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ORGANIZATION AND MODULATION OF NUCLEAR LAMINA STRUCTURE

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SUMMARY

The nuclear lamina is a protein meshwork associated with the nucleoplasmic surface of the inner nuclear membrane, that is suggested to be important for organizing nuclear envelope and interphase chromosome architecture. To investigate the structural organization of the lamina, we have analysed rat liver nuclear envelopes by various chemical extraction procedures. From these studies, we have defined conditions that yield a nuclear envelope subfraction that is both highly enriched in the lamina and devoid of pore complexes. This fraction contains mostly lamins A, B and C, the three major lamina polypeptides that are apparently arranged in a polymeric assembly. Our chemical extraction studies also indicate that lamin B has a stronger interaction with nuclear membranes than the other two lamins, and support the possibility that lamin B is important for attaching the lamina to the inner nuclear membrane.

We have examined the synthesis and assembly of the lamins during interphase in tissue-culture cells to investigate lamina structure by a second approach. We found that all three lamins are synthesized at similar rates throughout the cell cycle in synchronized Chinese hamster ovary cells, and that their biosynthesis is not temporally coupled to DNA replication. Our studies indicate that newly synthesized lamins are rapidly assembled into an insoluble lamina structure but that the apparent half-time for lamina insertion differs for individual lamins. We have also observed that lamin A is synthesized as an apparent precursor molecule that is converted to mature lamin A only after integration into the lamina structure.

The lamina is reversibly depolymerized during cell division, a process that may be mediated by enzymic phosphorylation of the lamins. To investigate this possibility further, we have analysed charge-altering modifications of the lamins on two-dimensional gels, and have found that phosphorylation is the only detectable modification of these proteins that occurs specifically during mitosis. Furthermore, we have determined that when the lamins are disassembled during metaphase, each lamin has approximately 2 moles of associated phosphate/mole lamin, a value that is four to sevenfold higher than the average interphase level. Considering this information, we discuss a model by which depolymerization and reassembly of the lamina can regulate the reversible disassembly of the nuclear envelope during mitosis.

INTRODUCTION

The nuclear envelope is a complex membrane organelle that delimits the boundary of the nuclear compartment in eukaryotic cells (reviewed by Franke, 1974; Fry, 1976; Franke, Scheer, Krohne & Jarasch, 1981). This membrane structure regulates the nucleocytoplasmic movement of large macromolecules (including many protein and RNA species), and may be important for organizing interphase chromosome architecture. The major structural components of the nuclear envelope include inner and outer nuclear membranes, pore complexes and the nuclear lamina.

Pore complexes, which occur at regions where the inner and outer membranes are joined, are elaborate protein assemblies that provide channels through the nuclear

envelope for nucleocytoplasmic molecular exchange (Franke, 1974; Maul, 1977). The nuclear lamina (Gerace & Blobel, 1982) is a protein meshwork closely opposed to the nucleoplasmic surface of the inner nuclear membrane that forms a shell-like structure at the nuclear periphery. The lamina is postulated to provide a framework for organization and regulation of nuclear envelope structure (Gerace, Blum & Blobel, 1978; Gerace & Blobel, 1982), and an attachment site in the interphase nucleus (Gerace *et al.* 1978; Hancock & Hughes, 1982; Lebkowski & Laemmli, 1982) for higher order chromatin domains (Benyajati & Worcel, 1976; Cook & Brazell, 1976). In this fashion, the lamina may be a major skeletal component of the interphase nucleus.

A nuclear lamina can be directly visualized by thin-section electron microscopy in a number of eukaryotic cells, where it appears as a discrete 30–100 nm layer interposed between the inner nuclear membrane and chromatin (Fawcett, 1966; for other references, see Gerace *et al.* 1978). In many cell types, however, the lamina is too thin to be detected ultrastructurally in intact cells or nuclei, and can be observed only after partial nuclear subfractionation (e.g. see Aaronson & Blobel, 1975; Scheer *et al.* 1976).

Chemical extraction studies indicate that the lamina of many cell types is a stable supramolecular protein assembly that remains intact during sequential treatment of isolated nuclear envelopes with buffers containing non-ionic detergents and high concentrations of monovalent salts (Dwyer & Blobel, 1976; Shelton *et al.* 1980; Krohne, Dabauvalle & Franke, 1981). A lamina-enriched fraction that has been isolated from rat liver nuclear envelopes by detergent and salt extraction (the 'pore complex–lamina fraction'; Dwyer & Blobel, 1976) contains three prominent 60 000–70 000 molecular weight polypeptides that comprise approximately 40 % of the total protein mass of this fraction. Immunocytochemical localization studies indicate that these polypeptides (lamins A, B and C) occur exclusively at the nuclear periphery during interphase (Gerace *et al.* 1978; Krohne *et al.* 1978*a*; Stick & Hausen, 1980), and that they are localized in the lamina and not in nuclear pore complexes (Gerace & Blobel, 1982; Gerace, Ottaviano & Kondor-Koch, 1982). These three lamins may be organized in a polymeric array that serves as a structural 'core' for the lamina (Gerace & Blobel, 1980).

Three analogous lamins have been detected by immunochemical and biochemical techniques in a variety of vertebrate somatic and tissue-culture cells (Shelton *et al.* 1980; Gerace & Blobel, 1982; Havre & Evans, 1983), indicating that these polypeptides are common constituents of the vertebrate nuclear lamina. The three lamins apparently represent members of a family of related proteins, since two of these polypeptides (lamins A and C) are structurally very similar, as shown by peptide mapping studies (Shelton *et al.* 1980; Gerace & Blobel, 1982; Kaufman, Gibson & Shaper, 1983), and since all three lamins cross-react with certain monoclonal antibodies (Burke, Tooze & Warren, 1983; Krohne *et al.* 1984). However, the functional specializations of individual lamins have not been precisely defined.

In contrast to many vertebrate somatic cells, which have three related lamins, certain higher eukaryotic cell types appear to have a lamina structure that contains

only one (in clam oocytes (Maul & Avdaloric, 1980) and amphibian oocytes (Krohne, Franke & Scheer, 1978*b*)) or two (in *Xenopus* erythrocytes (Krohne *et al.* 1981)) major lamin-related polypeptides. The biochemical composition of the lamina in evolutionarily lower organisms has not been precisely described. However, a single (or two closely related) approximately 75 000 M_r polypeptides found in *Drosophila* cells (Risau, Saumweber & Symmons, 1981; Fisher, Berrios & Blobel, 1982; Fuchs *et al.* 1983) are candidates for being *Drosophila* lamins.

Important information concerning the relationship of the lamina to nuclear architecture has been obtained from studies of tissue-culture cells in mitosis, the period of the cell cycle when the nucleus (and nuclear envelope) of higher eukaryotes is disassembled and subsequently reconstructed (see Fry, 1976). Immunocytochemical (Ely, D'Arcy & Jost, 1978; Gerace *et al.* 1978; Krohne *et al.* 1978*a*) and subcellular fractionation (Gerace & Blobel, 1980) methods have demonstrated that the lamins in these cells are disassembled to a monomeric state and dispersed throughout the cell during mitotic prophase, and subsequently reassembled at the surfaces of the daughter cell nuclei in telophase. These changes occur concomitantly with the processes of nuclear envelope disassembly and reformation, respectively. Based on the biochemical and structural characteristics described for the interphase lamina, the reversible mitotic depolymerization of the lamina is proposed to regulate the disassembly and reconstruction of the nuclear envelope during cell division (Gerace *et al.* 1978). Similarly, growth in the mass or surface area of the lamina during interphase (e.g. by synthesis and insertion of new lamins) may be a major factor in determining the increase in nuclear envelope surface area that occurs during the cell cycle.

In this paper, we describe recent progress that we have made in understanding the biochemical organization of the nuclear lamina and its association with nuclear membranes. Furthermore, we consider events associated with synthesis and assembly of the lamins during interphase, and the relationship of lamin phosphorylation to regulation of lamina structure during cell division. Finally, we discuss a mechanism by which reversible disassembly of the lamina polymer during mitosis can regulate the major physical events of nuclear envelope disassembly and reconstruction.

MATERIALS AND METHODS

Preparation and fractionation of rat liver nuclear envelopes

All fractionation procedures involving nuclear envelopes were performed at 0–4 °C. Rat liver nuclear envelopes were isolated by digesting purified rat liver nuclei with DNase I (Sigma, DN-EP grade) in a low ionic strength, low magnesium buffer as described (Dwyer & Blobel, 1976), except that all preparative solutions contained 0·0005 M-phenylmethylsulphonyl fluoride (PMSF) (freshly added to buffers from a 0·1 M-PMSF stock solution in ethanol) and 0·001 M-dithiothreitol (DTT). Also, RNase A (Sigma, type XII) was added during the two DNase digestion steps to a final concentration of 1 μg/ml. Stock solutions of DNase I (1 mg/ml) and RNase A (5 mg/ml) were prepared by dissolving enzymes in TKM buffer (Dwyer & Blobel, 1976) containing PMSF and incubating solutions for 1 h at 0 °C before dividing into samples and freezing in liquid N_2.

We refer to the 'D_2p' fraction of Dwyer & Blobel (1976) as 'crude nuclear envelopes' in this paper. Salt-washed nuclear envelopes were prepared by resuspending the crude nuclear envelope pellet to approximately 2 mg/ml protein in 10% (w/v) sucrose, 0·01 M-triethanolamine·HCl (pH 7·4),

0·0001 M-$MgCl_2$ and 0·001 M-DTT and adding an equal volume of 10% sucrose, 0·01 M-triethanolamine·HCl (pH 7·4), 2 M-KCl, 0·0001 M-$MgCl_2$, 0·001 M-DTT. Samples were then centrifuged for 15 min at 2500 rev/min in a Beckman JS 5·2 rotor through a cushion containing 30% sucrose, 0·01 M-triethanolamine·HCl (pH 7·4), 0·0001 M-$MgCl_2$ and 0·001 M-DTT, to yield a pellet of salt-washed nuclear envelopes.

For preparation of a lamina-enriched fraction (Fig. 1), salt-washed nuclear envelopes were resuspended to a concentration of approximately 1 mg/ml protein in 10% sucrose, 0·02 M-triethanolamine·HCl (pH 7·4), 0·005 M-$MgCl_2$ and 0·001 M-DTT, and to this sample was added an equal volume of 10% sucrose, 0·02 M-triethanolamine·HCl (pH 7·4), 0·6 M-KCl, 0·005 M-$MgCl_2$, 0·001 M-DTT. After a 30-min incubation, the sample was centrifuged for 15 min at 5000 rev./min in a Beckman JS 5·2 rotor to yield a pellet of lamina-enriched material. We observed that if a reducing agent such as DTT was omitted from solutions during nuclear envelope preparation and subfractionation, the solubilization of minor nuclear envelope polypeptides by the Triton/0·3 M-KCl step (Fig. 1) was incomplete, presumably due to *in vitro* oxidation of these polypeptides.

Chemical extraction of rat liver nuclear envelopes (Fig. 3) was performed by resuspending pellets of 0·5 M-KCl-washed nuclear envelopes in the appropriate extraction solutions to approximately 1 mg/ml protein, and subsequently centrifuging samples at 48 000 rev./min for 30 min in a Beckman 50 Ti rotor. $MgCl_2$ and sodium carbonate samples were incubated for 30 min before centrifugation, while the remaining samples were centrifuged immediately after resuspension. Extraction solutions contained: (1) 0·05 M-triethanolamine·HCl (pH 7·4), 0·25 M-$MgCl_2$, 0·001 M-DTT; (2) 0·1 M-sodium carbonate (pH 10·5), 0·001 M-DTT; (3) 0·02 M-NaOH, 0·001 M-DTT; or (4) 0·05 M-triethanolamine·HCl (pH 7·4), 1 M-guanidine·HCl.

Electron microscopy

To examine the ultrastructure of material derived from nuclear envelopes treated with Triton X-100 in low or high ionic strength solution (Fig. 2), pellets of crude nuclear envelopes were resuspended in 10% sucrose, 0·02 M-triethanolamine·HCl (pH 7·4), 0·005 M-$MgCl_2$ and 0·001 M-DTT at 2 mg/ml protein. We then added to separate samples of this material an equal volume of: (1) 2% Triton X-100, 0·02 M-triethanolamine·HCl (pH 7·4), 0·005 M-$MgCl_2$ and 0·001 M-DTT (low ionic strength sample) or (2) 2% Triton, 0·02 M-triethanolamine, 0·6 M-KCl, 0·005 M-$MgCl_2$ and 0·001 M-DTT (high ionic strength sample), mixed the capped tubes by gently inverting, and incubated samples for 30 min at 0 °C.

Subsequently, to these samples we added (with gentle mixing as above) an equal volume of solutions containing either: (1) 5% glutaraldehyde, 0·02 M-triethanolamine (pH 7·4), 0·005 M-$MgCl_2$ and 0·001 M-DTT (low ionic strength sample) or (2) 5% glutaraldehyde, 0·02 M-triethanolamine (pH 7·4), 0·3 M-KCl, 0·005 M-$MgCl_2$ and 0·001 M-DTT (high ionic strength sample), and fixed the material for 60 min at 0 °C. Samples were then pelleted at 10 000 ***g***, postfixed for 60 min at 0 °C with 1% OsO_4 in veronal acetate buffer (Farquhar & Palade, 1955), stained *en bloc* for 60 min at room temperature with 0·5% uranyl acetate in veronal acetate buffer, dehydrated with a graded ethanol series, and finally embedded in EMBED 812 (Polysciences). Thin sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Venable & Coggleshall, 1965) and examined at 80 kV in a Zeiss 10A electron microscope.

Cell culture and synchrony

Chinese hamster ovary (CHO) cells, obtained from R. Tobey (University of California at Los Alamos, New Mexico), and BRL cells, a rat liver cell line obtained from H. Coon (National Institutes of Health, Bethesda, MD) were grown in monolayer culture at 37 °C in modified (Tobey, Anderson & Petersen, 1967) Ham's F10 medium containing 10% (w/v) foetal calf serum (GIBCO), 0·02 M-HEPES buffer, 100 U/ml penicillin and 100 μg/ml streptomycin. HEPES buffer and antibiotics were also included in all culture media described below.

To obtain synchronized mitotic CHO cells for analysing biosynthesis of the lamins through the cell cycle (Fig. 4), we plated 5×10^6 cells/dish in T-150 culture flasks (Corning) and allowed cells to grow for approximately 30 h. Culture medium was then replaced with Ham's growth medium containing 0·002 M-thymidine (Bostock, Prescott & Kirkpatrick, 1971) to accumulate cells in early–mid *S* phase. After 11 h, the thymidine medium was replaced with normal Ham's growth medium lacking thymidine, and cultures were allowed to grow for another 5 h, when a wave of

mitotic cells began to appear. At this point, metaphase cell populations were obtained by selective mechanical detachment ('shake-off', Tobey *et al.* 1967). Cells were harvested from flasks every 15 min and were immediately chilled to 0 °C in an ice bath to arrest cell cycle progress. The pooled metaphase cells obtained over a total period of about 3 h were then divided and placed in 60 mm Petri dishes (Corning) and returned to 37 °C culture. With this synchrony procedure, virtually all cells attached to the Petri dishes and were in early G_1 phase 1 h after return to culture.

For pulse-labelling cells with [^{35}S] methionine, growth medium was aspirated from individual dishes at various times after metaphase, dishes were rinsed with modified Ham's F10 medium (methionine minus) +10 % dialysed foetal calf serum and medium was replaced with modified Ham's F10 medium (methionine minus) +10 % dialysed foetal calf serum +100 μCi/ml [^{35}S]-methionine (Amersham). Dishes were then returned to culture for 30 min, and labelled cells were subsequently harvested by scraping into 1 ml (for 2×10^6 cells) of 0·4 % sodium dodecyl sulphate (SDS), 0·05 M-triethanolamine (pH 7·4), 0·1 M-NaCl and 0·002 M-EDTA. To quantify the rate of total protein synthesis in cell populations at the various times, harvested cell samples were sonicated briefly, and a small portion of each solubilized sample (0·004 ml) was added to 1 ml of 1 mg/ml bovine serum albumin, which was then precipitated at 0 °C by the addition of 1 ml of 20 % trichloroacetic acid (TCA). Precipitates were subsequently collected on glass fibre disks (Whatman GF-C filters) for scintillation counting. The remainder of the labelled samples were frozen in liquid N_2 until use for immunoprecipitation.

The rate of DNA replication in cell populations at various times after mitosis was measured by labelling separate cell samples for 30 min with normal Ham's F10 medium +10 % foetal calf serum +1 μCi/ml [^{3}H]thymidine (Amersham). Labelled cells were scraped into phosphate-buffered saline (PBS), precipitated at 0 °C with 10 % TCA, and filtered onto glass fibre discs for scintillation counting as described above.

BRL cells were used for pulse–chase labelling of the lamins (Fig. 7). Exponentially growing cultures in 60 mm Petri dishes (approx. 2×10^6 cells/dish) were incubated in modified Ham's F10 (methionine minus) +10 % dialysed foetal calf serum for 30 min before pulse-labelling. Dishes were then pulse-labelled for 5 min in modified Ham's F10 medium (methionine minus) +10 % dialysed foetal calf serum +500 μCi/ml [^{35}S]methionine, and were subsequently chased for various periods of time (0–240 min) in Ham's F10 medium +10 % foetal calf serum +5 mM-L-methionine. Cells were then harvested by removing the medium, cooling dishes to 0 °C (all subsequent steps were at 0 °C), rinsing well with PBS, and scraping cells directly into 1 % Triton, 0·02 M-triethanolamine-HCl (pH 7·4), 0·13 M-NaCl, 0·0025 M-$MgCl_2$, 0·0005 M-PMSF and 0·005 M-iodoacetamide. After gentle vortexing for approximately 10 s, samples were immediately centrifuged for 5 min in an Eppendorf microfuge (approx. 15 000 g_{max}) to obtain supernatants and pellets. These samples were then precipitated for 1 h at 0 °C with 10 % TCA, pelleted, and extracted for 30 min at 0 °C with 90 % (v/v) acetone, 0·1 M-HCl (to remove Triton X-100). Following a second pelleting, samples were solubilized in SDS solution for immunoprecipitation analysis (see below).

Radioactively labelled CHO metaphase cells that were used for two-dimensional gel analysis (Fig. 6) were obtained by maintaining cultures for 11 h in modified Ham's F10 medium (methionine minus) containing 10 % non-dialysed foetal calf serum, 2·5 mg/l L-methionine (half the normal methionine concentration), 10 μCi/ml [^{35}S]methionine and 0·002 M-thymidine. After this period, the thymidine medium was removed and replaced for 5 h with radioactive Ham's medium lacking thymidine. Metaphase populations were then selected by shake-off as described above. Interphase cell populations for this experiment were obtained by maintaining exponentially growing cultures for 16 h in modified Ham's F10 medium (methionine minus) containing 10 % non-dialysed foetal calf serum, 2·5 mg/l L-methionine and 10 μCi/ml [^{35}S]methionine.

The absolute level of phosphorylation of the lamins during interphase and metaphase (Table 1) was determined using CHO cells labelled to steady state with [^{32}P]phosphate. For these experiments, we continuously labelled CHO cells for 48 h in Ham's F10 medium (phosphate minus) containing 20 % dialysed foetal calf serum, $1\cdot7\times10^{-4}$ M-sodium phosphate (pH 7·4) and 2·5 μCi/ml [^{32}P]phosphate (Amersham). Metaphase cells were obtained by shake-off synchronization from thymidine presynchronized labelled cultures (see above). In control experiments, we found the same apparent levels of lamin-associated phosphate (see below) in interphase cells that had been labelled for either 36 h or 60 h, indicating that we were at steady state for incorporation of labelled phosphate into the lamins in this experiment (48 h label).

Immunoprecipitation and gel analysis

For immunoprecipitation, we used guinea pig antibodies raised against electrophoretically purified rat liver lamins. Procedures for immunization and antigen preparation were similar to those previously described for chickens (Gerace *et al.* 1978), except that complete Freund's adjuvant was used for only the initial immunization, and incomplete Freund's adjuvant was used for all subsequent injections. Generally, antibodies from animals injected with either lamin A or lamin C reacted strongly on immunoblots with both lamins A and C, but only weakly with lamin B. These antibodies are designated 'anti-lamins (A and C)'. Similarly, antibodies from animals injected with lamin B reacted strongly with lamin B, but only weakly with lamins A and C. These are designated 'anti-lamin B'. All antibodies were affinity-purified by applying serum to a column consisting of SDS-solubilized rat liver pore complex–lamina proteins conjugated to Sepharose 4B, and eluting bound antibodies with glycine·HCl (pH 2·2) (Gerace *et al.* 1982).

Cells or cell fractions used for immunoprecipitation of the lamins in Figs 4, 5, and Table 1 were first solubilized by incubating samples in a boiling water bath for 3 min at a concentration of 4×10^6 cells/ml in a solution containing 0·4% SDS, 0·05 M-triethanolamine (pH 7·4), 0·1 M-NaCl and 0·002 M-EDTA. Samples were then cooled, sonicated briefly with a Branson sonifier (equipped with a microtip probe) to reduce sample viscosity, and centrifuged for 5 min in an Eppendorf microfuge to remove insoluble material. Next, to each 0·5 ml of solubilized cell sample we sequentially added: 0·05 ml of 20% Triton X-100, 0·005 ml of Trasylol, 0·001 ml of a 1 mg/ml leupeptin +1 mg/ml pepstatin solution (in dimethylsulphoxide), 10 μg of affinity-purified anti-lamin (A and C) antibodies and 5 μg of affinity-purified anti-lamin B antibodies. For the experiment shown in Fig. 5, we used anti-lamin (A and C) antibodies only. Samples were incubated for 5–16 h at 4 °C, and were subsequently mixed for 2–4 h at 4 °C with either 0·02 ml of packed protein A–Sepharose beads (Pharmacia), or with 0·02 ml of beads containing rabbit anti-guinea pig immunoglobulin G (IgG) antibodies conjugated to Sepharose 4B at a concentration of 10 mg IgG/ml beads (Gerace *et al.* 1982). Immunoprecipitate beads were then washed batchwise six times with 1 ml of 0·5% Triton X-100, 0·1% SDS, 0·05 M-triethanolamine (pH 7·4), 0·1 M-NaCl and 0·002 M-EDTA, and were washed twice more with a solution of 0·01 M-triethanolamine (pH 7·4). Finally, immunoprecipitates were eluted from immunoadsorbent beads by incubating beads for 15 min at 37 °C with 0·06 ml of 15% sucrose, 3% SDS, 0·05 M-Tris (pH 8·8), 0·002 M-EDTA and 0·01% Bromphenol Blue. Beads were then removed by centrifugation, and eluted samples were given an addition of 0·006 ml of 1 M-DTT, incubated for 3 min in a boiling water bath, and electrophoresed on SDS/7·5% to 15% polyacrylamide gels (Gerace *et al.* 1978). Gels were visualized by fluorography (Bonner & Laskey, 1974), and radioactivity in individual lamin gel bands from immunoprecipitates (Table 1 and Fig. 4) was quantified as described (Gerace & Blobel, 1980).

To measure the absolute level of lamin phosphorylation in immunoprecipitates of metaphase or interphase CHO cells labelled to steady state with [^{32}P]phosphate (see above), immunoprecipitated lamins from approximately 1×10^7 cells were electrophoresed in a single gel lane. The gel was then stained with Coomassie Blue, the amount of protein in each lamin band was quantified by eluting the bound dye from gel slices with 25% pyridine and reading the absorbance at 605 nm (Fenner, Trout, Mason & Wikman-Doffelt, 1975). Samples of bovine serum albumin electrophoresed on the same gel were used as protein standards. Following elution of dye from excised gel bands, the ^{32}P radioactivity in each slice was determined as described (Gerace & Blobel, 1980). Based on the specific activity of the [^{32}P]phosphate present in our growth medium, we were able to calculate the moles of phosphate/mole of lamin.

Samples that were immunoprecipitated for two-dimensional gel electrophoresis (Fig. 6) were initially solubilized in the 0·4% SDS solution (see above) by incubation for 10 min at 60 °C, instead of by boiling. The remainder of the immunoprecipitation procedure up to the final elution step was identical to that described above. Material (from 5×10^6 cells) was eluted from immunoadsorbent beads (0·05 ml) by incubation in 0·1 ml of 0·2% SDS for 15 min at 37 °C followed by centrifugation to remove the beads. Eluted samples were then brought to a final sample composition (using appropriate stock solutions) of 1% octylglucoside, 0·1% SDS, 0·1 M-Tris·HCl (pH 8·0), 0·005 M-$MgCl_2$, 1% Trasylol, 2 μg/ml leupeptin and 2 μg/ml pepstatin in a final volume of 0·2 ml. To one half of each sample *Escherichia coli* alkaline phosphatase (Sigma type III) was added to 200 μg/ml, using a 2 mg/ml stock solution. Subsequently, both the latter sample and a control

sample (minus alkaline phosphatase) were incubated at 37 °C for 3 h. Samples were then cooled to 0 °C, precipitated for 2 h in 20 % TCA and pelleted. After the pellets were rinsed with 5 % TCA, samples were prepared for two-dimensional gels. This involved incubating pellets in 0·025 ml of 1 % SDS, 9·5 M-urea, 2 % LKB ampholines (pH 3·5–10), 0·001 M-EDTA and 0·05 M-DTT for 15 min at 37 °C, followed by adding 0·025 ml of 10 % Nonidet-P40, 2 % ampholines (pH 3·5–10) and 9 M-urea. Samples were applied to the acidic end of a non-equilibrium pH gradient electrophoresis slab gel containing 2 % LKB 3·5–10 range ampholines (O'Farrell, Goodman & O'Farrell, 1977), and were separated at 150 V constant voltage for 21 h (3150 volt-hours). Second-dimensional SDS/polyacrylamide gel electrophoresis and fluorography were performed as described (Gerace & Blobel, 1980).

RESULTS AND DISCUSSION

Chemical fractionation of nuclear envelopes

We have examined the effects of a variety of chemical extraction procedures on isolated rat liver nuclear envelopes to investigate the organization and biochemical composition of the lamina. Previous studies have demonstrated that both nuclear pore complexes and the lamina remain morphologically intact (Aaronson & Blobel, 1974; Scheer *et al.* 1976; Unwin & Milligan, 1982) when treated with low ionic strength buffers containing Triton X-100 (which solubilize the nuclear membrane lipids). Correspondingly, many nuclear envelope polypeptides, including the lamins, are insoluble under these conditions and fractionate completely in the pellet derived from this Triton/low ionic strength treatment, as determined by SDS/polyacrylamide gel electrophoresis (Dwyer & Blobel, 1976). The pore complexes and lamina also appear to retain moderate ultrastructural integrity when treated with buffers containing high concentrations of monovalent salts (e.g. 1 M-NaCl) that lack Triton X-100 (Dwyer & Blobel, 1976; Unwin & Milligan, 1982).

In contrast to these conditions, we have determined that treatment of nuclear envelopes with buffers containing both Triton X-100 and high concentrations of monovalent salts results in preferential solubilization of nuclear pore complex structures, while the lamina remains preferentially intact (Figs 1 and 2). We have defined Triton/high salt extraction conditions (2 % Triton +0·3 M-KCl) under which most nuclear envelope polypeptides are completely solubilized and appear in the supernatant after centrifugation, while the lamins appear almost quantitatively in a 5000 ***g*** pellet (Fig. 1, 2 % Triton +0·3 M-KCl, s and p lanes). In solutions containing Triton and higher concentrations of monovalent salts (e.g. 0·5–1 M-KCl), the lamins themselves are partially or completely solubilized (data not shown) indicating that the rat liver lamina has a relative but not absolute resistance to chemical solubilization in Triton/high salt solutions compared to pore complexes.

The pellet fraction derived from treatment of nuclear envelopes with Triton +0·3 M-KCl (Fig. 1; 2 % Triton+0·3 M-KCl, p lanes) contains a number of minor bands in the 40 000–55 000 M_r range in addition to the lamins. These minor polypeptides are not insoluble by virtue of being physically associated with the lamina, since certain treatments of the Triton/0·3 M-KCl pellet fraction (e.g. Triton +1 M-KCl) result in preferential solubilization of the lamins, while these other bands sediment in the pellet (data not shown). These solubility characteristics are consistent with

the possibility that many or all of these minor bands represent polypeptides of intermediate filaments that contaminate our nuclear envelope fraction (see Fig. 1 legend).

While a supramolecular structure containing nuclear pore complexes attached to the lamina is clearly evident in electron micrographs of nuclear envelopes treated with 2% Triton in a low ionic strength buffer (Fig. 2A,B), only a lamina structure with no identifiable associated pore complexes results from incubation of nuclear envelopes in 2% Triton +0·3 M-KCl (Fig. 2C,D). At high magnification the lamina contained

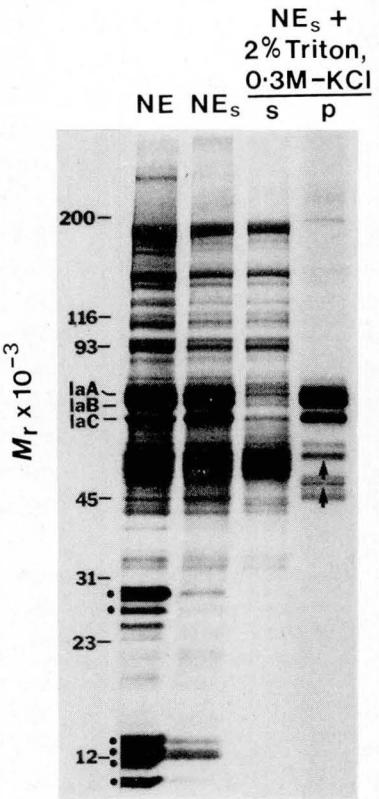

Fig. 1. Isolation of a lamina fraction by chemical extraction of rat liver nuclear envelopes. A crude rat liver nuclear envelope fraction (NE lane) contains numerous polypeptides resolved by SDS/polyacrylamide gel electrophoresis, including the three lamins (1a A, 1a B and 1a C), and histone contaminants (dots to left of NE lane). Extraction of this fraction with a solution containing 1 M-KCl yields salt-washed nuclear envelopes (NE_s lane) from which the histones are largely removed. Salt-washed nuclear envelopes were incubated in a solution containing 2% Triton +0·3 M-KCl and centrifuged at 5000 g_{max} yielding a supernatant and a pellet (NE_s+2% Triton, 0·3 M-KCl; s and p lanes, respectively). The three lamins are highly enriched in the pellet fraction, which also contains small amounts of putative intermediate filament polypeptides migrating in the 40 000–55 000 M_r region (see the text). Two of these bands (NE_s+2% Triton, 0·3 M-KCl; p lane, arrows) comigrate precisely with major cytokeratins present in samples of rat liver plasma membranes (provided by Dr Ann Hubbard; Hubbard & Ma, 1983). Samples were electrophoresed on a SDS/7·5% to 15% polyacrylamide gel and stained with Coomassie Blue.

in this insoluble fraction appears to comprise a meshwork of short (approx. 10–40 nm) interconnected fibrils when viewed in tangential section (Fig. 2D, double arrows). In transverse section, the lamina is seen as a structure of approximately 10–20 nm diameter (Fig. 2D, single arrows). These characteristic ultrastructural features have been noted previously in the lamina contained in the pore complex–lamina fraction (Dwyer & Blobel, 1976; Scheer *et al.* 1976).

Hence, this biochemical fractionation scheme appears to yield a nuclear envelope subfraction devoid of pore complexes that is highly enriched in a supramolecular assembly composed of the three lamins. These results support our previous immunoferritin-localization results (Gerace & Blobel, 1982; Gerace *et al.* 1982) indicating that the rat liver lamins occur exclusively in the lamina and not in pore complexes. The observation that most or all of the nuclear envelope polypeptides other than the lamins can be solubilized, while an apparently intact lamina structure remains, also supports the possibility that a polymeric assembly of the three lamins serves as a core element of the lamina structure.

In other studies (Krohne *et al.* 1978*a*), it was shown that treatment of *Xenopus* oocyte nuclear envelopes with Triton plus high concentrations of KCl results in considerable enrichment of a major approximately 66 000 M_r nuclear envelope band in the insoluble pellet fraction. However, in contrast to our results with rat liver nuclear envelopes, it was argued that oocyte pore complexes are preferentially stable to (and that lamina is preferentially extracted by) this chemical treatment (Krohne *et al.* 1978*b*, 1981), and that the 66 000 M_r band (which cross-reacts with the somatic lamins) is present in both lamina and pore complexes (Stick & Krohne, 1982).

The lamina has a tight physical interaction with the inner nuclear membrane (Dwyer & Blobel, 1976), a characteristic that is likely to be important for coordinating the structures of the lamina and nuclear membranes. To investigate the biochemical basis for this interaction, we have investigated whether any of the lamins has a preferentially strong association with the inner nuclear membrane (Fig. 3) by extracting nuclear envelopes with different chemical perturbants in the absence of non-ionic detergents (Steck & Yu, 1973). With the non-detergent chemical conditions that we have used, the lipid bilayer of nuclear membranes remains intact, although the membranes are induced to fragment into small structures that require ultracentrifugation to be completely pelleted. Treatment of nuclear envelope with 0·25–1·0 M-$MgCl_2$ results in selective extraction of lamins A and C from membranes, while lamin B preferentially occurs in the membrane pellet (Gerace & Blobel, 1982; Fig. 3, 0·25 M-$MgCl_2$, s and p lanes). Similarly, a preferentially strong association of lamin B with nuclear membranes is evident upon incubation of nuclear envelopes with 1 M-guanidine·HCl (Fig. 3, 1 M gu·HCl, s and p lanes), and in a pH 10·5 buffer (Fig. 3; pH 10·5, s and p lanes). Incubation of nuclear envelopes with higher concentrations of each of these protein perturbants, such as 0·02 M-NaOH (Fig. 3; 0·02 M-NaOH, s and p lanes) results in complete extraction of lamin B in addition to the other two lamins.

These results indicate that lamin B has a stronger physical interaction with nuclear membranes than the other two lamins, and may therefore have an important role in

attachment of the lamina to the nuclear envelope. In further support of this possibility, when the nuclear envelope is in a physiological state of disassembly during metaphase, biochemical fractionation studies suggest that lamin B may remain associated with membrane vesicles derived from the disassembled nuclear envelope, while lamins A and C are clearly non-membrane-associated (Gerace & Blobel, 1980).

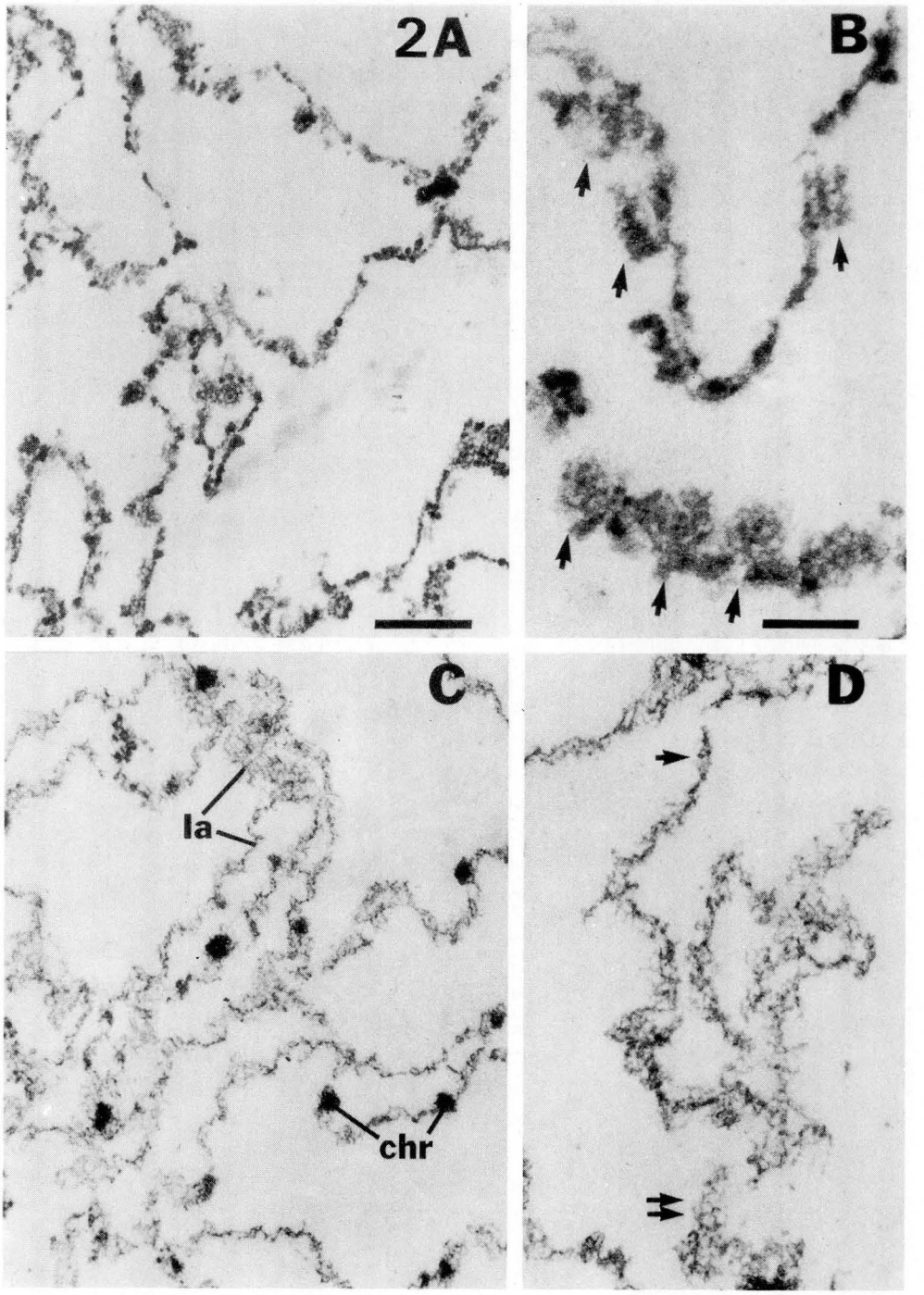

Fig. 2

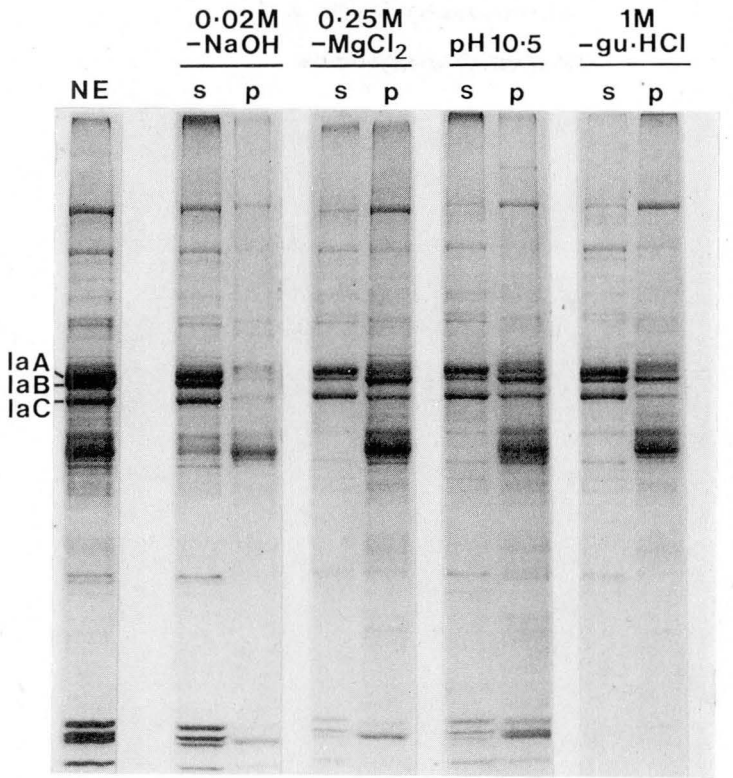

Fig. 3. Extraction of rat liver nuclear envelopes with chemical protein perturbants. Rat liver nuclear envelopes that had been washed with 0·5 M-KCl (NE) were incubated in solutions containing 0·02 M-NaOH, 0·25 M-$MgCl_2$, 0·1 M-sodium carbonate (pH 10·5); and 1 M-guanidine·HCl (1 M-gu·HCl); and sedimented at 200 000 g_{max} to yield supernatants (s) and membrane pellets (p). Samples were electrophoresed on a SDS/7·5 to 15 % polyacrylamide gel and stained with Coomassie Blue. Under the latter three chemical conditions, lamin B is preferentially resistant to extraction, although it can be completely solubilized by more-alkaline pH buffer conditions (0·02 M-NaOH lanes) as well as with 3 M-$MgCl_2$ and 3 M-guanidine·HCl (data not shown).

Fig. 2. Thin-section electron micrographs of rat liver nuclear envelopes extracted with Triton X-100 in low or high ionic strength buffers.A crude rat liver nuclear envelope fraction was incubated in buffers containing 0·02 M-triethanolamine (pH 7·4) and 2 % Triton (A,B) or 2 % Triton +0·3 M-KCl (C,D) and prepared for electron microscopy. Extraction of nuclear envelopes with Triton/low ionic strength conditions (A,B) yields an insoluble supramolecular assembly that contains a morphologically continuous lamina with attached pore complexes (arrows in Fig. 2B). Upon incubation of nuclear envelopes with Triton/high salt solutions (C,D), only the lamina (1a) remains structurally intact, while pore complexes are completely absent. Since we obtain better ultrastructural preservation of pore complexes in samples of crude nuclear envelopes (Fig. 1, NE lane) compared to 1 M-KCl-washed nuclear envelopes (Fig. 1, NE_s lane), we used crude nuclear envelopes for our starting material in this experiment. Chromatin contamination (chr) is visible as irregular darkly staining granular material associated with the lamina in A and B, and is aggregated into larger dense clumps in C and D. On SDS/polyacrylamide gels, the material shown in C and D has a very similar profile to Fig. 1 (NE_s +2 % Triton, 0·3 M-KCl, p lane), except that histone contamination is present (data not shown). A and C: bar, 375 nm; ×40 000: B and D: bar, 150 nm; ×100 000.

Immunofluorescence staining of *Drosophila* metaphase cells with monoclonal antibodies directed against a putative *Drosophila* lamin gives a distinctly punctate staining reaction in the cytoplasm (Fuchs *et al.* 1983), consistent with the possibility that this antigen is associated with partially fragmented nuclear membranes after nuclear disassembly. While lamin B of vertebrates may be primarily responsible for attachment of the lamina to the inner nuclear membrane, it is possible that lamins A and C have physiologically significant membrane interactions as well.

The lamin B–membrane interaction could be mediated either by an association of lamin B with a second intrinsic protein of the inner nuclear membrane, or by the direct physical association of lamin B (possibly through a hydrophobic domain) with the inner membrane bilayer. We have obtained no evidence for the existence of an intrinsic membrane protein associated with lamin B, based on studies of rat liver nuclear envelopes using chemical cross-linking and solubilization approaches (L. Gerace, unpublished). Therefore, we favour the possibility that lamin B itself directly mediates the lamina–membrane interaction. In the future it will be possible to study this question in more detail using a membrane reconstitution approach with purified lamin B.

It has been observed that lamin B in alkaline-extracted nuclear envelopes can be labelled with a hydrophobic photoaffinity reagent (Lebel & Raymond, 1984), which could indicate the presence of a lipid-integrated domain on this polypeptide. However, lamins A and C were apparently labelled to a similar proportional extent in this material, and labelling of native (non-extracted) nuclear envelopes was not described in this study.

Synthesis and assembly of the lamins during interphase

The surface area of the nuclear envelope, as well as the number of pore complexes, increases continuously during the cell cycle (Maul *et al.* 1972; Fry, 1976). Since the lamina forms a skeleton-like protein shell at the nuclear periphery, increase in its mass may be important for regulating growth of nuclear envelope surface area and increase in nuclear volume during interphase. To obtain insight into this question, we have examined the cell cycle timing of lamin biosynthesis. In these experiments we were interested in determining whether synthesis of the lamins occurs continuously throughout interphase (during G_1 and G_2 phases as well as during S), or whether biosynthesis of these proteins (which have a putative role in chromosome structure) is temporally coupled to DNA replication.

Metaphase CHO cell populations were obtained by selective mechanical detachment, and samples of cells were returned to culture for 1–17 h. At 2-h intervals during this period, dishes were pulse-labelled with [^{35}S]methionine, and the lamins were immunoprecipitated from solubilized cells and electrophoresed in SDS/polyacrylamide gels. This permitted quantitation of the relative rate of synthesis of these polypeptides at progressive stages of the cell cycle (Fig. 4).

In these synchronized populations (Fig. 4A), the earliest point at which a significant number of cells has reached S phase is 5 h, and cells begin entering mitosis by 13–15 h. It is evident from this analysis (Fig. 4B) that the lamins are synthesized

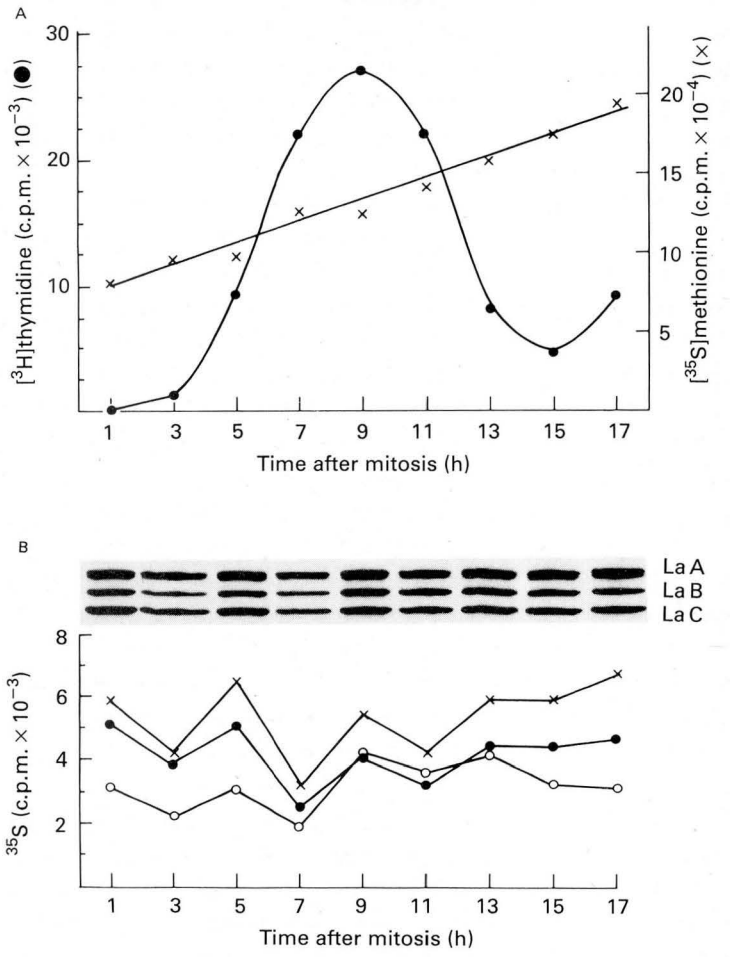

Fig. 4. Biosynthesis of the lamins through the cell cycle in CHO cells. Metaphase CHO cell populations were selected by mechanical shake-off, and cells were sampled and returned to culture. After a 1-h period at 37 °C, and at 2-h intervals thereafter, individual dishes were pulse-labelled for 30 min with either [^{3}H]thymidine or [^{35}S]methionine. [^{3}H]-thymidine-labelled samples ($0{\cdot}9\times10^6$ cells each) were precipitated with TCA to measure the rate of DNA replication in cell populations at various times (A). [^{35}S]methionine-labelled cells were directly solubilized in SDS, and a portion of this material ($1{\cdot}5\times10^4$ cells) was precipitated with TCA to measure the total rate of protein synthesis at progressive cell cycle stages (A), while the remaining sample ($1{\cdot}8\times10^6$ cells) was used for immunoprecipitation of the lamins. Immunoprecipitated material was electrophoresed on an SDS/polyacrylamide gel and visualized by fluorography (B, top). Individual lamin bands were then excised from this gel and ^{35}S incorporation was measured by scintillation counting (B, bottom). Each of the lamin A bands analysed in this experiment is actually a closely spaced doublet comprised of lamin A_o and lamin A (see Fig. 5). The cell population shows a peak in the rate of DNA replication at 9 h, with a small percentage of cells entering *S* phase of the subsequent cell cycle at 17 h (A). Mitotic cells were first observed in these cultures at 13 h, and reached a peak during the 15 to 17-h period. Each of the lamins has a roughly similar biosynthetic rate at all cell cycle times examined (B). The apparent decreases in lamin biosynthetic rate seen at 3 and 7 h in this experiment were not observed in a subsequent experiment, and were apparently due to incomplete sample recovery.

at similar rates in G_1-enriched (1–3 h) and G_2-enriched (13–15 h) cell populations, compared to *S* phase cells (e.g. 9 h). Therefore, unlike certain other nuclear structural proteins, such as histones, whose synthesis in many cell types occurs mainly during *S* phase (Kedes, 1979; Hereford, Osley, Ludwill & McLaughlin, 1981), biosynthesis of the lamins is not temporarily coupled to DNA replication in a rapidly growing tissue-culture line (CHO cells).

While the lamins must be synthesized on polyribosomes in the cytoplasmic compartment and subsequently transported into the nucleus for assembly into a lamina structure, several types of evidence indicate that this 'unassembled' cytoplasmic pool of lamins is small in many types of somatic and cultured cells. First, immunofluorescence staining of tissue sections or of growing tissue-culture cells (Gerace *et al.* 1978; Krohne *et al.* 1978*a*) with anti-lamin antibodies indicates that the lamins occur largely or entirely in a peripheral nuclear localization during interphase (presumably in an assembled lamina), with no clearly detectable levels of these polypeptides in a cytoplasmic configuration. Furthermore, immunoprecipitation studies have shown that when exponentially growing interphase cells are lysed in isotonic Triton-containing buffers (conditions where the isolated lamina is insoluble), the lamins are recovered almost entirely in a high-speed pellet (Gerace & Blobel, 1980). With the same detergent fractionation procedure, the disassembled metaphase lamins are almost quantitatively soluble (Gerace & Blobel, 1980).

Since isotonic Triton fractionation of tissue-culture cells should distinguish, to a first approximation, between unassembled and assembled lamins, we have used this procedure to investigate the average rate of assembly of newly synthesized lamins during interphase (Fig. 5). Exponentially growing cultures of a rat liver cell line (BRL cells) were pulse-labelled with [^{35}S]methionine, and after various chase periods with non-radioactive medium were fractionated by isotonic Triton lysis and centrifugation. The lamins were then immunoprecipitated from supernatants and pellets, and samples were analysed on SDS/polyacrylamide gels. After a 5-min pulse label, an antibody specific for lamins A and C immunoprecipitates two major polypeptides that occur exclusively in the supernatant fraction (Fig. 5; 0 min chase, s and p lanes). Compared to the lamin A and C species found in cells labelled continuously for 36 h (Fig. 5, 36-h lane), the upper of these two bands (designated lamin A_o) migrates approximately 2000 daltons more slowly than the steady state-labelled lamin A species, while the lower band comigrates precisely with lamin C. With progressive periods of chase, the immunoprecipitated lamin A_o and lamin C species shift to the Triton-insoluble pellet fraction (Fig. 5). The half-time for 'insertion' of lamin A_o into a Triton-insoluble structure is approximately 5 min, while for lamin C it is approximately 60 min. Finally, subsequent to the time that lamin A_o appears in the pellet fraction, it shifts in a precursor–product fashion to a faster-migrating form that comigrates with the steady-state labelled lamin A (Fig. 5; 60 and 120 min min chase, p lanes). When monospecific antibodies to lamin B were used to analyse this pulse–chase experiment, we found that the lamin B-reactive species initially appears (0 min chase) entirely in the supernatant fraction as a form that comigrates with steady-state labelled lamin B, and that this species is incorporated

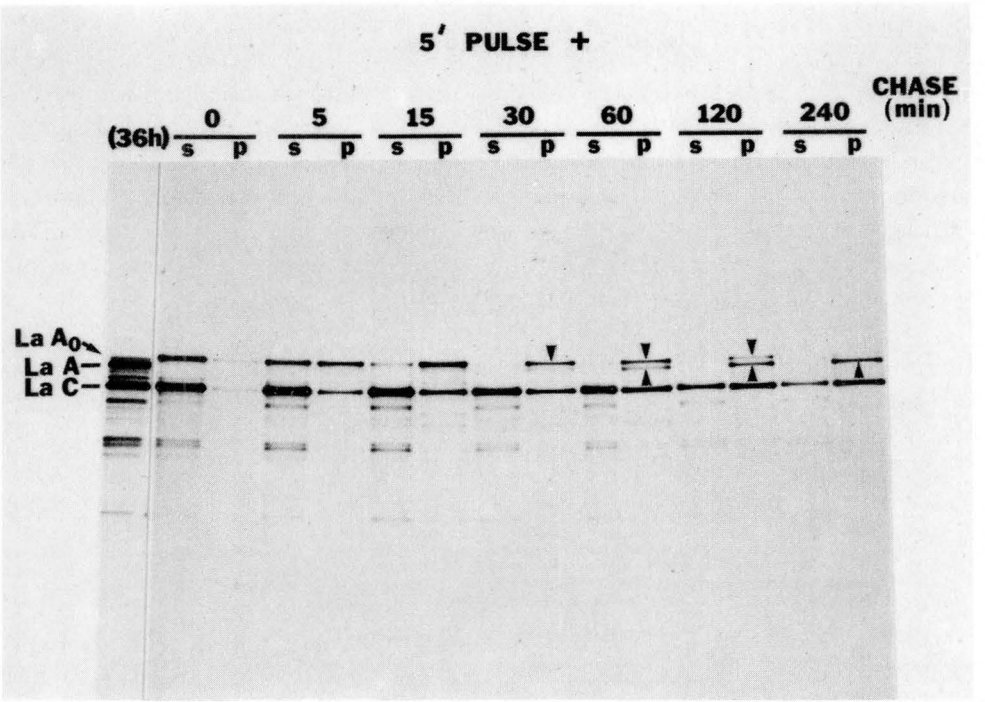

Fig. 5. Assembly of newly synthesized lamins into a Triton-insoluble structure. Exponentially growing cultures of a rat liver cell line (BRL cells) were pulse-labelled with [^{35}S]methionine for 5 min, and subsequently chased with non-radioactive methionine for 0–240 min. Cultures were then lysed in an isotonic Triton-containing buffer, and samples were sedimented in an Eppendorf microfuge to yield supernatants (s) and pellets (p). Lamins A and C were immunoprecipitated from these fractions and electrophoresed on an SDS/polyacrylamide gel before visualization by fluorography. Lamin A initially appears as a form (designated lamin A_0) that migrates approximately 2000 daltons more slowly than 36-h-labelled lamin A (compare 0-min chase lane to 36-h lane), and is gradually converted to a 'mature' lamin A form in a precursor–product fashion after it is integrated into a Triton-insoluble structure (arrows in 30, 60 and 120-min chase lanes).

with a Triton-insoluble structure with a half-time of approximately 60 min in BRL cells (data not shown).

We have examined the characteristics of lamin biosynthesis and assembly in other cell lines (CHO cells and MDBK cells) using this type of pulse–chase analysis, and have observed the same general features described above for BRL cells (L. Gerace & Y. Ottaviano, unpublished). First, lamin A is synthesized as a precursor molecule that migrates approximately 2000 daltons more slowly on SDS/polyacrylamide gels than the principal cellular form of lamin A, and is converted to a species that comigrates with lamin A subsequent to its appearance in a Triton-insoluble fraction. Second, lamin A_0 is incorporated into a Triton-insoluble structure considerably more rapidly than lamins B and C. However, the half-times for insertion of the lamins varied among different cell lines.

In vitro translation of mRNA isolated from BHK cells (Laliberte *et al.* 1984) and from rat liver (L. Gerace, unpublished) also yields an apparent precursor molecule

to lamin A that migrates approximately 2000 daltons more slowly on SDS/polyacrylamide gels than the major cellular form of lamin A in these cells. Hence, it is possible that the presence of a lamin A precursor is a widespread phenomenon. The physiological function for conversion of lamin A_o to lamin A is unclear, but since it apparently occurs after insertion of lamin A_o into the lamina structure, this conversion is probably not related to intracellular targeting of this polypeptide to the lamina. Clearly, the processed (mature) lamin A is competent to be assembled into a lamina structure, since it is quantitatively reutilized in telophase after mitotic disassembly in tissue-culture cells (Gerace & Blobel, 1980).

While lamins A and C have very similar tryptic peptide maps (Shelton *et al.* 1980; Kaufman *et al.* 1982), experiments involving *in vitro* translation of mRNA suggest that lamins A_o and lamin C are encoded by distinct messenger RNA species (Laliberte *et al.* 1984). This result is supported by the pulse–chase experiments described above (Fig. 5). These two messenger species could be encoded by separate genes or alternatively, could arise from post-transcriptional processing of a single precursor RNA molecule.

Regulation of lamina structure during mitosis

The lamins are phosphorylated during both interphase and mitosis (Gerace & Blobel, 1980), but these polypeptides have an approximately four to sixfold higher level of associated phosphate during metaphase, when they are disassembled, compared to interphase, when they are assembled. Furthermore, they lose much of this metaphase phosphate by telophase when the lamina has been reconstructed (Gerace & Blobel, 1980). This correlation suggests that enzymic phosphorylation of the lamins may be important for regulating mitotic disassembly of the lamina.

To investigate this possibility further, and to establish a framework for studying disassembly of the lamina *in vitro*, we have analysed CHO cells to compare phosphorylation of the lamins that occurs during mitotic prophase (the period when the lamina is disassembled) to that taking place during interphase (Ottaviano & Gerace, 1985). In these studies, we have found that all of the detectable phosphate associated with each lamin during interphase and mitosis occurs in a phosphomonoester linkage, predominantly as phosphoserine and, to a lesser extent, as phosphothreonine. Furthermore, lamins A and C are phosphorylated on partially distinct sets of tryptic peptides during mitosis, compared to interphase.

We have quantified the absolute level of phosphorylation of the lamins from metaphase and exponentially growing interphase cells by steady-state labelling of CHO cells with [^{32}P]phosphate (Table 1). This determination indicates that each of the lamins has 1·4–2·2 moles of associated phosphate per mole of lamin during metaphase, compared to 0·27–0·46 moles of phosphate per mole of protein in the average interphase state. Since protein mass was quantified in this experiment by Coomassie Blue dye binding, these values are to be considered approximate. The results of this analysis agree well with the relative levels of interphase and mitotic lamin phosphorylation that we determined previously (Gerace & Blobel, 1980).

Most postsynthetic protein modifications including phosphorylation induce

Table 1. *Steady-state levels of phosphate associated with the lamins*

	mol P/mol lamin		
	M	I	M/I
Lamin A	2·2	0·46	4·8
Lamin B	1·9	0·27	7·0
Lamin C	1·4	0·33	4·2

CHO cells were grown in medium containing [^{32}P]phosphate for 48 h, and the lamins were immunoprecipitated from exponentially growing interphase populations (I) or shake-off synchronized metaphase cells (M). After electrophoresis on an SDS/polyacrylamide gel and staining with Coomassie Blue, the protein mass in individual lamin bands was determined spectrophotometrically using bovine serum albumin as a standard (Fenner *et al.* 1975). Subsequently the mol phosphate in each lamin band was determined by scintillation counting. Values represent the average of two separate experiments (which in all cases differed by no more than 15 %).

changes in protein isoelectric point that are detectable on two-dimensional isoelectric focusing SDS/polyacrylamide gels (Wold, 1981). We have used two-dimensional gel electrophoresis of [^{35}S]methionine-labelled lamins to examine phosphorylation and other possible charge-altering modifications of the lamins that occur during interphase and mitosis (Fig. 6). When immunoprecipitates of lamins from interphase cells are analysed on two-dimensional gels (Fig. 6; I,−AP) two predominant charge isomers are detectable for lamins A and C, while a single major form is seen for lamin B. CHO cell lamins A and C are isoelectric at approximately pH 7–7·5, while lamin B has an isoelectric point near pH 6·0 (Gerace & Blobel, 1980). Lamins A and C show minor satellite spots adjacent to major charge species on our pH-gradient electrophoresis gels. (This phenomenon is especially evident in Fig. 6, +AP.) These satellite spots do not necessarily represent charge isomers of these polypeptides, but may be due, for example, to non-uniform binding of ampholytes to the proteins (Cann, 1979).

As previously demonstrated (Gerace & Blobel, 1980), the lamins from mitotic cells migrate as more acidic isoelectric species than those from interphase cells (Fig. 6; M,−AP). This acidic charge shift is consistent with the increased level of mitotic lamin phosphorylation that has been determined (Gerace & Blobel, 1980; Table 1). Mitotic lamin B occurs almost exclusively as a closely spaced doublet (the two components of which have slightly different motilities in both electrophoretic dimensions), while mitotic lamins A and C are predominantly one or two major charge isomers (Fig. 6; M,−AP).

We have determined that the phosphate that becomes associated with pulse-labelled lamins during interphase and mitosis can be almost quantitatively hydrolysed by treatment of immunoprecipitates with bacterial alkaline phosphatase (Ottaviano & Gerace, 1985). Taking advantage of this observation, we have analysed alkaline phosphatase-treated immunoprecipitates of [^{35}S]methionine-labelled interphase and mitotic lamins on two-dimensional gels to determine whether the mitotic

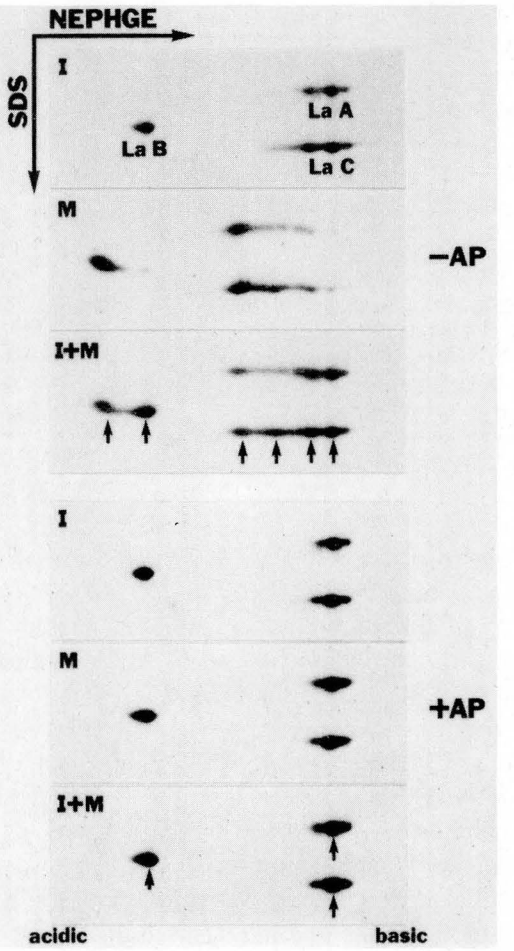

Fig. 6. Two-dimensional gel analysis of interphase and mitotic lamins. CHO cells were labelled with [^{35}S]methionine and metaphase cells were obtained by shake-off synchronization. These mitotic cells (M), and populations of labelled exponentially growing interphase cells (I) were immunoprecipitated with antibodies recognizing the three lamins. Immunoprecipitates were then separated on non-equilibrium pH-gradient electrophoresis (NEPHGE)/SDS/polyacrylamide two-dimensional gels, either without (−AP) or with (+AP) treatment with bacterial alkaline phosphatase before electrophoresis. For both −AP and +AP samples, we electrophoresed mixtures of interphase and mitotic immunoprecipitates (I+M) as well as separate interphase (I) and mitotic (M) samples. Major charge isomers of the lamins that are apparent in mixed immunoprecipitates of interphase and mitotic cells (−AP and +AP) are indicated by arrows. Any pair of adjacent charge isomers probably differs by one phosphate molecule.

charge shift of the lamins is entirely due to phosphorylation (Fig. 6, +AP panels). The interphase lamins A and C treated with alkaline phosphatase shift to a single predominant spot (Fig. 6; +AP, I panel), which comigrates with the more basic of the two charge isomers of these respective polypeptides in the interphase untreated sample (Fig. 6; −AP, I panel). The single interphase lamin B species is not shifted

in charge by alkaline phosphatase treatment, compared to lamin B of the untreated sample. All three mitotic lamins undergo a basic charge shift due to alkaline phosphatase treatment, and now occur as single major charge isomers (Fig. 6; +AP, M panel) that precisely comigrate with the corresponding spots of interphase alkaline phosphatase-treated samples. This demonstrates that phosphorylation is the only detectable charge-altering modification of the lamins that occurs specifically during mitosis. Since we find no evidence for other major mitotis-specific postsynthetic modifications of the lamins besides phosphorylation, this result strengthens our hypothesis (Gerace & Blobel, 1980) that phosphorylation of the lamins is important for mediating disassembly of the lamina during cell division. However, our analysis would not detect mitotis-specific modifications of the lamins that affect only a small percentage of the protein population, nor would it necessarily detect protein modifications that are labile in neutral pH buffers. However, the latter are uncommon (Wold, 1981).

Lamina and regulation of nuclear architecture during mitosis

Our working model for the organization of the lamina and its relationship to the mitotic dynamics of the nuclear envelope is shown diagrammatically in Fig. 7. This is an extension of our earlier discussions (Gerace *et al.* 1978; Gerace & Blobel, 1982), and is motivated by the structural, biochemical and physiological properties of the lamins that have been determined. The scheme presented in Fig. 7 depicts the lamina structure of vertebrate cell types that contain three distinct lamins, such as liver and tissue-culture cells (e.g. see Gerace & Blobel, 1982; see Introduction and discussion below).

During interphase, we envisage that the three lamins are organized in a polymeric array of which all three polypeptides are intrinsic components (Fig. 7, Interphase). We suggest that this lamin polymer provides the 'core' element (and probably most of the mass) of the lamina itself. This model is strongly supported by the observation that a lamina-like structure composed almost exclusively of the three lamins can be isolated by chemical extraction of rat liver nuclear envelopes (Figs 1 and 2).

Lamins A and C are depicted as being structurally and functionally similar (Fig. 7, Interphase), based on their extensive immunological and biochemical homology (Gerace *et al.* 1978; Shelton *et al.* 1980; Gerace & Blobel, 1982; Kaufman *et al.* 1982), but these polypeptides may not be functionally identical. Although lamin B has been shown to cross-react with lamins A and C, with certain monoclonal antibodies (Burke *et al.* 1982; Krohne *et al.* 1984), this polypeptide is in part biochemically different from the other two lamins (Shelton *et al.* 1980; Gerace & Blobel, 1982), and is proposed to have a specialized function for mediating the lamina–inner nuclear membrane interaction (Gerace & Blobel, 1982; and Fig. 3). Lamin B may interact with the inner nuclear membrane by virtue of a direct association with the inner membrane bilayer as indicated in Fig. 7, but other possibilities are not excluded at this time.

We also suggest (Gerace *et al.* 1978; and Fig. 7, Interphase) that the lamina functions to attach chromatin to the nuclear envelope (see Franke, 1974; Fry, 1976).

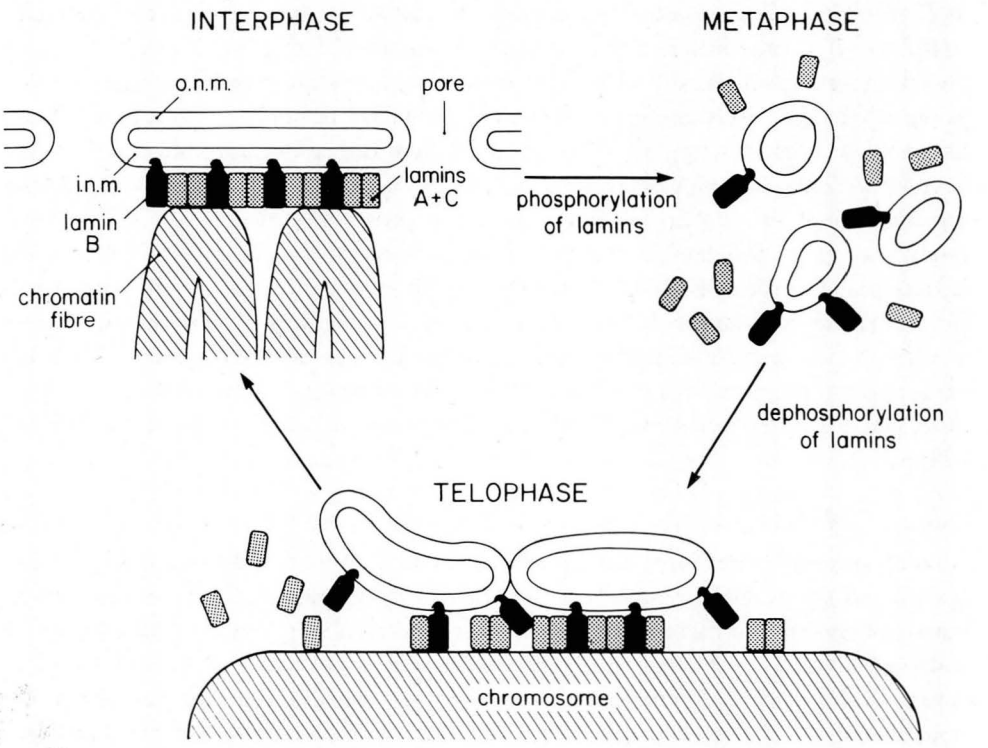

Fig. 7. Proposal for the organization of the interphase lamina, and the relationship between the lamina and the reversible mitotic disassembly of the nuclear envelope. The lamina of many vertebrate cell types is composed mainly of three related polypeptides (lamins A, B and C) that form a shell-like meshwork at the nuclear periphery, and that appear to be organized in a polymeric array (Interphase). The lamina has a strong interaction with the inner nuclear membrane, that may be mediated predominantly by lamin B. In addition, the lamina is proposed to provide a major anchoring site for chromatin in the nucleus, possibly serving as an organizing centre for higher order chromatin domains. By virtue of its dual interaction with the inner nuclear membrane and chromatin, the lamina may be a major skeletal element of the interphase nucleus. During mitotic prophase, the lamins are disassembled to a monomeric form and lose their association with chromatin, so that in metaphase lamins A and C appear to be soluble, while lamin B may retain an association with membrane fragments derived from the disassembled nuclear envelope (Metaphase). The process of lamina depolymerization is proposed to trigger the overall disassembly of the nuclear envelope during prophase, and is suggested to be mediated (at least in part) by enzymic phosphorylation of the lamins. During telophase, the lamina reassembles at the surfaces of mitotic chromosomes, a process that is proposed to result in association of membranes with chromosome surfaces and to promote reassembly of the double-membrane nuclear envelope structure. Lamina repolymerization is suggested to be mediated by dephosphorylation of the lamins during this period.

In this fashion the lamina may provide a major anchoring site for chromatin in the interphase nucleus, and may be important for organization of higher order chromatin domains (Benyajati & Worcell, 1976; Cook & Brazell, 1976). At present, the details of this putative interaction are unknown, and it is not apparent whether it involves an association of one or several of the lamins with DNA directly (Hancock & Hughes,

1982; Lebkowski & Laemmli, 1982), or with some additional chromosomal protein(s) (McKeon, Tuffanelli, Kobayashi & Kirschner, 1984).

This model for the structure of the interphase lamina of vertebrate cells emphasizes three sets of functional interactions for the lamins: those involved in polymeric self-association, those related to interaction with the inner nuclear membrane, and those involved in chromatin attachment. The lamina may in addition provide an anchoring site for pore complexes in the nuclear envelope (not indicated in Fig. 7; Aaronson & Blobel, 1975). While certain vertebrate cell types apparently contain only one or two lamin-related polypeptides (Krohne *et al.* 1981), it is clearly possible that many or all of the functions associated with the three rat liver lamins (which are structurally similar) could be encoded in the structure of a single related polypeptide in other cells of the same organism, as well as in other eukaryotic organisms.

At present the detailed molecular organization of the lamin polymer is not clearly understood. However, small homotypic oligomers of each lamin can be generated in nuclear envelopes by *in vitro* oxidation of intrinsic cysteine sulphydryls (Shelton & Cochran, 1978; Lam & Kasper, 1979; Kaufman *et al.* 1982), consistent with the possibility that a basic 'subunit' of lamina structure may consist of oligomers of each lamin. Considering the ultrastructural appearance of the lamina seen in thin-section electron microscopy, it is conceivable that the short fibrils visible in the isolated lamina structure represent lamin oligomers.

During mitosis, the lamina undergoes dramatic structural reorganizations that temporally coincide with the disassembly and reconstruction of the nuclear envelope (Fig. 7, Metaphase and Telophase). Biochemical and immunocytochemical investigations (Gerace *et al.* 1979; Krohne *et al.* 1978*a*; Gerace & Blobel, 1980) indicate that the lamins are disassembled to an apparently monomeric form during mitotic prophase and lose their interaction with chromatin (Fig. 7, Metaphase). Furthermore, lamin B (but not lamins A and C) may retain an interaction with disassembled nuclear membranes at metaphase (Gerace & Blobel, 1980). Considering the biochemical characteristics described for the metaphase lamins (Gerace & Blobel, 1980; and Table 1, Fig. 6), we suggest that specific enzymic phosphorylation of the lamins is important for mediating prophase lamina disassembly, and possibly for effecting the dissociation of lamins from chromatin. This process of lamina depolymerization is likely to be a major event that controls nuclear envelope disassembly, permitting the processes of nuclear membrane fragmentation to take place.

During telophase (Fig. 7, Telophase), the lamins are reassembled at or near the surfaces of the condensed mitotic chromosomes (Gerace *et al.* 1978; Krohne *et al.* 1978*a*). Studies of mitotic cells by electron microscopy have suggested that nuclear envelope reconstruction involves aggregation and fusion of membrane vesicles in direct contact with the surfaces of telophase chromosomes, which are often fused into a single continuous mass at this period (Robbins & Gonatas, 1964; Murray, Murray & Pizzo, 1965; Erlandsen & DeHarven, 1971; Roos, 1973). Considering the apparent membrane-associated state of lamin B in metaphase cells, lamina reassembly adjacent to chromatin would necessarily result in the association of membrane vesicles with the surfaces of the telophase chromosomes. This would provide appropriate topological

conditions for nuclear membrane reconstruction, and could also facilitate the membrane vesicle fusion that apparently results in reappearance of a continuous double membrane structure enclosing the chromosomes (Fry, 1976). We suggest that dephosphorylation of the lamins is important for regulating this reassembly process, with surfaces of chromosomes and, or, membrane vesicles serving as nucleating sites for this reaction. The observation (Forbes, Kirschner & Newport, 1983) that bacteriophage DNA microinjected into *Xenopus* eggs induces assembly of nuclear envelopes and lamina (apparently after the injected DNA is assembled into chromatin) supports the notion that chromatin surfaces are important for directing the topological specificity of lamina reassembly.

In this fashion the processes of lamina disassembly and reformation may be principal factors regulating changes in nuclear envelope architecture occurring during cell division. Our proposals on the relationship of the lamina to nuclear envelope structure are analogous to principles of membrane organization that have derived from study of the red cell membrane (reviewed by Steck, 1974; Branton, Cohen & Taylor, 1981), where it has been demonstrated that a membrane-associated protein meshwork (containing spectrin, actin, etc.) is directly involved in determining the membrane's physical and molecular properties. In the future it will be important to obtain a detailed biochemical understanding of the relationship of phosphorylation to the reversible depolymerization of the lamina during mitosis, and the nature of other processes that affect nuclear envelope structure during this period. This information will undoubtedly help to elucidate the mechanisms that control and coordinate the various mitotic events, and should also promote understanding of interphase nuclear envelope structure and physiology.

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REFERENCES

AARONSON, R. & BLOBEL, G. (1974). On the attachment of the nuclear pore complex. *J. Cell Biol.* **62**, 746–754.

AARONSON, R. & BLOBEL, G. (1975). Isolation of nuclear pore complexes in association with a lamina. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1007–1011.

BENYAJATI, C. & WORCELL, A. (1976). Isolation, characterization, and structure of the folded interphase genome of *Drosophila melanogaster*. *Cell* **9**, 393–407.

BONNER, W. & LASKEY, R. (1974). A film detection method for tritium-labeled proteins and nucleic acid in polyacrylamide gels. *Eur. J. Biochem.* **46**, 83–88.

BOSTOCK, C., PRESCOTT, D. & KIRKPATRICK, J. (1971). An evaluation of the double thymidine block for synchronizing mammalian cells at the *G1–S* border. *Expl Cell Res.* **68**, 163–168.

BRANTON, D., COHEN, C. & TYLER, J. (1981). Interaction of cytoskeleton proteins on the human erythrocyte membrane. *Cell* **24**, 24–32.

BURKE, B., TOOZE, J. & WARREN, G. (1983). A monoclonal antibody which recognizes each of the nuclear lamin polypeptides in mammalian cells. *EMBO J.* **2**, 361–367.

CANN, J. (1979). In *Electrokinetic Separation Methods* (ed. P. Righetti, C. Vanoss & J. Vanderhoff), pp. 369–387. New York: Elsevier.

COOK, P. & BRAZELL, I. (1976). Conformational constraints in nuclear DNA. *J. Cell Sci.* **22**, 287–302.

DWYER, N. & BLOBEL, G. (1976). A modified procedure for isolation of a pore complex–lamina fraction from rat liver nuclei. *J. Cell Biol.* **70**, 581–591.

ELY, S., D'ARCY, A. & JOST, E. (1978). Interaction of antibodies against nuclear envelope-associated proteins from rat liver nuclei with rodent and human cells. *Expl Cell Res.* **116**, 325–331.

ERLANDSON, R. & DEHARVEN, E. (1971). The ultrastructure of synchronized HeLa cells. *J. Cell Sci.* **8**, 353–397.

FARQUHAR, M. & PALADE, G. (1955). Cell junctions in amphibian skin. *J. Cell Biol.* **26**, 263–291.

FAWCETT, D. (1966). On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. *Am. J. Anat.* **119**, 129–146.

FENNER, C., TRAUT, R., MASON, D. & WIKMAN-DOFFELT, J. (1975). Quantification of Coomassie blue stained proteins in polyacrylamide gels based on analysis of eluted dye. *Analyt. Biochem.* **63**, 595–602.

FISHER, P., BERRIOS, M. & BLOBEL, G. (1982). Isolation and characterization of a proteinaceous sub-nuclear fraction composed of nuclear matrix, peripheral lamina, and nuclear pore complexes from embryos of *Drosophila melanogaster*. *J. Cell Biol.* **92**, 676–686.

FORBES, D., KIRSCHNER, M. & NEWPORT, J. (1983). Spontaneous formation of nucleus-like structures around bacteriophage DNA microinjected into *Xenopus* eggs. *Cell* **34**, 13–23.

FRANKE, W. (1974). Structure, biochemistry, and functions of the nuclear envelope. *Int. Rev. Cytol. Suppl.* **4**, 71–236.

FRANKE, W., SCHEER, U., KROHNE, G. & JARASCH, E. (1981). The nuclear envelope and the architecture of the nuclear periphery. *J. Cell Biol.* **91**, 39s–50s.

FRY, D. (1976). The nuclear envelope in mammalian cells. In *Mammalian Cell Membranes*, vol. 2 (ed. G. A. Jamieson & D. M. Robinson), pp. 197–265. Boston: Butterworth.

FUCHS, J., GILOH, H., KUO, C., SAUMWEBER, H. & SEDAT, J. (1983). Nuclear structure: determination of the fate of the nuclear envelope in *Drosophila* during mitosis using monoclonal antibodies. *J. Cell Sci.* **64**, 331–349.

GERACE, L. & BLOBEL, G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell* **19**, 277–287.

GERACE, L. & BLOBEL, G. (1982). Nuclear lamina and the structural organization of the nuclear envelope. *Cold Spring Harbor Symp. quant. Biol.* **46**, 967–978.

GERACE, L., BLUM, A. & BLOBEL, G. (1978). Immunocytochemical localization of the major polypeptides of the nuclear pore complex–lamina fraction. Interphase and mitotic distribution. *J. Cell Biol.* **79**, 546–566.

GERACE, L., OTTAVIANO, Y. & KONDOR-KOCH, C. (1982). Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* **95**, 826–837.

HANCOCK, R. & HUGHES, M. (1982). Organization of DNA in the interphase nucleus. *Biol. Cell* **44**, 201–212.

HAVRE, P. & EVANS, D. (1983). Disassembly and characterization of the nuclear pore complex–lamina fraction from bovine liver. *Biochemistry* **22**, 2852–2860.

HEREFORD, L., OSLEY, M., LUDWILL, J. & MCLAUGHLIN, C. (1981). Cell cycle regulation of yeast histone mRNA. *Cell* **24**, 367–375.

HUBBARD, A. & MA, A. (1983). Isolation of rat hepatocyte plasma membranes. II. Identification of membrane-associated cytoskeletal proteins. *J. Cell Biol.* **96**, 230–239.

KAUFMAN, S., GIBSON, W. & SHAPER, J. (1983). Characterization of the major polypeptides of rat liver nuclear envelopes. *J. biol. Chem.* **258**, 2710–2719.

KEDES, L. (1979). Histone genes and histone messages. *A. Rev. Biochem.* **48**, 837–870.

KROHNE, G., DABAUVALLE, M. & FRANKE, W. (1981). Cell type-specific differences in protein composition of nuclear pore complex–lamina in oocytes and erythrocytes of *Xenopus laevis*. *J. molec. Biol.* **151**, 121–141.

KROHNE, G., DEBUS, E., OSBORN, M., WEBER, K. & FRANKE, W. (1984). A monoclonal antibody against nuclear lamina proteins reveals cell-type specificity in *Xenopus laevis*. *Expl Cell Res.* **150**, 47–59.

KROHNE, G., FRANKE, W., ELY, S., D'ARCY, A. & JOST, E. (1978*a*). Localization of a nuclear envelope-associated protein by indirect immunofluorescence microscopy using antibodies against a major polypeptide from rat liver fractions enriched in nuclear envelope-associated material. *Cytobiologie* **18**, 22.

KROHNE, G., FRANKE, W. & SCHEER, U. (1978*b*). The major polypeptides of the nuclear pore complex. *Expl Cell Res.* **116**, 85–102.

LALIBERTE, J., DAGENAIS, A., FILION, M., BIBOR-HARDY, V., SIMARD, R. & ROYAL, A. (1984). Identification of distinct messenger RNAs for nuclear lamin C and a putative precursor of nuclear lamin A. *J. Cell Biol.* **96**, 980–985.

LAM, K. & KASPER, C. (1979). Electrophoretic analysis of three major nuclear envelope polypeptides. *J. biol. Chem.* **254**, 11713–11720.

LEBEL, S. & RAYMOND, Y. (1984). Lamin B from rat liver nuclei exists both as a lamina protein and as an intrinsic membrane protein. *J. biol. Chem.* **259**, 2693–2696.

LEBKOWSKI, J. & LAEMMLI, U. (1982). Non-histone proteins and long-range organization of HeLa interphase DNA. *J. molec. Biol.* **156**, 325–344.

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

MAUL, G. & AVDALORIC, N. (1980). Nuclear envelope proteins from *Spisula solidissima* germinal vesicles. *Expl Cell Res.* **130**, 229–240.

MAUL, G., MAUL, H., SCOGNA, J., LIEBERMAN, M., STEIN, G., HSU, B. & BORUN, T. (1972). Time sequence of nuclear pore formation in phytohemagglutinin-stimulated lymphocytes and in HeLa cells during the cell cycle. *J. Cell Biol.* **55**, 433–447.

MCKEON, F., TUFFANELLI, F., KOBAYASHI, S. & KIRSCHNER, M. (1984). The redistribution of a conserved nuclear envelope protein during the cell cycle suggests a pathway for chromosome condensation. *Cell* **36**, 83–92.

MURRAY, R., MURRAY, A. & PIZZO, A. (1965). The fine structure of mitosis in rat thymic lymphocytes. *J. Cell Biol.* **26**, 601–619.

O'FARRELL, P., GOODMAN, H. & O'FARRELL, P. (1977). High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12**, 1133–1142.

OTTAVIANO, Y. & GERACE, L. (1985). *J. biol. Chem.* (in press).

RISAU, W., SAUMWEBER, H. & SYMMONS, P. (1981). Monoclonal antibodies against a nuclear membrane protein of *Drosophila*. *Expl Cell Res.* **133**, 47–54.

ROBBINS, E. & GONATAS, N. (1964). The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**, 429–463.

ROOS, U. (1973). Light and electron microscopy of rat kangaroo cells in mitosis. I. Formation and breakdown of the mitotic apparatus. *Chromosome* **40**, 43–82.

SCHEER, U., KARTENBECK, J., TRENDELENBURG, M., STADLER, J. & FRANKE, W. (1976). Experimental disintegration of the nuclear envelope. Evidence of pore-connecting fibers. *J. Cell Biol.* **69**, 1–18.

SHELTON, K. & COCHRAN, D. (1978). *In vitro* oxidation of intrinsic sulfhydryl groups yields polymers of the two predominant polypeptides in the nuclear envelope fraction. *Biochemistry* **17**, 1212–1216.

SHELTON, K., HIGGINS, L., COCHRAN, D., RUFFOLO, D. & EGLE, P. (1980). Nuclear lamins of erythrocyte and liver. *J. biol. Chem.* **255**, 10978–10983.

STECK, T. (1974). The organization of proteins in the human red blood cell membrane. *J. Cell Biol.* **62**, 1–19.

STECK, T. & YU, J. (1973). Selective solubilization of proteins from red cell membranes by protein perturbants. *J. supramolec. Struct.* **1**, 220–231.

STICK, R. & HAUSEN, D. (1980). Immunological analysis of nuclear lamina proteins. *Chromosoma* **80**, 219–236.

STICK, R. & KROHNE, G. (1982). Immunological localization of the major architectural protein associated with the nuclear envelope of the *Xenopus laevis* oocyte. *Expl Cell Res.* **138**, 319–330.

TOBEY, R., ANDERSON, E. & PETERSEN, D. (1967). Properties of mitotic cells prepared by mechanically shaking monolayer cultures of Chinese hamster cells. *J. cell. Physiol.* **70**, 63–68.

UNWIN, P. & MILLIGAN, R. (1982). A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* **93**, 63–75.

VENABLE, J. & COGGESHALL, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**, 407–408.

WATSON, M. (1958). Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. biochem. Cytol.* **4**, 475–478.

WOLD, F. (1981). *In vivo* chemical modifications of proteins (posttranslational modification). *A. Rev. Biochem.* **50**, 783–814.