Calcium influx in mammalian eggs

Chunmin Wang and Zoltan Machaty

Department of Animal Sciences, Purdue University, 915 West State Street, West Lafayette, Indiana 47907, USA

Correspondence should be addressed to Z Machaty; Email: zmachaty@purdue.edu

Abstract

Calcium (Ca^{2+}) signals are involved in the regulation of oocyte maturation and play a critical role during fertilization. In the egg, Ca^{2+} is stored in the lumen of the endoplasmic reticulum and a signal is generated when the stored Ca^{2+} is released through specialized channels in the membrane of the endoplasmic reticulum to elevate the free Ca^{2+} concentration in the cytoplasm. Extracellular Ca^{2+} is also important, indicated by the fact that the mobilization of luminal Ca^{2+} is typically followed by Ca^{2+} entry across the plasma membrane. The transmembrane Ca^{2+} flux replenishes the endoplasmic reticulum, and thus, it is essential to sustain prolonged Ca^{2+} signals. It also seems to be responsible for the stimulation of important signaling cascades required for complete egg activation. Characterization of the pathway that mediates Ca^{2+} entry implies that its major components include STIM1, a protein that senses the filling status of the stores, and ORAI1, a channel protein located in the plasma membrane. Defining the mechanism and functions of Ca^{2+} entry will not only lead to a better understanding of egg physiology but may also help improving the efficiency of a number of assisted reproductive technologies.

Reproduction (2013) **145** R97–R105

Introduction

The calcium ion (Ca^{2+}) is the most universal signaling messenger that regulates a wide array of cellular processes (Berridge *et al.* 1998). It carries messages by reversibly attaching to Ca^{2+} -binding sites of proteins; Ca^{2+} binding changes the shape and charge of proteins and confers new functions to them. A series of proteins and signaling molecules then make up a signaling cascade that transduces signals between or within cells. Because prolonged exposure to high Ca^{2+} concentration is detrimental, cells maintain cytosolic Ca^{2+} levels low, ~20 000-fold lower than that in the extracellular space. For this purpose, Ca^{2+} can be chelated by specialized proteins, stored in intracellular compartments or pumped out the cell. For signaling, the Ca^{2+} concentration in the cytosol is elevated briefly and this can be achieved by releasing Ca^{2+} from the intracellular stores or letting external Ca^{2+} in through ion channels located in the plasma membrane. Cells employ a set of signaling, homeostatic, and sensory mechanisms to shape Ca^{2+} signals in space and time (Bootman *et al.* 2001). In the female gamete, Ca^{2+} signals drive important biological processes and Ca^{2+} released from the endoplasmic reticulum contribute primarily to the generation of the signals. Although extracellular Ca^{2+} is also essential for proper signaling, relatively little is known about the mechanism that mediates the entry of Ca^{2+} across the plasma membrane. The aim of this review is to summarize the

information that is available about Ca^{2+} entry in mammalian eggs with special emphasis on the most recent findings.

The source of Ca^{2+} during signaling

In some cell types, Ca^{2+} signaling depends primarily on the entry of extracellular Ca^{2+} (Clapham 2007). In excitable cells, certain external stimuli are able to depolarize the plasma membrane. Membrane depolarization then activates voltage-gated Ca^{2+} channels, thus creating an influx of Ca^{2+} across the plasma membrane. The Ca^{2+} that enters the cell will initiate various cellular events either directly by increasing intracellular Ca^{2+} levels or indirectly by inducing Ca^{2+} release from the sarcoplasmic reticulum.

Cells that cannot use membrane depolarization to trigger a Ca^{2+} influx rely on the release of Ca^{2+} from intracellular stores to elevate their intracellular free Ca^{2+} concentrations (Van Den Brink *et al.* 1999). Stimulation of cell surface receptors in such cells activates the phosphoinositide signaling system leading to Ca^{2+} release from the endoplasmic reticulum and the generation of a Ca^{2+} signal. These cells, however, can also use the external source of Ca^{2+} during signaling. The release of Ca^{2+} from the internal store is generally followed by a Ca^{2+} influx across the plasma membrane. The Ca^{2+} that enters the cells is believed to be necessary to replace Ca^{2+} pumped out of the cell during the period

of high Ca^{2+} levels (Rink & Hallam 1989); it refills the intracellular stores, provides Ca^{2+} for long-lasting plateau signals, and also sustains repetitive Ca^{2+} oscillations (reviewed by Prakriya (2009)).

The origin of Ca^{2+} during signaling may vary depending on a number of factors. During fertilization for example, eggs of many species including most marine invertebrates, fish, and frogs show an action potential and Ca^{2+} influx as a result of this depolarization is part of the signaling mechanism responsible for their activation. Some of these (e.g. the echinuran worm *Urechis*) rely almost entirely on the Ca^{2+} entry for successful fertilization; in others such as sea urchins, the initial influx is followed by a release of Ca^{2+} from internal stores and the mobilized intraluminal Ca^{2+} provides the primary trigger for activation (Stricker 1999). The situation in mammalian eggs is slightly different. Hamster and mouse eggs do not show depolarization at fertilization; also, their resting membrane potential of -30 to -40 mV inactivates voltage-gated Ca^{2+} channels (Miyazaki & Igusa 1981a, Igusa *et al.* 1983). Due to these reasons, in such eggs, the fertilization Ca^{2+} signal is primarily provided by Ca^{2+} release from the intracellular stores and not by Ca^{2+} entry across the plasma membrane. Nevertheless, an increasing line of evidences now indicates that Ca^{2+} influx in mammalian eggs does have an important role to play.

Ca^{2+} influx during oocyte maturation

Before ovulation, mammalian oocytes are arrested at the first meiotic prophase in the ovarian follicles (Edwards 1965). During the control of meiosis, a high level of cAMP in the oocyte cytoplasm that originates from the surrounding granulosa cells is probably critical for maintaining the first meiotic arrest (Dekel & Beers 1978). Under physiological conditions, oocyte maturation is triggered by an LH surge (or it can occur spontaneously, after the removal from the follicle environment) that leads to the disruption of gap junctions between the oocyte and the granulosa cells (Gilula *et al.* 1978) and a drop in cAMP levels (Eppig & Downs 1988). cAMP is known to suppress the activity of the M-phase promoting factor (MPF) through cAMP-dependent protein kinase A (PKA); thus, a decrease in cAMP concentrations leads to the activation of MPF. Active MPF then stimulates the progression of the cell cycle from the G2 to the M-phase and germinal vesicle breakdown (GVBD) occurs (reviewed by Downs (2010)). Additional factors such as downregulation of cGMP levels (Norris *et al.* 2009, Vaccari *et al.* 2009) and activation of the MAP kinase cascade (Fan *et al.* 2009) are also believed to be crucial in the induction and progression of meiosis. A great deal of experimental data suggests, however, that Ca^{2+} signals also play a regulatory role in meiotic maturation. Early observations

in the mouse indicated that extracellular Ca^{2+} was essential for meiotic resumption (De Felici & Siracusa 1982, Racowsky 1986). It was then reported that immature mouse and hamster oocytes showed a series of spontaneous Ca^{2+} oscillations after being released from antral follicles and the oscillations lasted until GVBD (Carroll & Swann 1992, Fujiwara *et al.* 1993). Although the Ca^{2+} oscillations in mouse oocytes were caused by an increase in inositol 1,4,5-trisphosphate (InsP_3), Ca^{2+} influx inhibitors rapidly blocked the repetitive Ca^{2+} transients indicating the involvement of Ca^{2+} entry in the signaling process. Interestingly, the spontaneous oscillations did not serve as a trigger for meiotic resumption as inhibition of the signals with a Ca^{2+} chelator had no effect on the onset of GVBD (Carroll & Swann 1992). Other studies led to similar conclusions: GVBD was Ca^{2+} independent and proceeded without interruption in the absence of extracellular Ca^{2+} but completion of the first meiotic division did not occur without Ca^{2+} in the holding medium (Paleos & Powers 1981, Tombes *et al.* 1992). This again indicated the importance of Ca^{2+} entry across the plasma membrane in meiotic maturation. Species-specific differences clearly exist, as in bovine (Homa 1991) and pig oocytes (Kaufman & Homa 1993), chelating Ca^{2+} inhibited GVBD; in the pig, an influx of Ca^{2+} was also a prerequisite for development beyond the metaphase I stage (Kaufman & Homa 1993).

The mechanism that mediates Ca^{2+} entry during maturation is not known in detail. The result of early studies indicated the involvement of unspecific voltage-gated calcium channels, and an increase in the number of such channels together with a gradual depolarization of the plasma membrane was also shown to occur during oocyte growth when the female gamete gains meiotic competence (Murnane & De Felice 1993). This implies that such channels may carry a Ca^{2+} current to control the onset of maturation. Another study described the presence of functional L-type voltage-gated Ca^{2+} channels in mouse oocytes that were missing from GVBD-arrested oocytes, suggesting that such channels were necessary for maturation (Lee *et al.* 2004). In addition, inward currents through L-type voltage-gated channels were detected in immature bovine (Tosti *et al.* 2000) and sheep (Boni *et al.* 2005) oocytes, although in cattle their activity decreased by the end of maturation. Finally, in pig oocytes, P-type voltage-gated Ca^{2+} channels were identified and their activation by membrane depolarization led to a prolonged increase in cytoplasmic Ca^{2+} levels and eventually maturation of the oocyte (Gioia *et al.* 2005). These findings may indicate the involvement of Ca^{2+} influx via voltage-gated channels in the G2/M transition.

Similarly, there is no complete agreement on the exact function of Ca^{2+} as a transducer of the maturation signal. As mentioned earlier, cAMP is a crucial molecule in the cascade that controls the maintenance of the arrest

at prophase of the first meiotic cell division. It activates PKA that in turn inhibits MPF through the phosphorylation of mediating proteins (Downs 2010, Luciano *et al.* 2011). The question is whether Ca^{2+} signals exert any control over the downregulation of cAMP levels at the beginning of maturation. In spontaneously maturing mouse oocytes, the phosphoinositide signaling pathway may play a regulatory role (Lefevre *et al.* 2007), whereas gonadotropin-induced oocyte maturation was suggested to be Ca^{2+} independent (Mehlmann *et al.* 2006). What seems to support the regulatory function of Ca^{2+} is the observation that the preovulatory LH surge generates a Ca^{2+} signal in the cumulus cells that is transmitted to the oocyte (Mattioli *et al.* 1998, Webb *et al.* 2002). This Ca^{2+} signal may be involved in controlling intracytoplasmic cAMP levels by modulating the activity of Ca^{2+} -sensitive adenylate cyclases during both spontaneous and gonadotropin-induced oocyte maturation (Silvestre *et al.* 2011).

Ca^{2+} influx during fertilization

At the time of ovulation, mammalian eggs are arrested at the metaphase stage of their second meiotic cell division. They are released from this second meiotic arrest and embryo development is stimulated during fertilization, when the sperm triggers a prolonged series of Ca^{2+} oscillations in the egg cytoplasm. Research has shown that the oscillations are the result of the release of Ca^{2+} from the egg's intracellular stores; the release is induced by the protein phospholipase C ζ (PLC ζ) that diffuses from the sperm into the ooplasm after gamete fusion (Saunders *et al.* 2002). The released Ca^{2+} binds calmodulin and Ca^{2+} -bound calmodulin activates Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) that stimulates a signaling cascade leading to the downregulation of the MPF and eventually exit from meiosis (Whitaker 2006).

The series of sperm-induced Ca^{2+} transients were first demonstrated indirectly, in hamster eggs, as periodic hyperpolarizations of the egg's plasma membrane (Miyazaki & Igusa 1981a). The hyperpolarizations were caused by a potassium conductance, which in turn was stimulated by the transient elevations in the intracellular free Ca^{2+} levels. The transients were then observed directly in mouse eggs using the Ca^{2+} -sensitive photoprotein aequorin (Cuthbertson *et al.* 1981, Cuthbertson & Cobbold 1985). The series of Ca^{2+} transients stopped upon the removal of extracellular Ca^{2+} and its frequency also depended on the Ca^{2+} concentration outside of the cell (Igusa & Miyazaki 1983). Based on these observations, it was hypothesized that Ca^{2+} entry was a key element of the spiking mechanism in mammalian eggs. Subsequent findings that identified InsP_3 as a Ca^{2+} -mobilizing second messenger (Streb *et al.* 1983), the demonstration that its level increased during fertilization (Turner *et al.*

1984), and the discovery that its injection into the egg stimulated parthenogenetic activation (Whitaker & Irvine 1984) established that InsP_3 was the molecule that had a key role in generating the fertilization Ca^{2+} signal by triggering the release of Ca^{2+} from intracellular stores. The sperm-induced Ca^{2+} transients seemed to be associated with a continuous Ca^{2+} influx, and a linkage between the Ca^{2+} influx and Ca^{2+} release from the intracellular stores was also suggested. However, the identity of the transport system through which Ca^{2+} passed through the plasma membrane was not known. Voltage-gated Ca^{2+} channels present in the plasma membrane of hamster eggs (Miyazaki & Igusa 1981b) were ruled out mostly because the frequency of the hyperpolarization responses increased on hyperpolarization. A model of Ca^{2+} oscillations was later proposed where the fertilizing sperm increased the permeability of the plasma membrane and the Ca^{2+} influx was essential not only for providing favorable conditions for the discharge of Ca^{2+} but also for the refilling of the Ca^{2+} pools to release Ca^{2+} again (Miyazaki 1991).

The dependence of the oscillations on extracellular Ca^{2+} was also demonstrated in mouse eggs. By using fluorescent Ca^{2+} indicators to monitor intracellular free Ca^{2+} levels, these experiments revealed that the sperm-induced Ca^{2+} oscillations rapidly stopped or their frequency significantly decreased upon chelation of external Ca^{2+} (Kline & Kline 1992, Shiina *et al.* 1993). In addition, depleting the intracellular stores with thapsigargin, a plant-derived inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, activated a Ca^{2+} influx across the plasma membrane in unfertilized eggs and suppressed the train of Ca^{2+} spikes in fertilized ones (Kline & Kline 1992). This indicated that a mechanism known in somatic cells as store-operated Ca^{2+} entry was functional in mouse eggs as well and implied that it was essential to refill the stores and maintain the Ca^{2+} oscillations. Further evidence regarding the existence of Ca^{2+} influx during fertilization came from experiments using the sulfhydryl reducing agent dithiothreitol (DTT). In unfertilized mouse eggs, DTT increased the rate of divalent cation influx while in fertilized eggs it was able to accelerate the frequency of the sperm-induced Ca^{2+} transients (Cheek *et al.* 1993). This again implied that the influx of external Ca^{2+} played an important part in signaling during fertilization.

The precise linkage between the Ca^{2+} influx and the train of Ca^{2+} spikes was determined using the manganese (Mn^{2+})-quench technique (McGuinness *et al.* 1996). When added to the extracellular medium, Mn^{2+} acts as a Ca^{2+} surrogate: it can cross the plasma membrane and bind the Ca^{2+} indicator dye fura-2. Binding quenches the fluorescence of fura-2 and this enables the measurement of divalent cation influx. This study revealed that in fertilized mouse eggs, the rising phase of each Ca^{2+} transient was followed by the

stimulation of a Ca^{2+} influx across the plasma membrane. In addition, a smaller yet notable increase in Ca^{2+} influx persisted during the periods between the spikes. Again, although the presence of voltage-gated Ca^{2+} channels had previously been reported in mouse eggs (Murnane & De Felice 1993, Day *et al.* 1995), these cells show only negligible hyperpolarizations during Ca^{2+} transients (Igusa *et al.* 1983). Thus, the authors hypothesized that the spike-associated Ca^{2+} influx was probably controlled by the filling status of the internal stores. A similar conclusion was reached by Mohri *et al.* (2001) after injecting a sperm extract into mouse eggs to stimulate repetitive Ca^{2+} oscillations. The injections were followed by a persistent Ca^{2+} influx that was activated during the initial Ca^{2+} release from the intracellular stores, possibly by a store-operated mechanism. The continuous Ca^{2+} influx was apparently responsible for refilling the stores and sustaining the Ca^{2+} oscillations.

The Ca^{2+} entry that is stimulated by the filling status of the Ca^{2+} store seems to be under the control of protein kinase C (PKC). It was known for quite some time that phorbol esters such as 12-*O*-tetradecanoylphorbol acetate (TPA) or phorbol-12-myristate-13-acetate (PMA), potent stimulators of PKC, caused low-amplitude Ca^{2+} oscillations, ZP2 modification, zona hardening, and cortical granule exocytosis in mouse eggs (Cuthbertson & Cobbold 1985, Endo *et al.* 1986, Colonna *et al.* 1989, Ducibella *et al.* 1991). Similarly, 1-oleyl-2-acetyl-sn-glycerol (OAG), a synthetic analog of endogenous diacylglycerol (DAG, the physiological activator of PKC) promoted mouse egg activation (Colonna *et al.* 1989). Further evidence came from the observation that during Ca^{2+} oscillations in fertilized mouse eggs, fluorescently labeled PKCs translocated to the egg plasma membrane repeatedly and the pattern of translocation followed that of the Ca^{2+} transients and also the periodic increases in the rate of Ca^{2+} influx (Halet *et al.* 2004). In addition, inhibition of PKCs with bisindolylmaleimide I (BIM) not only blocked Ca^{2+} influx after store depletion induced by thapsigargin but also terminated the sperm-induced Ca^{2+} oscillations. On the other hand, the PKC agonist PMA stimulated store-operated Ca^{2+} entry and promoted the long-lasting Ca^{2+} signal at fertilization. In similar studies, PKC activation promoted Ca^{2+} influx and repetitive Ca^{2+} oscillations (Yu *et al.* 2008) and constitutively active PKC constructs triggered a persistent elevation in cytosolic Ca^{2+} levels after the mobilization of luminal Ca^{2+} (Madgwick *et al.* 2005). It was hypothesized that the Ca^{2+} entry channel or some accessory proteins at the plasma membrane became phosphorylated by PKC that in turn caused an increase in Ca^{2+} entry. Interestingly, the store depletion-activated Ca^{2+} influx in somatic cells was inhibited, rather than stimulated, by PKC activation (Parekh & Penner 1995, Haverstick *et al.* 1997), and later it was demonstrated that PKC suppressed Ca^{2+} entry by phosphorylating

ORAI1, the protein that forms the store-operated Ca^{2+} entry channel (Kawasaki *et al.* 2010). The reason for this difference is not clear. It is possible that proteins of the store-operated Ca^{2+} entry cascade are not substrates for PKC in eggs (other isoforms of the ORAI protein such as ORAI2 and ORAI3 are not targeted by PKC inhibitory phosphorylation and mouse eggs may use such proteins as the pore-forming subunit as their store-operated Ca^{2+} channel) and PKC stimulates Ca^{2+} influx through a completely different mechanism. Alternatively, differences in the various PKC subtypes or variations in the molecular composition of store-operated Ca^{2+} entry channels in different cell types may explain the diverse effects of PKC on Ca^{2+} entry. In addition, the level of PKC activation may also be important: in *Xenopus* oocytes, low level of PKC activation promoted store-operated Ca^{2+} entry while high degree of PKC activity had an inhibitory effect (Petersen & Berridge 1994). Overall, these findings suggested that PKC controlled cytoplasmic Ca^{2+} levels in eggs, and it may do so through a store-operated entry mechanism.

The onset of a Ca^{2+} influx triggered by the depletion of the intracellular stores was later demonstrated in pig (Machaty *et al.* 2002) and human (Martín-Romero *et al.* 2008) eggs as well. This further extended the list of mammalian eggs in which the mechanism of store-operated Ca^{2+} entry was shown to be functional. The molecular components of the pathway were clarified soon after the identity of these proteins became known in somatic cell types. Limited RNAi screens in human HeLa (Liou *et al.* 2005) and *Drosophila* S2 (Roos *et al.* 2005) cells identified stromal interacting molecules (STIM) in the endoplasmic reticulum. STIM proteins (STIM1 and STIM2) are single transmembrane proteins that sense Ca^{2+} content with a canonical EF hand directed toward the lumen of the Ca^{2+} store. Upon store depletion, STIM1 translocates to the plasma membrane and activates Ca^{2+} influx; STIM2 is activated by smaller decreases in luminal Ca^{2+} and therefore functions to stabilize basal Ca^{2+} levels in the cytoplasm and the endoplasmic reticulum (Brandman *et al.* 2007). The proteins that form the channel pore were discovered through whole genome RNAi screens in *Drosophila* S2 cells (Feske *et al.* 2006, Vig *et al.* 2006, Zhang *et al.* 2006) and named ORAI after the gatekeepers of heaven in Greek mythology. Of the three ORAI proteins (ORAI1–3) identified, ORAI1 is the most potent; it is located in the plasma membrane and when stimulated by STIM1, it lets Ca^{2+} flow into the cytoplasm from the extracellular space.

The presence of STIM1 has been demonstrated in pig (Koh *et al.* 2007) and mouse (Gómez-Fernández *et al.* 2009) eggs. In the pig, STIM1 expression was shown by PCR amplification using oligonucleotide primers based on a porcine EST sequence that showed high similarity to human and mouse STIM1. In resting cells, STIM1 co-localized with the endoplasmic reticulum and

pharmacological store depletion triggered its redistribution in regions close to the plasma membrane. STIM1 overexpression led to an increase in Ca^{2+} influx after depletion of luminal Ca^{2+} whereas the influx was inhibited in the presence of store-operated Ca^{2+} channel blockers or after suppression of STIM1 expression with siRNAs (Koh *et al.* 2009). Additional experiments implicated the involvement of STIM1 in the signaling process during fertilization. The protein rapidly re-distributed at the plasma membrane following fertilization (Gómez-Fernández *et al.* 2009). In addition, downregulation of STIM1 expression using siRNAs in pig eggs completely abolished the repetitive Ca^{2+} oscillations at fertilization and had a negative effect on subsequent embryo development. Ionomycin added to fertilized eggs that stopped oscillating due to down-regulated STIM1 levels was able to mobilize only a small amount of Ca^{2+} compared with that in control eggs, indicating that the stores were not properly refilled in the absence of STIM1. Restoring STIM1 levels in these eggs following fertilization did not rescue embryo development, suggesting that it was at earlier stages when the function of STIM1 was necessary. Finally, as expected, STIM1 downregulation was without effect on egg activation when development was stimulated parthenogenetically, by triggering a single rise in the egg's cytosolic free Ca^{2+} levels (Lee *et al.* 2012). These results

seem to suggest that STIM1 is essential to maintain the repetitive Ca^{2+} signal by mediating store-operated Ca^{2+} entry during fertilization.

The channel component of the store-operated Ca^{2+} entry cascade, ORAI1, is also present and functional in pig and mouse eggs. Both indirect immunocytochemistry and overexpression of fluorescently tagged ORAI1 revealed that ORAI1 was present mostly in the cell cortex consistent with plasma membrane localization. Expression of ORAI1 decreased in pig eggs during maturation with STIM1 levels remaining fairly constant (Wang *et al.* 2012); this was different in mouse eggs where ORAI1 expression levels were unaltered during maturation while STIM1 was expressed only at very low levels in the GV-stage oocyte (Gómez-Fernández *et al.* 2012). Despite these species-specific differences in the expression levels of these signaling proteins, the effect of the changes seems to be the same in both species: to enhance the small store-operated Ca^{2+} influx measured in GV-stage oocytes to a much higher level by the time the oocytes complete maturation and are ready for fertilization. Downregulation of ORAI1 expression with siRNAs blocked Ca^{2+} entry induced by pharmacological depletion of the intracellular stores and also abolished the repetitive Ca^{2+} oscillations in fertilized eggs. Furthermore, embryo development stimulated by the abnormal fertilization Ca^{2+} signals in eggs with

Figure 1 Schematic of Ca^{2+} entry during fertilization. Sperm-derived PLC ζ cleaves PIP $_2$ and generates InsP $_3$ that triggers Ca^{2+} release from the endoplasmic reticulum. Results in pig oocytes seem to indicate that STIM1 senses store depletion and transmits the empty signal to the plasma membrane where it opens ORAI1 channels and induces store-operated Ca^{2+} entry (Lee *et al.* 2012, Wang *et al.* 2012). Recent data from mouse oocytes did not support the involvement of a store-operated entry mechanism (Miao *et al.* 2012, Takahashi *et al.* 2013). Nevertheless, Ca^{2+} entering the cell is pumped back into the store and another Ca^{2+} transient will be generated. The other product of PIP $_2$ hydrolysis, DAG, may activate PKC that promotes Ca^{2+} influx through a yet-to-be identified Ca^{2+} entry channel.

downregulated ORAI1 was severely impaired. On the other hand, overexpression of ORAI1 together with STIM1 resulted in a huge increase in Ca^{2+} influx induced by artificial depletion of the intracellular stores. These data suggest that the cascade that mediates store-operated Ca^{2+} entry involves STIM1 and ORAI1 and it is essential to sustain the long-lasting Ca^{2+} oscillations during fertilization.

Interestingly, inhibiting store-operated Ca^{2+} entry in mouse eggs using known inhibitors or by the expression of protein fragments that interfere with STIM1–ORAI1 interaction did not prevent the sperm-induced Ca^{2+} oscillations raising the possibility that Ca^{2+} entry other than that regulated by the stores is responsible for sustaining the train of sperm-induced Ca^{2+} spikes (Miao *et al.* 2012, Takahashi *et al.* 2013). This may indicate species-specific differences and suggest that, at least in mouse eggs, ORAI1 proteins are not essential to provide Ca^{2+} influx in order to maintain the long-lasting Ca^{2+} signal. Potential candidates for this role may include trp channels that in several cell types mediate Ca^{2+} entry. Such channels are present in eggs (Petersen *et al.* 1995, Machaty *et al.* 2002) and certain types can be sensitized via phosphorylation by PKC (Hardie 2007), which would explain the above-mentioned stimulatory effect of PKC on the fertilization Ca^{2+} signal.

The potential mechanism that may operate during fertilization to sustain the repetitive Ca^{2+} signal is described in Fig. 1. The sperm delivers PLC ζ that hydrolyzes PIP_2 into InsP_3 and DAG. InsP_3 binds to its receptor on the surface of the endoplasmic reticulum and triggers Ca^{2+} release from the intracellular store. Ca^{2+} is pumped out of the cell or bound to buffer proteins in the cytoplasm, thus replenishing of the store requires extracellular Ca^{2+} . This may be mediated by STIM1: when it senses store depletion, STIM1 moves closer to the plasma membrane and opens store-operated Ca^{2+} channels that let Ca^{2+} flow into the cell. SERCA pumps load Ca^{2+} back into the endoplasmic reticulum, so it will be available when another Ca^{2+} transient is generated. DAG produced by PIP_2 hydrolysis may activate PKC that promotes Ca^{2+} influx through the entry channel and thereby facilitates store refilling. However, additional experiments are clearly needed to clarify the exact mechanism involved in the process.

The importance of transmembrane Ca^{2+} fluxes has been further highlighted by the results of a study mentioned earlier (Miao *et al.* 2012). In those experiments using mouse eggs, Ca^{2+} influx was found necessary not only to maintain the sperm-induced Ca^{2+} oscillations but also for the activation of critical signaling pathways. Following IVF or ICSI, blocking Ca^{2+} influx inhibited late activation events such as second polar body emission even in the presence of multiple Ca^{2+} transients. In addition, after a single sperm-induced Ca^{2+} spike, Ca^{2+} influx itself was

sufficient to induce polar body extrusion and pronuclear formation. A similar phenomenon was observed before in somatic cells where only Ca^{2+} transients with accompanying Ca^{2+} influxes through store-operated Ca^{2+} channels were able to stimulate gene expression (Di Capite *et al.* 2009). These results are unexpected and seem to contradict earlier studies where the number of Ca^{2+} oscillations was the primary determinant of the temporal order of egg activation events (Ducibella *et al.* 2002, Tóth *et al.* 2006). They also mean, if confirmed, that subcellular localization of the Ca^{2+} elevation is important for successful initiation of embryo development and further strengthens the notion that Ca^{2+} influx is an integral part of the Ca^{2+} signaling mechanism in the egg.

Concluding remarks

It is generally accepted that transient elevations in the intracellular free Ca^{2+} concentration in the cytoplasm of the female gamete play important regulatory roles during development. The origin of Ca^{2+} is mostly intracellular, but influx across the plasma membrane is also important to shape the signal and determine its spatial and temporal dynamics. Efforts to characterize the Ca^{2+} influx pathway involved received impetus in recent years; however, a number of important questions remain to be clarified. PKC is known to regulate Ca^{2+} fluxes across the plasma membrane but the pathway through which it operates is not known. It is not clear whether it controls store-operated Ca^{2+} entry or alternatively its action is mediated by an entirely different mechanism. Transient receptor potential (trp) channels are also present in eggs; in somatic cell types, such channels are capable of mediating transmembrane Ca^{2+} fluxes and they might have a potential role in eggs as well. Additional experiments are needed to determine the contribution of store-operated Ca^{2+} entry to the fertilization Ca^{2+} signal in other species and to resolve the apparent discrepancies in recent published results. Finally, the exact function of the Ca^{2+} influx during fertilization also needs to be verified: whether it is needed simply for replenishing the stores or has other roles such as activating additional signaling cascades during egg activation. The information obtained through such studies will deepen our general understanding about Ca^{2+} signaling in the egg and may improve the efficiency of assisted reproductive technologies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

The experiments conducted to investigate store-operated Ca^{2+} entry in pig eggs were supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-67015-30006 from the USDA National Institute of Food and Agriculture.

Acknowledgements

The authors wish to thank Dr Karl Swann for providing helpful feedback during the preparation of the manuscript.

References

- Berridge MJ, Bootman MD & Lipp P 1998 Calcium – a life and death signal. *Nature* **395** 645–648. (doi:10.1038/27094)
- Boni R, Cocchia N, Silvestre F, Tortora G, Lorizo R & Tosti E 2005 Plasma membrane electrical properties and intracellular calcium stores in immature and *in vitro* matured adult and juvenile sheep oocytes. *Reproduction, Fertility, and Development* **17** 279. (doi:10.1071/RDv17n2Ab279)
- Bootman MD, Collins TJ, Peppiatt CM, Prothero LS, MacKenzie L, De Smet P, Travers M, Tovey SC, Seo JT, Berridge MJ *et al.* 2001 Calcium signaling – an overview. *Seminars in Cell & Developmental Biology* **12** 3–10. (doi:10.1006/scdb.2000.0211)
- Brandman O, Liou J, Park WS & Meyer T 2007 STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell* **131** 1327–1339. (doi:10.1016/j.cell.2007.11.039)
- Carroll J & Swann K 1992 Spontaneous cytosolic calcium oscillations driven by inositol trisphosphate occur during *in vitro* maturation of mouse oocytes. *Journal of Biological Chemistry* **267** 11196–11201.
- Cheek TR, McGuinness OM, Vincent C, Moreton RB, Berridge MJ & Johnson MH 1993 Fertilisation and thimerosal stimulate similar calcium spiking patterns in mouse oocytes but by separate mechanisms. *Development* **119** 179–189.
- Clapham DE 2007 Calcium signaling. *Cell* **131** 1047–1058. (doi:10.1016/j.cell.2007.11.028)
- Colonna R, Tatone C, Malgaroli A, Eusebi F & Mangia F 1989 Effects of protein kinase C stimulation and free Ca^{2+} rise in mammalian egg activation. *Gamete Research* **24** 171–183. (doi:10.1002/mrd.1120240205)
- Cuthbertson KS & Cobbold PH 1985 Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+} . *Nature* **316** 541–542. (doi:10.1038/316541a0)
- Cuthbertson KS, Whittingham DG & Cobbold PH 1981 Free Ca^{2+} increases in exponential phases during mouse oocyte activation. *Nature* **294** 754–757. (doi:10.1038/294754a0)
- Day M, Johnson MH & Cook D 1995 Changes in inward peak Ca^{2+} current during progression of the cell cycle in early embryos. *Proceedings of the Physiological Society* **489** 52P.
- De Felici M & Siracusa G 1982 Survival of isolated, fully grown mouse ovarian oocytes is strictly dependent on external Ca^{2+} . *Developmental Biology* **92** 539–543. (doi:10.1016/0012-1606(82)90198-1)
- Dekel N & Beers WH 1978 Rat oocyte maturation *in vitro* relief of cAMP inhibition by gonadotropins. *PNAS* **75** 4369–4373. (doi:10.1073/pnas.75.9.4369)
- Di Capite J, Ng SW & Parekh AB 2009 Decoding of cytoplasmic Ca^{2+} oscillations through the spatial signature drives gene expression. *Current Biology* **19** 853–858. (doi:10.1016/j.cub.2009.03.063)
- Downs SM 2010 Regulation of the G2/M transition in rodent oocytes. *Molecular Reproduction and Development* **77** 566–585. (doi:10.1002/mrd.21175)
- Ducibella T, Duffy P, Kurasawa S, Kopf GS & Schultz RM 1991 The cortical reaction and modifications of the zona pellucida are stimulated by protein kinase C agonists in the mouse egg. *Journal of Cell Biology* **115** 461a. (doi:10.1083/jcb.115.2.461)
- Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S & Ozil JP 2002 Egg-to-embryo transition is driven by differential responses to Ca^{2+} oscillation number. *Developmental Biology* **250** 280–291. (doi:10.1006/dbio.2002.0788)
- Edwards RG 1965 Maturation *in vitro* in mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* **208** 349–351. (doi:10.1038/208349a0)
- Endo Y, Kopf GS & Schultz RM 1986 Stage-specific changes in protein phosphorylation accompanying meiotic maturation of mouse oocytes and fertilization of mouse eggs. *Journal of Experimental Zoology* **239** 401–409. (doi:10.1002/jez.1402390311)
- Eppig JJ & Downs SM 1988 The role of purines in the maintenance of meiotic arrest in mammalian oocytes. *Progress in Clinical Biological Research* **267** 103–113.
- Fan HY, Liu Z, Shimada M, Sterneck E, Johnson PF, Hedrick SM & Richards JS 2009 MAPK3/1(ERK1/2) in ovarian granulosa cells are essential for female fertility. *Science* **324** 938–941. (doi:10.1126/science.1171396)
- Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M & Rao A 2006 A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* **441** 179–185. (doi:10.1038/nature04702)
- Fujiwara T, Nakada K, Shirakawa H & Miyazaki S 1993 Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. *Developmental Biology* **156** 69–79. (doi:10.1006/dbio.1993.1059)
- Gilula NB, Epstein ML & Beers WH 1978 Cell-to-cell communication and ovulation. A study of the cumulus–oocyte complex. *Journal of Cell Biology* **78** 58–75. (doi:10.1083/jcb.78.1.58)
- Gioia L, Barboni B, Turriani M, Capacchietti G, Ioannoni A, Lucidi P, Pistilli G & Mattioli M 2005 P-type Ca channel activation by membrane depolarisation induces maturation in pig oocytes. *Veterinary Research Communications* **29** (Suppl 2) 195–198. (doi:10.1007/s11259-005-0041-1)
- Gómez-Fernández C, Pozo-Guisado E, Gañán-Parra M, Perianes MJ, Alvarez IS & Martín-Romero FJ 2009 Relocalization of STIM1 in mouse oocytes at fertilization: early involvement of store-operated calcium entry. *Reproduction* **138** 211–221. (doi:10.1530/REP-09-0126)
- Gómez-Fernández C, López-Guerrero AM, Pozo-Guisado E, Álvarez IS & Martín-Romero FJ 2012 Calcium signaling in mouse oocyte maturation: the roles of STIM1, Orai1 and SOCE. *Molecular Human Reproduction* **18** 194–203. (doi:10.1093/molehr/gar071)
- Halet G, Tunwell R, Parkinson SJ & Carroll J 2004 Conventional PKCs regulate the temporal pattern of Ca^{2+} oscillations at fertilization in mouse eggs. *Journal of Cell Biology* **164** 1033–1044. (doi:10.1083/jcb.200311023)
- Hardie RC 2007 TRP channels and lipids: from *Drosophila* to mammalian physiology. *Journal of Physiology* **578** 9–24. (doi:10.1113/jphysiol.2006.118372)
- Haverstick DM, Dicus M, Resnick MS, Sando JJ & Gray LS 1997 A role for protein kinase C β in the regulation of Ca^{2+} entry in Jurkat T cells. *Journal of Biological Chemistry* **272** 15426–15433. (doi:10.1074/jbc.272.24.15426)
- Homa ST 1991 Neomycin, an inhibitor of phosphoinositide hydrolysis, inhibits the resumption of bovine oocyte spontaneous meiotic maturation. *Journal of Experimental Zoology* **258** 95–103. (doi:10.1002/jez.1402580111)
- Igusa Y & Miyazaki S 1983 Effects of altered extracellular and intracellular calcium concentration on hyperpolarizing responses of the hamster egg. *Journal of Physiology* **340** 611–632.
- Igusa Y, Miyazaki S & Yamashita N 1983 Periodic hyperpolarizing responses in hamster and mouse eggs fertilized with mouse sperm. *Journal of Physiology* **340** 633–647.
- Kaufman ML & Homa ST 1993 Defining a role for calcium in the resumption and progression of meiosis in the pig oocyte. *Journal of Experimental Zoology* **265** 69–76. (doi:10.1002/jez.1402650110)
- Kawasaki T, Ueyama T, Lange I, Feske S & Saito N 2010 Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca^{2+} level via the store-operated Ca^{2+} channel. *Journal of Biological Chemistry* **285** 25720–25730. (doi:10.1074/jbc.M109.022996)

- Kline D & Kline JT 1992 Thapsigargin activates a calcium influx pathway in the unfertilized mouse egg and suppresses repetitive calcium transients in the fertilized egg. *Journal of Biological Chemistry* **267** 17624–17630.
- Koh S, Lee K, Wang C & Machaty Z 2007 Characterization of STIM1 gene expression in porcine oocytes. *Biology of Reproduction* **77** (Suppl 1) 139.
- Koh S, Lee K, Wang C, Cabot RA & Machaty Z 2009 STIM1 regulates store-operated Ca^{2+} entry in oocytes. *Developmental Biology* **330** 368–376. (doi:10.1016/j.ydbio.2009.04.007)
- Lee JH, Yoon SY & Bae IH 2004 Studies on Ca^{2+} -channel distribution in maturation arrested mouse oocyte. *Molecular Reproduction and Development* **69** 174–185. (doi:10.1002/mrd.20162)
- Lee K, Wang C & Machaty Z 2012 STIM1 is required for Ca^{2+} signaling during mammalian fertilization. *Developmental Biology* **367** 154–162. (doi:10.1016/j.ydbio.2012.04.028)
- Lefevre B, Pesty A, Courtot AM, Martins CV, Broca O, Denys A, Arnault E, Poirot C & Avazeri N 2007 The phosphoinositidephospholipase C (PI-PLC) pathway in the mouse oocyte. *Critical Reviews in Eukaryotic Gene Expression* **17** 259–269. (doi:10.1615/CritRevEukarGeneExpr.v17.i4.10)
- Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr & Meyer T 2005 STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Current Biology* **15** 1235–1241. (doi:10.1016/j.cub.2005.05.055)
- Luciano AM, Franciosi F, Modina SC & Lodde V 2011 Gap junction-mediated communications regulate chromatin remodeling during bovine oocyte growth and differentiation through cAMP-dependent mechanism(s). *Biology of Reproduction* **85** 1252–1259. (doi:10.1095/biolreprod.111.092858)
- Machaty Z, Ramsoondar JJ, Bonk AJ, Bondioli KR & Prather RS 2002 Capacitative calcium entry mechanism in porcine oocytes. *Biology of Reproduction* **66** 667–674. (doi:10.1095/biolreprod.66.3.667)
- Madgwick S, Levasseur M & Jones KT 2005 Calmodulin-dependent protein kinase II, and not protein kinase C, is sufficient for triggering cell-cycle resumption in mammalian eggs. *Journal of Cell Science* **118** 3849–3859. (doi:10.1242/jcs.02506)
- Martín-Romero FJ, Ortíz-de-Galisteo JR, Lara-Laranjeira J, Domínguez-Arroyo JA, González-Carrera E & Alvarez IS 2008 Store-operated calcium entry in human oocytes and sensitivity to oxidative stress. *Biology of Reproduction* **78** 307–315. (doi:10.1095/biolreprod.107.064527)
- Mattioli M, Gioia L & Barboni B 1998 Calcium elevation in sheep cumulus-oocyte complexes after luteinizing hormone stimulation. *Molecular Reproduction and Development* **50** 361–369. (doi:10.1002/(SICI)1098-2795(199807)50:3<361::AID-MRD13>3.0.CO;2-7)
- McGuinness OM, Moreton RB, Johnson MH & Berridge MJ 1996 A direct measurement of increased divalent cation influx in fertilised mouse oocytes. *Development* **122** 2199–2206.
- Mehlmann LM, Kalinowski RR, Ross LF, Parlow AF, Hewlett EL & Jaffe LA 2006 Meiotic resumption in response to luteinizing hormone is independent of a Gi family G protein or calcium in the mouse oocyte. *Developmental Biology* **299** 345–355. (doi:10.1016/j.ydbio.2006.07.039)
- Miao YL, Stein P, Jefferson WN, Padilla-Banks E & Williams CJ 2012 Calcium influx-mediated signaling is required for complete mouse egg activation. *PNAS* **109** 4169–4174.
- Miyazaki S 1991 Repetitive calcium transients in hamster oocytes. *Cell Calcium* **12** 205–216. (doi:10.1016/0143-4160(91)90021-6)
- Miyazaki S & Igusa Y 1981a Fertilization potential in golden hamster eggs consists of recurring hyperpolarizations. *Nature* **290** 702–704. (doi:10.1038/290702a0)
- Miyazaki S & Igusa Y 1981b Ca-dependent action potential and Ca-induced fertilization potential in golden hamster eggs. In *The Mechanism of Gated Calcium Transport Across Biological Membranes*, pp 305–311. Eds ST Ohnishi & M Endo. New York: Academic Press.
- Mohri T, Shirakawa H, Oda S, Sato MS, Mikoshiba K & Miyazaki S 2001 Analysis of $\text{Mn}^{2+}/\text{Ca}^{2+}$ influx and release during Ca^{2+} oscillations in mouse eggs injected with sperm extract. *Cell Calcium* **29** 311–325. (doi:10.1054/ceca.2000.0196)
- Murnane JM & De Felice LJ 1993 Electrical maturation of the murine oocyte: an increase in calcium current coincides with acquisition of meiotic competence. *Zygote* **1** 49–60. (doi:10.1017/S0967199400001295)
- Norris RP, Ratzan WJ, Freudzon M, Mehlmann LM, Krall J, Movsesian MA, Wang H, Ke H, Nikolaev VO & Jaffe LA 2009 Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. *Development* **136** 1869–1878. (doi:10.1242/dev.035238)
- Paleos GA & Powers RD 1981 The effect of calcium on the first meiotic division of the mammalian oocyte. *Journal of Experimental Zoology* **217** 409–416. (doi:10.1002/jez.1402170312)
- Parekh AB & Penner R 1995 Depletion-activated calcium current is inhibited by protein kinase in RBL-2H3 cells. *PNAS* **92** 7907–7911. (doi:10.1073/pnas.92.17.7907)
- Petersen CC & Berridge MJ 1994 The regulation of capacitative calcium entry by calcium and protein kinase C in *Xenopus* oocytes. *Journal of Biological Chemistry* **269** 32246–32253.
- Petersen CC, Berridge MJ, Borgese MF & Bennett DL 1995 Putative capacitative calcium entry channels: expression of *Drosophila* trp and evidence for the existence of vertebrate homologues. *Biochemical Journal* **311** 41–44.
- Prakriya M 2009 The molecular physiology of CRAC channels. *Immunological Reviews* **231** 88–98. (doi:10.1111/j.1600-065X.2009.00820.x)
- Racowsky C 1986 The releasing action of calcium upon cyclic AMP-dependent meiotic arrest in hamster oocytes. *Journal of Experimental Zoology* **239** 263–275. (doi:10.1002/jez.1402390214)
- Rink TJ & Hallam TJ 1989 Calcium signalling in non-excitable cells: notes on oscillations and store refilling. *Cell Calcium* **10** 385–395. (doi:10.1016/0143-4160(89)90064-X)
- Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD et al. 2005 STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *Journal of Cell Biology* **169** 435–445. (doi:10.1083/jcb.200502019)
- Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K & Lai FA 2002 PLC ζ : a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* **129** 3533–3544.
- Shiina Y, Kaneda M, Matsuyama K, Tanaka K, Hiroi M & Doi K 1993 Role of the extracellular Ca^{2+} on the intracellular Ca^{2+} changes in fertilized and activated mouse oocytes. *Journal of Reproduction and Fertility* **97** 143–150. (doi:10.1530/jrf.0.0970143)
- Silvestre F, Boni R, Fissore RA & Tosti E 2011 Ca^{2+} signaling during maturation of cumulus-oocyte complex in mammals. *Molecular Reproduction and Development* **78** 744–756. (doi:10.1002/mrd.21332)
- Streb H, Irvine RF, Berridge MJ & Schulz I 1983 Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306** 67–69. (doi:10.1038/306067a0)
- Stricker SA 1999 Comparative biology of calcium signaling during fertilization and egg activation in animals. *Developmental Biology* **211** 157–176. (doi:10.1006/dbio.1999.9340)
- Takahashi T, Kikuchi T, Kidokoro Y & Shirakawa H 2013 Ca^{2+} influx-dependent refilling of intracellular Ca^{2+} stores determines the frequency of Ca^{2+} oscillations in fertilized mouse eggs. *Biochemical and Biophysical Research Communications* **430** 60–65. (doi:10.1016/j.bbrc.2012.11.024)
- Tombes RM, Simerly C, Borisy GG & 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. (doi:10.1083/jcb.117.4.799)
- Tosti E, Boni R & Cuomo A 2000 Ca^{2+} current activity decreases during meiotic progression in bovine oocytes. *American Journal of Physiology. Cell Physiology* **279** C1795–C1800.
- Tóth S, Huneau D, Banrezes B & Ozil JP 2006 Egg activation is the result of calcium signal summation in the mouse. *Reproduction* **131** 27–34. (doi:10.1530/rep.1.00764)
- Turner PR, Sheetz MP & Jaffe LA 1984 Fertilization increases the polyphosphoinositide content of sea urchin eggs. *Nature* **310** 414–415. (doi:10.1038/310414a0)
- Vaccari S, Weeks JL II, Hsieh M, Menniti FS & Conti M 2009 Cyclic GMP signaling is involved in the luteinizing hormone-dependent meiotic maturation of mouse oocytes. *Biology of Reproduction* **81** 595–604. (doi:10.1095/biolreprod.109.077768)

- Van Den Brink GR, Bloemers SM, Van Den Blink B, Tertoolen LG, Van Deventer SJ & Peppelenbosch MP 1999 Study of calcium signaling in non-excitable cells. *Microscopy Research and Technique* **46** 418–433. (doi:10.1002/(SICI)1097-0029(19990915)46:6<418::AID-JEMT9>3.0.CO;2-0)
- Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R & Kinet JP 2006 CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* **312** 1220–1223. (doi:10.1126/science.1127883)
- Wang C, Lee K, Gajdócsi E, Bali Papp A & Machaty Z 2012 Orai1 mediates store-operated Ca^{2+} entry during fertilization in mammalian oocytes. *Developmental Biology* **365** 414–423. (doi:10.1016/j.ydbio.2012.03.007)
- Webb RJ, Marshall F, Swann K & Carroll J 2002 Follicle-stimulating hormone induces a gap junction-dependent dynamic change in [cAMP] and protein kinase A in mammalian oocytes. *Developmental Biology* **246** 441–445. (doi:10.1006/dbio.2002.0630)
- Whitaker M 2006 Calcium at fertilization and in early development. *Physiological Reviews* **86** 25–88. (doi:10.1152/physrev.00023.2005)
- Whitaker M & Irvine RF 1984 Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature* **312** 636–639. (doi:10.1038/312636a0)
- Yu Y, Halet G, Lai FA & Swann K 2008 Regulation of diacylglycerol production and protein kinase C stimulation during sperm- and PLC ζ -mediated mouse egg activation. *Biology of the Cell* **100** 633–643. (doi:10.1042/BC20080033)
- Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA & Cahalan MD 2006 Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *PNAS* **103** 9357–9362. (doi:10.1073/pnas.0603161103)

Received 7 December 2012

First decision 14 January 2013

Accepted 18 February 2013