MITOTIC CHROMOSOME CONDENSATION IN THE SPERM NUCLEUS DURING POSTFERTILIZATION MATURATION DIVISION IN *URECHIS* EGGS

NIRMAL K. DAS and CAROLYN BARKER. From the Department of Cell Biology, College of Medicine, University of Kentucky, Lexington, Kentucky 40506

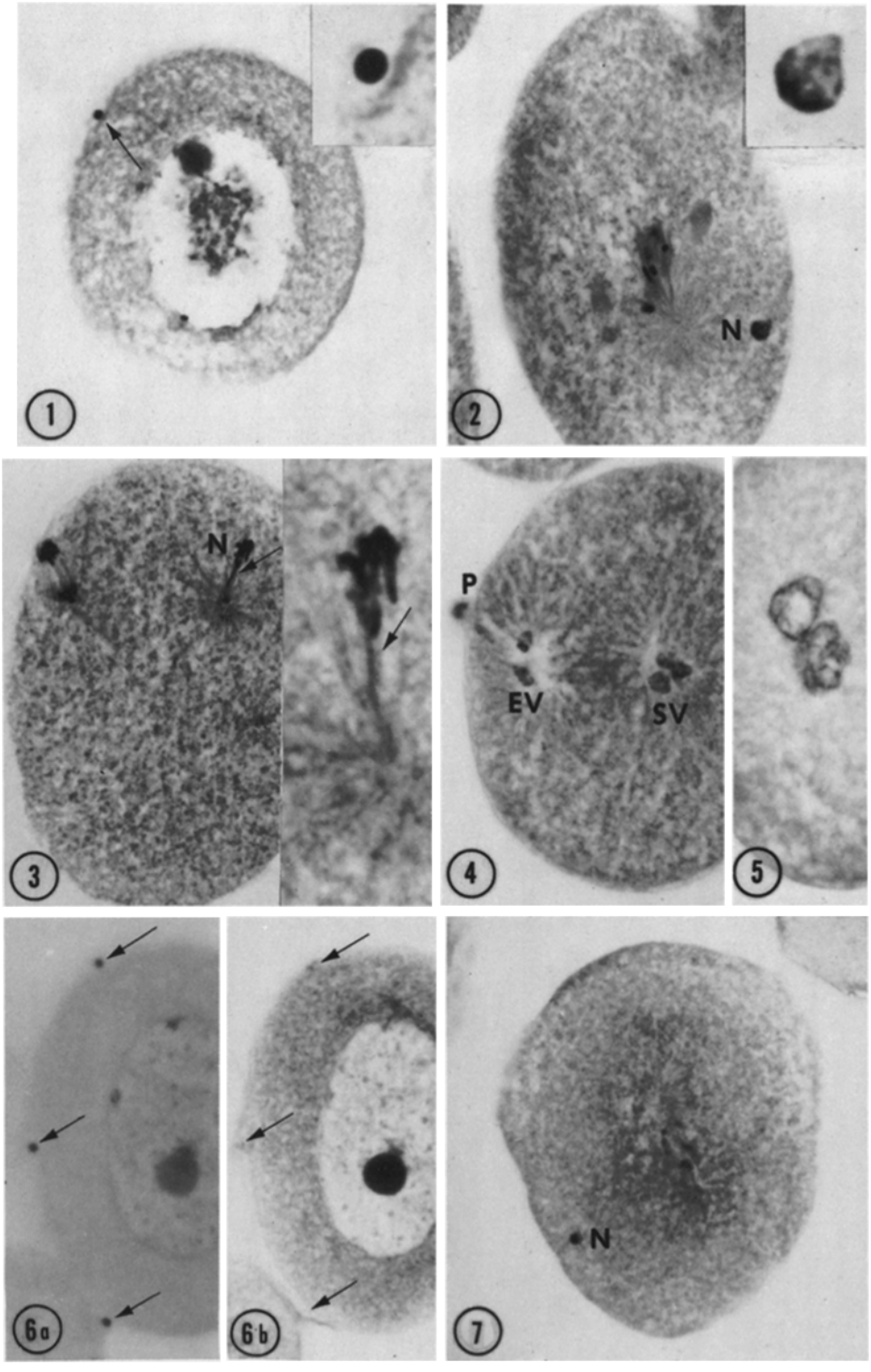
Changes in the morphology of the sperm nucleus in the egg cytoplasm are mong the immediate events in nucleocytoplasmic interactions during early embryogenesis. Soon after its entrance into the egg cytoplasm, the sperm nucleus of various organisms increases in size with the transformation of condensed chromatin to a diffuse state, resembling the chromatin of an interphase nucleus (2, 13, 15, 16). This is followed by a close association or fusion of male and female pronuclei (2, 13, 15, 16). Cytoplasmic influences on nuclear morphology have also been demonstrated clearly in nuclear transplantation and cell fusion studies (10, 11). Reactivation of the nucleus, such as the transplanted brain nucleus in *Xenopus* egg cytoplasm or the hen erythrocyte nucleus in interphase cytoplasm of HeLa cells, is accompanied by nuclear enlargement and chromatin dispersion (10, 11). However, premature mitotic-like chromosome condensation takes place in the nuclei of sperm or interphase cells fused with mitotic cells (9, 12). Thus, chromosome dispersion and condensation seem to depend on the state of the cytoplasm in which the nucleus is present. These observations imply that the initial morphological changes in the sperm nucleus after fertilization may very well be dependent on the state of maturation of eggs at the time of sperm entry. Unfertilized eggs of *Urechis caupo*, a marine echiuroid worm, are stored at the diakinesis stage. These eggs complete maturation division after insemination, and this is followed by fusion of male and female pronuclei (5, 8). Therefore, *Urechis caupo* is a suitable organism in which to study the response of the sperm nucleus to the changing state of the egg cytoplasm during and after postfertilization maturation division.

MATERIALS AND METHODS

Eggs of *Urechis caupo* were fertilized at 16–18°C in sea water (5). Samples were fixed in 10% neutral Formalin or acetic acid-alcohol at various times after fertilization. Eggs were washed throughly in distilled water, and squash preparations or 10-μm paraffin sections were made. Slides were stained with Feulgen-acid fast green or Feulgen-hematoxylin. Some slides were also stained for histones by alkaline fast green (AFG) (1) and for total proteins by acid fast green or Millon's reaction (3). Changes in the size of the sperm nucleus, after fertilization, were determined from nuclear diameters.

RESULTS

The sperm nucleus of *Urechis* is highly condensed and nearly spherical in shape (Fig. 1). Such nuclei exhibit mitotic chromosome condensation in the cytoplasm of eggs undergoing postfertilization maturation division. Squash preparations and sections of eggs, stained with Feulgen-acid fast green or Feulgen-hematoxylin, show the beginning of such chromosome condensation by 20 min after fertilization (Fig. 2). Eggs are in the first meiotic division during this time. Sperm chromosomes become more distinct in appearance 40–50 min later when eggs are in the second meiotic division (Fig. 3). In many cases, sperm chromosomes appear to be stretched out at one end, giving an impression that they are pulled by sperm astral fibers (Fig. 3). However, no typical metaphase or anaphase sperm chromosome configurations are seen at any time after fertilization. In many eggs, after the completion of maturation division, both paternal and maternal chromosomes become diffuse at the same time and form vesicles or karyomeres of varying numbers (Fig. 4). The



detection of paternal chromosome vesicles is an indication of some degree of separation of these chromosomes from one another during the maturation division of the egg. Chromosome vesicles coalesce into highly enlarged male and female pronuclei (Fig. 5), typical of many organisms (2, 13, 15, 16). The pronuclei fuse 75–85 min after fertilization.

The initiation of mitotic chromosome condensation in the sperm nucleus is preceded by replacement of protamine-type proteins by adult histones and nuclear enlargement. Sperm nuclei of *Urechis*, containing protamine-type protein, do not stain with AFG (6, 7). However, they stain well within 10–15 min after fertilization (cf. Figs. 6 and 7; see also reference 7). This stainability of sperm nuclei is blocked by acetylation, suggesting that adult histones are involved in the staining reaction (3). The volume of the sperm nucleus increases during the time of transition to histones and up to 30 min after fertilization (Fig. 8). Sections of fertilized eggs stained for total proteins by Millon's reaction or by acid fast green show a qualitatively similar staining intensity in sperm nuclei at various stages of enlargement. Therefore, the protein content of the sperm nucleus seems to be increasing corresponding with the increase of its volume. Most of these proteins are not synthesized immediately before or after fertilization. Autoradiographs of eggs exposed to [^{3}H]leucine for 20 h, fertilized, and then sampled 10–50 min later show little or no labeled proteins in the sperm nucleus (7). Under similar experimental conditions, migration of labeled egg RNA into sperm nuclei also could not be detected.

DISCUSSION

The influence of egg cytoplasm on morphological and cytochemical events in the sperm nucleus, after fertilization, is further demonstrated by the present results (Fig. 8). The response of the *Urechis* sperm nucleus to the egg cytoplasm is similar to that of the egg nucleus. Mitotic-like sperm chromosome condensation is induced at the time of postfertilization maturation division of eggs. Such an effect of the egg cytoplasm on sperm nuclei after normal fertilization has not yet been reported, although the haploid sperm nuclei have been observed to enter "mitosis" when they are present in the cytoplasm of mitotic cells (9, 12) and developing oocytes (4). In sea urchins, in which the egg is at the haploid interphase stage, the sperm chromatin decondenses into the interphase condition shortly after fertilization (13); however, premature mitotic sperm chromosome condensation occurs in the cytoplasm of eggs in which the maternal chromosome cycle is turned on by NH_4OH before sperm entry (14). Thus, the highly compact sperm nucleus can undergo mitotic-like

FIGURES 1–5 Feulgen-acid fast green stained sections (10 μm) of eggs fixed at various times after fertilization. *Insets* (in Figs. 1–3) are higher magnifications of sperm nuclei. × 700. *Insets:* × 2700.

FIGURE 1 5 min after fertilization; sperm nucleus (arrow) attached to egg surface.

FIGURE 2 20 min after fertilization; first meiotic metaphase; beginning of mitotic-like sperm chromosome (*N*) condensation.

FIGURE 3 65 min after fertilization; second meiotic anaphase; sperm chromosomes (*N*) appear more distinct and seem to be pulled by sperm astral fibers (arrow).

FIGURES 4 and 5 75 min after fertilization; vesicle (Fig. 4) and pronuclear (Fig. 5) stages; female chromosomal vesicles (*EV*) may be distinguished from male chromosomal vesicles (*SV*) because of their close proximity to the polar body (*P*). In Fig. 5 the male and female pronuclei cannot be distinguished.

FIGURES 6 *a* and *b* 5 min after fertilization; egg section stained with hematoxylin (Fig. 6 *a*) to show sperm nuclei (arrows) attached to the egg surface; the same section was then stained with AFG staining procedures (Fig. 6 *b*) specific for histones. Note that these sperm nuclei do not stain with AFG. Hematoxylin stain is lost during the hot trichloroacetic acid hydrolysis used to extract DNA before AFG staining. × 700.

FIGURE 7 15 min after fertilization; sperm nucleus (*N*), present in the egg cytoplasm, stain well with AFG. × 700.

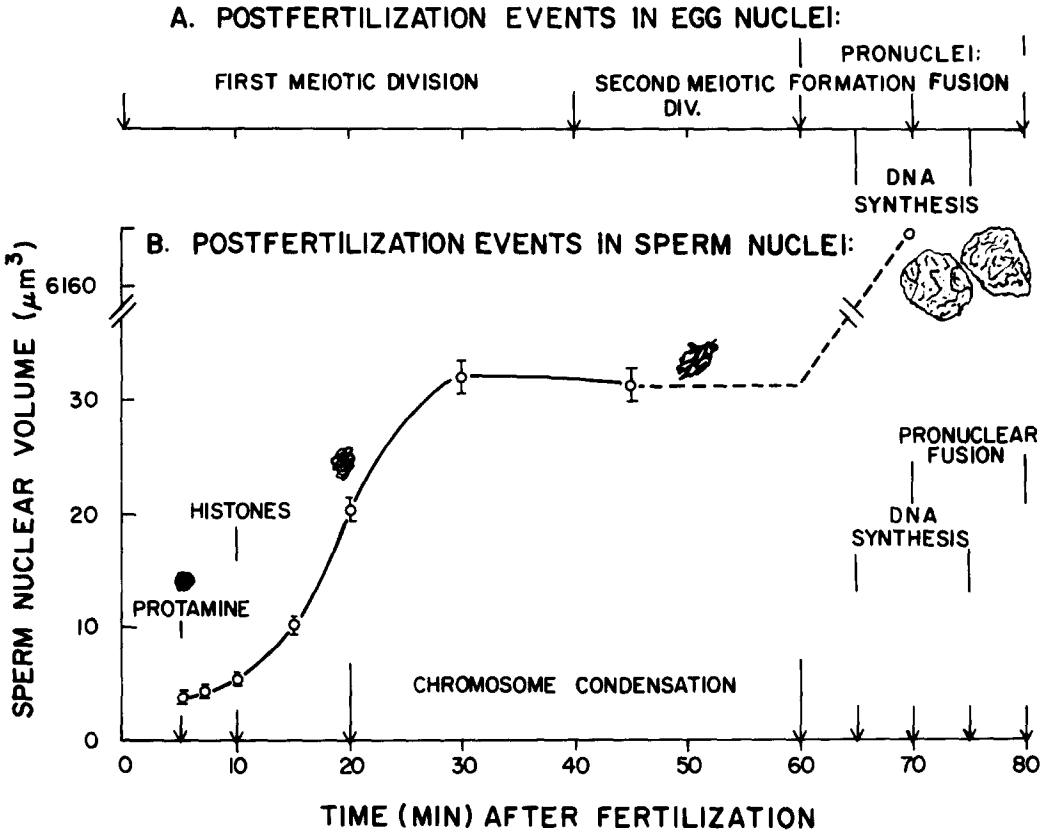


FIGURE 8 Summary of morphological and cytochemical events in *Urechis* sperm and egg nuclei after fertilization. The graph illustrates changes in the volume of sperm nuclei (25 at each point) with time. The size and morphology of the sperm nucleus are illustrated by India ink drawings at several points. Nuclear volumes cannot be determined between 50–70 min after fertilization when chromosomes are condensed and irregularly arranged, a stage that is followed by formation of chromosomal vesicles.

chromosome condensation without first going through the diffuse interphase stage and DNA synthesis in the cytoplasm of dividing cells.

The significance of the postfertilization mitotic behavior of *Urechis* sperm chromosomes, in relation to their reactivation, is not known. Perhaps it bears some relation to the proposal that telophase of mitosis is the stage when reprogramming of chromosomes could occur (10). In many eggs after maturation division groups of both sperm and egg chromosomes form vesicles which fuse to form greatly swollen male and female pronuclei. This is followed by DNA synthesis in both pronuclei (7). The postfertilization reactivation of sperm nuclei of various organisms, as well as the reactivation of somatic nuclei after transplantation or cell fusion, also accompanies telophase-like nuclear enlargement and chromatin dispersion (9–11). If reprogramming does occur, it probably is related to the kinds of proteins acquired during nuclear enlargement.

Little is known about the molecular basis of the morphological changes in the sperm nucleus after fertilization. As indicated above, the *Urechis* sperm nucleus contains protamine-type protein. Transition of protamine-type protein to adult histones and nuclear enlargement precede chromosome condensation (Fig. 8). We have also observed that pronuclear enlargement and DNA synthesis in *Urechis* are associated with the migration of some egg proteins into these nuclei (7). These observations may indicate that the mitotic-like chromosome condensation and the subsequent chromosome reactivation in *Urechis* sperm nuclei require replacement of protamine by histones and association of nonhistone egg proteins with chromosomes.

SUMMARY

The sperm nucleus of *Urechis caupo* undergoes mitotic-like chromosome condensation in the cytoplasm of the egg in postfertilization maturation division. The maturation division is followed by concomitant formation of sperm and egg chromosomal vesicles and coalescence of these vesicles into enlarged male and female pronuclei. These morphological changes are preceded by the replacement of protamine-type protein of the sperm nucleus by adult histones and perhaps also by the migration into the sperm nucleus of some nonhistone egg proteins.

We would like to dedicate this report to the memory of Professor Berwind P. Kaufmann (1897–1975) who was the senior author's teacher, friend, and inspiration. We thank Drs. Lester Goldstein and Jesse E. Sisken for constructive comments on the manuscript.

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