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Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent

Keith T. Jones*, John Carroll, Julie A. Merriman, David G. Whittingham and Tomohiro Kono†

Medical Research Council Experimental Embryology and Teratology Unit, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE, UK

*Author for correspondence
†Present address: NODAI Research Institute, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan

SUMMARY

Mature mouse oocytes are arrested at metaphase of the second meiotic division. Completion of meiosis and a block to polyspermy is caused by a series of repetitive Ca^{2+} transients triggered by the sperm at fertilization. These Ca^{2+} transients have been widely reported to last for a number of hours but when, or why, they cease is not known. Here we show that Ca^{2+} transients cease during entry into interphase, at the time when pronuclei are forming. In fertilized oocytes arrested at metaphase using colcemid, Ca^{2+} transients continued for as long as measurements were made, up to 18 hours after fertilization. Therefore sperm is able to induce Ca^{2+} transients during metaphase but not during interphase. In addition metaphase II oocytes, but not pronuclear stage 1-cell embryos showed highly repetitive Ca^{2+} oscillations in response to microinjection of inositol trisphosphate. This was explored further by treating in vitro maturing oocytes at metaphase I for 4-5 hours with cycloheximide, which induced nuclear progression to interphase (nucleus formation) and subsequent re-entry to metaphase (nuclear envelope breakdown). Fertilization of cycloheximide-treated oocytes revealed that continuous Ca^{2+} oscillations in response to sperm were observed after nuclear envelope breakdown but not during interphase. However interphase oocytes were able to generate Ca^{2+} transients in response to thimerosal. This data suggests that the ability of the sperm to trigger repetitive Ca^{2+} transients in oocytes is modulated in a cell cycle-dependent manner.

Key words: cycloheximide, mitosis, meiosis, fertilization, mouse oocyte, Ca^{2+} transients

INTRODUCTION

Mature mouse oocytes are arrested at metaphase of the second meiotic division (MII) and complete meiosis following sperm entry (Wassarman, 1988). Meiotic arrest at MII is maintained by high levels of maturation promoting factor (MPF) (Kubiak et al., 1993), which is stabilised by the action of cytostatic factor (CSF). A series of repetitive Ca^{2+} transients occur at fertilization (Cuthbertson et al., 1981; Cuthbertson and Cobbold, 1985; Igusa and Miyazaki, 1986; Taylor et al., 1993) which are responsible for the destruction of MPF activity and re-entry into the cell cycle (Kline and Kline, 1992).

Parthenogenetic activation can be stimulated by artificially raising Ca^{2+} levels (Fulton and Whittingham, 1978; Cuthbertson, 1983; Vincent et al., 1992; Kline and Kline, 1992) or by inhibiting protein synthesis (Siracusa et al., 1978; Clarke et al., 1988), which presumably inhibits the production of MPF and CSF. Activating agents that cause monotonic rises in Ca^{2+} (Cuthbertson, 1983) or protein synthesis inhibition (Kubiak, 1989) are inefficient activators of freshly ovulated oocytes, which are only activated at high rates by agents that cause repetitive Ca^{2+} transients, such as sperm and strontium-containing medium (Kline and Kline, 1992; Bos-Mikich et al., 1995). Inhibition of protein synthesis in oocytes at metaphase I (MI) for 9 hours, does not induce parthenogenetic activation (Clarke and Masui, 1983). Instead chromosome decondensation and nucleus formation is followed by chromosome recondensation to a state similar to MII after removal of the protein synthesis inhibitor. During maturation oocytes enter MI, then extrude the first polar body, and the chromosomes, which remain condensed, align on a second meiotic spindle. Therefore, normally, there is no interphase between MI and MII. Inhibition of protein synthesis at MI effectively induces a period of interphase between MI and MII (Fig. 1).

Using in vitro maturation of oocytes we have previously shown that the ability of sperm to trigger a series of long lasting Ca^{2+} transients is dependent upon the stage of maturation (Jones et al., 1995). Repetitive Ca^{2+} transients were seen only in MII oocytes at 12-14 hours, and in spontaneously arrested MI oocytes at 14 hours after release from the follicle. Oocytes at MI, 8-10 hours after release, did not show repetitive transients. This suggests that the ability of sperm to generate continuous Ca^{2+} oscillations develops late in oocyte maturation and is dependent on cytoplasmic maturation between MI and MII.

The aim of the present study was to determine whether Ca^{2+} oscillations induced by sperm are regulated in a cell cycle-dependent manner. To investigate this we correlated the

cessation of sperm-induced Ca^{2+} transients with the stage of the first mitotic cell cycle. We found that Ca^{2+} transients cease at a specific point in the cell cycle and Ca^{2+} oscillations can be made to continue indefinitely by blocking cell cycle progression. Furthermore interphase oocytes made by treating MI oocytes with cycloheximide (Clarke and Masui, 1983) fail to generate repetitive Ca^{2+} transients in response to sperm. Thus sperm are unable to induce Ca^{2+} oscillations during interphase. This suggests that the MII oocyte is at a unique position in the cell cycle to respond to sperm with continuous Ca^{2+} oscillations.

MATERIALS AND METHODS

All compounds were from Sigma unless stated otherwise and of tissue culture grade where appropriate.

Gamete culture and in vitro fertilization

The mice used in this study were 21- to 23-day-old female F_1 B6CB (C57Bl/6JLac × CBA/CaLac) hybrids. Oocytes were collected in medium M2 (Fulton and Whittingham, 1978). Immature germinal vesicle (GV) stage oocytes were collected from ovaries 48 hours after the intraperitoneal injection of 7.5 i.u. pregnant mares' serum gonadotrophin (PMSG). Mature oocytes were collected from PMSG primed mice 15 hours after injection of 5 i.u. human chorionic gonadotrophin (hCG). The cumulus cells were removed by a brief incubation in hyaluronidase (0.3 mg/ml).

Sperm were collected from known fertile mice and capacitated for 90 minutes in medium T6 at a concentration of $1\text{-}2\times10^6$ sperm/ml (Quinn et al., 1982; Glenister et al., 1987). For in vitro fertilization, cumulus-free oocytes were incubated with capacitated sperm in medium T6 for up to 4 hours as previously described (Glenister et al., 1987).

In vitro maturation of oocytes and addition of cycloheximide

GV stage oocytes were released from antral follicles using a sterile needle and the cumulus-intact oocytes collected. Oocytes were cultured in Waymouth's medium MB752/1 (Gibco) supplemented with 0.33 mM pyruvate and 5% fetal calf serum. At 9 hours after release from the follicle, cumulus cells were removed from oocytes by pipetting through a fine-bore pipette. Cumulus-free MI oocytes were cultured for a further 3-6 hours in Waymouth's medium containing 10 μg/ml cycloheximide or 0.1% dimethyl sulphoxide (control). After cycloheximide treatment the oocytes were washed and cultured in medium M16 (Whittingham, 1971).

Staining of chromatin

The chromatin structure of in vitro maturing oocytes previously treated with cycloheximide for 3-6 hours was assessed using Hoechst 33258 at 17 hours after release from the follicle (2-5 hours following removal of cycloheximide; Clarke and Masui, 1983). Oocytes were fixed in 2% formalin for 10 minutes and the chromatin stained by incubation with 10 μg/ml Hoechst 33258 (Calbiochem-Behring). Chromatin organization was observed using a Nikon Diaphot microscope with epi-fluorescence.

Ca^{2+} measurement

Measurements were made using Indo-1. Oocytes were loaded with 50 μM of the acetoxymethyl ester (AM) form of Indo-1 plus 0.02% Pluronic (Molecular Probes) and readings were made using a Nikon Diaphot inverted microscope fitted for epi-fluorescence, with an excitation wavelength of 350 nm and emission recorded at 405 nm and 490 nm (Carroll and Swann, 1992). A Umans software package was used to record Ca^{2+} readings. Ratio readings were converted to intracellular Ca^{2+} levels using a cell free calibration kit (Molecular Probes C-3008) and the method of Grynkiewicz et al. (1985).

Oocytes that had been incubated with sperm for 1-2 hours were loaded with Indo-1 AM and placed on a glass coverslip base of a heated metal chamber (33-36°C) in a 50 μl drop of medium M2 covered with paraffin oil to avoid evaporation. For recording of Ca^{2+} transients immediately following sperm addition, the zona pellucida was removed from Indo-1 loaded oocytes by a brief exposure to acid Tyrode's solution. The zona-free oocytes were transferred to the heated chamber containing 500 μl of M2 without bovine serum albumin (BSA). The oocytes were allowed to attach to the base in protein-free M2 before the addition of 500 μl of M2 containing fraction V BSA (7mg/ml final concentration). For the immediate recording of Ca^{2+} changes at fertilization 10 μl of capacitated sperm suspension was added to the chamber.

Inositol trisphosphate microinjection

Oocytes and embryos were loaded with Indo-1 AM, the zona removed and placed on the stage of the microscope for Ca^{2+} measurement as described above. D-myo-inositol 1,4,5-trisphosphate (IP_3, Alexis Corporation, Nottingham, UK) in 120 mM KCl, 20 mM Hepes, pH 7.5 was microinjected using broken tipped micropipettes. Pipettes were inserted into cells by overcompensation of the negative capacitance on an electrophysiological amplifier used to monitor membrane potential. A bolus injection of IP_3 corresponding to 1% of the total cell volume was achieved by using a Pneumatic Picopump (World Precision Instruments).

RESULTS

Ca^{2+} transients following fertilization cease at pronuclear formation

Mature oocytes collected 15 hours post hCG were fertilized in vitro and the intracellular Ca^{2+} levels recorded using the Ca^{2+} sensitive dye, Indo-1 AM. Intermittently ultraviolet illumination, necessary for Ca^{2+} measurement, was interrupted and under bright-field illumination using Nomarski optics, the formation of the second polar body, approximately 1-2 hours following fertilization, and the pronucleus, at about 4 hours were observed.

As found by ourselves and others, Ca^{2+} oscillations lasting a number of hours followed sperm entry. To determine when sperm-induced Ca^{2+} oscillations cease, oocytes were incubated with capacitated sperm for 1-2 hours before loading with Indo-1 AM and recording Ca^{2+}. Ca^{2+} transients continued after extrusion of the second polar body (n=10) in all oocytes

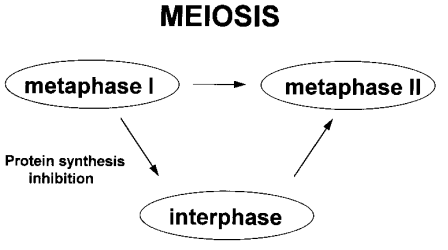


Fig. 1. Protein synthesis inhibition of MI oocytes induces nuclear progression to interphase. Normally during meiotic maturation oocytes progress from MI to MII without an intervening interphase. However, when protein synthesis is inhibited at MI, nuclear progression to interphase is induced.

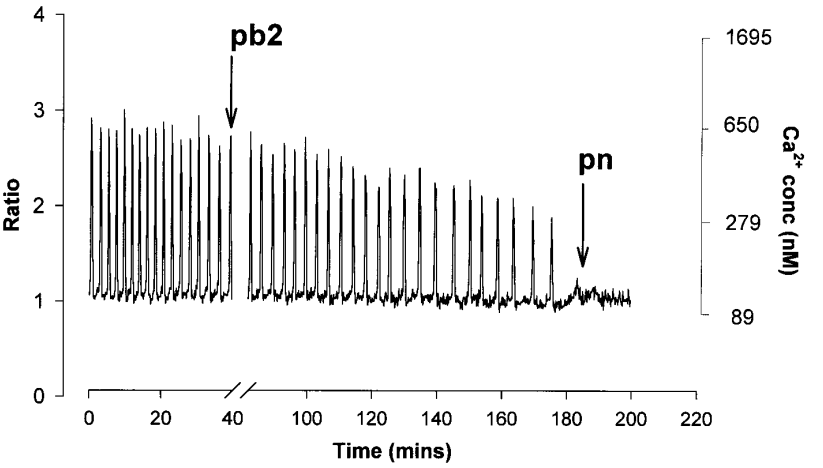


Fig. 2. Sperm-induced Ca^{2+} oscillations cease at pronuclear formation. Following in vitro fertilization oocytes were loaded with the Ca^{2+}-sensitive dye Indo-1 AM and intracellular Ca^{2+} recorded. In the Ca^{2+} recording shown, second polar body extrusion (pb2) occurred 2 hours after sperm addition and pronucleus formation (pn) 2.5 hours later. Note that there was a decrease in the amplitude and frequency of the Ca^{2+} oscillations before pronucleus formation.

studied (Fig. 2). The amplitude of these oscillations decreased shortly before pronuclear formation and ceased when the pronucleus was clearly visible (Fig. 2). The formation of pronuclei lasted 10 minutes and in all oocytes studied Ca^{2+} oscillations had ceased when pronuclear formation was complete. Ca^{2+} levels were recorded in pronuclear stage embryos for a number of hours and no further Ca^{2+} transients were observed (n=10).

To determine whether sperm-induced Ca^{2+} transients cease at a pre-set time after fertilization coincident with pronuclear formation, oocytes were arrested at MII by addition of the microtubule polymerisation inhibitor colcemid during incubation with sperm. Fertilized oocytes treated with 100 ng/ml colcemid remained arrested at MII due to disruption of the metaphase spindle which is required for exit from metaphase (Kubiak et al., 1993). Treated oocytes were loaded with Indo-1 AM at various times after fertilization. Repetitive Ca^{2+} oscillations at a low frequency were observed in all oocytes studied (n=7), several hours after the pronuclei would normally have formed (Fig. 3). In metaphase-arrested oocytes the sperm-induced Ca^{2+} oscillations continued for as long as intracellular Ca^{2+} measurements were made, up to 18 hours after the addition of sperm.

IP_3-induced Ca^{2+} release in oocytes and embryos

The above results suggested that pronuclear stage oocytes had become insensitive to sperm-induced Ca^{2+} release following fertilization at interphase. This was examined by comparison of IP_3-induced Ca^{2+} release in MII oocytes and 1-cell pronuclear stage embryos. In MII oocytes (n=9), IP_3 induced a dose-dependent series of high frequency Ca^{2+} oscillations lasting tens of minutes (Fig. 4). However in the embryos (n=11) IP_3 failed to induce a series of oscillations (Fig. 4).

Cycloheximide treatment of MI oocytes

The cessation of sperm-induced Ca^{2+} transients at interphase and the inability of interphase embryos to generate Ca^{2+} oscillations with IP_3 would suggest interphase is a period of insensitivity to Ca^{2+} release mechanisms. To examine this further, MI oocytes were induced to enter interphase before MII by treatment with cycloheximide for 3-6 hours (Clarke and Masui, 1983). After removal of cycloheximide and further culture, cycloheximide-treated oocytes had a visible nucleus by 17 hours after release from the follicle and underwent nuclear envelope breakdown (NEBD) by 23 hours. Oocytes were therefore in interphase for about 6 hours.

Previously, puromycin had been added to MI oocytes for 9 hours to cause nucleus formation (Clarke and Masui, 1983). The shortest incubation time necessary to achieve nucleus formation in the present culture system was firstly determined. Nuclear progression to interphase at 17 hours after release from the follicle was assessed by the presence of a nucleus in MI oocytes treated with cycloheximide for 3-6 hours. Following a 6 hour incubation with cycloheximide, 95% of oocytes had a nucleus (Fig. 5) and 99% of these underwent NEBD (n=139). However a 4 hour incubation with cycloheximide resulted in 89% of oocytes showing a nucleus and 98% of these underwent NEBD (n=142). In further studies, therefore, MI oocytes were cultured with cycloheximide for 4 hours.

Cycloheximide addition did not block extrusion of the first

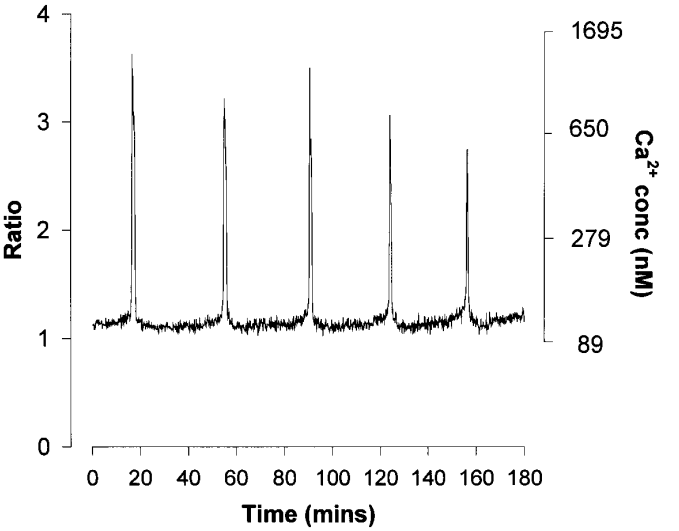


Fig. 3. Sperm-induced Ca^{2+} oscillations continue in oocytes arrested in metaphase. Mature oocytes were incubated with 100 ng/ml colcemid and sperm. In the Ca^{2+} trace shown, the oocyte had been incubated with sperm 13 hours previously. In the continuous presence of colcemid, fertilized oocytes showed Ca^{2+} oscillations for as long as measurements were recorded.

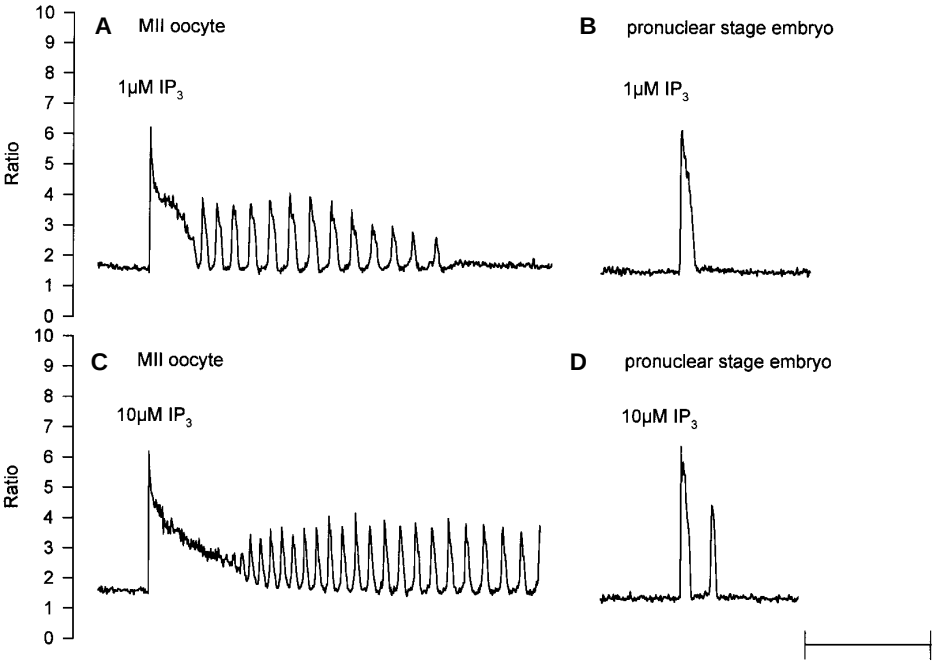


Fig. 4. IP_3 microinjection into oocytes and embryos. In MII oocytes (A,C) IP_3 induced a dose-dependent series of Ca^{2+} oscillations at 1 μM (A) and 10 μM (C), which lasted for several minutes. However in 1-cell pronuclear stage embryos (B, D) the response to IP_3 was ablated with 1 μM generating one transient (B) and 10 μM generating two transients (D). Concentrations are cell concentrations calculated with a 1% v/v injection. The bar represents 10 minutes.

polar body, which occurred between 9-10 hours after release from the follicle, but no second polar body extrusion was observed (Fig. 5) as assessed after staining with the DNA binding fluorochrome Hoechst 33258. Thus all oocytes treated with cycloheximide at MI were assumed to have a single diploid nucleus, similar to those treated with puromycin (Clarke and Masui, 1983).

Sperm addition to cycloheximide-treated oocytes

The ability of sperm to induce repetitive Ca^{2+} transients was examined in cycloheximide-treated oocytes that had progressed to interphase and in those that had undergone NEBD and so re-entered metaphase. When sperm were added to treated oocytes during interphase (n=9) only one to three Ca^{2+} oscillations were observed (Fig. 6A). However, in oocytes that had undergone NEBD (n=8), repetitive Ca^{2+} oscillations were observed (Fig. 6B), similar to oocytes that had been matured in vitro for 15 hours (n=8) and fertilized at MII (Fig. 6C).

These results showed that cycloheximide-treated oocytes, which were in interphase, did not generate repetitive Ca^{2+} transients in response to sperm. As control experiments we therefore examined the ability of cycloheximide to inhibit Ca^{2+} release mechanisms in both MII oocytes and in oocytes at MI. Cycloheximide was added for 4 hours to in vitro matured oocytes at 15 hours after isolation from the follicle. These oocytes did not parthenogenetically activate and oocytes remained at MII. Ca^{2+} oscillations in response to sperm were similar in oocytes treated for 4 hours with cycloheximide (Fig. 6D) and untreated MII oocytes (Fig. 6C). Sperm-induced Ca^{2+} oscillations in cycloheximide-treated MII oocytes continued for the duration of the Ca^{2+} measurement and therefore cycloheximide incubation had no inhibitory effect on the Ca^{2+} release mechanisms of the MII oocyte.

To demonstrate further that cycloheximide did not affect the ability of oocytes to generate repetitive Ca^{2+} oscillations, in vitro maturing oocytes were blocked at MI by the addition of 100 ng/ml colcemid at 8 hours after removal from the follicle. Therefore cycloheximide added at 9 hours after removal, for a period of 4 hours, as for previous experiments, failed to induce nucleus formation. When sperm were added to these oocytes (n=6) Ca^{2+} oscillations were observed (Fig. 6E). This data therefore demonstrates that cycloheximide did not affect Ca^{2+} release mechanisms in both MII and MI oocytes.

Thimerosal-induced Ca^{2+} changes in cycloheximide-treated oocytes

The thiol reagent thimerosal has been shown to cause repetitive Ca^{2+} transients in both mature and immature oocytes (Swann, 1991; Carroll and Swann, 1992). The ability of interphase oocytes, treated with cycloheximide, to respond to thimerosal was therefore examined to determine whether these oocytes were capable of Ca^{2+} oscillations in response to other stimuli. Unlike sperm, 100 μM thimerosal induced repetitive Ca^{2+} oscillations (Fig. 7) in all interphase oocytes studied (n=11).

DISCUSSION

Repetitive Ca^{2+} transients have been observed during the fertilization of mammalian oocytes (Cuthbertson et al., 1981; Cuthbertson and Cobbold, 1985; Igusa and Miyazaki, 1986; Kline and Kline, 1992; Taylor et al., 1993). The present study shows that normally these transients continue until pronuclear formation but if metaphase arrest is maintained, by the addition of colcemid, the Ca^{2+} oscillations continue indefinitely. This suggests that cessation of Ca^{2+} oscillations does not occur at a pre-set time after sperm entry, but rather, is associated with events in the cell cycle. Further we find that in cycloheximide-treated oocytes sperm trigger repetitive Ca^{2+} oscillations during metaphase but not during interphase. Therefore inter-

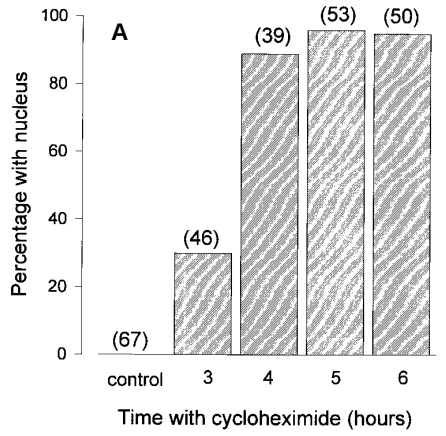
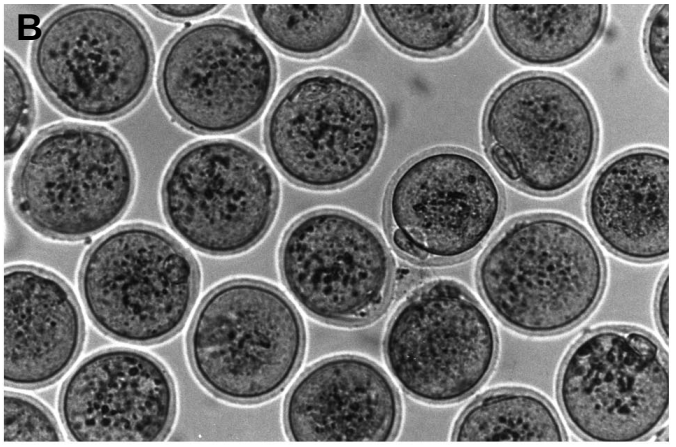
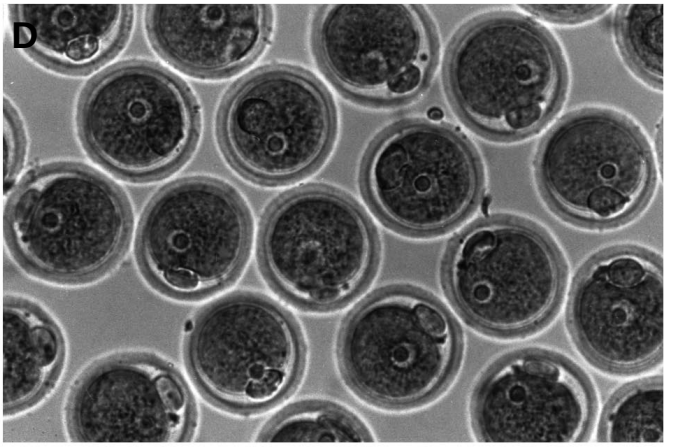
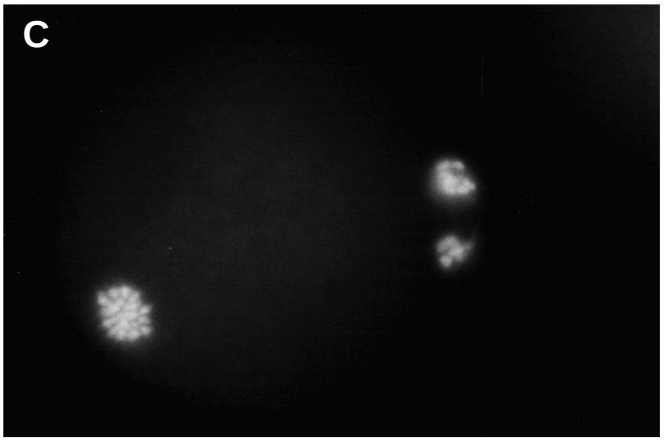
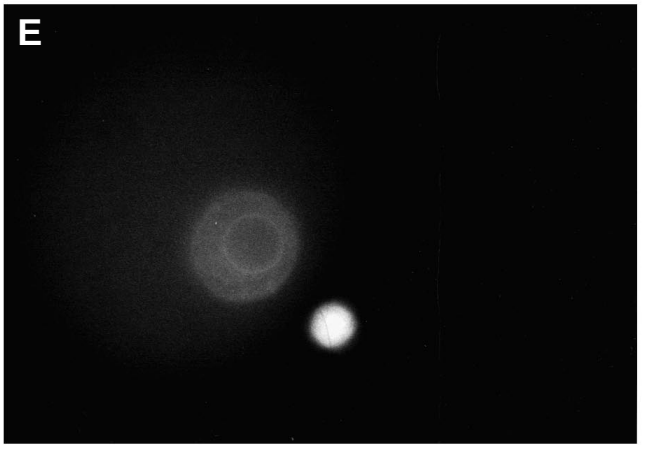


Fig. 5. Cycloheximide-induced nucleus formation in MI oocytes. In vitro maturing oocytes, at 9 hours after release from the follicle, were cultured with 10 μg/ml cycloheximide for 3-6 hours, washed and further cultured in medium M16. Formation of a nucleus was assessed, in Hoechst stained oocytes, at 17 hours after release from the follicle. The percentage of oocytes with a nucleus increased with time in cycloheximide (A). The number of oocytes examined are in parentheses. Under control conditions (B,C) (0.1% dimethyl sulphoxide) MI oocytes matured and arrested at MII (B) as assessed by Hoechst staining (C). A 4 hour incubation with cycloheximide (D,E) was necessary to induce formation of a nucleus (D) in the majority of oocytes. The chromosomes were decondensed within the nucleus (E).









phase marks a period of insensitivity to sperm-induced Ca^{2+} changes.

Sperm-induced Ca^{2+} oscillations are cell cycle dependent

Ca^{2+} oscillations induced by sperm continued past second polar body extrusion, decreasing in frequency before, and stopping at, pronuclear formation. Furthermore in oocytes arrested at MII with colcemid, sperm-induced Ca^{2+} transients continued for as long as Ca^{2+} was recorded. This suggests that there is a cell cycle-dependent change in the Ca^{2+} release mechanism activated by sperm at fertilization. Further work is required to determine whether this change is seen universally in mitosis and is not just specific to meiotic MII and the first embryonic interphase. However, the generation of Ca^{2+} transients during mitosis in fibroblasts (Kao et al., 1990), sea

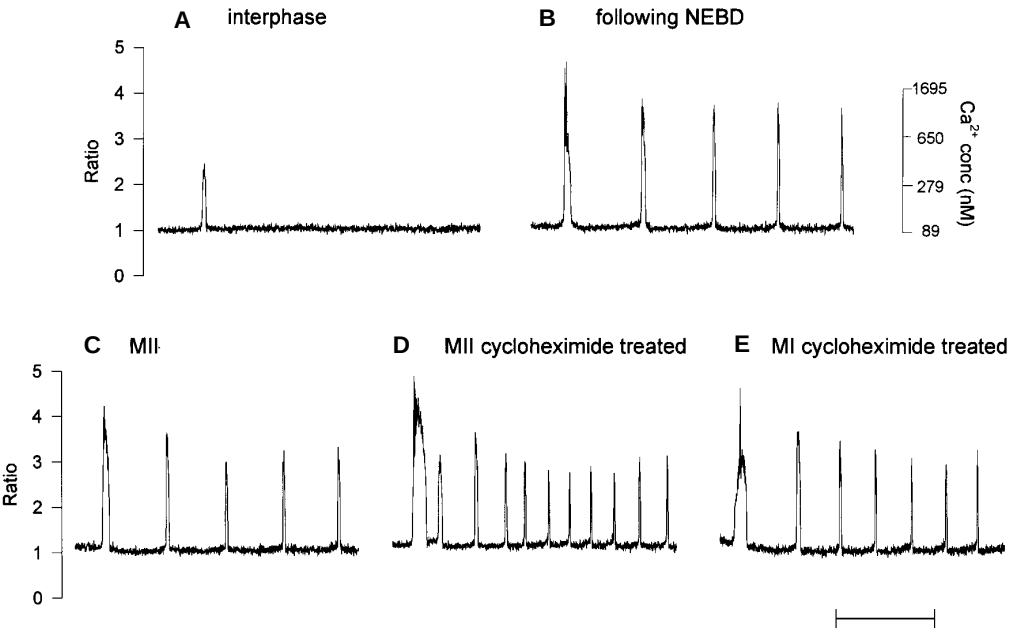


Fig. 6. Sperm-induced Ca^{2+} oscillations are cell cycle dependent. The addition of cycloheximide, for 4 hours, to maturing oocytes at 9 hours after release from the follicle, induced nuclear progression to interphase. Interphase oocytes did not generate repetitive Ca^{2+} oscillations in response to sperm (A). However, repetitive Ca^{2+} oscillations were seen when cycloheximide-treated oocytes underwent NEBD and returned to metaphase (B). In vitro matured MII oocytes 15 hours after release from the follicle gave repetitive Ca^{2+} oscillations in response to sperm (C). Incubation with cycloheximide did not block repetitive Ca^{2+} transients when added for 4 hours in both MII (D) and MI oocytes (E). MII oocytes were matured in vitro for 15 hours and in vitro maturing oocytes were arrested at MI with colcemid at 8 hours following isolation from the follicle. Time bar represents 30 minutes.

urchin embryos (Poenie et al., 1985) and mouse embryos (Tombes et al., 1992) support the idea that Ca^{2+} release mechanisms are modulated in a cell cycle-dependent manner.

The mechanism underlying the cell cycle changes in Ca^{2+} sensitivity is not known. However, in sea urchin embryos there is an increased turnover of IP_3 during mitosis which may act to trigger the observed Ca^{2+} transients during this period (Ciapa et al., 1994). In somatic cells it has been reported that the regulation of Ca^{2+} homeostasis differs between mitosis and interphase such that agonists induce different Ca^{2+} responses in mitosis and interphase (Preston et al., 1991). Also in preimplantation mouse embryos there are cell cycle related changes in membrane potential and ion channel conductance (Day et al., 1993). Our data similarly shows that the ability of sperm to stimulate repetitive Ca^{2+} transients in oocytes is cell cycle regulated. The inability of IP_3 to generate highly repetitive Ca^{2+} transients in interphase embryos in the present study suggests there is a decrease in the regenerative nature of Ca^{2+} release following pronuclear formation.

One possible reason why sperm-induced Ca^{2+} oscillations stop at pronuclear formation is suggested by Ca^{2+} measurements made on mouse oocytes following nuclear transfer (Kono et al., 1995). In that study Ca^{2+} transients were observed when the nuclei of fertilized embryos were transferred to MII oocytes. Ca^{2+} transients began following NEBD of the transferred nucleus. NEBD happens shortly after exposure to the oocyte cytoplasm due to high MPF levels. This Ca^{2+} releasing activity may be within the nucleus itself or associated with perinuclear material, such as endoplasmic reticulum. In the results presented here it was found that sperm-induced Ca^{2+} oscillations cease at pronucleus formation. This may be due to the Ca^{2+} releasing activity introduced by the sperm becoming localized to the pronucleus, as observed for other proteins localized to the nucleus during interphase (Millar et al., 1991; Ookata et al., 1992; Hepler et al., 1994) or by inactivation of the mechanism of sperm-induced Ca^{2+} release during interphase. During mitosis the Ca^{2+} releasing activity may be released in an active form from the nucleus or the Ca^{2+} release mechanisms may be re-activated allowing the generation of repetitive Ca^{2+} transients.

Sperm- and thimerosal-induced Ca^{2+} release

Both the thiol reagent, thimerosal, and sperm are able to generate repetitive Ca^{2+} oscillations in MII mouse oocytes (Swann, 1992; Cheek et al., 1993). However, in this study we find that thimerosal stimulates Ca^{2+} release in interphase oocytes while sperm do not. This suggests there are different

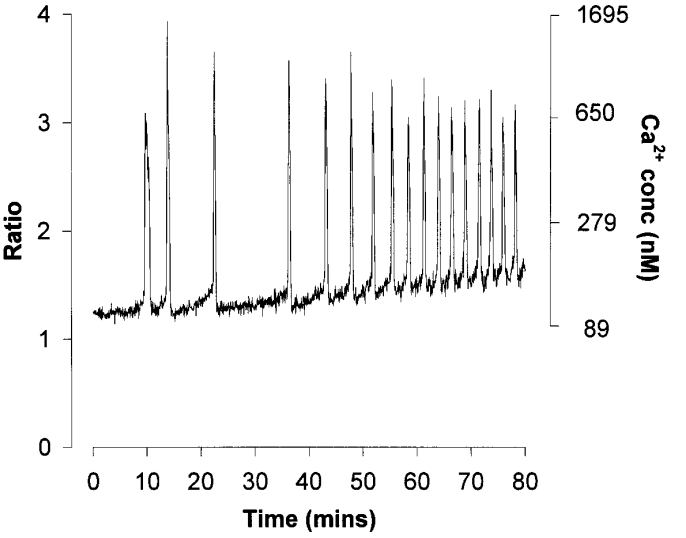


Fig. 7. Cycloheximide-treated oocytes during interphase generated repetitive Ca^{2+} transients in response to thimerosal. Addition of 100 μM thimerosal to interphase oocytes gave continuous Ca^{2+} oscillations in all oocytes studied.

mechanisms for thimerosal- and sperm-induced Ca^{2+} release. Also in immature oocytes thimerosal and TP_3 are able to generate repetitive Ca^{2+} transients (Carroll and Swann, 1992) whereas sperm do not (Jones et al., 1995). A difference in the mechanism of Ca^{2+} release by sperm and thimerosal has previously been shown in mouse MII oocytes by their sensitivity to the reducing agent dithiothreitol, which inhibits thimerosal but not sperm-induced Ca^{2+} oscillations (Cheek et al., 1993). These data suggest that the Ca^{2+} release mechanisms induced by thimerosal and sperm are not equivalent and that only Ca^{2+} oscillations in response to sperm are cell cycle dependent.

The development of repetitive sperm-induced Ca^{2+} oscillations between MI and MII

Previously we have shown that the ability of the oocyte to generate repetitive Ca^{2+} oscillations in response to sperm occurs late during in vitro maturation and is independent of nuclear progression from MI to MII (Jones et al., 1995). In the present study it was found that oocytes arrested at MI with colcemid and cultured for 4 hours with cycloheximide showed repetitive Ca^{2+} oscillations (Fig. 6E). This demonstrates that a post-translational event during progression from MI to MII, rather than new protein synthesis, is likely to be responsible for the modifications that support sperm-induced Ca^{2+} oscillations.

The mechanism of repetitive Ca^{2+} transients at fertilization

The mechanism whereby sperm induce Ca^{2+} oscillations in MII oocytes remains unresolved. The two most favoured hypotheses have been a soluble sperm factor that is released into the oocyte (Dale et al., 1985; Swann, 1990) or, alternatively, a receptor mediated pathway that may involve G-proteins (Miyazaki, 1988; Jaffe, 1990). The present findings that sperm-induced Ca^{2+} oscillations can continue for 18 hours in the presence of colcemid, are not in keeping with a G-protein-mediated pathway as a non-hydrolysable G-protein analogue microinjected into mouse oocytes only induces Ca^{2+} oscillations lasting for a few minutes (Swann, 1992). As yet it is unclear what mechanism is present in the oocyte at MII that is responsible for repetitive Ca^{2+} transients lasting for several hours after sperm entry.

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