Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4727–4731, July 1985
Cell Biology

Nuclear lamins and peripheral nuclear antigens during fertilization and embryogenesis in mice and sea urchins

(development/karyoskeleton/mitosis/nuclear envelope)

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Communicated by J. Herbert Taylor, March 25, 1985

ABSTRACT **Nuclear structural changes during fertilization and embryogenesis in mice and in sea urchins have been followed by using antibodies against the nuclear lamins A/C and B and against antigens at the periphery of nuclei and chromosomes. Lamins are found on all pronuclei and nuclei during mouse fertilization, but with a diminished intensity on the second polar body nucleus. On sperm in both systems, lamins are reduced and detected only at the acrosomal and centriolar fossae. In sea urchin eggs, lamins are found on both pronuclei. Unlike in other dividing cells, the mitotic chromosomes of sea urchin eggs and embryos retain an association with lamins. The peripheral antibodies delineate each chromosome and nucleus except the mature mouse sperm nucleus. A dramatic change from the expected lamin distribution occurs during early development. In mouse morulae or blastocysts, lamins A/C are no longer recognized, although lamin B remains. In sea urchins both lamins A/C and lamin B, as detected with polyclonal antibodies, are lost after the blastula stage, although a different lamin A/C epitope emerges as recognized by a monoclonal antibody. These results demonstrate that pronucleus formation in both systems involves a new association or exposure of lamins, that the polar body nucleus is largely restricted from the cytoplasmic pool of lamins, and that mitotic chromosomes in the rapidly proliferating sea urchin egg retain associated lamins. They also suggest that changes in the expression or exposure of different lamins are a common feature of embryogenesis.**

Fertilization requires several dramatic changes in nuclear organization. The architecture of the nuclear surface (reviewed in refs. 1–4) involves the nuclear lamins, which are typically three proteins subjacent to the inner nuclear membrane (5, 6), and nuclear peripheral proteins referred to as "P1" (7) or "Perichromin" (8), which probably reside between the chromatin and the nuclear lamins. During mitosis in somatic cells, the lamins dissociate from the nuclear envelope at prophase and reappear with the reconstituting envelope at telophase (9). Unlike the behavior of the lamins at mitosis, the peripheral antigens separate from the nuclear periphery and ensheathe the condensing chromosomes before nuclear envelope breakdown and dissolution of the lamins during mitosis (7, 8). During spermatogenesis the nuclear lamins are lost or vastly reduced (10–12), whereas during oogenesis the lamina may be comprised of only a single lamin (13–16), which differs from somatic lamins (17).

In this study the presence and distribution of nuclear lamins and of the nuclear and chromosomal P1 peripheral antigens are traced during fertilization and embryogenesis in mice and in sea urchins. These two systems represent extremes in fertilization mechanisms. The sea urchin egg is spawned as a mature egg with a female pronucleus, and pronuclear fusion or syngamy occurs shortly after sperm incorporation. The ovulated mouse oocyte is arrested at second meiotic metaphase; since pronuclear fusion does not occur, fertilization is only completed at first mitosis, when the parental chromosomes align at metaphase.

Table 1. Distribution of nuclear lamins and peripheral nuclear antigens during fertilization and embryogenesis in mice and sea urchins

	Lamins			Peripheral nuclear Ag
	B	A/C		
	pAb	mAb	pAb	P1 mAb
Mouse				
Sperm	+/−	S	−/*	−
Oocytes; GV	+	+	+	+
Meiotic chromosomes, unfertilized	−	−	−	+
Pronucleus				
Male	+	+	+	+
Female	+	+	+	+
Polar body nucleus	−	−	−	+
Mitotic chromosomes	−	−	−	+
Nuclei				
Blastomere	+	+	+	+
Morula	+	−	−	+
Blastocyst	+	−	−	+
Adult somatic cells (3T3)	+	+	+	+
Sea urchin				
Sperm	S	S	S	+
Pronucleus				
Female, unfertilized	+	−	+	+
Male, fertilized	+	−	+	+
Female, fertilized	+	−	+	+
Mitotic chromosomes				
First	J	−	J	+
Morula	J	−	J	+
Nuclei				
Gastrula	−	+	−	+
Pluteus	−	+	−	+
Adult cells (coelomocytes)	+	−	+	+

mAb, Monoclonal antibody; pAb, polyclonal antibody; Ag, antigen; GV, germinal vesicles; *, apparent only after extraction with DNase and 2 M NaCl; S, lamins are localized only at the acrosomal and centriolar fossae in sperm; J, lamins localized at chromosome peripheries.

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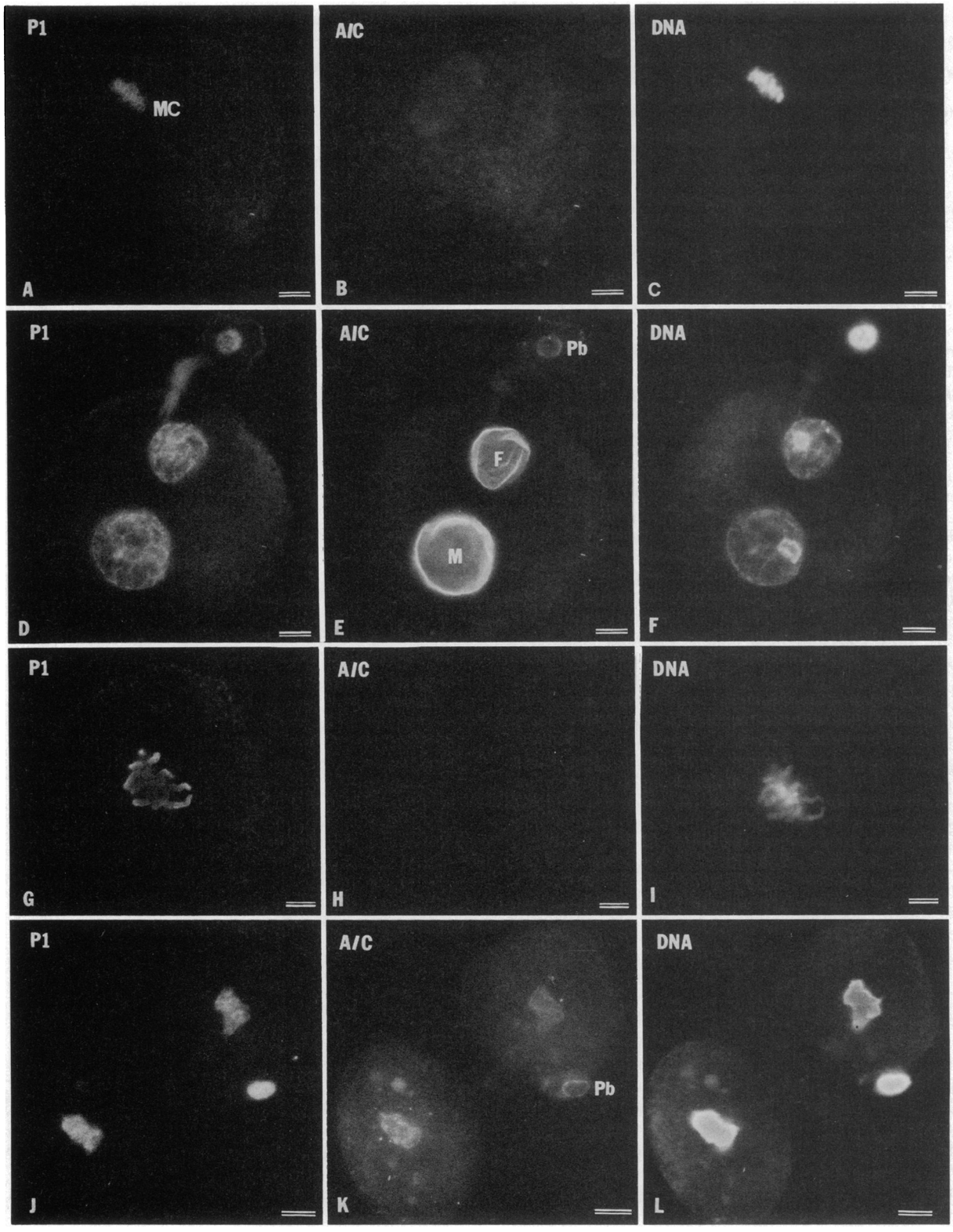

FIG. 1. Nuclear lamins and peripheral nuclear antigens during mouse fertilization and early development. (*A–C*) Unfertilized oocyte. (*A*) The P1 peripheral antigens ensheathe the surface of each meiotic chromosome (MC). (*B*) Lamin staining is lost in the ovulated oocyte, which is arrested at the second meiotic metaphase (lamins A/C). (*C*) Hoechst DNA fluorescence. (*D–F*) Pronucleate egg. (*D*) The peripheral antigens are associated with the rims of the male and female pronuclei and with the polar body nucleus. (*E*) The lamins A/C reassociate with the nuclear surface, and characteristically the polar body nucleus (Pb) stains only weakly. (*F*) Hoechst DNA fluorescence. (*G–I*) Mitotic egg. (*G*) At prophase, the P1 antibody against the peripheral antigens is redistributed from the pronuclear surfaces to cover each chromosome. (*H*) The lamins dissociate from the mitotic chromosomes. (*I*) Hoechst DNA fluorescence. (*J–L*) Cleavage. (*J*) As the daughter nuclei reform after first division, the peripheral antigens dissociate from the decondensing chromosomes and reassociate with the nuclear periphery (P1 antigen in *J*). (*K*) The lamins associate with the reformed nuclear envelope. (*L*) Hoechst DNA fluorescence. (Bars = 10 μm.)

MATERIALS AND METHODS

Gamete Collection and Fertilization. Virgin female CD-1 mice (Charles River Breeding Laboratories) were superovulated with 10 units of human chorionic gonadotropin, followed 48 hr later with 10 units of pregnant mare's serum gonadotropin (18). The oocytes were collected from the oviduct and fertilized *in vitro* by the methods of Whittingham (19). Morulae and blastocysts were collected from mated females 61, 66, and 84 hr after the estimated time of ovulation.

Gametes from the sea urchin *Lytechinus variegatus* were collected by intracoelomic stimulation with 0.5 M KCl. Eggs were spawned into Millipore-filtered (0.22 μm) sea water; sperm was kept "dry" on ice.

Antibodies. Four different antibodies to karyoskeletal antigens were used in this study (Table 1). Mouse monoclonal antibodies to nuclear lamins A/C have been described by Newmeyer (20) and by Maul *et al.* (16) and cross-react with two proteins in the 60–70 kDa range. Monoclonal antibody to P1, generated against nuclear matrix antigens and detecting nuclear and chromosomal peripheral antigens (7), cross-reacts with a triplet of proteins of 27, 30, and 32 kDa. Human autoimmune antibodies to lamins A/C were derived from a patient with linear scleroderma (LS-1; ref. 21), and those against lamin B were from a patient with systemic lupus erythematosus (SLE-50; G. G. Maul, T. Pinkus, A. E. Carrera, S. Jimenez, and G. Schatten, personal communication).

Immunofluorescence Microscopy. Mouse gametes were permeabilized in 25% glycerol/50 mM KCl/0.5 mM $MgCl_2$/0.1 mM EDTA/1 mM EGTA/1 mM 2-mercaptoethanol/50 mM imidazole, pH 6.7/1% Triton X-100 (22), and sea urchin gametes were extracted in 25% glycerol/25 mM 2-(*N*-morpholino)ethanesulfonic acid/10 mM EGTA/0.55 mM $MgCl_2$/25μM phenylmethylsulfonyl fluoride/1% Nonidet P-40 (23). The cells then were affixed to polylysine-coated coverslips (24) and fixed in methanol at −10°C. The cell extracts were incubated with the primary antibodies, washed with phosphate-buffered saline, and incubated with a second fluorescent antibody (Cappel Laboratories, Cochranville, PA). After a final rinse in phosphate-buffered saline, the coverslips were mounted over glycerol and sealed with nail polish. Zeiss epifluorescence microscopy equipped for Hoechst 33258 (American Hoechst, San Diego, CA), fluorescein, or rhodamine was used; cells were photographed with Tri-X film at an effective ASA of 1600, which was developed in Diaphine (Accuphine, Chicago, IL).

RESULTS

Mouse Fertilization: Lamins Appear on Pronuclei but Not on Chromosomes While Peripheral Antigens Are Present on Both. In the unfertilized mouse oocyte, peripheral antigens ensheathed each meiotic chromosome (labeled MC in Fig. 1*A*), and the lamins were not detected (Fig. 1*B*, see Table 1 for results with other tested antibodies). Hoechst DNA fluorescence of the chromosomes is shown in Fig. 1*C*.

During fertilization, the developing male and female pronuclei acquired lamins A/C (Fig. 1*E*), and the peripheral antigens redistributed to the nuclear periphery (Fig. 1*D*). The polar body nucleus (labeled Pb in Fig. 1*E*) was only dimly labeled with lamin antibody.

At mitosis the peripheral antigens condensed around each chromosome (Fig. 1*G*) as the lamins dissociated from the chromosome mass (Fig. 1*H*). After first division, the peripheral antigens again redistributed to the periphery of each nucleus (Fig. 1*J*), and the lamins reappeared on the recon-

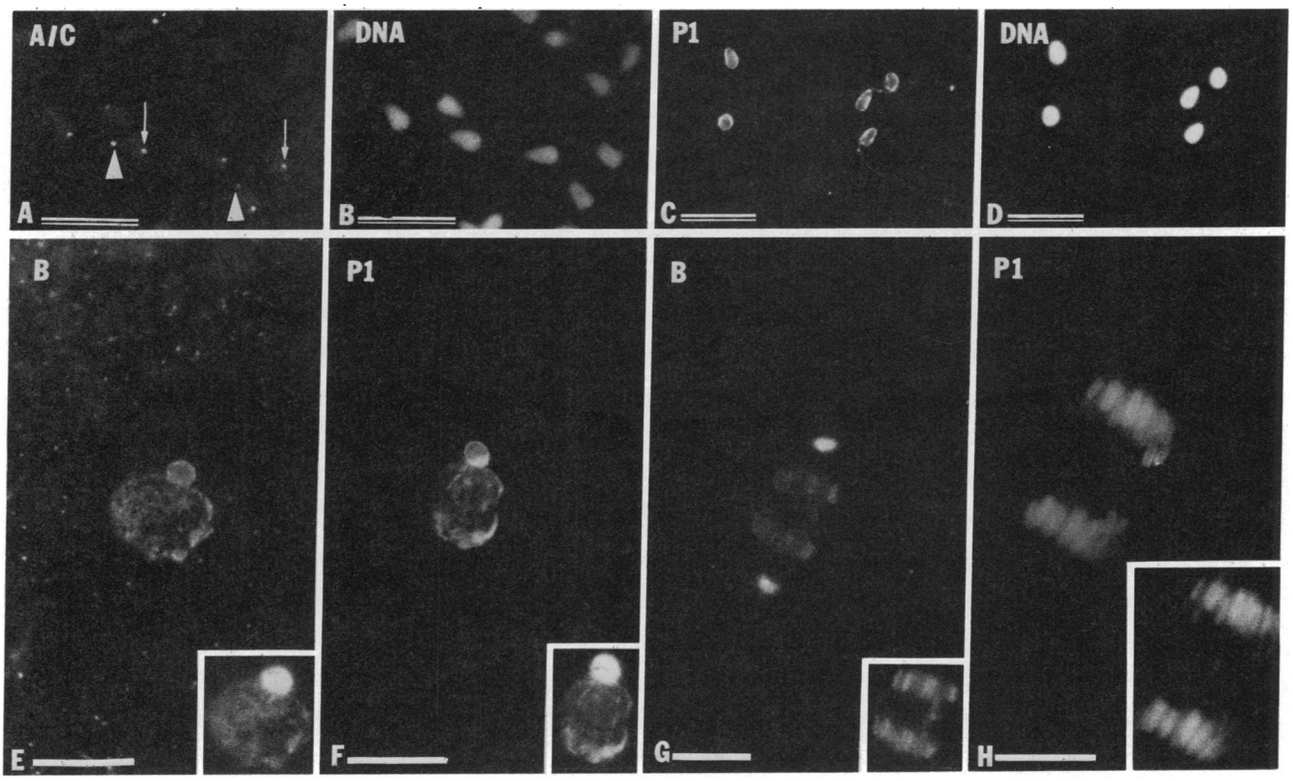

FIG. 2. Nuclear lamins and peripheral antigens during sea urchin fertilization and early development. (*A*) In sperm lamin antibodies label only the acrosomal (triangles) and centriolar (arrows) fossae. (*C*) Entire sperm nuclei are outlined with P1 antibody against the peripheral antigens. (*B* and *D*) DNA fluorescence. (*E* and *F*) During sea urchin fertilization, pronuclei bind lamin (*E*) and P1 antibodies against peripheral antigens (*F*). At syngamy these nuclear structural proteins are found along both pronuclear surfaces. (*G*) At mitosis, the lamins dissociate from the nuclear surface and are found on each chromosome; the centrosomes are frequently detected. (*H*) The peripheral antigens are redistributed to delineate each chromosome (P1 antigen in *H*). (*Insets E–H*) Hoechst DNA fluorescence. (Bars = 10 μm.)

stituted blastomere nuclei (Fig. 1*K*). Table 1 summarizes these localizations.

Sperm: Only Lamin Remnants Are Present. Lamins were reduced in sea urchin sperm to only the acrosomal and centriolar fossae (Fig. 2*A*), while the peripheral antigens were present around the sea urchin sperm nucleus (Fig. 2*C*). Mouse sperm bound lamin antibodies regionally and only sparsely and apparently do not contain the peripheral antigens (ref. 12; Table 1).

Sea Urchin Fertilization and Mitosis: Lamins and Peripheral Antigens Are Present on Both Pronuclei and Chromosomes. The female pronucleus of the unfertilized sea urchin egg was spawned with lamins and peripheral antigens (Table 1). After sperm incorporation, the decondensing male pronucleus displayed both sets of nuclear structural proteins (lamin B: Fig. 2*E*; peripheral antigens: Fig. 2*F*). At syngamy or pronuclear fusion (Fig. 2 *E* and *F*), the lamins and peripheral antigens remained at the nuclear surface and coalesced to form the zygote nucleus.

The lamins behave in an unusual fashion during mitosis in sea urchins. Unlike mouse zygotes (Fig. 1) and somatic cells (9), where lamins are never observed on chromosomes, nuclear lamins delineated each of the sea urchin mitotic chromosomes (Fig. 2*G*); frequently the centrosomes were also detected. The peripheral antigens were found to ensheathe each chromosome (Fig. 2*H*).

Embryogenesis: Detection of Different Lamins. Mouse and sea urchin embryogenesis displayed an unexpected appearance and disappearance of lamin epitopes (Table 1). In the mouse, staining of lamins A/C with either monoclonal or polyclonal antibody was unrecognizable at the morula and blastocyst stages (Fig. 3*B*), though lamin B staining was retained (Fig. 3*A*). Lamin B dissociated from the nuclear region during mitosis, as expected (labeled M in Fig. 3*A*). Sea urchin embryogenesis displayed a similar phenomenon with the loss of lamin recognition by human autoimmune antibodies in blastula, gastrula (lamin B: Fig. 3*D*), and plutei and with the new recognition of a monoclonal lamin antibody at these stages (lamins A/C: Fig. 3*F*) that did not bind to egg or morula nuclei (Table 1).

DISCUSSION

Fertilization in both mice and sea urchins involves the new appearance of nuclear lamins and rearrangements of the

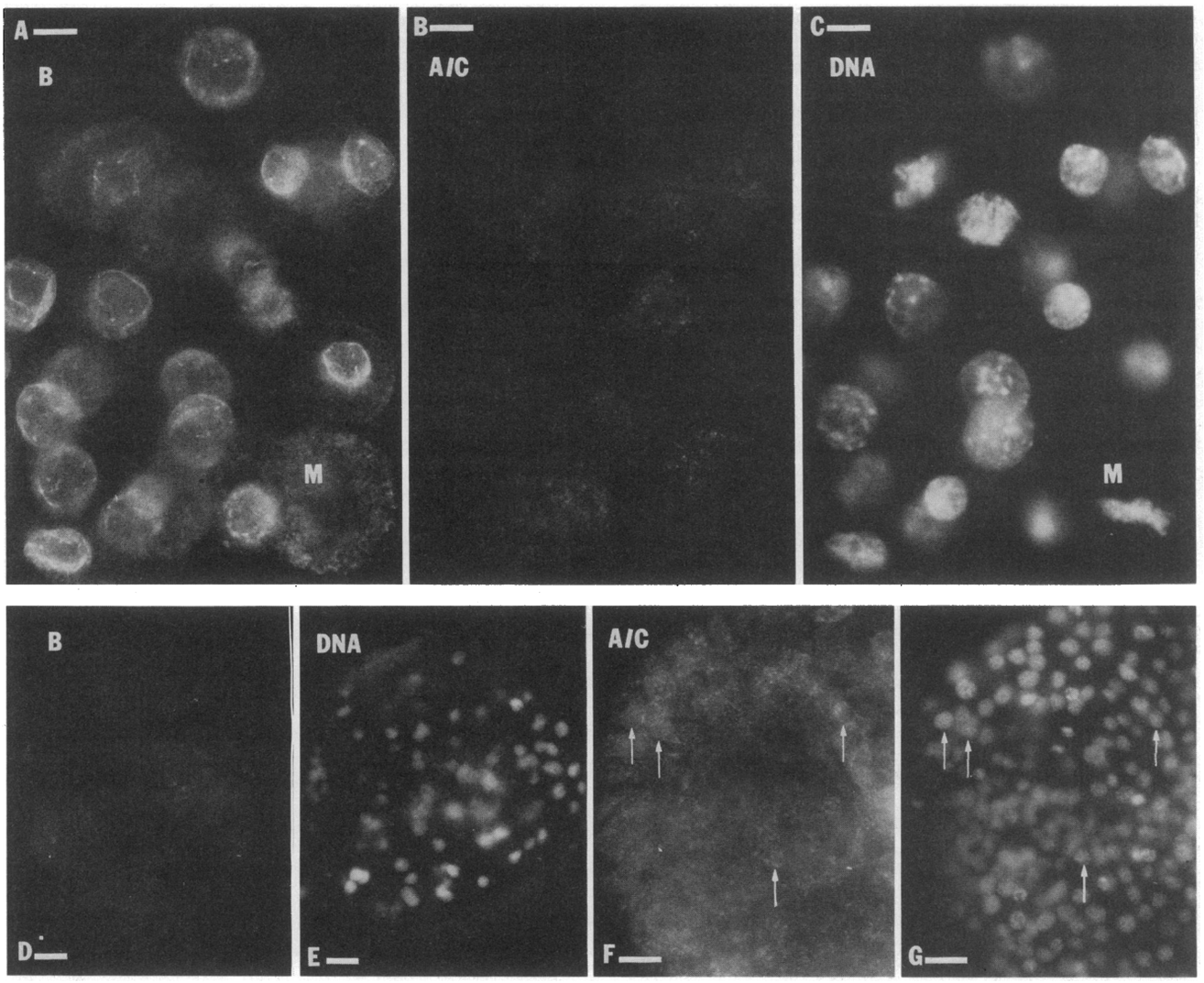

FIG. 3. Differential nuclear lamin appearance during embryogenesis in mice and in sea urchins. (*A* and *B*) In mouse morulae and blastocysts, lamin B is retained (*A*), while the staining with antibodies against lamins A/C is not (*B*). (*D* and *F*) In sea urchin blastulae, gastrulae (lamin B) (*D*), and plutei, staining with the human autoimmune antibodies is lost, whereas the mouse monoclonal antibody against lamins A/C, which did not label early nuclei, now binds to the perinuclear area (*F*). Arrows denote corresponding nuclei in *F* and *G*. (*C*, *E*, and *G*) DNA fluorescence. (Bars = 10 μm.) M, mitotic cell.

peripheral nuclear antigens. Detectable lamins are vastly reduced in sperm, and the formation of the male pronucleus in both systems, involving dramatic biochemical (reviewed in refs. 25 and 26) and ultrastructural rearrangements (27, 28), is coupled with the appearance of lamins associated with the male pronuclear envelope. In mouse oocytes, lamins appear on the female pronucleus as it develops after the completion of meiosis. The polar body nucleus has a reduced lamin complement, as judged by fluorescence intensity, perhaps because of its restriction from the cytoplasmic pool; this suggests a possible pathway leading to its ultimate degeneration.

Fertilization in the mouse is only formally completed at first division when the parental chromosomes intermix. At prophase, the lamins dissociate from the pronuclei, and the peripherals ensheathe each chromosome in a pattern typical for somatic cells (7–9). After telophase when diploid nuclei first form, the lamins and peripheral antigens redistribute to the nuclear surface.

The sea urchin egg is spawned with a mature female pronucleus, which already has associated lamins. The sperm nucleus after incorporation into the egg quickly expands with the concomitant uptake of lamins and peripheral antigens, probably of maternal origin. True pronuclear fusion occurs, and the lamins and peripheral antigens retain their association with the nuclear peripheries and merge to form the zygote nucleus. In contrast to division in somatic cells (9) and in the mouse zygotes, the lamins are retained in or around the chromosomes during the first few mitoses in sea urchins. This retention of lamins around the chromosomes could be essential for the swift nuclear envelope reconstitution during the rapid cell cycles in a pattern that is not dissimilar to that observed in *Drosophila* embryos, where the lamins have been shown to remain near the mitotic spindle (29). Whether the lamins remain attached to the sea urchin mitotic chromosomes or are present together with the membrane vesicles (reviewed in ref. 30) at or near the chromosomes remains to be established.

During embryogenesis in both systems, specific lamins may be replaced. Lamins A and C, closely related proteins (4, 31), are apparently absent in mouse morulae and blastocysts but reappear later in somatic cells. In sea urchin embryos, both lamins A/C and lamin B, as detected with polyclonal antibodies, are lost after the blastula stage, although a different lamin A/C epitope emerges as recognized by a monoclonal antibody. The differential disappearance of the lamins during embryogenesis occurs after the first divisions in the mouse and only at the blastula stage in sea urchins.

In both systems detection of the lamins by immunocytochemical methods can be influenced by several parameters. The lamins may be extracted if less tightly bound at certain stages. They may be diluted out during division to a level not recognizable by the assay methods, or secondary modifications may reduce the epitopes recognized by the antibodies. In contrast, the appearance of a new antigen on the nuclear envelope during embryogenesis might result from synthesis, new association of a stored but previously extracted protein, uncovering of epitopes, or an increase of the new lamins to the threshold for detection at a specific site. In this context it is of interest that Donovan *et al.* (32) report the disappearance and later reappearance of lamins in the cytoplasm during mouse egg activation. The recent findings that the single lamin present in *Xenopus* oocytes differs antigenically from the lamins in their nucleated erythrocytes (17) and that a specific lamin is present in mouse spermatids (G. Maul, personal communication) support the contention that we detect different lamins during embryogenesis.

Although the lamins undergo fluctuations in staining patterns during the cell cycle and during development, the P1 peripheral antigens are present on all nuclei and chromosomes, except the mature mouse sperm. These results support the suggestion of Chaly *et al.* (7) and McKeon *et al.* (8) that these peripheral nuclear antigens might be involved in maintaining chromatin/chromosome order.

In summary, the nuclear lamins lost during spermatogenesis are restored at fertilization probably from maternal sources, though the new exposure of paternal proteins cannot yet be excluded. The peripheral antigens associate with the surface of chromosomes during meiosis and mitosis, and with the periphery of pronuclei and nuclei during interphase; sea urchin sperm nuclei also have a coating of the peripheral antigens, though it appears to be absent in mature mouse sperm nuclei. In the mammalian system, the nuclear lamins behave during mitosis as observed in somatic cells: they undergo dissolution at late prophase and reassemble at telophase. In contrast, nuclear lamins are retained on the chromosomes during mitosis in sea urchin eggs. During embryogenesis, specific lamins are differentially expressed or exposed in both systems studied. Changes in the architecture of the nuclei that participate in fertilization and embryogenesis may prove crucial for later events leading to development and differentiation.

It is our pleasure to acknowledge the generous donation of human autoimmune antibodies to nuclear lamins A and C by Dr. Frank McKeon (University of California, San Francisco) and the support of this research by the National Institutes of Health (Grants HD12913 and RR1466 to G.S., Grants GM21615 and CA10815 to G.M., and Research Career Development Award HD363 to G.S.), the National Science Foundation (PCM 8315900 to G.S.), and the Medical Research Council of Canada (D.L.B.).

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