Calcium in sea urchin egg during fertilization

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ABSTRACT Calcium plays a strikingly important role in two of the major events in developmental biology: cell activation and differentiation. In this review we begin with the location and quantity of intracellular calcium in sea urchin oocytes, and then discuss the changes that occur during fertilization and egg activation, placing special emphasis on the mobilization and redistribution of intracellular calcium. We also discuss the propagation of the calcium wave and the role of the burst of calcium on the process of reorganizing the egg cortex at fertilization.

KEY WORDS: *sea urchin, egg, embryo, calcium, fertilization*

Introduction

Calcium is of fundamental importance in numerous biological processes as an exchangeable ion, an intracellular second messenger, a modulator of enzyme activity and polymerization reactions, a mediator of cellular interactions and a major constituant of skeletal components.

Since the 1900's, the work on sea urchin gametes and embryos has contributed significantly to these domains. The choice of this biological material comes from the large number of gametes and synchronous embryos available, the access to a broad background of knowledge concerning the biology of fertilization, early development, cell interactions, skeleton formation and the historical role that calcium played in these processes.

A little history

Research on sea urchin eggs and embryos has proceeded in steps that have constituted landmarks for the role of calcium in cell activation and differentiation. Pioneering studies on sea urchin have often stimulated research on other eggs and somatic cells. We can cite in chronological order:

- The discovery that calcium was essential for cell adhesion (Herbst, 1900)
- The first measurements of intracellular calcium that drew attention to its important role in fertilization and in the initiation of the cell cycle (Mazia, 1937)
- The demonstration that calcium was necessary for exocytosis of submembranous vesicles (cortical granules) with the plasma membrane (Moser, 1939)
- The description of the genesis of an embryo skeleton made of calcium carbonate crystals (Okazaki, 1956)
- The discovery that intracellular calcium was released at fertilization (Steinhardt *et al.*, 1977)
- The realization that a coherent sequence of ionic events was implicated in cell activation (Epel, 1978)
- The demonstration that injection of IP_3 and GTP γ S led to cell activation (Whitaker and Irvine, 1984; Turner *et al.*, 1986)
- The experiments that first linked nuclear breakdown and mitosis to cytosolic calcium signals (Poenie *et al.*, 1985).

At present, studies on sea urchin eggs are still at the forefront of research concerning the sequestration and release of intracellular calcium, its effect on key enzymatic steps and the reorganization of the cytoskeleton and the triggering of mitosis. In addition, the contribution of calcium to the formation of an organized embryonic skeleton is actively thought.

The scope of this review

In this report, we essentially review what is known about the role of calcium during fertilization. We do not discuss what happens to the spermatozoid (see Epel and Mastroianni, 1988 and Garbers, 1989 for review) or what happens during cell division and early development. We have made minimal references to work already well reviewed and have aimed at providing access to recent sources in the field as well as introducing some of our recent results.

We address a series of questions such as the following: What is the calcium content of eggs and how does it vary? Where is calcium localized? How is it pumped, sequestered and released?

Perhaps the most striking event of fertilization and of cell activation in general is the transient elevation of the intracellular calcium level. This phenomenon has been well examined in the egg of the sea urchin where the fertilizing spermatozoid triggers a wave of elevated calcium that traverses the egg. We discuss what is

Abbreviations used in this paper: IP3, inositol 1, 4, 5-triphosphate; GTPγS, guanosine triphosphate; DAG, diacylglycerol.

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known of the wave and of its propagation. Finally we examine the role of this burst of calcium on the reorganization of the egg cortex at fertilization.

Calcium compartments in eggs and embryos

How much calcium is in eggs and embryos?

Since the pioneering experiments of Page (1927), the total calcium content of eggs and its variation after fertilization has been examined by several workers: Lord Rothschild and Barnes (1953) like Azarnia and Chambers (1976) have reviewed the values measured in many species. Our recent study (Gillot *et al.*,1989b) confirms that eggs contain up to millimolar concentrations of total calcium (between 3 and 38 mmoles/l egg in the different species). The variations between species are not due to the methodology, since using two independent methods, we have shown that eggs of *Arbacia lixula* contain twice as much calcium as eggs of *Paracentrotus lividus* (11.3 and 7.3 mmoles/l egg). Upon fertilization a loss of total calcium has been measured that amounts to 30% according to Azarnia and Chambers (1976) and 20% according to Walter *et al.* (1989). Our latest results suggest that this decrease could be attributed to the exocytosis of the calcium rich cortical granules (Gillot *et al.*, submitted, and next paragraphs). Surprisingly there is no measurement of calcium content during development. One would expect to find an increase before spicule formation.

Cytosolic free calcium concentration and calcium buffering capacity of the egg cytoplasm

Using the Ca^{2+} sensitive fluorescent dye Fura-2 and the egg of *Lytechinus pictus*, Poenie *et al.* (1985), Swann and Whitaker (1986) and Steinhardt and Alderton (1988) have estimated the remaining level of free calcium to be between 144 and 277 nM. At fertilization, it is estimated that free calcium concentration rises 10 to 20 times (see further).

Any transient change in Ca^{2+} concentration in the cytosol is set against intracellular buffering and reflects a balance between mechanisms that allow calcium to move toward the cytosol and mechanisms that tend to sequester or bind the calcium as soon as it appears. According to Swann and Whitaker (1986) who recorded free cytosolic calcium after microinjection of calcium-EGTA buffers, the buffering capacity of sea urchin egg cytoplasm is comparable to that of squid axoplasm. In giant axons, Baker (1986) determined a calcium buffering activity of about 0.9 mmole/kg of fresh tissue when calcium concentration of injected buffers increased from 10^{-7} to 10^{-6} M, i.e. a rise comparable to that measured during egg activation. Assuming that the average values of the total calcium content of the egg is of 10 mmoles/l egg (Gillot *et al.*, 1989b), mobilization of about 10% of total intracellular cell calcium would account for the burst of free calcium at fertilization.

Where is calcium localized?

It has been shown that an influx of calcium from the surrounding sea water is not essentiel for fertilization (Schmidt *et al.*,1982) and that calcium sequestered in intracellular stores is the major source of free calcium liberated during activation (Steinhardt *et al.*, 1977, Baker and Whitaker, 1978, Schmidt *et al.*, 1982). On the other hand, in the unfertilized egg, we know that more than 99.9 % of the total calcium, reaching millimolar concentrations, is bound or sequestered (Mazia, 1937, Nakamura and Yasumasu, 1974). It is therefore important to understand how and where calcium is sequestered.

We have reviewed the existing literature on calcium localization by electron microscopy (Table 1). Most studies have used pyroantimonate precipitation of calcium. This qualitative method, which is subject to criticism, repeatedly yielded calcium precipitates on cortical granules, endoplasmic reticulum, mitochondria and pigmented or acidic vesicles. Only recently has it been possible to make quantitative measurements of calcium content of organelles by X-ray microprobe analysis (Gillot *et al.*, 1989b). Our measurements clearly show that cortical granules contain much higher concentrations of calcium than the other large vesicular compartments. This result implies that the egg periphery would contain more calcium than the interior. In fact, based on measurements of calcium present in whole eggs and isolated cortices, we recently calculated that although this cortical layer contains only 1% of total egg proteins, it sequesters 14% of the egg calcium (Gillot *et al.*, submitted). Whereas the calcium content of several organelles has been analyzed, it has unfortunately been impossible until now to measure the calcium content of the endoplasmic reticulum by quantitative methods. It is probable that the endoplasmic reticulum is one of the major stores of calcium since it contains the calcium-binding protein calsequestrin and constitutes the bulk of crude microsomal fractions that pump and release calcium (Oberdorf *et al.*, 1988; Henson *et al.*, 1989). In addition, the presence of calcium in the cortical reticular network has recently been directly visualized with a calcium-sensitive dye (Terasaki and Sardet, 1989).

An important question is: what happens at fertilization and in particular what stores are involved in calcium release? Three different approaches to this question have been described. They have implicated endoplasmic reticulum and mitochondria as the major participating source and sink of free calcium released at fertilization (Eisen and Reynolds, 1985; Ohara and Sato, 1986; Poenie and Epel, 1987). These studies are however too qualitative and preliminary to settle the matter.

How is calcium handled by these different compartments?

We have seen that there are many intracellular stores of calcium in the egg. It is important to know what the dynamics are of calcium sequestered in these various compartments.

This question has been approached by isotopic flux measurement on several preparations: egg homogenates and homogenate fractions (cell free systems), eggs permeabilized by electroporation or digitonin treatment, and isolated cortices. Permeabilized eggs provide a convenient preparation to study the contributions of all the organelles by controlling free Ca^{2+} concentrations, substrates and ATP levels, etc. By comparing total calcium content and the ^{45}Ca accumulation in digitonized eggs, we found recently that the exchangeable calcium represented about a third of total cell calcium (Girard *et al.*, submitted).

Egg homogenates and crude microsomes display the ability to accumulate ^{45}Ca in an ATP dependent manner (Inoue and Yoshioka, 1982; Oberdorf *et al.*, 1986; Clapper *et al.*, 1987). Exchangeable calcium accessible with ^{45}Ca is fully released by the calcium ionophore A23187 (Oberdorf *et al.*, 1986), trifluoroperazine at high concentration (Oberdorf *et al.*, 1986) and partly released by the calcium mobilizing agent IP_3 (Clapper and Lee, 1985; Clapper *et al.*, 1987) or pyridine nucleotides metabolites (Clapper *et al.*, 1987).

It is also possible to prepare isolated cortical lawns, which are

TABLE 1

LOCALIZATION OF TOTAL CALCIUM IN UNFERTILIZED EGG

AUTHORS	METHOD	SPECIES	RESULTS					
			CG	PV or AV	ER	M	YG	cell-surface
Cardasis *et al.*, 1978	Pyroantimonate precipitation associated with qualitative X-ray microanalysis	*A. punctulata*	+	+	+	+	+	+
		S. purpuratus	+	+	+	+	+	+
Ornberg and Reese, 1981	X-ray microanalysis of frozen and freeze-substituted eggs in acetone+ O_3O_4	*S. purpuratus*	+					
Poenie *et al.*, 1982	Calcium precipitants	*S. purpuratus*			+	+		
Poenie *et al.*, 1983	Calcium precipitants	*S. purpuratus*	+	+	+			
Sardet and Chang, 1985	Fluoride-pyroantimonate precipitation associated with qualitative X-ray microanalysis	*P. lividus*	+	+	+			+
Poenie and Epel, 1987	Fluoride-pyroantimonate precipitation associated with qualitativeX-ray microanalysis	*S. purpuratus*	+	+	+	-		
Gillot *et al.*, 1989a	QuantitativeX-ray microanalysis of frozen, freeze-substituted and embedded eggs	*P. lividus*	30	+		+	+	
		A. lixula	130	+		+	+	

CG: Cortical granule; PV or AV: Pigmented vesicles or acidic vesicles; ER: endoplasmic reticulum; M: Mitochondria; YG: Yolk granule.
+: detected but not measured; if measured: in mmoles/kg of dry weight; -: not detected. Empty square: not examined.

constituted by the egg plasma membrane to which cortical granules, pigmented vesicles and a part of the endoplasmic reticulum remain attached (Vacquier, 1975; Sardet, 1984; Sardet and Chang, 1985, 1987). This preparation is also able to sequester calcium in the presence of ATP (Oberdorf *et al.*, 1986; Payan *et al.*, 1986). Such ^{45}Ca preloaded cortices release the pumped calcium upon the addition of calcium ionophores (A23187, ionomycin) and trifluoroperazine. On the other hand, IP_3, even used at high concentrations, releases no more than 10% to 30% of the total exchangeable pool of calcium (Oberdorf *et al.*,1986; Payan *et al.*,1986). Cortical granules may be selectively removed from cortices as digitonin treatment leads to their swelling and bursting (Zimmerberg and Liu, 1988). Under these conditions 30% of the exchangeable calcium is lost and the remaining organelles (mainly the endoplasmic reticulum network) still pump calcium in the presence of ATP. This subcortical network of endoplasmic reticulum can be directly visualized by imaging cortices loaded with the fluorescent calcium-sensitive dye Fluo 3 (Terasaki and Sardet, 1989). This should provide a means for studying directly how the endoplasmic reticulum handles calcium.

There have been a few attempts to study these compartments after fertilization (Petzelt and Wülfroth, 1984; Suprynowicz and Mazia, 1985). Suprynowicz and Mazia (1985), by monitoring the Ca^{2+} uptake by electroporated eggs, observed that the non-mitochondrial Ca^{2+} sequestering activity varied through the successive phases of the cell cycle before the first cleavage. Therefore, little is known about the mechanisms by which organelles pump or release Ca^{2+} and particularly, how they are modified during fertilization and early development.

There is clearly a need to define the contribution of each of the compartments (vesicular compartments, mitochondria, endoplasmic reticulum network) by flux measurements on well-fractionated organelle preparations as well as on open cell preparations with imaging techniques using calcium-sensitive dyes or photoproteins.

The calcium wave at fertilization

In 1977, Steinhardt *et al.*, on *Lytechinus pictus*, first reported that fertilization provoked a rise in intracellular calcium. Almost simultaneously, Gilkey *et al.* (1978) on the medaka fish egg showed that calcium was in fact first elevated at the site of sperm entry and that it traveled through the egg as a wave (Steinhardt *et al.*, 1977; Eisen *et al.*, 1984; Swann and Whitaker, 1986; Yoshimoto *et al.*, 1986; Hafner *et al.*, 1988). Since then the wave has been observed in several species of sea urchin eggs (see Table 2) and many other deuterostomian eggs (Jaffe, 1983)

What are the characteristics of the wave?

From published values based on the measurement of aequorin luminescence and fluorescent emission of Ca^{2+} sensitive dyes, it is clear that peak calcium level (1-4.5 µM) and wave velocity (≈ 5µm/sec) are quite similar to those reported for other deuterostomian eggs (Jaffe, 1983, 1985, 1988; Berridge and Irvine, 1989). Interestingly Swann and Whitaker (1986) estimated that the Ca^{2+} concentration at the site of sperm entry reached higher levels – on the order of 8 µM – whereas the mean free Ca^{2+} during the wave is 1.3µM to 2.5µM (Hafner *et al.*, 1988). The main characteristics of the calcium wave in various species are presented in Table 2.

How is the calcium wave initiated?

Due to measurements of the capacity of sperm to increase its calcium when activated (Schackmann *et al.*, 1978) it was sug-

TABLE 2

CHARACTERISTICS OF THE CA^{2+} PEAK AND WAVE IN SEA URCHIN EGGS AND SAND DOLLARS

PEAK LEVEL	PEAK BEGINNING	RISING PHASE	PROPAGATION TIME	WAVE SPEED	Total duration of Ca SPIKE	SPECIES	AUTHORS AND METHODS
(μM)	seconds	seconds	seconds	μm/sec	seconds		
2.5-4.5	≥45 *	n.d.	n.d.	n.d.	n.d.	*Lytechinus pictus*	Steinhardt *et al.*, 1977 calcium-aequorin luminescence
≈1 (40 sec Δ)	23 ± 3 Δ	n.d.	6-10	n.d.	15-60	*Arbacia punctulata*	Eisen *et al.*, 1984 calcium-aequorin luminescence
1.3 (20sec PF)	n.d.	21.0 ± 0.8	15-25	5	n.d.	*Lytechinus pictus*	Swann and Whitaker, 1986 calcium-aequorin luminescence
n.d.	n.d.	20 ± 3	20 over the entire egg	n.d.	78 ± 4	*Scaphechinus mirabilis*	Yoshimoto *et al.*, 1986 calcium-aequorin luminescence
n.d.	n.d.	27 ± 3	n.d.	n.d.	126 ± 6	*Clypeaster japonicus*	Yoshimoto *et al.*, 1986 calcium-aequorin luminescence
2.5 ± 0.2	10 PF	n.d.	n.d.	n.d.	n.d.	*Lytechinus pictus*	Hafner *et al.*, 1988 Fura-2

n.d.: not determined. PF: Post-fertilization. *: response latency after sperm addition. Δ: after fertilization potential.

gested that the fertilizing sperm may act as a calcium bomb (Jaffe, 1983, 1985, 1988). The calcium thus locally released in the egg at the site of sperm entry would be responsible for the calcium wave. A variation on this theme has been presented by Dale *et al.* (1985) who hypothesized that a soluble factor present in sperm activates the egg when sperm egg fusion occurs. This factor could be IP_3 itself since activated sperm contains high levels of this polyphosphoinositide (Domino and Garbers, 1988). Alternatively it has been proposed that sperm interaction with a specific membrane receptor rather than fusion is the key event in egg activation (Turner and Jaffe, 1989, for review). This latter mechanism would involve the same cascade of events as that described for other cells where stimulation of cell surface receptors leads to the hydrolysis of polyphosphoinositides located in the plasma membrane (reviewed by Berridge and Irvine, 1989). These alternate mechanisms are depicted in Fig. 1.

Polyphosphoinositides and calcium (see mechanism n°2, Fig. 1)

An increased turnover of polyphosphoinosotides (Turner *et al.*, 1984) coupled with a production of two intracellular messengers, inositol trisphosphate (IP_3) and diacyglycerol (DAG), accompany fertilization of the sea urchin egg (Kamel *et al.*, 1985; Ciapa and Whitaker, 1986). IP_3 releases calcium from intracellular stores (Whitaker and Irvine, 1984; Clapper and Lee, 1985; Oberdorf *et al.*, 1986; Payan *et al.*, 1986; Turner *et al.*, 1986), while DAG increases the cytosolic pH by activating the protein kinase C and thence Na^+/H^+ exchange (Swann and Whitaker, 1984). At the level of cell surface calcium may interact with the polyphosphoinositide cycle either by activating PI and PIP kinases (Oberdorf *et al.*, 1989) to produce respectively PIP and PIP_2, or by activating phospholipase C to produce IP_3 and DAG (Whitaker and Aitchison, 1985). Oberdorf *et al.* (1989) imagine the following sequence of events taking place at fertilization: the sperm acts by stimulating a G-protein (Turner *et al.*, 1986, 1987) probably by way of a plasma membrane receptor which still remains to be characterized. The active form of the G-protein, by means of the phospholipase C, mediates the hydrolysis of PIP_2 resulting in an increase of IP_3 and DAG (Ciapa and Whitaker, 1986; Turner *et al.*, 1986). IP_3 causes the release of calcium from intracellular stores (Whitaker and Irvine, 1984). This calcium then stimulates phospholipase C (Whitaker and Aitchison, 1985) to further hydrolyze PIP_2. Simultaneously, the increased Ca^{2+} would stimulate both the PI and PIP kinases (Oberdorf *et al.*, 1989) resulting in a further supply of phospholipase C substrates.

Role of calcium flux during fertilization.

Although the intracellular calcium store can largely account for the calcium peak at fertilization the role of extracellular Ca^{2+} has also been examined. From studies of calcium uptake by whole eggs (Paul and Johnston, 1978; Schmidt *et al.*, 1982; Walter *et al.*, 1989) it appears that influx is rapidly increased after fertilization. Calcium influx was effectively measured at fertilization using brief pulse labeling to limit radioactive backflux (Walter *et al.*, 1989). Using this method, we have shown that in *Paracentrotus lividus*, calcium influx was transiently stimulated 15-fold soon after fertilization and then plateaued at a level 10 times higher than in unfertilized eggs. The increase in calcium uptake occurs in two successive phases:

1) the first phase occurs within one minute following fertilization and is a major component of the fertilization potential (Chambers and De Armendi, 1979; Obata and Kuroda, 1987). It does not however participate in the inhibition of multiple sperm entry via an electrogenic mechanism involving a Na^+-dependent fast block to polyspermy (Jaffe, 1976; Hulser and Schatten, 1982; Schmidt *et al.*, 1982).

2) The second phase consists of a sustained calcium influx lasting 2 to 5 min (Azarnia and Chambers, 1976; Paul and Johnston,

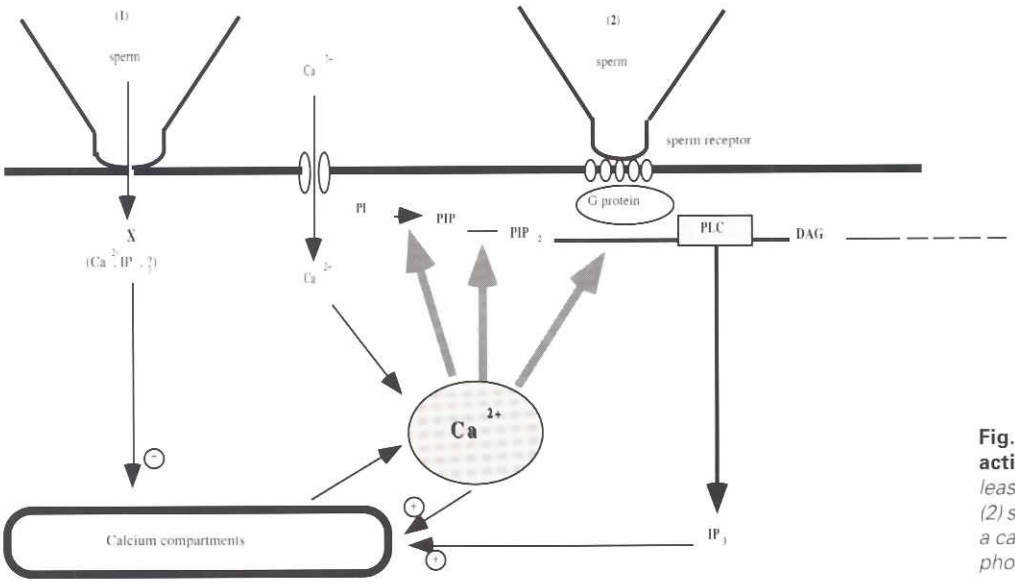

Fig. 1. Two alternative mechanisms for egg activation by sperm. *(1) Sperm acts by releasing an activating factor X (Ca^{2+}, IP_3 or other); (2) sperm acts via specific sperm receptors and a cascade of events linked to the hydrolysis of phosphoinositides.*

1978; Schmidt *et al.*, 1982; Walter *et al.*, 1989). The calcium influx does not play an important role since sperm fusion and mitosis occur normally in Ca^{2+} free-ASW. However, Schmidt *et al.* (1982) noted that under these conditions the cortical reaction was slowed down. Thus, we can only speculate on the role of calcium influx after fertilization. We have shown that during the first ten minutes following fertilization calcium influx represents an amount of calcium corresponding at about 10% of total calcium content (Walter *et al.*, 1989). Although calcium efflux also increases, we cannot estimate its exact value since we do not know the specific activity of internal calcium (Steinhardt and Epel, 1974). From published experiments, it appears that after fertilization the remaining free Ca^{2+} level remains significantly higher than in unfertilized eggs (Poenie *et al.*, 1985; Hafner *et al.*, 1988). We therefore suggest that the simultaneous increase in both influx and efflux could result in the calcium ions recycling through the plasma membrane that would set a new level for the cytosolic free calcium (see review by Alkon and Rasmussen, 1988 for a development of this idea).

How does the calcium wave propagate?

Considering the high buffering and sequestering capacity of the egg cytoplasm, propagation of a wave throughout the egg needs a self-regenerating system. Two mechanisms have been envisioned: an IP_3-induced Ca^{2+}-release loop as proposed by Swann and Whitaker (1986) or a Ca^{2+}-induced Ca^{2+}-release loop similar to that originally proposed by Gilkey *et al.* (1978) and Busa *et al.* (1985) in fish and amphibian eggs. At present in sea urchin egg the available experiments are compatible with the first mechanism schematized in Fig. 2 for the following reasons: 1) The Ca^{2+}-dependence of the phospholipase C hydrolysing PIP_2 is within the micromolar range of the free Ca^{2+} peak at fertilization (Whitaker and Irvine, 1984). 2) IP_3 has a markedly higher mobility than Ca^{2+} in egg cytoplasm compatible with the speed of the wave (Whitaker and Irvine, 1984). 3) Our recent experiments show that Ca^{2+} accumulated in an ATP-dependent manner in the non-mitochondrial compartment of digitonized eggs or isolated cortices is not released by addition of up to 10 μM Ca^{2+} (Girard *at al.*, submitted). In both cases (Ca^{2+}-induced Ca^{2+}-release or IP_3-induced Ca^{2+}-release) the target is thought to be the endoplasmic reticulum. The fact that in sea urchin egg the endoplasmic reticulum is a continuous tubular network containing a calsequestrin-like protein and is tightly attached to the plasma membrane suggests that it may have analogous properties with the endoplasmic reticulum of muscle cells (Sardet and Terasaki, 1988; Henson *et al.*, 1989). Since the endoplasmic network is continuous throughout the cell it should also be considered that propagation of the wave could be an intrinsic property of the endoplasmic reticulum network itself.

How is calcium resequestered?

The transient elevation of Ca^{2+} at fertilization implies that cytosolic free Ca^{2+} must be resequestered. From early experiments it appears that the calcium store cannot be recharged and unloaded again for up to 40 minutes after fertilization (Zucker *et al.*, 1978). The compartment toward which Ca^{2+} is pumped is still being debated. Two distinct cellular sites have been proposed as sinks for the Ca^{2+} releasing during activation. From their experiments using injected aequorin luminescence in stratified eggs, Eisen and Reynolds (1985) suggest that mitochondria buffers the cytoplasmic Ca^{2+}. These authors do not exclude the participation of additional organelles. On the contrary, Ohara and Sato (1986) analyzing the location of chlortetracycline fluorescence in stratified eggs claimed that the endoplasmic reticulum is the main sink for Ca^{2+}. Our recent investigations support the finding of Eisen and Reynolds (1985) attributing a role for mitochondria in calcium resequestration, since in digitonin-permeabilized *Paracentrotus lividus* egg, the succinate-dependent ^{45}Ca uptake rises significantly several minutes after fertilization (Girard *et al.*,submitted).

The role of calcium in egg activation

The reorganization of the egg surface

From the point of sperm entry a wave of cortical granule exocytosis traverses the egg to the antipode slightly lagging behind the calcium wave. The content of the granule mixes with the egg's

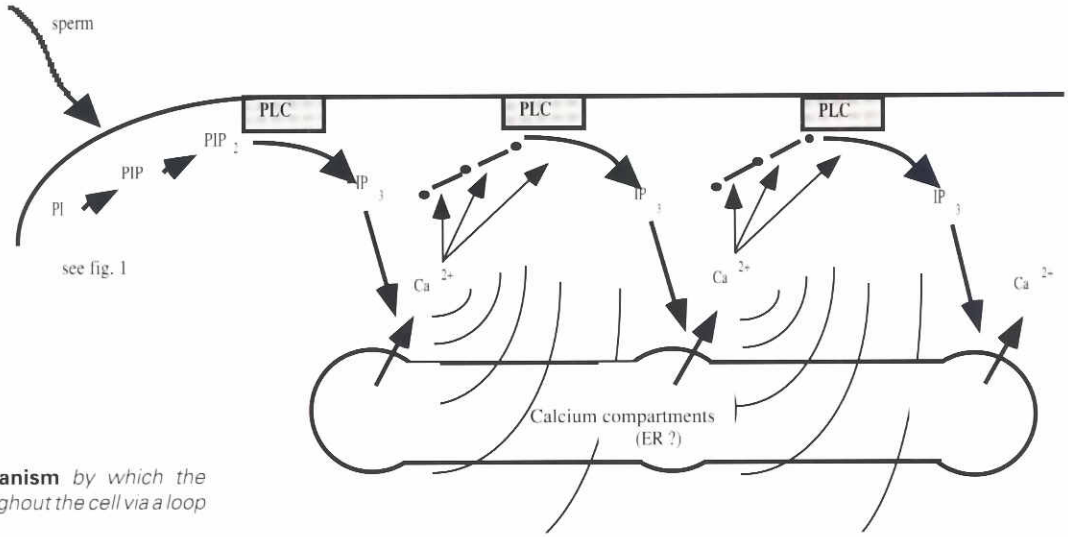

Fig. 2. The auto-catalytic mechanism *by which the calcium wave could propagate throughout the cell via a loop of IP_3-induced Ca^{2+}-release.*

vitelline coat and soon after, a fertilization membrane assembles away from the egg surface while an extracellular layer stabilized by calcium coats the egg surface.

The reorganization also involves the elongation of microvilli, the polymerization of actin filaments, a burst of coated vesicle endocytosis over the entire surface of the egg and the migration of pigmented or acidic vesicles beneath the plasma membrane (see Eddy and Shapiro, 1976; Vacquier, 1981; Kay and Shapiro, 1985; Schuel, 1985; Sardet and Chang, 1987; and Longo, 1989 for review).

Cortical granule exocytosis

After the discovery that cortical granule fusion was associated with a rise in intracellular ionized calcium (Moser, 1939; Steinhardt *et al.*, 1977) many workers have approached the question of the exact role of calcium in exocytosis in whole eggs microinjected with calcium buffers (Mohri and Hamaguchi, 1989), or exposed locally to calcium ionophores (Chambers and Hinkley, 1979), eggs permeabilized by electroporation (Baker *et al.*, 1980) or on preparations of isolated egg cortices, also called cortical granule lawns. In this latter case one obtains a preparation consisting of a plasma membrane sheet to which thousands of cortical granules are attached, ready to fuse (Vacquier, 1975; Sardet, 1984). From these various preparations the following conclusions have been reached: depending on the composition of the milieu, 1 to 12 μM calcium is necessary for exocytosis (Vacquier, 1975; Steinhardt *et al.*, 1977; Baker and Whitaker, 1978; Zucker and Steinhardt, 1978, 1979; Sasaki and Epel, 1983). Calcium is the only ion required (Schön and Decker, 1981; Zimmerberg and Liu, 1988) and probably acts by favoring an osmotically driven fusion step during exocytosis (Zimmerberg *et al.*,1985, Zimmerberg and Whitaker, 1985, Whitaker, 1987). The fact that isolated cortical granules do not swell and burst in the presence of micromolar calcium suggests that the adherent plasma membrane is necessary for the swelling activation of the cortical granule content (Zimmerberg and Liu, 1988). It is likely that this process will be elucidated soon since it is now possible to reassemble isolated plasma membrane and granules into functional fusing partners (Crabb *et al.*, 1987; Whalley and Whitaker, 1988).

Extracellular fertilization membrane and cell coat assembly

Components of the cortical granules and of the vitelline layer are first modified by hydrolytic enzymes, then reorganized and stabilized by crosslinking enzymes released from the cortical granules to form a hardened glycoprotein barrier surrounding the embryo (Kay and Shapiro, 1985). Schön and Decker (1981) provided the first electron microscopic evidence indicating that calcium is required for fertilization membrane thickening. Since then, Weidman *et al.*, (1985) have identified and isolated a protein called proteoliaisin that appears to mediate the insertion of ovoperoxidase, a fertilization membrane component and cross linker into the fertilization membrane. Battaglia and Shapiro (1988), using eggs from the sea urchin *Strongylocentrotus purpuratus*, indicate that an egg surface transglutaminase also participates in an early event of fertilization membrane assembly. This transglutaminase is Ca^{2+}-dependent and its maximal activity occurs within the first minutes of activation, when hardening of the fertilization membrane is achieved.

Ultimately, the embryo must escape from this extremely resistant envelope at the blastula stage. This process is mediated by a specific enzyme referred to as the hatching enzyme. The enzyme is a Ca^{2+}-activated protease (Edwards *et al.*, 1977). Recents results of Lepage and Gache (1989) show that the enzyme is optimally actived by Ca^{2+} at the calcium concentration of 10 mM – that of sea water.

A thick extracellular coat made of proteins previously sequestered in cortical granules and other vesicles assembles at the egg surface after fertilization and plays an important role in blastomere adhesion and cell morphogenesis. The presence of calcium is essential to the integrity of this layer. One of its main components, hyalin, a high molecular weight protein (or proteins) seems to be first secreted as a wave from a minority of cortical granules. A little later the rest is released and patches of hyalin coalesce forming a continuous extracellular layer that is subsquently modified (Mc Clay and Fink, 1982; Alliegro and Mc Clay, 1988).

Microvilli elongation and endocytosis

Just as there is a wave of cortical granule exocytosis, there is also a wave of cortical actin polymerization from the point of sperm entry (Yonemura and Mabuchi, 1987). It takes about two minutes

to propagate over the entire cortex. At about this time vericosed microvilli appear on the entire surface of the egg. They organize and extend over the next 5 minutes into finger-like projections (Chandler and Heuser, 1981). By manipulating the external Na content and activating the egg with weak amine bases to affect intracellular calcium rise and alkalinization of the cytoplasm, Begg *et al.*, (1982) and Carron and Longo (1982) showed that a rise in cytoplasmic pH was sufficient for actin bundle formation. However, the stiffening and full elongation of microvilli presumably required the elevation of cytoplasmic calcium. Many actin-modulating proteins have been isolated in recent years from sea urchin eggs. At least four of them are calcium sensitive: an egg alpha-actinin (Mabuchi *et al.*, 1985), a 100-kD actin-fragmenting protein (Hosoya *et al.*, 1986), a 45-kD protein (Ohnuma and Mabuchi, 1988) that is similar to gelsolin and an egg spectrin which is a Ca^{2+}-regulated actin cross-linking protein (Kuramochi *et al.*, 1986; Fishkind *et al.*, 1987). How these proteins regulate actin bundle formation and elongation is not yet clear.

Two others major processes complete the reorganization of the cortical layer: a burst of coated vesicle endocytosis (3-5 minutes after fertilization) and a migration of large pigmented or acidic vesicles from the interior of the egg beneath the egg's plasma membrane (Fisher and Rebhun, 1983; Lee and Epel, 1983; Sardet, 1984). The endocytosis appears to be linked to prior exocytosis of cortical granules and is therefore indirectly triggered by the calcium wave although it is not known if this endocytotic burst occurs also as a wave (Fisher *et al.*, 1985).

The role of calcium in metabolic activation

This subject has already been amply reviewed previously. The relative role of Ca^{2+} and pH in activating protein synthesis and various key enzymes has been discussed (Epel, 1982; Whitaker and Steinhardt, 1982, 1985; Epel, 1989). In this field new approaches are necessary to dissect the complex steps of activation and study key enzymes (kinases, phospholipases, phosphatases, etc...) *in situ*. In that sense the type of approach undertaken to study the activity of the G6PDH in permeabilized eggs and embryos seems to hold promise for the future (Swezey and Epel, 1988).

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