Immature mouse oocytes undergo spontaneous meiotic maturation when released from antral follicles into culture media. The first sign of meiotic resumption is germinal vesicle breakdown (GVB). Cytosolic free Ca\(^{2+}\) was measured in mouse oocytes during spontaneous maturation by monitoring fluorescence of indo-1 or fluo-3. The majority of oocytes showed a series of Ca\(^{2+}\) oscillations that continued for 1–3 h. Repetitive Ca\(^{2+}\) increases occurred every 1–3 min and lasted for 10–60 s. The Ca\(^{2+}\) oscillations appeared to be caused by an increase in inositol 1,4,5-trisphosphate (InsP\(_3\)) because once they ceased, similar oscillations were triggered by injection of exogenous InsP\(_3\). Also, injection of the InsP\(_3\) receptor antagonist heparin (final concentration, 100 \(\mu\)g/ml) blocked the spontaneous Ca\(^{2+}\) oscillations. In contrast, Ca\(^{2+}\) oscillations induced by thimerosal were not inhibited by heparin. Treating oocytes with media containing 20 \(\mu\)M BAPTA/AM abolished Ca\(^{2+}\) oscillations in oocytes but did not affect the rate of GVB. The data show that cytosolic Ca\(^{2+}\) oscillations apparently caused by polyphosphoinositide turnover occur during mammalian oocyte maturation. However, the spontaneous oscillations do not appear to trigger GVB. Also, the data indicate that there are two separate Ca\(^{2+}\) release mechanisms in mouse oocytes, one sensitive to InsP\(_3\), the other to thimerosal.

Oscillations in the concentration of intracellular free calcium (Ca\(^{2+}\) oscillations) occur in many types of somatic cells in response to hormones and/or growth factors. The oscillations are thought to control an array of cellular processes including cell metabolism, exocytosis, and cell cycle progression. Generally, Ca\(^{2+}\) oscillations appear to be caused by the activation of phosphoinositide C by receptor-coupled G-proteins or tyrosine kinases resulting in the production of the InsPs receptor antagonist heparin (final concentration, 100 \(\mu\)g/ml) blocked the spontaneous Ca\(^{2+}\) oscillations. In contrast, Ca\(^{2+}\) oscillations induced by thimerosal were not inhibited by heparin. Treating oocytes with media containing 20 \(\mu\)M BAPTA/AM abolished Ca\(^{2+}\) oscillations in oocytes but did not affect the rate of GVB. The data show that cytosolic Ca\(^{2+}\) oscillations apparently caused by polyphosphoinositide turnover occur during mammalian oocyte maturation. However, the spontaneous oscillations do not appear to trigger GVB. The data indicate that there are two separate Ca\(^{2+}\) release mechanisms in mouse oocytes, one sensitive to InsP\(_3\), the other to thimerosal.

Another review suggests the existence of two separate Ca\(^{2+}\) stores; one is sensitive to InsPs which releases Ca\(^{2+}\) into a second store that generates oscillatory Ca\(^{2+}\) release by a process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR).

Mammalian oocytes are a useful model to study the mechanism of Ca\(^{2+}\) oscillations (4). In mature oocytes Ca\(^{2+}\) oscillations occur at fertilization (5, 6) or after injecting InsP\(_3\) (7) or non-hydrolyzable GTP analogues (8, 9). The increase in intracellular Ca\(^{2+}\) at fertilization or after parthenogenetic activation stimulate the mature oocyte to complete the second meiotic division and begin development (10). Fewer studies on changes in intracellular Ca\(^{2+}\) have been made in the immature oocyte. Immature germinal vesicle (GV) stage oocytes are arrested in the dictyate stage of the first meiotic division (equivalent to G2) and when released from antral follicles undergo spontaneous meiotic maturation (11, 12). The first morphological sign that meiosis has resumed is germinal vesicle breakdown (GVB) which in the mouse occurs within 2 h of release from the follicle. The cellular messengers that are responsible for triggering the resumption of meiosis in immature oocytes are not known. Some studies on immature amphibian and invertebrate oocytes have suggested a role for Ca\(^{2+}\) in meiotic resumption (see Ref. 13 for review). However, others have suggested that changes in intracellular Ca\(^{2+}\), although they may occur, are not essential for GVB (14–16). In mammals there is indirect evidence to suggest that Ca\(^{2+}\) changes are involved in the resumption of meiosis. In bovine oocytes, intracellular BAPTA, a Ca\(^{2+}\) chelator, inhibits spontaneous GVB (17). Neomycin and heparin, which inhibit InsP\(_3\) production and inositol phosphate-induced Ca\(^{2+}\) release, respectively, inhibit GVB (17, 18). Also, inhibition of GVB in mouse and hamster oocytes by increasing the concentration of intracellular cAMP is antagonized by high extracellular Ca\(^{2+}\) (20 mM) or Ca\(^{2+}\) ionophore (19, 20) while Ca\(^{2+}\) channel blockers verapamil and tetracaine enhance the inhibitory effect of BtcAMP (19). However, direct measurements of intracellular Ca\(^{2+}\) in mammalian oocytes during the resumption of meiosis have not been reported.

In this study intracellular Ca\(^{2+}\) was measured in mouse oocytes during spontaneous meiotic maturation using the calcium-sensitive fluorescent dyes indo-1 and fluo-3. We report that immature mouse oocytes show spontaneous Ca\(^{2+}\) transients during the resumption of meiosis in vitro. Apparently the oscillations are driven by spontaneous PI turnover since when they cease similar transients are triggered by the injection of InsP\(_3\). Also, the spontaneous oscillations, but not those induced by the sulphhydryl reagent thimerosal, were inhibited by the injection of heparin suggesting that two different calcium release mechanisms exist in mouse oocytes. The potential role of these Ca\(^{2+}\) changes in meiotic maturation is considered.

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Materials and Methods

Oocyte Recovery—Oocytes were obtained from naturally cycling 6-8-week-old random bred MF1 mice or 21-24-day-old B6CB (C57BL/6Jlac x CBA/CaLac) F1 hybrids that were given 5 IU pregnant mares' serum gonadotropin 44-45 h previously. Ovaries were removed and transferred to Medium M2 (21). Antral follicles were punctured to release oocytes, and those with adhering granulosa cells were collected. Granulosa cells were removed by drawing them in and out of fine-bore pipettes. Oocytes with an intact GV were washed in M2 and used for in vitro maturation experiments or for the direct measurement of calcium.

In Vitro Maturation—The ability of oocytes to resume meiosis was assessed by the presence or absence of an intact GV. Oocytes were released from antral follicles into M2 (controls), or into M2 supplemented with various drugs as described, and incubated for up to 4 h at 37°C. The incubation was in the continual presence of the agents used except in the case of BAPTA/AM (20 μM) where the oocytes were transferred to fresh M2 after 15 min of incubation in the BAPTA/AM. Heparin was microinjected (as described below) to a final concentration of 100 μg/ml. Injections were completed within 30 min after release from the follicle. Oocytes were examined for GVB at regular intervals. The oocyte was scored as having undergone GVB when no sign of the GV was visible in the cytoplasm.

Measurement of Intracellular Calcium—Intracellular Ca2+ was measured using the Ca2+-sensitive dyes indo-1 (22) or fluo-3 (23, 24). For loading, oocytes were incubated for 15 min at 37°C with 50 μM of the acetoxymethyl (AM) form of the dyes made up in M2 with 0.02% pluronic F-127 (25). After loading, oocytes were placed in 50-400-μl drops of M2 under paraflin oil in a dish with a polylsine (100 μg/ml)-coated coverslip as its base. The dish was transferred to a heated stage (34-37°C) of an inverted epifluorescence microscope (Nikon Diaphot for indo-1 or Leitz Diavert for fluo-3). Fluorescence was measured with 992B photomultiplier tubes connected to current to voltage A1 amplifiers (Thorn EMI, UK). analogue voltage signals from indo-1 were collected, recorded, and analyzed on a computer using the UMANS system (C. Regan, Urbana, IL) while those from fluo-3 were recorded on a chart recorder. Base-line fluo-3 fluorescence from loaded oocytes decreased with time, but since the indo-1 recordings suggested a fairly constant base-line level of intracellular Ca2+, the decrease in fluo-3 fluorescence was judged to be due to loss of dye. The diminishing signal was therefore prevented, and a constant level of base-line fluorescence was maintained by the addition of 0.25 μM fluo-3 AM to the solution containing the oocytes.

Microinjection—Microinjection was carried out using broken tipped micropipettes (50-150 MΩ resistance) filtered with solution of 120 mM KCl buffered with 20 mM Hepes (pH 7.5) (9). Fluo-3 potas-
sium salt (~2 pl of 10 mM fluo-3) and heparin (~2 pl of 10 mg/ml heparin; M, 4000-6000) were pressure-injected (26). Injection of InsP3 was performed as described previously (9).

Results

Spontaneous Oscillations of Intracellular Calcium Occur during the Resumption of Meiosis—GV intact mouse oocytes show sustained Ca2+ oscillations after release from antral follicles (Fig. 1). Ca2+ oscillations were seen in oocytes incubated in indole-1 AM (A) or fluo-3 AM (B). The majority of oocytes released from antral follicles showed oscillations in intracel-
ular Ca2+ (49/57) that continued for 1-3 h. Each Ca2+ transient had a slow pacemaker rise followed by a characteristic rapid upstroke and decline. Repeated Ca2+ increases occurred every 1-3 min and consisted of 450-960 nM peaks above a 170-380 nM base-line level that lasted for 10-60 s. With increasing time after release from the follicle fewer oocytes showed Ca2+ oscillations, and after GVB (about 2 h after release from the follicle), oscillations occurred in only 15/61 oocytes. The oscillations that were seen after 2 h occurred at irregular intervals and were more sinusoidal with smaller oscillations than seen in GV intact oocytes shortly after release from the follicle (Fig 1).

Removal of the granulosa cells or the AM loading techniques did not affect the pattern of Ca2+ oscillations observed in immature oocytes after release from the antral follicle. When granulosa cell intact and granulosa cell free oocytes were injected with the fluo-3 salt, Ca2+ oscillations were observed before (4/4 oocytes) and after removal of the surrounding follicle cells (3/4 oocytes) (Fig. 2). The Ca2+ oscillations were not caused by microinjection per se because inserting pipettes containing injection buffer did not trigger Ca2+ changes in non-oscillating fluo-3 AM-loaded oocytes (3/ 

3 oocytes). The membrane potential, as recorded through the fluo-3 injection pipette, was ~20 to ~50 mV, and sometimes during spontaneous Ca2+ oscillations small hyperpolarizations (~5 mV) were seen (data not shown).

Reinitiation of Calcium Oscillations by InsP3 and Thimerosal—In oocytes that had stopped oscillating spontaneously the continuous injection of InsP3 initiated new Ca2+ oscillations in all cases (7/7). The InsP3-induced oscillations were similar in size and frequency to those seen in spontaneously oscillating oocytes (Fig 3A). In addition, carbachol (100 μM), which increases InsP3 production in other cell types (27), elevated the oscillation frequency in oocytes where the spontaneous oscillations had ceased to oscillate (21/21 oocytes) (Fig 3C). However, the thimerosal-induced transients were larger than the sponta-
eneous oscillations and consisted of 778-1702 nm peaks that occurred at a frequency of one transient every 2-5 min compared with 450-960 nM every 1-3 min.

Inhibition of Spontaneous Oscillations by Heparin—The injection of the InsP3 receptor antagonist heparin (final concentration, 100 μg/ml) rapidly blocked the spontaneous Ca2+ oscillations in immature mouse oocytes. Oocytes were released from antral follicles, denuded of adhering granulosa cells, and loaded with indo-1 AM (A) or fluo-3 AM (B). Both traces start about 30 min after the oocyte was released from the follicle, and the time taken from release to undergo GVB is marked on each trace. Recording was continuous for each oocyte. Where the record has been broken the minutes left out are marked on each trace. In trace A the calcium concentrations were calibrated according to Grykiewicz et al. (22). In this and subsequent traces of indo-1 fluorescence the calcium concentrations of 92, 285, 555, and 963 nM correspond to 340/380 ratio values of 0.75, 1.5, 2.25, and 3.0, respectively. Fluor-3 (FL) records are uncalibrated; the fluorescence scale is arbitrary.

FIG. 1. Spontaneous calcium oscillations in immature mouse oocytes. Oocytes were released from antral follicles, denuded of adhering granulosa cells, and loaded with indo-1 AM (A) or fluo-3 AM (B). Both traces start about 30 min after the oocyte was released from the follicle, and the time taken from release to undergo GVB is marked on each trace. Recording was continuous for each oocyte. Where the record has been broken the minutes left out are marked on each trace. In trace A the calcium concentrations were calibrated according to Grykiewicz et al. (22). In this and subsequent traces of indo-1 fluorescence the calcium concentrations of 92, 285, 555, and 963 nM correspond to 340/380 ratio values of 0.75, 1.5, 2.25, and 3.0, respectively. Fluor-3 (FL) records are uncalibrated; the fluorescence scale is arbitrary.
Inhibition of GVB Does Not Affect Spontaneous Calcium Oscillations—It is established that medium containing BtzcAMP (0.25 mM) (29) or activators of protein kinase C such as phorbol myristate acetate (PMA, 10 ng/ml) (30, 31) inhibit the spontaneous maturation of mouse oocytes. This was confirmed in the present study (Table I). Their effect on Ca^{2+} oscillations was investigated. The addition of 0.25–0.5 mM BtzcAMP (n = 6) or 10–100 ng/ml PMA (n = 3) to the media containing the oocytes caused no measurable change in the pattern of spontaneous oscillations (Fig. 5). Thus it seems unlikely that either CAMP or protein kinase C inhibits GVB by affecting intracellular Ca^{2+} oscillations.

Inhibition of Calcium Oscillations Does Not Affect GVB—To determine whether there was a direct correlation between the occurrence of Ca^{2+} transients and GVB, oocytes were incubated in BAPTA/AM (20 μM for 15 min or 10 μM for 30 min). In all oocytes (n = 24) incubated with BAPTA/AM immediately after release from antral follicles no Ca^{2+} oscillations were seen (Fig. 6A). The addition of BAPTA/AM (10–20 μM) to oocytes that were spontaneously undergoing Ca^{2+} oscillations abolished responses within 2 min (Fig. 6B). Despite these effects, GVB still occurred in 11/12 BAPTA/AM-loaded oocytes that showed no Ca^{2+} changes for 2–3 h after the commencement of BAPTA/AM treatment. In parallel experiments, where intracellular Ca^{2+} was not measured, BAPTA/AM loading of oocytes immediately on release from the follicle did not affect the timing of GVB (Table I). The addition of nickel (Ni^{2+}, 5 mM), an inhibitor of Ca^{2+} influx, rapidly inhibited spontaneous Ca^{2+} oscillations (3/3 oocytes) (Fig. 6C). Similar to the BAPTA experiments, release of oocytes from follicles into medium containing Ni^{2+} did not affect GVB (Table I).

In the present study (Table I), the occurrence of Ca^{2+} transients and GVB, oocytes were incubated in BAPTA/AM loading of oocytes immediately on release from the follicle did not affect GVB (Table I). The rapid inhibition of spontaneous Ca^{2+} oscillations was investigated. The addition of nickel (Ni^{2+}, 100 μM) to the medium containing the oocytes of mouse oocytes did not affect GVB (Table I). The inhibition of Ca^{2+} influx, rapidly inhibited spontaneous Ca^{2+} oscillations (3/3 oocytes) (Fig. 6C). Similar to the BAPTA experiments, release of oocytes from follicles into medium containing Ni^{2+} did not affect GVB (Table I).

Fig. 3. Stimulation of Ca^{2+} oscillations in oocytes when spontaneous oscillations have stopped or slowed down. Calcium oscillations were stimulated in fluo-3-(FL) or indo-1-loaded oocytes 50–90 min after release from the follicle by (A) inserting a pipette containing 1 mM InsP_{3} (9), or by addition to the medium containing the oocytes of (B) carbachol (100 μM) or (C) thimerosal (100 μM). In traces B and C some low frequency spontaneous oscillations were seen prior to addition of drugs. Note the carbachol-induced oscillations (B) are similar in size to the spontaneous oscillations, but thimerosal-induced oscillations (C) are larger. The simulated oscillations continued while the agonist was present. The time scale for B and C is the same.

Fig. 4. The effect of heparin on spontaneous oscillations in mouse oocytes. Spontaneously oscillating fluo-3-loaded (FL) oocytes were injected with heparin. Trace A shows that injection of heparin (100 μg/ml final concentration) inhibits spontaneous oscillations. After removal of the pipette used to inject heparin a brief calcium transient occurred (arrow), but this did not trigger further Ca^{2+} oscillations. In traces B and C the injection pipette was left in the oocyte. Trace B shows that thimerosal (100 μM) induces a series of Ca^{2+} oscillations larger than the spontaneous oscillations previously inhibited by injection of heparin. The dashed line indicates the fluorescence record has gone off scale. Trace C shows that control injections of de-N-sulfated heparin (100 μg/ml final concentration), after an initial delay at the time of injection, do not inhibit spontaneous oscillations.

Table I

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect on Ca^{2+} oscillations</th>
<th>% of oocytes that underwent GVB at 120 min (n)</th>
<th>Treated</th>
<th>Controls</th>
</tr>
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<tr>
<td>BtzcAMP (0.5 mM)</td>
<td>None</td>
<td>0 (17)</td>
<td>100 (5)</td>
<td></td>
</tr>
<tr>
<td>10 nM</td>
<td></td>
<td>0 (15)</td>
<td>86 (7)</td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td></td>
<td>0 (23)</td>
<td>86 (7)</td>
<td></td>
</tr>
<tr>
<td>BAPTA/AM (20 μM)</td>
<td></td>
<td>Inhibits</td>
<td>70 (82)</td>
<td></td>
</tr>
<tr>
<td>Nickel (10 mM)</td>
<td></td>
<td>Inhibits</td>
<td>90 (10)</td>
<td></td>
</tr>
<tr>
<td>Heparin (100 μg/ml)</td>
<td></td>
<td>Inhibits</td>
<td>13 (8)</td>
<td></td>
</tr>
</tbody>
</table>

Spontaneous Calcium Oscillations

![Figure 2](image2.png)

Fig. 2. Ca^{2+} oscillations after injection of fluo-3. Fluo-3 (FL) was injected into oocytes 10–25 min after release from the follicle at the time indicated by the arrow. In trace A the oocyte was injected after removal of attached follicular cells, and in B follicular cells were not removed.

![Figure 4](image4.png)

FIG. 4. The effect of heparin on spontaneous oscillations in mouse oocytes. Spontaneously oscillating fluo-3-loaded (FL) oocytes were injected with heparin. Trace A shows that injection of heparin (100 μg/ml final concentration) inhibits spontaneous oscillations. After removal of the pipette used to inject heparin a brief calcium transient occurred (arrow), but this did not trigger further Ca^{2+} oscillations. In traces B and C the injection pipette was left in the oocyte. Trace B shows that thimerosal (100 μM) induces a series of Ca^{2+} oscillations larger than the spontaneous oscillations previously inhibited by injection of heparin. The dashed line indicates the fluorescence record has gone off scale. Trace C shows that control injections of de-N-sulfated heparin (100 μg/ml final concentration), after an initial delay at the time of injection, do not inhibit spontaneous oscillations.
The data show clearly that there are spontaneous Ca\textsuperscript{2+} oscillations in immature GV stage oocytes after release from antral follicles that last for up to 2 h. It is well established that Ca\textsuperscript{2+} oscillations occur in mature mammalian eggs after fertilization, but this is the first report that Ca\textsuperscript{2+} oscillations also occur in immature oocytes during the resumption of meiosis. The Mechanism of Spontaneous Calcium Oscillations—Several lines of evidence suggest that the trigger for the spontaneous Ca\textsuperscript{2+} oscillations is increased production of InsP\textsubscript{3}. In oocytes that had stopped oscillating spontaneously, increasing the level of intracellular InsP\textsubscript{3} directly by microinjection reinitiated Ca\textsuperscript{2+} oscillations that were similar in size and frequency to the spontaneous oscillations. Further, carbachol, which increases InsP\textsubscript{3} production in other cell types (27), also potentiated the Ca\textsuperscript{2+} oscillations. Previously it was shown that acetylcholine caused small and refractory membrane potential changes in mouse oocytes (32). Our observations suggest that an acetylcholine and carbachol receptor is linked to PI turnover in the mouse oocyte. The idea that the spontaneous oscillations are due to increased InsP\textsubscript{3} production is also strongly supported by our finding that heparin, a competitive inhibitor of InsP\textsubscript{3}, rapidly inhibited spontaneous oscillations. Control injections of de-N-sulfated heparin (which does not bind the InsP\textsubscript{3} receptor) (28) caused only a brief interruption at the time of injection, before spontaneous oscillations returned to normal. The results suggest that during the resumption of meiosis, in vitro PI turnover, which is not agonist-induced, leads to a series of spontaneous Ca\textsuperscript{2+} oscillations.

Our findings that Ca\textsuperscript{2+} oscillations driven by InsP\textsubscript{3} occur spontaneously in mouse oocytes are in contrast to most other cell types where oscillations in intracellular Ca\textsuperscript{2+} are induced by an agonist (1) or some other external stimulus such as fertilization (4). Spontaneous Ca\textsuperscript{2+} transients also occur during the first embryonic cell cycle (33), but the mechanism of generation of these transients is unknown. Spontaneous Ca\textsuperscript{2+} oscillations have also been reported in some somatic cell types including neutrophils (34), somatotrophs (35), and rat chromaffin cells (36). In rat chromaffin cells, where the spontaneous Ca\textsuperscript{2+} oscillations have been characterized, they are based upon the process of CICR and appear to be independent of PI turnover (36). The present study provides the first evidence suggesting that Ca\textsuperscript{2+} changes can be triggered by the spontaneous generation of InsP\textsubscript{3}. The putative spontaneous PI turnover in mouse oocytes may occur continuously in oocytes in intact follicles, or alternatively, PI turnover may be stimulated in oocytes upon release from the follicle. This might be due to the removal of an endogenous inhibitor of PI turnover, since agonists that inhibit PI turnover have been described in other types of cells (37).

Several different models have been proposed to explain how InsP\textsubscript{3} causes Ca\textsuperscript{2+} oscillations. The models rely on the processes of InsP\textsubscript{3}- or Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release. Both InsP\textsubscript{3}- and Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release mechanisms are present in immature mouse oocytes (38, 39). It has been suggested that CICR occurs in a separate Ca\textsuperscript{2+} store from the InsP\textsubscript{3}-sensitive store (2, 40, 41). Our observations support the hypothesis of two separate Ca\textsuperscript{2+} stores. Intracellular injection of heparin has been used previously to dissociate InsP\textsubscript{3}-sensitive and InsP\textsubscript{3}-insensitive Ca\textsuperscript{2+} release mechanisms (2, 42). The fact that thimerosal triggers a series of Ca\textsuperscript{2+} oscillations in oocytes previously injected with heparin demonstrates the presence of an InsP\textsubscript{3}-insensitive Ca\textsuperscript{2+} store or release mechanism. In mature hamster oocytes, thimerosal causes Ca\textsuperscript{2+} oscillations by sensitizing a CICR mechanism (25). It seems likely, therefore, that a similar CICR mechanism accounts for thimerosal-induced InsP\textsubscript{3}-independent Ca\textsuperscript{2+} release in mouse oocytes. The idea that thimerosal causes Ca\textsuperscript{2+} oscillations by affecting CICR is also supported by the observations that thimerosal-induced Ca\textsuperscript{2+} oscillations were consistently larger than the InsP\textsubscript{3}-induced oscillations. The fact that thimerosal causes Ca\textsuperscript{2+} release after the injection of heparin argues against the suggestion that thimerosal triggers Ca\textsuperscript{2+} release by sensitizing the InsP\textsubscript{3} receptor to endogenous levels of InsP\textsubscript{3} (43).

Are the Calcium Oscillations Involved in GVB?—The question of whether Ca\textsuperscript{2+} oscillations are a signal for causing GVB is controversial. A number of our observations suggest that the repetitive spontaneous oscillations in mammalian oocytes do not play a causal role in GVB. 1) Treatment with BAPTA/AM or Ni\textsuperscript{2+} rapidly inhibited the spontaneous Ca\textsuperscript{2+} oscillations, but GVB was not affected. 2) Inhibitors of GVB (Bt2cAMP, PMA) had no detectable effect on Ca\textsuperscript{2+} oscillations. 3) In a significant proportion of oocytes Ca\textsuperscript{2+} oscillations continued well after GVB had occurred. 4) Oocytes that showed no sign of spontaneous Ca\textsuperscript{2+} oscillations underwent...
Spontaneous Calcium Oscillations

Although the spontaneous Ca\textsuperscript{2+} oscillations we describe do not appear to be necessary for GVB in mouse oocytes they may be important for other processes such as the modifications of the cytoplasm associated with meiotic maturation (cytoplasmic maturation). During in vitro maturation many structural and biochemical modifications (46, 47) in the organization of the cytoplasm occur, including exocytosis, secretion, changes in protein synthesis, and phosphorylation, all of which have been shown to be modulated by intracellular Ca\textsuperscript{2+} in other systems (3). Cytoplasmic maturation is essential for normal fertilization and subsequent development to occur (48–50). In different species the conditions for in vitro maturation that are necessary to produce viable oocytes differ considerably. Mouse oocytes matured in vitro are viable after maturation in minimal conditions (50) whereas the oocytes of domestic species often require somatic cell and hormonal support (49). The ability of mouse oocytes to undergo spontaneous Ca\textsuperscript{2+} oscillations in the absence of granulosa cells or hormonal stimulation may provide an explanation for the different requirements for successful maturation in vitro. In vivo, meiosis is resumed in response to a surge of gonadotrophins, luteinizing hormone and follicle-stimulating hormone. Luteinizing hormone and follicle-stimulating hormone cause Ca\textsuperscript{2+} changes in luteal cells and granulosa cells, respectively (61, 52). Our current findings raise the possibility that the preovulatory surge of gonadotrophins may trigger Ca\textsuperscript{2+} oscillations in follicle-enclosed oocytes, perhaps via the gap junction-mediated communication between the follicular cells and the oocyte (53, 54).

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REFERENCES
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