

Ca²⁺ signalling during fertilization of echinoderm eggs

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The Ca²⁺ rise at fertilization of echinoderm eggs is initiated by a process requiring the sequential activation of a Src family kinase, phospholipase C γ , and the inositol trisphosphate receptor/channel in the endoplasmic reticulum. The consequences of the Ca²⁺ rise include exocytosis of cortical granules, which establishes a block to polyspermy, and inactivation of MAP kinase, which functions in linking the Ca²⁺ rise to the reinitiation of the cell cycle.

Key words: calcium / echinoderm / fertilization

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In some species including mammals, multiple Ca²⁺ rises occur, over a period of hours. The functions of the Ca²⁺ rise in various species include the opening of ion channels and the exocytosis of cortical granules, both of which are important for polyspermy prevention;^{6–9} the Ca²⁺ rise also stimulates the resumption of the cell cycle (see below). This paper reviews recent evidence concerning the signal transduction pathway that leads from sperm–egg interaction to the Ca²⁺ rise, and from the Ca²⁺ rise to the resumption of the cell cycle, focusing on echinoderm (sea urchin and starfish) eggs. Related papers in this issue review Ca²⁺ signalling in mammalian fertilization,¹⁰ and in the regulation of the cell cycle.¹¹

Introduction

The concept of Ca²⁺ as a signal for activating the egg to begin development at fertilization arose from early studies of artificial activation,¹ but it was not until 50 years later that an increase in free Ca²⁺ in a fertilizing egg was first measured.^{2,3} A Ca²⁺ rise has since been shown to be a universal event of fertilization among all species examined, and generally occurs in the form of a regenerative wave starting at the site of sperm–egg fusion.⁴ In sea urchin eggs, the Ca²⁺ rise results from intracellular Ca²⁺ release, since it is not reduced by lowering extracellular Ca²⁺ (Ref. 3); Ca²⁺ reaches a peak of about 2 μ M,⁵ and remains elevated for a period of several minutes.

IP₃ and Ca²⁺ release from the egg's endoplasmic reticulum

Fertilization stimulates the phosphatidylinositol cycle and the production of inositol 1,4,5-trisphosphate (IP₃) within 15 s after insemination in sea urchin eggs,^{12,13} and injection of IP₃ into unfertilized eggs causes Ca²⁺ release.¹⁴ These results, in conjunction with studies showing the necessity for IP₃ production (see below), support the hypothesis that the IP₃ rise causes the Ca²⁺ rise at fertilization. Injection of echinoderm eggs with a number of other small molecules, including cGMP,¹⁵ cyclic ADP-ribose (cADPR),¹⁶ or nicotinic acid adenine dinucleotide phosphate (NAADP),¹⁷ or application of nitric oxide (NO),¹⁸ can also cause a Ca²⁺ rise, but whether these small molecules could, along with IP₃, function in releasing Ca²⁺ at fertilization, has been a subject of considerable controversy. Like IP₃, cGMP has been measured to increase in sea urchin eggs at fertilization,¹⁹ but whether or not increases occur in cADPR, NAADP or NO is unknown, and inhibitor studies argue against a role in fertilization for these alternative Ca²⁺-releasing molecules. In particular, inhibition of cGMP- or NO-induced Ca²⁺ release by use

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of a cGMP analogue does not inhibit Ca^{2+} release at fertilization, even in the presence of heparin to partially inhibit IP_3 -induced Ca^{2+} release.^{18,20} An inhibitor of cADPR-induced Ca^{2+} release, 8-NH₂-cADPribose, inhibits Ca^{2+} release at fertilization when injected into sea urchin eggs together with heparin, but has no inhibitory effect by itself.¹⁶ Although this result was interpreted as evidence for dual roles for cADPR and IP_3 in releasing Ca^{2+} at fertilization, the possibility of an additive effect of the two injected inhibitors on IP_3 -induced Ca^{2+} release was not definitively excluded. Arguing against a role for NAADP in releasing Ca^{2+} at fertilization, the Ca^{2+} rise in response to injection of NAADP is reduced to a low level by a prior injection of thio-NADP, but thio-NADP injection does not block the fertilization response.¹⁷ It has also been suggested that Ca^{2+} itself might initiate Ca^{2+} release at fertilization,²¹ by entering the egg from the external solution. However, this possibility is not consistent with the finding that Ca^{2+} release can occur in the absence of external Ca^{2+} (Ref. 22).

Until recently, the key difficulty in testing whether the IP_3 increase was the cause of the Ca^{2+} release at fertilization in echinoderm eggs was the lack of specific inhibitors.⁹ In mammalian eggs, an antibody against the IP_3 receptor was shown to completely inhibit Ca^{2+} release at fertilization,²³ but no corresponding antibody is available for echinoderms. The problem of finding a specific inhibitor of IP_3 -induced Ca^{2+} release was overcome by use of a dominant negative form of the IP_3 -generating enzyme phospholipase C γ . In the presence of this inhibitor, Ca^{2+} release at fertilization of starfish eggs,²⁴ as well as sea urchin eggs^{25,26} could be completely prevented (see next section). These findings not only established that IP_3 causes Ca^{2+} release at fertilization of echinoderm eggs, but also showed that phospholipase C γ is required.

The IP_3 -sensitive store in echinoderm eggs is the endoplasmic reticulum (ER),²⁷ and fertilization of centrifugally stratified sea urchin eggs shows Ca^{2+} release occurring from the zone containing the ER.²⁸ In echinoderm eggs, the release of Ca^{2+} is accompanied by fragmentation of the ER, as detected by the loss of pathways for diffusion of molecules in the ER lumen or membrane.^{29,30} ER continuity is regained as Ca^{2+} is resequestered. The return of cytosolic Ca^{2+} to a low level after fertilization results at least in part from its resequestration into the ER, since injection of IP_3 into sea urchin eggs at 20 min after fertilization causes Ca^{2+} release comparable to that at fertilization.³¹ However, uptake into mitochondria also appears to occur.²⁸

Phospholipase C γ and Ca^{2+} release

IP_3 is produced from phosphatidylinositol 4,5-bisphosphate by the phospholipase C (PLC) family of enzymes.³² The function of a PLC in causing the Ca^{2+} rise during fertilization of sea urchin eggs has been suggested by the use of the general PLC inhibitor, U73122, although this inhibitor also has actions unrelated to PLC.³³ Of the three isoforms of PLC, β , γ and δ , echinoderm eggs contain PLC γ ,^{34,35} and probably PLC β as well;²⁴ it is not known if PLC δ is present.

In sea urchin eggs, an increase in PLC γ activity occurs by 30 s post-insemination.³⁵ A requirement for PLC γ activation for the initiation of Ca^{2+} release at fertilization of echinoderm eggs was demonstrated by injecting eggs with a dominant negative fragment of the PLC γ protein.^{24–26} PLC γ is activated when it is phosphorylated by a tyrosine kinase; the interaction with the tyrosine kinase occurs by way of two tandem Src homology 2 (SH2) domains present in the PLC γ sequence, which recognize a phosphotyrosine-containing sequence in the tyrosine kinase.³² Microinjection of mammalian cells with excess PLC γ SH2 domains can inhibit PLC γ activation in response to tyrosine kinase stimulation, presumably by interacting with the tyrosine kinase and blocking access of endogenous full-length PLC γ .^{36,37} Likewise, microinjection of PLC γ SH2 domains into eggs of starfish²⁴ and sea urchin^{25,26} inhibits the cytoplasmic Ca^{2+} increase that occurs during fertilization. The PLC γ SH2 domains affect the initiation and amplitude of the Ca^{2+} rise in a concentration-dependent manner. At lower concentrations, the SH2 domains increase the time between sperm–egg fusion and the initiation of the Ca^{2+} rise, and reduce the amplitude of the resulting Ca^{2+} increase. At higher concentrations, the SH2 domains completely inhibit the Ca^{2+} rise. Sperm entry into these eggs occurs normally, indicating that the PLC γ SH2 domains do not interfere with sperm–egg fusion.

Microinjection of IP_3 bypasses the block of Ca^{2+} release by the PLC γ SH2 domains, confirming that the SH2 domains act upstream of IP_3 production.^{24–26} Ca^{2+} signalling through PLC β , cGMP, or cADPR is not inhibited by PLC γ SH2 domains, indicating the specificity of the inhibitor.^{24,25} Additional evidence for specificity comes from experiments in which mutant PLC γ SH2 domains, which are deficient in binding to phosphotyrosine (and thus inactive), were found to have no effect on the Ca^{2+} rise during fertilization.^{24,25} Furthermore, SH2 domains from several other signalling proteins (with one exception, see below) have no effect on Ca^{2+} release during fertilization.^{24–26,38,39}

Similar experiments have demonstrated that PLC γ is also required for the Ca²⁺ increase that occurs during fertilization of ascidian eggs,⁴⁰ but in vertebrate eggs, the mechanisms leading to IP₃ production and the Ca²⁺ rise during fertilization are unknown. In frog and mouse eggs, the non-specific PLC inhibitor U73122 has an inhibitory effect on the Ca²⁺ release at fertilization.^{41,42} However, neither PLC γ SH2 domains, nor a function-blocking antibody against G_q family G-proteins, which prevents the activation of PLC β , have any effect on Ca²⁺ release at fertilization in these species.^{37,43,44}

Src family kinases and Ca²⁺ release

The finding that the γ isoform of PLC is responsible for the production of IP₃ at fertilization of echinoderm eggs allows several predictions about the signalling components that are upstream of PLC γ in the egg activation pathway. Since PLC γ enzymatic activity is commonly regulated by tyrosine phosphorylation,³² one can predict that a protein tyrosine kinase(s) will be activated at fertilization. A second prediction is that the interaction of the activated protein tyrosine kinase and PLC γ should be fertilization dependent and rapid (occurring prior to Ca²⁺ release), and should result in the tyrosine phosphorylation of PLC γ . Finally, activation of the tyrosine kinase should be necessary for normal Ca²⁺ release at fertilization and sufficient to cause Ca²⁺ release in the absence of a fertilizing sperm.

In echinoderm eggs, an increase in tyrosine kinase activity has been detected by 15 s post-insemination,⁴⁵ and the general protein tyrosine kinase inhibitor genistein delays the onset of Ca²⁺ release.⁴⁶ One group of tyrosine kinases that participates, directly or indirectly, in the activation of PLC γ is the Src family.⁴⁷ Src family kinases are present in echinoderm eggs,^{39,48–50} and *in vitro* kinase assays of proteins immunoprecipitated with a Src family kinase antibody have indicated that in sea urchin eggs, a Src family kinase is activated within 30 s after insemination.³⁹

In starfish eggs, fertilization results in the specific binding of a Src family kinase to the SH2 domains of PLC γ .⁵¹ The interaction of the Src family kinase with PLC γ SH2 domains is detected within 15 s of insemination, and correlates with an increase in the level of tyrosine kinase activity bound to the PLC γ SH2 domains. Related experiments using the SH2 domain of the Src family kinase Fyn, or a construct including the Fyn SH3 and unique domains, showed that PLC γ protein and a corresponding phospholipase activity from

fertilized sea urchin egg extracts specifically bind these Fyn protein domains.⁵² These results indicated a rapid interaction between a Src family kinase and PLC γ during fertilization of echinoderm eggs. An increase in tyrosine phosphorylation of PLC γ at fertilization has not been detected, but the increase could be very small if it occurs locally at the site of sperm–egg interaction.^{34,35}

The requirement for a Src family kinase in initiating Ca²⁺ release at fertilization was investigated using a dominant negative approach similar to that used with the PLC γ SH2 domains (discussed above). Microinjection of starfish,³⁸ and sea urchin^{39,52} eggs with dominant negative Src family kinase SH2 domains results in a concentration-dependent delay or complete inhibition of Ca²⁺ release in response to a fertilizing sperm. Injection of control SH2 domains from three non-Src family kinases is not inhibitory, and injection of a point-mutated form of the Src SH2 domain, which binds phosphotyrosine with reduced affinity, shows reduced inhibition of Ca²⁺ release at fertilization. In addition, PP1, a pharmacological inhibitor of Src family kinases, significantly delays Ca²⁺ release in sea urchin eggs.³⁹ These results provide evidence that SH2 domain-mediated interactions with a Src family kinase are a requisite component of the pathway leading to Ca²⁺ release at fertilization in echinoderm eggs.

The sufficiency of Src kinase activity to initiate Ca²⁺ release in the absence of fertilization was established with the observation that Ca²⁺ release is initiated when starfish eggs are microinjected with a form of Src that has high kinase activity.⁴⁷ This response depends on the phosphorylation state of the Src protein, as only the kinase active form is able to initiate Ca²⁺ release. Moreover, as was observed at fertilization, Ca²⁺ release in response to Src protein injection is delayed or blocked by a prior injection of the dominant negative PLC γ SH2 domains. However, unlike fertilization, a prior injection of the dominant negative Src SH2 domains has no inhibitory effect on Ca²⁺ release in response to active Src protein injection. These findings allow us to propose a model (Figure 1), in which fertilization results in the SH2 domain-mediated activation of a Src family kinase. Once the Src family kinase is activated, it then directly or indirectly causes the phosphorylation and activation of PLC γ . This activation step requires the binding of PLC γ SH2 domains, as both fertilization and injection of active Src protein are inhibited by excess dominant negative PLC γ SH2 domains.

Experiments with ascidian eggs support a similar model.⁴⁰ In vertebrates, experiments with frog eggs also indicate the involvement of a Src family kinase in the fertilization signalling pathway.⁴¹ Pharmacological

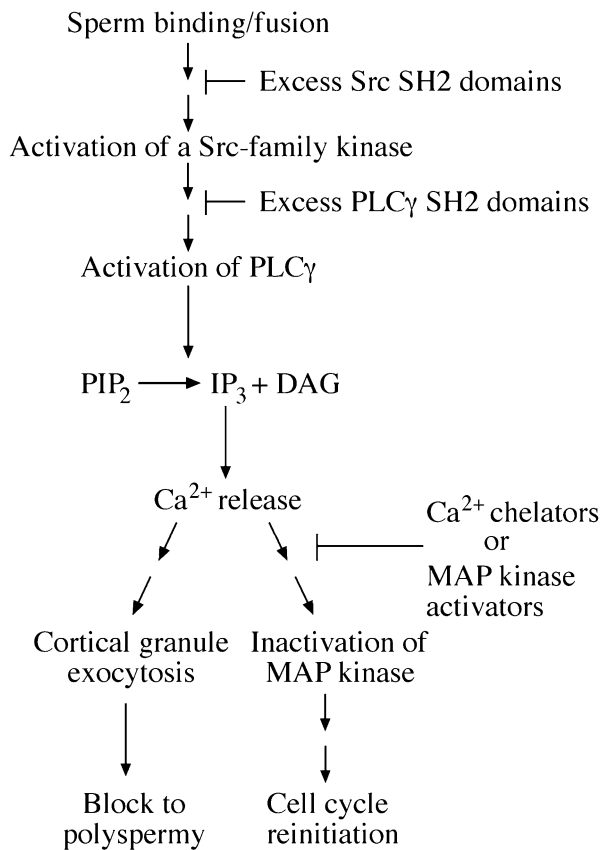


Figure 1. A model of the signal transduction pathway that leads from sperm–egg interaction to the Ca^{2+} rise, and from the Ca^{2+} rise to the resumption of the cell cycle during echinoderm fertilization. Introduction of SH2 domains of Src and $\text{PLC}\gamma$, Ca^{2+} chelators, and MAP kinase activators interferes with signalling at the indicated points.

inhibitor studies suggest the function of a tyrosine kinase in mouse fertilization, but this remains to be established definitively.⁴² Although the fertilization signalling pathway that leads to IP_3 production may differ somewhat between echinoderms and vertebrates, a tyrosine kinase may be a common feature.

A major unanswered question is how the contact and/or fusion of the sperm and egg plasma membranes results in the activation of a Src family kinase and the ensuing events (see discussion in References 9, 10 and 40). One hint comes from a recent study of ascidians, in which Ca^{2+} release in response to either fertilization or injection of an extract of sperm was found to be inhibited by SH2 domains of $\text{PLC}\gamma$ or of a Src family kinase.⁴⁰ These results support the hypothesis that in ascidians at least, Ca^{2+} release at fertilization is initiated by a soluble factor from the sperm cytoplasm (presumably a protein, since it

is inactivated by heat). This protein might be a regulator, directly or indirectly, of a Src family kinase in the egg. A variation of this hypothesis is that a protein from the sperm membrane serves as an activator of Src in the egg cytoplasm, when the sperm membrane comes in contact with the egg cytoplasm as a consequence of sperm–egg fusion. Alternatively, a sperm membrane protein might activate Src as a consequence of binding to a protein in the egg membrane.

The molecule that activates Src at fertilization of echinoderm eggs appears to do so by way of the SH2 domain of Src.⁴⁷ In somatic cells, proteins that activate Src by binding to its SH2 domain include the PDGF receptor, antigen receptors, and the focal adhesion kinase FAK; these proteins serve as Src activators only when they themselves are tyrosine phosphorylated.^{47,48} Possibly sperm–egg interaction results in the tyrosine phosphorylation of such a protein in the egg, allowing it to bind to and activate Src. Alternatively sperm–egg fusion might introduce an already phosphorylated Src activator into the egg cytoplasm from the sperm. Either of these mechanisms would be consistent with the requirement for the Src SH2 domain in the interaction leading to the activation of the Src-like kinase in the egg at fertilization.

Ca^{2+} and the reinitiation of the cell cycle at fertilization

At fertilization, the quiescent egg resumes the cell division cycle, leading to the formation of a new multicellular organism. Ca^{2+} appears to be a universal regulator of this response, although eggs of different species are arrested, and then restarted, at different stages of the meiotic or mitotic cell cycle. In vertebrates, the Ca^{2+} rise at fertilization reinitiates the cell cycle from second meiotic metaphase.^{7,8,53} In echinoderms, fertilization normally occurs at first metaphase (starfish) or after the completion of meiosis (sea urchins). However, in both starfish and sea urchins, meiosis is completed independently of fertilization, and the Ca^{2+} rise at fertilization serves to stimulate the egg to proceed into first mitosis. In sea urchins,^{25,54,55} and some starfish,^{56–58} the control point is before DNA synthesis (from G1 arrest), while in some other starfish, the primary control point is after DNA synthesis (from G2 arrest).⁵⁹ The evidence for regulation by Ca^{2+} is that experimental elevation of Ca^{2+} stimulates the resumption of the cell cycle as occurs at fertilization,^{53–55,58,59} while injection of Ca^{2+} chelators or $\text{PLC}\gamma$ SH2 domains, which prevent the Ca^{2+} rise at fertilization, prevent cell cycle resumption.^{25,55,59}

How Ca²⁺ reinitiates the cell cycle at fertilization is only beginning to be understood. The signalling pathway differs from that in somatic cells responding to mitogens in that new protein synthesis is not required for sea urchin eggs to enter the first S phase.⁶⁰ Recent evidence indicates that for both sea urchins and starfish, inactivation of mitogen-activated protein kinase (MAPK) may be one of the links between the Ca²⁺ rise to the reactivation of the cell cycle (Figure 1). In unfertilized eggs, MAPK is in its phosphorylated and active state; after fertilization, it becomes dephosphorylated and therefore inactive^{25,55–57,59,61} (but see Reference 62, and discussion in Reference 55). In sea urchin eggs, these changes in MAPK are detected within 5–15 min after insemination,⁵⁵ and DNA synthesis begins at about 15–20 min after insemination.⁶⁰ If MAPK activity is experimentally maintained, by injection of kinases that maintain its phosphorylated state, the fertilized egg does not re-enter the cell cycle.^{56,59} Conversely, inactivation of MAPK in an unfertilized egg, by injection of MAPK specific phosphatases or application of an inhibitor of MAPK kinase, results in cell cycle resumption without fertilization.^{55–57,59} The conclusion that Ca²⁺ causes the inactivation of MAPK at fertilization is supported by evidence that raising Ca²⁺ with the Ca²⁺ ionophore A23187 or injection of buffered Ca²⁺ inactivates MAPK, and that preventing the Ca²⁺ rise by injection of Ca²⁺ chelators or PLC γ SH2 domains prevents the inactivation of MAPK at fertilization^{25,55,59} (but see Reference 62). The links between the Ca²⁺ rise at fertilization, the inactivation of MAPK, and the resumption of the cell cycle are unknown.

Ca²⁺ is also a regulator of cell cycle reinitiation at fertilization in vertebrate eggs.^{7,8,63} As in echinoderms, MAPK is inactivated at fertilization in frog eggs,^{41,64} and this appears to result from the Ca²⁺ rise.⁶³ Based on the kinetics of the degradation of the MAPK kinase kinase, mos, MAPK inactivation does not appear to cause the reinitiation of meiosis from second metaphase arrest in vertebrate eggs, but it may function in the initiation of DNA synthesis.^{65,66} For further discussion of the role of Ca²⁺ in cell cycle regulation, see the paper by Whitaker and Larman, this issue.¹¹

Conclusions

For almost 25 years, it has been known that fertilization causes a release of Ca²⁺ from intracellular stores in the egg, and that the initiation of embryonic development depends on this rise in cytosolic Ca²⁺. Yet it remains

unknown how the contact and/or fusion of the sperm and egg plasma membranes initiates the Ca²⁺ release. In sea urchin and starfish eggs, these early plasma membrane interactions somehow cause the activation of a Src family kinase, which leads to the activation of phospholipase C γ , which produces IP₃, which releases Ca²⁺ from the ER. However, it is unknown what stimulates IP₃ production in vertebrate eggs. An apparently universal consequence of the Ca²⁺ rise at fertilization is the resumption of the cell cycle, and recent evidence indicates a role for the inactivation of MAP kinase in this process. However, it is unknown how Ca²⁺ causes MAP kinase inactivation, and how MAP kinase inactivation causes DNA synthesis. Thus, despite considerable progress, fertilization continues to present a wealth of questions about Ca²⁺ signalling.

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Note added in proof

A recent paper⁶⁷ has proposed that nitric oxide synthase may be the factor from sea urchin sperm that causes Ca²⁺ release in sea urchin eggs at fertilization. However, as noted above, this hypothesis is difficult to reconcile with the finding that Rp-8-pCPT-cGMPs, which is an inhibitor of cGMP-dependent protein kinase, inhibits NO-induced Ca²⁺ release,¹⁸ but not fertilization-induced Ca²⁺ release.²⁰ It will be important to determine whether a specific inhibitor of nitric oxide synthase inhibits Ca²⁺ release at fertilization, and whether known inhibitors of Ca²⁺ release at fertilization, such as the SH2 domains of PLC γ and Src, inhibit Ca²⁺ release in response to NO.

References

1. Heilbrunn LV, Young RA (1930) The action of ultra-violet rays on Arbacia egg protoplasm. *Physiol Zool* 3:330–341
2. Ridgway EB, Gilkey JC, Jaffe LF (1977) Free calcium increases explosively in activating medaka eggs. *Proc Natl Acad Sci USA* 74:623–627
3. Steinhart R, Zucker R, Schatten G (1977) Intracellular calcium release at fertilization in the sea urchin egg. *Dev Biol* 58:185–196
4. Stricker SA (1999) Comparative biology of calcium signaling

- during fertilization and egg activation in animals. *Dev Biol* 211:157–176
5. Crossley I, Whalley T, Whitaker M (1991) Guanosine 5'-thiotriphosphate may stimulate phosphoinositide messenger production in sea urchin eggs by a different route than the fertilizing sperm. *Cell Regulation* 2:121–133
 6. Zucker RS, Steinhardt RA (1978) Prevention of the cortical reaction in fertilized sea urchin eggs by injection of calcium-chelating ligands. *Biochem Biophys Acta* 541:459–466
 7. Kline D (1988) Calcium-dependent events at fertilization of the frog egg: injection of a calcium buffer blocks ion channel opening, exocytosis, and formation of pronuclei. *Dev Biol* 126:346–361
 8. Kline D, Kline JT (1992) Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Dev Biol* 149:80–89
 9. Jaffe LA (1996) Egg membranes during fertilization, in *Molecular Biology of Membrane Transport Disorders* (Schultz SG, Andreoli T, Brown A, Fambrough D, Hoffman J, Welsh M, eds) pp. 367–378. Plenum, New York
 10. Carroll J (2000) The initiation and regulation of Ca^{2+} signalling at fertilisation in mammals. *Sem Cell Dev Biol* 12:37–43
 11. Whitaker M, Larman MG (2001) Calcium and mitosis. *Sem Cell Dev Biol* 12:53–58
 12. Turner PR, Sheetz MP, Jaffe LA (1984) Fertilization increases the polyphosphoinositide content of sea urchin eggs. *Nature* 310:414–415
 13. Ciapa B, Whitaker M (1986) Two phases of inositol polyphosphate and diacylglycerol production at fertilization. *FEBS Lett* 195:347–351
 14. Whitaker M, Irvine RF (1984) Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature* 312:636–639
 15. Whalley T, McDougall A, Crossley I, Swann K, Whitaker M (1992) Internal calcium release and activation of sea urchin eggs by cGMP are independent of the phosphoinositide signaling pathway. *Mol Biol Cell* 3:373–383
 16. Lee HC, Aarhus R, Walseth TF (1993) Calcium mobilization by dual receptors during fertilization of sea urchin eggs. *Science* 261:352–355
 17. Perez-Terzic CM, Chini EN, Shen SS, Dousa TP, Clapham DE (1995) Ca^{2+} release triggered by nicotinate adenine dinucleotide phosphate in intact sea urchin eggs. *Biochem J* 312:955–959
 18. Willmott N, Sethi JK, Walseth TF, Lee HC, White AM, Galione A (1996) Nitric oxide-induced mobilization of intracellular calcium via the cyclic ADP-ribose signaling pathway. *J Biol Chem* 271:3699–3705
 19. Ciapa B, Epel D (1996) An early increase in cGMP follows fertilization of sea urchin eggs. *Biochem Biophys Res Comm* 223:633–636
 20. Lee S-J, Christenson L, Martin T, Shen SS (1996) The cyclic GMP-mediated calcium release pathway in sea urchin eggs is not required for the rise in calcium during fertilization. *Dev Biol* 180:324–335
 21. Créton R, Jaffe LF (1995) Role of calcium influx during the latent period in sea urchin fertilization. *Dev Growth Differ* 37:703–709
 22. Schmidt T, Patton C, Epel D (1982) Is there a role for the Ca^{2+} influx during fertilization of the sea urchin egg? *Dev Biol* 90:284–290
 23. Miyazaki S, Yuzaki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S, Mikoshiba K (1992) Block of Ca^{2+} wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5- trisphosphate receptor in fertilized hamster eggs. *Science* 257:251–255
 24. Carroll DJ, Ramarao CS, Mehlmann LM, Roche S, Terasaki M, Jaffe LA (1997) Calcium release at fertilization in starfish eggs is mediated by phospholipase $\text{C}\gamma$. *J Cell Biol* 138:1303–1311
 25. Carroll DJ, Albay DT, Terasaki M, Jaffe LA, Foltz KR (1999) Identification of $\text{PLC}\gamma$ -dependent and independent events during fertilization of sea urchin eggs. *Dev Biol* 206:232–257
 26. Shearer J, De Nadai C, Emily-Fenouil F, Gache C, Whitaker M, Ciapa B (1999) Role of phospholipase $\text{C}\gamma$ at fertilization and during mitosis in sea urchin eggs and embryos. *Development* 126:2273–2284
 27. Terasaki M, Sardet C (1991) Demonstration of calcium uptake and release by sea urchin egg cortical endoplasmic reticulum. *J Cell Biol* 115:1031–1037
 28. Eisen A, Reynolds GT (1985) Source and sinks for the calcium released during fertilization of single sea urchin eggs. *J Cell Biol* 100:1522–1527
 29. Terasaki M, Jaffe LA (1991) Organization of the sea urchin egg endoplasmic reticulum and its reorganization at fertilization. *J Cell Biol* 114:929–940
 30. Terasaki M, Jaffe LA, Hunnicutt GR, Hammer JA (1996) Structural change of the endoplasmic reticulum during fertilization: Evidence for loss of membrane continuity using the green fluorescent protein. *Dev Biol* 179:320–328
 31. Twigg J, Patel R, Whitaker M (1988) Translational control of InsP_3 -induced chromatin condensation during the early cell cycles of sea urchin embryos. *Nature* 332:366–369
 32. Rhee SG, Bae YS (1997) Regulation of phosphoinositide-specific phospholipase C isozymes. *J Biol Chem* 272:15045–15048
 33. Lee SJ, Shen SS (1998) The calcium transient in sea urchin eggs during fertilization requires the production of inositol 1,4,5-trisphosphate. *Dev Biol* 193:195–208
 34. De Nadai C, Cailliau K, Epel D, Ciapa B (1998) Detection of phospholipase $\text{C}\gamma$ in sea urchin eggs. *Dev Growth Differ* 40:669–676
 35. Rongish BJ, Wu W, Kinsey WH (1999) Fertilization-induced activation of phospholipase C in the sea urchin egg. *Dev Biol* 215:147–154
 36. Roche S, McGlade J, Jones M, Gish GD, Pawson T, Courtneidge SA (1996) Requirement of phospholipase $\text{C}\gamma$, the tyrosine phosphatase Syp and the adaptor proteins Shc and Nck for PDGF-induced DNA synthesis: Evidence for the existence of Ras-dependent and Ras-independent pathways. *EMBO J* 15:4940–4948
 37. Mehlmann LM, Carpenter G, Rhee SG, Jaffe LA (1998) SH2 domain-mediated activation of phospholipase $\text{C}\gamma$ is not required to initiate Ca^{2+} release at fertilization of mouse eggs. *Dev Biol* 203:221–232
 38. Giusti AF, Carroll DJ, Abassi YA, Terasaki M, Foltz KR, Jaffe LA (1999) Requirement of a Src family kinase for initiating calcium release at fertilization of starfish eggs. *J Biol Chem* 274:29318–29322
 39. Abassi YA, Carroll DJ, Giusti AF, Belton RJ, Foltz KR (2000) Evidence that Src-type tyrosine kinase activity is necessary for initiation of Ca^{2+} release at fertilization in sea urchin eggs. *Dev Biol* 218:206–219
 40. Runft LL, Jaffe LA (2000) Sperm extract injection into ascidian eggs signals Ca^{2+} release by the same pathway as fertilization. *Development* 127:3227–3236
 41. Sato K, Tokmakov AA, Iwasaki T, Fukami Y (2000) Tyrosine kinase-dependent activation of phospholipase $\text{C}\gamma$ is required for calcium transient in *Xenopus* egg fertilization. *Dev Biol* 224:453–469
 42. Dupont G, McGuinness OM, Johnson MH, Berridge MJ, Borgese F (1996) Phospholipase C in mouse oocytes: Characterization of β and γ isoforms and their possible involvement in sperm-induced Ca^{2+} spiking. *Biochem J* 316:583–591
 43. Williams CJ, Mehlmann LM, Jaffe LA, Kopf GS, Schultz RM (1998) Evidence that Gq family G proteins do not function in mouse egg activation at fertilization. *Dev Biol* 198:116–127
 44. Runft LL, Watras J, Jaffe LA (1999) Calcium release at fertilization of *Xenopus* eggs requires type I IP_3 receptors, but not SH2 domain-

- mediated activation of PLC γ or Gq mediated activation of PLC β . *Dev Biol* 214:399–411
45. Ciapa B, Epel D (1991) A rapid change in phosphorylation on tyrosine accompanies fertilization of sea urchin eggs. *FEBS Lett* 295:167–170
 46. Shen SS, Kinsey WH, Lee SJ (1999) Protein tyrosine kinase-dependent release of intracellular calcium in the sea urchin egg. *Dev Growth Differ* 41:345–355
 47. Giusti AF, Xu W, Hinkle B, Terasaki M, Jaffe LA (2000) Evidence that fertilization activates starfish eggs by sequential activation of a Src-like kinase and phospholipase C γ . *J Biol Chem* 275:16788–16794
 48. Abram CL, Courtneidge SA (2000) Src family tyrosine kinases and growth factor signaling. *Exp Cell Res* 254:1–13
 49. Kinsey WH (1997) Tyrosine kinase signaling at fertilization. *Biochem Biophys Res Commun* 240:519–522
 50. Onodera H, Kobari K, Sakuma M, Sato M, Suyemitsu T, Yamasu K (1999) Expression of a *src*-type protein tyrosine kinase gene, *AcSrc1*, in the sea urchin embryo. *Dev Growth Differ* 41:19–28
 51. Giusti AF, Carroll DJ, Abassi YA, Foltz KR (1999) Evidence that a starfish egg Src family tyrosine kinase associates with PLC- γ 1 SH2 domains at fertilization. *Dev Biol* 208:189–199
 52. Kinsey WH, Shen SS (2000) Role of the Fyn kinase in calcium release during fertilization of the sea urchin egg. *Dev Biol* 225:253–264
 53. Steinhardt RA, Epel D, Carroll EJ, Yanagimachi R (1974) Is calcium ionophore a universal activator for unfertilized eggs? *Nature* 252:41–43
 54. Steinhardt RA, Epel D (1974) Activation of sea urchin eggs by a calcium ionophore. *Proc Natl Acad Sci USA* 71:1915–1919
 55. Carroll DJ, Albay DT, Hoang KM, O'Neill FJ, Kumano M, Foltz KR (2000) The relationship between calcium, MAP kinase, and DNA synthesis in the sea urchin egg at fertilization. *Dev Biol* 217:179–191
 56. Tachibana K, Machida T, Nomura Y, Kishimoto T (1997) MAP kinase links the fertilization signal transduction pathway to the G₁/S-phase transition in starfish eggs. *EMBO J* 16:4333–4339
 57. Sadler KC, Ruderman JV (1998) Components of the signaling pathway linking the 1-methyladenine receptor to MPF activation and maturation in starfish oocytes. *Dev Biol* 197:25–38
 58. Nomura A, Nemoto S (1998) DNA replication cycle in parthenogenetically developing eggs of the starfish *Asterina pectinifera*. *Dev Growth Differ* 40:377–386
 59. Fisher D, Abrieu A, Simon M-N, Keyse S, Vergé V, Dorée M, Picard A (1998) MAP kinase inactivation is required only for G2-M phase transition in early embryogenesis cell cycles of the starfishes *Marthasterias glacialis* and *Astropecten aranciacus*. *Dev Biol* 202:1–13
 60. Zhang H, Ruderman JV (1993) Differential replication capacities of G₁ and S-phase extracts from sea urchin eggs. *J Cell Sci* 104:565–572
 61. Chiri S, De Nadai C, Ciapa B (1998) Evidence for MAP kinase activation during mitotic division. *J Cell Sci* 111:2519–2527
 62. Philipova R, Whitaker M (1998) MAP kinase activity increases during mitosis in early sea urchin embryos. *J Cell Sci* 111:2497–2505
 63. Lorca T, Cruzalegui FH, Fesquet D, Cavadore J-C, Méry J, Means A, Dorée M (1993) Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of *Xenopus* eggs. *Nature* 366:270–273
 64. Ferrell JE, Wu M, Gerhart JC, Martin GS (1991) Cell cycle phosphorylation of p34^{cdc2} and a microtubule-associated protein kinase homolog in *Xenopus* oocytes and eggs. *Mol Cell Biol* 11:1965–1971
 65. Sagata N (1996) Meiotic metaphase arrest in animal oocytes: its mechanisms and biological significance. *Trends Cell Biol* 6:22–28
 66. Gross ST, Schwab MS, Taieb FE, Lewellyn AL, Qian Y-W, Maller JL (2000) The critical role of the MAP kinase pathway in meiosis II in *Xenopus* oocytes is mediated by p90^{Rsk}. *Curr Biol* 10:430–438
 67. Kuo RC, Baxter GT, Thompson SH, Stricker SA, Patton C, Bonaventura J, Epel D (2000) NO is necessary and sufficient for egg activation at fertilization. *Nature* 406:633–636