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RESEARCH NEWS

The fast block to polyspermy breaks with convention

Ben Short

JGP study (Komondor et al. 2023. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213258) reveals that conventional PLC activation pathways are not required for the fertilization-induced depolarization of *Xenopus* eggs that prevents the entry of additional sperm.

When it comes to fertilization, the winner takes it all. Fertilization of an egg by more than one sperm (polyspermy) leads to chromosomal abnormalities and lethal defects in embryonic development, so animals have evolved various mechanisms to prevent the entry of additional sperm after the initial sperm penetrates the egg.

Nearly all animal species have a "slow" block to polyspermy, in which fertilization triggers the exocytosis of cortical granules to form a physical barrier around the zygote. But species that undergo external fertilization, such as frogs and sea anemones, have also evolved a "fast" block to polyspermy, where the fertilizing sperm induces a rapid depolarization of the egg plasma membrane, preventing the entry of additional sperm (1). The signaling pathway leading from fertilization to depolarization relies on the enzyme phospholipase C (PLC). In this issue of *JGP*, however, Komondor et al. reveal that none of the conventional PLC activation pathways are required for this process (2).

Anne Carlson's group at the University of Pittsburgh previously found that, in the African clawed frog *Xenopus laevis*, PLC is activated following fertilization to generate the second messenger inositol trisphosphate (IP_3), which induces the release of Ca^{2+} from the endoplasmic reticulum. The rise in cytoplasmic Ca^{2+} levels then activates the Cl^- channel TMEM16A, leading to Cl^- efflux and membrane depolarization (3, 4). "So, now we wanted to figure out how fertilization activates PLC," Carlson says.

Though there is conflicting evidence, the most likely model was that fertilization triggers the phosphorylation of a critical tyrosine residue, Y776, on PLCγ1. Carlson and colleagues,

(Left to right) Anne Carlson, Kayla Komondor, Rachel Bainbridge, and colleagues found that the fertilization-induced depolarization that mediates the fast block to polyspermy in *Xenopus* eggs does not require the conventional pathways of PLC activation. Compared to a control (A), a PLC inhibitor prevents fertilization-induced depolarization (B). But depolarization proceeds normally in the presence of drugs that block either tyrosine kinase-mediated activation of PLCγ1 (C) or $G\alpha_q$-mediated activation of PLCβ (D). This suggests that PLC is activated by a non-canonical pathway.

including co-first authors Kayla Komondor and Rachel Bainbridge, set out to test this model directly by identifying two tyrosine kinase inhibitors that block the canonical phosphorylation and activation of PLCγ1 (2). Surprisingly, the researchers found that neither of these drugs prevented the fertilization-induced depolarization of *Xenopus* eggs. Moreover, Western blotting with a phosphospecific antibody indicated that PLCγ1 isn't phosphorylated on Y776 in response to fertilization.

Though PLCγ1 is the most abundant PLC in *Xenopus* eggs, two other isoforms, PLCβ1 and PLCβ3, are present at lower levels (5). PLCβs are conventionally activated by $G\alpha_q$ proteins, with $G\alpha_{11}$ being the sole member of this family present in *Xenopus* oocytes. Similar to their previous approach investigating the activation of PLCγ1, Carlson and colleagues identified an inhibitor of $G\alpha_{11}$'s ability to activate PLCβ1 and PLCβ3, but found that this compound failed to suppress fertilization-induced depolarization.

"Our data suggest that the existing model is not correct and, instead, an egg PLC is activated by a different, non-canonical pathway," Carlson says.

This could involve cytoplasmic Ca^{2+}, because elevated Ca^{2+} levels have been shown to directly activate many PLC isoforms, including PLCγ1 and PLCβs. "So, the sperm might enter the egg and release Ca^{2+} in some way, which then activates PLC to release further Ca^{2+} and induce depolarization," Carlson explains. "That's what we're looking at now."

References

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bshort@rockefeller.edu.