

## Expression of *Drosophila* lamin C is developmentally regulated: analogies with vertebrate A-type lamins

Dieter Riemer<sup>1,\*</sup>, Nico Stuurman<sup>2,\*†</sup>, Miguel Berrios<sup>2</sup>, Cecil Hunter<sup>2</sup>, Paul A. Fisher<sup>2</sup> and Klaus Weber<sup>1,‡</sup>

<sup>1</sup>Max-Planck-Institute for Biophysical Chemistry, Department of Biochemistry, PO Box 2841, D-37018 Goettingen, FRG

<sup>2</sup>Department of Pharmacological Sciences, SUNY at Stony Brook, Stony Brook, NY 11794-8651, USA

\*The first two authors contributed equally to this study

†Present address: M. E. Müller Institut at the Biozentrum of the University Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

‡Author for correspondence

### SUMMARY

Vertebrate nuclear lamins form a multigene family with developmentally controlled expression. In contrast, invertebrates have long been thought to contain only a single lamin, which in *Drosophila* is the well-characterized lamin Dm<sub>0</sub>. Recently, however, a *Drosophila* cDNA clone (pG-IF) has been identified that codes for an intermediate filament protein which harbors a nuclear localization signal but lacks a carboxy-terminal CAA<sub>X</sub> motif. Based on these data the putative protein encoded by pG-IF was tentatively called *Drosophila* lamin C. To address whether the pG-IF encoded protein is expressed and whether it encodes a cytoplasmic intermediate filament protein or a nuclear lamin we raised antibodies against the recombinant pG-IF protein. The antibodies decorate the nuclear envelope in *Drosophila* Kc tissue culture cells as well as in salivary and accessory glands demonstrating that pG-IF encodes a nuclear lamin (lamin C). Antibody decoration, in situ

hybridization, western and northern blotting studies show that lamin C is acquired late in embryogenesis. In contrast, lamin Dm<sub>0</sub> is constitutively expressed. Lamin C is first detected in late stage 12 embryos in oenocytes, hindgut and posterior spiracles and subsequently also in other differentiated tissues. In third instar larvae lamins C and Dm<sub>0</sub> are coexpressed in all tissues tested. Thus, *Drosophila* has two lamins: lamin Dm<sub>0</sub>, containing a CaaX motif, is expressed throughout, while lamin C, lacking a CaaX motif, is expressed only later in development. Expression of *Drosophila* lamin C is similar to that of vertebrate lamin A (plus C), which loses its CaaX motif during incorporation into the lamina.

Key words: developmental control, *Drosophila*, intermediate filament protein, lamin expression, nuclear lamin

### INTRODUCTION

The nuclear lamina is a proteinaceous meshwork underlying the inner nuclear membrane (Aebi et al., 1986; Moir and Goldman, 1993). Its major constituents, the nuclear lamins, constitute a special class of intermediate filament proteins with two topogenic sequences: a nuclear localization signal which directs lamins to the nucleus and a carboxy-terminal CaaX motif, which functions in targeting the lamins to the nuclear envelope membrane (Loewinger and McKeon, 1988; Holtz et al., 1989; Krohne et al., 1989; Kitten and Nigg, 1991; Monteiro et al., 1994). The lamina is thought to be involved in maintaining nuclear envelope integrity, anchoring nuclear pore complexes and organizing interphase chromatin three-dimensionally (Aebi et al., 1986; Newport et al., 1990; Ludérus et al., 1992; Glass et al., 1993). In addition, some lamins may play a role in DNA replication (Meier et al., 1991; Nigg, 1992; Fuchs and Weber, 1994; Moir et al., 1994).

Vertebrate lamins are encoded by a multigene family (Nigg, 1992). There are several B-type lamins as well as the larger A-type lamin, which has a unique subdomain in the carboxy-terminal tail region. A differentially spliced version of lamin

A, lacking the extended tail domain including the CaaX motif, is called lamin C. It has so far only been observed in mammals (Stick, 1992; Lin and Worman, 1993). While lamins B<sub>1</sub> and B<sub>2</sub> are constitutively expressed, the expression of lamin A/C is developmentally regulated and usually restricted to differentiated cells (Lehner et al., 1987; Stewart and Burke, 1987; Röber et al., 1989; Höger et al., 1990).

Interestingly, vertebrate A- and B-type lamins differ not only in expression patterns, but also show a striking difference during mitosis. Upon breakdown of the nuclear envelope, lamin A becomes soluble and is distributed throughout the cell, while lamins B<sub>1</sub> and B<sub>2</sub> remain bound to nuclear envelope-derived membrane vesicles (Gerace and Blobel, 1980; Stick et al., 1988). This difference may arise in part from the fate of the post-translational hydrophobic modifications in mature A and B lamins. All lamins, except the mammalian lamin C, have a carboxy-terminal CaaX box, which is subjected to an extensive posttranslational modification (addition of a farnesyl group, proteolytic trimming of the terminal 3 residues and carboxylmethylation of the cysteine; Clarke, 1992; Sinensky and Lutz, 1992) but only lamin A is involved in a further maturation step, the proteolytic removal of the modified carboxy-

terminal end (Vorburger et al., 1989; Weber et al., 1989a; Hennekes and Nigg, 1994; Sinensky et al., 1994). This occurs after incorporation of lamin A into the lamina (Lehner et al., 1986). In addition, the permanent membrane binding of B-type lamins may also involve the action of specific lamin B receptors in the nuclear envelope (see for instance Meier and Georgatos, 1994).

Given the wealth of information on vertebrate lamins, it is surprising how little is known about lamins from other eukaryotes. Molecular information is restricted to three invertebrates and involves molecules which by their size reflect B-type lamins. Thus the CeLam-1 gene of the nematode *Caenorhabditis elegans* encodes a protein with a nuclear localization signal and a CaaX box. However, this lamin is atypical since it lacks the consensus cdc2 phosphorylation site in the head domain (Riemer et al., 1993). An extensive biochemical and immunological search suggested the existence of only a single lamin protein in the mollusc *Spisula soldissima* (Dessev and Goldman, 1990). Also, the arthropod *Drosophila melanogaster* has long been thought to contain only a single nuclear lamin (Gruenbaum et al., 1988). Lamin Dm<sub>0</sub> is expressed in all cells except for mature sperm (Whalen et al., 1991 and this study) and occurs in multiple post-translationally modified isoforms (Smith et al., 1987; Smith and Fisher, 1989).

Bossie and Sanders (1993) recently isolated a *Drosophila* cDNA clone, called pG-IF, that encodes a 70 kDa intermediate filament protein. The predicted protein contains six additional heptads in the coil 1b domain, a feature found in all nuclear lamins and in the cytoplasmic intermediate filament proteins of nematodes and molluscs (Weber et al., 1989b; Fuchs and Weber, 1994). The predicted pG-IF protein has a putative nuclear localization signal and the consensus cdc2 kinase phosphorylation site, but lacks a CaaX box (Bossie and Sanders, 1993). Although a homologous mRNA was detected in cultured Kc and Schneider cells (Bossie and Sanders, 1993; Riemer and Weber, 1994) it is not known whether the corresponding protein is expressed and whether it behaves as a cytoplasmic intermediate filament protein or as a nuclear lamin. In the latter case, the protein product of pG-IF could be described in analogy to mammalian lamin C as *Drosophila* lamin C, since both lack the CaaX box.

We have generated three different antibodies specific for the protein predicted by pG-IF and ascertained their lack of cross-reactivity on *Drosophila* lamin Dm<sub>0</sub>. These antibodies localize the protein to the nuclei of cultured Kc cells and thus demonstrate that pG-IF encodes a lamin. Our results show that pG-IF expression is developmentally controlled. In contrast, parallel experiments confirm that lamin Dm<sub>0</sub> is expressed throughout *Drosophila* development.

## MATERIALS AND METHODS

### *Drosophila* growth and maintenance

*D. melanogaster* strain Oregon R was maintained in mass culture (Allis et al., 1977). For northern and western analysis, specimens at various developmental stages were harvested, frozen in liquid nitrogen, and stored at -70°C until use.

### Expression of pG-IF (lamin C) and its tail domain in *Escherichia coli*

For expression of the full length pG-IF polypeptide (Bossie and

Sanders, 1993), the cDNA insert from the pG-IF clone (a kind gift from Dr M. M. Sanders, Piscataway, NJ) was inserted into pET17xb (Novagen, Madison, WI) to generate a plasmid (pETDmLC) which drives expression of full length pG-IF polypeptide in *E. coli* BL21(DE3). Isolation of inclusion bodies provided partially purified recombinant pG-IF-polypeptide.

To produce the pG-IF tail domain, PCR-amplified pG-IF cDNA was cloned in pUC18 and a *Bam*HI/*Eco*RI fragment was ligated into similarly digested pRSETA (Invitrogen, Leek, The Netherlands). This construct codes for a fusion protein between an oligohistidine tag and the entire pG-IF tail domain starting at glycine 448. The expressed fusion protein was isolated by affinity purification on a Ni<sup>2+</sup>NTA column (Diagen GmbH, Hilden, Germany) in buffer containing 8 M urea (Invitrogen). The pG-IF tail domain was further purified by preparative gel-electrophoresis.

### Generation and purification of antibodies

Rabbits were immunized with full length pG-IF polypeptide (to generate R $\alpha$ LC-FL) or the pG-IF tail domain (for R $\alpha$ LC-tail) according to standard methods. Specific antibodies were affinity purified using the gel-purified antigen bound to nitrocellulose strips or to glutaraldehyde activated beads (Boehringer, Mannheim, Germany). In addition, a murine monoclonal antibody (LC28) was raised against full length pG-IF using standard methods. Specificity of all antibodies was investigated by western blotting and immunofluorescence (see below). The monoclonal antibody ADL67, specific for *Drosophila* lamin Dm<sub>0</sub>, has been described elsewhere (N. Stuurman et al., unpublished).

### Immunofluorescence microscopy

Kc cells were grown on glass coverslips in D22 medium (Sigma, Deisenhofen, Germany) supplemented with 5% fetal calf serum (Gibco, Eggenstein, Germany). Cells were fixed and incubated with antibodies according to standard methods. For incubation with primary antibodies affinity purified R $\alpha$ LC-tail was used undiluted, R $\alpha$ LC-FL at 4  $\mu$ g/ml, LC28 as undiluted tissue culture supernatant and ADL67 tissue culture supernatant at 1:200 dilution, followed by incubation with secondary antibodies (Dako Immunochemicals, Klostrup, Denmark; diluted 50-fold) and Hoechst 33342 (Hoechst, Frankfurt, Germany).

To stain sections of 3rd instar larvae, larvae were transferred individually into a large drop of Tissue-Tek (OCT Compound, Miles, Elkhaart, IN) and frozen in isopentane. Sections were cut, air dried and fixed in acetone. Larval sections were incubated with primary and secondary antibodies and Hoechst 33342 as described above for Kc cells.

Accessory glands were prepared by extrusion of nuclei from adult male *Drosophila* accessory gland cells. Labeled specimens were examined with an Odyssey confocal laser scanning system (Noran Instruments, Middleton, WI) as previously described (Meller et al., 1995).

### Immunostaining of embryos and isolated larval organs

Whole mount embryo labeling was essentially according to Mitchison and Sedat (1983). Embryos were collected after 19 hours, dechorionated and fixed. Isolated salivary glands were fixed in 3.7% formaldehyde in PBS for 1 hour at 4°C. Rehydrated embryos were incubated overnight at 4°C with primary antibody (affinity purified R $\alpha$ LC-tail; 1:400 dilution of ADL67). Bound antibodies were visualized using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Dehydrated embryos were mounted in Araldite (Serva, Heidelberg, Germany). Photographs were taken under Nomarski optics on a Zeiss Axiophot microscope.

### In situ hybridization

Whole mount in situ hybridization of embryos was conducted using the pG-IF specific 1,009 bp *Sac*I/*Kpn*I fragment (Riemer and Weber,

1994). The lamin Dm<sub>0</sub> specific probe was a PCR amplified 1,170 bp fragment coding for amino acids alanine 40 to asparagine 430 (Gruenbaum et al., 1988). Hybridization with digoxigenin labeled DNA probes followed the protocol of Tautz and Pfeifle (1989).

#### CNBr-cleavage, protein gel-electrophoresis and western blotting

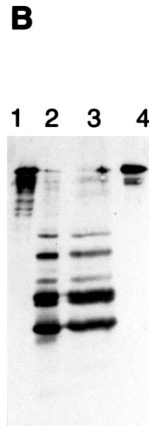
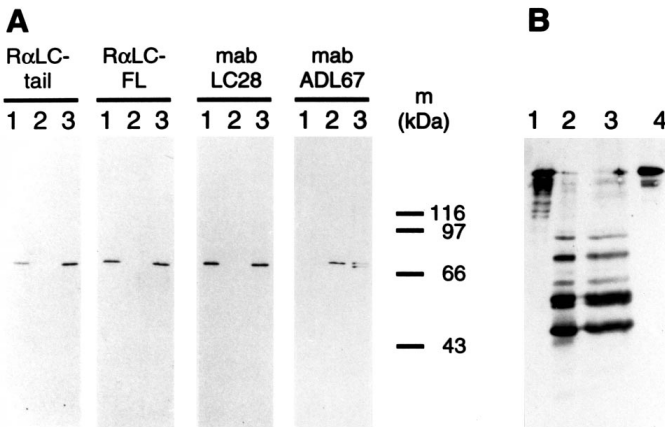
CNBr-cleavage was carried out as described (Smith et al., 1987). Polypeptides separated by SDS-PAGE were transferred to nitrocellulose or Immobilon-P (Millipore, Bedford, MA). Blots were incubated with primary antibodies (affinity purified R $\alpha$ LC-tail at 1:1,000 dilution; R $\alpha$ LC-FL at 0.1  $\mu$ g/ml pre-incubated with 50  $\mu$ g/ml bac-

terially expressed lamin Dm<sub>0</sub>), or culture supernatants from hybridomas secreting *Drosophila* lamin antibodies at a 1:10 dilution) for 2-16 hours at room temperature. Bound antibodies were detected using secondary antibodies conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD).

#### RNA isolation and northern blotting

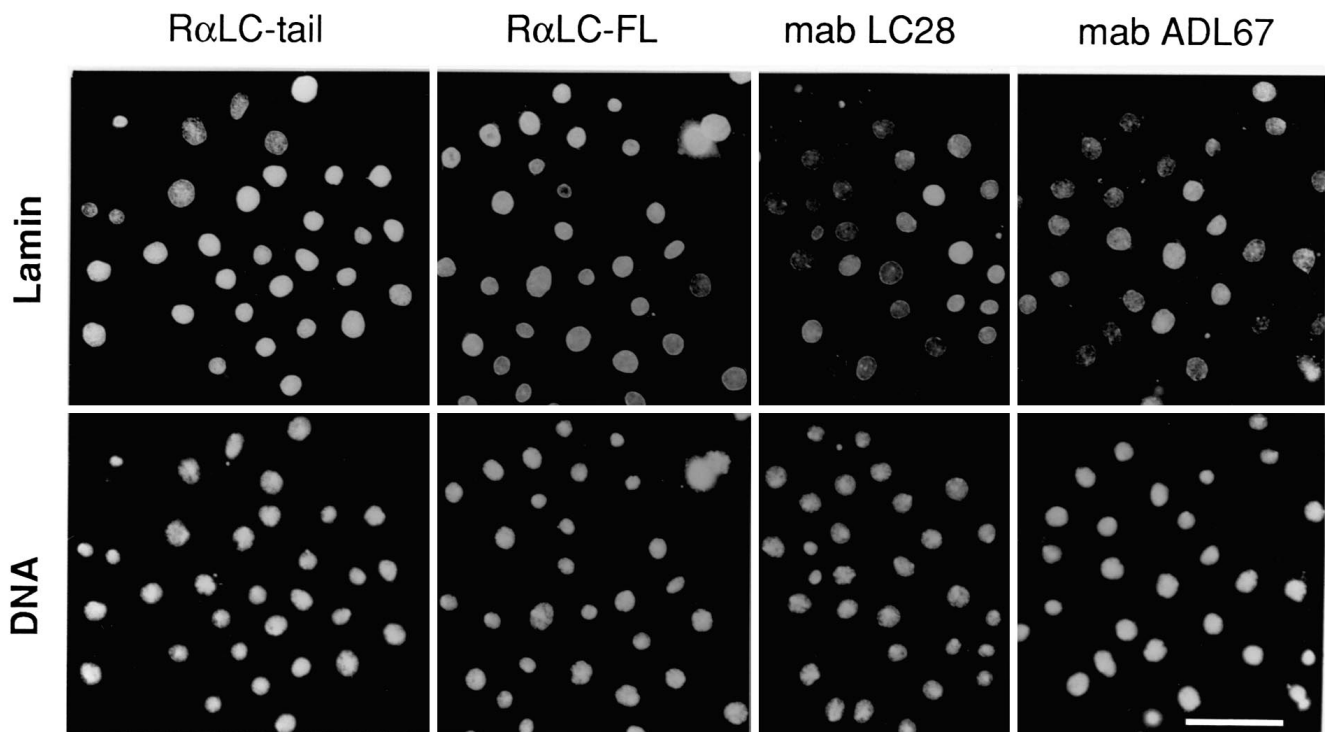
Preparation of total cellular RNA from 0.5 grams of *Drosophila* from various developmental stages was as described (Riemer et al., 1993). Poly(A)<sup>+</sup> RNA was isolated by affinity chromatography on oligo(dT)-cellulose (Pharmacia, Uppsala, Sweden).

Poly(A)<sup>+</sup> RNAs (1.5  $\mu$ g each) were separated under denaturing con-

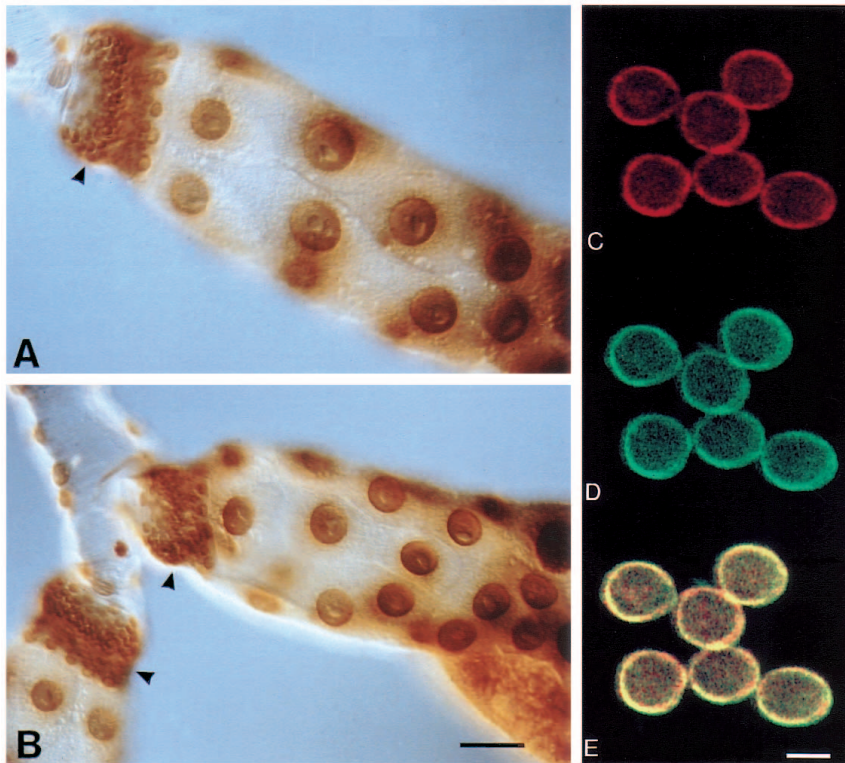


**Fig. 1.** Specificity of antibodies. (A) Bacterially expressed *Drosophila* pG-IF protein (0.25 ng, lanes 1), bacterially expressed *Drosophila* lamin Dm<sub>0</sub> (0.25 ng, lanes 2) or an extract from 19-22 hour *Drosophila* embryos (lanes 3) were subjected to SDS-8% PAGE and blotted to Immobilon-P. Blots were incubated with affinity purified antibodies raised against affinity purified pG-IF polypeptide tail (R $\alpha$ LC-tail), bacterially expressed full length pG-IF polypeptide (R $\alpha$ LC-FL), a murine monoclonal antibody against bacterially expressed pG-IF polypeptide (LC28), or a murine monoclonal antibody specific for *Drosophila* lamin Dm<sub>0</sub> (ADL67). (B) CNBr fingerprints of bacterially expressed pG-IF polypeptide and the immunoreactive protein from *Drosophila*. Untreated bacterially expressed pG-IF polypeptide (30 ng, lane 1), CNBr treated bacterially expressed pG-IF polypeptide (300 ng, lane 2), CNBr treated nuclei from 19-22-hour-old embryos (1  $\mu$ g, lane 3), and untreated nuclei (100 ng, lane 4) from 19-22-hour-old embryos were subjected to SDS-

15% PAGE and blotted to nitrocellulose. The blot was probed with the pG-IF specific R $\alpha$ LC-FL antibody. Fingerprints of bacterially expressed pG-IF polypeptide and pG-IF polypeptide present in 19-22-hour-old *Drosophila* embryos are identical.



**Fig. 2.** pG-IF protein (lamin C) of *Drosophila* Kc cells is localized in the nucleus. Kc cells were processed for indirect immunofluorescence microscopy with the indicated antibodies. DNA staining of the same cells, using Hoechst 33342, is shown in the lower panels. Both lamin C (antibodies R $\alpha$ LC-tail, R $\alpha$ LC-FL, and LC28) and lamin Dm<sub>0</sub> (ADL67) are present in the nuclei of all cells. Note the higher concentration of staining at the rim of the nuclei ('ring-like' staining) for both lamins. Bar, 10  $\mu$ m.



**Fig. 3.** Lamins C and Dm<sub>0</sub> are co-expressed in cells of salivary glands and accessory glands. (A,B) Salivary glands were incubated with polyclonal lamin C antibody R $\alpha$ LC-tail (A), or the Dm<sub>0</sub> specific mAb ADL67 (B). Arrowheads point to salivary gland imaginal rings. Bar in B, 20  $\mu$ m, and applies to A and B. (C,D,E) Double labeling of accessory gland cells with lamin C specific R $\alpha$ LC-FL (C) and lamin Dm<sub>0</sub> specific mAb ADL67 (D) in confocal scanning laser microscopy. Squashed accessory gland cells were fixed and incubated with R $\alpha$ LC-FL (C) and mAb ADL67 (D). Bound antibodies were detected with the appropriate TRITC labeled (C) or FITC labeled (D) secondary antibodies. Single optical sections midway through the nuclei show the separate lamin Dm<sub>0</sub> (C, red) and lamin C (D, green) images; a computer generated image reveals the overlap between the two lamins (E, yellow). Bar, 2.5  $\mu$ m, and applies to C-E.

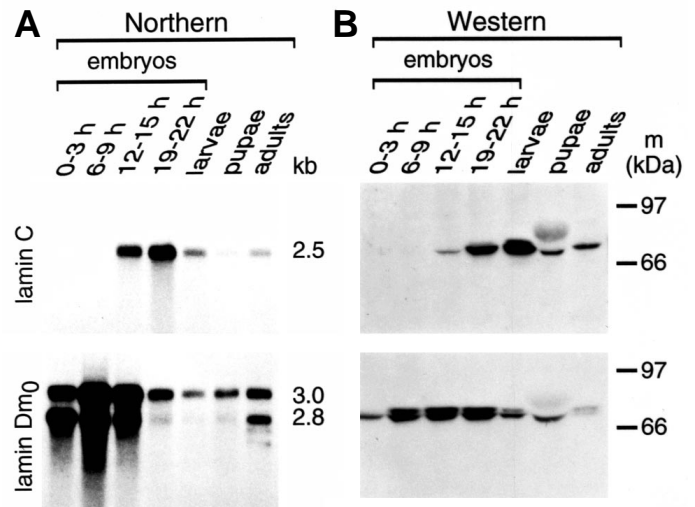
ditions on a 1.5% agarose gel. Blot transfer and hybridization at high stringency were as described (Riemer and Weber, 1994). Two identical blots were hybridized with the pG-IF specific 1,009 bp *SacI/KpnI* genomic DNA fragment and the PCR-amplified 1,170 bp fragment of lamin Dm<sub>0</sub> (see above). Both probes were <sup>32</sup>P-labeled by nick-translation to similar specific activities.

## RESULTS

### Antibodies specific for *Drosophila* pG-IF protein (lamin C)

To determine whether a protein product predicted by the cDNA clone pG-IF is expressed in *Drosophila*, we generated antibodies specific for the pG-IF polypeptide. Two rabbit sera were raised: one by immunization with the bacterially expressed pG-IF tail domain (R $\alpha$ LC-tail), the other by immunization with the recombinant full length pG-IF protein (R $\alpha$ LC-FL). Specific IgGs were obtained by affinity purification (see Materials and Methods). In addition, a monoclonal antibody (LC28) specific for pG-IF was generated using full length pG-IF polypeptide as antigen.

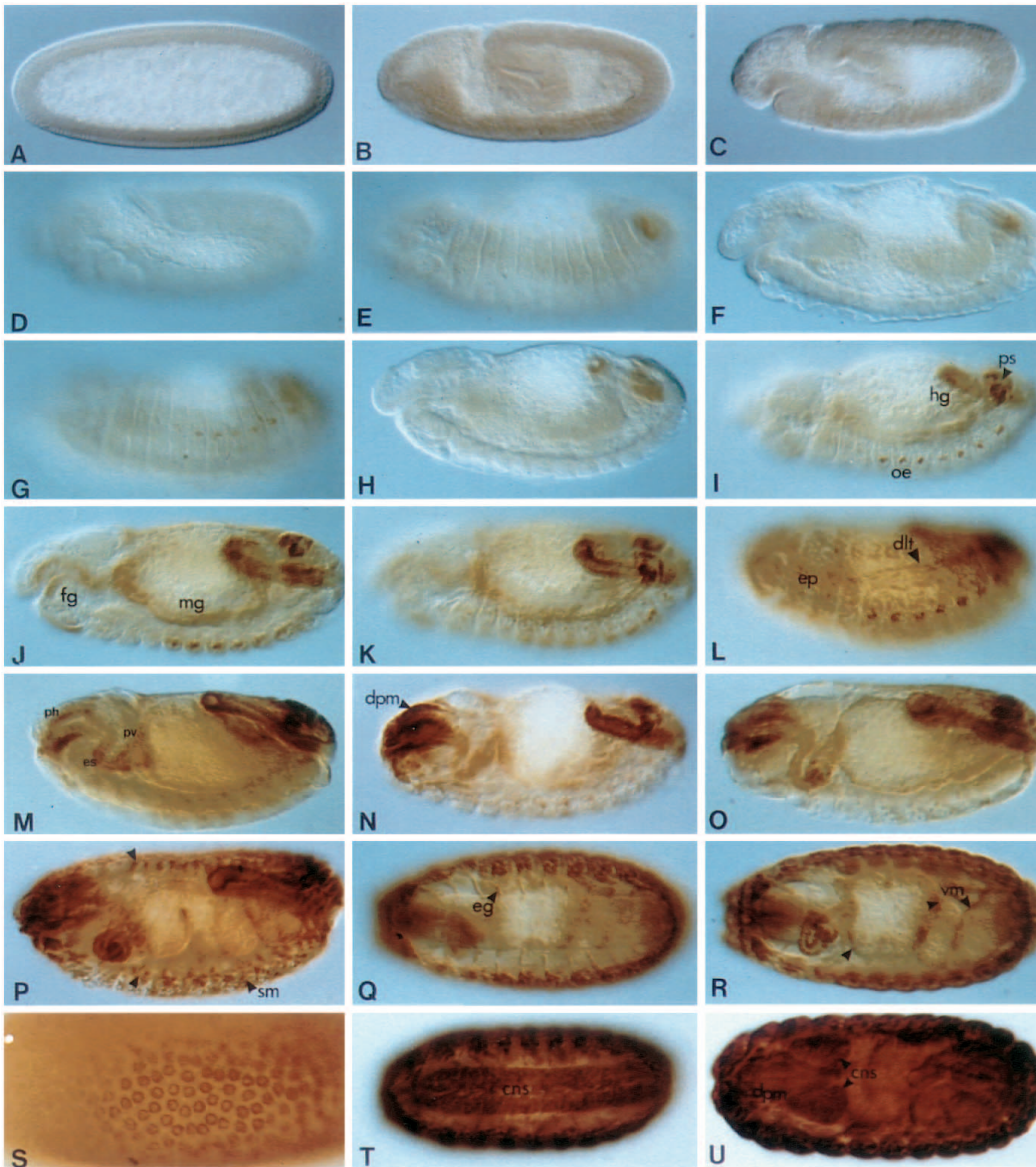
Since the predicted protein sequence of the pG-IF polypeptide is 52% identical to that of lamin Dm<sub>0</sub> (Bossie and Sanders, 1993) it was of special concern to ascertain that antibodies to pG-IF protein did not cross-react with lamin Dm<sub>0</sub>. In western blotting experiments all three antibodies bound to recombinant pG-IF protein but did not bind to lamin Dm<sub>0</sub> (Fig. 1A, lanes 1 and 2). Moreover, all three pG-IF specific antibodies bound to a single polypeptide in an extract of 19-22-hour-old *Drosophila* embryos (Fig. 1A, lanes 3). In contrast, monoclonal antibody ADL67 specific for lamin Dm<sub>0</sub>, bound to bacterially expressed lamin Dm<sub>0</sub> (Fig. 1A, lane 2) but not to recombinant pG-IF protein (Fig 1A, lane 1). It recognized two



**Fig. 4.** Lamin C expression is regulated during *Drosophila* development at the transcriptional level. Specimens from the indicated developmental periods were collected and used for either northern blot analysis (A) or western blot analysis (B). Blots were probed with either a lamin C-specific probe (upper panel) or a lamin Dm<sub>0</sub>-specific probe (lower panel). Both probes had the same specific activity. Approximate molecular sizes in kb are indicated. For western blot analysis 20  $\mu$ g protein were separated by SDS-8% PAGE and blotted to nitrocellulose. Immunodetection was either with lamin C-specific R $\alpha$ LC-FL (upper panel) or with lamin Dm<sub>0</sub>-specific affinity purified rabbit antibodies (Smith and Fisher, 1984, lower panel). Positions of both lamins are indicated on the right hand side. Larvae were a homogeneous population of second instar larvae.

bands in the embryo extract. These are the previously characterized lamin Dm<sub>0</sub> derivatives Dm<sub>1</sub> and Dm<sub>2</sub> (Smith et al., 1987). Longer development of similar blots did not show





**Fig. 5.** Localization of lamin C protein during embryonic development: 0-22 hour embryos were fixed and stained with R $\alpha$ LC-tail for lamin C (A-R), or with ADL67 for lamin Dm<sub>0</sub> (S-U). Embryos are oriented with anterior pole to the left and dorsal side up except when indicated. Embryonic stages are those of Campos-Ortega and Hartenstein (1985). No lamin C staining is visible in embryos of stage 5 (A), stage 8 (B), stage 9 (C), or stage 10 (D, lateral view). This contrasts strikingly with lamin Dm<sub>0</sub> staining which is found in all nuclei at all stages (for example: S, stage 5; T, stage 16 ventral view; U, stage 16 dorsoventral view at midplane). In late stage 12 embryos (E and F, different optical planes) the first tissues to express lamin C can be identified as oenocytes (oe) in abdominal segments A1-7, hindgut (hg), and posterior spiracles (ps), but no staining is visible in the foregut and dorsal longitudinal trunks. At stage 12/13 (G, lateral view; H, midsagittal section) the concentration of lamin C in these tissues increases. Staining of these tissues is most easily discerned in stage 13 embryos (I). At stage 14 (J and K) the foregut (fg) is also stained, whereas the tissues stained previously increase in intensity. In stage 15 embryos (L, lateral view; M, midsagittal section)

the epidermis (ep), and dorsal longitudinal trunk (dlt) acquire lamin C. In the foregut, the pharynx (ph), esophagus (es), and proventriculus (pv) can be discerned. The diffuse staining throughout the embryo in M is caused by out of focus stain located in the epidermis. At late stage 15 (N and O) the dorsal pharyngeal musculature (dpm) acquires lamin C. In stage 16 embryos (P-R; Q and R are dorsoventral views) staining of the somatic musculature (sm) increases and exit glia (eg) now express lamin C. Staining is visible around the midgut, this is, however, visceral mesoderm (vm) whereas the midgut itself seems negative. The central nervous system (cns) does not show detectable levels of lamin C (compare with lamin Dm<sub>0</sub> in T). For midgut (mg) see Results.

specific reaction with additional protein bands (data not shown, for lamin Dm<sub>0</sub> specific antibodies see also Smith and Fisher, 1989).

The polypeptide detected by the pG-IF specific antibodies in 19-22 hour *Drosophila* embryos migrates at a similar position to that of the recombinant protein. To ascertain that this polypeptide is the authentic pG-IF protein we compared CNBr-generated peptide maps of both proteins. CNBr digests of recombinant pG-IF polypeptide and of nuclei from 19-22-hour-old embryos were compared by western blotting using the pG-IF specific R $\alpha$ LC-FL antibody. As shown in Fig. 1B, the CNBr fingerprints of both proteins are identical, showing that the protein recognized by the pG-IF specific antibodies in embryo extracts is indeed the authentic pG-IF polypeptide.

### The pG-IF protein localizes to the nucleus

Immunofluorescence microscopy of Kc cells showed nuclear staining with all three pG-IF specific antibodies and with the lamin Dm<sub>0</sub> specific monoclonal antibody ADL67 (Fig. 2). Often, a ring-like image can be seen, suggesting that pG-IF protein is concentrated in the nuclear lamina. Because of these results, we refer to pG-IF protein in the remainder of the text as lamin C (as previously suggested by Bossie and Sanders, 1993).

To ascertain that lamin C is a nuclear protein not only in Kc cells but also in authentic *Drosophila* tissues, we stained salivary glands and squashed accessory glands with lamin C antibodies. Salivary gland nuclei (Fig. 3A,B) and accessory gland nuclei (Fig. 3C-E) contained both lamins C and Dm<sub>0</sub>.

The confocal microscopic images through the double labeled accessory gland cells (lamin C, red; lamin Dm<sub>0</sub>, green) showed that both lamin C and lamin Dm<sub>0</sub> were localized mainly in the nuclear rim (Fig. 3C,D). Although there was considerable overlap between the two lamins (yellow in Fig. 3E) this overlap was not complete. Thus, consistent with its nuclear location signal (Bossie and Sanders, 1993), lamin C is a nuclear protein which localizes mainly to the nuclear periphery. In cell fractionation experiments using 18-22 hour embryos we found lamin C almost exclusively in the nuclear fraction (not shown), further corroborating that lamin C is a nuclear protein.

### Expression of lamin C during development

Expression of the lamin C mRNA and protein was monitored throughout the *Drosophila* life cycle by northern and western analysis (Fig. 4, upper panels). Northern blot analysis revealed a single poly(A)<sup>+</sup> mRNA species. This mRNA had an apparent size (2.5 kb) comparable to that predicted from the cDNA sequence (Bossie and Sanders, 1993, and Fig. 4). The lamin C mRNA is barely detectable (after prolonged exposure of the northern blot, data not shown) in early embryos (up to 9 hours of embryonal development) but is present in large amounts in late (19-22 hour) embryos and in larvae (Fig. 4, upper panel). For comparison a similar blot was probed with a lamin Dm<sub>0</sub>-specific probe (Fig. 4, lower panel). The lamin Dm<sub>0</sub> mRNAs (2.8 and 3.0 kb; Gruenbaum et al., 1988) are already detected in 0-3 hour embryos. As was reported, the 3.0 kb form remains present throughout the *Drosophila* life cycle (Gruenbaum et al., 1988). Both the lamin C and lamin Dm<sub>0</sub> mRNAs decrease in abundance after the larval stages.

Expression of the lamin C protein follows the profile found for the mRNA. No lamin C is detected in 0-3- and 6-9-hour-old embryos. Lamin C is expressed in 19-22-hour-old embryos and peaks in larvae, after which it declines in pupae and adults (Fig. 4, upper panel). This contrasts with the lamin Dm<sub>0</sub> gene products which are already present in early embryos (Fig. 4, lower panel, and Whalen et al., 1991). Thus, lamin C expression is regulated mainly at the transcriptional level.

To determine whether lamin C expression is tissue-specific we labeled *Drosophila* embryos with a lamin C-specific antibody (Fig. 5). No lamin C-specific staining was discerned up to late stage 12 (about 9 hours of embryonic development, Fig. 5A-D). Lamin Dm<sub>0</sub> was readily identifiable in such embryos (for example Fig. 5S; also see Whalen et al., 1991). The lamin C protein was first detected in late stage 12 embryos in oenocytes, hindgut and posterior spiracles (Fig. 5E,F). At stage 15 foregut, epidermis, and dorsal longitudinal trunks have acquired lamin C (Fig. 5I-M). By stage 16 the dorsal pharyngeal musculature, exit glia, and visceral mesoderm were stained with lamin C antibody (Fig. 5N-R). Other tissues, most strikingly the central nervous system, did not express lamin C until the end of embryogenesis. This can be most easily appreciated by comparison with lamin Dm<sub>0</sub> staining which was found in all nuclei (Fig. 5T,U).

To analyze lamin C expression by an independent method we performed in situ hybridization on *Drosophila* embryos (Fig. 6). DNA probes specific for lamins Dm<sub>0</sub> and C were used in parallel. Lamin Dm<sub>0</sub> transcripts occurred at all stages and in all cells while lamin C transcripts were first detected in oenocytes in late stage 12 embryos (data not shown). In stage 13 embryos, lamin C was exclusively expressed in oenocytes,

foregut, hindgut and dorsal longitudinal trunks (Fig. 6C,E). The pharynx showed a remarkably high expression of lamin C in comparison with other tissues (Fig. 6E). In contrast to the very limited number of tissues expressing lamin C, lamin Dm<sub>0</sub> was constitutively expressed in the entire embryo (Fig. 6D,F). Notably, the brain as part of the embryonic central nervous system that begins to differentiate at stage 13 (Goodman and Doe, 1993), showed a high accumulation of Dm<sub>0</sub> mRNA (Fig. 6D,F) but lacked lamin C transcripts (Fig. 6C,E). The midgut of stage 13-15 embryos was stained diffusely in a non-nuclear fashion by the R $\alpha$ LC-tail antibody (Fig. 5I-K,N,O). However, in situ hybridization clearly demonstrated the absence of lamin C transcripts in the midgut during embryonic development (Fig. 6C), strongly suggesting that antibody staining of the midgut is artefactual. The combined results of immunostaining and in situ hybridization show that during embryogenesis lamin C expression is spatially and temporally regulated. While lamin Dm<sub>0</sub> is found in all cells, lamin C is detected only in various differentiating tissues. Fig. 7 summarizes those tissues of the embryo which display lamin C expression.

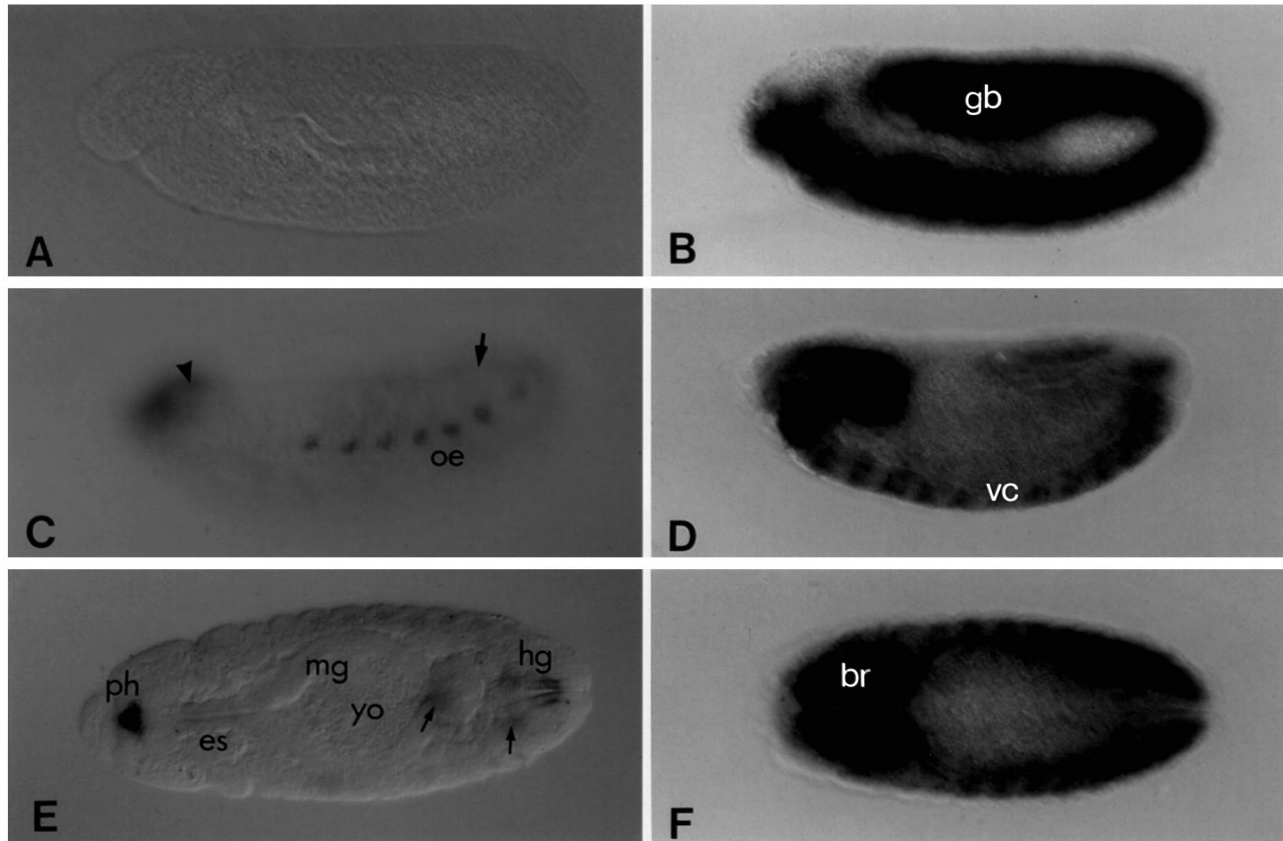
Expression of lamin C was also investigated in larval tissues. Cryosections of third instar larvae (L3) were double-labeled with lamin C- and lamin Dm<sub>0</sub>-specific antibodies for immunofluorescence microscopy. Nuclei of all cells in the entire third instar larvae seemed to be positive for lamin C and lamin Dm<sub>0</sub>. However, the level of lamin C expression showed marked differences among different tissues. The apparent amount of lamin C ranged from high in nuclei of salivary gland cells to very low in some imaginal discs (Fig. 8A). In contrast, lamin Dm<sub>0</sub> was more uniformly expressed (Fig. 8B). Isolated imaginal discs of mature L3 stages whole-mount stained with R $\alpha$ LC-tail and ADL67 antibodies also displayed coexpression of lamins C and Dm<sub>0</sub> (not shown, but see salivary gland imaginal rings in Fig. 3A and B, and wing and eye-antennal discs in Fig. 8).

### DISCUSSION

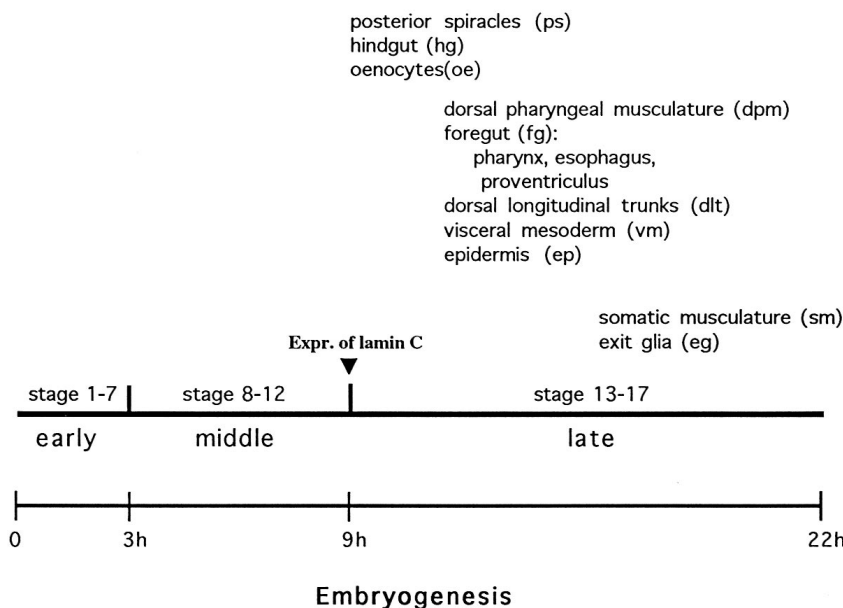
Until recently, it was thought that invertebrates contain only a single nuclear lamin, which in the case of *Drosophila* is the well characterized lamin Dm<sub>0</sub> (for references see Introduction). However, in 1993, Bossie and Sanders isolated a *Drosophila* cDNA clone, pG-IF, which from its sequence could either code for a potential second lamin (lamin C) or for a cytoplasmic intermediate filament protein. Here, we have shown that this protein is expressed in *Drosophila*, that it is indeed a nuclear lamin and that its expression is developmentally controlled.

Two different rabbit antisera and a murine monoclonal antibody, raised against the recombinant protein or its tail domain, specifically recognize lamin C in immunoblotting experiments and do not bind lamin Dm<sub>0</sub> (Fig. 1). These antibodies decorate the nuclear lamina in salivary and accessory glands as well as in cultured Kc cells (Figs 2, 3), which also contain lamin Dm<sub>0</sub>. This firmly establishes that the protein product of pG-IF is indeed a nuclear lamin, i.e. lamin C.

Analysis of lamin expression at selected time points of *Drosophila* development by northern and western blots, in situ hybridization and immunostaining shows that lamin C is developmentally controlled, while lamin Dm<sub>0</sub> is constitutively expressed. Lamin C transcription starts at the earliest, after 6 to 9 hours of embryonic development (Fig. 4). The onset of lamin

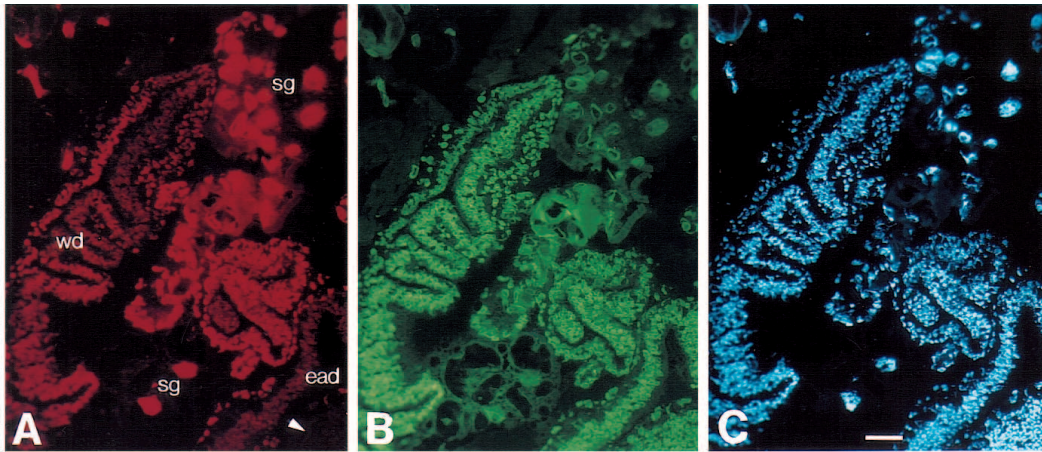


**Fig. 6.** Localization of lamin C and lamin Dm<sub>0</sub> transcripts during embryonic development. Whole mount in situ hybridization with lamin C specific (A,C,E) and lamin Dm<sub>0</sub> specific (B,D,F) DNA probes. Stage 7 embryos show no detectable transcripts of lamin C (A) whereas lamin Dm<sub>0</sub> is expressed uniformly in the germ band (gb in B). (C) In late stage 13 embryos lamin C expression is observed in oenocytes (oe in abdominal segments A1-7) and in the pharynx (ph, see E) of the foregut (here shown out of focus). The roof-like staining at the anterior tip of the embryo belongs to the pharyngeal musculature (arrowhead). Additional lamin C transcripts are also found in the hindgut (arrow). (D) At the same time (late stage 13) lamin Dm<sub>0</sub> expression is ubiquitous with a prominent accumulation of transcripts in the brain (br, see F) and the ventral cord (vc). (E,F) Dorsoventral view of late stage 13 embryos at mid-focal plane. (E) The pharynx (ph, see E) shows a high concentration of lamin C transcripts. Esophagus (es) has much fewer transcripts. The hindgut (hg, arrows) shows diffuse staining at this focal plane. Note that the midgut (mg), enclosing the central yolk (yo), shows no detectable levels of lamin C mRNA. (F) In marked contrast to the lamin C expression pattern lamin Dm<sub>0</sub> is expressed constitutively from the anterior to the posterior pole of the embryo.



**Fig. 7.** Summary of tissue specific expression of lamin C during embryonic development. Expression of lamin C is first detected at the protein (Fig. 5) and mRNA (Fig. 6) level in late stage 12 embryos (9 hours after the start of embryonic development at 22°C). At this stage oenocytes, hindgut and posterior spiracles are positive with lamin C antibodies. Based on immunological data the figure shows which other tissues acquire lamin C until the end of embryogenesis (stage 17).





**Fig. 8.** Lamin C expression in larval tissues. Longitudinal cryosections of third instar larvae were double labeled with lamin C specific R $\alpha$ LC-tail and lamin Dm<sub>0</sub> specific ADL 67 antibodies. Sections A and B show the distribution of the respective lamins in the thoracic region of the larva by immunofluorescence microscopy. (A) Lamin C expression is high in nuclei of salivary glands (sg) and significantly lower in nuclei of wing discs (wd) and eye-antennal discs (ead). Note that the levels of expression seems

to vary between nuclei in different parts of the same disc (for example ead). In contrast, lamin Dm<sub>0</sub> was found in all nuclei at approximately the same concentration (B). Staining of DNA with Hoechst 33342 revealed that lamins C and Dm<sub>0</sub> are coexpressed in nuclei of all cells (C). Bar, 10  $\mu$ m, and applies to all panels.

C transcription was verified independently by in situ hybridization, which documented the absence of lamin C transcripts in embryonic stages earlier than late stage 12 (Fig. 6). Lamin C transcription increases continuously during the subsequent 13 hours of embryogenesis until it reaches a maximum in 19- to 22-hour-old embryos. At this stage, the concentration of lamin C mRNA exceeds that of lamin Dm<sub>0</sub>. Complementary results, obtained by in situ hybridization, show that an increasing number of tissues acquire lamin C between stages 13 and 17. Oenocytes, dorsal longitudinal trunks and the hindgut are the first tissues in which lamin C specific mRNA can be detected. Parallel expression of lamins C and Dm<sub>0</sub> occurs during larval development. Northern analysis of second instar larvae shows mRNA signals for both lamins of almost the same intensity (Fig. 4). Cryosections of third instar larvae show that most nuclei containing lamin Dm<sub>0</sub> also display lamin C (Fig. 8). Differences in the intensity of lamin C staining observed in nuclei of salivary glands and the surrounding tissue and in nuclei of imaginal discs, may indicate that lamin C can be expressed at different levels in the developing larva (Fig. 8). Northern and western blots showed that lamin C and Dm<sub>0</sub> specific mRNAs and protein are also present in pupae and adults. Thus both lamins are also expressed during the final stages of *Drosophila* development.

The level of lamin Dm<sub>0</sub> mRNA follows closely the pattern of mitotic activity during development. Most somatic cells in the embryo undergo 16 cycles of mitosis in the first 7 hours and then stop dividing (Campos-Ortega and Hartenstein, 1985; Foe, 1989). Correspondingly, the highest lamin Dm<sub>0</sub> mRNA concentrations were found in samples from the first 12-15 hours of development with the maximal amount in the sample taken from 6 to 9 hour embryos.

Immunostaining of embryos confirms the developmentally controlled expression of lamin C and also reveals a tissue-specific pattern of staining (Fig. 5). Lamin C is first observed in late stage 12 embryos and involves the oenocytes and the hindgut. At later stages, it appears in a variety of other differentiating tissues. The most prominent decoration involves the entire foregut, posterior spiracles, dorsal longitudinal trunks and epidermis (Figs 5, 7). In marked contrast to the ectodermally derived foregut and hindgut, the embryonic midgut, which is of endodermal origin (Costa et al., 1993; Skaer, 1993),

seemed to show no detectable levels of lamin C transcripts by in situ hybridization. The visceral mesoderm surrounding the midgut as simple epithelium was positive for lamin C in immunostaining and in situ hybridization. Since the primordia of the foregut and hindgut on one side and the midgut on the other side originate from different germ-layers, we speculate that the differences observed for lamin C expression in the entire gut might reflect differences in the origin and development of the alimentary tract in *Drosophila* embryos.

The onset of lamin C expression observed by immunostaining in late stage 12 embryos seems to correlate with the loss of mitotic activity and morphological changes in the developing tissues. When lamin C is first detected in stage 12/13 embryos, most cells have stopped dividing and are arrested in G<sub>1</sub> of the mitotic cycle 17 (Foe et al., 1993). Most organ primordia begin to differentiate and become histologically defined (Hartenstein, 1993). Prominent among the tissues that remain negative for lamin C, even at later stages, is the embryonic central nervous system (CNS) comprising the brain and ventral cord. The ventral neuroblasts and the cells of the malpighian vessels, which also lack lamin C, continue to divide after stage 12/13. Currently, it is unknown whether the absence of lamin C in the CNS is related to the continuing mitotic activity of the differentiating cells. It is interesting to note that also in the mouse embryo the developmentally controlled lamin species (lamin A; see below) appears essentially postnatally in the CNS (Röber et al., 1989). Immunostaining of embryos showed that lamin Dm<sub>0</sub> is ubiquitously expressed. This finding confirms and extends previous studies (Whalen et al., 1991) which found that only *Drosophila* sperm lack lamin Dm<sub>0</sub>. Independent proof for the developmental control of lamin C expression comes from in situ hybridization experiments (Fig. 6). They show that lamin C is first recognized in embryos of stage 12. The in situ hybridization patterns and the immunostained patterns are remarkably similar (Figs 5, 6).

Our results show that *Drosophila* lamins Dm<sub>0</sub> and C can be considered as the analogues of vertebrate lamins B<sub>1</sub>/B<sub>2</sub> and A/C, respectively. *Drosophila* lamin Dm<sub>0</sub> is constitutively expressed (Figs 5, 6) as are the vertebrate B-type lamins (Lehner et al., 1987; Stewart and Burke, 1987; Röber et al., 1989; Höger et al., 1990). Consistent with this functional analogy, *Drosophila*



lamin Dm<sub>0</sub> has approximately the same size as B-type lamins. Interestingly, the same size also holds for *Drosophila* lamin C, which is now identified as an analogue of vertebrate lamins A/C due to the developmental control which governs the expression of these two lamins (for vertebrate lamin A see Lehner et al., 1987; Stewart and Burke, 1987; Röber et al., 1989). Since the vertebrate lamin C is a special splice form of lamin A (Stick, 1992; Lin and Worman, 1993) and is only detected in mammals (for references see Introduction) the analogue of *Drosophila* lamin C is actually the large vertebrate lamin A. In line with this analogy during embryonic development, the *Drosophila* lamin C cDNA does not encode a CaaX box (Bossie and Sanders, 1993), while the vertebrate lamin A loses this sequence after its incorporation into the lamina by a special maturation event based on proteolytic trimming (Vorbürger et al., 1989; Weber et al., 1989a; Hennekes and Nigg, 1994; Sinensky et al., 1994). Although the overall sequence identity between *Drosophila* lamin C and either vertebrate A- or B-type lamins is about the same (35%), one wonders whether there are special sequence motifs shared by vertebrate lamin A and *Drosophila* lamin C, which are absent from lamins Dm<sub>0</sub> and B<sub>1</sub>/B<sub>2</sub>. Inspection of the sequence alignment in Fig. 6 of Bossie and Sanders (1993) shows that major sequence differences between *Drosophila* lamins Dm<sub>0</sub> and C involve the amino-terminal head domain, which is rather variable in all lamins, and the carboxy-terminal 27 residues. In the latter region, lamin Dm<sub>0</sub> is closely related to vertebrate lamins B<sub>1</sub> and B<sub>2</sub>, while *Drosophila* lamin C displays an entirely different sequence, which is rich in serine/threonine and glycine. Interestingly, the extra domain of lamin A, which is absent in the smaller vertebrate B-type lamins, is also rich in these amino acids and is thought to contribute to lamin-chromatin interactions in vitro (Höger et al., 1991).

The finding that differentiated cells in vertebrates and in *Drosophila* contain both a constitutively expressed lamin and a developmentally regulated one, again raises the question of a specific function for the developmentally controlled lamin. Previously, it has been speculated that the differential expression of a lamin may relate to differences in the organization of interphase chromatin in different cell types (Nigg, 1989; Röber et al., 1989). However, the constitutive, ectopic expression of chicken lamin A in an undifferentiated murine embryonal carcinoma cell line, which does not normally express lamin A, does not alter the differentiation state or the differentiation potential (Peter and Nigg, 1991). Thus, elimination of individual lamin genes may be necessary to explore a special function for developmentally controlled nuclear lamins.

We are grateful to Dr Marcos Gonzales-Gaitan for help with in situ hybridizations and Drs Mary Osborn and Herbert Jäckle for helpful comments and suggestions. Susanne Isenberg provided expert technical assistance. The gifts of Kc tissue culture cells (from Dr H. Saumweber, Free University of Berlin) and the plasmid pG-IF (from Dr M. M. Sanders, Piscataway, NJ) are appreciated. N.S. was supported by a Long Term Fellowship from the Human Frontier Science Organization. This work was supported by a research grant from the US NIH (to P.A.F.).

## REFERENCES

Aebi, U., Cohn, J., Buhle, L. and Gerace, L. (1986). The nuclear lamina is a meshwork of intermediate-type filaments. *Nature* **323**, 560-564.  
 Allis, C. D., Waring, G. D. and Mahowald, A. P. (1977). Mass isolation of pole cells from *Drosophila melanogaster*. *Dev. Biol.* **56**, 372-381.

Bossie, C. A. and Sanders, M. M. (1993). A cDNA from *Drosophila melanogaster* encodes a lamin C-like intermediate filament protein. *J. Cell Sci.* **104**, 1263-1272.  
 Campos-Ortega, J. A. and Hartenstein, V. (1985). The pattern of embryonic cell divisions. In *The Embryonic Development of Drosophila melanogaster*, pp. 165-171. Springer Verlag, Berlin.  
 Clarke, S. (1992). Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. *Annu. Rev. Biochem.* **61**, 355-386.  
 Costa, M., Sweeton, D. and Wieschaus, E. (1993). Gastrulation in *Drosophila*: cellular mechanisms of morphogenetic movements. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 425-465. Cold Spring Harbor Laboratory Press, NY.  
 Dessev, G. and Goldman, R. (1990). The oocyte lamin persists as a single major component of the nuclear lamina during embryonic development of the surf clam. *Int. J. Dev. Biol.* **34**, 267-274.  
 Foe, V. E. (1989). Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* **107**, 1-22.  
 Foe, V. E., Odell, G. E. and Edgar, B. A. (1993). Mitosis and morphogenesis in the *Drosophila* embryo: Point and counterpoint. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 149-300. Cold Spring Harbor Laboratory Press, NY.  
 Fuchs, E. and Weber, K. (1994). Intermediate filaments: Structure, dynamics, function and disease. *Annu. Rev. Biochem.* **63**, 345-382.  
 Gerace, L. and Blobel, G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell* **19**, 277-287.  
 Glass, C. A., Glass, J. R., Taniura, H., Hasel, K. W., Blevitt, J. M. and Gerace, L. (1993). The alpha-helical rod domain of human lamins A and C contains a chromatin binding site. *EMBO J.* **12**, 4413-4424.  
 Goodman, C. S. and Doe, C. Q. (1993). Embryonic development of the *Drosophila* central nervous system. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 1131-1206. Cold Spring Harbor Laboratory Press, NY.  
 Gruenbaum, Y., Landesman, Y., Drees, B., Bare, J. W., Saumweber, H., Paddy, M. R., Sedat, J. W., Smith, D. E., Benton, B. M. and Fisher, P. A. (1988). *Drosophila* nuclear lamin precursor Dm<sub>0</sub> is translated from either of two developmentally regulated mRNA species apparently encoded by a single gene. *J. Cell Biol.* **106**, 585-596.  
 Hartenstein, V. (1993). *Atlas of Drosophila Development*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.  
 Hennekes, H. and Nigg, E. A. (1994). The role of isoprenylation in membrane attachments of nuclear lamins. A single point mutation prevents proteolytic cleavage of the lamin A precursors and confers membrane binding properties. *J. Cell Sci.* **107**, 1019-1029.  
 Höger, T. H., Zatloukal, K., Waizenegger, I. and Krohne, G. (1990). Characterization of a second highly conserved B-type lamin present in cells previously thought to contain only a single B-type lamin. *Chromosoma* **99**, 379-390.  
 Höger, T. H., Krohne, G. and Kleinschmidt, J. A. (1991). Interaction of *Xenopus* lamins A and LII with chromatin in vitro mediated by a sequence element in the carboxyterminal domain. *Exp. Cell Res.* **197**, 280-289.  
 Holtz, D., Tanaka, R. A., Hartwig, J. and McKeon, F. (1989). The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. *Cell* **59**, 969-977.  
 Kitten, G. T. and Nigg, E. A. (1991). The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B<sub>2</sub>. *J. Cell Biol.* **113**, 13-23.  
 Krohne, G., Waizenegger, I. and Höger, T. H. (1989). The conserved carboxy-terminal cysteine of nuclear lamins is essential for lamin association with the nuclear envelope. *J. Cell Biol.* **109**, 2003-2011.  
 Lehner, C. F., Furstenberger, G., Eppenberger, H. M. and Nigg, E. A. (1986). Biogenesis of the nuclear lamina: in vivo synthesis and processing of nuclear protein precursors. *Proc. Nat. Acad. Sci. USA* **83**, 2096-2099.  
 Lehner, C. F., Stick, R., Eppenberger, H. M. and Nigg, E. A. (1987). Differential expression of nuclear lamin proteins during chicken development. *J. Cell Biol.* **105**, 577-587.  
 Lin, F. and Worman, H. J. (1993). Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *J. Biol. Chem.* **268**, 16321-16326.  
 Loewinger, L. and McKeon, F. (1988). Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO J.* **7**, 2301-2309.  
 Ludérus, M. E., de Graaf, A., Mattia, E., den Blaauwen, J. L., Grande, M. A., de Jong, L. and van Driel, R. (1992). Binding of matrix attachment regions to lamin B<sub>1</sub>. *Cell* **70**, 949-959.

- Meier, J., Campbell, K. H., Ford, C. C., Stick, R. and Hutchison, C. J. (1991). The role of lamin LIII in nuclear assembly and DNA replication, in cell-free extracts of *Xenopus* eggs. *J. Cell Sci.* **98**, 271-279.
- Meier, J. and Georgatos, S. D. (1994). Type B lamins remain associated with the integral nuclear envelope protein p58 during mitosis: implications for nuclear reassembly. *EMBO J.* **13**, 1888-1898.
- Meller, V. H., Fisher, P. A. and Berrios, M. (1995). Intranuclear distribution of DNA topoisomerase II and chromatin. *Chromosome Res.* **3**, 255-260.
- Mitchison, T. J. and Sedat, J. (1983). Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99**, 261-264.
- Moir, R. D. and Goldman, R. D. (1993). Lamin dynamics. *Curr. Opin. Cell Biol.* **5**, 408-411.
- Moir, D. M., Montag-Lowy, M. and Goldman, R. D. (1994). Dynamic properties of nuclear lamins: Lamin B is associated with sites of DNA replication. *J. Cell Biol.* **125**, 1201-1212.
- Monteiro, M. J., Hicks, C., Gu, L. and Janicki, S. (1994). Determinants for cytoplasmic sorting of cytoplasmic and nuclear intermediate filaments. *J. Cell Biol.* **127**, 1327-1343.
- Newport, J. W., Wilson, K. L. and Dunphy, W. G. (1990). A lamin-independent pathway for nuclear envelope assembly. *J. Cell Biol.* **111**, 2247-2259.
- Nigg, E. A. (1989). The nuclear envelope. *Curr. Opin. Cell Biol.* **1**, 435-440.
- Nigg, E. A. (1992). Assembly-disassembly of the nuclear lamina. *Curr. Opin. Cell Biol.* **4**, 105-109.
- Peter, M. and Nigg, E. A. (1991). Ectopic expression of an A-type lamin does not interfere with differentiation of lamin A-negative embryonal carcinoma cells. *J. Cell Sci.* **100**, 589-598.
- Riemer, D., Dodemont, H. and Weber, K. (1993). A nuclear lamin of the nematode *Caenorhabditis elegans* with unusual structural features; cDNA cloning and gene organization. *Eur. J. Cell Biol.* **62**, 214-223.
- Riemer, D. and Weber, K. (1994). The organization of the gene for *Drosophila* lamin C: limited homology with vertebrate lamin genes and lack of homology versus the *Drosophila* lamin Dm<sub>0</sub> gene. *Eur. J. Cell Biol.* **63**, 299-306.
- Röber, R. A., Weber, K. and Osborn, M. (1989). Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. *Development* **105**, 365-378.
- Sinensky, M. and Lutz, R. J. (1992). The prenylation of proteins. *BioEssays* **14**, 25-31.
- Sinensky, M., Fantle, K., Trujillo, M., McLain, T., Kupfer, A. and Dalton, M. (1994). The processing pathway of prelamin A. *J. Cell Sci.* **107**, 61-67.
- Skaer, H. (1993). The alimentary canal. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez Arias), pp. 941-1012. Cold Spring Harbor Laboratory Press, NY.
- Smith, D. E. and Fisher, P. A. (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., Gruenbaum, Y., Berrios, M. and Fisher, P. A. (1987). Biosynthesis and interconversion of *Drosophila* nuclear lamin isoforms during normal growth and in response to heat shock. *J. Cell Biol.* **105**, 771-790.
- Smith, D. E. and Fisher, P. A. (1989). Interconversion of *Drosophila* nuclear lamin isoforms during oogenesis, early embryogenesis, and upon entry of cultured cells into mitosis. *J. Cell Biol.* **108**, 255-265.
- Stewart, C. and Burke, B. (1987). Teratocarcinoma stem cells and early mouse embryos contain only a single major lamin polypeptide closely resembling lamin B. *Cell* **51**, 383-392.
- Stick, R., Angres, B., Lehner, C. F. and Nigg, E. A. (1988). The fates of chicken nuclear lamin proteins during mitosis: evidence for a reversible redistribution of lamin B2 between inner nuclear membrane and elements of the endoplasmic reticulum. *J. Cell Biol.* **107**, 397-406.
- Stick, R. (1992). The gene structure of *Xenopus* nuclear lamin A: a model for the evolution of A-type from B-type lamins by exon shuffling. *Chromosoma* **101**, 566-574.
- Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.
- Vorbürger, K., Lehner, C. F., Kitten, G. T., Eppenberger, H. M. and Nigg, E. A. (1989). A second higher vertebrate B-type lamin. cDNA sequence determination and in vitro processing of chicken lamin B2. *J. Mol. Biol.* **208**, 405-415.
- Weber, K., Plessmann, U. and Traub, P. (1989a). Maturation of nuclear lamin-A involves a specific carboxy-terminal trimming, which removes the polyisoprenylation site from the precursor - implications for the structure of the nuclear lamina. *FEBS Lett.* **257**, 411-414.
- Weber, K., Plessmann, U. and Ulrich, W. (1989b). Cytoplasmic intermediate filament proteins of invertebrates are closer to nuclear lamins than are vertebrate intermediate filament proteins; sequence characterization of two muscle proteins of a nematode. *EMBO J.* **8**, 3221-3227.
- Whalen, A. M., McConnell, M. and Fisher, P. A. (1991). Developmental regulation of *Drosophila* DNA topoisomerase II. *J. Cell Biol.* **112**, 203-213.

(Received 17 May 1995 - Accepted 24 July 1995)