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COMMENTARY

The fast block to polyspermy: New insight into a century-old problem

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One of the central questions about fertilization is how only one sperm fertilizes each egg. For most species, the formation of a mechanical barrier is a key component. However, because this process takes time, some species use a fast electrically mediated block to protect the egg in the intervening period (Jaffe and Cross, 1986; Iwao and Izaki, 2018). Two papers in the *Journal of General Physiology* (see Wozniak, Phelps, et al., and Wozniak, Tembo, et al., in this issue) provide new insights about how this occurs.

Almost exactly 100 yr ago, E.E. Just (1919) observed that in the ∼30-s period between insemination of a sand dollar egg and elevation of the mechanical barrier, many sperm reach the egg surface, but only one succeeds in entering. Just concluded that "before the membrane begins lifting at the site of sperm entry, sperm can no longer enter at any point on the egg." Soon thereafter, J. Gray (1922) suggested that an electrical mechanism might be responsible. However, it was not until later that Tyler et al. (1956) reported the first successful microelectrode recording during fertilization and showed that the starfish egg membrane indeed depolarizes. 20 yr later, as a graduate student in the laboratory of S. Hagiwara, I demonstrated that this depolarization, or "fertilization potential," provides a fast block to polyspermy in sea urchin eggs (Jaffe, 1976).

Since that time, electrical polyspermy blocks have been described in many species, including frogs. In frog eggs, the depolarization results from a calcium-mediated increase in chloride permeability, allowing chloride to move from the cytosol to the low chloride "pond water" environment where fertilization occurs (Cross and Elinson, 1980; Grey et al., 1982; Kline, 1988). The calcium rise is caused by the opening of IP_3-gated calcium channels in the endoplasmic reticulum (Runft et al., 1999). In two companion papers, Wozniak et al. (2018a,b) show directly that IP_3 mediates the fertilization potential in eggs from the frog *Xenopus laevis*, and they identify the chloride channel that is responsible as TMEM16A.

To investigate the source of the calcium that activates the chloride channel, Wozniak et al. (2018b) used existing proteomic and RNA sequencing datasets to identify candidate calcium channels in the plasma membrane and intracellular membranes of the *Xenopus* egg. Two plasma membrane candidates were identified, but broad spectrum plasma membrane calcium channel inhibitors did not inhibit the fertilization potential, indicating that extracellular calcium is not needed. However, an IP_3 receptor antagonist (Xestospongin C) and a phospholipase C inhibitor (U73122) completely suppressed the fertilization potential and induced polyspermy, indicating that IP_3-induced calcium release is responsible for the fertilization potential.

To investigate which chloride channel gives rise to the calcium-induced depolarization at fertilization, Wozniak et al. (2018a) again used proteomic and RNA sequencing databases to identify candidates. The pharmacological properties of these candidate channels were characterized by expressing them exogenously in axolotl oocytes, which lack endogenous calcium-activated chloride channels. Inhibitors of the chloride channel xTMEM16a were then shown to inhibit the fertilization-induced depolarization in *Xenopus* eggs and induce polyspermy, revealing that xTMEM16a is the calcium-activated chloride channel responsible for the depolarization.

Two major questions about the electrical polyspermy block remain to be elucidated. First, how does the sperm–egg interaction result in IP_3 production and calcium release in the egg? And second, how does the voltage across the egg plasma membrane regulate fusion of the sperm and egg membranes?

With regard to the first of these questions, fertilization of *Xenopus* eggs activates IP_3 production by a pathway involving phosphorylation and activation of phospholipase Cγ (PLCγ; Sato et al., 2000), although this signaling pathway is not mediated by the SH2 domains of PLCγ as in other systems (Runft et al., 1999). In mammalian eggs, IP_3 is also produced in response to fertilization. Part, but not all, of the mammalian mechanism for stimulating IP_3 production is delivery of phospholipase C (PLCζ) from the sperm to the egg as a consequence of fusion of the sperm and egg plasma membranes (Saunders et al., 2002; Hachem et al.,

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2017; Nozawa et al., 2018). Whether *Xenopus* sperm also deliver PLCζ, or another phospholipase C, to the *Xenopus* egg remains to be determined.

The question of what confers voltage sensitivity to sperm–egg fusion has been elusive for decades. Early studies supported the hypothesis that voltage sensitivity might result from insertion of a positively charged molecule from the sperm into the egg membrane as part of the cell–cell fusion process (Jaffe and Cross, 1986; Iwao and Jaffe, 1989). Recent studies have identified a matrix metalloproteinase, MMP2, that is expressed on the *Xenopus* sperm surface and includes a positively charged peptide domain that may contribute to the voltage dependence of sperm–egg fusion (Iwao et al., 2014).

Fusion of viruses with their target cells has provided an important model for studies of sperm–egg fusion. The fusion of virus membranes with cell membranes is mediated by the insertion of a "fusion protein" from the virus, and for "class 2" and "class 3" viruses, this process is inhibited if the target cell membrane is clamped at a positive voltage (Markosyan and Cohen, 2010).

Sperm of flowering plants such as *Arabidopsis thaliana* express a transmembrane protein, HAP2, that has a structure similar to that of viral fusion proteins and is required for sperm–egg fusion (von Besser et al., 2006; Valansi et al., 2017; Fédry et al., 2018). Arabidopsis HAP2 has an extracellular loop that is thought to insert into the egg membrane to mediate fusion, containing charged amino acids interspersed with nonpolar residues (Valansi et al., 2017; Fédry et al., 2018). Conceivably, these charges, or those in other regions of the HAP2 protein (Wong et al., 2010), could confer voltage sensitivity to sperm–egg fusion, although it is unknown whether membrane fusion mediated by HAP2 shows the voltage dependency seen with viral fusion proteins. It is also unknown whether the Arabidopsis egg membrane depolarizes at fertilization, or whether sperm–egg fusion in Arabidopsis is voltage dependent. HAP2 is expressed in gametes of other species as well, including some protozoa and animals. Whether HAP2 or another a similar fusion protein is present in sperm from species in which fertilization is known to be voltage dependent, such as *Xenopus*, remains to be determined.

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