

The dynamics of calcium oscillations that activate mammalian eggs

KARL SWANN* and YUANSONG YU

Department of Obstetrics and Gynaecology, School of Medicine, Cardiff University, Cardiff, UK

ABSTRACT It has been known for some time that mammalian eggs are activated by a series of intracellular calcium oscillations that occur shortly after sperm egg membrane fusion. Recent work has identified a novel sperm specific phospholipase C zeta as the likely agent that stimulates the calcium oscillations in eggs after sperm-egg membrane fusion. PLCzeta is stimulated by low intracellular calcium levels in a manner which suggests that there is a regenerative feedback of calcium release and PLCzeta induced inositol 1,4,5-trisphosphate (InsP₃) production in eggs. This implies calcium oscillations in fertilizing mammalian eggs are driven by underlying oscillations of InsP₃. This model of oscillations is supported by the response of mouse eggs to sudden increases in InsP₃. The cellular targets of calcium oscillations include calmodulin-dependent protein kinases, protein kinase C and mitochondria. There is evidence that eggs might be best activated by multiple calcium increases rather than a single calcium rise. As yet we do not fully understand how the target of calcium in a mammalian egg might decode the patterns of calcium changes that can occur during egg activation.

KEY WORDS: *fertilization, egg, sperm, calcium, oscillations*

Introduction

At fertilization the egg is activated to begin development. Egg activation can involve a multitude of cellular changes depending upon the species. The most widely observed changes in mammals are the completion of meiosis, the exocytosis of cortical granules, changes in the pattern of protein synthesis and the formation of pronuclei indicating the start of the first zygotic cell cycle (Ducibella *et al.*, 2006; Stricker, 1999; Runft *et al.*, 2002; Whitaker 2006). A role for Ca²⁺ in directly causing the events egg activation at fertilization was first indicated when it was reported that application of this Ca²⁺ ionophore, to causes a rise in cytosolic Ca²⁺, was able to induce the early events of activation in eggs of a wide range of species including those from hamsters (Steinhardt *et al.*, 1974). Subsequently, it was also shown that microinjection of Ca²⁺ ions alone could trigger embryo development up to the blastocyst stage in the mouse (Fulton and Whittingham, 1978). The importance of Ca²⁺ changes was then later demonstrated by showing that chelation of Ca²⁺ ions using BAPTA could block all signs of activation in mouse eggs (Kline and Kline, 1992a). These data suggest that a Ca²⁺ increase in the egg is a universal trigger for the activation of development in mammals. The first measurements of Ca²⁺ at fertilization were in medaka fish eggs, and then

sea urchin eggs, where the Ca²⁺ sensitive photoprotein aequorin was used to show that a wave of cytosolic free Ca²⁺ increase crosses the egg after sperm interaction (Ridgway *et al.*, 1977; Steinhardt *et al.*, 1977). These studies suggested that a single Ca²⁺ increase that lasts several minutes is the cause of egg activation in these species.

In 1981 two papers were published that suggested that the free Ca²⁺ changes in mammalian eggs might be more complex than in fish or sea urchin eggs. Firstly measurements of the membrane potential in fertilizing hamster eggs showed a series of repetitive hyperpolarizations within 10 seconds of sperm egg interaction (Igusa and Miyazaki 1981). Since the hyperpolarizations appeared to be due to a Ca²⁺-activated K⁺ conductance the data suggested that hamster eggs underwent period Ca²⁺ changes at fertilization (Igusa and Miyazaki, 1982). Other studies using aequorin to measure Ca²⁺ directly also suggested that fertilization in mouse eggs was accompanied by periodic increases in cytosolic free Ca²⁺ (Cuthbertson *et al.*, 1981; Cuthbertson and Cobbold, 1985). These data in mouse and hamster eggs were backed up

Abbreviations used in this paper: InsP₃, inositol triphosphate; PLC, phospholipase C; PLCζ, PLC zeta.

***Address correspondence to:** Karl Swann, Department of Obstetrics and Gynaecology, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, U.K. Fax: +44-2920-744399. e-mail: Swannk1@Cardiff.ac.uk

Published online: 30 May 2008

0214-6282/2008/\$35.00

© UBC Press
Printed in Spain

by further studies which confirmed that a series of repetitive Ca^{2+} oscillations occurred for several hours following sperm egg interaction. Studies in many other mammalian species using fluorescent dyes to measure Ca^{2+} have subsequently confirmed that a prolonged series of Ca^{2+} oscillations with a wavelike onset is a characteristic feature of mammalian egg fertilization (Miyazaki *et al.*, 1993). Fig. 1A shows an example of a mouse egg undergoing Ca^{2+} oscillations at fertilization.

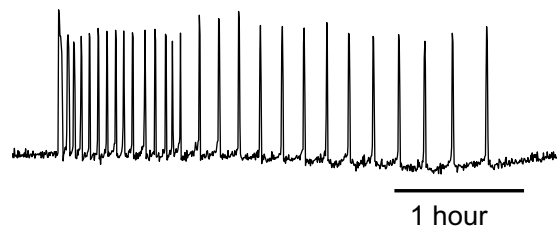
We now know that Ca^{2+} oscillations are not unique to mammalian eggs since fertilizing ascidian oocytes also demonstrate a series of Ca^{2+} oscillations at fertilization (Dumollard *et al.*, 2004a). However, the response in mammalian eggs is distinctive in that the oscillations lasts for several hours and involve relatively low frequency, large amplitude Ca^{2+} increases. The mechanism and consequences of these Ca^{2+} oscillations is pivotal for our understanding of how a sperm activates development. This review will focus on the dynamic aspects of Ca^{2+} signalling and discuss how oscillations are generated, and how they may be transformed into a response by the egg. Some of the potential downstream targets of Ca^{2+} signals in eggs, such as myosin light chain kinase, or src family protein kinases (Matsun *et al.*, 2006; McGinnis *et al.*, 2007), are not discussed since there is no explicit dynamic data on their activity at fertilization.

Ca^{2+} release at fertilization in mammalian eggs is generated by InsP_3 production

The Ca^{2+} oscillations in mammalian eggs appear to be a result of Ca^{2+} release via the InsP_3 receptor (predominantly type 1) that is both the receptor for InsP_3 and a Ca^{2+} release channel (Kurakawa *et al.*, 2004). Studies in mouse and hamster eggs demonstrated that blocking the InsP_3 receptor with a specific antibody inhibits Ca^{2+} oscillations at fertilization (Miyazaki *et al.*, 1993). Furthermore, injecting adenophostin to cause the down-regulation of InsP_3 receptors before fertilization, can block all Ca^{2+} oscillations in subsequently fertilized eggs (Brind *et al.*, 2000; Jellerette *et al.*, 2000). Reducing the expression of InsP_3 receptors by injection of siRNA can also inhibit subsequent Ca^{2+} oscillations in fertilizing mouse eggs (Xu *et al.*, 2003). Other Ca^{2+} releasing agents such as cyclic ADP ribose, nitric oxide or NAADP do not appear to cause Ca^{2+} release in mouse eggs and so other Ca^{2+} release channels are probably not directly involved in generating Ca^{2+} oscillations. These data all show that the InsP_3 receptor is the essential messenger for Ca^{2+} release in mammalian eggs fertilization.

The essential role of the InsP_3 receptor implies that increased InsP_3 production occurs at fertilization in eggs. Increases in phosphoinositide turnover and InsP_3 production have been demonstrated from biochemical and radiolabelling assays in sea urchin and frog eggs (Whitaker, 2006). However, this is impractical in mammalian eggs because thousands of eggs are required. However, a useful probe for measuring InsP_3 production in single cells is GFP (green fluorescent protein) tagged to the PH (pleckstrin homology) domain of PLC δ 1 (Hirose *et al.*, 1999). The translocation of this probe away from the phosphatidyl-inositol biphosphate (PIP_2) in plasma membrane into the cytoplasm can be caused by increase InsP_3 production and so this translocation can be used to indicate InsP_3 production (Hirose *et al.*, 1999). When introduced into mouse eggs the GFP-PH domain this probe under-

A Fertilization



B Injection of PLC ζ

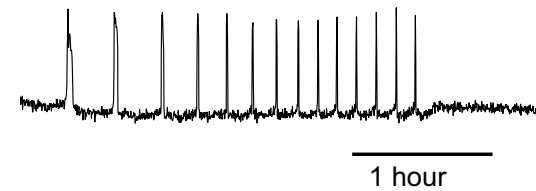


Fig. 1. Intracellular Ca^{2+} oscillations in mouse eggs measured by the fluorescence of a Ca^{2+} sensitive dye (Oregon green BAPTA dextran). See Campbell and Swann (2006) for experimental details. In (A), an egg is shown in response to fertilization, and in (B), the response of an egg is shown after microinjection of cRNA for mouse PLC ζ (Campbell and Swann, 2006; Saunders *et al.*, 2002). The fluorescence F on the y-axis is in arbitrary units, so the Ca^{2+} levels are not calibrated.

goes an *increase* in plasma membrane labelling with each of the early Ca^{2+} increases (Halet *et al.*, 2002). This is paradoxical since, if fertilization triggers an increase in InsP_3 production, the probe would be expected to detach from the plasma membrane and translocate to the cytosol. In fact the data suggest that the probe does not respond to InsP_3 changes in mouse eggs, but instead this probe is relocating due to an increase in PIP_2 in the plasma membrane (Halet *et al.*, 2002).

Although it has proved difficult to use fluorescent InsP_3 indicators in fertilizing mouse eggs (but see later), sperm induced InsP_3 production in mammalian eggs can be inferred from the finding that fertilization leads to a marked down-regulation in the number of InsP_3 receptors (Brind *et al.*, 2000; Jellerette *et al.*, 2000). This down-regulation is not due to the Ca^{2+} increase and can only be induced by agents that bind to the InsP_3 receptor on the InsP_3 binding site. This data clearly suggest that InsP_3 does increase at fertilization in mammalian eggs. The key question is how the sperm produces the increase in InsP_3 levels.

Signalling by a sperm factor

There have been many different ideas for how sperm may cause InsP_3 production and Ca^{2+} release in eggs. These are covered in many previous reviews (Stricker 1999, Kurakawa *et al.*, 2004; Runft *et al.*, 2002; Swann *et al.*, 2006; Whitaker, 2007). A consensus of opinion has emerged that Ca^{2+} signalling in mammalian fertilization is initiated by the introduction of a sperm factor into the egg after sperm-egg membrane fusion. This fusion event occurs a few minutes before the first Ca^{2+} release in the mouse egg and this means there is ample time for a factor to

diffuse into the egg (Lawrence *et al.*, 1997; Jones *et al.*, 1998a). The initial direct evidence for the existence of such a soluble sperm factor came from the observation that injecting soluble sperm extracts could trigger Ca^{2+} oscillations similar to those seen at fertilization in hamster and mouse eggs (Swann, 1990). Such a sperm factor has now been shown in other species such as pigs and humans (Kurakawa *et al.*, 2004; Swann *et al.*, 2006). Important clues to the nature of the sperm factor came from studies using the sea urchin egg homogenate. It was found that the mammalian sperm factor causes a large increase in $InsP_3$ and Ca^{2+} release from intracellular vesicles in this egg homogenate (Jones *et al.*, 1998b). Similarly injection of the sperm factor into frog eggs has been shown to cause and increase in $InsP_3$ that can be measured by bioassay (Wu *et al.*, 2001). Injecting the sperm protein factor into mouse eggs has also been shown to cause down regulation of $InsP_3$ receptors which is a sign that an $InsP_3$ increase has occurred (Lee *et al.*, 2006). The work in sea urchin egg homogenates also showed that the sperm extract themselves possessed a high PLC activity (Jones *et al.*, 1998b; Rice *et al.*, 2000). This suggested that the sperm factor was itself a phosphoinositide (PI) specific PLC. The problem with this hypothesis was that injecting the known isoforms of PI specific PLC did not cause Ca^{2+} oscillations in eggs (Jones *et al.*, 2000), or else where they were reported to cause Ca^{2+} oscillations, such as with $PLC\gamma$, the concentrations of PLC were hundreds of times greater than the PLC activity found in a single sperm (Mehlmann *et al.*, 2001). The resolution to this conundrum came with discovery of a novel, sperm specific, PLC isoform.

PLC ζ and Ca^{2+} release at fertilization

PLC zeta (PLC ζ) was first identified as a novel form of PLC expressed exclusively in testis (Saunders *et al.*, 2002). It has a molecular mass of ~70kDa which makes it the smallest of the mammalian PI specific PLCs (Rebecchi and Pentylala 2000). It is closest in primary sequence to the δ class of PLCs. PLC ζ consist of four EF hand domains that bind Ca^{2+} , a C2 domain, and X and Y catalytic domains found in all mammalian PLCs. It is notably different from most other mammalian phosphoinositide specific PLCs in lacking a PH domain. When the cRNA for PLC ζ or recombinant PLC ζ protein is injected into mouse eggs it triggers Ca^{2+} oscillations very similar to those seen at fertilization (Saunders *et al.*, 2002; Kouchi *et al.*, 2004). Fig. 1B shows an example of a mouse egg undergoing Ca^{2+} oscillations after injection of PLC ζ cRNA. Immunodepletion of PLC ζ from sperm extracts abolishes their ability to cause Ca^{2+} oscillations in mouse eggs. This suggests that the previously described sperm factor is PLC ζ (Swann *et al.*, 2006; Saunders *et al.*, 2007).

There is every indication that PLC ζ is involved in causing Ca^{2+} oscillations in fertilizing mammalian eggs. A PLC ζ isoform has now been identified in at eight mammalian species and in many cases it has been shown to trigger Ca^{2+} oscillations in eggs (Swann *et al.*, 2006). The amount of PLC ζ that is required to trigger Ca^{2+} oscillations is within the range of PLC ζ in a single sperm (Saunders *et al.*, 2002). The release of PLC ζ from sperm probably occurs well within the first hour after sperm-egg fusion and this correlates with the time over which the Ca^{2+} oscillations occur (Yoon and Fissore, 2007). There also now evidence that PLC ζ is the factor responsible for causing Ca^{2+} oscillation after

intracytoplasmic sperm injection (Fujimoto *et al.*, 2004).

Only one study to date has directly addressed the requirement of PLC ζ in normal fertilization. The knockdown of PLC ζ in sperm using a transgenic RNAi approach has been shown to reduce the number of Ca^{2+} oscillations and reduce the activation rates at fertilization (Knott *et al.*, 2005). This transgenic approach generates mosaic expression in spermatogenic cells, and some sperm would still contain some PLC ζ that could account for why some sperm can still cause some Ca^{2+} oscillations and egg activation. One noteworthy feature, however, is that the transgene was never passed on from males to the next generation. This implies that sperm carrying the transgene, where PLC ζ would be most reduced, are not able to trigger an egg to activate and develop. This implies that PLC ζ is required for normal fertility in male mice (Knott *et al.*, 2005). The actions of PLC ζ appears to be specific to egg since transgenic expression of PLC ζ in somatic cells appears to have little effect. In the ovary PLC ζ expression it leads to spontaneous egg activation and the subsequent development of ovarian teratocarcinomas (Yoshida *et al.*, 2007).

The mechanism of action and localization of PLC ζ in eggs is unclear. Studies using a Venus-tagged (hence highly fluorescent) version of PLC ζ have suggested that it is not specifically localized in the plasma membrane (Yoda *et al.*, 2004). The apparent lack of specific plasma membrane localization could be due to the fact that PLC ζ lacks a PH domain which localizes the closely related PLC $\delta 1$ to the PIP_2 in plasma membrane (Saunders *et al.*, 2002). The parts of PLC ζ that may be involved in localization to a source of PIP_2 are the C2 domain and a region between the X and Y catalytic domains called the X-Y linker (Kouchi *et al.*, 2005; Nomikos *et al.*, 2005). The X-Y linker region has several basic residues that could help anchor it to PIP_2 which is a very negatively charged phospholipid (Nomikos *et al.*, 2007). However, it is still unclear whether the PIP_2 that PLC ζ binds to is in the plasma membrane or an internal organelle. The only statement we can make about its localization is that PLC ζ enters the pronuclei as they form after mouse egg activation (Larman *et al.*, 2004; Yoda *et al.*, 2004; Yoon and Fissore, 2007). This localization appears to involve a nuclear targeting region in the X-Y linker region of the protein that is the same as that proposed to bind to PIP_2 . The localization of PLC ζ in pronuclei is associated with the termination of Ca^{2+} oscillations and may act in conjunction with modifications to the $InsP_3$ receptor to terminate Ca^{2+} signals at fertilization (Larman *et al.*, 2004; Lee *et al.*, 2006). More extensive reviews of PLC ζ can be found elsewhere (Swann *et al.*, 2006; Saunders *et al.*, 2007).

How does Ca^{2+} oscillate in an egg?

The discovery of PLC ζ provides a foundation for understanding how Ca^{2+} oscillations are generated at fertilization. One of the features that distinguishes PLC ζ from other mammalian PLCs is the extraordinary sensitivity to Ca^{2+} ions due to the presence of EF domains that bind Ca^{2+} (Kouchi *et al.*, 2005; Nomikos *et al.*, 2005). The high sensitivity to Ca^{2+} was observed previously for the sperm factor associated PLC activity (Rice *et al.*, 2000). Most, if not all, phosphoinositide specific PLCs show some stimulation by Ca^{2+} ions, but the level of Ca^{2+} required to stimulate the isolated enzyme is in the micromolar range which is well above the resting levels in cells (Rebecchi and Pentylala 2000). In contrast recom-

binant PLC ζ is stimulated by Ca $^{2+}$ in the 100nM-800nM range *in vitro*. This means that once PLC ζ is in the egg it is expected to generate InsP $_3$ at resting Ca $^{2+}$ concentrations in eggs (~100nM). Furthermore, when Ca $^{2+}$ levels start to increase there will be a further increase in InsP $_3$ production that will enhance the size of the Ca $^{2+}$ increase. It is possible that egg-derived PLCs, such as PLC β , could also contribute to InsP $_3$ generation during the peak of such Ca $^{2+}$ rises (Igirashi *et al.*, 2007). This all implies a positive feedback of increased Ca $^{2+}$ and InsP $_3$ during the rising phase of each Ca $^{2+}$ increase. Such positive feedback plays a central role in models of Ca $^{2+}$ oscillations in some somatic cells (Meyer and Stryer, 1988; Harootunian *et al.*, 1991; Hirose *et al.*, 1999; Politi *et al.*, 2006). The only explicit mathematical model of Ca $^{2+}$ signals in eggs at fertilization to date is in ascidians where the best way of explaining repetitive Ca $^{2+}$ waves is to assume that a highly Ca $^{2+}$ sensitive PLC generated InsP $_3$ induced Ca $^{2+}$ release (Dupont and Dumollard, 2004). Such an idea of Ca $^{2+}$ dependent InsP $_3$ production in eggs dates back to one proposed to account to the singular Ca $^{2+}$ wave at fertilization in the sea urchin egg (Whitaker and Irvine, 1983). It is possible that this idea could also account for waves and oscillations in mammalian eggs.

Although regenerative InsP $_3$ production is a plausible mechanism in eggs exact mechanism of Ca $^{2+}$ oscillations has remained

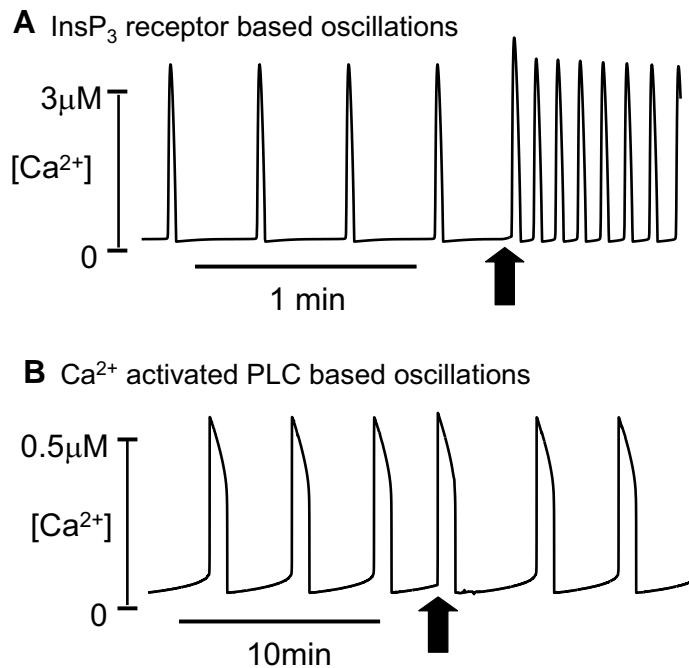


Fig. 2. Different models of Ca $^{2+}$ oscillations respond differently to pulses of InsP $_3$. In (A), a simulation is shown for a model in which positive and negative feedback on the InsP $_3$ receptor generate the oscillations. A sudden increase in InsP $_3$ (indicated by the arrow) causes an increase in the frequency of oscillations. For the sake of illustration, in this case, InsP $_3$ is slowly metabolized and so the increase lasts for some time, but a pulse of InsP $_3$ in this model leads to an increase in the frequency of oscillations (Sneyd *et al.*, 2006). In (B), a simulation is shown for oscillations generated by Ca $^{2+}$ induced feedback on InsP $_3$ production. A sudden increase in InsP $_3$ leads to a Ca $^{2+}$ transient that resets the oscillation cycle, with no increase in frequency. The model and parameters are described in Sneyd *et al.* (2006).

unresolved because there is another popular model of Ca $^{2+}$ oscillations that involves positive and negative feedback loops of Ca $^{2+}$ acting directly on the InsP $_3$ receptor (De Young and Keizer, 1992). In this model at low Ca $^{2+}$ levels the InsP $_3$ receptor is stimulated by Ca $^{2+}$, but at higher Ca $^{2+}$ levels the InsP $_3$ receptor is closed by Ca $^{2+}$ (Bezprozvanny *et al.*, 1991). If this model is correct the InsP $_3$ does not oscillate but instead it provides constant stimulation to the InsP $_3$ receptor that undergoes oscillatory openings. This idea has also received experimental verification in somatic cells. A fluorescent resonance energy transfer (FRET) indicator called IRIS-1, based upon the InsP $_3$ -binding domain of the InsP $_3$ receptor, has been used to show that InsP $_3$ does not oscillate during metabotropic glutamate receptor stimulation of Ca $^{2+}$ oscillations in HeLa cells (Matsu-ura *et al.*, 2006). This model is supported in eggs by the finding that sustained injection of InsP $_3$ or its non hydrolysable derivative, or adenosphostin, or generating a sustained small InsP $_3$ increase by photorelease, can all lead to a series of Ca $^{2+}$ oscillations in unfertilized hamster or mouse eggs (Swann *et al.*, 1989; Swann, 1994; Galione *et al.*, 1994; Nixon and Jones, 2000; Jellerette *et al.*, 2000). This suggests that the InsP $_3$ receptor alone can generate oscillations and that InsP $_3$ levels do not need to oscillate.

One way to try and resolve the mechanism of Ca $^{2+}$ oscillations is to measure InsP $_3$ during fertilization. As, noted above, the GFP-PH indicator used in many somatic cells is not useful for measuring InsP $_3$ in mammalian eggs. However, a different FRET based fluorescent indicator called fretino has been used to study the dynamics of InsP $_3$ in mouse eggs (Shirakawa *et al.*, 2006). Like IRIS, this indicator is based upon InsP $_3$ binding to the binding-domain of the InsP $_3$ receptor which contains two attached fluorescent proteins (Shirakawa *et al.*, 2006). At fertilization this probe suggested there is an increase InsP $_3$, but the signal change is very small and not obviously oscillatory. However, after PLC ζ cRNA injection the Ca $^{2+}$ oscillations eventually become associated with distinct oscillations in InsP $_3$ (Shirakawa *et al.*, 2006). One interpretation of these data is that the indicator is not sensitive enough to measure InsP $_3$ oscillations at fertilization but when PLC ζ is introduced into the egg via RNA injection there is a gradual increase in PLC ζ levels to that somewhat above physiological. This high level of PLC ζ is then sufficient to generate enough InsP $_3$ to be measurable as oscillations. However, it is also possible to argue that physiological levels of PLC ζ at fertilization are insufficient to lead to the InsP $_3$ oscillations, or that InsP $_3$ oscillations are an epiphenomena and not central to the oscillatory mechanism.

Distinguishing between different models of Ca $^{2+}$ oscillations

It is difficult to distinguish between the two different models for Ca $^{2+}$ oscillations. Either one can apply depending upon the cell type and stimulus. One method proposed to distinguish between mechanisms is based upon introducing a sudden increase in InsP $_3$ using, for example, photo-release of a caged InsP $_3$ (Sneyd *et al.*, 2006). The effect of a sudden pulse of InsP $_3$ has very different effects depending upon the model of Ca $^{2+}$ oscillations. If the model has InsP $_3$ as a control parameter (InsP $_3$ receptor based oscillations) then any transient increase in InsP $_3$ leads to an increase in the frequency of Ca $^{2+}$ oscillations. If, however, InsP $_3$

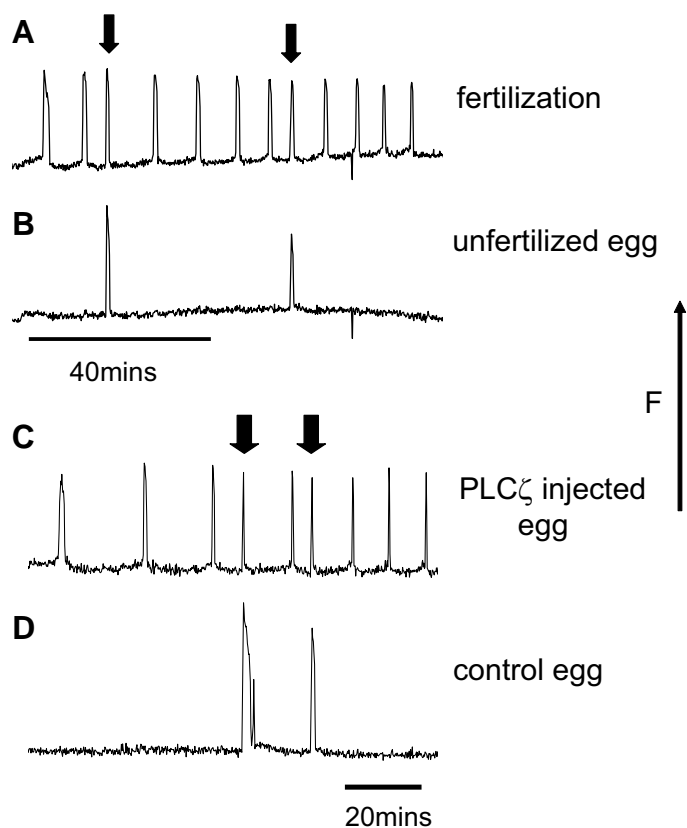


Fig. 3. Photorelease of caged $InsP_3$ in mouse eggs. All eggs were injected with caged $InsP_3$ (0.5 mM in pipette) and Oregon green BAPTA dextran and conditions are as in Fig. 1. In (A), a fertilizing egg was exposed to (5s) pulses of UV light from a mercury lamp (with a UG11 filter) at the times indicated by the arrows. In (B), an egg that was in the same dish but had not fertilized was exposed to the same UV pulses at the same time. In (C), an egg injected with PLC ζ cRNA was exposed to a (10s) UV pulse during oscillations, and (D) shows an uninjected egg in the same dish that also responded to the UV pulses. In all cases a large Ca^{2+} rise followed the photo-release of $InsP_3$. In eggs undergoing oscillations, there was no obvious increase in the frequency of Ca^{2+} oscillations either at fertilization (example from 5 eggs), or after PLC ζ injection (example from 12 eggs).

is a dynamic component of the oscillation mechanism (regenerative $InsP_3$ production) then a sudden pulse increase in $InsP_3$ will cause a Ca^{2+} transient that resets the oscillation cycle and does not increase the frequency Ca^{2+} transients. This difference in response to perturbation is entirely robust with respect to other details of the mathematical model and it has been confirmed with 13 different models of Ca^{2+} oscillations (Sneyd *et al.*, 2006). It provides a simple and qualitative way of discriminating which model of oscillations is operative in a cell.

In Fig. 2 some example simulations are shown for the two different types of mathematical model of Ca^{2+} oscillations (for details see Sneyd *et al.*, 2006). In the case where oscillations occur via $InsP_3$ receptor feedback the sudden increase in $InsP_3$ leads to a marked increase in frequency of Ca^{2+} oscillations (Fig. 2A). In the case where $InsP_3$ undergoes regenerative increases, the sudden increase in $InsP_3$ causes a large Ca^{2+} increase but this does not lead to extra oscillations, but instead resets the oscillation

cycle (Fig. 2B). Fig. 3 shows examples of experiments where $InsP_3$ was increased suddenly in a fertilizing, or PLC ζ injected, mouse eggs by photo-release of caged $InsP_3$. In each case there is a sudden rise of Ca^{2+} that is similar to that seen in control unfertilized eggs. However, this does not lead to any sign of extra Ca^{2+} oscillations. Instead it resets the period of Ca^{2+} oscillations and the next transient occurs with a delay similar to the period prior to the pulse of $InsP_3$. These results are consistent with the model of oscillations in which Ca^{2+} induced $InsP_3$ production is involved in each Ca^{2+} rise (Hirose *et al.*, 2002).

To reconcile the data in Fig. 3 with previous data on $InsP_3$ induced Ca^{2+} oscillations, we suggest that two mechanisms of Ca^{2+} oscillations exist in mammalian eggs in a manner illustrated in Fig. 4. In an unfertilized mammalian egg the $InsP_3$ receptor alone provides the mechanism for generating Ca^{2+} oscillations that are seen, for example, in response to sustained $InsP_3$ increases (Swann *et al.*, 1989; Swann 1994; Nixon and Jones, 2000). This mechanism could also explain the spontaneous Ca^{2+} oscillations that are seen in immature mouse oocytes shortly after isolation from the ovary (Carroll and Swann, 1992; Nixon and Jones, 2000). After fertilization the sperm will have introduced PLC ζ and as a result oscillations are generated by a different mechanism involving a regenerative rise in $InsP_3$ during each Ca^{2+} transient. The regenerative and Ca^{2+} dependent $InsP_3$ production in Fig. 4B also offers an explanation for the enhanced sensitivity of the eggs to ' Ca^{2+} induced Ca^{2+} release' (Igusa and Miyazaki, 1983). The so called ' Ca^{2+} induced Ca^{2+} release' could actually be Ca^{2+} induced $InsP_3$ production which is only seen after fertilization because of the presence of PLC ζ (Shirakawa *et al.*, 2006)

In support of the mechanisms proposed in Fig. 4 it is noteworthy that models involving just the kinetics of the $InsP_3$ receptor tend to produce high frequency short duration oscillations with the time periods of less than a minute (Politi *et al.*, 2006). This is why the mathematical model of Ca^{2+} oscillations based upon the $InsP_3$ receptor in Fig. 2 are rather high frequency. This relatively high frequency response is similar to what is observed in immature oocytes, and in unfertilized hamster and mouse eggs injected with agents that stimulate the $InsP_3$ receptor (Carroll and Swann, 1992; Swann *et al.*, 1989; Galione *et al.*, 1994; Swann, 1994; Jones and Nixon, 2000; Jellerette *et al.*, 2000). In contrast models involving positive feedback of Ca^{2+} on $InsP_3$ production can produce a much greater range of Ca^{2+} oscillations including those with time periods of many minutes as seen with fertilizing eggs (Politi *et al.*, 2006). There could also be Ca^{2+} dependent metabolism of $InsP_3$ which would mean that $InsP_3$ oscillations would be even more strongly coupled to Ca^{2+} oscillations (Politi *et al.*, 2006).

Although most of the debate over different ideas for oscillations revolves around the rising phase of each Ca^{2+} transient, there are various Ca^{2+} pumps such as the SERCA pumps that play an important role in returning Ca^{2+} to the Ca^{2+} stores (Kline and Kline, 1992b). There is also some Ca^{2+} flux out of the cell due to a plasma membrane Ca^{2+} ATPase, and a Na^+/Ca^{2+} exchanger (Georgou *et al.*, 1998; Carroll 2000). In compensation for the efflux, a small influx of Ca^{2+} into the egg appears to occur during each Ca^{2+} transient (McGuinness *et al.*, 1996). Ca^{2+} uptake into mitochondria occurs (Dumollard *et al.*, 2007), and it is possible that a cycle of Ca^{2+} through other compartment, such as the mitochondria,

could play a role in setting up the frequency of Ca^{2+} oscillations (Ishii *et al.*, 2006). However, the Ca^{2+} dynamics inside the various compartments in eggs has not yet been reported.

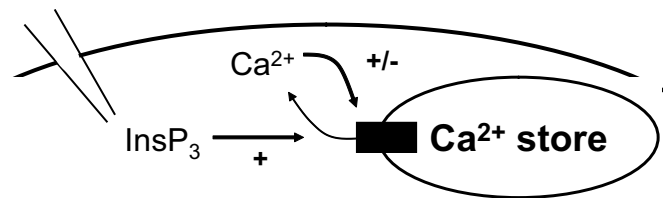
How might an egg decode Ca^{2+} oscillations?

The multiple increases in Ca^{2+} trigger all the major events at fertilization. Furthermore, with each Ca^{2+} increase there is a progressive stimulation of each process (Ducibella *et al.*, 2006). This is shown most directly by using repetitive electroporation to drive different numbers of Ca^{2+} increases in eggs and induce parthenogenetic activation (Ozil and Hunaeu 2001). Some events such as meiotic resumption are stimulated early by a single Ca^{2+} transient, whilst other events such as the decreases in activity of cell cycle protein kinases requires more Ca^{2+} transients (Ducibella *et al.*, 2002). Exocytosis occurs in a stepwise manner with each pulse of Ca^{2+} stimulating a loss of cortical granules from the egg (Ducibella *et al.*, 2002). This suggests that, during normal fertilization, mammalian eggs respond in an integral way to pulses of Ca^{2+} release. In turn this implies that some downstream components in the egg may read out the number, or frequency, of Ca^{2+} increases in eggs (Dupont and Goldbeter, 1998). It is of particular interest, therefore, to examine enzymes or signalling modules that can be seen to be 'decoding' the oscillatory signal.

One of the immediate targets of the Ca^{2+} increases in eggs is the cell cycle machinery that keeps the egg arrested in metaphase of the second meiosis. The resumption of meiosis is stimulated through a series of changes in the activity of protein kinases (Carroll, 2001; Jones 2007). This results in the stimulation of the anaphase promoting complex/cyclosome which leads to proteolysis of cyclin B that is required to maintain the CDK1 (cell division cycle kinase) activity responsible for arrest in meiosis II (Carroll 2001; Jones 2007). The dynamics of cyclin B destruction has been measured at fertilization using GFP-tagged cyclin B and it has been shown that cyclin B is rapidly destroyed at the onset of Ca^{2+} oscillations (Nixon *et al.*, 2002). The early studies suggested that each Ca^{2+} spike might lead to a distinct increment in cyclin B destruction (Nixon *et al.*, 2002). However, subsequent studies suggest a non oscillatory, steady decline in cyclin B following the onset of Ca^{2+} oscillations (Marangos and Carroll, 2004). The kinetics of CDK1 activity during fertilization have yet to be determined with the time resolution applied to cyclin B.

Most recent attention has focussed on calmodulin dependent protein kinase II, or CaMKII, as a transducer of the Ca^{2+} signal at fertilization. CaMKII activity is stimulated at fertilization (Markoulaki *et al.*, 2004), and this is significant because it can phosphorylate Emi2 which targets it for destruction and sets in train the activation of the anaphase promoting complex (Jones 2007). The destruction of Emi2 has also been measured with a fluorescent protein tag and its destruction during Sr^{2+} induced egg activation precedes cyclin B destruction, but it is not clear if the destruction is oscillatory (Madgwick *et al.*, 2006). The full effects of CaMKII in eggs have been directly assessed by injecting a constitutively activate form of CaMKII (CA-CaMKII). The injection of cRNA for CA-CaMKII into mouse eggs leads to meiotic resumption and initiation of the first cell cycles and development up to the blastocyst stage (Madgwick *et al.*, 2005; Knott *et al.*, 2006). These data suggest that CaMKII is the major transducer of the Ca^{2+} oscillations in fertilizing mammalian eggs. This is of particular

A Injecting InsP_3 into an unfertilized egg



B After fertilization

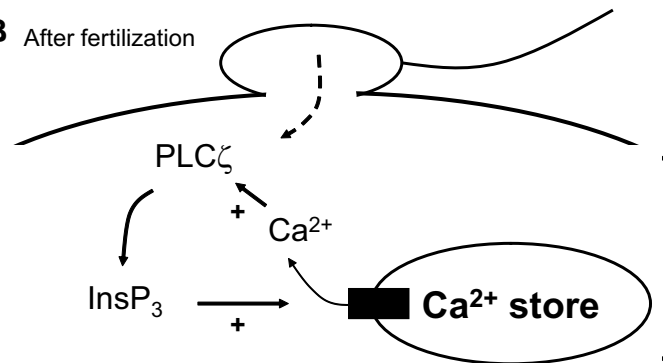


Fig. 4. A schematic diagram to illustrate the different oscillates that are proposed to exist in mammalian eggs. In both cases, Ca^{2+} release occurs from an intracellular store via the InsP_3 receptors (the black rectangle). **(A)** When InsP_3 , or its derivatives, are microinjected into an unfertilized eggs the oscillations are proposed to be solely due to the feedback properties of the InsP_3 receptor. **(B)** In contrast, after fertilization the introduction of $\text{PLC}\zeta$ introduced a new positive feedback loop which takes over as the mechanism of oscillations.

interest because CaMKII can undergo auto-activation and stay active after a decrease in cytosolic Ca^{2+} levels (De Koninck and Schulman 1998). It can show a level of activity that is a 'read out' of the frequency of Ca^{2+} oscillations (De Koninck and Schulman, 1998; Dupont and Goldbeter, 1998). However, the data that shows that CaMKII activity acts as such an analogue transducer of Ca^{2+} oscillations in neurons, for example, involves very high frequency Ca^{2+} pulses (>1 Hz) compared with those in mammalian eggs (<0.005 Hz) (De Koninck and Schulman, 1998; Dupont and Goldbeter, 1998). When the auto-activation of CaMKII is measured in mouse eggs, it undergoes an increase, and then a decrease in activity, essentially in phase with each Ca^{2+} transient (Markoulaki *et al.*, 2004). In other words CaMKII activity does not show any long term memory of each Ca^{2+} pulse. So CaMKII itself, in eggs, does not appear to act as the 'analogue output' of Ca^{2+} oscillations in the way suggested in neurons (De Koninck and Schulman, 1998). If there is a decoder of Ca^{2+} oscillations in eggs it probably involves downstream protein substrates of CaMKII that could be reversibly phosphorylated (Dupont and Goldbeter, 1998; Ducibella *et al.*, 2006).

As well as CaMKII, other protein kinases may be involved in turning the Ca^{2+} signals into cellular responses. For example a number of different protein kinase C isoforms are expressed in mammalian eggs, including both the conventional isoforms that can be activated by the Ca^{2+} and diacylglycerol (Halet 2004).

PLC ζ is expected to produce DAG as well as InsP_3 , so this could act in concert to stimulate PKC. Indeed at fertilization there is an increase in PKC activity (Tatone *et al.*, 2003), and different isoforms undergo translocation to either the plasma membrane or meiotic spindle (Tatone *et al.*, 2003; Page Baluch *et al.*, 2004). The stimulation of PKC could play a number of roles at fertilization since phorbol esters, which can mimic the effects of DAG stimulation in stimulating PKC, has been reported to cause egg activation and even trigger small Ca^{2+} oscillations (Cuthbertson and Cobbold, 1985; Colonna *et al.*, 1989). However, the injection of a constitutively activated form of PKC α does not cause Ca^{2+} oscillations or meiotic resumption in mouse eggs so its role in activation is unclear (Madgwick *et al.*, 2004). The role of PKC is made more complex to understand by the existence of non-conventional PKC isoforms that are not stimulated by Ca^{2+} , and the finding that the increase in PKC activity in eggs is not much affected by inhibiting Ca^{2+} transients at fertilization (Tatone *et al.*, 2003; Halet 2004). On the other hand PKC stimulation has the potential to alter Ca^{2+} oscillations at fertilization since either phorbol esters, or a constitutively activate PKC, can enhance Ca^{2+} influx into mouse eggs (Halet *et al.*, 2004; Madgwick *et al.*, 2005).

Of the different isoforms PKC γ is of particular interest with respect to Ca^{2+} oscillations. Rather like CamKII it has been suggested to act a 'decoding machine' for the frequency of Ca^{2+} oscillations (Oancea and Meyer, 1998). The only studies that have addressed the dynamics of Ca^{2+} oscillations and PKC activation have used plasma membrane translocation of GFP linked to either the whole of PKC γ or PKC α , or GFP linked to the C2 or C1 domains of PKC γ (Halet *et al.*, 2004). In many cellular systems the translocation of PKC via its specific domains is linked to enzyme activation so translocation is a surrogate assay for PKC stimulation. These studies have shown that PKC translocation occurs with each Ca^{2+} spike, with most activation occurring during the first Ca^{2+} transient which tends to be larger than subsequent responses (Halet *et al.*, 2004). There are some indications that PKC does undergo incremental translocation during the series of rapid (~30s) Ca^{2+} oscillations that occur on top of the initial Ca^{2+} transient at fertilization (Halet *et al.*, 2004). However, there is no incremental translocation in response to the low frequency Ca^{2+} oscillations that occur over several hours. As with CamKII, the PKC activity appears to track the overall pattern of Ca^{2+} changes and there is no obvious 'decoding' of the frequency of Ca^{2+} oscillations at this level. Again it is assumed that some substrates of PKC could act to integrate the pulses in PKC activity.

A different kind of target for Ca^{2+} oscillations at fertilization are the mitochondria. Mammalian eggs typically contain >100,000 mitochondria and rely on oxidative phosphorylation for ATP production throughout fertilization and the early cleavage divisions (Dumollard *et al.*, 2004). During the Ca^{2+} oscillations in fertilizing mouse eggs it has been shown that there are a series of oscillatory increases in reduction of FAD and NAD (Dumollard *et al.*, 2004b). It appears that Ca^{2+} increases in the cytosol result in Ca^{2+} increases in the mitochondrial matrix and this leads to stimulation of mitochondrial dehydrogenases. The direct stimulation of the mitochondria reduction could also explain the finding that the sperm-induced Ca^{2+} oscillations lead to a distinct and transient increase in ATP levels as monitored by firefly luciferase

luminescence (Campbell and Swann, 2006).

Mitochondria have also been proposed to act as device for decoding Ca^{2+} oscillations in cells where the output is measured in terms of reduction mitochondrial NAD (Hajnoczky *et al.*, 1995). There are indication that mitochondria in eggs can act as a unit to decode Ca^{2+} oscillations into an analogue response. Ca^{2+} oscillations are probably causing Ca^{2+} pulses in the eggs mitochondria (Dumollard *et al.*, 2007). The Ca^{2+} stimulated NADH/FADH reduction that follows is also oscillatory but there is clearly some degree of memory and integration in that each increase in FADH, for example, outlasts each Ca^{2+} transient (Dumollard *et al.*, 2004b; Dumollard *et al.*, 2006). By the time we look at the ATP in the cytosol we find that these Ca^{2+} dependent events have lost their oscillatory pattern and instead there is a steady increase in ATP that is maintained for the duration of oscillations (Campbell and Swann, 2006). This is a rare example where we can trace the dynamics of a form of digital to analogue conversion in the egg's biochemistry. Unfortunately it is not known if a Ca^{2+} stimulated ATP increase has a direct function. It may be more significant that the ATP is not allowed to fall during the increased energy demands associated with Ca^{2+} pumping during Ca^{2+} oscillations. A transient fall in ATP levels in mouse oocytes has been shown to cause a decrease in the rate developmental to the expanded blastocyst stage after fertilization (Van Blerkom *et al.*, 1995).

Why does Ca^{2+} oscillate in an egg?

One question that often arises with regards to the Ca^{2+} signals in mammalian eggs is why there are oscillations at all. It has been known for some time that Ca^{2+} ionophore, or ethanol can activate mammalian eggs by causing a single large rise in Ca^{2+} (Colonna *et al.*, 1989; Ozil and Swann, 1995). Furthermore, so much of the Ca^{2+} signal seems to be transduced via CamKII and yet egg activation and development can be achieved with a CA-CamKII, which is presumably constant in activity. So oscillations in CamKII are not necessary for egg activation and the question remains as to why Ca^{2+} levels normally oscillate?

One possibility is that the oscillations are simply a consequence of the non linear feedback loop of Ca^{2+} activated InsP_3 production that is intrinsic to PLC ζ . This may well be the case, but it should be noted that many eggs such as those of sea urchins or frogs have many of the same signalling components and yet display a single Ca^{2+} increase at fertilization (Stricker 1999). The difference is not likely to be the absence or presence of PLC ζ in mammalian eggs because even injection of the sperm factor into a frog egg (Wu *et al.*, 2001) causes only a single large Ca^{2+} increase. So the Ca^{2+} release machinery, or toolkit, can be put together in a way that does not lead to oscillations.

One reason why a single Ca^{2+} increase of the type seen in frog or sea urchins eggs is not seen in mammalian fertilization probably has to do with efficiency. A single Ca^{2+} increase generated by Ca^{2+} ionophore, or by electroporation to keep Ca^{2+} high for 5-10 minutes is generally not very efficient at activating eggs. Agents that cause such a single Ca^{2+} increase work best with mammalian eggs that are aged *in vivo*. When eggs are treated with a single large Ca^{2+} increase the activation rate of freshly ovulated mouse eggs is very low (Ozil and Swann, 1995; Toth *et al.*, 2006). A much better protocol for activation is to expose eggs to a single large Ca^{2+} increase and then follow this with a series

of extra smaller pulse of Ca^{2+} , that in effect resemble the pattern seen at fertilization (Toth *et al.*, 2006). A single 5-10 minute Ca^{2+} increase is often a poor stimulus for activation because, despite cyclin B degradation and polar body emission after one Ca^{2+} transient, the continued synthesis of cyclin B can lead to a return of cyclinB/CDK1 activity and the re-establishment of meiotic arrest in some eggs (Ducibella *et al.*, 2002). This is why the most general protocol for egg activation uses a Ca^{2+} ionophore plus either a protein synthesis inhibitor, or a protein kinase inhibitor, which block either the re-synthesis of cyclin B or activity of CDK1.

Whilst it is clear that a relatively short singular Ca^{2+} stimulus (5-10mins) is not always effective in activating eggs, a more difficult question is raised when a longer duration, but sustained, Ca^{2+} rise is applied to eggs. For example, electroporation can be used to cause prolonged elevations of Ca^{2+} in the egg and if the period of a Ca^{2+} increase is extended to more than 20 minutes, the egg activation can be effectively triggered (Ozil *et al.*, 2005). In fact it has been suggested that it is the total sum of elevated Ca^{2+} this is important in stimulating egg activation rather than any particular pattern of Ca^{2+} pulses (Ozil *et al.*, 2005; Toth *et al.*, 2006). This suggests that stimulating an egg to go through the first cell cycle is less dependent upon the form of Ca^{2+} increase so long as enough Ca^{2+} is released.

The answer to this second issue may be more subtle and connected with later events in development. The eggs that are activated by a single Ca^{2+} increase do not develop as well after implantation as ones activated by multiple Ca^{2+} pulses (Ozil *et al.*, 2005). This is consistent with studies that have shown that the pattern of Ca^{2+} oscillations during activation influences the size and morphology of post-implantation parthenogenetic rabbit embryos, or fertilized mouse embryos (Ozil and Huneau, 2001; Ozil *et al.*, 2006). In a similar vein the protocol of exposing mouse eggs to different duration Sr^{2+} treatments, to induced different durations of Ca^{2+} oscillations, has been shown to effect the cell composition of blastocysts (Bos-Mikich *et al.*, 1997). These data all imply that there might be some range of patterns of Ca^{2+} oscillations that is best for embryo development in mammals and that this is not connected with the immediate task of getting the embryo through the first cell cycle. This idea is supported by the development of eggs that are activated without any Ca^{2+} increase at all. Inhibitors of protein synthesis such as cycloheximide, or inhibitors of CDK1, such as roscovtine, can activate mouse eggs and stimulate them to proceed through the first couple of cell cycles without causing any Ca^{2+} increase (Rogers *et al.*, 2006). However, embryo development to the blastocyst stage is very poor compared to embryos activated by Sr^{2+} that causes Ca^{2+} oscillations (Rogers *et al.*, 2006). It is far from clear how the presence or absence of different patterns of Ca^{2+} oscillations can influence later development in mouse. It has been shown that either the absence or presence of different patterns of Ca^{2+} oscillations can influence the pattern of gene expression in mouse embryos (Rogers *et al.*, 2006; Ozil *et al.*, 2006). This serves to repose the question in terms of how Ca^{2+} changes during activation can influence later gene expression. Further progress on this and related questions will require that more of the downstream effects of Ca^{2+} are monitored with the same temporal resolution that we can apply to Ca^{2+} itself.

Acknowledgements

We thank Tony Lai, John Carroll, Guillaume Halet and Keith Jones for

comments on the manuscript. Our work is supported by the BBSRC and the Wellcome Trust.

References

- BEZPROZVANNY I, WATRAS J AND EHRlich BE (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* 351:751-754.
- BRIND S, SWANN K, AND CARROLL J. (2000) Inositol 1,4,5-trisphosphate receptors are downregulated in mouse oocytes in response to sperm and adenophostin A but not to and increase in intracellular Ca^{2+} or egg activation. *Dev. Biol.* 223: 251-265
- BOS-MIKICH A, WHITTINGHAM DG AND JONES KT (1997) Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. *Dev. Biol.* 182: 172-179
- CARROLL J (2000) Na^{+} - Ca^{2+} exchange in mouse oocytes: modifications in the regulation of intracellular free Ca^{2+} during oocyte maturation. *J. Reprod. Fertil.* 118: 337-342
- CARROLL J. (2001) The initiation and regulation of Ca^{2+} signalling at fertilization in mammals. *Semin Cell Dev Biol.* 12: 37-43
- CARROLL J AND SWANN K (1992) Spontaneous cytosolic calcium oscillations driven by inositol trisphosphate occur during in vitro maturation of mouse oocytes *J. Biol. Chem.* 267: 11196-1201
- CAMPBELL, K. & SWANN, K. (2006) Ca^{2+} oscillations stimulate an ATP increase during fertilization of mouse eggs. *Dev. Biol.* 298: 225-233
- COLONNA R, TATONE C, MALGAROLI A, EUSEBI F AND MANGIA F (1989) Effects of protein kinase C stimulation and free Ca^{2+} rise in mammalian egg activation. *Gamete Res.* 24: 171-183
- CUTHBERTSON KS, WHITTINGHAM DG AND COBBOLD (1981) Free Ca^{2+} increases in exponential phases during mouse oocyte activation. *Nature* 294: 754-757
- CUTHBERTSON KS AND COBBOLD PH (1985) Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+} . *Nature* 316: 541-542
- DEKONICK P AND SCHULMAN H (1998) Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* 279: 227-230
- DEYOUNG GW AND KEIZER J (1992) A single pool inositol 1,4,5 trisphosphate receptor based model for agonist stimulated oscillations in Ca^{2+} concentration. *Proc Natl Acad Sci.* 89: 9895-9899
- DUCIBELLA T, HUNEAU DS, ANGELICHIO E, XU Z, SCHULTZ RM, KOPG GS, FISSORE RA, MADOUX S AND OZIL JP. (2002) Egg to embryo transition is driven by differential responses to Ca^{2+} oscillation number. *Dev. Biol.* 250: 280-291
- DUCIBELLA T, SCHULTZ RM AND OZIL JP (2006) Role of calcium signals in early development. *Sem. Cell Dev. Biol.* 17: 324-332
- DUMOLLARD R, MCDOUGHALL A, ROUVIERE C AND SARDET C (2004a) Fertilisation calcium signals in the ascidian egg. *Biol. Cell.* 96: 29-36
- DUMOLLARD, R., MARANGOS, P., FITZHARRIS, G., SWANN, K., DUCHEN, M. AND CARROLL, J. (2004b) Sperm-triggered [Ca^{2+}] oscillations and Ca^{2+} homeostasis in the mouse egg have an absolute requirement for mitochondrial ATP production. *Development* 131: 3057-3067
- DUMOLLARD R, DUCHEN M AND CARROLL J (2007) The role of mitochondrial function in the oocyte and embryo. *Curr. Top. Dev. Biol.* 77: 21-49
- DUPONT G AND GOLDBETER A (1998) CamKII as frequency decoder of Ca^{2+} oscillations. *Bioessays* 20: 607-610
- DUPONT G AND DUMOLLARD R (2004) Simulation of calcium waves in ascidian eggs: insights into the origin of the pacemaker sites and the possible nature of the sperm factor. *J. Cell Science* 117: 4313-4323
- FUJIMOTO S, YOSHIDA N, FUKUI T, AMANAI M, ISOBE T, ITAGAKI C, IZUMI T, PERRY A C F. (2004) Mammalian phospholipase C α induces oocyte activation from the sperm perinuclear matrix. *Dev. Biol.* 274: 370-383
- FULTON, BP AND WHITTINGHAM DG (1978) Activation of mammalian oocytes by intracellular injection of calcium. *Nature* 273: 149-151
- GALIONE A, SWANN K, GEORGIU P AND WHITAKER M. (1994) Regenerative

- and non-regenerative calcium transients in hamster eggs triggered by inositol 1,4,5-trisphosphate. *J. Physiol.* 480: 465-474
- GEORGOU,P., HOUSE,C.R., MCNIVEN,A.I. AND YOSHIDA,S. (1988) On the mechanism of a pH induced rise in membrane potassium conductance in hamster eggs. *J. Physiol.* 402: 121-138
- HAJNOCCZY G, ROBB-GASPERS LD, SEITZ MB AND THOMAS AP (1995) Decoding of cytosolic Ca²⁺ oscillations in the mitochondria. *Cell*82: 415-424
- HALET G, TUNWELL R, BALLAT, SWANN K, AND CARROLL J. (2002) The dynamics of plasma membrane PtdIns(4,5)P₂ at fertilization of mouse eggs. *J Cell Sci.* 115: 2139-2149
- HALET, G., TUNWELL, R., PARKINSON, S. J. AND CARROLL, J. (2004) Conventional PKCs regulate the temporal pattern of Ca²⁺ oscillations at fertilisation in mouse eggs. *J. Cell Biol.* 164: 1033-1044
- HALET,G. (2004) PKC signalling at fertilization in mammalian eggs. *Biochem. Biophys. Acta.* 1742: 185-189
- HAROOTUNIAN AT, KAO JP, PARANJAPE S AND TSEIN RY (1991) Generation of calcium oscillations in fibroblasts by positive feedback between calcium and IP3. *Science* 251: 75-78
- HIROSE K, KADOWAKI S, TANABE M, TAKESHIMA H AND IINO M (1999) Spatialtemporal dynamics of inositol 1,4,5 trisphosphate that underlies complex Ca²⁺ mobilization patterns. *Science* 284: 1527-1530
- IGIRASHI H, KNOTT JG, SCHULTZ RM AND WILLIAMS CJ (2007) Alterations of PLCbeta1 in mouse eggs change calcium oscillatory behavior following fertilization. *Dev. Biol.* (Epub ahead of print)
- IGUSA Y AND MIYAZAKI S (1981) Fertilization potential in golden hamster eggs consists of recurring hyperpolarizations. *Nature* 290: 702-704
- IGUSA Y AND MIYAZAKI S (1982) Ca-mediated activation of a K current at fertilization of golden hamster eggs. *Proc. Natl. Acad. Sci.* 79: 931-935
- IGUSA Y AND MIYAZAKI S (1983) Effects of altered extracellular and intracellular calcium concentration on hyperpolarizing responses of the hamster egg. *J. Physiol.* 340, 633-647
- ISHII K, HIROSE K AND IINO M (2006) Ca²⁺ shuttling between endoplasmic reticulum and mitochondria underlying Ca²⁺ oscillations. *EMBO Rep.* 7: 390-396
- JELLERETTE, T., HE, C.L., WU, H., PARYS, J.B. AND FISSORE, R.A. (2000) Down-regulation of the inositol 1,4,5-trisphosphate receptor in mouse eggs following fertilization or parthenogenetic activation. *Dev. Biol.* 223: 238-250
- JONES KT, SOELLER C AND CANNELL MB (1998a) The passage of Ca²⁺ and fluorescent markers between the sperm and egg after fusion in the mouse. *Development* 125: 4627-4635
- JONES KT, CRUTTWEEL C, PARRINGTON J, AND SWANN K. (1998b). A mammalian sperm cytosolic phospholipase C activity generates inositol trisphosphate and causes Ca²⁺ release in sea urchin egg homogenates. *FEBS Lett* 437:297-300
- JONES,K.T., MATSUDA,M., PARRINGTON,J., KATAN,M. AND SWANN,K. (2000) Different Ca²⁺ releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenate and mouse eggs. *Biochemical Journal* 346: 743-749.
- JONES,K.T. (2007) Intracellular calcium in the fertilization development of mammalian eggs. *Clin. Exper. Pharm. Physiol.* 34: 1084-1089
- JONES KT, SOELLER C AND CANNELL MB (1998) The passage of Ca²⁺ and fluorescent markers between the sperm and egg after fusion in the mouse. *Development* 125: 4627-4635
- KLINE D, AND KLINE JT. (1992a) Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Dev Biol.* 149: 80-89.
- KLINE D AND KINE JT (1992b) Thapsigargin activates a calcium influx pathway in the unfertilized mouse egg and suppresses repetitive calcium transients in the fertilized egg. *J. Biol. Chem.* 267: 17624-30.
- KNOTT JG, KUROKAWA M, FISSORE RA, SCHULTZ RM, AND WILLIAMS CJ (2005) Transgenic RNAi reveals role for mouse sperm phospholipase C ζ in triggering Ca²⁺ oscillations during fertilization. *Biol. Reprod.* 72: 992-996
- KNOTT,J.G., GARDNER,A.J., MADGWICK,S.,JONES,K.T., WILLIAMS,C.J. AND SCHULTZ,R. (2006) Calmodulin-dependent protein kinase II triggers mouse egg activation and embryo development in the absence of Ca²⁺ oscillations. *Dev. Biol.* 296: 388-395
- KOUCHI Z, FUKAMI K, SHIKANOT, ODA S, NAKAMURA Y, TAKENAWA T, AND MIYAZAKI S. (2004) Recombinant phospholipase C ζ has high Ca²⁺ sensitivity and induces Ca²⁺ oscillations in mouse eggs. *J. Biol Chem.* 279: 10408-10412
- KOUCHIZ, SHIKANOT, NAKAMURAY, SHIRAKAWA,H, FUKAMI K, AND MIYAZAKI S. (2005) The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase C ζ . *J. Biol Chem.* 280: 21015-21021
- KURAKAWA M, SATO, K AND FISSORE RA (2004) Mammalian fertilization: from sperm factor to PLC ζ . *Biol. Cell*96: 37-45
- LARMAN MG, SAUNDERS CM, CARROLL J, LAI FA, AND SWANN K. (2004) Cell cycle-dependent Ca²⁺ oscillations in mouse embryos are regulated by nuclear targeting of PLC ζ . *J. Cell Science* 117: 2513-2521
- LAWRENCE,Y., WHITAKER,M. AND SWANN,K. (1997) Sperm-egg fusion is the prelude to the initial Ca²⁺ increase at fertilization in the mouse. *Development* 124: 223-241
- LEE B, YOON SY AND FISSORE RA (2006) Regulation of fertilization-initiated [Ca²⁺]_i oscillations in mammalian eggs: a multi-pronged approach. *Semin Cell Dev Biol.* 17: 274-84.
- MADGWICK S, LEVASSEUR M AND JONES KT (2005) Calmodulin-dependent protein kinase II, and not protein kinase C, is sufficient for triggering cell-cycle resumption in mammalian eggs. *J. Cell Science* 118: 3849-3859
- MADGWICK S, HANSEN DV, LEVASSEUR M, JACKSON PK AND JONES KT (2006) Mouse Emi2 is required to enter meiosis II by reestablishing cyclin B1 during interkinesis. *J. Cell Biol.* 174: 791-801
- MARANGOS P AND CARROLL J (2004) Fertilization and InsP3-induced Ca²⁺ release stimulate a persistent increase in the rate of degradation of cyclin B1 specifically in mature mouse oocytes. *Dev. Biol.* 272: 26-38
- MARKOULAKI, S., MATSON, S. AND DUCIBELLA, T. (2004) Fertilisation stimulates long lasting oscillations of CaMKII activity in mouse eggs. *Dev. Biol.* 272: 15-25
- MATSON,S, MARKOULAKI S AND DUCIBELLA T. (2006) Antagonists of myosin light chain kinase and of myosin II inhibit specific events of egg activation in fertilized mouse eggs. *Biol. Reprod.* 74: 169-176
- MATSU-URA T, MICHIKAWA T, INOUE T, MIYAWAI A, YOSHIDA M AND MIKOSHIBA K (2006) Cytosolic inositol 1,4,5-trisphosphate dynamics during intracellular calcium oscillations in living cells. *J Cell Biol.* 173, 755-765
- MCGUINNESS OM, MORETON RB, JOHNSON MH AND BERRIDGE MJ (1996) A direct measurement of increases divalent cation influx in fertilised mouse oocytes. *Development* 122: 2199-2206
- MCGINNIS LK, ALBERTINI DF AND KINSEY WH (2007) Localized activation of Src-family protein kinases in the mouse egg. *Dev. Biol.* 306: 241-254
- MEHLMANN LM, CHATTOPADHYAY A, CARPENTER G, AND JAFFE LA. (2001) Evidence that phospholipase C from the sperm is not responsible for initiating Ca²⁺ release at fertilization in mouse eggs. *Dev. Biol.* 236: 492-501
- MEYER T AND STRYER L (1988) Molecular model for receptor stimulated calcium spiking. *Proc. Natl. Acad. Sci.* 85: 5051-5055
- MIYAZAKI S, SHIRAKAWA H, NAKADA K, AND HONDA Y. (1993) Essential role of the inositol 1,4,5-trisphosphate/Ca²⁺ release channel in Ca²⁺ waves and Ca²⁺ oscillations at fertilization of mammalian eggs. *Devel Biol.* 58: 62-78
- NIXON VL AND JONES KT (2000) Sperm-induced Ca(2+) oscillations in mouse oocytes and eggs can be mimicked by photolysis of caged inositol 1,4,5-trisphosphate: evidence to support a continuous low level production of inositol 1, 4,5-trisphosphate during mammalian fertilization. *Dev. Biol.* 225, 1-12
- NIXON VL, LEVASSUER M, MCDOUGHALL A AND JONES KT (2002) Ca⁽²⁺⁾ oscillations promote APC/C-dependent cyclin B1 degradation during metaphase arrest and completion of meiosis in fertilizing mouse eggs. *Curr Biol.* 12: 746-50.
- NOMIKOS M, MULGREW-NESBITT A, PALLAVI P, MIHALYNE G, ZAITSEVA I, SWANN K, LAI FA, MURRAY D AND MCLAUGHLIN S (2007) Binding of phosphoinositid-specific phospholipase C ζ (PLC ζ) to phospholipid membranes. *J. Biol. Chem.* 282: 16644-16653
- NOMIKOS, M., BLAYNEY, L.M., LARMAN, M.G., CAMPBELL, K., ROSSBACH, A., SAUNDERS, C.M., SWANN, K. AND LAI, F.A. (2005) Role of phospholipase C-(zeta) domains in Ca²⁺-dependent phosphatidylinositol 4,5-bisphosphate hy-

- drololysis and cytoplasmic Ca^{2+} oscillations. *J. Biol. Chem.* 280: 31011-31018.
- OANCEA E AND MEYER T (1998) Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* 95: 307-318
- OZIL JP, AND HUNEAU D. (2001) Activation of rabbit oocytes: the impact of the Ca^{2+} signal regime on development. *Development* 128: 917-28
- OZIL, J.P. AND SWANN, K. (1995) Stimulation of repetitive calcium transients in mouse eggs. *Journal of Physiology* 483: 331-346
- OZIL JP, MARKOULAKI, S., TOTH S, MATSON S, BANREZES B., KNOTT JG, SCHULTZ RM, HUNEAU D AND DUCIBELLA T. (2005) Egg activation events are regulated by the duration of a sustained $[\text{Ca}^{2+}]_{\text{cyt}}$ signal in the mouse. *Dev. Biol.* 282: 39-54
- OZIL JP, BANREZES B, TOTH S, PAN H AND SCHULTZ RM (2006) Ca^{2+} oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Dev. Biol.* 300: 534-544
- PAGE BALUCH D, KOENEMAN BA, HATCH KR, MCGUAGHEY RW AND CAPCO DG (2004) PKC isotypes in post-activated and fertilized mouse eggs: association with the meiotic spindle. *Dev. Biol.* 274: 45-55
- POLITI A, GASPERIS LD, THOMAS AP AND HOFER T. (2006) Models of IP3 and Ca^{2+} oscillations: Frequency encoding and identification of underlying feedbacks. *Biophys. J.* 90: 3120-3133
- RUNFT LL, JAFFE LA, AND MEHLMANN LM (2002) Egg activation at fertilization: where it all begins. *Devel Biol.* 245: 237-254
- RICE A, PARRINGTON J, JONES KT, AND SWANN K. (2000) Mammalian sperm contain a Ca^{2+} sensitive phospholipase C activity that can generate InsP_3 from PIP_2 associated with intracellular organelles. *Devel Biol.* 227: 125-135
- REBECCHI MJ AND PENTYALA SN (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol Rev.* 80: 291-335.
- RIDGWAY EB, GILKEY JC AND JAFFE, LF (1977) Free calcium increases explosively in activating medaka eggs *Proc. Natl. Acad. Sci.* 74: 623-627
- ROGERS, N.T., HALET, G., PIAO, Y., CARROLL, J., KO, M.S.H. AND SWANN, K. (2006) The absence of a Ca^{2+} signal during mouse egg activation can affect parthenogenetic preimplantation development, gene expression patterns, and blastocyst quality. *Reproduction* 132: 45-57
- RUNFT LL, JAFFE LA AND MEHLMANN LM (2002) Egg activation at fertilization: where it all begins. *Dev. Biol.* 245: 237-254
- SAUNDERS CM, LARMAN MG, PARRINGTON J, COX LJ, ROYSEJ, BLAYNEY LM, SWANN K, AND LAI FA (2002) $\text{PLC}\zeta$: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* 129: 3533-3544.
- SAUNDERS CM, SWANN K AND LAI FA (2007) $\text{PLC}\zeta$, a sperm specific PLC and its potential role in fertilization. *Biochem. Soc. Symp.* 74: 23-26
- SNEYD J, TSANEVA-ATANASOVA K, REZNIKOV V, BAI Y, SANDERSON MJ AND YULE DI (2006) A method for determining the dependence of calcium oscillations on inositol trisphosphate oscillations. *Proc. Natl. Acad. Sci.* 103: 1675-1680
- SHIRAKAWA H, ITO M, SATO M, UEZAWA Y AND MIYAZAKI S. (2006) Measurement of intracellular IP3 during Ca^{2+} oscillations in mouse eggs with GFP based FRET probe. *Biochem. Biophys. Res. Comm.* 345: 781-788
- STEINHARDT, R.A., EPEL, D., CARROLL, E.J. AND YANAGIMACHI, R. (1974) Is calcium ionophore a universal activator for unfertilised eggs. *Nature* 252: 41-43
- STEINHARDT, R.A., ZUCKER, R. AND SCHATTEN, G. (1977) Intracellular calcium release at fertilization in the sea urchin egg. *Dev. Biol.* 58: 185-196
- STRICKER SA. (1999) Comparative biology of calcium signaling during fertilization and egg activation in animals. *Dev. Biol.* 211: 157-76
- SWANN K. (1990) A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster oocytes. *Development* 110: 1295-1302
- SWANN K (1994) Ca^{2+} oscillations and sensitization of Ca^{2+} release in unfertilized eggs injected with a sperm factor. *Cell Calcium* 15: 331-339
- SWANN, K., IGUSA, Y. AND MIYAZAKI, S. (1989) Evidence for an inhibitory effect of protein kinase C on G-protein mediated repetitive calcium transients in hamster eggs. *EMBO J.* 8: pp 3711-3781
- SWANN, K., SAUNDERS, C.M., ROGERS, N. AND LAI, F.A. (2006) $\text{PLC}\zeta$ (zeta): A sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals. *Sem. Cell Dev. Biol.* 17: 264-73
- TATONE C, DELLE MONARCHE S, FRANCIONE A, GIOIA L, BARBONI B AND COLONNA R (2003) Ca^{2+} -independent protein kinase C signalling in mouse eggs during the early phases of fertilization. *Int. J. Dev. Biol.* 47: 327-33.
- TOTH S, HUNEAU D, BANREZEES B AND OZIL JP (2006) Egg activation si the result of signal summation in the mouse. *Reproduction* 131: 27-34
- VAN BLERKOM J, DAVIS PW AND LEE J (1995) ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. *Human Reprod.* 10: 415-424
- WHITAKER, M. (2006) Calcium at fertilization and in early development. *Physiol. Rev.* 86: 25-88
- WHITAKER M AND IRVINE RF (1983) Inositol 14,5-trisphosphate microinjection activates sea urchin eggs. *Nature* 312: 636-639
- WU H, SMYTH J, LUZZI V, FUKAMI K, TAKENAWA T, BLACK SL, ALLBRITTON NL AND FISSORE RA (2001) Sperm factor induces intracellular free calcium oscillations by stimulating the phosphoinositide pathway. *Biol. Reprod.* 64: 1338-1349
- XU Z, WILLIAMS CJ, KOPF GS AND SCHULTZ RM (2003) Maturation associated increase in IP3 receptor subtype 1: role in conferring increased IP3 sensitivity and Ca^{2+} oscillatory behaviour in mouse eggs. *Dev. Biol.* 254: 163-171
- YODA A, ODA S, SHIKANO T, KOUCKI Z, AWAJI T, SHIRAKAWA H, KINOSHITA K AND MIYAZAKI S (2004) Ca^{2+} oscillation-inducing phospholipase C zeta expressed in mouse eggs is accumulated to the pronucleus during egg activation. *Dev. Biol.* 268: 245-57.
- YOON SY AND FISSORE RA (2007) Release of phospholipase C zeta and Ca^{2+} oscillation inducing activity during mammalian fertilization. *Reproduction* 134: 695-704
- YOSHIDA N, AMANAI M, FUKIUT, KAJIKAWA E, BRAHMAJOSYULAM, IWAHORI A, NAKANO Y, SHOJI S, DIEBOLD J, HESSEL H, HUSS R AND PERRY AC (2007) Broad, ectopic expression of the sperm protein $\text{PLC}\zeta 1$ induces parthenogenesis and ovarian tumours in mice. *Development* 134: 3941-52.

Related, previously published *Int. J. Dev. Biol.* articles

See our recent Special Issue *Developmental Biology in Poland* edited by Tarkowski, Maleszewski and Kloc at:
<http://www.ijdb.ehu.es/web/contents.php?vol=52&issue=2-3>

See our recent Special Issue *Ear Development* edited by Fernando Giraldez and Bernd Fritsch at:
<http://www.ijdb.ehu.es/web/contents.php?vol=51&issue=6-7>

Regionalized calcium signaling in zebrafish fertilization

Dipika Sharma and William H. Kinsey
Int. J. Dev. Biol. (2008) 52: 561-570

Defective calcium release during in vitro fertilization of maturing oocytes of LT/Sv mice

Karolina Archacka, Anna Ajduk, Pawel Pomorski, Katarzyna Szczepanska, Marek Maleszewski and Maria A. Ciemerych
Int. J. Dev. Biol. (2008) 52: doi: 10.1387/ijdb.072397ka

Ca²⁺-independent protein kinase C signalling in mouse eggs during the early phases of fertilization.

Carla Tatone, Simona Delle Monache, Antonella Francione, Luisa Gioia, Barbara Barboni and Rosella Colonna
Int. J. Dev. Biol. (2003) 47: 327-333

CpZPC, a new ZPC molecule, localizes to the inner surface of the egg envelope.

Youko Makabe-Kobayashi, Etsuko Kudaira, Akihiko Watanabe and Kazuo Onitake
Int. J. Dev. Biol. (2003) 47: 51-58

Association of egg zona pellucida glycoprotein mZP3 with sperm protein sp56 during fertilization in mice.

N Cohen and P M Wassarman
Int. J. Dev. Biol. (2001) 45: 569-576

Fertilization triggers activation of Fyn kinase in the zebrafish egg.

W Wu and W H Kinsey
Int. J. Dev. Biol. (2000) 44: 837-841

Sperm-egg interaction at fertilization: glycans as recognition signals.

F Rosati, A Capone, C D Giovampaola, C Brettoni and R Focarelli
Int. J. Dev. Biol. (2000) 44: 609-618

A two-component cytoskeletal system of *Xenopus laevis* egg cortex: concept of its contractility.

L V Ryabova and S G Vassetzky
Int. J. Dev. Biol. (1997) 41: 843-851

Electrically fused-egg induction and its development in the goldfish, *Carassius auratus*.

E Yamaha and F Yamazaki
Int. J. Dev. Biol. (1993) 37: 291-298

Calcium in sea urchin egg during fertilization.

I Gillot, P Payan, J P Girard and C Sardet
Int. J. Dev. Biol. (1990) 34: 117-125

2006 ISI **Impact Factor = 3.577**

