Abstract. We have examined the nuclear localization of isoprenylated proteins in CHO-K1 cells labeled with [14C]mevalonate. Nuclear proteins of 68, 70, and 74 kD, posttranslationally modified by an isoprenoid, are also components of a nuclear matrix–intermediate filament preparation from CHO cells. Furthermore, the 68-, 70-, and 74-kD isoprenylated polypeptides are immunoprecipitated from cell extracts with two different anti–lamin antisera. Based on exact two-dimensional comigration with lamin B, both from rat liver lamin and CHO nuclear matrix–intermediate filament preparations, and its immunoprecipitation with anti–lamin antisera, we conclude that the 68-kD isoprenylated protein found in nuclei from [14C]mevalonate-labeled CHO cells is lamin B. The more basic 74-kD isoprenylated nuclear protein is similar in molecular mass and isoelectric pH variants to the lamin A precursor polypeptide reported by others. Starving cells for mevalonate results in a dramatic accumulation of a polypeptide that comigrates on two-dimensional, non-equilibrium pH gradient electrophoresis (NEPHGE) gels with the 74-kD isoprenylated protein. The 70-kD isoprenylated protein, which is resolved on NEPHGE gels as being higher in molecular mass and slightly more basic than lamin B, has not yet been identified.

The nuclear lamina, nuclear pore complex, and the inner and outer nuclear membranes are the major structural components of the nuclear envelope. Sequential extraction of isolated nuclear envelopes with nucleases, non-ionic detergents, and high salt yields an insoluble fraction enriched in the structure known as the nuclear lamina (12, 18, 25). The three major polypeptide components of nuclear lamina from mammalian cells are designated lamins A, B, and C (16), and comprise ~75% of the lamina mass (17). Lamins have been described in a range of vertebrates and invertebrates (15, 19, 22, 38). Although there are variations in the type of lamins present in the nuclear lamina of some species, these proteins appear to be reasonably well conserved (22, 38). Additional protein components of the lamina are also present in insect, avian, and rodent cells. These include lamin B subtypes or variants (10, 30, 31, 48), precursor forms of lamins A and B (19, 26, 32), and Drosophila nuclear lamin isoforms (51).

At mitosis, lamins undergo a transient and reversible depolymerization (6, 16, 52). During this process, lamins A and C become soluble and nonmembrane associated (6, 16). In contrast, lamin B remains associated with nuclear membrane vesicles (6, 17, 25, 29), a phenomenon that may be due to hydrophobic properties of lamin B itself (19). Newly synthesized lamin A has been reported to exist as a short-lived precursor molecule (19, 26, 32, 40). Lamin A appears to be processed to a mature form after being inserted into a Triton X-100-insoluble, membrane-associated fraction (19, 32, 40).

We are currently investigating the subcellular distribution of a class of proteins that are posttranslationally modified by a product of mevalonate metabolism (5, 34, 44, 49). These proteins are characterized by incorporation of radioactive mevalonate labeled at either the 5-3H position or the 2-14C position but not the 1-14C position (44), consistent with the hypothesis that the actual substituent is an isoprenoid. Further evidence consistent with conversion of mevalonate to an isoprenoid, before incorporation into these proteins, is its physical properties after release from proteins by acid hydrolysis (5). These observations have led others to refer to proteins posttranslationally modified by a product of mevalonate metabolism as isoprenylated proteins (5, 34, 44), a practice we will also follow in this report.

The requirement for a specific nonsterol product, or products, of mevalonate metabolism in maintaining cellular viability and regulating cell cycle progression has been well documented (20, 42, 49) and we have proposed (49) that one or more isoprenylated proteins fulfill this function. Because of our interest in the possible role that one or more isoprenylated proteins might play in mitosis, we elected to examine mammalian nuclei for the presence of such proteins. In this report, we present evidence that specific proteins found in nuclei and nuclear matrix–intermediate filament preparations (33) from CHO-K1 cells are isoprenylated, that the 68-kD isoprenylated polypeptide is lamin B, and the
74-kD isoprenylated polypeptide is the precursor form of laimin A.1

Materials and Methods

Cells and Antisera

The CHO-K1 cell line, met-18b-2 (13), was a gift of Dr. J. Faust (Tufts University, Boston, MA). This cell line incorporates labeled mevalonate 50 times more efficiently into isoprenylated proteins than wild-type CHO-K1 cells. Guinea pig antiserum (3) against lamins (A+B+C) was a gift of Dr. G. Krohne (Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany). Anti-lamin (A+C) autoimmune serum (33) was a gift of Dr. M. Kirschner (Department of Biochemistry and Biophysics, University of California, San Francisco, CA). Normal human and guinea pig sera were obtained from Rockland, Inc. (Gibertsville, PA).

Cell Culture and Radiosotopic Labeling

The CHO-K1 cell line, met-18b-2, was grown as described (13). Cells (6 × 10^6 per 150-mm culture dish) were labeled in the presence of 5 μg/ml mevinolin (2), a potent inhibitor of mevalonate biosynthesis in mammalian cells (supplied by A. Alberts, Merck and Co., Rahway, NJ), with 2-10 μCi/ml R-(2-14C) (2.10 GBq/mmol; Amersham Corp., Arlington Heights, IL) mevalonic acid lactone for 13-24 h in the appropriate growth medium containing 5% dialyzed feral calf serum without exogenous mevalonate. When appropriate, parallel cultures of cells were labeled with 10-30 μCi/ml of [35S]methionine (Trans35S-label; 1104 Ci/mmol; ICN Radiochemicals, Inc., Irvine, CA) as described (41).

Gel Electrophoresis

SDS-PAGE was performed on 18 × 16 × 0.12 cm gels, containing a gradient of 7.5-15% acrylamide (30:0.8 ratio of acrylamide to bis-acrylamide and 5-15% sucrose, using the buffer system of Laemmli [27]). Samples were dient of 7.5-15% acrylamide (30:0.8 ratio of acrylamide to bis-acrylamide concentration of 0.4% each to the swollen cells with gentle vortexing. After an additional 15 s of vortexing, the cells were incubated on ice for 10 min. An equivalent volume of 500 μmol sucrose in TKM/PMSF/PIM was added to return the extraction buffer to isotonicity and the nuclei were pelleted by centrifugation for 10 min in a Sorvall HB-4 rotor (E. 1. Du Pont de Nemours & Co., Newtown, CT) at 365 g (1,500 rpm). The nuclei were gently resuspended in 250 mM sucrose in TKM/PMSF/PIM followed by dropwise addition of NP-40 and sodium deoxycholate to a final concentration of 0.4% each. The nuclei were then vortexed for 30 s and pelleted as before. This wash step was repeated twice, followed by two washes with 250 mM sucrose in TKM/PIM without nonionic detergents.

Peptide Mapping

Nuclear lamina preparations were subjected to two-dimensional NEPHGE analysis and the location of component polypeptides visualized by Coomassie Blue staining. Gel slices containing individual polypeptide spots were

1. While this manuscript was being revised, it was reported that lamin B is posttranslationally modified by a derivative of mevalonate (Wolda, S. L., and J. A. Glomset. 1988. Evidence for modification of lamin B by a product of mevalonic acid. J. Biol. Chem. 263:5997-6000).

2. Abbreviations used in this paper: NEPHGE, nonequilibrium pH gradient electrophoresis; PIM, protease inhibitor mixture; TKM buffer, 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM MgCl2, 5 mM NaClO4.

Preparation of Cytosolic Extract from CHO Cells

Labeled cells were harvested from culture dishes by scraping in ice-cold PBS (I containing 1 mM phenylmethylsulfonyl fluoride (PMSF; freshly prepared in acetone: ethanol [1:1]) and added immediately before scraping the cells. The cells were washed twice in PBS, and the cell pellet, at a density of 10^9 cells per ml of buffer, was resuspended by gentle vortexing in Triton lysis buffer. Triton lysate buffer contains 50 mM Tris-HCl, pH 7.5, 5 mM imidazole, 5 mM EDTA, 20 mM KCl, 50 mM NaCl, 0.25% Triton X-100 (Surfact-Amps X-100; Pierce Chemical Company, Rockford, IL), 1 mM PMSF (added immediately prior to use), and contains a protease inhibitor mixture (PIM), consisting of 5 μg/ml each (final buffer concentration) of leupeptin, pepstatin A, chymostatin, bestatin, antipain, and aprotinin (Sigma Chemical Co.). The cells were then incubated for 30 min on ice and nuclei and cellular debris removed by a 15-min centrifugation at 4°C in an Eppendorf microfuge (Brinkman Instruments, Inc., Westbury, NY). The supernatant was transferred to a fresh microfuge tube and incubated with 100 μg/ml RNase A (type X-A, protease-free; Sigma Chemical Co.) at 37°C for 30 min to digest isopentenyl tRNA (49). The RNase-treated, cytosolic extract was stored in aliquots at ~80°C.

Preparation of Nuclei from CHO Cells

Labeled cells were washed five times with ice-cold PBS and harvested by trypsinization. After two additional washes with centrifugation, the cells were resuspended at a density of 1.5 × 10^7 per ml in 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM MgCl2 (TKM buffer), with 1 mM PMSF and PIM, and allowed to swell on ice for 15 min. A 10% NP-40 (vol/vol) and 10% (wt/vol) sodium deoxycholate mixture was added dropwise to a final concentration of 0.4% each to the swollen cells with gentle vortexing. After an additional 15 s of vortexing, the cells were incubated on ice for 10 min. An equivalent volume of 500 μmol sucrose in TKM/PMSF/PIM was added to return the extraction buffer to isotonicity and the nuclei were pelleted by centrifugation for 10 min in a Sorvall HB-4 rotor (E. 1. Du Pont de Nemours & Co., Newtown, CT) at 365 g (1,500 rpm). The nuclei were gently resuspended in 250 mM sucrose in TKM/PMSF/PIM followed by dropwise addition of NP-40 and sodium deoxycholate to a final concentration of 0.4% each. The nuclei were then vortexed for 30 s and pelleted as before. This wash step was repeated twice, followed by two washes with 250 mM sucrose in TKM/PIM without nonionic detergents.

Preparation of Nuclear Matrix–Intermediate Filament Fraction from CHO Cells

The procedure of McKeon et al. (33) was used with modifications to prepare a nuclear matrix–intermediate filament fraction enriched in lamins from CHO cells. Radiolabeled cells were washed five times with PBS containing 2 mM EDTA, harvested by trypsinization, and incubated on ice in PBS containing 1 mM PMSF for 3-4 h. The cells were then pelleted by centrifugation in an IEC clinical centrifuge (Damon/IEC, Needham Heights, MA) for 15 min on ice in 15 mM Tris-HCl, pH 7.5, 5 mM EDTA, 300 mM NaCl, 1 mM PMSF and PIM, and washed three times with PBS, 0.2% NaClO4, 30 mM 2-mercaptoethanol, 1 mM PMSF and PIM, 20 mM CaCl2, 1 mg/ml DNase I (Code DP; CooperBiomedical Inc.) and 200 μg/ml RNase A (Code RASE; CooperBiomedical Inc.). After 20 min at 25°C, the nuclear envelope preparation was pelleted by centrifugation in an HB-4 rotor at 4°C for 10 min at 365 g (1,500 rpm). The supernatant was removed by aspiration and the pellet was resuspended for 15 min on ice with 15 mM Tris-HCl, pH 7.5, 5 mM EDTA, 80 mM KCl, 3 M NaCl, 1.05% Triton X-100, 15 mM 2-mercaptoethanol, 0.5 mM PMSF, and PIM. The pellet was resuspended in 10 mM PMSF and PIM, and subjected to a nuclear suspension by the addition of an equal volume of PBS, 0.2% NaClO4, 30 mM 2-mercaptoethanol, 1 mM PMSF and PIM, 20 mM CaCl2, 1 mg/ml DNase I (Code DP; CooperBiomedical Inc.) and 200 μg/ml RNase A (Code RASE; CooperBiomedical Inc.). After 10 min at 25°C, the nuclear envelope preparation was pelleted by centrifugation in an HB-4 rotor at 4°C for 10 min at 365 g (1,500 rpm). The supernatant was removed by aspiration and the pellet was resuspended for 15 min on ice with 15 mM Tris-HCl, pH 7.5, 5 mM EDTA, 80 mM KCl, 3 M NaCl, 1.05% Triton X-100, 15 mM 2-mercaptoethanol, 0.5 mM PMSF, and PIM. After a 10-min centrifugation at 4°C in an HB-4 rotor at 2,000 rpm (3,500 rpm), the nuclear matrix–intermediate filament preparation was incubated on ice a second time with the same buffer, and pelleted for 10 min at 10,000 g (8,000 rpm). The supernatant was removed and the final nuclear matrix–intermediate filament preparation was solubilized in 63 mM Tris-HCl, pH 7.5, 2% SDS, 5% 2-mercaptoethanol, 8 M urea, and stored in aliquots at ~80°C.
Preparation of Rat Liver Lamina

The protocol used in the preparation of rat liver lamina was adapted from published procedures. Nuclei were prepared from the livers of adult, male, Sprague-Dawley rats according to the procedure of Blobel and Potter (4), pelleted a second time through a 2.3 M sucrose cushion (4), and washed twice by centrifugation in 10 mM Tris-HCl (pH 7.2), 400 mM sucrose, and 1 mM PMSF (23). The procedure of Krohne and Franke (23) was used to prepare nuclear envelopes from rat liver nuclei. However, the PMSF concentration was increased to 1 mM, leupeptin and pepstatin (at a final concentration of 5 μg/ml each) were added to all solutions, and TKM buffer was substituted for Tris-HCl during the nuclear swelling step. The final pore complex-lamina fraction was resuspended in 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.5 mM PMSF, 2 mM dithiothreitol, 5 μg/ml pepstatin and leupeptin, and 100 mM NaCl, precipitated with 3 vol of -20°C absolute ethanol (12), and stored at -20°C.

Immunoprecipitation of [14C]Mevalonate and [35S]Methionine-labeled Nuclear Matrix Proteins from CHO Cells

The immunoprecipitation procedure of Ottaviano and Gerace (40) was followed with minor modifications. CHO-K1 (met-18b-2) cells (6 x 10⁶ per 150-mm plate) were labeled for 17 h with 14C]-mevalonate or [35S]methionine as described, and solubilized in 500 μl of 55 mM triethanolamine, pH 7.4, 110 mM NaCl, 0.44%, SDS, 2.2 mM EDTA, 1 mM PMSF and PIM for 5 min at 100°C. Samples were cooled on ice, sonicated briefly, and cellu- 
lar debris removed by centrifugation in an Eppendorf microtube Beck et al. (4) was adapted from 

Figure 1. Identification of CHO isoprenylated proteins by SDS-PAGE and fluorography. CHO-K1 cells (met-18b-2) were labeled with R-[2-14C]mevalonate in Ham's F12 medium containing 5% dialyzed fetal calf serum and 8 μg/ml mevinolin. The cells were then washed exhaustively with PBS and harvested by trypsinization. Cytosolic extract, nuclei, nuclear matrix-intermediate filament fraction, and rat liver lamina were prepared as described in Materials and Methods. SDS-PAGE analysis of these fractions was performed on slab gels containing a combinator of 7.5-15% acrylamide and a 5-15% sucrose gradients in the buffer system of Laemmli. After electrophoresis, the gel was stained with Coomassie Brilliant Blue (A, lanes a-c), and 14C-isoprenylated polypeptides visualized by fluorography (B, lanes a-c) using preflashed Kodak X-Omat AR film. Fluorographic exposure was for 45 d. (A) lane a, cytosolic extract; lane b, nuclei; lane c, nuclear matrix-intermediate filament fraction; lane d, rat liver lamina. The corre- 
spanding fluorogram (B) presents the radioactive banding patterns of 14C-isoprenylated polypeptides: lane a, cytosolic extract; lane b, nuclei; lane c, nuclear matrix-intermediate filament fraction. The solid triangles to the right of A indicate the location of rat liver lamins A, B, and C. The bars to the right of B indicate the location of the 68-, 70-, and 74-kD 14C-isoprenylated polypeptides. Molecular mass standards are presented at the left of A.

Results

Identification of Isoprenylated, Nuclear Proteins, Using One-dimensional SDS-PAGE

To determine whether isoprenylated proteins are components of the cell nucleus, we prepared nuclei from R-[2-14C]mevalonate-labeled CHO cells. Labeled nuclei were then subjected to SDS-PAGE and [14C]isoprenylated proteins visualized by fluorography. A representative Coomas-sie Blue-stained gel (Fig. 1 A) and the corresponding autoradiogram (Fig. 1 B) generated by 14C-labeled isoprenylated polypeptides are presented in Fig. 1. Comparison of the au-

toradiographic banding patterns of 14C-isoprenylated polypeptides obtained from cytosolic extract and nuclei (Fig. 1 B, lanes a vs. b), demonstrates that several isoprenylated pro-

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ated polypeptides was suggestive of nuclear proteins known as lamins. To examine this possibility further, we prepared a nuclear matrix-intermediate filament fraction enriched in proteins of the nuclear lamina from [14C]mevalonate-labeled CHO cells and examined this preparation by SDS-PAGE and fluorography. Coomassie Blue-staining polypep-
tides from the SDS-PAGE analysis (Fig. 1 A, lane c) shows a nuclear matrix-intermediate filament preparation highly enriched in lamins A (72 kD), B (68 kD), and C (62 kD), as well as the intermediate filament protein vimentin (56 kD), and actin (43 kD), as has been reported for such prepa-
Two-dimensional NEPHGE gel analysis of rat liver lamin and CHO nuclear matrix—intermediate filament preparations. Rat liver lamin and nuclear matrix—intermediate filament preparations from CHO-K1 (met-18b-2) cells labeled with [35S]methionine were prepared as described in Materials and Methods. A trace (non-Coomassie Blue-stainable) amount of the 35S-labeled CHO nuclear matrix—intermediate filament fraction was added to rat liver lamin preparation and the mixture analyzed on two-dimensional NEPHGE gels. 3,500 Vh of electrophoresis was used for the first (NEPHGE) dimension. SDS-PAGE consisted of 8% acrylamide and 10% glycerol using the buffer system of Laemmli. After SDS-PAGE, the gel was stained with Coomassie Brilliant Blue to identify the rat liver lamins (A). The gel was then processed for fluorography to visualize the [35S]methionine-labeled CHO nuclear matrix polypeptides (B). Letters indicate the positions of lamins A, B, C, vimentin (v), and actin (a).

Examination of the autoradiographic banding pattern of the nuclear matrix—intermediate filament preparation (Fig. 1 B, lane c) reveals three distinct labeled isoprenylated polypeptides of 68, 70, and 74 kD which comigrate with isoprenylated polypeptides of identical molecular masses in nuclei. The most highly labeled, 68-kD polypeptide, precisely comigrates with CHO and rat liver lamin B. The 70-kD band displays a relative mobility intermediate between lamins A and B, while the largest of these isoprenylated polypeptides...
migrates as a polypeptide of 74-kD, banding at a position just above CHO and rat liver lamin A (compare Fig. 1 B, lane c, with Fig. 1 A, lane d).

**Two-dimensional Polyacrylamide Gel Analysis of the 14C-isoprenylated Nuclear Matrix–Intermediate Filament Proteins**

To further examine the possibility that one or more of the 68–74-kD isoprenylated proteins of CHO nuclei were part of the nuclear lamina, we compared rat liver lamin and radiolabeled CHO nuclear matrix–intermediate filament preparations by two-dimensional NERHEGE gels and fluorography. Analysis of these isoprenylated proteins was greatly facilitated by their enrichment in nuclear matrix–intermediate filament preparations from whole cells. NERHEGE gel analysis on rat liver lamin mixed with trace amounts of a nuclear matrix–intermediate filament preparation from [35S]-methionine-labeled CHO cells is presented in Fig. 2. Precise two-dimensional comigration afforded us additional certainty in our identification of the CHO lamins in such preparations as previously reported (21, 47). The Coomassie Blue–staining pattern of a two-dimensional NERHEGE gel analysis of rat liver lamins (Fig. 3 A) was then compared with the Coomassie Blue–staining pattern (Fig. 3 B) and the corresponding fluorogram pattern (Fig. 3 C) of the 14C-labeled isoprenylated proteins present in CHO nuclear matrix–intermediate filament preparations. Prominent Coomassie Blue–staining proteins in the CHO nuclear matrix–intermediate filament preparations (Fig. 3 B) are lamin A (72 kD) and lamina C (62 kD), as well as actin (43 kD) and vimentin (56 kD). Present in the acidic portion of the NERHEGE gel (Fig. 3 B), at molecular masses between those of lamins A and C, are two Coomassie Blue–staining polypeptides. The more acidic of these polypeptides, with a molecular mass of 68 kD and an isoelectric point nearly identical to that of vimentin, corresponds to the 68-kD CHO nuclear protein which comigrates with rat liver lamin B on SDS-PAGE (Fig. 1 A, lanes c and d) and two-dimensional NERHEGE gels (Fig. 2). Also resolved is an additional Coomassie Blue–staining spot higher in molecular mass (70 kD) and slightly more basic than lamin B. Several minor Coomassie Blue–staining spots of unknown identity are also present in these nuclear matrix–intermediate filament preparations.

Fig. 3 C presents the fluorographic pattern obtained from the Coomassie Blue–stained gel presented in Fig. 3 B. The three 14C-isoprenylated polypeptide bands seen in Fig. 1 B,
polypeptide (Fig. 3 C, open triangle), which migrates to a
ylated polypeptide migrates to the basic region of the
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dioactive polypeptide bands (Fig. 5, lane b) on the autoradio-
anti-lamin (A+B+C) antiserum, generates a pattern of ra-
CHO cells were immunoprecipitated with lamin-specific an-
extracts from [~4C]mevalonate- or [35S]methionine-labeled
immunoprecipitated [35S]methionine-labeled CHO lamin B (lanes
Molecular mass standards are presented to the left of
radioimmune precipitation with anti-lamin (A+B+C) anti-
sen is presented in Fig. 5, lane c. Three [14C]-isoprenylated polypeptides are recognized by the anti-lamin antise-
immunoprecipitated radioactive band of 68 kD precisely comigrates with the Coomassie Blue–staining band of
radiation and fluorography as described in Materials and Methods. Fluorographic exposure was for 30 [14C]-isoprenylated polypeptides) and 3 d (for [35S]methionine-labeled polypeptides).
[35S]Methionine-labeled (lane c, bars) immunoprecipitated with guinea pig anti-lamin (A+B+C) serum. [35S]Methionine-labeled (lane f, open triangles) and [14C]-isoprenylated polypeptides (lane e, open triangles) and [14C]-isoprenylated (lane f, bars) polypeptides immunoprecipitated with human anti-lamin (A+C) autoimmune serum. [35S]Methionine-labeled polypeptides nonspecifically bound to preimmune guinea pig (lane a) and human (lane g) serum. Shown in lane d are Coomassie Blue–stained rat liver lamins A, B, and C (solid triangles). Molecular mass standards are presented to the left of the figure.

Immune Precipitation of the Isoprenylated Proteins
with Anti-Lamin Antisera

Two-dimensional NEPHGE analysis of the isoprenylated nu-
clear matrix–intermediate filament proteins provided clear evidence that these isoprenylated proteins have molecular masses and isoelectric pH values characteristic of proteins of the nuclear lamina. We attempted to further substantiate these assignments by immunological techniques. Whole cell extracts from [14C]mevalonate- or [35S]methionine-labeled CHO cells were immunoprecipitated with lamin-specific antiserum and analyzed by SDS-PAGE and fluorography. Radioimmunoprecipitation of [35S]methionine-labeled cells with anti-lamin (A+B+C) antiserum, generates a pattern of radioactively labeled peptides bands (Fig. 5, lane b) on the autoradiogram that comigrate with rat liver lamins A, B, and C (Fig. 5, lane d).

The radioactive banding pattern obtained from CHO cells labeled with [14C]mevalonate and subjected to a parallel

Figure 5. SDS-PAGE and fluorographic analysis of isoprenylated lamin polypeptides from CHO after immunoprecipitation with anti–lamin antisera. Whole cell extracts of CHO-K1 (met-18b-2) cells, labeled with 3 μCi/ml [35S]methionine or 10 μCi/ml R-[2-
NEPHGE analysis (unpublished observations), it was pos-
possible that the comigration in the NEPHGE dimension of la-
m A and the lamin A-like, 74-kD [14C]-isoprenylated polypep-
was an electrophoretic artifact. Accordingly, we repeated the two-dimensional NEPHGE analysis of the [14C]-
isoprenylated nuclear matrix–intermediate filament prepara-
tion for 1,580 and 2,200 (nonequilibrium), and 3,240 (near-
equilibrium) Vh of electrophoresis in the first (NEPHGE) dimension. In this series of two-dimensional NEPHGE gels (Fig. 4, A–C), we observe increasing resolution of the family of Coomassie Blue–staining pH isovariants for lamins A and C as the time of electrophoresis in the NEPHGE dimension is increased from 1,580 Vh (Fig. 4 A) to 3,240 Vh (Fig. 4 C). The corresponding series of fluorograms (Fig. 4, D–F) demonstrates that the 74-kD [14C]-isoprenylated polypeptide (solid triangle) also contains a family of pH isovariants that are more clearly resolved in the basic dimension with increasing time of electrophoresis. Furthermore, the migration pattern of the 74-kD [14C]-isoprenylated pH isovariants is ex-
ctly the same as the pattern of the Coomassie Blue–staining
72-kD lamin A pH isovariants. Therefore, lamin A and the
74-kD isoprenylated polypeptide have an identical family of
isolectric pH variants, differing by ~2 kD.

Immune Precipitation of the Isoprenylated Proteins
with Anti-Lamin Antisera

Two-dimensional NEPHGE analysis of the isoprenylated nu-
clear matrix–intermediate filament proteins provided clear evidence that these isoprenylated proteins have molecular masses and isoelectric pH values characteristic of proteins of the nuclear lamina. We attempted to further substantiate these assignments by immunological techniques. Whole cell extracts from [14C]mevalonate- or [35S]methionine-labeled CHO cells were immunoprecipitated with lamin-specific antiserum and analyzed by SDS-PAGE and fluorography. Radioimmunoprecipitation of [35S]methionine-labeled cells with anti-lamin (A+B+C) antiserum, generates a pattern of radioactively labeled peptides bands (Fig. 5, lane b) on the autoradiogram that comigrate with rat liver lamins A, B, and C (Fig. 5, lane d).

The radioactive banding pattern obtained from CHO cells labeled with [14C]mevalonate and subjected to a parallel
Figure 6. Accumulation of the precursor form of CHO lamin A in cells starved for mevalonate by treatment with mevinolin. CHO-K1 cells were plated at a density of $6 \times 10^6$ cells per 150-mm culture dish and allowed to attach overnight. The growth medium was then removed and the cells washed with PBS. To block endogenous mevalonate synthesis, fresh growth medium containing 2 μg/ml mevinolin (MVN) was added to half of the culture dishes. Normal growth medium was added to the remaining culture dishes. After an additional 17-h incubation, nuclear matrix-intermediate filament preparations were prepared as described in Materials and Methods. The nuclear matrix-intermediate filament preparations obtained from control and mevalonate starved cells were then analyzed on two-dimensional NEPHGE gels using the experimental conditions described in the legend to Fig. 2, and visualized by silver staining. Indicated are lamins A, B, C; the precursor form of lamin A, (Ao, solid triangle); the 70-kD isoprenylated polypeptide (open triangle); vimentin (v); and actin (a). (A) Nuclear matrix-intermediate filament preparation from the control CHO cells. (B) Nuclear matrix-intermediate filament preparation from mevinolin-treated (mevalonate starved) CHO cells. Note the accumulation of the precursor form of lamin A in the mevinolin-treated CHO cells (B), and the absence of lamin Ao in the control (nonmevinolin treated) CHO cells.

Pattern from $^{35}$S)methionine or $^{14}$C)mevalonate-labeled CHO cells is obtained using anti-lamin (A+C) autoimmune serum (Fig. 5, lanes e and f). Immunoprecipitation by anti-lamin antisera is evidence that these $^{14}$C-isoprenylated proteins are components of the nuclear lamina.

**Peptide Mapping of the 74-kD Polypeptide**

In comparing two-dimensional gel patterns of nuclear matrix-intermediate filament preparations of CHO-K1 cells starved for mevalonate by mevinolin treatment with those from untreated cells, we noted a marked accumulation of material visualizable by Coomassie Blue staining at the same migration position as the 74-kD isoprenylated polypeptide (Fig. 6, compare solid triangle in panel A vs. B). The 74-kD isoprenylated polypeptide and lamin A were excised from the gel, and subjected to peptide mapping (9) by partial hydrolysis with *Staphylococcus aureus* V-8 protease. The resultant peptide fragment patterns are compared in Fig. 7. Peptide mapping data indicate substantial similarity between the 74-kD isoprenylated polypeptide and lamin A and are consistent with the hypothesis that the 74-kD polypeptide is prelamin A.

**Discussion**

Using a combination of biochemical and immunological analyses, we have identified isoprenylated nuclear proteins of 68, 70, and 74 kD in nuclei and nuclear matrix-intermediate filament preparations from CHO cells. These proteins display the characteristic insolubility of lamins upon treatment with nucleases, nonionic detergents, and high salt. One- and two-dimensional gel analyses (Figs. 1 and 3) of $^{14}$C-isoprenylated nuclear matrix-intermediate filament preparations have demonstrated that the 68-kD isoprenylated polypeptide and lamin B from CHO cells and rat liver exactly comigrate. The 70-kD isoprenylated polypeptide appears on two-dimensional gels (Fig. 3) as a polypeptide of slightly higher molecular mass and more basic isoelectric pH than lamin B. The 74-kD isoprenylated nuclear protein has the same isovariant pH pattern (Figs. 4 and 6) and partial peptide mapping pattern (Fig. 7) as does CHO lamin A, but is larger by ~2 kD.

Further evidence for the association of these isoprenylated proteins with the nuclear lamina was made through radioimmune precipitation studies using anti-lamin antisera. We have compared the autoradiographic patterns generated by SDS-PAGE of immunoprecipitated $^{14}$C-isoprenylated polypeptides (Fig. 5) with nuclear matrix-intermediate filament preparations (Fig. 1) from $^{14}$C)mevalonate-labeled CHO cells. The results of these experiments demonstrate that the three $^{14}$C-isoprenylated polypeptides of 68, 70, and 74 kD found in CHO nuclei are identical to those immunoprecipitated from $^{14}$C)mevalonate-labeled cell extracts with anti-lamin antisera.

The 68-kD isoprenylated polypeptide can be identified as lamin B on the basis of its immunoprecipitation by anti-lamin antisera and its exact comigration on two-dimensional gels.
because of its solubility characteristics and because it can be immunoprecipitated with anti-lamin antisera. This polypeptide displays a family of isoelectric pH variants identical to those known for the 74-kD isoprenylated polypeptide with mature position (32), rat liver cells (19), and in CHO cells (17, 40). These data are consistent with the hypothesis that the 74-kD isoprenylated polypeptide is a lamin A precursor. Therefore, assignment of homology between the 74-kD isoprenylated polypeptide, an apparent molecular weight ~2 kD greater than lamin A and exists as a transient component of the nuclear matrix. In CHO cells, the lamin A precursor (lamin A0) becomes membrane associated and Triton X-100 insoluble within 5 min of synthesis, and it is in this Triton X-100-insoluble form that lamin A0 is processed to the mature form of lamin A (19). In contrast, the precursor form of lamin A in chicken embryo fibroblasts is Triton X-100 soluble, becoming membrane associated and detergent insoluble only after being processed to the mature form of lamin A (32). In preliminary turnover studies, we have found that the half-life of isoprenylation for the 74-kD protein is much shorter than that for the 68- and 70-kD isoprenylated nuclear proteins (unpublished results), as might be expected for a precursor polypeptide.

The accumulation of the 74-kD protein in mevinolin-treated cells is reminiscent of another isoprenylated precursor protein found in the yeast Tremella mesenterica (36, 37). This 28-kD protein is a precursor to an isoprenylated 1,480-D polypeptide mating pheromone known as Tremerogen A-10. When Tremella are treated with an inhibitor of mevalonate biosynthesis, the nonisoprenylated precursor accumulates. The observations in this system and for prelamin A in this report suggest that isoprenylation may act as a signal for proteolytic maturation of proteins.

The presence of the 70-kD polypeptide in nuclear matrix-intermediate filament preparations and its immunoprecipitation by anti-lamin antisera suggest that this protein is also a component of the CHO-K1 nuclear lamina. Nuclear lamina proteins migrating on two-dimensional gels as a larger and more basic polypeptide than lamin B, as is the case for the 70-kD isoprenylated polypeptide, have either been reported or can be identified in two-dimensional gels in published studies on rat liver (21, 29, 30, 47), avian (32, 48), and baby hamster kidney cells (10, 26).

Lehnert et al. (31) demonstrated that avian liver, in addition to the major acidic lamin protein (lamin B1), contains at least one minor acidic pore-complex lamina protein (lamin B2), not previously thought to be associated with the nuclear matrix. They also demonstrated that lamin B1 is immunologically related to mammalian lamin B whereas lamin B2 is more closely related immunologically to mammalian and chicken lamin A. Therefore, assignment of homology between the 70-kD isoprenylated protein and other lamins, based only on its two-dimensional gel migration position reported here, cannot be made.

It is interesting to speculate on the function of lamin B isoprenylation. It has been clearly demonstrated (6, 17, 25, 29) that lamin B is more tightly associated with the nuclear membrane than lamins A or C, and that a significant proportion of lamin B exists as an intrinsic nuclear membrane protein (29). Lamin B, therefore, must possess physical properties either in its primary sequence or via posttranslational modification that promotes sequestration into the hydrophobic lipid bilayer of the inner nuclear membrane. It has been proposed (19) that this interaction might be due to a hydrophobic domain of lamin B. An equally valid hypothesis to explain affinity of lamin B for the nuclear membrane, is that this interaction is mediated by an isoprenoid substituent. A review of the literature (45) reveals the existence of a large class of proteins covalently modified with lipids, whose membrane-binding ability is absolutely dependent upon cotranslational or posttranslational modification by lipids. It

Figure 7. Peptide maps of CHO lamin A and the 74-kD isoprenylated polypeptide. Gel slices containing CHO lamins A and the 74-kD isoprenylated precursor form of lamin A were cut from a two-dimensional NEPHGE gel identical to that shown in Fig. 6. Partial proteolysis with Staphylococcus aureus V8 protease (2.5 ng per lane) and concomitant SDS-PAGE performed in 15% acrylamide gels according to the method of Cleveland et al. (9). After electrophoresis (20 h, 7 mA, constant current), the gel was silver stained. Lane a, CHO lamin A; lane b, lamin A0. Molecular mass standards are presented at the left of the figure.

with lamin B from CHO cells and rat liver. Lamin B, therefore, is posttranslationally derivatized by an isoprenoid.

We conclude that the nuclear isoprenylated polypeptide of 74-kD is a component of the nuclear lamina of CHO cells because of its solubility characteristics and because it can be immunoprecipitated with anti-lamin antisera. This polypeptide displays a family of isoelectric pH variants identical to lamin A. Furthermore, partial digestion of CHO lamin A and the 74-kD isoprenylated polypeptide with Staphylococcus aureus V8 protease generates similar peptide profiles. These data are consistent with the hypothesis that the 74-kD polypeptide is a lamin A precursor.

Similar lamin A-like proteins, shown to be lamin A precursors, have been described in Drosophila cells (31), baby hamster kidney cells (10, 26), chicken embryo fibroblasts (32), rat liver cells (19), and in CHO cells (17, 40). In each of these cell lines, the lamin A precursor has, as does the 74-kD isoprenylated polypeptide, an apparent molecular mass ~2 kD greater than lamin A and exists as a transient component of the nuclear matrix. In CHO cells, the lamin A precursor (lamin A0) becomes membrane associated and Triton X-100 insoluble within 5 min of synthesis, and it is in this Triton X-100-insoluble form that lamin A0 is processed to the mature form of lamin A (19). In contrast, the precursor form of lamin A in chicken embryo fibroblasts is Triton X-100 soluble, becoming membrane associated and detergent insoluble only after being processed to the mature form of lamin A (32). In preliminary turnover studies, we have found that the half-life of isoprenylation for the 74-kD protein is much shorter than that for the 68- and 70-kD isoprenylated nuclear proteins (unpublished results), as might be expected for a precursor polypeptide.

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has also been proposed (54) that fatty acylation might mediate binding of acylated proteins to various subcellular membranes. Another relevant lipid/protein interaction occurs with intermediate filaments of the vimentin type. Here, different classes of nonpolar lipids can strongly interact with both the non-α-helical headpiece and the α-helical rod domain of vimentin in a noncovalent fashion (53). This interaction facilitates the insertion of intermediate filament proteins into the phospholipid bilayer of lipid vesicles. Exhaustive proteolytic digestion of CHO ~4C-isoprenylated proteins (unpublished results and reference 44) releases ~4C-isoprenylated peptides that are soluble in organic solvents. The relative hydrophobicity of these isoprenylated moieties lends credence to a postulated membrane binding function for the isoprenylation of lamins, particularly in light of the well-documented correlation between lipid modification of proteins and membrane association.

Considerable evidence exists to support a key role for the nuclear lamins in mitosis. In interphase cells, the lamina forms a fibrous scaffoldlike structure positioned between the inner nuclear membrane and chromatin (1, 12). The lamina interconnects at the pore complexes where it may form attachment sites for chromatin, stabilizing cell cycle dependent chromatin structure (14). When postmitotic soluble lamins are sequestered through microinjection of anti-lamin antibodies, chromosomes are arrested in a telophase-like configuration (3). Furthermore, in vitro immunodepletion of CHO cell extracts for lamina A and C or lamin B blocks reassembly of nuclear envelopes (6). These reports support the premise that lamins are required for the postmitotic reassembly of the nuclear envelope. Thus, it is possible that one or more of the isoprenylated proteins of the nuclear lamina described in this report may be responsible for the cell cycle arrest observed in cells starved for mevalonate (20, 42, 49).

We thank Dr. Robert Evans for his many helpful suggestions, Dr. Sherry Leonard for her critical reading of this manuscript, and Ramesh Pandey for his excellent technical assistance.

This research has been supported by American Cancer Society Grants BC-516 and BC-516A.

Received for publication 2 February 1988, and in revised form 1 June 1988.

References

1. Aaronson, R. P., and G. Blobel. 1975. Isolation of nuclear pore complexes in association with a lamina. Proc. Natl. Acad. Sci. USA. 72:1007–1011.
2. Alberts, A. W., J. Chen, G. Kuron, V. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Alberts-Schonberg, O. Hensens, J. Hirsbfield, K. Hoogsteen, J. Lisch, and J. Springler. 1980. Mevinolin: a highly potent competitive inhibitor of HMG-CoA reductase and a cholesterol-lowering agent. Proc. Natl. Acad. Sci. USA. 77:3957–3961.
3. Benavente, R., and G. Krohne. 1985. Involvement of nuclear lamins in postmitotic reorganization of chromatin as demonstrated by microinjection of lamin antibodies. J. Cell. Biol. 103:1847–1854.
4. Blobel, G., and V. R. Potter. 1966. Nuclei from rat liver: Isolation method and ~4C in polyacrylamide gels by fluorography. Proc. Natl. Acad. Sci. USA. 59:980–985.
5. Bruegger, E., and H. C. Rilling. 1986. Prenylated proteins from kidney. Biochem. Biophys. Res. Commun. 139:209–214.
6. 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.
7. Cabral, F., and M. Gottesman. 1978. The determination of similarities in protein sequences. J. Biol. Chem. 253:5134–5140.
8. Chua, N.-H., and P. Bennoun. 1975. Thylakoid membrane polypeptides of Chlamydomonas reinhardtii: wild type and mutant strains deficient in Photosystem II reaction center. Proc. Natl. Acad. Sci. USA. 72:2175–2179.
38. Newport, J. W., and D. J. Forbes. 1987. The nucleus: Structure, function and dynamics. *Annu. Rev. Biochem.* 56:535–565.

39. O’Farrell, P. Z., H. M. Goodman, and P. H. O’Farrell. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell.* 12:1133–1142.

40. Ottaviano, Y., and L. Gerace. 1985. Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* 260:624–632.

41. Peffley, D., and M. Sinensky. 1985. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase synthesis by a non-sterol mevalonate-derived product in Mev-1 cells: apparent translational control. *J. Biol. Chem.* 260:9949–9952.

42. Quesney-Huneues, V., M. H. Wiley, and M. D. Siperstein. 1979. Essential role for mevalonate synthesis in DNA replication. *Proc. Natl. Acad. Sci. USA.* 76:5056–5060.

43. Scheer, U., J. Kartenbeck, M. F. Trendelenburg, J. Stadler, and W. W. Franke. 1976. Experimental disintegration of the nuclear envelope. *J. Cell Biol.* 69:1–18.

44. Schmidt, R. A., C. J. Schneider, and J. A. Glomset. 1984. Evidence for post-translational incorporation of a product of mevalonic acid into Swiss 3T3 cell proteins. *J. Biol. Chem.* 259:10175–10180.

45. Sefton, B. M., and J. E. Bus. 1987. The covalent modification of eukaryotic proteins with lipid. *J. Cell Biol.* 104:1449–1453.

46. Sinensky, M., R. Torget, and P. A. Edwards. 1981. Radioimmune precipitation of 3-hydroxy-3-methylglutaryl coenzyme A reductase from Chinese hamster fibroblasts: Effect of 25-hydroxycholesterol. *J. Biol. Chem.* 256:11774–11779.

47. Shelton, K., L. Higgins, D. Cochran, J. Raffolo, Jr., and P. Egle. 1980. Nuclear lamins of erythrocyte and liver. *J. Biol. Chem.* 255:10978–10983.

48. Shelton, K. R., P. M. Egle, and D. L. Cochran. 1981. Nuclear envelope proteins: Identification of lamins B subtypes. *Biochem. Biophys. Res. Commun.* 103:975–981.

49. Sinensky, M., and J. Logel. 1985. Defective macromolecule biosynthesis and cell-cycle progression in a mammalian cell starved for mevalonate. *Proc. Natl. Acad. Sci. USA.* 82:3257–3261.

50. Skinner, M. K., and M. D. Griswold. 1983. Fluorographic detection of radioactivity in polyacrylamide gels with 2,5-diphenyl-oxazole in acetic acid and its comparison with existing procedures. *Biochem. J.* 209:281–289.

51. Smith, D. E., Y. Gruenbaum, M. Berrios, and P. A. Fisher. 1987. Biosynthesis and interconversion of *Drosophila* nuclear lamin isoforms during normal growth and in response to heat shock. *J. Cell Biol.* 105:771–790.

52. Suprynowicz, F. A., and L. Gerace. 1986. A fractionated cell-free system for analysis of prophase nuclear disassembly. *J. Cell Biol.* 103:2073–2081.

53. Traub, P., G. Perides, S. Kühn, and A. Scherbarth. 1987. Efficient interaction of nonpolar lipids with intermediate filaments of the vimentin type. *Eur. J. Cell Biol.* 43:55–64.

54. Wilcox, C. A., and E. N. Olson. 1987. The majority of cellular fatty acid acylated proteins are localized to the cytoplasmic surface of the plasma membrane. *Biochemistry.* 26:1029–1036.