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When a sperm meets an egg: Block to polyspermy

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Abstract

Embryonic development is initiated after the fertilizing spermatozoon enters the egg and triggers a series of events known as egg activation. Activation results in an increase in intracellular calcium concentration, cortical granule exocytosis (CGE), cell cycle resumption and recruitment of maternal mRNA. CGE is an evolutionary developed mechanism that causes modification of the zona pellucida to prevent penetration of additional spermatozoa, ensuring successful egg activation and embryo development. The egg CGE is a unique and convenient mammalian model for studying the different proteins participating at the membrane fusion cascade, which, unlike other secretory cells, occurs only once in the egg's lifespan. This article highlights a number of proteins, ascribed to participate in CGE and thus the block to polyspermy. CGE can be triggered either by a calcium dependent pathway, or via protein kinase C (PKC) activation that requires a very low calcium concentration.

In a recent study, we suggested that the filamentous actin (F-actin) at the egg's cortex is a dynamic network. It can be maneuvered towards allowing CGE by activated actin associated proteins and/or by activated PKC and its down stream proteins, such as myristoylated alanine-rich C kinase substrate (MARCKS). MARCKS, a protein known to cross-link F-actin in other cell types, was found to be expressed and colocalized with actin in non-activated MII eggs. We further demonstrated MARCKS dissociation from actin after activation by ionomycin, a process that can lead to the breakdown of the actin network, thus allowing CGE. The more we know of the intricate process of CGE and of the proteins participating in it, the more the assisted reproductive procedures might benefit from that knowledge.

Keywords: Signal transduction; Cortical granules; Zona pellucida; Fertilization; Parthenogenetic activation; Protein kinase C; Calcium

1. Introduction

A successful mammalian fertilization process culminates with fusion between the membranes of one sperm and one egg, causing activation of the egg and generation of a diploid zygote, which undergoes several cleavages to form an early embryo. One of the very early cellular events observed following egg activation is an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) followed by Ca^{2+} oscillations. It is hypothesized that the long-lasting series of $[Ca^{2+}]_i$ oscillations ensures a complete escape from meiotic arrest (Jones et al., 1995a). However, the precise mechanism of sperm-induced Ca^{2+}-release is not fully understood. It has been proposed that an egg-receptor sperm-ligand signaling cascade is activated to switch on an egg phospholipase C (PLC; Evans and Kopf, 1998). An alternative hypothesis is that a "sperm factor" diffuses into the egg after gamete fusion and generates egg activation. A sperm specific zeta isoform of PLC can trigger in mouse eggs Ca^{2+} oscillations of the same nature as those triggered by the "sperm factor" (Cox et al., 2002; Saunders et al., 2002; Kurokawa et al., 2004). It is believed that Ca^{2+} release involves activation of the phosphoinositide pathway leading to increased production of inositol 1,4,5-triphosphate (IP_3; Stricker, 1999; Runft et al., 2002) and diacylglycerol (DAG) by hydrolysis of phosphatidylinositol 4, 5 biphosphate (PIP_2). The two cleavage products serve as secondary messengers during egg activation: IP_3 at the pathway leading to the release of Ca^{2+} from intracellular stores, and DAG at the pathway leading to activation of protein kinase C (PKC). It is accepted that the initial rise in $[Ca^{2+}]_i$ is both necessary and sufficient for triggering the consecutive events of egg activation.

An early event in egg activation is the cortical granule exocytosis (CGE) followed by alteration of the zona pellucida (ZP) glycoproteins, thus establishing a block to polyspermy (reviewed by Wassarman et al., 2001; Talmor-Cohen et al., 2002; Sun, 2003). CGE is an evolutionary developed mechanism preventing penetration of additional spermatozoa and ensuring successful egg activation and embryo development. The cortical granules (CGs), residing just beneath the plasma membrane of metaphase II (MII) arrested eggs, secrete their

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content into the perivitelline space causing the ZP-block to polyspermy. Although the content of CGs is poorly characterized, it is proposed that a CG-derived glycosidase modifies ZP3, probably by its glycosylation (Miller et al., 1993), abolishing the ZP's ability to bind spermatozoa and induce acrosome reaction (AR; Wassarman, 1990). A CG-derived protease has been suggested to cleave ZP2 to ZP2f, which can no longer interact with ZP-bound and acrosome-reacted spermatozoa (Moller and Warssaman, 1989).

Later events of egg activation include exit from the arrest at the MII stage, completion of meiosis, pronuclear formation, and the early mitotic cleavages. Prior to egg activation, MII arrest is maintained by an active complex of cell cycle regulators, including $p34^{cdc2}$ kinase, cyclin B and cytostatic factor (CSF; Yanagimachi, 1994; Taieb et al., 1997; Beckhelling and Ford, 1998). Fertilization induces Ca^{2+}-dependent inactivation of $p34^{cdc2}$ kinase, via cyclin B degradation and subsequent resumption of the second meiotic division (Lorca et al., 1993). Changes in the activity of other kinases appear to play an important role in chromosome separation at anaphase II, extrusion of the second polar body and pronuclear formation (Moos et al., 1995, 1996; Johnson et al., 1998). Many signal molecules have been shown to play an important role in CGE and in resumption of the second meiotic division during the egg activation process. The current article focuses on the signaling pathways involved in the process of CGE and block to polyspermy.

2. Ca^{2+} and CGE

CGs originate from the Golgi; during oocyte growth they increase in number and start migrating towards the cortex (Ducibella et al., 1988). The final movements to the periphery of the egg, as well as formation of a CG-free domain overlying the MII spindle, take place during maturation of the rodent eggs (Ducibella et al., 1988). The ability of eggs to undergo CGE is developed after germinal vesicle breakdown, but this ability is not fully developed until MII stage or near the time of ovulation (Abbott et al., 2001; Ducibella, 1996; Wang et al., 1997). The egg acquires its competence to be activated by a fertilizing spermatozoon and responds by secreting its CGs content, concomitantly with obtaining its ability to release $[Ca^{2+}]_i$ and respond to it (Abbott et al., 2001). CGE occurs in a matter of seconds as a response to an increase in $[Ca^{2+}]_i$ (Kline and Stewart-Savage, 1994). Moreover, inhibition of pathways involved in $[Ca^{2+}]_i$ release, results in a block in CGE and the absence of the modification of the egg's ZP (Xu et al., 1994; Shilling et al., 1994; Carroll et al., 1997, 1999). Similar results were obtained by injecting Ca^{2+} chelators, that prevent the rise in free $[Ca^{2+}]_i$, into mammalian egg cytoplasm, prior to fertilization or egg activation (Xu et al., 1996; Kline and Kline, 1992).

How is the calcium signal translated into a stimulus that evokes a biological effect such as CGE? Many Ca^{2+}-dependent proteins were identified by Western blot analysis, immunofluorescence or reverse transcriptase polymerase chain reaction (RT-PCR). Among them are calmodulin (CaM), Ca^{2+}/CaM-dependent protein kinase II (CaMKII) the soluble *N*-ethylmalameide-sensitive factor attachment protein receptor (SNARE) proteins and some others.

2.1. CaM

CaM is a 17 kDa ubiquitous intracellular protein that contains a conserved Ca^{2+} binding motif (EF hand) and plays a vital role in many aspects of cell signaling; it transduces Ca^{2+} signals generated by various signaling pathways (Berridge, 1993). CaM accounts for approximately 0.3% of total protein in mouse eggs and is considered as an important Ca^{2+} effector at fertilization. Due to its abundance in eggs and considering its numerous important substrates, CaM has been hypothesized to regulate both CGE and meiotic cell cycle (reviewed by Abbott and Ducibella, 2001). The role of CaM in promoting CGE emerged from research performed using spermatozoa, eggs and secretory cells. AR requires Ca^{2+} influx through voltage dependent Ca^{2+}channels (Darszon et al., 1999). Being localized to the acrosome region (Fouquet et al., 1991) and redistributed during AR, CaM is believed to participate in AR (Hernandez et al., 1994). Furthermore, CaM antagonists inhibit the AR in sea urchin (Sano, 1983; Guerrero and Darszon, 1989) and in mouse sperm (Lopez-González et al., 2001).

CaM antibodies (Steinhardt and Alderton, 1982) and trifluoroperazine, a CaM antagonist, inhibited the fusion of CGs with sea urchin eggs plasma membrane (Haggerty and Jackson, 1983), whereas, W7 a different CaM antagonist, did not block CGE, though it delayed both the fertilization-associated decrease in histone H1 kinase activity and the emission of the second polar body (PBII: Xu et al., 1996). The opposite responses to CaM antagonist exhibited by sea urchin and mouse eggs could be attributed to species differences or to different technical methodology employed. It should be noted that the mouse eggs were inseminated in the absence of W7. Electrophysiological studies demonstrated that CGE in hamster eggs commenced as early as 4 s after binding of sperm to the egg membrane, while resumption of the second meiotic division was observed only 20 min after sperm binding (Kline and Stewart-Savage, 1994). In light of these observations, the presence of an inhibitor during egg activation period is obligatory, as even a brief absence of the inhibitor from the culture medium during activation can commence CGE. In other cell types, CaM binds to secretory granules and secretion of the granules content is sensitive to CaM inhibitors and antibodies (reviewed in Trifaro et al., 1992).

All the aforementioned observations point to a potentially important role of the Ca^{2+}–CaM signaling pathway in regulating CGE, although the nature of the Ca^{2+}–CaM signal that regulates CGE is not yet well understood. CaM has been shown to be associated with a large number of proteins including CaMKII, a serine–threonine protein kinase.

2.2. CaMKII

CaMKII, one important target protein of CaM, is present in all somatic cell types examined (Schulman and Hanson,