Chapter 3
Fertilization in Starfish and Sea Urchin: Roles of Actin

Jong Tai Chun, Filip Vasilev, Nunzia Limatola, and Luigia Santella

Abstract Marine animals relying on "external fertilization" provide advantageous opportunities to study the mechanisms of gamete activation and fusion, as well as the subsequent embryonic development. Owing to the large number of eggs that are easily available and handled, starfish and sea urchins have been chosen as favorable animal models in this line of research for over 150 years. Indeed, much of our knowledge on fertilization came from studies in the echinoderms. Fertilization involves mutual stimulation between eggs and sperm, which leads to morphological, biochemical, and physiological changes on both sides to ensure successful gamete fusion. In this chapter, we review the roles of actin in the fertilization of starfish and sea urchin eggs. As fertilization is essentially an event that takes place on the egg surface, it has been predicted that suboolemmal actin filaments would make significant contributions to sperm entry. A growing body of evidence from starfish and sea urchin eggs suggests that the prompt reorganization of the actin pools around the time of fertilization plays crucial regulatory roles not only in guiding sperm entry but also in modulating intracellular Ca^{2+} signaling and egg activation.

3.1 Introduction

Conceptually, fertilization is a simple event: union of sperm and egg from the same species to produce an offspring. The actual fertilization process, however, involves a series of preparatory changes that have to be exquisitely coordinated in both gametes. For example, before reaching the egg, spermatozoa should gain their cytological competence by undergoing acrosome reaction and acquire apt motility for successful fertilization to occur. The substance on the egg surface may induce these changes and emit the cues for chemotaxis. On the other hand, the oocytes

J. T. Chun (✉) · F. Vasilev · N. Limatola · L. Santella (✉)
Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy
e-mail: chun@szn.it; santella@szn.it

M. Kloc, J. Z. Kubiak (eds.), *Marine Organisms as Model Systems in Biology and Medicine*, Results and Problems in Cell Differentiation 65,
https://doi.org/10.1007/978-3-319-92486-1_3

should be matured into fertilization-competent eggs before fertilization. The sequential programs that lead to gamete maturation, chemotaxis, acrosome reaction, and the binding of sperm to eggs may fundamentally vary depending on the phyla and species, as has been reviewed elsewhere (Trimmer and Vacquier 1986; Hirohashi et al. 2008; Santella et al. 2012). Upon sperm's binding and fusion, the fertilized egg, which should be now called a zygote, undergoes a series of changes. The first detectable change is a swift shift of the membrane potential, which is accompanied by other cytological modifications such as cytoskeletal alteration and the increase of cytosolic pH and Ca^{2+} that are known to play important roles for the resumption of cell cycle and subsequent embryonic development. The intracellular Ca^{2+} increase in fertilized eggs is achieved mainly by two mechanisms, i.e., influx from the extracellular sources through voltage-gated ion channels and the ligand-gated release from the intracellular stores. The contribution and pattern of these two modes of Ca^{2+} increase may also differ widely depending on the species (Jaffe 1993; Miyazaki et al. 1975; Miyazaki 2006; Stricker 1999; Santella et al. 2004, 2012, 2015; Whitaker 2006). Hence, fertilization involves fine regulation of ion channel activities and cytoskeleton, as well as other cell signaling events that usher in a new life cycle in the zygote. In this chapter, we review how the basic cytoskeletal protein actin is utilized in the fertilization of starfish and sea urchin eggs.

3.2 Actin in Sperm Acrosome Reaction

In the research of fertilization, sea urchin and starfish have traditionally served as important animal models since sperm's entry into eggs was first viewed in the echinoderm species: *Echinus esculentus* (Dufossé 1847), *Paracentrotus lividus* (Hertwig 1876), and *Asterias glacialis* (Fol 1879). While sperm in animal kingdom conspicuously display vast interspecific diversities in their morphology and the strategy to reach and fuse with the eggs (Pitnick et al. 2009), echinoderm spermatozoa generally tend to use filamentous projection from the head. Owing to this characteristic morphological feature and the timely development of phase-contrast microscopy, acrosome reaction was convincingly demonstrated in starfish sperm (Dan 1952). When exposed to the homologous egg water, sperm from diverse species of starfish (*Asterina pectinifera*, *Asterias amurensis*, and *Astropecten scoparius*) readily extended long, slender, and rigid filaments (Dan et al. 1954). This so-called acrosomal process of starfish sperm can reach 25 μm in length, which is much longer than the one produced by sea urchin sperm (Fig. 3.1), and may serve as a tether to draw the sperm into the fertilized egg (Dan et al. 1954; Puppo et al. 2008). The activated sperm of another echinoderm, sea cucumber can extend even longer acrosomal process than starfish sperm. In the case of *Thyone briareus*, the extending acrosomal process can reach over 70 μm in length within just 7 s. The exceedingly fast extension of the acrosomal process turned out to be due to the rapid polymerization of actin monomers that are reserved between the acrosomal vesicle and the nucleus of the sperm (Tilney et al. 1973; Tilney and Inoué 1982). This

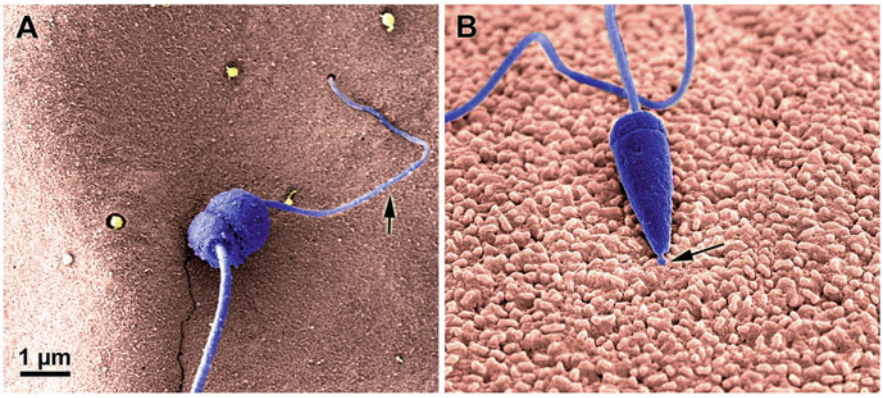

Fig. 3.1 Fertilizing sperm on the egg surface viewed by scanning electron microscopy. (**a**) Starfish (*A. pectinifera*). Several well-like holes are open in the egg vitelline coat. Sperm acrosomal process (arrow) pierces into the hole and gains access to egg microvilli. (**b**) Sea urchin (*Paracentrotus lividus*). Note that the acrosomal process extending from the sea urchin sperm head (arrow) is much shorter than that of the starfish sperm. Image (**b**) adapted from Embryogenesis Explained (Gordon and Gordon 2016), Copyright@2016 World Scientific Publishing, Singapore

phenomenon was one of the first examples suggesting that actin polymerization per se can provide protrusive force to push the cell boundary (Footer et al. 2007) and rendered insights into the mechanisms of cell motility and plasticity through the changes in specialized actin-based subcellular structures such as lamellipodia, filopodia, microspikes, and growth cones (Welch et al. 1997; Luo 2002; Svitkina 2018). Owing to this long acrosomal process, which traverses the egg jelly coat, starfish spermatozoa can trigger the initial responses in the fertilized eggs, such as swift membrane depolarization, and Ca^{2+} increases while the sperm head still remains outside of the jelly coat (Puppo et al. 2008).

In contrast to the sperm head, sperm tail (flagellum) is predominantly enriched with microtubules that are organized in the highly conserved "9 + 2" arrangement together with the molecular motor dyneins to constitute the axoneme. While the flagella of echinoderm sperm do not contain detectable level of actin (Tilney et al. 1973), F-actin has been intermittently visualized in the sperm of some other species including crane fly and mammals (Behnke et al. 1971; Dvoráková et al. 2005; Cohen et al. 2004; Correa et al. 2007). It has been reported that actin inhibitors such as cytochalasin B and D can deform the tail of macaque sperm and moderately diminish sperm motility in a dose-dependent manner (Correa et al. 2007). However, the presence of actin in the sperm tail has not been clearly demonstrated for echinoderms, and accordingly its contribution to sperm motility has not been much explored.

3.3 Actin in Oocyte Maturation

Toward the end of oogenesis, fully grown oocytes in the ovary are arrested in certain phases of meiotic cell cycle until they are utilized. For fertilization, these oocytes should be converted to fertilization-competent eggs. In starfish, this transition (release from the arrest at the first prophase) is initiated by the oocyte maturation hormone, which was identified as 1-methyladenine (1-MA) (Kanatani et al. 1969). Upon binding to its yet unidentified receptor, which is presumably present in the plasma membrane (Kanatani and Hiramoto 1970), 1-MA induces a series of biochemical and cytological modifications in the oocytes, such as intracellular Ca^{2+} increase (within 1–2 min), reorganization of the actin cytoskeleton, and changes in protein phosphorylation and membrane potential. With the progression of these changes, the nucleus of the oocyte (germinal vesicle, GV) loses its envelope (GV breakdown), and the nucleoplasm intermixes with cytoplasm eventually to render the cell physiologically suitable for fertilization (Moreau et al. 1978; Dale et al. 1979; Dorée et al. 1981; Schroeder and Stricker 1983; Meijer and Guerrier 1984; Lim et al. 2001). It has been shown that, following GV breakdown, the nuclear zone of the starfish oocyte forms a contractile actin network that captures the chromosomes and delivers them within the reach of microtubule asters (Lénárt et al. 2005). Thus, actin is expected to assist in the subsequent progression of meiotic progression.

The surface of oocytes is covered with countless projections of F-actin bundles called microvilli and with the meshwork of subplasmalemmal actin filaments. Both microvilli and the cortical actin meshwork undergo drastic reorganization during the meiotic maturation of oocytes. It is quite conspicuous that the 1-MA-induced reorganization of the actin cytoskeleton, visualized by the structural changes of the microvilli on the surface of oocyte, is as prompt as the Ca^{2+} increase (Schroeder and Stricker 1983; Santella and Kyozuka 1994; Kyozuka et al. 2008). Thus, in response to 1-MA, the number of microvilli inserting into vitelline coat becomes significantly reduced (Hirai et al. 1971), leading to the selective loss of microvilli-residing K^+ channels and the consequent shift in membrane potential during the course of meiotic maturation (Dale et al. 1979; Moody and Bosma 1985).

One crucial cytoplasmic change that takes place during meiotic maturation is the translocation of cortical granules from the inner cytoplasm to the subplasmalemmal zone. This process appears to be heavily dependent upon actin dynamics both in starfish and sea urchin, as judged by its inhibition by the agents interfering with actin dynamics (Santella et al. 1999; Wessel et al. 2002). Cortical granules that are now apposed to the egg plasma membrane are more readily exocytosed and elevate the vitelline layer when the Ca^{2+} wave arrives during fertilization. In addition, both GV and endoplasmic reticulum (ER) appear to be bound and modulated by the actin cytoskeleton (Stricker and Schatten 1991; Terasaki 1994). ER is the major Ca^{2+} store in the egg, which is studded with ion channels such as $InsP_3$ receptor and ryanodine receptor. The actin-based rearrangement of ER in the maturing oocytes may in part contribute to the progressive sensitization of the maturing oocytes to $InsP_3$ (Chiba

et al. 1990; Lim et al. 2003). This phenomenon, in which the same amount of exogenous $InsP_3$ evokes a much larger Ca^{2+} increase in the matured eggs than in the immature oocytes, can be precluded by latrunculin A (LAT-A), a drug promoting actin depolymerization (Lim et al. 2003).

Starfish oocytes reentering meiotic cell cycle by the action of 1-MA manifest intracellular Ca^{2+} increases at two different time points: the early response readily induced by the hormone and the late Ca^{2+} increases at the time of GV breakdown. The earlier response takes place within 1–2 min after the hormone addition, and it comprises spatiotemporally coordinated Ca^{2+} release from internal stores that starts from the vegetal hemisphere and propagates as a single wave along the subplasmalemmal zone (Moreau et al. 1978; Santella and Kyozuka 1994; Dorée and Kishimoto 1981; Kyozuka et al. 2008). The few minutes' time lag required for the Ca^{2+} wave to respond to 1-MA implies that the signal transduction involved in this pathway is quite efficient. Experimental evidence has indicated that 1-MA-induced meiotic resumption is mediated by ßɣ subunit of heterotrimeric G-protein (Chiba et al. 1993; Jaffe et al. 1993). Indeed, metabolically stable GTP (GTPɣS) microinjected into starfish oocytes mimicked 1-MA by readily inducing a similar Ca^{2+} wave, while GDPßS inhibited the GV breakdown, the hallmark of meiotic maturation of oocytes (Kyozuka et al. 2009). Interestingly, it has been shown that alteration of the actin cytoskeleton by LAT-A, jasplakinolide (JAS), or heparin suppresses the 1-MA-induced Ca^{2+} increase (Kyozuka et al. 2008, 2009). For example, pretreatment of *A. pectinifera* oocytes with 12 μM JAS nearly blocked the 1-MA-induced Ca^{2+} wave (Fig. 3.2). However, these oocytes retained intact activities of $InsP_3$ receptor, as judged by the peak amplitude of the Ca^{2+} increase triggered by $InsP_3$ uncaging. On the other hand, oocytes of *A. pectinifera* are known not to respond to cyclic ADP-ribose with a Ca^{2+} increase (Nusco et al. 2002). These and other observations led to the suggestion that the Ca^{2+} increase induced by 1-MA may be mediated by a mechanism distinct from $InsP_3$ receptor or ryanodine receptor (Kyozuka et al. 2008). The late-stage Ca^{2+} increases in maturing oocytes of starfish are due to the influx from outside through the L-type Ca^{2+} channels, which is detected as a train of Ca^{2+} spikes taking place around the time of GV breakdown (Limatola et al. 2015) when the oocytes undergo continual depolarization and swift shift of membrane potential (Dale et al. 1979). This late stage of Ca^{2+} response to 1-MA, which had not been previously reported, was also shown to be dependent upon the status of cortical actin cytoskeleton. Exposure of the maturing oocytes to 1 μM LAT-A 8 min after the hormone addition (thus after the early Ca^{2+} wave has subsided) led to the significant reduction in the frequency of the Ca^{2+} spikes, while the average amplitude of the Ca^{2+} spikes was significantly enhanced by the LAT-A-induced actin depolymerization (Limatola et al. 2015). Taken together, these observations suggest that the reorganization of the cortical actin cytoskeleton and microvilli readily induced by 1-MA is instrumental in fine-tuning other structural and functional changes that take place in maturing eggs, in particular the optimization of the intracellular Ca^{2+} stores and ion channels.

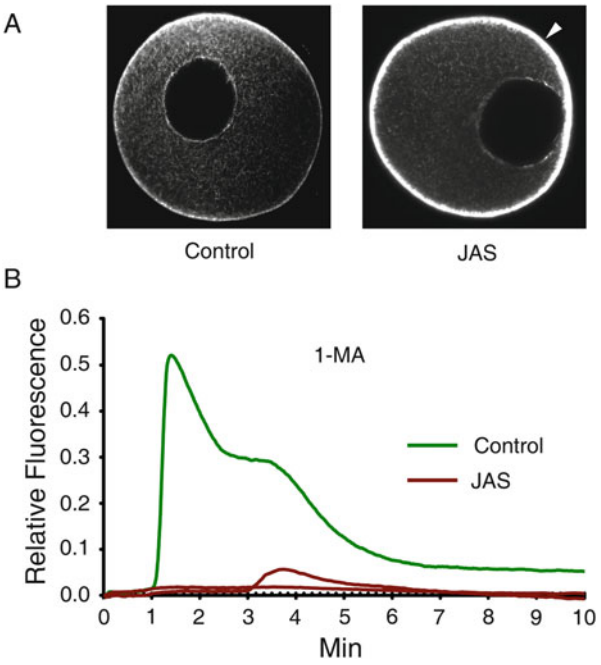

Fig. 3.2 The Ca^{2+} increase induced by 1-MA in the starfish (*A. pectinifera*) oocytes is suppressed by JAS. (**a**) Actin filaments visualized by Alexa Fluor 568 phalloidin in live oocytes with or without (control) preincubation with 12 μM JAS. Note that the subplasmalemmal actin is hyperpolymerized by the incubation with JAS (arrow). (**b**) Quantified Ca^{2+} responses after 1-MA stimulation. Adapted from Kyozuka et al. (2009) PLoS One 4: e6296

3.4 Actin in Fertilized Eggs

Fertilization requires successful gamete recognition, adhesion, and fusion. As aforementioned, the egg and sperm influence each other to induce morphological and physiological changes in a sequential manner, which involves multiple bilateral receptor-ligand couplings (Ohlendieck and Lennarz 1996; Vacquier 2012). In starfish, the egg jelly coat provides at least three signaling substances for homologous spermatozoa to induce acrosome reaction: (1) acrosome reaction-inducing substance (ARIS), which is a sulfated proteoglycan-like molecule with >1000 repeating units; (2) Co-ARIS, sulfated steroidal saponins; and (3) asterosap, a peptide. The cognate receptors on sperm mediate a cascade of biochemical changes affecting acrosomal reaction (Matsumoto et al. 2008). Similarly, sea urchin spermatozoa possess the specific receptors (e.g., suREJ1) for egg jelly protein that are activated to trigger the acrosome reaction (Moy et al. 1996). Exocytosis of the acrosomal vesicle as a result of the Ca^{2+} increase in the sperm may now expose species-specific adhesion/fusion molecules such as bindin on the surface of the acrosomal process (Vacquier and Moy 1977). Two bindin receptors have been identified in sea urchin eggs, which might provide protease activity as well (Kamei and Glabe 2003; Mah et al. 2005). Because an earlier study showed that the 350-kDa bindin receptor is predominantly localized in the tip of microvilli (Ohlendieck et al. 1994), it seems probable that the microvilli may play a crucial role in intercellular fusion during fertilization, as well as in the pathogenic processes and in the immune responses (Wilson and Snell 1998). Indeed,

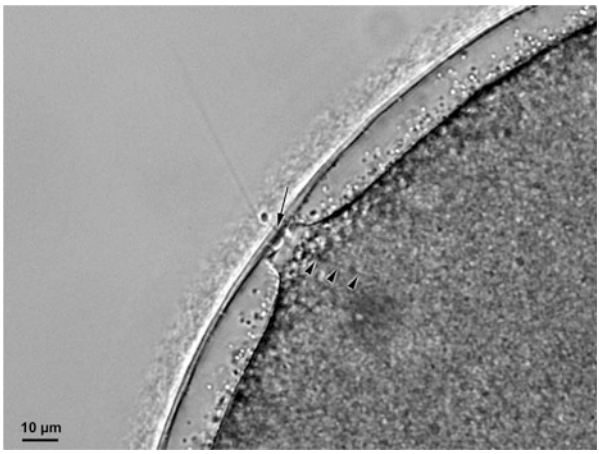

Fig. 3.3 A moment before sperm entry into the fertilized egg of starfish (*A. aranciacus*). The acrosomal process of sperm pierces the vitelline coat, which is about to elevate (arrow), and is adjoined by the egg microfilaments in the fertilization cone. Apparently, the connection continues to a tubular structure in the inner cytoplasm (arrowheads). Adapted from Puppo et al. 2008 PLoS One 3: e3588

lymphocytes are also covered with oocyte-like microvilli (Majstoravich et al. 2004). The actin-filled protrusions from both gametes, i.e., the acrosomal process of activated sperm and the microvilli of eggs, are likely to subserve sperm-egg fusion and the subsequent entrance of sperm into eggs. Analyses of the ultrastructure of sea urchin eggs at fertilization suggested that several microvilli at the sperm fusion site may undergo lateral association to produce a fertilized cone (Tilney and Jaffe 1980). Adjoining of the acrosomal process of incoming sperm with the fertilization cone apparently forms a thick "tether" that can be seen even in the light microscope due to its characteristic optical density (Fig. 3.3). After entering the egg, the sperm head is now attached to several thick bundles of actin filaments in the egg cytoplasm (Fig. 3.4, also see Santella et al. 2016).

Following fertilization, starfish and sea urchin eggs elongate their microvilli as the fertilization envelope is formed. The elongated microvilli traversing the entire depth of the perivitelline space may mechanically contribute to the elevation of the vitelline layer or to the accommodation of the plasma membrane whose area is greatly increased as a result of cortical granule exocytosis and the membrane fusion (Carron and Longo 1982; Chun et al. 2010). Below microvilli, portions of subplasmalemmal actin filaments in the fertilized eggs undergo centripetal translocation (Terasaki 1996; Vasilev et al. 2012), while other filaments remain locally and become reorganized to form a new surface boundary of the zygote (Chun et al. 2010). Although precise roles of the inwardly translocating actin filaments are not known, it is conceivable that their centripetal migration might also assist in the concomitant sperm entry into the egg cortex. Although it is known that the migration of the male and female pronuclei toward each other is mediated by microtubule-based movement, the centrosome separation has been attributed to the actin filaments (Schatten et al. 1988; Holy and Schatten 1991).

Being heavily involved in gamete fusion and sperm entry, actin is thought to play an important role in controlling the number of sperm incorporated into the egg. In line with earlier observation that interference with actin dynamics using cytochalasin

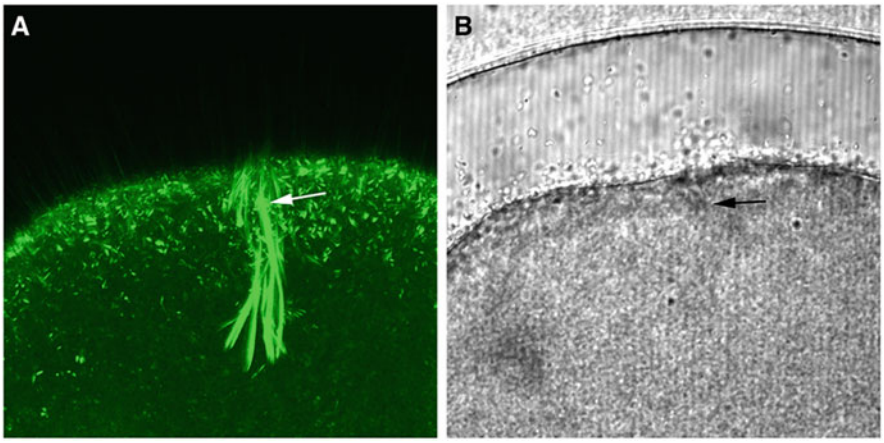

Fig. 3.4 A moment after sperm entry into the fertilized egg of starfish (*A. aranciacus*). Actin filaments were visualized by Alexa Fluor 568 phalloidin in live eggs at fertilization. (**a**) Confocal image. (**b**) Bright field view. While microvilli of the fertilized egg prolong and traverse the perivitelline space, thick actin bundles are now formed and attached to the egg-incorporated sperm head (arrow)

caused polyspermy in the fertilized eggs of clams (Ziomek and Epel 1975), starfish and sea urchin eggs treated with actin-inhibiting drugs manifested either increased polyspermy rates or failure of sperm entry. Hyperpolymerization of subplasmalemmal actin induced by JAS and heparin in starfish egg (*A. aranciacus*) led to supernumerary sperm-egg interactions that induced multiple initiations of Ca^{2+} release (Puppo et al. 2008). The same effect was observed when endogenous depactin, an actin-depolymerizing protein, was inhibited in the eggs of *A. aranciacus* by a specific function-blocking antibody (Chun et al. 2013). In sea urchin (*P. lividus*), pretreatment of eggs with moderate dose of cytochalasin B (400 nM) or phalloidin (3 μM) increased the rate of polyspermy, but sperm entry was suppressed by higher dose of the same drugs (10 and 30 μM, respectively). Likewise, 10 nM of LAT-A and 12 μM of JAS severely inhibited sperm entry, as judged by Hoechst-33342-stained spermatozoa (Chun et al. 2014). Taken together, these observations suggested that fine regulation of egg surface actin filaments is a prerequisite for correct gametes interaction and fusion.

The egg actin cytoskeleton also appears to contribute to the regulation of exocytosis. In the early studies utilizing the "cortical lawn" prepared from fractured sea urchin eggs, negative data showing no effect of actin or tubulin inhibitors on exocytosis led to the suggestion that Ca^{2+} is the sole modulator of cortical granule exocytosis (Whitaker and Baker 1983; Whitaker 1994). However, later studies with intact eggs led to a different conclusion. Pretreatment of starfish eggs with an actin drug JAS did not apparently affect the intracellular Ca^{2+} increases in response to $InsP_3$ uncaging or fertilizing sperm, but the elevation of the vitelline layer was severely inhibited as a sign of impaired cortical granule exocytosis (Kyozuka et al.

2008; Puppo et al. 2008; Santella and Chun 2011). Similarly, sea urchin eggs pretreated with JAS or other actin drugs often failed to elevate the vitelline layer at fertilization, although the Ca^{2+} response at fertilization was unaffected or comparable with that in the control eggs (Chun et al. 2013). Hence, in live eggs, the subplasmalemmal actin cytoskeleton surrounding cortical granules also seem to serve as a decisive factor controlling exocytosis.

3.5 Modulation of Intracellular Ca^{2+} Signaling by the Actin Cytoskeleton

The subplasmalemmal actin filaments also affect the spatiotemporal pattern of intracellular Ca^{2+} signaling. Whereas Ca^{2+} signals affect structural organization and function of the actin cytoskeleton through the action of Ca^{2+}-dependent actin-binding proteins such as gelsolin, the status of actin filaments can also influence the Ca^{2+} signals in return. This reciprocal relationship may comprise a feedback loop and contribute to Ca^{2+} homeostasis inside cells (Santella et al. 2008; Chun and Santella 2009a, b). Actin may influence intracellular levels of Ca^{2+} at least in two different ways. Firstly, actin is a highly abundant protein, and the monomeric actin displays an unusually high binding affinity to free Ca^{2+} with the K_d value being estimated around 2×10^{-9} M (Gershman et al. 1986; Carlier et al. 1986). This implied that actin can serve as a highly efficient Ca^{2+} buffer inside the cell and led to the hypothesis that polymerizing actin can absorb excess Ca^{2+} ions into the microfilament, which can serve as a different kind of Ca^{2+} store. At depolymerization, these filaments can now release free Ca^{2+} (Lange 1999). For the same reason, monomeric actin and the treadmilling actin filaments can serve as a diffusion barrier for free Ca^{2+}. Indeed, the diffusion of individual Ca^{2+} ions is known to be conspicuously limited in cells like oocytes (Allbritton et al. 1992). All these physical parameters of actin may serve as a direct mechanism to regulate intracellular Ca^{2+} levels. Secondly, the subplasmalemmal and cortical actin filaments may set the tone to the microenvironment of the ion channels that are studded in the plasma membrane or on the membranes of organelles and vesicles such as ER. For these reasons, polymerization status of the actin filaments and the dynamics of their rearrangements were expected to influence the efficiency of ion channel activities. It has long been known that the $InsP_3$ receptor binds to actin filaments, which may modulate its Ca^{2+}-transmitting activities (Fujimoto et al. 1995; Fukatsu et al. 2004; Bose and Thomas 2009). In addition to the Ca^{2+} release through $InsP_3$ receptor, it has been shown that Ca^{2+} uptake through the pump on ER is under the regulation of the actin cytoskeleton in neurons (Wang et al. 2002).

Starfish and sea urchin eggs are supposed to be suitable in addressing the questions regarding the causal relationship between the actin cytoskeleton and intracellular Ca^{2+} signaling. Certainly, large eggs with resilient surface and transparent cytoplasm have an advantage in tackling these questions in live cells by

microinjection of fluorescent dyes and imaging technology. Indeed, the first experimental models in which intracellular Ca^{2+} wave was demonstrated were medaka fish and sea urchin eggs at fertilization (Ridgway et al. 1977; Steinhardt et al. 1977). The biochemical and electrical signals transmitted by a fertilizing sperm have to be transduced at the plasma membrane/cytoskeleton interface in the egg which is covered by microvilli and is crowded with actin filaments. With starfish eggs, several methodological strategies have been adopted to alter the F-actin structure and dynamics: microinjection of actin-binding protein cofilin (Nusco et al. 2006), metabolically stable guanidine nucleotides (Kyozuka et al. 2009), recombinant protein sequestering PIP2 (Chun et al. 2010), and so on. In each case, certain aspects of Ca^{2+} influx (cortical flash) and intracellular Ca^{2+} release have been observed to be significantly changed by the alteration of the actin cytoskeleton. For example, starfish eggs microinjected with human cofilin, which binds and severs actin filaments, led to enhancement of the Ca^{2+} wave at fertilization with the concomitant suppression of the cortical flash (Nusco et al. 2006). Corroborating this observation, inhibiting the endogenous activity of depactin (structural homologue of cofilin in starfish) with microinjection of the function-blocking antibody suppressed the Ca^{2+} wave in starfish eggs at fertilization (Chun et al. 2013). The modulation of intracellular Ca^{2+} signaling by the actin cytoskeleton is not a peculiar phenomenon restricted to starfish. When sea urchin eggs (*P. lividus*) were pretreated with actin drugs prior to fertilization, the spatiotemporal pattern of the Ca^{2+} increase was changed. For example, two actin drugs LAT-A and cytochalasin B, which promote actin depolymerization but by different mechanisms, displayed similar effects. In both cases, the fertilized eggs required longer time lag to generate the Ca^{2+} wave after firing cortical flashes (Chun et al. 2014). The exact physiological significance of this interval between the cortical flash (sperm-induced egg membrane depolarization) and the onset of the Ca^{2+} wave at fertilization is not clear (Dale and De Santis 1981; Santella et al. 2015). It might represent the time required for the egg to receive the sperm-borne signal and convert it into the synthesis of Ca^{2+}-mobilizing second messengers. While the exact mechanism by which a fertilizing sperm initiates Ca^{2+} wave in the egg is not fully established and probably varies depending on the animal species (Parrington et al. 2007), it is noteworthy that disassembly of subplasmalemmal actin filaments with LAT-A or cytochalasin induces both fertilization-like Ca^{2+} waves and depolarization of the membrane potential in starfish (*A. aranciacus*) eggs (Lim et al. 2002; Moccia 2007). Our recent finding that the Ca^{2+} increase induced by LAT-A is caused by de novo synthesis of $InsP_3$ by stimulating PLCγ (Vasilev et al. 2018) suggests that the subplasmalemmal actin filaments also modulate the activity of a key enzyme involved in intracellular Ca^{2+} signaling.

3.6 Concluding Remarks

The old and new experimental data obtained from the oocytes and eggs of starfish and sea urchin that we have reviewed here suggest that actin is functionally involved in nearly all aspects of fertilization both in sperm and eggs: maturation, interaction, and fusion of the gametes. Actin provides both rigidity and plasticity for the membranes of sperm and eggs. The actin cytoskeleton is highly dynamic as exemplified by the elongating acrosomal process of sperm and the microvilli extending into the perivitelline space of the fertilized eggs. The actin pools in the sperm head or in the egg cortex maintain the spatiotemporal balance between F-actin and G-actin, which can be swiftly shifted as needed. In this review, our emphasis has been focused on the role of actin filaments in the subplasmalemmal space, which has also been referred to as "ectoplasm" (Just 1939). Conceptually, the actin filaments in the sperm acrosomal process and egg microvilli also fall into this category in a sense that they are subjacent to the plasma membrane. The actin filaments located in this tight zone of plasma membrane/cytoskeleton interface bear utmost importance not only for fertilization but also for all intercellular signaling. Although fertilization takes place on the egg surface, the subplasmalemmal zone is the locus where all the signal-transducing events take place. Whereas many aspects of fertilization may considerably vary among species, what we have reviewed here for echinoderms, and the other findings reported in the literature, support the idea that the actin meshwork in the subplasmalemmal zone may serve as a scaffold on which receptors, signaling molecules such as G-proteins, and protein kinases, as well as pertinent enzymes and ion channels, are functionally and perhaps physically interlinked together through the self-remodeling framework of the actin cytoskeleton.

Acknowledgment The authors are grateful to D. Caramiello for maintenance of *A. aranciacus* and *P. lividus* and to R. Graziano, F. Iamunno, and G. Lanzotti at the AMOBIO Unit of the *Stazione Zoologica Anton Dohrn* who prepared samples for scanning electron microscopy. We also acknowledge G. Gragnaniello for the preparation of the figures and C. Caccavale for her graphic design of Fig 3.1.

References

Allbritton NL, Meyer T, Stryer L (1992) Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. Science 258:1812–1815

Behnke O, Forer A, Emmersen J (1971) Actin in sperm tails and meiotic spindles. Nature 234:408–410

Bose DD, Thomas DW (2009) The actin cytoskeleton differentially regulates NG115-401L cell ryanodine receptor and inositol 1,4,5-trisphosphate receptor induced calcium signaling pathways. Biochem Biophys Res Commun 379:594–599

Carlier M-F, Pantaloni D, Korn ED (1986) The exchangeability of actin-bound calcium with various divalent cations to high-affinity and low-affinity binding sites on ATP-G-actin. J Biol Chem 261:10778–10784

Carron CP, Longo FJ (1982) Relation of cytoplasmic alkalinization to microvillar elongation and microfilament formation in the sea urchin egg. Dev Biol 89:128–137
Chiba K, Kado RT, Jaffe LA (1990) Development of calcium release mechanisms during starfish oocyte maturation. Dev Biol 140:300–306
Chiba K, Kontani K, Tadenuma H, Katada T, Hoshi M (1993) Induction of starfish oocyte maturation by the ßɣ subunit of starfish G protein and possible existence of the subsequent effector in cytoplasm. Mol Biol Cell 4:1027–1034
Chun JT, Santella L (2009a) The actin cytoskeleton in meiotic maturation and fertilization of starfish eggs. Biochem Biophys Res Commun 384:141–143
Chun JT, Santella L (2009b) Roles of the actin-binding proteins in intracellular Ca^{2+} signalling. Acta Physiol (Oxf) 195:61–70
Chun JT, Puppo A, Vasilev F, Gragnaniello G, Garante E, Santella L (2010) The biphasic increase of PIP2 in the fertilized eggs of starfish: new roles in actin polymerization and Ca^{2+} signaling. PLoS One 5:e14100
Chun JT, Vasilev F, Santella L (2013) Antibody against the actin-binding protein depactin attenuates Ca^{2+} signaling in starfish eggs. Biochem Biophys Res Commun 441:301–307
Chun JT, Limatola N, Vasilev F, Santella L (2014) Early events of fertilization in sea urchin eggs are sensitive to actin-binding organic molecules. Biochem Biophys Res Commun 450:1166–1174
Cohen G, Rubinstein S, Gur Y, Breitbart H (2004) Crosstalk between protein kinase A and C regulates phospholipase D and F-actin formation during sperm capacitation. Dev Biol 267:230–241
Correa LM, Thomas A, Meyers SA (2007) The macaque sperm actin cytoskeleton reorganizes in response to osmotic stress and contributes to morphological defects and decreased motility. Biol Reprod 77:942–953
Dale B, De Santis A (1981) The effect of cytochalasin B and D on the fertilization of sea urchins. Dev Biol 83:232–237
Dale B, de Santis A, Hoshi M (1979) Membrane response to 1-methyladenine requires the presence of the nucleus. Nature 282:89–90
Dan J (1952) Studies on the acrosome. I. Reaction to egg-water and other stimuli. Biol Bull 103:54–66
Dan JC, Kitahara A, Kohri T (1954) Studies on the acrosome. II. Acrosome reaction in starfish spermatozoa. Biol Bull 107:203–218
Dorée M, Kishimoto T (1981) Calcium-mediated transduction of the honnonal message in 1-methyladenine-induced meiosis reinitiation of starfish cocytes. In: Goem J, Peaud-Lendel C (eds) Metabolism and molecular activities of cytokinins. Springer, Berlin, pp 338–348
Dorée M, Kishimoto T, Le Peuch CJ, Demaille JG, Kanatani H (1981) Calcium-mediated transduction of the hormonal message in meiosis reinitiation of starfish oocytes: modulation following injection of cholera toxin and cAMP-dependent protein kinase. Exp Cell Res 135:237–249
Dufossé A (1847) Observations sur le developpement des oursins (*Echinus esculentus*). Comptes Rendus Hebdomadaires des Seances del Academie des Sciences 24:15–18
Dvoráková K, Moore HD, Sebková N, Palecek J (2005) Cytoskeleton localization in the sperm head prior to fertilization. Reproduction 130:61–69
Fol H (1879) Recherches sur la fécondation et le commencement de l'hénogénie chez divers animaux. Mem Soc Phys Hist Nat Genève 26:392–397
Footer MJ, Kerssemakers JW, Theriot JA, Dogterom M (2007) Direct measurement of force generation by actin filament polymerization using an optical trap. Proc Natl Acad Sci U S A 104:2181–2186
Fujimoto T, Miyawaki A, Mikoshiba K (1995) Inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae is linked to actin filaments. J Cell Sci 108:7–15
Fukatsu K, Bannai H, Zhang S, Nakamura H, Inoue T, Mikoshiba K (2004) Lateral diffusion of inositol 1,4,5-trisphosphate receptor type 1 is regulated by actin filaments and 4.1N in neuronal dendrites. J Biol Chem 279:48976–48982

Gershman LC, Selden LA, Estes JE (1986) High affinity binding of divalent cations to actin monomers is much stronger than previously reported. Biochem Biophys Res Commun 135:607–614

Gordon NK, Gordon R (2016) Embryogenesis explained. World Scientific Press, Singapore, p 657

Hertwig O (1876) Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. Morphol Jahr 1:347–452

Hirai S, Kubota J, Kanatani H (1971) Induction of cytoplasmic maturation by 1-methyladenine in starfish oocytes after removal of the germinal vesicle. Exp Cell Res 68:137–143

Hirohashi N, Kamei N, Kubo H, Sawada H, Matsumoto M, Hoshi M (2008) Egg and sperm recognition systems during fertilization. Develop Growth Differ 50(Suppl 1):S221–S238

Holy J, Schatten G (1991) Spindle pole centrosomes of sea urchin embryos are partially composed of material recruited from maternal stores. Dev Biol 147:343–353

Jaffe LF (1993) Classes and mechanisms of calcium waves. Cell Calcium 14:736–745

Jaffe LA, Gallo CJ, Lee RH, Ho YK, Jones TL (1993) Oocyte maturation in starfish is mediated by the beta gamma-subunit complex of a G-protein. J Cell Biol 121:775–783

Just EE (1939) The biology of the cell surface. P. Blakiston's Son, Philadelphia, pp 75–103

Kamei N, Glabe CG (2003) The species-specific egg receptor for sea urchin sperm adhesion is EBR1, a novel ADAMTS protein. Genes Dev 17:2502–2507

Kanatani H, Hiramoto Y (1970) Site of action of 1-methyladenine in inducing oocyte maturation in starfish. Exp Cell Res 61:280–284

Kanatani H, Shirai H, Nakanishi K, Kurokawa T (1969) Isolation and identification on meiosis inducing substance in starfish *Asterias amurensis*. Nature 221:273–274

Kyozuka K, Chun JT, Puppo A, Gragnaniello G, Garante E, Santella L (2008) Actin cytoskeleton modulates calcium signaling during maturation of starfish oocytes. Dev Biol 320:426–435

Kyozuka K, Chun JT, Puppo A, Gragnaniello G, Garante E, Santella L (2009) Guanine nucleotides in the meiotic maturation of starfish oocytes: regulation of the actin cytoskeleton and of Ca^{2+} signaling. PLoS One 4:e6296

Lange K (1999) Microvillar Ca^{++} signaling: a new view of an old problem. J Cell Physiol 180:19–34

Lénárt P, Bacher CP, Daigle N, Hand AR, Eils R, Terasaki M, Ellenberg J (2005) A contractile nuclear actin network drives chromosome congression in oocytes. Nature 436:812–818

Lim D, Kyozuka K, Gragnaniello G, Carafoli E, Santella L (2001) $NAADP^+$ initiates the Ca^{2+} response during fertilization of starfish oocytes. FASEB J 15:2257–2267

Lim D, Lange K, Santella L (2002) Activation of oocytes by latrunculin A. FASEB J 16:1050–1056

Lim D, Ercolano E, Kyozuka K, Nusco GA, Moccia F, Lange K, Santella L (2003) The M-phase-promoting factor modulates the sensitivity of the Ca^{2+} stores to inositol 1,4,5-trisphosphate via the actin cytoskeleton. J Biol Chem 278:42505–42514

Limatola N, Chun JT, Kyozuka K, Santella L (2015) Novel Ca^{2+} increases in the maturing oocytes of starfish during the germinal vesicle breakdown. Cell Calcium 58:500–510

Luo L (2002) Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu Rev Cell Dev Biol 18:601–635

Mah SA, Swanson WJ, Vacquier VD (2005) Positive selection in the carbohydrate recognition domains of sea urchin sperm receptor for egg jelly (suREJ) proteins. Mol Biol Evol 22:533–541

Majstoravich S, Zhang J, Nicholson-Dykstra S, Linder S, Friedrich W, Siminovitch KA, Higgs HN (2004) Lymphocyte microvilli are dynamic, actin-dependent structures that do not require Wiskott-Aldrich syndrome protein (WASp) for their morphology. Blood 104:1396–1403

Matsumoto M, Kawase O, Islam MS, Naruse M, Watanabe SN, Ishikawa R, Hoshi M (2008) Regulation of the starfish sperm acrosome reaction by cGMP, pH, cAMP and Ca^{2+}. Int J Dev Biol 52:523–526

Meijer L, Guerrier P (1984) Maturation and fertilization in starfish oocytes. Int Rev Cytol 86:129–196

Miyazaki S (2006) Thirty years of calcium signals at fertilization. Semin Cell Dev Biol 17:233–243

Miyazaki SI, Ohmori H, Sasaki S (1975) Action potential and non-linear current-voltage relation in starfish oocytes. J Physiol 246:37–54
Moccia F (2007) Latrunculin A depolarizes starfish oocytes. Comp Biochem Physiol A Mol Integr Physiol 148:845–852
Moody WJ, Bosma MM (1985) Hormone-induced loss of surface membrane during maturation of starfish oocytes: differential effects on potassium and calcium channels. Dev Biol 112:396–404
Moreau M, Guerrier P, Doree M, Ashley CC (1978) Hormone-induced release of intracellular Ca^{2+} triggers meiosis in starfish oocytes. Nature 272:251–253
Moy GW, Mendoza LM, Schulz JR, Swanson WJ, Glabe CG, Vacquier VD (1996) The sea urchin sperm receptor for egg jelly is a modular protein with extensive homology to the human polycystic kidney disease protein, PKD1. J Cell Biol 133:809–817
Nusco GA, Lim D, Sabala P, Santella L (2002) Ca^{2+} response to cADPr during maturation and fertilization of starfish oocytes. Biochem Biophys Res Commun 290:1015–1021
Nusco GA, Chun JT, Ercolano E, Lim D, Gragnaniello G, Kyozuka K, Santella L (2006) Modulation of calcium signalling by the actin-binding protein cofilin. Biochem Biophys Res Commun 348:109–114
Ohlendieck K, Lennarz WJ (1996) Molecular mechanisms of gamete recognition in sea urchin fertilization. Curr Top Dev Biol 32:39–58
Ohlendieck K, Partin JS, Stears RL, Lennarz WJ (1994) Developmental expression of the sea urchin egg receptor for sperm. Dev Biol 165:53–62
Parrington J, Davis LC, Galione A, Wessel G (2007) Flipping the switch: how a sperm activates the egg at fertilization. Dev Dyn 236:2027–2038
Pitnick S, Hosken DJ, Birkhead TR (2009) Sperm morphological diversity. In: Birkhead TR et al (eds) Sperm biology: an evolutionary perspective. Academic, London, pp 69–149
Puppo A, Chun JT, Gragnaniello G, Garante E, Santella L (2008) Alteration of the cortical actin cytoskeleton deregulates Ca^{2+} signaling, monospermic fertilization, and sperm entry. PLoS One 3:e3588
Ridgway EB, Gilkey JC, Jaffe LF (1977) Free calcium increases explosively in activating medaka eggs. Proc Natl Acad Sci U S A 74:623–627
Santella L, Chun JT (2011) Actin, more than just a housekeeping protein at the scene of fertilization. Sci China Life Sci 54:733–743
Santella L, Kyozuka K (1994) Reinitiation of meiosis in starfish oocytes requires an increase in nuclear Ca^{2+}. Biochem Biophys Res Commun 203:674–680
Santella L, De Riso L, Gragnaniello G, Kyozuka K (1999) Cortical granule translocation during maturation of starfish oocytes requires cytoskeletal rearrangement triggered by $InsP_3$-mediated Ca^{2+} release. Exp Cell Res 248:567–574
Santella L, Lim D, Moccia F (2004) Calcium and fertilization: the beginning of life. Trends Biochem Sci 29:400–408
Santella L, Puppo A, Chun JT (2008) The role of the actin cytoskeleton in calcium signaling in starfish oocytes. Int J Dev Biol 52:571–584
Santella L, Vasilev F, Chun JT (2012) Fertilization in echinoderms. Biochem Biophys Res Commun 425:588–594
Santella L, Limatola N, Chun JT (2015) Calcium and actin in the saga of awakening oocytes. Biochem Biophys Res Commun 460:104–113
Santella L, Limatola N, Chun JT (2016) The fertilization process: a new way to look at an old phenomenon. Atlas of Science another view on science. https://atlasofscience.org/the-fertilization-process-a-new-way-to-look-at-an-old-phenomenon/
Schatten H, Walter M, Biessmann H, Schatten G (1988) Microtubules are required for centrosome expansion and positioning while microfilaments are required for centrosome separation in sea urchin eggs during fertilization and mitosis. Cell Motil Cytoskeleton 11:248–259
Schroeder TE, Stricker SA (1983) Morphological changes during maturation of starfish oocytes: surface ultrastructure and cortical actin. Dev Biol 98:373–384

Steinhardt R, Zucker R, Schatten G (1977) Intracellular calcium release at fertilization in the sea urchin egg. Dev Biol 58:185–196
Stricker SA (1999) Comparative biology of calcium signaling during fertilization and egg activation in animals. Dev Biol 211:157–176
Stricker SA, Schatten G (1991) The cytoskeleton and nuclear disassembly during germinal vesicle breakdown in starfish oocytes. Develop Growth Differ 33:163–171
Svitkina T (2018) The actin cytoskeleton and actin-based motility. doi: https://doi.org/10.1101/cshperspect.a018267.
Terasaki M (1994) Redistribution of cytoplasmic components during germinal vesicle breakdown in starfish oocytes. J Cell Sci 107:1797–1805
Terasaki M (1996) Actin filament translocations in sea urchin eggs. Cell Motil Cytoskeleton 34:48–56
Tilney LG, Inoué S (1982) Acrosomal reaction of *Thyone* sperm. II. The kinetics and possible mechanism of acrosomal process elongation. J Cell Biol 93:820–827
Tilney LG, Jaffe LA (1980) Actin, microvilli, and the fertilization cone of sea urchin eggs. J Cell Biol 87:771–782
Tilney LG, Hatano S, Ishikawa H, Mooseker MS (1973) The polymerization of actin: its role in the generation of the acrosomal process of certain echinoderm sperm. J Cell Biol 59:109–126
Trimmer JS, Vacquier VD (1986) Activation of sea urchin gametes. Annu Rev Cell Biol 2:1–26
Vacquier VD (2012) The quest for the sea urchin egg receptor for sperm. Biochem Biophys Res Commun 425:583–587
Vacquier VD, Moy GW (1977) Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. Proc Natl Acad Sci U S A 74:2456–2460
Vasilev F, Chun JT, Gragnaniello G, Garante E, Santella L (2012) Effects of ionomycin on egg activation and early development in starfish. PLoS One 7:e39231
Vasilev F, Limatola N, Park DR, Kim UH, Santella L, Chun JT (2018) Disassembly of subplasmalemmal actin filaments induces cytosolic Ca^{2+} increases in *Astropecten aranciacus* eggs. Cell Physiol Biochem (under review)
Wang Y, Mattson MP, Furukawa K (2002) Endoplasmic reticulum calcium release is modulated by actin polymerization. J Neurochem 82:945–952
Welch MD, Mallavarapu A, Rosenblatt J, Mitchison TJ (1997) Actin dynamics in vivo. Curr Opin Cell Biol 9:54–61
Wessel GM, Conner SD, Berg L (2002) Cortical granule translocation is microfilament mediated and linked to meiotic maturation in the sea urchin oocyte. Development 129:4315–4325
Whitaker M (1994) Exocytosis in sea urchin eggs. Ann N Y Acad Sci 710:248–253
Whitaker M (2006) Calcium at fertilization and in early development. Physiol Rev 86:25–88
Whitaker MJ, Baker PF (1983) Calcium-dependent exocytosis in an in vitro secretory granule plasma membrane preparation from sea urchin eggs and the effects of some inhibitors of cytoskeletal function. Proc R Soc Lond B Biol Sci 218:397–413
Wilson NF, Snell WJ (1998) Microvilli and cell-cell fusion during fertilization. Trends Cell Biol 8:93–96
Ziomek CA, Epel D (1975) Polyspermy block of *Spisula* eggs is prevented by cytochalasin B. Science 189:139–141