Development 102, 271–278 (1988)
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Expression of nuclear lamins during mouse preimplantation development

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Summary

The expression of nuclear lamins during mouse preimplantation development was studied by immunofluorescence, immunoblotting and immunoprecipitation. Two sera were used, specific either for lamin B or lamins A and C. Both sera gave a positive staining of the nuclear periphery throughout preimplantation development (fertilized eggs to late blastocysts). Immunoblots revealed that the three lamins were present in eggs and blastocysts. However, lamin A from eggs was found to have a higher apparent M_r than lamin A from blastocysts and other mouse cells. Using immunoprecipitation, synthesis of lamin A was detected in eggs while synthesis of lamin B was detected in 8-cell embryos and blastocysts, indicating that at least some of the lamins used during early development do not come from a store in the egg. These results are discussed in relation to the possible role of lamins during cell differentiation.

Key words: nuclear lamins, nucleus, mouse, preimplantation, immunofluorescence.

Introduction

The nuclear lamina is a proteic meshwork associated with nuclear pores and apposed to the inner nuclear membrane. Together with the internal scaffold (or matrix), it constitutes the nucleoskeleton. In most mammalian cells, it is composed of three related proteins called lamins A, B and C with relative molecular masses of 60000–78000 (for reviews see Gerace, 1986; Krohne & Benavente, 1986). The filaments of the nuclear lamina are closely related to intermediate filaments, as shown by analysis of the genes' sequences (McKeon *et al.* 1986; Fisher *et al.* 1986) and self-association properties (Aebi *et al.* 1986; Goldman *et al.* 1986). During mitosis, the lamina is disassembled in parallel with hyperphosphorylation of the lamins (Gerace & Blobel, 1980; Ottaviano & Gerace, 1985). The nucleoskeleton is thought to play an important role in nuclear physiology. Three major nuclear functions, replication, transcription and control of gene activity, have been suggested to depend upon the anchorage of DNA loops to the nucleoskeleton (Cockerill & Garrard, 1986; Gasser & Laemmli, 1986*a*,*b*; Heck & Earnshaw, 1986; Jackson & Cook, 1985, 1986; Mirkovitch *et al.* 1984).

A differential expression of lamins has been described during early development in *Xenopus*, one lamin (L_{III}) being present in oocytes whereas two others (L_I and L_{II}) appear later during development (Benavente *et al.* 1985; Krohne *et al.* 1981; Stick & Hausen, 1985). Differential lamin expression may also occur during development in other species. In *Drosophila*, a 74000 polypeptide is predominantly expressed during early stages of embryogenesis (2–4 h) whereas in older embryos (6–21 h) a 76000 polypeptide is also present (Smith & Fisher, 1984). In mammals, it has been reported using immunofluorescence that lamins A, B and C are present in fertilized eggs and early cleavage blastomeres. In

morulae and blastocysts, only lamin B was detected (Schatten *et al.* 1985). In order to understand further the possible role of these proteins during development, we have reinvestigated the expression of the nuclear lamins during mouse preimplantation development using immunofluorescence, immunoblotting and immunoprecipitation.

Materials and methods

Recovery of embryos

MF1 (Central Animal Services, Cambridge, UK), F_1 (C57/B1 × CBA; bred in our laboratories) or Swiss (Animalerie Spécialiseé de Villejuif, CNRS, France) female mice (3–6 weeks) were superovulated by injections of 5–7·5 i.u. of pregnant mare's serum gonadotrophin (PMSG; Intervet) and human chorionic gonadotrophin (hCG; Intervet) 48 h apart. Unfertilized eggs were recovered at 14–16 h post-hCG and freed of their cumulus cells by brief exposure to 0·1 M-hyaluronidase (Sigma). In order to obtain embryos, the females were paired overnight with HC-CFLP (Hacking & Churchill, UK) or Swiss (Animalerie Spécialisée de Villejuif, CNRS, France) males and inspected for vaginal plugs the next day. 1- and 2-cell embryos were recovered by flushing fertilized eggs at 16–18 h post-hCG followed by culture in Medium 16 containing 4 mg ml^{-1} bovine serum albumin (M16 + BSA; Whittingham & Wales, 1969) under oil at 37 °C in 5 % CO_2 in air. Later stage embryos were recovered by flushing late 2-cell/early 4-cell embryos at 46–50 h post-hCG or 8-cell embryos at 65–70 h post-hCG, followed by culture in M16 + BSA to the appropriate stage.

Parthenogenetic activation

Oocytes were activated by a 4·5 min exposure of the cells to 7 % ethanol solution as described in Webb *et al.* (1986).

Cell fixation and immunocytological staining

After the removal of the zona pellucida by brief exposure to acid Tyrode's solution (Nicolson *et al.* 1975), eggs and embryos were placed in specially designed chambers exactly as in Maro *et al.* (1984) except that the chambers were coated first with a solution of 0·1 mg ml^{-1} Concanavalin A and the samples centrifuged in them at 450 *g* for 10 min at 30 °C. Tissue culture cells were grown on glass coverslips. Samples were then fixed in one of three ways. (1) Cells were fixed for 30 min at 20 °C with 1·8 % formaldehyde in phosphate-buffered saline (PBS), washed for 10 min in PBS containing 50 mM-NH_4Cl, extracted for 10 min in PBS containing 0·25 % Triton X-100 and finally washed three times in PBS. (2) Cells were fixed for 6 min at −20 °C in methanol, then rehydrated and washed in PBS. (3) Cells were washed quickly in PHEM buffer (10 mM-EGTA, 2 mM-$MgCl_2$, 60 mM-Pipes, 25 mM-Hepes, pH 6·9; derived from Schliwa *et al.* 1981) extracted for 5 min in PHEM buffer containing 0·25 % Triton X-100, washed in PHEM buffer and fixed for 30 min with 1·8 % formaldehyde in PHEM buffer.

Immunocytological staining was performed as described in Maro *et al.* (1984) using either an anti-lamin B serum (Guilly *et al.* 1987) or an anti-lamin A+C human serum (McKeon *et al.* 1983) followed by fluorescein-labelled anti-human immunoglobulin antibodies (Miles). In order to stain chromosomes, fixed embryos and cells were incubated in Hoechst dye 33258 (5 μg ml^{-1} in PBS) for 45 min.

Photomicroscopy

The coverslips were removed from the chambers and samples were mounted in 'Citifluor' (City University, London) and viewed on Leitz Ortholux II and Dialux 20 microscopes with filter sets L2 for FITC-labelled reagents, and A for Hoechst dye. Photographs were taken on Kodak Tri-X or Ilford HP5 films using Leitz Vario-Orthomat photographic systems.

Immunoblotting

Embryos were washed in Medium 2 (Fulton & Whittingham, 1978) containing 4 mg ml^{-1} polyvinylpyrrolidone (M2 + PVP), then lysed in boiling SDS sample buffer (Laemmli, 1970) and analysed on a 10 % SDS–polyacrylamide gel (Laemmli, 1970). Proteins were transferred to nitrocellulose by electrophoresis (Towbin *et al.* 1979; Burnette, 1981). Immobilized proteins were incubated with a 1:500 dilution of anti-lamin B and a 1:1000 dilution of anti-lamin A+C, then with ^{125}I-Protein A (Amersham) or with alkaline-phosphatase-labelled anti-human immunoglobulins (Promega Biotec, Madison, USA).

Immunoprecipitation

Embryos were labelled for 3 h with 670 μCi ml^{-1} of [^{35}S]methionine (specific activity: 1100–1300 mCi mM^{-1}; Amersham) in M16 + BSA and washed twice in M16 + BSA and three times in M2 + PVP. Embryos were lysed in 100 mM-Tris–HCl, pH 8·3, 2 mM-EDTA, 0·5 % SDS, 0·5 % deoxycholate (DOC), 0·5 % NP40, 5 mM-iodacetamide, 1 μg ml^{-1} leupeptin, 1 μg ml^{-1} pepstatin, 100 μg ml^{-1} aprotinin, 1 mM-PMSF and dispersed by sonication. After a 15 min centrifugation at 10000 *g*, the supernatant was incubated overnight with a 1:50 dilution of anti-lamin B and a 1:25 dilution of anti-lamin A+C serum or a 1:25 dilution of control mixed human sera. Samples were then mixed with Protein A–Sepharose 4B beads (Pharmacia) and incubated for 2 h at 4 °C under continuous agitation. Beads were then washed five times by centrifugation with DOC buffer, once in 100 mM-Tris–HCl pH 6·8 and finally boiled in SDS sample buffer. The eluted proteins were analysed using 10 % SDS–PAGE and revealed by fluorography (Bonner & Laskey, 1974).

Cell lines

Three mouse teratocarcinoma cell lines (Jakob & Nicolas, 1987), PYS-2, F9 and 3-TDM1 were used in this study. L929 (mouse) and HeLa (human) cells were used as controls in the immunoblot and immunoprecipitation described above. For immunoprecipitation, subconfluent cells were labelled for 6 h with 100 μCi ml^{-1} of [^{35}S]methionine (specific activity: 1100–1300 mCi mM^{-1}; Amersham) in methionine-free RPMI (Seromed) containing 10 % fetal calf serum. Extracts of cells were prepared in the same way as for the embryos.

Results

Lamin B was detected by immunofluorescence in fertilized eggs and at all subsequent stages of preimplantation development (Fig. 1A–D). It was not possible to distinguish between lamins A and C by immunofluorescence since the antibody used does not discriminate between these two proteins. Using this serum, all stages showed a positive staining of the nuclear periphery (Fig. 1E–H). Similar results were obtained in mice from strains with different genetic backgrounds (Swiss, MF1 × CFLP, F_1(C57/B1 × CBA) × CFLP, F_1(C57/B1 × CBA) × Swiss) and using three different fixation procedures (data not shown).

In order to check for the presence of each individual lamin, we used a biochemical approach. Total proteins of fertilized eggs and blastocysts were separated by SDS–PAGE, transferred to nitrocellulose paper and probed with a mixture of both anti-lamin sera. The results show that three lamins were present at these stages of development (Fig. 2). Lamin A from blastocysts was found to display a lower apparent M_r than lamin A from eggs or HeLa cells (Fig. 2). This change in mobility may reflect a real difference in the polypeptide or may be an artifact linked to the presence of large amounts of bovine serum albumin (BSA), which migrates very close to the lamins, in the culture medium. We tested this possibility by mixing HeLa cell extracts with various amounts of BSA: the presence of BSA ($1\,mg\,ml^{-1}$) did not modify the migration of lamin A (data not shown). Moreover, in cumulus cells, liver nuclei and various mouse cell lines, a lamin pattern similar to the blastocyst pattern was observed (Figs 2, 3). In those cell lines, we also checked that the antigens detected were located at the nuclear periphery (Fig. 4). This higher mobility lamin A was also detected using the anti-lamin A+C serum alone in 8-cell embryos, blastocysts and cumulus cells (data not shown) showing that it belongs to the lamin A+C family.

We also ascertained that the signals obtained from our unfertilized eggs were unlikely to be caused by cumulus cell contamination. It was not possible to detect lamins in protein extracts made from 10^4 cumulus cells (this would correspond to a cumulus cell-to-egg ratio of 5:1 in our egg sample whereas the maximum number of cumulus cells present did not exceed 2–3 cumulus cells per egg). Moreover, we did not detect the higher mobility lamin A (present in the cumulus cells) in the egg sample.

The change in mobility for lamin A in eggs may be due to the fact that eggs are arrested in second metaphase of meiosis. In metaphase, lamins are soluble and hyperphosphorylated (Gerace & Blobel, 1980; Ottaviano & Gerace, 1985), while they are

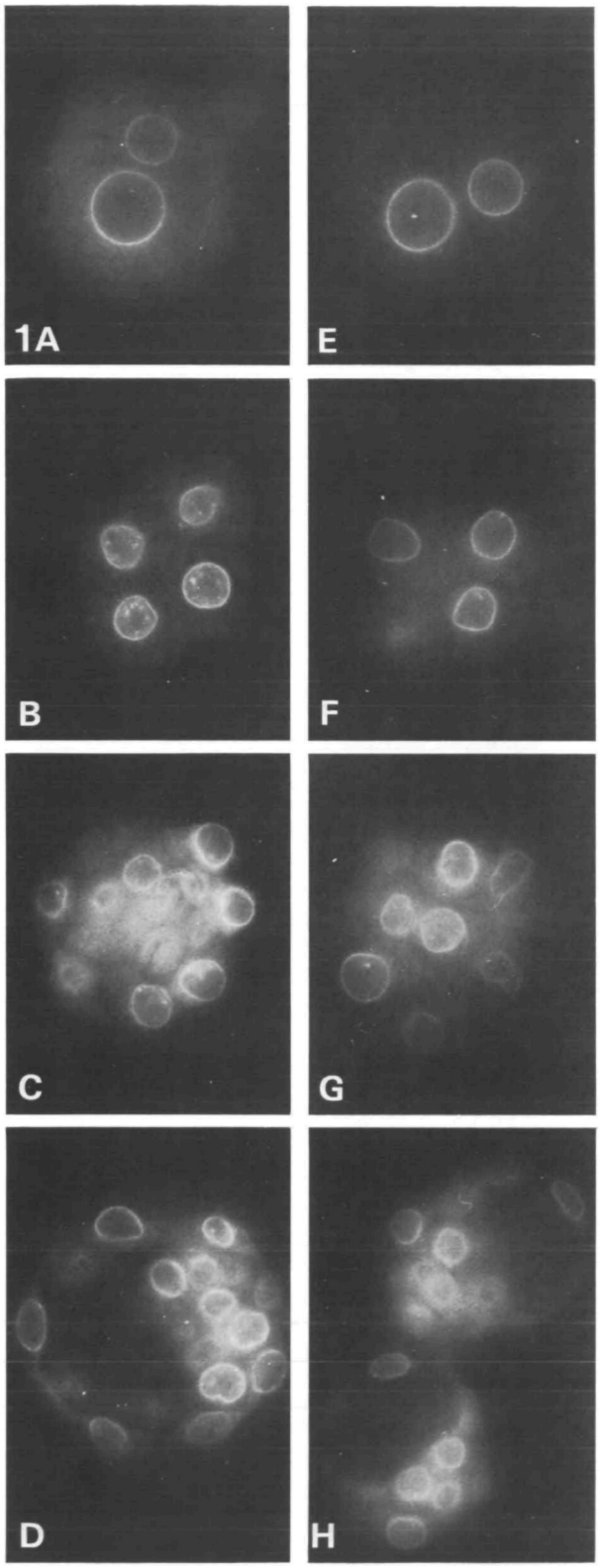

Fig. 1. Distribution of nuclear lamins in preimplantation mouse embryos. Embryos were stained either with an anti-lamin B antibody (A–D) or with an anti-lamin A+C antibody (E–H). Fertilized eggs (A,E); 8-cell embryos (B,F); morulae (16–32 cells; C,G); blastocysts (D,H). ×250.

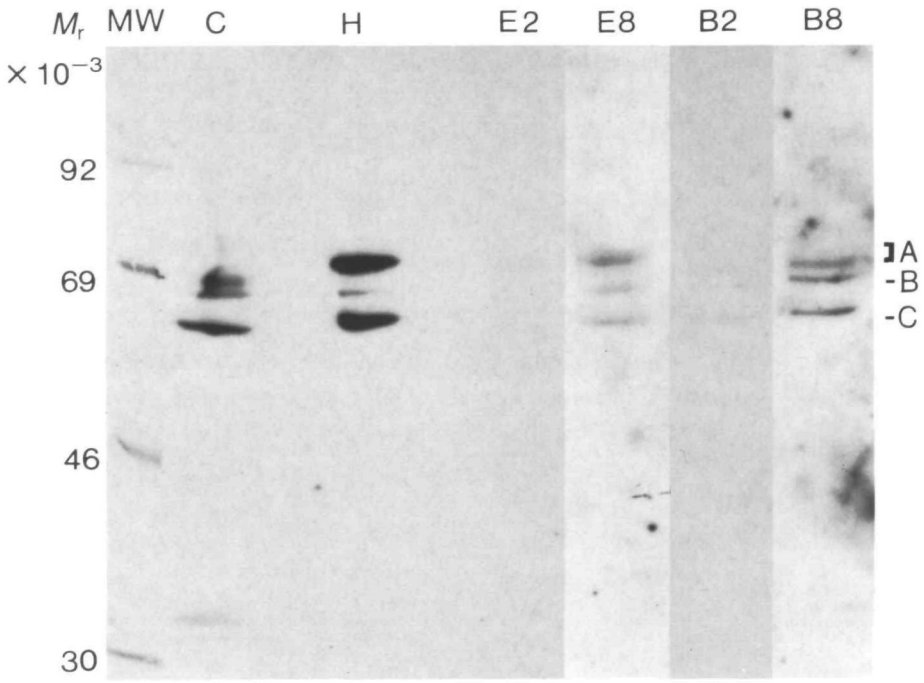

Fig. 2. Immunoblotting of nuclear lamins in mouse preimplantation embryos. Cellular proteins were probed with a mixture of anti-lamin B and anti-lamin A+C sera (see Materials and methods). C, cumulus cells; H, HeLa cells; E, 2000 unfertilized eggs; B, 600 blastocysts. A, B, C, lamins A, B and C; MW, molecular weight markers. MW, C, H, E2, B2, 2 days exposure time; E8 and B8, 8 days exposure time.

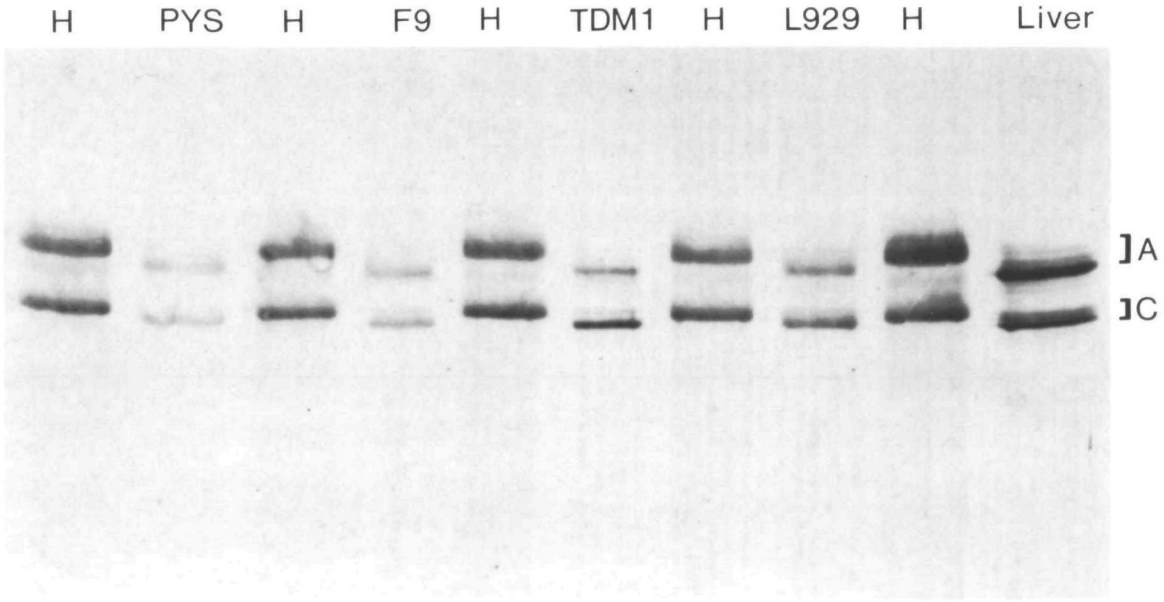

Fig. 3. Immunoblotting of nuclear lamins in mouse cell lines. Cellular proteins were probed with an anti-lamin A+C serum (see Materials and methods). H, HeLa (human); PYS, PYS-2 cells (mouse parietal endoderm); F9, F9 spontaneously differentiated in tissue culture conditions (mouse endoderm); TDM1, TDM1 cells (mouse trophoblast); Liver, mouse liver nuclei. A, C, lamins A and C.

insoluble, located at the nuclear periphery and less phosphorylated in interphase cells (as those found in later stage embryos). It is known that many proteins are phosphorylated during meiosis and that hyper-phosphorylation may induce a change in the mobility of some of these proteins as shown in mouse oocytes (Howlett, 1986). When unfertilized eggs (in mitosis) and ethanol-activated eggs (in interphase) were probed by immunoblotting, lamins A and C migrated with the same mobility in both samples (Fig. 5). Thus the difference in mobility cannot be accounted for by a cell-cycle-dependent mechanism.

Immunoprecipitation experiments were performed in order to find out whether the lamins present in early blastomeres come entirely from a store in the egg or if synthesis of these proteins takes place during early development. Lamin B was synthesized in 8-cell-stage embryos and blastocysts while synthesis of

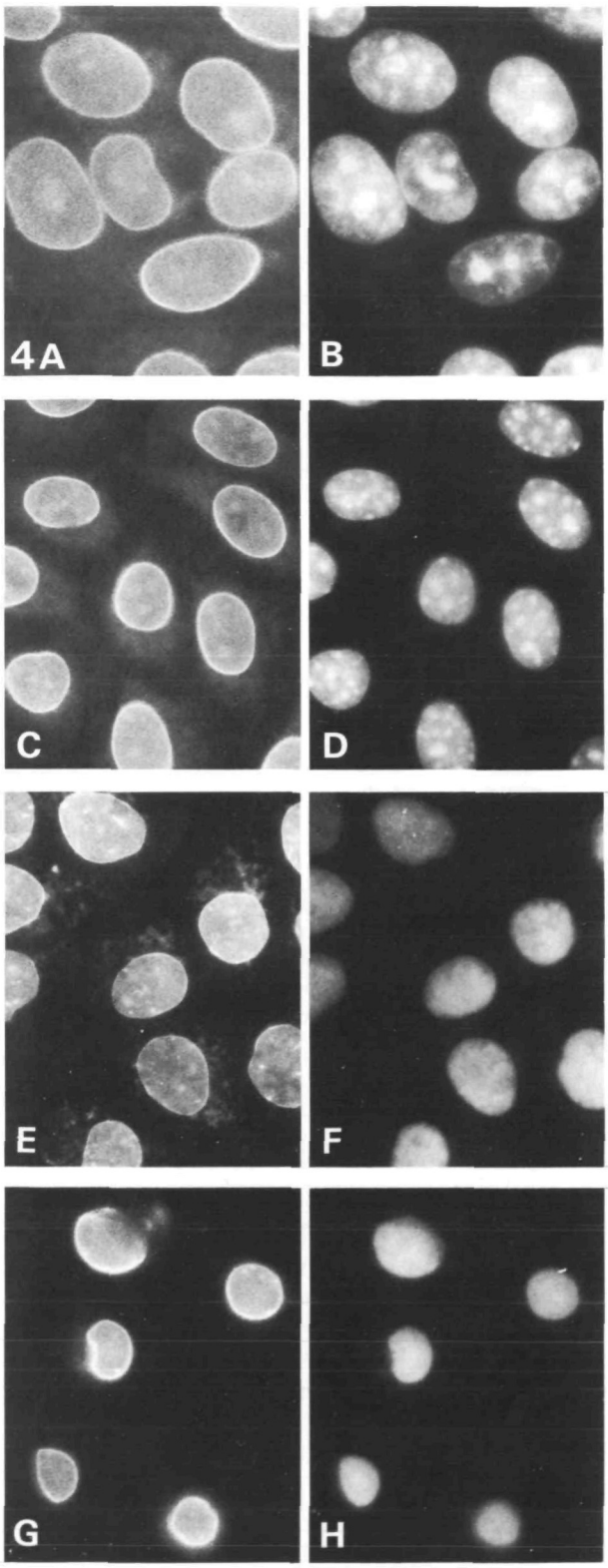

Fig. 4. Distribution of nuclear lamins in various cell lines. Cells were stained with an anti-lamin A+C antibody (A,C,E,G) and with Hoechst dye 33258 to stain the chromatin (B,D,F,H). (A,B) HeLa; (C,D) L929; (E,F) PYS (16- to 32-cells); (G,H) TDM1. ×1000.

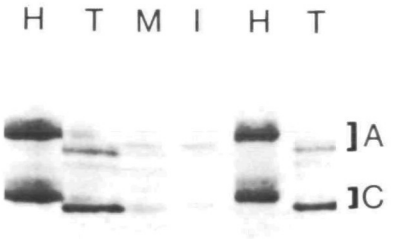

Fig. 5. Immunoblotting of nuclear lamins in mouse oocytes. Cellular proteins were probed with an anti-lamins A+C serum (see Materials and methods). H, HeLa cells (human); T, TDM1 cells (mouse); M, 2000 unfertilized eggs in metaphase; I, 1250 activated eggs in interphase. A, C, lamins A and C.

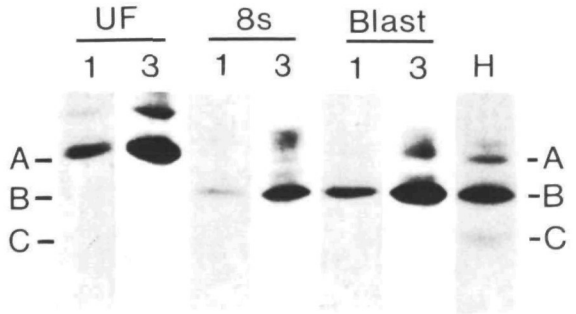

Fig. 6. Immunoprecipitation of nuclear lamins in mouse preimplantation embryos. Cellular proteins were probed with a mixture of anti-lamin B and anti-lamin A+C sera (see Materials and Methods). UF, 2000 unfertilized eggs; 8s, 1000 8-cell embryos; Blast, 700 Blastocysts; H, HeLa cells. A, B, C, lamins A, B and C. 1, 1 week exposure time; 3, 3 weeks exposure time.

lamin A was only detected in unfertilized eggs (Fig. 6). We were not able to immunoprecipitate lamins A and C from any of the later stages studied whereas our serum was able to precipitate lamins A and C from mouse cells (Fig. 7). Thus lamin synthesis takes place in mouse oocytes and in early embryos, lamin A being the predominant lamin synthesized in eggs whereas lamin B is the main one synthesized in cleavage-stage blastomeres.

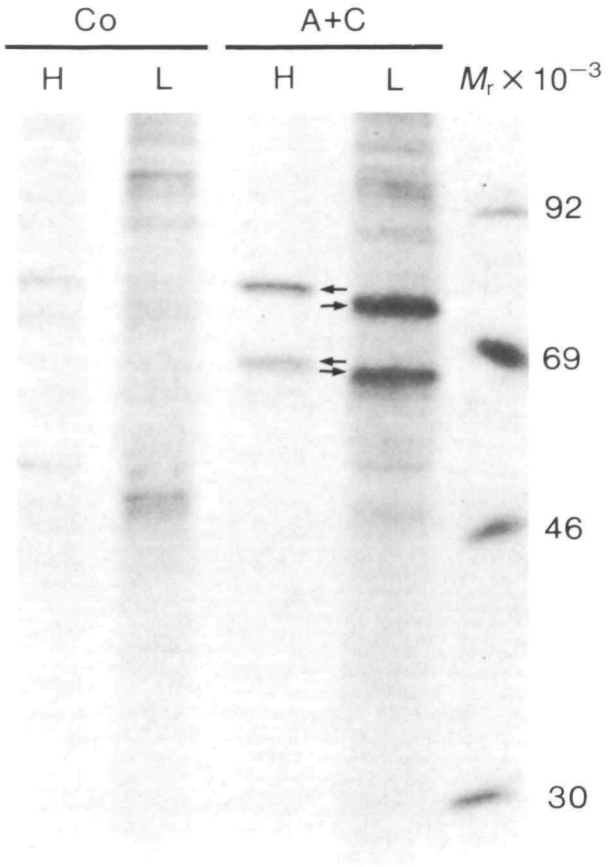

Fig. 7. Immunoprecipitation of nuclear lamins in mouse and human cells. Cellular proteins were probed with an anti-lamin A+C serum (see Materials and methods). H, HeLa cells (human); L, L929 cells (mouse); MW, molecular weight markers; Co, control serum; A+C, anti-lamin A+C serum. 4 days exposure time.

Discussion

The morphological and biochemical data presented in this report show that three lamins are present at all stages of mouse preimplantation development. A positive staining of the nuclear periphery was observed by immunofluorescence at all stages of the mouse preimplantation development using both an anti-lamin B serum and an anti-lamin A+C serum. Moreover, we were able to detect lamins A, B and C in eggs, 8-cell embryos and blastocysts by immunoblotting. However, the mobility of lamin A from blastocysts, 8-cell embryos and many mouse cell types on SDS gels is higher than that of lamin A from oocytes or human cells. Comparison of the lamin patterns in oocytes and in activated eggs demonstrates that this change in mobility is not linked to a cell-cycle-dependent mechanism but rather to a developmentally regulated mechanism. It remains to be seen whether an alternative lamin A gene is transcribed in mouse oocytes or whether a lamin-A-specific post-translational modification accounts for the difference. Resolution of this problem is hampered by the small amount of material available from early mouse embryos, which makes many types of biochemical analysis such as the identification of minor lamin components (Lehner *et al.* 1986) very difficult in this system. We have demonstrated by immunoprecipitation that the lamins present in early blastomeres do not derive entirely from a store in the egg. Lamin B was synthesized in 8-cell-stage embryos and blastocysts whereas only lamin A was synthesized in unfertilized eggs. We were not able to detect a synthesis of lamin C from any of the stages studied. This absence of signal for lamin C may be due either to the insensitivity of this technique when limited by the small amount of material available and the necessary high stringency (0·5 % SDS) of our immunoprecipitation conditions or to an absence of synthesis of this protein. We hope to be able to answer this question at the RNA level by use of cDNA probes. Nevertheless, it should be noted that the observed change in relative synthesis of lamins A and B coincides with the transition between transcription from the maternal to zygotic genomes (Young *et al.* 1978; Levey *et al.* 1978).

Taken together, the results from our three approaches indicate that the three adult mouse lamins are expressed during early mouse development although a lamin A variant is detected in oocytes. In a previous work, an absence of immunofluorescent staining for lamins A and C in morulae and blastocysts was reported (Schatten *et al.* 1985). Using the same polyclonal antibody as these authors, we obtained a positive staining of the nuclear periphery at all stages of early development with a variety of different fixation procedures and different mouse strains (see Materials and Methods and Fig. 1). Thus, we concluded that this discrepancy is probably due to differences in the handling of the embryos.

The results shown in this report indicate that there are no major changes in the pattern of nuclear lamins during mouse early development. This contrasts with the situation in *Xenopus*, a developmental system where the expression of lamins has been extensively studied. In *Xenopus* oocytes, only one lamin (L_{III}) is detectable, while somatic cells express two (L_I and L_{II}) or three (L_I, L_{II} and L_{III}) lamins (Benavente *et al.* 1985; Krohne *et al.* 1981; Stick & Hausen, 1985). During development, the amount of L_{III} decreases gradually while L_I appears at the midblastula transition and L_{II} at the gastrula stage. During that period, the *Xenopus* embryo cleaves very rapidly, the cell cycle being very short (30 min) and reduced to a succession of S and M phases (for review, see Kirschner *et al.* 1985). The nuclear membrane has to assemble and disassemble very rapidly and DNA synthesis may occur during M phase (Newport &

Kirschner, 1984; for review see Ford, 1985). In *Xenopus* embryos, significant amounts of RNA synthesis do not occur until the midblastula transition (Kirschner *et al.* 1985). In contrast, the cell cycles of the mouse embryo during cleavage are long (12–20 h) and consist of the full succession of G_1, S, G_2 and M phases (Howlett & Bolton, 1985; for review see Johnson & Maro, 1987). In addition, the activation of the embryonic genome takes place very early in development, at the 2-cell stage (Young *et al.* 1978; Levey *et al.* 1978). Since it has been shown that DNA loops anchored to the nucleoskeleton are involved in major nuclear functions (Jackson & Cook, 1985, 1986; Gasser & Laemmli, 1987), we suggest that the existence of a different set of lamins during cleavage stages in *Xenopus* may be correlated to the absence of transcription and/or to the very short cell cycle time. In contrast, transcription and replication in the cells of cleaving mouse embryos and in somatic cells are very similar and so major changes in the composition of nuclear lamins may not be necessary.

We thank Josette Bergès, Gin Flach and Susan Pickering for their expert technical assistance, Martin Johnson for stimulating discussions and critical reading of the manuscript, Marc Kirschner and Frank McKeon for the gift of the anti-lamin A+C serum, Hedwige Jakob for the gift of the mouse cell lines, Françoise Danon for the gift of the anti-lamin B serum. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (864010) to Bernard Maro, from the Association pour la Recherche sur le Cancer to Jean-Claude Courvalin and from the Medical Research Council and Cancer Research Campaign to M. H. Johnson. Evelyn Houliston received a fellowship from the European Molecular Biology Organisation and a studentship from the Medical Research Council.

References

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

Benavente, R., Krohne, G. & Franke, W. W. (1985). Cell type-specific expression of nuclear lamina proteins during development of *Xenopus laevis*. *Cell* **41**, 177–190.

Bonner, W. M. & Laskey, R. A. (1974). A film detection method for tritium-labelled proteins an nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**, 83–88.

Burnette, W. N. (1981). "Western-blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-iodinated protein A. *Anal. Biochem.* **112**, 195–203.

Cockerill, P. N. & Garrard, W. T. (1986). Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* **44**, 273–282.

Fisher, D. Z., Chauhary, N. & Blobel, G. (1986). cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6450–6454.

Ford, C. C. (1985). Maturation promoting factor and cell cycle regulation. *J. Embryol. exp. Morph.* **89 Supplement**, 271–284.

Fulton, B. P. & Whittingham, D. G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature, Lond.* **273**, 149–151.

Gasser, S. M. & Laemmli, U. K. (1986*a*). Cohabitation of scaffold binding regions with upstream enhancer elements of three developmentally regulated genes of *D. melanogaster*. *Cell* **46**, 521–530.

Gasser, S. M. & Laemmli, U. K. (1986*b*). The organization of chromatin loops: characterization of a scaffold attachment site. *EMBO J.* **5**, 511–518.

Gasser, S. M. & Laemmli, U. K. (1987). A glimpse at chromosomal order. *TIG* **3**, 16–22.

Gerace, L. (1986). Nuclear lamina and organization of nuclear architecture. *TIBS* **11**, 443–446.

Gerace, L. & Blobel, G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell* **19**, 277–287.

Goldman, A. E., Maul, G., Steinert, P. M., Yang, H. Y. & Goldman, R. D. (1986). Keratin-like proteins that coisolate with intermediate filaments of BHK-21 cells are nuclear lamins. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3839–3843.

Guilly, M.-N., Danon, F., Brouet, J.-C., Bornens, M. & Courvalin, J.-C. (1987). Autoantibodies to nuclear lamin B in a patient with thrombopenia. *Eur. J. Cell Biol.* **43**, 266–272.

Heck, M. M. S. & Earnshaw, W. C. (1986). Topoisomerase II: a specific marker for cell proliferation. *J. Cell Biol.* **103**, 2569–2581.

Howlett, S. K. (1986). A set of proteins showing cell cycle dependent modification in the early mouse embryo. *Cell* **45**, 387–396.

Howlett, S. K. & Bolton, V. N. (1985). Sequence and regulation of morphological and molecular events during the first cell cycle of mouse embryogenesis. *J. Embryol. exp. Morph.* **87**, 175–206.

Jackson, D. A. & Cook, P. R. (1985). Transcription occurs at a nucleoskeleton. *EMBO J.* **4**, 919–925.

Jackson, D. A. & Cook, P. R. (1986). Replication occurs at a nucleoskeleton. *EMBO J.* **5**, 1403–1410.

Jakob, H. & Nicolas, J. F. (1987). Mouse teratocarcinoma cells. *Methods in Enzymology* **151**, 66–81.

Johnson, M. H. & Maro, B. (1987). Time and space in the early mouse embryo: a cell biological approach to cell diversification. In *Experimental Approaches to Mammalian Embryonic Development* (ed. J. Rossant & R. Pedersen), pp. 35–65. Cambridge University Press.

KIRSCHNER, M., NEWPORT, J. & GERHART, J. (1985). The timing of early developmental events in *Xenopus*. *TIG* **1**, 41–47.

KROHNE, G. & BENAVENTE, R. (1986). The nuclear lamins: a multigene family of proteins in evolution and differentiation. *Expl Cell Res.* **162**, 1–10.

KROHNE, G., DABAUVALLE, M. C. & FRANKE, W. W. (1981). Cell type-specific differences in protein composition of nuclear pore-lamina structures in oocytes and erythrocytes of *Xenopus laevis*. *J. molec. Biol.* **151**, 121–141.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, Lond.* **227**, 11 713–11 720.

LEHNER, C. F., KURER, V., EPPENBERGER, H. M. & NIGG, E. A. (1986). The nuclear lamin protein family in higher vertebrates: identification of quantitatively minor lamin proteins by monoclonal antibodies. *J. biol. Chem.* **261**, 13 293–13 301.

LEVEY, I. L., STULL, G. B. & BRINSTER, R. L. (1978). Poly(A) and synthesis of polyadenylated RNA in the preimplantation mouse embryo. *Devl Biol.* **64**, 140–148.

MARO, B., JOHNSON, M. H., PICKERING, S. J. & FLACH, G. (1984). Changes in the actin distribution during fertilisation of the mouse egg. *J. Embryol. exp. Morph.* **81**, 211–237.

MCKEON, F., KIRSCHNER, M. W. & CAPUT, D. (1986). Homologies in both primary and secondary structure between nuclear envelope and intermediate filament protein. *Nature, Lond.* **319**, 463–468.

MCKEOWN, F. D., TUFFANELLI, D. L., FUKUYAMA, K. & KIRSCHNER, M. (1983). Autoimmune response against conserved determinants of nuclear envelope proteins in a patient with linear sclerodermia. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4374–4378.

MIRKOVITCH, J., MIRAULT, M. E. & LAEMMLI, U. K. (1984). Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. *Cell* **39**, 223–232.

NEWPORT, J. W. & KIRSCHNER, M. W. (1984). Regulation of the cell cycle during early Xenopus development. *Cell* **37**, 731–742.

NICOLSON, G. L., YANAGIMACHI, R. & YANAGIMACHI, H. (1975). Ultrastructural localisation of lectin binding sites on the zonae pellucidae and plasma membranes of mammalian eggs. *J. Cell Biol.* **66**, 263–274.

OTTAVIANO, Y. & GERACE, L. (1985). Phosphorylation of the nuclear lamins during interphase and mitosis. *J. biol. Chem.* **260**, 624–632.

SCHATTEN, G., MAUL, G. G., SCHATTEN, H., CHALY, N., SIMERLY, C., BALCZON, R. & BROWN, D. L. (1985). Nuclear lamins and peripheral nuclear antigens during fertilization and embryogenesis in mice and sea urchins. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4727–4731.

SCHLIWA, M., EUTENEUER, U., BULINSKY, J. C. & IZANT, J. G. (1981). Calcium lability of cytoplasmic microtubules and its modulation by microtubule associated proteins. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1037–1041.

SMITH, D. E. & FISHER, P. A. (1984). Identification, developmental regulation, and response to heat shock to two antigenitically 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.

STICK, R. & HAUSEN, P. (1985). Changes in the nuclear lamina composition during early development of *Xenopus laevis*. *Cell* **41**, 191–200.

TOWBIN, H., STAELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354.

WEBB, M., HOWLETT, S. K. & MARO, B. (1986). Parthenogenesis and cytoskeletal organisation in ageing mouse eggs. *J. Embryol. exp. Morph.* **95**, 131–145.

WHITTINGHAM, D. G. & WALES, R. G. (1969). Storage of two-cell mouse embryos in vitro. *Austr. J. biol. Sci.* **22**, 1065–1068.

YOUNG, R. J., SWEENEY, K. & BEDFORD, J. M. (1978). Uridine and guanosine incorporation by the mouse one-cell embryo. *J. Embryol. exp. Morph.* **44**, 133–148.

(Accepted 13 October 1987)