Egg Activation at Fertilization: Where It All Begins

Linda L. Runft, Laurinda A. Jaffe, and Lisa M. Mehlmann

Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06030

A centrally important factor in initiating egg activation at fertilization is a rise in free Ca\(^{2+}\) in the egg cytosol. In echinoderm, ascidian, and vertebrate eggs, the Ca\(^{2+}\) rise occurs as a result of inositol trisphosphate-mediated release of Ca\(^{2+}\) from the endoplasmic reticulum. The release of Ca\(^{2+}\) at fertilization in echinoderm and ascidian eggs requires SH2 domain-mediated activation of a Src family kinase (SFK) and phospholipase C (PLC). Though some evidence indicates that a SFK and PLC may also function at fertilization in vertebrate eggs, SH2 domain-mediated activation of PLC\(\gamma\) appears not to be required. Much work has focused on identifying factors from sperm that initiate egg activation at fertilization, either as a result of sperm–egg contact or sperm–egg fusion. Current evidence from studies of ascidian and mammalian fertilization favors a fusion-mediated mechanism; this is supported by experiments indicating that injection of sperm extracts into eggs causes Ca\(^{2+}\) release by the same pathway as fertilization. © 2002 Elsevier Science (USA)

Key Words: fertilization; calcium; egg activation.

INTRODUCTION

At fertilization, the sperm activates the egg to reenter the cell cycle and begin embryonic development. The cell cycle stage at which the egg is paused until it is fertilized varies among species; for example, first meiotic prophase in the clam Spisula and in the marine worm Urechis, first meiotic metaphase in ascidians, second meiotic metaphase in almost all vertebrates, and G1 of the first mitosis in sea urchins (Stricker, 1999). In plants, the cell cycle pause and subsequent resumption at fertilization always occur after the egg has completed meiosis (Mogensen et al., 1995; Friedman, 1999). An analogous activation of the cell cycle also occurs during mating in the yeast Saccharomyces cerevisiae; the haploid cells are paused at G1, and mitosis resumes after the two cells fuse (Kim et al., 2000).

A universal feature of the egg-activation process is an increase in cytosolic free Ca\(^{2+}\) within the egg. This was first demonstrated 25 years ago in fish and sea urchin eggs (Ridgway et al., 1977; Steinhardt et al., 1977), and has subsequently been observed at fertilization in eggs of all other animal and plant species studied (Jaffe, 1985; Stricker, 1999; Antoine et al., 2000; Samuel et al., 2001). Ca\(^{2+}\) levels in the egg increase from ~0.1 to 1 \(\mu\)M, and in almost all species, this occurs as a wave or waves that cross the egg (Gilkey et al., 1978; Jaffe, 1985; Stricker, 1999).

The Ca\(^{2+}\) increase at fertilization is necessary and sufficient for restarting cell cycle events in eggs of ascidians (Sensui and Morisawa, 1996; Russo et al., 1996), vertebrates (Steinhardt et al., 1974; Kline, 1988; Kline and Kline, 1992; Yamamoto et al., 1999), and echinoderms (Steinhardt and Epel, 1974; Nomura and Nemoto, 1998; Carroll et al., 1999, 2000).

Abbreviations used: ADAM, a disintegrin and metalloprotease; ER, endoplasmic reticulum; ICSI, intracytoplasmic sperm injection; IP\(_3\), inositol 1,4,5-trisphosphate; NO, nitric oxide; PDGF, platelet derived growth factor; PIP\(_2\), phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; SFK, Src family kinase; SH2, Src homology 2.

To whom correspondence should be addressed at MSI, Trailer 342, Lagoon Rd., UCSB, Santa Barbara, CA 93106. Fax: 805-893-8062. E-mail: runft@lifesci.ucsb.edu.
The Ca\(^{2+}\) increase causes ascidian and vertebrate eggs to enter anaphase and complete meiosis, and causes sea urchin eggs, which have already completed meiosis, to undergo DNA synthesis (see Jaffe et al., 2001, for a discussion of the more complex case of starfish). These conclusions have been established by artificially elevating Ca\(^{2+}\) in eggs and, conversely, by inseminating eggs that have been injected with Ca\(^{2+}\) buffers to prevent the Ca\(^{2+}\) increase. The pathway connecting the Ca\(^{2+}\) increase to the occurrence of anaphase is only partly understood, but appears to involve a calmodulin-dependent protein kinase (Lorca et al., 1993; Johnson et al., 1998), which somehow leads to the proteolysis of securin and cyclin, which results in the proteolysis of the cohesin that holds the chromosomes together (Kawahara and Yokosawa, 1994; Aizawa et al., 1996; Peter et al., 2000; Stemmann et al., 2001). The pathway connecting the Ca\(^{2+}\) increase to DNA synthesis in echinoderm eggs appears to involve the inactivation of MAP kinase (Tachibana et al., 1997; Carroll et al., 1999, 2000; see Jaffe et al., 2001, for further discussion), but how Ca\(^{2+}\) causes this inactivation and how MAP kinase inactivation leads to DNA synthesis have not been determined.

Another incompletely understood issue is how far the embryonic cell cycle can proceed in response to artificially elevating Ca\(^{2+}\). Rabbit eggs treated with a series of electric shocks to cause Ca\(^{2+}\) elevations proceed into mitosis and about a third of the way through embryonic development (Ozil and Huneeu, 2001). However, the electrical stimuli could have consequences in addition to elevating Ca\(^{2+}\). Evidence from studies of starfish and frogs has supported the conclusion that the continuation of embryonic cell cycles, beyond the initial events triggered by Ca\(^{2+}\), requires the introduction of a centrosome, as a consequence of fertilization or experimental manipulation (Nomura and Nemoto, 1998; Tournier and Bornens, 2001).

In addition to reinitiating the cell cycle, the Ca\(^{2+}\) increase at fertilization causes other egg activation events; in particular, in many species, it causes cortical granule exocytosis, which establishes a mechanical block to polyspermy (Zucker and Steinhardt, 1978; Kline, 1988; Kline and Kline, 1992). However, some other events of fertilization are not caused by Ca\(^{2+}\); for example, in sea urchin eggs, sperm entry and pronuclear formation (Carroll et al., 1999), as well as the initial membrane conductance increase that contributes to polyspermy prevention (McCullough et al., 2000), occur independently of the Ca\(^{2+}\) rise.
Egg Activation at Fertilization

In echinoderms, ascidian, and vertebrate eggs, the Ca\(^{2+}\) increase at fertilization (Fig. 1) is caused primarily by inositol 1,4,5-trisphosphate (IP\(_3\))-mediated Ca\(^{2+}\) release from the endoplasmic reticulum (ER) (see Table 1). Other small molecules such as cyclic GMP (cGMP) (Whalley et al., 1993), cyclic ADP-ribose (cADP ribose) (Shen and Buck, 1992), and nitric oxide (Willmott et al., 1998) can also cause Ca\(^{2+}\) release in sea urchin eggs, but current evidence (see below) indicates that IP\(_3\) is the primary initiator of intracellular Ca\(^{2+}\) release at fertilization (see also Jaffe et al., 2001). It is possible that these other small molecules act in concert with IP\(_3\) to regulate the Ca\(^{2+}\) rise after it has been initiated.

This paper reviews studies of the signaling pathways that lead to IP\(_3\) production and Ca\(^{2+}\) release at fertilization and how these pathways are initiated by sperm in eggs of echinoderms, ascidians, and vertebrates. Since IP\(_3\) is generated by enzymes of the phospholipase C (PLC) family, which cleave the lipid phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) to generate IP\(_3\) and diacylglycerol (Rhee, 2001), recent studies have examined whether PLCs and PLC regulatory pathways function in mediating egg activation during fertilization. Hypotheses as to how Ca\(^{2+}\) release at fertilization is initiated include sperm contact with a protein in the egg plasma membrane (Fig. 2A), or introduction of a factor(s) into the egg as a consequence of sperm–egg fusion. One possibility is that this factor is passed from the sperm membrane to the egg membrane (e.g., ion channels, membrane tyrosine kinases, or other membrane proteins or lipids) (Fig. 2B). Studies of mating in yeast provide an example of such a mechanism; as a consequence of fusion of the two yeast cells, a G-protein-linked receptor (Ste3p) from one cell membrane interacts with a transmembrane protein (Asg7p) from the other cell membrane, and this membrane protein interaction reinitiates the mitotic cycle in the diploid zygote, although it is unknown whether the process involves a Ca\(^{2+}\) rise (Roth et al., 2000). Possibly, an analogous interaction of membrane proteins from the sperm and egg occurs at fertilization. Alternatively, the egg-activating factor could be passed from the sperm cytosol to the egg cytosol (e.g., Ca\(^{2+}\), PLC enzymes, or cytoplasmic kinases) (Figs. 2C and 2D).

Table 1: Signaling Components that Mediate Ca\(^{2+}\) Release in Eggs at Fertilization

<table>
<thead>
<tr>
<th>Echinoderms</th>
<th>Ascidians</th>
<th>Amphibians</th>
<th>Mammals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inositol trisphosphate (^{a})</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phospholipase C(_{\gamma})</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine kinase (^{b})</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


Echinoderms Ascidians Amphibians Mammals

© 2002 Elsevier Science (USA). All rights reserved.
domains are sequences of phosphotyrosine-containing sequence (Rhee, 2001). SH2 of its two tandem Src homology 2 (SH2) domains with a tyrosine kinase (or linker proteins) via a specific interaction with the activated SH2 domains of the protein. Furthermore, injection of the IP3-binding region of the PLCγ1 SH2 domains do not inhibit sperm entry, have no effect on the ability of IP3 to stimulate Ca2+ release at fertilization (Carroll et al., 1997, 1999; Shearer et al., 1999), and do not prevent PLCγ-mediated Ca2+ release in starfish eggs (Carroll et al., 1997). These results indicate that the PLCγ SH2 domains prevent Ca2+ release at fertilization by specifically inhibiting SH2-mediated activation of PLCγ.

Additional studies have shown that IP3, levels increase in echinoderm eggs at fertilization (Ciapa and Whitaker, 1986; Lee and Shen, 1998; Kuroda et al., 2001), and that injection of heparin (a nonspecific inhibitor of the IP3 receptor) can completely block the Ca2+ rise at fertilization (Mohri et al., 1995). Furthermore, injection of the IP3-binding region of the mouse IP3 receptor, which sequesters IP3, greatly reduces Ca2+ release at fertilization in starfish eggs (Iwasaki et al., 2001). Taken together with the SH2 domain experiments, these findings argue that Ca2+ release at fertilization in echinoderm eggs is mediated mainly by IP3 produced by PLCγ. cGMP and cADP ribose have also been considered as possible mediators of Ca2+ release at fertilization in sea urchin eggs, since injection of these compounds into eggs can stimulate Ca2+ release (Whalley et al., 1992; Galione et al., 1993; Lee et al., 1993, 1996), and their levels rise in eggs at fertilization before Ca2+ release (Ciapa and Epel, 1996; Kuroda et al., 2001). However, the ability of cGMP and cADP ribose is limited.
cADP ribose to induce a Ca²⁺ rise is not inhibited by PLCγ SH2 domains, unlike the Ca²⁺ rise at fertilization (Carroll et al., 1999). Also, a cGMP analog, Rp-8-pCPT-cGMP, inhibits cGMP-induced Ca²⁺ release, but not Ca²⁺ release at fertilization (Lee et al., 1996). These findings indicate that the Ca²⁺ rise at fertilization is probably not initiated by cGMP or cADP ribose.

Further characterization of how PLCγ functions in echinoderm fertilization has shown that PLCγ protein is present in sea urchin eggs, that PLCγ activity increases by 30 s after insemination, and that the PLCγ protein translocates from cytosolic to membrane fractions by 60 s after insemination (De Nadai et al., 1998; Rongish et al., 1999). In the PLCγ activity assays, the amount of sperm protein in the sample was calculated to be too small to contribute significantly to the assay, indicating that activation of an egg PLCγ accounts for the activity increase (Rongish et al., 1999). Although increased tyrosine phosphorylation of PLCγ has not been detected in sea urchin eggs after insemination, the pool of PLCγ undergoing an increase in tyrosine phosphorylation at fertilization may be very small and difficult to detect (Rongish et al., 1999). Alternatively, PLCγ may be activated by an SH2-mediated mechanism, such as translocation to the plasma membrane (Matsuda et al., 2001; Wang et al., 2001), that does not necessarily involve tyrosine phosphorylation of PLCγ itself.

Since cytoplasmic tyrosine kinases of the Src family often function in pathways leading to activation of PLCγ, by phosphorylating both PLCγ and/or linker proteins that associate with PLCγ (Rhee, 2001), a role for a Src family kinase (SFK) in initiating Ca²⁺ release at fertilization in echinoderm eggs has been examined. If the activity of a SFK mediates Ca²⁺ release at fertilization via a pathway involving PLCγ, several criteria should be fulfilled. A SFK should associate (directly or indirectly) with PLCγ in a fertilization-responsive manner, SFK activity should increase at fertilization, inhibition of SFK activity should prevent the Ca²⁺ rise at fertilization, and artificially raising SFK activity in the egg should lead to Ca²⁺ release. If the SFK operates upstream of PLCγ in the Ca²⁺ release pathway, then PLCγ SH2 domains should inhibit the Ca²⁺ rise in response to artificially raising SFK activity in the egg.

In starfish egg extracts, a 58-kDa protein that is recognized by a vertebrate SFK antibody associates with mammalian PLCγ SH2 domains by 15 s postinsemination (Giusti et al., 1999a). Tyrosine kinase activity also associates with the PLCγ SH2 domains by 15 s postinsemination. In extracts of fertilized sea urchin eggs, active PLCγ protein binds to domains of the SFK Fyn (Kinsey and Shen, 2000). These results indicate that a SFK and PLCγ directly or indirectly interact with one another at fertilization.

An increase in protein tyrosine kinase activity at fertilization of sea urchin eggs was first reported almost 20 years ago (Dasgupta and Garbers, 1983). In this initial study, the earliest time point examined was at 1 h after insemination. Subsequent studies have detected an increase in tyrosine kinase activity at earlier and earlier time points, up to as early as 15 s after insemination (Ciapa and Epel, 1991; Kinsey, 1997). More specifically, immunoprecipitation studies using an antibody raised against a peptide present in mammalian Src have shown that SFK activity increases by 30 s after insemination in sea urchin eggs (Abassi et al., 2000). Controls showed that this increase could not be accounted for by the addition of SFK present in nonfertilizing sperm in the sperm–egg mixture.

The general tyrosine kinase inhibitor genistein (Shen et al., 1999), and the SFK inhibitor PP1 (Abassi et al., 2000), both significantly delay Ca²⁺ release in sea urchin eggs at fertilization. Though genistein and PP1 can have nonspecific effects (Moore and Kinsey, 1995; Glahn et al., 1999; Sato et al., 2000; see Abram and Courtenedge, 2000), these findings hint that SFK activity may be required for the Ca²⁺ rise at fertilization. Src family SH2 domains have also been used as inhibitors of processes mediated by SFK activity (Roche et al., 1995). Injecting echinoderm eggs with Src family SH2 domains can completely inhibit the Ca²⁺ rise at fertilization, while control SH2 domains from non-Src family cytoplasmic tyrosine kinases have no effect (Giusti et al., 1999b; Abassi et al., 2000; Kinsey and Shen, 2000). This indicates that SFK activity is required for the Ca²⁺ rise at fertilization.

Injecting active mammalian SFK protein into starfish eggs stimulates intracellular Ca²⁺ release, and this release is inhibited by PLCγ SH2 domains, supporting the hypothesis that a SFK operates upstream of PLCγ at fertilization (Giusti et al., 2000). Related experiments have examined how the SFK may be activated. Though SFK SH2 domains can block the Ca²⁺ rise at fertilization, they do not inhibit Ca²⁺ release in response to injecting starfish eggs with an
Another possibility is that egg activation is due to an influx of extracellular Ca\(^{2+}\) (Armendi, 1979), which is seen as a small transient Ca\(^{2+}\) rise (Chambers and de Chambers, 1984; Lynn and Chambers, 1992), supporting the idea that sperm–egg fusion occurs at the same time as a membrane conductance increase (McCulloh and Chambers, 1992). Within milliseconds, the conductance increase causes a Ca\(^{2+}\) action potential (Chambers and de Chambers, 1979), which is seen as a small transient Ca\(^{2+}\) rise in the egg (see Fig. 1A). Thus, the conductance increase or the action potential provides a marker of the time of sperm–egg fusion, which can be compared with the time of Ca\(^{2+}\) release from the ER (see Fig. 1A). Based on such Ca\(^{2+}\) records, it has been concluded that sperm–egg fusion precedes Ca\(^{2+}\) release in echinoderm eggs by about 4–12 s (Mohri et al., 1995; Carroll et al., 1997, 1999; Giusti et al., 1999b, 2000).

One observation that favors the fusion hypothesis is that clamping the sea urchin egg's membrane potential at a positive level prevents both sperm–egg fusion, as indicated by sperm entry into the egg cytoplasm, and Ca\(^{2+}\) release, as indicated by cortical granule exocytosis, without having an obvious effect on sperm–egg binding (Jaffe, 1976). Voltage clamp studies over a range of membrane potentials have shown that Ca\(^{2+}\) release (cortical granule exocytosis) is only seen when there is at least a transient sperm–egg fusion event (as indicated by a conductance increase) (Lynn and Chambers, 1984; Lynn et al., 1988; McCulloh and Chambers, 1992), supporting the idea that sperm–egg fusion is a necessary step in the events leading to Ca\(^{2+}\) release in echinoderm eggs.

**TABLE 2**

| Experiment Examining the Initiation of Ca\(^{2+}\) Release in Eggs at Fertilization |
|---------------------------------|-------------------------------|-----------------|-----------------|-----------------|
| **Echinoderms** | **Ascidians** | **Amphibians** | **Mammals** |
| Contact with a protease, lectin, or integrin-binding peptide can cause Ca\(^{2+}\) release | + | + | + | + |
| Extracellular Ca\(^{2+}\) influx is required for Ca\(^{2+}\) release at fertilization | – | – | – | – |
| NO is required for Ca\(^{2+}\) release at fertilization | ? | – | ? | – |
| Sperm extract injection can cause Ca\(^{2+}\) release/egg activation | + | + | + | + |
| Sperm extract causes Ca\(^{2+}\) release via the same pathway as fertilization | ? | + | ? | + |


Active SFK (Giusti et al., 2000). This indicates that the step in the fertilization process that is interfered with by the SFK SH2 domains is upstream of Src activation.

In summary, evidence from echinoderm studies indicates that fertilization stimulates the SH2-mediated activation of a SFK, which either directly or indirectly activates PLC\(_\gamma\) by a SH2-mediated mechanism (Table 1, Fig. 3). PLC\(_\gamma\) activation then leads to IP\(_3\) production and Ca\(^{2+}\) release. It should be noted that the SFK SH2 and PLC\(_\gamma\) SH2 domains used in the echinoderm fertilization studies described above were derived from vertebrate proteins. Further studies using echinoderm-specific SH2 domains would be valuable.

**Initiation of the Ca\(^{2+}\) Release Pathway in Echinoderm Eggs: An Unanswered Question**

Although several components of the signal transduction pathway leading to intracellular Ca\(^{2+}\) release at fertilization have been identified, it is unknown whether sperm–egg contact or sperm–egg fusion initiates the signaling pathway (Table 2; Fig. 2). In support of the contact hypothesis (Fig. 2A) is the observation that application of proteases to echinoderm eggs can stimulate early events of egg activation, including Ca\(^{2+}\) release (Steinhardt et al., 1971; Carroll and Jaffe, 1995). In support of the fusion hypothesis (Fig. 2D), injection of an extract of sea urchin sperm into sea urchin eggs leads to egg activation (Dale et al., 1985). However, it is unknown whether proteases or sperm extract cause Ca\(^{2+}\) release by the same pathway used at fertilization. Since PLCs can be activated by 1–10 \(\mu M\) Ca\(^{2+}\) (Wahl et al., 1992; Jhon et al., 1993; Hwang et al., 1996; Rhee, 2001), another possibility is that egg activation is due to an influx of extracellular Ca\(^{2+}\) into the egg after sperm–egg fusion through Ca\(^{2+}\) channels located in the sperm membrane (Fig. 2C; see Créton and Jaffe, 1995). This idea, however, is not consistent with the observation that echinoderm eggs can be activated by fertilization in a low-Ca\(^{2+}\) medium (44 nM Ca\(^{2+}\)) (Schmidt et al., 1982).

Experiments examining the timing of fertilization events in sea urchin eggs have indicated that both contact and fusion precede the release of intracellular Ca\(^{2+}\), such that either of these events could be the cause of Ca\(^{2+}\) release. The time of sperm–egg fusion has been determined by measurements of membrane capacitance, which is proportional to membrane surface area, and these measurements have indicated that sperm–egg fusion occurs at the same time as a membrane conductance increase (McCulloh and Chambers, 1992). Within milliseconds, the conductance increase causes a Ca\(^{2+}\) action potential (Chambers and de Armandi, 1979), which is seen as a small transient Ca\(^{2+}\) rise in the egg (see Fig. 1A). Thus, the conductance increase or the action potential provides a marker of the time of sperm–egg fusion, which can be compared with the time of Ca\(^{2+}\) release from the ER (see Fig. 1A). Based on such Ca\(^{2+}\) records, it has been concluded that sperm–egg fusion precedes Ca\(^{2+}\) release in echinoderm eggs by about 4–12 s (Mohri et al., 1995; Carroll et al., 1997, 1999; Giusti et al., 1999b, 2000).
Recently, Kuo et al. (2000) presented evidence that the sperm-induced Ca\(^{2+}\) rise in sea urchin eggs at fertilization may be mediated by nitric oxide (NO), produced in the egg at fertilization due to the introduction of NO synthase from the sperm. NO is believed to stimulate Ca\(^{2+}\) release in sea urchin eggs via a cGMP/protein kinase G-mediated pathway that leads to cADP ribose production; cADP ribose may stimulate Ca\(^{2+}\) release from ryanodine receptors (a type of Ca\(^{2+}\) channel found in the ER of some cells) (Galione et al., 1993; Lee et al., 1993; Willmott et al., 1996). Kuo et al. (2000) showed that, based on an increase in fluorescence of dianino fluorescein, NO rises in the egg at fertilization, that NO donors stimulate egg activation, and that oxyhemoglobin (an NO scavenger) reduces the amplitude of the Ca\(^{2+}\) rise at fertilization (Kuo et al., 2000). However, other findings are not consistent with the conclusion that NO mediates the Ca\(^{2+}\) rise at fertilization. The protein kinase G inhibitor, Rp-8-pCPT-cGMPs, blocks Ca\(^{2+}\) release in sea urchin eggs in response to NO (Willmott et al., 1996), but has no effect on the Ca\(^{2+}\) rise at fertilization (Lee et al., 1996).

ASCIDIANs

Ca\(^{2+}\) Release in Ascidian Eggs Occurs by a Pathway Similar to That in Echinoderms

Ascidians are marine invertebrates that are grouped with vertebrates in the phylum Chordata. Experiments like those described above for echinoderm eggs have indicated that the pathway leading to Ca\(^{2+}\) release at fertilization in ascidian eggs also involves a SFK, PLC\(\gamma\), and IP\(_3\) (Table 1; Fig. 3). In particular, IP\(_3\) is produced at fertilization (Tortonan and Yokosawa, 1995), introducing IP\(_3\) causes Ca\(^{2+}\) release (McDougall and Sardet, 1995; Albrieux et al., 1997; Yoshida et al., 1998), and injecting PLC\(\gamma\) SH2 or Src family SH2 domains completely and specifically inhibits Ca\(^{2+}\) release at fertilization (Runft and Jaffe, 2000). In addition, ruthenium red, an inhibitor of the ryanodine receptor, does not inhibit Ca\(^{2+}\) release at fertilization in ascidian eggs (Wilding and Dale, 1998; Yoshida et al., 1998), consistent with an IP\(_3\)-mediated pathway. The requirement for tyrosine kinase activity is further supported by the inhibition of egg activation events at fertilization by the tyrosine kinase inhibitor erbstatin (Ueki and Yokosawa, 1997).

Initiation of the Ca\(^{2+}\) Release Pathway in Ascidian Eggs: Evidence for an Activator Protein from the Sperm Cytosol

In ascidians, current evidence favors the fusion hypothesis for how the sperm initiates Ca\(^{2+}\) release at fertilization (Table 2; Fig. 2). Although externally applied lectins can stimulate Ca\(^{2+}\) release and other early events of egg activation (Zalokar, 1980; Speksnijder et al., 1990; Flannery and Epel, 1998), there is no evidence as to whether such a contact-mediated mechanism operates at fertilization. Extracellular Ca\(^{2+}\) influx has also been considered as a possible initiator of Ca\(^{2+}\) release, but is not likely since asidian eggs can be activated by fertilization in very low Ca\(^{2+}\)-containing media (Speksnijder et al., 1989, 1990; Sensui and Morisawa, 1996).

In support of the fusion hypothesis, injecting an extract of asidian sperm into asidian eggs stimulates Ca\(^{2+}\) release that is temporally and spatially similar to the Ca\(^{2+}\) rise that occurs at fertilization (Dale, 1988; Wilding and Dale, 1998; Kyozuka et al., 1998; McDougall et al., 2000; Nixon et al., 2000; Runft and Jaffe, 2000). Extract from approximately one to three sperm is sufficient to stimulate egg activation (Kyozuka et al., 1998; Runft and Jaffe, 2000). Injecting PLC\(\gamma\) SH2 domains or Src family SH2 domains into asidian eggs blocks Ca\(^{2+}\) release in response to sperm extract, indicating that fertilization and sperm extract injection use the same signaling molecules to initiate Ca\(^{2+}\) release (Table 2). These findings support the hypothesis that asidian egg activation at fertilization is initiated by a cytosolic factor introduced from the sperm into the egg following sperm–egg fusion (Fig. 2D).

The activating factor(s) from asidian sperm has been partially characterized. It is heat- and trypsin-sensitive and is therefore most likely a protein (Kyozuka et al., 1998; Runft and Jaffe, 2000; McDougall et al., 2000). It has a molecular weight between 30 and 100 kDa, and its activity is not reduced by high-speed centrifugation of the sperm extract or by protease inhibitors in the extraction buffer, indicating that it is soluble and probably not a protease (Wilding and Dale, 1998; Runft and Jaffe, 2000; Hyslop et al., 2001). The activating factor from the sperm extract appears to operate via a cytoplasmic mechanism only, as applying it to the surface of asidian eggs does not cause Ca\(^{2+}\) release (Runft and Jaffe, 2000). It also appears to be sperm-specific, because injecting asidian eggs with an extract of asidian ovary does not stimulate Ca\(^{2+}\) release (Runft and Jaffe, 2000). However, the activating factor may not be species-specific, as injecting human sperm extract into asidian eggs also stimulates egg activation (Wilding et al., 1997).

Since NO synthase has been proposed as an activating factor from sperm in sea urchin fertilization (Kuo et al., 2000), the role of NO in asidian fertilization has recently been examined. Though NO can stimulate Ca\(^{2+}\) release in asidian eggs, a rise in NO has not been detected at fertilization (Hyslop et al., 2001). Furthermore, treating asidian eggs with an inhibitor of the NO-mediated Ca\(^{2+}\) release signaling pathway (N\(^{\text{2-}}\)-nitro-L-arginine methyl ester) has no effect on Ca\(^{2+}\) release at fertilization (Hyslop et al., 2001). These results indicate that Ca\(^{2+}\) release at fertilization in asidian eggs is probably not mediated by NO.

Because Src family SH2 domains can inhibit Ca\(^{2+}\) release induced by sperm extract (Runft and Jaffe, 2000), the activating factor in the extract may be a regulator, directly or indirectly, of an egg SFK. Thus, SFK regulators known from studies of somatic cells, including kinases and phosphatases, and molecules that bind the SH2 and SH3 domains of
SFKs (see Abram and Courtneidge, 2000) are possible candidates for the activating factor in ascidian sperm.

VERTEBRATES

The Pathway Leading to Ca\(^{2+}\) Release at Fertilization in Vertebrate Eggs Requires IP\(_3\) but Not SH2-Mediated Activation of PLC \(\gamma\)

As in echinoderms and ascidians, intracellular Ca\(^{2+}\) release at fertilization in vertebrate eggs is mediated by IP\(_3\) (Table 1). IP\(_3\) levels rise in Xenopus eggs at fertilization (Stith et al., 1993; Snow et al., 1996; Sato et al., 2000), and injecting IP\(_3\) into frog, fish, and mammalian eggs can stimulate a rise in intracellular Ca\(^{2+}\) (Busa et al., 1985; Iwamatsu et al., 1988; Miyazaki, 1988). A monoclonal antibody directed against the C-terminal domain of the mouse type I IP\(_3\) receptor can completely block Ca\(^{2+}\) release and other events of egg activation at fertilization of hamster and mouse eggs (Miyazaki et al., 1992, 1993; Xu et al., 1994). A similar mammalian IP\(_3\) receptor antibody largely inhibits the Ca\(^{2+}\) rise at fertilization of Xenopus eggs, though small, localized rises in Ca\(^{2+}\) do occur (Fontanilla and Nuccitelli, 1998; Runft et al., 1999). It is unclear whether these local Ca\(^{2+}\) rises result from an IP\(_3\)-independent pathway or because the antibody is not a complete inhibitor; the latter is suggested by the finding that the antibody only partially inhibits Ca\(^{2+}\) release in response to injected IP\(_3\) (Runft et al., 1999). The importance of IP\(_3\) in mediating Ca\(^{2+}\) release at fertilization in vertebrate eggs indicates that PLCs function in this pathway, and consistently, the PLC inhibitor U73122 has an inhibitory effect on Ca\(^{2+}\) release at fertilization in mouse and frog eggs (Dupont et al., 1996; Sato et al., 2000). However, U73122 can have nonspecific effects as discussed above (see Echinoderms).

Signaling pathways for both G-protein-PLC\(\beta\)-mediated and tyrosine kinase-PLC\(\gamma\)-mediated intracellular Ca\(^{2+}\) release are present in vertebrate eggs, as indicated by the ability of frog and mouse eggs to activate after the stimulation of exogenously expressed receptors that function through these pathways (Kline et al., 1988; Williams et al., 1992; Moore et al., 1993; Yim et al., 1994; Mehlmann et al., 1998; Runft et al., 1999). To examine whether a G-protein is required for Ca\(^{2+}\) release at fertilization, a general inhibitor of GTP-dependent events, GDP-\(\beta\)-S, was used initially. GDP-\(\beta\)-S prevents Ca\(^{2+}\) release and other early events of egg activation in hamster and mouse eggs (Miyazaki, 1988; Moore et al., 1994), but whether this is due to inhibition of a heterotrimeric G-protein or some other GTP-dependent process is uncertain. In Xenopus, attempts to test the requirement for a G-protein using GDP-\(\beta\)-S were inconclusive due to nonspecific effects (Kline et al., 1990). More recently, mouse and Xenopus eggs have been treated with more specific inhibitors of G-proteins, including pertussis toxin (which inhibits G\(_{i}\) family members G\(_{i}\) and G\(_{o}\)), and an antibody that blocks G\(_{o}\) activation by 7-transmembrane receptors. Although pertussis toxin and the G\(_{o}\) antibody were shown to be effective inhibitors of the corresponding G-proteins in the egg, neither has an effect on Ca\(^{2+}\) release at fertilization (Kline et al., 1991; Moore et al., 1994; Williams et al., 1998; Runft et al., 1999). Likewise, using phosducin or excess G-protein \(\alpha\) subunits in the GDP-bound form to sequester G-protein \(\beta\gamma\) subunits does not inhibit early events of egg activation, including the ZP2-to-ZP2, transition in mouse (Moore et al., 1994) and Ca\(^{2+}\) release in frog (Runft et al., 1999). These results argue against a function for a heterotrimeric G-protein and PLC\(\beta\) in mediating Ca\(^{2+}\) release at fertilization in mouse and frog eggs.

In frog eggs, PLC\(\gamma\) activity increases significantly within 2 min after insemination and PLC\(\gamma\) tyrosine phosphorylation also appears to increase (Sato et al., 2000), but one uncertainty is the possible contribution of nonfertilizing sperm in the sperm-egg mixture to these assays (see Rongish et al., 1999). Whether this increase in PLC\(\gamma\) activity is required for Ca\(^{2+}\) release at fertilization in frog eggs is unknown. Although PLC\(\gamma\) SH2 domains block PLC\(\gamma\)-mediated Ca\(^{2+}\) release in response to platelet-derived growth factor (PDGF) in mouse and frog eggs expressing PDGF receptors, the PLC\(\gamma\) SH2 domains do not inhibit Ca\(^{2+}\) release at fertilization in these eggs, even at a 10\(^{\times}\) higher concentration than that used to inhibit PDGF-induced Ca\(^{2+}\) release (Mehlmann et al., 1998; Runft et al., 1999). These findings demonstrate that the mechanism of Ca\(^{2+}\) release at fertilization in vertebrate eggs differs from that in echinoderms and ascidians in that it does not require SH2-mediated activation of PLC\(\gamma\) (Table 1). Coinjecting frog eggs with both PLC\(\gamma\) SH2 domains and the G\(_{o}\) antibody does not inhibit the Ca\(^{2+}\) rise at fertilization either, arguing against the possibility that G\(_{o}\)-mediated activation of PLC\(\beta\) and SH2-mediated activation of PLC\(\gamma\) operate redundantly at fertilization (Runft et al., 1999). PLC\(\gamma\) activity can also be stimulated by its translocation to the plasma membrane by a pathway requiring phosphatidylinositol-3-kinase (PI3 kinase) (Rhee, 2001). However, in mouse eggs, fertilization-induced Ca\(^{2+}\) release is not inhibited by the PI3 kinase inhibitor wortmannin (Mehlmann et al., 2001).

In summary, though IP\(_3\) is required for Ca\(^{2+}\) release at fertilization in vertebrate eggs and studies using the general PLC inhibitor U73122 suggest a role for PLC activity in the Ca\(^{2+}\) release pathway, which PLC subtype is involved has not been conclusively determined.

Evidence That a Tyrosine Kinase Functions in Egg Activation at Fertilization in Vertebrates

In zebrafish eggs, a SFK is activated at fertilization, as demonstrated by increased tyrosine kinase activity in immunoprecipitates of the SFK Fyn from inseminated eggs (Wu and Kinsey, 2000). This increase occurs within 30 s postinsemination, and cannot be accounted for by Fyn activity present in nonfertilizing sperm in the insemination mixture. Likewise, studies of Xenopus eggs indicate that
the kinase activity of a partially purified SFK called Xyk increases by 10 min postinsemination (K. Sato et al., 1996, 1999). One approach to examine whether Xyk functions in causing Ca\(^{2+}\) release at fertilization in frog eggs has been to immunoprecipitate PLC\(\gamma\) from frog egg lysates at various times after insemination, and to examine whether active Xyk coimmunoprecipitates (Sato et al., 2000). These experiments showed that tyrosine kinase activity was present in PLC\(\gamma\) immunoprecipitates made from eggs at 2 min postinsemination, and suggested that the activity was due to Xyk.

Studies using tyrosine kinase inhibitors have provided further evidence that tyrosine kinase activity is required for the Ca\(^{2+}\) rise at fertilization in Xenopus eggs. The tyrosine kinase inhibitor lavendustin inhibits Ca\(^{2+}\) release at fertilization in frog eggs (Glahn et al., 1999), as do two inhibitors that are considered to be relatively specific for Src family kinases, the peptide A7 and the pharmacological inhibitor PP1 (K. Sato et al., 1999, 2000). However, A7 and PP1 also inhibit sperm–egg fusion, raising the question of whether the action of these inhibitors is on egg activation or on an earlier sperm function. Furthermore, it remains uncertain how a tyrosine kinase might lead to Ca\(^{2+}\) release at fertilization in frog eggs, since PLC\(\gamma\) SH2 domains do not inhibit Ca\(^{2+}\) release at fertilization in this species (Runft et al., 1999).

In mouse eggs, several tyrosine kinase inhibitors delay Ca\(^{2+}\) release at fertilization and reduce the number of Ca\(^{2+}\) oscillations (Dupont et al., 1996). However, since these inhibitors were applied extracellularly, the delay could result from an effect on sperm function prior to egg activation. A further indication that a tyrosine kinase, and in particular a SFK, may function in mammalian fertilization comes from a study showing that Fyn is localized to the region of sperm entry in rat eggs observed at 1 h after fertilization (Talmor et al., 1998). Although these findings provide hints that a SFK may function in mammalian fertilization, further studies are needed to determine whether and how this might occur.

What Initiates Ca\(^{2+}\) Release at Fertilization in Amphibian Eggs?

Both sperm-contact and soluble sperm factor hypotheses (Table 2; Fig. 2) have been examined in amphibian eggs. Although there is, as yet, no answer as to which of these mechanisms operates at fertilization, results supporting each are summarized below. In support of the contact hypothesis (Fig. 2A), the protease cathepsin B causes an increase in intracellular Ca\(^{2+}\) when applied to Xenopus eggs (Mizote et al., 1999). A protease from sperm of the newt Cynops also induces early events of egg activation when applied to Xenopus eggs (Mizote et al., 1999). A similar protease is present in Xenopus sperm extract (Mizote et al., 1999; see Iwao, 2000), but has not been applied to Xenopus eggs. Fertilization in Xenopus can be blocked by incubating eggs with protease inhibitors or with substrate peptides for the Cynops sperm protease (Mizote et al., 1999). These inhibitors may prevent the sperm protease from acting on egg molecules involved in activation; it is also possible, however, that these inhibitors block molecules involved in the acrosome reaction and/or other sperm functions prior to egg activation.

Other evidence suggests that Ca\(^{2+}\) release is initiated in frog eggs when a disintegrin molecule on the sperm surface contacts an integrin on the egg surface (see Shilling et al., 1998). Applying peptides containing the integrin-binding amino acid sequence RGDS to Xenopus eggs can induce Ca\(^{2+}\) release, cortical contraction, and resumption of meiosis (Iwao and Fujimura, 1996; K. Sato et al., 1999). A cDNA encoding a metalloprotease/disintegrin termed xMDC16 has been isolated from Xenopus testis (Shilling et al., 1997), and applying a peptide derived from the disintegrin region of xMDC16 to Xenopus eggs can either induce intracellular Ca\(^{2+}\) release or block fertilization, depending on the peptide concentration used (Shilling et al., 1997; 1998). Though the protease, RGDS, and xMDC16 studies show that Xenopus eggs are capable of being activated by a contact-stimulated mechanism, the egg molecules with which the examined proteases and disintegrins interact have not been identified, and whether a protease or disintegrin on the sperm surface operates at fertilization is unknown.

Xenopus eggs can activate when fertilized in a Ca\(^{2+}\)-free medium (Wilkinson et al., 1998), arguing against egg activation by means of extracellular Ca\(^{2+}\) influx through sperm Ca\(^{2+}\) channels after sperm–egg fusion (Table 2; Fig. 2C). In support of the idea that sperm–egg fusion introduces an activating factor from the sperm cytoplasm into the egg (Fig. 2D), is a study showing that injection of newt sperm extract into newt eggs stimulates a Ca\(^{2+}\) rise similar both temporally and spatially to that at fertilization (Yamamoto et al., 2001). Although an extract of Xenopus sperm can stimulate Ca\(^{2+}\) release when injected into mouse eggs, this extract has not been injected into Xenopus eggs (Dong et al., 2000). The identity of the factor in the newt or Xenopus sperm extract that causes a Ca\(^{2+}\) rise in eggs, and the necessity of this factor for fertilization, have yet to be investigated.

What Initiates Ca\(^{2+}\) Release at Fertilization in Mammalian Eggs?

In the past several years, much progress has been made in identifying fertilization-related molecules in the plasma membranes of mammalian sperm and eggs. In particular, current evidence indicates that the binding of the sperm and egg membranes may involve the binding of an ADAM (a disintegrin and metalloprotease) family protein in the sperm (see Cho et al., 1998; Nishimura et al., 2001) to an unknown receptor in the egg (see Miller et al., 2000). The subsequent fusion of the sperm and egg membranes depends on the presence in the egg membrane of CD9, a member of the tetraspanin family of proteins (Chen et al., 1999; Le Naour et al., 2000; Miyado et al., 2000; Kaji et al., 2000; Miller et al., 2000). A glycosyl-phosphatidylinositol
anchored protein in the egg membrane also appears to be 
required for fusion (Coonrod et al., 1999). However, there is 
no evidence that any of these proteins have a signaling 
function in initiating Ca\(^{2+}\) release in eggs.

Investigations of how fertilization activates Ca\(^{2+}\) release 
in mammalian eggs have pointed to a mechanism in which 
an activating protein from the sperm cytoplasm is intro-
duced into the egg cytoplasm. One exception to this con-
clusion is a study in which application of a peptide contain-
ing the RGD sequence recognized by integrins caused two 
Ca\(^{2+}\) transients in bovine eggs (Campbell et al., 2000). 
Although this finding supports the possibility that sperm– 
egg contact could cause Ca\(^{2+}\) release, evidence reviewed 
below argues more strongly for a fusion-mediated mecha-
nism.

**Insights from ICSI**

The technique of intracytoplasmic sperm injection (ICSI) 
has become widely used in assisted reproduction and has 
provided some information about the basic science of 
fertilization. Mammalian eggs injected with whole mam-
malian sperm can exhibit normal events of egg activation, 
including Ca\(^{2+}\) release (Tesarik et al., 1994; Nakano et al., 
1997; Meng and Wolf, 1997; M. S. Sato et al., 1999), and 
can develop to term (Palermo et al., 1992; Van Steirteghem et 
al., 1993; Kimura and Yanagimachi, 1995a; Kuretake et al., 
1996; Kimura et al., 1998). This suggests that contact of 
sperm and egg plasma membranes is not a critical step for 
egg activation. Studies using ICSI have shown that only 
the sperm head is critical for egg activation and subsequent 
embryonic development (Kuretake et al., 1996; Meng and 
Wolf, 1997; Kimura et al., 1998). Injecting sperm at various 
stages of spermiogenesis has demonstrated that elongating 
spermatids can activate eggs, whereas round spermatids and 
secondary spermatocytes cannot (Ogura et al., 1994; 
Kimura and Yanagimachi, 1995b; Sousa et al., 1996; 
Tesarik, 1998; Sato et al., 1998; Sakurai et al., 1999). Egg 
activation and subsequent development following ICSI are 
sperm-specific and not the result of artifactual introduction 
of Ca\(^{2+}\) into the egg from the outside medium; while there 
is often an initial release of Ca\(^{2+}\) upon insertion of the 
injectation pipet, neither this initial Ca\(^{2+}\) release nor injecting 
culture medium alone activates eggs (Tesarik et al., 1994; 
Dozortsev et al., 1995a; Laybaert et al., 1996; Nakano et al., 
1997; M. S. Sato et al., 1999). ICSI initiates Ca\(^{2+}\) oscillations in eggs resembling those 
seen at fertilization, beginning 4–10 h after sperm injection 
into human eggs (Tesarik et al., 1994) and 15–30 min after 
sperm injection into mouse eggs (Nakano et al., 1997; M. S. 
Sato et al., 1999). Interestingly, injected sperm are often 
more effective at causing resumption of meiosis if the 
sperm plasma membrane is first disrupted or removed, 
perhaps allowing an activating substance (see below) to 
diffuse from the sperm more easily (Tesarik and Sousa, 
1995; Fishel et al., 1995; Dozortsev et al., 1995b; Kasai et 
al., 1999). In summary, the ability of an injected sperm to 
cause egg activation is consistent with the hypothesis that 
a substance from the sperm is transferred to the egg follow-
ing sperm–egg fusion. However, it is not certain that these 
mechanisms are the same as those that operate during 
natural fertilization.

**Sperm-Egg Fusion and Activation of Mammalian 
Eggs**

In in vitro fertilization, sperm–egg fusion precedes mam-
nalian egg activation. Dye transfer studies that can deter-
mine the time of sperm–egg fusion, in parallel with moni-
toring intracellular Ca\(^{2+}\) levels, show that sperm–egg fusion 
occurs ~1–5 min before the initial rise in Ca\(^{2+}\) in mouse 
eggs (Lawrence et al., 1997; Jones et al., 1998a). The idea 
that the sperm contributes to the initial Ca\(^{2+}\) rise by acting 
as a conduit to allow an influx of Ca\(^{2+}\) into the egg through 
the sperm membrane at sperm–egg fusion (Fig. 2C) is ruled 
out by the observations that there is no localized increase of 
Ca\(^{2+}\) in the mouse egg near the site of sperm–egg fusion, 
and that normal Ca\(^{2+}\) release occurs in eggs fertilized at very 
low (13 nM) extracellular Ca\(^{2+}\) concentrations (Jones et al., 
1998a). Therefore, Ca\(^{2+}\) release following sperm–egg fusion 
does not appear to be the result of an increased plasma 
membrane permeability to Ca\(^{2+}\). A more likely explanation 
for how the sperm causes Ca\(^{2+}\) release in mammalian eggs is 
that it transfers a Ca\(^{2+}\)-releasing substance(s) to the egg 
following sperm–egg fusion.

In mammalian eggs, the “fusion hypothesis”— 
specifically, the idea that the sperm introduces a soluble 
activating substance into the egg upon sperm–egg fusion 
(Fig. 2D)—has been studied considerably in the last decade. 
The first evidence in support of this hypothesis came from 
experiments demonstrating that injection of extracts pre-
pared from hamster or boar sperm induced Ca\(^{2+}\) oscillations 
in hamster eggs similar to those seen at fertilization 
(Swann, 1990). Since then, many groups have confirmed 
that sperm extracts from a number of mammalian species 
cause Ca\(^{2+}\) release in eggs similar to those seen at fertiliza-
tion (Swann, 1990, 1994; Homa and Swann, 1994; Sousa et 
al., 1996; Wu et al., 1997; Palermo et al., 1997; Sakurai et 
al., 1999). In addition to causing Ca\(^{2+}\) release, sperm 
extracts fully activate eggs, causing cortical granule exocy-
tosis, second polar body formation, pronuclear formation, 
and cleavage (Meng and Wolf, 1997; Wu et al., 1998; Sakurai et 
al., 1999; Gordo et al., 2000). The Ca\(^{2+}\)-releasing ability 
of sperm extracts is not species-specific and is abolished after 
heating and protease treatment, indicating that the active 
substance is a protein (Swann, 1990; Wu et al., 1997).

Despite widespread agreement that sperm extracts con-
tain a protein(s) that causes egg activation when injected 
into eggs, two major issues remain to be resolved. First, this 
protein(s) has not been identified; and second, it still re-
mains to be shown whether this protein(s) is the physiolog-
ical activator of eggs at fertilization. The finding that a 
function-blocking antibody against the IP₃ receptor blocks 
sperm extract-induced Ca\(^{2+}\) release in mouse eggs (Oda et
In Search of the Elusive Sperm Factor

There have been several candidates suggested for the egg-activating protein from the sperm. The first was a ~33-kDa protein that had high sequence homology with the bacterial enzyme glucosamine-6-phosphate isomerase. This protein was partially purified from hamster sperm lysate and was termed "oscillin" (Parrington et al., 1996). Oscillin is found in fractions of hamster sperm lysate that contain Ca\(^{2+}\)-releasing activity (Parrington et al., 1996; Swann and Lai, 1997; Wolny et al., 1999). Oscillin is also present in sperm of other species, including human (Wolny et al., 1999; Montag et al., 1998).

However, recombinant oscillin, though enzymatically active, does not cause Ca\(^{2+}\) release when injected into mouse eggs (Wolosker et al., 1998; Shevchenko et al., 1998; Wolny et al., 1999). In addition, immunodepletion of oscillin from sperm extract fails to abolish the Ca\(^{2+}\)-releasing ability of the extract (Wolny et al., 1999). Furthermore, oscillin is expressed in a wide variety of tissues (Nakamura et al., 2000; Amireault and Dubé, 2000), whereas the protein from the sperm that causes Ca\(^{2+}\) release appears to be sperm-specific, as injection of tissue extracts from brain (Wu et al., 1997; Jones et al., 2000), kidney, and liver (Jones et al., 2000) into eggs fails to cause Ca\(^{2+}\) release. Therefore, although oscillin is present in mammalian sperm, it is not likely to be the egg-activating protein.

Another protein proposed to be the activating factor in sperm extract is the truncated form of the c-kit tyrosine kinase receptor (tr-kit) (Sette et al., 1997, 1998). Tr-kit is a 90-kDa protein that accumulates in elongating spermatids during spermiogenesis (Albanesi et al., 1996). Injecting extracts of COS cells expressing recombinant tr-kit into mouse eggs has been reported to cause egg activation events, such as cortical granule exocytosis, second polar body formation, and pronuclear formation. These events are inhibited by preincubating eggs in BAPTA-AM, suggesting that tr-kit is acting by causing Ca\(^{2+}\) release (Sette et al., 1997). Tr-kit protein stimulates PI\(_3\) hydrolysis and tyrosine phosphorylation of PLC\(\gamma_1\) when transfected in COS cells (Sette et al., 1998), adding to its attraction as a candidate for the sperm Ca\(^{2+}\)-releasing protein. When coexpressed with the SH3 domain of PLC\(\gamma_1\), the ability of tr-kit to activate eggs is significantly reduced, suggesting that tr-kit activates PLC\(\gamma_1\) in some way that involves PLC\(\gamma_1\)'s SH3 domain. However, injecting the PLC\(\gamma_1\) SH3 domain into mouse eggs at a much higher concentration does not inhibit Ca\(^{2+}\) release at fertilization (Mehlmann et al., 1998), indicating that tr-kit releases Ca\(^{2+}\) by a pathway differing from that operating at fertilization. Recombinant tr-kit protein has not yet been purified and shown to cause Ca\(^{2+}\) oscillations when injected into eggs; such experiments need to be done in order to test whether tr-kit has a role in initiating Ca\(^{2+}\) release at fertilization.

More recently, PLC, the enzyme that produces IP\(_3\), has been implicated as the Ca\(^{2+}\)-releasing factor from sperm. In support of this hypothesis, injection of recombinant PLC\(\gamma_1\) protein into mouse eggs causes Ca\(^{2+}\) oscillations that closely resemble those seen at fertilization (Mehlmann et al., 2001). PLC\(\gamma_1\), PLC\(\gamma_2\), and PLC\(\beta_1\) are present in mammalian sperm (Dupont et al., 1996; Tomes et al., 1996; Mehlmann et al., 1998; Wu et al., 2001; Fukami et al., 2001), and there is at least one report that PLC\(\beta_1\) is present as well (Walesky and Snyder, 1995; but see Mehlmann et al., 2001). Other PLC isoforms have not yet been identified in sperm. Sperm lysates have measurable PLC activity (Ribbes et al., 1987; Tomes et al., 1996; Jones et al., 2000; Rice et al., 2000; Mehlmann et al., 2001), and boar sperm extracts cause the production of IP\(_3\) when added to sea urchin egg homogenates (Jones et al., 1998b; Rice et al., 2000). The PLC inhibitor, U73122, inhibits Ca\(^{2+}\)-releasing activity in boar sperm lysates (Jones et al., 2000; Wu et al., 2001). However, these results are difficult to interpret because the concentration of U73122 used was higher than those concentrations shown to have nonspecific effects (see Echinoderms).

Of the PLCs present in sperm, PLC\(\gamma_1\), PLC\(\gamma_2\), and PLC\(\beta_1\) are not present in fractions of sperm extracts containing Ca\(^{2+}\)-releasing ability (Heyers et al., 2000; Wu et al., 2001). In addition, sperm from mice lacking PLC\(\beta_1\) cause Ca\(^{2+}\) release when injected into mouse eggs (Fukami et al., 2001). It has been reported that the PLC activity in a single boar sperm is sufficient to generate enough IP\(_3\) to activate an egg, based on incubation of sperm extract with PI\(_3\) and measurements of the IP\(_3\) produced (Rice et al., 2000). However, the concentration of PI\(_3\) present in the reaction mixture was not specified to be the same as that present in an egg, and may have been considerably higher. In another study, measurements of the PLC activity in a single mouse sperm were compared with the PLC activity in the minimum amount of PLC\(\gamma\) protein needed to cause Ca\(^{2+}\) release in mouse eggs. The PLC activity per sperm was found to be ~500–900 times less than the PLC activity in the amount of recombinant PLC\(\gamma\) required for Ca\(^{2+}\) release, indicating that sperm-derived PLC is not responsible for initiating Ca\(^{2+}\) release at fertilization (Mehlmann et al., 2001).

Another potential candidate for the soluble sperm protein is NO synthase, which could cause production of NO in the egg and cause Ca\(^{2+}\) release through a cGMP pathway (Willmott et al., 1996). One report suggests that NO might have a role in fertilization of sea urchin eggs (Kuo et al., 2000; see Echinoderms). However, a subsequent study that examined a role for NO at fertilization of mammalian eggs indicates that NO is not the Ca\(^{2+}\)-releasing agent (Hyslop et al., 2001).

One of the potential shortcomings of the aforementioned studies of a soluble egg-activating protein from the sperm is that the extracts are prepared from sperm that have not been incubated in conditions that promote the acrosome activation at fertilization (Table 2).
reaction. Therefore, these extracts contain acrosomal contents that would not normally be present in the natural fertilization setting.

Recent experiments showing that injection of demembranated sperm heads activates mouse eggs have suggested that the sperm-derived egg-activating protein may be associated with the sperm perinuclear material (Kuretake et al., 1996; Kimura et al., 1998). Sperm heads treated with the detergent Triton X-100 are completely demembranated and lack the acrosomal vesicle and cytoplasmic contents, though they retain the perinuclear matrix that surrounds the nucleus. Injection of such sperm heads into mouse eggs causes polar body and pronuclear formation, followed by normal development; live offspring have been obtained in this way. Sperm heads treated with Triton X-100 followed by trypsin or SDS, which remove the perinuclear matrix (but might also inactivate a Ca\(^{2+}\)-releasing protein), are incapable of activating eggs (Kimura et al., 1998). These results support the hypothesis that the factor from the sperm that activates eggs is associated with the sperm perinuclear material, but the active component in this material has not been identified (see Perry et al., 1999, 2000). It is possible that the sperm contains both soluble proteins as well as perinuclear matrix-associated proteins that serve as redundant mechanisms to ensure that the sperm will be able to activate the egg. Alternatively, it is possible that the same egg-activating protein is present in both fractions.

In summary, current evidence favors the hypothesis that, during mammalian fertilization, a protein present in the sperm enters the egg cytoplasm as a consequence of sperm–egg fusion, and causes egg activation. However, the identification of this protein remains a problem for the next century of fertilization research.

ACKNOWLEDGMENTS

We thank Dave Carroll, Kathy Foltz, and Becky Kalinowski for useful discussions and comments on the manuscript. This work was supported by a grant from the N.I.H. to L.A.J., and by a postdoctoral fellowship from the Lalor Foundation to L.M.M.

REFERENCES


Egg Activation at Fertilization


Egg Activation at Fertilization

is not required for the rise in calcium during fertilization. Dev. Biol. 180, 324–335.


Received for publication July 16, 2001
Revised January 4, 2002
Accepted January 4, 2002
Published online April 16, 2002

© 2002 Elsevier Science (USA). All rights reserved.