The fertilization process: a new way to look at an old phenomenon

The union of the egg with the sperm at fertilization has fascinated many philosophers and scientists. Most of our knowledge on fertilization derives from studies on marine organisms that release eggs and spermatozoa into the sea water. Thus, starfish and sea urchin have been used as ideal experimental models for more than a hundred years.

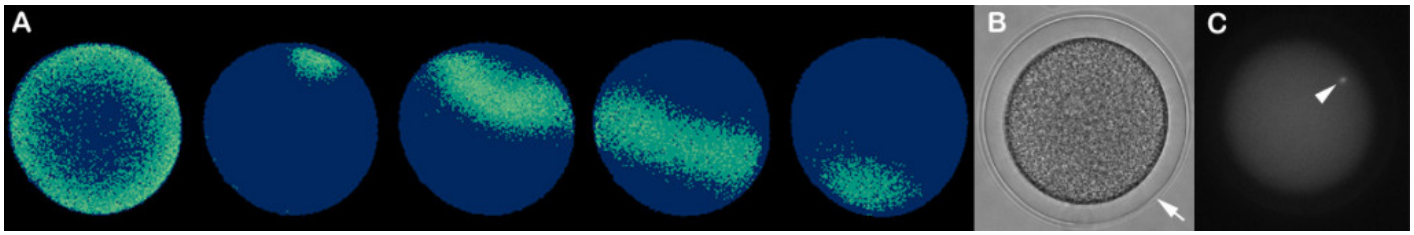

Fig. 1. Fertilization of sea urchin eggs. (A) Intracellular calcium increases. After the cortical flash, the calcium wave spreads from the sperm interaction site to the opposite pole. (B) A light microscopy image of the fertilization envelope (arrow). (C) Visualization of the Hoechst 33342-stained sperm (arrowhead) incorporated into the egg (10 minutes after insemination).

Already in 1876, the penetration of eggs by spermatozoa and the subsequent union of the male and female pronuclei were demonstrated in sea urchin by use of light microscopy. With the advancement of modern technologies that record the early events taking place in fertilized eggs, many new findings were made on the rapid structural and physicochemical changes on the surface of fertilized eggs. Microinjection of fluorescent dyes has demonstrated that fertilized eggs mobilize free calcium, which is widely used as a second messenger in many cell functions. The calcium increase in fertilized eggs may take the form of a rapid influx into the periphery of the cell (cortical flash) and a wave originated by calcium coming from internal stores (Fig. 1). The nature of the calcium stores in the egg from which calcium is released is still debated. In addition, the fertilizing sperm alters the physical state of the egg surface, the most drastic example in echinoderm eggs being the detachment of the vitelline envelope from the plasma membrane as a result of the exocytosis of cortical granules and the formation of actin spikes.

The calcium increase and the structural reorganization of the actin cytoskeleton are precisely coordinated in time and space. Our Laboratory has demonstrated that intracellular calcium level and the state of the actin cytoskeleton are mutually affected by each other. Besides modulating the intracellular calcium signaling, the cortical actin cytoskeleton appears to play a central role as a functional scaffold on which many physiological events are processed during fertilization; namely cortical granules exocytosis and sperm entry.

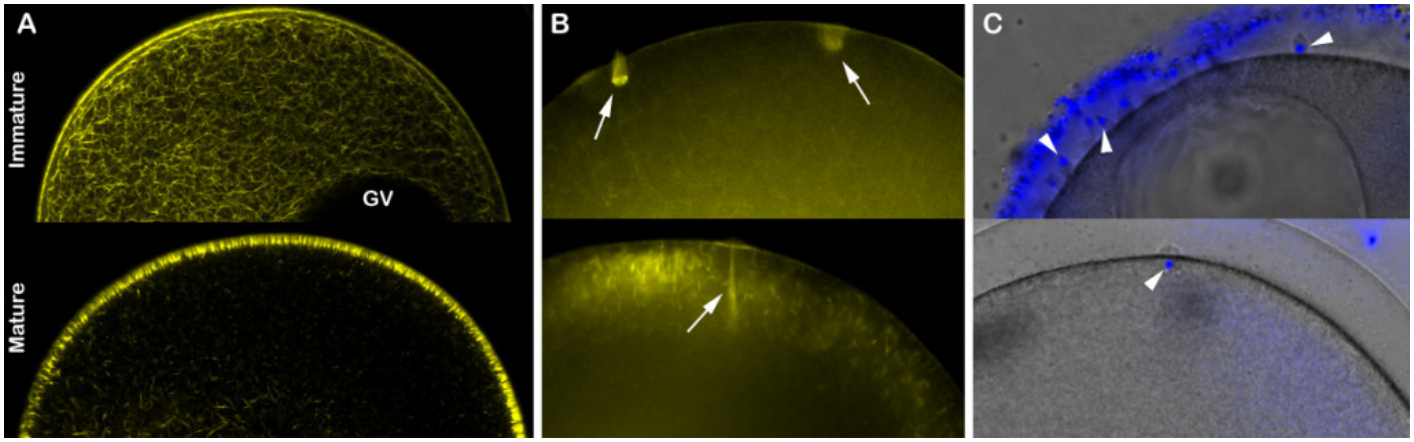

Fig. 2. Changes of the actin cytoskeleton following maturation and fertilization. Immature oocytes (top panels) and mature eggs (bottom panels) of starfish were microinjected with Alexa-Fluor 568 conjugated phalloidin. During maturation starfish oocytes reach a maturity stage through a structural organization of the actin cytoskeleton, microvilli retraction, and proper positioning of cortical granules beneath the plasma membrane. Such changes are reflected in the actin filaments in the cortex of the mature eggs being perpendicularly oriented to the plasma membrane. (A) Confocal microscopy image of F-actin before fertilization. (B) Epifluorescence microscopy images showing the multiple F-actin sperm entry sites of the polyspermic immature oocytes (arrows) and F-actin translocation from the fertilized mature egg surface to the center. F-actin bundles are visualized around the egg-engulfed sperm (arrow). (C) Hoechst 33342-stained sperm incorporated into an immature oocyte and a mature egg (arrowheads).

The actin cytoskeleton of immature oocytes not exposed to the maturing hormone 1-methyladenine exhibit a strikingly different configuration from the mature eggs, and these oocytes are physiologically penetrated by numerous spermatozoa (polyspermy) (Fig. 2). On the other hand, perturbation of the cortical actin in mature eggs by poking or treating with pharmacological agents makes the eggs to react slowly, and polyspermic. Furthermore, simply with time, the eggs overripe after hormone treatment (>3 hours) often manifest considerably altered structure of the actin cytoskeleton, and these eggs display polyspermy at insemination despite full elevation of the fertilization envelope. This finding has raised doubts on the general assumption that the fertilization envelope acts as a mechanical barrier against the entry of supernumerary spermatozoa. Likewise, eggs with altered actin cytoskeleton often fail to elevate the fertilization envelope despite the seemingly normal calcium increase. Thus, the structural and functional integrity of the actin cytoskeleton in the egg cortex is indicative of physiological competence of the egg, whose implication could be further extended to the problems with reproduction in humans.

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