# Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study

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## Summary

In mouse embryos, acquisition of the nuclear lamin polypeptides A/C varies according to developmental stage and tissue type. In order to determine the precise time points and cell types in which lamin A/C are first observed, we have used two monoclonal antibodies in immunofluorescence studies of different tissues of developing mouse embryos and of young mice. One antibody (mAB346) is specific for lamins A and C, while the other (PKB8) detects lamins A, B and C. Dividing uterine development into three phases – germ layer formation, organogenesis and tissue differentiation – our results show that lamin A/C expression in the embryo proper is not observed until the third phase of development. Lamin A/C first appears at embryonic day 12 in muscle cells of the trunk, head and the appendages. Three days later it is also seen in cells of the epidermis where its appearance coincides with the time of stratification. In the simple epithelia of lung, liver, kidney and intestine, as well as in heart and brain, lamins A/C do not appear until well after birth. Embryonal carcinoma (EC) cells express lamin B but not lamin A/C. Lamin A/C expression is noted in some EC cells after they are induced to differentiate and in several differentiated teratocarcinoma cell lines. Our results suggest that commitment of a cell to a particular pathway of differentiation (assayed by cell-type-specific expression of intermediate filament proteins) usually occurs prior to the time that lamin A/C can be detected. Thus lamin A/C expression may serve as a limit on the plasticity of cells for further developmental events.

Key words: lamins A/C, mouse embryogenesis, tissue differentiation, embryonal carcinoma cells.

## Introduction

The nuclear lamina is a protein meshwork lining the nucleoplasmic face of the inner nuclear membrane. It consists of one or more related structural proteins, the lamins, which may play a role in the organization of nuclear architecture (Gerace, 1986). In mammalian somatic cells three major lamins – A, B and C – are clearly distinguished (Gerace & Blobel, 1980), and there is some evidence for additional minor lamin polypeptides in birds (Lehner et al. 1986). As mammalian lamins A and C differ only in their carboxy-terminal tail domain they could arise from the same transcript by differential splicing (McKeon et al. 1986; Fisher et al. 1986). Mammalian lamin B segregates differently from lamins A/C in mitosis and, as recently shown for Xenopus (Krohne et al. 1987), is the product of a distinct gene. All lamins display the domain organization found in the structural proteins of the various cytoplasmic intermediate filaments (IF) (McKeon et al. 1986; Fisher et al. 1986 for lamins, Geisler & Weber, 1982 for IFs) and at least detergent-extracted nuclear envelopes of Xenopus oocytes reveal a tetragonal mesh of IF in electron micrographs obtained after metal shadowing (Aebi et al. 1986).

Different gene programs regulate the expression of the multiple nuclear lamins (Benavente et al. 1985; Stick & Hausen, 1985; Stewart & Burke, 1987; Lehner et al. 1987) and the various cytoplasmic IF proteins (see for instance Osborn et al. 1985; Quinlan et al. 1985). Cell-type-specific expression of different lamins was first noted in Xenopus (for review see Krohne & Benavente, 1986). In the early mouse embryo from the blastocyst stage till embryonic day 8, the embryo proper seems to express only lamin B (Stewart & Burke, 1987). In the chick embryo, lamin A acquisition has been monitored by Lehner et al. (1987). Although a detailed study was not performed, they clearly document that during embryonic development lamin A becomes increasingly prominent with the extent and developmental timing of this change showing pronounced tissue-specific variation. The aim of our study was to try to define the precise time points and the cell types where lamin A/C is observed in different tissues of the mouse embryo and the young animal.

## Materials and methods

### Gel electrophoresis and immunoblotting

The crude lamin preparation and the cell extracts were analysed by one-dimensional polyacrylamide gel electrophoresis on 10% gels. For two-dimensional gel electrophoresis, proteins were first separated by nonequilibrium pH gradient electrophoresis (NEPHGE) using ampholines in the pH range 3·5–10 (LKB, FRG). 10% gels were used in the second dimension. For immunoblotting polypeptides were electrophoretically transferred to nitrocellulose sheets. After staining with Ponceau S, the positions of the major spots or bands were marked with a needle. Immunoblotting was by standard procedures using peroxidase-labelled second antibodies.

### Embryos and tissues

The embryos were from spontaneous matings of NMRI mice. The presence of a vaginal plug indicating a successful mating was regarded as embryonic day 0. At day 8 and on subsequent days, mice were killed by cervical dislocation. The uterine horns were removed and dissected. At day 9, embryos together with their decidua were placed on dry ice and frozen. From day 10 to 17, only the embryo itself was frozen on dry ice. Individual organs (brain, heart, lung, liver, kidney, intestine, skeletal muscle) were dissected from postnatal day 1 (newborn), 5, 10, 15, and from adult mice. These tissues were frozen by immersion in isopentane cooled with liquid nitrogen to −140°C. Embryos and tissue samples were stored at −70°C. 5 μm thick transverse or longitudinal cryostat sections were prepared. After air-drying they were processed for indirect immunofluorescence microscopy as described below.

### Cell lines

The origin of embryonal-carcinoma-(EC) and teratocarcinoma-derived cell lines used in this study, as well as the growth media have been described previously (Paulin et al. 1982 and Table 2). The EC cell lines were induced to differentiate by allowing cells to attach to 12 mm glass coverslips. Retinoic acid was added the next day (final concentration $5 \times 10^{-7}$ M) and dibutyrylcyclic-AMP after a further 48 h (final concentration 1 mM).

RMCD cells are a fibroblast-like line derived from rat mammary tissue. CHO are Chinese hamster ovary cells. HK22 is a mouse cell line derived from the LDBH line originating from a spontaneous lymphoma (Holtkamp et al. 1978). HS27 is a human fibroblastic line derived from a human mammary carcinoma.

### Antibodies

#### (1) Lamins

The mouse monoclonal antibody PKB8 recognizing lamins A, B and C in immunoblotting has been described (Krohne et al. 1984; Osborn & Weber, 1987). Lamin antibody 346 is described below. The lamin-B-specific rabbit antibody was a kind gift of Dr Robert Shoeman and Dr Peter Traub (Traub et al. 1988).

#### (2) Intermediate filament proteins

A polyclonal guinea pig antibody specific for vimentin was used (Osborn et al. 1980). Keratin filaments were positively identified using either the rat monoclonal antibody Troma 1 (Brûlet et al. 1980) or the mouse monoclonal antibodies LE41 or LE61 (Lane, 1982). Troma 1 was a kind gift from Dr R. Kemmler. LE41 and LE61 were kindly provided by Dr Birgit Lane.

#### (3) Titin

This rabbit antibody (503) was raised against titin purified from chicken skeletal muscle (Fürst et al. 1988).

#### (4) Section antibodies

FITC-labelled goat anti-mouse IgGs, FITC-labelled goat anti-rat IgGs, and rhodamine-labelled goat anti-rabbit IgGs (Cappell Laboratories, Cochraneville, PA, USA) were used at a dilution of 1:40–1:60 (approximately $0.5$ mg ml$^{-1}$).

### Immunofluorescence microscopy

Tissue sections and cells grown on coverslips were first fixed at −10°C for 5 min in methanol, then for 10 min at −10°C in acetone and then air-dried (Krohne et al. 1984). Specimens were incubated at 37°C for 45 min with the primary antibody. Subsequently they were washed three times in phosphate-buffered saline (137 mM-NaCl, 7 mM-Na$_2$HPO$_4$, 1·5 mM-KH$_2$PO$_4$, 2·7 mM-KCl pH 7·1) and then incubated with the appropriate rhodamine- or fluorescein-coupled second antibody. After a further wash with phosphate-buffered saline, cells and sections were stained for 10 min at room temperature with the Hoechst dye no. 33258 to visualize nuclei. Samples were mounted without further washing with Mowiol 4.88 (Hoechst AG, Frankfurt, FRG).

### Immunoperoxidase

The procedure was as above for fluorescence, except that peroxidase-labelled rabbit anti-mouse IgGs (Dako, Klostrup, Denmark) were used as the second antibodies.

## Results

### Isolation and characterization of lamin monoclonal antibodies

A crude nuclear lamin fraction was prepared from rat liver using the treatments with DNAse, Triton-X-100 and high salt described by Dwyer & Blobel (1976). BALBc mice were immunized by standard procedures and the murine myeloma line PAI was used as fusion partner. Interesting hybridoma clones were identified by using aliquots of the culture supernatant in immunofluorescence microscopy on rat RMCD cells. Hybridomas revealing the peripheral nuclear staining characteristic of lamins were subcloned.

Lamins A, B and C of mammalian cells differ in molecular mass and in isoelectric point (for review see Krohne & Benavente, 1986). Monoclonal antibodies were characterized by immunoblotting of a rat nuclear fraction separated by one- and two-dimensional gel electrophoretic systems. A typical two-dimensional polypeptide profile of a crude rat liver lamin fraction is shown in Fig. 1A. Coomassie blue staining shows the characteristic lamin profile with lamins A and C in a more basic position than lamin B. In the corresponding immunoblot, mAB346 reacted strongly with lamins A and C and not with lamin B (Fig. 1B). In contrast PKB8, which was used as a complementary probe to mAB346, reacted with all three lamins (Fig. 1C see also Krohne et al. 1984; Osborn & Weber, 1987). It reacts strongly with lamin A as well as with lamin B and shows a somewhat weaker reaction with lamin C. Dot blots were performed on lamin A/C and on lamin B purified
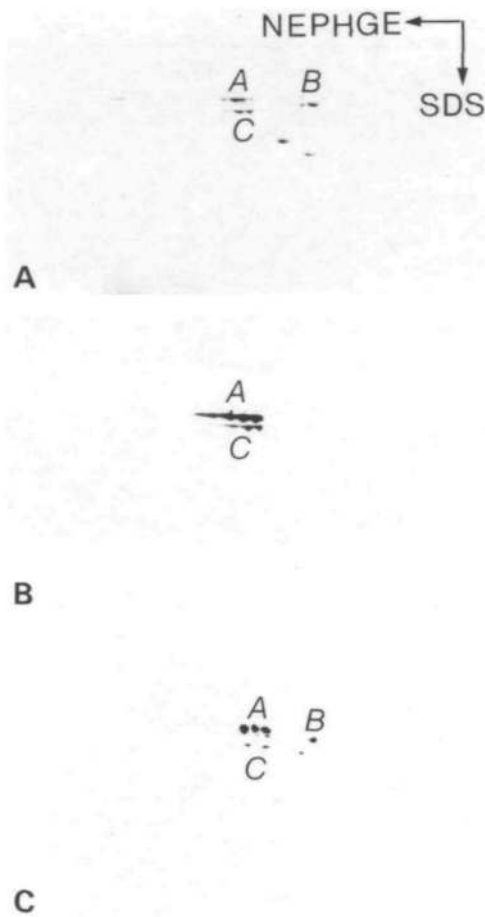
**Fig. 1.** A–C. Two-dimensional (2D) blots of rat lamins with mAB346 and PKB8. (A) Coomassie Blue staining of a crude lamin fraction; (B,C) immunoblots of the same preparation with mAB346 hybridoma supernatant (B), with PKB8 hybridoma supernatant (C) using the immunoperoxidase technique. mAB346 reacts only with lamins A and C while PKB8 reacts with all three lamin polypeptides.

from rat liver and kindly provided by Dr S. Georgatos, Rockefeller University. mAB346 reacted only with the lamin A/C fraction and not with lamin B, while PKB8 reacted with all three lamins (data not shown).

Although the mAB346 clearly recognizes several lamin A/C isotypes, we cannot exclude the possibility that it does not recognize all A/C like isotypes during development. In particular we have not investigated its reaction on embryos before day 9. We also cannot exclude that epitope masking could occur (cf. failure to stain lamins in several cell types in immunofluorescence with the anti-IFA antibody in Osborn & Weber, 1987). However, we stress that where immunofluorescence and immunoblotting have been performed on the same specimens (see below, and Lehner *et al.* 1987) they seem to yield equivalent results. It should be noted that as mAB346 detects both lamins A and C we cannot distinguish, short of immunoblotting, whether a positive cell type expresses one of the two lamins preferentially or both. This has been indicated by the use of the

term 'lamins A/C'. A potential uncertainty exists concerning lamin B expression. As long as a cell displays no reactivity with mAB346 the reaction with PKB8 establishes the presence of lamin B, since PKB8 detects all three known lamins. However, once cells express lamins A/C, reactivity with PKB8 does not prove lamin B expression. In some instances, we have therefore additionally used a rabbit antibody specific for lamin B (Traub *et al.* 1988).

*Lamin expression during mouse embryonic development*

The gestation period of mice can vary from 18 to 21 days, but it is usually 19 days. Intrauterine development falls into three consecutive phases, each lasting about 6 days: (a) from fertilization until germ layer derivation; (b) basic organogenesis and (c) tissue differentiation (Rugh, 1968). Immunofluorescent studies on lamin expression were conducted on cryostat sections of whole embryonic mice from day 9 until birth and of single organs from 5-, 10- and 15-day-old animals. Organs from adult mice were also examined. The immunofluorescence studies with mAB346 summarized in Table 1 suggest that lamin A/C expression in the embryo proper is not seen until the third phase of intrauterine development and that in some organs lamins A/C do not appear until well after birth. However, once a tissue has turned on lamin A/C expression it continues to express lamin A/C at later developmental stages. In contrast mABPKB8, which reacts with lamins A, B and C in immunoblots, faithfully detects the nuclei of cells in the same organs at all stages of development as monitored by the DNA-specific stain Hoechst 33258.

*Embryonic days 9, 10, 11*

Organogenesis becomes greatly accelerated at $8\frac{1}{2}$ to 9 days. At day 9, the embryo has 9 to 20 somites. A transverse section is shown in the sketch in Fig. 2E. The embryo proper is surrounded by membranes. When sections through the day-9 embryo proper and its surrounding membranes are stained with the mAB346 the most prominent feature is the strong positivity displayed by nuclei of cells in the decidua and the trophoblast. This is illustrated by the sections shown in Fig. 2A–D which are taken from the area enclosed by the box in Fig. 2E and include the otic vesicle, a characteristic feature of this stage of development. Nuclei of cells in the decidua and trophoblast show a classic lamin stain (Fig. 2A). The majority of yolk sac nuclei at days 9 and 10 remains negative with mAB346, although we could not exclude that an occasional nucleus of this special endoderm displayed a weak reactivity. In contrast nuclei in the embryo proper (Fig. 2B) show no staining by mAB346, although all such nuclei are stained with both the PKB8 antibody (Fig. 2C) and with the Hoechst DNA stain. Interestingly, staining of the embryonic tissues by PKB8 seems somewhat weaker than in the corresponding decidua, perhaps because tissues of the embryo proper have only lamin B at this stage, whereas the

decidua has in addition lamin A/C. At day 10, even though the embryo has 26 to 28 somites and formation of the gastrointestinal tract, heart, brain and sense organs is occurring, still only the trophoblast and decidua are positive with mAB346 (Table 1). At day 11 and on subsequent days, only the embryo proper was tested with mAB346. At this stage, the thyroid, meso- and metanephros are in an early stage of formation. Diffuse staining in the cytoplasm of a few mesenchymal cells and of a few presumptive myoblasts in limb buds (see below) was seen.

## Embryonic days 12, 13, 14

By day 12, the embryo has 43 to 48 somites and in a transverse section through the heart region resembles the sketch in Fig. 2F. Development of organs such as heart, esophagus, trachea and spinal cord continues. Examination of Table 1 shows that it is at this time that the first nuclei in the embryo proper become positive with mAB346. Most prominent are certain cells in the limb buds, (Fig. 2G,H) in the trunk and in the region where later the eye muscle will form. Only a minority of nuclei in these regions show a classic lamin pattern when assayed with the mAB346. As shown below these nuclei probably belong to myoblasts. Very occasionally

**Fig. 2.** A–H. Immunofluorescence microscopy with different lamin monoclonal antibodies, or with Hoechst stain on sections of 9-day (A–D) and 12-day (G,H) mouse embryos. Sketches of the approximate developmental stage for the 9 day (E) and a transverse section through the heart region for the 12 day embryo (F) are shown for comparison (modified from Rugh 1968). (A–D) Sections in the region of the otic vesicle (box in E). A shows strong staining of decidua and trophoblast cells with mAB346 specific for lamin A/C. (B) No reaction of the embryo proper is seen with mAB346. (C) In contrast, nuclei in the embryo proper are stained with the PKB8 antibody. (D) Overview of embryo in its decidua stained with Hoechst to show nuclei. d, decidua; t, trophoblast; my, myelencephalon; ov, otic vesicle. (G) Immunofluorescence microscopy with mAB346; lamin-A/C-positive cells in myoblasts in the forelimb of a 12-day mouse embryo. (H) Counterstain of the same section as in G with Hoechst to show nuclei. Note that at this stage in development only a very few cells are positive with mAB346. Magnifications A–C,G,H ×250; D ×60.

additional single cells in the mesenchymal tissue are positive (<1 %). Very weak staining of nuclei in cells of large vessels including aorta is also seen at day 12. At day 13, development proceeds to the 49- to 60-somite stage; a transverse section through the optic region is shown in Fig. 3A. At day 13, the same cell types are

**Table 1.** *Lamin A/C expression as judged by positive staining with mAB346*

| | Embryonic day | | | | | | | | | Postnatal day | | | | |
| | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 1 | 5 | 10 | 15 | Adult |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Decidua | +++ | +++ | | | | | | | | | | | | |
| Trophoblast | +++ | +++ | | | | | | | | | | | | |
| **EMBRYO PROPER** | | | | | | | | | | | | | | |
| Mesenchyme/ | − | − | d | s | s | m | +++ | +++ | +++ | | | | | |
| Connective tissue* | | | | | | | | | | +++ | +++ | +++ | +++ | +++ |
| **Muscle†** | | | | | | | | | | | | | | |
| Appendages† | − | − | d | m | +++ | +++ | +++ | +++ | +++ | | | | | |
| Trunk† | − | − | − | m | +++ | +++ | +++ | +++ | +++ | | | | | |
| Eye muscle† | − | − | − | m | +++ | +++ | +++ | +++ | +++ | | | | | |
| Skeletal muscle | | | | | | | | | | +++ | +++ | +++ | +++ | +++ |
| Heart | − | − | − | − | − | − | − | − | − | − | m | ++ | +++ | +++ |
| **Vessels‡** | | | | | | | | | | | | | | |
| Umbilical | − | − | − | + | + | + | + | ++ | ++ | | | | | |
| Aorta | − | − | − | (+) | + | + | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| **Epithelia** | | | | | | | | | | | | | | |
| Epidermis | − | − | − | − | − | d | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Tongue | | | | | | | | | ++ | | | | | ++ |
| Intestine | − | − | − | − | − | − | − | − | − | − | − | − | − | +§ |
| Liver | − | − | − | − | − | − | − | − | − | − | m | +++ | ++ | +++ |
| Lung | − | − | − | − | − | − | − | − | − | m | +++ | +++ | +++ | +++ |
| Kidney tubule | − | − | − | − | − | − | − | − | − | − | m | ++ | +++ | |
| Nasopharynx | − | − | − | − | − | − | − | − | − | − | m | + | ++ | |
| Brain | − | − | − | − | − | − | − | − | − | − | m | m | +++ | +++ |
| Spleen | | | | | | | | | | | | | m | m |
| Thymus | | | | | | | | | | | | | m | m |
| Bone marrow | | | | | | | | | | | | | | m |

Symbols used in Table 1 refer to the staining patterns seen with mAB346: −, no staining seen; d, diffuse staining usually confined to the cytoplasm of a minority of cells; s, occasional cells or m, a minority of cells (usually 20–50 %) or + → +++ almost all cells giving a characteristic lamin pattern. (+), +, ++ and +++ refer to the intensity of staining seen with mAB346 i.e. (+) very weak, +++ very strong. All the cell types marked with − in the table in addition stain positively with PKB8 and therefore contain lamin B. Cell types marked with other symbols also stain positively with PKB8 but because they contain lamin A/C such staining does not necessarily imply lamin B positivity. *Connective tissue restricted to newborn/adult mouse, † up to day 13 myoblasts; after day 13 myotubes; ‡ vascular smooth muscle cells; § in adult intestine not all epithelial cells are positively stained by mAB346.
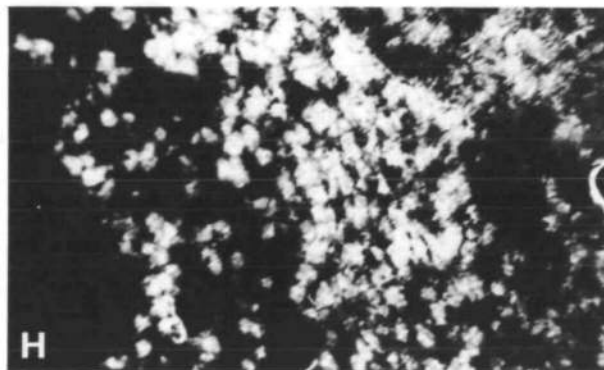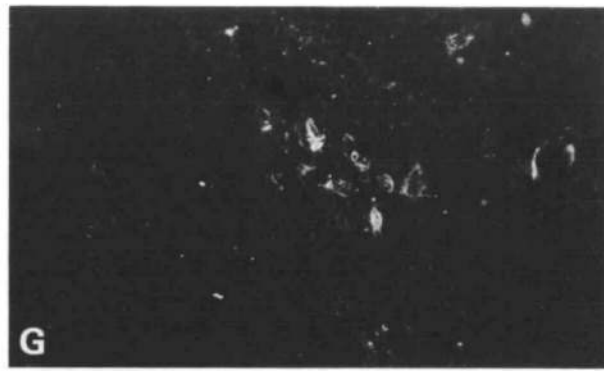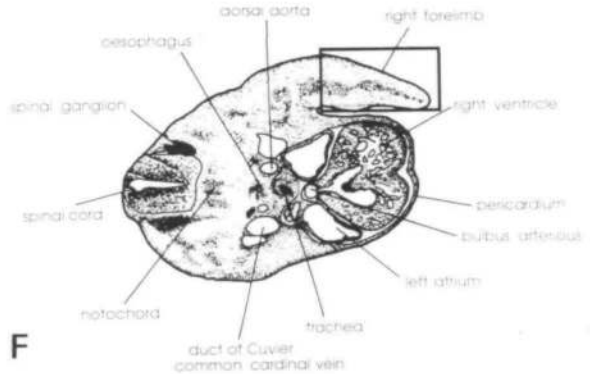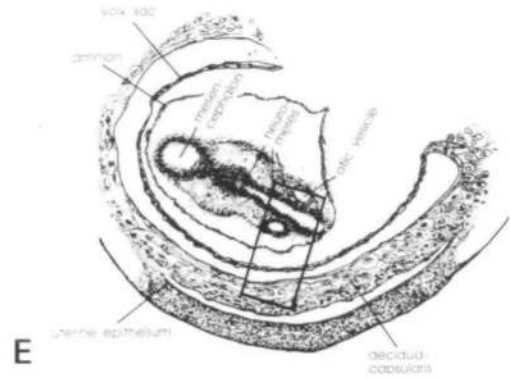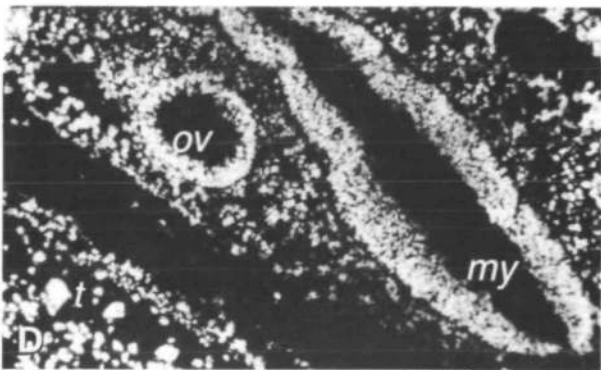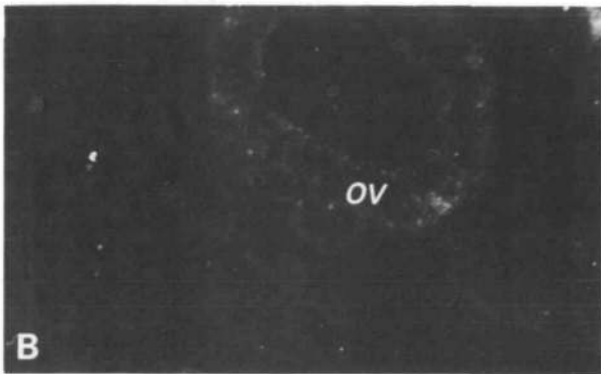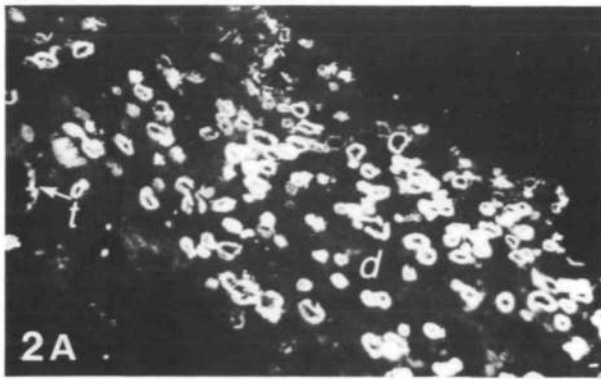
stained by the mAB346 as on earlier days (see above and Table 1). Such cells can be positively identified with a rabbit antibody specific for the high molecular weight protein titin. Titin antibodies, specific for sarcomeric muscle (Hill & Weber, 1986; Fürst *et al.* 1988) decorate structures in the cytoplasm of the presumptive eye muscle cells (Fig. 3D). A careful comparison of Fig. 3B, stained with mAB346, and Fig. 3D, which shows the same section stained with the titin antibody in double label immunofluorescence microscopy, confirms that lamin-A/C-positive nuclei belong to cells committed to skeletal muscle differentiation. Thus these lamin-A/C-

positive cells are titin positive but not all titin-positive cells present in this section are lamin A/C positive. These lamin-A/C-positive cells are probably early myotubes. Even though in the 13-day embryo the circulation is largely formed and the heart started to beat on day 9, cardiac muscle cells in the 13-day embryo remain negative for mAB346. All epithelial cells tested in Table 1 as well as the different cell types of the brain are also lamin A/C negative at day 13. At day 14, no striking changes in lamin A/C distribution were detected with mAB346. The number of 'mesenchymal' cells stained with mAB346 increases as does the number of muscle cells. A diffuse stain in the cytoplasm of a few cells in the single-layered epidermis is additionally seen.

### Embryonic day 15

At this stage, somite formation is largely complete (64–65 somites) but differentiation of tissues and organs continues. The striking feature of the lamin A/C distribution in the 15-day embryo is that, for the first time, a lamin pattern is seen in an epithelial cell type with the mAB346. As shown in Fig. 3F, relatively weak staining of the epidermis is seen with the lamin-A/C-specific antibody. In the example given in Fig. 3F, the staining of the epidermal cells is still weaker than of mesenchymal cells and of the muscle cells identified by the titin stain in Fig. 3H. Interestingly, the different cell types present in the epidermis show different degrees of positivity with mAB346. The cells in the periderm or outer layer are more strongly stained than those in the basal layer. The central cells of the epidermal primordia, which have just begun to develop, are not stained by mAB346 (Fig. 3F), while in a parallel section incubated with antibody PKB8 such cells showed clear lamin staining (Fig. 3G). Nuclei in other epithelia such as intestine, liver and lung do not stain with mAB346. Cell types positive at earlier stages with mAB346 remain positive while nuclei in heart and brain remain negative for lamin A/C.

### Embryonic days 16 and 17

Somite formation is complete. The differential staining patterns seen with mAB346 do not change appreciably at days 16 and 17. Nuclei in skeletal muscle as well as mesenchyme and epidermis show characteristic lamin-staining patterns seen also at earlier stages with mAB346. Tongue epidermis is also positive for mAB346 at day 17. Although we have not investigated mAB346 staining of tongue at earlier days, it is interesting that a second stratified epithelium is positive at day 17, a time when several simple epithelia tested (intestine, liver, lung, and kidney tubuli) as well as the nasopharyngeal pseudostratified epithelia remain negative. Heart and brain show no staining with mAB346 at day 17.

### Postnatal day 1

No strong change in the mAB346 patterns takes place at birth. At postnatal day 1, a few cells in the bronchial epithelium stain for the first time positively. Brain still remains negative as can be seen by the immunoperoxi-

Fig. 3. A–H. Immunofluorescence with different lamin antibodies, titin antibodies or with Hoechst stain on tissues of 13-day (B–E) or 15-day (F–H) mouse embryos. A schematic drawing of a transverse section through the optic region of the 13-day mouse embryo is shown in A for comparison (modified from Rugh, 1968). B–E show sections through the region shown in the box in A. B Some cells are positive with mAB346 and therefore express lamin A/C. In contrast not only these cells but also all the surrounding cells are decorated by PKB8 in a parallel section. D identifies the lamin-A/C-positive cells in B as myoblasts by the use of a rabbit titin antibody in double label immunofluorescence microscopy on the same section as B. E shows a section stained with Hoechst to reveal nuclei photographed at lower magnification. em, eye muscle; r, retina. (F–H) Epidermis stained with either mAB346 (F) PKB8 (G) or rabbit titin antibody (H). F and H show a double label on the same section, G staining of an adjacent section. Nuclei of all cells present are positive with PKB8. While nuclei of mesenchymal cells and of the titin-positive muscle cells are strongly stained with mAB346, nuclei of epidermal cells are more weakly stained. The most differentiated outer layer of the epidermis is relatively strongly stained, the basal layer less strongly while cells of the epidermal primordia are not stained. e, epidermis; p, primordia. Magnifications: B–D,F–H ×250; E ×60.

dase stain of a longitudinal section of the upper half of a postnatal day-1 mouse shown in Fig. 4A. By contrast, other tissues present in the same section such as epidermis and skeletal muscle are strongly positive. A comparison of mAB346, PKB8 and Hoechst staining in the brain of a 1-day-old mouse is shown in Fig. 4B–D. Whereas PKB8 stains the nuclei of all cells in brain and the adjacent connective tissue, mAB346 revealed only the nuclei in the connective tissue cells and not in the brain proper. Since a detailed study of the different regions of brain was not done we cannot exclude that certain specialized cell types present in particular areas of the brain at low frequency might show lamins A/C already at this stage of development.

### Postnatal days 5–20

At postnatal day 5, certain tissues previously negative for lamins A/C begin to show staining with mAB346. Thus a minority of cells in liver, kidney tubuli and nasopharynx now show a lamin pattern with mAB346. A similar phenomenon is seen in heart and brain, where the first lamin-A/C-positive cells are recognized at postnatal day 5. Assay of the same organs at postnatal days 10 and 15 shows that by postnatal day 15 almost all cells in heart as well as in different epithelial tissues listed in Table 1 with the exception of intestine now express lamins A/C. In brain still only a minority of cells express lamin A/C at postnatal day 10 but almost all cells do so by postnatal day 15. In the intestinal epithelium, lamin A/C positivity was first detected in some cells at postnatal day 20. Thymus and spleen were examined at postnatal day 15. Only a minority of cells in these complex tissues were stained by the mAB346 at postnatal day 15.

3A
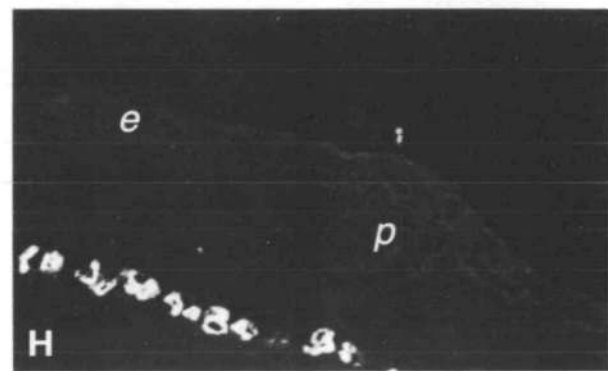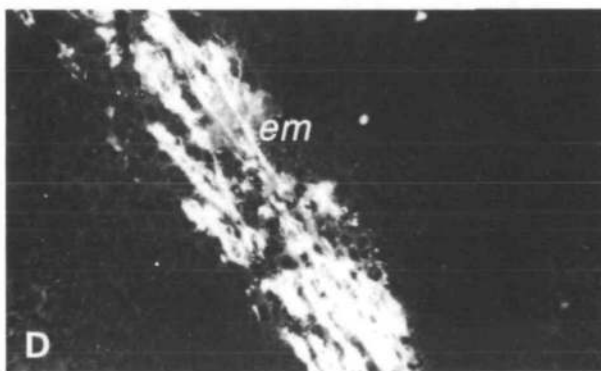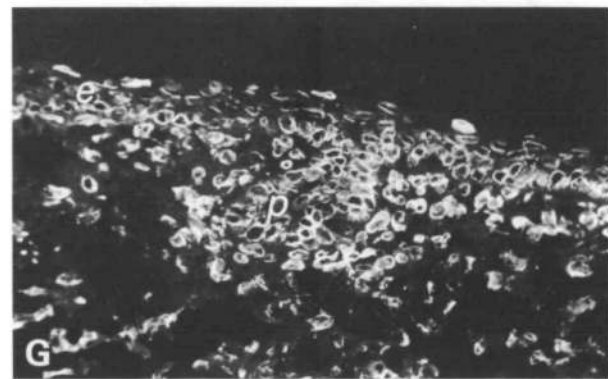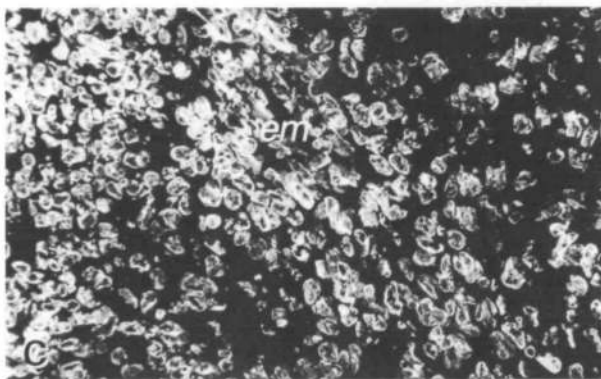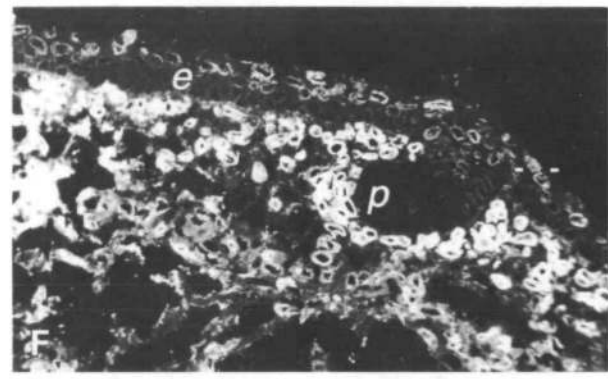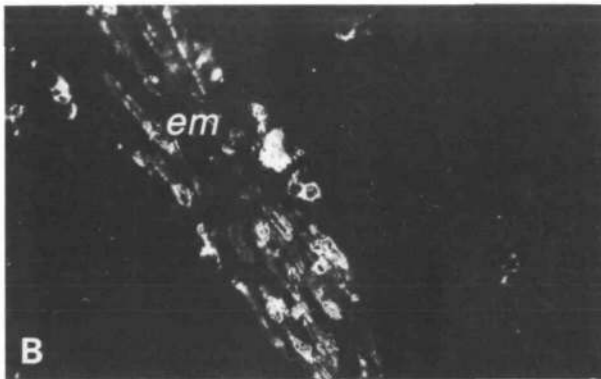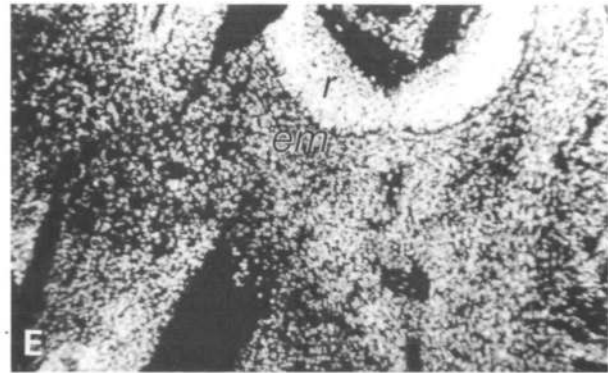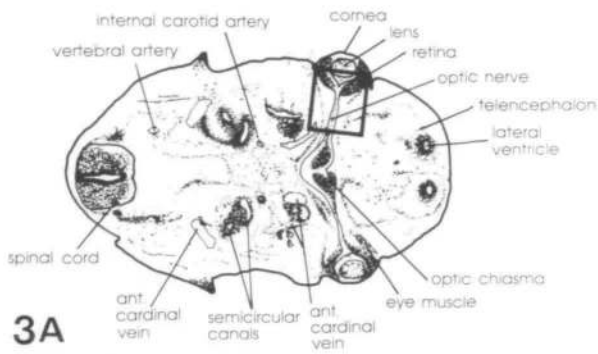




*Adult*

In the adult mouse, lamins A/C were assayed in all tissues listed in Table 1. In heart (Fig. 4E), muscle (Fig. 4F), lung (Fig. 4G), liver (Fig. 4H) and brain (Fig. 4J), almost all nuclei were lamin A/C positive. In intestine (Fig. 4I), the percentage of positive nuclei, as well as the intensity of staining with mAB346 was greater than in the 20-day-old mouse. However, decoration of the epithelial cell nuclei in adult intestine was always much weaker than that seen in the other cell types present in the same section (Fig. 4I). Thymus and spleen continued to show large numbers of cells that
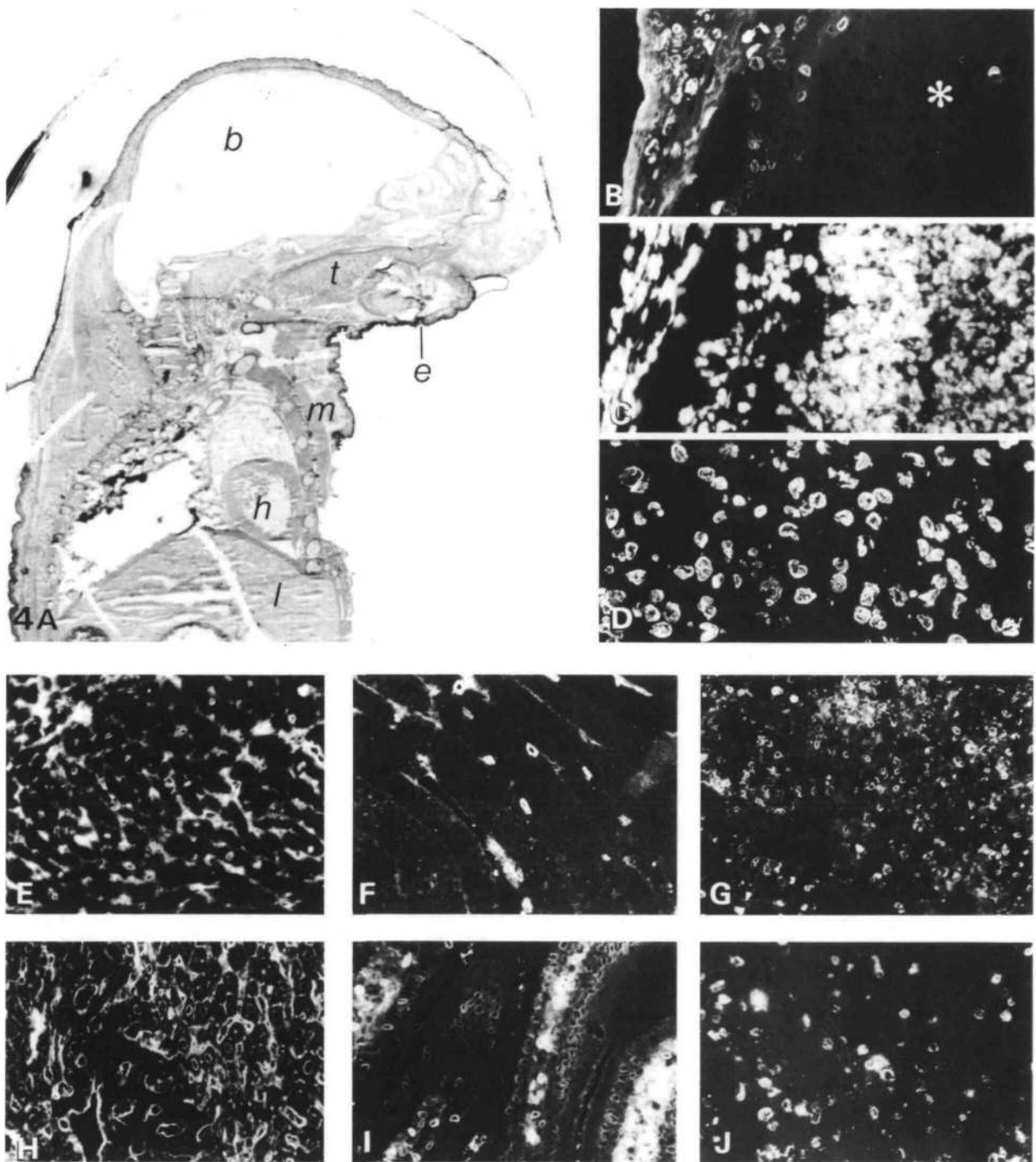
**Fig. 4.** A–J. Immunoperoxidase (A) and immunofluorescence (B–J) with different lamin antibodies on tissues of postnatal day-1 (A–D) and adult mice (E–J). A shows a longitudinal section through the upper half of the embryo. Positive staining with the mAB346 is apparent for some tissues such as epidermis, muscle and connective tissue (see table), but no staining is seen in brain. *b*, brain; *t*, tongue; *e*, epidermis; *m*, muscle; *h*, heart; *l*, liver. B–D show sections through brain at higher power stained with either mAB346 (B) Hoechst (C) or PKB8 (D). Note the lamin-A/C-negative nuclei in cells in the brain (*) adjacent to the lamin-A/C-positive nuclei in cells of the connective tissue (the section in D is taken from a brain area equivalent to that shown with a* in B). (E–J) mAB346 staining of heart (E) skeletal muscle (F) lung (G) liver (H) intestine (I) and brain (J). Nuclei in essentially all cells are positively stained in these tissues of adult mice. Magnifications: (A) ×60; (B–D) ×250; (E–J) ×180.

**Table 2.** *Expression of lamins A/C and of IF proteins in EC cell lines and their derivatives*

| | | | % of cells decorated with antibody against | | | |
| | | | lamins | | IFs | |
| Cell line | Origin | Cell type | 346 (A/C) | PKB8 (A,B,C) | Vimentin | Keratin |
|---|---|---|---|---|---|---|
| EC cell lines | | | | | | |
| PCC3 | OTT6050 | EC, P | <5+++ | >95 | >98 | <3 |
| F9 | OTT6050 | EC | <3+++ | >98 | >98 | <3 |
| Differentiated cell lines | | | | | | |
| PYS-2 | OTT6050 | differentiated parietal yolk sac endoderm | *(+) | >98 | >98 | >98 |
| 3/A1/D3 | *in vitro* PCC3/A/1 | differentiated fibroblastic | >95++ | >98 | >98 | 0 |
| C17-S1-T-984+ clone 10 | *in vivo* tumour C17-S1 | differentiated skeletal myoblast | >95+++ | >98 | >98 | 0 |
| After retinoic and cAMP treatment of EC cell lines | | | | | | |
| PCC3 | | | 5–20+++ | >98 | >98 | 50–80 |
| F9 | | | 10–20+++ | >98 | >98 | 40–80 |

EC, embryonal carcinoma; P, pluripotent line. For original references to cell line derivation see Paulin *et al.* 1982.
* PYS-2 shows very weak positivity in some cells.
(+)→ +++ refers to the intensity of staining seem with mAB 346. (+) = very weak. +++ = very strong. Time of treatment varied in different experiments between 3 days retinoic acid, 1 day cAMP, and 6 days retinoic acid, 4 days cAMP.

were negative when tested with mAB346. In bone marrow from adult mice, only some cells were decorated by mAB346.

### Lamin B expression

Embryos of different ages (embryonic days 10, 11, 13, 15 and 18) were assayed with the lamin-B-specific rabbit antibody. The results showed that all lamin-A/C-negative cells were lamin B positive, as expected from the results described above with PKB8. To cite only one example in the day-18 embryo, >95 % of cells in the thymus, spleen, gonads, kidneys and brain stained positively with the lamin-B-specific rabbit antibody. More importantly, use of this antibody showed that for those tissues tested lamin-A/C-positive cells did not lose lamin B i.e. such cells were also positively stained by the lamin-B-specific rabbit antibody. Thus in liver, heart, muscle, brain, thymus and spleen from adult animals >95 % of cells were stained by the lamin-B-specific rabbit antibody.

### Expression of lamins A and C in EC and differentiated mouse teratocarcinoma cell lines

The lamin content and the intermediate filament (IF) types in embryonal carcinoma (EC) cells and their differentiated derivatives are listed in Table 2. While more than 95 % of the cells in the EC cell lines PCC3 and F9 reacted with PKB8, less than 5 % and 3 %, respectively, were positive with mAB346. We assume that this low percentage of mAB346-positive cells represents differentiated cells that passage with the EC cells (cf. Paulin *et al.* 1982). In contrast, all nuclei of the fibroblast line 3/A1/D3 stain positively with mAB346 and PKB8, as do those of the myoblast cell line (Fig. 5K,L). PYS-2 a cell line derived from parietal endoderm shows very weak staining of some nuclei with mAB346, while all nuclei stain with PKB8. These results are confirmed by immunoblotting results (Fig. 6). While no reaction is seen with mAB346 on uninduced PCC3 or F9 EC cells, a strong positive reaction is obtained in the lamin A and C positions on extracts of the established fibroblast and myoblast cell lines. With PYS-2 cells only very weak bands at the A/C positions were detected, and these could not be photographed.

Lamin and IF expression was also monitored after EC cells were induced to differentiate by retinoic acid and cyclic AMP. The percentage of cells showing lamin A/C expression increased to 5–20 % depending on the time of treatment. The number of cells showing lamin A/C expression in an individual experiment was always

**Table 3.** *Characterization of lamin monoclonal antibodies*

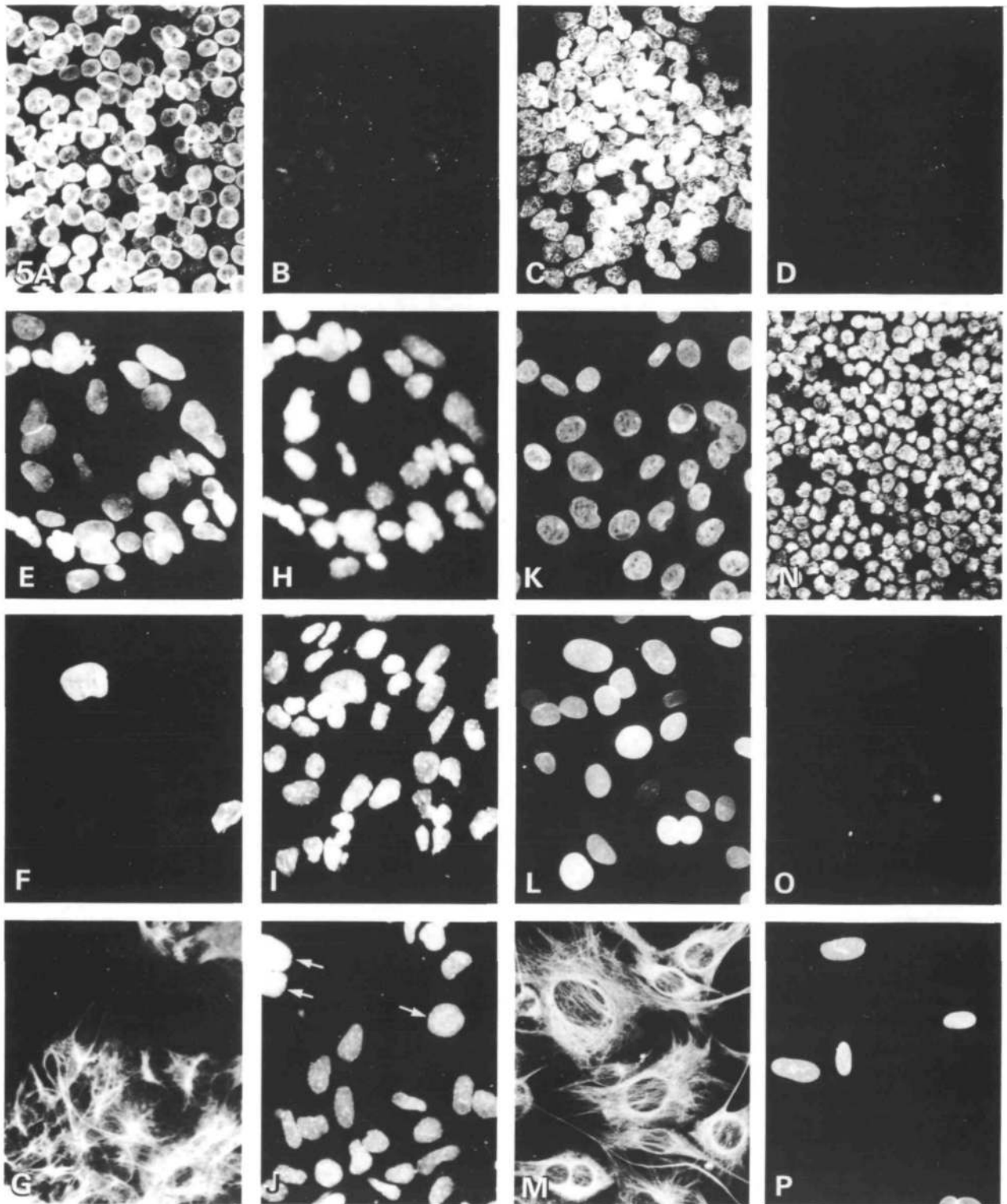| | | | Immunofluorescence on cell lines | | | | | | |
| | | | Human | | | Animal | | | |
| mAB | Isotype | Immunoblot | Hela | TR146 | HS27 | RMCD | CHO | SW3T3 | HK22 |
|---|---|---|---|---|---|---|---|---|---|
| 346 | IgM | A,C | + | + | + | + | + | + | − |
| PKB8 | IgG1 | A,B,C | + | + | + | + | + | + | + |

**Fig. 5.** Immunofluorescence of uninduced embryonal carcinoma cells and of differentiated cell lines. For cell line derivation see Table 2. (A,B) PCC3 cells stained with PKB8 (A) or mAB346 (B). (C,D) F9 cells stained with PKB8 (C) and with 346 (D). (E-G) PCC3 after incubation (5 days retinoic acid 3 days cyclic AMP) stained with either PKB8 (E), mAB346 (F) or the Troma 1 keratin antibody (G). Staining of the same fields with Hoechst is shown in H-J. (K-M) Myoblast cell line styained with (K) PKB8 (L) mAB346 (M) vimentin antibody. (N,O) Mouse HK22 lymphoma cells stained with PKB8 (N) and MAB346 (O). (P) Human HS27 fibroblast cells stained with mAB346. Magnifications: A-M,P ×250; N,O, ×220.
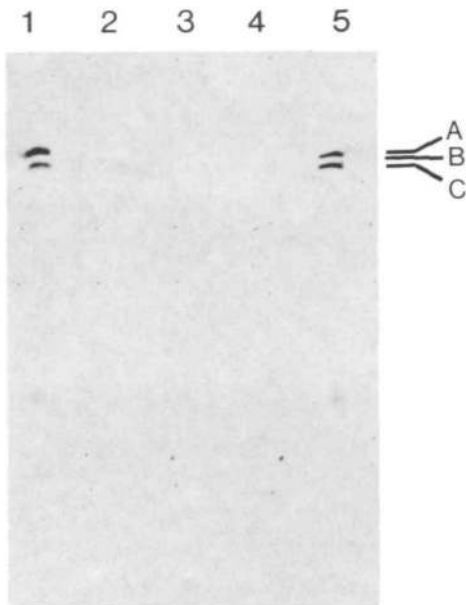
**Fig. 6.** One-dimensional blots of extracts of EC cells and of differentiated cell lines immunoblotted with mAB346. Lane 1, myoblast; lane 2, PYS-2 parietal yolk sac; lane 3, PCC3 EC cells; lane 4, F9 EC cells; lane 5, fibroblast line. Note that the myoblast and fibroblast cell line extracts show bands at the positions of lamins A and C but that the PCC3 and F9 cells do not. A very faint band at the A and C positions was visible in the original blot of the PYS-2 cells. ABC indicate the positions of lamins A, B and C taken from a parallel blot with PKB8 antibodies.

much lower than the number of keratin-positive cells (Table 2).

*Species and cell type specificity*

The lamin monoclonal antibodies have been further characterized on the human and animal cell lines listed in Table 3. On human cell lines, mAB346 was positive on Hela cells and TR146 cells, both of which are of epithelial origin, and on the human fibroblast cell line HS27. On human and animal cell lines, PKB8 gives characteristic lamin staining, but in addition on the human cell lines listed shows weak staining of some intermediate filament types (cf. Krohne *et al.* 1984). mAB346 shows characteristic lamin staining on CHO cells and on mouse 3T3 cells but interestingly does not stain the mouse HK22 cell line which originates from a spontaneous lymphoma.

**Discussion**

Our results show that at days 9–11 all tissues of the developing mouse embryo essentially lack lamins A/C; that expression of lamins A/C is first observed in embryonic tissue at about the time that tissue differentiation starts; that lamins A/C are not simultaneously expressed in all organs but that the time at which they are first observed depends on the tissue, with some organs not showing full lamin A/C expression until well after birth.

*From days 9–11 only lamin B seems expressed in the mouse embryo proper*

Our survey of mouse embryonic development covers embryonic day 9 to birth and the postnatal development to the adult. At embryonic days 9, 10 and 11, the nuclei in all tissues of the embryo proper listed in Table 1 lack lamin A/C reactivity. Therefore, the positive reaction with mABPKB8 indicates the exclusive expression of lamin B as this antibody cross-reacts with all three lamins. These results complement and extend the studies of Schatten *et al.* (1985), who noted lamin B but not lamin A/C expression in the mouse blastocyst, and of Stewart & Burke (1987) who reported that lamin B is the only lamin species expressed in mouse embryos from the blastocyst stage to the 8-day embryo. The latter authors noted, as did we, the staining of trophoblast nuclei by lamin A/C antibodies as well as the presence of lamins A/C and B in the maternal decidua. Prior to the blastocyst stage, data on lamins A/C in the mouse are conflicting and this time period has not been the focus of our study. Schatten *et al.* (1985) originally suggested that lamins A/C were not present, but more recent results of Maul *et al.* (1987), Houliston *et al.* (1988) and of Stewart & Burke (1987) indicate, that lamins A/C or immunologically related molecules may still be present in the zygote and during the first few cleavages. At least some of this lamin A/C may be of maternal origin. Lamin expression has also been studied in early embryological stages of other species. In the chicken, lamin A appears to be absent from early embryos (Lehner *et al.* 1987). In *Xenopus* developmental expression of the lamin B equivalent, $L_I$, occurs at mid blastula (Benavente *et al.* 1985; Stick & Hausen, 1985). For expression of lamin A/C equivalents in *Xenopus* see Krohne & Benavente 1986, but note the recent discovery of a third somatic lamin A for which the distribution in embryonal development has not yet been studied (Wolin *et al.* 1987).

*Timing of the onset of murine lamin A/C expression is tissue dependent*

Striking tissue-dependent differences in lamin A/C expression are observed in later stages of mouse embryological development. Lamin A/C expression is first observed at about the time when tissue differentiation starts, i.e. days 12 to 13. However, examination of the figures and of Table 1 shows that different organs start to express lamins A/C at quite different times. Thus individual cells destined to form the muscles of eye, limbs and trunk reveal lamin A/C expression clearly at day 13 as do certain vascular smooth muscle cells. In striking contrast, the myocytes of the embryonic heart lack lamins A/C and the expression of these lamins in cardiac muscle seems essentially a postnatal event, which starts at day 5 and then accelerates. Similarly, the acquisition of lamins A/C in the brain occurs essentially only several days past birth. This rather late onset of lamin A/C expression in the brain is not without precedence. Certain cytoskeletal proteins such as the large neurofilament polypeptide NF-H (Shaw & Weber, 1982; Pachter & Liem, 1984), some tau proteins (Mar-

eck et al. 1980) as well as the glial-specific intermediate filament protein (GFAP) (Raju et al. 1981; D. Fürst, this laboratory) are primarily acquired past birth. The first display of lamins A/C in epithelia was noted at embryonic day 15 for the epidermis while lamins A/C are present at day 17 in the tongue. Lamin A/C expression in the embryonic epidermis coincides with the formation of the stratum germinativium, i.e. the time at which stratification begins. Interestingly the periderm continues to express simple epithelial keratins while the keratinocytes form under this monolayer (Moll et al 1982; Schweizer & Winter, 1982). In the simple epithelia, liver and lung, lamins A/C are only acquired several days past birth, while in the intestinal epithelium lamin A/C acquisition seems to occur even later, being present in only some cells at postnatal day 20. Even in the adult mouse, many cells in spleen, thymus and bone marrow, as well as some intestinal epithelial cells, appear lamin A/C negative.

At least superficially during embryological development there may be an inverse correlation between lamin A/C expression and the percentage of proliferating cells. For instance, in cardiac muscle the percentage of proliferating cells decreases gradually from 80–90 % in the early stages to an almost zero rate at postnatal day 21 in muscle (Erokhina & Rumyantsev, 1986). Those cell types expressing only lamin B in the adult have to be further examined to see if they can be characterized as stem cells (cf. Stewart & Burke, 1987).

Our data on later embryological stages can be compared with those obtained in other species. Although a detailed day-by-day analysis has not been performed for the embryonic chicken, the general delay in lamin A expression has been documented by Lehner et al. (1987). In chicken, a differential expression of lamins A and B has been noted using both immunofluorescence and immunoblotting techniques as well as by a direct demonstration of the lamins in Coomassie-blue-stained gels in several instances. Liver and skeletal muscle are positive with lamin A antibodies already at chicken embryonic day 10, while even the adult brain seems to have a reduced complement of lamin A. Thus the apparent order in which the different tissues express lamin A may be somewhat different to that described for the mouse. In Xenopus, differential expression of lamins $L_I$–$L_{IV}$ (Krohne & Benavente, 1986) and of the newly discovered lamin A (Wolin et al. 1987) is well documented in somatic cells of the adult amphibian. The order in which lamin A is expressed relative to the other A/C-like lamins has not yet been assayed.

## Lamin expression in cell lines

Our results on EC cells and teratocarcinoma-derived cell lines confirm and extend those of others (Stewart & Burke, 1987; Lebel et al. 1987; Houliston et al. 1988). The combined results show that uninduced EC cells do not express lamins A/C and reveal only lamin B. Our data further show that when EC cells are induced to differentiate in vitro, some increase in lamin A/C expression is seen paralleling previous results of Lebel et al. (1987) who noted an increase in lamin A/C

expression in immunoblots of F9 cells induced to differentiate by retinoic acid. Immunofluorescence shows that only some of the differentiated cells express lamins A/C, with the percentage of cells showing lamin A/C expression being always much lower than the percentage of cells expressing keratin. When differentiated cell lines derived from EC cells are studied, all differentiated derivatives (with the exception of PYS-2) express lamin A/C in >95 % of the cells. Thus also in the EC system, differentiation as indicated by expression of particular IF types occurs prior to lamin A/C expression.

Only a very few cell lines in culture express lamin B exclusively. Thus the human T lymphoblastic cell line KE37 lacks lamin A/C when assayed on gels (although a weak reaction is observed in immunofluorescence with A/C-specific antibodies) (Guilly et al. 1987). Our results showed that the mouse cell line HK22, which is derived from a spontaneous lymphoma (Holtkamp et al. 1978), also appears to lack lamins A/C. In contrast, in many other established cell lines of animal origin including mouse 3T3 cells, CHO cells and rat mammary cells (RMCD) are lamin A/C and lamin B positive (Table 3).

## Biological significance

The data documented here and elsewhere argue that a single lamin (lamin B) is sufficient to form a nuclear lamina at least in some cell types. Acquisition of lamins A/C during development may be accompanied by changes in nuclear architecture and in the interactions of lamins with chromatin. Whether this could lead to the activation of different transcription programs remains to be shown. Our results on the embryonic and postnatal mouse and on EC cells show that commitment of a cell to a particular pathway of differentiation, as assayed by cell- and tissue-type-specific expression of intermediate filament proteins, occurs before lamin A/C expression is detected (for reviews on intermediate filament expression see for instance Osborn et al. 1985; Quinlan et al. 1985). To cite only two particularly striking examples, desmin expression starts at embryonic day 8 in the presumptive heart muscle (D. Fürst, this laboratory), while lamin A/C expression in most cardiac muscle cells is not seen until postnatal day 10 (Table 1). Likewise neurofilament proteins are expressed in the neural tissue and brain at embryonic day 9 in the mouse (Cochard et al. 1982) while lamin A/C expression first occurs in most brain cells well after birth. Thus the much later acquisition of lamins A/C versus lamin B may signal an aspect of consolidation in the differentiation programs and possibly a limitation in the plasticity for further developmental pathways. Finally those cell types that seem to express only lamin B i.e. EC cells and some cells of spleen, thymus, bone marrow and intestine in the adult mouse may fall into the 'stem cell' category, but a much more careful correlation using specific stem cell markers is needed to critically test this hypothesis.

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