Transformation of Sperm Nuclei to Metaphase Chromosomes in the Cytoplasm of Maturing Oocytes of the Mouse

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Abstract. Zona-free oocytes of the mouse were inseminated at prometaphase I or metaphase I of meiotic maturation in vitro, and the behavior of the sperm nuclei within the oocyte cytoplasm was examined. If the oocytes were penetrated by up to three sperm, maturation continued during subsequent incubation and became arrested at metaphase II. Meanwhile, each sperm nucleus underwent the following changes. First, the chromatin became slightly dispersed. By 6 h after insemination, this dispersed chromatin had become coalesced into a small mass, from which short chromosomal arms later became projected. Between 12 and 18 h after insemination, each mass of chromatin became resolved into 20 discrete metaphase chromosomes. In contrast, if oocytes were penetrated by four to six sperm, oocyte meiosis was arrested at metaphase I, and each sperm nucleus was transformed into a small mass of chromatin rather than into metaphase chromosomes. If oocytes were penetrated by more than six sperm, the maternal chromosomes became either decondensed or pycnotic, and the sperm nuclei were transformed into larger masses of chromatin. As control experiments, immature and fully mature metaphase II oocytes were inseminated. In the immature oocytes, which were kept immature by exposure to dibutyryl cyclic AMP, no morphological changes in the sperm nucleus were observed. On the other hand, in the fully mature oocytes, which were activated by sperm penetration, the sperm nucleus was transformed into the male pronucleus. Therefore, the cytoplasm of the maturing oocyte develops an activity that can transform the highly condensed chromatin of the sperm into metaphase chromosomes. However, the capacity of an oocyte is limited, such that it can transform a maximum of three sperm nuclei into metaphase chromosomes. Furthermore, the presence of more than six sperm causes a loss of the ability of the oocyte to maintain the maternal chromosomes in a metaphase state.

THE initiation of meiotic maturation in vertebrate oocytes is marked by the dissolution of the nuclear membrane of the germinal vesicle and the condensation of the chromosomes to a metaphase state. The oocytes undergo the first meiotic division and then become arrested at metaphase II. Thus, after germinal vesicle breakdown (GVBD),[1] the oocyte chromosomes remain condensed until the oocytes are fertilized (reviewed in reference 24).

The mechanisms that initiate and maintain chromosome condensation within the maturing oocyte have been investigated by introducing the nuclei from interphase cells into the cytoplasm of maturing oocytes. When a follicle cell, blastomere, thymocyte, or immature oocyte is fused to the cytoplasm of a maturing oocyte of the mouse, the chromatin of the nucleus is rapidly transformed into metaphase chromosomes (1, 2, 8, 39). In amphibians, the cytoplasm of the maturing oocyte can transform the chromatin of injected brain nuclei into metaphase chromosomes (14, 46). Furthermore, cytoplasm or cytoplasmic extracts prepared from frog (23, 32, 34, 43) or mouse (36) oocytes arrested at metaphase II will induce GVBD and chromosome condensation when injected into frog immature oocytes. All these results indicate that, in a wide variety of species, maturing oocytes possess a cytoplasmic activity that induces the transformation of interphase nuclei to metaphase chromosomes.

However, when maturing oocytes of several mammalian species were either inseminated or injected with sperm nuclei, the nuclei did not form metaphase chromosomes. Instead, they either became enlarged or formed a pronucleus (mouse [17, 18]; hamster [41]; rat [30, 40]; dog [22]). From these results, it appears that, in contrast to its effect on somatic nuclei, the cytoplasm of maturing oocytes of mammals cannot induce the transformation of sperm nuclei to metaphase chromosomes. The experiments described below were done to reconcile the discrepancy between the results obtained when somatic nuclei and sperm nuclei are exposed to the cytoplasm of mammalian oocytes.

Materials and Methods

Collection and Culture of Ovarian Oocytes

Sexually mature female mice (CD-1, Charles River Breeding Laboratories, Inc., Wilmington, MA) were given an injection of 5 IU pregnant mares' serum

[1] *Abbreviations used in this paper:* dbcAMP, dibutyryl cyclic AMP; GVBD, germinal vesicle breakdown.

(Sigma Chemical Co., St. Louis, MO), and killed 44–48 h later. Their ovaries were removed and placed in modified L-15 medium (20) that contained neither amino acids nor vitamins and to which 50 μg/ml of dibutyryl cyclic AMP (dbcAMP) had been added. The ovarian follicles were punctured using the sharp tips of forceps, thus releasing immature oocytes that were partly or completely free of cumulus cells. The dbcAMP prevented spontaneous GVBD during collection of these oocytes (6). The oocytes were transferred to modified minimum essential medium (35) or Brinster's medium (5), and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

GVBD occurred after ~2 h of incubation, and the first polar body was formed ~10 h later. Depending on the experiment, the oocytes were incubated for 2 h (prometaphase I oocytes), 8–9 h (metaphase I oocytes), or 18–21 h (metaphase II oocytes) before insemination.

In some experiments, immature oocytes were incubated for 2 h in medium supplemented with 100 μg/ml dbcAMP, and then were inseminated. This 2-h exposure to dbcAMP did not affect the subsequent events of meiosis in these oocytes after they were transferred to dbcAMP-free medium after insemination.

Removal of Zonae pellucidae

To increase the chance of sperm penetration into the oocytes, the zonae pellucidae were removed shortly before insemination. In most experiments, the oocytes were exposed to 0.05% α-chymotrypsin (Sigma Chemical Co.) in Dulbecco's modified Eagle's medium (11) at 37°C for less than 1 min, until the zona around each oocyte had swelled and thinned. When the oocytes were transferred, using a micropipette, from the enzyme solution to L-15, the zona usually fell away, together with any cumulus cells that had been enclosing the oocytes. Since longer enzyme treatments caused a loss of oocyte fertilizability, in some experiments the zonae were removed by incubation for several minutes in acidified (pH 3.0) Tyrode's medium (29). However, when this method was used, small fragments of the zona and cumulus cells often remained on the surface of the oocyte, as seen using the dissecting microscope. For this reason, the enzymatic method of zona removal was used in most experiments. Only zona-free oocytes that were completely free of cumulus cells were used in all insemination experiments. These oocytes were rinsed in L-15 medium and in F medium (27) and transferred to a dish (Falcon 3037, Falcon Labware, Oxnard, CA) that contained 0.5 ml of F medium. This modified Krebs-Ringer solution (16) was used for sperm capacitation and for insemination.

Collection of Sperm

Vas deferens-cauda epididymus complexes were dissected out of a sexually mature male and placed in F medium. Fat adhering to the caudae was removed, and the complexes were transferred to a dish that contained 0.5 ml of F medium. The sperm were squeezed out of each vas deferens using forceps, proceeding from the epididymal end of the vas deferens to its cut end. The extruded sperm were dispersed, the empty vasa were cut away from the caudae and discarded, and the caudae were shredded to release the sperm inside. The minced tissue was left in the dish along with the sperm from the vasa. This concentrated sperm suspension was placed in the CO_2 incubator for 15 min. Taking care not to include pieces of minced caudae, 75 μl of the concentrated suspension then was taken from the dish and diluted with 0.5 ml of F medium. This dilute sperm suspension was placed in the CO_2 incubator for 45 min to allow capacitation.

Insemination and Subsequent Incubation

A measured volume of the dilute sperm suspension was added to the 3037 dishes containing the oocytes suspended in 0.5 ml of F medium. Different dishes received different volumes of sperm suspension, with the aim of producing a wide range of polyspermy in each experiment. Generally, each dish contained 20–50 oocytes and received 0.5–50 μl of dilute sperm suspension. These volumes of sperm suspension may be expected to produce a final sperm concentration of 10^3–10^5 sperm/ml, based on previous measurements (26). After the sperm were added, the dishes were gently swirled to mix the oocytes and sperm, and returned to the incubator. 45 min later, the oocytes were transferred to Whitten's medium (44), which was modified by reducing the NaCl from 88 mM to 71 mM, and kept for 3–30 h.

Fixation and Staining

In some cases, the oocytes were fixed for several minutes in a fixative that consisted of 8 vol ethanol/3 vol water/3 vol acetic acid, then transferred along with a drop of fixative onto a glass slide and air-dried. For more detailed chromosome examination, the method of Tarkowski (38) was used. Living oocytes were exposed for several minutes to a 1% (wt/vol) solution of Na-citrate (BDH Chemicals, Poole, UK) in water, then transferred into a drop of fixative on a slide, and air-dried. All of the fixed oocytes were stained using a 2% (vol/vol) solution of Giemsa (BDH Chemicals) in 0.02 M phosphate buffer (pH 6.8).

Results

Insemination of Fully Mature Oocytes (Normal Fertilization)

To determine whether zona-free oocytes that had undergone meiotic maturation in vitro could be activated and support pronuclear formation after fertilization, immature oocytes were incubated for 18–21 h, and those that underwent GVBD and gave off a polar body were freed of the zona and inseminated. By 12 h after insemination, 74% (114/155) of the oocytes that had been penetrated by a sperm had emitted the second polar body, and had developed a female pronucleus and one or more male pronuclei. The rest of the sperm-penetrated oocytes had failed to develop a male pronucleus, though some of these oocytes had emitted the second polar body and developed a female pronucleus. These results show that oocyte activation and development of male and female pronuclei could occur in oocytes that had undergone meiotic maturation in vitro.

Insemination of Maturing Oocytes

Effects on the Progression of Oocyte Meiosis. MONOSPERMY. Fully grown immature oocytes of the mouse advance through meiosis from prophase I to metaphase II in 12 h when they are placed in culture (10, 42). We confirmed this observation and investigated whether penetration of the maturing oocyte by sperm would interfere either with the progression of meiosis or with its arrest at metaphase II. Oocytes were inseminated at prometaphase I or at metaphase I, and incubated for different lengths of time. Then the maternal chromosomes were examined to determine the meiotic stage that each oocyte had reached. Oocytes that were not penetrated by sperm during the insemination served as controls.

Of the oocytes that had been inseminated at prometaphase I, only 19% (5/27) of those that were penetrated by a sperm reached metaphase II during the first 9 h of incubation after insemination, compared with 44% (18/41) of the unpenetrated control oocytes. However, by 12 h of incubation, 82% (31/38) of the sperm-penetrated oocytes and 89% (32/36) of the control oocytes had reached metaphase II. These results indicate that sperm penetration at prometaphase I may have retarded meiosis slightly, but did not otherwise impair the ability of the oocytes to complete maturation and become arrested at metaphase II. When oocytes had been inseminated at metaphase I, over 90% of both the penetrated and control oocytes reached metaphase II by 9 h after insemination. Thus, sperm penetration at metaphase I did not delay the first meiotic division.

When fixed 18 h after insemination, most of the oocytes that had been penetrated by a sperm contained maternal chromosomes morphologically similar to those of control oocytes (Fig. 1 *a*). These metaphase II chromosomes were slender and appeared to be coiled. However, in some sperm-penetrated oocytes, the maternal chromosomes became shortened and thickened, though to differing degrees in different oocytes. Fig. 1 *b* shows a typical example of this morphological change. Apparently, in these cases, sperm penetration had an effect on the condensation of the maternal chromosomes.

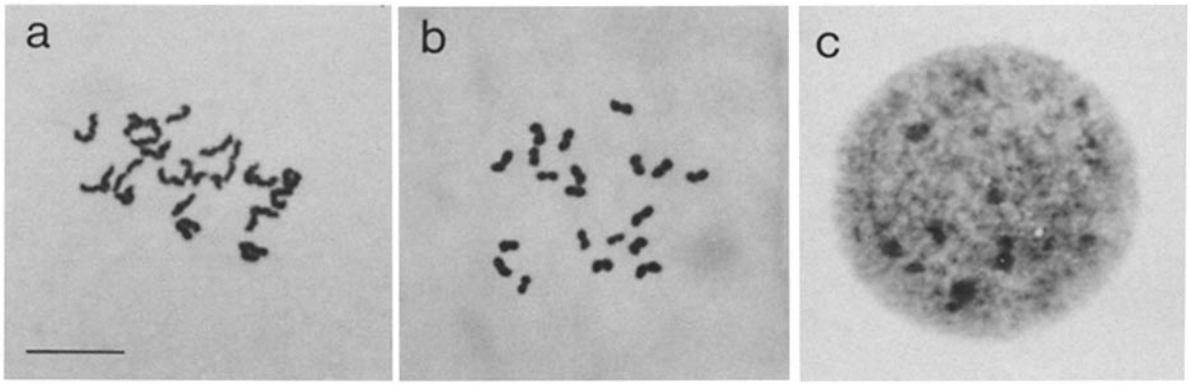

Figure 1. Morphology of the maternal chromosomes in oocytes inseminated at prometaphase I and incubated for 18 h. (*a*) Control oocyte; (*b*) monospermic oocyte; (*c*) highly polyspermic oocyte (7 sperm). Bar, 10 μm.

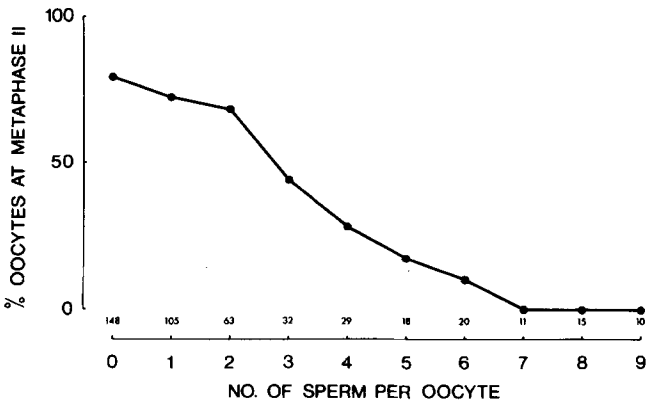

Figure 2. Effect of polyspermy on oocyte meiosis. Oocytes at prometaphase I were inseminated and incubated for ≥18 h. Oocytes were classified as metaphase II if they contained one or two groups of 20 dyads. The number of oocytes examined are shown along the abscissa.

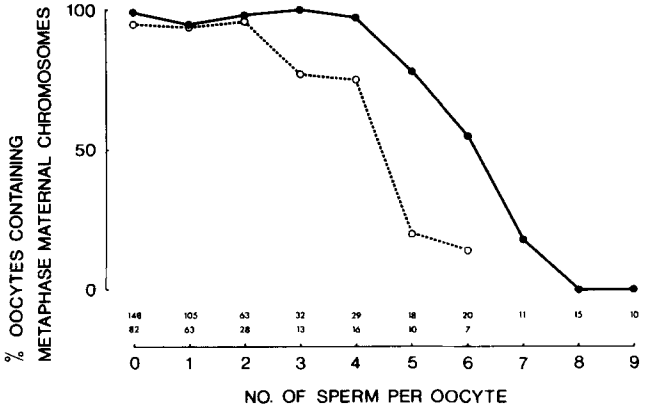

Figure 3. Effect of polyspermy on maternal chromosome morphology. Oocytes at prometaphase I (solid line) or metaphase I (dotted line) were inseminated and incubated for ≥18 h. The numbers of oocytes examined are shown along the abscissa.

POLYSPERMY. The effects of polyspermy on oocyte meiosis and the morphology of the maternal chromosomes were analyzed also, using the oocytes that had been inseminated at prometaphase I and incubated for 18 h. Oocytes that had been penetrated by more than two sperm were inhibited in a dose-dependent manner from completing meiosis to metaphase II (Fig. 2). Those oocytes that had been penetrated by three, four, or five sperm usually were arrested at metaphase I. The shortening and thickening of the maternal chromosomes, which was observed in some monospermic oocytes, occurred more frequently in these polyspermic oocytes. In some cases, the chromosomes had become extremely contracted, so that individual chromatids could not be distinguished.

In highly polyspermic oocytes, which had been penetrated by more than six sperm, the maternal chromosomes underwent drastic morphological changes. Discrete metaphase chromosomes could be seen 6 h after insemination, but were no longer present 3 h later. In some cases, the chromosomes had instead become decondensed, forming a granular or fibrous mass of chromatin (Fig. 1*c*). This decondensed chromatin was confined to a clearly circumscribed area, which suggests that it might have been surrounded by a nuclear envelope. However, these chromatin structures usually were not as large as a germinal vesicle or a pronucleus. Furthermore, whereas the germinal vesicle and pronucleus both contain nucleoli that are visible in the living oocyte, no nucleoli were visible in either living or fixed highly polyspermic oocytes. In other highly polyspermic oocytes, the maternal chromosomes became coalesced into a darkly stained dot of pycnotic chromatin. As seen in Fig. 3, the decondensation or pycnosis of the maternal chromosomes could be induced by fewer sperm if the oocytes were inseminated at metaphase I than if the oocytes were inseminated at prometaphase I. It may be concluded that a high degree of polyspermy inhibited the ability of the oocyte to maintain the metaphase state of the maternal chromosomes.

Effects on Sperm Nuclear Morphology. MONOSPERMY. To examine the changes that occurred in the sperm nucleus within the oocyte cytoplasm, the oocytes were fixed at 3-h intervals after insemination. If immature oocytes were inseminated, and then were incubated in the presence of dbcAMP to prevent GVBD, the sperm nucleus appeared as a compact, darkly stained structure (Fig. 4*a*), which did not undergo any morphological changes. In contrast, if prometaphase I or metaphase I oocytes were inseminated, or if the inseminated immature oocytes were allowed to undergo GVBD, a series of changes was observed in the sperm nucleus.

In these oocytes, by 3 h after insemination, the chromatin of the sperm nucleus had become dispersed slightly (Fig. 4*b*), but not as extensively as the chromatin of a male pronucleus. Unlike a typical pronucleus, the shape of the dispersed chromatin was not spherical. Instead, it retained the oblong shape

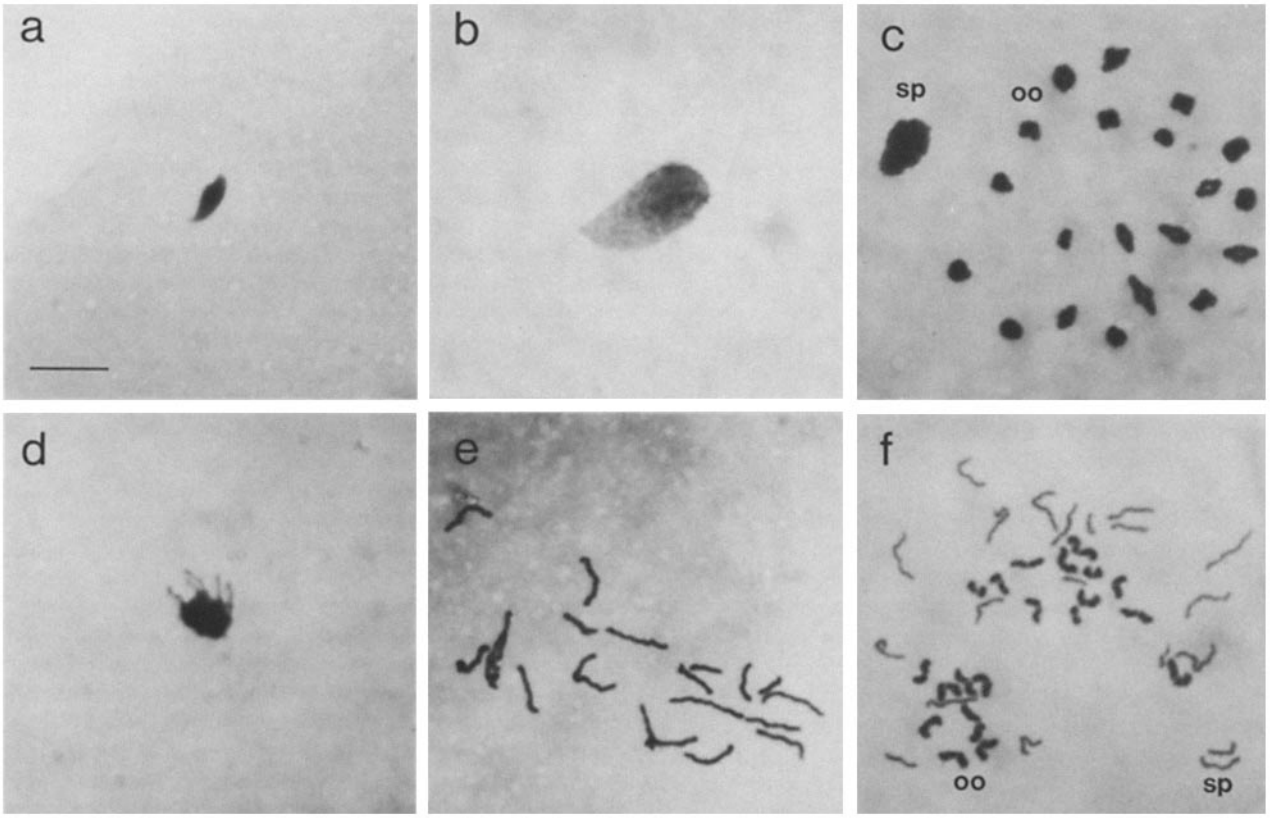

Figure 4. Changes in the morphology of the sperm chromatin in monospermic oocytes, inseminated at prometaphase I and incubated for the indicated lengths of time. (*a*) Untransformed sperm nucleus (0 h); (*b*) dispersed chromatin (3 h); (*c*) mass of recondensed chromatin (*sp*), with maternal chromosomes (*oo*) at metaphase I (9 h); (*d*) chromosomal arms projecting from the mass (12 h); (*e*) individually separated sperm chromosomes (18 h); (*f*) individually separated sperm chromosomes (*sp*), mingled with darker-stained maternal chromosomes (*oo*) at metaphase II (18 h). Bar, 10 μm.

of the intact sperm. Usually, the center of the dispersed chromatin and a small area at one end were more darkly stained than the rest of the mass, which suggests that the chromatin in these regions may have been more condensed than elsewhere (25). By 6 h after insemination, the dispersed chromatin had become more condensed, so that a small darkly stained mass of chromatin was seen (Fig. 4*c*). This mass was arrowhead-shaped and had a bumpy contour. In most cases, the mass was uniformly stained, but areas of lighter and darker stain could be seen in some cases. The chromatin remained as a condensed mass for several hours. Then, small arms could be seen to project from the mass (Fig. 4*d*). The number and length of these arms varied among oocytes. By 12–18 h after insemination, discrete chromosomes could be seen (Fig. 4, *e* and *f*). In Fig. 4*e*, 20 chromosomes can be counted, though more or fewer were counted in other oocytes.

The sperm chromosomes were easily distinguished from the maternal chromosomes, even when the two sets of chromosomes had become mingled together, as seen in Fig. 4*f*. First, each sperm chromosome consisted of a single chromatid, whereas each maternal chromosome joined two chromatids. Second, the sperm chromosomes were longer and thinner than the maternal chromosomes, which suggests that they were less condensed. However, the appearance of the sperm chromosomes varied among oocytes. The sperm chromosomes shown in Fig. 4, *e* and *f* are well-separated, and the contour of each is wavy. In other oocytes, the chromosomes were closely clustered, or more condensed with a smooth contour. In fact, if the oocytes were incubated for longer periods of time (e.g., 30 h), the sperm chromosomes were found to be as condensed as the maternal chromosomes.

Fig. 5 shows the temporal sequence of these changes in the morphology of the sperm nucleus within maturing oocytes. In this figure, masses of recondensed sperm chromatin with projecting arms, as well as discrete sperm chromosomes, have been classified as metaphase chromosomes. It can be seen that by 18 h after insemination, sperm metaphase chromosomes were present in over 90% of the penetrated oocytes. The formation of these chromosomes may have occurred slightly faster in oocytes inseminated at metaphase I than in oocytes inseminated at prometaphase I. However, the sperm chromosomes found in oocytes inseminated at prometaphase I tended to be more condensed and better-separated from each other than those found in oocytes inseminated at metaphase I, even though the oocytes were incubated for the same length of time after insemination.

In the oocytes inseminated at prometaphase I, mingling of the sperm and maternal chromosomes (Fig. 4*f*) was observed in most (~2/3) cases, whether the oocytes were incubated for a short (15 h) or long (24 h) period after insemination. In contrast, in oocytes inseminated at metaphase I, the sperm and maternal chromosomes were found as separate sets in most oocytes incubated for 15–18 h after insemination, but

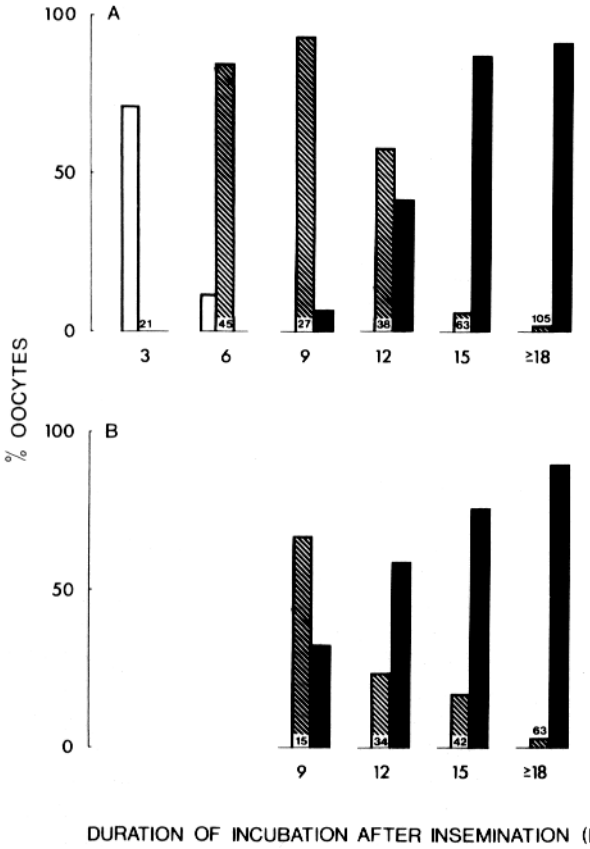

Figure 5. Sequence of changes in sperm chromatin morphology in monospermic oocytes. Oocytes at prometaphase I (*A*) or metaphase I (*B*) were inseminated and incubated for 3–18 h. The sperm chromatin was classified according to criteria described in the text. The bars do not sum to 100% at every time point, since the sperm in some oocytes could not be classified into any of these categories. The numbers of oocytes examined are shown along the abscissa. □, Dispersed chromatin; ▩, mass of recondensed chromatin; ■, metaphase chromosomes.

had become mingled in most cells by 20–24 h after insemination. These results suggest that, once transformed to metaphase chromosomes, sperm and oocyte chromatin both are subject to an influence of the oocyte cytoplasm that brings metaphase chromosomes into the same location within the cell. Similar observations of metaphase chromosomes becoming mingled have been reported after fusion of maturing oocytes either to each other or to blastomeres (1, 7, 37, 39).

POLYSPERMY. When more than one sperm penetrated an oocyte, all of the sperm nuclei within an oocyte underwent the same morphological changes. This indicates that the oocyte cytoplasm exerted a uniform effect on all of the sperm nuclei that were present within it. In every oocyte, regardless of the number of sperm that had penetrated it, each of the sperm nuclei had become enlarged, and resembled that shown in Fig. 4*b*, by 3–6 h after insemination. However, the pattern of subsequent changes in the nuclei was dependent on the number of sperm in the oocyte.

When two or three sperm penetrated an oocyte, the nuclei underwent the same changes as were observed in monospermic oocytes; thus, each was transformed into metaphase chromosomes (Fig. 6*a*). This transformation occurred at the same rate in dispermic oocytes as in monospermic oocytes, but appeared to be retarded in the trispermic oocytes. In some cases, the sperm chromosomes were found in the cytoplasm as a group of 40 or 60, whereas in others, the chromosomes derived from each sperm nucleus were grouped separately. One or more of the sperm chromosome sets were mingled with the maternal chromosomes in many oocytes, as was described in the monospermic cases. In some oocytes that contained two sets of metaphase II chromosomes, presumably due to failure of first polar body emission, sperm chromosomes were found mingled with each set of maternal chromosomes.

When four sperm penetrated an oocyte, these nuclei were transformed into metaphase chromosomes in only ⅓ of the cases (Fig. 7). In the remaining ⅔ of the oocytes, and in all oocytes penetrated by more than four sperm, none of the

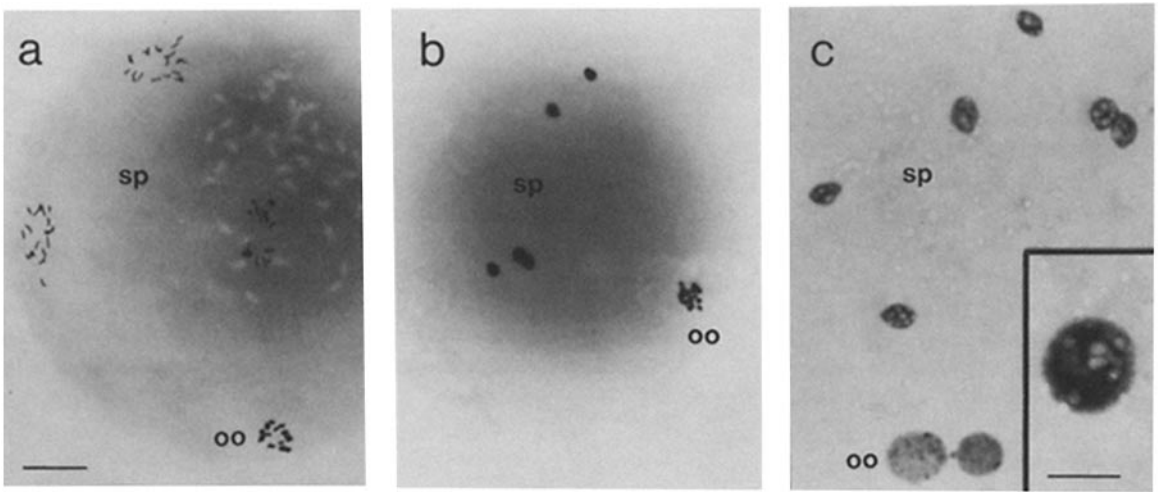

Figure 6. Morphology of sperm chromatin in polyspermic oocytes, inseminated at prometaphase I and incubated for ≥18 h. (*a*) Oocyte penetrated by three sperm. Metaphase sperm (*sp*) and maternal (*oo*) chromosomes are visible. (*b*) Oocyte penetrated by five sperm. Sperm chromatin masses (*sp*) and metaphase maternal chromosomes (*oo*) are visible. (*c*) Oocyte penetrated by seven sperm. Six sperm chromatin masses (*sp*) and decondensed maternal chromatin (*oo*) are visible. Bar, 25 μm. (*Inset*) Higher magnification of sperm chromatin within another highly polyspermic oocyte. Bar in inset, 10 μm.

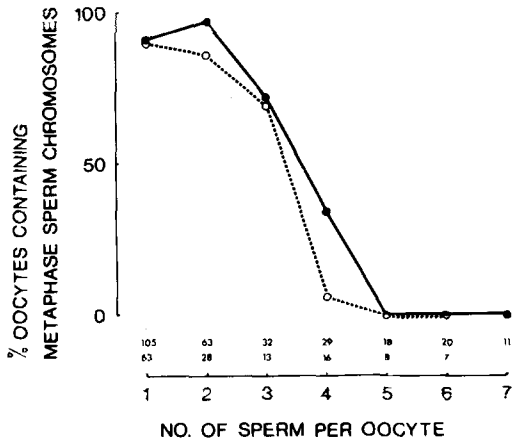

Figure 7. Effect of polyspermy on transformation of sperm nuclei into metaphase chromosomes. Oocytes at prometaphase I (solid line) or metaphase I (dotted line) were inseminated and incubated for ≥18 h. Within each oocyte, either all or none of the sperm nuclei had formed metaphase chromosomes. The numbers of oocytes examined are shown along the abscissa.

sperm nuclei was transformed into metaphase chromosomes. Instead, each nucleus was transformed into a mass of chromatin. These masses were rarely found to be mixed with the maternal chromosomes. Even if the oocytes that had been penetrated by more than four sperm were incubated for 24 h, instead of 18 h, none of the nuclei was transformed to metaphase chromosomes. Therefore, the transformation of the nuclei was not simply delayed in these oocytes, as it had been delayed in the trispermic oocytes. Rather, it appears that if oocytes incorporated more than four sperm, transformation of any of the sperm nuclei into metaphase chromosomes was completely inhibited.

Fig. 6, *b* and *c* shows the sperm chromatin in oocytes that failed to transform these nuclei to metaphase chromosomes. In oocytes penetrated by four to six sperm (Fig. 6*b*), the sperm nuclei formed condensed masses of chromatin, which individually resembled that shown in Fig. 4*c*. However, in highly polyspermic oocytes, which had been penetrated by more than six sperm, the sperm nuclei formed chromatin masses that were larger and more lightly stained (Fig. 6*c*). Examined using the light microscope, the lightly stained sperm chromatin masses often appeared to contain vacuole-like structures. In most of the highly polyspermic oocytes, it was also noted that the maternal chromatin became either decondensed or pycnotic, as described above (Fig. 1*c*). However, no vacuole-like structures were observed in these maternal chromatin bodies.

Discussion

Behavior of Sperm Nuclei Introduced into Maturing Oocytes

Our results have shown that the chromatin of the sperm nucleus underwent a series of changes within the cytoplasm of a maturing oocyte. Initially, the chromatin became sightly dispersed within the cytoplasm. Several hours later, this dispersed chromatin became recondensed into a small mass. Eventually, this mass was resolved into discrete condensed chromosomes, which we designated as metaphase chromosomes. This process of sperm nuclear transformation required 12–18 h of residence in the cytoplasm. In many oocytes, the sperm chromosomes became mingled with the maternal chromosomes, which suggests that they may have become integrated into the meiotic spindle. In contrast, when immature oocytes were inseminated and kept in an immature state, or when fully mature oocytes were fertilized, the sperm nucleus did not form metaphase chromosomes. Instead, the nucleus remained unchanged in immature oocytes and developed into a pronucleus in fully mature oocytes. It may be concluded that the mouse maturing oocyte possesses a cytoplasmic activity that induces the transformation of a sperm nucleus into metaphase chromosomes.

A single oocyte could transform a maximum of three or four sperm nuclei into metaphase chromosomes. Thus, there was a limit to the capacity of the activity that induced sperm chromosome formation. This could be explained by supposing that there is an oocyte cytoplasmic factor required for metaphase chromosome formation that reacts stoichiometrically with sperm chromatin, and whose supply in each oocyte is limited to the amount that allows the transformation of up to three or four sperm into metaphase chromosomes. According to this hypothesis, this cytoplasmic factor must not accumulate during incubation of the inseminated oocytes, since, even when they were incubated for up to 24 h after insemination, the capacity of a single oocyte to transform sperm into metaphase chromosomes never increased.

However, even though oocytes contained enough of the factor to transform up to four sperm nuclei to metaphase chromosomes, those oocytes that were penetrated by more than four sperm never transformed any of the nuclei to metaphase chromosomes. This could mean that the factor was equally distributed among all of the sperm nuclei within the oocyte cytoplasm, so that none of the nuclei was provided with enough of the factor to allow the appearance of discrete chromosomes. In oocytes penetrated by four to six sperm, all of the sperm nuclei instead were transformed into small masses of condensed chromatin, resembling those observed before the chromatin is transformed into metaphase chromosomes in monospermic oocytes. This suggests that the initial dispersion and recondensation of the chromatin may have occurred in polyspermic oocytes, as well as in monospermic oocytes, regardless of whether the subsequent transition to metaphase chromosomes could occur.

In highly polyspermic oocytes, not only did the sperm nuclei fail to become transformed into metaphase chromosomes, but the maternal chromosomes became either decondensed or pycnotic. Thus, the presence of an excess of sperm nuclei could deprive an oocyte not only of its ability to induce sperm chromosome formation, but also the ability to maintain its own chromosomes in a metaphase state.

Comparison with the Behavior of Somatic Cell Nuclei in Oocyte Cytoplasm

Our observation that the cytoplasm of a maturing oocyte contains an activity that transforms a sperm nucleus into metaphase chromosomes may be compared with results of previous investigators, who had observed such an activity manifested on somatic nuclei within maturing oocytes (see the introduction). However, the cytoplasmic chromosome condensation activity is manifested differently on sperm nuclei, as compared with other types of nuclei. First, sperm

chromosome formation required at least 12 h in our experiments, whereas the transformation of somatic or oocyte nuclei into metaphase chromosomes within the mouse oocyte requires <2 h (2, 7, 8, 39). Second, the number of sperm nuclei that can be transformed into metaphase chromosomes within one oocyte is limited to three or four, whereas up to 15 thymocyte nuclei can form metaphase chromosomes after fusion to a mouse oocyte (8).

Structural and molecular differences between sperm and somatic chromatin (reviewed in references 3 and 4) may account for these differences. Sperm chromatin is extremely condensed, and according to our observations undergoes an initial dispersion before recondensation to metaphase begins. In contrast, the chromatin within somatic nuclei is decondensed; thus, its condensation to a metaphase state might begin immediately on exposure to the oocyte cytoplasm. In addition, whereas the chromatin of somatic nuclei contains histones, sperm chromatin contains protamines, which perhaps must be replaced by histones for the transformation to metaphase chromosomes to occur (9). These differences may explain why the transformation of sperm nuclei to metaphase chromosomes requires more time within the oocyte cytoplasm and more oocyte cytoplasmic factors than does the transformation of somatic nuclei.

Comparison with the Behavior of Sperm Nuclei in Fully Mature Oocytes

The transformation of the sperm chromatin into metaphase chromosomes in the cytoplasm of maturing oocytes may be contrasted with its behavior after fertilization of fully mature oocytes. In these oocytes, the sperm chromatin becomes extremely decondensed within the male pronucleus, which indicates that the activity that induces the transformation to metaphase chromosomes is absent. This may be explained by the fact that the fully mature oocyte has been activated by sperm penetration, as indicated by the development of the female pronucleus, whereas the maturing oocyte has not been activated by penetration, since the maternal chromosomes remain condensed.

Even though the sperm chromatin in activated oocytes is not transformed into metaphase chromosomes, its earliest morphological changes appear to be the same as those that we observed in the sperm chromatin within maturing oocytes. Thus, after oocyte activation, the sperm chromatin initially becomes dispersed, and then condensed into a small mass (12, 19; Clarke, H. J., and Y. Masui, unpublished observations) before its transformation into a pronucleus. In activated oocytes, these early morphological changes are correlated with the replacement of the sperm chromatin protamines by histones present in the oocyte cytoplasm (31, 33). It may be speculated that, in the maturing oocytes also, the early morphological changes in the sperm chromatin might be accompanied by a similar biochemical modification.

Another similarity between these two transformations is that, in polyspermic activated oocytes, only a limited number of sperm nuclei can be transformed into well-developed pronuclei (45). This suggests that, during the transformation of the sperm into a pronucleus, as well as into metaphase chromosomes, it must react with a threshold amount of a cytoplasmic factor whose supply is limited (15, 45).

Previous Studies of Sperm Nuclear Behavior in Maturing Oocytes

It has been reported previously that the cytoplasm of maturing oocytes of *Urechis* (9) and amphibians (13, 28), and cytoplasmic extracts prepared from unactivated amphibian eggs in the presence of EGTA (21), can induce the transformation of sperm nuclei into metaphase chromosomes. In contrast, except for this paper, sperm chromosome formation has not been observed in the cytoplasm of maturing mammalian oocytes (see the introduction). The purpose of most of the studies using mammalian oocytes was to determine whether oocytes at specific stages of maturation could induce sperm pronuclear formation. Consequently, the inseminated oocytes generally were incubated <9 h, which would be long enough for pronuclear formation to occur in activated oocytes. But, in maturing oocytes, metaphase chromosomes derived from sperm do not appear until at least 12 h after insemination (this paper). In fact, Iwamatsu and Chang (18) reported that when maturing oocytes were inseminated and incubated between 10 and 18 h, the sperm nuclei formed small masses of chromatin and occasionally spindle-like structures that frequently were found near the maternal metaphase chromosomes. These results are very similar to ours, except that individual chromosomes were not developed. Thus, the fact that no sperm chromosome formation in mammalian maturing oocytes was observed by previous investigators may be attributed to the relatively brief incubation of the inseminated oocytes.

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