Calcium wave pacemakers in eggs

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Summary

During the past 25 years, the characterization of sperm-triggered calcium signals in eggs has progressed from the discovery of a single calcium increase at fertilization in the medaka fish to the observation of repetitive calcium waves initiated by multiple meiotic calcium wave pacemakers in the ascidian. In eggs of all animal species, sperm-triggered inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] production regulates the vast array of calcium wave patterns observed in the different species. The spatial organization of calcium waves is driven either by the intracellular distribution of the calcium release machinery or by the localized and dynamic production of calcium-releasing second messengers. In the highly polarized egg cell, cortical endoplasmic reticulum (ER)-rich clusters act as pacemaker sites dedicated to the initiation of global calcium waves. The extensive ER network made of interconnected ER-rich domains supports calcium wave propagation throughout the egg. Fertilization triggers two types of calcium wave pacemakers depending on the species: in mice, the pacemaker site in the vegetal cortex of the egg is probably a site that has enhanced sensitivity to Ins(1,4,5)P_3; in ascidians, the calcium wave pacemaker may rely on a local source of Ins(1,4,5)P_3 production apposed to a cluster of ER in the vegetal cortex.

Key words: Fertilization, Egg, Calcium oscillations, Calcium wave pacemaker, Ins(1,4,5)P_3, Endoplasmic reticulum, Cortex

Introduction

Egg cells from every animal and plant species studied to date elicit single or repetitive Ca^{2+} transients in response to fertilizing sperm. As an important second messenger, Ca^{2+} triggers a broad range of cellular reactions, including contraction, secretion and gene expression (Berridge, 1997; Bootman et al., 2001). In eukaryotic cells, a calcium wave starts with an initial increase in Ca^{2+} concentration in a restricted region of the cell (a pacemaker site), which then propagates, leading to a global Ca^{2+} wave (Berridge, 1997; Bootman et al., 2001; McDougall et al., 2000; Marchant and Parker, 2001). Several factors are important in determining the transition from non-propagated elementary Ca^{2+} release events (Ca^{2+} 'puffs') to the initiation and propagation of a global Ca^{2+} wave. They include the magnitude and kinetics of Ca^{2+} release during each elementary event, the Ca^{2+} sensitivity of the Ca^{2+} release channels, the spatial organization of release sites, Ca^{2+} sequestration and Ca^{2+} diffusion, as well as Ca^{2+} buffering within the cytosol (Berridge, 1997; Marchant et al., 1999; Marchant and Parker, 2001; Bootman et al., 2001). In somatic cells such as hepatocytes, acinar cells or HeLa cells, all or some of these factors contribute to create a subcellular region of higher sensitivity to the Ca^{2+}-releasing second messengers, which becomes the pacemaker site (Rooney et al., 1990; Lee et al., 1997; Petersen et al., 1999; Thomas et al., 1999; Ito et al., 1999). In eggs, global Ca^{2+} waves always initiate in the cortex and then propagate through the cortex or the whole cytoplasm (reviewed in Sardet et al., 1998; Stricker, 1999; McDougall et al., 2000; Kline et al., 1999; Deguchi et al., 2000). After initiation of the Ca^{2+} wave in the cortex, propagation is due to the sequential activation by Ca^{2+} of Ca^{2+}-release channels at the front of the wave (Berridge, 1997; Bootman et al., 2001). The Ca^{2+} wave speed, which mainly depends on the rate of passive Ca^{2+} diffusion between Ca^{2+} release sites, can be influenced by the spatial organization of these Ca^{2+} release sites (Bugrim et al., 1997).

In eggs, Ca^{2+} waves triggered by sperm entry result mainly from the release of Ca^{2+} from intracellular stores by inositol 1,4,5 trisphosphate [Ins(1,4,5)P_3]-induced Ca^{2+} release (IICR) (reviewed in Miyazaki et al., 1993; Stricker, 1999; McDougall et al., 2000). The mechanism underlying Ins(1,4,5)P_3 production in eggs at the time of fertilization is still intensely debated. Similarly, the nature of the sperm factor(s) inducing Ca^{2+} release at fertilization remains elusive, although several competing groups agree that it must be a protein, possibly a form of phospholipase C or an activator of it (see Stricker, 1999; Swann and Parrington, 1999; Parrington et al., 2000; McDougall et al., 2000; Nixon et al., 2000; Runft and Jaffe, 2000; Mehlmann et al., 2001; Jaffe et al., 2001; Carroll, 2001; Runft et al., 2002). Very recently, a mammalian sperm factor was characterized and found to be a new form of PLC [PLCζ (Saunders et al., 2002)]. In most species, it seems reasonable to assume that the entering sperm delivers a factor into the egg and that this factor generates Ins(1,4,5)P_3 either directly or indirectly.

The first wave, which we will refer to as the 'fertilization Ca^{2+} wave', is generally the largest and longest-lasting wave, and, in some species, it is followed by repetitive Ca^{2+} waves of lower amplitude and shorter duration. Ca^{2+} wave pacemakers elicit waves for minutes (20-30 minutes in

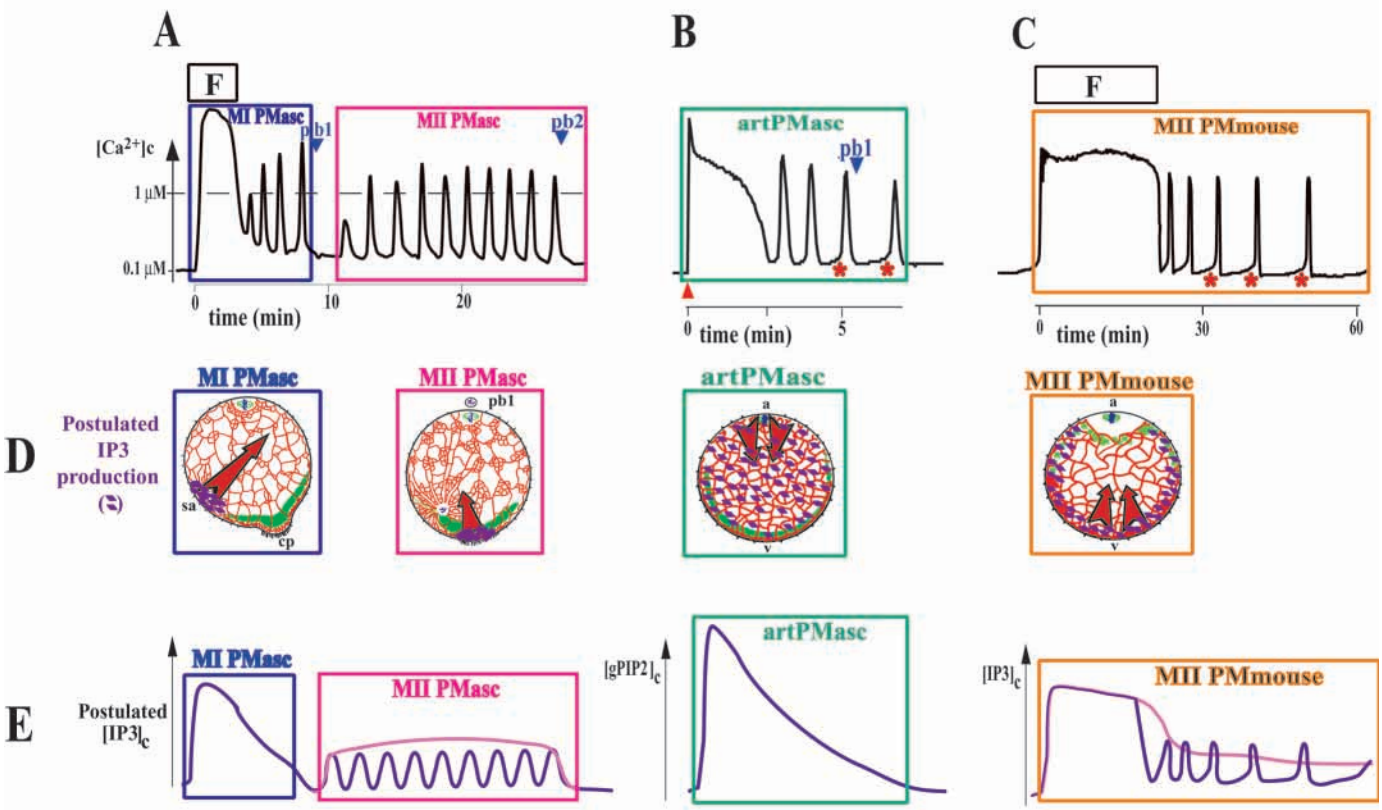

Fig. 1. Cortical Ca^{2+} wave pacemakers in the ascidian and mouse egg. (A) Sperm-triggered Ca^{2+} waves in ascidians: the meiotic Ca^{2+} waves, composed of a fertilization wave (F) followed by repetitive Ca^{2+} waves, are initiated by two pacemakers (MI PM_{asc} and MII PM_{asc}). (B) An artificial pacemaker (artPM_{asc}, red arrowhead) can be induced in the animal pole of the egg (a) by global UV photorelease of cgPtdIns(4,5)P_2. The Ca^{2+} waves emitted by this pacemaker are preceded by a pacemaker Ca^{2+} rise (red asterisks). (C) The mouse egg is fertilized at metaphase II and thus possesses only a MII pacemaker (MII PM_{mouse}). After the fertilization wave (F) starting from the point of sperm entry, repetitive calcium waves emanate from the vegetal cortex of the egg. Each calcium wave is preceded by a pacemaker calcium rise (red asterisks). (D) The drawings represent schematically the organization in the ascidian (MI, MII and artPM_{asc}) and mouse egg (MII PM_{mouse}) of the ER network (in red) and mitochondria (in green). Red arrows indicate the direction of the waves, whereas the postulated sites of Ins(1,4,5)P_3 production are symbolized as purple dots. sa: sperm aster; cp: contraction pole. (E) Postulated temporal variations of $[Ins(1,4,5)P_3]_c$, which may underlie the activity of the meiotic Ca^{2+} wave pacemakers. Two possibilities remain for the ascidian and mouse MII pacemakers: a sustained Ins(1,4,5)P_3 production (pink trace) or oscillatory Ins(1,4,5)P_3 production (purple trace).

ascidians, 45-60 minutes in some molluscs, 90 minutes in nemerteans) or hours (4 hours in mammals), and they stop operating at the end of the meiotic cell cycles (except in mammals, in which they stop several hours after completion of meiosis, at the time of pronuclei formation). The pacemaker site can be fixed in the cortex or undergo dramatic movements as the cortex is reorganized in preparation for development (Sardet et al., 2002). Ca^{2+} wave pacemakers are either located in a region of enhanced sensitivity to Ins(1,4,5)P_3 or reside in the vicinity of a local source of Ins(1,4,5)P_3. Here, we briefly examine how the subcellular organization of the Ca^{2+} release machinery may create stable Ca^{2+} wave pacemakers in the egg. We also discuss how spatially and temporally regulated production of Ins(1,4,5)P_3 can give rise to multiple calcium wave pacemakers in a single egg cell.

Different calcium wave pacemakers in different species

Two main types of egg can be distinguished with regards to their patterns of sperm-triggered Ca^{2+} signals. Eggs of sea urchins, amphibians, cnidarians, nematode and fish display a single Ca^{2+} increase upon fertilization. Conversely, eggs of nemerteans, some molluscs, annelids, ascidians and mammals display repetitive Ca^{2+} waves. In most of these eggs, the oscillations following the fertilization Ca^{2+} wave all emanate from cortical sites distinct from the initial sperm entry site (Eckberg and Miller, 1995; Kline et al., 1999; Deguchi et al., 2000) (reviewed in Sardet, 1998; Stricker, 1999). Perhaps the most elaborate of these examples is the egg of ascidians (urochordates at the base of the vertebrate line). The mature ascidian egg is arrested in metaphase I before fertilization, and sperm entry induces two series of Ca^{2+} waves, driving, successively, the completion of meiosis I and meiosis II (Fig. 1). The first series originates from the mobile meiosis-I-associated Ca^{2+} wave pacemaker [the MI pacemaker (McDougall and Sardet, 1995)]. The second meiotic cycle is entrained by a second pacemaker (the MII pacemaker) stably located in the vegetal cortex (Speksnijder, 1990b; McDougall and Sardet, 1995; Dumollard and Sardet, 2001) (Figs 1 and 2).

We do not completely understand why some eggs display repetitive Ca^{2+} waves whereas others exhibit only a single

wave. Recent work on ascidian and mouse eggs reveals that arresting the egg in meiotic metaphase is both sufficient and necessary to sustain sperm-triggered Ca^{2+} oscillations (for details, see Jones, 1998; Nixon et al., 2000; Carroll, 2001). Meiotic 'M-phase' thus favors repetitive Ca^{2+} waves (as in nemerteans, some molluscs, annelids, ascidians and mammals). By contrast, only a single large fertilization Ca^{2+} wave is observed when fertilization causes a rapid transition to an interphasic cytoplasm (<20 minutes, as in fish or amphibians) and when fertilization takes place during interphase (as in cnidarians or sea urchins). It remains to be seen whether eggs of fish or amphibians can be made to undergo Ca^{2+} oscillations when blocked in meiotic M-phase after fertilization. The data relating an M-phase stage of the cell cycle to the ability to generate multiple Ca^{2+} transients is compelling in ascidian and mouse eggs (reviewed in Nixon et al., 2000; Carroll, 2001). In these eggs, regulation of the Ca^{2+} release machinery by cell cycle factors probably participates in determining the temporal pattern of the fertilization Ca^{2+} signals. However, whether such regulation proves to be universal requires further research.

The calcium signalling hardware in eggs

The major organelles contributing to the regulation of intracellular Ca^{2+} levels are the endoplasmic reticulum (ER), the plasma membrane and mitochondria. Eggs also possess large numbers of specific vesicular organelles (yolk platelets, pigmented vesicles and cortical granules) that contain Ca^{2+} (Gillot et al., 1991); however their role in Ca^{2+} homeostasis is unknown.

The egg cortex and cytoplasm are filled with an extensive and continuous ER network (Speksnijder et al., 1993; Jaffe and Terasaki, 1993; Terasaki et al., 1996; Terasaki et al., 2001; Stricker et al., 1998; Kline et al., 1999). The ER network contains the intracellular Ca^{2+} channels – the Ins(1,4,5)P_3 receptors (IP3Rs) and ryanodine receptors (RyRs) – as well as the sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) that pump calcium back into the ER.

Among the three known isoforms of IP3R found in somatic cells (Taylor et al., 1999), IP3R1 is the most prevalent and functionally important isoform in the egg [*Xenopus* (Runft et al., 1999); mammals (Miyazaki et al., 1993; Fissore et al., 1999; Brind et al., 2000); ascidian (Kyozuka et al., 1998)]. Low levels of IP3R2 and IP3R3 have been reported in mouse eggs (Fissore et al., 1999), but their physiological roles remain unclear (Brind et al., 2000; Jellerette et al., 2000). The other family of Ca^{2+} release channels (RyRs) is present on the cortical ER of sea urchin eggs (McPherson et al., 1992) and in ascidian (Albrieux et al., 2000) and mouse eggs (Ayabe et al., 1995). However, except for sea urchins, the involvement of RyRs in the initiation and propagation of sperm-triggered calcium waves appears to be minor, and their role remains unclear (reviewed in McDougall et al., 2000).

Eggs from all animal phyla seem principally to use Ca^{2+} release from internal stores to generate single or repetitive Ca^{2+} waves at fertilization. In some species, external Ca^{2+} is also used for the fertilization wave [in molluscs (Deguchi et al., 1996) (reviewed in Sardet et al., 1998)] or contributes to the maintenance of the repetitive Ca^{2+} waves [in mice (McGuiness et al., 1996) (reviewed in Stricker, 1999)]. In many species, voltage-operated Ca^{2+} channels [VOCC (Arnoult and Villaz, 1994; Leclerc et al., 2000)] and Ca^{2+}-release-activated Ca^{2+} (CRAC) channels that mediate so-called 'capacitative Ca^{2+} entry' (Arnoult et al., 1996; Jaconi et al., 1997; Csutora et al., 1999; Machaca et al., 2000; Putney et al., 2001) are also present, but their role at fertilization is still ill defined.

In the mature mouse egg, the physiological Ca^{2+} load is primarily cleared via SERCAs and plasma membrane Ca^{2+} ATPases (PMCAs). A minor contribution may also be provided by the plasma membrane Na^+/Ca^{2+} exchanger (Carroll, 2000). PMCAs are probably responsible for Ca^{2+} efflux from ascidian eggs after each Ca^{2+} wave (Kuthreiber et al., 1993) as well as for the loss of total Ca^{2+} content after fertilization in sea urchin eggs (Gillot et al., 1991).

In the past few years, mitochondria have been shown to be major regulators of Ca^{2+} signals (reviewed in Rutter and Rizzuto, 2000; Rizzuto et al., 2000; Duchen, 2000). Sequestration of Ca^{2+} by mitochondria has two regulatory effects on IICR, suppressing positive and negative Ca^{2+} feedback on the opening of the IP3R. In addition, ATP production by mitochondria might provide a further means of modulating Ca^{2+} signals: ATP^{4-} sensitizes the IP3R (Mak et al., 1999; Mak et al., 2001), whereas Mg^{2+}-complexed ATP is consumed to refill the ER Ca^{2+} stores. Mitochondria can thus provide negative or positive feedback on Ins(1,4,5)P_3-mediated Ca^{2+} signals. Such negative feedback has been reported in a wide range of somatic cells. For example, initiation of global Ca^{2+} waves in myocytes preferentially occurs in mitochondrion-poor regions of the cell (Boitier et al., 1999). A positive feedback effect of mitochondria on Ins(1,4,5)P_3-mediated signals has been reported only in oligodendrocytes, in which Ca^{2+} wave initiation and amplification sites are found in mitochondrion-rich regions of the cell (Simpson et al., 1997).

Except in sea urchins, in which mitochondria are a sink for cytosolic Ca^{2+} (Eisen and Reynolds, 1985; Girard et al., 1991), the role mitochondria play in Ca^{2+} signalling in eggs remains largely obscure. In mouse eggs, collapsing mitochondrial potential impairs Ca^{2+} clearance from the cytosol (Liu et al., 2001), but no picture of the regulation of Ca^{2+} oscillations by mitochondria can be drawn from only this study. In ascidian eggs, mitochondria contribute to the activity of the second Ca^{2+} wave pacemaker both by buffering cytosolic Ca^{2+} and by locally providing ATP (R.D., unpublished). Nevertheless, an understanding of the role of mitochondria in regulating Ca^{2+} wave pacemakers will require measurement of the local intracellular Ca^{2+} concentration and the local mitochondrial ATP production in the vicinity of the IP3Rs. The recent development and subcellular targeting of GFP-based Ca^{2+} and Ins(1,4,5)P_3 indicators as well as luciferase-based ATP indicators should allow the direct measurement of mitochondrial Ca^{2+} levels, intracellular ATP concentration and the Ins(1,4,5)P_3 concentration in the living zygote (Hirose et al., 1999; Rutter and Rizzuto, 2000).

Ca^{2+} wave pacemakers in eggs reside in cortical ER-rich domains

Given the central role played by Ca^{2+} release from intracellular stores, the organization of the ER in eggs has received much attention. In eggs that display repetitive Ca^{2+} waves, the

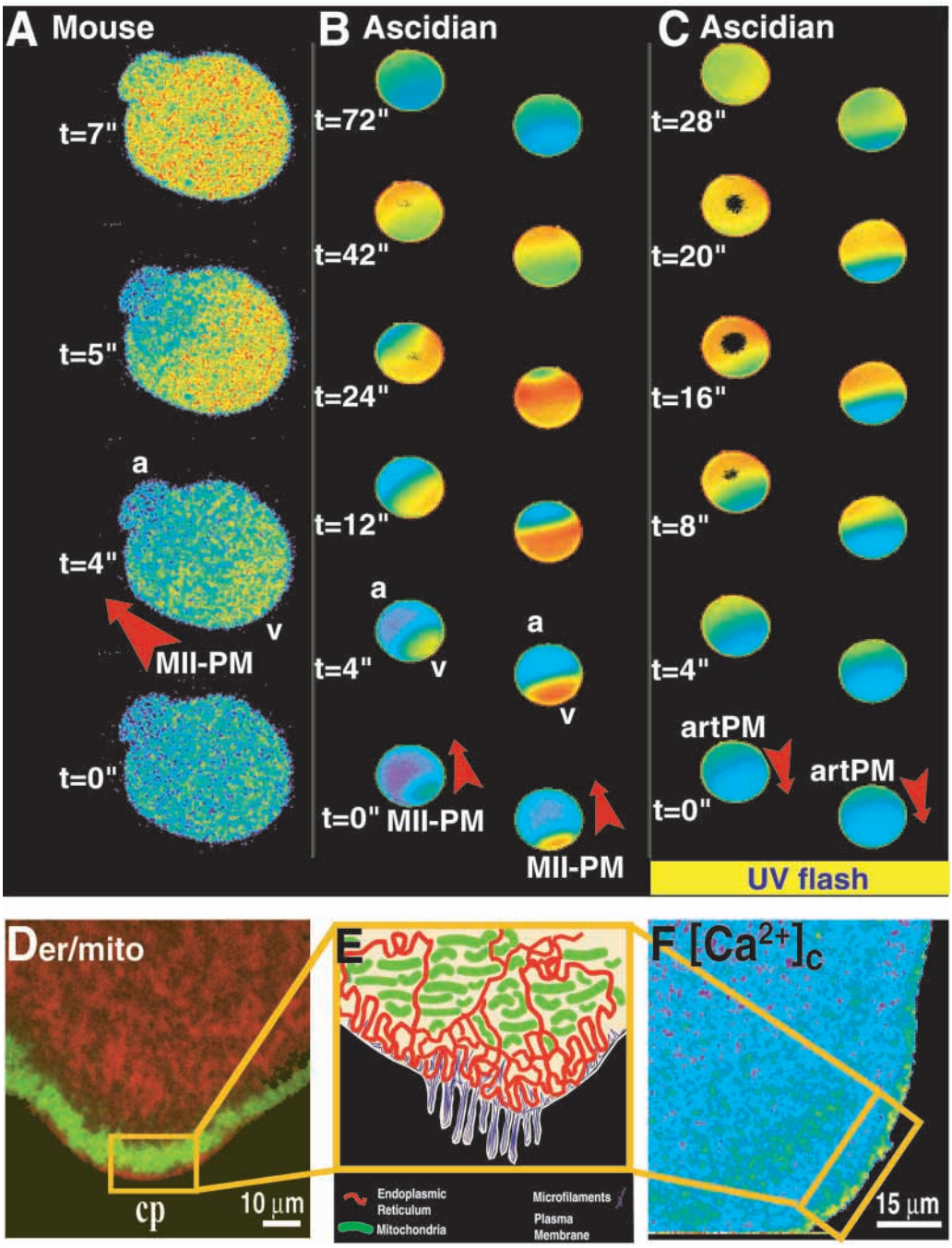

Fig. 2. Ca^{2+} wave pacemakers in eggs. (A) A sequence showing a Ca^{2+} wave initiated by the meiosis II pacemaker (MII PM) in the vegetal pole (v) of a mouse egg injected with sperm extracts. The second polar body is visible in the animal pole (a). (B) A sequence showing two examples of fertilized ascidian eggs displaying Ca^{2+} waves initiated by the MII calcium wave pacemaker located in the vegetal contraction pole (MII PM). (C) Two examples of a UV flash releasing cgPtdIns(4,5)P_2 applied between two waves, the fertilized ascidian eggs respond by eliciting a wave from the animal pole of the egg (artPM). (D) The distribution of ER and mitochondria in the contraction pole (cp). A layer of cortical ER in the cortical most layer can be seen (in red) juxtaposed to the mitochondria-rich sub-cortical region (in green). (E) Schematic representation of the contraction pole showing the microvillated plasma membrane, microfilaments (in blue), as well as the ER-rich domains in the cortex (red) and the mitochondria-rich subcortical domain (green). (F) Calcium Green/Texas Red ratiometric image of $[Ca^{2+}]_c$, showing the initiation of a Ca^{2+} wave elicited by the MII pacemaker in the contraction pole.

interconnected network of ER sheets and tubes is organized into ER-rich domains (also called ER clusters). The cytoplasm also has ER-poor domains, which contain high densities of mitochondria and/or other vesicular organelles (Speksnijder et al., 1993; Stricker et al., 1998; Kline et al., 1999; Dumollard and Sardet, 2001). The ER clusters are made of densely packed tubes and sheets of ER membrane (Speksnijder et al., 1993; Fissore et al., 1999; Terasaki et al., 2001; Dumollard and Sardet, 2001). In oscillating eggs [nemertean (Stricker et al., 1998); mouse (Kline et al., 1999); ascidian (Dumollard and Sardet, 2001; Sardet et al., 2002)] as well as in *Xenopus* eggs (Terasaki et al., 2001), ER-rich domains are concentrated in the 2-6 μm thick layer beneath the plasma membrane and are more dispersed in the deeper cytoplasm (deeper than 5 μm). In ascidian eggs, meiosis II Ca^{2+} waves initiate in a large cortical disc of concentrated ER tubes and sheets (20 μm in diameter and 2-5 μm thick) located in the vegetal contraction pole (Fig. 2B-F) (Speksnijder, 1992; McDougall and Sardet, 1995; Dumollard and Sardet, 2001). In mouse and nemerteans eggs, ER clusters also line the vegetal cortex of the egg where meiotic Ca^{2+} waves are initiated (Stricker et al., 1998; Kline et al., 1999).

In *Xenopus* and mouse eggs, at least, these ER-rich domains are rich in IP3R1s (Terasaki et al., 2001; Mehlmann et al., 1996; Fissore et al., 1999). The appearance of cortical ER-rich domains during maturation correlates with an increase in

sensitivity to Ins(1,4,5)P_3 and to sperm-induced Ca^{2+} release (Chiba et al., 1990; Shiraishi et al., 1995; Mehlmann and Kline, 1994; Terasaki et al., 2001) (reviewed in Sardet et al., 2002). Local injections of Ins(1,4,5)P_3 and sperm extracts in mouse eggs have revealed that the egg cortex is a region of higher sensitivity to Ins(1,4,5)P_3 and to sperm extracts (Oda et al., 1999). Indeed, although the abundance of ER in the egg cortex renders this region more sensitive to Ins(1,4,5)P_3, it is also exposed to the highest concentrations of Ins(1,4,5)P_3 as it is closest to the source of PtdIns(4,5)P_2 in the plasma membrane (Halet et al., 2002; Sardet et al., 2002).

The organization of the ER network may regulate the Ca^{2+} wave pacemakers

In several somatic cells, the location of the Ca^{2+} wave pacemakers corresponds to the area of the cell that is most sensitive to Ins(1,4,5)P_3 (Ito et al., 1999; Thomas et al., 1999; Petersen et al., 1999), and Ins(1,4,5)P_3, which diffuses rapidly in the cytoplasm, is thought to act as a global messenger (Albritton et al., 1992; Kasai and Petersen, 1994).

The mouse MII pacemaker appears to be an example of this type of pacemaker. In the mature mouse egg, ER-rich domains are larger in the vegetal cortex (Kline et al., 1999), whereas mitochondria are more abundant in the animal hemisphere (Calarco, 1995; Van Blerkom et al., 2002). The MII pacemaker of the mouse egg resides in the ER-enriched vegetal cortex, which is probably a site of enhanced sensitivity to Ins(1,4,5)P_3. Therefore, similarly to the somatic cell Ca^{2+} wave pacemakers, the mouse MII pacemaker site appears to be determined by the organization of the Ca^{2+} stores of the egg, with Ins(1,4,5)P_3 acting as global messenger (Fig. 1, Fig. 2A).

Interestingly, an artificial pacemaker can be induced in the ascidian egg by global uncaging of caged Ins(1,4,5)P_3 (cIns(1,4,5)P_3) or its poorly metabolised analogue cgPtdIns(4,5)P_2 [caged 1-(a-Glycerophosphoryl)-D-myo-inositol 4,5-bisphosphate, P4(5)]. This artificial pacemaker, localized in the animal pole of the ascidian egg, functions under globally elevated Ins(1,4,5)P_3 levels and thus resides in the region of highest sensitivity to Ins(1,4,5)P_3 (Dumollard and Sardet, 2001) (Figs 1 and 2). In common with the mouse MII pacemaker, the location of this artificial pacemaker can be explained by asymmetries in the distribution of the ER along the animal-vegetal axis of the ascidian egg. In these eggs, the ER-rich domains invade the whole egg except for the vegetal subcortex, where most mitochondria accumulate (Fig. 2) (Dumollard and Sardet, 2001). The corollary of this is that the sperm-triggered MII pacemaker in the ascidian egg, located in the vegetal pole (the site opposite the artificial pacemaker), is not at a site of enhanced Ins(1,4,5)P_3 sensitivity. This indicates that the general organization of the ER stores in these eggs is not sufficient to determine the pacemaker site. The pacemakers in the ascidian egg may then rely on mechanisms other than a global increase in Ins(1,4,5)P_3 levels.

Local and dynamic production of Ins(1,4,5)P_3 defines the Ca^{2+} wave pacemaker site

In ascidians, the MI pacemaker stimulated by sperm entry resides in a cortical ER-rich domain that forms rapidly around the sperm nucleus and centrosome and moves with them towards the vegetal pole (Fig. 1) (Dumollard and Sardet, 2001). This suggests that the MI pacemaker of the ascidian relies on a localised moving source of Ins(1,4,5)P_3. The ascidian MII Ca^{2+} wave pacemaker does not reside in a region of enhanced sensitivity to Ins(1,4,5)P_3 (Fig. 2) (Dumollard and Sardet, 2001); it might thus require local production of Ins(1,4,5)P_3 in the vegetal contraction pole. The contraction pole possesses numerous microvilli and is thus rich in PtdIns(4,5)P_2 (Figs 1 and 2) (Sardet et al., 2002). Therefore, the ascidian MII pacemaker may be different from the characterized pacemakers of somatic cells and the mouse MII pacemaker, since it would rely on the apposition of cortical ER-rich clusters to a local source of Ins(1,4,5)P_3 (Fig. 1) (Dumollard and Sardet, 2001).

Hypothesizing local production of Ins(1,4,5)P_3 even in the large egg cell raises the question of how such a gradient is maintained. Indeed, as Ins(1,4,5)P_3 diffuses rapidly through the cytosol, locally produced Ins(1,4,5)P_3 would quickly invade the whole cell, making Ins(1,4,5)P_3 gradients energy consuming to maintain without dynamic Ins(1,4,5)P_3 production. Theoretically, repetitive Ca^{2+} waves can result from either a sustained increase in Ins(1,4,5)P_3 levels or an oscillating production of Ins(1,4,5)P_3 (Jacob, 1990). In ascidians, a single, large and sustained Ins(1,4,5)P_3 increase (achieved by uncaging cgPtdIns(4,5)P_2 in the whole egg, Fig. 1) mimics the first series of Ca^{2+} oscillations, indicating that the ascidian MI pacemaker is driven by a continuous moving source of Ins(1,4,5)P_3 induced by sperm entry (Dumollard and Sardet, 2001). Similarly in mouse eggs, a slow and continuous uncaging of cIns(1,4,5)P_3 can reproduce the sperm-triggered Ca^{2+} oscillations (Jones and Nixon, 2000). Therefore, the mouse MII Ca^{2+} wave pacemaker can also be regulated by a single and sustained increase in Ins(1,4,5)P_3 levels (Fig. 1). Furthermore, the Ca^{2+} transients triggered by the ascidian MI and artificial pacemakers, as well as those triggered by the mouse MII pacemaker, are all preceded by a characteristic slow rise in Ca^{2+} levels called a 'pacemaker Ca^{2+} rise' (Fig. 1) (Jones and Nixon, 2000; Dumollard and Sardet, 2001). This 'pacemaker Ca^{2+} rise' is a hallmark of low-frequency (period >20 seconds) Ca^{2+} oscillations generated under constantly elevated Ins(1,4,5)P_3 levels (Jacob, 1990; Marchant and Parker, 2001), which further suggests that a single and sustained Ins(1,4,5)P_3 increase regulates the ascidian MI pacemaker and the mouse MII pacemaker.

By contrast, the ascidian MII pacemaker cannot be reproduced by a long-lasting increase in Ins(1,4,5)P_3 levels in the egg, and no 'pacemaker Ca^{2+} rise' precedes these Ca^{2+} transients (Fig. 1) (Dumollard and Sardet, 2001). An oscillating production of Ins(1,4,5)P_3 from the contraction pole might underlie the activity of the ascidian Ca^{2+} wave MII pacemaker (Fig. 1). The recent finding that the mammalian sperm factor is possibly a Ca^{2+}-activated phospholipase C (PLC) (Rice et al., 2000; Saunders et al., 2002) argues in favor of Ins(1,4,5)P_3 oscillations driving sperm-triggered Ca^{2+} oscillations in eggs. Indeed, the prolonged stimulation of a Ca^{2+}-activated PLC can result in Ca^{2+} oscillations regulated by an oscillating production of Ins(1,4,5)P_3 (for details, see Meyer and Stryer, 1988). In addition, Ins(1,4,5)P_3 oscillations regulating repetitive Ca^{2+} waves during prolonged exposure to agonists have now been observed in several types of somatic cells (Hirose et al., 1999; Nash et al., 2001). The issue of Ins(1,4,5)P_3 oscillations is an intensively debated topic in cell

physiology, and the Ca^{2+} wave pacemakers in eggs could provide an invaluable experimental system to resolve such questions in the future.

A role for vegetal Ca^{2+} wave pacemakers in development

Even though the ascidian and the mouse Ca^{2+} wave pacemakers seem to rely on different mechanisms, they are both located in the vegetal cortex of the egg (as is the pacemaker in eggs of the primitive nemertean). This suggests that the location the Ca^{2+} wave pacemaker may have developmental significance.

The polarized nature of the calcium signals may in itself influence embryonic patterning by regulating early embryonic cleavages. In ascidians, nemerteans and mouse, the egg cortex is polarized along the animal-vegetal axis and, in ascidians, this polarity amplifies after fertilization through actomyosin-driven cortical contractions (Sardet et al., 2002). Is the generation of repetitive Ca^{2+} waves from the vegetal cortical pacemaker a mechanism used to prime the vegetal pole region for later developmental events such as cleavage or gastrulation, which, in nemerteans and ascidians, takes place in the vegetal/dorsal pole of the embryo? Mouse embryos were long thought to have no significant polarity until the late cleavage stage, but recent marking experiments show that in fact, as in ascidians and nemerteans, although regulation can override this polarity, there is a relationship between the animal-vegetal axis, the sperm entry point and the developmental axes of pre- and post-implantation embryos (reviewed in Lu et al., 2001).

Finding out whether Ca^{2+} wave patterns play a role in later development will require studies that interfere with the normal spatio-temporal pattern of Ca^{2+} waves without perturbing mitosis and cleavage. The rather simple ascidian embryo, which displays two different meiotic Ca^{2+} wave pacemakers and develops into a swimming tadpole within a day, is particularly suited to studies of the relationship between meiotic Ca^{2+} waves and development (Fig. 2) (Dumollard and Sardet, 2001). It should be possible in the future to relate patterns of Ca^{2+} waves and phenotypic differences in embryos.

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