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Ca²⁺ oscillations and the cell cycle at fertilisation of mammalian and ascidian eggs

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At fertilisation of mammalian and ascidian eggs the sperm induces a series of Ca²⁺ oscillations. These Ca²⁺ oscillations are triggered by a sperm-borne Ca²⁺-releasing factor whose identity is still unresolved. In both mammals and ascidians Ca²⁺ oscillations in eggs are associated with the period leading up to exit from meiosis and entry into the first embryonic cell cycle. Thus, in mammals Ca²⁺ oscillations continue for several hours but are complete by within 30 min in the ascidian. In mammals and ascidians Ca²⁺ oscillations stop at around the time when pronuclei form in the 1-cell embryo. There is evidence to show that cell cycle factors are important in regulating the fertilisation Ca²⁺ signal. If the formation of pronuclei is blocked either in mammals (by spindle disruption) or in ascidians (by clamping maturation promoting factor levels high) then Ca²⁺ oscillations continue indefinitely. Here, we explore the nature of the sperm Ca²⁺-releasing factor and examine the relationship between cell cycle resumption and the control of Ca²⁺ oscillations at fertilisation.

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egg / fertilisation / cell cycle / calcium

1. INTRODUCTION

The process of fertilisation is initiated by gamete fusion and can be considered to include all the events associated with egg activation until the time when the egg is committed to embryonic development. For nearly all species it is a rise in the cytosolic free Ca²⁺ concentration that acts as the trigger for the events associated with fertilisation.

In most species the egg arrests during meiosis before fertilisation. Notable exceptions include sea urchin, which completes meiosis before arresting, and starfish, which does not seem to show any arrest at all at the time the eggs are normally fertilised (for review see Whitaker, 1996). For this review we will concentrate on

mammals, specifically mouse and hamster, where most of the experimental work has been performed, and ascidians. Mammalian and ascidian eggs share many similarities; both arrest at metaphase, mammalian eggs arrest at metaphase of the second meiotic division (MII), while ascidian eggs arrest at metaphase of the first meiotic division (MI). They also both show a series of Ca²⁺ oscillations when fertilised, rather than a single Ca²⁺ transient. For the purposes of this review we will define 'egg' as the female gamete at the time it is normally fertilised. Neither mammalian nor ascidian eggs have completed meiosis at the time of fertilisation and therefore should strictly be called oocytes rather than eggs.

In this review we will try to address how the sperm triggers Ca²⁺ oscillations at fertilisation and how the egg regulates these Ca²⁺ oscillations. Our main hypothesis is that a Ca²⁺-releasing factor in sperm induces egg

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activation and this factor is regulated by events in the cell cycle. Thus Ca^{2+} oscillations stop when they are no longer needed, when the egg has become committed to embryonic development.

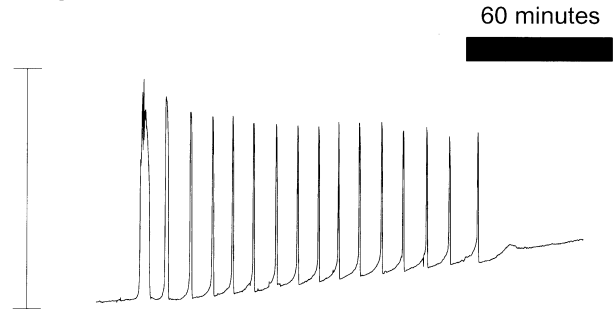
2. THE SPATIOTEMPORAL ASPECTS OF THE Ca^{2+} SIGNAL AT FERTILISATION

A sperm-induced increase in the egg intracellular Ca^{2+} concentration was first observed in medaka eggs over 20 years ago (Ridgway et al., 1977). Such an increase has since been found in nearly every animal species and even during fertilisation of species in the plant kingdom (Digonnet et al., 1997).

The precise temporal dynamics of the Ca^{2+} signal in the egg at fertilisation varies between species. In medaka (Ridgway et al., 1977), sea urchin (Steinhardt et al., 1977) and frog (Busa and Nuccitelli, 1985) Ca^{2+} propagates as a single wave, originating from the point of sperm entry. However, in mammalian eggs the initial wave of Ca^{2+} , which also propagates from the point of sperm fusion (Miyazaki et al., 1986), is followed by a long-lasting series of Ca^{2+} oscillations (Miyazaki and Igusa, 1981; Cuthbertson and Cobbold, 1985). This has been documented in murine (Cuthbertson and Cobbold, 1985), hamster (Miyazaki et al., 1986), bovine (Fissore et al., 1992), porcine (Sun et al., 1992), rabbit (Fissore and Robl, 1993), rat (Ben-Yosef et al., 1993) and human (Taylor et al., 1993). Such Ca^{2+} oscillations last for several hours (figure 1a). In ascidians the sperm-induced Ca^{2+} signal is also initiated from the point of sperm fusion (Speksnijder et al., 1989a). The sperm generates a series of Ca^{2+} oscillations that last for about 30 min (Speksnijder et al., 1989b). Intriguingly the pattern of Ca^{2+} release is composed of two distinct phases of oscillations separated by a gap period (figure 1b). The gap period corresponds to when the first polar body is extruded.

Some 'variations' on the above 'theme' (that sperm induce a Ca^{2+} rise) have been observed in a number of animal eggs. For example in zebrafish, the increase in Ca^{2+} occurs before fusion with the sperm, when the egg is released into freshwater (Lee et al., 1999). In the shrimp (*Sicyonia ingentis*) and prawn (*Palaemon serratus*) Mg^{2+} in the seawater acts to raise intracellular Ca^{2+} (Lindsay et al., 1992; Goudeau and Goudeau, 1996). However, in the prawn, sperm trigger an additional calcium signal at fertilisation (Lindsay et al., 1992). Such activations are certainly worthy of study, but should not distract from the more general finding that it is only the sperm that raises intracellular Ca^{2+} following gamete fusion.

a. mouse fertilisation



b. ascidian fertilisation

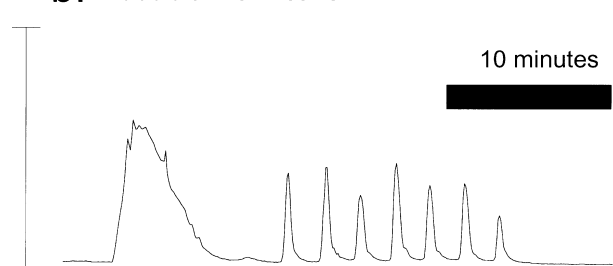


Figure 1. Fertilisation in mammalian and ascidian eggs. Fertilisation of MF1 mouse eggs (a) results in a long-lasting series of Ca^{2+} oscillations that stop about the time when pronuclei form in the developing embryo. The Ca^{2+} oscillations associated with fertilisation of ascidian (*Ascidella aspersa*) eggs occur over a much shorter timeframe (note different timescale in a and b). In ascidians the Ca^{2+} oscillations consist of two phases separated by a gap period that corresponds to extrusion of the first polar body. In both eggs Ca^{2+} is recorded using Fura2, for details of Ca^{2+} recording see Levasseur and McDougall (2000). y-axis is 1 ratio unit (see Levasseur and McDougall, 2000).

3. FUNCTION OF Ca^{2+} AT FERTILISATION

All the events associated with fertilisation in mouse are either inhibited by buffering Ca^{2+} action (Kline and Kline, 1992) or induced by microinjecting Ca^{2+} into eggs (Fulton and Whittingham, 1978). These events include cortical granule (CG) exocytosis, essential for preventing polyspermy (Kline and Kline, 1992; Ducibella et al., 1993; Xu et al., 1994), and meiotic resumption (reviewed in Whitaker, 1996; Jones, 1998). The exact signal transduction pathways involved in CG release and meiotic resumption remain to be fully understood. Temporally CG release occurs quickly, as would be physiologically expected, while meiotic resumption takes a few hours to complete. This has led to the suggestion of 'early' and 'late' events of egg activation (Xu et al., 1994). In ascidian there is no CG release but polyspermy can be inhibited by two other

mechanisms. There is a fast electrical block whereby positive membrane potentials block sperm–egg fusion (Goudeau et al., 1994). This is followed by a slower block to sperm–egg binding, associated with the release of an egg and follicular cell surface N-acetyl glucosaminidase (Lambert, 1989; Robert et al., 1999) in a Ca^{2+} -dependent manner (McDougall et al., 1995). However, in ascidian, like mammals, the Ca^{2+} oscillations are involved in inducing meiotic maturation (McDougall and Sardet, 1995; Arnoult et al., 1996; Russo et al., 1996; Yoshida et al., 1998).

For eggs which arrest at metaphase II (and likely MI also), meiotic arrest is achieved through the concerted action of maturation (or M-phase) promoting factor (MPF) and cytotstatic factor (CSF). MPF is a universal trigger for entry from interphase into mitosis/meiosis (Nurse, 1990). MPF was first described in the amphibian by its ability to trigger oocyte maturation (Masui and Markert, 1971; Smith and Ecker, 1971) and was later identified as being composed of a regulatory component, cyclin B (Evans et al., 1983; Labbe et al., 1989), and a homologue of the fission yeast protein p34^{cdc2} (Nurse and Thuriaux, 1980). A flurry of interest in 1988 convincingly demonstrated that the catalytic component of MPF was p34^{cdc2}, now renamed CDK1 for cyclin-dependent kinase (Dunphy et al., 1988; Gautier et al., 1988; Lohka et al., 1988; see review by Morgan, 1997). MPF is inactivated by polyubiquitination of its cyclin B component, which targets it for destruction (Murray et al., 1989). However, in eggs MPF activity is stabilised by a cytotstatic factor (CSF), such that cell cycle arrest is achieved before fertilisation. CSF was first defined by its ability to trigger metaphase arrest when injected into blastomeres of developing embryos (Masui and Market, 1971; Masui, 1974). It is now generally regarded that the product of the *c-mos* gene has CSF activity (reviewed by Masui, 1991; Sagata, 1997). Consistent with this, mice with a *c-mos* knockout fail to arrest in MII and parthenogenetically activate (Colledge et al., 1994; Hashimoto et al., 1994). However, unlike MPF, the components of CSF are still not fully determined (Ciemerych and Kubiak, 1999).

The signal transduction pathway from the Ca^{2+} signal to meiotic resumption in the frog egg involves calmodulin-dependent protein kinase II (CaMKII). CaMKII induces ubiquitin-dependent degradation of the MPF cyclin B component (Lorca et al., 1993), targeting it for proteolysis by the proteasome. Evidence now exists that CaMKII is also activated in mammals by a Ca^{2+} rise (Winston and Maro, 1995; Tatone et al., 1999) and a CaMKII inhibitor can block meiotic resumption but not cortical granule release of mouse eggs (Inagaki et al., 1997). The involvement of Ca^{2+} in meiotic resumption is sketched in figure 2. It is clear that many of the exact details still need to be examined

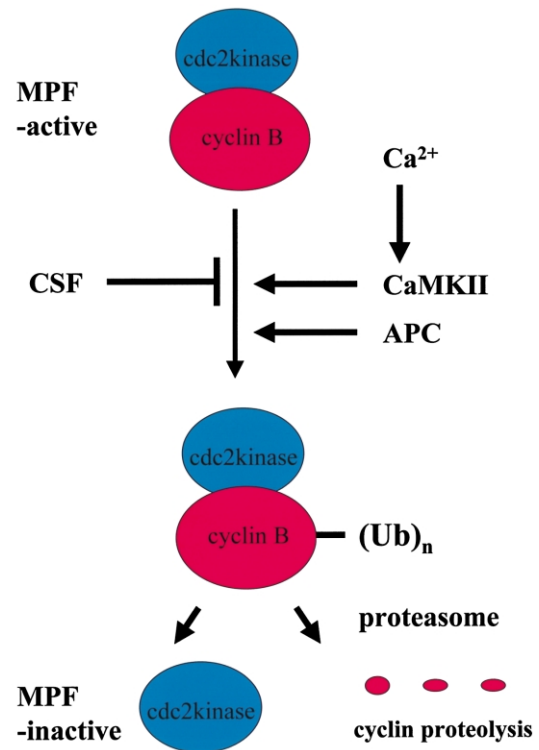


Figure 2. Interaction of the Ca^{2+} signal at fertilisation with CSF and MPF. MPF activity is clamped high before fertilisation by the presence of CSF. MPF is known to be composed of a catalytic cdc2 kinase subunit and a regulatory cyclin B subunit. Cyclin B is polyubiquitinated (Ub)_n by the anaphase promoting complex (APC), a ubiquitin ligase. This ubiquitination targets it for destruction by the proteasome. However, in eggs this is prevented by CSF, a factor whose exact identity is not fully resolved. The product of the *c-mos* gene has CSF activity and *c-mos* is believed to have MAP kinase kinase activity, implicating the MAP kinase pathway in CSF-induced egg arrest. In both amphibians and mouse, activation of MPF destruction by Ca^{2+} has been shown to be mediated by CaMKII. The precise signalling pathway that involves CaMKII is not known. Neither is the mechanism by which CSF is degraded.

since our knowledge of the actual pathways involved is rudimentary.

4. THE MECHANISM RESPONSIBLE FOR THE Ca^{2+} TRANSIENTS

Knowledge of the mechanism employed by the sperm to trigger Ca^{2+} transients is also important for our understanding of fertilisation. The precise mechanism operating for any species is unknown but we will consider the models in terms of the ‘conduit’, ‘contact’ and ‘content’ models as first coined by Jaffe (1991).

In the 'conduit' model the sperm acts as a conduit for Ca^{2+} entry from the extracellular medium. Alternatively, the source of the Ca^{2+} could be the sperm itself, the so-called 'Ca²⁺ bomb' hypothesis. Ca^{2+} entry would overload intracellular stores and so induce Ca^{2+} release. Such a phenomenon of Ca^{2+} influx causing store overload has been proposed to occur in the sea urchin egg at fertilisation (Creton and Jaffe, 1995) and in muscle cells (Fabiato, 1985; Wier and Blatter, 1991). In support of this model, it is well known that extracellular Ca^{2+} is needed for the maintenance of oscillations in the mouse egg (McGuinness et al., 1996) and that blocking the influx of the Ca^{2+} results in inhibition of the oscillations (e.g., Igusa and Miyazaki, 1983). However, the influx of Ca^{2+} is associated with capacitative Ca^{2+} influx following a Ca^{2+} transient, rather than initiating it (McGuinness et al., 1996). Furthermore, direct measurement of sperm and egg Ca^{2+} levels in the region of gamete fusion failed to detect any evidence of a Ca^{2+} influx from the fertilising sperm (Jones et al., 1998a). Finally, the sperm-triggered calcium oscillations all appear to be waves that originate in the vegetal hemisphere of the oocyte irrespective of the site of sperm-egg fusion (Kline et al., 1999; Deguchi et al., 2000). Therefore, in mammals at least, this model appears untenable.

The second model proposes that 'contact' between a sperm ligand and an egg receptor induces activation of an egg phospholipase C (PLC) (Foltz and Shilling, 1993; Evans and Kopf, 1998). This model predicts that the second messenger inositol 1,4,5-trisphosphate (InsP_3), produced by egg PLC hydrolysis of phosphatidylinositol 4-, 5-bisphosphate, would be responsible for Ca^{2+} oscillations. There is very strong experimental evidence for this model in mammals. Much of the signal transduction pathway necessary in this model is present and functions in mammalian eggs. InsP_3 can induce Ca^{2+} oscillations in both mouse and hamster oocytes (Swann et al., 1989, Swann, 1994; Jones and Whittingham, 1996). Furthermore, methods to block this pathway invariably block fertilisation. For example, the fertilisation-dependent Ca^{2+} increase in hamster and mouse eggs is blocked by a functionally inhibitory antibody to the type 1 InsP_3 receptor (Miyazaki et al., 1992, 1993), which is the predominant receptor isoform in mammalian eggs (Parrington et al., 1998; He et al., 1999). In addition the PLC inhibitor U73122 blocks Ca^{2+} oscillations in mouse (Dupont et al., 1996). Despite this evidence, no reported sperm ligand or egg receptor has been identified to cause Ca^{2+} changes in mammalian eggs. In fact, so far, molecules believed to be important in gamete binding and fusion do not seem to be involved in triggering egg activation and hence Ca^{2+} release (Cho et al., 1998; LeNaour et al., 2000; Miyado et al., 2000).

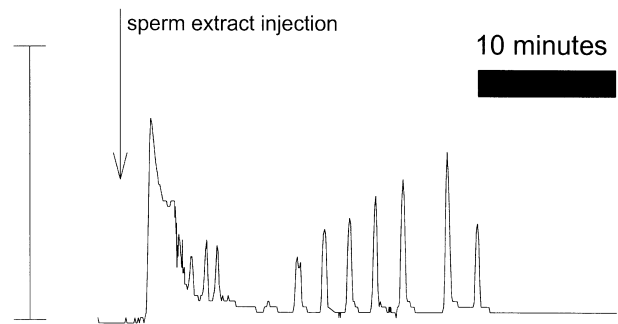


Figure 3. Sperm extract microinjection into ascidian eggs mimics the Ca^{2+} oscillations seen at fertilisation. Ascidian sperm extract (7.8 mg/mL) microinjected into *A. aspersa* gives Ca^{2+} oscillations that are identical to fertilisation. Because of this ascidian offers the best evidence for the physiological relevance of the sperm factor at fertilisation.

The 'content' model proposes that a soluble sperm protein factor is released directly into the egg following gamete fusion (Swann, 1990). This model is based on the finding that soluble extracts from sperm in a number of mammalian and non-mammalian species cause Ca^{2+} oscillations identical to fertilisation when microinjected into eggs (Swann, 1990; Homa and Swann, 1994; Stricker, 1997; Wu et al., 1997, 1998b; Kyozuka et al., 1998; Tang et al., 2000). The activity of the factor is lost following heat or trypsin treatment. The ascidian egg is the most striking example of how sperm extracts can mimic fertilisation (figure 3) (Kyozuka et al., 1998). Since sperm extracts are able to mimic the fertilisation Ca^{2+} signal so precisely, we believe the sperm factor theory is the most probable mechanism by which sperm trigger Ca^{2+} release. Interestingly, most evidence for the existence of a sperm factor comes from species in which a series of Ca^{2+} oscillations are observed at fertilisation (mammals, ascidians, nemerteans). As yet the significance of this observation is not known.

5. CANDIDATES FOR THE TITLE OF SPERM FACTOR

The concept of the sperm factor is not new, being suggested at least 15 years ago (Whitaker and Irvine, 1984) and was first described by Dale et al. (1985) in sea urchin. Since that time the existence of a sea urchin sperm factor has not been the subject of extensive studies, although more recent data suggest that sea urchin sperm do contain a Ca^{2+} -releasing factor that can activate mammalian eggs (Wakayama et al., 1997). Better evidence for a soluble sperm factor has come from the pioneering work of Swann in mammals. Several groups have now reported the existence of

such a soluble mammalian factor (Wu et al., 1997; Wolosker et al., 1998; Oda et al., 1999; Tang et al., 2000) but its identity remains unknown. The existence of the mammalian sperm factor is based on the ability of soluble extracts to mimic the fertilisation-associated Ca^{2+} changes (see figure 4 for preparation of extracts). It is well established that the Ca^{2+} -releasing ability of such extracts is lost when extracts are heat- or trypsin-treated, suggesting the factor is proteinaceous (Swann, 1990). Furthermore, the active factor can be fractionated using FPLC and this has been the principal method employed for purifying the sperm protein.

A protein, which was named oscillin, isolated from hamster by FPLC was the first proposed sperm factor candidate (Parrington et al., 1996). Oscillin had sequence homology with a bacterial deaminase. It was found exclusively in the equatorial region of the sperm head (Parrington et al., 1996), which is where the sperm fuses with the egg (reviewed in Yanagimachi, 1994). However, further studies from the same group and by others have conclusively shown that oscillin is not the sperm factor. Recombinant oscillin has no Ca^{2+} releasing ability (Wolosker et al., 1998). Furthermore, fractionated sperm extracts can be obtained which lack any oscillin but still have Ca^{2+} oscillation-inducing activity (Wu et al., 1998b; Parrington et al., 1999). The opposite is also true. Fractionated extracts which lack oscillin can have Ca^{2+} oscillation-inducing activity (Wu et al., 1998b; Parrington et al., 1999). The group leading the mammalian sperm factor purification failed to purify the correct protein, a fact that must highlight the difficulties in purifying this protein by FPLC. Obviously fractions which are active at inducing Ca^{2+} oscillations in mammalian eggs contain a factor which is not readily discernible by SDS-PAGE (figure 1, fractions 4 and 5; Parrington et al., 1996).

Following oscillin there are far more promising candidates that have potential in being the correct sperm factor. A 23-kDa truncated form of the c-kit receptor, which is involved in germ cell migration, termed tr-kit, has been shown to trigger full egg activation following microinjection into mouse eggs. It is specifically expressed late during spermatogenesis and when either tr-kit mRNA or protein is microinjected into eggs all the events of egg activation are induced (Sette et al., 1997). Critically, microinjection of control mRNA or protein fails to induce egg activation. Importantly tr-kit has also been shown to increase InsP_3 production by activating $\text{PLC}\gamma 1$ (Sette et al., 1998). It may seem trivial that it is still to be shown that tr-kit can induce Ca^{2+} oscillations when microinjected into eggs. However, it has been demonstrated that sperm extracts which lack tr-kit retain Ca^{2+} oscillation-inducing activity (Wu et al., 1998b). In fact, tr-kit is localised mainly to the residual body of the sperm mid-piece (Sette et al., 1997) and this region does not activate eggs following micro-

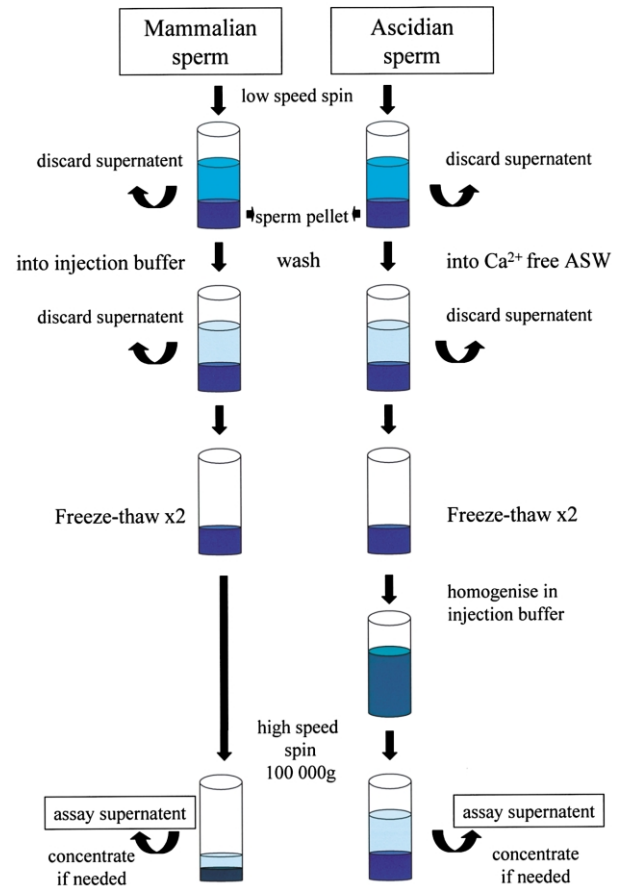


Figure 4. Preparation of mammalian and ascidian sperm extracts. Both mammalian and ascidian sperm extracts are made by a simple protocol. For ascidian sperm we use a freeze–thaw cycle and an homogenisation step to ensure release of the sperm factor (as found by Kyozyuka et al., 1998). However, in mammals freezethawing alone is adequate to liberate the Ca^{2+} -releasing factor. To aid protein recovery we suggest the inclusion of protease inhibitors (PMSF and leupeptin). It is necessary to perform all steps at 4°C except in mammalian sperm for the initial washes. For more details on preparing mammalian sperm extracts see Swann (1990) and Jones et al. (1998b). A more intricate procedure is described by Kyozyuka et al. (1998) for preparing ascidian sperm extracts. Final supernatants may be concentrated on ultrafiltration membranes if found to be very dilute (e.g., Centricons, Amicon, UK).

injection (Kimura et al., 1998). Physiologically tr-kit may actually switch on a sperm PLC rather than an egg PLC, during either spermiation, capacitation or even the acrosome reaction. If the PLC is activated prior to sperm fusion this may explain why SH2 constructs fail to block fertilisation in the mouse (Mehlmann et al., 1998) but do so in other species (Carroll et al., 1997, 1999; Shearer et al., 1999). SH2 domains

Table I. Sperm extract equivalents injected into eggs. This table shows the species in which sperm extracts have been injected into eggs and calculations made as to the number of sperm equivalents being introduced. A sperm factor activity has been described in eggs of the protostome clade (Nemertean worms) suggesting it is not limited to animals in the chordate phylum.

Animal from which sperm extracts prepared	Sperm extracts mimic fertilisation?	Number of sperm equivalents injected
Nemertean worm	Yes	5.3–20.9 ^a
Ascidian	Yes	1.8 ^d
Bovine	Yes	2–3 ^{c,1}
Hamster	Yes	8 ^{b,1}
	Yes	7 ^{e,2} 1–10 ^f
Mouse	Yes	2–3 ^c

¹ Sperm extracts were assayed in mouse eggs. ² Sperm extract responses were noted as similar to the response seen with polyspermy, suggesting high doses of sperm extracts were used. ^a Stricker, 1997; ^b Oda et al., 1999; ^c Tang et al., 2000; ^d Kyojuzuka et al., 1998; ^e Swann, 1990; ^f Swann, 1993.

block by competing with PLC γ SH2 domains required for PLC activation. If tr-kit were involved in PLC activation in the sperm prior to fertilisation there would be no critical need for tr-kit to be in active fractions. There is good support for a model in which tr-kit induces activation of a sperm PLC since sperm do contain active PLC (Jones et al., 1998b). Following FPLC of sperm extracts this sperm PLC activity remains associated with fractions capable of inducing Ca²⁺ oscillations when microinjected into mouse eggs (Parrington et al., 1999). At present it is not known if the sperm PLC is a member of the PLC γ , PLC β or PLC δ family.

6. ARGUMENTS AGAINST THE SOLUBLE SPERM FACTOR THEORY

Does the soluble sperm factor act alone at fertilisation in activating the egg? While it is clear that a soluble sperm factor is able to induce all the events associated with egg activation (Stice and Robl, 1990; Wu et al., 1998a), it could be argued that multiple mechanisms operate to ensure activation. At this stage it is not possible to rule out this scenario. Non-soluble perinuclear material from mouse sperm heads can cause Ca²⁺ oscillations (and so egg activation) when microinjected into eggs (Perry et al., 2000). Although we favour this as evidence that in the mouse the sperm factor is not entirely soluble, Perry et al. (2000) suggest that there are multiple sperm components (so-called SOAF-Is in combination with SOAF-II). This supposition is based on their ability to recombine 'inactive' fractions to produce an active one (Perry et al., 2000). At this stage it is not entirely clear whether such an effect could be attributed to dose alone. Until the identity of these components are known little can be said about their merit. The more obvious sperm factor candidate is the one that is extractable (in the most part) and which causes full egg activation, as established by a number of independent groups.

Once the sperm factor has been identified, either a mouse knockout or an immunoprecipitating/functionally inhibitory antibody may go some way to addressing the above point. Alternatively, in the meantime it is possible that an egg receptor/sperm ligand interaction will be found that complements the action of the sperm factor.

Another important issue is the amount of sperm extracts being injected into eggs. Could it be that a high number of sperm equivalents are being microinjected such that we are observing a non-physiological phenomenon? *Table I* highlights the calculated sperm equivalents injected to induce Ca²⁺ oscillations for various species. These sperm equivalents are not high. It must be noted that incomplete recovery of the sperm factor from sperm and loss of activity of the sperm factor with time are two parameters not easily quantified. Therefore it would be highly unlikely to recover 100% sperm factor activity and maintain 100% activity in the extracts.

7. CALCIUM AND THE CELL CYCLE

We have addressed how the sperm probably initiates Ca²⁺ release, but once initiated how are the Ca²⁺ oscillations controlled? In mammals it seems probable that long-lasting Ca²⁺ oscillations ensure exit from meiosis. If freshly ovulated MII eggs are treated with ethanol, to induce a Ca²⁺ transient, then the eggs start to activate. They extrude a second polar body but then the egg re-arrests at metaphase (in a so-called MIII state) (Kubiak, 1989). Thus MPF activity comes back. MPF similarly returns in eggs given a single Ca²⁺ transient through electrical activation (Collas et al., 1993, 1995). Therefore, without a long-lasting series of Ca²⁺ oscillations the egg does not escape meiosis. It is certainly the case that eggs can be fully activated by a single Ca²⁺ transient or without Ca²⁺ at all (Whittingham, 1980). However, this is usually so in eggs that are aged and have passed the window in which they are

normally fertilised. Protein synthesis inhibitors and agents that cause a monotonic Ca^{2+} rise are very effective activating agents in aged eggs (Whittingham, 1980 and references therein). Thus with age it is presumed that the ability of the egg to maintain MPF and CSF activity declines. Obviously if MPF and CSF activity decline below a threshold necessary to keep the egg arrested, then activation will ensue, independent of any Ca^{2+} signal.

The influence that cell cycle resumption has on mammalian eggs is seen in the finding that Ca^{2+} oscillations stop around the time that pronuclei form in the newly formed zygote (Jones et al., 1995; Deguchi et al., 2000). Interestingly, Parrington et al. (1998) and He et al. (1999) both reported that the type 1 InsP_3 receptor is down-regulated following fertilisation. Such a down-regulation in receptor number requires InsP_3 binding to its receptor (Zhu et al., 1999). This provides further support to the suggestion that a PLC (sperm or egg) generates InsP_3 at fertilisation and also may give us a simple model to explain the cessation of Ca^{2+} oscillations. Simply, Ca^{2+} oscillations stop when the InsP_3 receptor has been critically down-regulated. However, if meiotic progression is blocked by disrupting the integrity of the metaphase spindle (spindle checkpoint) using microtubule disrupting agents and the egg remains locked in meiosis, then Ca^{2+} oscillations continue indefinitely (Jones et al., 1995). Pronuclei are formed by 6 h following sperm addition in control eggs whilst in colcemid-treated eggs no pronuclei form and we can observe Ca^{2+} oscillations continuing some 18 h post fertilisation. It is possible that the down-regulation of the InsP_3 receptor is blocked in colcemid-treated eggs or alternatively that cell cycle components are involved in maintaining/stopping the Ca^{2+} oscillations. In support of a cell cycle component, zygotes during interphase do not show Ca^{2+} oscillations but do when they undergo entry into first mitosis (Tombs et al., 1992; Kono et al., 1996). Furthermore, Ca^{2+} oscillations have been reported to last the entire period of M-phase (Kono et al., 1996). Therefore, it is suggested that the sperm factor (still present in the zygote from fertilisation) may be reactivated by factors present in the mitotic embryo, which are common to meiosis. Candidates that could induce reactivation include MPF and MAP kinase. Very recent evidence (Deng and Shen, 2000) shows that roscovitine, an inhibitor of MPF activity, can reversibly block fertilisation-associated Ca^{2+} changes in mouse eggs, implying that a high MPF activity is needed to maintain Ca^{2+} oscillations.

In the ascidian there is very good evidence that cyclin B1-dependent kinase, the principal kinase component of MPF, controls the pattern of Ca^{2+} release at fertilisation. This is based on the observation that sperm-triggered Ca^{2+} oscillations do not stop in a background of elevated MPF and low MAP kinase

activity (Levasseur and McDougall, 2000). The straightforward conclusion to be drawn from these data is that the MPF activity of the fertilised egg positively regulates the Ca^{2+} oscillations. The molecular details of how MPF accomplishes this task are not yet known. There are, however, two main possibilities that encompass most (perhaps all) of the specific ideas. These are that the MPF activity could affect the Ca^{2+} signalling system of the egg or that the MPF activity could affect the sperm factor. The first of these two hypotheses has been tested. Although there is evidence that the Ca^{2+} signalling system desensitises as the ascidian egg exits meiosis (McDougall and Levasseur, 1998), it is noteworthy that the desensitisation of the InsP_3 receptor does not completely follow the same temporal profile of the MPF activity. This was shown in the observation that the InsP_3 receptor remains sensitive during the period when the first polar body is extruded and the MPF activity is low (McDougall and Levasseur, 1998). Although these data are a useful indicator to distinguish between the two main possibilities noted above, they are not conclusive since the sensitivity of the InsP_3 receptors in the Ca^{2+} wave pacemaker was not measured. However, the observations that adenophostin B, a non-metabolisable agonist of the InsP_3 receptor, gives one long series of Ca^{2+} oscillations without a noticeable alteration in frequency (Yoshida et al., 1998), also suggest that the sensitivity of the Ca^{2+} signalling system does not completely follow the activity of MPF. Adenophostin B may be useful to determine the sensitivity of the InsP_3 receptor since the frequency of the Ca^{2+} oscillations triggered by adenophostin B decreased approximately 25 min following injection, at the time the sperm-triggered Ca^{2+} oscillations would normally stop (Yoshida et al., 1998). This is likely to indicate the desensitisation of Ca^{2+} signalling system that accompanies meiotic exit (McDougall and Levasseur, 1998). Although there are indications that the Ca^{2+} signalling system does not completely follow the activity of MPF, we still do not know for certain whether the MPF activity affects the Ca^{2+} signalling system or the sperm factor protein in fertilised ascidian eggs. To further test between the possibilities, it will be useful to examine the sensitivity of the Ca^{2+} signalling system in fertilised eggs that contain persistently elevated MPF activity.

8. CONCLUSION

This review has concentrated on the role of Ca^{2+} at fertilisation. We argue strongly that a sperm-borne Ca^{2+} -releasing factor is released into the egg following gamete fusion and it is most likely a PLC or else a PLC activator. The Ca^{2+} oscillations observed in both mammalian and ascidian eggs at fertilisation seem regulated by cell cycle factors. The relationship between the

cell cycle and Ca^{2+} oscillations at fertilisation may only be fully understood once we have identified the sperm factor.

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