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Biophysical Chemistry 72 (1998) 131–140

Biophysical Chemistry

Calcium waves and oscillations in eggs

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Revision received 16 January 1998; accepted 13 February 1998

Abstract

Eggs from several protostomes (molluscs, annelids, nemerteans, etc.) and two deuterostomes (mammals and ascidians) display repetitive calcium signals. Oscillations in the level of intracellular calcium concentration are occasionally triggered by maturing hormones (as in some molluscs) and mostly observed after fertilization which occurs at different stages of the meiotic cell cycle (oocytes are arrested in prophase, metaphase I or metaphase II). In most eggs examined so far, calcium oscillations last until the end of meiosis just before male and female pronuclei form. This ability depends on the sensitivity of InsP3 channels and on the permeability of the plasma membrane to extracellular calcium. In eggs that undergo cytoplasmic reorganization at fertilization (annelids, nemerteans, ascidians, etc.) the repetitive calcium signals are waves that originate from localized cortical sites that become calcium waves pacemakers. In ascidians we have identified the site of initiation of repetitive calcium waves as an accumulation of endoplasmic reticulum sandwiched between the plasma membrane and an accumulation of mitochondria. We compare and discuss the generation of calcium signals in the different eggs, their relationship with the cell cycle and the possible roles they play during development.

Keywords: Calcium; Oscillations; Waves; Fertilization; Meiosis; Ascidians; Mammals

1. Introduction

Eggs can be activated by sperm or a variety of parthenogenetic means. This activation is thought to be the result of a large transient rise in intracellular calcium concentration.

More than 10 years ago, it was further discovered that activation of mouse eggs resulted in periodic elevations of the free intracellular calcium concentration [1]. Such oscillatory calcium signals were later reported in other eggs and many somatic cell types including plant cells (see Refs. [2–4] for reviews). A large single transient rise in intracellular calcium concentration either followed or not by oscillations, is associated with activation of most cell types [3,4]. The calcium changes are known to trigger transformations of the cell cortex and cytoplasm as well as to stimulate many enzymatic and metabolic processes [3,4].

Animal groups in whom oocytes display calcium oscillations during meiotic maturation and/or fertilization range from protostomes (several bivalve molluscans [5–8] the nemertean *Cerebratulus* [9,10] and the polychaete worm *Chaetopterus* [11]) to deuterostomes (several mammals [12–15] and ascidians [16–19]).

We first review what is known of these oscillatory calcium signals in protostomes, then briefly summar-

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0301-4622/98/$19.00
PII S0301-4622(98)00129-X

ize the extensive work in mammalian eggs and finally present in greater detail the observations we and others have made on ascidians eggs. Ascidians are marine urochordates that lie at the base of the vertebrate line [20]. They have provided excellent models for experimental embryology since the days of Chabry and Conklin at the beginning of the 20th century and present remarkably stereotyped repetitive calcium signals.

1.1. Calcium oscillations in eggs of the protostome

Embryos of protostomes (molluscs, annelids, nemerteans, etc.) are generally characterized by spiral cleavage patterns and in the way they form their body cavity and their mouth region before their anal region.

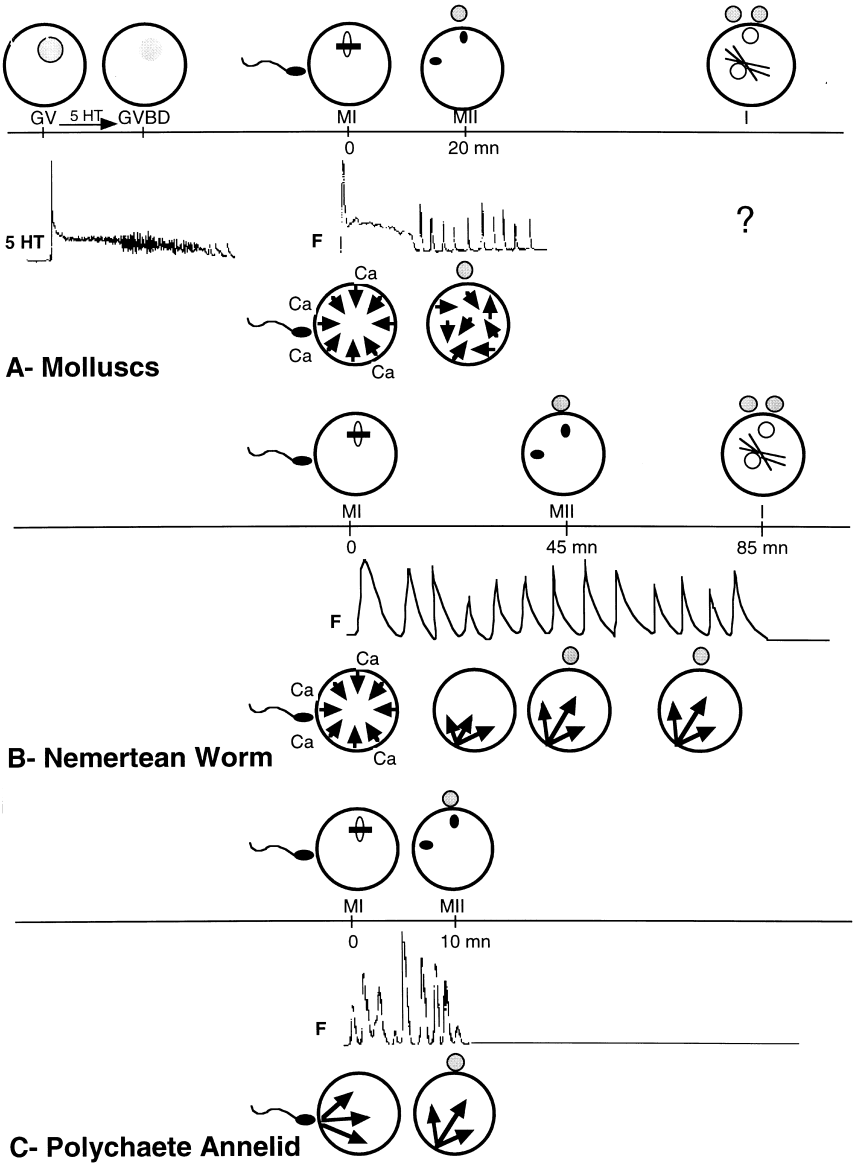
In the mollusc *Hiatella flaccida* (a bivalve) the oocyte is arrested in the prophase stage of meiosis (germinal vesicle stage) (Fig. 1A). The hormone serotonin (5-hydroxytryptamine; 5HT) promotes entry into the meiotic cell cycle (and rupture of the large germinal vesicle) and causes a series of small calcium transients [5–7]. In this species, the stimulated oocyte stops in the first metaphase of meiosis (MI) and, as in the other mollusc examined (*Mytilus)*, fertilization results in another bout of calcium signaling characterized by a large activation pulse, followed by a 10–20-min period of sustained elevation in intracellular calcium concentration during which the first polar body is emitted (Fig. 1A). Small repetitive calcium transients precede the emission of the second polar body and the completion of the meiotic cell cycle [5–8]. The early rise in intracellular calcium apparently occurs simultaneously over the whole egg and clearly depends on external calcium (10 mM in natural sea water) flowing through plasma membrane calcium channels. These voltage-operated channels are apparently L-type calcium channels [21,22]. The later oscillations in free intracellular calcium level depend on InsP3-sensitive internal calcium stores. No clear waves are observed during the entire period of calcium signaling [5–7].

In the nemertean worm *Cerebratulus lactus* the oocyte is arrested in MI and the sperm reinitiates the meiotic cell cycle, causing 7–18 periodic calcium oscillations that stop just before the emission of the second polar body (85 min after fertilization) [9] (Fig. 1B). As in molluscan oocytes the first calcium transient depends on extracellular calcium. The initial signal imaged in the confocal microscope is described as a cortical flash while the other periodic calcium signals are at first incomplete waves originally starting from the sperm entry site. Later waves traverse the whole egg, and originate from a site located in the

Fig. 1. Calcium oscillations in eggs of protostomes. (A) Calcium signals during maturation and fertilization of molluscan eggs. The top drawings show 5 stages of meiotic maturation and fertilization of molluscan eggs (*Hiatella flaccida* and *Mytilus edulis)*.The hormone serotonin (5-hydroxytryptamine; 5HT) triggers maturation of the oocyte arrested at the germinal vesicle (GV; large gray nucleus) stage and germinal vesicle breakdown (GVBD). Fertilization (F) occurs in molluscan eggs arrested at the metaphase I stage (MI). At that stage the egg's chromosomes (bar inside the egg) are aligned on the metaphase spindle. About 20 min after fertilization half of the egg's chromosomes are expelled in a polar body (small gray circle on top of the egg). Condensed male and female chromosomes (small black dots inside the egg) are present in the egg at the metaphase II stage (MII). Male and female pronuclei (large open circles within the egg) then form and meet in the center of the egg (interphase I). By that time two polar bodies have been extruded (2 gray circles on top of the egg). The calcium traces below the top drawings represent the variations in intracellular calcium during meiotic maturation and after fertilization. They are from the work of Deguchi and Osanai [6–8]. The pattern of calcium signals after MII have not been reported (?). The drawings below the calcium traces show the spatial characteristics of the calcium signals after fertilization. At fertilization (F), extracellular calcium enters the egg and intracellular calcium rises homogeneously in the egg starting from the egg surface (short peripheral arrows). Repetitive calcium signals that do not require external calcium occur during MII. They do not seem to be waves (randomly distributed short arrows within the egg). 'Ca' outside the eggs indicates requirement for extracellular calcium. (B) Calcium signals in the egg of the nemertean worm *Cerebratulus lacteus*. The top drawings in this panel show fertilization occurring in the metaphase I stage (MI) and the completion of meiosis (symbols as in panel (A)). The calcium traces beneath are drawn from Stricker's work [9,10]. The reader is encouraged to consult the experimental traces in the original papers [9,10] to appreciate the variations in calcium oscillations in different eggs. The drawings below the calcium traces show that as in molluscs, the initial calcium signals in *Cerebratulus* require external calcium. Later calcium signals are repetitive waves initiated from a point source near the surface (arrows within the egg). These calcium oscillations do not require external calcium and last until meiosis is completed (about 85 min). (C) Calcium signals in the egg of the polychaete annelid *Chaetopterus pergamentaceus*. (Symbols used are the same as in panels (A) and (B)). In these eggs, fertilization (F) also occurs in metaphase I (MI). The fertilizing sperm triggers a series of calcium transients that propagate as waves. The calcium trace shown in this panel is from the work of Eckberg and Miller [11] and the reader should consult the original reference to appreciate variations in calcium signals in different eggs.

vegetal hemisphere (calcium wave pacemaker) [9,10]. All these later signals depend on internal calcium stores and presumably on the presence of InsP3-sensitive channels in the endoplasmic reticulum (ER). In more recent experiments it was observed that injected sperm extracts were able to trigger these repetitive waves [10]. As in fertilization these calcium waves originate from a pacemaker region in the vegetal hemisphere. Even though the injected sperm extract was able to induce the completion of meiosis it could not lead to full parthenogenesis, presumably because the injected extract lacked sperm nucleus and centrosome. In these experiments no extracellular calcium was needed to generate the repetitive calcium signals [10].

Finally, oscillations in intracellular calcium concentration have also been described in the polychaete annelid *Chaetopterus pergamentaceus* by imaging the light emission of injected aequorin [11]. In these eggs arrested in MI, fertilization triggers an activation



wave of calcium release starting from the sperm entry site and followed by a few other calcium transients (Fig. 1C). In contrast to the other protostomes, all calcium oscillations in this organism appear to be complete or incomplete waves first starting from a single site on the surface (the sperm entry site). Again as in *Cerebratulus* the latter calcium waves appear to originate from a cortical 'pacemaker' which has not been identified. Both *Chaetopterus* and *Cerebratulus* are known to undergo large cytoplasmic and cortical reorganizations that participate in the establishment of the embryonic axes and are probably at the origin of the establishment of the calcium wave 'pacemaker' [23,24]. In contrast to what has been described in the molluscan and *Cerebratulus* eggs where calcium oscillations last through meiosis, the periodic oscillations in *Chaetopterus* terminate well before the completion of meiosis.

1.2. Calcium oscillations in eggs of deuterostomes

Deuterostomes (such as echinoderms and chordates) are organisms whose embryos often exhibit radial cleavages and form their mouth region after the anal region. All deuterostomes eggs examined exhibit large calcium signals initiated from the sperm entry point that traverse the whole egg as waves propagating at an average speed of 10 μm/s

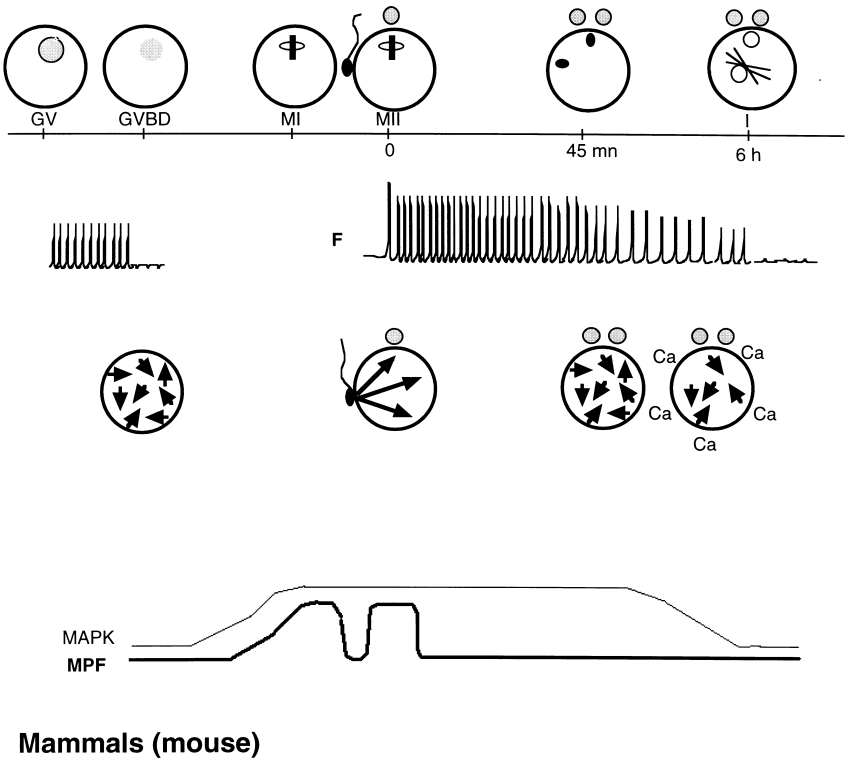


Fig. 2. Calcium oscillations in mouse eggs. The top drawings show five different stages of maturation and fertilization in mouse eggs. The symbols used are the same as in Fig 1A. In the mouse, fertilization (F) occurs in eggs arrested in metaphase II (MII) and the completion of meiosis takes several hours. The calcium traces below the top drawing are drawn from representative experimental traces published and provided by different authors [14,27,30] and J P Ozil. The reader is invited to consult the original papers to appreciate the variabilities in calcium patterns. The drawings below the calcium traces show that clear wave(s) are only observed at fertilization (long arrows starting at sperm entry site) and that most calcium signals (short arrows within the oocyte and fertilized egg) appear randomly and simultaneously in the egg. Later oscillations require external calcium ('Ca' outside the egg) and decrease in amplitude near the completion of meiosis. The traces at the bottom of this panel depict the levels of MAP kinase (MAPK) and maturation promoting factor (MPF) during the different phases of maturation, fertilization and meiosis completion. They are drawn according to Kubiak et al. (J. Cell Sci. 104 (1993) 102).

[25]. Among the deuterostomes eggs examined to date, only mammals (vertebrates) and ascidians (urochordates) display an activation wave of calcium followed by calcium oscillations that last for the entire period of meiosis completion [17,26,27].

Mammalian eggs (particularly mouse and hamster eggs but also human eggs) have been best studied and are treated in detail in another manuscript in the journal (see Ozil in this issue) and several reviews [28,29]. Based on the extensive work done in mouse and hamster oocytes and eggs, we can briefly summarize and schematize the essential features of calcium oscillations in the mammal (Fig. 2). In many mammals and rodents, during a period of meiotic maturation that lasts about 10–12 h, small calcium transients that emanate from locations throughout the entire oocyte are observed. During this period, the oocyte gradually develops a capacity to release intracellular calcium (assessed as sensitivity to InsP3 injection) [14,30, 31]. Normally mouse or hamster oocytes arrest at the second meiotic metaphase stage (MII). The fertilizing sperm reinitiates the meiotic cell cycle by starting a propagating wave of intracellular calcium release from the sperm entry site possibly through introduction of sperm macromolecules [32,33]. A few other waves initiated from the same site propagate through the egg followed by oscillatory calcium signals that seem to emanate synchronously from the entire egg cytoplasm. These periodic signals last for 4–6 h diminishing in frequency and intensity as meiosis is being completed and as male and female pronuclei reform (Fig. 2). Although both InsP3 receptors (InsP3R) and ryanodine receptors (RyR) are present in the mouse and hamster eggs, experiments using perfusion of InsP3 and an antibody against the InsP3R demonstrate that the bulk of calcium signaling is tied to the receptor/channel sensitive to InsP3 [31,34]. It is clear that an increase in InsP3R and a reorganization of the endoplasmic reticulum (ER) network into cortical domains occurs during maturation, fertilization and meiosis but the significance of these events for the spatial and temporal patterns of calcium signaling is not yet known [31,35,36].

Finally, there exists a relationship between these oscillations in calcium levels in eggs and the levels of the factors that control entry and regulation of the cell cycle (MPF, MAP kinase, etc.) [37]. The best evidence for this is that arresting mouse eggs in MII of meiosis (using a microtubule inhibitor) results in the persistence of calcium oscillations [27].

1.3. Calcium signaling in ascidians

Ascidians are sessile marine organisms (tunicates/urochordates) that metamorphose from simple swimming tadpoles that resemble those of vertebrates [20]. In 1989 we reported that the eggs of two species of ascidians (*Ciona intestinalis* and *Phallusia mammillata*) exhibited periodic contractions during meiosis coupled with oscillations in the level of intracellular calcium [17,38]. These oscillatory calcium signals and coupled permeability changes together with the cell cycle changes have now been investigated by several groups in Europe and Japan [17,18]. It is now possible to offer a good chronology of the calcium events coupled with the stereotyped transformations of the egg after fertilization (Fig. 3). Typically, binding and fusion of the sperm with the egg triggers a large release of calcium from intracellular stores (7–12 μM) [16,17]. A wave of intracellular calcium release propagates from the sperm entry site to the antipode. The wave passes through the entire egg cytoplasm at an average speed of 5 μm/s [26]. The large calcium signal triggers a wave of cortical contraction which reorganizes the entire egg cortex and cytoplasm such that it relocates and also forms distinct cytoplasmic and cortical domains (see Refs. [39–41]).

The eggs of *Ciona* and *Phallusia* are arrested at the first meiotic metaphase stage (MI) prior to fertilization and the extrusion of the first polar body (Fig. 3). In eggs of many species, sperm triggers only one large calcium wave which is sufficient to cause the resumption of the meiotic cell cycle. As in amphibian eggs, the calcium signals in ascidians may also act via the activation of a CaM kinase (see Ref. [42]). In ascidians, the activation wave of calcium is immediately followed by a first series of calcium waves (amplitude: 1–4 μM frequency: 1–2 waves/min) whose sites of initiation are progressively localized from the site of sperm entry to a region situated within the constriction generated by the cortical contraction [26,38,43] (Fig. 3; waves B1–B3). This pole of contraction forms within 5 min of fertilization in the vegetal hemisphere. Then, a new series of calcium waves (Fig. 3; waves C1–C10) is initiated from the contraction pole region with a variable delay with

respect to the first train of localizing waves. This phase is characteristically composed of a train of 10–20 oscillations rising in amplitude then decreasing in a symmetrical fashion [17,18,38] (Fig. 3).

Experiments with centrifuged eggs (in which organelles and cytoplasmic domains are stratified) and with confocal microscopy (particularly imaging the source of the initiation of calcium wave with line scanning) indicate that a zone of accumulation of endoplasmic reticulum and apposed plasma membrane in the contraction pole acts as the site of initiation of calcium waves thus acting as a calcium waves pacemaker [26,43]. At present we do not know how this pacemaker functions and what the respective roles of the endoplasmic reticulum and of the plasma membrane are in triggering the repetitive waves from a point source.

It is remarkable that the second train of calcium oscillations in ascidian egg (the waves that repeatedly originate from the contraction pole) propagate preferentially in the egg cortex and not through the center of the egg [26]. These oscillations always cease just as

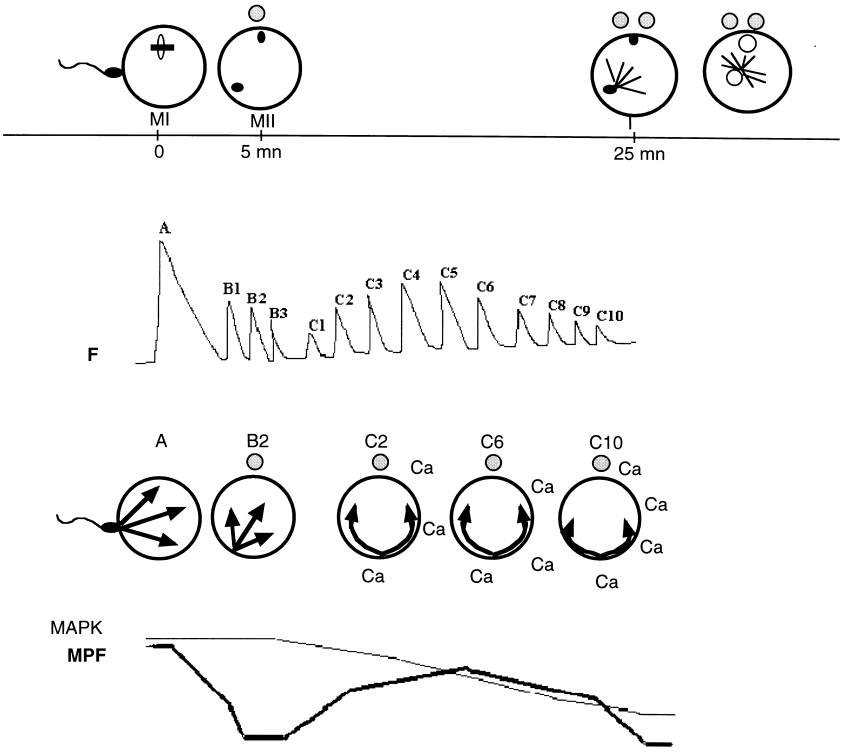


Fig. 3. Calcium oscillations in ascidian eggs. The top drawings show different stages of meiotic completion in ascidian eggs (*Phallusia mammillata* or *Ciona intestinalis*). The symbols are the same as in Figs. 1 and 2. Fertilization (F) occurs in eggs arrested in metaphase I (MI) and meiosis completion is achieved within 20–30 min as two polar bodies (small gray circles on top of the egg) are extruded. The calcium traces below the top drawing are drawn from our experimental observations [17,38]. Three phases (A–C) of calcium signaling are always observed during fertilization and meiosis completion. (A) Activation peak. (B) Oscillations before and during first polar body extrusion (5 min). (C) Oscillations that show progressive increase and then decreasing in amplitude between first polar body and second polar body extrusion. The drawings below the calcium traces show that all calcium oscillations in ascidian eggs are calcium waves (arrows within the eggs) first originating from the sperm entry site. The calcium waves initiation site is progressively localized to a pacemaker site situated in the vegetal hemisphere of the egg. These later oscillations apparently require extracellular calcium (Ca outside the egg) and propagate cortically. At the bottom of the panel we have schematically represented the variations in the levels of MPF and MAP kinase (MAPK) during the different phases of calcium signaling (from the work of Russo [19] and unpublished data by A. McDougall).

the second polar body is emitted and the male and female pronuclei form (Fig. 3). No other calcium signals are detected during either pronuclear migration and fusion, first mitosis and the following mitotic division cycles.

The different phases of calcium signaling described above in the ascidian egg appear to depend on different mechanisms. The activation wave and the following localizing calcium waves (first train of oscillations) do not require the presence of calcium in the extracellular medium whereas the second train of oscillations (later waves originating from a pacemaker) apparently requires the presence of extracellular calcium [18,44,45]. Injection of ascidian sperm extract and/or human sperm extract can trigger the initial activation wave and the first phase of calcium oscillations but not the second phase [46]. Only the first polar body is emitted in this case.

There are numerous plasma membrane permeability changes associated with fertilization and the repetitive calcium waves [16,44,45]. The first detectable change after insemination is a large depolarization in membrane potential that precedes the onset of calcium release near the sperm entry site by about 20 s. The cell maintains its depolarized state throughout the meiotic cell cycle but oscillations in the membrane potential that tend to repolarize the cell occur in synchrony with calcium oscillations. Of particular interest is the fact that fertilization also causes a rapid decrease in the egg plasma membrane depolarization-activated calcium current simultaneous with but independent of the large calcium release produced by sperm. The appearance of this current could depend in part on the insertion of new membrane to the egg plasma membrane [45]. These phenomena depend in turn on the presence of receptors (RYR) sensitive to nanomolar concentrations of ryanodine and cADP ribose [45].

Although these experiments indicate that a RYR is present in the egg, the bulk of calcium signaling in ascidian eggs clearly depends on the presence of an InsP3-sensitive receptor/calcium channel (InsP3R) probably located in the ER. Injection of InsP3 (10 μM) is sufficient enough to produce a rise in intracellular calcium, activate the egg and provoke the extrusion of one polar body [26]. Repeated generation of InsP3 via photolysis of caged InsP3 [26] or continuous perfusion of InsP3 (10 μM) through a patch pipette [45] entrain both calcium oscillations and the completion of meiosis (assayed by second polar body formation). Interestingly, after the second polar body is emitted, continued perfusion of InsP3 ceases to cause calcium oscillations suggesting that the InsP3R is desensitized as the egg enters a new phase of the cell cycle (interphase/low MPF/low MAP kinase). In fact, experiments done by Russo et al. [19] and by ourselves (unpublished data) that measure critical cell cycle factors (MPF/MAP kinase) during normal and perturbed calcium signaling, indicate that an obligatory link exists between these multiple calcium signals and the completion of the meiotic cell cycle. It is not yet clear what type of causal relationship exists since a single exaggerated calcium signal caused by very high concentration of injected InsP3, injected calcium buffer above a threshold, or the use of calcium ionophores can also lead to the completion of the meiotic cell cycle without producing calcium oscillations [18,26].

Finally, we must stress that in ascidian eggs, there are striking changes in the organization of the egg cortex and cytoplasm that are triggered by the activation of the egg by sperm or parthenogenetic agents. These changes accompany and probably regulate in turn the observed calcium signals. Local elevation of intracellular calcium (through release of caged InsP3) leads to a local contraction of the egg cortex [41]. Full contraction requires a threshold level of InsP3 (or calcium release). It is possible that the cortex which contracts massively at fertilization in the direction of the vegetal pole, carries in that direction calcium-releasing factors that the sperm has introduced providing an explanation for the progressive localization of the initiation site of the first series of calcium waves (the introduced sperm nucleus and centrosomes also move vegetally during that period) [39,41].

After the ascidian egg cortex has fully contracted (3 min), distinct layers of the organelles constituting the bulk of dynamic calcium stores in the egg (ER and mitochondria) are concentrated in the vegetal hemisphere, centered around the newly formed contraction pole [40]. This region then becomes the calcium wave pacemaker [39–41].

During this period of calcium oscillations (Fig. 3, waves C1–C10), the egg contracts and relaxes in phase with the repetitive passages of each calcium wave [39]. The contraction pole whose exact location

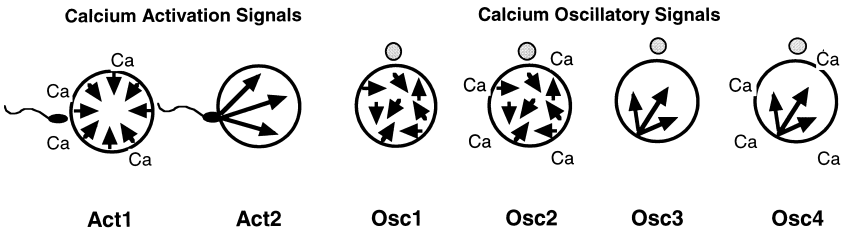


Fig. 4. Different types of calcium signaling in eggs of protostomes and deuterostomes. *Calcium activation signals.* Type Act1, sperm induced depolarization of the plasma membrane triggers entry of extracellular calcium quasi-simultaneously over the entire egg surface (e.g. protostomes). Type Act2, release of sperm factors within the egg and/or activation of transduction pathways by sperm triggers of wave of intracellular calcium release from intracellular stores starting from the sperm entry site (e.g. deuterostomes). *Calcium oscillatory signals.* Type 0sc1, intracellular calcium release occurs quasi-simultaneously throughout the entire egg. No extracellular calcium is needed (i.e. protostomes, e.g. molluscs). Type 0sc2, intracellular calcium release occurs quasi-simultaneously throughout the entire egg. Extracellular calcium is needed (i.e. deuterostomes, e.g. mammals). Type 0sc3, intracellular calcium release occurs repetitively from a localized cortical site (pacemaker) and propagates as a wave. No extracellular calcium is needed (i.e. protostomes, e.g. nemerteans). Type 0sc4, intracellular calcium release occurs repetitively from a localized cortical site (pacemaker) and propagates as a wave. Extracellular calcium is needed (i.e. deuterostomes, e.g. ascidians).

in the vegetal hemisphere is determined by the direction of the calcium wave and the site of sperm entry will become the site of gastrulation [41]. By the time the oscillations cease the embryo has acquired its axial organization along the dorsoventral axis.

2. Discussion

2.1. Calcium signals and oscillations

There is a whole phenomenology of calcium signals. They have been given suggestive names; puffs, sparks, waves, etc. that describe the temporal and spatial features of the signals. These signals can be either localized, elemental calcium release events, or massive propagating floods of the cell's cytoplasm with calcium coming from the extracellular medium or released from organelles (ER and/or mitochondria) where it is normally sequestered [2–4].

We can distinguish several types of calcium signals in eggs. Eggs of many species are arrested in interphase after meiosis is completed and activated by a large (above 1 μM) single transient rise in intracellular calcium (i.e. echinoderms, cnidarians, etc. reviewed in Ref. [47]). Some eggs are arrested in metaphase of meiotic division (i.e. amphibians fishes, ect.) are also activated by a single wave of intracellular calcium release (essentially from intracellular stores) and do not display any other major repetitive calcium signals. These eggs display calcium activation signals of the type Act1 shown in Fig. 4 that traverse the entire egg or its periphery at a characteristic speed (about 10 μm/s see Refs. [3,4,25,47]). In other eggs (such as molluscan and nemertean eggs), the initial calcium signal (or signals) are associated with a sperm-induced depolarization of the plasma membrane and the opening of calcium channels (type Act2 shown in Fig. 4). This leads to quasi-synchronous calcium entry from the surface and a rise in cytoplasmic calcium that is sufficient to activate the egg. It is not clear yet why the third protostome examined so far (the polychaete *Chaetopterus)* does not fit with a picture that was predicted years ago by Jaffe [47]. In mammalian and ascidian eggs, as in other deuterostome eggs cited above (amphibians, sea urchins, fishes, etc.), the calcium activation signal ('calcium wave') is initiated at the site of sperm entry either by factors introduced by sperm [32,33] and/or signal transduction pathways involving G proteins or tyrosine kinase-linked pathways that stimulate the production of InsP3 [4,28]. In both mammals and ascidians, repetitive calcium signals follow that initial activation wave. They somehow require calcium from the external medium to be sustained (Fig. 4: oscillatory calcium signals of type 0S4). In protostomes eggs examined so far (several molluscans and a nemertean) once primed by extracellular calcium entry, calcium signaling apparently relies on intracellular calcium stores to continue its oscillations [7–9] (Fig. 4: type 0S1 or 0S2).

The spatial characteristics of the various calcium signals are also interesting to compare among different species. They are the result of multiple quasi-simultaneous events either starting from the entire egg surface (as in molluscans, Fig. 4: type Act1) or originating from multiple cytoplasmic foci firing quasi-simultaneously (as in mammals, Fig. 4: type 0S2). Alternatively they may originate from a point source (Fig. 4: type Act2, 0S3, 0S4). These point sources (or calcium wave pacemakers) may represent the accumulation of calcium release factors introduced by the fertilizing sperm or they may be generated by the congregation of channels and/or particular organelles (as in ascidians) or a combination of both (the sperm factor acting as the trigger and the accumulations of channels and organelles as detonators). It is particularly remarkable that the calcium wave pacemakers initiating calcium waves exist in both deuterostomes (ascidians) and protostomes (nemertean, polychaete) eggs that are known to undergo drastic reorganizations of their cortical and cytoplasmic components at fertilization [23,24,39]. Only in ascidians is there a good correlation between the particular accumulation of calcium regulating organelles (layers of endoplasmic reticulum and mitochondria accumulated in the contraction pole) and the repetitive initiation of calcium waves. The exact nature of the pacemaker however still remains a mystery. It could be that:

- a high density of InsP3R exists in the accumulation of endoplasmic reticulum;
- calcium channels which periodically allow influx of external calcium, are segregated in the plasma membrane overlying the region of ER accumulation;
- close proximity of the mitochondria-rich layer plays a role in the frequency or amplitude modulation of the repetitive calcium waves;
- all of these factors are working together.

There are other examples of repetitive calcium transients that originate from a specific area of a cell. The best example is that of the pancreatic acinar cell where the calcium waves are initiated from a trigger zone [48]. Hepatocytes, megacaryocytes and even growing tips of plant cells exhibit characteristic calcium waves [49–52]. The precise nature of the calcium oscillators and pacemakers in these cells still remains unknown. Their definition will require a better understanding of the cellular and molecular mechanisms that underly calcium oscillations, a subject still hotly debated [2,3, 53].

In terms of modeling repetitive calcium signals in eggs, ascidian eggs may provide a particularly favorable experimental model since the spatial and temporal characteristics of the calcium oscillations are remarkably stereotyped. The fact that the egg can be properly orientated to observe the initiation site and the propagation of a dozen repetitive waves of progressively increasing and then progressively decreasing amplitudes is of particular interest in this regard [17,19,26,38].

2.2. Calcium oscillations and the cell cycle

There is a clear temporal correlation between the ability of eggs to generate calcium oscillations and the process of meiotic maturation although this is not an obligatory relationship (for example amphibian eggs fertilized in MII display a single activation wave). The fact that under certain conditions, such as in parthenogenetically activated mouse eggs, oscillations can be triggered during mitosis suggests that the levels of activity of critical cell cycle factors are somehow linked to an endogenous increase in calcium release activity which in turn, leads to the generation of repetitive calcium transients [30]. Alternatively calcium oscillations could in some cases drive the cell cycle machinery. A clear understanding of the links between calcium signals and oscillations and the cell cycle will require the simultaneous monitoring of calcium signals and cell cycle factors (such as MPF, MAP kinase and MOS) in the eggs that display calcium oscillations, as well as experimental manipulations of these signals.

2.3. The role of calcium oscillations

It is thought that the strength of the activation stimulus is conveyed to the cell by the frequency rather than the amplitude of the calcium oscillations [53]. In this way mitochondria probably decode cytosolic calcium oscillations (i.e. activation of mitochondrial energy production is coupled to the frequency of calcium spiking [54]) and in certain cells, there is evi-

dence that calcium oscillations control secretory activity [55].

In terms of differentiation and development, not much is known about the role of calcium oscillations. As discussed above, they are apparently essential for the completion of meiosis. In addition, in mammalian eggs there are indications that calcium oscillations are necessary for proper development [56,57]. These aspects are treated in detail by J.P. Ozil in an accompanying article in this issue.

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