Drosophila Nuclear Lamin Precursor Dm₀ Is Translated from Either of Two Developmentally Regulated mRNA Species Apparently Encoded by a Single Gene

Yosef Gruenbaum,§ Yosef Landesman,§ Barry Drees,* John W. Bare,* and Harald Saumweber;‖ Michael R. Paddy,* and John W. Sedat;* and David E. Smith,‡ Bret M. Benton,‡ and Paul A. Fisher‡

*Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143; ‡Department of Pharmacological Sciences, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794; §Department of Genetics, The Hebrew University, Jerusalem, Israel 91904; and ‖Max Planck Institut fur Entwicklungsbiologie, 7460 Tubingen, West Germany

Abstract. A cDNA clone encoding a portion of *Drosophila* nuclear lamins Dm_1 and Dm_2 has been identified by screening a λ-gt11 cDNA expression library using *Drosophila* lamin-specific monoclonal antibodies. Two different developmentally regulated mRNA species were identified by Northern blot analysis using the initial cDNA as a probe, and full-length cDNA clones, apparently corresponding to each message, have been isolated. In vitro transcription of both full-length cDNA clones in a pT7 transcription vector followed by in vitro translation in wheat germ lysate suggests that both clones encode lamin Dm_0, the polypeptide precursor of lamins Dm_1 and Dm_2. Nucleotide sequence analyses confirm the impression that both cDNA clones code for the identical polypeptide, which is highly homologous with human lamins A and C as well as with mammalian intermediate filament proteins. The two clones differ in their 3′-untranslated regions. In situ hybridization of lamin cDNA clones to *Drosophila* polytene chromosomes shows only a single locus of hybridization at or near position 25F on the left arm of chromosome 2. Southern blot analyses of genomic DNA are consistent with the notion that a single or only a few highly similar genes encoding *Drosophila* nuclear lamin Dm_0 exist in the genome.

THREE biochemically distinguishable forms of nuclear lamin have been identified in *Drosophila melanogaster* (Smith et al., 1987). A single primary translation product, lamin Dm_0 (apparent mass of 76 kD), is synthesized and processed rapidly to lamin Dm_1 (apparent mass of 74 kD) in the cytoplasm. We have suggested that this processing event may be proteolytic. Newly synthesized lamin Dm_1 is then assembled into the nuclear envelope at which point, posttranslational phosphorylation occurs. Two sorts of phosphorylation events have been recorded. One does not alter the one-dimensional SDS-PAGE mobility of lamin Dm_1; the second results in a shift in apparent mass back up to 76 kD. This form, designated lamin Dm_2, comigrates upon one-dimensional SDS-PAGE with lamin Dm_0 but can be distinguished from lamin Dm_0 by two-dimensional gel analysis as well as by peptide mapping.

The identification of what is in effect, only a single nuclear lamin in *Drosophila melanogaster* embryos, although similar to results that have been obtained with the surf clam (Maul et al., 1984), another invertebrate, is in apparent contrast to observations made in a variety of vertebrate systems (for recent reviews, see Krohne and Benavente, 1986; Gerace, 1986; Franke, 1987). In mammals, three lamins, desig-

nated A, B, and C, have been identified. In *Xenopus*, four polypeptides, designated lamins I-IV, have been reported. Of potentially more significance than merely the greater number of lamins, vertebrate lamins appear to be divided into at least two discrete classes. Biochemically, mammalian lamins A and C share considerable primary sequence homology both with each other and with mammalian intermediate filament proteins such as vimentin (McKeon et al., 1986; Fisher et al., 1986). Lamin B is largely distinct on the basis of immunochemical analyses and peptide map comparisons (Lam and Kasper, 1979; Gerace and Blobel, 1982; Kaufmann et al., 1983). Lamins A and C have relatively neutral isoelectric points, whereas lamin B is quite acidic (see e.g., Gerace and Blobel, 1980). With respect to isoelectric point, *Xenopus* lamins II, III, and IV resemble lamins A and C, whereas lamin I has a similar pI to lamin B (Benavente et al., 1985; Benavente and Krohne, 1985).

It has also been proposed that lamins of the A/C class can be distinguished from B-type lamins functionally. Burke and Gerace (1986) as well as others (Lebel and Raymond, 1984) have proposed that lamin B is important in mediating the interactions between the nuclear lamina and the inner nuclear membrane, whereas lamins A and C are involved in anchor-

ing chromosomes to the nuclear envelope. Developmental regulation of lamin type has also been reported in *Xenopus*, chicken, and mouse (Benavente et al., 1985; Stick and Hausen, 1985; Benavente and Krohne, 1985; Lehner et al., 1987; Schatten et al., 1985).

Attempted comparisons between *Drosophila* lamins and those of vertebrates have been relatively unrewarding. The *Drosophila* lamins Dm_1 and Dm_2 exhibit weak immunochemical homology with all three mammalian lamins (Fuchs et al., 1983; Fisher, P., unpublished observation). The pI of the *Drosophila* lamins of ∼6 is intermediate between lamin B and lamins A/C. Preliminary attempts at protein sequencing analysis revealed a short stretch of near-perfect homology (12 out of 13 residues) with mammalian vimentin (Slaughter, C., and P. Fisher, unpublished observation). Our recent identification of an extremely short-lived *Drosophila* lamin precursor (Smith et al., 1987) is reminiscent of a putative lamin B precursor that has been described in avian fibroblasts (Lehner et al., 1986).

The availability of a number of highly specific antibodies directed against the *Drosophila* lamins as well as suitable *Drosophila* cDNA expression libraries offered the opportunity to screen for cDNA clones encoding these proteins. The identification of such clones would initially facilitate the direct and systematic comparison of the *Drosophila* lamins with cloned lamins from other species. The availability of such clones would also offer unique opportunities for in vivo genetic analyses that are afforded by *Drosophila melanogaster*. Here we report the identification of full-length cDNA clones coding for the *Drosophila* lamin precursor Dm_0, and initial results of in situ localization and characterization of the *Drosophila* nuclear lamin gene. Nucleotide sequence determinations reveal strong homologies of both primary and secondary amino acid structure with human lamins A and C as well as with mammalian intermediate filament proteins.

Materials and Methods

The sources of most of the materials and much of the methodology has been described previously (Smith et al., 1987). Additional materials and methods are as follows.

The genomic library of *Drosophila* enl in Charon 34 phage was a gift of Z. Ali and T. Kornberg (University of California, San Francisco). The *Drosophila* λ-gtl0 library, made from 3–12-h-old embryos was a gift from L. Kauvar and T. Kornberg (University of California, San Francisco). The *Drosophila* cDNA library in λ-gtll phage made from 1–5.5-h-old embryos was a generous gift from P. Mohanan and D. Brutlag (Stanford University). The *Drosophila* histone gene complex clone, cDM500 (Karp and Hogness, 1976) was a gift of D. Hogness (Stanford University). The actin gene clone 5C was a gift from S. Tobin and J. Fristrom (University of California, Berkeley).

Screening of the λ-gtll Expression Library for Lamin Fusion Protein

The screening of the λ-gtll *Drosophila* cDNA expression library for the lamin clone was done as suggested by T. St. John, J. Rosen, and H. Gershenfeld (personal communication). In brief, after infecting *E. coli* KM392 cells with the phage, two 150-mm plates containing 25,000 lytic phage each were grown for 8 h at 37°C. Nitrocellulose filters were placed on top of plates and incubated overnight at 37°C. All of the following steps were done in 140 mM NaCl, 10 mM KHPO₄, pH 7.5 (PBS) containing 5% nonfat dry milk and 0.1% NP-40 at 4°C. The filters were incubated for 16 h with both monoclonal antibodies B9 and T40 (40 μg/ml) followed by three washes and a 4-h incubation with 500,000 cpm/ml iodinated goat anti-mouse Fab'. Antibodies were labeled with ¹²⁵I to a specific activity of 5×10^7 cpm/μg protein using the iodogen technique (Markwell and Fox, 1978). The single posi-

tive plaque was purified further by three more dilution-streaking cycles and was shown to react separately with B9 and T40 monoclonals.

A lysogen containing the lamin-β-galactosidase fusion protein was prepared as described by Huynh et al. (1985). *E. coli* Y1089 cells were used as the bacterial host for the lysogen. After growing the cells at 30°C to an optical density at 600 nm of 0.5, the temperature was quickly shifted to 42°C for 20 min. 10 mM isopropyl β-D-thiogalactopyranoside (IPTG;[1] Bethesda Research Laboratories, Gaithersburg, MD) was added to the solution, and the cells were transferred to 37°C for 35 min. A total protein extract of the bacteria was prepared by boiling the cells in 62.5 mM Tris-HCl pH 6.8, 10% SDS, 20 mM dithiothreitol (DTT) for 2–4 min.

In Situ Hybridization

In situ hybridization was by a modification of the procedure of Bonner and Pardue (1976), as follows. Salivary glands were removed from third instar larvae in 45% acetic acid, fixed in fresh 45% acetic acid for 2 min, and squashed between a coverslip and slide. The preparation was quick-frozen in liquid nitrogen, the coverslip removed, and the slide immersed in ice cold 95% ethanol for 10 min. The slide was then air dried. Heat, RNase, and sodium hydroxide treatments were done according to Bonner and Pardue (1976). Hybridization was carried out with nick-translated probe ([³H]thymidine) at a specific activity of 10^7–10^8 cpm/μg of DNA. The hybridization solution consisted of 50% formamide, 3.4× SSC (1× SSC is 0.3 M NaCl, 0.3 M sodium citrate), 150 μg/ml sheared calf thymus DNA, and 1.2 μg/ml of probe; 2 μl of hybridization solution was applied under an 18-mm coverslip, which was then sealed with Carter's rubber cement. Hybridization was carried out at 37°C for 20 h. Slides were then washed 4 × 15 min in 2× SSC at 32°C, washed with ethanol, and air dried. Autoradiography and staining were done according to Pardue and Gall (1975).

Isolation of Staged Egg Chambers

Mature *Drosophila* females were dissected on ice in modified Shields' medium buffered with Pipes (Smith et al., 1987) containing 100 μg/ml polyvinyl-pyrollidone. Egg chambers were isolated in this buffer on ice and staged according to Mahowald and Kambysellis (1980). Staged egg chambers were washed with ice cold buffer and stored at −70°C until use; RNA was extracted as was described for embryos (Smith et al., 1987).

Northern Blot Analysis

Northern blot analyses were performed either according to Poole et al. (1985) or as follows. RNA samples prepared as described (Smith et al., 1987) were subjected to electrophoresis in 0.8% agarose gels containing 6% formaldehyde and using buffer that included 50 mM boric acid, 5 mM sodium borate, 10 mM sodium sulfate, and 10 mM tetrasodium EDTA, plus 3% formaldehyde. Electrophoresis was at constant voltage for 700–1,000 V-h. RNA sample preparation was as follows. To the RNA (in H₂O) was added formaldehyde to 6%, formamide to 50%, and electrophoresis buffer to the same concentration as in the gel. This solution was heated to 60°C for 5 min and then quickly cooled on ice. To this was added 0.25 vol of loading solution (50% glycerol, 50% formamide, 0.05% bromphenol blue). After electrophoresis, the gel was rinsed 2 × 15 min with 10× SSC and blotted to nitrocellulose with 20× SSC as described by Thomas (1980). After transfer, the blot was probed with ³²P-labeled nick-translated probe (5×10^7–1×10^8 cpm/μg) in 50% formamide, 5× SSC, 10 mM NaHPO₄ pH 7.0, 5 mM EDTA, 5× Denhardt's solution (1× is 0.02% Ficoll, 0.02% polyvinyl-pyrollidone, 0.02% BSA), 200 μg/ml sonicated salmon sperm DNA, and 0.1% SDS. Hybridization was for 18 h at 42°C. The blot was then washed 2 × 15 min with 2× SSC, 0.1% SDS at room temperature, and then 4 × 30 min in 0.5× SSC, 0.1% SDS at 65°C. The filter was exposed to Kodak X-OMAT XAR film for the times indicated in the individual figure legends.

Southern Blot Analysis

Conditions used were essentially as described by Southern (1975) as follows. Hybridization mixtures contained 50% formamide, 5× SSC, 25 mM NaHPO₄, 5× Denhardt's solution, 2 mg/ml Herring testis DNA, and ³²P-labeled hybridization probe (5×10^7 cpm/0.2 μg DNA). Hybridizations were done overnight at 42°C. After hybridization, the nitrocellulose filters were first washed twice in 2× SSC, 0.1% SDS, at room temperature, followed by four 30-min washes with 0.1× SSC, 0.1% SDS at 52°C.

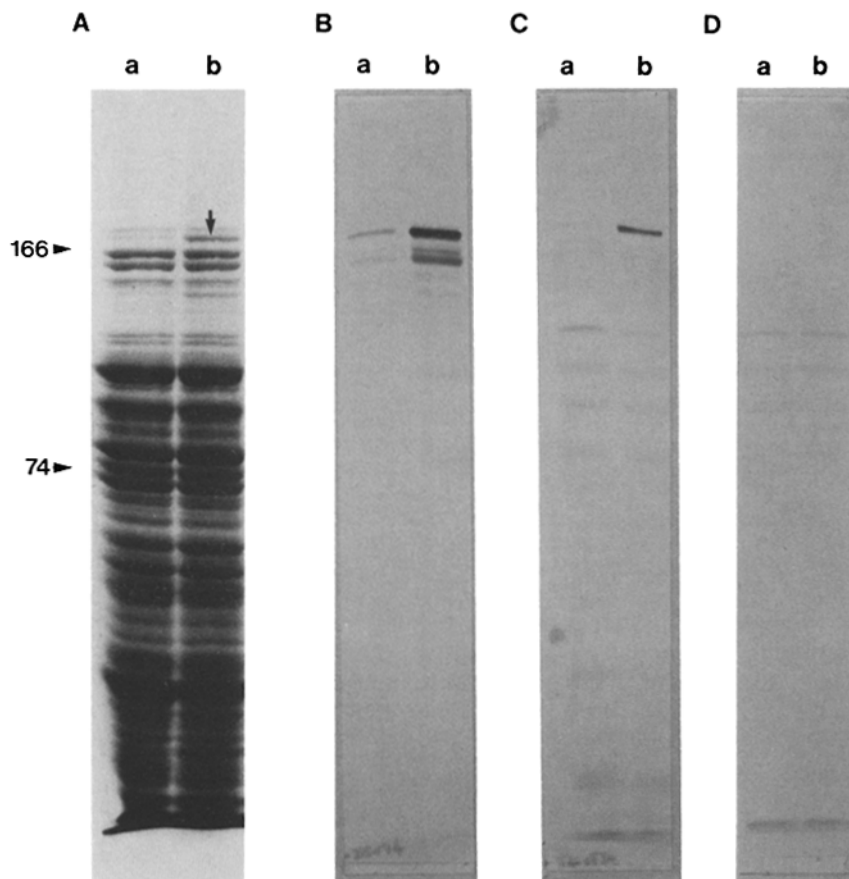1. *Abbreviation used in this paper:* IPTG, isopropyl β-D-thiogalactopyranoside.

Figure 1. Synthesis of the lamin-β-galactosidase fusion protein in *E. coli.* A lysogen harboring the λ-gt11 recombinant clone was prepared as described by Huynh et al. (1985). Cell lysates of the lysogen derived from 1×10^9 cells (*A*) or 2×10^8 cells (*B–D*) were subjected to electrophoresis on an SDS-7% polyacrylamide gel. Lanes *a* were loaded with lysate derived from the non-induced lysogen; lanes *b* were loaded with lysate from the IPTG-induced cells. (*A*) Coomassie Blue–stained gel. (*B–D*) Immunoblots prepared from parallel gel segments. (*B*) Blot was probed with affinity purified anti–lamin antibodies diluted at 1:2,000 relative to the specific antibody concentration of the unfractionated anti–lamin antiserum. (*C*) Blot was probed with ammonium sulfate purified monoclonal antibody T40 diluted at 1:100 relative to the specific antibody concentration of the unfractionated mouse ascites fluid. (*D*) Blot was probed with preimmune serum diluted at 1:2,000. *Arrow* in *A*, lane *b* designates the IPTG-induced lamin-β-galactosidase fusion protein.

Gel Purification of RNA From Methyl Mercury Hydroxide Gels

Electrophoresis was using the buffer system as described above for the formaldehyde gels except the gel contained 10 mM methyl mercury hydroxide. The gel was cast using low melting agarose (Weislander, 1979), and recovery of the RNA was as described by Maniatis et al. (1982) as follows; 300 μg total embryo RNA in H_2O was made 1× electrophoresis buffer, 10 mM methyl mercury hydroxide, and incubated at room temperature for 30 min. The gel was of preparative scale containing a single wide lane. After electrophoresis the gel was soaked for 30 min in 100 mM 2-mercaptoethanol and cut into 3-mm slices starting just above the 18 S ribosomal RNA, which was visualized by UV illumination of a parallel gel strip. The gel slices were melted at 65°C in 4 vol of 0.5 M ammonium acetate, 1% SDS with 50 μg calf thymus tRNA added as carrier. Once the gel slices were melted, they were extracted once with phenol and twice with chloroform at room temperature. The RNA was then ethanol precipitated overnight, dried, resuspended in 40 μl H_2O, and stored at −70°C; aliquots of this RNA were used for subsequent Northern blot analysis and in vitro translation as indicated in the figure legend.

DNA Nucleotide Sequence Analysis

DNA sequence analysis was by the dideoxynucleotide chain termination method of Sanger et al. (1977). Lamin cDNA clones cDNL2800 and cDNL3000 were cloned in both orientations in pUC118 (designed by J. Viera [Rutgers University] and kindly provided by Y. Kassir [Hebrew University]). Overlapping deletions were produced from both strands of the gene by limited digestion with DNase I.

Results

Isolation of Lamin cDNA

When DNA fragments are inserted into a λ-gt11 phage expression system, hybrid proteins are produced from the fu-

sion of the β-galactosidase gene and the inserted DNA sequences in the transfected *E. coli.* Antibodies can then be used to identify plaques that contain phage expressing specific gene products. Two monoclonal antibodies directed against the *Drosophila* lamins, B9 (Fuchs et al., 1983) and T40 (Risau et al., 1981; see also Smith and Fisher, 1984) were combined and used to screen a λ-gt11 cDNA expression library constructed from early *Drosophila* embryonic mRNA (1–5.5 h after oviposition). Screening a total of 5 × 10^4 phage grown on two 150-mm plates yielded a single plaque that bound both T40 and B9 monoclonals individually after transfer to nitrocellulose filters. Immunoreactivity of this plaque was confirmed with affinity-purified polyclonal anti–lamin antibodies. The size of the cDNA insert as judged by agarose gel electrophoresis was ~1,700 bp, and it was designated cDNL1700.

The cDNL1700 Clone Encodes a Portion of the Drosophila Lamin

One line of evidence that the cDNA clone encodes a portion of the lamin protein is the fact that its protein product reacts specifically with anti–lamin antibodies. The protocol of Huynh et al. (1985) was used to make a lysogenic strain in *E. coli* Y1089 containing the cDNL1700 phage. After induction with IPTG, a 170-kD fusion protein between the cDNA protein product and β-galactosidase was identified upon SDS-PAGE of a lysate of the lysogen. These results are shown in Fig. 1. Lanes *a* are before induction with IPTG. Lanes *b* are after induction with IPTG. A Coomassie Blue–stained
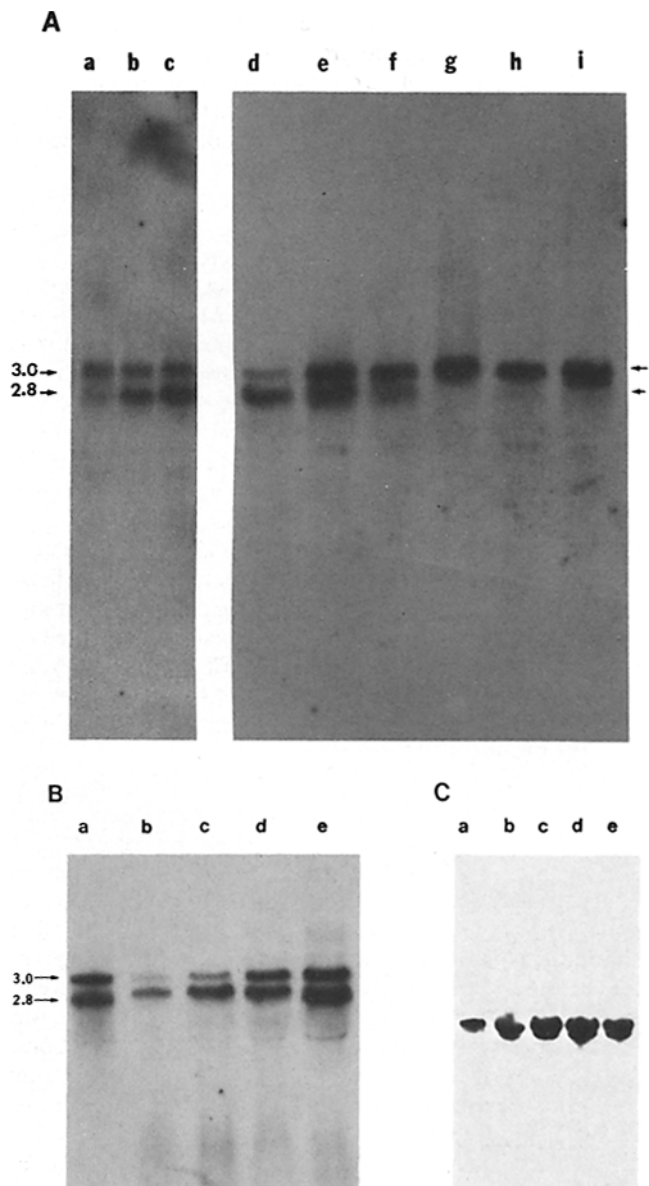
A

B   **C**

Figure 2. Differential expression of lamin mRNAs during oogenesis and embryogenesis. (*A*) Mature female flies were chilled on ice and their ovaries were excised and staged into three groups: lane *a*, germarium to stage 8; lane *b*, stages 9 and 10; lane *c*, stages 11–14. Embryos were collected for 2.5 h from a well-fed population and aged at 25°C to generate subpopulations of the following ages: lane *d*, 0–2.5 h; lane *e*, 2.5–5 h; lane *f*, 5–7.5 h; lane *g*, 7.5–10 h; lane *h*, 10–12.5 h; lane *i*, 12.5–15 h. Visual inspection of the dechorionated embryos confirmed that >90% of the embryos in each population were of the desired developmental age. Total RNA was extracted from each population of oocytes or embryos; 15 µg RNA, lanes *a–c*, and 30 µg RNA, lanes *d–i* was subjected to electrophoresis on a formaldehyde-1% agarose gel and blotted to nitrocellulose. Blots were probed with nick-translated 1.7-kb lamin cDNA clone in pEMBL vector with a specific activity of 5 × 10⁷ cpm/µg. Blots were processed as described (Materials and Methods). Fluorography was for 18 h at −70°C. (*B* and *C*) Expression of lamin mRNAs early in embryogenesis. The developmental stages of dechorionated embryos were determined according to Foe and Alberts (1983). For each developmental window, total RNA was prepared from 200 embryos. Lanes *a*, poly(A)⁺ RNA from 0–4-h-old embryos; lanes *b*, total RNA from pre-stage 9 embryos; lanes *c*, stages 10–12 embryos; lanes *d*, stages 13 and 14 embryos; lanes *e*, mid-gastrula

gel is shown in Fig. 1 *A*. Immunoblot analyses of this whole protein extract are shown in *B–D*. The blot in *B* was probed with affinity-purified polyclonal anti–lamin antibodies. The blot in *C* was probed with monoclonal antibody T40. The blot in *D* was probed with preimmune serum. In both *B* and *C*, a specific and intense band of immunoreactivity was seen to comigrate with the IPTG-induced fusion protein identified by Coomassie Blue staining of the parallel gel segment (*A*). Some expression of the fusion protein was apparent before IPTG induction.

Two Lamin mRNA Species are Differentially Expressed during Development

Northern blot analyses performed using cDNL1700 as hybridization probe revealed the existence of what were apparently two poly(A)⁺ *Drosophila* lamin mRNAs of 2.8 and 3.0 kb, respectively. A profile of the two mRNAs during oogenesis and embryogenesis is shown in Fig. 2 *A*. Early in oogenesis, both mRNAs could be identified and were of similar abundance. However, in the later stages of oogenesis and early in embryogenesis, the 2.8-kb message predominated. Between 2 and 3 h after oviposition, however, there was a shift such that the 3.0-kb message became the more abundant. By the later stages of embryogenesis in the experiment shown (Fig. 2 *A*), the 2.8-kb message was essentially undetectable. In other experiments, small amounts (∼20%) of the 2.8-kb form were seen at the later time points (not shown). Examination of later stages in organismal development (larvae, pupae, adults) showed predominance of the 3.0-kb mRNA at all points examined; however, small amounts (10–20%) of the 2.8-kb message were also detectable (not shown).

A more precise profile of transcription in early embryos was obtained by separating embryos according to four specific developmental windows: from oviposition to stage 9, stages 10–12, stages 13 and 14 (cellular blastoderm), and mid-gastrulation. Stages 10–14 correspond to ∼1.5–3 h of embryogenesis. Each lane on the gel was loaded with total RNA extracted from 200 staged embryos that were hand-selected using a dissecting microscope. As shown in Fig. 2 *B*, both transcripts were present during all early embryonic stages. Before stages 13 and 14 (Fig. 2 *B*, lanes *b* and *c*), the 2.8-kb transcript was approximately four times more abundant than the 3.0-kb transcript. In the mid-gastrula stage (lane *e*), levels of both transcripts were approximately equal. The total amount of both the 2.8- and 3.0-kb lamin mRNAs per embryo increased during progressive stages of embryogenesis. In stages 10–12 (Fig. 2 *B*, lane *c*), the RNA hybridization signal of both transcripts was doubled relative to oviposition to stage 9 (Fig. 2 *B*, lane *b*). In stages 13 and 14 (Fig. 2 *A*, lane *d*), the hybridization signal of the 3.0-kb transcript was about three times stronger than in stages 10–12. Each lane contains RNA from the same number of embryos; the increase in lamin RNA from pre-stage 10 to stages 10–12 presumably represents new synthesis. Thus, it appears that transcription of the lamin gene may be initiated at least as early as stages 10–12, before cellular blastoderm. Hybridization of similar Northern blots with the actin gene sequence (Fig. 2 *C*) or the histone gene cluster sequence (not shown)

stage embryos. Northern blots were probed as follows. (*B*) Lamin cDNA clone cDNL1700; (*C*) actin 5C clone.

A

a   b   c   d   e   f   g

3.0→
2.8→

B

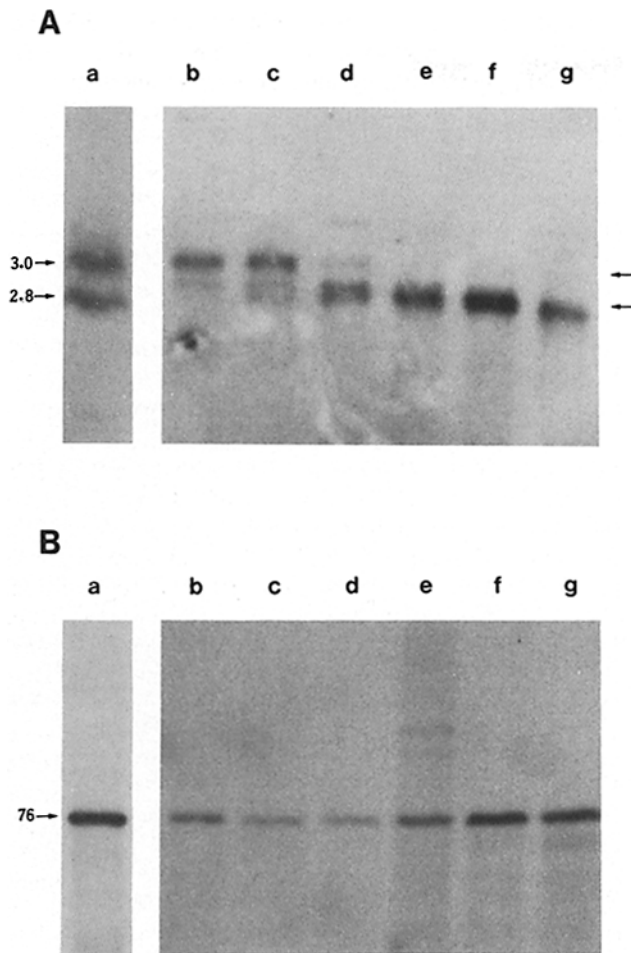a   b   c   d   e   f   g

76→

Figure 3. In vitro translation of gel-purified lamin mRNAs in wheat germ lysate. Total RNA (300 μg) from 3–5-h-old embryos was subjected to electrophoresis on a preparative methyl mercury hydroxide-0.8% agarose gel. The gel was sliced into fractions through the region where the two lamin mRNA species were known to migrate and RNA was recovered from each slice. (*A*) Northern blot analysis of RNA fractions. (*B*) In vitro translation of the RNA fractions characterized in *A* in wheat germ lysate. Lanes *a*, unfractionated 3–5-h-old embryo RNA; lanes *b–g*, gel purified lamin mRNA fractions, displayed from larger to smaller, left to right. Northern analysis (*A*) was as described (Materials and Methods); in vitro translation with immunoprecipitation and SDS-PAGE (*B*) was as detailed previously (Smith et al., 1987). Fluorographic exposure was for 4 wk at −70°C.

shows that the total amounts of RNA from these two genes were roughly constant over this developmental period and thus serves as an internal control for the amount of RNA loaded in each lane.

Both the 2.8- and the 3.0-kb Drosophila Lamin mRNAs Code for the Same Polypeptide, Lamin Dm_0

The differential pattern of mRNA expression observed during embryogenesis appeared remarkably similar to the differential expression of lamins Dm_1 and Dm_2 during the same period of development (Smith and Fisher, 1984). However, we have gone to considerable lengths to show that neither lamin Dm_1 nor Dm_2 represents a primary translation

product but rather, that both are derived posttranslationally from a single polypeptide precursor, lamin Dm_0 (Smith et al., 1987). It was therefore essential that to confirm these observations at the mRNA level, we be able to demonstrate that the 2.8- and the 3.0-kb lamin mRNAs both coded for lamin Dm_0.

Two approaches were used to address this problem. First, an RNA fraction from early *Drosophila* embryos was subjected to electrophoresis on a methyl mercury-agarose gel to resolve the two mRNAs. Approximately equal amounts of each of the two messages were identified by Northern blot analysis after electrophoresis (Fig. 3 *A*, lane *a*). The gel was then cut into several fractions through the region where the lamin mRNAs were found to migrate, and the RNA was eluted from each slice. An aliquot of each RNA fraction was subjected to electrophoresis on a second gel and analyzed by Northern blot. These results are shown in Fig. 3 *A*, lanes *b–g* and demonstrate the relative enrichment of the various gel eluate fractions in either the 3.0-kb message (lanes *b* and *c*) or the 2.8-kb form (lanes *d–g*). Aliquots of each of these mRNA fractions were then translated in the wheat germ lysate and the in vitro translation products were analyzed by immunoprecipitation and SDS-PAGE (Fig. 3 *B*). It appears from this analysis that both mRNAs do indeed code for the same primary translation product, lamin Dm_0, and with about the same efficiency.

When unfractionated *Drosophila* lamin mRNA was translated in rabbit reticulocyte lysate, two polypeptide forms were identified (Smith et al., 1987). However, we were able to show that the 74-kD form (lamin Dm_1) was processed from the 76-kD form (lamin Dm_0) posttranslationally. To complete our analysis of the gel-purified mRNAs, a similar experiment to that shown in Fig. 3 was performed using rabbit reticulocyte lysate for in vitro translation. *Drosophila* embryo mRNA was subjected to electrophoresis, the gel was fractionated in the region of interest and mRNA fractions, taken as described in the legend of Fig. 3, were eluted from the gel. When these RNA fractions were translated in the rabbit reticulocyte lysate, it was apparent that both the 2.8- and the 3.0-kb messages directed the synthesis of both the 76- and the 74-kD lamin (not shown).

The second approach to proving the specificity of the two lamin mRNAs identified by Northern analysis was as follows. The cDNL1700 clone was used to screen a *Drosophila* cDNA library made in λ-gt10 (Poole et al., 1985). Two of the clones isolated, cDNL2800 and cDNL3000, had cDNA insert sizes of ∼2,800 and 3,000 bp, respectively. These corresponded exactly to the sizes of the two different lamin mRNAs. These inserts were cloned into the pT7 in vitro transcription vector (Tabor and Richardson, 1985). mRNA was transcribed from each clone using purified T7 RNA polymerase and used to program a wheat germ in vitro translation reaction. Both in vitro transcripts apparently coded for lamin Dm_0 (76 kD) and moreover, both primary translation products could be processed to lamin Dm_1 (74 kD) by addition of the appropriate *Drosophila* embryo lysate (Smith et al., 1987) (not shown).

The insertion of cDNL2800 and cDNL3000 into pT7 afforded us the opportunity to further compare the in vitro translation products encoded by the two clones by peptide mapping. We were also able to compare these cloned proteins with the in vitro translation product of authentic *Dro-*

CCCCCCAGTCTCTGAAACCATCGCAACAAATTCGATTACACGTTCCAGCGTCGGCCGCGTTTTCCTGCTGCAAGATACTTCATTTTCAAATTTGTGCATT 100

TTCCGTTCGTTATAAAAAGTCAGCGTGAAC ATG TCG AGC AAA TCC CGA CGT GCT GGC ACC GCC ACG CCG CAG CCC GGC AAC ACC TCC ACC CCC CGG CCC 199
                               Met Ser Ser Lys Ser Arg Arg Ala Gly Thr Ala Thr Pro Gln Pro Gly Asn Thr Ser Thr Pro Arg Pro

GCC ATC GGG TCC GCA GCC GCC GCC GCC GTC CAC TCA CTC GCA GAC GCC TCC AGC CCC CTC AGC CCC ACC CGG CAC TCG CGC GTG GCC GAG AAG GTG GAG 298
Ala Ile Gly Ser Ala Ala Ala Ala Ala Val His Ser Leu Ala Asp Ala Ser Ser Pro Leu Ser Pro Thr Arg His Ser Arg Val Ala Glu Lys Val Glu
Pst I
CTG CAG AAC CTG AAC GAT CGC CTG GCC ACC TAC ATT GAC CGG GTG CGC AAC CTG GAG ACG GAG AAC TCC CGC CTC ACC ATC GAG GTG CAG ACC ACC AGG 397
Leu Gln Asn Leu Asn Asp Arg Leu Ala Thr Tyr Ile Asp Arg Val Arg Asn Leu Glu Thr Glu Asn Ser Arg Leu Thr Ile Glu Val Gln Thr Thr Arg

GAC ACG GTC ACA CGC GAG ACC ACC AAC ATC AAG AAC ATC TTC GAG GCC GAG CTG CTG GAG ACG CGC CGT CTG CTC GAT GAC ACA GCT AGG GAT CGC GCT 496
Asp Thr Val Thr Arg Glu Thr Thr Asn Ile Lys Asn Ile Phe Glu Ala Glu Leu Leu Glu Thr Arg Arg Leu Leu Asp Asp Thr Ala Arg Asp Arg Ala

CGT GCC GAG ATC GAT ATC AAG CGT CTC TGG GAG AGG AAC GAG GAG CTC AAG AAC AAG CTG GAC AAG AAG ACC AAG GAG TGC ACC ACT GCT GAG GGC AAT 595
Arg Ala Glu Ile Asp Ile Lys Arg Leu Trp Glu Arg Asn Glu Glu Leu Lys Asn Lys Leu Asp Lys Lys Thr Lys Glu Cys Thr Thr Ala Glu Gly Asn
                                                                                                Hind III
GTC CGC ATG TAC GAG TCG CGC GCC AAC GAG CTG AAC AAC AAA TAC AAC CAG GCC AAC GCC GAT CGG AAG AAG CTT AAC GAA GAC CTG AAT GAG GCG CTA 694
Val Arg Met Tyr Glu Ser Arg Ala Asn Glu Leu Asn Asn Lys Tyr Asn Gln Ala Asn Ala Asp Arg Lys Lys Leu Asn Glu Asp Leu Asn Glu Ala Leu
                                                                                              Hinc II
AAG GAG CTG GAG AGA CTG CGT AAG CAG TTC GAG GAA ACG CGG AAG AAC CTG GAA CAG GAG ACA CTG TCG CGC GTT GAC CTG GAG AAC ACC ATT CAG AGT 793
Lys Glu Leu Glu Arg Leu Arg Lys Gln Phe Glu Glu Thr Arg Lys Asn Leu Glu Gln Glu Thr Leu Ser Arg Val Asp Leu Glu Asn Thr Ile Gln Ser

CTG CGC GAG GAG CTC TCG TTC AAG GAT CAG ATC CAT TCG CAG GAG ATC AAT GAG TCG CGC CGC ATC AAA CAG ACA GAG TAT AGC GAG ATC GAC GGG TCG 892
Leu Arg Glu Glu Leu Ser Phe Lys Asp Gln Ile His Ser Gln Glu Ile Asn Glu Ser Arg Arg Ile Lys Gln Thr Glu Tyr Ser Glu Ile Asp Gly Ser
                                                     Pst I
CCT CAG CTC CGA GTA CGA TGC CAG TTG AAG CAG TCG CTG CAG GAC GTG CGC GCC CAG TAC GAG GAG CAG ATG CAG ATT AAT CGC GAT GAA ATC CAG TCC 991
Pro Gln Leu Arg Val Arg Cys Gln Leu Lys Gln Ser Leu Gln Asp Val Arg Ala Gln Tyr Glu Glu Gln Met Gln Ile Asn Arg Asp Glu Ile Gln Ser

CTC ATC GAG GAC AAG ATC CAA CGA CTG CAA GAG GCC GCC GCA CGC ACA TCC AAT TCC ACG CAC AAG TCC ATC GAG GAG CTG CGC TCC ACT CGT GTG CGT 1090
Leu Ile Glu Asp Lys Ile Gln Arg Leu Gln Glu Ala Ala Ala Arg Thr Ser Asn Ser Thr His Lys Ser Ile Glu Glu Leu Arg Ser Thr Arg Val Arg
                                                                        BamH I   Pvu II
ATC GAT GCG CTC AAC GCC AAT ATC AAC GAA CTG GAG CAA GCC AAT GCC GAC CTC AAT GCG CGG ATC CGT GAT CTG GAG CGC CAG CTG GAC AAC GAT CGC 1189
Ile Asp Ala Leu Asn Ala Asn Ile Asn Glu Leu Glu Gln Ala Asn Ala Asp Leu Asn Ala Arg Ile Arg Asp Leu Glu Arg Gln Leu Asp Asn Asp Arg
                                          Xho I
GAA CGC CAC GGT CAA GAG ATA GAC CTT CTC GAG AAG GAG CTC ATT CGG CTG CGC GAA GAG ATG ACG CAA CAG CTC AAG GAG TAC CAG GAC CTT ATG GAC 1288
Glu Arg His Gly Gln Glu Ile Asp Leu Leu Glu Lys Glu Leu Ile Arg Leu Arg Glu Glu Met Thr Gln Gln Leu Lys Glu Tyr Gln Asp Leu Met Asp

ATC AAG GTC TCC CTG GAT TTG GAA ATC GCC GCA TAC GAC AAG CTG CTG GTG GGC GAG GAG GCT CGT TTG AAC ATC ACC CCA GCC ACC AAC ACG GCC ACA 1387
Ile Lys Val Ser Leu Asp Leu Glu Ile Ala Ala Tyr Asp Lys Leu Leu Val Gly Glu Glu Ala Arg Leu Asn Ile Thr Pro Ala Thr Asn Thr Ala Thr

GTG CAG TCC TTT AGC CAG TCG CTG CGC AAC TCC ACG CGA GCC ACG CCA TCG CGT CGC ACT CCC TCT GCT GCC GTG AAG CGC AAA CGC GCC GTG GTC GAC 1486
Val Gln Ser Phe Ser Gln Ser Leu Arg Asn Ser Thr Arg Ala Thr Pro Ser Arg Arg Thr Pro Ser Ala Ala Val Lys Arg Lys Arg Ala Val Val Asp

GAG TCG GAG GAT CAC AGC GTC GCC GAT TAC TAT GTG TCC GCC AGT GCC AAG GGC AAC GTG GAG ATC AAG GAG ATC GAT CCC GAG GGC AAG TTC GTA AGG 1585
Glu Ser Glu Asp His Ser Val Ala Asp Tyr Tyr Val Ser Ala Ser Ala Lys Gly Asn Val Glu Ile Lys Glu Ile Asp Pro Glu Gly Lys Phe Val Arg
                                                    Pvu II   Pst I
CTG TTC AAC AAG GGC AGC GAG GAG GTG GCC ATC GGT GGC TGG CAG CTG CAG CGG CTA ATC AAC GAG AAA GGT CCT TCG ACC ACT TAC AAG TTC CAT CGA 1684
Leu Phe Asn Lys Gly Ser Glu Glu Val Ala Ile Gly Gly Trp Gln Leu Gln Arg Leu Ile Asn Glu Lys Gly Pro Ser Thr Thr Tyr Lys Phe His Arg

TCG GTG AGG ATC GAG CCA AAT GGC GTG ATC ACC GTT TGG TCG GCG GAC ACC AAG GCC TCG CAC GAG CCG CCA TCG AGC CTT GTG ATG AAG TCA CAG AAG 1783
Ser Val Arg Ile Glu Pro Asn Gly Val Ile Thr Val Trp Ser Ala Asp Thr Lys Ala Ser His Glu Pro Pro Ser Ser Leu Val Met Lys Ser Gln Lys

TGG GTC TCC GCC GAC AAC ACT AGG ACG ATT TTG CTG AAC TCC GAG GGC GAG GCC GTG GCC AAT CTG GAT CGC ATC AAG CGC ATT GTG TCC CAA CAC ACA 1882
Trp Val Ser Ala Asp Asn Thr Arg Thr Ile Leu Leu Asn Ser Glu Gly Glu Ala Val Ala Asn Leu Asp Arg Ile Lys Arg Ile Val Ser Gln His Thr

TCC TCC TCC CGG CTG AGT CGT CGT CGC AGC GTG ACC GCC GTG GAC GGC AAT GAG CAG CTC TAC CAC CAG CAG GGC GAT CCT CAG CAG TCA AAC GAG AAG 1981
Ser Ser Ser Arg Leu Ser Arg Arg Arg Ser Val Thr Ala Val Asp Gly Asn Glu Gln Leu Tyr His Gln Gln Gly Asp Pro Gln Gln Ser Asn Glu Lys

TGC GCC ATT ATG TAA 1996
Cys Ala Ile Met ***

AATCAAACGCAGCACAACACAACTCTTTCCTCTTTGCTGAACAAGACAAACAAATAAGCACACACGAAGATCATAATTTAGAACCCAAAAACACACACACCGAGGTACAGAGATTTATTATTCAGCTAAGT 2128
TATTTTTTGTGGCCGCAGCGCCAATTATTTAATCAAAAACATTTGTGTAGAAGACATCCTGAATCTTCGTTTCGTTTGTACACTTTCGTTTTCCCTTTCTTAACAAATTCTATAGTTTATTGTTTCGATGTT 2260
TTGTATTCCGCGTTAACTAATCTATGTAAACTTTATTTGGTATAAACTGGAGAGAGCATGTTGCCTCTTTTTTATGCCACATAGAATTTACGTAAGACGTTCACTTCTTGTATTCGCGGCGGCAAGAACTTT 2392
GAAAATATTGCAAGAAAAACAATATTTTCGATGTACTTACGCTCCACCTACATATTTAGTAAATTAGTTTTTAAGCTATATCCCAGATAAACCAGTCGACGTCAGCAAACAACAACAAAAAAAAAACAACGA 2524
GCACGTAGTGAGTGATTTATAAGATACAGGTCAAGAGGATTAACTAAAGAAAACAAACGCTCCTGACAACAGACATATTTATTTAAGTATTTTTTGTACAATCAACATAAAATACATTTATACATTATACATA 2656
CATATACATACATACATTATATATATAGACTCATGCCTACGGAAGTGACAACCAGCCAGCAATATATATTTTTAGCCATGGCCATAGGGTTTACGATCCACCAAAACGGCTTTCTCCCGGTTTAGACCGGTT 2788
CTTGAGCTTCTGCAGACATTTTACCGGACGAGCAACAATAAGAACAGCAACAACAACAACAGCATTAACAGCAACAACACAAATGTACAACAATTACAAAAGCAACAAAGAAGAAAAA 2907

Figure 4. Translated cDNA sequence of cDNL3000 encoding *Drosophila* nuclear lamin precursor Dm$_0$. cDNL3000 was sequenced completely from both strands as described (Materials and Methods). Translation was started at the first in-phase methionine and continued through to nucleotide 1993. A stop codon was encountered beginning at nucleotide 1994. cDNL2800 was sequenced similarly to determine the 3'-untranslated sequence and was found to be identical with cDNL3000 up to its point of termination at nucleotide 2702. The last 10 nucleotides of cDNL2800 are underlined and the final nucleotide is designated by a downpointing arrowhead. Sequence analysis of cDNL2800 at the 5' end, partial sequence analysis throughout the coding region, and detailed restriction mapping suggested that cDNL2800 was otherwise identical with cDNL3000. Restriction sites designated above the DNA sequence were experimentally determined for both clones. The unlabeled downpointing arrowhead at nucleotide 2274 indicates the presence of a Hinc II site that was common to both cDNL2800 and cDNL3000 in the 3'-untranslated region.

sophila lamin mRNA and with authentic lamin protein synthesized in the organism. Within the technical limits of the procedure, these experiments showed all of these variously derived lamins to be identical (Smith et al., 1987).

Nucleotide Sequence Analysis of Drosophila Lamin cDNA Clones cDNL2800 and cDNL3000

The complete nucleotide sequence of cDNL3000 is shown in Fig. 4. The cDNA has been translated from the first ATG
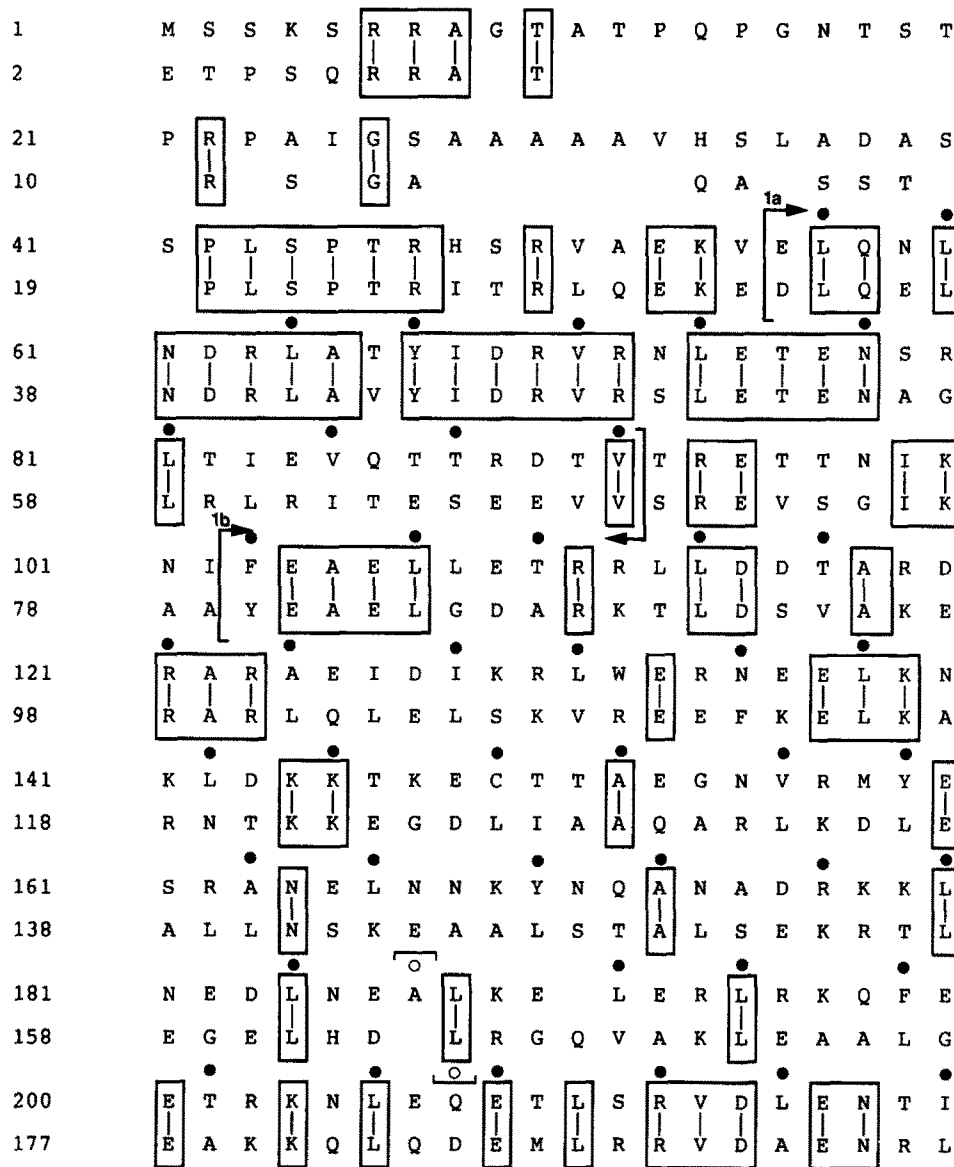
Figure 5. Comparison of amino acid sequences between *Drosophila* lamin Dm₀ and human lamin A. The complete cDNL-3000-predicted amino acid sequence of *Drosophila* lamin Dm₀ was matched to the sequence of human lamin A. Significant homology was recorded throughout; identical amino acids are "boxed." Characteristic coiled domains (1a, 1b, and 2) of intermediate filament proteins are as designated and coincide with those reported for human lamins A and C (McKeon et al., 1986; Fisher et al., 1986). The a and d positions of the heptad repeats within the coiled domains are also designated above the *Drosophila* lamin sequence (●). An apparent disparity in this regard between *Drosophila* and human at amino acids 187 and 164, respectively, is designated by the open circles (○) and brackets. This discrepancy is an artifact of the alignment program resulting from the introduction of single-amino acid gaps in both the *Drosophila* and the human sequences in this immediate region. There are two phase shifts in the heptad repeat pattern of coil 2 evident at amino acids 288/289 and 352/353 of the *Drosophila* lamin sequence. These coincide with similar phase shifts in the human lamin sequence (McKeon et al., 1986; Fisher et al., 1986).

```
  1  M S S K S R R A G T A T P Q P G N T S T
  2  E T P S Q R R A   T T

 21  P R P A I G S A A A A A V H S L A D A S
 10    R S   G A               Q A   S S T       1a

 41  S P L S P T R H S R V A E K V E L Q N L
 19    P L S P T R I T R L Q E K E D L Q E L

 61  N D R L A T Y I D R V R N L E T E N S R
 38  N D R L A V Y I D R V R S L E T E N A G

 81  L T I E V Q T T R D T V T R E T T N I K
 58  L R L R I T E S E E V V S R E V S G I K   1b

101  N I F E A E L L E T R R L L D D T A R D
 78  A A Y E A E L G D A R K T L D S V A K E

121  R A R A E I D I K R L W E R N E E L K N
 98  R A R L Q L E L S K V R E E F K E L K A

141  K L D K K T K E C T T A E G N V R M Y E
118  R N T K K E G D L I A A Q A R L K D L E

161  S R A N E L N N K Y N Q A N A D R K K L
138  A L L N S K E A A L S T A L S E K R T L

181  N E D L N E A L K E   L E R L R K Q F E
158  E G E L H D L R G Q V A K L E A A L G

200  E T R K N L E Q E T L S R V D L E N T I
177  E A K K Q L Q D E M L R R V D A E N R L
```
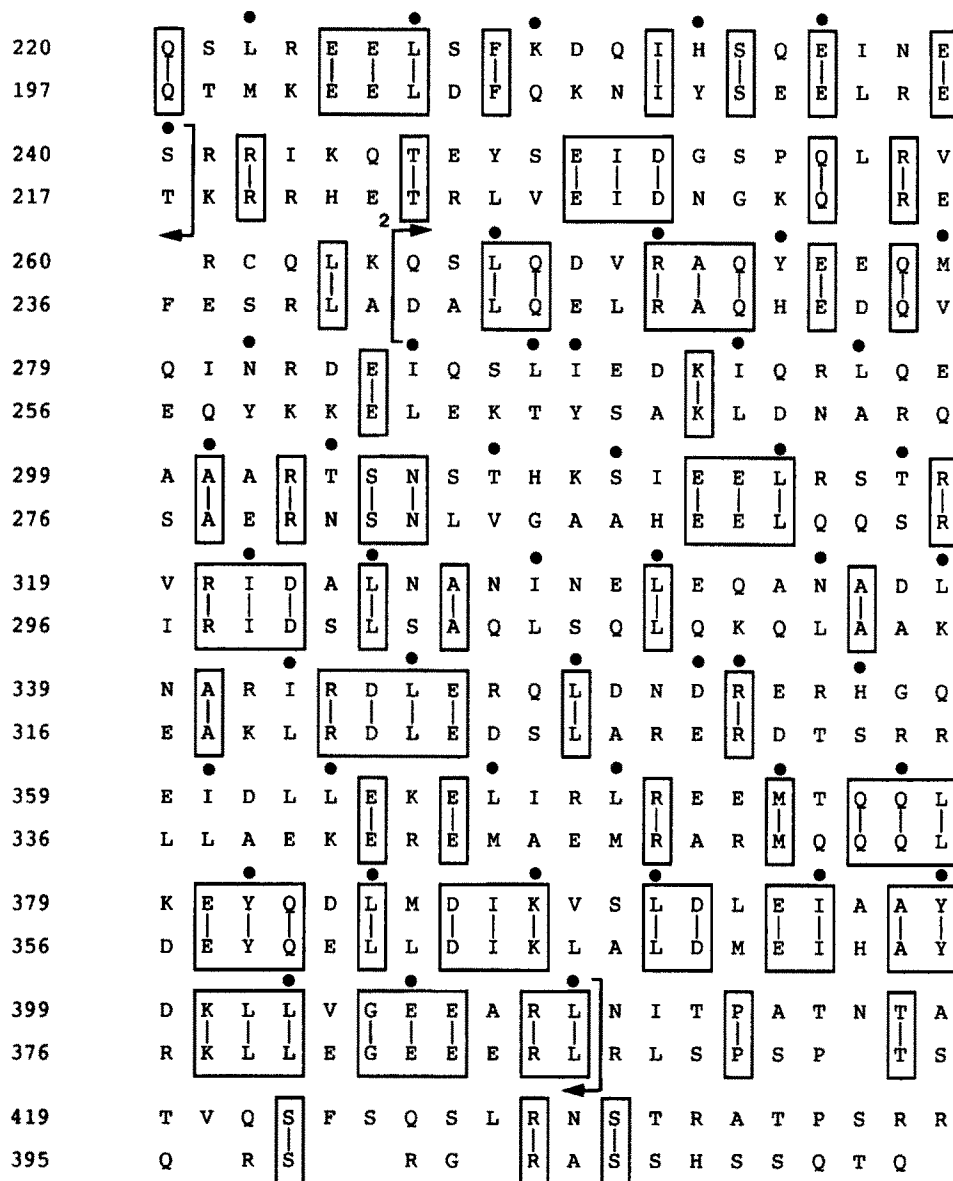
codon beginning at nucleotide 131. Translation terminates after nucleotide 1993. The primary translation product, lamin Dm₀ is therefore 621 amino acids in length and has a calculated mass of 70,974. This is in reasonable agreement with the SDS-PAGE estimated mass of 76 kD, particularly given the demonstrable tendency of the *Drosophila* lamin to change SDS-PAGE mobility after posttranslational modification.[2] This calculated mass is intermediate in size between human lamins A and C (Fisher et al., 1986).

Detailed restriction mapping and partial nucleotide sequencing of cDNL2800 indicate that cDNL2800 and cDNL3000 share identical 5' termini and protein coding regions (not shown). This latter observation confirms the impression derived from peptide mapping studies of pT7cDNL-

2800 and pT7cDNL3000 clone-encoded lamins transcribed and translated in vitro (Smith et al., 1987). At the 3' end, cDNL2800, terminates with the sequence CTACGGAAGT as indicated in Fig. 4, and is otherwise identical to cDNL-3000 up to this point.

An amino acid sequence comparison of *Drosophila* lamin Dm₀ with human lamin A is shown in Fig. 5. Regions of similarity can be identified throughout the molecule. Overall, the *Drosophila* lamin shows 35% identity of amino acid sequence with the human lamin. Moreover, the *Drosophila* lamin shows the characteristic secondary structural features of intermediate filament proteins (see review by Steinert et al., 1985). The coil 1a, coil 1b, and coil 2 regions are designated with explicit reference to the human lamins as previously reported (McKeon et al., 1986; Fisher et al., 1986). The region of greatest homology between *Drosophila* and human lamins runs from amino acid 42 to 81 of the *Drosophila* lamin. In this region, 29 out of 40 amino acids are identical for a sequence homology of 72.5%. Other values include 30% sequence identity in coil 1b, 41% identity in coil 2, and 45% identity in the COOH-terminal domain between amino

2. We might also note that *Drosophila* lamin Dm₁ comigrates with rat liver lamin A on SDS-polyacrylamide gradient gels (Fisher et al., 1982). Rat lamin A has a reported mass of 70 kD (see e.g. Gerace et al., 1978). We therefore suggest that the difference in mass between the authentic *Drosophila* lamin and the cDNA sequence-derived value is within the error of SDS-PAGE mass determinations given variations of protein standards used and exact conditions of electrophoresis.

acids 479 and 571 of the *Drosophila* lamin. A putative nuclear localization signal similar to that reported for the human lamins is found beginning at amino acid 446 (lys-arg-lys-arg-ala-val) of the *Drosophila* lamin. Striking homology is also observed at the extreme COOH terminus between human lamin A and *Drosophila* lamin Dmo (lys-cys-ala-ile-met versus asn-cys-ser-ile-met, respectively). This is not represented in the alignment shown in Fig. 5.

Comparison of *Drosophila* lamin Dmo with a number of mammalian intermediate filament proteins also revealed the expected similarities both of secondary structure as discussed above as well as primary amino acid sequence (not shown). For example, in the coil 1a region, *Drosophila* lamin Dmo exhibits a 49% sequence identity with hamster vimentin. Similar results (45% identity) have been found for human lamins A and C (McKeon et al., 1986; Fisher et al., 1986).

Characterization of the Lamin Gene in the Drosophila Genome

Three different experiments were done to estimate the num-

ber of lamin genes and their location in the *Drosophila* genome. First, observations in other biological systems show that diverged copies of very similar genes can often be distinguished from each other by variability in nonconserved restriction sites within introns and flanking sequences. Southern analyses of total genomic DNA digests were performed using several restriction enzymes with 6-bp recognition sequences. As shown in Fig. 6, after probing the genomic blot with cDNL3000, most restriction enzymes produce either a single high molecular weight band or a few lower molecular weight bands. The number of bands recognized by genomic Southern analysis was consistent with results obtained by digestion of the cDNA clones by the same enzyme (not shown).

Second, the genomic region of the lamin gene was cloned from a λ-Charon 34 phage genomic library using cDNL1700 as the probe. Of the nine positive clones, seven contained a single 9.8-kb or smaller EcoR1 fragment, which hybridized to the cDNA. All seven shared at least one genomic EcoR1 fragment, which did not hybridize to the cDNA. The two remaining clones have a 10.8-kb EcoR1 fragment, which hy-

439	T	P	S	A	A	[V	K	R	[K	R]	A	V	V	D	E	S	[E]	D	H	[S]
410	G	G	G	S		V]	T	K	[K	R]	K	L		E	S	T	[E]	S	R	[S]

```
439   T  P  S  A  A  |V| K  R  |K  R| A  V  V  D  E  S  |E| D  H  |S|
                     |V|       |K  R|                   |E|       |S|
410   G  G  G  S     |V| T  K  |K  R| K  L     E  S  T  |E| S  R  |S|

459   V  A  D  Y  Y  V  S  |A| S  A  K  |G| N  |V| E  I  K  |E| I  |D|
                          |A|          |G|    |V|          |E|    |D|
428      S     F  S  Q  H  |A| R  T  S  |G| R  |V| A  V  E  |E| V  |D|

479   P  |E  G  K  F  V  R  L| F  |N  K| G  S  |E| E  V  A  I  |G| G
         |E  G  K  F  V  R  L|    |N  K|       |E|             |G|
446   E  |E  G  K  F  V  R  L| R  |N  K| S  N  |E| D  Q  S  M  |G| N

499   |W  Q| L  Q  |R| L  I  |N| E  K  G  |P| S  T  |T  Y| K  |F| H  R
      |W  Q|       |R|       |N|          |P|       |T  Y|    |F|
466   |W  Q| I  K  |R|    Q  |N| G  D  D  |P| L  L  |T  Y| R  |F| P  P

519   S  V  R  I  E  P  N  G  |V| I  |T| V  |W| S  |A| D  T  K  |A| S
                             |V|    |T|    |W|    |A|          |A|
485   K  F  T  L  K  A  G  Q  |V| V  |T| I  |W| A  |A| G  A  G  |A| T

539   |H| E  |P  P| S  S  |L  V| M  |K| S  |Q| K     |W| V  S  A  D  N
      |H|    |P  P|       |L  V|    |K|    |Q|       |W|
505   |H| S  |P  P| T  D  |L  V| W  |K| A  |Q| N  T  |W| G  C  G  N  S

558   T  |R  T| I  |L| L  |N  S| E  |G  E| A  |V  A| N  L  D  |R| I  |K|
         |R  T|    |L|    |N  S|    |G  E|    |V  A|          |R|    |K|
525   L  |R  T| A  |L| I  |N  S| T  |G  E| E  |V  A|    M     |R|    |K|

578   R  I  |V| S  Q  H  T  S  S  S  R  L  S  R  R  R  S  V  T  A
            |V|
542      L  |V|    R  S  V  T  V     V  E           D  D  E  D

598   V  |D  G| N  E  Q  |L| Y  |H| Q  Q  |G| D  P  Q  Q  S  N  E  K
         |D  G|          |L|    |H|       |G|
555   E  |D  G| D  D  L  |L| H  |H| H  H  |G| S     H     C  S  S  S

618   C  A  I  M

574   G  D  P  A
```

Discussion

bridized to cDNL1700. Southern blots of *Drosophila* embryo DNA cut with EcoR1 and hybridized with the lamin cDNA clones showed only the 9.8-kb band, but not the 10.8-kb band. We therefore think it likely that the 10.8-kb fragment is an artifact of cloning.

Third, in situ hybridization to polytene chromosomes with the cDNL1700 clone, labeled with tritiated thymidine, resulted in the identification of a single site at or near 25F on the left arm of the second chromosome after 4 d of exposure (Fig. 7). Even after 28 d of exposure of the emulsion to the hybridized chromosomes, no additional sites were observed. The single hybridization site was consistently observed in >100 squashed nuclei analyzed on five different slides. cDNL3000 was also used for in situ hybridization and gave the same result as cDNL1700. While in situ hybridization does not measure the number of copies of the gene at a specific site, the experiment does demonstrate that copies of the lamin gene are not at dispersed loci.

The Southern analysis, which indicates a single EcoR1 fragment, the similarity of the genomic clones, and the single chromosomal locus of the gene, all indicate that there are one or conceivably, a few adjacent similar copies of the lamin gene.

The identification and characterization of full-length cDNA clones coding for the *Drosophila* nuclear lamin precursor Dm₀ represents a significant advance in our efforts to understand the structure and functions of the nuclear envelope and lamina. Novel information regarding developmental regulation of lamin gene transcription has already been obtained, and preliminary studies of genome organization suggest only one or a few highly similar copies exist in the organism. Comparable results have recently been reported for the human lamins A and C (McKeon et al., 1986; Fisher et al., 1986).

The implications of observed sequence homologies between nuclear lamins and cytoplasmic intermediate filament proteins have already been discussed in detail (McKeon et al., 1986; Fisher et al., 1986). Moreover, the intermediate filament–like structure of nuclear lamina fibrils assembled in vitro or revealed in situ has recently been demonstrated (Aebi et al., 1986). The results presented in this article establish *Drosophila* lamins Dm₁ and Dm₂ as members of this distinctive class of proteins and further substantiate the identification of these *Drosophila* nuclear envelope components as lamins.
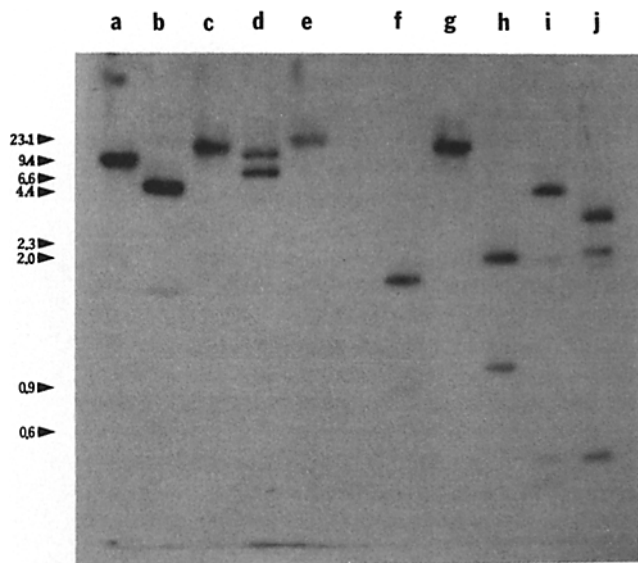
Figure 6. Southern blot analysis of lamin genomic DNA. Total genomic DNA was prepared from 2–16-h-old embryos by a modification of the procedure by Marmur (1961). 10 µg of DNA was digested with 50 U of each of the restriction enzymes at 37°C for 4 h. The digested DNA fragments were separated by gel electrophoresis on a 1% agarose gel and transferred to nitrocellulose; 0.2 µg of ^{32}P-labeled nick-translated cDNL3000 DNA (5×10^8 cpm/µg) was used as radiolabeled probe. Restriction enzymes used: Lane *a*, EcoRI; lane *b*, HindIII; lane *c*, SmaI; lane *d*, Bam HI; lane *e*, KpnI; lane *f*, MspI; lane *g*, XbaI; lane *h*, SalI; lane *i*, SstI; lane *j*, PvuII. Molecular weight markers to left of figure are in kilobases. Autoradiographic exposure was for 5 d.

The observation that two messages appear to code for the same polypeptide (lamin Dm$_0$) is not unprecedented, nor is what seems to us the most likely explanation for this observation, i.e., different sites of transcription termination (see e.g., Nevins and Wilson, 1981). It is also plausible that these two species arise as a result of differential splicing. Given the pattern of mRNA expression during development, it seems possible that the 2.8-kb species is primarily a specialized oocyte "storage" form evolved to program lamin biosynthesis during early embryogenesis. The 3.0-kb form predominates at most other developmental stages. It is nevertheless apparent from the data shown in Fig. 2 that both mRNAs are expressed by the embryo, but that the 3.0-kb form is preferred. It is also clear that both forms of the message are competent to program in vitro translation of nuclear lamin Dm$_0$ in either wheat germ or rabbit reticulocyte lysate.

To date, it is still not certain whether *Drosophila* lamins Dm$_0$, Dm$_1$, and Dm$_2$ are more "A/C"-like or more "B"-like in their nature. Circumstantial evidence regarding isoelectric point and immunochemical homologies has been inconclusive. On the basis of data reported previously on the processing of lamin Dm$_0$ in *Drosophila* tissue culture cells (Smith et al., 1987), we now feel that the majority of such evidence points more toward similarity with lamin B than with either A or C. However, the homologies of primary and secondary structure between *Drosophila* lamin Dm$_0$ and human lamins A and C are striking. Moreover, sedimentation analyses of depolymerized *Drosophila* lamins obtained either from

mitotic cell extracts or early embryos have failed to demonstrate any sort of membrane association (Smith, D., unpublished results) comparable to that reported for mammalian lamin B by Burke and Gerace (1986). It will therefore be necessary to compare the sequences of *Drosophila* lamin Dm$_0$ and a B-type lamin from a vertebrate source before any definitive conclusions can be reached. To date, no such sequence has been reported in the literature.

We might also note in this regard that in aligning the sequences of *Drosophila* lamin Dm$_0$ with human lamins A and C, we find that there are an extra 23 amino acids in the *Drosophila* sequence immediately preceding the region of maximum homology near the NH$_2$ terminus. We have proposed that the processing of lamin Dm$_0$ to Dm$_1$ involves NH$_2$-terminal proteolysis of $\sim$2 kD of protein (Smith et al., 1987). It is conceivable that this 23 amino acid NH$_2$-terminal extension of *Drosophila* lamin Dm$_0$ represents the portion that is cleaved in the cytoplasmic processing to lamin Dm$_1$. Processing of mammalian lamin A$_0$ has been suggested to take place at the COOH terminus (Fisher et al., 1986).

In conclusion, we would like to point out that *Drosophila* is practically unique among higher eukaryotes in its amenability to genetic analysis. Through site-directed mutagenesis of the cloned lamin gene, it may be possible to directly analyze the role of various structural domains within the protein in contributing to in vivo function. It may for example, be possible to elucidate the biological significance of the precursor form of the *Drosophila* lamins by mutating in the relevant region of the polypeptide. It may also be possible to investigate the structural and functional interactions of mutant lamins with other molecular components of the nuclear envelope. Finally, precise elucidation of the role of phosphorylation in regulating nuclear lamina plasticity during interphase (Smith et al., 1987) and disassembly of the nucleus during mitotis (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985; for a review, see Fisher, 1987) may ultimately be forthcoming.

Note Added in Proof: Deduced amino acid sequences have recently been published for *Xenopus* lamins L$_I$ (Krohne, G., S. L. Wolin, F. D. McKeon, W. W. Franke, and M. W. Kirschner. 1987. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3801–3808) and A (Wolin, S. L., G. Krohne, and M. W. Kirschner. 1987. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3809–3818). (*Xenopus* L$_I$ is thought to be a member of the lamin B subfamily.) Comparison of *Drosophila* lamin Dm$_0$ with these two *Xenopus* proteins results in the following ob-
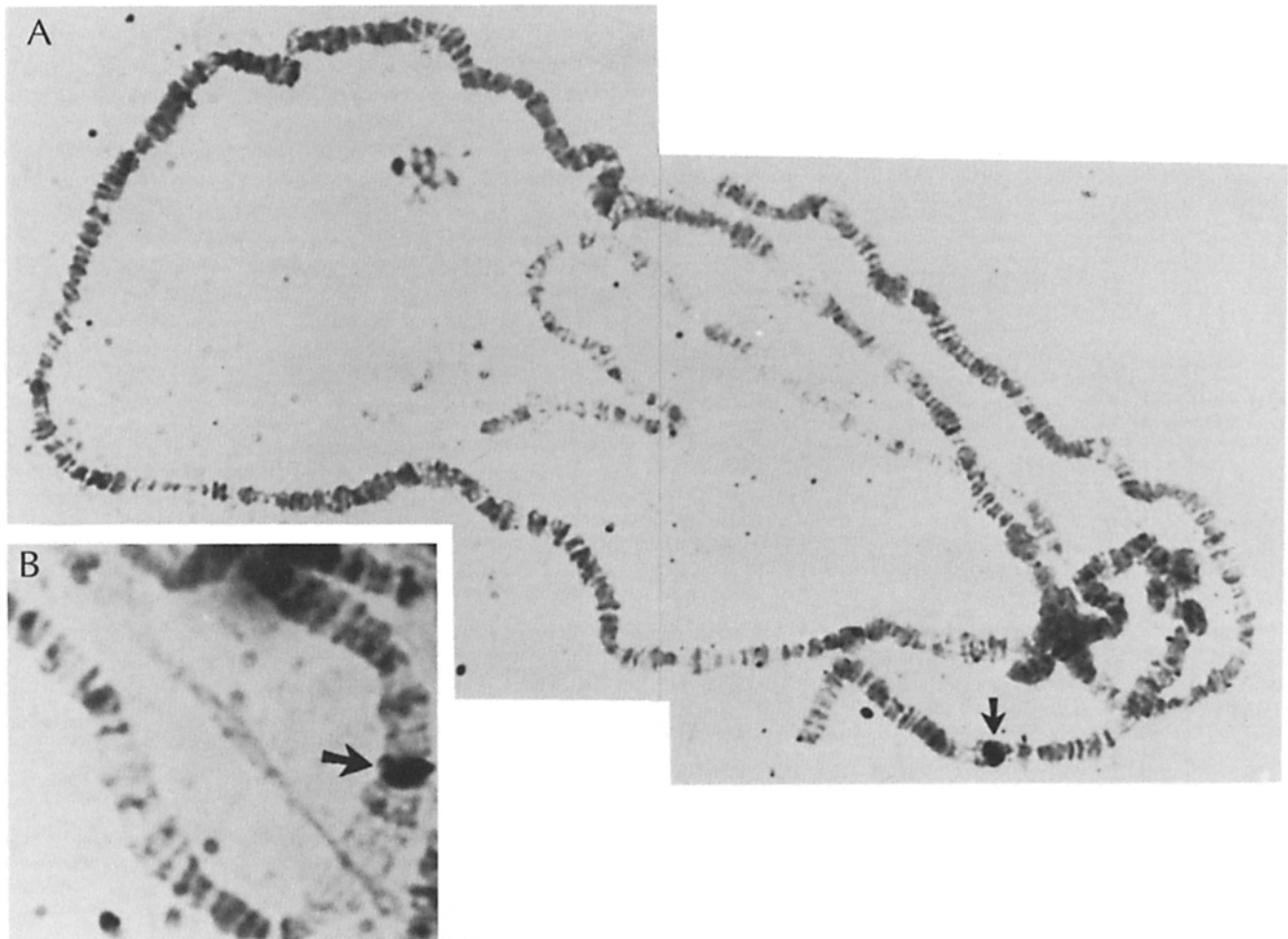
Figure 7. In situ hybridization of labeled lamin cDNA to polytene chromosomes. The hybridization conditions, and autoradiography procedures were as described (Materials and Methods). Two separate experiments are shown. *A* shows the complete polytene chromosome spread. The probe hybridized to a single site (*arrow*) at or near position 25F on the left arm of chromosome 2. *B* shows a higher magnification of the hybridization of the probe (*arrow*) to this same region. Autoradiographic exposure time was 4 d. Exposure for 28 d gave the same results.

servations: 32% sequence identity with lamin A, 35.3% identity with lamin L_I; highly similar putative nuclear localization signals between lamin Dm_0 (lys-arg-lys-arg-ala-val) and lamin L_I (lys-arg-lys-arg-ile-asp); lack of an oligo-histidine rich stretch in the COOH-terminal tail region of lamin Dm_0, distinct from lamin A and similar to lamin L_I; identical four amino acids at the COOH terminus between lamin Dm_0 and lamin L_I, three out of four identical COOH-terminal amino acids between lamin Dm_0 and lamin A. These observations are consistent with our suggestion that *Drosophila* lamin Dm_0 is more "B-like" in nature.

References

Aebi, U., J. Cohn, L. Buhle, and L. Gerace. 1986. The nuclear lamina is a meshwork of intermediate-type filaments. *Nature (Lond.).* 323:560-564.

Benavente, R., and G. Krohne. 1985. Changes of karyoskeleton during spermatogenesis of *Xenopus*: expression of lamin L_{IV}, a nuclear lamina protein specific for the male germ line. *Proc. Natl. Acad. Sci. USA.* 82:6176-6180.

Benavente, R., G. Krohne, and W. W. Franke. 1985. Cell type-specific expression of nuclear lamina proteins during development in *Xenopus laevis. Cell.* 41:177-190.

Bonner, J. J., and M. L. Pardue. 1976. Ecdysone-stimulated RNA synthesis in imaginal discs of *Drosophila melanogaster. Chromosoma (Berl.).* 58:87-99.

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.

Fisher, P. A. 1987. Disassembly and reassembly of nuclei in cell-free systems.

Cell. 48:175-176.

Fisher, P. A., M. Berrios, and G. Blobel. 1982. Isolation and characterization of a proteinaceous subnuclear fraction composed of nuclear matrix, peripheral lamina, and nuclear pore complexes from embryos of *Drosophila melanogaster. J. Cell Biol.* 92:674-686.

Fisher, D., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci. USA.* 83:6450-6554.

Foe, V., and B. M. Alberts. 1983. Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell. Sci.* 6:31-70.

Franke, W. W. 1987. Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. *Cell.* 48:3-4.

Fuchs, J. P., H. Giloh, C.-H. Kuo, H. Saumweber, and J. W. Sedat. 1983. Nuclear structure: determination of the fate of the nuclear envelope in *Drosophila* during mitosis using monoclonal antibodies. *J. Cell Sci.* 64:331-349.

Gerace, L. 1986. Nuclear lamina and organization of the nuclear architecture. *Trends Biochem. Sci.* 11:443-446.

Gerace, L., and G. Blobel. 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell.* 19:277-287.

Gerace, L., and G. Blobel. 1982. Nuclear lamina and the structural organization of the nuclear envelope. *Cold Spring Harbor Symp. Quant. Biol.* 46:967-978.

Gerace, L., A. Blum, and G. Blobel. 1978. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. Interphase and mitotic distribution. *J. Cell Biol.* 79:546-566.

Huynh, T. V., R. A. Young, and R. W. Davis. 1985. DNA Cloning: A Practi-

cal Approach. Vol. 1. D. M. Glover, editor. IRL Press, Washington DC. 49–78.

Karp, R., and D. S. Hogness. 1976. Isolation and mapping of histone genes contained in cloned segments of Drosophila melanogaster. Fed. Proc. 35:1623.

Kaufmann, S. H., W. Gibson, and J. H. Shaper. 1983. Characterization of the major polypeptides of the rat liver nuclear envelope. J. Biol. Chem. 258:2710–2719.

Krohne, G., and R. Benavente. 1986. The nuclear lamins. A multigene family of proteins in evolution and differentiation. Exp. Cell Res. 162:1–10.

Lam, K. S., and C. B. Kasper. 1979. Electrophoretic analysis of three major nuclear envelope polypeptides. Topological relationship and sequence homology. J. Biol. Chem. 254:11713–11720.

Lebel, S., and Y. Raymond. 1984. Lamin B from rat liver exists as both a lamina protein and as an intrinsic membrane protein. J. Biol. Chem. 259:2693–2696.

Lehner, C. F., G. Furstenberger, H. M. Eppenberger, and E. A. Nigg. 1986. Biogenesis of the nuclear lamina: in vivo synthesis and processing of nuclear protein precursors. Proc. Natl. Acad. Sci. USA. 83:2096–2099.

Lehner, C. F., R. Stick, H. M. Eppenberger, and E. A. Nigg. 1987. Differential expression of nuclear lamin proteins during chicken development. J. Cell Biol. 105:577–587.

Mahowald, A. P., and M. P. Kambysellis. 1980. Oogenesis: organization of the adult ovary. In The Genetics and Biology of Drosophila. Vol 2d. M. Ashburner and T. R. F. Wright, editors. Academic Press, Inc., New York. 149–157.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Recovery of RNA from agarose gels containing methylmercuric hydroxide. In Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 203.

Markwell, M. A., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eukaryotic cells using 1, 3, 4, 6-tetrachloro-3-alpha-6-alpha-diphenylglycoluril. Biochemistry. 17:4807–4817.

Marmur, J. 1961. Procedure for isolation of deoxyribonucleic acids from microorganisms. J. Mol. Biol. 3:208–218.

Maul, G. G., F. A. Baglia, D. D. Newmeyer, and B. M. Ohlsson-Wilhelm. 1984. The major 67,000 molecular weight protein of the clam oocyte nuclear envelope is lamin-like. J. Cell Sci. 67:69–85.

McKeon, F. D., M. W. Kirschner, and D. Caput. 1986. Primary and secondary structural homologies between the major nuclear envelope and cytoplasmic intermediate filament proteins. Nature (Lond.). 319:463–468.

Moss, B., and E. N. Rosenblum. 1972. Hydroxylapatite chromatography of protein-sodium dodecyl sulfate complexes. A new method for the separation of polypeptide subunits. J. Biol. Chem. 247:5194–5198.

Nevins, J. R., and M. C. Wilson. 1981. Regulation of adenovirus-2 gene expression at the level of transcriptional termination and RNA processing. Nature (Lond.). 290:113–118.

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

Pardue, M. L., and J. Gall. 1975. Nucleic acid hybridization to the DNA of cytological preparations. Methods Cell Biol. 10:1–17.

Poole, S. J., L. M. Kauvar, B. Drees, and T. Kornberg. 1985. The engrailed locus of Drosophila: structural analysis of an embryonic transcript. Cell. 40:37–43.

Risau, W., H. Saumweber, and P. Symmons. 1981. Monoclonal antibodies against a nuclear membrane protein of Drosophila: localization by indirect immunofluorescence and detection of antigen using a new protein blotting procedure. Exp. Cell Res. 133:47–54.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463–5467.

Schatten, G., G. G. Maul, H. Schatten, N. Chaly, C. Simerly, R. Balczon, and D. L. Brown. 1985. Nuclear lamins and peripheral nuclear antigens during fertilization and embryogenesis in mice and sea urchins. Proc. Natl. Acad. Sci. USA. 82:4727–4731.

Smith, D. E., and P. A. Fisher. 1984. Identification, developmental regulation and response to heat shock of two antigenically related forms of a major nuclear envelope protein in Drosophila embryos. Application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J. Cell Biol. 99:20–28.

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.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

Steinert, P. M., A. C. Steven, and D. R. Roop. 1985. The molecular biology of intermediate filaments. Cell. 42:411–419.

Stick, R., and P. Hausen. 1985. Changes in nuclear lamina composition during early development of Xenopus laevis. Cell. 41:191–200.

Tabor, S., and C. C. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlling exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA. 82:1074–1078.

Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA. 77:5201–5205.

Weislander, L. 1979. A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. Anal. Biochem. 98:305–309.