

Novel Signalling Pathways Mobilizing Ca²⁺ and F-actin in Starfish and Sea Urchin Eggs

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Abstract

Together with sea urchins, starfish have served as an excellent animal model for studying mechanisms of meiotic maturation, fertilization, and embryonic development. Being large and transparent, echinoderm oocytes and eggs have facilitated experimental approaches requiring microinjection. Indeed, electrical recordings and fluorescence imaging of Ca²⁺ and other molecules can be performed in this living cell in real-time. This proposal focuses on regulation of intracellular Ca²⁺ signalling and actin dynamics in the echinoderm oocyte and egg. Stimulated with the maturation-inducing hormone 1-methyladenine (1-MA), starfish oocytes display a cortical Ca²⁺ wave that is generated in the vegetal hemisphere and eventually reaches the nucleus. Concomitantly, microvilli, the cortical actin cytoskeleton, and associated cortical granules are drastically remodeled. Similar but distinct changes take place when the eggs are fertilized. Within a few seconds after the fertilizing sperm fuses with the egg, a synchronized but short-lived Ca²⁺ influx takes place over the entire surface of the egg (cortical flash), and a Ca²⁺ wave is generated at the sperm interaction site to propagate to the antipode. Meanwhile, the cortical actin cytoskeleton is drastically reorganized with an increase of intracellular pH. While the cortical flash is mediated by voltage-gated Ca²⁺ channels, and the Ca²⁺ wave by the intracellular receptor ion channels responding to their cognate second messenger ligands (e.g. InsP₃, cADPr, and NAADP), several questions remain:

1. How do 1-MA and sperm produce the Ca²⁺ signals?
2. What is the causal relationship between the Ca²⁺ influx and Ca²⁺ wave?
3. How do 1-MA and sperm trigger changes of the actin cytoskeleton in the oocytes and eggs?
4. How are actin filaments anchored to the plasma membrane in starfish oocytes?
5. Can the intracellular pH increase serve as a cell signal during actin cytoskeletal remodeling?

To tackle these questions, we will microinject oocytes and eggs with recombinant proteins that interfere with specific Ca²⁺ and actin signaling pathways. We will utilize the transcriptome data assembled from the local starfish (*Astropecten aranciacus*) to produce fusion proteins containing the intermolecular interaction sites of the target protein. The study is mainly focused on starfish oocytes, which are easier to microinject, but the key findings will also be tested in sea urchin eggs (*Paracentrotus lividus*). As Ca²⁺ and actin are under the control of exquisite mechanisms in many cell types, new findings from this study deciphering the signalling pathways mobilizing Ca²⁺ and F-actin are likely to provide universal insights for other cell biology disciplines.