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Ca^{2+} release and the development of Ca^{2+} release mechanisms during oocyte maturation: a prelude to fertilization

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Oogenesis involves the production of an oocyte that can undergo fertilization and support early development. The stimulus that initiates embryogenesis is an increase in the concentration of intracellular Ca^{2+} in the cytoplasm of the oocyte at the time of fertilization. The development of the ability of the oocyte to release Ca^{2+} in response to the fertilizing spermatozoon is an essential step in the process of oogenesis. Mammalian oocytes are particularly useful for studying the development of Ca^{2+} signalling systems, owing to the series of Ca^{2+} oscillations generated at fertilization, compared with the monotonic Ca^{2+} increase seen in non-mammalian species. Recent evidence has revealed that Ca^{2+} release mechanisms are modified during oogenesis. The maximal sensitivity of Ca^{2+} release is reached in the final stages of oocyte maturation, just before the optimal time for fertilization. In this review, we consider the mechanism underlying Ca^{2+} release in mammalian oocytes and discuss how the release mechanisms are modified during oocyte maturation. The tight co-ordination of the differentiation of the Ca^{2+} signalling system with the development of the oocyte provides a means of ensuring successful activation at the time of fertilization. Finally, we consider the consequences for embryo development in circumstances in which the co-ordination is lost.

Fertilization marks the end of oogenesis and the beginning of embryogenesis. The legacy of oogenesis is, however, maintained by virtue of the stored nutritional and regulatory molecules that fuel, and in many cases direct, early development. The signal that stimulates the transition from oocyte to embryo is a sperm-induced increase in the concentration of intracellular free Ca^{2+}. Thus successful initiation of embryogenesis hinges on the ability of the oocyte to respond to the fertilizing spermatozoa with a suitable increase in intracellular Ca^{2+}. How the signal transduction systems differentiate during oogenesis to ensure a successful transition from oocyte to embryo is the subject of this review.

Normally fertilization occurs at the end of the final stage of oogenesis, that is, oocyte maturation (see Fig. 1). Oocyte maturation in mammals consists of nuclear (cell cycle) progression from the first meiotic prophase through to metaphase of the second meiotic division where the oocyte arrests until fertilization. In addition, there are cytoplasmic changes that are necessary to support early developmental events (Fig. 1). The process of oocyte maturation *in vivo* is stimulated by the preovulatory surge of gonadotrophins, while *in vitro* it occurs spontaneously when the fully grown oocyte is removed from the inhibitory follicular environment to a suitable culture medium.

Fertilization triggers two key events that signal the oocyte has been activated and has entered the programme of embryonic development. First, exocytosis of the cortical granules, which prevents the penetration of supernumerary spermatozoa and, second, the completion of meiosis and entry into the first embryonic mitosis. The explosive increase of intracellular Ca^{2+} at fertilization is responsible for triggering both of these events (Kline and Kline, 1992; Whitaker and Swann, 1993). Before considering how the Ca^{2+} release systems develop during oocyte maturation, we will discuss how intracellular Ca^{2+} is regulated in mature oocytes.

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Mechanisms of Ca^{2+} signalling in oocytes

Basic properties of Ca2+ stores and channels in mouse oocytes

In oocytes and most other somatic cells, cytosolic free Ca^{2+} is increased by the release of Ca^{2+} from intracellular Ca^{2+} stores in the endoplasmic reticulum (ER). Typically Ca^{2+} increases from about 100 nmol l^{-1} at rest to micromolar concentrations at the time of cell stimulation. The Ca^{2+} stores are gated by two families of Ca^{2+} channels, the inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) and the ryanodine receptor (RyR) (Lai *et al.*, 1988; Furuichi *et al.*, 1989). There are a number of different isoforms of both families of Ca^{2+} channels and these are expressed in a tissue-specific manner (Ross *et al.*, 1992; De Smedt *et al.*, 1994; Giannini *et al.*, 1995). Further heterogeneity is provided by a number of splice variants (Danoff *et al.*, 1991; Nakagawa *et al.*, 1991; Berridge, 1993 for review). These receptors are sensitive to cytosolic Ca^{2+} concentration such that low concentrations stimulate opening while high concentrations inhibit the channels (Bezprovzanny *et al.*, 1991; Iino and Endo, 1992). The stimulatory effect of low Ca^{2+} concentrations causes the phenomena of Ca^{2+}-induced Ca^{2+}-release (CICR). This positive feedback mechanism leads to a regenerative mechanism of Ca^{2+}

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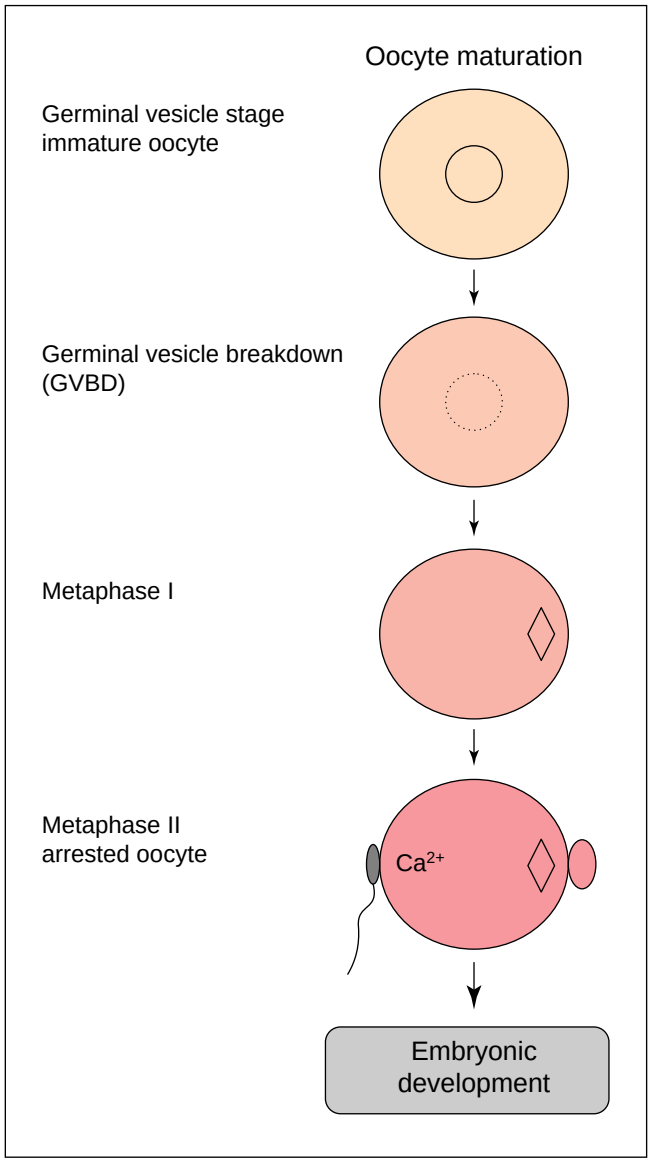

Fig. 1. Oocyte maturation consists of cell cycle progression and cytoplasmic changes necessary for early embryonic development. The first indication that oocyte maturation has been initiated is germinal vesicle breakdown (GVBD). The oocyte then progresses through metaphase I before arresting at metaphase of the second meiotic division. An increase in intracellular free Ca^{2+} stimulates the completion of meiosis and the initiation of early embryonic development. One of the cytoplasmic changes necessary for early development to proceed is a modification in the Ca^{2+} signalling pathway.

release which is responsible for amplifying small Ca^{2+} changes into global Ca^{2+} oscillations and waves. The sensitivity of the Ca^{2+} channels to Ca^{2+} release is regulated by association with other messenger molecules such as $InsP_3$ for the $InsP_3R$ (Berridge, 1993) and cyclic ADP-ribose for the RyR (Galione *et al.*, 1991).

Experiments using oocytes from a variety of species have contributed much to our understanding of the control of intracellular Ca^{2+}. There is physiological and molecular evidence that both families of Ca^{2+} release channels are present in mammalian oocytes. Physiological evidence for $InsP_3R$ is provided by the widespread finding that microinjection of $InsP_3$ stimulates Ca^{2+} oscillations (Whitaker and Swann, 1993 for review). It is not surprising therefore that $InsP_3R$ has been identified in hamster oocytes by western analysis and immunocytochemistry (Miyazaki *et al.*, 1992). Evidence for RyRs is not as clear. Agonists of RyRs, caffeine and cyclic ADP-ribose, release Ca^{2+} in bovine oocytes (Yue *et al.*, 1995) but have no effect on mouse or hamster oocytes (Whitaker and Swann, 1993). However, ryanodine itself can increase intracellular Ca^{2+} and block agonist-induced Ca^{2+} transients in mouse oocytes, suggesting the presence of a ryanodine-sensitive Ca^{2+} release mechanism (Swann, 1992; Jones *et al.*, 1995a). This contention is supported by the finding that mouse oocytes express mRNA for two (types 2 and 3) of the three known RyR isoforms. The protein was also detected by immunoprecipitation and western blots of mouse oocyte proteins (Ayabe *et al.*, 1995; J. Carroll, unpublished). In hamster oocytes similar experiments failed to detect RyR protein (Miyazaki *et al.*, 1992). It is not clear whether this was due to the complete absence of protein or low amounts of expression since, in the mouse, at least 1200 oocytes are required to obtain a signal on a western blot (J. Carroll, unpublished). Also, in smooth muscle, expression of RyR is tenfold less than that of $InsP_3Rs$ (Wibo and Godfraind, 1994). This difference in quantity may not be reflected in function, as the RyR is thought to have a conductance about tenfold higher than that of the $InsP_3R$. Thus, while both families of Ca^{2+} release channels appear to be present in mouse oocytes, it is not clear which of the family members are present, and more importantly, which contribute to the physiologically relevant Ca^{2+} signals.

An example of the different approaches organisms have evolved to regulate intracellular Ca^{2+} is seen in *Xenopus* oocytes and sea urchin eggs. *Xenopus* oocytes are thought to use one $InsP_3R$ exclusively, which is homologous to the mammalian type 1 $InsP_3R$, for regulation of intracellular Ca^{2+} (Parys *et al.*, 1992; Kume *et al.*, 1993; Parys and Bezprovzanny, 1995). To date, biochemical, physiological and molecular approaches have not revealed any evidence for RyRs in *Xenopus* oocytes (Parys *et al.*, 1992; Kume *et al.*, 1993). In contrast, sea-urchin eggs release Ca^{2+} in response to $InsP_3$, cADP-ribose and caffeine, suggesting a role for both $InsP_3Rs$ and RyRs in the maintenance of intracellular Ca^{2+} homeostasis (Galione *et al.*, 1991; Whitaker and Swann, 1993).

Which Ca^{2+} channels are activated at fertilization?

In mammalian oocytes evidence suggests that Ca^{2+} release at fertilization is mediated via Ca^{2+} stores gated by the $InsP_3R$. The most convincing evidence for this is the fact that a functionally inhibitory monoclonal antibody (18A10) raised against the channel forming region of the type 1 mouse $InsP_3R$ inhibits fertilization induced Ca^{2+} transients in mouse and hamster oocytes (Miyazaki *et al.*, 1992). Despite this compelling evidence, there are a number of observations suggesting an $InsP_3$-insensitive Ca^{2+} release mechanism also plays a role. Microinjection of heparin, a competitive antagonist of $InsP_3$ binding to the $InsP_3R$, delays Ca^{2+} transients only at fertilization of hamster oocytes (Miyazaki *et al.*, 1993) and has no effect on the ability of spermatozoa to activate cow oocytes

(Sun *et al.*, 1994). Inhibition of Ca^{2+} release by the 18A10 antibody in the absence of inhibition by heparin suggests different mechanisms of inhibition. The effectiveness of the antibody suggests that the $InsP_3R$ is important for Ca^{2+} release at fertilization, while the insensitivity to heparin suggests that $InsP_3$ binding is not critical. Thus, in a number of mammals it appears that $InsP_3$ sensitive and insensitive mechanisms of Ca^{2+} release play a role in the generation of Ca^{2+} transients at fertilization.

A role for RyR in the generation of Ca^{2+} oscillations at fertilization remains controversial. Ryanodine induces Ca^{2+} release after external application or microinjection (Swann, 1992; Jones *et al.*, 1995a). Also, ryanodine preincubation attenuates sperm-induced Ca^{2+} transients (Jones *et al.*, 1995a). However, some workers have not seen an increase in Ca^{2+} in response to ryanodine and have failed to detect any effect on fertilization as assayed by oocyte activation (Kline and Kline, 1994; Ayabe *et al.*, 1995). Some of the confusion between these studies may be accounted for by the different end-points used. The use of oocyte activation as the end-point shows that the Ca^{2+} signal generated by spermatozoa in ryanodine-treated oocytes is sufficient to cause oocyte activation (Ayabe *et al.*, 1995). This is not surprising since it is well established that monotonic Ca^{2+} increases can be sufficient to induce oocyte activation (Tombes *et al.*, 1992). In addition, oocyte activation may be induced, or at least enhanced, by nonspecific effects of agents that deplete intracellular Ca^{2+} stores and thereby inhibit protein synthesis (Bos-Mikich *et al.*, 1995). However, assaying Ca^{2+} release shows clearly that ryanodine attenuates the Ca^{2+} signal produced by the spermatozoa, implicating a role for RyR in the physiological response to the fertilizing spermatozoa (Jones *et al.*, 1995a).

In *Xenopus* oocytes it is widely accepted that the type 1 $InsP_3R$ is responsible for Ca^{2+} release at fertilization. In addition to the intuitive argument that no other intracellular Ca^{2+} channels have been found in *Xenopus* oocytes, there is good experimental evidence. Functionally inhibitory antibodies and anti-sense oligonucleotides specific for *Xenopus* $InsP_3R$ inhibit oocyte activation at fertilization (Kume *et al.*, 1993). In sea-urchin eggs, which have both $InsP_3R$ and RyR, inhibition of either channel does not prevent the Ca^{2+} wave at fertilization (Galione *et al.*, 1993; Lee *et al.*, 1993), although its kinetics may be significantly slowed (Mohri *et al.*, 1995). Complete inhibition of Ca^{2+} is apparent when both channels are inhibited (Galione *et al.*, 1993; Lee *et al.*, 1993). Thus oocytes and eggs from a variety of species use different intracellular Ca^{2+} stores for the generation of Ca^{2+} signals at fertilization. There appears to be variation in the type of Ca^{2+} channel used in different species but the end-point, an increase in intracellular Ca^{2+}, is similar. How do the Ca^{2+} release mechanisms in oocytes develop so that the ability to generate Ca^{2+} transients is co-ordinated with the time that the transients are needed?

Development of Ca^{2+} release mechanisms during oogenesis

A clear demonstration that the Ca^{2+} signalling system is developmentally regulated is that the ability of spermatozoa to cause Ca^{2+} transients depends on the stage of maturation of the oocyte (Fig. 2). This was first seen in starfish oocytes in which the

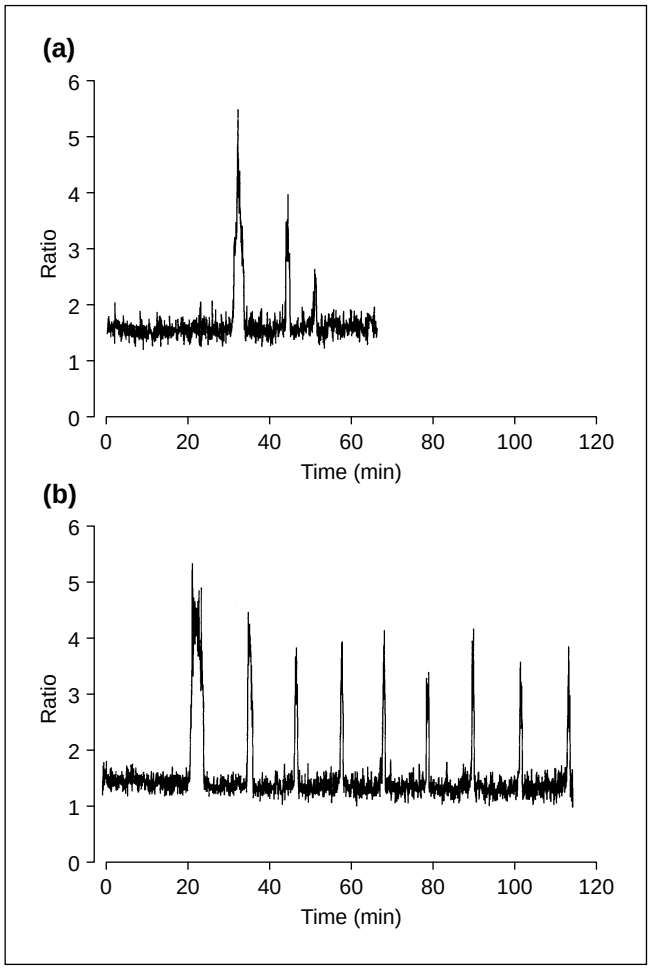

Fig. 2. Fertilization-induced Ca^{2+} release is modified during oocyte maturation. In response to spermatozoa, immature oocytes generate two–three Ca^{2+} transients (a) compared with 30–40 in the mature oocyte (b). The Ca^{2+} transients generated in immature oocytes are smaller, of shorter duration and have a slower rate of rise than those in the mature oocytes. To obtain the fluorescence records oocytes were loaded with the Ca^{2+} -sensitive fluorescent dye, indo-1. The concentration of Ca^{2+} is expressed as a ratio of emission at 405 and 490 nm. The Ca^{2+} concentration increases from about 150 nmol l^{-1} at rest to 1000 nmol l^{-1} at the peak of the Ca^{2+} transient.

sperm-induced Ca^{2+} transient increased during maturation (Chiba *et al.*, 1990). In mouse oocytes, maturation-associated effects on Ca^{2+} signals were first seen in response to a semi-purified sperm extract that caused Ca^{2+} oscillations similar to those of spermatozoa (Carroll *et al.*, 1994). In mature oocytes, Ca^{2+} transients were larger, had a faster rate of rise and continued for longer than in immature oocytes. In addition, the spatial organization of the Ca^{2+} signals were different, such that mature oocytes often showed rapid Ca^{2+} waves, while a wave-front was never visible in immature oocytes. Similar effects on the kinetics and spatial organization of Ca^{2+} transients at the time of fertilization have been reported in hamster oocytes (Fujiwara *et al.*, 1993). In immature mouse oocytes there is also a similar lower Ca^{2+} response to spermatozoa (Mehlmann and Kline, 1994; Jones *et al.*, 1995a; Fig. 2), although there are

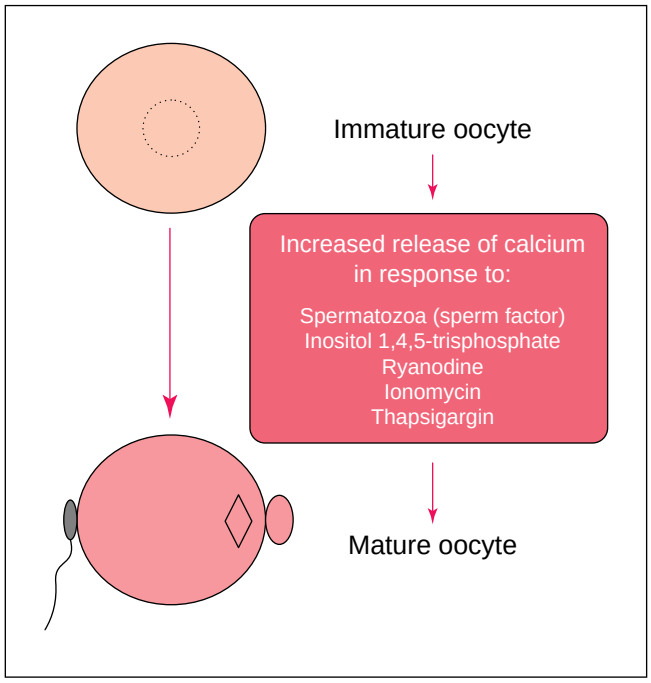

Fig. 3. During oocyte maturation Ca^{2+} release in response to a number of factors is modified. The ability of spermatozoa and sperm factors to stimulate Ca^{2+} oscillations and Ca^{2+} waves is modified during oocyte maturation as described in the text and depicted in Fig. 2. The ability of other Ca^{2+}-releasing agents to stimulate Ca^{2+} release is also modified during maturation. These include agents that act directly on the Ca^{2+} channels, $InsP_3$ and ryanodine, as well as probes for the Ca^{2+} store, such as thapsigargin, which inhibits Ca^{2+} pumps, and the Ca^{2+} ionophore, ionomycin.

some differences between studies in how the Ca^{2+} signal appears to be modified. The first sperm-induced Ca^{2+} transient is significantly smaller with a slower rate of rise in immature compared with mature oocytes (Mehlmann and Kline, 1994; Jones *et al.*, 1995a). In addition to these changes, Jones *et al.* (1995a) found that the immature oocyte generated only up to four Ca^{2+} transients compared with the 20–30 seen in mature oocytes (Fig. 2). Similar to the first transient, the subsequent transients in immature oocytes also had a lower peak Ca^{2+} and a slower rate of rise. Furthermore, it was not until late in the maturation process, close to metaphase II (MII), that the Ca^{2+} profile typical of mature oocytes was first seen (Jones *et al.*, 1995a). The explanation for such differences between studies is not immediately obvious; nevertheless, the overall consensus taken from a wide variety of species is that there are marked changes in the spatial and kinetic properties of Ca^{2+} release during oocyte maturation.

How is the Ca^{2+} release mechanism modified during oocyte maturation so that the fertilizing spermatozoon initiates a Ca^{2+} signal sufficient to stimulate oocyte activation (Fig. 3)? Changes may be brought about by modifications in the sensitivity of Ca^{2+} release through the $InsP_3R$. The sensitivity of $InsP_3$ mediated Ca^{2+} release increases during oocyte maturation in starfish, hamster and mouse oocytes (Chiba *et al.*, 1990; Fujiwara *et al.*, 1993; Mehlmann and Kline, 1994; Fig. 3). In hamster oocytes the time course of sensitization has been studied closely. First, there is a gradual increase during the first 5 h of maturation. The second and more marked increase is revealed at a lower dose of $InsP_3$ and takes place 10–12 h after the initiation of maturation, between prometaphase II and metaphase II (Fujiwara *et al.*, 1993). This correlates with the time during maturation when spermatozoa generate repetitive Ca^{2+} transients (Jones *et al.*, 1995a), suggesting a similar mechanism may underlie the increased response to both agonists.

The underlying mechanism for the increased sensitivity of Ca^{2+} release through the $InsP_3R$ may be revealed in experiments examining the spatial organization of Ca^{2+} release in immature and mature oocytes. In mature oocytes a regenerative Ca^{2+} wave is triggered by a very low dose of $InsP_3$. In contrast, similar doses injected into immature oocytes cause only a local increase at the site of injection (Fujiwara *et al.*, 1993; Fig. 4). It is arguable whether a regenerative Ca^{2+} wave is ever generated in immature oocytes. When a Ca^{2+} wave is generated in response to high concentrations of $InsP_3$ it is, as Fujiwara *et al.* (1993) point out, likely to result from the diffusion of $InsP_3$ rather than from a regenerative mechanism. Thus the component of the calcium release mechanism that is modified during maturation is apparently the regenerative mechanism itself, that is, CICR (Fig. 4) .

It is not yet known how CICR is modified during oocyte maturation. Changes in the inherent properties of Ca^{2+} channels is a likely possibility. The majority of these very large proteins ($InsP_3R$, 330 kDa; RyR, 550 kDa) consists of an N-terminal region that projects into the cytoplasm, thereby providing ample possibilities for regulation. Ca^{2+} channels are known to be regulated by phosphorylation (Nakade *et al.*, 1994) and changes in redox state (Salama *et al.*, 1992) as well as by interacting with cytoplasmic proteins such as ankyrin (Bourgguignon *et al.*, 1993) and FK506-binding protein (Brillantes, 1994; Cameron *et al.*, 1995). Ca^{2+} channels are also thought to be regulated by the amount of Ca^{2+} in the lumen of the ER. For example, in smooth muscle an increase in store filling enhances the effect of Ca^{2+} on CICR through the $InsP_3R$ (Iino and Endo, 1992). In oocytes there is evidence that the Ca^{2+} store increases during maturation. The amount of Ca^{2+} released in response to ionomycin and thapsigargin increases several fold during oocyte maturation (Tombes *et al.*, 1992; Mehlmann and Kline, 1994; Jones *et al.*, 1995a; Fig. 3). However, this does not appear to be mediated entirely through the $InsP_3$-sensitive Ca^{2+} store, since maximal doses of $InsP_3$ produce Ca^{2+} transients of similar size in mouse and hamster oocytes (Fujiwara *et al.*, 1993; Mehlmann and Kline, 1994). During maturation of mouse oocytes, the ability to release Ca^{2+} in response to ryanodine increases (Jones *et al.*, 1995a). Irrespective of which Ca^{2+} stores are involved, an increase in the size of these stores may contribute to the increase in CICR apparent during maturation (Fig. 4). An additional mechanism by which an increase in luminal Ca^{2+} may influence the properties of Ca^{2+} release is by increasing the unitary amount of Ca^{2+} released through individual Ca^{2+} channels, as was recently observed in cardiac cells (Cheng *et al.*, 1996). This would have the effect of increasing the probability of activating neighbouring Ca^{2+} channels and thereby initiating a regenerative Ca^{2+} wave (Fig. 4).

In both mouse and hamster oocytes, the ER undergoes marked changes in organization during maturation. This involves dispersal of ER throughout the oocyte cortex (Ducibella *et al.*, 1988; Mehlmann *et al.*, 1995; Shiraishi *et al.*, 1995). This

reorganization is associated with a redistribution of $InsP_3R$ in hamster (Shiraishi *et al.*, 1995) and *Xenopus* (Kume *et al.*, 1993) oocytes. It is not immediately obvious how this reorganization may lead to an increase in the sensitivity of Ca^{2+} release, but the modified distribution may alter the properties of the Ca^{2+} channels or the Ca^{2+} store itself.

One further possibility is that the sensitivity of Ca^{2+} release may be a result of an increase in the expression of one or both families of Ca^{2+} channels during maturation. Recent work suggests that there is an increase in $InsP_3R$ during maturation (D. Kline, personal communication; J. Carroll, unpublished). In sea urchin oocytes a protein that reacts with a RyR antibody becomes localized in the oocyte cortex during maturation (McPherson *et al.*, 1992). An increase in the number of Ca^{2+} channels would decrease the distance required for Ca^{2+} to diffuse in order to activate neighbouring Ca^{2+} channels, thereby increasing the ability to generate Ca^{2+} waves (Fig. 4). Such behaviour is predicted by a recent model in which a direct relationship between wave velocity and the density of Ca^{2+} channels is demonstrated (Parys and Bezprovzanny, 1995).

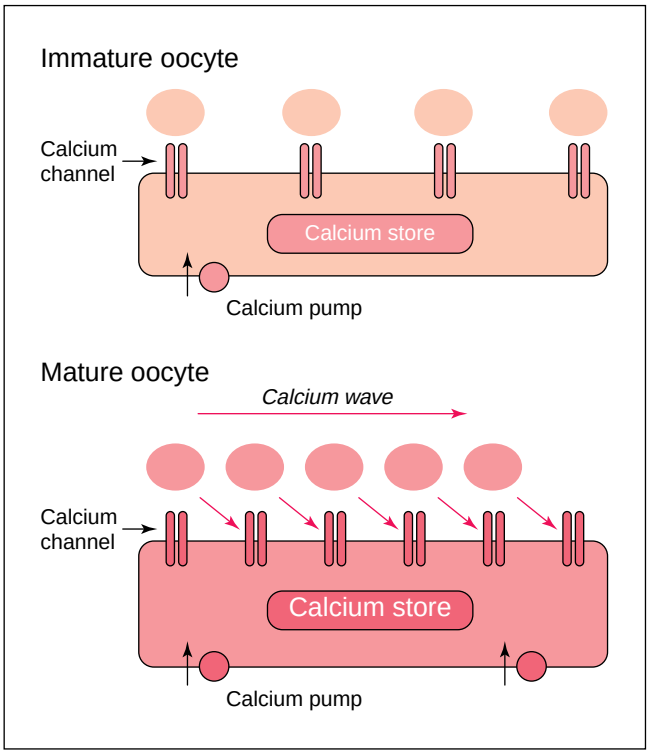

Fig. 4. A summary of changes that may be responsible for modifications in Ca^{2+} release mechanisms during oocyte maturation. One of the most marked differences between immature and mature oocytes is the greater propensity of mature oocytes for the generation of Ca^{2+} waves. This is the case in response to microinjection of sperm factor and inositol 1,4,5-trisphosphate ($InsP_3$). On the basis of changes known to occur in response to other agonists and properties of Ca^{2+} release in other cell types, there are a number of possibilities that may lead to an increase in the properties of regenerative Ca^{2+} release. This generic diagram shows the essential components of Ca^{2+} release in cells; the Ca^{2+} store itself, the Ca^{2+} channels, and Ca^{2+} pumps, and how changes in them during maturation may modify the properties of Ca^{2+} release in the mature oocyte. The main changes proposed are: a modification in the inherent conducting properties of the Ca^{2+} channels ($InsP_3$ receptors and ryanodine receptors) which may be mediated via specific regulatory mechanisms. One such mechanism may be an increase in the size of the Ca^{2+} store which in itself may lead to an increase in the amount of Ca^{2+} released from the channels. An increase in the density of Ca^{2+} channels is also predicted to lead to an increase in the rate of Ca^{2+} release and a decrease in the distance required for Ca^{2+} to diffuse and activate neighbouring channels. Thus the inherent properties of the Ca^{2+} channels, the size of the Ca^{2+} store and the density of the Ca^{2+} channels are all expected to increase the sensitivity of regenerative Ca^{2+} release.

Ca^{2+}, the cell cycle and the cytoplasm

There are some indications that the regulation of Ca^{2+} release mechanisms may be related to cell cycle changes in the oocyte. As the oocyte grows it becomes competent to undergo germinal vesicle breakdown and it is at this stage that the oocyte first generates spontaneous Ca^{2+} transients (Carroll and Swann, 1992; Carroll *et al.*, 1994). In addition, the two phases of increased $InsP_3$ sensitivity during maturation correlate with development from prometaphase to metaphase in each of the two meiotic divisions (Fujiwara *et al.*, 1993). This finding suggests that changes in the cytoplasm are linked to the nuclear cell cycle. However, it is possible to dissociate the cytoplasmic changes that occur between MI and MII from this nuclear transition. Oocytes arrested at MI that fail to progress to MII still acquire the ability to generate Ca^{2+} transients and undergo egg activation in response to spermatozoa (Eppig *et al.*, 1994; Jones *et al.*, 1995b). This finding suggests that the ability to generate Ca^{2+} transients is independent of progression from MI to MII. The oocyte does apparently have to be in metaphase to acquire the ability to respond to spermatozoa, since inducing interphase and nuclear formation after MI prevents the oocyte generating a normal Ca^{2+} signal at fertilization. It is not until the oocyte re-enters metaphase that the ability to generate Ca^{2+} oscillations is acquired (Jones *et al.*, 1995b). These studies suggest that the cell cycle plays an important role in the acquisition of maturation-associated changes in Ca^{2+} release and that the oocyte is required to be in metaphase to undergo this change.

What is it about metaphase that is necessary for the development of the Ca^{2+} release mechanisms? In different cell types, including early mouse and sea urchin embryos and fibroblasts, mitotic Ca^{2+} transients are apparent (Kao *et al.*, 1990; Whitaker and Patel, 1990; Tombes *et al.*, 1992), suggesting an increase in the sensitivity of Ca^{2+} release during this period. Strontium also causes Ca^{2+} release specifically during mitosis and not in interphase of the first embryonic cell cycle (Kono *et al.*, 1996). The transport of Ca^{2+} across the plasma membrane is also modified (Preston *et al.*, 1991) such that capacitative Ca^{2+} influx does not appear to operate during mitosis. This finding raises the possibility that Ca^{2+} cycling between the intracellular stores and the cytoplasm may be increased during mitosis. These changes may be brought about by changes in the organization of the ER, as described above. It is also possible that Ca^{2+} channels are regulated by mitotic cell cycle control proteins or by factors released from the nucleus that control Ca^{2+} release during mitosis (Kono *et al.*, 1995). Clearly the identification of the mechanism for the cell cycle-associated changes in the properties of Ca^{2+} release may help in elucidating the maturation-associated changes in Ca^{2+} release.

Functional role of maturation-associated changes in Ca^{2+} release

The differentiation of the Ca^{2+} signalling pathway during oocyte maturation may be required for the stimulation of Ca^{2+}-mediated events at fertilization, including cortical granule exocytosis and resumption of the cell cycle. Like the maturation of the Ca^{2+} release system, the ability of oocytes to undergo cortical granule exocytosis also develops during maturation. In fact, the ability to undergo global cortical granule exocytosis in response to spermatozoa occurs between MI and MII (Ducibella and Buetow, 1994), similar to the time that the oocyte generates repetitive Ca^{2+} transients in response to spermatozoa (Jones *et al.*, 1995a) and shows regenerative Ca^{2+} release in response to $InsP_3$ (Fujiwara *et al.*, 1993). Before this, the fertilizing spermatozoon produces a local loss of cortical granules over the site of insemination which may be caused by localized Ca^{2+} release or by cortical reorganization in response to the sperm chromatin (Ducibella and Buetow, 1994). The ability to release cortical granules in response to agents that release Ca^{2+} also increases during the maturation of *Xenopus* oocytes (Bement, 1992). In immature oocytes of both *Xenopus* and mice, activation of protein kinase C induces exocytosis, whereas agents that increase Ca^{2+} fail to do so (Bement, 1992; Ducibella *et al.*, 1993). This indicates that the exocytotic machinery can be activated in immature oocytes and that the spermatozoon is not providing the normal signal. It is possible that this signal is Ca^{2+}.

The finding that the ability of the oocyte to respond to spermatozoa with Ca^{2+} transients develops during oocyte maturation may help to explain some cases of human infertility. Recently it was shown that about 20% of apparently unfertilized oocytes are penetrated by spermatozoa but have arrested at an early stage of the fertilization process (Van Blerkom *et al.*, 1994). Arrest at this stage of development may be due in part to paternal factors such as oscillin, a sperm-derived protein proposed to cause Ca^{2+} release at fertilization (Parrington *et al.*, 1996), or abnormalities in the paternal centrosome (Simerly *et al.*, 1995), but it may also be caused by abnormalities in oocyte maturation (Schmiadi and Kentenich, 1989; Angell *et al.*, 1991; Calafell *et al.*, 1991). In these cases the fertilizing spermatozoon would be unable to trigger a normal series of Ca^{2+} oscillations, with the result that oocyte activation is inhibited in its early stages and the transition from oocyte to embryo does not take place successfully.

References

Key references are identified by asterisks.

Angell RR, Ledger W, Yong EL, Harkness L and Baird DT (1991) Cytogenetic analysis of unfertilized human oocytes *Human Reproduction* **6** 568–573

Ayabe T, Kopf GS and Schultz RM (1995) Regulation of mouse egg activation: presence of ryanodine receptors and effects of microinjected ryanodine and cyclic ADP ribose on uninseminated and inseminated eggs *Development* **121** 2233–2244

Bement WM (1992) Signal transduction by calcium and protein kinase C during egg activation *Journal of Experimental Zoology* **263** 382–397

***Berridge MJ** (1993) Inositol trisphosphate and calcium signalling *Nature* **365** 388–389

Bezprovzanny I, Watras J and Ehrlich BE (1991) Bell shaped calcium-response curves of $Ins(1,4,5)P_3$-and calcium gated channels from endoplasmic reticulum of cerebellum *Nature* **351** 751–754

Bos-Mikich A, Swann K and Whittingham DG (1995) Calcium oscillations and protein synthesis inhibition synergistically activate mouse oocytes *Molecular Reproduction and Development* **41** 84–90

Bourgguignon LYW, Jin H, Iida N, Brandt NR and Zhang SH (1993) The involvement of ankyrin in the regulation of inositol 1,4,5-trisphosphate receptor-mediated internal Ca^{2+} release from C storage vesicles in mouse T-lymphoma cells *Journal of Biological Chemistry* **268** 7290–7297

Brillantes AMB, Ondrias K, Scott A, Scott A, Kobrinsky E, Ondriasova E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE and Marks AR (1994) Stabilization of calcium-release channel (ryanodine receptor) function by FK506 binding protein *Cell* **77** 513–523

Calafell JM, Badenas J, Egozcue J and Santalo J (1991) Premature chromosome condensation as a sign of oocyte immaturity *Human Reproduction* **6** 1017–1021

Cameron AM, Steiner JP, Sabatini DM, Kaplin AI, Walensky LD and Snyder SH (1995) Immunophilin FK506 binding protein associated with inositol 1,4,5-trisphosphate receptor mediates calcium influx *Proceedings of the National Academy of Sciences USA* **92** 1784–1788

Carroll J and Swann K (1992) Spontaneous cytosolic Ca^{2+} oscillations driven by inositol trisphosphate occur during *in vitro* maturation of mouse oocytes *Journal of Biological Chemistry* **267** 11196–11201

***Carroll J, Swann K, Whittingham DG and Whitaker MJ** (1994) Spatiotemporal dynamics of intracellular Ca^{2+} oscillations during the growth and meiotic maturation of mouse oocytes *Development* **120** 3507–3517

Cheng H, Lederer MR, Lederer WJ and Cannell MB (1996) Calcium sparks and calcium waves in cardiac myocytes *American Journal of Physiology* **270** C148–C159

***Chiba K, Kado RT and Jaffe LA** (1990) Development of calcium release mechanisms during starfish oocyte maturation *Developmental Biology* **140** 300–306

Danoff SK, Ferris CD, Donath C, Fischer GA, Munemitsu S, Ullrich A, Snyder S and Ross CA (1991) Inositol 1,4,5-trisphosphate receptors: distinct neuronal and nonneuronal forms derived by alternative splicing differ in phosphorylation *Proceedings of the National Academy of Sciences USA* **88** 2951–2955

De Smedt H, Missiaen L, Parys JB, Bootman MD, Mertens L, Van Den Bosch L and Casteels R (1994) Determination of relative amounts of inositol trisphosphate receptor mRNA isoforms by ratio polymerase chain reaction *Journal of Biological Chemistry* **269** 21691–21698

Ducibella T and Buetow J (1994) Competence to undergo normal, fertilization-induced cortical activation develops after metaphase I of meiosis in mouse oocytes *Developmental Biology* **165** 95–104

Ducibella T, Rangarjan S and Anderson E (1988) The development of mouse oocyte cortical reaction competence is accompanied by a major change in cortical vesicles and not cortical granule depth *Developmental Biology* **130** 789–792

Ducibella T, Kurasawa S, Duffy P, Kopf GS and Schultz RM (1993) Regulation of polyspermy block in the mouse egg: maturation-dependent differences in cortical granule exocytosis and zona pellucida modifications induced by inositol 1,4,5-trisphosphate and an activator of protein kinase C *Biology of Reproduction* **48** 1251–1257

Eppig JJ, Schultz RM, O'Brien M and Chesnel F (1994) Relationship between the developmental programmes controlling nuclear and cytoplasmic maturation of mouse oocytes *Developmental Biology* **164** 1–9

***Fujiwara T, Nakade K, Shirakawa H and Miyazaki S** (1993) Development of inositol trisphosphate-induced calcium release mechanisms during maturation of hamster oocytes *Developmental Biology* **156** 69–79

Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N and Mikoshiba K (1989) Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400} *Nature* **342** 32–38

Galione A, Lee HC and Busa WB (1991) Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: modulation by cyclic ADP-ribose *Science* **253** 1143–1146

Galione A, McDougall A, Busa WB, Willmott N, Gillot I and Whitaker M (1993) Redundant mechanisms of calcium-induced calcium release underlying calcium waves during fertilization of sea urchin eggs *Science* **261** 348–352

***Giannini G, Conti A, Mammarella S, Scrobogna M and Sorrentino V** (1995) The ryanodine receptor/calcium release channel genes are widely and differentially expressed in murine brain and peripheral tissues *Journal of Cell Biology* **128** 893–904

Iino M and Endo M (1992) Calcium-dependent immediate feedback control of inositol 1,4,5-trisphosphate-induced Ca^{2+} release *Nature* **360** 76–78

***Jones KT, Carroll J, and Whittingham DG** (1995a) Ionomycin, thapsigargin, ryanodine and sperm sensitive calcium release increase during meiotic maturation of mouse oocytes *Journal of Biological Chemistry* **270** 6671–6677

Jones KT, Carroll J, Merriman JA, Whittingham DG, and Kono T (1995b) Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell-cycle dependent *Development* **121** 3259–3266

Kao JPY, Alderton JM, Tsien RY and Steinhardt RA (1990) Active involvement of Ca^{2+} in mitotic progression of Swiss 3T3 fibroblasts *Journal of Cell Biology* **111** 183–196

Kline D and Kline JT (1992) Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg *Developmental Biology* **149** 80–89

Kline D and Kline JT (1994) Regulation of intracellular Ca^{2+} release in the mouse egg: evidence for inositol trisphosphate-induced calcium release but not calcium-induced calcium release *Biology of Reproduction* **50** 193–203

Kono T, Carroll J, Swann K and Whittingham DG (1995) Nuclei from fertilized one-cell mouse embryos have calcium releasing activity *Development* **121** 1123–1128

Kono T, Jones KT, Bos-Mikich A, Whittingham DG and Carroll J (1996) A cell cycle-associated change in Ca^{2+} releasing activity leads to the generation of Ca^{2+} transients in mouse embryos during the first mitotic division *Journal of Cell Biology* **132** 915–923

Kume S, Muto A, Aruga J, Nakagawa T, Michikawa T, Furichi T, Nakade, S, Okano H, and Mikoshiba K (1993) The *Xenopus* $InsP_3$ receptor: structure, function, and localization in oocytes and eggs *Cell* **73** 555–570

Lai FA, Erickson HP, Rousseau E, Lui QY and Meissner G (1988) Purification and reconstitution of the calcium release channel from skeletal muscle *Nature* **331** 315–319

Lee HC, Aarhus R and Walseth TF (1993) Calcium mobilization by dual receptors during fertilization of sea urchin eggs *Science* **261** 352–355

McPherson SM, McPherson PS, Mathews L, Campbell KP and Longo FJ (1992) Cortical localization of a calcium release channel in sea urchin eggs *Journal of Cell Biology* **116** 1111–1121

***Mehlmann LM and Kline D** (1994) Regulation of intracellular calcium in the mouse egg: calcium release in response to sperm or inositol trisphosphate is enhanced after meiotic maturation *Biology of Reproduction* **51** 1088–1098

Mehlmann LM, Terasaki M, Jaffe L and Kline D (1995) Reorganization of the endoplasmic reticulum during meiotic maturation of the mouse oocyte *Developmental Biology* **170** 607–615

***Miyazaki S, Yazuki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S and Mikoshiba K** (1992) Block of Ca^{2+} wave and Ca^{2+} oscillations by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs *Science* **257** 251–255

Miyazaki S, Nakade K and Shirakawa H (1993) Signal transduction of gamete interaction and intracellular calcium release mechanism at fertilization of mammalian eggs. In *Biology of the Germ Line* pp 125–143 Ed. H Mohri, M Takahashi and C Tachi. Japan Scientific Societies Press, Tokyo

Mohri T, Ivonnet PI and Chambers EL (1995) Effect of sperm-induced activation current and increase of cytosolic Ca^{2+} by agents that modify the mobilization of $[Ca^{2+}]_i$ *Developmental Biology* **172** 130–157

Nakade S, Rhee SK, Hamanaka H and Mikoshiba K (1994) Cyclic AMP-dependent phosphorylation of an immunoaffinity purified homotetrameric inositol 1,4,5-trisphosphate receptor (type 1) increases Ca^{2+} flux in reconstituted lipid vesicles *Journal of Biological Chemistry* **269** 6735–6742

Nakagawa T, Okano H, Furuichi T, Aruga J and Mikoshiba K (1991) The subtypes of the mouse inositol 1,4,5-trisphosphate receptor are expressed in a tissue specific and developmentally specific manner *Proceedings of the National Academy of Sciences USA* **88** 6244–6248

Parrington J, Swann K, Shevchenko IV, Sesay A and Lai FA (1996) Calcium oscillations in mammalian eggs triggered by a soluble sperm protein *Nature* **379** 364–368

***Parys JB and Bezprovzanny I** (1995) The inositol trisphosphate receptor of *Xenopus* oocytes *Cell Calcium* **18** 353–363

Parys JB, Sernett SW, DeLisle S Snyder PM, Welsh MJ and Campbell KP (1992) Isolation, characterization and localization of the inositol 1,4,5-trisphosphate receptor protein in *Xenopus laevis* oocytes *Journal of Biological Chemistry* **267** 18776–18782

Preston SF, Sha'afi R and Berlin RD (1991) Regulation of calcium influx and depletion during mitosis: Ca^{2+} influx and depletion of intracellular Ca^{2+} stores are coupled in interphase but not mitosis *Cell Regulation* **2** 915–925

Ross CA, Danoff SK, Schell MJ, Snyder SH and Ulrich A (1992) Three additional inositol 1,4,5-trisphosphate receptors: molecular cloning and differential localization in brain and peripheral tissues *Proceedings of the National Academy of Sciences USA* **89** 4265–4269

Salama G, Nigam M, Shome K, Finkel MS, Lagenaur C and Zaidi NF (1992) Ryanodine-affinity chromatography purifies 106 kDa Ca^{2+} release channels from skeletal and cardiac sarcoplasmic reticulum *Cell Calcium* **13** 635–647

Schmiadi H and Kentenich H (1989) Premature chromosome condensation after *in vitro* fertilization *Human Reproduction* **4** 689–695

***Shiraishi K, Okada A, Shirakawa H, Nakanishi S, Mikoshiba K and Miyazaki S** (1995) Developmental changes in the distribution of the endoplasmic reticulum and inositol 1,4,5-trisphosphate receptors and the spatial pattern of Ca^{2+} release during maturation of hamster oocytes *Developmental Biology* **170** 594–606

Simerly C, Wu G-J, Zoran S, Ord T, Rawlins R, Jones J, Navara C, Gerrity M, Rinehart J, Binor Z, Asch R and Schatten G (1995) The paternal inheritance of the centrosome, the cell's microtubule-organizing centre, in humans, and the implications for infertility *Nature Medicine* **1** 47–52

Sun FZ, Bradshaw JP, Galli C and Moor RM (1994) Changes in calcium concentration in bovine oocytes following penetration by spermatozoa *Journal of Reproduction and Fertility* **101** 713–719

Swann K (1992) Different triggers for calcium oscillations in mouse eggs involve a ryanodine sensitive calcium store *Biochemical Journal* **287** 79–84

Tombes RM, Simerly C, Borisy B and Schatten G (1992) Meiosis, egg activation, and nuclear envelope breakdown are differentially reliant on Ca^{2+}, whereas germinal vesicle breakdown is Ca^{2+} independent in the mouse oocyte *Journal of Cell Biology* **117** 799–811

Van Blerkom J, Davis PW and Merriam J (1994) A retrospective analysis of unfertilized and presumed parthenogenetically activated human oocytes demonstrates a high frequency of sperm penetration *Human Reproduction* **9** 2381–2388

Whitaker M and Patel R (1990) Calcium and cell cycle control *Development* **108** 525–542

***Whitaker M and Swann K** (1993) Lighting the fuse at fertilization *Development* **117** 1–12

Wibo M and Godfraind T (1994) Comparative localization of inositol 1,4,5-trisphosphate and ryanodine receptors in intestinal smooth muscle: an analytical subfractionation study *Biochemical Journal* **297** 415–423

Yue C, White KL, Reed WA and Bunch TD (1995) The existence of inositol 1,4,5-trisphosphate receptors and ryanodine receptors in mature bovine oocytes *Development* **121** 2645–2654