

Elements of the Sperm Activate Mammalian Eggs

The sperm is often thought of as a nucleus with a propulsion system. The material in the minute amount of sperm cytoplasm is usually ignored. However, the sperm cytoplasm has recently been found to contain enzymes that activate egg metabolism, as well as RNA fragments that may alter gene expression (Sharma et al. 2016; Tarozzi et al 2021). As in every other animal studied, a transient rise in cytoplasmic Ca^{2+} is necessary for egg activation in mammals (Yeste et al. 2017; Kashir et al. 2018). The sperm induces a series of Ca^{2+} waves that can last for hours, terminating in egg activation (i.e., resumption of meiosis, cortical granule exocytosis, and release of the inhibition on maternal mRNAs) and the formation of the male and female pronuclei. And, again as in sea urchins, fertilization triggers intracellular Ca^{2+} release through the production of IP_3 by the enzyme phospholipase C (PLC) (Swann et al. 2006; Igarashi et al. 2007).

PLC ζ

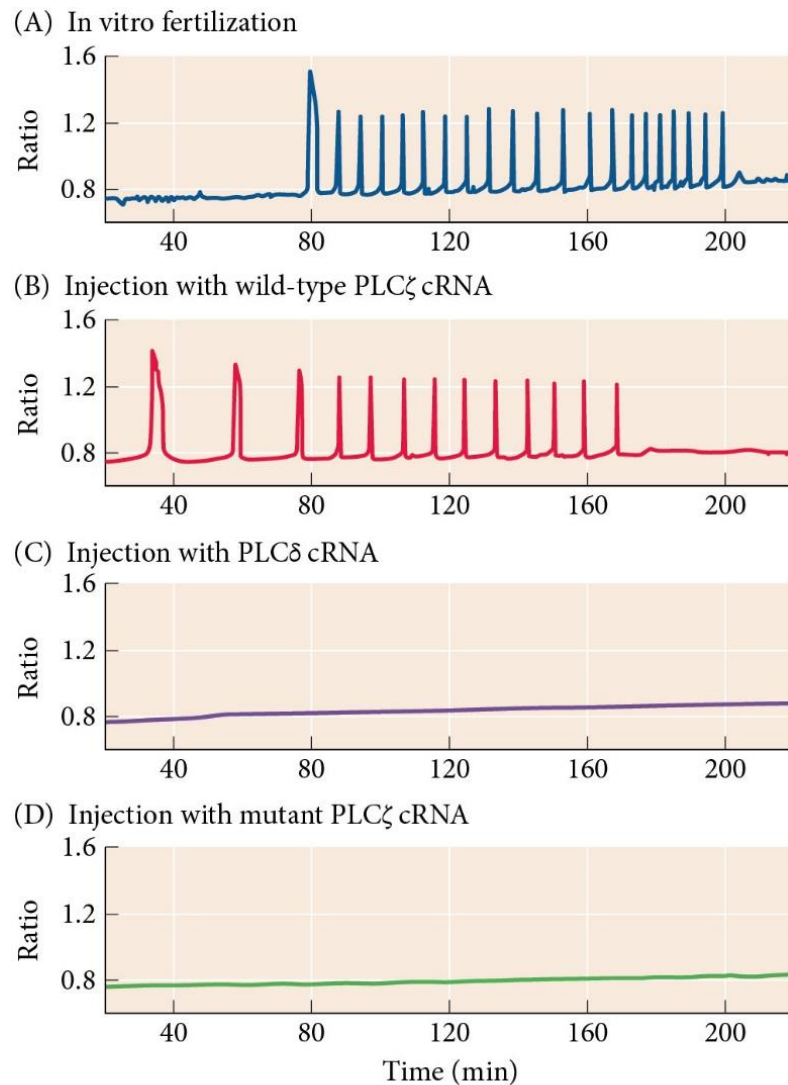
As mentioned in the textbook (Chapter 7), the mammalian PLC responsible for egg activation and pronucleus formation may in fact come from the sperm cytoplasm rather than from the egg. Some of the first observations for a sperm-derived PLC came from studies of intracytoplasmic sperm injection (ICSI), an experimental treatment for curing infertility. Here, sperm are directly injected into oocyte cytoplasm, bypassing any interaction with the egg cell membrane. To the surprise of many biologists (who had assumed that sperm *binding* to an egg receptor protein was critical for egg activation), this treatment worked. The human egg was activated, and pronuclei

formed. Injecting mouse sperm into mouse eggs will also induce fertilization-like Ca^{2+} oscillations in the egg and lead to complete development (Kimura and Yanagimachi 1995).

It appeared that an activator of Ca^{2+} release was stored in the sperm head. This activator turned out to be a soluble sperm PLC enzyme, **PLC ζ** (zeta), which is delivered to the egg by gamete fusion. In mice, expression of PLC ζ mRNA in the egg produces Ca^{2+} oscillations, and removing PLC ζ from mouse sperm (by antibodies or RNAi) abolishes the sperm's calcium-inducing activity, as shown in Figure 1. (Saunders et al. 2002; Yoda et al. 2004; Knott et al. 2005). PLC ζ is also responsible for initiating the membrane block to polyspermy by removing Juno from the egg cell membrane (Nozawa et al. 2018). Human sperm that are unsuccessful in ICSI have been shown to have little or no functional PLC ζ . In fact, normal human sperm can activate Ca^{2+} oscillations when injected into mouse eggs, but sperm lacking PLC ζ do not (Yoon et al. 2008).

Whereas sea urchin eggs usually are activated as a single wave of Ca^{2+} crosses from the point of sperm entry, the mammalian egg is traversed by numerous waves of Ca^{2+} (Miyazaki et al. 1992; Ajduk et al. 2008; Ducibella and Fissore 2008). The extent (amplitude, duration, and number) of these Ca^{2+} oscillations appears to regulate the timing of mammalian egg activation events (Ducibella et al. 2002; Ozil et al. 2005; Toth et al. 2006). In this way, cortical granule exocytosis occurs just before the nucleus resumes meiosis and much before the translation of maternal mRNAs.

In mammals, the Ca^{2+} released by IP_3 binds to a series of proteins, including calmodulin-activated protein kinase (which will be important in eliminating the inhibitors of mRNA translation), MAP kinase (which allows the resumption of meiosis), and synaptotagmin (which helps initiate cortical granule fusion). Unused Ca^{2+} is pumped back into the endoplasmic reticulum, and additional Ca^{2+} is acquired from outside the cell. This recruitment of extracellular Ca^{2+} appears to be necessary for the egg to complete meiosis. If Ca^{2+} influx is blocked, the second polar body does not form; instead, the result is two nonviable (triploid) egg pronuclei (Maio et al. 2012; Wakai et al. 2013).



After C. M. Saunders et al. 2002. *Development* 129: 3533–3544.

FIGURE 1

Importance of PLCζ-induced Ca^{2+} oscillations. Ca^{2+} fluxes across the mouse oocyte were monitored after in vitro fertilization with mouse sperm (A) or after microinjection with cRNA encoding wild-type PLCζ at 0.02 mg/ml (B), PLCδ1 at 2 mg/ml (C), or a mutated form of PLCζ at 2 mg/ml (D). The Ca^{2+} fluxes were produced by the PLCζ but not by the mutated PLCζ or the relatively similar enzyme PLCδ1.

Centrioles

In addition, the sperm cytoplasm contains centrioles, and these are essential for fertilization and subsequent development (Nigg and Raff 2009). The oocyte loses its centrioles into the polar bodies (Sathanathan et al 2006). When the sperm and egg fuse, the sperm centrioles enter the oocyte cytoplasm, where they organize the microtubules that bring the pronuclei together. Defects in male centrioles are seen as causes for

developmental arrest and infertility (Avidor-Reiss et al 2019; Kahraman et al 2020).

It appears, then, that two factors from the sperm cytoplasm-- PLC ζ and centrioles--are needed for fertiliation to occur in mammals.

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