Developmental and mitotic behaviour of two novel groups of nuclear envelope antigens of *Drosophila melanogaster*

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Summary

Two novel groups of nuclear envelope antigens have been identified using monoclonal antibodies. On immunoblots the antigens correspond to distinct sets of polypeptides in the $175\times10^3$ molecular weight range. The antigens are enriched in a nuclear matrix–pore complex–lamina fraction of *Drosophila* tissue culture cells. We have studied the cellular distribution of these antigens throughout oogenesis and early embryo development.

Immunoblots show that one group of the $175\times10^3M_r$ antigens is maternally transmitted to the embryo. This had already been observed for the $74/76\times10^3M_r$ *Drosophila* lamins described previously, and we showed that a large proportion of the lamins is localized in the interior of the oocyte nucleus. We have also followed the fate of the high molecular weight antigens during mitosis. Each of the antigens uses a different pathway for its distribution to the daughter nuclei. These observations may give clues to the molecular mechanisms involved in the disassembly–reassembly process of the nuclear envelope.

Key words: *Drosophila*, nuclear envelope antigens, development.

Introduction

The multilayered structure of the nuclear envelope separates the chromatin from the cytoplasmic compartment of the eukaryotic cell. It is thought that it acts both as a barrier controlling the exchange of larger molecules between the nuclear interior and the cytoplasm (Paine *et al.* 1985) and as a structural support for the ordered attachment of the chromatin during the interphase of the cell cycle (for reviews, see Franke *et al.* 1981; Hancock & Boulikas, 1982).

The nuclear envelope is composed of an outer and an inner membrane separated by the perinuclear space, except at the level of the nuclear pores where both membranes are continuous. The nuclear pores are surrounded by an octagonal array of eight protein granules (the nuclear pore complex) and seem to provide aqueous channels across the membrane for the transport of larger molecules. The outer membrane with the ribosomes on its surface and the perinuclear space are thought to be continuous with the endoplasmic reticulum and its lumen, respectively. The inner membrane is associated with the proteinaceous lamina underneath (Hancock & Boulikas, 1982), which appears to provide a framework for the nuclear envelope structure and contact points for the attachment of the chromatin.

In most eukaryotes the lamina is composed of related lamin proteins with molecular weights between 60 and $80\times10^3$ (Gerace & Blobel, 1980). In mammals, lamins A and C are most closely related immunologically and in their cDNA sequences (McKeon *et al.* 1986). In *Drosophila*, polypeptides analogous to the lamin proteins with molecular weights at $74/76\times10^3$ have been described (Risau *et al.* 1981; Fisher *et al.* 1982; Smith *et al.* 1987).

Little is known, however, about the integral components of the nuclear membrane. Gerace *et al.* (1982) identified a 190K ($K = 10^5M_r$) glycoprotein as a constituent of rat liver nuclear pore complexes, which was later shown to correspond to a glycoprotein of similar molecular weight found in *Drosophila* (Filson *et al.* 1985). Other glycoproteins considered to be components of the pore complex have been demonstrated in rat liver (Davis & Blobel, 1986; Snow *et al.* 1987). In addition, Berrios *et al.* (1983) demonstrated the presence of a distinct 188K protein contained in the nuclear
pore complex–lamina fraction of *Drosophila* and other species with an ATPase activity. (The apparent molecular weight of the ATPase was first reported to be 174K and later revised to 188K (Berrios & Fisher, 1986).) Other authors identified a different 46K protein with a NTPase activity, which they suggested to be involved in the active export of RNA from the nucleus (Clawson et al. 1984).

We report here the immunobiochemical identification of two different types of nuclear envelope antigens of *Drosophila* using monoclonal antibodies. We have followed the distribution of these antigens in oogenesis and early embryonic development, and studied their behaviour during the cell cycle. Each of the two groups shows a peculiar distribution during development and mitosis. Their patterns of distribution are also different from the developmental and mitotic patterns of the *Drosophila* lamin antigens (Fuchs et al. 1983). An unusual feature of the *Drosophila* lamins on the other hand is the fact that they are present not only in the nuclear envelope but also in large amounts in the interior of oocyte nuclei.

**Materials and methods**

**Materials**

For all experiments the *Drosophila melanogaster* Oregon R P2 strain (Allis et al. 1977) was used. Unless stated otherwise, all chemicals were purchased from Merck (Darmstadt, FRG).

**Source of monoclonal antibodies and cell culture**

The generation of monoclonal antibodies directed against the *Drosophila* lamins (T40-ag) has been described (Risau et al. 1981; Kuo et al. 1982). The other monoclonal antibodies described in this study were obtained against a nuclear fraction of *Drosophila* embryos. The fractionation procedure has been described in detail (Frasch, 1985).

Briefly, nuclei from 0–5 h embryos were prepared (Mayfield et al. 1978), digested with RNase A ( Worthington; 25 μg ml⁻¹, 60 min, 0°C), and extracted with 200 mM-NaCl. The nuclear pellet was sonicated in the presence of 450 mM-NaCl and solubilized chromatin was further fractionated on a 10% to 30% linear sucrose gradient. Gradient fractions containing the bulk of the soluble chromatin were pooled and fractionated on a BioRex 70 column (BioRad) according to Augenlicht & Baserga (1973). Bx34-ab, Bx95-ab, and most of the antibodies (ab) detecting the *Drosophila* lamins were obtained by immunizing with the flow through fractions of this column. The Bx38-ab was obtained by immunizing with a chromatin fraction of the sucrose gradient directly. Methods for establishment and screening of clonal antibodies secreting cell lines and the maintenance of the *Drosophila* cell line Kc have been described (Saumweber et al. 1980).

**Immunoblotting**

For immunoblots embryos of the desired age were collected on apple juice/agar plates from 200 cm³ cages containing of the order of 200 well-fed flies. After dechorionation the embryos were sonicated in Eppendorf tubes in a small volume of water three times for 15 s, with cooling intervals on ice. The protein concentration was determined from a sample, according to Bradford (1976). Equal amounts of protein were separated on a 9–17% SDS–polyacrylamide gel (Laemmli, 1970), electrophoretically transferred to a nitrocellulose filter and incubated with the antibodies as described (Dequin et al. 1984). The concentration of monoclonal antibodies was adjusted to 5 μg ml⁻¹ using cell culture medium. As secondary antibodies we used peroxidase-conjugated or alkaline phosphatase-conjugated (Fab)₂ goat anti-mouse IgG (H+L) (Jackson ImmunoResearch, USA) at a 1:3000 (v/v) dilution in PBS containing 0·3 % haemoglobin and 0·05 % Tween 80 (Serva, FRG). For peroxidase staining, the filter was developed in 50 mM-Tris·HCl, pH 7·6, containing 0·6 mg ml⁻¹ chloronaphthol (Sigma, USA) and 0·015 % H₂O₂. For alkaline phosphatase staining we developed the filter in 0·1 M-sodium carbonate, pH 10·2, containing 0·5 mg ml⁻¹ 5-bromo-4-chloro-3-indoxylphosphate toluidine salt (Serva, FRG). Marker proteins were visualized on a nitrocellulose strip treated in parallel according to Wojtkowiak et al. (1983).

**Indirect immunofluorescence**

Indirect immunofluorescence on sectioned embryos was done essentially as described by Dequin et al. (1984). Ovaries from adult females were manually dissected in buffer A (15 mM-Tris·HCl, pH 7·4, 60 mM-KCl, 15 mM-NaCl, 1·5 mM-spermine, 0·5 mM-spermidine) and ovarioles were separated from each other. The tissue was fixed for 10 min at ambient temperature with 3·7 % freshly prepared formaldehyde in buffer A containing 0·1 % Triton X-100. After washing three times for 30 min in buffer A, the tissue was incubated overnight with the monoclonal antibodies at a concentration of 5 μg ml⁻¹ in cell culture medium. All incubations were done at 4°C. After washing four times for 1 h in buffer A the ovarioles were incubated with rhodamine-conjugated goat anti-mouse IgG H+L (Cappel, USA), which was diluted 200-fold in buffer A. After washing four times for 1 h in buffer A the ovarioles were stained for 10 min with 0·5 μg ml⁻¹ bisbenzimide H33258 (Serva, FRG) in buffer A, washed once in buffer A and prepared for sectioning as described (Dequin et al. 1984). The preparations were viewed in a Zeiss standard microscope with epifluorescence illumination. Zeiss filter systems ultraviolet G365 (487701) and green (487714) were used for Hoechst and rhodamine fluorescence, respectively. Photographs were taken on a Kodak 2415 technical film developed with Kodak developer D19 for 4 min at 20–22°C.

**Preparation of nuclear matrix–pore complex–lamina (NMPCL) fraction**

*Drosophila* Kc nuclei were prepared according to Biessmann et al. (1976) with minor modifications. For the preparation of the NMPCL fraction we followed the protocol of Fisher et al. (1982) from their step of purified nuclei onwards, omitting protease inhibitors except phenylmethylsulphonyl fluoride (PMSF).
Results

Different types of nuclear envelope antigens are detected by monoclonal antibodies

We have previously described libraries of monoclonal antibodies that had been established using fractionated nuclear proteins of *Drosophila* Kc-cells and *Drosophila* early embryos as antigens (Saumweber et al. 1980; Frasch, 1985). In screening these libraries, we identified several antibodies that in indirect immunofluorescence on Kc-cells or early embryos showed a ring-like staining of the nuclear periphery reminiscent of the nuclear envelope. The results of an immunoblotting experiment using these monoclonal antibodies is shown in Fig. 1.

Bx34 antibody detects two polypeptides of 182K and 170K, respectively (Fig. 1, lane b; Table 1). To date we have identified three antibodies that by immunoblot analysis detect the same polypeptides as Bx34 antibody. In addition, there is one antibody (Bv38-ab) that in many respects has the same properties as Bx34-ab (see following sections and Table 1). However, this antibody is essentially negative on immunoblots.

The Bx95 antibody detects four major polypeptide bands 220K, 180K, 150K and 143K, and two minor bands, one in the molecular weight range near 300K, the other at 155K (Fig. 1, lane c; Table 1). In nuclei obtained from late embryos we observed an additional protein at 57K.

The T40 antibody, which was included as a control in our experiments, detects two proteins of 74 and 76K, respectively (see Smith & Fisher, 1984). These polypeptides have been designated *Drosophila* lamins Dm1 and Dm2 (Smith et al. 1987). In addition, the T40 antibody binds more weakly to polypeptides of lower molecular weights (60K, 52K, 50K; Fig. 1, lane d; Table 1). To date we have obtained 35 monoclonal antibodies that on immunoblot analysis detect the *Drosophila* lamins. The 3-5-H-2 antibody, which has been described by Fuchs et al. (1983), also belongs to this group.

The signals on immunoblots obtained both with Bx34 and Bx95 antibodies are of about the same intensity but significantly weaker than the signals obtained with the anti-lamin antibody. A multiple polypeptide band pattern was consistently obtained under a variety of conditions, even when proteins were prepared in the presence of protease inhibitors (1 mM PMSF, 1-5 mM N-tosyl-1-phenylalanine chloromethyl ketone (TPCK), 2.5 mM N-ethylmaleimide (MalNEt)) or when intact cells were lysed directly in electrophoresis sample buffer containing 6% SDS (data not shown).

The fate of the nuclear envelope antigens during oogenesis and early embryonic development

Early in the development the *Drosophila* embryo undergoes a series of rapid nuclear divisions (Zalokar & Erk, 1976) with an increasing demand for rapid delivery of the components of the nuclear envelope. We were interested in knowing how these antigens might be provided to the embryo during development. Therefore, we used indirect immunofluorescence and
immunobiochemical methods to detect and examine the distribution of the nuclear envelope antigens during oogenesis and early development.

In all cell types of the follicle, the Bx95 antibody shows a ring-like nuclear staining. This is demonstrated in Fig. 2a,b, which shows a follicle at about stage 9 of development (King, 1970). In addition, we also observed significant staining of the nurse cell cytoplasm. The Bx34 antibody stains the nuclear envelopes of all cell types of the follicle as well, there is no cytoplasmic staining detectable, but in addition there is a significant amount of antigen detected inside the nuclei of nurse and follicle cells (Fig. 2C,D).

Table 1. Antigens described in this study

<table>
<thead>
<tr>
<th>Names of antigens</th>
<th>$M_r$ ($\times 10^{-3}$)</th>
<th>Monoclonal antibodies</th>
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<tr>
<td>Bx34 antigens</td>
<td>182</td>
<td>Bx34</td>
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<td></td>
<td>170</td>
<td>Bx38</td>
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<td>150</td>
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<td></td>
<td>143; (weak: 300, 155)</td>
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<tr>
<td>T40 antigens</td>
<td>76</td>
<td>T40, T50, U25</td>
</tr>
<tr>
<td>(Drosophila lamins)</td>
<td>74; (weak: 60, 52, 50)</td>
<td>3-5-H-2</td>
</tr>
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<td></td>
<td></td>
<td>35 different</td>
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<td></td>
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<td>(Frasch, 1985, and</td>
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T40, T50 and U25, see Risau et al. (1981); for 3-5-H-2, see Kuo et al. (1982). The $M_r$ values were determined by immunoblotting using the marker proteins listed in Fig. 1. For the T40 antigens, Smith & Fisher (1984) reported $M_r$ values of 74 and 76K. In our laboratory a value of 80K was determined (Risau et al. 1981) due to the use of a different gel system and less appropriate molecular weight markers.

At early stages of follicle development the T40 antibody (anti-lamin) shows the typical ring-like staining of the nuclei of all cell types comprising the follicle. However, beginning with stage 4 of oocyte development, when the chromatin of the oocyte nucleus has condensed into the karyosome (King, 1970), the oocyte nucleus shows exceptional staining. The T40 antibody now stains both the nuclear envelope and the whole interior of the oocyte nucleus without any apparent substructures. In contrast, the nurse-cell and follicle-cell nuclei still show ring-like staining. This difference in staining remains throughout the following stages of follicle development as demonstrated in Fig. 3 for two consecutive sections of a follicle at stage 10 of development. Other anti-lamin antibodies show essentially the same staining.

During embryogenesis, the T40 antigen is present in the nuclear envelope from the earliest stages of development onwards. Yet in cleavage and early syncytial blastoderm stages the nuclear interior also appears to be stained. This staining is not seen at later stages (Fig. 4).

The Bx95 antibody also stains the nuclear envelope from the beginning of embryo development. In contrast, staining of the nuclear envelope by Bx34 is not detectable at early cleavage stages. However, at nuclear cycle 9 of embryo development, when the nuclei begin to migrate towards the embryo periphery (Zalokar & Erk, 1976), we observe a significant staining of the nuclear envelope, which reaches its final intensity in the early syncytial blastoderm (see also following sections).

Some of the antigens are present as maternally derived components

To obtain a more quantitative comparison of the amounts of antigens present at different stages of embryo development, we analysed the antigens on immunoblots (Fig. 5).

The Bx95 antigens are present in substantial amounts very early in embryo development. The total amount of these polypeptides begins to increase at about 2-3 h of development (blastoderm stage). In late embryos (12-20 h) we observed the appearance of a strong signal at 57K and a fainter additional band at 100K, respectively (Fig. 5A).

In contrast to the Bx95 antigens, the Bx34 antigens are not detectable at the beginning of embryogenesis (Fig. 5B, 0-5 h). In 1-hour cleavage embryos we detected only a faint signal of the polypeptide doublet at 175K. The amount of the antigens appears to increase substantially until 3-5 h of development and then remains approximately constant. (The additional band seen at 57K in Fig. 5B, lane 20 has not consistently been observed with the Bx34-ab, and probably results from a crosscontamination by the Bx95-ab during the washing steps.) This observation is consistent with the results obtained by indirect immunofluorescence (see preceding section).

We stained the same filters in the second step with T40 antibody (cf. Smith & Fisher, 1984). The amount of both T40 and Bx95 antigen clearly do not follow the exponential increase in number of nuclei generated during early embryo development. Thus it appears that substantial amounts of these proteins are maternally derived and stored in the embryo for their later use.

The behaviour of the antigens during the cell cycle is different

The usefulness of Drosophila embryos as a system for the study of distribution of specific proteins during mitosis has been discussed along with a detailed description of the mitotic behaviour of the Drosophila lamins (using 3-5-H-2-ab; Fuchs et al.). Here we
Fig. 2. Distribution of Bx95-ag and Bx34-ag in Drosophila follicles. Indirect immunofluorescence of cryosections of D. melanogaster follicles stained by Bx95-ab (A) and Bx34-ab (C). B, D. Corresponding DNA staining by Hoechst dye. ocn, oocyte nucleus; ncn, nurse cell nucleus; fcn, follicle epithelial cell nuclei. Bar, 50 μm.

Nuclear envelope antigens of Drosophila
Fig. 3. Distribution of T40-ag in Drosophila follicles. A,C. Indirect immunofluorescence of two consecutive cryosections of the same D. melanogaster follicle at stage 10 of development stained by T40-ab. Note the bright staining of the oocyte nucleus. B,D. Corresponding DNA staining by Hoechst dye. For abbreviations see legend to Fig. 2. Bar, 50 μm.
extend these studies in describing the distribution of the Bx95 and the Bx34 antigens throughout nuclear cycle 10.

At interphase the Bx95 antibody stains the nuclear envelope in a similar way to that reported for 3-5-H-2 antibody (Fuchs et al. 1983; and Fig. 6A,B). We observed a ring-like staining, which on closer inspection consists of granular staining material. In prophase (Fig. 6C,D) and during metaphase this structure is progressively broken down and becomes more diffuse in appearance. The bulk of the antigen remains in the area of the chromosomes (Fig. 6E,F). Early in anaphase the Bx95 antigen is primarily found in the centre of the spindle region. The staining of this region gets gradually less intense towards the spindle poles (Fig. 6G,H). During middle and late anaphase there is still a substantial amount of the antigen in a more condensed state found in the centre of the spindle region (Fig. 6I,K; see also inset showing a different plane of focus). Otherwise the staining becomes pro-

**Fig. 4.** Indirect immunofluorescence staining of embryonic nuclei of different developmental stages by T40-ab. Surface views of whole-mount embryos at nuclear cycle 12 (A) and at nuclear cycle 14 (C) of embryo development stained by T40-ab. B,D. Corresponding DNA staining by Hoechst dye. Bar, 20 μm.
Fig. 5. Immunoblot of nuclear envelope antigens at different times during early embryo development. Embryos were collected for the times listed at the bottom of each lane: 0–5, 0–0.5 h, cleavage stage; 1, 0.5–1 h, cleavage stage; 2, 1–2 h, syncytial blastoderm; 3, 2–3 h, cellular blastoderm and gastrulation; 4, 3–4 h, germ band elongation; 7, 4–7 h, germ band elongation; 12, 7–12 h, germ band shortening and segmentation; and 20, 12–20 h, head involution and dorsal closure. Each 120 μg of total embryo protein was separated on a 9–17% SDS-polyacrylamide gel, transferred to nitrocellulose and incubated with the antibodies as described in Materials and methods. The antibody incubation and detection was done sequentially in A using Bx95-ab followed by T40-ab; and in B using Bx34-ab followed by T40-ab. The polypeptide antigens detected by the different antibodies correspond to the bands included in brackets labelled Bx95, Bx34 and T40, respectively.

 progression similar to the staining observed for the 3-S-H-2 antibody (Fig. 6L,M; cf. Fuchs et al. 1983, fig. 3). The two daughter nuclei are covered by cup-like structures growing towards the centromere region (compare Fig. 6L–I) and the diffuse staining in the region between the separating nuclei is substantially reduced. Finally, in telophase both daughter nuclei are completely coated by the antigen in a ring-like fashion (Fig. 6N,O) and there is no antigenic material left in between. We observed a temporarily higher concentration of Bx95 antigen near the centromeres, which becomes evenly distributed along the nuclear rim during the following interphase.

As a representative of the Bx34 antibodies the Bv38-ab has been chosen. In indirect immunofluorescence it gives essentially the same results as the Bx34 antibody, with the advantage of having a somewhat better signal-to-noise ratio. Although not detected on immunoblots, the antigen detected by both antibodies is probably identical, since it also copurifies with Bx34-ag (antigen) on a SDS-hydroxyapatite column, as could be shown by a solid-phase assay (ELISA; data not shown). In interphase Bv38 antibody stains the nuclei in a very granular fashion (Fig. 7A,B). The bulk of these granules appears to be located at the nuclear rim. In addition, we find some less-intensely stained granules in the nuclear interior. The staining of both the nuclear envelope and the nuclear interior has also been observed in developing follicles and Kc-cells (see previous section). However, the data shown in Fig. 7 suggest that the bulk of the antigen is located in the nuclear envelope region (compare the distribution in number and intensity of granules in tangential and cross-sections of the nuclei shown in Fig. 7).

In prophase the circumferential staining shown by Bv38-ab is completely disrupted (Fig. 7C,D). Most of the granular material is now found inside the nuclear compartment. This is in contrast to the Drosophila lamins, which are excluded from the nuclear interior (Fuchs et al. 1983). Later the granular material is concentrated in the area of the metaphase plate (Fig. 7E,F) where it remains during the ensuing anaphase (Fig. 7G,H). It appears as if the granules were condensed around two centres with less staining material between them. Up to early anaphase the bulk of the antigen remains in this area, as judged by the unchanged intensity of the fluorescence signal in this region. However, later in anaphase the staining of this region gets less intense. Concomitantly, we observed granules stained by the antibody in the surrounding
cytoplasm. This is illustrated for late anaphase in Fig. 71, K. At this stage the bipartite nature of the central structure becomes readily apparent. At telophase most of the antigen is distributed as granular material in the cytoplasm. It seems as if most of the granules are located in the space between the daughter nuclei and the embryo surface. The Bx38 antigen is not assembled in the nuclear envelope before interphase of the following nuclear cycle (data not shown).

The antigens are enriched in a nuclear matrix–pore complex–lamina fraction

The results obtained in indirect immunofluorescence strongly suggested that the antigens described are components of the nuclear envelope. In order to test this by an independent method we prepared the nuclear matrix–pore complex–lamina fraction (NMPCL fraction) from Drosophila Kc cells according to established protocols (Fisher et al. 1982; therein cited as DSNF 1) and tested this fraction on immunoblots for the presence of the antigens. The results of this experiment are shown in Fig. 8. Comparable amounts of protein from whole nuclei, from the combined protein fractions removed during the preparation of the NMPCL fraction, and from the NMPCL fraction have been run on a polyacrylamide gel, transferred to nitrocellulose and probed with Bx34 and Bx95 antibodies. Clearly, both antigens are enriched in the NMPCL fraction and are not removed in significant amounts during preparation when compared with the whole nuclei used as a starting material. A large proportion of the Drosophila lamins are also present in the NMPCL fraction; however, substantial amounts are extracted during preparation, consistent with the observations made by Filson et al. (1985).

Discussion

Two different groups of polyepitide antigens have been detected by monoclonal antibodies as constituents of the nuclear envelope of D. melanogaster. The ring-like staining of the nuclei observed in indirect immunofluorescence strongly suggests that the bulk of the antigens is located at the nuclear periphery. The significance and function of a small amount of the Bx34 antigen found in the nuclear interior has yet to be established. However, antigens with similar distribution have been identified in the periphery and in the interior of mouse fibroblast cells (Chaly et al. 1984). The finding that these antigens are enriched in a nuclear matrix–pore complex–lamina fraction of Drosophila is consistent with the idea that these antigens are integral components of the nuclear envelope.

Polypeptide antigens and antibodies

Several high molecular weight polypeptides have been identified as constituents of the nuclear envelope of vertebrates and Drosophila. A glycoprotein with a molecular weight of 190K has been found to be a component of the nuclear pore complex in vertebrate nuclei (Gerace et al. 1982). In Drosophila, a corresponding glycoprotein of 188K (Filson et al. 1985) and a dATPase of similar molecular weight have been described by Berrios et al. (1983) as constituents of the NMPCL fraction. However, the Bx34 and Bx95 antigens are clearly distinct from both of these proteins. This was evident from immunoblot studies in which Bx34-ab and Bx95-ab have been tested in parallel with monoclonal antibodies against the glycoprotein (Filson et al. 1985) and an antiserum against the ATPase (the antibodies were kindly provided by Dr P. Fisher). Both the glycoprotein and the ATPase antigens migrate somewhat more slowly on SDS–polyacrylamide gels than the Bx34 polypeptides, and none of them co-migrates with one of the Bx95 polypeptides (data not shown). Antibodies have been described in rat liver cells, which show a punctate distribution in the nuclear envelope, reminiscent of the pattern observed with the Bx34-ab. One of these antigens is clearly different in molecular weight (Davis & Blobel, 1986). The other group of antigens (Snow et al. 1987), although only slightly different in molecular weight, appears to be different in immunofluorescence staining. Cytoplasmic dispersion of granular material during mitosis is much more prominent with these antigens when compared with Bx34-ag. The molecular weight reported for this group of antigens agrees even better with our estimates for the Bx95 polypeptides; however, the distribution of the Bx95-ag appears to be clearly different. Thus Bx34-ag and Bx95-ag represent two novel groups of nuclear envelope antigens.

The fact that each of the antibodies detects a set of polypeptide bands on immunoblots may be surprising and it is unlikely to result from in vitro degradation, since it was also observed using whole cells directly lysed in sample buffer or when nuclear proteins were treated with various protease inhibitors. In the case of the Drosophila lamins, the two major polypeptide antigens are apparently derived post-translationally from a single primary translation product (Smith et al. 1987). The additional bands of lower molecular weight might be other crossreacting proteins. Intermediate filament proteins with polypeptides of similar molecular weight have been found to be homologous to eukaryotic lamin proteins (McKeon et al. 1986).

At present we have no explanation for the double band pattern obtained with the Bx34 antibody and the even more complex pattern shown by the Bx95 antibody. Since we may exclude effects of in vitro degradation (see above), the antibodies either detect a structurally and functionally related family of high molecular weight proteins, or they bind to an epitope
Fig. 6. Fate of the Bx95 antigen during mitosis in early Drosophila embryos. A,C,E,G,I,L,N. Indirect immunofluorescence on cryosections of embryos in nuclear cycle 10 stained by Bx95-ab; B,D,F,H,K,M,O, corresponding DNA staining by Hoechst dye. A,B. Interphase; C,D, prophase; E,F, metaphase; G,H, early anaphase; I,K, mid-anaphase (inset in I shows a different focal plane); I,M, late anaphase; N,O, telophase. Bar, 10 μm.

common to otherwise unrelated proteins (see Snow et al. 1987). Antibodies against different epitopes of these proteins could help us to decide between the two possibilities.

We have independently obtained a number of monoclonal antibodies that react with the Drosophila lamins, suggesting that these proteins are highly antigenic. To date only a few antibodies have been obtained that detect the Bx34 antigen, and only one antibody detects the Bx95 antigen. Bv38 antibody may deserve special consideration, since in indirect immunofluorescence it shows essentially the same behaviour as the Bv38 antibody. In addition, both antigens copurify on SDS-hydroxyapatite columns, when tested by enzyme-linked immunosorbent assay (ELISA; data not shown). Yet the Bv38-ab is essentially negative on
Fig. 7. Fate of the Bv38 and Bx34 antigen during mitosis in early Drosophila embryos. A,C,E,G,I. Indirect immunofluorescence on cryosections of embryos stained by Bv38-ab; L, stained by Bx34-ab; B,D,F,H,K,M, corresponding DNA staining by Hoechst dye. A,B. Interphase stage 13; C,D, prophase stage 10; E,F, metaphase stage 10; G,H, anaphase stage 10; I,K, late anaphase stage 10; L,M, anaphase stage 10 stained by Bx34-ab. Bar, 10 μm.
Fig. 8. Enrichment of the nuclear envelope antigens in a subnuclear fraction of Drosophila (NMPCL). Subnuclear NMPCL fraction was prepared from Drosophila Kc cell nuclei, equal amounts of protein (75 μg/slot) of the starting Kc cell nuclear proteins (lanes a, b, e), the pooled wash fractions (lanes c, f) and the NMPCL fraction (lanes d, g, h) were separated on a 9–17% SDS-polyacrylamide gel, transferred to nitrocellulose and incubated with the antibodies as described in Materials and methods. Lanes a, c. Gel stained by Coomassie Brilliant Blue; lanes b, c, d, immunoblot stained by Bx34-ab, lanes e, f, g, immunoblot stained by Bx95-ab. The polypeptide antigens detected correspond to the bands labelled for Bx34 (•), Bx95 (○) and T40 (---), respectively.

immunoblots. We suggest that both antibodies detect different epitopes on the same antigen, the Bx38 epitope being sensitive to the immunoblotting treatment.

The antigens during development

The results of immunofluorescence and immunoblot studies indicate that both the Drosophila lamins and the Bx95 antigens are maternally transmitted to the early embryo. The large amount of lamin antigens that accumulated in the interior of the oocyte nucleus appears surprising. It is not known which of the described lamin polypeptide forms (Smith et al. 1987) accumulated. For technical reasons we could not decide whether this accumulation persists up to the stage of the mature egg, since at later stages the vitelline membrane is laid down and the oocytes become inaccessible to antibody staining by the whole-mount technique employed. In particular, we do not know how the T40 antigen is distributed following the breakdown of the nuclear envelope of the oocyte nucleus in the mature egg. However, the data are consistent with the idea that the intact oocyte nucleus is used as a storage vesicle for the T40 antigens. It is tempting to speculate that it would be used as vehicle to transport these maternally derived antigens into the embryos, to the site where they are concentrated for later use in the early rapid nuclear divisions. It should be mentioned that several other antigens of unknown function are also enriched to a similar extent in the oocyte nucleus (Frasch, 1985). We suggest a more general storage and transport function for the oocyte nucleus, which would be consistent with its extraordinary size compared to a normal somatic cell nucleus. In Xenopus, the oocyte nucleus is also exceedingly large and serves as a store for nuclear protein (Dreyer et al. 1981; Kleinschmidt & Franke, 1982). In this case, however, the lamin proteins are stored as constituents of the nuclear envelope of the germinal vesicle (Stick & Krohne, 1982; Benavente et al. 1985). Thus, in different organisms there exist different mechanisms for the storage and maternal transmission of the lamin proteins.

From fertilization to cellular blastoderm the combined surface area of nuclei increases several thousand-fold. If all the maternally derived lamins were contained in the nuclear envelope during that time, the nuclear envelopes of early embryos should contain a large excess of the T40 antigen when compared with blastoderm nuclei. However, this is not the case, since the staining of early embryo nuclear envelopes is of about the same intensity as of those at blastoderm. The staining of the nuclear interior at early stages, which later decreases towards the cellular blastoderm stage, may account to some extent for this extra amount of antigen. Yet in the early embryo a significant amount of the T40 antigen could be stored in the cytoplasm. This would be difficult to detect by immunofluorescence if it were evenly distributed in a large cytoplasmic volume.

The Bx95 antigens also appear to be maternally derived. In follicles the staining of the nurse cell cytoplasmic compartment indicates that the antigens are synthesized by the nurse cells and transported to the oocyte cytoplasm where they may be stored for later use. In embryos up to early syncytial blastoderm the increase in total amount of the antigens detected on immunoblots does not correlate with the large increase in the combined surface area of the nuclei. The intensity in immunofluorescence staining of early and late nuclear envelopes by Bx95-ab remains approxi-
mately constant and there is no Bx95 antigen detected in the nuclear interior. Therefore, we suggest that in early embryos the extra amount of Bx95 antigen might be stored in the cytoplasm.

The Bx34 antigens on the other hand appear to be synthesized by the developing embryo. In follicles there is neither prominent staining of the oocyte nucleus nor significant staining of the cytoplasm. In embryos, the antigens are not detectable on immunoblots at the earliest time point tested (however, due to the limited sensitivity of our assay the synthesis of Bx34-ag could already have been started). They begin to appear at early syncytial blastoderm and increase in amount up to cellular blastoderm formation. This is consistent with the observations made by indirect immunofluorescence and would be expected if the amount of the antigen were to increase in parallel with the number of nuclei.

Mitosis

The mitotic behaviour of the Drosophila lamins has been described (Fuchs et al., 1983). It has been suggested that during the rapid divisions in the early Drosophila embryo these antigens are never completely dispersed throughout the embryo cytoplasm. This also seems to be true for the Bx34 and Bx95 antigens, although each of them appears to rely on a different mechanism.

In contrast to the T40 antigens, the Bx34 antigens enter the former nuclear interior upon breakdown of the nuclear envelope. (The term Bx34 has been used interchangeably with Bv38-ab. However, both antibodies are very similar in indirect immunofluorescence and may detect the same antigen.) During metaphase they are concentrated in the chromosomal area and later during anaphase they appear to be confined to the centre of the spindle region. The bipartite nature of their distribution in this region could be explained by their location around the dense stem body (Stafstrom & Staehelin, 1984) from which these antigens might be excluded. The reassembly of Bx34-ag in the nuclear envelope between late anaphase and early interphase is still not fully understood.

It is interesting that the Bx95 antigen follows a mitotic disassembly-reassembly process, which appears to be a combination of those employed by the lamins and the Bx34 antigens. Following the breakdown of the nuclear envelope, the antigen starts to accumulate in the nuclear interior during metaphase with the highest concentration around the centre of the spindle (like Bx34-ag). During anaphase it starts to envelope the chromosomes in a way typical of Drosophila lamin antigens (Fuchs et al., 1983). Thus, one could speculate that there exist some underlying basic transport mechanisms that are used to various extents by passenger molecules in order to reach their final destinations. This speculation is strengthened by the observation that several other nuclear antigens of unknown function show a similar distribution in the spindle area during mitosis (Frasch et al., 1986). One mechanism could be their adherence to the spindle fibres, in order to be maintained in the nuclear region and then to be transported to the daughter nuclei; another could involve adherence to and the subsequent migration along the retracting chromosome arms as a means of transport. Stafstrom & Staehelin (1984) identified in an electron-microscopic study a double-layered envelope structure persisting throughout mitosis of blastoderm nuclei. Clearly, none of our nuclear envelope antigens is continuously associated with such a spindle envelope (Figs 6, 7). Rather, this spindle envelope might border the compartiment around the chromosomes, in which the Bx34 and Bx95 antigens are located from prophase and metaphase onwards. It is interesting that the disappearance of the Bx95 antigens and their reappearance in an envelope structure at late anaphase have similar kinetics to those that these authors found for the nuclear pore complexes. Although it may be difficult to correlate the antigens with distinct substructures in the nuclear envelope using indirect immunofluorescence, the antibodies may be useful tools for studies at the electron-microscopic level.

We thank Dr F. Bonhoeffer and Dr D. M. Glover for helpful suggestions and reading the manuscript. Technical assistance by G. Thoma and M. Wild and help by K. Ralitsky in typing this manuscript are also gratefully acknowledged.

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(Received 4 January 1988 – Accepted 24 February 1988)