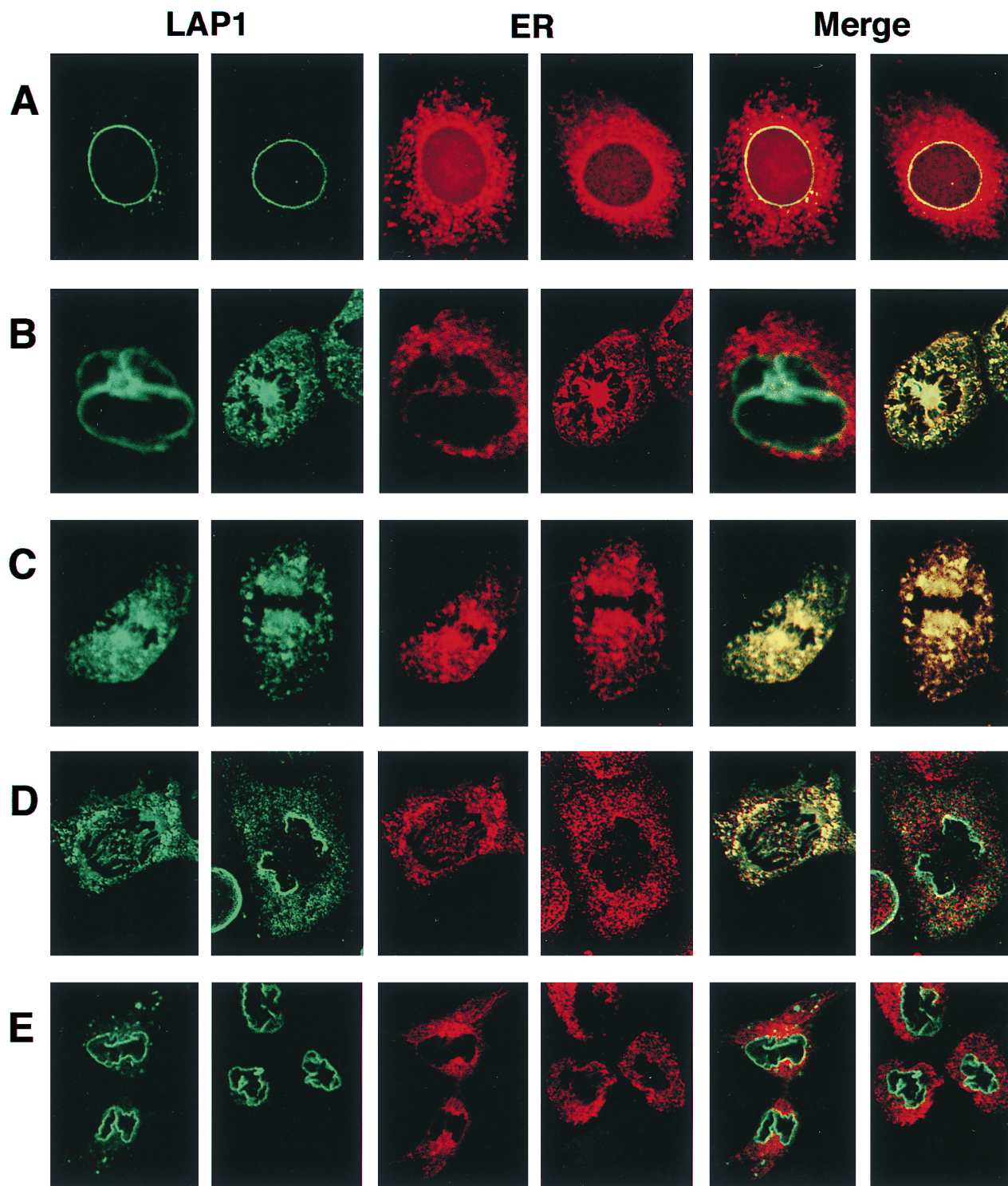
LAP1 was exclusively perinuclear by telophase when it was separated from all peripheral ER membranes (Fig. 2 *E*). It should be noted that LAP1 has a higher concentration in the telophase NE relative to the ER label than in the interphase NE (i.e., the NE appears green in the merged images of Fig. 2 *E*, while it appears yellow in the merged images of Fig. 2 *A*). This very likely is due to a substantial increase in the surface area of the NE from telophase to early G1 without concomitant synthesis of new NE proteins, thereby decreasing the relative LAP1 concentration.

We obtained comparable localization results for LAP1 during mitosis using either a monoclonal antibody to LAP1 (shown in Fig. 2) or a polyclonal antibody raised against the nucleoplasmic domain of LAP1 (data not shown; see Materials and Methods), lending confidence to our findings. These results indicate that LAP1 becomes essentially randomized throughout the ER by prometaphase when the NE is disassembled and again becomes concentrated in a discrete subdomain of the ER in late anaphase when the NE reassembles.

We next extended this analysis to LAP2, another integral membrane protein of the inner nuclear membrane, and gp210, an integral protein of the nuclear pore membrane. Similar to the results seen with LAP1, LAP2 and gp210 appeared to be dispersed throughout all ER membranes in mitosis. In prometaphase (data not shown) and metaphase (Fig. 3) cells, virtually all ER membranes were labeled in a roughly uniform fashion with antibodies to LAP2 (Fig. 3 *A*) and gp210 (Fig. 3 *B*). The proteins were resegregated to the nuclear periphery at the end of mitosis (Gerace et al., 1982; Foisner and Gerace, 1993). Comparable results on the localization of LAP2 in mitotic cells were obtained using either monoclonal antibodies (shown in Fig. 3) or polyclonal antibodies raised against a fragment of the nucleoplasmic domain of this protein (data not shown; see Materials and Methods). As observed previously (Chaudhary and Courvalin, 1993), we found that assembly of LAP1 and LAP2 around chromosomes, which occurred in late anaphase (Foisner and Gerace, 1993), preceded the assembly of the majority of gp210 (data not shown).

As a control, we analyzed the localization in mitotic cells of α-mannosidase II, an integral membrane protein of the Golgi complex (Velasco et al., 1993). Golgi membranes are known to remain separate from the ER during mitosis (Warren and Wickner, 1996) and therefore should present a distribution distinct from ER membranes in confocal microscopy. As expected, in mitotic cells the antibody to α-mannosidase II labeled a set of membrane structures that largely did not overlap with ER membranes, even though both membranes were extensively dispersed throughout the cytoplasm (Fig. 3 *C*). This indicates that our microscope procedure would be able to clearly distinguish hypo-

Figure 2. Comparison of the localizations of LAP1 and ER membranes throughout mitosis. NRK cultures enriched in mitotic cells were fixed and examined by double immunofluorescence microscopy after labeling with the LAP1-specific monoclonal antibody RL13 and a polyspecific anti-ER antibody. Shown is the labeling with RL13 (*left column*), the anti-ER antibody (*center column*), and the merge of the two fluorescent images (*right column*). The figure presents representative examples of cells (two different cells in each row) in inter-phase (*A*), prophase (*B*, *left cell*), prometaphase (*B*, *right cell*), metaphase (*C*), mid-anaphase (*D*, *left cell*), late anaphase (*D*, *right cell*) and telophase (*E*). The NE in the prophase cell shown in *B* (*left cell*) is deformed due to invagination by the mitotic spindle. Bar, 10 μm.
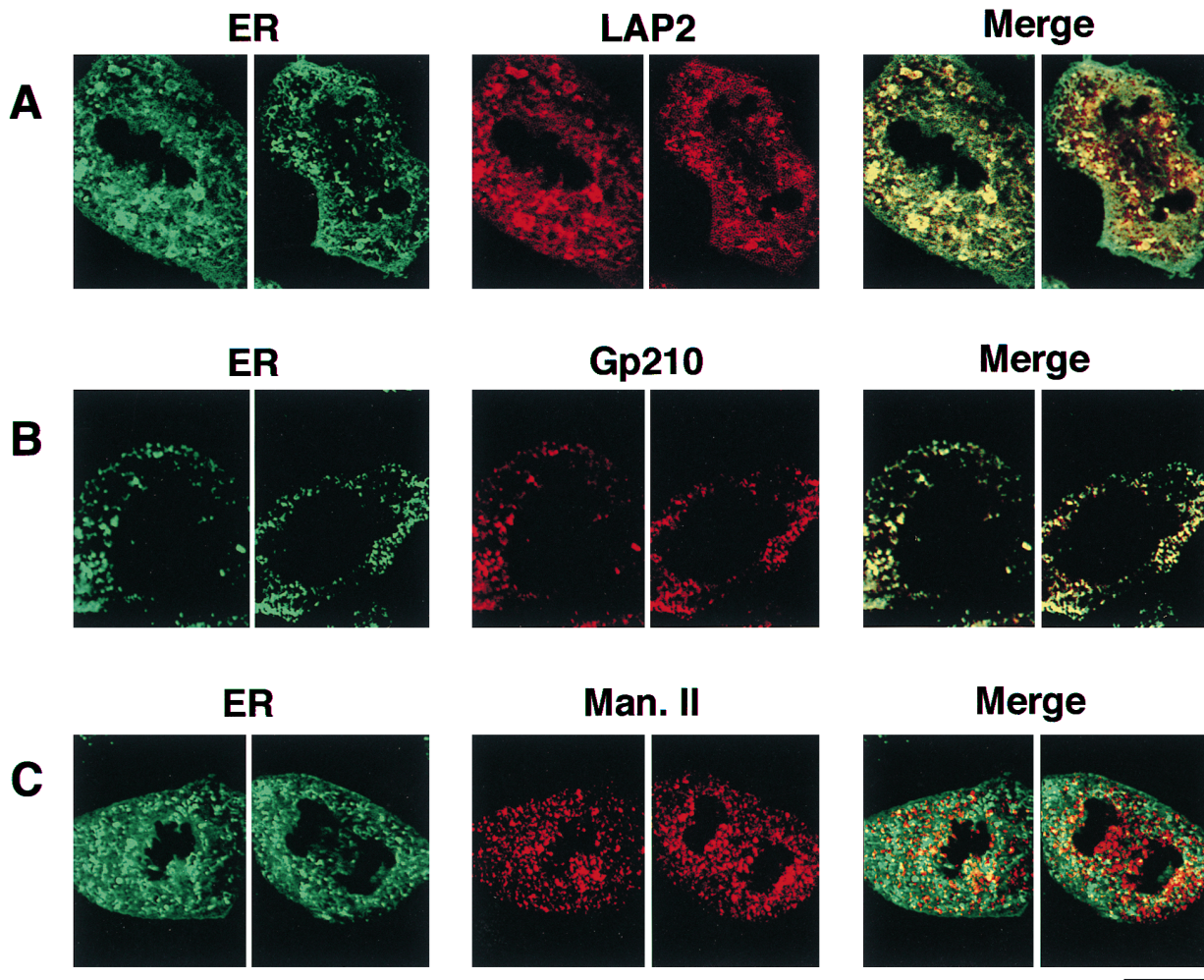
Figure 3. Comparison of the localization of ER membranes to the distributions of LAP2, gp210, and α-mannosidase II in metaphase cells. NRK cultures enriched in mitotic cells were fixed and examined by double labeling with DiOC$_6$ and the LAP2-specific monoclonal antibody RL29 (*A*), DiOC$_6$ and the gp210-specific monoclonal antibody RL20 (*B*), or R6 and a polyclonal antibody against the Golgi enzyme α-mannosidase II (*C*). Shown is the labeling with the ER probes (*left column*), antibody probes (*center column*), and a merge of the two fluorescent images (*right column*). The green/red color representation for the antibody and R6 was computationally reversed in the images presented in *C* for consistency. In *A* and *B*, two different metaphase cells are shown, and in *C*, a metaphase (*left cell*) and anaphase (*right cell*) are shown. Bar, 10 μm.

thetical NE-specific membranes, if they existed, as a population separate from bulk ER membranes.

To directly visualize the mitotic dynamics of LAPs, we expressed chimeras consisting of LAP1C and LAP2 fused to green fluorescent protein (GFP) in cultured mammalian cells and examined the GFP fluorescence by confocal light microscopy (Yang, L., and L. Gerace, unpublished observations). The GFP chimeras were localized to the NE in interphase cells and were dispersed throughout ER membranes during mitosis, in agreement with the results of immunofluorescence localization. Unfortunately, because the fluorescence intensity of membrane structures labeled with the GFP–LAP fusion proteins was strongly diminished after the proteins were distributed throughout the ER in mitosis, we were not able to carry out a real time analysis of the mitotic dynamics of LAPs in the NE.

In summary, our results indicate that integral membrane proteins of both the inner nuclear membrane (LAP1 and

LAP2) and nuclear pore membrane (g210) are dispersed throughout the ER during mitosis in NRK cells, at the resolution of light microscopy. We have obtained similar results in several other cultured mammalian cell lines (CHO, COS, and HeLa cells; data not shown), and we believe that the phenomenon we have described is a general property of these NE proteins during mitosis.

Localization of NE and ER Membranes in Mitotic Cells by Immunogold Electron Microscopy

To confirm and extend the results we obtained with confocal light microscopy, we carried out double immunogold labeling of digitonin-permeabilized mitotic NRK cells to localize LAP1 and ER membranes at the EM level (Fig. 4). The populations used for this analysis were selected from nocodazole-arrested cultures and were highly enriched in metaphase-like cells. ER membranes were la-
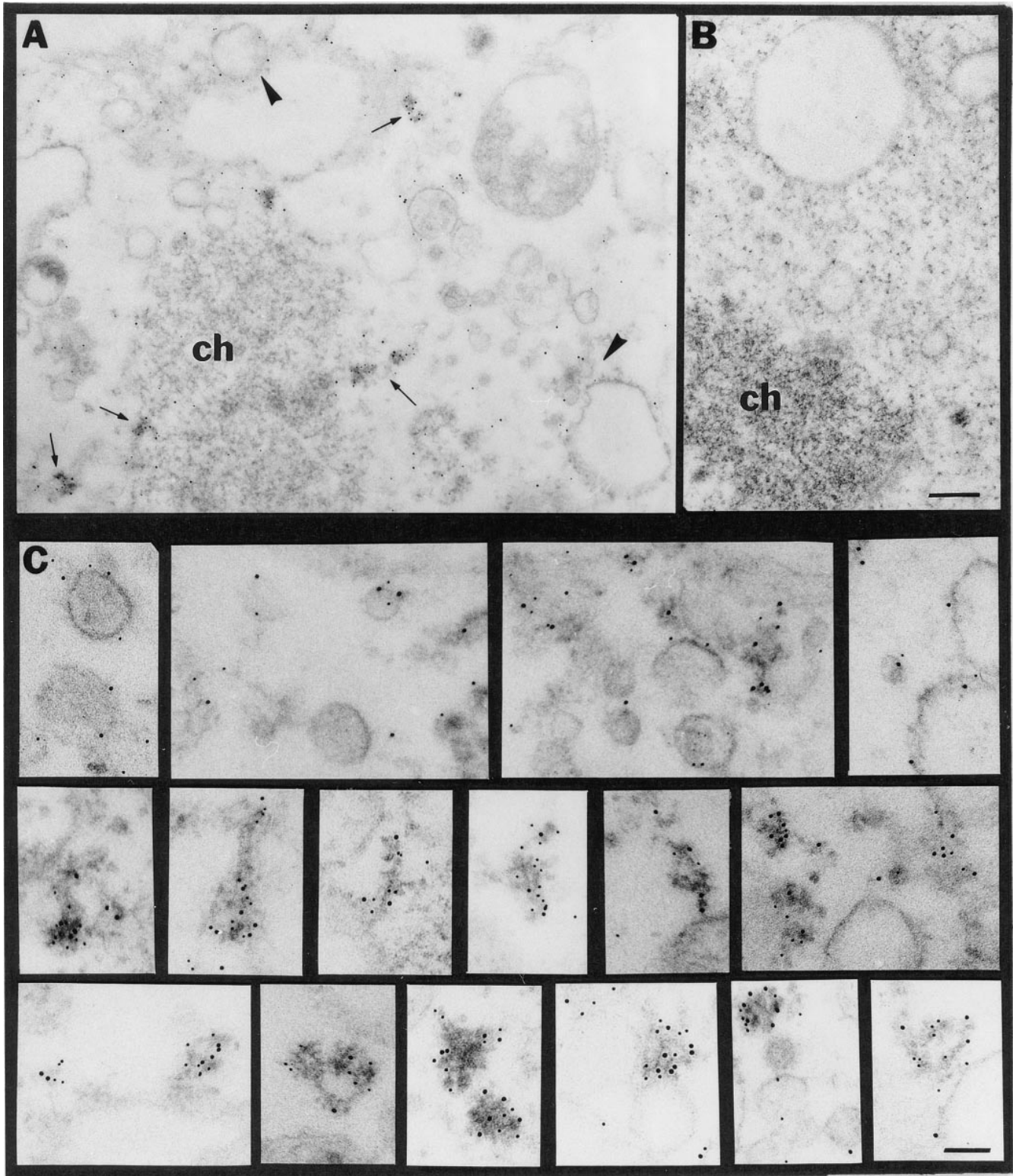
Figure 4. Comparison of the localizations of LAP1 and ER membranes in metaphase cells by double immunogold labeling. Populations of nocodazole-arrested metaphase NRK cells were processed for double immunogold labeling by incubation with the LAP1-specific monoclonal antibody RL13 and a polyspecific anti-ER antibody, followed by 10-nm antibody-coupled gold (to detect the LAP1 probe) and 5-nm antibody-coupled gold (to detect the ER probe). Shown are images of cells incubated with anti-LAP1 and anti-ER antibodies followed by secondary gold-coupled antibodies (*A* and *C*) or cells incubated with only the secondary gold-coupled antibodies and without the primary antibodies (*B*). Examples of mitotic chromosomes (*ch*) used to identify mitotic cells are designated. *A* indicates examples of the two classes of antibody-labeled structures: large discrete vesicles (*large arrowheads*) and densely staining aggregates of small vesicles and tubules (*small arrows*). *C* shows a gallery of the antibody-labeled membranes: large discrete vesicles (*top row*) and aggregates of small vesicles and tubules (*bottom two rows*). Bars: (*A* and *B*) 300 nm; (*C*) 100 nm.

beled with a rabbit polyclonal anti-ER antibody and 5-nm gold coupled to a secondary antibody, and LAP1 was detected with a mouse monoclonal antibody and 10-nm gold coupled to a secondary antibody. The antibody concentrations were adjusted so that similar labeling densities were obtained with the 5- and 10-nm gold particles. Both antibodies labeled two categories of intracellular membrane structures: discrete, relatively large (usually 50–500 nm in diameter) vesicles with an obvious lumen (Fig. 4 *A*, *large arrowheads*; Fig. 4 *C*, *top row*) and densely-staining aggregates that contained thin tubules and clusters of small vesicles (Fig. 4 *A*, *small arrows*; Fig. 4 *C*, *bottom two rows*). The density of gold labeling was considerably higher for the latter category of structures than for the former. At least in part, this probably reflects the larger amount of membrane surface per unit area in aggregates of thin tubules and small vesicle clusters as compared to large, single vesicles.

Most membrane structures of both classes (i.e., discrete vesicles and aggregates of thin tubules/small vesicles) that were labeled with anti-ER antibodies also were labeled with anti-LAP1 antibodies (Fig. 4 *A*, *arrows and arrowheads*, and gallery in Fig. 4 *C*). The gold labeling with anti-ER and -LAP1 antibodies was specific, as very little labeling of membranes was obtained in samples incubated with gold-coupled secondary antibodies alone (Fig. 4 *B*). Furthermore, very little labeling of the peripheral ER was obtained with anti-LAP1/10-nm gold particles in interphase cells (data not shown), where LAP1 is undetectable in the peripheral ER by immunofluorescence microscopy (Fig. 2). Finally, the anti-ER and -LAP1 antibodies labeled only a fraction of all membrane structures in the permeabilized cells (e.g., Fig. 4 *A*).

We confirmed the close colocalization of the ER and LAP1 probes by quantitative analysis. In one analytical method, a field containing circular windows with a diameter of 100 nm was randomly placed on prints of electron micrographs. We found that 82% of the windows that contained at least two 5-nm gold particles (ER probe) also contained at least one 10-nm particle (LAP1 probe) ($n = 55$). By contrast, only 3.8% of all random windows contained at least one 10-nm gold particle ($n = 500$). In a second method of analysis, we measured the distance from each 5-nm gold particle (ER probe) to the nearest 10-nm gold particle (LAP1 probe). We found that 68.2% of all 5-nm gold particles had a 10-nm gold particle localized within a radius of 100 nm ($n = 197$). Considered together, these results indicate that LAP1 is located close to most of the ER label. If LAP1 were restricted to a minor subset of ER membranes, a much smaller fraction of the ER label would be expected to have closely associated LAP1. In conclusion, the findings from immunogold EM are in close agreement with the results from confocal light microscopy and confirm that LAP1 is dispersed throughout ER membranes during mitosis.

Order of Lamin and LAP Assembly at the End of Mitosis

Our localization studies suggest that binding interactions are likely to be important for localizing integral membrane proteins to the reforming NE at the end of mitosis (see

Discussion). In principle, nuclear lamins could provide binding sites to promote this process if lamins were to assemble around chromosomes at the same time as integral membrane proteins. Although recent immunofluorescence localization studies with conventional light microscopy showed that LAP1 and LAP2 (Foisner and Gerace, 1993) and p58/LBR (Chaudhary and Courvalin, 1993) become concentrated around chromosomes at the end of mitosis before most lamins, these studies could not exclude the possibility that a fraction of lamins associates with the chromosome surfaces at the same time as the inner membrane proteins. Lamins exist in a large stoichiometric excess over integral membrane proteins of the inner nuclear membrane, and the high concentration of disassembled lamins in the cytosol would make it difficult to detect chromosome-associated lamins by conventional light microscopy (see Gerace and Foisner, 1994).

To reinvestigate this question, we carried out double immunofluorescence localization of lamin A and LAPs in cultured NRK cells during late mitosis and examined specimens using confocal light microscopy to enhance the ability to visualize chromosome-associated lamins in the presence of disassembled cytosolic lamins. As shown in Fig. 5, when LAP1 (*A*) and LAP2 (*C*) started to become concentrated at parts of the chromosome surfaces in late anaphase, some lamin A also was concentrated in the same regions of the chromosomes. By early telophase, we observed that virtually all LAPs were concentrated at the chromosome surfaces, while a significant fraction of lamins remained unassembled (Foisner and Gerace, 1993; and data not shown), while by mid-late telophase (Fig. 5, *B* and *D*), most of the lamin pool had reassembled as well. These data indicate that lamin and LAPs associate with the chromosome surfaces in late anaphase in a temporally and spatially coordinated fashion, even though much lamin remained unassembled in early telophase when the assembly of LAPs was essentially completed (Foisner and Gerace, 1993). Thus, even though lamin and LAPs begin to associate with chromosome surfaces at the same time in late anaphase, the half-time of assembly of the lamin pool appears to be longer than that of LAPs.

Discussion

The Fate of Nuclear Membranes in Mitotic Cells

We have used light and electron microscope immunolocalization to investigate the fate of several integral membrane proteins of the NE during mitosis in cultured mammalian cells. We have examined two integral membrane proteins of the inner nuclear membrane, LAP1 and LAP2, and an integral protein of the nuclear pore membrane, gp210. By immunofluorescence staining and confocal microscopy, we found that the three NE markers are localized throughout all ER membranes after NE disassembly in prometaphase and remain dispersed until the time of NE reassembly in late anaphase. In agreement with the results of light microscopy, we found by immunogold EM that LAP1 is detectable in most ER membranes in metaphase cells. To a first approximation, the NE proteins appear to be uniformly dispersed throughout the mitotic ER. However, our light and EM localization is not sensitive enough
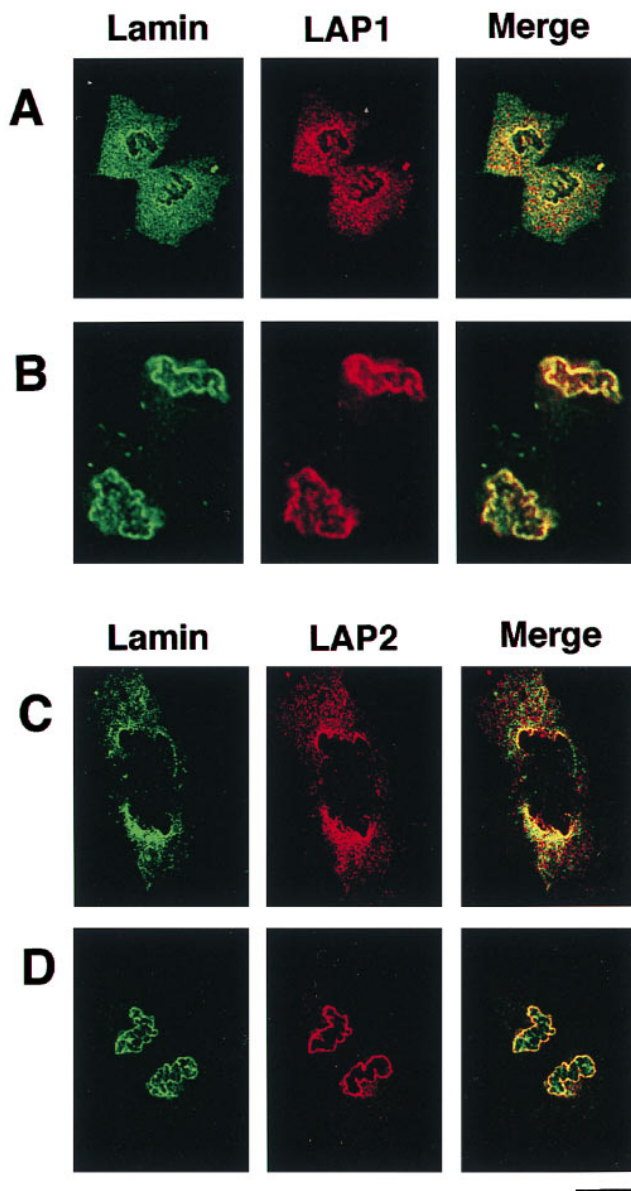
Figure 5. Comparison of the localization of lamins to the distribution of LAPs at the end of mitosis. NRK cultures enriched in mitotic cells were labeled for double immunofluorescence microscopy with an antibody against lamin A and the LAP1-specific monoclonal antibody RL13 (*A* and *B*) or the LAP2-specific monoclonal antibody RL29 (*C* and *D*). Shown are images of the lamin labeling (*left column*), the LAP1 or LAP2 labeling (*center column*), or a merge of the two fluorescent images (*right column*). Each row presents representative images of cells in late anaphase (*left cell*) or mid-late telophase (*right cell*). Bar, 15 μm.

to rule out the possibility that one or more of the markers we have analyzed have a somewhat higher concentration in certain elements of the ER than others (see discussion of gp210 below). Nevertheless, it is clear that the NE markers are not restricted to hypothetical NE-derived vesicles in mitotic cells since the NE comprises only ~5% of the surface area of the interphase ER in BHK cells, a typical cultured mammalian cell line (Griffiths et al., 1989).

We consider it likely that the three proteins we have

studied are representative of most if not all NE-specific integral membrane proteins since these three proteins have the same problems of biogenesis and compartmentalization as do other proteins of the inner nuclear membrane and nuclear pore membrane. Therefore, our data indicate that NE membranes lose their identity as a distinct subcompartment of the ER during mitosis, and that disassembled nuclear membrane proteins are distributed throughout ER membranes in a relatively nonselective fashion.

In contrast to the findings of the present study, a number of authors have speculated previously that *Xenopus* eggs (meiotic cells) and mitotic mammalian cells contain a separate population of NE-derived vesicles that is distinct from most ER membranes. However, these proposals have been based on largely circumstantial evidence rather than direct localization of specific proteins in intact cells, and we now reconsider these previous findings in light of our present results.

Experiments analyzing in vitro nuclear assembly in extracts from *Xenopus* eggs demonstrated that only ~20% of ER membrane markers became assembled into nuclei when the ability to form nuclear membranes was saturated by the addition of a large amount of chromatin substrate to the extracts (Wilson and Newport, 1988). The authors interpreted this to mean that the membrane proteins involved in NE assembly are present in a discrete population of ER vesicles (Wilson and Newport, 1988). However, since ER membranes continuously undergo fusion in the assembly extracts (Newport and Dunphy, 1992) and the NE is continuous with the ER, these results also are consistent with the possibility that NE proteins initially are dispersed throughout ER vesicles and are subsequently depleted from most ER elements during the assembly reaction by diffusion through the ER and binding to sites in the reassembling NE (see below). Other studies with *Xenopus* nuclear assembly extracts showed that two separate detergent-sensitive particulate fractions were involved in NE assembly in this system, prompting the conclusion that two separate vesicle populations are involved in nuclear pore and membrane assembly (Vigers and Lohka, 1991). However, only one of the fractions could be prepared as isolated membranes, raising the possibility that the active component of the second fraction is a nonmembranous, detergent-sensitive particulate structure.

Immunological studies of two lamin isotypes in *Xenopus* eggs showed that a minor fraction of these lamins is membrane-associated in egg extracts and that the two lamin isotypes present in the membrane-associated pool are bound to different-sized vesicles (Lourim and Krohne, 1993). We have found that the membrane-associated B-type lamins of CHO cells redistribute between a membrane and a soluble state in cell homogenates (Gerace, L., unpublished). This raises the possibility that the membrane associations observed for the different *Xenopus* egg lamins could in part result from in vitro redistribution during membrane isolation. Immunolocalization of the membrane binding sites for the lamins (which presumably are nonexchangeable integral proteins) in whole cells would be useful in this situation.

Studies with mammalian cultured cells showed that the NPC protein gp210 accumulates in the reforming NE at the end of mitosis subsequent to reassembly of the inner

membrane protein p58/LBR (Chaudhary and Courvalin, 1993). The authors interpreted this to mean that gp210 and p58/LBR are present in distinct vesicles in mitotic cells (Chaudhary and Courvalin, 1993). However, the data also are consistent with the possibility that these proteins are distributed throughout ER membranes in mitosis and accumulate in the NE asynchronously because of the asynchronous appearance of their appropriate binding sites in the reforming NE. Most pore complex assembly occurs during telophase and early G1 after assembly of nuclear membranes (e.g., Maul, 1977), and it is possible that the binding sites for gp210 appear in the NE only at the time of pore complex assembly. Immunoblot analysis indicated that the relative amounts of gp210 in a heavy vs light membrane fraction from metaphase cells were different from the relative amounts of p58/LBR in the same two membrane fractions (Chaudhary and Courvalin, 1993). Although these results could suggest that the two proteins differ somewhat in relative concentrations in heavy and light membrane vesicles in mitotic cells, they do not demonstrate that they are present in physically distinct vesicles. Similarly, although lamin B and p58/LBR appear to be more enriched in vimentin-associated vesicles in mitotic cells extracts compared to free vesicles (Maison et al., 1993), these results do not demonstrate the existence of an NE-specific vesicle population. In conclusion, previous studies have not clearly addressed the fate of NE proteins during mitosis. We believe that direct localization of NE proteins in intact cells, as we have presented in this study, is the only approach that can resolve this issue. It should be noted that integral membrane proteins of the NE conceivably may not be distributed throughout the ER in *Xenopus* eggs as we have found in mitotic mammalian cells because of the size differences between the two cells or some other reason.

Mechanism of Nuclear Envelope Disassembly and Reformation during Mitosis

Our findings directly demonstrate that integral membrane proteins of the NE are dispersed throughout the ER during mitosis, as summarized in Fig. 6. Two processes (alone or in combination) could lead to dispersion of NE proteins throughout the ER during prometaphase. In one scenario, the lamina and pore complexes could undergo disassembly before NE vesiculation and loss of morphological continuity between the NE and the peripheral ER. Upon release of integral membrane proteins from their binding sites at the NPC and lamina, the integral proteins could rapidly diffuse throughout the membrane bilayer of the continuous NE/ER system. In a second scenario, the NE could lose connections with the peripheral ER before disassembly of the NPC and lamina. In this case, integral proteins of the NE could become dispersed throughout the ER by a continuous fusion and fission among the disassembled membranes of the NE and ER, similar to the continuous fusion that occurs among tubular and cisternal elements of the interphase ER (Lee and Chen, 1988).

Our observations do not support models in which reassembly of the NE occurs by selective targeting of hypothetical NE-specific vesicles to the chromosome surfaces (Wilson and Newport, 1988; Chaudhary and Courvalin,
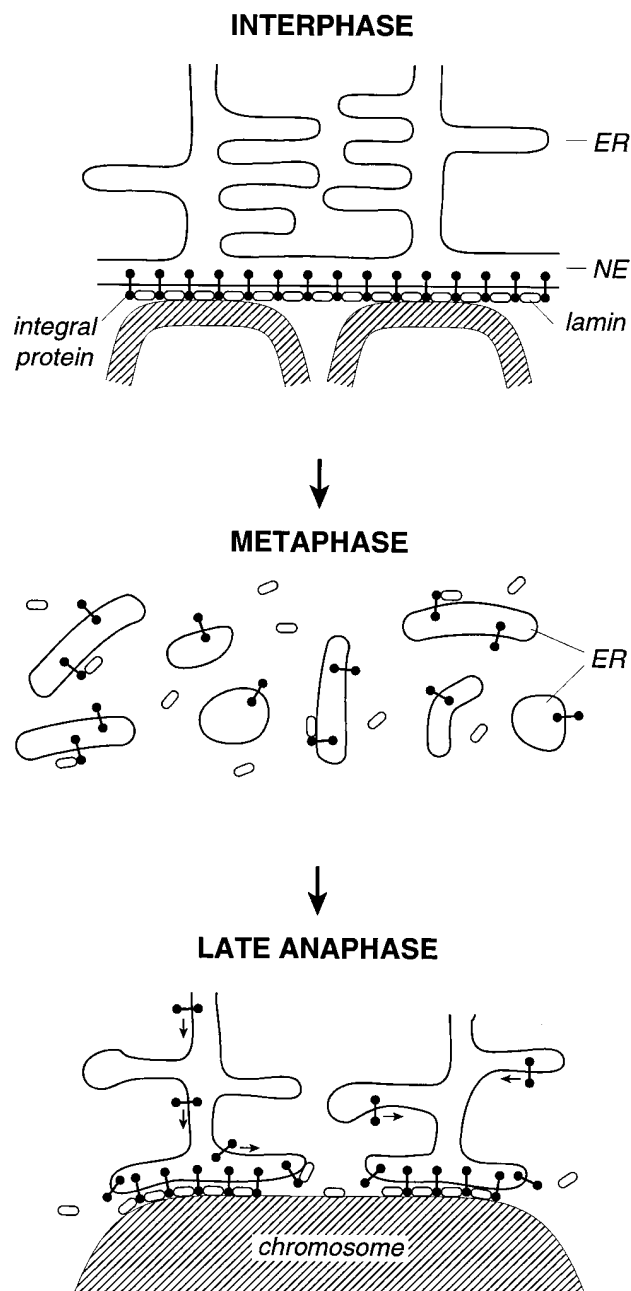

Figure 6. Model depicting the dynamics of integral membrane proteins of the NE during mitosis. The interphase NE is morphologically continuous with the peripheral ER but is a specialized ER subcompartment that contains integral membrane proteins specific to the inner nuclear membrane (*top*) and nuclear pore membrane (not shown). Integral proteins of the inner nuclear membrane may be bound to lamins, lamina-associated proteins, or chromatin. During mitosis when the NE is disassembled, integral membrane proteins of the NE are dispersed throughout all ER membranes, and the NE loses its identity as an ER subcompartment (*middle*). We propose that integral proteins are resorted to the NE during late anaphase by diffusion through a functionally continuous ER and subsequent association with binding sites at the chromosomes (*bottom*). The binding sites for inner membrane proteins may include lamins and chromatin (*bottom*); those for nuclear pore membrane proteins may include other pore complex components (not shown). These binding interactions would result in the net collection of nuclear membrane proteins at the reforming NE (*bottom, arrows*). Reformation of the NE may involve cooperative assembly of lamins and integral membrane proteins of the inner nuclear membrane (*bottom*).

1993). Rather, they strongly support the possibility that binding interactions at the chromosome surfaces account for the accumulation of integral membrane proteins at the reforming NE in late anaphase (Fig. 6). In a process analogous to that of NE disassembly (see above), integral membrane proteins could reach the reforming NE by two different mechanisms, working either separately or in concert. In one case, the ER could exist as a continuous reticulum in late anaphase, and integral proteins could accumulate in the reforming NE by rapid diffusion through the continuous ER bilayer to binding sites at the chromosome surfaces. Morphological evidence supports the existence of a highly interconnected ER reticulum in late mitotic cells (Robbins and Gonatas, 1964; Roos, 1973; Zeligs and Wollman, 1979; Stracke and Martin, 1991). Alternatively, a number of separate ER elements could exist in late anaphase cells, and integral proteins of the NE could be rapidly exchanged between these elements by continuous fusion/fission. After reaching the chromosome-associated nuclear membranes, integral nuclear membrane proteins could be removed from the diffusionally free pool by binding interactions.

We obtained evidence that in some late anaphase cells where LAPs are partially assembled around chromosomes, a fraction of LAPs becomes concentrated in localized regions of the peripheral ER away from the chromosome surfaces. However, we do not know the quantitative significance of this phenomenon, or whether LAPs are stably concentrated in these regions or are undergoing dynamic disassembly/assembly. Among other possibilities, LAPs could become concentrated in localized regions of the peripheral ER by a low level of lamin assembly at the ER membrane surface. NE proteins from these regions, either as aggregates or monomers, could become concentrated at the NE by the diffusion/binding model depicted in Fig. 6.

A diffusion/binding mechanism also could be responsible for assembly of integral membrane proteins at the inner nuclear membrane during interphase after their synthesis on the rough ER (see Wiese and Wilson, 1993). Integral membrane proteins have the capacity to rapidly move between outer and inner nuclear membranes (discussed by Gerace and Burke, 1988; see also Powell and Burke, 1990). Morphological studies have indicated that this diffusional exchange may occur around nuclear pore membranes, since pore complexes appear to contain ~10-nm-diam diffusional channels immediately adjacent to the pore membrane (Hinshaw et al., 1992). Diffusion of integral proteins to the inner membrane via the nuclear pore membrane may occur during the later stages of NE assembly at the end of mitosis as well as during interphase.

The accumulation of integral proteins of the inner nuclear membrane at the chromosome surfaces during NE reformation could be directed by binding to lamins, lamina-associated proteins, or chromatin. All three of the well-characterized inner nuclear membrane proteins of mammalian cells (LAP1, LAP2, and p58/LBR) interact directly with lamins, and two of these (LAP2 and p58/LBR) also appear to bind to chromatin directly (see introduction). In this study, we used confocal microscopy to refine the results of previous immunofluorescence microscopy on nuclear lamin reassembly and found that a portion of

the lamin pool assembles around late anaphase chromosomes at the same time as integral membrane proteins of the inner nuclear membrane. Thus, lamins, which themselves bind chromatin directly, are present at the reforming NE at an appropriate time to provide binding sites for integral membrane proteins (and vice versa).

Considered together, these observations indicate that NE reassembly in late anaphase is likely to be a highly cooperative process involving interactions of lamins and integral membrane proteins with the chromosome surfaces and each other (Fig. 6). From this perspective it seems likely that multiple proteins contribute to nuclear membrane assembly around chromosomes, and that individually many of these may be dispensable for this process. Antibody inhibition studies have indicated that lamins facilitate the association of membrane with chromosome surfaces (Burke and Gerace, 1986; Ulitzur et al., 1992), although the question of whether lamins are essential for this process is not resolved (discussed in Gerace and Foisner, 1994; Lourim and Krohne, 1994). It is likely that the abundance of individual inner membrane proteins and their binding affinities for chromosomes and other NE proteins will determine their relative importance in the pathway of NE reassembly.

In conclusion, our data indicate that the NE loses its identity as a specialized subcompartment of the ER during mitosis, when integral membrane proteins that are highly concentrated in the NE during interphase become dispersed throughout ER membranes. Our findings strongly support the possibility that NE reassembly involves diffusion of integral membrane proteins through a functionally continuous ER and subsequent accumulation at the chromosome surfaces by binding interactions. It is plausible that a similar diffusion/binding mechanism is used by cells to reconstitute other subcompartments of the ER (e.g., rough and smooth ER) that may become disassembled during mitosis.

References

Burke, B. 1990. On the cell-free association of lamins A and C with metaphase chromosomes. *Exp. Cell Res.* 186:169–176.

Burke, B., and L. Gerace. 1986. A cell-free system to study reassembly of the nuclear envelope at the end of mitosis. *Cell.* 44:639–652.

Chaudhary, N., and J.C. Courvalin. 1993. Stepwise reassembly of the nuclear envelope at the end of mitosis. *J. Cell Biol.* 122:295–306.

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

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

Georgatos, S.D., J. Meier, and G. Simos. 1994. Lamins and lamin-associated proteins. *Curr. Opin. Cell Biol.* 6:347–353.

Gerace, L., and B. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335–374.

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

Gerace, L., Y. Ottaviano, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826–837.

Glass, J.R., and L. Gerace. 1990. Lamins A and C bind and assemble at the surface of mitotic chromosomes. *J. Cell Biol.* 111:1047–1057.

Greber, U.F., A. Senior, and L. Gerace. 1990. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large lumenal domain and a small cytoplasmic tail. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1495–1502.

Griffiths, G., S.D. Fuller, R. Back, M. Hollinshead, S. Pfeiffer, and K. Simons. 1989. The dynamic nature of the Golgi complex. *J. Cell Biol.* 108:277–297.

Guan, T., S. Muller, G. Klier, N. Pante, J.M. Blevitt, M. Haner, B. Paschal, U. Aebi, and L. Gerace. 1995. Structural analysis of the p62 complex, an assembly of O-linked glycoproteins that localizes near the central gated channel of the nuclear pore complex. *Mol. Biol. Cell.* 6:1591–1603.

Hallberg, E., R.W. Wozniak, and G. Blobel. 1993. An integral membrane protein of the pore membrane domain of the nuclear envelope contains a nucleoporin-like region. *J. Cell Biol.* 122:513–521.

Hinshaw, J., B. Carragher, and R. Milligan. 1992. Architecture and design of the nuclear pore complex. *Cell.* 69:1133–1141.

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

Lee, C., and L.B. Chen. 1988. Behavior of endoplasmic reticulum in living cells. *Cell.* 54:37–46.

Lourim, D., and G. Krohne. 1993. Membrane-associated lamins in *Xenopus* egg extracts: identification of two vesicle populations. *J. Cell Biol.* 123:501–512.

Lourim, D., and G. Krohne. 1994. Lamin-dependent nuclear envelope reassembly following mitosis: an argument. *Trends Cell Biol.* 4:314–318.

Louvard, D., H. Reggio, and G. Warren. 1982. Antibodies to the Golgi complex and the rough endoplasmic reticulum. *J. Cell Biol.* 92:92–107.

Maison, C., H. Horstmann, and S.D. Georgatos. 1993. Regulated docking of nuclear membrane vesicles to vimentin filaments during mitosis. *J. Cell Biol.* 123:1491–1505.

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

Maul, G.G. 1977. The nuclear and the cytoplasmic pore complex: structure, dynamics, distribution and evolution. *Int. Rev. Cytol.* 6(Suppl.):75–186.

Melchior, F., T. Guan, N. Yokoyama, T. Nishimoto, and L. Gerace. 1995. GTP hydrolysis by Ran occurs at the nuclear pore complex in an early step of protein import. *J. Cell Biol.* 131:1–11.

Newport, J.W., and W. Dunphy. 1992. Characterization of the membrane binding and fusion events during nuclear envelope assembly using purified components. *J. Cell Biol.* 116:295–306.

Nigg, E. 1992. Assembly-disassembly of the nuclear lamina. *Curr. Opin. Cell Biol.* 4:105–109.

Padan, R., S. Nainudel-Epszteyn, R. Goitein, A. Fainsod, and Y. Gruenbaum. 1990. Isolation and characterization of the *Drosophila* nuclear envelope otefin cDNA. *J. Biol. Chem.* 265:7808–7813.

Porter, K.R., and R.D. Machado. 1960. Studies on the endoplasmic reticulum IV: its form and distribution during mitosis in cells of onion root tip. *J. Biophys. Biochem. Cytol.* 7:167–180.

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

Robbins, E., and N.K. Gonatas. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* 21:429–463.

Roos, U.-P. 1973. Light and electron microscopy of rat kangaroo cells in mitosis 1: formation and breakdown of the mitotic apparatus. *Chromosoma (Berl.).* 40:43–82.

Rout, M.P., and S.R. Wente. 1994. Pores for thought: nuclear pore complex proteins. *Trends Cell Biol.* 4:357–365.

Senior, A., and L. Gerace. 1988. Integral membrane proteins specific to the inner nuclear membrane and associated with the nuclear lamina. *J. Cell Biol.* 107:2029–2036.

Stracke, S., and R. Martin. 1991. Postmitotic reassembly of the cell nucleus in whole cells: on electron-spectroscopic study. *J. Cell Sci.* 100:541–550.

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

Terasaki, M., and T.S. Reese. 1992. Characterization of endoplasmic reticulum by co-localization of BiP and dicarbocyanine dyes. *J. Cell Sci.* 101:315–322.

Ulitzur, N., A. Harel, N. Feinstein, and Y. Gruenbaum. 1992. Lamin activity is essential for nuclear envelope assembly in a *Drosophila* embryo cell-free extract. *J. Cell Biol.* 119:17–25.

Vaux, D., J. Tooze, and S. Fuller. 1990. Identification by anti-idiotype antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. *Nature (Lond.).* 345:495–502.

Velasco, A., L. Hendricks, K.W. Moremen, D.R.P. Tulsiani, O. Touster, and M.G. Farquhar. 1993. Cell type–dependent variations in the subcellular distribution of α-mannosidase I and II. *J. Cell Biol.* 122:39–51.

Vigers, G.P.A., and M.J. Lohka. 1991. A distinct vesicle population targets membranes and pore complexes to the nuclear envelope in *Xenopus* eggs. *J. Cell Biol.* 112:545–556.

Ware, F.E., A. Vassilakos, P.A. Peterson, M.R. Jackson, M.A. Lehrman, and D.B. Williams. 1995. The molecular chaperone calnexin binds $Glc_1Man_9GlcNAc_2$ oligosaccharide as an initial step in recognizing unfolded glycoproteins. *J. Biol. Chem.* 270:4697–4704.

Warren, G., and W. Wickner. 1996. Organelle inheritance. *Cell.* 84:395–400.

Wiese, C., and K.L. Wilson. 1993. Nuclear membrane dynamics. *Curr. Opin. Cell Biol.* 5:387–394.

Wilson, K.L., and J. Newport. 1988. A trypsin-sensitive receptor on membrane vesicles is required for nuclear envelope formation in vitro. *J. Cell Biol.* 107: 57–68.

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

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

Wozniak, R.W., E. Bartnik, and G. Blobel. 1989. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. *J. Cell Biol.* 108: 2083–2092.

Ye, Q., and H.J. Worman. 1996. Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to *Drosophila* HP1. *J. Biol. Chem.* 271:14653–14656.

Yuan, J., G. Simos, G. Blobel, and S.D. Georgatos. 1991. Binding of lamin A to polynucleosomes. *J. Biol. Chem.* 266:9211–9215.

Zeligs, J.D., and S.H. Wollman. 1979. Mitosis in rat thyroid epithelial cells in vivo 1: ultrastructural changes in cytoplasmic organelles during the mitotic cycle. *J. Ultrastruct. Res.* 66:53–77.