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Review

Cell cycle arrest and activation of development in marine invertebrate deuterostomes

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ABSTRACT

Like most metazoans, eggs of echinoderms and tunicates (marine deuterostomes, there is no data for the cephalochordates) arrest awaiting fertilization due to the activity of the Mos/MEK/MAPK cascade and are released from this cell cycle arrest by sperm-triggered Ca2+ signals. Invertebrate deuterostome eggs display mainly three distinct types of cell cycle arrest before fertilization mediated by potentially different cytostatic factors (CSF): one CSF causes arrest during meiotic metaphase I (MI-CSF in tunicates and some starfishes), another CSF likely causes arrest during meiotic metaphase II (amphioxus), and yet another form of CSF causes arrest to occur after meiotic exit during G1 of the first mitotic cycle (G1-CSF). In tunicates and echinoderms these different CSF activities have been shown to rely on the Mos//MAPK pathway for establishment and on Ca2+ signals for their inactivation. Despite these molecular similarities, release of MI-CSF arrest is caused by APC/C activation (to destroy cyclin B) whereas release from G1-CSF is caused by stimulating S phase and the synthesis of cyclins. Further research is needed to understand how both the Mos//MAPK cascade and Ca2+ achieve these tasks in different marine invertebrate deuterostomes.

Another conserved feature of eggs is that protein synthesis of specific mRNAs is necessary to proceed through oocyte maturation and to maintain CSF-induced cell cycle arrest. Then activation of development at fertilization is accompanied by an increase in the rate of protein synthesis but the mechanisms involved are still largely unknown in most of the marine deuterostomes. How the sperm-triggered Ca2+ signals cause an increase in protein synthesis has been studied mainly in sea urchin eggs.

Here we review these conserved features of eggs (arrest, activation and protein synthesis) focusing on the non-vertebrate deuterostomes.

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1. Introduction

The mature oocyte (or egg) contains all the factors necessary to support early development but embryonic development is halted until fertilization. It has been known for more than 30 years that transient Ca2+ increases in the egg are the ubiquitous signals used

Abbreviations: MAPK, mitogen-activated protein kinase; MPF, maturation promoting factor; APC/C, anaphase promoting complex; CSF, cytostatic factor; eIF2 – eukaryotic initiation factor 2, 4E-BP – eIF4E (eukaryotic initiation factor 4E) binding protein.

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to stimulate development in metazoans [1]. Such cell cycle arrest of the egg is mediated by an activity discovered in *Rana pipens* amphibian oocytes called cytostatic factor [2]. Despite the universality of CSF and its inactivation by Ca2+, CSF arrest occurs at different time points during maternal meiosis: metaphase II in vertebrates and cephalochordates [21], metaphase I in many protostomes and tunicates, and interphase (mostly G1) in cnidarian, echinoderms and some protostomes (mollusks).

Because of these differences in cell cycle arrest at the end of oocyte maturation there are inconsistencies in the terminology surrounding oocytes/eggs/fertilized eggs (and zygotes). For example, some authors, including the entire mammalian field, use the criteria that exit from the meiotic cell cycle (when the pronucleus forms) defines the transition from oocyte to egg. Thus the metaphase II oocyte awaits fertilization in the mammalian field and the fertilized oocyte becomes a zygote following pronuclear fusion. However, other authors prefer to use "egg" to describe the female gamete that can be fertilized regardless of the state of meiotic maturation. Thus, in the mammalian field, the fertilized egg becomes a zygote following pronuclear fusion (the karyogamy). Here we will use the egg nomenclature to refer to the mature female gamete at the moment of fertilization. Likewise there are some differences in the usage of zygote. Like Wessell we prefer to use zygote to define the moment of union between the male and female pronuclei rather than fusion between the sperm and the egg [3].

Eggs and embryos of many marine invertebrate deuterostome species have very favorable optical properties and are easy to manipulate in the laboratory (i.e., external fertilization and development). For these reasons eggs of marine invertebrate deuterostomes have been used in embryological studies for more than a century. While first documented embryonic development was of sea urchin embryos [4], the first experimental approaches in embryology were performed using ascidian embryos [5] (reviewed by Fischer [6]). The precise details of ascidian embryogenesis were documented in depth later by Conklin [7]. Furthermore, thanks to the copious amount of gametes recovered, these eggs have been useful material for biochemical studies since the late 40's (sea urchin [8], starfish [9]) and several processes of oocyte maturation, CSF arrest and meiotic cell cycle were first characterized using starfish oocytes ([10], reviewed in [11]) before being confirmed in vertebrates. What was once useful for biochemistry is now useful for proteomic studies for similar reasons. Today, there are genomic and transcriptomic resources for several species of tunicates (ascidians: www.anisse d.cnrs.fr, appendicularian:http://www.genoscope.cns.fr/externe/GenomeBrowser/Oikopleura/) or echinoderms (sea urchin: www.spbase.org/SpBase/, starfish: *Patiria miniata*, www.echinobase.org/Echinobase/PmBase) which have opened the way to specifically modify each gene via morpholino oligonucleotide injections or TALEN-mediated deletions/mutations [12]. Finally, the success of transgenesis in these species permits the visualization of the localization and/or activity of GFP-tagged proteins and to use fluorescent molecular imaging techniques in the living egg or embryo.

Oocyte development is devoted to exchange genetic material between homolog chromosomes and decrease chromosomal number during meiosis but more importantly to producing and storing all constituent necessary to support early development until zygotic transcription can take over. During vitellogenesis, active transcription and massive protein synthesis generate stockpiles of maternal mRNAs and proteins. Then transcription ceases at the resumption of oocyte maturation and tightly regulated translation of these maternal mRNAs drives the oocyte through meiosis until CSF arrest occurs. The egg thus hosts the maternal proteome and transcriptome that will drive cleavage of the early embryo. For example, artificially activating enucleated sea urchin eggs (thus, activation without sperm or gynogenesis) can remarkably result in cell division and formation of a blastula stage embryo [13]. Furthermore, inhibiting transcription in *Xenopus* embryos does not affect development up to the blastula stage [14]. These experiments clearly reveal the maternal nature of early embryonic development. Because of the ease of maturing starfish oocytes *in vitro*, the starfish model has contributed enormously to the understanding of such processes in metazoans [15–17]. Unfortunately no other marine invertebrate deuterostome oocytes are so easily amenable to *in vitro maturation*, however despite this limitation there are some articles detailing oocyte maturation in ascidians [18,20], and sea urchins [19].

Oocyte maturation and the onset of development are two developmental windows during which regulated mRNA translation is of utmost importance. Indeed protein translation can both drive progression through meiotic (and early mitotic) divisions and arrest cell cycle progression in the mature egg. Even though the regulation of CSF by the Mos//MAPK(ERK1/2) pathway is universal [23], large divergences have emerged downstream of ERK1/2 to result in the different outcomes of CSF observed in echinoderm or tunicate eggs. Further diversity in CSF arrest points is found among starfishes suggesting an important selective pressure on the different starfish species which adapted by deploying different life cycles.

In this review, we will first describe the different types of CSF arrest observed in marine invertebrate deuterostome eggs. Then we will briefly describe how fertilization Ca2+ signals start and stop. We will then emphasize how Ca2+ inactivates CSF to trigger a mitotic cell cycle oscillator repressed in the egg. Finally we will discuss some aspects of the regulation of mRNA translation in the egg. Biological models such as the mouse, *Xenopus* or *Drosophila* are well documented in recent reviews [24–27]. Here we will address advances on egg activation mechanisms provided by studies on marine deuterostomes such as echinoderms and several chordates (mainly tunicates).

2. CSF arrest in marine deuterostomes is established and maintained by the Mos//MAPK pathway

Fig. 1 depicts all the CSF arrests observed in marine invertebrate deuterostomes. CSF arrest point is very conserved in sea urchin species which arrest at G1 (*Paracentrotus lividus* [28], *Sphaerechinus granularis* [29], *Lytechinus variegatus*, *L. pictus*, *Strongylocentrotus purpuratus* [30]) and in all tunicates, which arrest at metaphase I (*Ciona intestinalis* [48], *Ascidiella aspersa* [59], *Phallusia mammillata* [41], *Boltenia villosa* [20], *Phallusia nigra* [74], *Halocynthia roretzi*, *Styela plicata*). In contrast, CSF arrest points diverge much more within asteroids with some starfish species (*Asterina pectinifera* [31,32], *P. miniata* [33]) arresting at G1, while *Astropecten aranciacus* and *Marthasterias glacialis* [34,35] arrest at G2 (reviewed in [15]). The pacific purple starfish *Pisaster ochraceus* and the Japanese starfish *A. pectinifera* even display two cell cycle arrests : one at metaphase I and another at G1 [36–38]. Metaphase arrest is characterized by high MPF activity and the APC/C must be blocked to prevent destruction of cyclin, MPF inactivation and completion of meiosis. In contrast G1 arrested eggs are preloaded with all the initiation/replication complexes and are ready to proceed through S phase [28,32], but they are prevented from doing so because of the lack of cdc45 in the nucleus [31]. Beyond S phase progression, entry into mitosis is blocked by a separate mechanism that down regulates synthesis of cyclins [32].

Despite such divergence in the actions of CSF, CSF arrest relies on the Mos/MEK/MAPK(ERK1/2) pathway in almost all metazoan eggs studied (Fig. 1). Mos is a kinase signaling upstream of the MEK/MAPK pathway (Fig. 1) initially described as c-Mos, a proto-oncogene (moloney sarcoma). Its role as part of CSF was first revealed in *Xenopus* [40]. For an extended overview of CSF signaling in metazoans see [25–27,42,43]. Mos and its associated MAPK