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How to make a good egg! The need for remodeling of oocyte Ca^{2+} signaling to mediate the egg-to-embryo transition

Nancy Nader, Rashmi P. Kulkarni, Maya Dib, Khaled Machaca*

Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar (WCMC-Q), Education City, Qatar Foundation, Qatar

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ABSTRACT

The egg-to-embryo transition marks the initiation of multicellular organismal development and is mediated by a specialized Ca^{2+} transient at fertilization. This explosive Ca^{2+} signal has captured the interest and imagination of scientists for many decades, given its cataclysmic nature and necessity for the egg-to-embryo transition. Learning how the egg acquires the competency to generate this Ca^{2+} transient at fertilization is essential to our understanding of the mechanisms controlling egg and the transition to embryogenesis. In this review we discuss our current knowledge of how Ca^{2+} signaling pathways remodel during oocyte maturation in preparation for fertilization with a special emphasis on the frog oocyte as additional reviews in this issue will touch on this in other species.

1. Introduction

One of the most fundamental developmental transitions occurs at fertilization when two haploid gametes join to initiate the development of a multicellular organism. Throughout phylogeny Ca^{2+} has emerged as the messenger of choice to instruct the egg following sperm entry to initiate its developmental transition into embryogenesis [1]. This involves cellular events, including the block to polyspermy and the completion of meiosis, that need to occur in a sequential fashion to be effective. Polyspermy needs to be prevented before the completion of the meiotic cell cycle otherwise the zygote is doomed even if meiosis is completed. Hence not only does the fertilization Ca^{2+} signal need to instruct specific cellular events, it needs to do so in a sequential fashion. This is accomplished using the broad temporal and spatial dynamics that Ca^{2+} signaling is endowed with. Therefore, the Ca^{2+} signal at fertilization has information encoded in its spatial, temporal and amplitude dynamics that mediate the egg-to-embryo transition. In fact egg activation in all sexually reproducing species investigated to date, occurs in response to a specialized Ca^{2+} transient at fertilization [1–4]. Importantly fully grown oocytes in the ovary are incapable of producing this specialized fertilization-specific Ca^{2+} transient. The competency of eggs to produce this particular calcium transient is acquired during a complex cellular differentiation pathway referred to as "oocyte maturation". Oocyte maturation encompasses the reductionist DNA division leading to the production of a haploid gamete, and in addition dramatic cellular differentiation at the structural and signaling levels that prepare the egg for fertilization. *Xenopus laevis* has provided a rich model system to understand how Ca^{2+} signals remodel during oocyte maturation. This special issue focuses on the role and regulation of Ca^{2+} signaling in gametes in select species across phylogeny. Herein we focus on the mechanisms controlling Ca^{2+} signaling differentiation during *Xenopus* oocyte maturation and its implications on fertilization and the egg to embryo transition.

* Corresponding author at: Weill Cornell Medical College in Qatar, PO Box 24144, Doha, Qatar. Fax: +974 4492 8422.
E-mail address: khm2002@qatar-med.cornell.edu (K. Machaca).

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2. The *Xenopus* oocyte – a rich model system

Xenopus laevis oogenesis was described 40 years ago resulting in the classification of oocytes in six stages (I–VI) with the largest fully grown stage VI oocytes reaching a diameter of ~1.3 mm [5]. Their large sizes made these oocytes an excellent experimental system for studying different aspects of cell biology and physiology, such as elucidating the kinase cascade that drives the meiotic M-phase [6] and even synchronize it with physiological events at the single cell level [7]. In addition, their ease of handling and amenability to imaging and electrophysiological techniques made them an attractive model system for studying the molecular regulation of Ca^{2+} signaling especially during oocyte maturation and fertilization [8–10], as well as for expression cloning of several channels and transporters [11]. *Xenopus* oocytes are polarized cells with a dark animal hemisphere and a clear vegetal hemisphere that is enriched in yolk platelets. The nucleus, known as the germinal vesicle, localizes to the animal hemisphere. Based on these morphological aspects, oocyte maturation can be easily followed visually. In response to progesterone, the widely used hormone inducer, fully grown "immature" stage VI oocytes undergo maturation and are identified by the appearance of a white spot on the animal pole. This white spot is due to the breakdown of the nuclear envelope – referred to as germinal vesicle breakdown (GVBD) – in the "egg" leading to the dispersion of the dark pigment granules. Moreover because of their large size and their high capacity for protein synthesis, *Xenopus* oocytes are extensively used in manipulating the expression levels of different proteins of interest using injection of in vitro synthesized RNA or anti-sense approaches. Due to the specific characteristics mentioned above, *Xenopus* oocytes were pillars in unfolding critical steps leading to egg activation such as the discovery of the maturation promoting factor (MPF) in the mid-1970s [12] and Ca^{2+} remodeling during oocyte maturation [13,14].

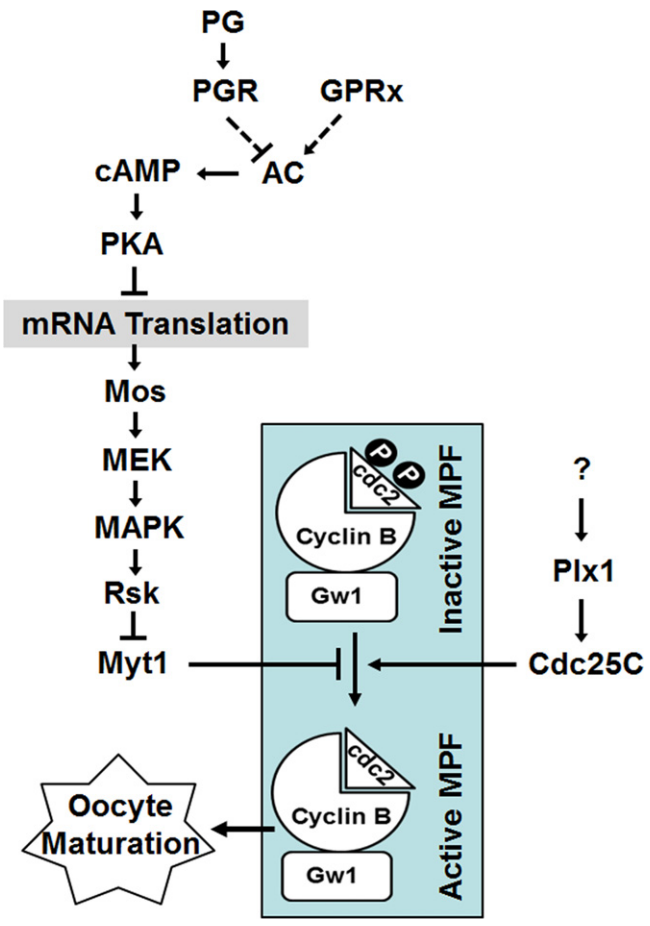

Fig. 1. Signaling cascades controlling *Xenopus* oocyte maturation. See text for details.

3. Meiotic arrest

Fully grown vertebrate oocytes are naturally arrested in a G2-like state at prophase of meiosis I (MI) for prolonged periods of time before resuming meiosis in a process termed "maturation" that prepares the egg for fertilization [15,16]. During this meiotic arrest the oocyte grows and stores macromolecular components that are necessary for future development [17]. While the molecular details of how meiotic arrest is sustained are still not fully elucidated, current data argue that G-protein coupled receptors (GPCRs) are key players in the process. In contrast to mammals, meiotic arrest in *Xenopus* oocytes is completely cell-autonomous, since isolating oocytes away from their follicles does not induce maturation, highlighting the role for endogenous mechanisms responsible for meiotic arrest. Indeed, mounting evidence shows that in different species studied, including *Xenopus*, oocytes are held in meiotic arrest by constitutively active GPCR signaling that elevates cyclic adenosine monophosphate (cAMP) levels through stimulating adenylyl cyclase (AC). High cAMP results in tonic inhibition of the cascade that culminates in the activation of MPF (cyclinB–Cdc2 complex) the key driver of meiosis (Fig. 1) [18–26]. Treatments that increase cAMP levels and/or activate its downstream effector Protein Kinase A (PKA) block *Xenopus* oocyte maturation, and in contrast inhibiting the cAMP–PKA cascade induces oocyte maturation (Table 1) [18,27–31]. More recently, the long-chain acyl-CoA synthetase (ACSL) that is involved in lipid metabolism, was shown to be required for maintaining meiotic arrest in *Xenopus* oocytes through $G\alpha_S$ palmitoylation [32]. Palmitoylation is a post-translational modification that anchors Gα to the membrane and is important for its functions [33]. Mehlmann et al. first described a constitutively active GPCR, GPR3, as a crucial component of maintaining meiotic arrest in mouse oocytes [34]. In 2008, two groups cloned and characterized GPRx, a related protein to mammalian GPR12/GPR3, as the main GPCR in *Xenopus* oocytes that sustains meiotic arrest. GPRx is thought to activate both Gαs and Gβγ with potentially a more pronounced role for Gβγ signaling, since sequestration of Gβγ inhibits GPRx activity [34–37]. Table 1 lists representative experimental interventions that modulate oocyte meiotic arrest in *Xenopus*.

Table 1
Regulation of oocyte meiotic arrest.[a]

	References
Examples of interventions that release meiotic arrest	
1. Inhibition of PKA	[29]
2. Decrease Gαs expression	[23,276]
3. Injection of Gαs antibodies	[23]
4. Sequestration of Gβγ heterodimer	[24,26,37]
5. Blocking of exocytosis	[51]
Examples of interventions that inhibit progesterone-induced oocyte maturation	
1. Increasing cAMP levels by forskolin	[31,277]
2. Phosphodiesterase inhibitors	[30]
3. Injection of PKA catalytic subunit	[18]
4. Overexpression of GPRx	[35,37]
5. Blocking clathrin-dependent endocytosis	[51]
6. Activation of Gαs by cholera toxin	[44,46,278]
7. Overexpression of Gαs	[276]
8. Overexpression of Gβγ heterodimer	[24,26]
No effect on progesterone-induced oocyte maturation	
1. Inhibition of Gαi by pertussis toxin	[279,50]

[a] Examples given are representative and are not meant to be exhaustive.