# Incorporation of a Product of Mevalonic Acid Metabolism into Proteins of Chinese Hamster Ovary Cell Nuclei

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*Abstract.* We have examined the nuclear localization of isoprenylated proteins in CHO-K1 cells labeled with [$^{14}$C]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 [$^{14}$C]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, nonequilibrium 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, nonionic 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-$^{3}$H position or the 2-$^{14}$C position but not the 1-$^{14}$C 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

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74-kD isoprenylated polypeptide is the precursor form of lamin 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. (Gilbertsville, PA).

### Cell Culture and Radiosotopic Labeling

The CHO-K1 cell line, met-18b-2, was grown as described (13). Cells ($6 \times 10^6$ per 150-mm culture dish) were labeled in the presence of 8 μg/ml mevinolin (2), a potent inhibitor of mevalonate biosynthesis in mamalian cells (supplied by A. Alberts, Merck and Co., Rahway, NJ), with 2–10 μCi/ml R-(2-$^{14}$C) (2.10 GBq/mmol; Amersham Corp., Arlington Heights, IL) mevalonic acid lactone for 13–24 h in the appropriate growth medium containing 5% dialyzed fetal calf macroserum without exogenous mevalonate. When appropriate, parallel cultures of cells were labeled with 10–30 μCi/ml of [$^{35}$S]L-methionine (Tran$^{35}$S-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 extracted with 6 vol of −20°C acetone-$NH_4OH$ (5.3:0.3) (7) by mixing on a Labquake Mixer (Labindustries, Inc., Berkeley, CA) for 30 min at 4°C. The insoluble protein component was pelleted by centrifugation at 15,000 rpm (12,800 *g*) in an Eppendorf microfuge (Bio-Rad Laboratories, Richmond, CA) for 15 min at 4°C and dried under vacuum. Samples were then solubilized in an SDS sample buffer (8) (30 mM Trsi-HCl, pH 7.0, 50 mM $Na_2CO_3$, 3% SDS, 2.5% 2-mercaptoethanol, 4 M urea [molecular biology grade; Sigma Chemial Co., St Louis, MO], 15% sucrose, and 0.04% bromophenol blue) by heating for 4 min at 100°C. After electrophoresis (routinely 5–7 mA, constant current for 12–16 h), gels were soaked overnight in methanol–acetic acid–water (50:7:43), 0.25% (wt/vol) Coomassie Brilliant Blue R-250, and destained in methanol–acetic acid–water (30:7:63) (8). Fluorography was performed using the procedure of Skinner and Griswold (50) and the dried films exposed to preflashed (28) Kodak X-OMAT AR film at −70°C.

For two-dimensional NEPHGE analysis, the samples were extracted with acetone-$NH_4OH$ as described above. The dried protein pellet was then solubilized as described by Ottaviano and Gerace (40). Two-dimensional nonequilibrium pH gradient electrophoresis (NEPHGE)[2] analysis was performed as described by O'Farrell et al. (39) at a constant voltage of 150 V for 22–24 h (3,200–3,500 Vh). Second dimension SDS-PAGE was performed as described on 8% acrylamide gels containing 10% glycerol. Fluorographic analysis was performed as described.

### 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 excised and subjected to partial proteolysis (9) with *Staphylococcus aureus* V8 protease (CooperBiomedical Inc., Malvern, PA). After SDS-PAGE, gels were soaked overnight at 4°C in 10% TCA and peptide maps visualized by silver staining (Silver Stain DPC; Integrated separation Systems, Hyde Park, MA).

### Preparation of Cytosolic Extract from CHO Cells

Labeled cells were harvested from culture dishes by scraping in ice-cold, PBS (11) 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 $10^7$ cells per ml of buffer, resuspended by gentle vortexing in Triton lysis buffer. Triton lysis buffer consists of 50 mM Tris-HCl, pH 7.5, 5 mM dithiothreitol, 5 mM EDTA, 20 mM KCl, 50 mM NaF, 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 by centrifugation, the cells were resuspended at a density of $1.5 \times 10^7$ per ml in 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$ (TKM buffer) (4), 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 equal volume of 500 mM 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. I. 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 10 min at 140 *g* (900 rpm) and resuspended in PBS containing 1 mM PMSF/PIM. Next, an equal volume of PBS containing 0.2% NP-40, 30 mM 2-mercaptoethanol, 1 mM PMSF and PIM, was added and the cell suspension incubated on ice for 10 min. The cells were then pelleted as before, resuspended in PBS, 1.0 mM PMSF and PIM, and subjected to a mixed nuclease digestion by the addition of an equal volume of PBS, 0.2% NP-40, 30 mM 2-mercaptoethanol, 1 mM PMSF and PIM, 20 mM $CaCl_2$, 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 extracted for 15 min on ice with 15 mM Tris-HCl, pH 7.5, 5 mM EDTA, 80 mM KCl, 3 M NaCl, 1.0% 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 *g* (3,500 rpm), the nuclear matrix–intermediate filament preparation was extracted on ice a second time with the same buffer, and pelleted for 10 min at 10,400 *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.

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 $MgCl_2$.

### *Preparation of Rat Liver Lamin*

The protocol used in the preparation of rat liver lamin 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-$H_2O$ 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 [$^{14}$C]Mevalonate and [$^{35}$S]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 ($6 \times 10^6$ per 150-mm plate) were labeled for 17 h with [$^{14}$C]mevalonate or [$^{35}$S]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 cellular debris removed by centrifugation in an Eppendorf microfuge (Brinkman Instruments, Inc.) at 4°C for 10 min. After clarification, PIM, 100 μl of 10% Triton X-100, and, depending on the source of antiserum to be added later, an equivalent amount of normal human or guinea pig serum was added. Samples were incubated 2–8 h with mixing, followed by 2–4 h of mixing with a 50 μl slurry of protein A–Sepharose CL-4B (Sigma Chemical Co.) prewashed (46) with BSA (RIA grade; Sigma Chemical Co.). The protein A–Sepharose beads were then removed by centrifugation in an Eppendorf microfuge (Brinkman Instruments, Inc.) for 5 min at 25°C.

After centrifugation, the supernatant fraction was transferred to a fresh microfuge tube and either 2 μl of anti-lamin (A+C) LS-1 human autoimmune serum (33), or 15 μl of guinea pig anti-lamin (A+B+C) immune serum (3) was added. The samples were then mixed for 4–16 h at 4°C, followed by an additional 2–4 h of mixing after adding a second 50 μl slurry of protein A–Sepharose beads. The samples were centrifuged for 5 min in an Eppendorf microfuge (Brinkman Instruments, Inc.) and the supernatant removed. The immunoabsorbed protein A–Sepharose beads were washed as described (40) in the presence of 0.1 mM PMSF and PIM. Immunoabsorbed protein was eluted from the beads by a 10-min incubation at 37°C in 100 mM triethanolamine-HCl pH 7.5, 2 mM EDTA, 2% 2-mercaptoethanol, 6% SDS, and 2 M urea, extracted with 6 vol of −20°C acetone/$NH_4OH$, dried under vacuum, and solubilized in SDS-PAGE sample buffer for electrophoresis.

## *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-$^{14}$C]mevalonate-labeled CHO cells. Labeled nuclei were then subjected to SDS-PAGE and [$^{14}$C]isoprenylated proteins visualized by fluorography. A representative Coomassie Blue–stained gel (Fig. 1 *A*) and the corresponding autoradiogram (Fig. 1 *B*) generated by $^{14}$C-labeled isoprenylated polypeptides are presented in Fig. 1. Comparison of the autoradiographic banding patterns of $^{14}$C-isoprenylated polypeptides obtained from cytosolic extract and nuclei (Fig. 1 *B*, lanes *a* vs. *b*), demonstrates that several isoprenylated proteins appear to be common to both cytosol and nuclei and that the 68-, 70-, and 74-kD isoprenylated proteins are enriched in nuclear preparations.

The molecular mass range (68–74-kD) of these isoprenylated 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 [$^{14}$C]mevalonate-labeled CHO cells and examined this preparation by SDS-PAGE and fluorography. Coomassie Bue–staining polypeptides 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-

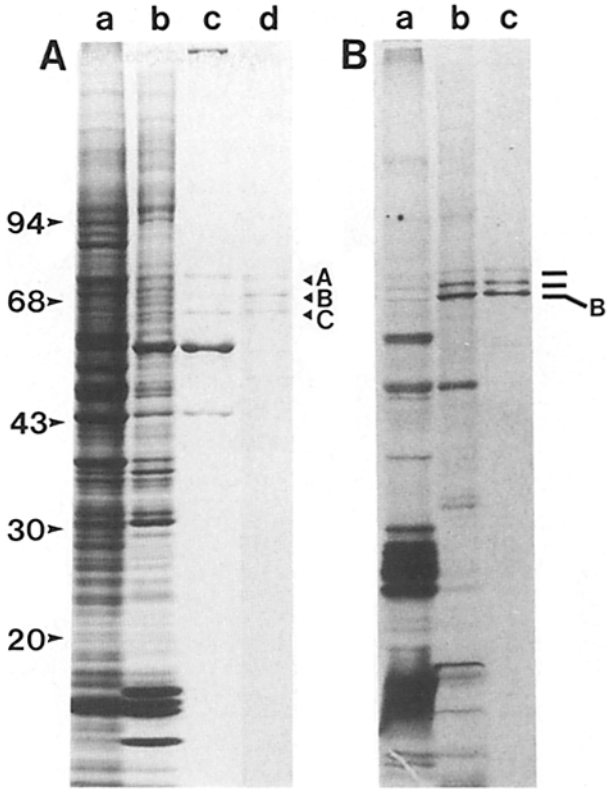


*Figure 1.* Identification of CHO isoprenylated proteins by SDS-PAGE and fluorography. CHO-K1 cells (met-18b-2) were labeled with R-[2-$^{14}$C]mevalonate in Ham's F12 medium containing 5% dialyzed fetal calf macroserum and 8 μg/ml mevinolin. The cells were then washed exhaustively with PBS and harvested by trypsinization. Cytosolic extract, nuclei, nuclear matrix–intermediate filament fraction, and rat liver lamin were prepared as described in Materials and Methods. SDS-PAGE analysis of these fractions was performed on slab gels containing a combinatin 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–d*), and $^{14}$C-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 lamin. The corresponding fluorogram (*B*) presents the radioactive banding patterns of $^{14}$C-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 $^{14}$C-isoprenylated polypeptides. Molecular mass standards are presented at the left of *A*.

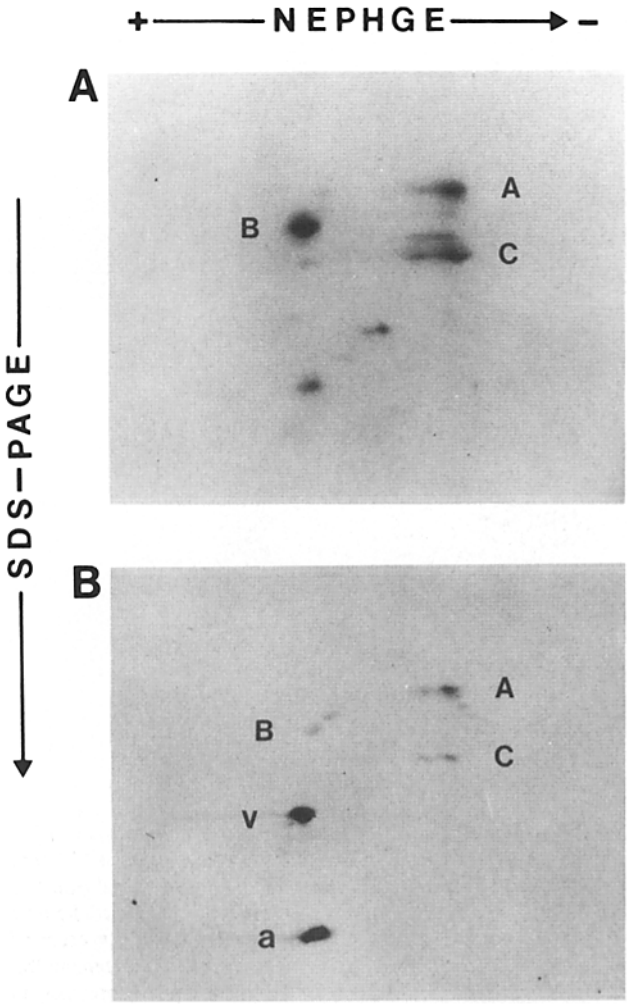


*Figure 2.* Two-dimensional NEPHGE gel analysis of rat liver lamin and CHO nuclear matrix–intermediate filament preparations. Rat liver lamin and nuclear matrix–intermediate filament preparations from CHO-K1 (met-18b-2) cells labeled with [$^{35}$S]methionine were prepared as described in Materials and Methods. A trace (non-Coomassie Blue–stainable) amount of the $^{35}$S-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 electrphoresis 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 [$^{35}$S]methionine-labeled CHO nuclear matrix polypeptides (*B*). Letters indicate the positions of lamins A, B, C, vimentin (*v*), and actin (*a*).

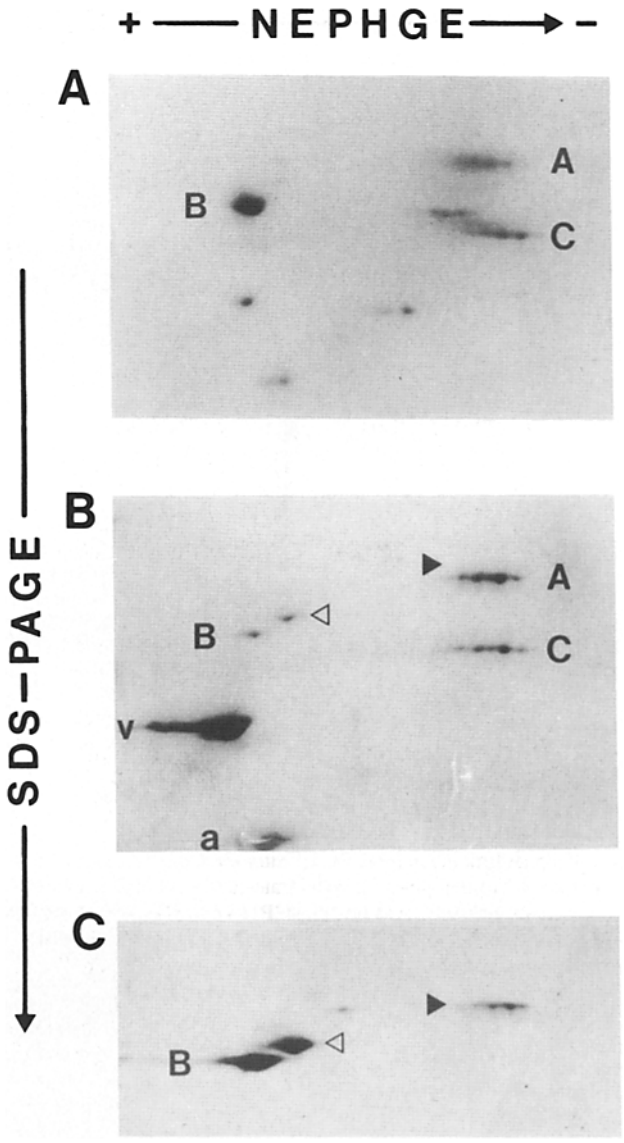


*Figure 3.* Identification of isoprenylated CHO nuclear matrix–intermediate filament polypeptides by two-dimensional NEPHGE gel electrophoresis and fluorography. Rat liver lamin and nuclear matrix–intermediate filament from R-[2-$^{14}$C]mevalonate-labeled CHO-K1 cells (met-18b-2) were prepared as described in Materials and Methods. Two-dimensional NEPHGE and fluorographic analysis were performed using the experimental conditions described in the legend to Fig. 2. (*A*) Coomassie Blue–stained rat liver lamin; (*B*) Coomassie Blue–stained $^{14}$C-isoprenylated CHO nuclear matrix–intermediate filament preparation; (*C*) fluorogram of the NEPHGE gel in *B*. Only the relevant portion of the fluorogram is shown. Fluorographic exposure was for 57 d. Indicated are lamins A, B, C; vimentin (*v*); actin (*a*); 74-kD isoprenylated polypeptide (*solid triangle*); and 70-kD isoprenylated polypeptide (*open triangle*). The solid triangle in *B* indicates the relative position of the 74-kD isoprenylated polypeptide in *C* as determined by superimposing the fluorogram on the Coomassie Blue–stained gel.

rations by McKeon et al. (33). It has previously been shown that rat liver lamins comigrate with lamins from other species (25, 35) on SDS-PAGE. Comigration with rat liver lamins (Fig. 1 *A*, lane *d*) is consistent with the assignment of the three polypeptides in the molecular mass range of 60–75 kD in Fig. 1 *A*, lane *c*, as CHO-K1 lamins.

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

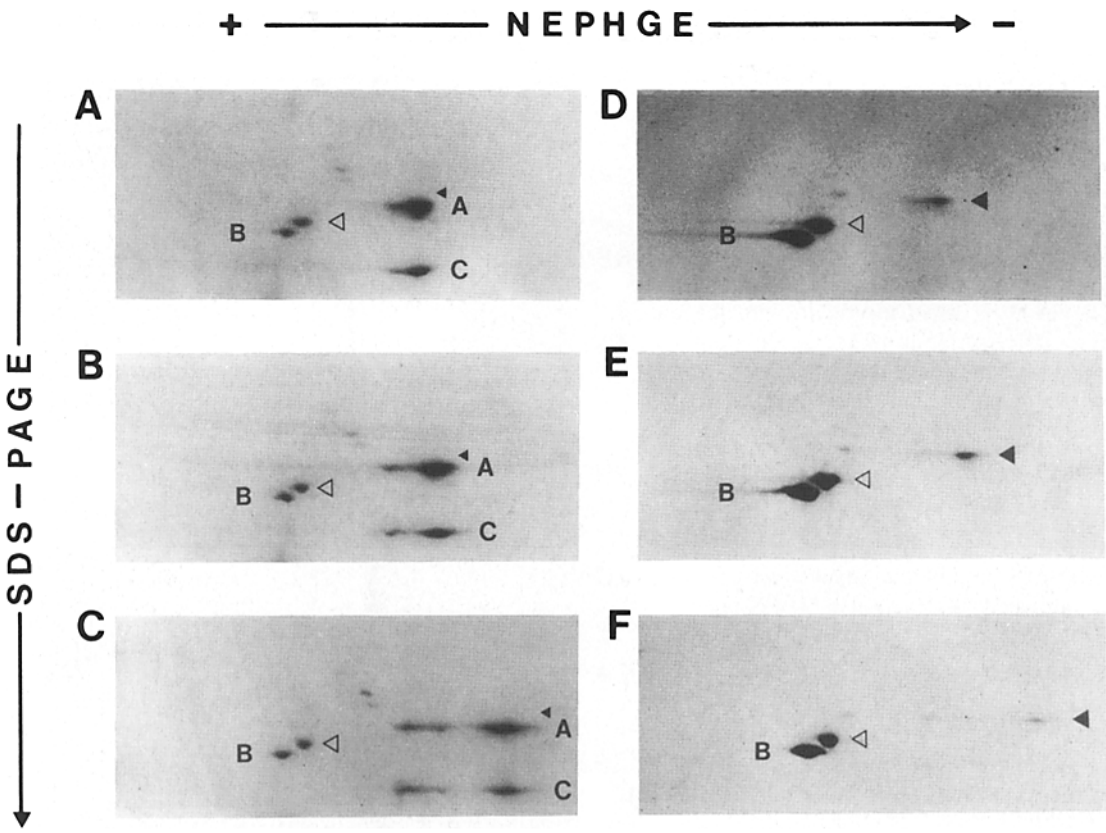


*Figure 4.* Two-dimensional NEPHGE time course analysis of the isoprenylated 74-kD pH isovariants. Nuclear matrix–intermediate filament was prepared from R-[2-$^{14}$C]mevalonate-labeled CHO-K1 (met-18b-2) cells as described in Materials and Methods. Identical aliquots were subjected to two-dimensional NEPHGE analysis for 1,580 and 2,200 (nonequilibrium), and 3,240 (near-equilibrium) Vh in the first (NEPHGE) dimension. SDS-PAGE and fluorography were performed as described in the legend to Fig. 2. Only the relevant portion of the two-dimensional NEPHGE gels are shown. (*A*, *B*, and *C*) Coomassie Blue–stained gels; (*D*, *E*, and *F*) fluorograms produced by the corresponding NEPHGE gels in *A*, *B*, and *C*, respectively. Fluorographic exposure was for 32 d. Length of electrophoresis (volt hours) in the first dimension: (*A* and *D*) 1,580; (*B* and *E*) 2,200; (*C* and *F*) 3,240. Indicated are lamins A, B, and C; 70-kD isoprenylated polypeptide (*open triangle*); 74-kD isoprenylated lamin A pH isovariants (*solid triangle*).

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 $^{14}$C-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 NEPHGE gels and fluorography. Analysis of these isoprenylated nuclear proteins was greatly facilitated by their enrichment in nuclear matrix–intermediate filament preparations from whole cells. NEPHGE gel analysis on rat liver lamin mixed with trace amounts of a nuclear matrix–intermediate filament preparation from [$^{35}$S]-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 NEPHGE 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 $^{14}$C-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 lamin C (62 kD), as well as actin (43 kD) and vimentin (56 kD). Present in the acidic portion of the NEPHGE 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 NEPHGE 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 $^{14}$C-isoprenylated polypeptide bands seen in Fig. 1 *B*,

lane *c*, are now resolved on the basis of isoelectric pH and molecular mass. Two major $^{14}$C-isoprenylated polypeptides are located at an acidic pH, while the 74-kD, $^{14}$C-isoprenylated polypeptide migrates to the basic region of the NEPHGE gel. Comparison of Figs. 3, *B* and *C* demonstrates that the more acidic of these two $^{14}$C-isoprenylated polypeptides precisely comigrates on two-dimensional gels with the 68-kD Coomassie Blue–staining polypeptide identified in Fig. 2 as lamin B. Likewise, the 70-kD, $^{14}$C-isoprenylated polypeptide (Fig. 3 *C*, open triangle), which migrates to a more basic pH than lamin B, is superimposable with the Coomassie Blue–staining polypeptide in the corresponding position in Fig. 3 *B*.

The basic $^{14}$C-isoprenylated polypeptide seen in Fig. 3 *C* (*closed triangle*), migrating with a molecular mass of 74 kD but displaying no visible Coomassie Blue–staining mass (Fig. 3 *B*, *solid triangle*), has an isovariant pH pattern identical to that of lamin A, yet a molecular mass that is ~2 kD larger. Since only the acidic proteins have reached their isoelectric pH under the electrophoretic conditions used in this NEPHGE analysis (unpublished observations), it was possible that the comigration in the NEPHGE dimension of lamin A and the lamin A–like, 74-kD $^{14}$C-isoprenylated polypeptide was an electrophoretic artifact. Accordingly, we repeated the two-dimensional NEPHGE analysis of the $^{14}$C-isoprenylated nuclear matrix–intermediate filament preparation 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 $^{14}$C-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 $^{14}$C-isoprenylated pH isovariants is exactly 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 isoelectric pH variants, differing by ~2 kD.

### *Immune Precipitation of the Isoprenylated Proteins with Anti-Lamin Antisera*

Two-dimensional NEPHGE analysis of the isoprenylated nuclear 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 [$^{14}$C]mevalonate- or [$^{35}$S]methionine-labeled CHO cells were immunoprecipitated with lamin-specific antisera and analyzed by SDS-PAGE and fluorography. Radioimmune precipitation of [$^{35}$S]methionine-labeled cells with anti-lamin (A+B+C) antiserum, generates a pattern of radioactive polypeptide 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 [$^{14}$C]mevalonate and subjected to a parallel radioimmune precipitation with anti-lamin (A+B+C) antiserum is presented in Fig. 5, lane *c*. Three $^{14}$C-isoprenylated polypeptides are recognized by the anti-lamin antiserum. The immunoprecipitated radioactive band of 68 kD precisely comigrates with the Coomassie Blue–staining band of lamin B from rat liver, (Fig. 5, lanes *c* vs. *d*) and immunoprecipitated [$^{35}$S]methionine-labeled CHO lamin B (lanes *c* vs. *b*). Likewise, the $^{14}$C-isoprenylated polypeptides of 70 and 74 kD exhibit identical mobilities on SDS-PAGE whether immunoprecipitated (Fig. 5, lane *c*) or isolated as nuclear matrix (Fig. 1 *B*, lane *c*). A similar autoradiographic banding

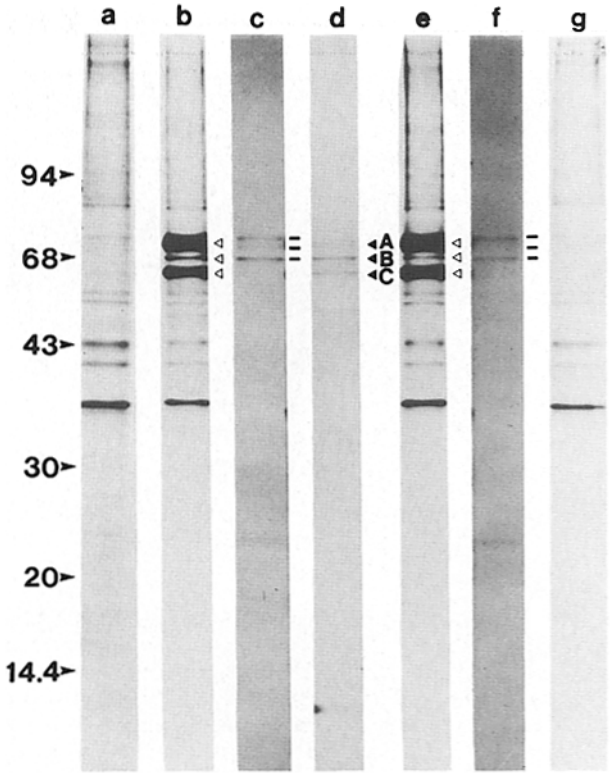


*Figure 5.* SDS-PAGE and fluorographic analysis of isoprenylated lamin polypeptides from CHO after immunoprecipitation with anti-lamin antisera. Whole cell extracts of CHO-K1 (met-18b-2) cells, labeled with 3 μCi/ml [$^{35}$S]methionine or 10 μCi/ml R-[2-$^{14}$C]mevalonate, were precleared by incubation with nonimmune guinea pig or human sera followed by the addition of protein A–Sepharose beads and centrifugation. The resulting supernatants were incubated with either guinea pig anti-lamin (A+B+C) antiserum or human anti-lamin (A+C) autoimmune serum as described in Materials and Methods. Antigen–antibody complexes were then bound to protein A–Sepharose beads, washed, eluted, and examined by SDS-PAGE and fluorography as described in Materials and Methods. Fluorographic exposure was for 30 ($^{14}$C-isoprenylated polypeptides) and 3 d (for [$^{35}$S]methionine-labeled polypeptides). [$^{35}$S]Methionine-labeled (lane *b*, *open triangles*) and $^{14}$C-isoprenylated polypeptides (lane *c*, *bars*) immunoprecipitated with guinea pig anti-lamin (A+B+C) serum. [$^{35}$S]Methionine-labeled (lane *e*, *open triangles*) and $^{14}$C-isoprenylated (lane *f*, *bars*) polypeptides immunoprecipitated with human anti-lamin (A+C) autoimmune serum. [$^{35}$S]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.

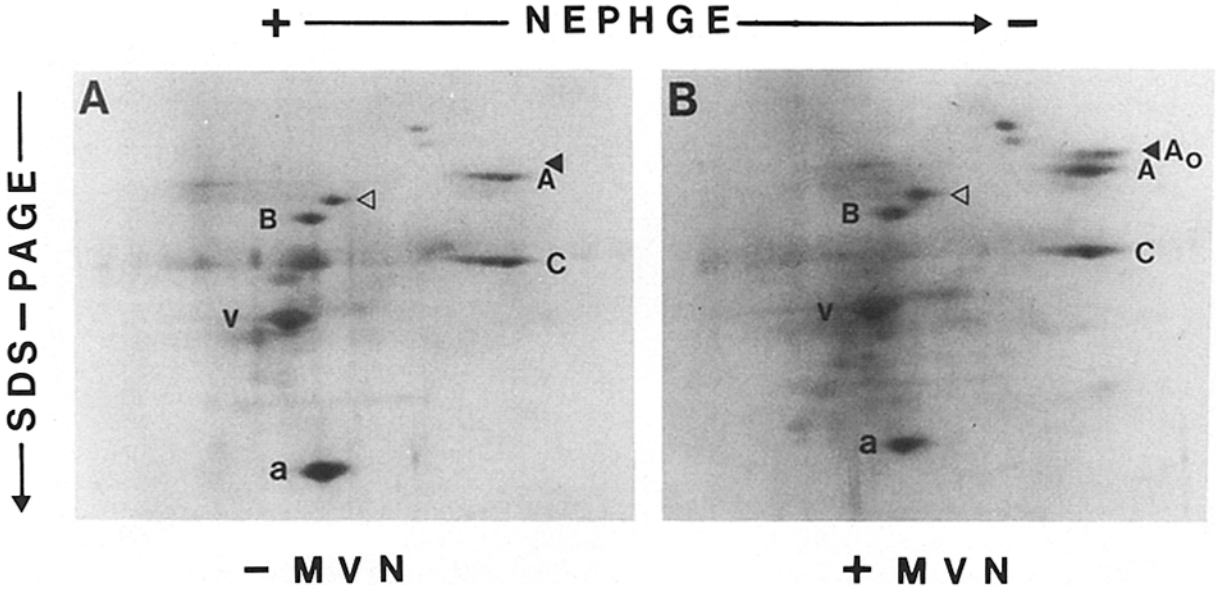


*Figure 6.* Accumulation of the precursor form of CHO lamin A in cells starved for mevalonate by treatment with mevinolin. CHO-K1 cells were plated at a density of $6 \times 10^6$ cells per 150-mm culture dish and allowed to attach overnight. The growth medium was then removed and the cells washed with 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 fractions 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, ($A_o$, *solid triangle*); the 70-kD isoprenylated polypeptide (*open triangle*); vimentin (*v*); and action (*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 (*solid triangle*) in the mevinolin-treated CHO cells (*B*), and the absence of lamin $A_o$ 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

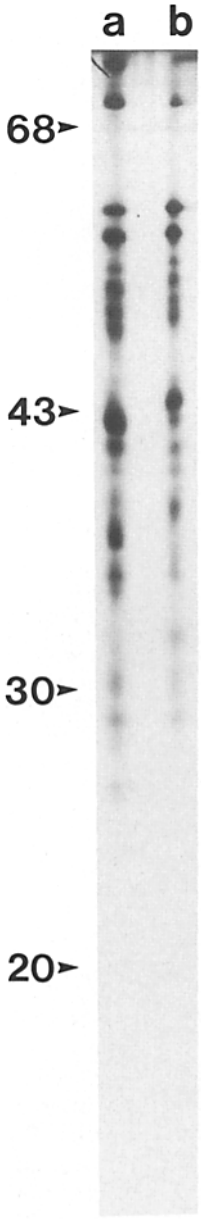


*Figure 7.* Peptide maps of CHO lamin A and the 74-kD isoprenylated polypeptide. Gel slices containing CHO lamins A and the 74-kD isoprenylated precursor form of lamin A were cut from a two-dimensional NEPHGE gel identical to that shown in Fig. 6. Partial proteolysis with *Staphylococcus aureus* V8 protease (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 $A_o$. 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 (51), 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 $A_o$) 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 $A_o$ 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). Lehner et al. (31) demonstrated that avian liver, in addition to the major acidic lamin protein (lamin $B_2$), contains at least one minor acidic pore-complex lamina protein (lamin $B_1$), not previously thought to be associated with the nuclear matrix. They also demonstrated that lamin $B_1$ is immunologically related to mammalian lamin B whereas lamin $B_2$ 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

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 $^{14}$C-isoprenylated proteins (unpublished results and reference 44) releases $^{14}$C-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 lamin B, 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 immunological depletion of CHO cell extracts for lamins 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).

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