Cell Calcium 53 (2013) 2–9

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Calcium signaling surrounding fertilization in the nematode *Caenorhabditis elegans*

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ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form 2 November 2012
Accepted 3 November 2012
Available online 4 December 2012

Keywords:
Spermiogenesis
Meiotic maturation
Ovulation
Sheath cells
Spermatheca
Membranous organelle (MO)
Inositol triphosphate (IP3)
ITR-1
Store operated calcium entry (SOCE)
Ca^{2+} release-activated Ca^{2+} channel
SPE-38
TRP-3/SPE-41
Calcium wave

ABSTRACT

Calcium plays a prominent role during fertilization in many animals. This review focuses on roles of Ca^{2+} during the events around fertilization in the model organism, *Caenorhabditis elegans*. Specifically, the role of Ca^{2+} in sperm, oocytes and the surrounding somatic tissues during fertilization will be discussed, with the focus on sperm activation, meiotic maturation of oocytes, ovulation, sperm–egg interaction and fertilization.

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1. Introduction

Calcium plays a ubiquitous role in biological pathways. Fertilization is one such process that exploits Ca^{2+} mediated signals in several phases and many species across the animal kingdom seem to rely extensively upon Ca^{2+} signal for this purpose [1].

Caenorhabditis elegans is an attractive model system to study the biology of fertilization [2]. The transparent nature of the body permits the live examination of several key events of fertilization [3]. Especially, monitoring the influx of Ca^{2+} ions into oocytes enables one to directly correlate the Ca^{2+} signaling with the hallmarks of fertilization in an intact animal.

C. elegans exist as sexually dimorphic males and hermaphrodites. Hermaphrodites are predominantly found in natural population and under normal culture conditions in the lab. Hermaphrodites produce both sperm and oocytes, which enable the animal to self-fertilize to produce self-progeny. Males are found very rarely in nature and produce only sperm. Males can mate with hermaphrodite to sire cross-progeny [4].

The reproductive system in *C. elegans* is consists of a simple U shaped tube (Fig. 1) [5]. At the distal most part of the gonad reside germ cells, which undergo mitotic proliferation, followed by meiotic division to give rise to gametes. During the initial phase of gametogenesis, hermaphrodites produce a limited number of sperm. Thereafter, it continues to produce oocytes only [6].

The diploid spermatocytes undergo complete meiotic division to give rise to haploid spermatids through a process called spermatogenesis [7–9]. The spermatids are round in shape, immotile and are incapable of fertilizing oocyte. Spermatids are rapidly activated to form mature spermatozoa through a process called spermiogenesis or sperm activation (Fig. 2) [8]. During the activation of sperm, pseudopods are formed from one side of the sperm, which enable the sperm to become motile and fertilization-competent. *C. elegans* sperm contain a specialized Golgi-derived vesicle called membranous organelle (MO). In spermatids, these organelles are sequestered inside the cytosol. During or following sperm

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0143-4160/$ – see front matter. Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.ceca.2012.11.009

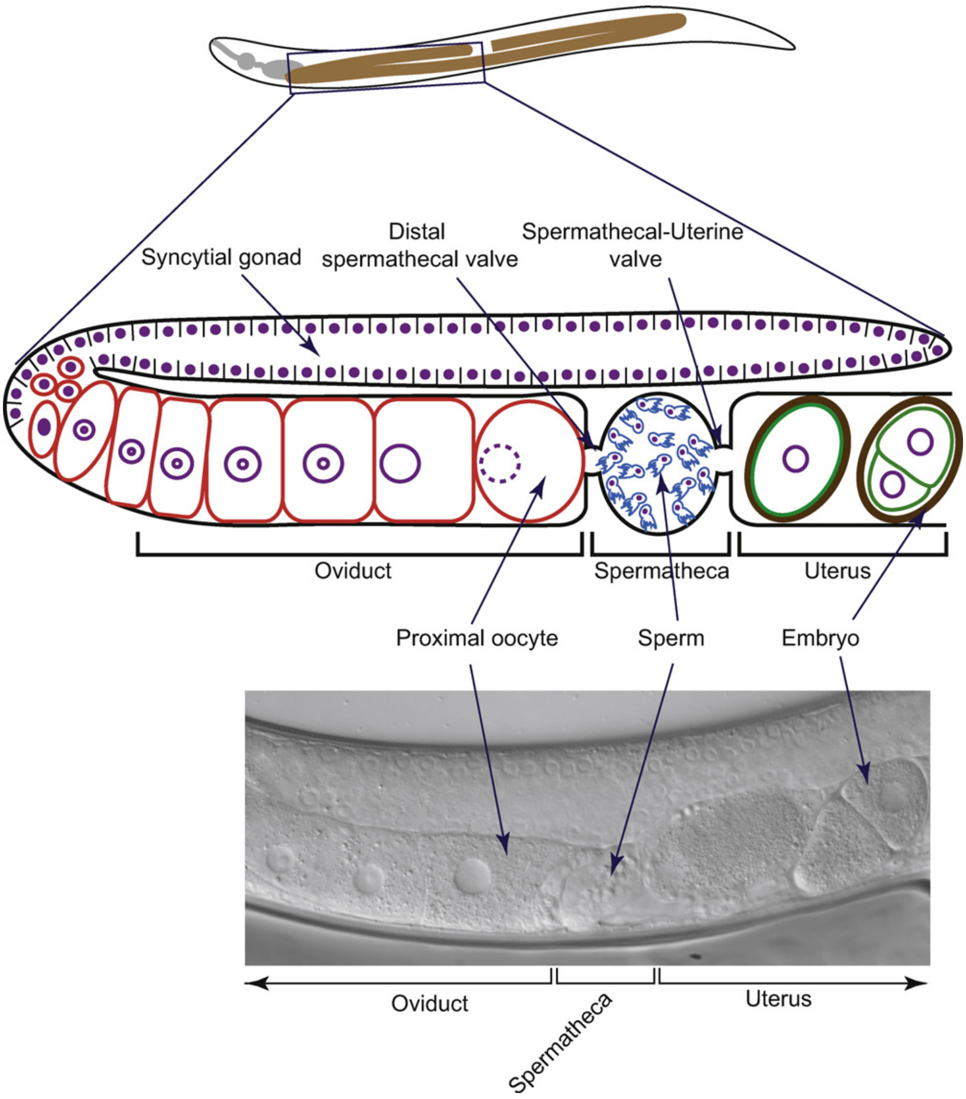

Fig. 1. Schematic diagram showing the reproductive tract of adult hermaphrodite (top). Oocytes mature and enter one by one into spermatheca, which is the site of sperm storage and fertilization. Fertilized eggs enter into uterus where early embryonic development takes place. Eventually eggs are pushed out through vulva (not shown). DIC micrograph showing part of the adult gonad (bottom).

activation, MOs fuse with plasma membrane and release their contents outside [9].

Oocytes undergo several cytological changes in preparation for fertilization and enter into spermatheca serially (see Fig. 1). The process of entry of oocyte into spermatheca is called ovulation [10]. Contraction of gonadal sheath cells accompanied by the dilation of distal spermatheca valve propels the oocyte into spermatheca [3]. Fertilization takes place in spermatheca, which can house both male derived and hermaphrodite derived sperm. Newly fertilized eggs then enter into the uterus, where early embryonic development takes place. Eventually eggs are pushed out on to the surrounding media through the vulva.

2. Roles of Ca^{2+} and Ca^{2+} binding proteins during sperm activation

Depletion of extracellular Ca^{2+} by treating spermatids with EDTA or EGTA does not compromise sperm activation, which suggests that Ca^{2+} from extracellular environment may not play a role in sperm activation [11]. In contrast, buffering intacellular Ca^{2+} by treating the spermatids with BAPTA-AM (a membrane permeable Ca^{2+} chelator) causes reduction in MO fusion, suggesting that intracellular Ca^{2+} is necessary for MO fusion and sperm activation [12].

The requirement of intracellular Ca^{2+} for the complete activation of *C. elegans* sperm raises an interesting question: is the intracellular pool of Ca^{2+} regulated in sperm? Most eukaryotic cells sequester Ca^{2+} in the endoplasmic reticulum (ER) in order to ensure the regulated delivery of Ca^{2+} into the cytosol [13]. However, most of the organelles, including ER, are discarded during spermatogenesis in *C. elegans* [14]. Therefore, it is less likely that ER could play a prominent role in regulating Ca^{2+} homeostasis in *C. elegans* sperm. Surprisingly, calreticulin, a Ca^{2+} buffering protein that resides in ER, is detected in *C. elegans* sperm and plays a role in fertility in *C. elegans* [15]. Since calreticulin has the propensity to retrotranslocate from ER to cytosol [16], it is likely that the observed CRT-1 staining in *C. elegans* sperm corresponds to the pool of cytosol-localized CRT-1 [15]. *In vitro* activated *crt-1* mutant spermatozoa are smaller than wild-type sperm, display shorter pseudopods and the nuclei are positioned off center [15]. Since larger sperm outcompete smaller sperm in *C. elegans* [17], the function of CRT-1 might be critical for the reproductive success of the worm.

In addition to ER, other organelles such as mitochondria, Golgi apparatus, lysosome and nucleus also stores Ca^{2+} [18]. Since sperm