

Does a Soluble Sperm Factor Trigger Calcium Release in the Egg at Fertilization?

Review

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Changes in intracellular calcium (Ca^{2+}) constitute one of the central signaling mechanisms utilized by living cells. One important physiological process in which Ca^{2+} signaling plays a central role is the activation of an egg by a sperm at fertilization. In all species studied, egg activation is triggered by a rise in Ca^{2+} levels within the egg (Jaffe, 1983; Whitaker and Swann, 1993; Stricker, 1999). In the overwhelming majority of species, a sperm induces a release of Ca^{2+} from an egg's intracellular stores (Whitaker and Swann, 1993; Stricker, 1999). In only a few species does the Ca^{2+} rise appear to be due to a direct influx from the extracellular fluid (Stephano and Gould, 1997) or to be triggered by agents other than the sperm (Lee et al, 1999). Not only is a Ca^{2+} rise necessary for egg activation, it is also sufficient to trigger the process. Thus, blocking the endogenous Ca^{2+} rise at fertilization with chelators inhibits egg activation (Whitaker and Steinhardt, 1982; Kline and Kline, 1992), while agents that artificially raise intracellular Ca^{2+} induce the events associated with the process (Whittingham, 1980; Whitaker and Steinhardt, 1982; Swann and Ozil, 1994; Schultz and Kopf, 1995). Without a rise in Ca^{2+} , there is no cortical granule release to prevent polyspermy, and no meiotic resumption and subsequent entry into the embryonic cell cycle.

Although a rise in intracellular Ca^{2+} appears to be a universally employed signal for egg activation, the exact form taken by the Ca^{2+} rise varies significantly between species. Thus, in sea urchins, frogs, and fish, a single, explosive wave of Ca^{2+} crossing the egg is observed (Jaffe, 1983; Whitaker and Swann, 1993; Stricker, 1999). In contrast, in mammals, nemertean worms, and ascidians, the sperm triggers a series of periodic increases in intracellular Ca^{2+} , which have been termed Ca^{2+} oscillations (Miyazaki et al, 1993a; Swann and Ozil, 1994; Stricker,

1999). In mammals, these oscillations can last from 2 to 6 hours after sperm-egg fusion and appear to stop at around the time that pronuclei form (Cuthbertson and Cobbold, 1985; Swann and Ozil, 1994; Jones et al, 1995).

The discovery that in some species, including mammals, the pattern of Ca^{2+} release takes the form of oscillations, has raised the question of what functional role this oscillatory signal fulfills. One possibility in mammals is that Ca^{2+} oscillations, rather than a single Ca^{2+} transient, are required for efficient egg activation *in vivo* (Jones, 1998; Swann and Parrington, 1999). Although mammalian eggs can be parthenogenetically activated by Ca^{2+} injection or ionophores, the efficiency of activation is often low and dependent on the postovulatory age of the oocyte (Whittingham, 1980; Swann and Ozil, 1994; Jones, 1998). However, activation rates are improved if multiple Ca^{2+} transients are artificially triggered in eggs by means of an electroporation device (Ozil, 1990; Vitullo and Ozil, 1992; Ozil and Swann, 1995; Jones, 1998). Conversely, in a study in which the number of Ca^{2+} oscillations triggered at fertilization were restricted by means of the heavy metal ion chelator, N,N,N',N'-Tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN), it was found that a minimum number of Ca^{2+} transients were required for all the eggs to activate (Lawrence et al, 1998).

An important question still to be fully resolved is whether the Ca^{2+} changes observed at fertilization play a role in determining or modifying later events in the development of the zygote (Ozil and Swann, 1995; Jones, 1998; Ozil, 1998). There have been some suggestions that in mammals, this may indeed be the case. In parthenogenetic egg activation studies, the pattern of repetitive Ca^{2+} transients was shown to affect the size and implantation rate of rabbit embryos (Ozil, 1990, 1998) and the proportion of cells forming the inner cell mass versus the trophectoderm in mouse blastocysts (Bos-Mikich et al, 1997).

How could changes in intracellular Ca^{2+} exert a more long-term influence on the development of the early embryo? One intriguing possibility would be that Ca^{2+} signals influence the timing and extent of zygotic gene activation. Such a possibility has been suggested by recent findings in somatic cells. It has been known for some time that changes in intracellular Ca^{2+} can stimulate gene expression in a variety of cell types (Ghosh et al, 1994; Roche and Prentki, 1994; Rosen et al, 1995). The recent

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findings go further in showing that gene activation can be highly sensitive to the precise pattern of Ca^{2+} signals in a cell (Dolmetsch et al, 1997, 1998; Li et al, 1998). It appears that different transcription factors are differentially sensitive both to the amplitude of Ca^{2+} signals (Dolmetsch et al, 1997) and the frequency of Ca^{2+} oscillations (Dolmetsch et al, 1998). A variety of mechanisms may be employed by cells to translate Ca^{2+} signals into changes in gene expression. One potential route would be via a Ca^{2+} -sensitive protein kinase such as the multifunctional Ca^{2+} /calmodulin-dependent kinase (CaM kinase II), the activity of which has recently been shown to be highly sensitive to the temporal pattern of Ca^{2+} transients (De Koninck and Schulman, 1998), suggesting that such a kinase may act as a cellular “frequency decoder” of Ca^{2+} oscillations (Dupont and Goldbeter, 1998). Determining whether mechanisms such as these are operating in the zygote will no doubt provide some exciting avenues of study in the future.

How do Sperm Trigger Intracellular Ca^{2+} Release in Eggs?

Even though Ca^{2+} has been known to be the essential trigger of egg activation for several decades, the mechanism by which a sperm causes intracellular Ca^{2+} release remains to be resolved. Three main models have been proposed to explain how a sperm induces Ca^{2+} release (Figure 1; Whitaker and Swann, 1993; Schultz and Kopf, 1995; Evans and Kopf, 1998; Swann and Parrington, 1999). The first is the “ Ca^{2+} bomb” or “ Ca^{2+} conduit” model, which proposes that Ca^{2+} is introduced by or channeled through the sperm (Jaffe, 1983, 1991; Creton and Jaffe, 1995). The second is the “membrane receptor” model, in which a sperm surface ligand is proposed to bind to an egg membrane receptor, which activates a signaling cascade, which leads to generation of the second messenger inositol trisphosphate (InsP_3), which causes Ca^{2+} release through the IP_3 receptor (Jaffe, 1990; Schultz and Kopf, 1995; Evans and Kopf, 1998). The third is the “soluble sperm factor” model, which proposes that a sperm Ca^{2+} -releasing factor is introduced into the egg at sperm-egg fusion (Whitaker and Swann, 1993; Swann and Ozil, 1994; Swann and Lai, 1997; Fissore et al, 1998; Parrington et al, 1998; Swann and Parrington, 1999).

Sperm Ca^{2+} as the Trigger of Egg Activation

One of the first and most straightforward suggestions as to how a sperm triggers Ca^{2+} release at fertilization proposed that Ca^{2+} flows into the egg from or via the sperm itself (Jaffe, 1983; 1991; Creton and Jaffe, 1995). In the first version of the model, it was proposed that the sperm contained a bolus of Ca^{2+} , which entered the egg at sperm-egg fusion (Jaffe, 1983). In a later version, Ca^{2+} channels in the sperm membrane were believed to allow

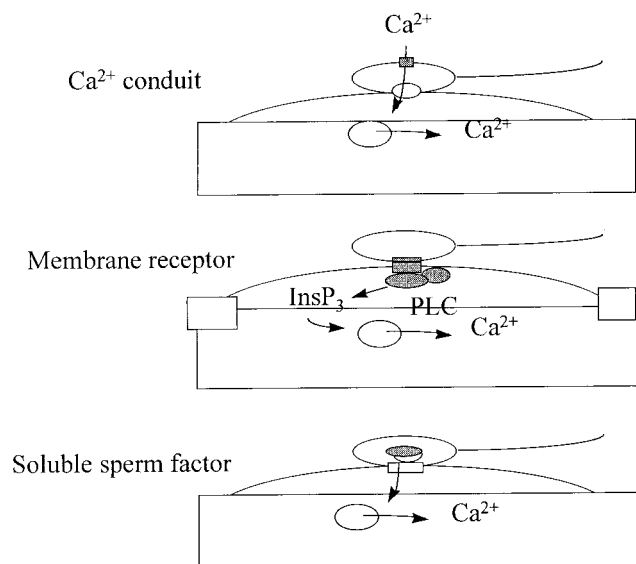


Figure 1. The three main models of egg activation: The ‘ Ca^{2+} bomb’ or ‘ Ca^{2+} conduit’ model, in which Ca^{2+} is introduced into the egg by, or channeled into the egg through, the sperm. The ‘membrane receptor’ model, in which a sperm surface ligand binds to an egg membrane receptor, which activates a signaling cascade, leading to generation of the second messenger inositol trisphosphate (InsP_3). The ‘soluble sperm factor’ model, in which a sperm Ca^{2+} releasing factor is introduced into the egg at sperm-egg fusion.

the sperm to act as a conduit through which Ca^{2+} from the extracellular medium could pass into the egg (Jaffe, 1991; Creton and Jaffe, 1995). Ca^{2+} introduced into the egg in this way could act as a trigger for Ca^{2+} release by Ca^{2+} -induced Ca^{2+} release (CICR; Jaffe, 1991).

A number of early findings appeared to support this model. It is consistent with the fact that sperm-egg fusion in the sea urchin and mouse always precedes egg Ca^{2+} release (McCulloch and Chambers, 1992; Lawrence et al, 1997). In addition, sperm from many species take up Ca^{2+} prior to fusion (Jaffe, 1983, 1991). Experimental evidence is provided by the finding that in frog and fish eggs, injection of Ca^{2+} can lead to the generation of a Ca^{2+} wave (Nuccitelli, 1991). However, more recently, a number of findings have argued against the idea that Ca^{2+} entering via the sperm is the trigger of egg activation. Injection of Ca^{2+} into sea urchin or ascidian eggs does not cause Ca^{2+} release (Whitaker and Swann, 1993). In mammals, introduction of Ca^{2+} into the egg in a variety of ways fails to initiate Ca^{2+} oscillations (Swann and Ozil, 1994). The most recent piece of evidence against this model comes from a Ca^{2+} imaging study (Jones et al, 1998a). If the sperm acts as a Ca^{2+} conduit, one would expect to see an elevation in the local cytoplasmic Ca^{2+} concentration. Yet in the mouse, at sperm-egg fusion, no such elevation was observed. In addition, removal of extracellular Ca^{2+} failed to block Ca^{2+} release in the egg (Jones et al, 1998a). Thus, if any Ca^{2+} influx via the sperm does take place at fertil-

ization, it now appears unlikely that it plays a primary role in triggering Ca^{2+} release and egg activation.

A Surface Interaction as the Trigger of Egg Activation

The most popular explanation, at least until recently, of how Ca^{2+} release is triggered at fertilization is that it is the consequence of an interaction between a sperm ligand and a receptor on the surface of the egg (Jaffe, 1990; Schultz and Kopf, 1995; Evans and Kopf, 1998). In this model, the receptor then activates an egg phosphatidylinositol-specific phospholipase C (PLC), which generates InsP_3 , leading to Ca^{2+} release from internal stores. In line with such a proposal, there are numerous pieces of evidence indicating that Ca^{2+} release at fertilization is triggered by generation of InsP_3 in the egg. Thus, if the binding of InsP_3 to the InsP_3 receptor is blocked by injecting the InsP_3 antagonist, heparin, or a functionally inhibiting InsP_3 -receptor antibody, Ca^{2+} release at fertilization and egg activation is also blocked (Miyazaki et al, 1993b). There also appears to be an increased turnover of phosphoinositide lipids and an increase in InsP_3 levels in sea urchin and frog eggs at fertilization (Nuccitelli, 1991; Ciapa et al, 1992; Snow et al, 1996; Lee and Shen, 1998). Further evidence for the involvement of a PLC during fertilization in sea urchins and mice comes from the finding that Ca^{2+} release and egg activation can be inhibited in both species with the PLC inhibitor, U73122 (Dupont et al, 1996; Lee and Shen, 1998).

In the surface-mediated model of egg activation, the surface interaction is assumed to lead to the activation of a $\text{PLC}\beta$ by a G protein mechanism (Jaffe, 1990; Schultz and Kopf, 1995) or, alternatively, a $\text{PLC}\gamma$, which is activated by tyrosine phosphorylation (Schultz and Kopf, 1995; Evans and Kopf, 1998). Mammalian eggs have been shown to contain both $\text{PLC}\beta$ and $\text{PLC}\gamma$ isoforms (Dupont et al, 1996; Mehlmann et al, 1998). One of the attractions of this model is that the mechanism proposed is similar to that found in many somatic cells, where it allows cells to receive signals across their cell membranes (Berridge, 1993). However, as sperm-egg fusion is now known to precede Ca^{2+} release and egg activation in sea urchins and mice (McCulloch and Chambers, 1992; Lawrence et al, 1997), the egg and sperm form a single cell at this time and one cannot necessarily assume that egg activation is a conventional transmembrane signaling problem. Over the last few years, the most convincing evidence that an egg PLC plays a role at fertilization has come from studies in starfish and sea urchins. In mammals, however, such a link with an egg PLC has been less forthcoming.

Initially, a role for an egg $\text{PLC}\beta$ in triggering Ca^{2+} release in the mouse egg was suggested by the finding that the nonhydrolyzable analogue, $\text{GTP}\gamma\text{S}$, stimulates Ca^{2+} release when injected into mouse eggs (Miyazaki,

1988; Fissore and Robl, 1994). Conversely, injection of $\text{GDP}\beta\text{S}$, which inhibits G proteins, blocks Ca^{2+} release at fertilization in hamster eggs (Miyazaki et al, 1993b). However, in mice and frogs, inhibition of members of the Gi, Gs, and Gq families fails to inhibit Ca^{2+} release at fertilization (Moore et al, 1994; Williams et al, 1998; Runft et al, 1999), thus arguing against an involvement of an egg $\text{PLC}\beta$ during vertebrate egg activation.

Interest over the last few years has instead focused on the alternative possibility that egg activation is triggered by an egg $\text{PLC}\gamma$ (Evans and Kopf, 1998). The main evidence for this is the finding that injection of recombinant proteins containing the SH2 domains of bovine $\text{PLC}\gamma$, block Ca^{2+} release in both starfish (Carroll et al, 1997) and sea urchin (Carroll et al, 1999; Shearer et al, 1999) eggs at fertilization. The SH2 domains are believed to compete with the SH2 domains of the endogenous egg $\text{PLC}\gamma$, blocking its access to a tyrosine kinase, which is presumed to be upstream of the PLC in a signaling cascade operating at fertilization. This may be an Src family kinase, as injection of inhibitors of this kinase family into starfish eggs results in a delay in Ca^{2+} release at fertilization (Abassi et al, 2000), while injection of Src protein into starfish eggs triggers Ca^{2+} release (Giusti et al, 2000). Although these studies have built up a convincing case for the involvement of an egg $\text{PLC}\gamma$ in egg activation in echinoderms, their significance for vertebrates is currently far from clear. Tyrosine kinase inhibitors have been shown to inhibit Ca^{2+} increases in the eggs of frogs and mice (Dupont et al, 1996; Glahn et al, 1999); however, studies in these species using $\text{PLC}\gamma$ SH2-domain-containing proteins, similar to those described earlier, have failed to show any inhibitory effect on Ca^{2+} release at fertilization (Mehlmann et al, 1998; Runft et al, 1999).

A more general concern for this model of egg activation is that it still remains to be demonstrated that a surface-mediated interaction between a sperm ligand and an egg receptor is indeed triggering the activation of a PLC in the egg. Sperm-egg interaction is believed to be mediated by egg integrins binding to their complementary disintegrin ligand on the sperm (Myles, 1993; Foltz, 1995; Myles and Primakoff, 1997; Evans, 1999). In frogs, a peptide from a disintegrin domain triggers egg activation (Shilling et al, 1997, 1998). In mammals, the sperm ligand involved has been proposed to be fertilin, a sperm surface protein of the ADAM family (so-called because they contain a disintegrin and metalloprotease domain; Myles, 1993; Foltz, 1995; Wolfsberg and White, 1996; Myles and Primakoff, 1997; Evans, 1999; Primakoff and Myles, 2000). The question of fertilin's exact role at fertilization is currently a matter of some debate (Frayne and Hall, 1999; Primakoff and Myles, 2000). For our purpose here, a significant recent finding is that in mice that lack functional fertilin β protein, sperm-egg binding and fu-

sion are significantly reduced; however, in these mice, Ca^{2+} release and egg activation at fertilization are unaffected (Cho et al, 1998).

Finally, it remains possible that even in species in which an egg PLC is activated at fertilization, it may be via another mechanism than a surface-mediated interaction between sperm and egg. For instance, an activating factor may enter the egg from the sperm during gamete fusion, a possibility we shall return to shortly.

A Soluble Sperm Factor as the Trigger of Egg Activation

One of the most controversial suggestions concerning the mechanism of egg activation has been the proposal that Ca^{2+} release at fertilization is triggered by a soluble sperm factor (Dale et al, 1985; Stice and Robl, 1990; Swann, 1990; Swann and Ozil, 1994; Fissore et al, 1998; Stricker, 1999; Swann and Parrington, 1999). Over the last few years, however, this model has become increasingly accepted as the mechanism most likely to be operating in mammals (Fissore et al, 1998; Swann and Parrington, 1999) and probably in other species such as ascidians and nemertean worms (Stricker, 1997; Kyojuka et al, 1998; Runft and Jaffe, 2000). What still remains a major unresolved question is the identity of the soluble sperm factor. Like the calcium "bolus" or "conduit" model, the proposal that a soluble sperm factor mediates egg activation is compatible with the finding that egg Ca^{2+} release and activation always occur after gamete fusion in sea urchins and mice (McCulloch and Chambers, 1992; Lawrence et al, 1997). Indirect evidence for the existence of an intracellular sperm factor comes from experiments in which sperm are directly injected into eggs. This procedure is used clinically and is referred to as intracytoplasmic sperm injection (ICSI). Besides leading to egg activation, during ICSI a series of Ca^{2+} oscillations are observed in the eggs of humans (Tesarik and Sousa, 1994; Tesarik et al, 1994), nemertean worms (Stricker, 1996), and mice (Nakano et al, 1997), similar to those seen at fertilization.

Most important, and unlike the previous 2 models, there is direct evidence for the existence of a soluble sperm factor. In mammals, injection of soluble sperm extracts from boars, hamsters, or humans can trigger Ca^{2+} oscillations similar to those seen at fertilization in mouse, hamster, human, and cow eggs (Swann, 1990, 1992, 1994; Homa and Swann, 1994; Palermo et al, 1997; Wu et al, 1997; Fissore et al, 1998). Sperm extracts have been shown to cause Ca^{2+} oscillations in nonmammals such as marine worms (Stricker, 1997) and ascidians (Kyojuka et al, 1998). Most recently, sperm extracts from frogs and chickens have been shown to trigger Ca^{2+} oscillations when injected into mouse eggs (Dong et al, 2000). In each case, injection of sperm extract closely mimics the particular characteristics of the pattern of Ca^{2+} release seen at fertilization. In hamsters, mice, and rabbits, one sees

an enhancement of CICR after fertilization (Igusa and Miyazaki, 1983; Swann, 1990, 1994; Fissore and Robl, 1994). A similar enhancement is seen after injection of sperm factor, but not with other Ca^{2+} -releasing agents (Swann and Ozil, 1994). In ascidians, Ca^{2+} oscillations at fertilization occur in 2 phases, 1 set directly after fertilization and another after the first polar body is extruded. The same temporal pattern can be mimicked by sperm extracts but not by other Ca^{2+} -releasing agents (Kyojuka et al, 1998; Runft and Jaffe, 2000).

Although the identity of the sperm factor remains to be resolved in all of the species in which it has been detected, a number of features characterizing it have emerged. It is evidently protein-based because it is both trypsin- and heat-sensitive (Swann, 1990; Wu et al, 1997). It can also be retained by ultrafiltration membranes, which indicate it has a molecular weight of at least 30 kd (Swann, 1990) and it can be resolved using protein separation methods (Parrington et al, 1996, 1999; Wu et al, 1998). The ability of sperm extracts to cause Ca^{2+} oscillations appears to be tissue-specific because soluble extracts prepared from other tissues do not cause Ca^{2+} oscillations when microinjected into eggs (Swann, 1990; Stricker 1997; Wu et al, 1997). The amount of sperm factor required to trigger Ca^{2+} oscillations in eggs appears to be between 1–10 sperm (Swann, 1990; Stricker 1997; Wu et al, 1997). This suggests that if one takes into account losses during extraction, the amount of sperm factor in a single sperm is within the range that would be expected if it is the physiological agent of egg activation.

Initial Candidates for the Soluble Sperm Factor

Undoubtedly, the major unresolved question of the soluble sperm factor model of egg activation is the identity of the factor. A related question is whether the sperm factor is conserved across species as different as nemertean worms, ascidians, and humans and other mammals, or whether the factor is different in each case. In fact, only in the case of the mammalian factor have particular proteins been suggested as candidates. The first candidate was a 33-kd hamster sperm protein, which correlated with ability to trigger Ca^{2+} oscillations in eggs over a series of chromatographic steps (Parrington et al, 1996). In line with the protein's proposed role, an antibody raised against the protein showed specific staining in the equatorial region of the sperm head, which is the first to come into contact with the egg cytoplasm during sperm-egg fusion (Yanagimachi, 1994). However, further experiments have shown that a recombinant version of the 33-kd protein does not generate Ca^{2+} oscillations when injected into mouse eggs (Shevchenko et al, 1998; Wolosker et al, 1998; Wolny et al, 1999). Furthermore, more extensive fractionation studies have shown that the 33-kd protein does not always correlate with Ca^{2+} -releasing ability in

eggs (Wu et al, 1998; Parrington et al, 1999). Thus, it now appears that the Ca^{2+} -releasing activity in the semi-purified extracts must have been due to another protein.

Another candidate for the sperm factor is the truncated form of the c-kit receptor, termed tr-kit, which is present in mouse sperm (Albanesi et al, 1996). When recombinant tr-kit messenger RNA (mRNA) or protein is micro-injected into mouse eggs it causes egg activation, apparently through the release of Ca^{2+} (Sette et al, 1997). Further studies suggested that tr-kit binds and activates an egg $\text{PLC}\gamma$ (Sette et al, 1998). Despite these initially suggestive findings, a number of subsequent findings have cast doubt on the idea that tr-kit is the sperm factor, or that it plays a role in egg activation. First, even though mouse sperm heads, and not tails, appear to contain the egg-activating factor (Kimura et al, 1998), tr-kit is located predominantly in the midpiece of the sperm tail and not the head (Sette et al, 1997). Second, tr-kit-induced egg activation is blocked by a recombinant protein containing the SH3 domain of $\text{PLC}\gamma$ (Sette et al, 1998), but this protein does not block egg activation at fertilization (Mehlmann et al, 1998). Finally, there has been an absence of reports showing that tr-kit can trigger Ca^{2+} oscillations in eggs, even though this has been the definitive assay for the soluble sperm factor.

The Case for the Mammalian Soluble Sperm Factor Being a Sperm-Derived Phospholipase C

Another reason for doubting that these initial sperm-factor candidates are correct is that there are now a number of pieces of evidence indicating that the soluble sperm factor in mammals is a sperm-derived PLC (Swann and Parrington, 1999). These findings link the sperm factor to an obvious mechanism of Ca^{2+} release; namely, generation of InsP_3 , and to the evidence already mentioned indicating that a PLC is involved at fertilization. At the same time, the PLC responsible appears to have some very distinctive and apparently sperm-specific properties. These recent findings were made using a different type of experimental approach to the egg injection assay. Instead, a bioassay based on the sea urchin egg homogenate; an egg-based, cell-free Ca^{2+} -release assay, was used. The sea urchin homogenate assay is well-characterized and has been used extensively to study Ca^{2+} -release mechanisms in eggs (Lee, 1997). Addition of boar and hamster sperm extract to the sea urchin egg homogenate triggers Ca^{2+} release with a characteristic delay (Galione et al, 1997; Jones et al, 1998b, 2000). Fractionation of sperm extract on a number of different chromatographic steps verified that the ability of fractions to release Ca^{2+} in the homogenate is associated with the ability to cause Ca^{2+} oscillations in intact mammalian eggs (Parrington et al, 1999), the previous defining feature of the sperm factor.

The study of the sperm factor response in a cell-free assay made it possible to dissect the mechanism of the response in a way that would be much more difficult in the intact egg. First, desensitization experiments demonstrated that the sperm factor mediates Ca^{2+} release through the InsP_3 receptor and that it generates InsP_3 in the homogenate, indicating involvement of a PLC (Jones et al, 1998b, 2000). Second, the sperm factor itself appears to be, or contains, a sperm-derived PLC, rather than it being another type of protein that activates a PLC in the egg. The reasons for believing this are based on a number of findings. First, the PLC inhibitors, U73122 and neomycin, block the sperm factor response in homogenates in a manner compatible with the sperm factor itself being a PLC, rather than it being an upstream activator of an egg PLC (Jones et al, 2000). Second, the ability of fractionated sperm extracts to cause Ca^{2+} release in homogenates and Ca^{2+} oscillations in mouse eggs correlates with ability to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP_2) at Ca^{2+} concentrations similar to those in the egg (Parrington et al, 1999; Jones et al, 2000; Parrington and Swann, unpublished results). This ability to hydrolyze PIP_2 at physiological Ca^{2+} concentrations may in fact be a distinctive feature of the sperm factor compared with PLCs found in soluble extracts from other tissues (Rice et al, 2000). It has been possible to quantify the PLC activity in sperm extracts and there appears to be a sufficient amount in a single sperm to account for the amount of Ca^{2+} release observed at fertilization (Rice et al, 2000).

One major question that now needs to be addressed is the identity of the PLC isoform that appears to be the active component of the mammalian sperm factor. Of the 3 PLC subtypes, $\text{PLC}\beta$, $\text{PLC}\gamma$, and $\text{PLC}\delta$ (Rhee and Bae, 1997), only the $\text{PLC}\beta$ and $\text{PLC}\gamma$ classes have previously been considered for a role at fertilization (Jaffe, 1990; Schultz and Kopf, 1995; Evans and Kopf, 1998). A number of $\text{PLC}\beta$ and $\text{PLC}\gamma$ isoforms have been detected in mouse sperm (Walensky and Snyder, 1995; Dupont et al, 1996; Mehlmann et al, 1998; Parrington and Swann, 1999). However, although some of these isoforms are clearly present in the soluble boar sperm extract, after fractionation none of them appear to correlate with Ca^{2+} -releasing activity (Parrington and Swann, 1999). In addition, recombinant $\text{PLC}\beta 1$, $\text{PLC}\gamma 1$, and $\text{PLC}\gamma 2$ are unable to mimic the sperm factor's ability to induce Ca^{2+} release in egg homogenates or intact eggs, even though they have higher specific PLC activities than sperm extract when measured using a standard PIP_2 hydrolysis assay (Jones et al, 2000). An alternative possibility is that the sperm factor is one of the $\text{PLC}\delta$ isoforms. Its apparent molecular weight after fractionation by gel filtration is in the range of 29 to 68 kd (Wu et al, 1998; Parrington, unpublished results), which is most compatible with the smaller $\text{PLC}\delta$ isoforms. The most well-characterized

PLC δ is PLC δ 1, however, recombinant PLC δ 1 does not cause Ca²⁺ release in egg homogenates or intact eggs, even though it has higher specific PLC activity than sperm extract when measured using a standard PIP₂ hydrolysis assay (Jones et al, 2000). The sperm factor may be one of the other PLC δ isoforms. Alternatively, it may be a novel PLC isoform or a variant of one of the known isoforms that is present only in sperm. This would be one possible explanation of the apparent tissue specificity of the sperm factor, which appears to be a feature both of the sperm factor protein (Swann, 1990; Wu et al, 1997; Jones et al, 2000) and of the one or more mRNAs that it appears to be generated from (Parrington et al, 2000; see later discussion).

The identity of the sperm factor in nonmammals has been less clear. There is currently no clear evidence that the sperm factor activities identified in nonmammalian species are due to a sperm PLC. As already mentioned, it remains a possibility that an egg PLC γ in sea urchins and starfish is activated, not by a surface interaction, but by a sperm protein that activates an egg PLC. The first report of a soluble sperm factor that could trigger some aspects of egg activation was made in sea urchins (Dale et al, 1985). The fact that at least 10 sperm are required for activation (Wakayama et al, 1997) may indicate that a single sea urchin sperm may contain less sperm factor than a mammalian sperm. If this were the case, it might be one reason why such a factor has been harder to detect.

The most direct evidence that in some species egg activation may be triggered by a sperm protein that activates an egg PLC comes from the recent finding that in ascidians, the Ca²⁺ oscillations triggered at fertilization and those induced by ascidian sperm extract are both inhibited by recombinant proteins containing SH2 domains from PLC γ and the Src family kinase, Fyn (Runft and Jaffe, 2000). This finding raises the possibility that in ascidians, and potentially also in sea urchins and starfish, egg activation may be triggered by a sperm protein that activates, directly or indirectly, an Src family kinase in the egg, and thus egg PLC γ . If this proves to be the case, we may be facing an intriguing scenario in which fertilization in both mammalian and nonmammalian species is found to be triggered by a sperm factor that induces Ca²⁺ release through a common mechanism, the phosphoinositide (PI) signaling pathway, but that in each case the sperm factor is a quite different kind of protein, entering at a different point in the pathway, depending on the species.

In contrast to the prospect of there being such a multiplicity of sperm factors, there are some suggestions from cross-species injection studies that a sperm factor with the qualities of the mammalian sperm factor may be conserved across very different species. We have already mentioned that injection of hamster, boar, and human sperm extracts into mouse eggs can all trigger Ca²⁺ os-

cillations similar to those seen at fertilization (Swann, 1990; Homa and Swann, 1994; Wu et al, 1997). In addition, injection of mammalian sperm extracts into ascidian eggs (Wilding et al, 1997) and chicken and frog sperm extracts into mouse eggs (Dong et al, 2000) triggers Ca²⁺ oscillations similar to those seen in the egg at fertilization. This suggests that although the pattern of Ca²⁺ release seen at fertilization may vary in these different species, the trigger of release may involve the same protein factor, possibly a sperm-derived PLC.

Finally, it remains possible that in some species a sperm factor is the initial trigger of Ca²⁺ release, but that the Ca²⁺ wave that crosses the egg is then carried by a different Ca²⁺-releasing agent (Whitaker and Swann, 1993). For instance, a sperm-derived PLC may be the initial trigger of Ca²⁺ release, with the subsequent wave of Ca²⁺ across the egg involving activation of an egg PLC. This possibility would be consistent with the finding that after injection of recombinant PLC γ SH2 domain proteins into sea urchin eggs, a local, nonpropagating Ca²⁺ increase is observed at the site of fusion (Shearer et al, 1999).

Unexplained Features of the Soluble Sperm Factor

Studies of the sperm factor over the last decade have uncovered a number of interesting, unexplained features. Undoubtedly, much of the explanation of these features will await the successful identification of the factor. However, these features may also provide potential clues now as to how the sperm factor is generated and activated in the sperm, its exact mechanism of action, and its post-fertilization regulation. If the sperm factor is already in an active state before sperm-egg fusion, then it must presumably be compartmentalized in some way to prevent it triggering Ca²⁺ release in the sperm. The factor appears to be already active in sperm extracts (Swann, 1990; Jones et al, 1998b), but this could be the result of artificial activation during extract preparation. In mouse sperm, an egg activating factor that may be the same as the sperm factor was found to be present in an insoluble form, apparently in the perinuclear matrix, and was only released under reducing conditions (Kimura et al, 1998; Perry et al, 1999, 2000). It is possible that after sperm-egg fusion, release of sperm factor from the sperm head during fertilization could be triggered by the transition from the highly oxidized environment of the sperm to the highly reduced one of the egg. This property may be most characteristic of the mouse sperm factor, as soluble extracts from hamster, boar, and human sperm are very effective at triggering Ca²⁺ oscillations and egg activation, while soluble extracts from mouse sperm are relatively poor (Stice and Robl, 1990; Swann, 1990). A greater ease of solubilization of the human sperm factor compared with that of the mouse may explain why, unlike in mice,

freeze-thawing of human sperm leads to a loss of their ability to activate eggs when introduced by ICSI (Rybouchkin et al, 1996).

The source of the sperm factor's apparent tissue specificity (Swann, 1990; Jones et al, 2000), and what relevance it has to the molecular identity of the sperm factor, also remains to be uncovered. Given that an essential component of the mammalian sperm factor appears to be a PLC, the apparent tissue specificity of the factor suggests that the PLC must either be a sperm-specific PLC isoform or, alternatively, that it is a PLC with a more general tissue distribution, which is combined with sperm-specific accessory proteins or modified in a sperm-specific manner. One recent study with potential relevance to this question found that injection of mRNA from hamster spermatogenic cells into mouse eggs triggers Ca^{2+} oscillations similar to those seen at fertilization (Parrington et al, 2000). The mRNA-induced oscillations resemble those seen at fertilization and after injection of sperm extracts, because they are of low frequency and large amplitude. The most obvious explanation of these findings is that the sperm factor is being generated from its mRNA or mRNAs by the translational machinery of the egg. In line with this, the response is blocked by cycloheximide and appears to be tissue-specific, similar to the protein sperm factor, as Ca^{2+} oscillations are not observed after injection of brain, liver, or muscle mRNA (Parrington et al, 2000). One explanation for these findings is that the sperm factor contains a sperm-specific protein as at least one of its components and that it requires no sperm-specific post-translational modifications for its activation.

Another unresolved question is whether the sperm factor plays a role in postfertilization events. In a number of species, Ca^{2+} release is not just observed at fertilization, but also during later stages of zygotic development. In mice, the Ca^{2+} oscillations observed at fertilization disappear at around the time that pronuclei form (Cuthbertson and Cobbold, 1985; Swann and Ozil, 1994; Jones et al, 1995), but then another series of Ca^{2+} oscillations occurs during the first mitotic division (Tombes et al, 1992; Kono et al, 1996). In sea urchins, mitotic Ca^{2+} transients are also observed, and are associated with specific mitotic events such as nuclear envelope breakdown and the metaphase-anaphase transition (Poenie et al, 1985; Whitaker and Patel, 1990). These Ca^{2+} transients appear to originate from the perinuclear region (Wilding et al, 1996). The possibility that both the Ca^{2+} oscillations seen at fertilization, and these mitotic Ca^{2+} oscillations, may be caused by the same soluble sperm factor, has been suggested by pronuclear transfer studies. Transfer of the female or male pronucleus from a fertilized mouse zygote has been shown to induce Ca^{2+} oscillations and egg activation (Kono et al, 1995). In contrast, pronuclei from unfertilized eggs, or from parthenogenetically activated eggs, did

not possess this capacity. However, if eggs were injected with sperm extract, and then pronuclear transfer was performed, Ca^{2+} oscillations were observed (Kono et al, 1995). This finding provides further evidence that a soluble sperm factor mimics the events associated with egg activation more faithfully than other parthenogenetic agents. It also suggests that the sperm factor may associate with the newly forming nucleus, either by interacting with the outer nuclear membrane, and thus with the endoplasmic reticulum, or by entering the nucleoplasm.

The Soluble Sperm Factor and Human Fertility

It has already been noted that sperm extracts from fertile human males trigger Ca^{2+} oscillations similar to those seen at fertilization when injected into human and mouse eggs. It is conceivable that certain types of human infertility could be caused by lack of active sperm factor. Although only affecting a minority of cases, one reason for failure of ICSI appears to be due to failure of the egg to activate (Flaherty et al, 1995; Battaglia et al, 1997; Rybouchkin et al, 1997; Flaherty et al, 1998). In at least some of these cases, the fault appeared to be due to a defect in the sperm, as application of Ca^{2+} ionophore activated the eggs (Battaglia et al, 1997; Rybouchkin et al, 1997). It has been suggested that injection of an active sperm factor may overcome the problem of egg activation in such cases (Palermo et al, 1997). Studies investigating the clinical procedure of round spermatid injection (ROSI) in mice found that eggs generally failed to activate (Kimura and Yanagimachi, 1995; Sato et al, 1998), and there was no increase in intracellular Ca^{2+} (Sato et al, 1998), presumably because the spermatids lack active sperm factor. Coinjection of round spermatids with hamster sperm extract overcame this problem, leading to successful fertilization, embryonic development, and live offspring (Sakurai et al, 1999). This finding raises the interesting question of which gene expression control mechanisms in spermatogenic cells may be responsible for this apparent, late appearance of sperm factor activity. In fact, a variety of control mechanisms are utilized to ensure some sperm proteins are expressed only at a late stage of spermatogenesis (Erickson, 1993; Sassone-Corsi, 1997). Uncovering which of these are used to generate the sperm factor will probably be forthcoming only with the molecular identification of the sperm factor protein or proteins. However, it is possible that injection into mouse eggs of mRNA from spermatogenic cells fractionated into different stages of development could be used to show at what stage of spermatogenesis the sperm factor mRNA or mRNAs can be translated into protein (see earlier discussion).

It is also possible that there may be a more general relationship between sperm factor activity and variations in human fertility. To explore this question further, it

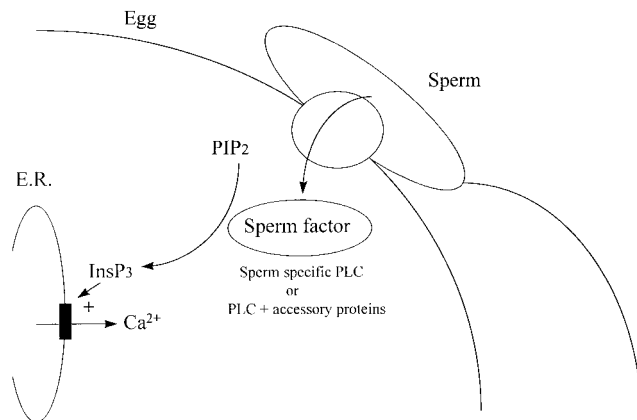


Figure 2. Model of how the sperm triggers Ca^{2+} release and egg activation in mammals: At sperm-egg fusion, a sperm-derived phospholipase C activity is introduced into the egg. The PLC is highly active and has distinctive properties. It may be a sperm specific PLC or a more generally expressed PLC modified in some way or combined with sperm-specific accessory proteins. The sperm PLC acts upon PIP₂ stores in the egg to generate InsP₃, leading to Ca^{2+} release from internal stores via the InsP₃ receptor.

would be necessary to measure sperm factor activity in a large number of human men with varying degrees of subfertility. One barrier to doing this has been the laborious nature of the sperm factor assay procedure most generally used—that is, injection of sperm extracts into intact eggs. The sea urchin egg homogenate assay is an easier, quicker, more sensitive, and more quantifiable assay than injection of the mammalian egg (Parrington et al, 1999) and would be better for screening large numbers of samples. Human sperm extract from normal fertile males triggers substantial Ca^{2+} release when added to homogenates (Parrington, unpublished results), making this a potentially useful clinical assay.

Conclusions

The mechanism by which a sperm induces Ca^{2+} release in an egg, and thus the activation of embryogenesis, remains an unsolved problem in fertilization research. While there is now evidence in sea urchins and starfish that an egg PLC plays an important role at fertilization, in mammals, demonstration of such an involvement has been unforthcoming. Instead, a number of lines of direct and indirect evidence suggest that in mammals, and potentially a number of other animal groups, a soluble cytosolic sperm factor enters the egg after gamete fusion, and it is this that causes Ca^{2+} release from internal stores. Recent studies suggesting that a sperm-derived PLC may be the active component of the mammalian sperm factor represent an important step forward in determining its exact mechanism of action (Figure 2). At the same time, the source of the mammalian sperm factor's apparent tissue specificity and distinctive properties remains unclear.

In some nonmammals, such as ascidians, a soluble sperm factor may be the trigger of egg activation but it may not be a sperm PLC. Instead, a recent finding suggests that the ascidian sperm factor may be an activator of a PLC in the egg. Full molecular characterization of the soluble sperm factors from mammalian and nonmammalian species remains an essential goal if we are to fully understand their physiological roles in this fundamental signaling event.

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