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Mouse Oocytes Gradually Develop the Capacity for Activation during the Metaphase II Arrest

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Metaphase II (M II) mouse oocytes were subjected to a parthenogenetic stimulus (8% ethanol) or fertilized *in vitro* at various times following the extrusion of the first polar body. The oocytes progressively develop the ability for full activation. Their responsiveness to activation stimuli not only increases, but also changes qualitatively with time. Newly arrested oocytes do not respond at all; then, when the ability to undergo meiotic anaphase II first develops, the response is defective: following extrusion of the second polar body (II PB), the oocyte does not enter interphase but arrests again at metaphase (M III-arrest). Finally, oocytes gain the ability for full activation including the entry to interphase. Depending on the type of activating stimulus, oocytes exhibit the capacity for full activation at different ages. The oocyte arrest in M III is similar to M II and can be released by subsequent activation. Such oocytes undergo anaphase III, extrude a third polar body (III PB), and form an aneuploid female pronuclei. © 1989 Academic Press, Inc.

INTRODUCTION

Under normal conditions mouse oocytes are fertilized while arrested at second meiotic metaphase. Sperm penetration causes activation of the oocyte leading to the completion of the second meiotic division, extrusion of the second polar body (II PB), and transition to the first or zygotic interphase. This transition is an essential aspect of oocyte activation since it starts a new cell cycle, enabling the genome to replicate and cleavage to occur. Iwamatsu and Chang (1972) have shown that maturing oocytes penetrated by spermatozoa are unable to undergo activation. The sperm nuclei do not form pronuclei, but instead undergo a morphological transformation into clumps of highly condensed chromatin. Clarke and Masui (1986, 1987) have recently extended these observations and demonstrated that penetration of a spermatozoon into prometaphase I or metaphase I (M I) oocytes leads to the formation of condensed chromosomes from the sperm nucleus. The behavior of both sperm and oocyte chromatin depends on the number of penetrating spermatozoa. High polyspermy induces "interphasing" of all nuclei, while monospermy or oligospermy may delay oocyte maturation but only slightly affects the eventual condensed morphology of oocyte chromosomes. Penetration of four to six spermatozoa causes an intermediate effect: maturation is most often blocked in M I, the oocyte chromosomes become highly contracted, and the sperm nuclei do not transform into chromosomes, but remain as masses of recondensed chromatin.

M I oocytes can be forced into interphase by inhibiting protein synthesis with puromycin or cycloheximide (Clarke and Masui, 1983). However, the nuclei induced by such a treatment do not initiate DNA replication, and upon removal of the drug the chromatin returns to a condensed state (Clarke and Masui, 1983). Prometaphase I oocytes penetrated by spermatozoa and then cultured in the presence of puromycin form interphase nuclei (Clarke and Masui, 1987), but their structure is abnormal (M. Szöllösi, D. Szöllösi and H. Rime, personal communication). Inhibition of protein synthesis in M II oocytes results in their true parthenogenetic activation (Siracusa *et al.*, 1978, Clarke and Masui, 1983). These data clearly demonstrate that the competence for activation develops in the mouse oocytes during the process of maturation. Iwamatsu and Chang (1972) concluded that "fertilizability of mammalian oocytes is acquired just before or at the completion of the first maturation division."

In the present study I show that the ability of mouse oocytes to activate develops gradually after they attain M II arrest and that nuclear and cytoplasmic maturation are two separable processes. The completion of the latter is expressed by the ability of the oocyte to undergo full activation. The time of acquisition of activatability depends on the mode of activation. It is exhibited earlier in response to sperm penetration than to the parthenogenetic action of ethanol.

MATERIALS AND METHODS

Oocyte Collection and Culture

F1 (CBA/HxC57Bl/10) (henceforth called F1) female mice were injected with 8 IU of pregnant mare serum

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gonadotropin (PMSG) and 48-52 hr later with 5 IU of human chorionic gonadotropin (HCG). Oocytes were collected either from ovaries by puncturing the follicles (10-10½ hr after HCG injection) or from ampullae of oviducts (starting at 11 hr post HCG). Oocytes were collected and cultured in M2 medium supplemented with 4 mg/ml BSA (Whittingham, 1971). Cumulus cells were removed with hyaluronidase (300 IU/ml) in phosphate-buffered saline (PBS) and the zona pellucida with acid Tyrode, pH 2,5 (Nicholson *et al.*, 1975) or α-chymotrypsin (Sigma) 30 μg/ml in M2 (for fertilization *in vitro*) or with 0.5% pronase (Sigma) (for parthenogenetic activation). The first polar bodies (I PB) of all oocytes were also removed by gentle pipetting.

Maturation in Vitro

Oocytes with the germinal vesicle intact were obtained by puncturing ovarian follicles of 4-week-old F1 females and were cultured in Whitten's medium (Whitten, 1971) under paraffin oil at 37°C in 5% CO_2.

Parthenogenetic Activation

Oocytes were treated with freshly prepared 8% ethanol (Merck) in M2 medium for 6½ min. (Cuthbertson, 1983) and then carefully washed in three large drops of M2 medium and cultured in this same medium under liquid paraffin oil at 37°C.

In Vitro Fertilization (IVF)

Sperm was squeezed out from the cauda epididymis of 4-5-month-old F1 males in Whittingham's medium as modified by Fraser and Drury (1975) and capacitated for 1 hr at 37°C under liquid paraffin in an atmosphere of 5% CO_2 in air. The sperm suspension was then diluted with the medium to a final concentration of 1 $\times$ 10^6 spermatozoa/ml and mixed with zona-free oocytes. After 10 min the oocytes were removed from the sperm suspension. To induce polyspermy the sperm suspension was diluted less or oocytes were incubated for a longer period (20-30 min). A higher density of sperm suspension was more effective in inducing polyspermy than was the prolonged incubation. Oocytes were cultured in M2 medium under liquid paraffin at 37°C in normal air atmosphere.

Drugs

Puromycin (Sigma) was diluted in DMSO (1 mg/ml) and stored at −20°C. The working dilution was 10 μg/ml, as recommended by Clarke and Masui (1983).

Preparations

Whole-mount preparations were stained with hematoxilin by the method of Tarkowski and Wróblewska (1967). Air-dried, Giemsa-stained preparations were made according to Tarkowski (1966).

RESULTS

When Do Mouse Oocytes Become Activable?

Most oocytes of F1 mice (described under Materials and Methods) extrude the first polar body (I PB) and enter M II arrest by 11 hr after HCG administration (my unpublished observations). Preovulatory oocytes used for experiments at this time point were obtained from ovaries 10-10½ hr after HCG administration. Only oocytes with the I PB extruded were used. Whole-mount preparations showed chromosome plates within the oocytes. Some of the plates were oriented parallel to the oocytes' surfaces, suggesting that their spindles had not yet rotated or fully developed. Since ovulation begins in F1 mice at 11½ hr after HCG injection (Fraser, 1979; my unpublished observations), oocytes for examination at later time points were obtained from oviducts after ovulation. The natural period of fertilization *in vivo* ranges from 13 to 18 hr after HCG injection in the mouse (Edwards and Gates, 1959). According to Donahue (1972) the process is definitely completed by 20 hr after HCG injection. The period chosen for the present experiments (11-21½ hr post HCG) thus fully covers the time interval when oocytes are normally fertilized.

Parthenogenetic activation. On the basis of cytological preparations made 5-7 hr after ethanol treatment (i.e., after a period sufficiently long to allow oocytes to form advanced pronuclei) I have distinguished six types of reactions of oocytes to the activation treatment (Fig. 1A). Some oocytes stayed in M II, and others extruded the II PB but passed to the next metaphase state both in the oocyte and the II PB (I propose to name this type of abortive activation metaphase III, M III) (Fig. 2A). As evident from air-dried preparations, the chromosomes of M III oocytes are unichromatid (Fig. 2B) in contrast to M II chromosomes consisting of two chromatids each (Fig. 2E). Their number was 20 in 12 of 19 successfully spreaded chromosome plates (Fig. 2B). In six cases hypohaploid plates (19, 18, and 17 chromatids) were found, and in one case a hyperhaploid (23 chromatids) was found. This demonstrates that M III oocytes are formed as the result of a true anaphase II. Karyotyping of the II PB was unsuccessful.

In some oocytes the II PB was withdrawn spontaneously 2-3 hr after extrusion. The oocytes contained invariably 40 chromatids per metaphase plate and a common spindle (this type of reaction is henceforth called a "40-chromatids stage"; Fig. 2C). Oocytes of these three reaction types retained the metaphase state despite the action of the parthenogenetic stimulus.

Three other types are characterized by their more or less successful transition to interphase. Some oocytes formed telophase-like, dense clumps of chromatin both

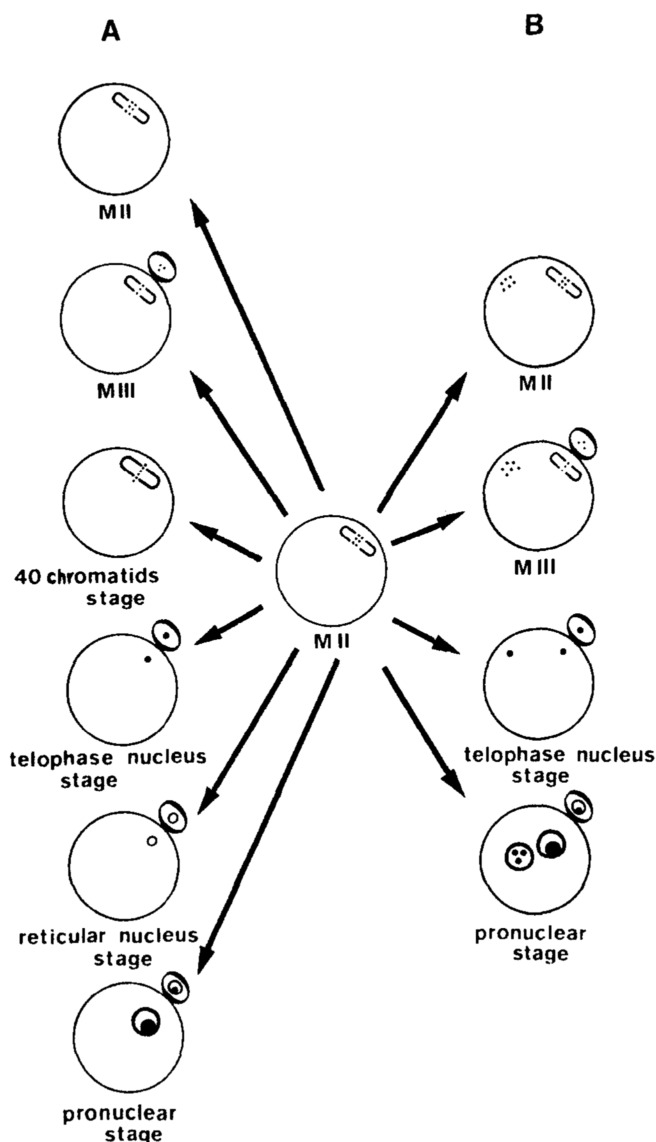

FIG. 1. Types of response of mouse M II oocytes to parthenogenetic stimulus (A) and sperm penetration (B). The drawings show oocytes as they were under the experimental conditions described in this paper, i.e., without the zona pellucida and without the first polar body.

in the oocyte and in the II PB ("telophase nucleus stage"; Fig. 2D). Others formed nuclei which were small, rounded, retarded in their enlargement, and had a characteristic reticular appearance of the chromatin ("reticular nucleus stage"). Finally, some oocytes formed normally enlarging pronuclei as well as normal nuclei in their II PB.

The frequencies of these types of reactions depended on the age of the oocytes when subjected to ethanol treatment (Table 1). The majority of oocytes treated at 11 hr after HCG injection did not react to parthenogenetic stimulus and stayed in M II (57.6%). The remaining oocytes extruded the II PB, but only a small portion formed normally enlarging pronuclei (3.4%), while the rest entered M III (35.6%). Ethanol treatment 13 hr after HCG administration resulted in a sharp decrease in the number of M II-arrested oocytes (3.6%) and a concomitant increase in the number of oocytes in M III (48.2%) and of oocytes forming normal pronuclei (45.6%). Alcohol treatment applied at later time points resulted in a further decrease in M II oocytes and abortively activated oocytes. During this period the number of normally reacting oocytes steadily increased. Thus, these three major types of oocyte reactions (M II, i.e., no response, M III, and pronuclei formation) show a clear age-dependence. The other and less frequent types of reactions occur with much less regularity in relation to the age of the oocytes (see Table 1). These data demonstrate that the capacity for normal activation is acquired gradually by mouse oocytes during the period of M II arrest. Initially they do not react and then they become able to complete meiosis II and to extrude the II PB, but are not yet able to undergo the transition to interphase and to form pronuclei. Still later the ability to react by entering interphase develops in the majority of oocytes. However, as late as $17\frac{1}{2}$ hr after HCG injection a significant portion of oocytes (12.5%) still reacts abortively to the parthenogenetic stimulus.

Fertilization in vitro. Because F1 oocytes which are fertilized *in vitro* at both 13 and 17 hr after HCG injection are known to form pronuclei (Fraser, 1979), I focused my attention on the behavior of oocytes fertilized between 11 and 13 hr after HCG injection. Fertilization *in vitro* of zona-free oocytes revealed that some "early" oocytes did not react to sperm by extruding a II PB but remained in M II. Some others extruded II PB and restored the metaphase state (M III). Also oocytes with telophase nuclei were observed among the fertilized oocytes, but none was found with reticular nuclei or 40 chromatids (Fig. 1B). The behavior of the sperm nucleus depended on the type of oocyte reaction. In M II and M III oocytes (i.e., in metaphase-arrested oocytes) sperm nuclei underwent decondensation, then recondensation and later formed distinct chromosomes. A similar behavior of the sperm chromatin has been described by Clarke and Masui (1986) in oocytes penetrated during prometaphase I and M I. However, they described long chromosomes (G1- or prophase-like) of sperm origin as a final stage of transformation rather than the fully condensed, unichromatid chromosomes observed in this study (Figs. 3A, 3B). When an oocyte reacts to form a telophase nucleus, the sperm nucleus also transforms into a dense clump of chromatin. These abortive types of reaction to sperm penetration were observed only in oocytes fertilized 11 and 12 hr after HCG injection. Penetration of oocytes by sperm 13 hr after HCG injection invariably caused normal activation (i.e., II PB extrusion and formation of male and female pronuclei). In the case of activation by fertilization the frequency of

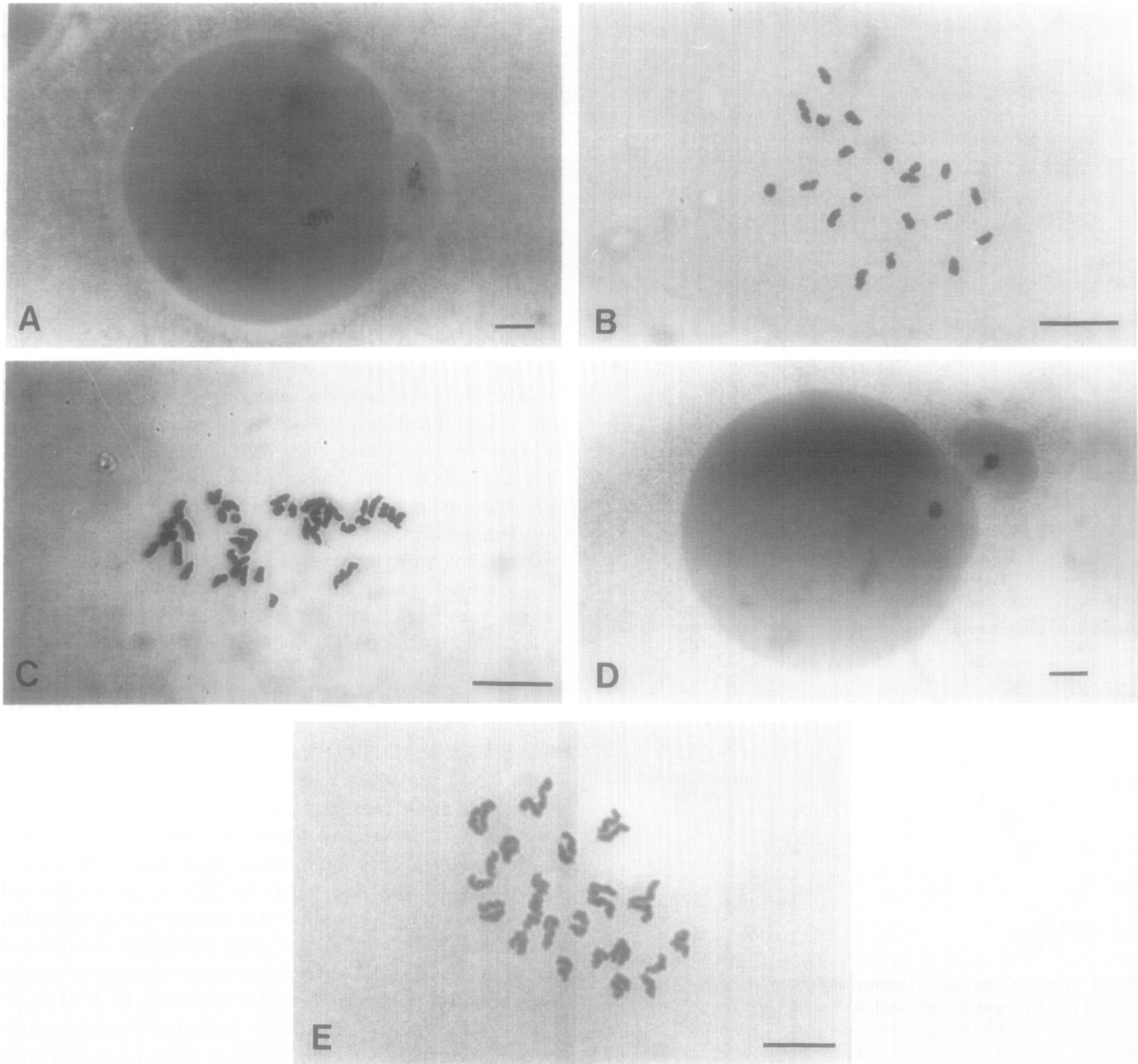

FIG. 2. Abortive reactions of mouse M II oocytes to a parthenogenetic stimulus (8% ethanol). The first polar bodies and the zonae pellucidae of all oocytes were removed as described under Materials and Methods. (A) M III oocyte. This oocyte and its second polar body have condensed chromosomes (whole-mount preparation). (B) Air-dried preparation of M III oocyte showing the presence of 20 unichromatid chromosomes within it. (C) Air-dried preparation of the oocyte in "40-chromatids stage." After ethanol treatment, this oocyte extruded a second polar body which was withdrawn spontaneously 2 hr later. (D) The oocyte in "telophase nucleus stage" 7 hr after ethanol treatment. The oocyte and its second polar body have deeply stained, telophase-like groups of chromatin (whole-mount preparation). (E) Air-dried preparation of M II oocyte. Each chromosome consists of two chromatids. Compare with the unichromatid chromosomes of M III (2B) and 40-chromatid stage (2C). Bars, 10 μm.

the types of abortive activation was clearly dependent on both the age of the oocytes and the number of penetrating spermatozoa (Table 2).

Monospermy. In contrast to oocytes parthenogenetically activated 11 hr after HCG administration the majority of oocytes penetrated at this time (65.6%) reacted normally, i.e., extruded II PB and formed two large pronuclei. However, 15.6% and 18.8% of oocytes fertilized at this time point stayed in M II or formed M III, respectively.

Fertilization at 12 hr after HCG injection resulted in the increase of normally activated oocytes (86.2%) and the decrease of abortive reactions. When fertilized at 13 hr after HCG, all oocytes underwent normal activation (i.e., extruded II PB and formed interphase pronuclei).

Dispermy. Dispermic oocytes revealed abortive types

TABLE 1
TYPES OF RESPONSE OF MOUSE OOCYTES TO A PARTHENOGENETIC STIMULUS (8% ETHANOL) APPLIED AT VARIOUS TIMES AFTER HCG INJECTION OF THE MOUSE DONOR

Age of oocytes (hr after HCG injection)	Types of reaction (%)						Total number of oocytes
	M II	40-chromatids stage	M III	Telophase nucleus stage	Reticular nucleus stage	Pronuclear stage	
11	57.6	1.7	35.6	1.7	—	3.4	59
13	3.6	1.3	48.2	1.3	—	45.6	224
16	1.9	—	7.6	13.3	7.7	69.5	105
17.5	—	1.8	3.5	5.4	1.8	87.5	56
21.5	—	—	—	—	—	100	91

of activation only when penetrated 11 hr after HCG administration and in a lower percentage than the monospermic cases (for details see Table 2). In contrast to the latter, in none of the abortively activated oocytes did the two sperm nuclei transform into distinct chromosomes. They only achieved a stage of recondensation (Fig. 3C).

Dispermic fertilization of oocytes at 12 and 13 hr after HCG injection invariably resulted in II PB extrusion and formation of one female and two male pronuclei.

Polyspermy. This was studied only in oocytes inseminated 11 hr after HCG. Among 57 oocytes penetrated by 3 or more spermatozoa (the highest number of spermatozoa per oocyte was 15) all but one underwent the transition to interphase. The only exception was a trispermic oocyte which arrested at M III, and in which sperm nuclei remained in a decondensed state 5 hr after insemination. Other tri- and tetraspermic oocytes formed enlarging pronuclei. When five or more spermatozoa penetrated a single oocyte, only one pronucleus (presumably female) was formed and the remaining sperm nuclei were blocked in development. They persisted in the oocyte cytoplasm as small, densely stained, oval nuclei.

These results provide further evidence of the gradual development of the ability of M II oocytes to undergo normal activation, and additionally demonstrate that the reaction of an oocyte depends on the nature and "strength" of the activating stimulus. It seems that polyspermy has a stronger effect on oocytes for achieving full activation than does penetration by one spermatozoon, and monospermic fertilization is more effective than a parthenogenetic agent (8% ethanol).

Characterization of the Metaphase-Arrested Types of Abortive Activation (M II, M III, and 40-Chromatids Stage)

The most intriguing type of abortive activation, the M III case, is achieved as a result of action of an activating stimulus on an oocyte which is able to resume anaphase II movement and extrude II PB, but is clearly unable to undergo transition to interphase.

Behavior of the II PB in M III oocytes. The characteristic feature of zona-free M III oocytes is that their II PB falls off easily 2–3 hr after extrusion. Even gentle touching with a pipet causes separation of the oocyte from its polar body. This property allows oocytes in M III to be distinguished easily from those representing telophase or reticular nucleus types of abortive activation, as well as pronucleate oocytes, long before the pronuclei become visible under the inverted microscope.

Is M III arrest similar in nature to M II arrest? To examine the nature of the M-phase arrest of M III oocytes, I reexposed them to 8% ethanol $2\frac{1}{2}$–4 hr after the first treatment. Only 3 of 43 of M III oocytes (7%) extruded a subsequent polar body (III PB). Two of these three oocytes formed a pronucleus and an interphase nucleus in the III PB, while the third one entered a subsequent metaphase (M IV) a few hours after the second ethanol treatment. The remaining 40 oocytes (93%) remained in M III. These results demonstrate a very limited ability of M III oocytes to proceed through anaphase, extrude the III PB, and undergo transition to interphase in response to the second treatment with 8% ethanol, i.e., the same parthenogenetic agent.

These results prompted me to investigate the effect of a repeated ethanol treatment on M II oocytes remaining in this state despite the first treatment. Fifty oocytes (matured *in vitro*) which had been subjected to 8% ethanol 14 hr after isolation from the ovary and which stayed in the M II stage, were treated again with ethanol 5 hr later (19 hr of culture *in vitro*). Only 4 (8%) of these oocytes extruded a II PB and formed M III; 3 (6%) formed telophase nuclei; 1 (2%) formed a reticular nucleus; 3 (6%) gave normal enlarging pronuclei; and 39 (78%) remained in M II. Of 51 control oocytes treated with ethanol after 19 hr of culture *in vitro*, the great majority (88.4%) activated fully. Only 1 (2%) formed M III, 3 (5.9%) formed telophase stage, and 2 (3.9%) re-

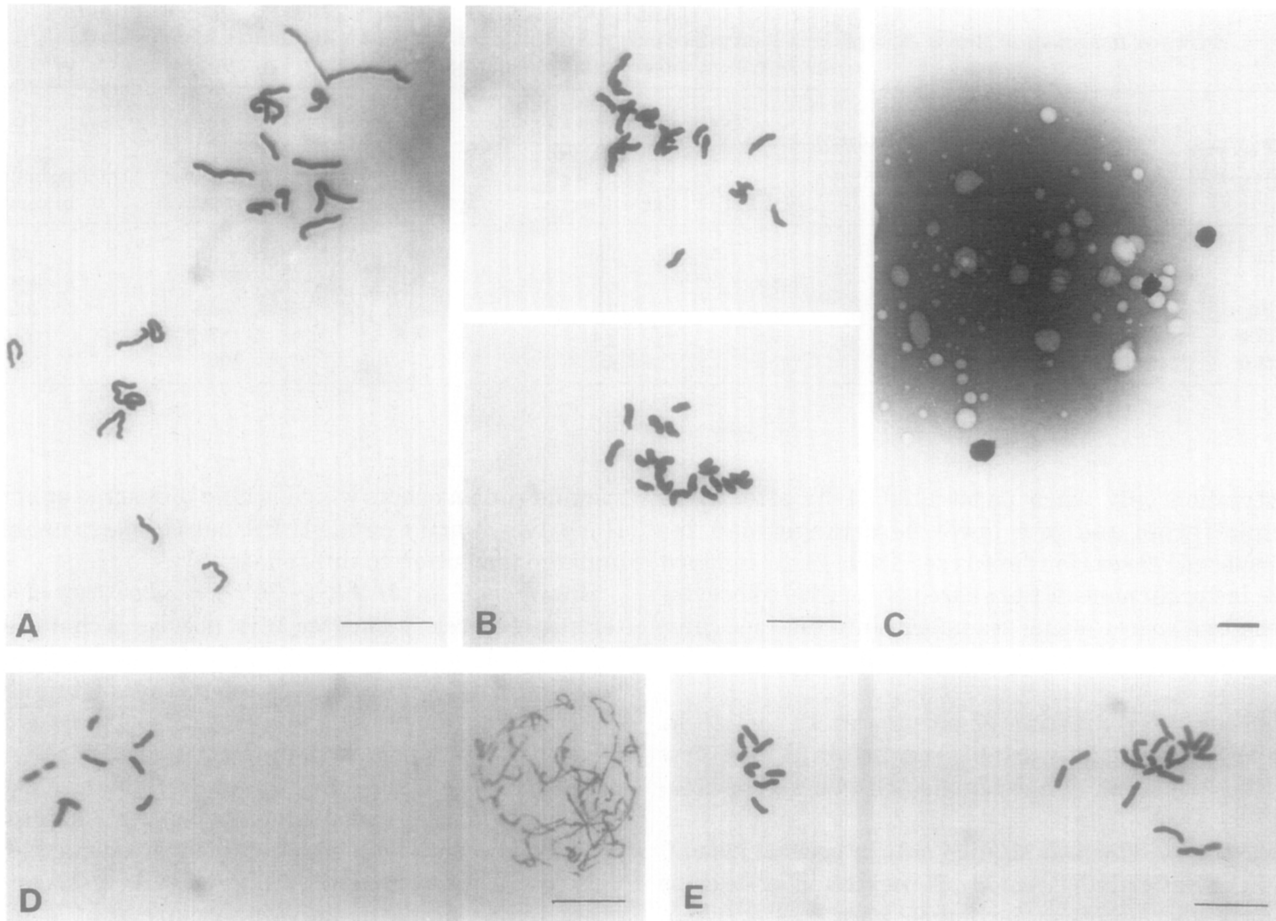

FIG. 3. Sperm-derived chromatin in mouse oocytes fertilized at the beginning of M II arrest, air-dried preparations (A–C). (A) Long, prophase-like chromosomes from a sperm nucleus 6 hr after fertilization. In the other part of the oocyte a group of short, M III chromosomes was present. The second polar body of this oocyte fell apart. (B) Two chromosome groups found in the oocyte 8 hr after fertilization. This oocyte had extruded a second polar body which fell off, indicating the M III type of reaction. The chromosomes of the two groups are unichromatid and the degree of their condensation is similar. Probably the upper one represents the sperm-derived chromosomes as judged by their slightly more elongated morphology. The lower one represents the M III plate of the oocyte origin. (C) Dispermic oocyte 8 hr after fertilization. Three clumps of similar, recondensed chromatin are present within the oocyte. Chromosomes of M III oocytes fertilized 5 hr after ethanol treatment. Penetration by spermatozoa caused the extrusion of a third polar body and formation of two pronuclei in each oocyte. The oocytes were cultured overnight in the presence of colcemid and their third polar bodies were removed before air-drying (D, E). (D) Left, 9 chromosomes originated from the aneuploid female pronucleus (M III derived); right, prophase group of the male pronucleus. Note the asynchrony of entering the first cleavage division by female and male pronuclei. (E) Left, 8 chromosomes of the aneuploid female pronucleus (M III derived); right, 20 chromosomes of the male pronucleus. Bars, 10 μm.

mained in M II. This experiment demonstrates that the efficiency of the repeated ethanol treatment is very low regardless of whether oocytes are in M II or M III.

To find out whether other agents can activate M III oocytes, 16 such oocytes were incubated in medium supplemented with 10 μg/ml puromycin. Almost all of them developed pronuclei, with just one remaining in M III after overnight culture in the presence of the inhibitor.

In another experiment, 22 M III oocytes were inseminated *in vitro* 4–5 hr after ethanol treatment. In all 16 penetrated oocytes (72.2%) the extrusion of a III PB was observed. The sperm nuclei underwent decondensation and then transformed into pronuclei. Four monospermic oocytes were cultured overnight in the presence of colcemid. Three of them showing dissolution of pronuclei were air-dried and Giemsa-stained. In these three cases two groups of chromosomes were present: one containing 20 chromosomes (originating from a male pronucleus) and one highly aneuploid containing 7, 8, and 9 chromosomes (originating from a female pronucleus of M III oocytes, Figs. 3D, 3E).

These results demonstrate that the M III arrest is very similar to the natural M II arrest and can be interrupted by sperm penetration or inhibition of protein synthesis. Activation of M III oocytes causes anaphase movement of unichromatid chromosomes and extrusion of the III PB.

TABLE 2
TYPES OF RESPONSE OF MOUSE OOCYTES TO FERTILIZATION *IN VITRO* AT VARIOUS TIMES AFTER HCG INJECTION

Age of oocytes (hr after HCG injection)	Types of reaction (%)				Total number of oocytes
	M II	M III	Telophase nucleus stage	Pronuclear stage	
Monospermy					
11	15.6	18.8	—	65.6	32
12	—	6.9	6.9	86.2	29
13	—	—	—	100	35
Dispermy					
11	5.9	11.8	—	82.3	17
12	—	—	—	100	4
13	—	—	—	100	22

DISCUSSION

The results presented here demonstrate that mouse oocytes develop the ability for activation progressively during their arrest at M II. The initial unresponsiveness of oocytes to activating stimuli may be related to the inability of the underdeveloped M II spindle to conduct an anaphase movement of chromosomes. However, the absence of the spindle/chromosome activities of anaphase II does not prevent the transition to interphase when M II oocytes are activated 17–19 hr after HCG injection in the presence of colcemid (my own unpublished observations). Thus, it seems that oocytes treated soon after I PB extrusion are unable to undergo this transition regardless of whether they are unable to proceed through the second meiotic anaphase. Since the transition between the first and the second meiotic metaphase proceeds without a definitive interphase (Donahue, 1968), it is difficult to judge whether oocytes treated just after I PB extrusion represent the real M II or just a prometaphase.

The occurrence of the M III type of abortive activation demonstrates even more directly the independence of anaphase movement and the transition to interphase. During a mitotic cycle, anaphase and then telophase are followed invariably by the transition to interphase of the daughter cells. The only exception to this rule is the first meiotic division during oogenesis (Donahue, 1968). The transition between M II and M III described in this paper provides another example of this kind.

Recent studies concerning the mechanisms governing transitions from metaphase to interphase and vice versa have revealed that solubilization of the nuclear lamina, nuclear envelope breakdown, and chromosome condensation are independent and separable events (Newport and Spann, 1987). Ghosh and Paweletz (1987a,b) have shown further that decondensation of chromosomes can be separated from nuclear envelope assembly. The present study demonstrates the independence of the anaphase movement and transition to interphase, events which usually occur in sequence. The M III example thus provides a unique model for investigating trigger mechanisms for those two separable processes.

It has been postulated by many authors that the mitotic anaphase movement as well as maturation promoting factor inactivation in the meiotic cycle are triggered by a transient increase in the concentration of free calcium ions in the cytoplasm (Lohka and Masui, 1984; Masui, 1985; Poenie *et al.*, 1986; Hepler and Callaham, 1988). Calcium transients have been demonstrated during activation of eggs of fish (Gilkey *et al.*, 1978), sea urchin (Eisen and Reynolds, 1985; Hafner *et al.*, 1988), starfish (Eisen and Reynolds, 1984), and mammals (Cuthbertson *et al.*, 1981, in the mouse; Miyazaki *et al.*, 1986, in the hamster). Recently, Kline (1988) has demonstrated that the prevention of this calcium burst during fertilization of *Xenopus laevis* eggs causes such eggs to retain their metaphase state; the entering spermatozoa behave similarly to those in mouse oocytes arrested in M II or M III (this paper). Thus, it might be interesting to examine whether the calcium transient appears in mouse M II oocytes undergoing transition to M III to try to dissect the role of calcium ions in anaphase and in the M-phase/interphase transition.

The M III spindle is also interesting for those investigating the organization of a division spindle and the mechanism of its function. It is believed that the metaphase spindle is stabilized due to symmetric forces acting on each kinetochore (Nicklas, 1975). Splitting in a centromere region (Lambert, 1980) results in triggering the anaphase movement of single chromatids which are pulled out to the opposite poles of the spindle due to the shortening of the kinetochore bundles (Nicklas, 1975). However, it would appear that this model cannot apply to the M III spindle. It is not known how the centromere region of a unichromatid chromosome behaves: how are the forces acting on such a single chromatid balanced, and how is anaphase triggered if the centromere cannot be split once again? To answer these questions further investigations of M III spindles are necessary.

Another feature of M III is an extremely weak adherence of the II PB to the oocyte. Usually the II PB of oocytes undergoing transition to interphase remains well attached to the oocyte. This is not the case with the II PB formed after transition to M III. Similar behavior is observed in the case of I PB which can be easily pipetted off 2–3 hr after extrusion. Perhaps the transition to interphase stabilizes the connection between the daughter cells. In the light of recent studies by Kidder *et al.* (1988) it seems possible that the midbody and its microtubules forming between the daughter cells could

be responsible for the stabilization of this connection. These authors showed that after each cleavage of the mouse embryo the midbodies persist only during the following interphase and disappear when blastomeres approach the next cleavage division. It seems likely that midbodies disappear due to the change in the cytoplasmic environment in cells entering M-phase. The influence of this environment on a midbody seems to be supported by the fact that in other types of abortive activation, i.e., telophase or reticular nucleus stages, the polar bodies are well attached. The two latter can be easily distinguished from M III solely by the behavior of their polar bodies.

The ability of young M II oocytes to undergo activation was investigated earlier by Fraser (1979), who fertilized oocytes 13 and 17 hr after HCG injection. She showed that both groups of oocytes are able to form pronuclei and that the development of pronuclei is faster in older oocytes. The present study shows that 13 hr after HCG injection mouse oocytes are indeed fully activable when penetrated by spermatozoa, but are not yet activable by a parthenogenetic stimulus. This demonstrates that the ability to undergo normal activation develops gradually in oocytes arrested in M II and achieves successive thresholds enabling full activation at various time points by various stimuli. This ability is first realized when triggered by the entrance of two (or more) spermatozoa (12 hr after HCG injection), then when only one spermatozoon penetrates (13 hr after HCG injection), and finally when treated with ethanol (16-17½ hr after HCG injection).

It seems that a similar, gradual development of activatability operates in oocytes of other mammals. Schmiady *et al.* (1986) observed the condensation of chromosomes from sperm nuclei in human M II oocytes fertilized *in vitro.* It seems likely that this phenomenon was due to fertilization of M II oocytes which had not yet developed the capacity for normal activation, i.e., were not fully cytoplasmatically matured despite completion of nuclear maturation.

In contrast to mouse oocytes, rat M II oocytes removed from the oviduct even shortly after ovulation react with abortive, parthenogenetic activation with no need for any special treatment. They undergo II PB extrusion and remain in an M III-like stage (Zeilmaker and Verhamme, 1974; Keefer and Schuetz, 1982). However, the chromosomes in such oocytes are usually dispersed in the cortex and only rarely form a true metaphase plate (M. Zernicka-Goetz, personal communication).

In mouse oocytes which remain in M II or proceed to M III, only one sperm nucleus per oocyte can undergo an effective condensation of chromosomes. This is in contrast with prometaphase I or M I oocytes in which up to four sperm nuclei are able to undergo such a transformation (Clarke and Masui, 1986). Also the time course of the sperm chromatin transformation is different in these two cases. Clarke and Masui (1986) observed chromosomes of sperm origin 12-18 hr after insemination, while in the present study I observed sperm chromosomes as early as 5 hr after insemination. Moreover, the sperm chromosomes can condense to a state almost indistinguishable from the M III chromosomes (see Fig. 3B). Further investigations are needed to understand the basis of these differences.

It seems that the full cytoplasmic maturation of oocytes, normally achieved during M II arrest, can develop independently from nuclear maturation. For instance ovulated M I oocytes of Lt/Sv mice can be activated spontaneously, by ethanol treatment (Kaufman and Howlett, 1986) or by fertilization (O'Neil and Kaufman, 1987). Parthenogenetic activation followed by development can be induced also in M I mouse oocytes incubated first with puromycin and then with dbcAMP (Clarke *et al.*, 1988). These oocytes never reach M II since they enter interphase directly from M I. The raised cAMP level in oocytes which are in such interphase seems to be sufficient to induce their activation. This demonstrates that activation proceeds regardless of the stage of the cell cycle (interphase vs metaphase) and of meiosis (M I vs M II).

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