

Interconversion of *Drosophila* Nuclear Lamin Isoforms during Oogenesis, Early Embryogenesis, and upon Entry of Cultured Cells into Mitosis

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Abstract. Two isoforms of a single nuclear lamin, distinguishable on one-dimensional SDS-polyacrylamide gels, have previously been identified in *Drosophila* nuclei during interphase. A third species, designated lamin Dm_{mit}, has now been identified as soluble in extracts of *Drosophila* tissue culture cells blocked in mitosis by drugs. An apparently identical form is the only lamin species detectable in late-stage egg chambers and early embryos. Phosphoamino acid analyses suggest that the conversion of lamins Dm₁ and Dm₂ to lamin Dm_{mit} is brought about by a specific rearrangement of phosphate groups rather than by dra-

matic net changes in the levels of lamin phosphorylation. The residues involved in these phosphorylation/dephosphorylation reactions have been tentatively mapped to a 17.8-kD cyanogen bromide fragment containing amino acids 385-547. This represents a potential "hinge" domain in the lamin structure between the end of coil 2 and the globular COOH terminus. These results have implications for understanding the regulation of nuclear envelope breakdown during mitosis and karyoskeletal dynamics during oogenesis and early embryogenesis.

THE nuclear lamina is a network of intermediate filament-like fibrils (Aebi et al., 1986; see also McKeon et al., 1986; Fisher et al., 1986; Franke, 1987) that lies subjacent to the inner nuclear membrane and surrounds the nuclear contents. In vertebrates, major polypeptides, termed lamins, have been identified and characterized (for reviews, see Krohne and Benavente, 1986; Gerace, 1986). During mitosis in higher eukaryotes, the nuclear envelope dissolves and molecular components such as the lamins are redistributed throughout the cell (Gerace et al., 1978; Krohne et al., 1978). Cell fractionation experiments indicate that redistribution correlates with depolymerization of the mammalian lamins; lamin depolymerization in turn correlates with transient hyperphosphorylation both in vivo (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985) and in vitro (Miake-Lye and Kirschner, 1985; Burke and Gerace, 1986; Suprynowicz and Gerace, 1986). Most recently, Chelsky et al. (1987) have suggested that lamin demethylation may also play a role in mitotic disassembly of the nuclear lamina.

All vertebrate species apparently possess multiple, developmentally regulated nuclear lamins (Schatten et al., 1985; Lebel et al., 1987; Stewart and Burke, 1987; Lehner et al., 1987; Benavente et al., 1985; Stick and Hausen, 1985; Bena-

vente and Krohne, 1985; Wolin et al., 1987). In mammalian cells, lamins A, B, and C have been distinguished with certainty. In chickens, lamins A, B₁, and B₂ have been identified (Lehner et al., 1986a, b). In *Xenopus*, five different nuclear lamins have been characterized.

Only a single nuclear lamin has thus far been identified in *Drosophila*. It occurs throughout development in a variety of cell and tissue types and in tissue culture cells. Two isoforms found in interphase nuclei, designated lamins Dm₁ and Dm₂, are distinguishable on one-dimensional SDS-polyacrylamide gels. Both are derived from a common polypeptide precursor, lamin Dm₀, apparently by proteolytic processing in the cytoplasm (Dm₀→Dm₁) followed by differential phosphorylation in the nuclear envelope (Dm₁↔Dm₂) (Smith et al., 1987). During interphase, the *Drosophila* lamins are phosphorylated to approximately 10 times the level of their mammalian counterparts (see for example Ottaviano and Gerace, 1985). During mitosis, the *Drosophila* lamins redistribute throughout the cell (Fuchs et al., 1983; Berrios et al., 1985).

Results of initial developmental analyses suggested that the ratios of lamins Dm₁ and Dm₂ varied during embryogenesis (Smith and Fisher, 1984). In early embryos, it appeared that only lamin Dm₁ was present and moreover, it was in soluble (i.e., nonnuclear) form. More recently, we noted that the soluble lamin isoform associated with early embryos was actually of intermediate SDS-PAGE mobility between lamins Dm₁ and Dm₂ and we decided to study it in more detail. In

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addition, we extended our developmental analysis to include oogenesis. Finally, we compared soluble lamins isolated from both oocytes and embryos with those extracted from tissue culture cells blocked in mitosis by drugs. Taken together, our results suggest that there is a novel lamin isoform characteristic of both mitotic and meiotic cells. This isoform, designated lamin Dm_{mit}, is soluble at low ionic strength and is apparently derived from lamins Dm₁ and Dm₂ by rearrangement of phosphate moieties, rather than by substantial net changes in the total levels of lamin phosphorylation.

Materials and Methods

Antibodies

Specific affinity-purified IgG fractions were from Cappel Laboratories Inc. (Cochranville, PA). Affinity-purified antilamin antibodies were prepared using a clone-encoded lamin- β galactosidase fusion protein (Gruenbaum et al., 1988) as recently described (Fisher and Smith, 1988).

Methods

Much of the methodology has been detailed previously. *Drosophila melanogaster* (Oregon R, P2 strain) were maintained in mass culture according to Allis et al. (1977). SDS-PAGE was according to Laemmli (1970); blot-transfer of proteins to nitrocellulose was passive (Fisher et al., 1982). Immunoblots were probed with specific IgG fractions and bands of reactivity visualized colorimetrically (Blake et al., 1984; Smith and Fisher, 1984). Two-dimensional gel electrophoresis was essentially according to O'Farrell (1975) exactly as recently described (Smith et al., 1987). Phosphoamino acid analyses and cyanogen bromide (CNBr)¹ digestions were also as recently described (Smith et al., 1987), except that CNBr digestions were carried to near completion by incubation with 50 mg/ml CNBr for 4 h at room temperature. Calf alkaline phosphatase was conjugated to affinity-purified goat anti-rabbit IgG according to Avrameas (1969); colorimetric detection was according to McGadey (1970). Additional details are provided in the figure legends.

Cell Culture Techniques

Schneider cells (line 2) were maintained in monolayer culture (Schneider, 1972) as previously described (Smith et al., 1987). To enrich for mitotic cells, exponentially growing monolayers ($\sim 1 \times 10^6$ cells/cm²) were incubated for 18–20 h in medium containing either 2 μ g/ml vinblastine sulfate or 1 μ g/ml colcemid. Cells were then washed in PBS and lysed as described below. For in vivo labeling of proteins with ³²P, cells were resuspended in low phosphate medium, with 2 μ g/ml vinblastine and 50–100 μ Ci/ml ³²P-orthophosphate.

Dissection and Staging of *Drosophila* Egg Chambers

Fertile, mature females were chilled on ice and their ovaries removed in 140 mM NaCl, 10 mM KH₂PO₄, pH 7.5 (PBS) containing 10 mg/ml polyvinylpyrrolidone. Complete ovaries were kept on ice until 12 were obtained. They were then dissected using 22-gauge needles to separate the follicles according to developmental stage. Follicles were either frozen at -70°C or used immediately.

Cell Fractionation and Immunoprecipitation

For biochemical analysis, dissected egg chambers, embryos, and Schneider 2 tissue culture cells were prepared as described (Smith et al., 1987). For generation of total lysates, samples were resuspended in extraction buffer (250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM MgCl₂, 1 mM PMSF, 2.5 mM *N*-ethyl maleimide, plus or minus 1% Triton X-100 as detailed in individual figure legends). Cells were lysed by Dounce homogenization with five strokes of a tight fitting pestle. For generation of

postnuclear supernatant and crude nuclear pellet fractions, homogenates were centrifuged at 10,000 *g* for 5 min. The nuclear pellet fraction was then resuspended in an equal volume of extraction buffer. Total lysates, supernatants, and pellets were SDS denatured by the addition of one-third volume of 20% SDS, 50 mM DTT, and then boiled. Immunoprecipitation of lamins from cell lysates was exactly as described previously (Smith et al., 1987).

Cryosectioning of Ovaries

For cryosectioning, ovaries were dissected in PBS containing 1% Triton X-100. Ovaries were then permeabilized and fixed by incubation for 10 min at room temperature in 1:1 solution of octane/PBS. The PBS contained in addition 3% paraformaldehyde, 0.1% glutaraldehyde. Ovaries were shaken in this solution for 10 min at room temperature, allowed to settle, and both octane and aqueous phases were then removed and ovaries were incubated in 3% paraformaldehyde, 0.1% glutaraldehyde in PBS for 15 min at 4°C. Ovaries were then transferred to a 3:1 solution of 45% acetic acid/95% ethanol, and incubated for 30 min at 4°C. After this incubation, ovaries were washed in PBS and equilibrated in PBS containing 30% sucrose at 4°C. Ovaries were finally embedded in OCT-embedding medium and quick-frozen by immersion in liquid nitrogen. 8- μ m sections were cut at -18°C and collected on gelatinized slides. Slides were stored at -70°C until use.

Immunofluorescence Microscopy

Ovary cryosections were probed with antilamin antibodies essentially as recently described for larval cryosections (McConnell et al., 1987) with modification as follows. Antibody incubations were done at room temperature for 2 h rather than at 37°C for 30 min. Sections were probed with antilamin IgG fractions at a final concentration of between 10 and 20 μ g per ml; similar concentrations of preimmune IgG were used. Sections were visualized with a Leitz Ortholux II epifluorescence microscope using a Zeiss 63 \times oil objective. Photography was done using Kodak Tri-X-Pan print film.

Glycerol Gradient Centrifugation

Vinblastine-treated Schneider Cells and 0–4-h-old embryos were lysed in extraction buffer and fractionated as described above. The supernatants were loaded onto 10–40% glycerol gradients containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl. All sedimentation experiments were performed both in the presence and absence of 0.5% Triton X-100. Gradients were formed in SW 60 tubes and centrifuged at 45,000 rpm for 18 h. 300- μ l fractions were collected and diluted with an equal volume of 50 mM Tris-HCl, pH 7.4, 20% SDS, 20 mM DTT. For generation of a sedimentation standard curve, 300- μ g each of cytochrome C, BSA, and catalase were centrifuged in separate tubes. The sedimentation positions of marker proteins were determined spectrophotometrically by absorbance at 280 nm.

Results

A Nuclear Lamin of Novel SDS-PAGE Mobility Predominates in Late-Stage Oocytes and Early Embryos

Our initial interest was to compare the soluble lamin isoform identified in early embryo extracts with any lamin species that might be present during oogenesis. Egg chambers were isolated by manual dissection and staged according to Mahowald and Kambyzellis (1980). Developmentally synchronous embryos were collected as previously described (Smith and Fisher, 1984) and embryonic stage was confirmed by visual inspection. In the experiment shown (Fig. 1), samples were lysed directly into boiling SDS and subjected to SDS-7% PAGE and immunoblot analysis using affinity-purified antilamin antibodies.

Early egg chambers (Fig. 1, lane *a*) were similar to mature embryos (Fig. 1, lane *g*) in that two lamin isoforms were identified in each. Electrophoresis of early oocyte and late embryo extracts in adjacent lanes (not shown) demonstrated

1. Abbreviation used in this paper: CNBr, cyanogen bromide.

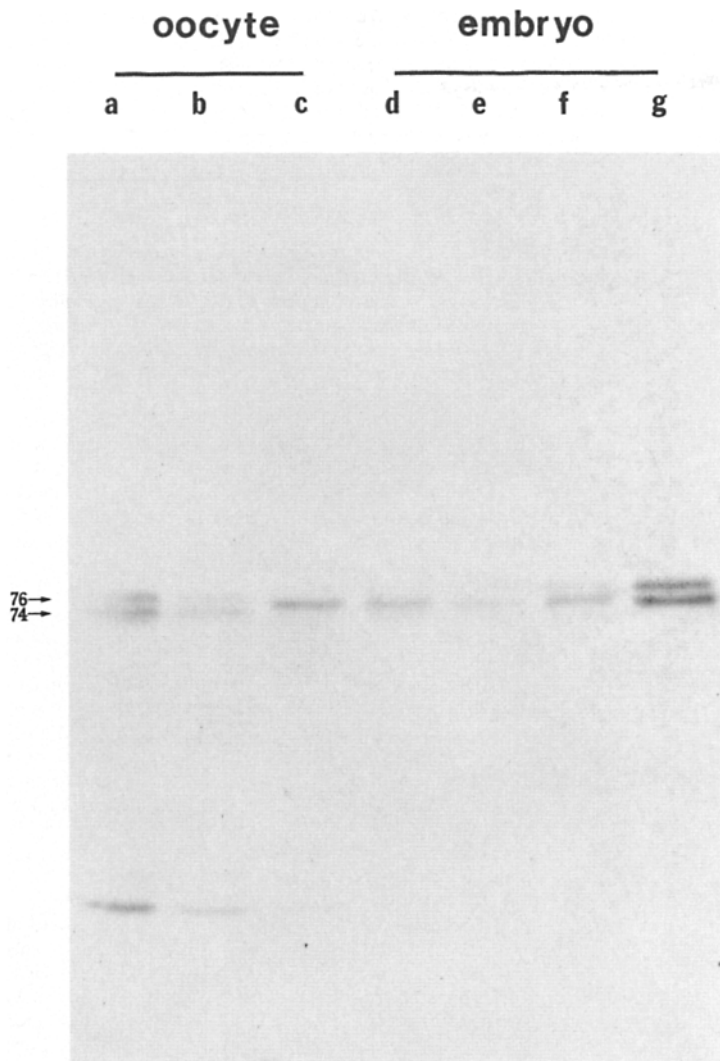


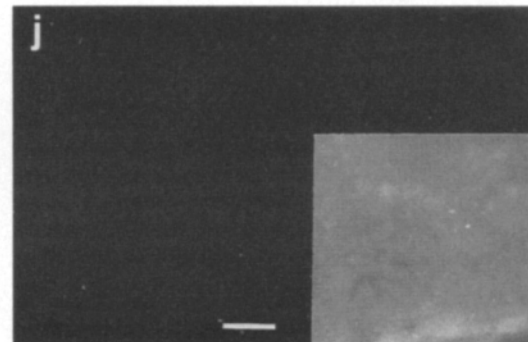
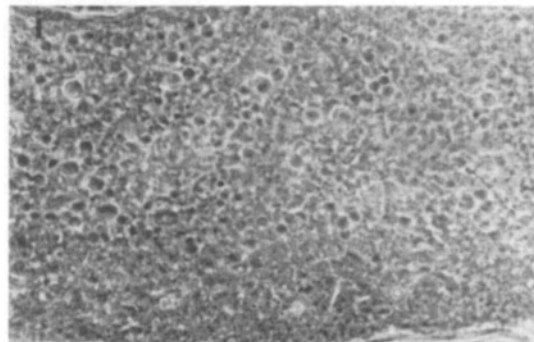
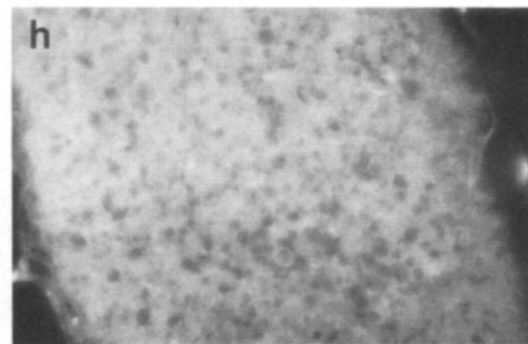
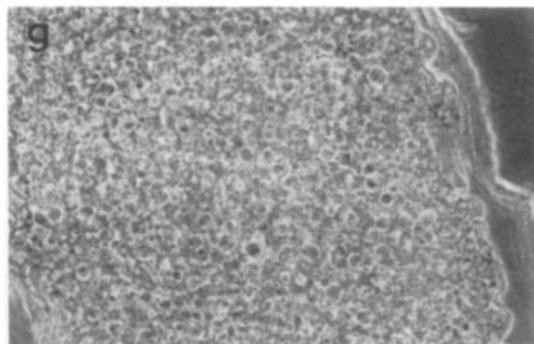
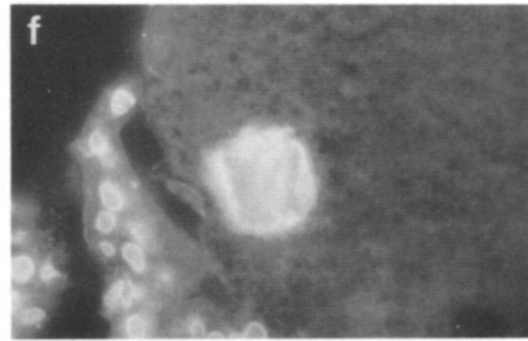
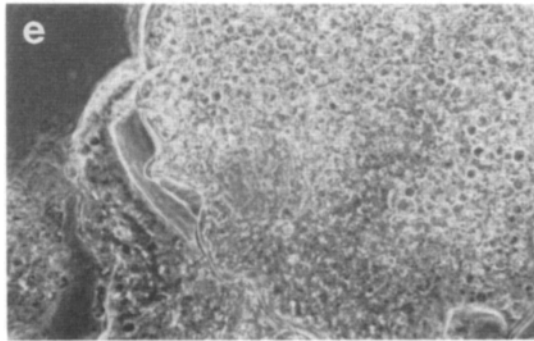
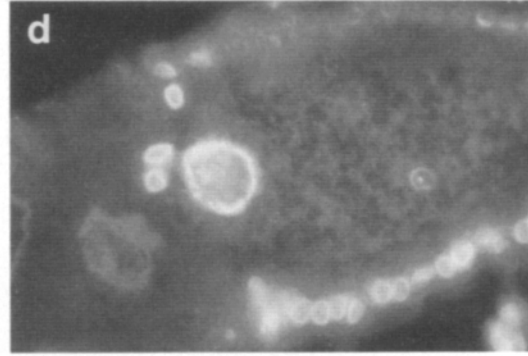
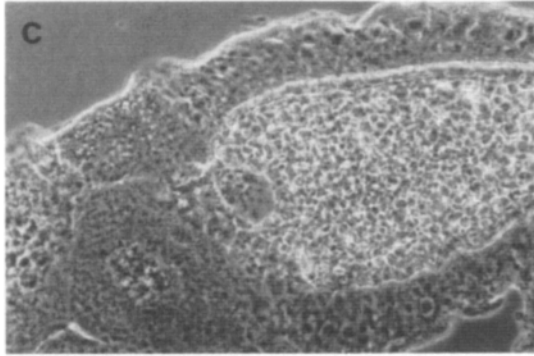
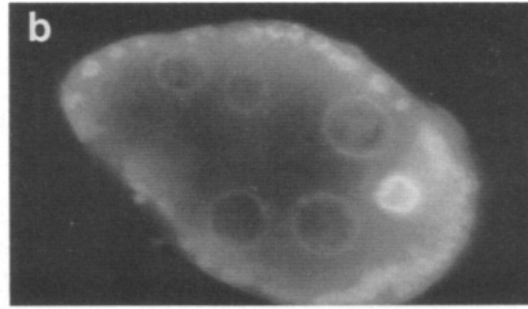
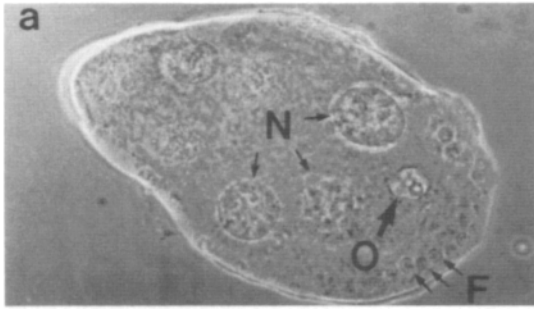
Figure 1. Steady-state levels of *Drosophila* nuclear lamin isoforms during oogenesis and early embryogenesis. SDS-PAGE was on 7% polyacrylamide gels; immunoblot analyses were with affinity-purified antilamin IgG diluted at 1:2,000, relative to the specific antibody titer of the unfractionated antiserum. Ovaries were dissected in PBS containing 10 mg/ml polyvinylpyrrolidone. Follicles were isolated and staged into three groups under a dissecting microscope. *oocyte*: lane *a*, germarium-stage 8; lane *b*, stages 9–10b; lane *c*, stages 11–14. Embryos were collected and aged so as to generate developmentally synchronous populations. *embryo*: lane *d*, 0–2.5 h; lane *e*, 2.5–5 h; lane *f*, 5.0–7.5 h; lane *g*, 6–18 h. Developmental synchrony was confirmed by visual inspection after dechorionation. Staged follicles and embryos were lysed by Dounce homogenization directly into boiling SDS. Approximately 100 μ g of protein were loaded in each lane.

comigration of both lamin species. This was consistent with the notion that the isoforms present in early egg chambers were in fact lamins Dm_1 and Dm_2 as they had been defined in embryos and Schneider 2 tissue culture cells (Smith et al., 1987). After stage 10b of oogenesis (Fig. 1, lane *c*) and during the early stages of embryogenesis (Fig. 1, lanes *d* and *e*), only a single species was identified. The form associated with late oocytes (Fig. 1, lane *c*) appeared to comigrate with that found in early embryos (Fig. 1, lane *d*) and was intermediate in SDS-PAGE mobility between lamins Dm_1 and Dm_2 .

In a separate experiment, egg chambers were subdivided into three groups, germarium through stage 10, stages 11–13, and stage 14. These were then lysed into a nondenaturing extraction buffer and separated by centrifugation at 10,000 *g* into a crude nuclear pellet and a postnuclear supernatant. When SDS-PAGE and immunoblot analyses were performed (not shown), it was found that the conversion of lamins Dm_1 and Dm_2 to a form of intermediate SDS gel mobility occurred gradually between stages 11 and 14 and was only complete at stage 14. At stage 14, ~70% of this lamin was recovered in the postnuclear supernatant fraction; ~30% remained in the 10,000-*g* pellet.

***In Situ* Analysis of Lamin Localization During Oogenesis**

Biochemical analyses of nuclear lamin form during oogenesis were complemented by immunocytochemical experiments. Cryosections of *Drosophila* ovaries were probed with affinity-purified antilamin antibodies. Results from four different stages of oogenesis are shown in Fig. 2. In Fig. 2, *a* and *b*, a stage-8 egg chamber is shown. Nurse cell nuclei, follicle cell nuclei, and the oocyte nucleus can be identified by phase-contrast microscopy (Fig. 2 *a*). Indirect immunofluorescence analysis (Fig. 2 *b*) revealed specific staining of all three types of nuclei with antilamin antibodies. Some qualitative differences were seen. Staining of nurse cell and follicle cell nuclei was relatively faint and typical in that it showed a characteristic pattern of “rim” fluorescence consistent with lamin localization at the nuclear periphery. In contrast, the oocyte nucleus was intensely stained. Moreover, although the fluorescence intensity was greatest at the nuclear perimeter, it was our initial impression that there was a relatively increased amount of internal nuclear staining as well. A high level of “background” staining was also observed in the oocyte cytoplasm. This cytoplasmic staining was of maximal intensity surrounding the oocyte nucleus and was not seen



with nonspecific primary antibodies (not shown, but see Fig. 2, *i* and *j*). This cytoplasmic reaction may reflect a small pool of soluble lamin Dm₁ that we identified in early-oocyte cell fractionation experiments (not shown).

A stage-10a egg chamber is shown in Fig. 2, *c* and *d*. Nurse cell nuclei are still identifiable although beginning to deteriorate morphologically. Follicle cell nuclei stain more intensely and continue to exhibit a typical rim pattern. The oocyte nucleus has increased in size dramatically, stains most intensely, and continues to show apparent intranuclear fluorescence. By stage 13 (Fig. 2, *e* and *f*), nurse cell nuclei have disappeared and the oocyte nucleus remains as the only large nucleus. The intranuclear staining of the oocyte nucleus is most prominent at this stage; note the greater relative intensity of the internal to rim staining in stage 13 (Fig. 2 *f*) as compared with stage 10a (Fig. 2 *d*). This impression regarding intranuclear staining of *Drosophila* oocyte nuclei with antilamin antibodies was confirmed by examination of serial sections of stage-13 egg chambers (not shown). Intranuclear staining was seen in all planes of section through a given nucleus.

A stage-14 egg chamber is shown in Fig. 2, *g* and *h*. At this, the final stage in *Drosophila* oogenesis, the oocyte is arrested in first meiotic metaphase and the oocyte nucleus is disassembled (see Mahowald and Kambyzellis, 1980). Reaction with antilamin antibodies revealed diffuse staining throughout the ooplasm. Parallel biochemical analysis indicated that this correlates with the complete conversion of lamins Dm₁ and Dm₂ to a largely soluble form of intermediate SDS-PAGE mobility (not shown). Follicle cell nuclei were no longer identifiable at this final stage. A control demonstrating that the diffuse ooplasmic staining at stage 14 represents diffuse lamin distribution is shown in Fig. 2, *i* and *j*. The specimen shown was probed with preimmune IgG at an identical concentration to the antilamin IgG used in *g* and *h*. The photomicrographic exposure, developing, and printing conditions for the print shown in Fig. 2 *j* were identical for that shown in *h*. Fig. 2 *j*, *inset* was printed from the same negative under conditions comparable to increasing the photomicrographic exposure time 5–10-fold; fluorescence intensity is still less than that seen in *h*.

Tissue Culture Cells Blocked in Mitosis by Drugs Accumulate a Soluble Lamin Species Apparently Identical with that Found in Late-Stage Oocytes and Early Embryos

The apparent redistribution of lamins throughout the ooplasm of stage-14 egg chambers, coincident with meiotic breakdown of the oocyte nucleus was reminiscent of in situ events observed upon mitotic dissolution of the nuclear enve-

lope in *Drosophila* somatic cells (Fuchs et al., 1983; Berríos et al., 1985) as well as a number of vertebrate species (see review by Gerace, 1986). The correlation between this morphologic change during oogenesis and the apparent conversion of lamins Dm₁ and Dm₂ to a soluble form of intermediate SDS-PAGE mobility suggested that the two phenomena might be related. We explored this possibility using *Drosophila* Schneider 2 tissue culture cells. Although we are unaware of protocols for synchronizing these cells, it has been reported that a significant proportion of the cells in a population can be blocked in mitosis by the drug vinblastine (Hanson and Hearst, 1973). We therefore examined the effects of this drug on the *Drosophila* lamins. The results of this experiment are shown in Fig. 3. Cells were grown for 20 h in the presence of 2 µg/ml vinblastine and then lysed into a non-denaturing extraction buffer. The lysate was fractionated into a crude nuclear pellet and postnuclear supernatant and the fractions were subjected to SDS-PAGE and immunoblot analysis. As anticipated, the nuclear pellet fraction (Fig. 3 *a*) contained both lamins Dm₁ and Dm₂. In contrast, the postnuclear supernatant (Fig. 3 *b*) contained a single immunoreactive form of intermediate SDS-PAGE mobility. This form was not identifiable in postnuclear supernatants from untreated cells (not shown), and comigrated with the soluble lamin isolated from early embryos (Fig. 3 *c*). Visual inspection of the cells used in these experiments after staining with the DNA-specific dye DAPI revealed mitotic indices of between 30 and 50% for the vinblastine-treated cells and <2% without addition of drug. Results qualitatively similar to those shown in Fig. 3 were obtained using the drug colcemid in place of vinblastine (not shown), although the efficiency of mitotic blockage was much reduced.

At this point, our tentative conclusion was that we had identified a new *Drosophila* lamin isoform, which was apparently soluble and was characteristic of cells which had undergone either meiotic or mitotic nuclear disassembly. Several preliminary controls were carried out before a detailed characterization of this isoform, henceforth designated lamin Dm_{mit}, was initiated. Two-dimensional gel analyses of lamin Dm_{mit} isolated from late oocytes, early embryos, and postnuclear supernatants of vinblastine-treated Schneider cells showed all three to be identical (not shown). One-dimensional SDS-PAGE peptide map comparison of lamin Dm_{mit} with lamins Dm₁ and Dm₂ also demonstrated identity except for the mobility differences among the three sets of fragments as reflected in the parent isoforms (not shown, see Smith et al., 1987 for a comparison of lamins Dm₁ and Dm₂; also see Fig. 5).

For further analyses, lamin Dm_{mit} was obtained either from early embryos or from postnuclear supernatants of vinblastine-treated Schneider cells. The first of these experiments,

Figure 2. In situ localization of nuclear lamins during oogenesis. Ovaries were manually dissected and processed for cryosectioning (Materials and Methods). Sections in *a–h* were probed with affinity-purified antilamin IgG; the section in *i* and *j* was probed with preimmune IgG. Detection of primary antibodies was with rhodamine-conjugated affinity-purified goat anti-rabbit IgG as previously described (McConnell et al., 1987). Phase contrast (*a, c, e, g,* and *i*) and fluorescence micrographs (*b, d, f, h,* and *j*) are shown. Follicles shown represent *a, b*, stage 8, labels in *a*: *F*, follicle cell nuclei; *N*, nurse cell nuclei; *O*, oocyte nucleus. (*c* and *d*) stage 10 a; (*e* and *f*) stage 13; (*g, h, i, j*) stage 14. For the preimmune control shown in *j*, film exposure time (1 min), printing, and developing conditions were identical to that for the experiment shown in *h*. For *j*, *inset* printing and developing conditions were adjusted so as to be equivalent to a 5–10-fold increase in film exposure time; the same negative was used. Bar, 20 µm.

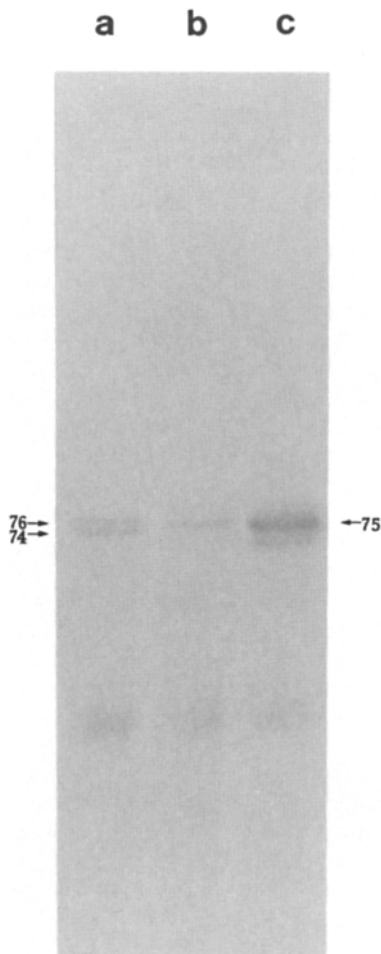


Figure 3. Immunoblot comparison of lamins derived from interphase nuclei, and postnuclear supernatant fractions from early embryos and tissue culture cells blocked in mitosis by vinblastine. Schneider cells were grown in medium containing 2 $\mu\text{g}/\text{ml}$ vinblastine for 20 h. At the end of this period, the mitotic index was estimated to be between 30 and 50% by microscopic inspection after DAPI staining. Postnuclear supernatant and pellet fractions were generated as described (Materials and Methods). A 0–3-h-old embryo population was collected and fractionated similarly. Lamins were immunoprecipitated before SDS-PAGE and immunoblot analysis in order to eliminate the potential for SDS-PAGE mobility artifacts resulting from comigration of lamins with major *Drosophila* proteins in the 70-kD-region of the gel. Immunoblots were prepared and probed as described in the legend to Fig. 1. Lane *a*, Schneider cell nuclei derived from approximately 5×10^6 vinblastine-treated cells; lane *b*, postnuclear supernatant from the same cells; lane *c*, postnuclear supernatant from early embryos. A minor species, migrating ahead of the major lamin band in lane *c*, was seen to a variable degree in supernatant fractions from both early embryos and vinblastine-treated Schneider cells (see also Fig. 4); we think it may represent a proteolytic breakdown product.

glycerol gradient sedimentation, is shown in Fig. 4. For the experiment shown in Fig. 4 *A*, a postnuclear supernatant from vinblastine-treated Schneider cells was subjected to sedimentation on a 10–40% glycerol gradient. After centrifugation, fractions were collected and subjected to SDS-PAGE and immunoblot analysis using affinity-purified anti-

lamin antibodies. An identical experiment was performed using a postnuclear supernatant fraction from early embryos; these results are shown in Fig. 4 *B*. In both cases, lamin Dm_{mit} behaved similarly with a sedimentation coefficient of ~ 4.5 – 5.0 S. Schneider cell lysis and glycerol gradient sedimentation were performed in the presence of 0.5% Triton X-100; early embryo lysis and sedimentation were in the absence of added detergents. For both tissue sources, similar results were obtained either in the presence or absence of detergents (not shown).

Changes in Phosphorylation Which Accompany the Interconversion between Interphase and Mitotic Lamins

To develop further insights into the biochemical differences between interphase and mitotic lamin isoforms in *Drosophila*, we performed CNBr peptide map analyses after *in vivo* ^{32}P -labeling of lamins in Schneider cells. In these experiments we were guided by the availability of the complete *Drosophila* lamin Dm_0 amino acid sequence as deduced from cDNA clones (Gruenbaum et al., 1988). Lamin Dm_0 contains a total of seven methionine residues including one at the NH_2 terminus and one at the COOH terminus. Six fragments are predicted with molecular masses of 17.8, 17.6, 14.5, 11.3, 8.2, and 1.4 kD. The 17.6-kD fragment derives from the NH_2 terminus, and we would expect it to be ~ 2 kD smaller in mature lamins Dm_1 and Dm_2 as a result of the posttranslational processing of lamin Dm_0 to Dm_1 (Smith et al., 1987). Cells were labeled to steady state in low phosphate medium as previously described (Smith et al., 1987), blocked in mitosis by vinblastine, and then lysed into a non-denaturing extraction buffer. Cell lysates were separated into postnuclear supernatant and nuclear pellet fractions and the separated fractions denatured by boiling in SDS. Immunoblot analyses indicated that there were approximately equal amounts of lamin protein for each of the three isoforms, Dm_1 , Dm_2 , and Dm_{mit} (see e.g., Fig. 3). Labeled lamins were then digested to near completion with CNBr (Materials and Methods), subjected to electrophoresis on an SDS 10–18% polyacrylamide gradient gel, and the gel fluorographed to reveal the presence of ^{32}P -labeled fragments. The results of this experiment are shown in Fig. 5 *A*. When a mixture of nuclear lamins Dm_1 and Dm_2 was digested, two major bands of ^{32}P -label were seen migrating between mobility standards at 18.4 and 20.4 kD (Fig. 5 *A*, lane *a*). Based on results obtained when lamins Dm_1 and Dm_2 were partially purified away from each other before CNBr digestion and subsequent analysis (Fig. 5 *B*), it is our impression that the two main bands seen in Fig. 5 *A*, lane *a* represent the same polypeptide fragment, phosphorylated to different degrees so as to give lamins Dm_1 and Dm_2 their characteristic SDS-gel mobilities. These two main bands of ^{32}P labeling correspond to the two largest polypeptide fragments in the CNBr map as determined empirically by immunoblot analysis of unlabeled lamins digested and subjected to electrophoresis similarly (not shown). Also seen in Fig. 5 *A*, lane *a* is weak ^{32}P labeling of some of the smaller fragments in the map, as well as what we believe to be labeled partial digestion products that apparently contain the major phosphorylated fragments migrating between mobility standards at 29 and 36 kD.

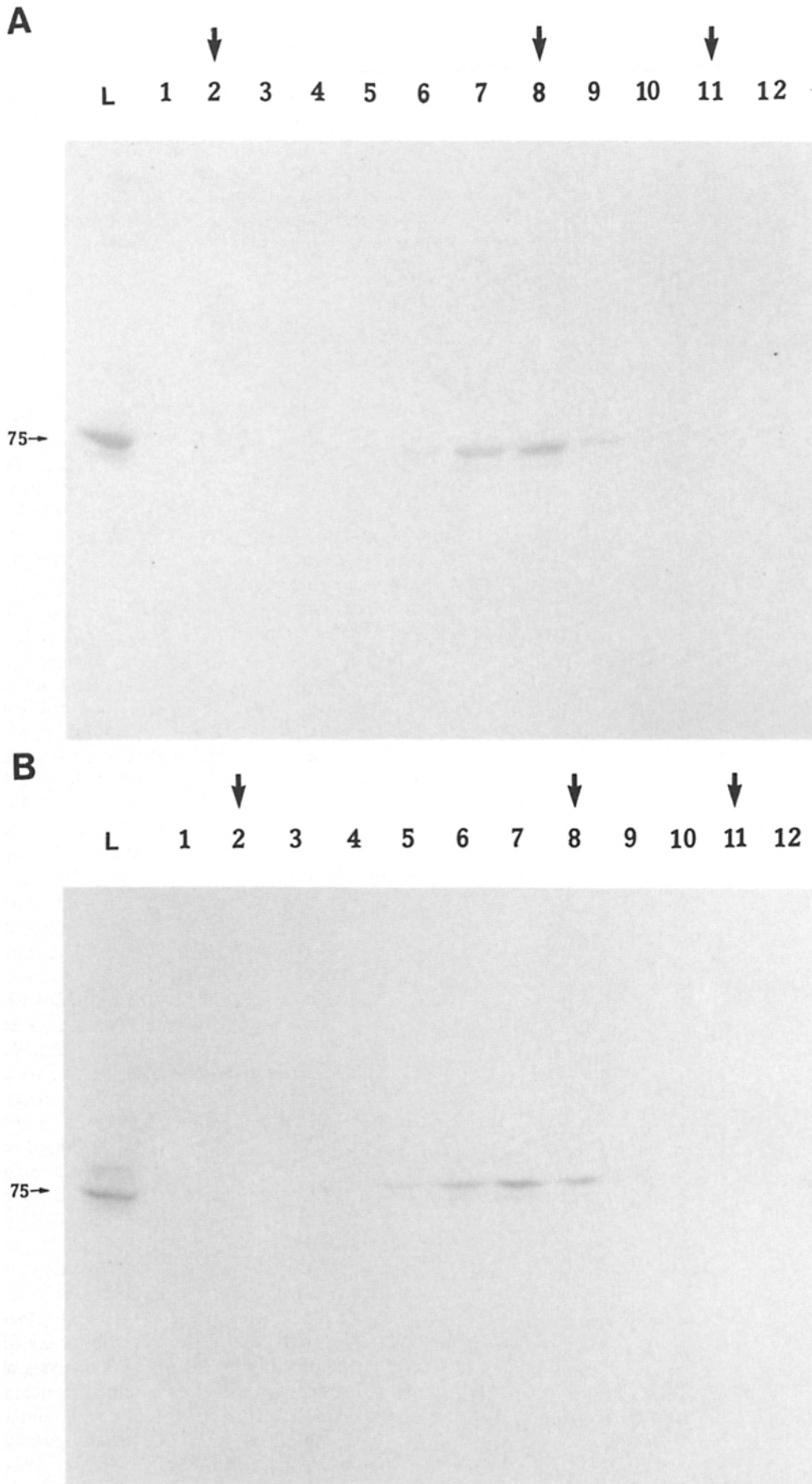


Figure 4. Glycerol gradient sedimentation of lamin D_{mit} derived from mitotically arrested tissue culture cells and early embryos. Postnuclear supernatant fractions of vinblastine treated Schneider cells (A) and early embryos (B) were generated as described in the legends to Figs. 1 and 3. Approximately 5 mg of total protein was loaded onto 3.75 ml 10–40% glycerol gradients in a final volume of 200 μ l. Gradient solutions contained, in addition, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4. Sedimentation was for 18 h at 4°C in an SW 60 rotor at 45,000 rpm. After centrifugation, 300- μ l fractions were collected and 75- μ l aliquots were subjected to standard SDS-PAGE and immunoblot analysis. Approximately 300 μ g each of catalase (11 S), BSA (4.3 S), and cytochrome C (2 S) were centrifuged in separate tubes and sedimentation positions were determined by measuring the OD₂₈₀ of fractions from each gradient. Marker positions are indicated by downpointing arrows.

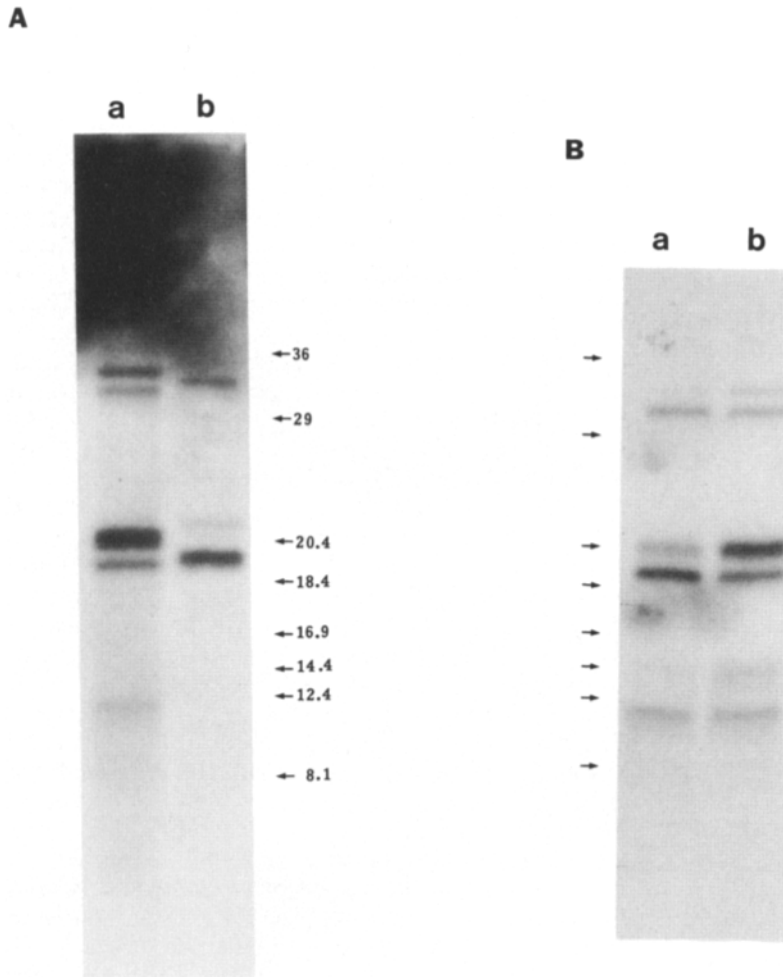


Figure 5. CNBr peptide map comparison of ^{32}P -labeled lamins Dm_1 , Dm_2 , and Dm_{mit} . (**A**) ^{32}P -labeled lamins were prepared from vinblastine-treated Schneider cells, purified by immunoprecipitation, and subjected to CNBr digestion and SDS-PAGE analysis on 10–18% polyacrylamide gradient gels. After electrophoresis, the gel was dried and applied to Kodak XAR film for 7 d at -70°C with an intensifier screen. Lane *a*, a mixture of lamins Dm_1 and Dm_2 derived from the nuclear pellet fraction; lane *b*, lamin Dm_{mit} derived from the postnuclear supernatant. (**B**) Schneider cell lamins Dm_1 and Dm_2 were labeled with ^{32}P -orthophosphate as in **A**, but in the absence of vinblastine; before CNBr digestion, lamins Dm_1 and Dm_2 were partially purified from each other by SDS-PAGE exactly as previously described (Smith et al., 1987). CNBr digestion, electrophoresis, and autoradiography were as in **A**. Lane *a*, a fraction enriched for lamin Dm_1 was analyzed; lane *b*, a fraction enriched for lamin Dm_2 was analyzed. Migration positions of protein mass standards were as indicated to the right of **A**; identical standards were used and positions are indicated to the left of **B**.

When ^{32}P -labeled lamin Dm_{mit} was analyzed (Fig. 5 *A*, lane *b*), a single major band was identified that migrated between the two major bands derived from lamins Dm_1 and Dm_2 (Fig. 5 *A*, lane *a*). A corresponding partial digestion product is also seen between standards at 29 and 36 kD. However, labeling of smaller fragments such as was seen with lamins Dm_1 and Dm_2 (Fig. 5 *A*, lane *a*; Fig. 5 *B*) was not apparent. Based on these initial studies, we conclude that the majority of interphase lamin phosphorylation, and virtually all phosphorylation of lamin Dm_{mit} takes place on a single CNBr fragment. Based on SDS-PAGE mobility, this fragment has been tentatively identified as the 17.8-kD polypeptide containing amino acids 385–547; this represents a potential “hinge” domain in the lamin structure between the end of coil 2 and the globular COOH terminus. The validity of this conclusion will have to be further evaluated by direct amino acid sequence analysis of the phosphorylated fragment.

To complete our initial comparison of phosphorylation of lamins Dm_1 and Dm_2 , and Dm_{mit} , we performed phosphoamino acid analysis. Labeled lamins were prepared from vinblastine-arrested Schneider cells as described for CNBr peptide map analyses, immunoprecipitated, subjected to electrophoresis on an SDS-7% polyacrylamide gel and subjected to phosphoamino acid analysis exactly as previously (Smith et al., 1987). The results of this analysis are shown

in Fig. 6. Consistent with previous reports, a mixed fraction containing both lamins Dm_1 and Dm_2 contained approximately equal amounts of phosphoserine and phosphothreonine; we had previously shown roughly comparable results when lamins Dm_1 and Dm_2 were analyzed separately (Smith et al., 1987). In contrast, lamin Dm_{mit} contained almost exclusively phosphoserine. By comparing the band intensities of ^{32}P -labeled lamins with immunoblots of lamin protein in each of the various isoforms used for this analysis, we could estimate relative degrees of phosphorylation: lamin $\text{Dm}_2 >$ lamin $\text{Dm}_{\text{mit}} >$ lamin Dm_1 (see also Fig. 5 *A*). Based on previous determinations for lamins Dm_1 and Dm_2 (Smith et al., 1987), we estimate that there are between 2 and 3 mol of phosphate per mole of lamin Dm_{mit} .

Discussion

Drosophila oogenesis is divided into 14 stages during which the oocyte develops from a primordial germ cell in the gerarium of the adult and arrests at first meiotic metaphase to await fertilization in the oviduct (see monograph by King, 1970; review by Mahowald and Kambyzellis, 1980). In the process, the egg is endowed with many of the components necessary for the rapid progression of early embryogenesis. From the results presented in this article and in a previous article (Smith and Fisher, 1984), it appears that nuclear lam-

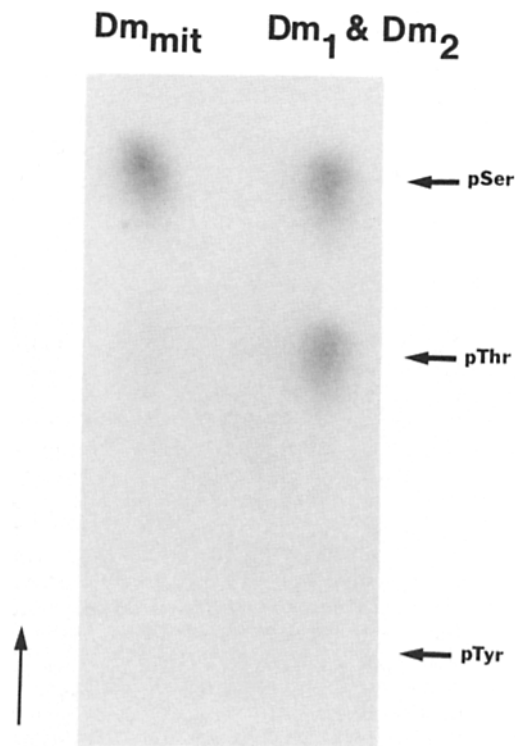


Figure 6. Phosphoamino acid analyses of lamins Dm_1 , Dm_2 , and Dm_{mit} . Schneider cells were labeled with ^{32}P -orthophosphate and fractionated as described in the legend to Fig. 5; lamins were further purified by immunoprecipitation. Immunoprecipitated lamins were recovered from the protein A-Sepharose by boiling in SDS, concentrated by TCA precipitation, and subjected to SDS-PAGE. Phosphoamino acid analyses on the SDS-PAGE-purified lamins were exactly as previously described (Smith et al., 1987). Migration positions of phosphoamino acid standards are indicated to the right of the figure: *p-Ser*, phosphoserine; *p-Thr*, phosphothreonine; *p-Tyr*, phosphotyrosine. Arrow in the lower left indicates the direction of paper electrophoresis. The paper was applied to preflashed Kodak XAR film for 11 d at $-70^\circ C$ with an intensifier screen.

ins are among these components. Moreover, the lamins are stored in a soluble, i.e., nonnuclear form. These results are analogous with previous reports regarding soluble lamin accumulation in *Xenopus* oocytes (Benavente et al., 1985) and are intuitively reasonable. The soluble lamin that is present in the mature egg has a characteristic SDS-PAGE mobility corresponding to an apparent mass of 75 kD, intermediate in size between lamins Dm_1 (74 kD) and Dm_2 (76 kD). This form persists into early embryogenesis.

Lamin Isoform Interconversion upon Entry of Cultured Cells into Mitosis

Insight into the significance of the soluble 75-kD lamin isoform found in late-stage egg chambers and early embryos was obtained using drugs to block Schneider 2 tissue culture cells in mitosis. A soluble lamin isoform with indistinguishable one- and two-dimensional gel mobilities, similar sedimentation properties, and identical one-dimensional CNBr peptide maps accumulated in these cells in direct proportion to the mitotic index of the cell population. Thus, it appears that this lamin isoform is characteristic of cells that have un-

dergone nuclear envelope breakdown either during mitosis or meiosis. On this basis, we have designated it lamin Dm_{mit} .

Perhaps the most surprising of our results regarding lamin Dm_{mit} concerns the relative levels of phosphorylation of lamins Dm_{mit} , Dm_1 , and Dm_2 . All three isoforms contain between 2 and 3 mol of phosphate per mole of protein; lamin Dm_{mit} is intermediate in this regard between lamins Dm_1 and Dm_2 . However, interphase lamins Dm_1 and Dm_2 are distinguishable in that they contain approximately equal amounts of phosphoserine and phosphothreonine whereas lamin Dm_{mit} predominantly contains phosphoserine. Thus it would seem that mitotic disassembly of the *Drosophila* nuclear lamina correlates not with absolute levels of lamin phosphorylation as has been suggested for mammalian cells (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985), but rather with a specific rearrangement of phosphate moieties.

Upon further comparison of results obtained in *Drosophila* with those from mammalian cells, it seems clear that the difference between the two systems is not actually between mitotic species. Both lamin Dm_{mit} and mitotic Chinese hamster ovary lamins (Ottaviano and Gerace, 1985) are phosphorylated to a similar degree and in both cases, phosphorylation occurs almost exclusively at serine residues. In contrast, comparison between interphase lamins derived from the different species reveals marked differences; these differences have recently been discussed in detail (Smith et al., 1987). Briefly, we noted that during interphase, *Drosophila* lamins are phosphorylated to ~ 10 times the level of their mammalian counterparts. We speculated that this may be in order to maintain nuclear envelope plasticity at the lower growth temperature of *Drosophila*. On the basis of our current observations, we can conclude that hyperphosphorylation in and of itself appears not to lead to nuclear lamina breakdown, at least in *Drosophila*. Rather, there may be specific sites of phosphorylation that regulate interphase plasticity versus mitotic dissolution, and/or there may be other sorts of posttranslational modification (e.g., demethylation [Chelsky et al., 1987]) that are also required for lamina disassembly. Both of these possibilities are under active consideration in our laboratory.

Our current results also suggest that growth of the interphase lamina by addition of monomer subunits to an essentially intact polymer is a fundamentally different process from concerted reassembly of the lamina at the end of mitosis. The substrate for interphase growth is newly synthesized, unphosphorylated lamin Dm_1 ; lamin Dm_1 is phosphorylated only coincident with or subsequent to incorporation into nuclei (Smith et al., 1987). In contrast, the substrate for concerted lamina reassembly at the end of mitosis is heavily phosphorylated lamin Dm_{mit} . Presumably, reassembly correlates with rearrangement of phosphate back to the interphase configuration.

Lamin Biosynthesis and Isoform Interconversion during Oogenesis

In conclusion, we wish to propose a model for the biosynthesis and accumulation of nuclear lamins during *Drosophila* oogenesis. Before stage 10, the majority of lamin protein is nuclear. Both isoforms Dm_1 and Dm_2 are present at similar levels. Between stages 11 and 13 of oogenesis, a new isoform, lamin Dm_{mit} , with an apparent mass of 75 kD appears. It is first detected as soluble, in contrast with lamins Dm_1 and Dm_2 which are primarily associated with the nuclear pellet.

The first appearance of lamin Dm_{mit} in extracts correlates with the breakdown of nurse cell nuclei as judged by indirect immunofluorescence. Hence, it is tempting to speculate that at least some of this material may be derived from the nurse cell lamina.

Throughout oogenesis up to stage 13, we note the apparent localization of lamin within the oocyte nucleus. This is the first observation of this sort of which we are aware. Examination of serial sections rules out the trivial explanation that the appearance of intranuclear lamin is an artifact of the plane of section. Rather, we think the localization of lamin within the oocyte nucleus may be reflective of a relatively high rate of lamin biosynthesis during oogenesis. Once the lamin polypeptide is synthesized in the cytoplasm of either the oocyte or surrounding nurse cells, we might expect the newly synthesized protein to be transported to the nucleus. It seems possible that transport is rapid but that once in the nucleus, assembly of lamin into the oocyte lamina is rate limiting, hence an accumulation of soluble intranuclear lamin is detected. This is in contrast with the situation in *Xenopus* where the oocyte germinal vesicle increases in size and apparently incorporates most or all of the lamin synthesized during oogenesis. This difference between *Drosophila* and *Xenopus* may reflect the relative rates of oogenesis in the two organisms, i.e., a few days (see for example Mahowald and Kambyzellis, 1980) versus several weeks (see for example Dumont, 1972), respectively.

At stage 14, the final stage of oogenesis, only lamin Dm_{mit} is identified and the majority is soluble. This correlates with the meiotic breakdown of the oocyte nucleus *in vivo* and the redistribution of lamin antigen throughout the ooplasm as determined by indirect immunofluorescence *in situ*. Lamin Dm_{mit} persists into early embryogenesis where it constitutes as much as 10–20% of the total lamin found in the fully developed embryo and presumably provides the karyoskeletal building blocks necessary for the efficient assembly of nuclei in the rapidly developing early embryo.

It is a pleasure to acknowledge Drs. Paul Bingham, Allan Spradling, and Sid Strickland for critical reading of the manuscript.

This work was supported by Research grant GM-33132 from the National Institutes of Health.

Received for publication 5 July 1988, and in revised form 12 October 1988.

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